UNIVERSITY OF LIVERPOOL

EARLY DETECTION OF DIABETIC MACULAR OEDEMA

Thesis submitted in accordance with the requirements of the University of Liverpool for the degree of Doctor in Philosophy

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STATEMENT OF ORIGINALITY

I declare that this thesis was composed by me and that the work contained therein is my own, except where explicitly stated otherwise in the text. The work within this thesis has not been submitted for any other degree or professional qualification.

ABSTRACT

Early Detection of Diabetic Macular Oedema (EDDMO) Study

PhD Candidate: Jae Yee Ku

Background

Diabetic retinopathy (DR) is the second leading cause of visual loss in working-age adults in the United Kingdom (UK) after inherited eye disease, and is asymptomatic in its early stages. Visual loss from DR is commonly due to diabetic macular oedema (DMO) which current screening methods cannot detect directly. The handheld radial shape discrimination (hRSD) test, has been approved by the US Food and Drug Administration (FDA) as a means of detecting metamorphopsia, and therefore maculopathy. There is also emerging evidence that DR is a neurodegenerative disease resulting in thinning of the ganglion cell complex detected by optical coherence tomography (OCT) in early DR. This thesis describes studies of people with diabetes (PWD) and healthy controls (HC) investigating two emerging approaches, namely hRSD and OCT in the early detection of DMO.

Methods

Macular function was measured using hRSD, distance and near visual acuity (VA) and macular structure was assessed using Heidelberg Spectralis OCT. Retinal layers segmentation and mean thicknesses were measured across all Early Treatment Diabetic Retinopathy Study (ETDRS) subfields using the Heidelberg auto-segmentation software with manual adjustment as needed. One eye from each participant was randomly selected for analysis.

Results

292 PWD (mean±SD 54±14 years, 175 males) referred from the local screening programme to hospital clinics as being at risk of DMO were recruited. 229 healthy participants (age 44±18 years; 94 males) were also recruited, of whom 50 (55±14 years, 26 males) were used as age-matched controls for the PWD.

Compared to HC, hRSD performance and distance VA were progressively worse in PWD with no or minimal DR, (hRSD logMAR: HC -0.77±0.11, no DR -0.68±0.18, minimal DR -

0.61±0.25, ANOVA $p<0.001$; distance VA logMAR: HC -0.08±0.12, no DR 0.03±0.15, minimal DR 0.06±0.16, ANOVA $p<0.001$).

Compared to HC there was a reduction in full retinal thickness across most subfields in PWD with no or minimal DR. This reduction was driven by thinning in the outer nuclear layer (ONL) in the central subfield (CSF), ganglion cell layer (GCL) and inner plexiform layer (IPL) in the inner subfields and retinal nerve fibre layer (RNFL) in the outer subfields compared to HC. In the outer subfields, there was also thinning in the retinal pigment epithelium (RPE) in PWD with no DR and thinning in the GCL and IPL in PWD with minimal DR.

Longitudinal data were available for 159 PWD (54±15 years, 97 males) who attended for a second visit after 191±86 days. In PWD with no or minimal DR, there was a significant decrease in GCL (visit 1 37.73±3.56µm, visit 2 37.27±3.84µm, $t=2.523$, $p=0.020$), IPL (visit 1 31.98±2.48µm, visit 2 31.61±2.69µm, $t=2.517$, $p=0.020$) and inner nuclear layer (INL) (visit 1 33.89±1.92µm, visit 2 32.96±1.11µm, $t=3.129$, $p=0.005$) between visits.

Conclusions

Functional and structural changes are detectable in the early pathogenesis of DR, consistent with neuroretinal thinning developing before microvascular abnormalities. Functional changes detected by the hRSD test in PWD with early DR have not been previously demonstrated. Findings from the Early Detection of Diabetic Macular Oedema (EDDMO) study add further support to the concept of pre-clinical retinopathy.

PUBLICATIONS AND CONFERENCES

Publications directly related to results presented in this thesis

Ku, J., Milling, A., Pitrelli Vazquez, N., et al. (2016) 'Performance, usability and comparison of two versions of a new macular vision test: the handheld Radial Shape Discrimination test', *PeerJ*, 4, pp. e2650.

Publications arising from the EDDMO study but not containing results presented in this thesis

Zhu W., **Ku J.**, Zheng Y., et al. (2020) 'Spatial linear mixed effects modelling for OCT images: SLME model', *J. Imaging*, 6(44).

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ACKNOWLEDGEMENTS

To reach mountaintops, I have walked through many valley lows, and Jesus, my LORD and saviour, has been with me.

I thank God that he has made me inadequate so that I may completely depend on him.

I thank God for giving me the best family possible who have nurtured and supported me through all my doubts and failures.

I thank God for excellent supervisors, especially Dr Paul Knox and Professor Simon Harding to whom I am deeply indebted to, and for exceptional collaborators, friends and colleagues who have made this journey possible.

Jesus replied, 'What is impossible with man is possible with God.'

Luke 18:27 NIV

LIST OF ABBREVIATIONS

ABBREVIATIONS	DEFINITIONS
ACCORD	Action to Control Cardiovascular Risk in Diabetes study
ACE	Angiotensin-converting enzyme
AD	Absolute difference
ADA	American Diabetes Association
AFC	Alternative forced choice
AG	Amsler grid
AGE	Advanced glycation end products
AMD	Age-related macular degeneration
ANOVA	Analysis of variance
ART	Automatic real-time tracking
ARVO	Association for Research in Vision and Ophthalmology
AUC	Area under the curve
BCVA	Best-corrected visual acuity
BMI	Body mass index
BP	Blood pressure
BRB	Blood-retinal barrier
CI	Confidence interval
CIMO	Centre involving macular oedema
CPT	Centre point thickness
CS	Contrast sensitivity
CSF	Central subfield
CSMO	Clinically significant macular oedema
CST	Central subfield thickness
CTMO	Centre threatening macular oedema
CWS	Cotton wool spots
DA	Disc area
DCCT	Diabetes Control and Complications Trial
DD	Disc diameter
DEC	Diabetic eye clinic
DMI	Diabetic macular ischaemia
DMO	Diabetic macular oedema
DNA	Deoxyribonucleic acid
DPPOS	Diabetes Prevention Program Outcomes study
DR	Diabetic retinopathy
DRAMA	Diabetic Retinopathy And the MyVisionTrack App study
DRCR	Diabetic Retinopathy Clinical Research network
DRIL	Disorganisation of the retinal inner layers
DRN	Diabetic retinal neuropathy
DRS	Diabetic Retinopathy Study
DRVS	Diabetic Retinopathy Vitrectomy Study
DS	Digital Surveillance

EDDMO	Early Detection of Diabetic Macular Oedema study
EDI	Enhanced depth imaging
EDIC	Epidemiology of Diabetes Interventions and Complications study
ELM	External limiting membrane
ENDESP	English Diabetic Eye Screening Programme
ERG	Electroretinogram
ERM	Epiretinal membrane
ETDRS	Early Treatment Diabetic Retinopathy Study
EUROCONDOR	European Consortium for the Early Treatment of Diabetic Retinopathy
FA	Fluorescein angiography
FAZ	Foveal avascular zone
FDA	Food and Drug Administration
FDT	Frequency doubling technology
FIELD	Fenofibrate Intervention and Event Lowering in Diabetes study
FPD	Fibrovascular proliferation disc
FPE	Fibrovascular proliferation elsewhere
FPR	False positive rate
GAPDH	Glycer-aldehyde-3 phosphate dehydrogenase
GCL	Ganglion cell layer
GP	General practitionersGP
HbA_{1c}	Glycated haemoglobin
HC	Healthy controls
HDL	High-density lipoprotein
HES	Hospital eye services
HMA	Haemorrhages and or microaneurysms
ICC	Intra-class correlation
ICO	International Council of Ophthalmology
IIM	Inferior inner macula
ILM	Internal limiting membrane
INL	Inner nuclear layer
IOM	Inferior outer macula
IOP	Intra-ocular pressure
IPL	Inner plexiform layer
IRMA	Intraretinal microvascular abnormalities
IS	Inner subfields
ISDR	Individualised Screening for Diabetic Retinopathy study
LDES	Liverpool Diabetic Eye Study
LDESP	Liverpool Diabetic Eye Screening Programme
logMAR	logarithm of the minimum angle of resolution
MODY	Maturity-onset diabetes of the young
MONARCH	Monitoring for nAMD Degeneration Reactivation at Home study
MRFIT	Multiple Risk Factor Intervention Trial
NA	Not available
nAMD	Neovascular age-related macular degeneration
NCTMO	Non-centre threatening macular oedema

NDESP	NHS Diabetic Eye Screening Programme
NHS	National Health Service
NICE	National Institute for Health and Care Excellence
NIM	Nasal inner macula
NMO	No macular oedema
NOM	Nasal outer macula
NPDR	Non-proliferative diabetic retinopathy
NT	Not treated
NVD	Neovascularisation at the optic disc
NVE	Neovascularisation elsewhere
NVI	Neovascularisation at the iris
OCT	Optical coherence tomography
OCTA	Optical coherence tomography angiography
ONL	Outer nuclear layer
OPL	Outer plexiform layer
OS	Outer subfields
PARP	Poly (ADP-ribose) polymerase
PDR	Proliferative diabetic retinopathy
PHP	Preferential hyperacuity perimetry
PKC	Protein kinase C
PRH	Pre-retinal haemorrhage
PRP	Peripheral retinal photocoagulation
PWD	People with diabetes
RAAS	Renin-angiotensin-aldosterone system
RAGE	Receptor for advanced glycation end products
RD	Relative difference
RDS	Routine digital screening
RNFL	Retinal nerve fibre layer
ROC	Receiver operating characteristic
ROS	Reactive oxygen species
RPE	Retinal pigment epithelial
hRSD	handheld radial shape discrimination
SD	Standard deviation
SD-OCT	Spectral-domain optical coherence tomography
SDH	Shape discrimination hyperacuity
SE	Study eye
SIM	Superior inner macula
SLBS	Slit-lamp biomicroscopy surveillance
SOM	Superior outer macula
SS-OCT	Swept-source optical coherence tomography
STDR	Sight-threatening diabetic retinopathy
TD-OCT	Time-domain optical coherence tomography
TIM	Temporal inner macula
TMV	Total macular volume
TOM	Temporal outer macula

TPR	True positive rate
TRD	Traction retinal detachment
TT	Treated
UK	United Kingdom
UKPDS	United Kingdom Prospective Diabetes Study
VA	Visual acuity
VB	Venous Beading
VEGF	Vascular endothelial growth factor
VH	Vitreous haemorrhage
VL	Venous loop
VMA	vitreomacular attachment
VMT	Vitreomacular traction
VR	Venous reduplication
WESDR	Wisconsin Epidemiologic Study of Diabetic Retinopathy study
YAG	yttrium aluminium garnet

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CHAPTER 1. THESIS INTRODUCTION

Diabetes has been known since antiquity and has been described in ancient Egyptian, Indian and Chinese medical literature (Karamanou et al., 2016). However, it was not until 1890 that Von Mering and Minkowski (1890) demonstrated that the pancreas was linked to glucose homeostasis. Their work paved the way for Banting et al. (1922) to discover insulin and treat their first patient with this new medication in 1922. This pioneering work and those of scientists since have contributed to the understanding and management of diabetes, saving countless lives from a disease that was historically thought incurable (Karamanou et al., 2016).

Diabetes affected around 463 million people globally in 2019 and this number is projected to increase to 578 million by 2030 (International Diabetes Federation, 2019). In the UK, approximately 7% of the population is living with diabetes (Whicher et al., 2020). Type 2 diabetes accounts for approximately 90% of diabetes worldwide while type 1 and gestational diabetes are the other main categories of diabetes (International Diabetes Federation, 2019). Diabetes causes microvascular complications of retinopathy, nephropathy and neuropathy, and macrovascular complications of cardiovascular and cerebrovascular disease (Forbes and Cooper, 2013). The National Health Service (NHS) spends at least £10 billion annually on diabetes, equivalent to 10% of its budget, of which 80% is spent on treating diabetes complications (Whicher et al., 2020).

Approximately 35% of PWD have DR (Yau et al., 2012). DR is asymptomatic in the early stages and is the leading cause of sight loss for working-age adults (International Diabetes Federation, 2019). In 1968, a group of experts met in a symposium in Airlie House, Virginia to discuss DR and they developed a standardised classification of DR (Goldberg and Jampol, 1987). This original classification was adapted and used in many major studies such as the Diabetic Retinopathy Study (DRS) (DRS, 1981), the ETDRS study (ETDRS, 1991e) and the Wisconsin Epidemiologic Study of Diabetic Retinopathy (WESDR) (Klein et al., 1984b). The ETDRS found that feature specific DR grading classifications enabled risk assessment of patients for developing proliferative DR (PDR) and clinically significant macular oedema (CSMO), which allowed timely treatment with scatter peripheral retinal photocoagulation (PRP) and macular (focal and grid) laser (ETDRS, 1985a). Based on these pivotal findings, early detection of DR by screening has been introduced in several countries and shown to improve the quality of life of patients and reduce healthcare burden (Lanzetta et al., 2020).

Over half a century has passed since the inception of the original Airlie House DR classification, which formed the basis of the current NHS Diabetic Eye Screening Programme (NDESP) protocols (NHS Diabetic Eye Screening Programme, 2012). The method of DR screening used in the UK based on fundus photography has changed little over the years. Meanwhile, imaging in ophthalmology has been transformed by OCT technologies introduced in 1991 (Huang et al., 1991) and the focus of DR care increasingly shifted to managing maculopathy and DMO. Treatment has been revolutionised with the introduction of anti-vascular endothelial growth factor (VEGF) therapy, which was first approved by the US FDA to treat neovascular age-related macular degeneration (nAMD) in 2004 (Gragoudas et al., 2004), and then for CSMO in 2012 (Brown et al., 2013). The National Institute for Health and Care Excellence (NICE) has since approved anti-VEGF therapy to treat nAMD and DMO in the UK (NICE, 2008, NICE, 2013).

OCT produces cross-sectional images of optical reflectivity in the retina and is now used routinely for diagnosing and monitoring DMO (Virgili et al., 2015). Anti-VEGF therapy can treat DMO effectively and it is particularly useful in eyes not amenable to macular laser (Heier et al., 2016, Romero-Aroca et al., 2014).

It has been recognised that feature specific grading in DR screening provides surrogate biomarkers for progression to CSMO, but that it cannot detect DMO directly (Bresnick et al., 2000, Olson et al., 2013). This has led to new issues in the interface between screening services and hospital eye services (HES). For example, one study found that in a local screening service only 119 out of 311 (38.3%) PWD referred to hospital services as maculopathy suspects had OCT evidence of macular oedema. This means that most of the participants in the study did not require a referral (Mackenzie et al., 2011).

Given the success of anti-VEGF therapy in treating DMO (Sharma, 2020), and the need to reduce unnecessary referral to hospital services, there has been a new impetus to improve the early detection of DMO in DR screening. The most obvious solution would be to introduce OCT alongside fundus photography. A health technology assessment concluded that introducing OCT would result in overall cost savings to the healthcare system (Olson et al., 2013). However, the initial capital costs, including workforce training, have prevented the adoption of this approach for routine use in the UK.

One practical way forward would be to introduce an additional test to complement photographic screening to improve the detection of DMO. Such a test would need to be

inexpensive, easy to complete from both a service and patient perspective, and to have good discriminative performance. The hRSD test has been shown to be able to detect the early stages of nAMD, but there is limited data on its ability to detect DMO (Pitrelli Vazquez et al., 2018, Wang et al., 2016). In this study, the ability of the hRSD test, distance and near VA to detect eyes with DMO, and discriminate between different levels of DMO, will be explored.

There has been much recent interest in the examination of the eyes as a non-invasive method to study the central nervous system in neurodegenerative diseases. Anatomically and developmentally, the retina is an extension of the central nervous system. Although the eyes are figuratively the window to the soul, they can biologically be considered as the windows to the brain (MacCormick et al., 2014). There has been a recognition that other neurodegenerative diseases are linked to diabetes. For example, Alzheimer's disease has been coined type 3 diabetes because of the shared molecular and cellular features between the two conditions (Kandimalla et al., 2017). There has been interest in using macular OCT angiography (OCTA) as a biomarker in detecting preclinical Alzheimer's disease (van de Kreeke et al., 2020).

Emerging evidence shows that DR is not only a microvascular disease but also a neurodegenerative condition and which may precede microvasculopathy (Liu et al., 2019, Reis et al., 2014, Sohn et al., 2016). The American Diabetes Association (ADA) has now defined DR as a highly tissue specific neurovascular complication of diabetes (Solomon et al., 2017). This has led to recently coined terms such as diabetic neuroretinopathy or diabetic retinal neuropathy (DRN). In PWD with no or minimal DR, there are electroretinogram (ERG), contrast sensitivity (CS), colour vision, dark adaptation and microperimetry abnormalities (Bearse et al., 2006, Hardy et al., 1992, Greenstein et al., 1993, Jackson et al., 2012).

Most studies on neurodegeneration in the eye have largely evaluated the retinal thickness of the ganglion cell complex, comprising the ganglion cell axons, cell bodies and dendrites, which reside in the RNFL, GCL and IPL respectively (Scuderi et al., 2020). This may be because glaucoma, which is also a neurodegenerative condition, has mainly used visual field tests and ganglion cell complex thinning detected by OCT as surrogate markers for disease progression (Kim and Park, 2018). Studies in PWD with no or early DR have also found thinning of the ganglion cell complex detected by OCT, thus supporting DR as a

neurodegenerative condition (van Dijk et al., 2009, van Dijk et al., 2010, Rodrigues et al., 2015, Vujosevic and Midena, 2013, Araszkiewicz et al., 2012). Therefore, studies on DRN have mainly examined the retinal thickness of the ganglion cell complex while the other layers remained unexplored.

Rapid improvement in OCT imaging technology has facilitated *in vivo* analysis of individual retinal layers. The Heidelberg Spectralis OCT used in this study, introduced retinal auto-segmentation software a few years ago. This has made the evaluation of different retinal thickness measurements across all ETDRS subfields an easier task. This new tool provides an excellent opportunity to examine retinal thickness changes in different retinal layers, especially in early DR.

This thesis aims to explore both functional and structural data in PWD by using hRSD, distance and near VA as functional assessments and by using OCT retinal thickness as structural measurements. Chapter 2 will provide a literature review on diabetes, DR screening and the hRSD test. Participants and general methods will be described in Chapter 3. A literature review on OCT will be presented in Chapter 4 with a discussion of OCT methods and definitions. Results obtained from large participant groups will be reported and discussed in Chapters 5 to 9 and the thesis will conclude with a general discussion in Chapter 10.

CHAPTER 2. LITERATURE REVIEW ON DIABETES, DIABETIC RETINOPATHY, DIABETIC RETINOPATHY SCREENING AND NEW APPROACHES TO DETECT DIABETIC MACULAR OEDEMA

2.1 CHAPTER INTRODUCTION

In this chapter, the literature on diabetes, DR, DR screening, DR management and new approaches to detecting DMO is reviewed. A critical appraisal of the existing literature on the hRSD test in the detection of DR is made. In this thesis, DR is used as a general term covering all categories and severities of diabetic eye diseases including DMO. Diabetes is characterised by hyperglycaemia due to insulin deficiency in type 1 diabetes or insulin resistance in type 2 diabetes. Type 1 diabetes is due to an autoimmune process, that destroys the pancreatic β cells in the islets of Langerhans. In type 2 diabetes, there is peripheral insulin resistance and secondary hypersecretion of insulin. In both cases, these lead to hyperglycaemia (Forbes and Cooper, 2013).

Despite many advances, the fundamental pathophysiology of diabetic complications including DR remains uncertain (Cohen and Gardner, 2016). However, it is becoming apparent that the onset of diabetes and the subsequent development of diabetes-related complications are multifactorial with genetic and environmental factors involved (Figure 2.1) (Forbes and Cooper, 2013).

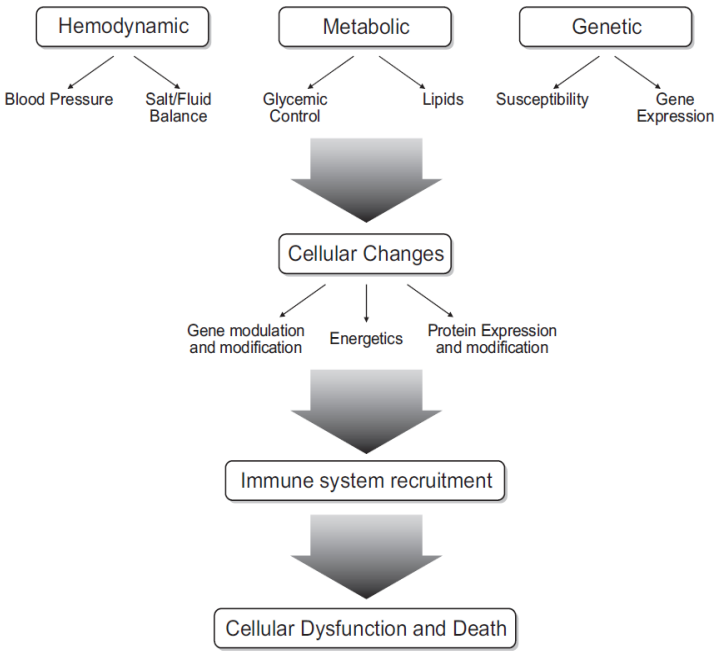


Figure 2.1. Schematic overview of the major pathways contributing to diabetic complications (Forbes and Cooper, 2013).

2.2 DIABETES

As diabetes is a systemic disease, a review of the medical diagnosis and management of diabetes is made followed by an exploration of how systemic risk factors such as hypertension contribute to the disease before moving onto how diabetes affects the eyes.

2.2.1 SCREENING, DIAGNOSIS AND MONITORING OF DIABETES

The National Institute of Health and Clinical Excellence (NICE) in the UK recommends screening high-risk individuals for diabetes. High-risk individuals include those with symptoms of diabetes, raised body mass index (BMI) and people of some ethnic backgrounds such as those of South Asian, Chinese and African. As part of this process, the NHS Health Check programme was established in 2009 as an integrated pathway to screen, identify and prevent diabetes, cardiovascular disease, stroke and kidney disease in people aged 40-74 (NICE, 2012). The diagnosis of diabetes is based on four blood tests, namely, fasting plasma glucose, oral glucose tolerance test, glycated haemoglobin (HbA_{1c}) and random plasma glucose. Based on these tests, individuals can be classified as having diabetes, impaired glucose tolerance or impaired fasting glucose. The values for these tests are shown in Figure 2.2 (International Diabetes Federation, 2019).

HbA_{1c} is used for both the diagnosis of diabetes and in the monitoring of glycaemic control. HbA_{1c} is formed when glucose in the peripheral blood is attached to haemoglobin. Because the lifespan of the haemoglobin is two to three months, HbA_{1c} values provide average blood glucose over that period (Inzucchi, 2012). For the diagnosis of diabetes, individuals need to have an HbA_{1c} of ≥ 48 mmol/mol (Table 2.1) (International Diabetes Federation, 2019). Some studies consider individuals with an HbA_{1c} of 42-47 mmol/mol (6.0-6.5%) as having impaired glucose tolerance, which is commonly termed pre-diabetes; these individuals will be at a high risk of developing diabetes with an incidence of 25-50% over 5 years (John, 2012, Zhang et al., 2010).

For the monitoring of glycaemic control, NICE recommends HbA_{1c} targets for adults with type 1 and 2 diabetes to be tailored to the individual. The HbA_{1c} target for most individuals with type 1 and type 2 diabetes is 48mmol/mol (6.5%). However, for patients with type 2 diabetes on medications associated with hypoglycaemia, the target is set at 53mmol/mol (7.0%). This target is more relaxed if the individual is elderly, frail or has other co-morbidities (NICE, 2015b, NICE, 2015c). For individuals needing tighter glycaemic

monitoring, such as those on insulin or prone to hypoglycaemia, they may perform regular glycaemic monitoring with a pinprick test, and then document the results for review with their healthcare professionals.

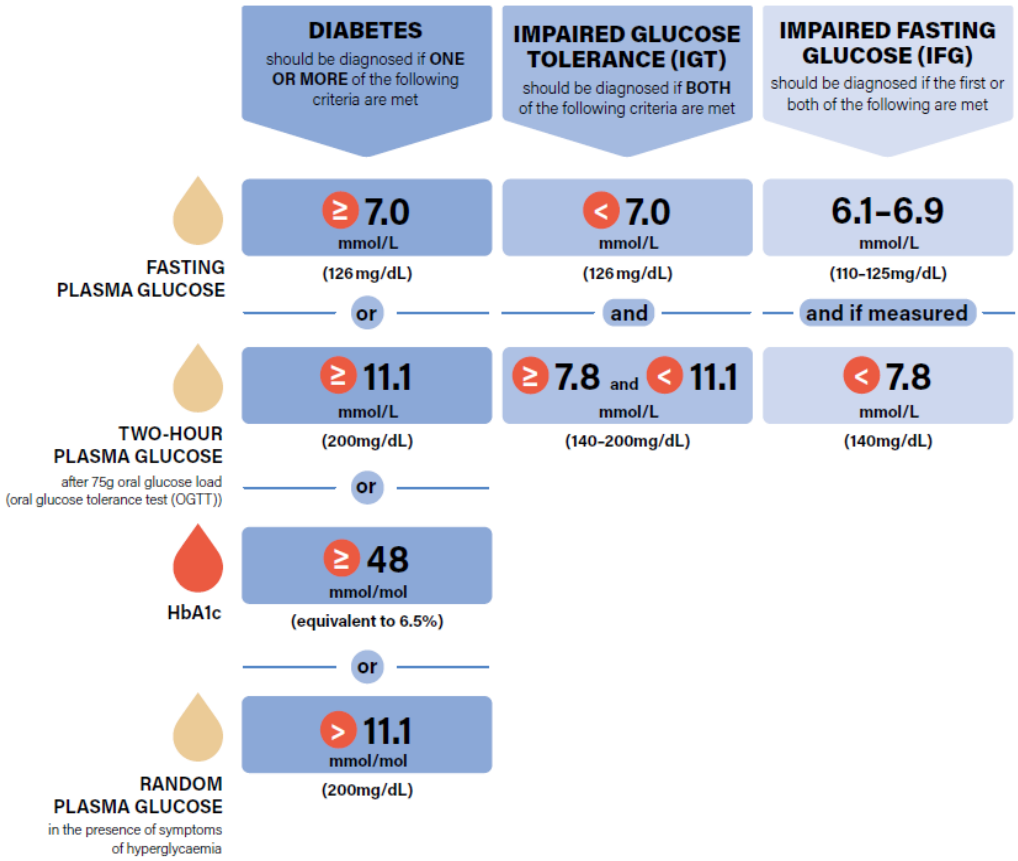


Figure 2.2. International Diabetes Federation diagnostic criteria for diabetes, impaired glucose tolerance and impaired fasting glucose (International Diabetes Federation, 2019).

2.2.2 ROLE OF HYPERGLYCAEMIA AND SYSTEMIC CONTROL

The benefits of intensive glycaemic control have been conclusively demonstrated in pivotal randomised clinical trials. The Diabetes Control and Complications Trial (DCCT) (Shamoon et al., 1993) and the Epidemiology of Diabetes Interventions and Complications (EDIC) study (EDIC., 1999), provided the key evidence upon which type 1 diabetes management is based. The DCCT studied 1441 patients with type 1 diabetes aged 13-39 years with no or mild DR at baseline over 6.5 years; the study showed that intensive blood sugar level control reduced the risk of diabetes complications such as retinopathy, nephropathy, and neuropathy by 35% to 90% compared with controls who received conventional treatment. Specifically, the DCCT found a 26% risk reduction in developing DMO with intensive glycaemic control. It also revealed that the timing of control was important: intensive

control was most effective before the onset of complications (Shamoon et al., 1993). The EDIC study followed up participants of the original DCCT study over 10 years; it showed that even when the intensive control was stopped, the rate of progression of complications remained less in the former intensive treatment group (EDIC., 1999). This phenomenon has been termed glycaemic memory, metabolic memory or legacy effect (Genuth et al., 2002). For type 2 diabetes, the pivotal randomised clinical trial was the United Kingdom Prospective Diabetes Study (UKPDS), which examined 3867 patients newly diagnosed with type 2 diabetes over 10 years; the study found that intensive glycaemic control reduces the risk of progression to DR by 17%, reduces the need for laser photocoagulation by 29%, reduces the development of vitreous haemorrhage by 23% and reduces the risk of legal blindness by 16% (Turner et al., 1998). Similar to the DCCT, the period of intensive control provided a legacy effect to produce a long-term reduction in other complications (Holman et al., 2008).

Hyperglycaemia affect cells differently. Most cells can regulate glucose transport during hyperglycaemia. However, cells that are unable to regulate their glucose transport efficiently during hyperglycaemia are vulnerable. These cells include the capillary endothelial cells in the retina, mesangial cells in the renal glomerulus and peripheral neurons and Schwann cells in the peripheral nerves (Brownlee, 2005).

In these vulnerable cells, hyperglycaemia causes mitochondrial overproduction of reactive oxygen species (ROS) causing oxidative stress (Figure 2.3). The resultant deoxyribonucleic acid (DNA) damage activates poly (ADP-ribose) polymerase (PARP), which is a family of proteins that plays a critical role in DNA repair. PARP modifies and decreases the activity of a key glycolytic enzyme glycer-aldehyde-3 phosphate dehydrogenase (GAPDH). This, in turn, leads to four major downstream mechanisms: 1. increased the flux of glucose and other sugars through the polyol pathway, 2. activation of protein kinase C (PKC) isoforms 3. overactivity of the hexosamine pathway and 4. increased intracellular formation of advanced glycation end (AGE) products (Brownlee, 2005, Giacco and Brownlee, 2010, Safi et al., 2014). These established principle pathways will now be discussed in detail.

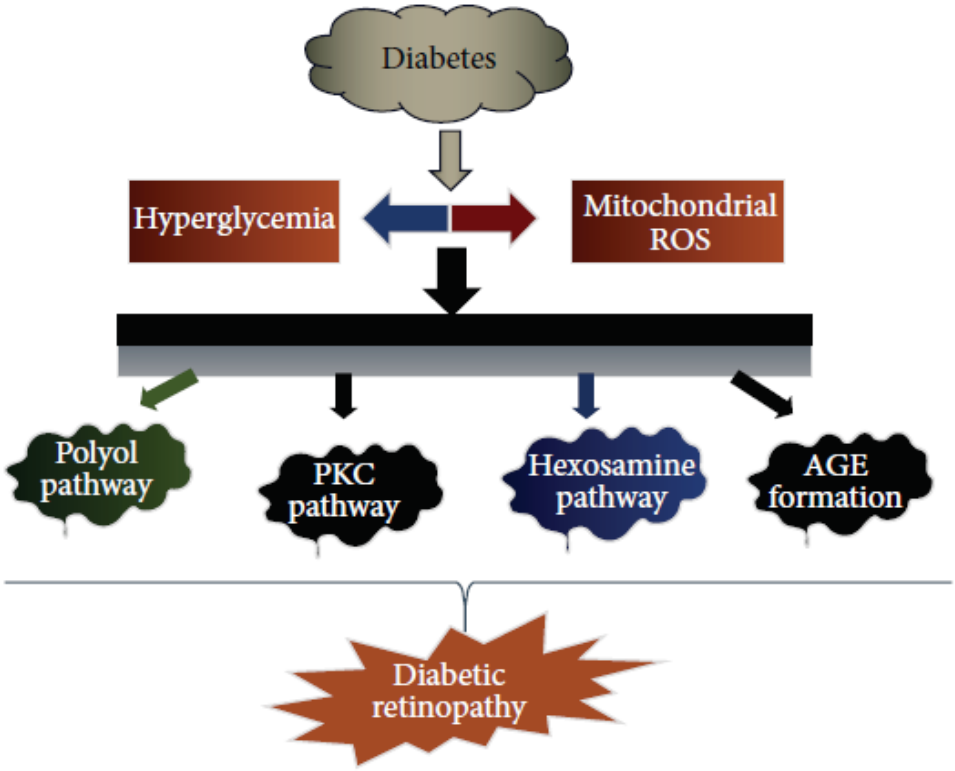


Figure 2.3. The four major mechanisms involved in the development of diabetic retinopathy are increase flux of glucose and other sugars in the polyol pathway, activation of protein kinase C (PKC) pathway, overactivity of the hexosamine pathway and increase the formation of advanced glycation end products (AGE) formation (Safi et al., 2014).

2.2.2.1 Increased polyol pathway flux

Cells usually obtain energy from glucose through phosphorylation using the enzyme hexokinase. During hyperglycaemia, hexokinase becomes saturated and excess glucose enters the polyol pathway instead. Aldose reductase is the main enzyme in the polyol pathway of glucose metabolism (Figure 2.4) (Safi et al., 2014). It normally functions to reduce toxic aldehydes in the cell to inactive alcohol but during hyperglycaemia, aldose reductase also converts glucose to sorbitol. Activation of the polyol pathway consumes the cofactor nicotinamide adenine dinucleotide phosphate (NADPH) that leads to decreased glutathione, nitric oxide and other anti-oxidants. This process generates AGEs, which are mediated by an AGE receptor (RAGE) (Giacco and Brownlee, 2010). Sorbitol can accumulate in cells, as it cannot diffuse out of them easily. The resultant osmotic forces cause water to diffuse into the cell causing damage (Yanoff, 2014).

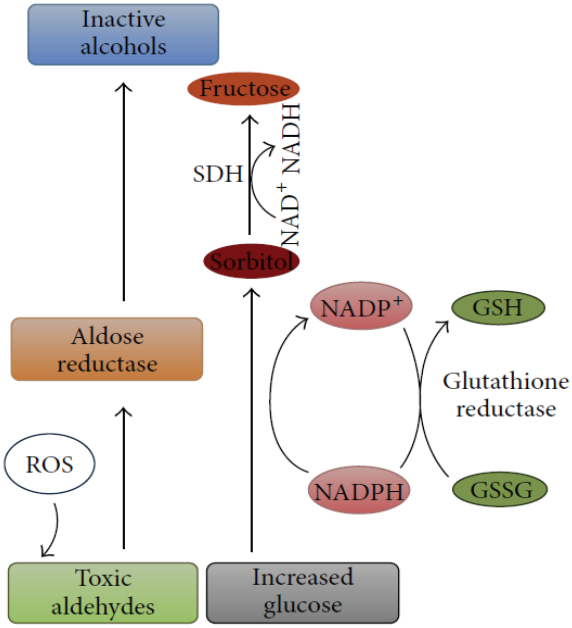


Figure 2.4. Hyperglycaemia increases flux through the polyol pathway. Aldose reductase reduces aldehydes generated by reactive oxygen species (ROS) into inactive alcohols and glucose into sorbitol using adenine dinucleotide phosphate (NADPH) as a cofactor. Glutathione (GSH), glutathione disulfide (GSSG), sorbitol dehydrogenase (SDH) (Safi et al., 2014).

2.2.2.2 Protein kinase C (PKC) activation

Hyperglycaemia increases ROS production, which in turn increases diacylglycerol. Diacylglycerol is a critical activating cofactor for PKC and its other isoforms (Giacco and Brownlee, 2010). This has multiple consequences such as increased VEGF that can cause neovascularisation in the eye (Figure 2.5; Section 2.3.5) (Brownlee, 2005).

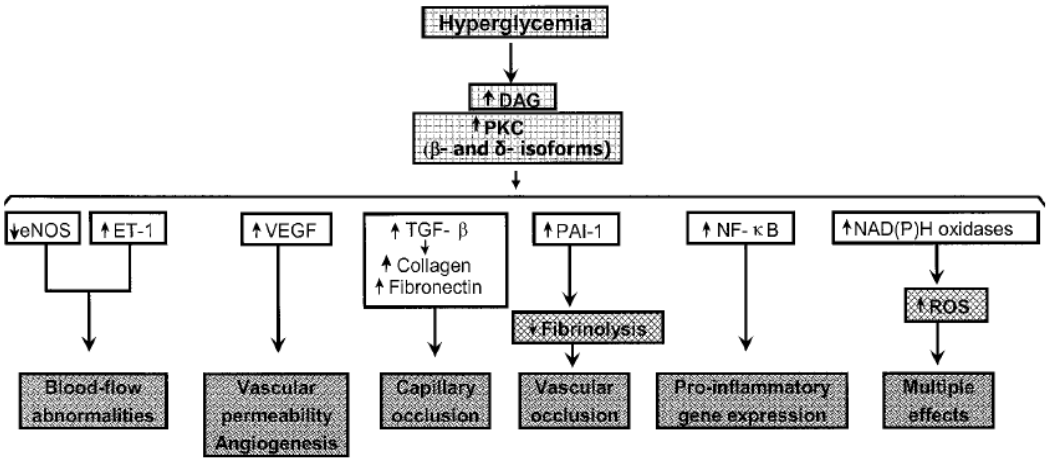


Figure 2.5. Consequences of hyperglycaemia-induced activation of protein kinase C (PKC) (Brownlee, 2005).

2.2.2.3 Increased hexosamine pathway activity

When glucose enters a cell, it is converted to fructose-6 phosphate. Most of this is metabolised via the glycolytic pathway but some enter the hexosamine pathway (Figure 2.6). Hyperglycaemia increases the flux of fructose 6-phosphate into the hexosamine pathway. In this pathway, the enzyme glutamine: fructose-6 phosphate amidotransferase (GFAT) converts fructose-6 phosphate to glucosamine-6 phosphate and eventually to uridine diphosphate (UDP) *N*-acetyl glucosamine (Brownlee, 2005). This disrupts gene transcription and cellular functions and lead to diabetic complications (Yumnamcha et al., 2020).

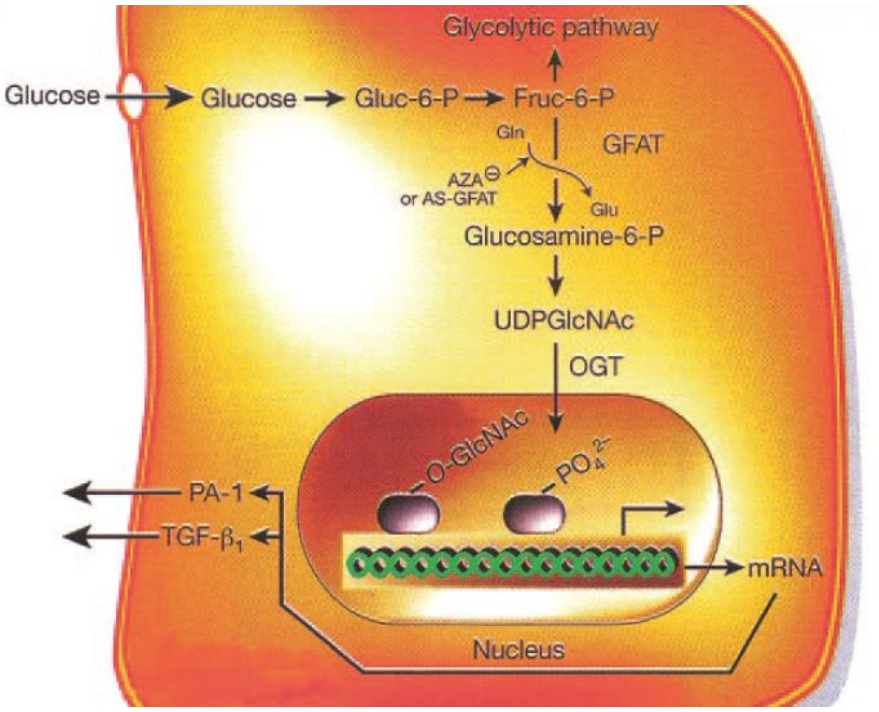


Figure 2.6. Hyperglycaemia increases flux through the hexosamine pathway (Brownlee, 2005).

2.2.2.4 Increased intracellular advanced glycation end products (AGE) formation

The resultant increase in AGE precursor formation can damage cells through three mechanisms (Figure 2.7). First, they can modify intracellular proteins including proteins involved in the regulation of gene transcription. Second, AGE precursors can diffuse out of cells and modify signalling between the matrix and the cell to cause cellular dysfunction. Third, AGE precursors can diffuse out of the cells and modify proteins in the blood such as albumin. These modified proteins can bind to RAGE and activate them to produce inflammatory cytokines and growth factors (Brownlee, 2005).

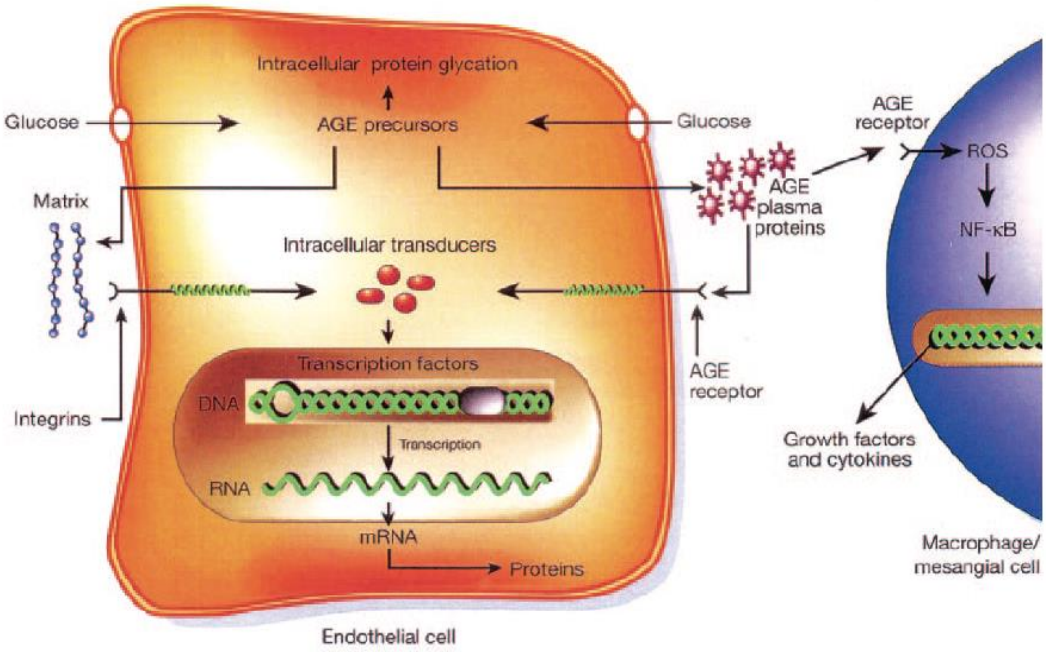


Figure 2.7. Increased production of advanced glycation end products (AGE) precursors and their pathologic consequences (Brownlee, 2005).

2.2.3 ROLE OF BLOOD PRESSURE (BP) CONTROL

The UKPDS study found that both glycaemic and blood pressure (BP) control have a large impact on the development of diabetes-related complications. In the study, improved glycaemic control or BP reduced the risk of major diabetic eye disease by one quarter, reduced serious deterioration of vision by nearly one half, reduced early renal damage by one third, reduced strokes by one third and reduced death from diabetes-related causes by one third (Turner et al., 1998, Leslie, 1999).

These results demonstrate that besides metabolic factors such as hyperglycaemia, haemodynamic factors such as hypertension play an important role in the accelerated progression of diabetic complications. This section examines the renin-angiotensin-aldosterone system (RAAS) that controls BP and fluid balance in the body (Figure 2.8).

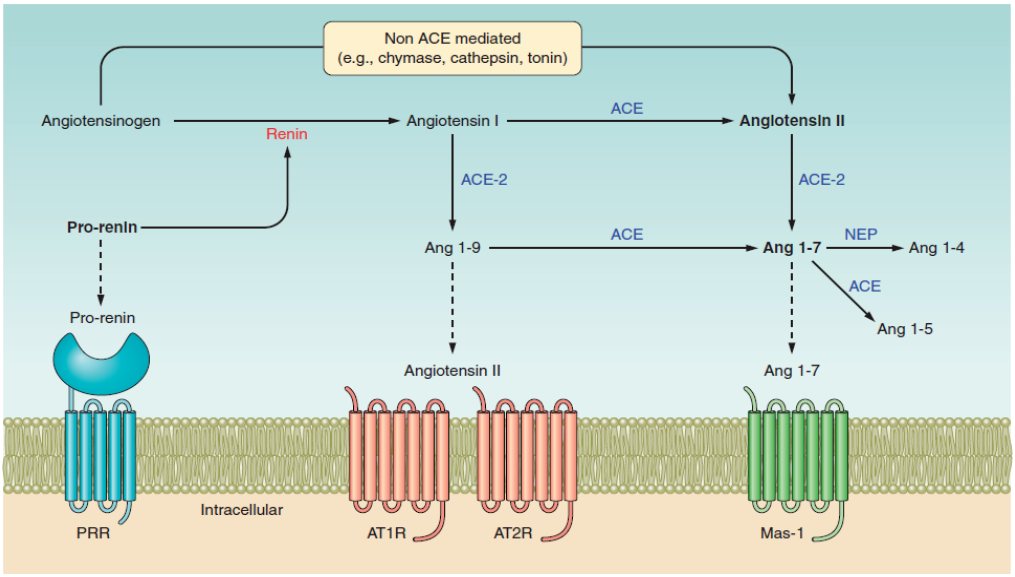


Figure 2.8. The renin-angiotensin cascade (Forbes and Cooper, 2013).

In the RAAS, angiotensinogen is converted to angiotensin I by the enzyme renin. Angiotensin I is then converted to angiotensin II by angiotensin-converting enzyme (ACE). Although angiotensinogen is mainly synthesised by the liver, it is expressed at other sites implicated in diabetic complications including the kidney and the heart (Forbes and Cooper, 2013). Excess angiotensinogen and its downstream products called angiotensin 1-7 causes renal damage in animal models (Liu et al., 2008, Shao et al., 2008). The RAAS causes the activation of the angiotensin receptors: AT1R and AT2R. These receptors are widely expressed at sites of diabetic complications. Activation of these receptors leads to the induction of cytokines such as VEGF (Forbes and Cooper, 2013). The role of VEGF and its role in the development of retinal neovascularisation is discussed in Section 2.3.5. The various angiotensins and changes in serum potassium concentrations cause the release of aldosterone from the adrenal glands. In the kidney, aldosterone promotes water and salt retention by the distal tubule that leads to increased blood volume and ultimately, elevation in BP (Briet and Schiffrin, 2011).

2.2.4 ROLE OF LIPID CONTROL

Patients with diabetes have an increased risk of cardiovascular disease. This may partly be due to dyslipidaemia. There is some evidence that dyslipidaemia may play a role in the pathogenesis of eye disease but its underlying mechanisms are not yet identified. In the DCCT and EDIC studies, the severity of DR was found to be positively associated with triglycerides and negatively associated with high-density lipoprotein (HDL) cholesterol

(Lyons et al., 2004). In the Fenofibrate Intervention and Event Lowering in Diabetes (FIELD) study, it was found that fenofibrate, a lipid-modifying agent reduces the need for laser treatment of DR (Keech et al., 2007). Interestingly, the Action to Control Cardiovascular Risk in Diabetes (ACCORD) study found no additional benefit of adding fenofibrate to patients already on a statin to prevent cardiovascular and cerebrovascular events (Ginsberg et al., 2010).

2.2.5 ROLE OF GENETICS

Genome-wide association studies have identified approximately 70 susceptibility genes associated with type 2 diabetes in different populations (Sun et al., 2014b). Type 2 diabetes is three to five times more prevalent in minority ethnic communities compared to the Caucasian population in the UK. People in these minority ethnic groups tend to develop the disease at a younger age (Goff, 2019) and they tend to progress from impaired glucose tolerance to diabetes faster than the Caucasian population (Webb et al., 2011). Having relatives (especially first degree) with type 2 diabetes also increases the risk of an individual developing diabetes (Olokoba et al., 2012). In addition, obesity, which is a risk factor for developing diabetes, is strongly inherited (Llewellyn et al., 2013). However, current genetic loci identified account for only about 10% of the overall heritability of type 2 diabetes suggesting that environmental factors play an important role (Sun et al., 2014b).

There are over 40 susceptibility genes associated with the development of type 1 diabetes (Noble and Erlich, 2012). In studies of identical twins, when one twin has type 1 diabetes, there is an increased chance of the other twin developing diabetes (Condon et al., 2008, Giwa et al., 2020). This suggests that while genetics play an important role in the development of type 1 diabetes, there are other contributing factors since the concordance rate is not 100% (Giwa et al., 2020).

Research on the genetics of diabetes has led to an increased understanding of the pathogenesis of the disease and potential intervention pathways compared to the past. Future research on identifying an individual's genetic susceptibility to diabetes may help with screening for the disease and personalising medicine for treatment (McCarthy, 2010, Giwa et al., 2020).

2.2.6 MANAGEMENT OF DIABETES

2.2.6.1 Lifestyle changes

Besides genetics, environmental or lifestyle factors that play a role in the development and management of diabetes. While individual genetics are not modifiable, some of the lifestyle factors that may be amenable to modification is examined in this section. This includes obesity contributed to by physical inactivity, and an unhealthy diet and cigarette smoking.

People who are overweight or obese are more likely to develop type 2 diabetes compared to people of normal weight. This risk rises as body weight increases (DECODE, 2002). In adults, a person with a BMI of 25 to 29.9 kg/m^2 is considered overweight while a person with a BMI of 30 kg/m^2 or over is considered obese. In 2020, 60% of women and 67% of men were overweight or obese in England (NHS Digital, 2020).

BMI does not distinguish between mass due to body fat and muscular physique, nor the distribution of fat. Therefore, waist circumference is measured to document abdominal obesity. For men, high waist circumference is 94-102cm and very high is ≥102cm. For women, high waist circumference is 80-88cm and very high is ≥88cm. The prevalence of diabetes among men and women with very high waist circumference is 14% and 10% respectively. In comparison, the prevalence of diabetes among men and women with a normal waist circumference is 5% and 4% respectively (NHS Digital, 2020).

One study suggested that a 1 kg/m^2 increase in BMI increases the risk of developing new-onset type 2 diabetes by 8.4% and the risk of impaired fasting glucose by 9.5%. A 1 cm increase in waist circumference increases the risk of type 2 diabetes and impaired fasting glucose by 3.2% and 3.5% respectively (Bombelli et al., 2011).

Physical inactivity and an unhealthy diet contribute to obesity. In 2019, 21% of adults were classified as being inactive (less than 30 minutes of physical activity weekly) in England. Inactivity levels increased with age. Those aged 19-24 were most active (74% active) while those over 85 years old were least active (31% active). Only 28% of adults ate the recommended 5 or more portions of fruit and vegetables a day, and women (30%) were more likely to do so than men (25%) (NHS Digital, 2020).

The 15-year results of the Diabetes Prevention Program Outcomes Study (DPPOS) found that lifestyle intervention and metformin reduced diabetes incidence by 27% (p<0.001) and

18% (*p*=0.001), respectively, compared to the placebo group. The lifestyle program included a 16-session curriculum with individual sessions aimed at achieving a 7% weight loss through a healthy low-fat, low-calorie diet and 150 minutes per week of moderate-intensity physical activity. After the initial 24 weeks, individual and group sessions were used to reinforce the lifestyle modification behaviours (Diabetes Prevention Program Research, 2015).

NICE recommends that people with type 2 diabetes have at least 150 minutes of moderate-intensity activity (e.g. brisk walking) or 75 minutes of vigorous-intensity activity (e.g. running) per week. Individuals are encouraged to increase dietary fibre and choose low fat, low calorie and low glycaemic-index food (NICE, 2012). For individuals with type 1 diabetes, NICE recommends that their blood sugar level and insulin therapy is monitored and adjusted according to their diet and physical activitity. However, NICE does not recommend a low glycaemic index diet for individuals with type 1 diabetes due to the risk of hypoglycaemia (NICE, 2015b).

Smoking is an independent and modifiable risk factor in the development of type 2 diabetes (Chang, 2012). One British study on middle-aged men found the relative risk of developing diabetes in smokers to be 1.6 after adjusting for confounding factors (Wannamethee et al., 2001). It has been found that smoking increases insulin resistance but the mechanism is unclear. Smoking has been widely associated with an increased risk of cardiovascular events. Smoking combined with diabetes leads to an exponential increase in microvascular and macrovascular complications (Chang, 2012).

2.2.6.2 Medications to manage diabetes

Many medications are available to manage diabetes with various novel therapies being developed. Patients with diabetes often have co-morbidities and they may present with multiple medications, some of which the ophthalmologist should be aware of. However, a detailed review of the current therapy for diabetes is beyond the scope of this thesis. Table 2.1 provides a summary of the medications in routine use in the UK for the management of diabetes (Olokoba et al., 2012, Diabetes UK, 2021).

Table 2.1. Medications used to manage diabetes and their mechanism of action (Olokoba et al., 2012, Diabetes UK, 2021)

Class of Medication	Mechanism of Action	Examples
Insulin and Insulin analogue	Hormone that regulates the metabolism of carbohydrates, fats and protein by absorbing blood glucose into cells	Glargine (Lantus®), insulin aspart (NovoRapid®, NovoMix®), insulin detemir (Levemir®), insulin lispro (Humalog®)
Biguanide	Suppress hepatic glucose production, increase insulin sensitivity, enhance peripheral glucose uptake	Metformin
Sulfonylurea	Stimulate endogenous insulin secretion	Gliclazide (Diamicron®), glimepride (Amaryl®), glibenclamide (Daonil®), glipizide (Glibenese®, Minodiab®), tolbutamide (Tolbutamide®)
Alpha glucosidase inhibitor	Limit absorption of dietary carbohydrates	Acarbose (Glucobay®)
Meglitinide	Stimulate endogenous insulin secretion	Repaglinide (Prandin®), nateglinide (Starlix®)
Thiazolidinedione (Glitazone)	Reduce insulin resistance and increase insulin sensitivity	Pioglitazone (Actos®)
Glucagon-like peptide 1 (GLP-1) agonist	Increase insulin secretion and inhibit glucagon release. Help glycaemic control and reduce weight	Liraglutide (Victoza®), exenatide (Byetta®, Bydureon®), lixisenatide (Lixumia®), dulaglutide (Trulicity®), semaglutide (Ozempic®)
Dipeptidyl-Peptidase (DPP) 4 inhibitor	Increase incretin levels which inhibit glucagon release	Saxagliptin (Onglyza®), sitagliptin (Januvia®), linagliptin (Trajenta®), vildagliptin (Galvus®), alogliptin (Vipidia®)
Sodium-glucose cotransporter 2 (SGLT2) inhibitor	Increase renal glucose elimination	Dapagliflozin (Forxiga®), canagliflozin (Invokana®), empagliflozin (Jardiance®), ertugliflozin (Steglatro®)

2.2.6.3 Management of hypertension

People with diabetes often have hypertension. There is likely an overlap in the aetiology of both conditions, which includes obesity, inflammation, oxidative stress and insulin resistance (Cheung and Li, 2012). NICE recommends a low-sodium diet and other lifestyle measures to control BP. However, if these measures do not reduce the BP to 140/80mmHg or less, then medications should be considered. The BP threshold is reduced to

130/80mmHg if there is retinal, renal or cerebrovascular damage (NICE, 2015c). ACE inhibitors are commonly used as a first-line anti-hypertensive treatment for patients with diabetes. ACE inhibitors preserve renal function and protect against cardiovascular disease (Ganesh and Viswanathan, 2011). β-blockers are another common class of anti-hypertensive medications. β-blockers modulate the sympathetic nervous system by competitive inhibition of catecholamine binding to β-adrenergic receptors. In the kidney, β-adrenergic receptors influence the secretion of renin which in turn alters BP. However, β-blockers must be used with caution in diabetic patients as there are concerns that they can mask the effects of hypoglycaemia (Dungan et al., 2019). Calcium channel blockers are also widely used. Pharmacologically, calcium channel blockers reduce the cellular uptake of calcium from intracellular stores. It is thought that calcium channel blockers reduce the responsiveness of the vasculature to angiotensin II. In effect, this class of medication reduces peripheral vascular resistance and thereby BP (Forbes and Cooper, 2013). Angiotensin 2 receptor antagonists, alpha-adrenoceptor blockers and diuretics are also sometimes used. In clinical practice, it is common for patients to need multiple groups of anti-hypertensive therapy to optimise control. In the UKPDS trial, 29% of the tight control group required three or more medications after nine years (Leslie, 1999).

2.2.6.4 Management of dyslipidaemia

Managing dyslipidaemia in patients with diabete is important. The Multiple Risk Factor Intervention Trial (MRFIT), found that mortality increases with serum cholesterol (Stamler et al., 1993). Mortality is three times higher in individuals with diabetes than in individuals with no diabetes, after adjusting for confounding factors (Stamler et al., 1993). NICE recommends taking a full lipid profile (total cholesterol, HDL cholesterol, non-HDL cholesterol and triglyceride) to assess dyslipidaemia. Patient intervention is considered if the total cholesterol concentration is more than 7.5mmol/litre. The initial management is to encourage lifestyle and dietary changes, such as a low-fat diet. If these changes fail improve dyslipidaemia, then statins is usually the first-line medication recommended (NICE, 2014).

The FIELD (Keech et al., 2007) and ACCORD studies (Chew et al., 2010) found that fenofibrate significantly slowed the progression of pre-existing DR in adults with type 2 diabetes. Fenofibrate is a peroxisome proliferator-activated receptor α (PPARα) agonist. Fenofibrate affects several pathways related to lipid metabolism, inflammation, apoptosis and angiogenesis. Importantly, it reduces VEGF and VEGF-receptor 2 expression. The FIELD

study of 9795 patients in Australia and Finland compared the effects of 5 years of oral fenofibrate vs. placebo treatment. Fenofibrate therapy was associated with an overall 37% reduction in the need for laser therapy for any DR (36% reduction for maculopathy and 38% for PDR) (Keech et al., 2007). In the ACCORD EYE sub-study, 1593 of 5518 patients in North America were treated with simvastatin, and either fenofibrate or matching placebo, for four years. Patients who received fenofibrate, instead of placebo, were associated with 40% less DR progression or laser therapy or vitrectomy (Chew et al., 2010). Based on these findings, there is a move for healthcare providers to prescribe fenofibrate in patients with type 2 diabetes with DR regardless of lipid profiles (Sharma et al., 2015). The Royal College of Ophthalmologists recommends adding fenofibrate to a statin for lipid management in patients with type 2 diabetes with non-PDR (Royal College of Ophthalmologists, 2012). It is important to note that DR was not a primary endpoint by design in either the FIELD or ACCORD EYE study. Neither study showed an improvement in VA outcomes despite showing a reduction in DR progression. The studies did not use any quantitative measurement of DMO such as OCT measurements, which are now part of routine practice (Sharma et al., 2015).

2.3 DIABETES AND THE EYE

Previous sections have discussed the systemic effect of diabetes, the cellular mechanisms and management of diabetes. This section focuses on the ocular complications of diabetes, namely DR. The section begins with a review of the anatomy of the healthy adult retina.

2.3.1 NORMAL RETINA ANATOMY

The retina is embryologically derived from the neural tube, which also forms the rest of the central nervous system. The eye consists of three layers, namely, the fibrous layer (corneoscleral), the vascular pigmented layer (uveal tract) and the neurological layer (Snell, 1998). The retina consists of an outer pigmented (RPE) layer and an inner neurosensory layer. The neurosensory layer consists of photoreceptors, bipolar cells, ganglion cells and

other cells that modulate and support their activities (Figure 2.9) (Snell, 1998, Gupta et al., 2016).

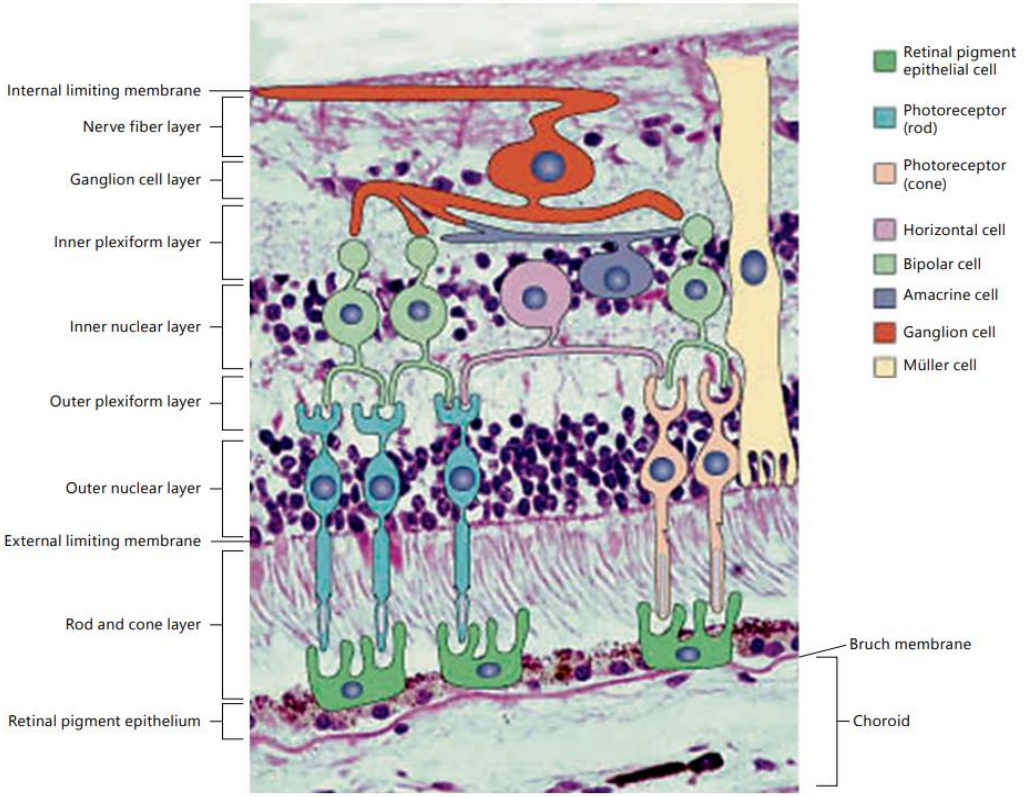


Figure 2.9. The 10 layers of the retina as seen in the histological section combined with a diagram of the pertinent retinal cells (Gupta et al., 2016).

The macula is an oval area in the centre of the posterior part of the retina. It measures approximately 5mm in diameter and lies 3mm temporal to the optic disc. The fovea is a depressed area in the centre of the macula. This depressed area is formed by the peripheral displacement of the nerve cells and fibres of the retinal inner layers to allow light to have greater access to the photoreceptors. The macula has the highest density of photoreceptors, most of which are cone photoreceptors, responsible for colour vision. This arrangement explains why the macula has the highest visual resolution in the retina and why macular pathology greatly affects vision (Snell, 1998, Gupta et al., 2016). The appearance of the macula area as imaged by OCT is discussed in Chapter 4.

2.3.2 COMPROMISE OF THE BLOOD RETINAL BARRIERS (BRB) IN DR

DR is a complication of diabetes that leads to characteristic changes in the retina. These changes occur at a cellular level and at a later stage manifests as changes visible during ocular examination. On a cellular level, the breakdown of the blood-retinal barriers (BRB)

due to hyperglycaemia plays an integral part in the development of DR (Fresta et al., 2020). In this section, the role of the BRB is discussed, and the impact on some specific cells secondary to its breakdown is discussed below.

Hyperglycaemia is the greatest risk factor for developing DR evidenced by several large clinical studies (Section 2.2.2). The precise mechanism of how DMO occurs as a result of chronic exposure to hyperglycaemia and other factors such as hypertension has been extensively studied. There is evidence that these exposures initiate a series of biochemical and physiological changes. Changes in the various biochemical pathways discussed in Section 2.2.2 result in increased loss of cell-to-cell barrier junctions, VEGF production, AGE-induced damage and oxidative stress, all of which contribute to the breakdown of the BRB and the development of DMO (Rudraraju et al., 2020).

There are two BRBs related to the two sources of blood supply to the retina. The inner two-thirds of the retina is supplied by capillaries from the central retinal artery. The outer third of the retina is supplied by the capillaries in the choroid, the choriocapillaris. The inner BRB is formed by the zonulae occludens, the tight junctions of the endothelial cells of the retinal capillaries in the inner retina. The outer BRB is formed by the adherens junctions and tight junctions between the cell membranes of the RPE cells that are situated between the fenestrated choriocapillaris and the outer retina. Both the inner and outer BRB play important roles by regulating fluid and electrolyte balance in the retina (Figure 2.10) (Forrester and Xu, 2012).

The regulation of the inner BRB that prevents leakage of molecules from the retinal capillaries depends on the integrity of the endothelial cell-to-cell junctions, a normal basement membrane and pericytes in the outer wall. In diabetes, alterations of the inner BRB cause well-documented changes such as a breakdown of the cell-to-cell junctions, thickening of the basement membrane and pericyte loss in the neurovascular unit. Further examination of the neurovascular unit is covered in Section 2.3.3.4 (Figure 2.11) (Das et al., 2015). The mechanism of how some of these cells are affected by the breakdown of the BRB is examined in the following section.

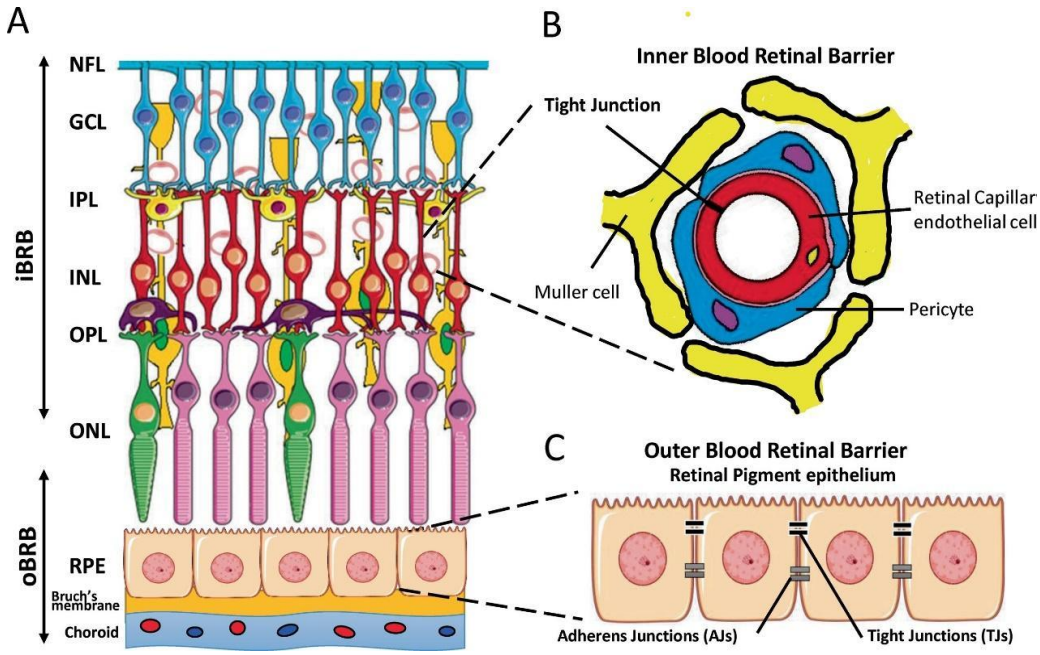


Figure 2.10. Diagram showing the retinal neurovascular unit comprising vascular cells, glia and groups of neurons (A), inner blood-retinal barrier (iBRB) composed of endothelial cells, pericytes and Müller cells (B) and the outer blood-retinal barrier (oBRB) consisting of retinal pigment epithelium (RPE) cells with adherens junctions and tight junctions (nerve fibre layer, NFL; ganglion cell layer, GCL; inner plexiform layer, IPL; inner nuclear layer, INL; outer plexiform layer, OPL; outer nuclear layer, ONL) (Rudraraju et al., 2020).

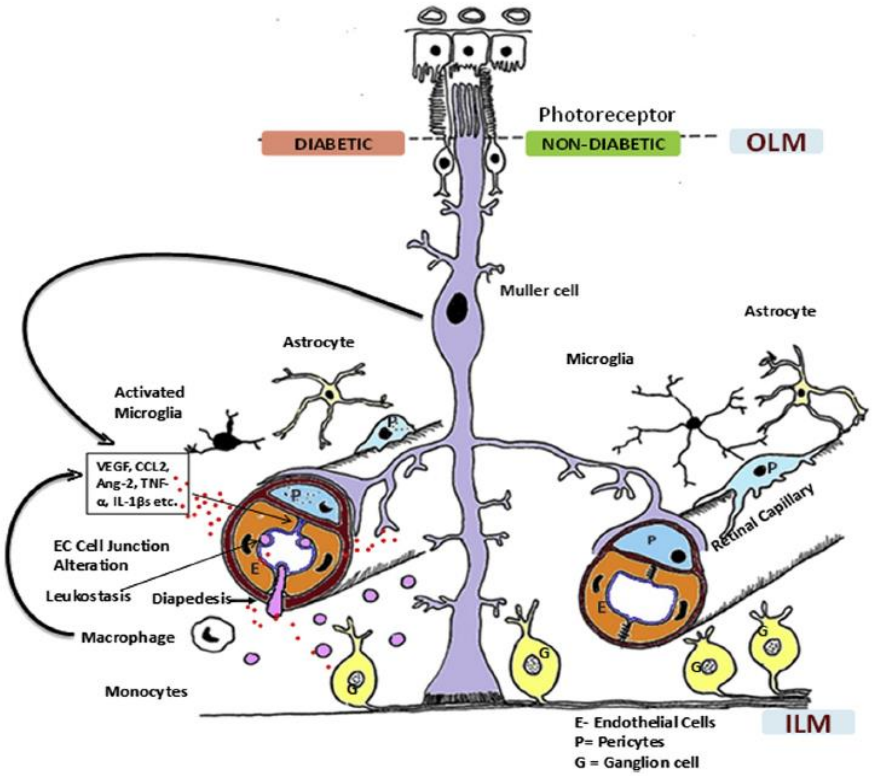


Figure 2.11. Neurovascular unit of the retina in diabetic and non-diabetic conditions (Das et al., 2015).

2.3.3 CELLS AFFECTED BY THE BREAKDOWN OF THE BRB

2.3.3.1 Endothelial cells

Endothelial cells in the retinal vessels play an important role in maintaining the BRB as they form a tight monolayer with surrounding pericytes, astrocytes, microglia and basal lamina. Endothelial cells have two pathways, namely the paracellular and transcellular pathways, to control the movement of molecules across the BRB. The paracellular pathway relies on three types of inter-endothelial junctions (tight, adherens and gap), which restrict the passage of water and water-soluble compounds. On the other hand, the transcellular pathway is important for the active transport of macromolecules via caveolae and receptor-mediated transport mechanisms (Klaassen et al., 2013). Endothelial junctions are dynamic structures that can be downregulated by many factors including elevated VEGF levels (Scheppke et al., 2008). When endothelial cells and pericytes undergo apoptosis in response to chronic hyperglycaemia, this forms acellular capillaries in a process known as vasoregression or vasodegeneration, which lead to ischaemic changes characteristic of DR (Mizutani et al., 1996, Hammes, 2018).

2.3.3.2 Pericytes

Pericytes are modified smooth muscle cells that have contractile properties (Das et al., 1988). Pericytes regulate retinal capillary blood flow. It is proposed that with hyperglycaemia and the subsequent accumulation of AGE and sorbitol in the pericytes, their ability to regulate retinal capillary blood flow decreases as DR progresses (Brownlee, 2005, Giacco and Brownlee, 2010). Lindahl et al. (1997) showed that platelet-derived growth factor-B which promotes proliferation and migration of pericytes may have a role in DR. Experimental studies in mice that were deficient in platelet-derived growth factor-B showed pericyte loss and microaneurysm formation similar to patients with DR (Lindahl et al., 1997). Orlidge and Damore (1987) showed that pericytes can suppress endothelial cell growth and subsequently affect capillary function. In DR, pericyte loss results in focal endothelial cell proliferation and may contribute to the formation of microaneurysms in the weakened walls of retinal capillaries. In response, the inner BRB can be damaged and macular oedema ensued (Das et al., 2015).

2.3.3.3 Basement membrane

The basement membrane surrounds the endothelial cells and encloses the pericytes completely. In addition to providing structural support, the basement membrane acts as a filtration barrier and regulates cell proliferation and differentiation. Thickening of the basement membrane is classically noted in DR, and there is evidence that this is secondary to inflammatory cytokines, alterations in Müller cell metabolism and non-enzymatic glycation (Kennedy and Baynes, 1984, Bianchi et al., 2016).

2.3.3.4 Neurovascular unit

DR has traditionally been thought of as a microvascular complication but there is emerging evidence that the wider retinal neurovascular unit is affected resulting in neurodegeneration. The neurovascular unit includes neural cells (ganglion, amacrine, horizontal and bipolar), glia (Müller cells and astrocytes), immune cells (microglia and macrophages) and vascular cells (endothelial cells and pericytes) (Figure 2.11) (Simo et al., 2018). There is a complex interaction between retinal neurons and glia that surround the retinal capillaries, which control fluid and metabolite transport in the neural tissue. Müller cells have an extensive network of villi that surround retinal capillaries. Müller cells release factors that induce the formation of zonula occludens in retinal vessels. Therefore, abnormalities in Müller cells may affect the BRB (Das et al., 2015).

Neural, microvascular and glia dysfunctions are interdependent and lead to the development of DR; the main features of neurodegeneration include glial activation (also known as reactive gliosis) and neural apoptosis (Simo et al., 2018). These changes in the neurovascular unit are in response to a hyperglycaemic environment and reactive metabolites (Hammes, 2018). The exact relationship between DR neurogeneration and microvascular changes is unknown. Simo et al. (2018) proposed that there is impaired cell-to-cell interaction within the neurovascular unit including alterations in endothelin-1 (ET-1) levels causing vasoconstriction and neurodegeneration (Figure 2.12). Increased extracellular glutamate due to downregulation of the glutamate aspartate transporter results in excitotoxicity and neuronal death. The subsequent progressive imbalance between neuroprotective factors plays a major role in neural apoptosis and glial activation in diabetes. On the other hand, microvascular impairment occurs due to an altered haemodynamic response, BRB breakdown and vasoregression.

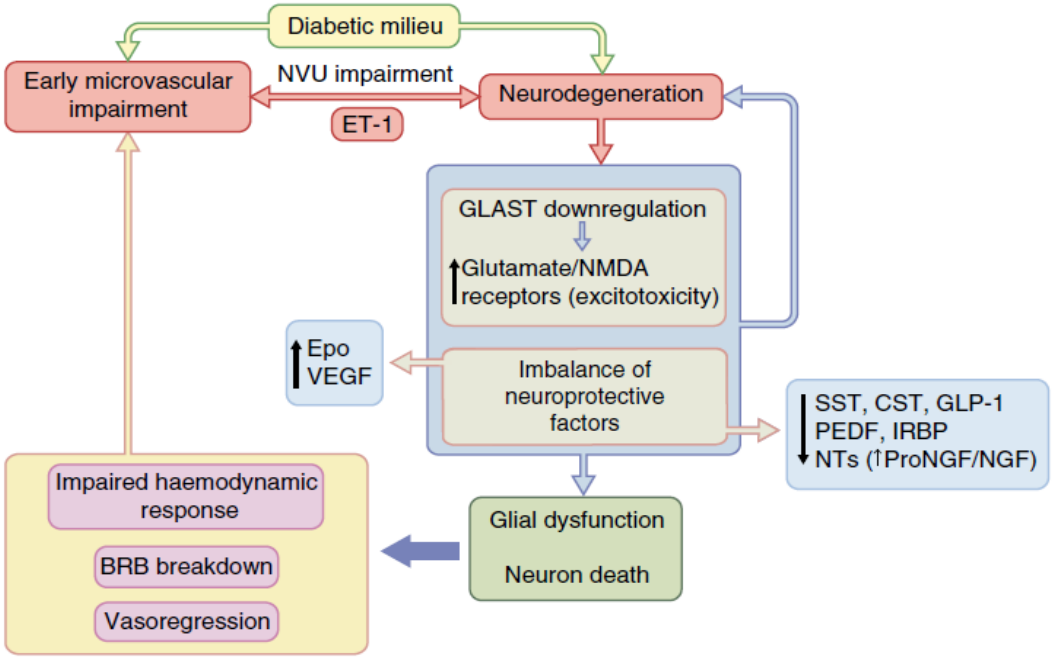


Figure 2.12 Main mechanisms involved between diabetic retinal neuropathy and microvascular impairment (neurovascular unit, NVU; endothelin-1, ET-1; glutamate aspartate transporter, GLAST; N-methyl-D-aspartate, NMDA; somatostatin, SST; cortistatin, CST; glucagon-like peptide 1, GCP-1; pigment epithelium-derived factor, PEDF; interphotoreceptor retinoid-binding protein, IRBP; neurotrophin, NT; nerve growth factor precursor, NGF; erythropoietin, Epo; vascular endothelial growth factor, VEGF; blood-retinal barrier, BRB) (Simo et al., 2018).

Using rat models and retinas of donors with diabetes, Barber et al. (1998) found that neural cell apoptosis occurred soon after the onset of diabetes and before overt microvascular changes; they also found thinning of the IPL and INL and decreased ganglion cells in rats with diabetes compared to controls. Jackson et al. (2012) found that in patients with non-proliferative DR (NPDR) who have good VA, impaired ganglion cell function can be measured using frequency doubling technology (FDT). The European Consortium for the Early Treatment of Diabetic Retinopathy (EUROCONDOR) study found multi-focal ERG deficits and decreased GCL-IPL thickness in people with minimal diabetes (ETDRS <20) compared to normal participants (Santos et al., 2017). Comparisons of retinal thickness in PWD with no or minimal DR and healthy participants is discussed in Chapter 7.

2.3.3.5 Photoreceptors

Photoreceptors are the most numerous cells in the retina and are very metabolically active. They depend on oxygen diffusion from retinal capillaries or the choriocapillaris (Arden,

2001). There is evidence that hyperglycaemia causes changes in the RPE due to mitochondrial dysfunction which leads to photoreceptor cell death (Yumnamcha et al., 2020).

2.3.3.6 Retinal pigment epithelium

Hyperglycaemia can cause alterations to the outer BRB by damaging the tight junctions between the RPE cells resulting in serum leakage from the choriocapillaris and retinal oedema (Figure 2.13). Eventually, capillary closure results in hypoxia, ischaemia and angiogenesis (American Academy of Ophthalmology, 2014, Xia and Rizzolo, 2017).

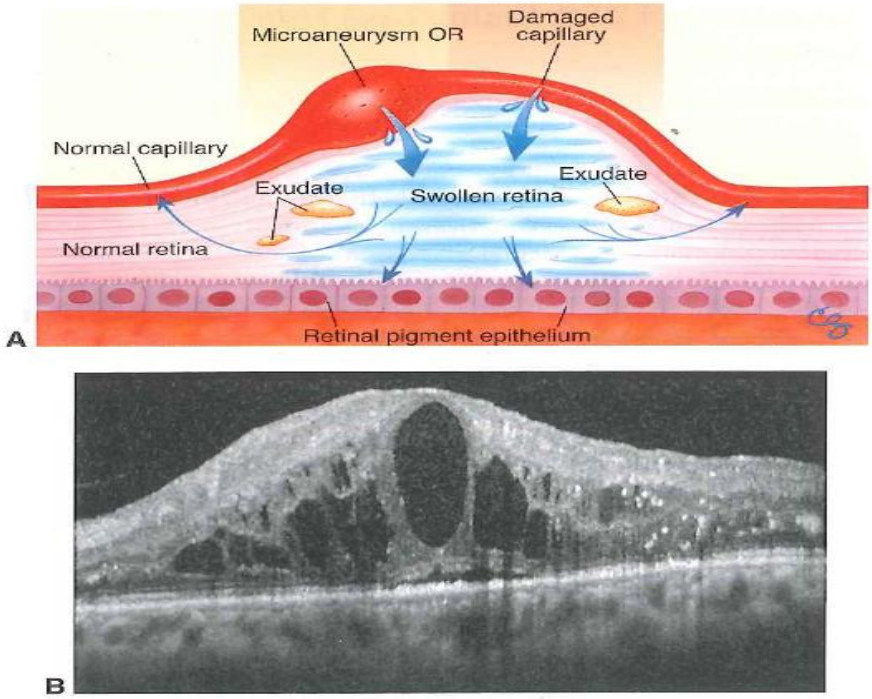


Figure 2.13 (A) Diagram of the mechanism of DMO (B) SD-OCT image of DMO (American Academy of Ophthalmology, 2014).

2.3.4 ROLE OF INFLAMMATION IN DR

There are many features of inflammation in DR such as tissue oedema, increased vascular permeability and blood flow, upregulation of cytokines, complement activation, microglial activation and macrophage infiltration (Das et al., 2015). DR has been described as a form of sustained, chronic inflammation with retinal leukostasis whereby abnormal intravascular leukocyte aggregation and clumping occurs. This increase in leukostasis can cause upregulation of intercellular adhesion molecules and increase vascular permeability leading to the characteristic vascular lesions in DR (Joussen et al., 2004). Monocytes are the largest

leukocytes and can differentiate into macrophages. It has been shown that increased numbers of monocytes and macrophages are brought into the extravascular tissue in diabetic mice (Rangasamy et al., 2014, Das et al., 2015). Macrophages in retinal tissue secrete a variety of cytokines and growth factors including angiopoietin, interleukin, tumour necrosis factor, nitric oxide and monocyte chemoattractant protein all of which can alter the BRB (Figure 2.11) (Rubsam et al., 2018).

2.3.5 ROLE OF ANGIOGENESIS LEADING TO RETINAL NEOVASCULARISATION

The development and maintenance of normal retinal vasculature requires a balance between proangiogenic and antiangiogenic factors. Ischaemia or inflammation can tip this balance leading to abnormal neovascularisation. Studies have found that hypoxia can lead to an upregulation of VEGF, which stimulates neovascularisation. Neovascularisation can cause visual loss due to proliferative changes at the iris (neovascularisation at the iris, NVI), optic disc (neovascularisation at the optic disc, NVD) or peripheral retina (neovascularisation elsewhere, NVE). This process can lead to vitreous haemorrhage, fibrovascular scarring and retinal traction. The sequence of events in retinal angiogenesis is described in Figure 2.14 below (Capitão and Soares, 2016). Now that the underlying pathophysiology of DR has been reviewed, the ocular manifestations of these processes evident on the clinical examination will be discussed.

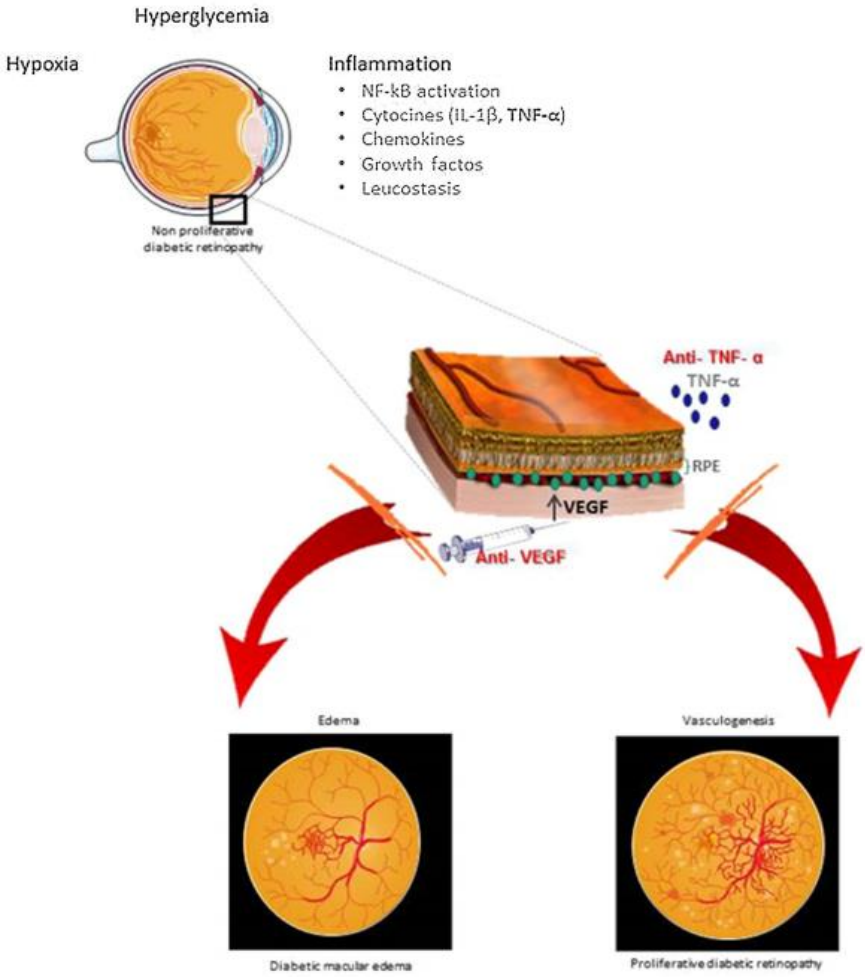


Figure 2.14. The sequence of events in angiogenesis in diabetic retinopathy (DR). The retinal pigment epithelium (RPE) produces vascular endothelial growth factor (VEGF) that is augmented by the maintained hyperglycaemic environment and up-regulated by tissue hypoxia and pro-inflammatory mediators. In DR, the progression of these processes leads to vasopermeability (diabetic macular oedema, DMO) and/or pathological angiogenesis (proliferative DR, PDR) (Capitão and Soares, 2016).

2.3.6 OCULAR MANIFESTATIONS OF DR

2.3.6.1 Clinical features of DR

Miroaneurysms are the earliest signs in DR that are visible on ophthalmological examination (Figure 2.15) (Antonetti et al., 2012). They are seen on ophthalmoscopy as a small red dot in the middle retinal layers. When the wall of a capillary is weakened, it may rupture and give rise to an intraretinal haemorrhage. If the haemorrhage is deep, for example in the INL or OPL, then it is usually round or oval in shape and is known as a dot or blot haemorrhage. It is clinically difficult to differentiate between a microaneurysm from a

dot haemorrhage. In contrast, if the haemorrhage is superficial, in the RNFL, it forms a flame or splinter shape and is described as a flame haemorrhage. These flame haemorrhages have the same appearance as those caused by hypertensive retinopathy (Yanoff, 2014).

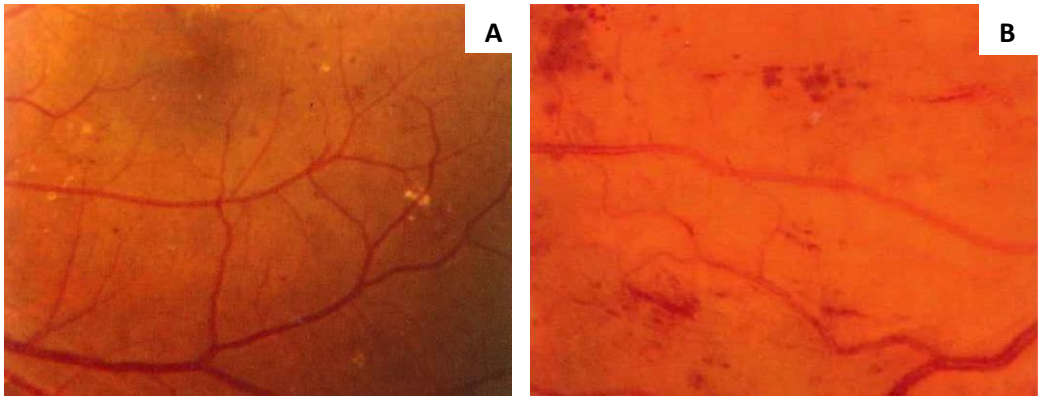


Figure 2.15. A. Microaneurysms and dot/blot haemorrhages B. Flame haemorrhages (Salmon and Bowling, 2015).

Exudates are caused by the breakdown of the BRB with subsequent precipitations of lipid components of the blood. They develop at the junction of the normal and oedematous retina. Exudates are composed of lipoprotein and lipid-filled macrophages located mainly in the OPL. They appear like waxy yellow lesions arranged in clumps or rings often with surrounding leaking microaneurysms (Figure 2.16). If the leakage stops, exudates can be reabsorbed spontaneously over months either into healthy surrounding capillaries or by phagocytosis (Salmon and Bowling, 2015). Exudates are used in feature-specific grading in DR screening which is discussed in Section 2.5. Retinal thickening is a second marker of BRB breakdown and is assessed on slit-lamp biomicroscopy or stereo photography. Retinal thickening can also occur in capillary non-perfusion.

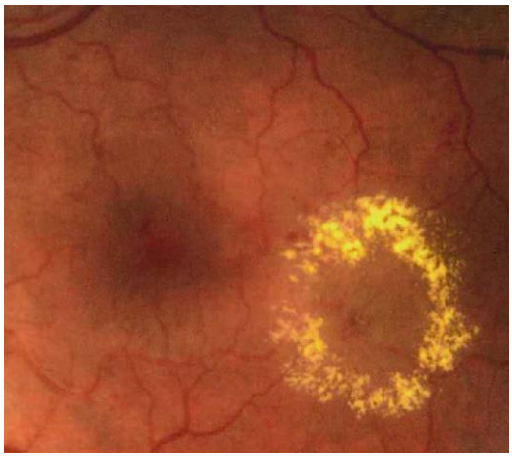


Figure 2.16. Ring of exudates temporal to the macula surrounding a zone of retinal thickening (Salmon and Bowling, 2015).

Cotton wool spots are accumulations of neuronal debris within the RNFL. They used to be known as soft exudates. They are caused by the ischaemic disruption of ganglion cell axons. They appear as fluffy white superficial lesions that are typically seen outside the macula (Figure 2.17). Cotton wool spots can be resolved by the removal of debris by autolysis and phagocytosis (Salmon and Bowling, 2015).

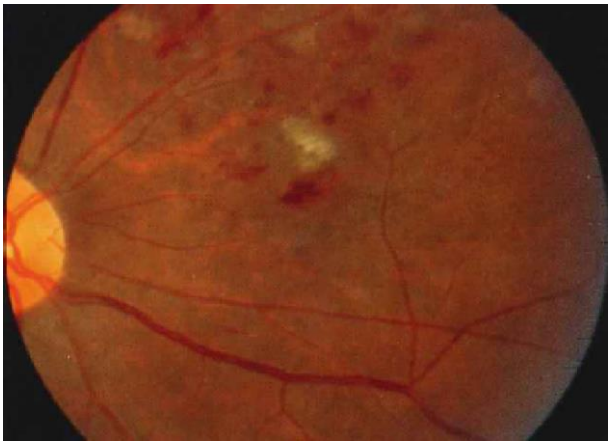


Figure 2.17. Cotton wool spots with flame haemorrhages (Salmon and Bowling, 2015).

Vascular changes in DR are due to ischaemic dysfunction and include venous changes, intraretinal microvascular abnormalities (IRMA) and arterial changes. Venous anomalies include generalised dilatation and tortuosity, looping, beading (focal narrowing and dilatation) and segmentation (Figure 2.18). Retinal areas with venous changes are well correlated to the development of proliferative disease. IRMA are arteriolar-venular shunts that run from retinal arterioles to venules, bypassing the capillary bed. As a result, they are often seen adjacent to areas of marked capillary hypoperfusion. IRMA are seen as fine, irregular intraretinal lines that run from arterioles to venules without crossing major blood vessels (Salmon and Bowling, 2015).

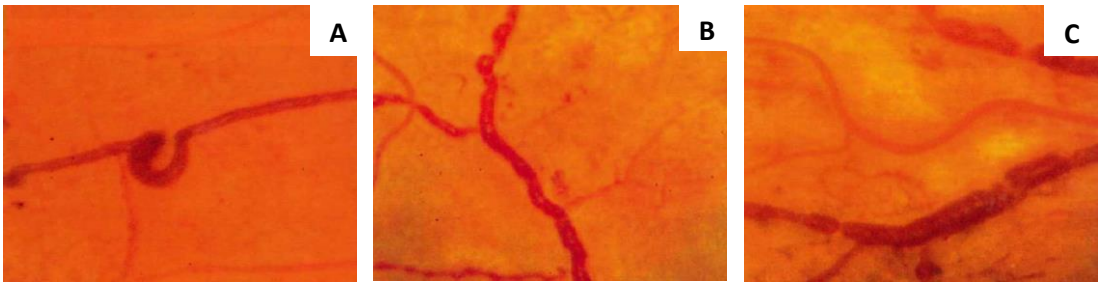


Figure 2.18. Venous changes A. Looping B. Beading C. Severe segmentation (Salmon and Bowling, 2015).

It has been estimated that over one-quarter of the retina needs to be non-perfused before PDR develops. Proliferative disease can be seen as NVD, NVE and NVI (Figure 2.19 A, B and

C). Bleeding of the NVD and NVE can cause preretinal or vitreous haemorrhage. Patients can also develop tractional retinal detachment due to progressive contraction of fibrovascular membranes across the retina (Figure 2.19D). Patients have advanced diabetic eye disease if they have total vitreous haemorrhage, NVI or tractional retinal detachment (Salmon and Bowling, 2015).

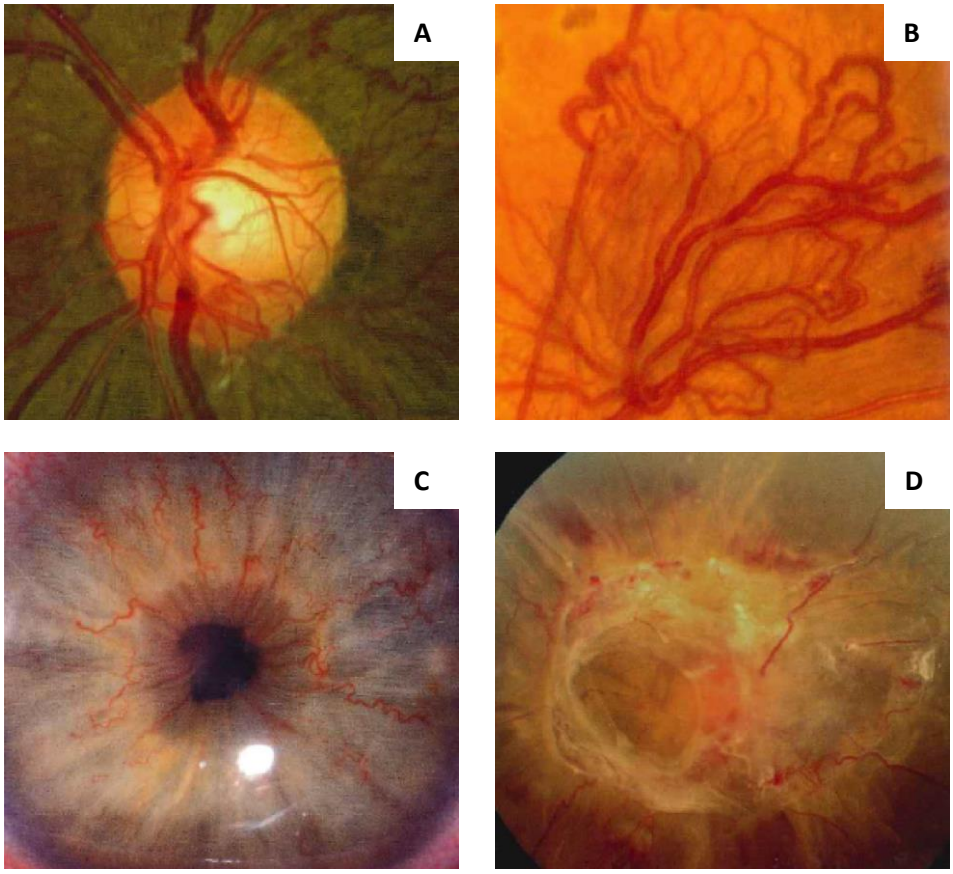


Figure 2.19. Signs of proliferative diabetic retinopathy (PDR) A. New vessels at the optic disc (NVD) B. New vessels elsewhere (NVE) C. New vessels on the iris (NVI) D. Tractional retinal detachment (Salmon and Bowling, 2015).

2.3.6.2 Diabetic maculopathy and DMO

Features of retinopathy involving the macula are termed diabetic maculopathy. Using NDESP maculopathy grading, these features are clinically significant and termed M1 if they meet the criteria described in Section 2.5 (Table 2.3). It is important to note that a PWD can have diabetic maculopathy that is not regarded as clinically significant (M0). PWD with diabetic maculopathy have a higher risk of developing DMO, which is described below.

DMO is caused by the breakdown of the BRB with the subsequent leakage of plasma and lipid at the macula that results in macular oedema (Antonetti et al., 2012). DMO is typically

described as focal or diffuse. Focal DMO is characterised by areas of focal leakage from microaneurysms and dilated capillary segments. Focal DMO is associated with exudates, typically in rings (Figure 2.16). In contrast, diffuse DMO is characterised by widespread retinal capillary leakage (American Academy of Ophthalmology, 2014). The fluid is thought to be initially located in the OPL and INL. Over time, it may also involve the IPL and RNFL until the entire thickness of the retina becomes oedematous (Salmon and Bowling, 2015). In clinical practice, it is often difficult to classify DMO into these two categories as they often overlap and DMO can present with mixed features of both types.

The ocular manifestations of DR discussed above are important for the grading of DR severity using feature specific grading. The categorisation of DR severity is important for prognosis and guiding management. DR screening and the grading of DR severity using feature specific grading is discussed in Section 2.5.

2.3.6.3 Diabetic macular ischaemia (DMI)

Diabetic macular ischaemia (DMI) is an important cause of visual impairment in patients with DR. In some patients, DMI can lead to irreversible visual loss (Sim et al., 2013). Although the underlying mechanisms of DMI are poorly understood, it has been postulated that the selective loss of pericytes and the thickening of the basement membrane in retinal capillaries occur because of chronic hyperglycaemia (Usman, 2018). DMI can be investigated using fluorescein angiography (FA) to identify the enlargement and disruption of the foveal avascular zone (FAZ) and the retinal capillary drop out that indicates a capillary loss in areas of the macula (ETDRS, 1991a). Recently, OCT angiogram (OCTA) has been used to evaluate DMI (Zhu et al., 2020). Some studies have found that in patients receiving treatment for DMO, coexisting DMI can limit the benefits of treatment (Jonas et al., 2005, Chung et al., 2008). OCTA is described in Section 2.4.1.4.

2.4 DIAGNOSIS AND TREATMENT OF DR

2.4.1 INVESTIGATIONS FOR DR

Standard clinical evaluation of DR includes fundus examination and imaging, FA and OCT, which will be covered in this section. OCTA has recently been introduced and is emerging as an important imaging modality in the investigation of DR, thus it will also be discussed here.

2.4.1.1 Fundus examination and retinal imaging

Several modalities that allow imaging of the retina are available. The most widespread is the digital optical fundus camera. The DRS protocol, later expanded by the ETDRS, used seven standard 30 degree stereoscopic colour fundus photographic images. When the seven standard fields are viewed collectively, they create an image that covers about 75 degrees of field of view (ETDRS, 1991e, Wessel et al., 2012). It is important to note that unless stereoscopic images are taken, slit-lamp biomicroscopy is still required to identify some features of DR such as retinal thickening.

The Retcam is a portable wide-angle camera system. The system does not capture images through media opacities well. However, it is well suited for paediatric patients because the patient can be supine and media opacities are rarely a problem in this population. The widest field lens provides a 130 degree field of view (Dai et al., 2011).

Scanning laser ophthalmoscopy is a more recent technology that uses laser light and the principles of confocal laser scanning microscopy. The Heidelberg Retina Angiograph system can be combined with a wide-angle lens such as the Staurenghi lens system to provide images with a field of view up to 150 degrees. FA can also be performed with the Heidelberg Retina Angiograph system (Staurenghi et al., 2005).

The Optos is an ultra-widefield system that also uses scanning laser ophthalmoscopy technology to capture images of the retina. The system can take retinal images with a 200 degree field. The system can also capture autofluorescence and FA images. The advantages of the Optos system include evaluating a wider area of the retina in less time, less impact on image quality due to media opacity because of the wider field of view and potentially less use of mydriatics. One study claimed that non-mydriatic Optos images compare favourably with dilated fundus examination in grading DR. The study found that the sensitivity and specificity of the Optos images for detecting DR diagnosed on ETDRS photographs were 99% and 100% respectively, but the study was based on a small cohort of patients (Silva et al., 2012). The Optos system has been criticised for its eyelash and other image artefacts, which can be troublesome. Although retinopathy may be readily detected, poor magnification of the macular area can make grading for maculopathy more difficult. In addition, the images are presented as pseudocolours and the interpretation of these images by graders, who are accustomed to grading traditional colour fundus photos,

can be challenging (Karmel, 2014). Given these issues, the Optos system is unlikely to be suitable for routine use in routine digital screening (RDS).

2.4.1.2 Fluorescein angiogram (FA)

FA has been the gold standard in clinical practice to visualise the retinal vasculature. FA relies on the principles of fluorescence whereby molecules that are stimulated by light of a shorter wavelength release light of a longer wavelength (Elkington et al., 1999). FA has been fundamental to the evaluation of areas of leakage and capillary non-perfusion in DR. Since FA requires the intravenous injection of fluorescein, which can cause systemic adverse effects ranging from nausea to anaphylaxis, it is only used in selected patients (Hope-Ross et al., 1994, Kwan et al., 2006).

2.4.1.3 Optical coherence tomography (OCT)

OCT was first introduced by Huang et al. two decades ago and now plays an integral part in the evaluation of DR (Huang et al., 1991). OCT is a non-invasive procedure that can be done without mydriasis and is widely used for the diagnosis and monitoring of DMO. There are currently three available OCT technologies, time-domain OCT (TD-OCT), spectral-domain OCT (SD-OCT) and swept-source OCT (SS-OCT). TD-OCT uses low coherence light that is split into two beams by a reflecting mirror, with one beam aimed at the tissue and the other at the moving reference arm. The beams then recombine at a photodetector, and the interference is assessed to determine the reflectance of the tissue (Lavinsky and Lavinsky, 2016). SD-OCT use light wavelengths instead of a time delay to determine the spatial location of reflected light. A diode laser acts as the light source while a spectrometer and camera detect the interference signals. Fourier transformation is used to produce the measurements. SD-OCT has superseded TD-OCT as the most commonly used OCT technology due to its superior image resolution and faster image acquisition speed (Schuman, 2008). The Royal Liverpool University Hospital, where this study was performed utilises the Heidelberg OCT system, which will be discussed in this section. The Heidelberg SD-OCT system has an enhanced depth imaging (EDI) mode that allows for the acquisition of higher resolution images at a greater depth, but this mode requires a longer scanning time. SS-OCT is the latest in OCT technologies and shows promise in anterior segment imaging. SS-OCT employs a frequency swept light source and a high-speed detector to detect the interference signal as a function of time, instead of a spectrometer and camera in SD-OCT (Grulkowski et al., 2012). The Heidelberg cataract and refractive imaging

platform currently utilise SS-OCT technology. For retinal imaging, SD-OCT currently offers a superior resolution in the inner retina. The Heidelberg retina and glaucoma imaging platforms currently uses SD-OCT (Barteselli et al., 2016).

The literature review on OCT is covered in Chapter 4 along with OCT methods because the existing literature has greatly informed the OCT methodology used in this thesis, including the Liverpool OCT definition of DMO, which is described in Section 4.9.

2.4.1.4 Optical coherence tomography angiography (OCTA)

OCTA is a novel non-invasive, non-mydriatic OCT technique that can potentially negate some of the limitations of structural OCT and FA. Although structural OCT is excellent in visualising the anatomical changes that may impact vision, it offers a poor contrast between small blood vessels and static tissue. Therefore, FA is used to identify vascular changes such as capillary dropout in DR (Gao et al., 2016). As mentioned previously, FA can have potential adverse effects and is time-consuming (Hope-Ross et al., 1994, Kwan et al., 2006). In addition, leakages seen on FA may obscure fine vascular structures that can be visualised on OCTA (Cole et al., 2016).

OCTA works by creating a decorrelation signal. The device compares the differences in the backscattered OCT signal intensity between sequential OCT B-scans obtained at a given cross-section (decorrelation signal) and uses this information to create a depth-resolved enface map of retinal and choroidal blood flow. The axial bulk motion due to patient movement between sequential OCT B-scans is eliminated, and the differences between repeated OCT B-scans are assumed to represent erythrocyte movement and therefore blood flow (de Carlo et al., 2015). The OCT2 is the latest platform available from Heidelberg Engineering. OCT2 combines a very high scan rate with a proprietary high-speed eye-tracking technology. This allows faster image acquisition with better alignment and repeatability compared to traditional OCT, which is required in OCTA (Heidelberg engineering, 2015). The four en-face zones captured on the OCTA in relation to retinal histology are shown in Figure 2.20 below.

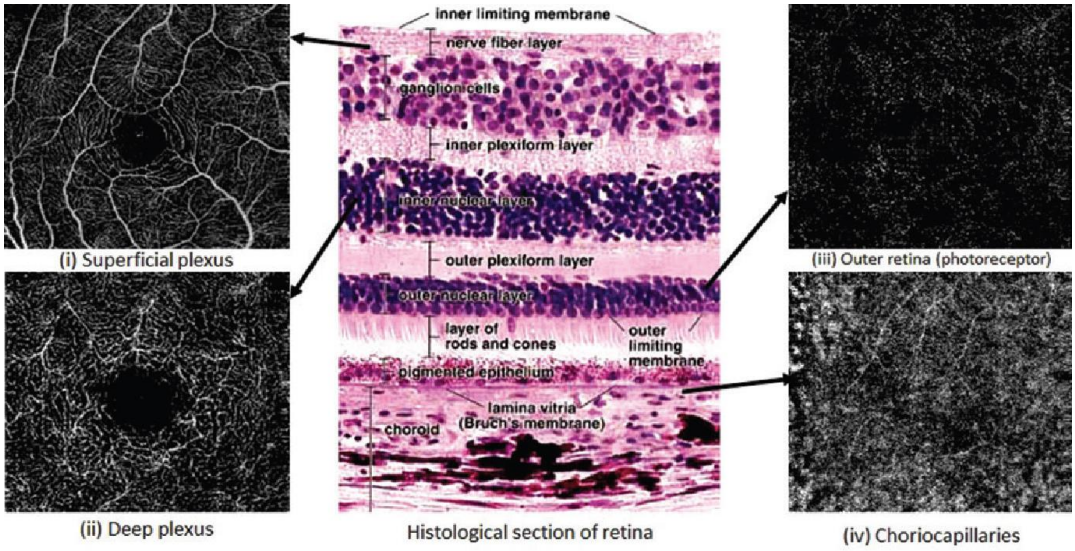


Figure 2.20. The location of different OCTA en-face zones in relation to retinal histology. The four en-face zones include (i) Superficial plexus (capillary network in the ganglion cell layer and the nerve fibre layer), (ii) Deep plexus (network of capillaries in the inner plexiform layer), (iii) Outer retina (photoreceptors), (iv) Choriocapillaries (choroid) (Chalam and Sambhav, 2016).

The OCTA could provide high yield information in DR. Hwang et al. (2015) found that the OCTA detected the enlargement and distortion of the FAZ and retinal capillary drop out. The areas of capillary loss obscured by fluorescein leakage on FA were better defined on the OCTA. They found that some areas of focal leakage on FA that were thought to be microaneurysms were small tufts of neovascularisation extending above the inner limiting membrane. Cennamo et al. (2017) examined 20 patients (31 eyes) with DMI using both OCTA and FA and found a good correlation between the two methods to define the FAZ. Images from the study (Figure 2.21) showed progressive enlargement of the FAZ with worsening DMI in both the superficial and deep capillary plexuses (Cennamo et al., 2017).

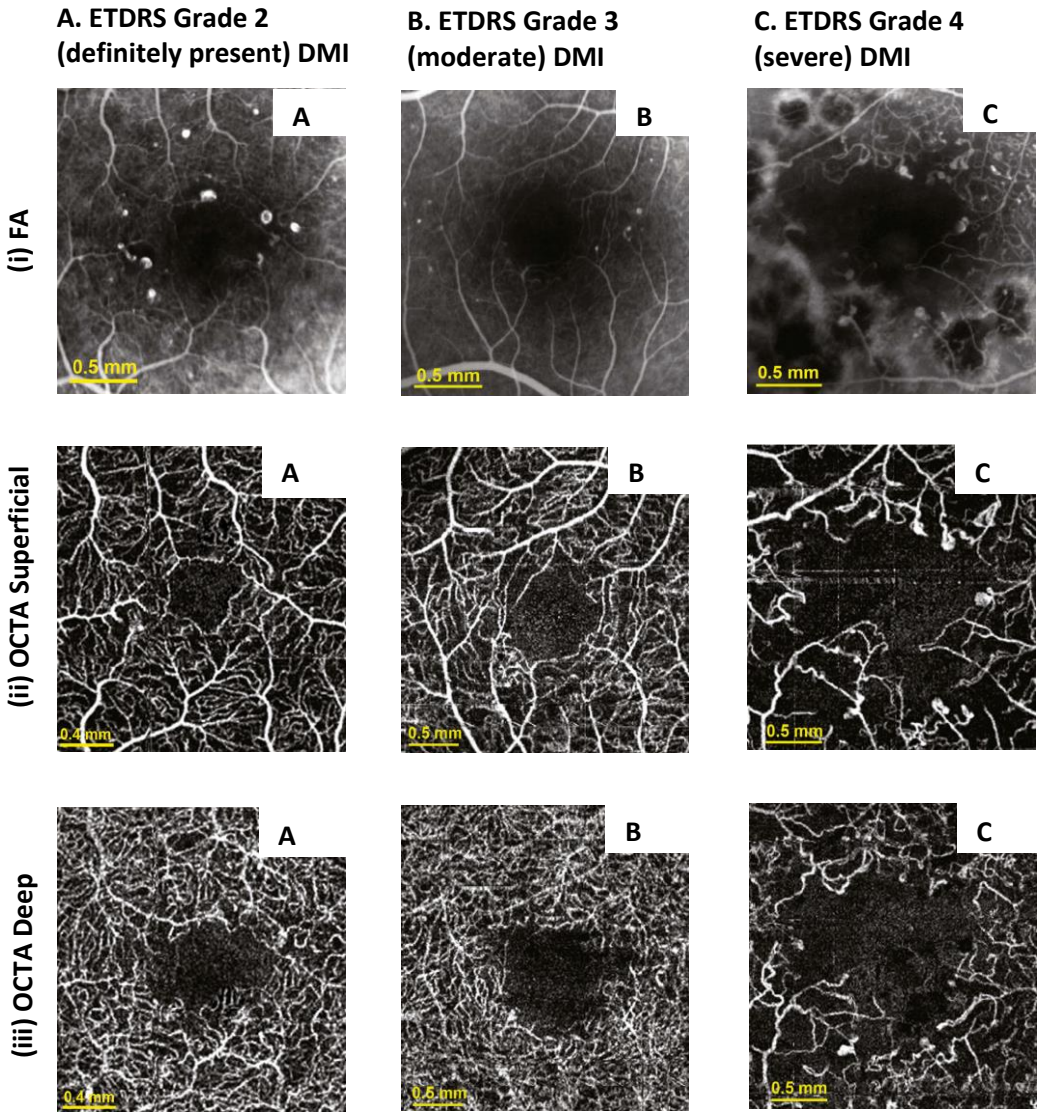


Figure 2.21. FA, OCTA superficial and deep capillary plexuses images of patients with diabetic macular ischaemia (DMI) (Cennamo et al., 2017).

Compared to FA, OCTA does not show leakage, staining or pooling, differentiate between arterioles and venules, or allow observation of areas of slow vascular flow such as micro-aneurysms (de Carlo et al., 2015, Heidelberg engineering, 2015, Gao et al., 2016). OCTA images can also have a range of artefacts, including shadow and motion artefacts, that interfere with image interpretation (de Carlo et al., 2015). OCTA has only recently been introduced, but it is already emerging as an important modality in multimodal imaging alongside OCT and FA. However, its potential to supersede FA remains to be seen. In the Royal Liverpool University Hospital, current workload constraints have precluded the routine use of OCTA on all patients referred to the diabetic eye clinic (DEC) clinics. Therefore it was not included as part of the EDDMO study.

2.4.2 OCULAR TREATMENT OF DR

The general management of diabetes has been discussed in Section 2.2. There are several treatment options to manage DR. In patients with DMO, the ETDRS showed that macular laser can prevent or slow loss of vision (ETDRS, 1991b). Aiello et al. (2010) found that macular laser can improve vision, and those with worse baseline VA showed the most improvement.

Many studies have found intravitreal anti-VEGF therapy to be beneficial in treating DMO (Stefanini et al., 2014). Current NICE guidelines recommend using intravitreal aflibercept or ranibizumab to treat DMO with a central subfield thickness (CST) of 400µm or more (NICE, 2015a). In DMO with a CST of less than 400µm, intravitreal bevacizumab and macular laser are recommended (NICE, 2013). Intravitreal brolucizumab has recently been approved by NICE for the treatment of nAMD (NICE, 2021); phase III clinical studies appear promising for its use in DMO (Garweg, 2020). The assessment of VA is also critical in making treatment decisions.

However, anti-VEGF therapy requires repeated injections. Elman et al. (2015) found that the median number of intravitreal ranibizumab injections needed over a 5-year period to treat DMO was 13 but that prompt macular laser could reduce the number of injections needed. Similar to anti-VEGF therapy in nAMD, there have been concerns that long-term anti-VEGF therapy can cause geographic atrophy (Chakravarthy et al., 2013, Lois et al., 2013).

DMO can also be treated with steroid injections. Intravitreal triamcinolone (Longo, 2006), dexamethasone (Ozurdex®) (Castro-Navarro et al., 2019) and fluocinolone acetonide (Iluvien®) (Campochiaro et al., 2011) can have an effect for 3 months, 6 months and 36 months respectively. Yet due to potential adverse effects such as intra-ocular pressure (IOP) rise, many ophthalmologists prefer to offer steroid injections in DMO refractory to other treatments (Syed, 2017).

PRP laser has been used in the management of PDR. There is evidence that earlier PRP at the severe NPDR stage may be more cost-effective than waiting until PDR has developed (Mistry et al., 2017). Vitrectomy may be needed in eyes with vitreous haemorrhage or tractional retinal detachment secondary to PDR. The Diabetic Retinopathy Vitrectomy Study (DRVS) found that early vitrectomy in eyes with vitreous haemorrhage that reduced vision to 5/200 (1.6 logMAR) or less had better visual outcomes than eyes that had

deferred vitrectomy (DRVS, 1985). Early vitrectomy also resulted in a greater chance of visual recovery in patients with type 1 diabetes, who were usually younger than patients with type 2 and had more severe PDR (DRVS, 1985).

2.5 SCREENING FOR DR

Screening for DR is important because most patients are asymptomatic until DMO or PDR develops. As mentioned in Section 2.4.2, current treatment options to manage DR are more beneficial in preventing visual loss than reversing reduced vision (Marozas and Fort, 2014). Therefore, early detection of DR prompting timely intervention is important to preserve vision in PWD. The following will introduce the principles of screening for diseases and then examine DR screening in Liverpool.

2.5.1 SCREENING

Wilson and Junger (1968) wrote a seminal article defining the principles of screenin,g which have been endorsed and promoted by the World Health Organisation. The following points represent principles of screening that are still relevant today:

1. The condition sought should be an important problem.

2. There should be an accepted treatment for patients with recognised disease.

3. Facilities for diagnosis and treatment should be available.

4. There should be a recognisable latent or early symptomatic stage.

5. There should be a suitable test or examination.

6. The test should be acceptable to the population.

7. The natural history of the condition, including development from latent to declared disease, should be adequately understood.

8. There should be an agreed policy on whom to treat as patients.

9. The cost of the case-finding programme (including the early diagnoses and treatment of patients diagnosed) should be economically balanced in relation to possible expenditure on medical care as a whole.

10. Case-finding should be a continuing process and not a one-time project.

Given DR's characteristics and the rising prevalence of individuals affected by diabetes in the world, DR is recognised as an important condition to screen for. DR screening aims to minimise visual impairment and to improve the quality of life in PWD through early detection and treatment. The following section discusses DR screening specifically.

2.5.2 DR SCREENING

The 1989 St Vincent Declaration, which aimed to reduce mortality and morbidity caused by diabetes and its complications, first encouraged DR screening in Europe (Diabetes Care and Research in Europe, 1990). The first UK National Workshop on mobile retinal screening, held in Exeter in 1994, established performance standards and guidelines for a planned DR screening programme. Some of these standards stated that screening should cover the whole of the designated population and that the method used should have a sensitivity of >80% and a specificity of >95% (Taylor et al., 1998). From 2002 to 2007, screening programmes for DR using digital retinal photography were implemented in England, Scotland, Wales and Northern Ireland (Royal College of Ophthalmologists, 2012). Each evolved nation in the UK has variations in their national screening policies despite national guidelines. The EDDMO study recruited patients from an English screening programme and this screening pathway is described in the following section. Because each screening programme is autonomous, there are some minor variations across England.

2.5.2.1 DR screening pathway

In Liverpool, general practitioners (GP) refer eligible patients with diabetes over 12 years old to the Liverpool Diabetic Eye Screening Programme (LDESP). Patients who have no perception of light in both eyes are considered ineligible. The patients are sent an invitation for screening within 3 months of referral from their GP. Screening occurs across seven locations in Liverpool (Figure 2.22).

Figure 2.23 shows an overview of the groups of patients within the screening service. Most patients have routine digital screening (RDS) annually. They are screened in the LDESP or the HES. Patients are suspended from routine screening if they are ineligible, opted out or are medically unfit. Those attending the HES for management of their DR do not undergo screening by their screening programme, but their screening data is included in annual reports. Digital Surveillance (DS) or Slit Lamp Biomicroscopy Surveillance (SLBS) are part of the screening programme. Patients are considered off the register for various reasons shown in Figure 2.23.

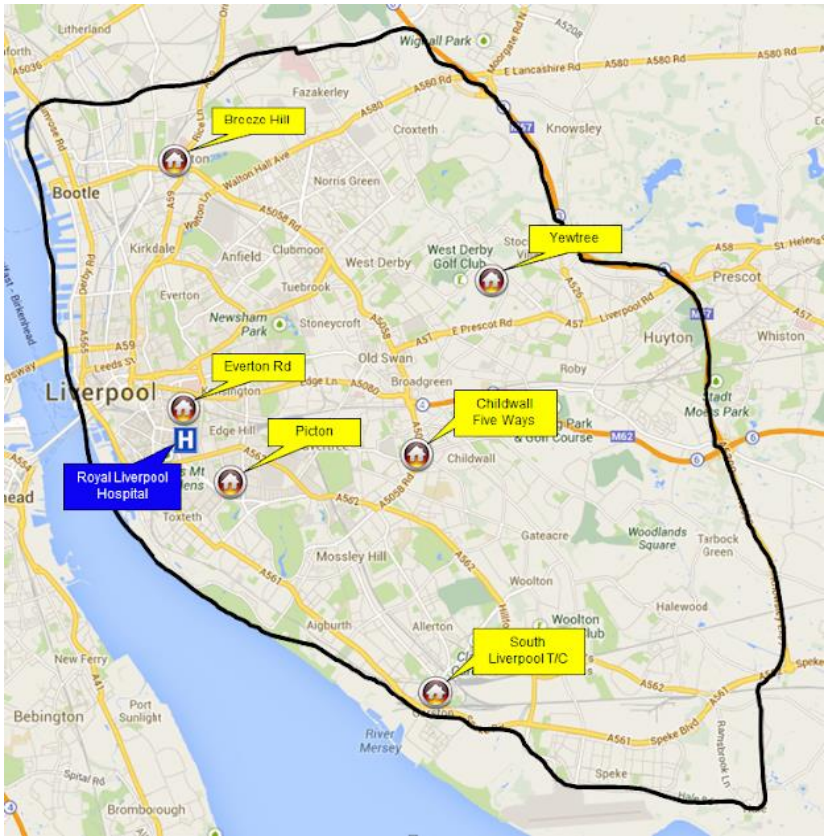


Figure 2.22. The Liverpool Diabetic Eye Screening Programme (LDESP) takes place over seven locations. Image courtesy of the LDESP.

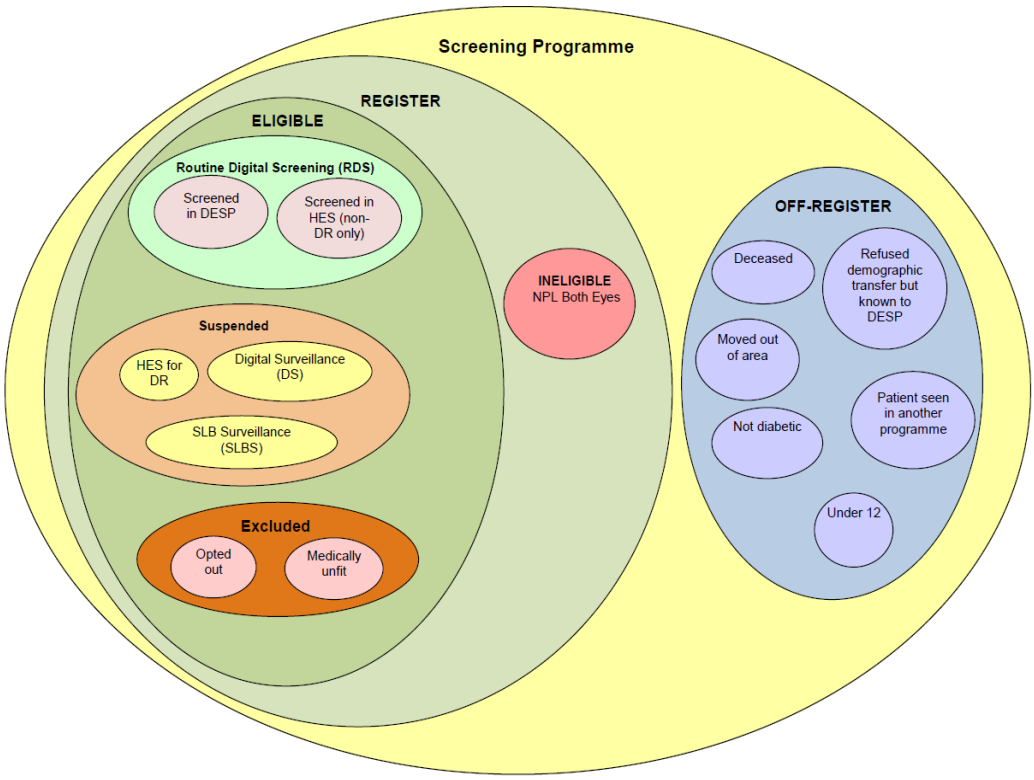


Figure 2.23. Diabetic eye screening cohort management in England and Wales. Source: https://www.gov.uk/government/publications/diabetic-eye-screening-cohort-management-overview/diabetic-eye-screening-cohort-management. Assessed 25 June 2021.

Patients are screen positive if their photographs show evidence of sight-threatening DR (STDR), are ungradeable or show other diseases. When patients are screened positive, they are referred for slit-lamp biomicroscopy by an ophthalmologist in the DEC in the Royal Liverpool University Hospital. After the review, possible patient outcomes include being discharged back to routine screening, continuing SLBS in DEC, referral for DS or referral to other services such as the glaucoma clinic as necessary. Patients remaining in the DEC are monitored or receive treatment such as laser or intravitreal therapy. DS is a special service whereby patients have their VA tested, dilated fundus photography and macular OCT. Patients are not seen by an ophthalmologist in DS. The DS pathway is best suited for patients with mild to moderate DR, and in particular, macular involvement, since a macular OCT is performed in addition to routine digital photography. The photographs and OCT images taken at DS are graded by trained graders. Depending on the outcome, these patients can either remain in DS, be referred back to DEC if there is evidence of deterioration or returned to RDS if there is evidence of improvement.

2.5.2.2 DR screening service visit

At the screening service visit, patients have their vision checked in each eye and the results are documented on the logarithm of the minimum angle of resolution (logMAR) scale. After pupillary dilation with tropicamide 1% in both eyes, patients have at least 2 fundus photographs (macular and retina photographs) taken for each eye. Liverpool uses non-stereoscopic photographs that are in line with the NHS Diabetic Eye Screening Programme (NDESP) protocols (NHS Diabetic Eye Screening Programme, 2012). These images are then graded by trained DR graders in the Royal Liverpool University Hospital (Figure 2.24). Patients and their GP receive a letter regarding the screening visit outcome.

Figure 2.24. Diabetic retinopathy screening visit in the Liverpool Diabetic Eye Screening Programme (LDESP). A. Visual acuity measurement B. Instillation of mydriatics. C. Colour fundus photographs. D. Grading of fundus photographs by trained graders. Image courtesy of the LDESP.

2.5.3 FEATURE SPECIFIC GRADING FOR DR

In 1968, a group of experts met in Airlie House, Virginia to discuss DR. During that symposium, they developed a standardised classification of DR (Goldberg and Jampol, 1987). This original classification was modified and used in many studies including the DRS

(DRS, 1981), the ETDRS (ETDRS, 1991e) and WESDR study (Klein et al., 1984a). Research protocols usually involved taking seven standard retinal photographs and grading retinal lesions in great detail. It was recognised that such detailed grading protocols were too time-consuming and impractical for routine clinical usage (Moss et al., 1989). These earlier research protocols are not discussed in detail here but the LDESP grading protocol is described in Section 2.5.4. However, the ETDRS provided a definition of CSMO (Figure 2.25), standardised photographs to grade DR (Figure 2.26) and introduced the 4-2-1 rule, which are still in widespread use. These are discussed below.

2.5.3.1 Definition of clinically significant macular oedema (CSMO)

CSMO is a clinical diagnosis based on slit-lamp biomicroscopy using a contact lens or +60D indirect lens and is defined as any one of these three features (Figure 2.25):

- Retinal thickening ≤ 500 µm of the centre of the fovea.
- Hard exudates ≤ 500 µm of the centre of the fovea if associated with adjacent retinal thickening.
- One or more disc diameters of retinal thickening, part of which is within one disc diameter of the centre of the fovea.

It is important to define CSMO because the ETDRS found that focal macular laser in eyes with CSMO reduced the risk of moderate visual loss by up to 50% (ETDRS, 1985b). Subsequent to the ETDRS, a seminal paper by Lee and Olk (1991) reported that modified macular grid laser was more effective in maintaining or improving VA in eyes with diffuse macular oedema.

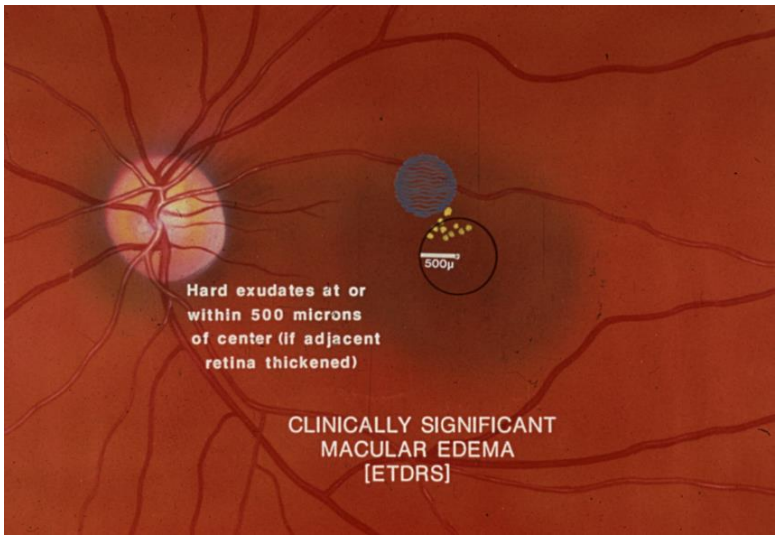


Figure 2.25. Definitions of clinically significant macular oedema (CSMO) (Cunha-Vaz et al., 2014).

2.5.3.2 The ETDRS 4-2-1 rule

An eye is diagnosed with severe NPDR if it meets one of the following three features (Figure 2.26):

- Retinal haemorrhages in four quadrants (≥ standard photograph 2A).
- Venous beading in ≥ two quadrants.
- Intra retinal microvascular abnormalities (IRMA) in ≥ one quadrant (≥ standard photograph 8A).

The ETDRS found that 17% of eyes at this severity level will develop PDR within 1 year and 40% of eyes will develop PDR within 3 years. If an eye has two out of three of the above features, it is considered to have very severe NPDR. 45% of eyes with very severe NPDR will develop PDR within 1 year and 65% will develop PDR within 3 years. Therefore, feature specific grading is valuable because it has prognostic capabilities (ETDRS, 1991e).

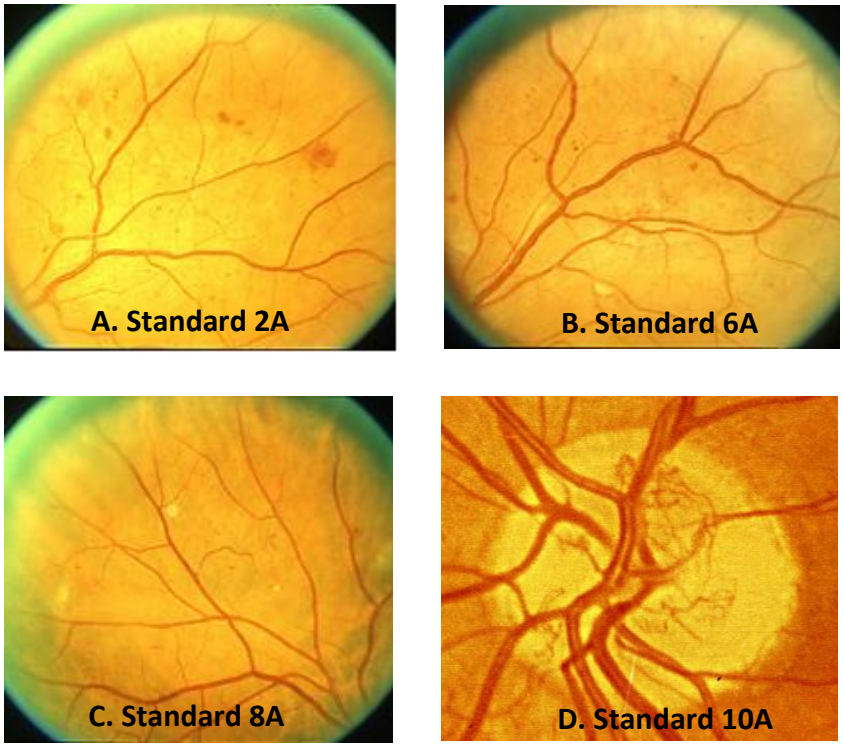


Figure 2.26. Early Treatment of Diabetic Retinopathy Study (ETDRS) standard photographs. A. Standard photograph 2A, the intermediate standard for haemorrhages and microaneurysms. B. Standard photograph 6A, less severe of two standards for venous beading. C. Standard photograph 8A, the standard for moderate IRMA. D. Standard photograph 10A, defines moderate new vessels at the optic disc (ETDRS, 1991e).

2.5.4 LIVERPOOL DR SCREENING PROGRAMME

In DR grading, protocols are organised to detect features of DR and surrogate markers of DMO and classify them according to retinopathy and maculopathy grades. Many international and national DR grading protocols exist. Although these protocols vary, they generally recognise the progression of low-risk to high-risk features, which determine management.

In 1991, the Liverpool Diabetic Eye Study (LDES) was established to develop an evidence base to support the introduction of screening for sight-threatening DR (STDR) (Harding et al., 1995). The LDES used a simplified version of the Wisconsin protocol in the ETDRS to grade the images by trained graders (ETDRS, 1991e) and established the LDESP.

In 2003, the Diabetic Retinopathy Grading and Disease Management Working Party proposed a simplification of the existing grading systems to form a coherent standard grading protocol. This standard is currently used by the NDESP in England, Wales and Northern Ireland. The party also proposed that a minimum of two fields of the retina could be photographed based on 45 degree or 50 degree nominal field sizes. One field of the retina should be centred on the optic disc and the other centred on the fovea. Images from each eye receive at least two grades, one for retinopathy (R0 to R3) and one for maculopathy (M0 or M1) (Harding et al., 2003). The images from each eye were given an additional grade if there was evidence of previous photocoagulation (P1). The most severe grade from either eye is assigned for the management of the patient. For example, if the patient was graded R1M1 in their right eye and R3M0 in their left eye, their most severe disease grade would be R3 and they would receive fast tracked referral to the HES.

The LDESP uses an amalgamation of the LDES (Harding et al., 1995) and the NDESP protocols for DR grading. The LDESP protocol has several differences compared to the NDESP protocol. For example, the LDESP protocol considers cotton wool spots (CWS) and venous reduplication while the NDESP protocol does not. Importantly, the RDS service is based on non-stereoscopic colour fundus photographs while DR grading during the DEC is based on ophthalmologist findings from slit-lamp biomicroscopy.

Tables 2.2 and 2.3 below shows the St Paul's Eye Unit at the Royal Liverpool University Hospital 2017 LDESP protocol mapped to the NDESP and ETDRS protocols (NHS Diabetic Eye Screening Programme, 2012), which is used in the EDDMO study. The identification of CSMO requires a three-dimensional stereoscopic view, which is possible using slit-lamp

biomicroscopy but not fundus photography obtained in screening. Therefore, CSMO is included as an M1 feature in DEC but not in LDESP (Table 2.4).

Table 2.2. Comparison of retinopathy grades in the NHS Diabetic Eye Screening Programme (NDESP), Early Treatment Diabetic Retinopathy Study (ETDRS) and the Liverpool Diabetic Eye Screening Programme (LDESP) protocols (Harding et al., 2003, NHS Diabetic Eye Screening Programme, 2012)

Feature specific grading	NDESP	ETDRS	LDESP	Narrative		NDESP Outcome
				LDESP	ETDRS	
No apparent retinopathy	R0	10	10	No retinopathy		Annual screening
HMA only < ETDRS photo 2A	R1	20/35	20	Background	Mild NPDR	
< 6 CWS in the absence of any other features	R0	20/35	30	Mild pre-proliferative	Mild NPDR	
< 6 CWS with HMA < ETDRS photo 2A	R1	20/35	30	Mild pre-proliferative	Mild NPDR	
Single VL	R1		40			
Any of: ● HMA ≥ ETDRS photo 2A in 1-3 quadrants ● ≥ 6 CWS ● 1 quadrant VB, VL or VR ● IRMA < ETDRS photo 8A	R2	43/47	40	Moderate pre-proliferative	Moderate NPDR	Refer to HES within 13 weeks
Any of: ● 4 quadrants HMA ≥ ETDRS photo 2A ● 2-4 quadrants VB, VL or VR ● ≥1 quadrant IRMA ≥ ETDRS photo 8A	R2	53	50	Severe pre-proliferative (4-2-1 rule)	Severe NPDR (4-2-1 rule)	
Any of: ● NVD < ETDRS photo 10A alone ● NVE < ½ DA alone ● NVE ≥ ½ DA in the absence of PRH or VH	R3a	61/65	60	PDR	Early PDR	Fast-tracked referral to HES within 2 weeks
NVD and/or NVE which in the opinion of the clinician are inactive	R3s			Stable treated DR		
FPD or FPE	R3s					
Any of: ● NVD ≥ 1/3 DA ETDRS photo 10A ● NVE ≥ ½ DA in the presence of PRH/VH	R3a	71,75	70	PDR with high-risk characteristics	High-risk PDR	Fast-tracked referral to HES within 2 weeks
VH precluding adequate view of fundus or TRD	R3a		80	Advanced PDR	Advanced PDR	
No previous photocoagulation	P0					
Previous photocoagulation	P1					
Ungradeable	U		90			Refer HES

NHS Diabetic Eye Screening Programme (NDESP), early treatment diabetic retinopathy study (ETDRS), Liverpool Diabetic Eye Screening Programme (LDESP), Hospital Eye Service (HES), Non-Proliferative Diabetic Retinopathy (NPDR), Haemorrhages and or Microaneurysms (HMA), Cotton Wool Spots (CWS), Venous Loop (VL), Venous Beading (VB), Venous Reduplication (VR), Intraretinal Microvascular Abnormalities (IRMA), New Vessels at the Optic Disc (NVD), New Vessels Elsewhere (NVE), Disc Area (DA), Pre-Retinal Haemorrhage (PRH), Vitreous Haemorrhage (VH), diabetic Retinopathy (DR), Proliferative diabetic Retinopathy (PDR), Fibrovascular Proliferation Disc (FPD), Fibrovascular Proliferation Elsewhere (FPE), Traction Retinal Detachment (TRD)

Table 2.3. Comparison of maculopathy grades in the NHS Diabetic Eye Screening Programme (NDESP) and the Liverpool Diabetic Eye Screening Programme (LDESP) protocols (Harding et al., 2003, NHS Diabetic Eye Screening Programme, 2012)

Definition	NDESP	LDESP	Outcome
Maculopathy based on exudates			
No maculopathy	M0	0	
Exudate(s) <1/2 DA >1 DD from foveal centre	M0	2	
Exudate within 1 DD of the centre of the fovea	M1	4	Refer HES within 13 weeks
A group of exudates with an area that is greater than or equal to half the DA and this area is all within the macular area	M1		
Any microaneurysm or haemorrhage within 1 DD of the centre of the fovea only if associated with a best VA of ≤ 0.30 logMAR (if no stereo photographs available)	M1	4	
Retinal thickening within 1 DD of the centre of the fovea (if stereo photographs available)	M1		
Ungradeable	U	90	
Maculopathy based on macular oedema			
No maculopathy	M0	0	
Macular thickening but not CSMO	M0	2	
Circinate ring but not CSMO	M1	3	Refer HES within 13 weeks
CSMO	M1	4	
Ungradeable	U	90	

Disc diameter (DD)

2.6 NEW APPROACHES TO DETECTING DMO

2.6.1 WHY ARE NEW APPROACHES NEEDED?

While PDR and DMO can both lead to visual impairment, DMO is the more common cause compared to PDR (Olson et al., 2013). However, current DR screening of diabetic maculopathy based on fundus photographs uses surrogate markers such as exudates to infer the presence of DMO but cannot detect DMO directly (Bresnick et al., 2000, Olson et al., 2013). In the past, the mainstay of treatment was PRP laser for PDR and treatment was limited to modified macular grid for DMO. As discussed in Section 2.4.2, various therapeutic options exist to manage DMO and preserve vision. The gap between the ability of the current RDS to detect DMO and the advancement in treatment options provides the motivation to seek alternative methods for the early detection of DMO, including tests that might be incorporated into screening, either as supplements to, or replacement for, retinal photography.

2.6.2 FUNCTIONAL MEASURES IN DETECTING DMO

The principle functional measure used in detecting DMO is best-corrected VA (BCVA). However, static VA only measures one aspect of visual function; there are other measurements that may be used and some are described below.

Contrast sensitivity (CS) has been shown to be affected in PWD before clinical evidence of DR (Krasny et al., 2007, Safi et al., 2017). Although early detection of DR is desirable, CS reduction does not specifically indicate DMO as CS reduction can be due to other pathologies such as cataracts (Howes et al., 1982). Microperimetry assesses photoreceptor function by mapping the pattern of a patient's retinal sensitivity onto an image of that individual's fundus (Midena and Vujosevic, 2011). Microperimetry has been used in a wide range of conditions from AMD to Stargardt macular dystrophy (Acton and Greenstein, 2013). Although the AG has been used in detecting DR, some disadvantages include a long test time and subsequent fatigue that makes it unsuitable as a screening test for DMO (Midena and Vujosevic, 2011, Acton and Greenstein, 2013).

Metamorphopsia is an important symptom that is associated with macular pathology of various types (Midena and Vujosevic, 2016). Therefore, a number of tests which can be used to detect metamorphopsia might have a role in the detection of DMO. One of the oldest and most familiar is the Amsler grid (AG) which is commonly provided to patients for self-monitoring of metamorphopsia in DMO (Kalinowska et al., 2018). Although it is inexpensive and easy to use, it has poor validity in detecting macular diseases (Schuchard, 1993, Achard et al., 1995). In addition, it is a subjective qualitative test based on patients' perceptions, making it difficult to monitor change.

Metamorphopsia charts (M-charts) assess metamorphopsia by using 19 dotted lines with dot sizes ranging from 0.2° to 2.0° visual angles. There is a fixation point of 0.3° in the centre of each line. The dotted lines are shown consecutively, ranging from fine lines to coarse lines, to the patient and the patient has to state when they think the distorted line appears straight; this endpoint is taken as the metamorphopsia score. The test is done in vertical and horizontal directions by rotating the charts 180° (Matsumoto, 2010). M-charts have shown promise in detecting nAMD and DMO (Nowomiejska et al., 2013, Achiron et al., 2015), but are not used widely. M-charts are unsuitable for patients with VA worse than 0.2 logMAR or large central or paracentral scotomata (Matsumoto, 2010).

Preferential hyperacuity perimetry (PHP) evaluates the central 14° of a patient's visual field. In the test, the patient is shown a line of dots whereby one of the dots is misaligned and the patient has to identify the misaligned dot. The line of dots is shown at various horizontal and vertical locations until a map of the patient's visual field is produced (Goldstein et al., 2005). When tested prospectively PHP had moderate sensitivity for detecting new nAMD (Do et al., 2012), and in a randomized trial of home monitoring, prompted patients to seek intervention sooner than in the control arm, preserving VA (Chew et al., 2014). PHP was approved by the FDA for home monitoring of nAMD (FDA, 2005). However, the PHP test relies on a patient's ability to view visual stimulus on a screen while manipulating a mouse that is out of their sight, and raises concerns that this could be a problem for elderly patients (Pitrelli Vazquez and Knox, 2015). In a study, which enrolled 109 AMD patients with a median age of 76 years, patients who failed the initial tutorial on using the test were excluded, and despite this, 13% of recruited participants were unable to produce reliable test results (Loewenstein et al., 2010). One study examined the ability of the PHP test to detect DMO in a small cohort of patients (N=33 patients, 66 eyes) and claimed the sensitivity and specificity of the PHP in detecting DMO to be 70.6% and 11.5% respectively (Matos et al., 2012).

As mentioned in Chapter 1, OCT would be an obvious option to detect DMO in a screening setting. A health technology assessment found that the introduction of OCT to RDS to detect DMO could reduce healthcare costs long-term. However, the additional set-up and running costs of OCT imaging and interpretation preclude this as a viable strategy in many health services for routine use in RDS at the present time (Olson et al., 2013).

2.6.3 HANDHELD RADIAL SHAPE DISCRIMINATION (HRSD) TEST

The handheld radial shape discrimination (hRSD) test has shown promise in detecting the earliest stages of nAMD (Pitrelli Vazquez et al., 2018). The following section examines the basis of the test and its potential suitability in detecting DMO.

2.6.3.1 Shape discrimination as a hyperacuity

Standard VA tests, such as the Snellen VA test, are based on the theory that the smallest object, which can be resolved by the eye, is limited by the spacing of the photoreceptors. The standard for normal VA is derived from the smallest distance separating two individual stimulated photoreceptors and is traditionally thought to be one minute of arc or logMAR 0.00 (Williams, 1985). However, the resolving power of the eye can be much greater

reaching 5 seconds of arc in some tasks. This hyperacuity is thought to be achieved by the retinal neuronal synaptic organisation and higher cortical processing (Hess et al., 1999, Bell and Badcock, 2008).

The natural world is full of curvatures and shapes, from clouds to flowers. The discrimination of different shapes is essential for human survival. Wilkinson et al. (1998) identified that humans are exquisitely sensitive in a particular aspect of shape processing, detecting and recognising small deviations from the circular form. Wilkinson et al. (1998) used radial frequency patterns with a circular contour and a cross-sectional luminance profile defined by a radial fourth derivative of a Gaussian (see the examples in Figure 2.27 and 2.28) and found participants to be extremely sensitive to deformations in these patterns. Detection thresholds as low as 2-4 seconds of arc were observed, which were in the hyperacuity range. They postulated that these results could not be explained by local mechanisms alone and that global pooling of contour information at higher levels of visual processing must be involved (Wilkinson et al., 1998). This further visual processing is most likely to involve extrastriate area V4 in the visual cortex, in which there are neurons known to be involved in shape discrimination (Wilkinson et al., 2000).

2.6.3.2 Shape discrimination hyperacuity (SDH) test

Wang and colleagues developed a test based on circular radial frequency patterns (Wang, 2001). The shape discrimination hyperacuity (SDH) test uses perfect and distorted circular contours as visual stimuli. Distortion from circularity is created by modulating the radius of the pattern sinusoidally. The main factors defining the stimulus pattern included: (1) mean radius (radius of the undistorted circular contour), (2) radial frequency (the number of modulation cycles around 360°), (3) amplitude of radial modulation (the amount of deformation), (4) peak spatial frequency of radial frequency patterns (determining the width of the contour), and (5) stimulus contrast (Wang, 2001, Wang et al., 2013). They found that the normal threshold for discriminating between perfect and distorted patterns was in the hyperacuity range (Wang, 2001).

SDH can be tested using either spatial or temporal order tasks. Wang et al. (2002) investigated both methods of testing on 20 patients with AMD, by presenting patterns on a computer monitor. In the spatial version of the task, patients were simultaneously presented with one distorted radial frequency shape and one undistorted radial frequency shape. In the temporal task, patients were presented with either the distorted or

undistorted radial frequency shape followed in time by the other shape for 0.5s each. In both the spatial and temporal tasks, patients were asked to select the distorted shape (2-alternative forced choice, 2AFC). In the spatial task, patients could control the testing pace and did not feel rushed. However, there was no control over fixation and it was difficult to determine which part of the retina the patient was using to complete the task. The temporal task provided more control over fixation, but at a test interval of 0.5s, patients could still have unintentionally used healthy retina to complete the task. In addition, two patients who were found to have severe deficits in the spatial task were unable to complete the temporal task. Essentially, the spatial task makes it easier to test patients with more severe retinal disease. Most subsequent SDH testing by Wang et al. (2002) was based on spatial AFC tasks.

2.6.3.3 Development and usage of the SDH test

Wang and colleagues developed a chart version followed by a desktop version of the task. More recently, a handheld version was developed in which the stimuli were presented on a small mobile device (Wang et al., 2009b, Chhetri et al., 2010). Wang et al. (2005)reported no significant difference between results obtained with the chart protocol compared with the desktop protocol. Similarly, the results of the desktop and handheld versions were highly correlated (Chhetri et al., 2010, He et al., 2010, Wang et al., 2012, Wang et al., 2013). In conjunction with the development of the different versions of the test, there were also variations in the number of patterns presented in different tests. In the 2-alternative forced-choice (2AFC) task, participants were presented with one distorted and one perfect pattern. In the 3-alternative forced-choice (3AFC) task, one distorted and two perfect circles were presented. In the 4-alternative forced-choice (4AFC) task, participants were presented with one distorted circle and three perfect circles (Figure 2.27). Participants were always asked to select the distorted circle from the simultaneously presented patterns.

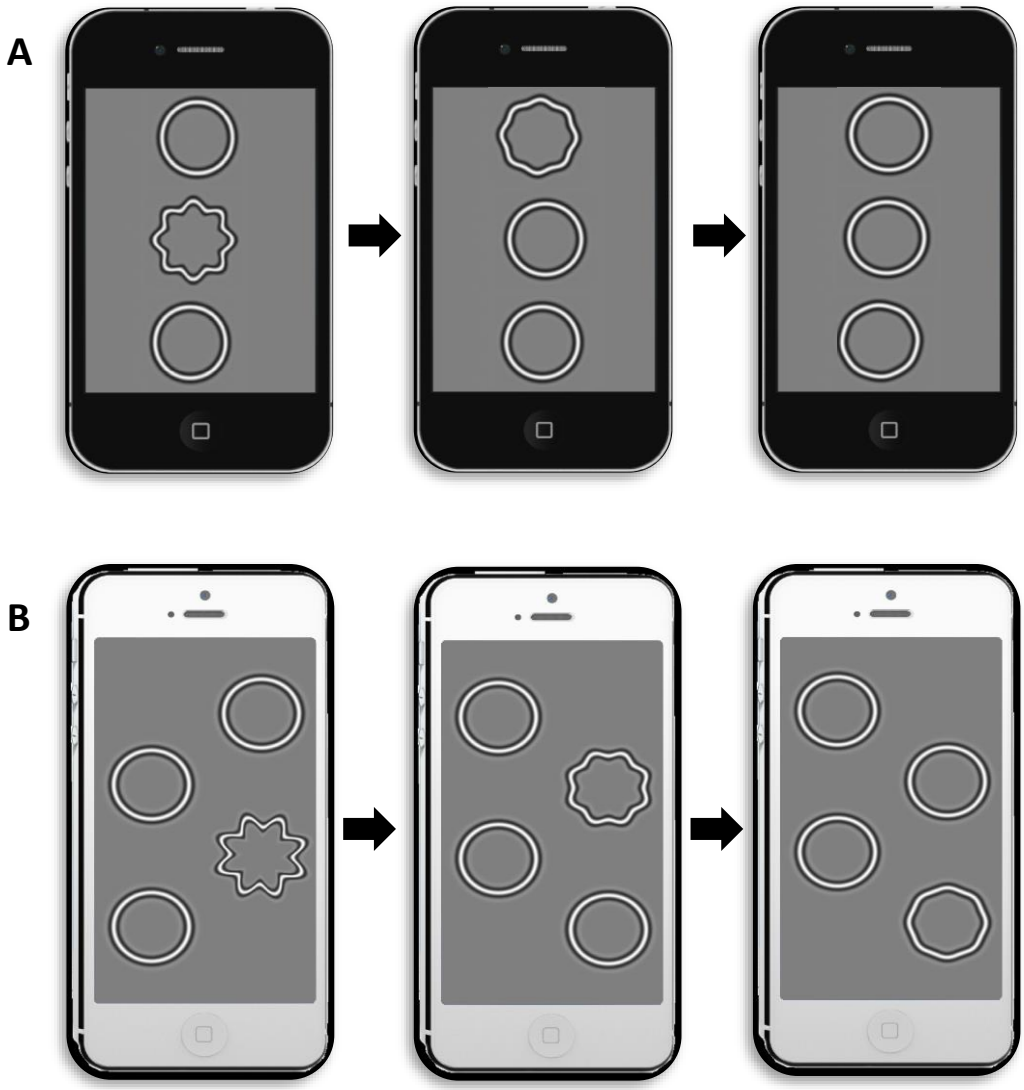


Figure 2.27. A. 3 Alternative Forced Choice hRSD test on iPod B. 3 Alternative Forced Choice hRSD test on an iPod (Bartlett et al., 2015).

The handheld version of the SDH test was finally developed as an application downloadable onto platforms such as iPods, iPhones and iPads called the myVisionTrack app. In this form, the test was passed by the FDA in the US for use in the monitoring of macular vision (FDA, 2015). In this thesis, the test will be referred to as the handheld Radial Shape Discrimination (hRSD) test. The mobile version makes the test more accessible and suitable for home testing (Kaiser et al., 2013). The rationale for this approach is that the hRSD test can be used in-between scheduled ophthalmologist visits in patients who are being monitored. If the hRSD threshold falls below a pre-set criterion, this would triggers an outpatient visit. In the case of nAMD, the system of home monitoring using hRSD might provide a convenient means of detecting either new onset of disease or reactivation. The potential benefits of using hRSD home monitoring might include increasing the duration

between scheduled visits and providing patients with assurance that their eyes are still being monitored in the meantime (Wang et al., 2013). The test follows a two-down, one-up staircase procedure whereby it decreases the modulation level (making the test harder) after two consecutive correct selections, and increases the modulation level (making the test easier) after each incorrect selection (Chhetri et al., 2010). A maximum-likelihood fitting procedure is used to fit a Weibull function to data obtained from each test. The estimated modulation threshold is defined as the stimulus level at the inflexion point of the Weibull psychometric function (Weibull, 1951, Nachmias, 1981, Wang et al., 2013). The threshold values are provided as a logMAR score (Chhetri et al., 2010).

In the hRSD test protocol for this thesis, the my Vision Track app was used to control and present test stimuli, and the app was installed on an Apple iPod Touch. Instructions for the hRSD test were provided by the tester and on-screen prompts. In the 3AFC hRSD test, the probability of making a correct selection by chance is 1/3. Since the user needs to make two correct selections in a row to advance to the next stimulus level, the probability of decreasing the level is 1/9. Correspondingly, in the 4AFC hRSD test, this probability is 1/16. Thus, the chance of a false negative in this test is very small (Chhetri et al., 2010). These two versions of the test is compared in Chapter 6.

2.6.3.4 hRSD thresholds in normal participants

To date, only a handful of studies and conference abstracts are available on the hRSD threshold in normal participants (Table 2.4) (Wang et al., 2002, Birch et al., 2000, Wang et al., 2009b, Wang et al., 2013, Bennett et al., 2016, Lott et al., 2021). The reported adult hRSD thresholds range from -0.69 logMAR to -0.86 logMAR. In the available studies, most have a limited number of participants and use different versions of the hRSD test (Table 2.4). However, adult normative hRSD thresholds appear to be generally comparable across different studies. The following section discusses the effects of ageing on the hRSD threshold.

Table 2.4. Summary of published papers of hRSD thresholds in healthy participants

Study	Number of participants	Age	Test version	Distance visual acuity (logMAR)	hRSD threshold
Birch et al. (2000)	31	4-12 months	Chart 2AFC spatial	4 months: 1.1 to 1.2	4 months: 1.1 to 1.2 logMAR
				9-12 months: 0.7	9-12 months: 0.3 logMAR
Wang et al. (2002)	10	Mean 70±9years (61-93years)	Desktop 2AFC temporal	0.03±0.03	19.6±1.8 arcsecs
Wang et al. (2009b)	236 (300 eyes)	4 months to 78 years	Chart 4AFC spatial or desktop 2AFC spatial	5 years: 0.0	3 months: 0.25 logMAR
				11 years to adult: -0.1	5.4 years: -0.56 logMAR
					Adults: -0.86 logMAR
Wang et al. (2013)	27	Mean 68.9±9.4 years (49-84 years)	iPod 3AFC spatial	0.02±0.07	-0.69 logMAR
Bennett et al. (2016)	10	35.5±6.0 years (16-66years)	iPad 4AFC spatial	Not available	-0.7±0.10 logMAR
Lott et al. (2021)	33 (56 eyes)	Mean 73.6±10.5years (56-90)	iPod 3AFC spatial	Not available	-0.69±0.14 (-0.94 to -0.33)

2.6.3.5 Effects of development and ageing on shape discrimination

Birch et al. (2000) examined the threshold for detecting distortions in infants aged 4 to 12 months using a preferential-looking protocol of radial frequency patterns presented on 2AFC charts. They found that hyperacuity improved rapidly during infancy to approximately 0.3 logMAR by 9 to 12 months of age but takes longer to reach the adult level in comparison to VA (Wang et al., 2009b, Birch et al., 2000).

Wang (2001) investigated the effects of ageing on shape discrimination on 76 adults (age range 15 to 78 years) using a 2AFC desktop version. He found that the detection threshold is not affected by contrast at low radial frequencies. In addition, hRSD showed much less change with normal ageing compared to CS and VA. Habak et al. (2009) also found the preservation of shape discrimination in ageing using radial frequency shapes. However, Weymouth and McKendrick (2012) found that shape discrimination was significantly worse in a relatively small group of older participants (N=14, mean age±SD 66±3, range 62-72 years) compared with younger participants (N=14, mean age±SD 28±6, range 19-39 years) (unpaired t=3.14, p<0.01). The main difference between Wang and Habak et al. studies and that of Weymouth and McKendrick was that Wang and Habak et al. asked participants to differentiate between radial frequency shapes with deformations from perfect circles while Weymouth and McKendrick asked participants to differentiate between radial frequency shapes with 3 deformation and 4 deformations, which is a more challenging task (Figure 2.28).

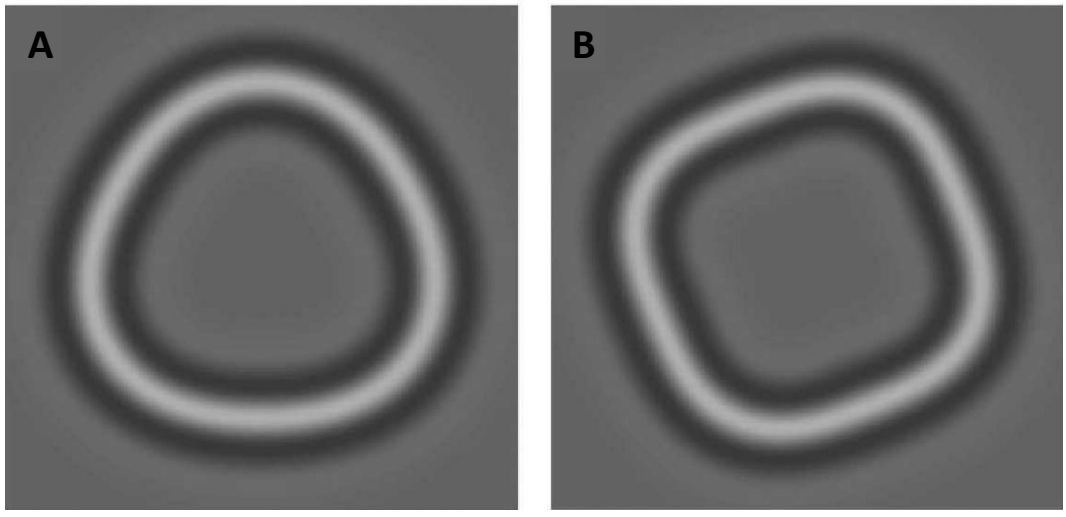


Figure 2.28. Examples of radial frequency shape with 3 deformations (A) and 4 deformations (B) used by Weymouth and McKendrick (2012).

Wang et al. (2009b) combined participants from previous studies (Wang, 2001, Birch et al., 2000) into a larger pool of participants to investigate hRSD thresholds that included 236 healthy controls (HC) ranging from 3 months to 78 years old. They found that the discrimination threshold was 0.25 logMAR at 3 months of age and it improved rapidly to -0.56 logMAR at 5.4 years of age but did not reach the mean adult level (-0.86 logMAR) until 21 years of age. Global hyperacuity then started to deteriorate from 55 years of age at the rate of 0.035 logMAR per decade. In addition, they also found that hRSD declined more slowly with ageing compared to VA with the rate of deterioration estimated to be 60% of the deterioration of VA. These test properties of hRSD would make it ideal for testing age-related eye diseases as it is less affected by normal ageing compared to VA. Data on the performance of healthy participants across the age range on the handheld version of the shape discrimination task will feature in this thesis (Chapter 5). The issue then is whether and to what extent it is sensitive to eye pathologies (Wang, 2001).

2.6.3.6 The effects of various macular pathologies on hRSD threshold

There are a small number of studies on hRSD threshold in various macular pathologies, mainly in participants with AMD and DR (Tables 2.5) (Wang et al., 2002, Wang et al., 2013, Pitrelli Vazquez et al., 2018). There is also a study on X-linked retinoschisis and one in Stargardt macular dystrophy, which is published as a conference abstract (Bennett et al., 2016, Wang et al., 2009a). Comparison of studies is complicated because different criteria for disease severity are used, and therefore, difficult to establish an approximate hRSD threshold for different disease states. However, it is clear that hRSD thresholds deteriorate as macular pathology progresses. The following section will discusses the studies involving hRSD thresholds in various macular pathologies in detail.

Table 2.5. Summary of published papers of hRSD thresholds in various macular pathologies

Authors	Number of participants	Mean age±SD years (range)	Test version	Macular Disease	Visual acuity (logMAR)	Mean hRSD threshold
Wang et al. (2002)	20 (34 eyes)	74±5 (65-81)	Desktop 2AFC temporal	AMD: - Drusen only - Drusen/hyperpigmentation - Dursen/hyperpigmentation, hypopigmentation - Extrafoveal geographic atrophy	 0.13±0.02 0.20±0.03 0.27±0.04 0.16±0.03	 37.8±3.7 arcsecs 53.8±17.8 arcsecs 60.7±7.3 arcsecs 127.1±34.4 arcsecs
Wang et al. (2013)	37 (37 eyes)	73.9±9.5 (50-93)	iPod 3AFC spatial	AMD: - Early (medium-size drusen) - Intermediate (large-size drusen or pigment change) - Advanced (geographic atrophy or exudation)	 0.11±0.20 0.23±0.20 0.41±0.18	 -0.67 logMAR * -0.36 logMAR * -0.13 logMAR *
	36 (36 eyes)	60.9±12 (40-83)	iPod 3AFC spatial	DR: - Mild to moderate NPDR - Severe to very severe NPDR or pre-PDR - PDR or NPDR affecting the fovea	 0.19±0.20 0.26±0.13 0.55±0.14	 -0.45 logMAR * -0.12 logMAR * -0.02 logMAR *
Bennett et al. (2016)	24	32.2±17.7 (9-79)	iPad 4AFC spatial	X-lined retinoschisis	0.5±0.3	-0.4±0.2 logMAR
Pitrelli Vazquez et al. (2018)	179	78±8 (52-93)	iPod 3AFC spatial	AMD: Intermediate AMD nAMD	 0.08±0.15 0.15*	 -0.53 logMAR -0.47 logMAR

*Estimated from the graphs. hRSD, handheld radial shape discrimination; alternate forced choice, AFC; age-related macular degeneration, AMD; neovascular age-related macular degeneration, nAMD; diabetic retinopathy, DR; non-proliferative diabetic retinopathy, NPDR.

Wang et al. (2002) tested 20 participants (40 eyes) with AMD using the 2AFC desktop version of the hRSD test. Of the 40 eyes, 5 eyes with VA worse than 20/50 (0.4 logMAR) and 1 eye with nAMD were excluded. Of the remaining 34 eyes, 13 had drusen only, 9 had drusen and hyperpigmentation, 7 had drusen, hyperpigmentation and hypopigmentation and 5 had extrafoveal geographic atrophy. Table 2.6 shows their VA and hRSD threshold. They found that radial frequency shape discrimination tasks could reveal visual deficits caused by AMD not identified by either VA or CS tests. They pointed out that given the density of the cone photoreceptors limit human foveal VA. According to the sampling theorem, to decrease resolution acuity by 50% (i.e. to reduce the spacing of the cones by 50%), the sampling density must be reduced by approximately 75% (Shannon, 1949). Therefore, the majority of the foveal photoreceptors must become dysfunctional before patients notice a significant loss of VA due to macular disease. Shannon (1949) postulated that in AMD, macular retinal abnormalities are inhomogeneous in early or intermediate disease. As a result, patients can still use small areas of the healthy retina to achieve normal VA. To support this, there is evidence that the human visual system can use information from surrounding intact areas of the retina to generate a perceptual filling-in of scotomas to generate a complete and undistorted perception through the scotoma. An example of this is the filling-in of the blind spot to provide an uninterrupted visual field (Zur and Ullman, 2003). However, metamorphopsia, commonly noticed by patients with macular pathologies, is due to the geometrical distortion of the normal retinal cell layers (Midena and Vujosevic, 2016). Wiecek et al. (2014) postulated that metamorphopsia is also due to the combination of retinal changes with changes in cortical processing, especially in patients with long-standing macular conditions. Wang et al. (2002) suggested that, as shape discrimination tasks require global visual processing, these tasks might reveal deficits in early macular disease not picked up by static letter acuity testing.

In a slightly larger study, Wang et al. (2013) examined 100 participants (37 AMD, 36 DR and 27 HC) using the 3AFC hRSD test presented on an iPhone app. One eye from each participant was used in the study. The study suggested that the hRSD threshold may be able to differentiate disease severity in both AMD and DR. One-way analysis of variance (ANOVA) showed that the mean hRSD threshold of the healthy participants, early AMD (medium-sized drusen, N=10), intermediate AMD (large-size drusen or pigment change, N=11) and advanced AMD (geographic atrophy or nAMD, N=16) were significantly different (p<0.001). Similarly, one-way ANOVA showed that the mean hRSD of HC, mild to moderate NPDR (N=11), severe to very severe NPDR (N=12) and PDR or NPDR affecting the fovea

(N=13) were significantly different (p<0.002). These results showed promise that the hRSD could be used to detect a change in disease severity in AMD or DR and could potentially be used to monitor treatment effects (Wang et al., 2013). However, the number of eyes in each category was small and some categories were combined for analysis (e.g. combining geographic atrophy and nAMD). Therefore, neither the numbers tested nor the categories used allowed an assessment of the diagnostic performance of the hRSD test. Interestingly, the study also found that a worse hRSD threshold significantly correlated with increased CST measured using OCT.

A small study published as an Association for Research in Vision and Ophthalmology (ARVO) abstract compared the diagnostic performance (in the form of calculating sensitivity and specificity) of hRSD, VA, CS and AG for discriminating between 24 eyes with high-risk intermediate AMD (large-size drusen) and 9 eyes with nAMD (Wang et al., 2011). The study found hRSD to have the best performance of all the tests in detecting nAMD (hRSD sensitivity 88.9%, specificity 79.2%; VA sensitivity 44.4%, specificity 66.7%; CS sensitivity 33.3%, specificity 83.3%; AG sensitivity 66.7%, specificity 41.7%) (Wang et al., 2011). However, using case-control cross-sectional analysis investigate diagnostic performance tends to flatter the performance of tests (Lijmer et al., 1999). Prospective designs avoid various types of bias. Therefore, Pitrelli Vazquez et al. (2018) used a prospective design to investigate the performance of the hRSD test in detecting the earliest stages of nAMD. Data was available from 179 patients who had unilateral nAMD with no nAMD in their other eye, which was followed prospectively as the study eye (SE). During the follow-up period, 19 SE's (10.6%) converted from having no nAMD to nAMD (converters). Converters were confirmed on OCT and with FA. The study found the hRSD threshold in the convertors to be -0.47 (95% CI -0.38 to -0.55) logMAR at the time of conversion, compared to -0.53 (95% CI -0.50 to -0.57) logMAR in the 160 non-converters. At an hRSD threshold of -0.60 logMAR, the sensitivity and specificity of the hRSD test to discriminate convertors from non-convertors (with presumed intermediate AMD) was 79% (95% CI 0.54–0.94) and 54% (95% CI 0.46–0.62) respectively. The differences in the reported sensitivity and specificity between these two studies were what would be expected given the differences in study designs (case-control vs prospective). In Wang et al. (2011) the nAMD patients had established disease as opposed to the first diagnosable stages of the disease, making discrimination easier. Interestingly, Pitrelli Vazquez et al. (2018) also found that the hRSD threshold began to decline 190 days prior to diagnosis of nAMD in the converters, while it was relatively stable

in the non-converters. This suggests that there may be a window for the early detection of nAMD, which has yet to be fully explored.

There were some studies published as ARVO abstracts which examined the effects of macular oedema on hRSD thresholds. Wang et al. (2010) tested 18 eyes with macular oedema secondary to nAMD or DR using the 2AFC desktop version. They found that eyes with macular oedema (-0.19±0.15 logMAR) had reduced mean hRSD thresholds compared to eyes with high-risk early AMD (-0.43±0.11 logMAR) and HC (-0.73±0.10 logMAR). The ability of the 4AFC hRSD test to monitor DMO was specifically examined by Wang et al. in the Diabetic Retinopathy And the MyVisionTrack App (DRAMA) study (Wang et al., 2015, Wang et al., 2016). 33 patients were monitored clinically every 3 months in a 12-month prospective study. At enrolment, the study eye was under monthly to bimonthly anti-VEGF treatments. Mean±SD hRSD threshold at the initial visit was -0.22±0.18 logMAR, which was significantly different to the hRSD thresholds at 3 months (-0.33±0.17 logMAR, p<0.003) and these values improved at 6 months (p<0.002) and 9 months (p<0.023). However, the mean±SD hRSD threshold at 12 months (-0.30±0.25 logMAR) was not significantly different from the initial visit. Interestingly, VA showed no significant change over the one-year period. These small studies suggest that hRSD can reveal visual function changes that VA fails to document in eyes with DMO.

2.6.3.7 Some advantages of the hRSD test

The hRSD test implemented on a touch screen device such as an iPod for visual function self-testing is intuitive and easy to use. Wang et al. (2013) reported high usability of the hRSD test among 46 participants with AMD or DR; 37% of the participants agreed and 63% strongly agreed that they understood how to use the test. 24% of patients agreed and 74% strongly agreed that the hRSD test was easy to use. In addition, 26% agreed and 72% strongly agreed that they felt confident they could test their vision with the hRSD test. Kaiser et al. (2013) investigated the feasibility of using the 3AFC hRSD test to monitor the eyes of 160 patients with nAMD remotely in a 16-week prospective study. Patients with nAMD in at least one eye and eligible for intravitreal ranibizumab were entered into the study. The patients were given training on how to use the hRSD test on an iPod and instructed to complete the test at least once daily. They found that 84.7% complied with daily testing while 98.9% also complied with weekly testing. Chhetri et al. (2010) reported the hRSD test as being easy to use with high rates of compliance in 28 HC. Wang et al. (2014) also reported high compliance with weekly self-testing over a 6-months period in 25

patients with DR (80±21%) and 9 patients with AMD (97±6%). In the UK, the hRSD test is currently being investigated as a home monitoring tool in AMD patients, particularly for the detection of lesion reactivation in nAMD (Ward et al., 2021). If the hRSD test is sensitive to the development of DMO, it might play a role in either screening in the diabetic population or as a remote monitoring tool in a relatively circumscribed at-risk population.

The hRSD test presented on a small, mobile, connected device is more practical than distance VA or CS tests. For protracted use, any letter chart is limited as most patients can memorise them over time. Perhaps more importantly, Wang et al. (2013) reported that the hRSD test is much less affected by the deterioration of the eye's optical system than VA and less affected by the effects of ageing compared to VA and CS. They argued that since the hRSD is a suprathreshold test, the stimulus shapes are easily visible and the test performance is less sensitive to the changes in ambient illumination, contrast and viewing distance (Wang et al., 2013).

2.7 CHAPTER CONCLUSION

In conclusion, this chapter has reviewed some of the literature on diabetes, DR, DR screening, DR management and new approaches to detecting DMO including the hRSD test. As described in Section 2.6.3, there is limited published data on the hRSD threshold in healthy participants and for various macular pathologies. Most of the available clinical information on the hRSD threshold has been collected on AMD patients with fewer studies on DR and DMO (Table 2.6). hRSD testing could play a role in either monitoring diabetic eye disease or in detecting DMO specifically. Given the relatively low cost and ease of use of the hRSD test, it could potentially be introduced as an adjuvant test in DR screening if it can detect DMO. Therefore, the EDDMO study aims to fill a gap in the literature on the ability of the hRSD test in the early detection of DMO.

In this thesis, hRSD test performance in both healthy participants and patients suspected of having or with DMO is explored along with distance and near VA. Given the critical role of OCT in the detection and monitoring of diabetic eye disease, macular OCT measurements and correlations with visual function, specifically measurements using the hRSD test, are investigated. The next chapter (Chapter 3) describes the general methods used in this thesis while Chapter 4 details the OCT literature review, analysis methods used and examine some of the definitional issues of DMO as seen on OCT. Results from this thesis serves to advance the current literature on the hRSD threshold with OCT findings in DR and DMO.

CHAPTER 3. PARTICIPANTS AND GENERAL METHODS

3.1 CHAPTER INTRODUCTION

In this thesis, the detection of DMO using both functional and structural means is investigated with particular reference to the hRSD test and macular OCT. This chapter covers the ethics approval, participants, inclusion and exclusion criteria, and standard operating procedures used in the various studies undertaken and described in this thesis. Details of the OCT acquisition protocol, image analysis and OCT classification of DMO are described in Chapter 4.

3.2 ETHICS STATEMENT

This study was performed in accordance with the ethical standards laid down in the Declaration of Helsinki. The Early Detection of Diabetic Macular Oedema study (16/NW/0163) was approved by the Health Research Authority, Northwest Research Ethics committee, covering the studies on PWD and age-matched HC. A protocol amendment to extend recruitment to a broader range of participants and perform additional procedures was later approved (16/NW/0163, V3). Additional healthy participants were obtained from the Assessment of visual functions, improving test procedures study (RETH000827) that was approved by the University of Liverpool Committee on Research Ethics and also from the Early Detection in Macular Disease study (13/NWEST/0449) that was approved by the Health Research Authority, Northwest Research Ethics committee. Written study information was provided to all participants, and their written informed consent was obtained (Appendices 1-4).

3.3 SAMPLE SIZE CALCULATIONS

There is no published information currently available on the sensitivity of the hRSD test for detecting DMO. Keenan et al. (2013) examined a large cohort of patients managed by the HES across 30 NHS trusts and found the prevalence of centre involving macular oedema (CIMO) to be 8.7-10%. To estimate recruitment targets, it was therefore assumed that 10% of the patients referred as M1 from the NDESP might be expected to have CIMO. On this basis, hRSD data from a minimum of 200 patients would allow the sensitivity of the test to detect CIMO to be established with a precision of ±20% (95% CI). As alluded to in Chapter 2 (Section 2.6), the type of study being conducted and the details of the discrimination

required, have a large influence on apparent test performance. Discriminating between CIMO and other levels of diabetic eye disease is clearly quite different from discriminating CIMO from performance in healthy participants. Further complicating the issue is that there are alternative ways of classifying the patients of interest in this study. Therefore, at the outset of the study, statistical advice was sought, and this suggested a recruitment target of 300 patients.

3.4 PARTICIPANTS

3.4.1 HEALTHY PARTICIPANTS

A total of 229 visually healthy participants were involved in the study, the majority of whom (N=213) were recruited as self-reported healthy participants from staff and students of the University of Liverpool, Royal Liverpool University Hospital and from the wider community. Participants with either self-reported ocular abnormalities or who were found to have abnormal ocular examinations were excluded from the study. Data was also available from a group of 16 older participants who had nAMD in one eye but no clinical evidence of retinal disease in their fellow, used here as the study eye. This was confirmed by macular OCT. Of the total 229 healthy participants, 80 participants completed the 3AFC hRSD test while 149 participants completed the 4AFC hRSD test. 106 participants completed both versions of the test that allowed a direct comparison (comparison group; Figure 3.1). Of the 229 participants, a group of 50 participants (control group) who did the 4AFC hRSD test were specifically recruited as a healthy age-matched comparison group for the PWD in the clinical part of this project, with the same age structure as the PWD group and these 50 participants had macular OCT.

Healthy Participants Inclusion Criteria: Minimum age 18 years old, able and willing to provide informed written consent.

Healthy Participants Exclusion Criteria: Previous or current ocular pathologies, amblyopia, concurrent health problems such as diabetes that may affect vision

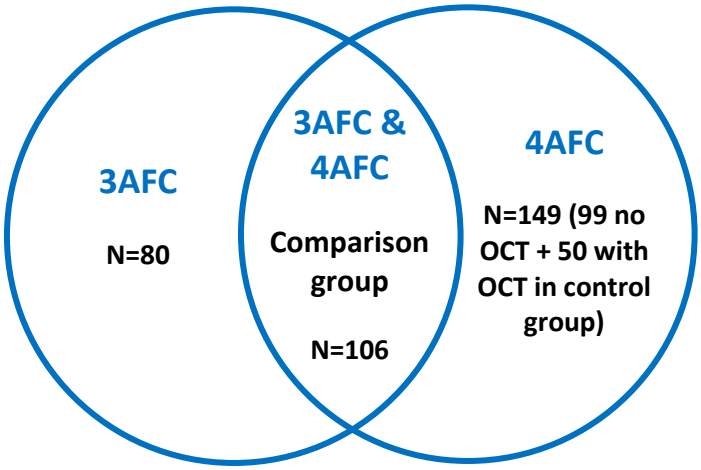


Figure 3.1 Total of 229 healthy participants; 80 participants did the 3AFC hRSD test while 149 participants did the 4AFC hRSD test. 106 participants in the comparison group did both versions of the test. Of the 149 participants who performed the 4AFC hRSD test, 99 had no OCT and the remaining 50 (control group) who had macular OCT were used as age-matched controls for the group of PWD.

3.4.2 PEOPLE WITH DIABETES

Participants were recruited from the DEC and they were referred to the DEC from two sources (Figure 3.2A). The first of these sources comprised PWD who attended LDESP for their routine screen event, which comprised of their digital fundus photography through dilated pupils and distance VA between October 2015 and July 2017. Initially, PWD with suspected diabetic maculopathy graded as R1M1 or R2M1 in either eye was eligible for recruitment. A protocol amendment (16/NW/0163, V3) was later approved by the ethics committee to extend recruitment to participants screened as R3M1 in either eye to allow the collection of data across a wider spectrum of disease severity. A total of 310 participants were recruited from the DEC in the Clinical Eye Research Centre in the Royal Liverpool University Hospital from May 2016 to August 2017 of whom 292 participants were eligible for the study. Of the 292 PWD, 272 were referred from RDS and 20 were referred from DS (Figure 3.2A). As this was an observational study, the necessity of subsequent visits was determined by the ophthalmologist based on clinical requirements. Ophthalmologists consisted of consultants, staff grade doctors and trainees. Participants' consent covered a second assessment where all elements of initial tests were repeated. 159 participants attended for a second visit in DEC (N=134) or DS (N=25) and were retested to provide longitudinal data (Figure 3.2B).

PWD Inclusion Criteria: Minimum age 18 years old, new referral from LDESP as M1 to DEC, able and willing to provide informed, written consent.

PWD Exclusion Criteria: Concurrent macular pathology such as intermediate to severe dry AMD, nAMD, epiretinal membrane (ERM), vitreomacular traction (VMT), macular holes, post-operative cystoid macular oedema, amblyopia, cerebral pathologies resulting in visual impairment.

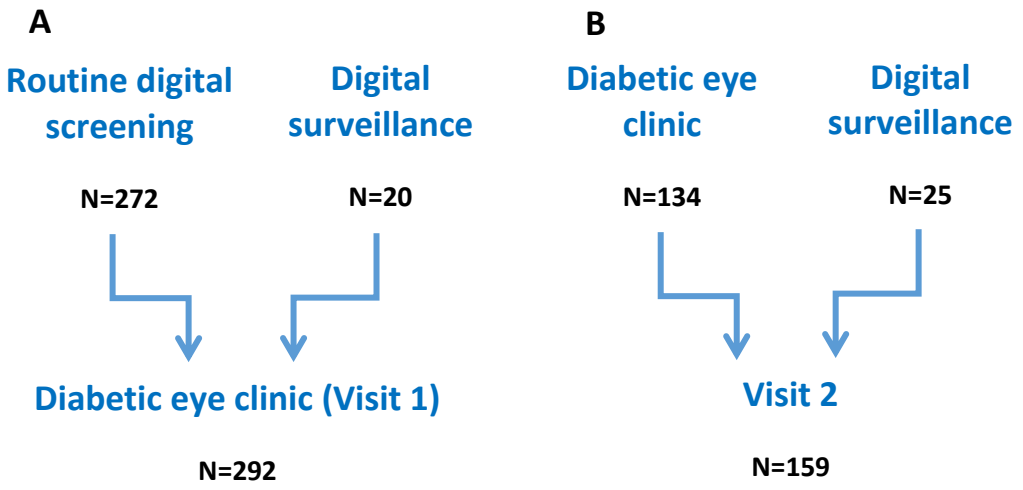


Figure 3.2 (A) In visit 1, a total of 292 eligible people with diabetes (PWD) were recruited, 272 were referred from routine digital screening (RDS) and 28 were referred from digital surveillance (DS) (B) 159 PWD had a second visit. 134 attended the diabetic eye clinic (DEC) and 25 attended DS.

3.5 PROCEDURES

3.5.1 HEALTHY PARTICIPANTS

All participants were questioned with regard to their ocular and general health. The majority (N=149) completed slit-lamp biomicroscopy and undilated fundoscopy, and the remainder had no clinical examination. Contrast sensitivity (CS; N=170) were also available for most participants. hRSD tests were performed using an Apple iPod Touch and the myVisionTrack (mVT) application (Vital Art & Science LLC, Richardson, Texas) described in Section 2.6. Testing was performed with the participants' habitual optical correction or age-appropriate near correction if they had forgotten to bring their glasses (Table 3.1) (Antona et al., 2008). The right eye was tested first with the fellow eye patched and vice versa. Previously published studies on the hRSD have used the 3AFC version, which has been superseded by the updated 4AFC version. To establish how comparable the hRSD threshold

was between the two versions, both versions were used in a group of participants (N=106; Figure 3.1).

In the 3AFC version of the test, two circular and one distorted radial frequency patterns were presented. The positions of the distorted and non-distorted patterns were randomised. In the 4AFC version of the test, one distorted and three non-distorted patterns were shown. Both the 3AFC and 4AFC versions of the hRSD test followed a 2-down, 1-up adaptive staircase procedure to determine the participants' threshold for detecting distortion. As part of the hRSD in-built test protocol, a third test was performed if there was a difference of ≥0.3 logMAR between the first two tests. At the end of the test, the results (the hRSD thresholds) were displayed on the iPod Touch screen and documented.

Table 3.1 Age expected near addition for presbyopia (Antona et al., 2008)

Age (years)	Additional power
40-42	+0.75
43-45	+1.00
46-47	+1.25
48-50	+1.50
51-52	+1.75
53-55	+2.00
56-57	+2.25
≥58	+2.50

A subgroup of participants undertook multiple 3AFC hRSD tests to provide test-retest data; these participants were recalls from an earlier study (RETH000827). 74 were tested twice within a single session, 30 of these participants returned for repeat testing within a few months, and 15 after a period of several years. Similarly, 149 participants undertook 4AFC tests and 7 returned for testing after several years.

Participants in the comparison group (N=106) were tested with both 3AFC and 4AFC versions of the hRSD test in a single test session to provide directly comparative data. The test order (3AFC vs 4AFC) was balanced across participants, and these participants also completed a 5 question usability survey asking them to explicitly compare the two versions of the test (Table 3.2). Near VA was measured using ETDRS 2000 series charts at 40cm, and their distance VA was measured using ETDRS or Bailey-Lovie logarithmic vision charts at 4m. CS was tested using the Pelli Robson CS chart at 1m and the results recorded as logCS units.

Table 3.2 hRSD usability survey questions

Questions	Possible responses
1. I understood how to use the hRSD test. 2. The hRSD test was easy to use. 3. The hRSD test did not take too long to do. 4. I could use the hRSD test to test my own vision.	1. Strongly disagree. 2. Disagree. 3. Neutral. 4. Agree. 5. Strongly agree.
5. Please select the statement that you most agree with.	1. The 3 choices hRSD test was much easier to use. 2. The 3 choices hRSD test was somewhat easier to use. 3. There was no difference in the ease of using either test. 4. The 4 choices hRSD test was somewhat easier to use. The 4 choices hRSD test was much easier to use.

A group of 50 participants were recruited as age-matched controls for the PWD (control group). The objective was to provide a group with the same age structure as the patient group. They performed 4AFC hRSD, near VA using the 40 cm ETDRS chart and distance VA using the 4m Bailey-Lovie Logarithmic vision chart. The protocol amendment (16/NW/0163, V3) approved by the ethics committee allowed autorefraction performed with a Nidek Ark 530A and biometry performed with a Zeiss IOL Master 07740, which were not available for the other healthy participants. These two tests were added to explore the relationship between axial length and retinal thickness. These participants also had macular OCT to exclude ocular pathologies and for further comparison with the PWD. Since this group of participants only used the 4AFC hRSD, they completed the first 4 questions of the usability survey and not the last question comparing the two versions of the test (Table 3.2).

3.5.2 PEOPLE WITH DIABETES

Eligible participants received a study information sheet along with their clinic appointment. On the day of their DEC appointment, participants were approached by the PhD candidate (J Ku) or another staff member, and if agreeable, written consent was obtained (Figure 3.3). Participants were asked about their ethnic background, ocular and general health. PWD were specifically asked about the type of diabetes they had, the duration of their diabetes and current medications. Near VA was measured using 40 cm ETDRS 2000 series charts and PWD had 4AFC hRSD testing as per the healthy participants. Testing was performed with the right eye followed by the left eye with the participants' habitual optical correction or

age-appropriate near correction if they have forgotten to bring their glasses (Table 3.1) (Antona et al., 2008). Similar to the control group, only participants recruited after the protocol amendment (16/NW/0163, V3) had the additional autorefraction performed with a Nidek Ark 530A and biometry performed with a Zeiss IOL Master 07740. As the PWD only used the 4AFC hRSD, they completed the first 4 questions of the usability survey and not the last question comparing the two versions of the test (Table 3.2).

All participants then proceeded to their scheduled DEC visit and received routine assessments that included measurements of distance VA using a 4m Bailey-Lovie logarithmic vision chart, BP, IOP and slit-lamp biomicroscopy including dilated fundoscopy examination (Figure 3.3). All participants had macular OCT. The OCT protocol is described in detail in Chapter 4. Figure 3.4 shows the Royal Liverpool University Hospital follow-up pathway for PWD who have been seen in the DEC.

HbA_{1c} results were obtained from the Integrated Clinical Environment software from the Royal Liverpool University Hospital. Only blood results within 3 months before their assessment were used because the life span of haemoglobin is 2 to 3 months and HbA_{1c} will only indicate glycaemic control within that time frame (Inzucchi, 2012). If there were multiple HbA_{1c} results available during the 3 months' timeframe, the result closest to their assessment date was used.

For participants who had a follow-up in DEC, all the same tests from their initial visit were repeated. For participants who were followed up in the DS clinic, they had the same tests repeated for the EDDMO study component but their subsequent clinic visit differed. The participants in DS had distance VA tested with a 4m Bailey-Lovie chart, macular OCT and were dilated for colour fundus photographs. Their macular OCT and colour fundus photographs were reviewed by an experienced grader to establish a clinical follow-up plan.

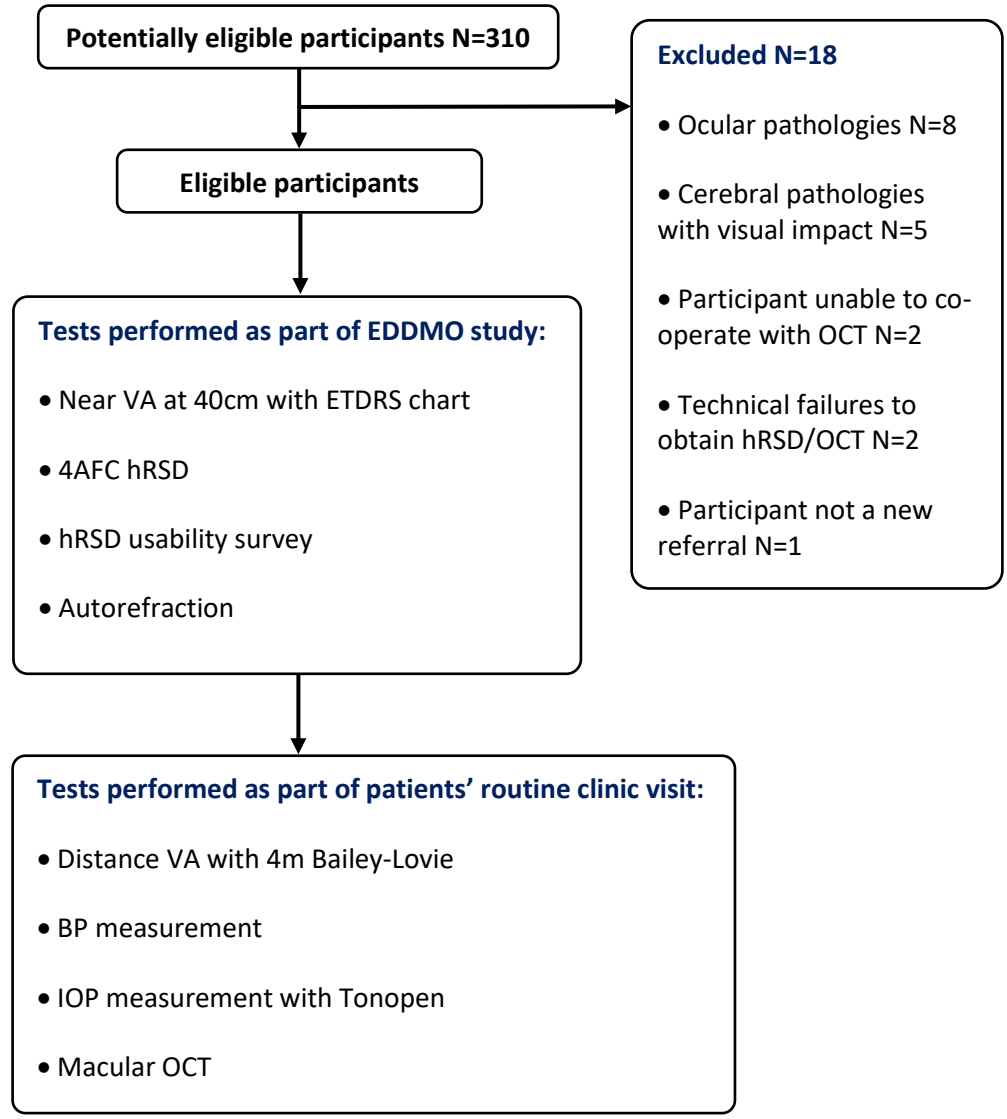


Figure 3.3. The flow of people with diabetes through the study.

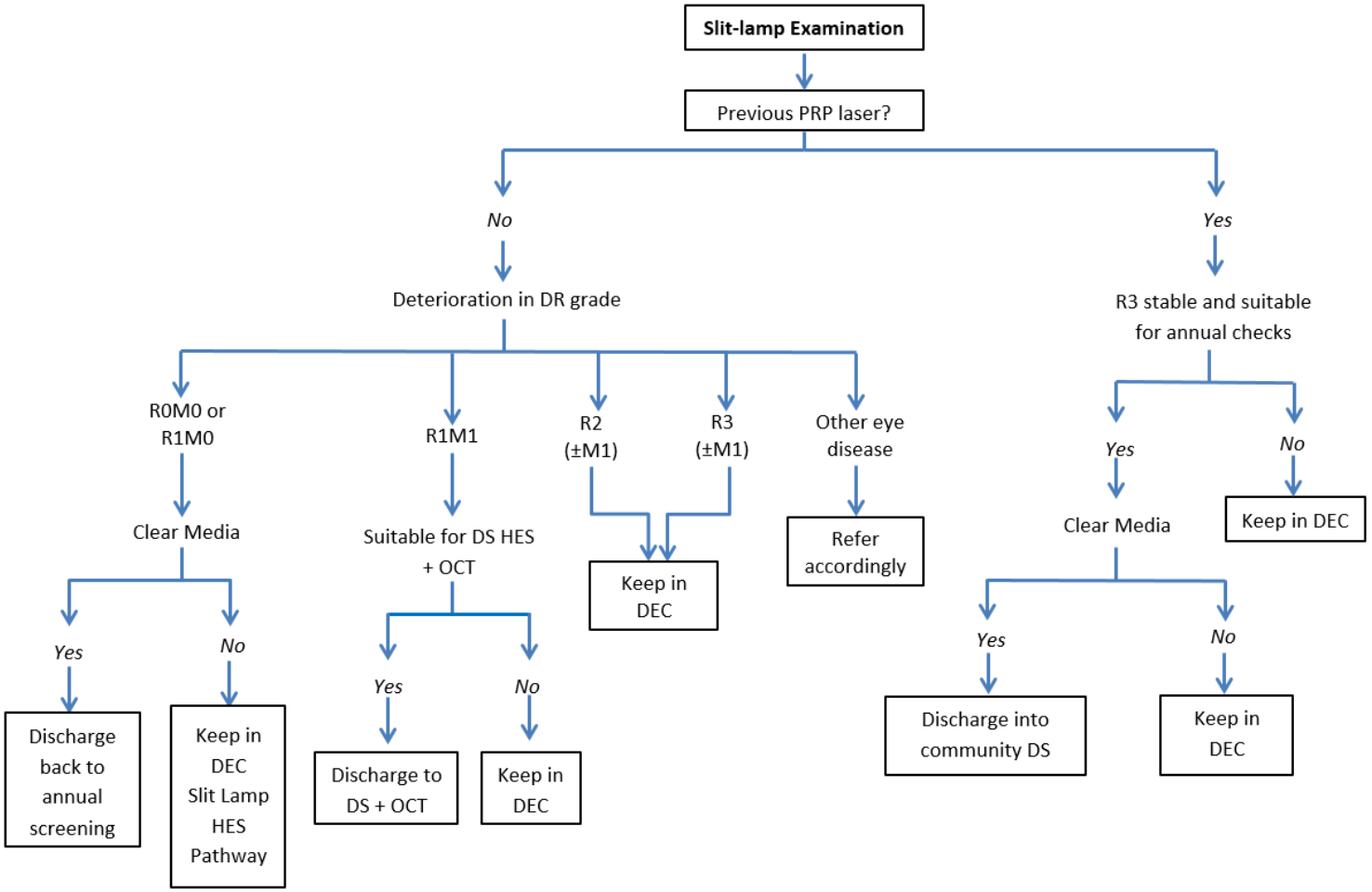


Figure 3.4 Royal Liverpool University Hospital follow-up pathway for people with diabetes (PWD) who have been seen in the diabetic eye clinic (DEC). Peripheral retinal photocoagulation (PRP), digital surveillance (DS), hospital eye service (HES).

3.6 DATA MANAGEMENT AND ANALYSIS

Study participants were allocated an anonymous participant code when they gave consent. All participant data were collected on paper then entered into a Microsoft Access 2016 database. All paper data were stored in locked cupboards at the University of Liverpool to be retained securely for 10 years upon the completion of the study, which is consistent with the University of Liverpool data policy. All computer files were stored on password-protected devices. Where data from both eyes were available, one eye was randomly selected for analysis unless specifically specified. Each results chapter has a data analysis section to describe the statistical approaches performed.

3.7 CHAPTER CONCLUSION

This chapter has covered the participants and general methods used in the EDDMO study. The following chapter contains a brief literature review on OCT and describes the OCT methods that are relevant to the EDDMO study.

CHAPTER 4. OCT LITERATURE REVIEW, PROTOCOLS AND GRADING METHODS

4.1 CHAPTER INTRODUCTION

Previous literature on OCT influenced the OCT methods used in the EDDMO study. This chapter commences with a brief literature review on ETDRS grids, and retinal thicknesses and volumes in healthy participants, and PWD with no or minimal DR. This sets a context for the description of the OCT acquisition protocol, image analysis and OCT classification of DMO in the chapter. OCT methods have been separated into this chapter for clarity because the information is different from the participants and general methods described in Chapter 3.

While there are several proposed OCT definitions of DMO, no general consensus on these has been reached (Panozzo et al., 2020, Parodi Battaglia et al., 2018, Panozzo et al., 2004, Kang et al., 2004, Kim et al., 2006, Koleva-Georgieva and Sivkova, 2008, Helmy and Atta Allah, 2013, Bolz et al., 2014, Reznicek et al., 2016). Therefore, a local definition was developed in the St Paul's Eye Unit in the Royal Liverpool University Hospital, and this will be discussed here.

The advent of OCT has allowed the examination of the effect of retinal disease on retinal structure, thickness and volume. The analysis of these measurements and other features has greatly contributed to the diagnosis and management of DR. Until a few years ago, Heidelberg Spectralis allowed only full retinal thickness measurements from the internal limiting membrane (ILM) to the posterior border of the RPE and Bruch's complex. With the introduction of Heidelberg retinal auto-segmentation software around 2017, it is now possible to obtain thickness values for different retinal layers (Li et al., 2017). The evaluation of particular layers could enhance the management of DR because the disease process can affect various retinal layers in different ways. For example, RNFL thinning has been associated with diabetic peripheral neuropathy (Shahidi et al., 2012). In DMO, fluid can initially accumulate in the INL and OPL due to breakdown of the BRB. Later, fluid can accumulate in the IPL and RNFL until the entire thickness of the retina becomes oedematous (Murakami and Yoshimura, 2013, Das et al., 2015). This chapter will start with the nomenclature used to describe different retinal layers seen on OCT.

4.2 NOMENCLATURE FOR NORMAL OCT TERMINOLOGY

Various terms have been used to describe the different retinal layers and features observed on OCT. In this thesis, the terms used are those proposed by the international nomenclature for OCT panel, which have gained widespread acceptance (Staurenghi et al., 2014) (Figure 4.1).

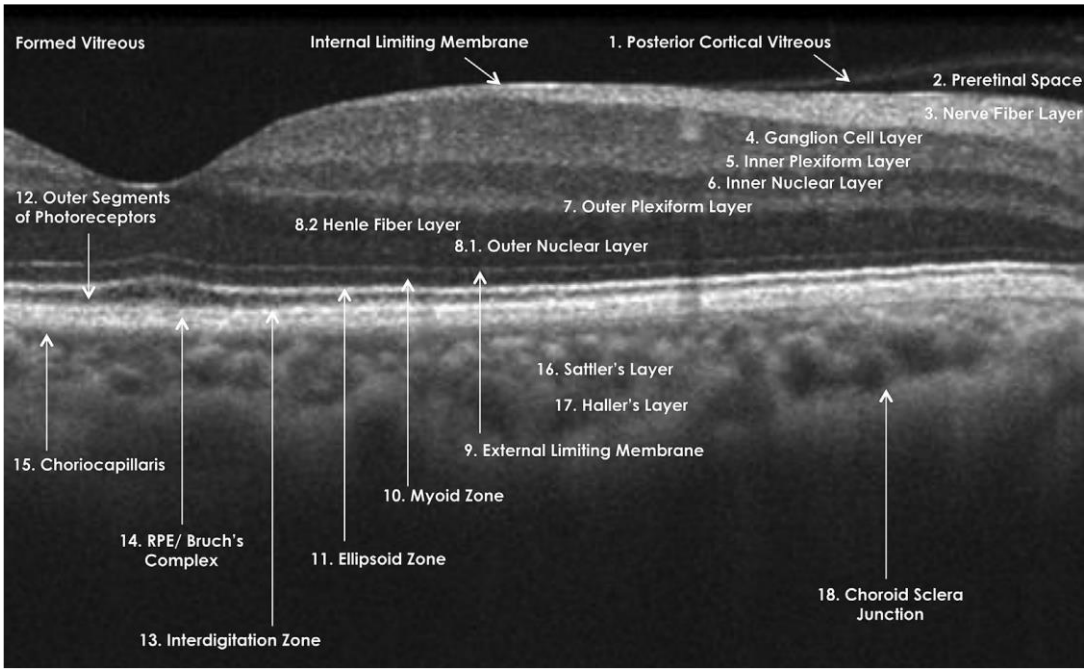


Figure 4.1. Nomenclature for normal anatomical landmarks seen on spectral-domain OCT images imaged using Heidelberg Spectralis; RPE (retinal pigment epithelium) (Staurenghi et al., 2014).

4.3 EARLY TREATMENT DIABETIC RETINOPATHY STUDY (ETDRS) GRIDS

By convention based on the ETDRS (ETDRS, 1991e), the macula is divided into nine subfields consisting of a circular central subfield (CSF; other subfields defined in Figure 4.2) 1mm in diameter that is centred on the fovea and surrounded by two further concentric regions with diameters of 3mm and 6mm (ETDRS, 1991e). The middle and outer regions are divided into four subfields, each as shown in Figure 4.2. It is important to note that some ETDRS publications have used a grid consisting of three concentric circles with diameters of 1mm, 2.22mm and 3.45mm, and some publications have obtained measurements for subfields based on this smaller ETDRS grid (ETDRS, 1991c). However, most publications, including this

thesis, use the larger ETDRS grid size (concentric circles measuring 1mm, 3mm and 6mm; Table 4.1).

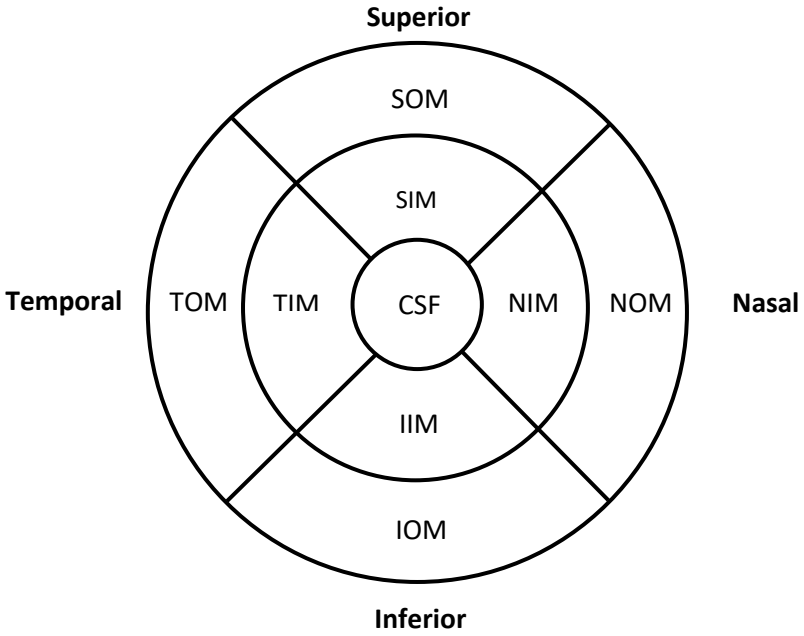


Figure 4.2. Depiction of the nine ETDRS subfields in the right eye (ETDRS, 1991e). CSF (central subfield), SIM (superior inner macula), NIM (nasal inner macula), IIM (inferior inner macula), TIM (temporal inner macula), SOM (superior outer macula), NOM (nasal outer macula), IOM (inferior outer macula), TOM (temporal outer macula).

Table 4.1. ETDRS grids subfields and diameters (ETDRS, 1991e)

Area	**Subfields**	**Smaller ETDRS grid diameter**	**Larger ETDRS grid diameter**
Foveal	Central	1mm	1mm
Parafoveal	Inner	2.22mm	3mm
Perifoveal	Outer	3.45mm	6mm

4.4 VARIATIONS BETWEEN OCT SYSTEMS

With each new generation of OCT instruments, there has been improved image resolution. There are variations of the outer retinal reference point of different OCT systems that can affect thickness measurements (DRCR network., 2012). For example, time-domain OCT, such as the Zeiss Stratus, measures retinal thickness as the distance from the ILM to the junction of the photoreceptor inner and outer segments while spectral-domain OCT, such

as the Heidelberg Spectralis measures retinal thickness as the distance from the ILM to the posterior border of the RPE and Bruch's complex (DRCR network., 2012) (Figure 4.3). Therefore, measurements of the mean CST from the Zeiss Stratus OCT and Heidelberg Spectralis OCT in healthy eyes differ by approximately 70µm (Zeiss Stratus OCT 200µm; Heidelberg Spectralis 270µm) (Kiernan, 2010, Grover et al., 2010). This is consistent with the 70µm difference between older DRCR network mean CST thresholds in DMO measured using the Zeiss Stratus OCT (≥250µm) compared to the more recent threshold for determining the presence of DMO based on the Heidelberg Spectralis OCT (≥320µm) (DRCR network., 2012, Bressler et al., 2008). As OCT data from the EDDMO study were obtained using Heidelberg Spectralis, full retinal thickness is measured from the ILM to the posterior border of the RPE and Bruch's complex, and comparison of retinal thickness measurements will mainly be made with studies that also used Heidelberg Spectralis.

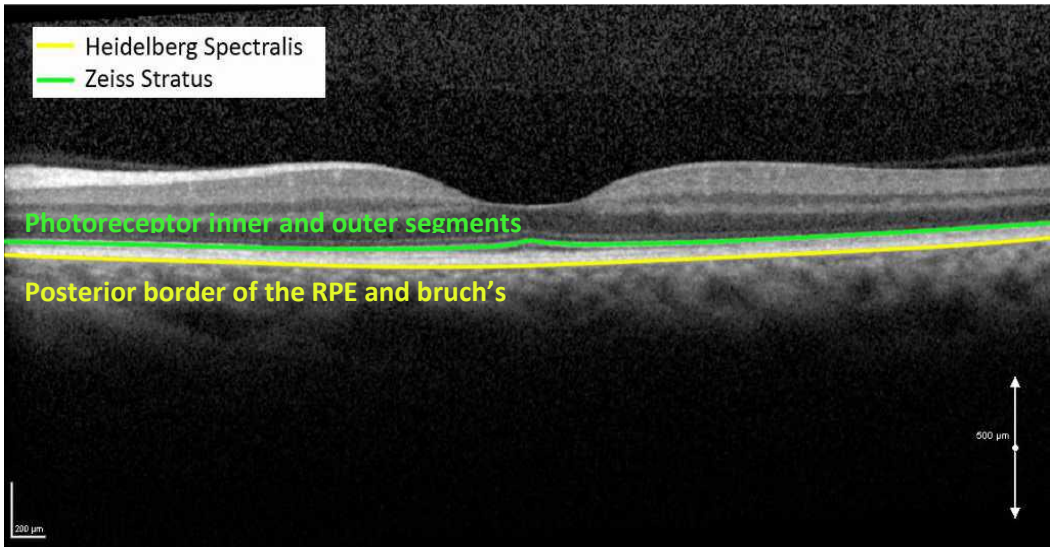


Figure 4.3. Outer retinal boundary lines for Status and Spectralis OCT. Time-domain OCT such as the Zeiss Stratus uses the junction of the photoreceptor inner and outer segments for this boundary (green line) while Spectral-domain OCT such as the Heidelberg Spectralis uses the posterior border of the RPE/Bruch's complex for retinal thickness measurements (yellow line) (DRCR network., 2012).

4.5 CENTRE POINT THICKNESS (CPT) AND CENTRAL SUBFIELD THICKNESS (CST)

When following patients longitudinally, it is important to document OCT measurements to assess whether there has been a change in retinal thickness between visits. This information is essential for monitoring, making management decisions and determining treatment effects. Centre point thickness (CPT) and CST are the OCT parameters commonly measured for the centre of the macula. The CPT is the average of the thickness values for the 6 radial scans at their point of intersection while the CST is the mean value of the 128 thickness values obtained in the CSF (Murthy et al., 2015). Recent studies have preferred CST over CPT to document changes due to DMO because measurements of the CPT have greater variability. This is because the CPT values are very dependent on centring the scan accurately while the CST thickness distributes measurements over a broader area. In addition, CPT is an average of 6 values while CST is an average of 128 values. Another advantage of CST over CPT is that it can be obtained from lower-quality scans more often (Chan et al., 2006). The EDDMO study collected both CPT and CST from all eyes. For the reasons described, CST values will mainly be used but CPT will also be reported in some sections of this thesis for completeness.

4.6 NORMATIVE ETDRS SUBFIELD THICKNESSES AND VOLUMES

The establishment of a retinal thickness normative database is essential as a comparison for the evaluation of any pathology. Unfortunately, Heidelberg does not provide access to a normative database of retinal thickness values (Kiernan, 2010, DRCR network., 2012), and there are limited studies providing normative data based on the Spectralis OCT. Some studies use the smaller ETDRS grid while others use the larger ETDRS grid described in Section 4.3. To add to this complexity, there are racial differences in OCT thickness measurements. It has been proposed that increased pigment in the RPE attenuates the OCT light signal that leads to an underassessment of retinal thickness in more pigmented retinas (Chauhan and Marshall, 1999). This section examines studies with normative retinal thickness based on the Heidelberg Spectralis focusing on studies with mainly Caucasian participants, as most of the participants whose results are reported in this thesis were Caucasian.

One of the earliest studies to establish a normative database was by Grover et al. (2009) involving 50 healthy participants (median age 43 years, range 20-84 years, 24 females, 26

males, 56% Caucasian). They reported the mean CPT±SD to be 227.3±23.2µm. Mean retinal thicknesses from the study for all the ETDRS subfields are shown in Table 4.2. The mean CSF in males (273.8±23µm) was thicker than females (266.3±21.9µm) but the study did not find any statistically significant differences in retinal thicknesses between genders or with age. Grover et al. (2009) proposed 315µm (two SDs above mean CST) as the upper limit of normal CST. Murthy et al. (2015) provided the ETDRS subfield volumes in the same group of participants in a subsequent paper. The mean retinal volumes for all subfields are shown in Table 4.2. The mean total macular volume (TMV) was 3.04±0.14mm^3. Both of the studies by Grover et al. (2009) and Murthy et al. (2015) obtained measurements based on the smaller ETDRS grid size with a diameter of 3.45mm. This accounts for the lower macular volumes reported by Murthy et al. (2015) compared to studies that used the larger ETDRS grid. In both the smaller and larger ETDRS grids, the CSF is 1mm in diameter. Therefore, values in the CSF in the smaller ETDRS grid used in the studies by Grover et al. (2009) and Murthy et al. (2015) are directly comparable with results from this thesis because CSF is the same. However, the values from the other subfields are not comparable. Subsequent references to ETDRS grid measurements in this thesis are based on the larger ETDRS grid of 6mm diameter unless specified otherwise. Chopovska et al. (2011) and Invernizzi et al. (2018) reported normative Caucasian retinal thicknesses obtained using Heidelberg Spectralis, and the ETDRS subfield thicknesses are shown in Table 4.3.

Table 4.2 Normative ETDRS subfields mean±SD thickness (Grover et al., 2009) and volumes (Murthy et al., 2015) based on smaller ETDRS grid

ETDRS subfield	Retinal thickness±SD (µm), 50 eyes	Retinal volume±SD (mm^3), 50 eyes
CSF	270.2±22.5	0.21±0.02
SIM	336.0±20.6	0.26±0.01
NIM	335.0±19.3	0.26±0.02
IIM	334.9±16.7	0.26±0.01
TIM	322.6±16.5	0.25±0.01
SOM	329.6±16.4	0.45±0.02
NOM	339.5±16.9	0.47±0.02
IOM	325.4±16.6	0.45±0.02
TOM	320.1±15.4	0.44±0.02

Table 4.3 Normative Caucasian ETDRS subfield thickness from Chopovska et al. (2011) and Invernizzi et al. (2018)

ETDRS subfield	Retinal thickness±SD (µm)	
	Chopovska et al. (2011), 34 eyes	Invernizzi et al. (2018), 200 eyes
CSF	277.4±16.8	280.1±17.5
SIM	348.6±14.9	346.9±14.0
NIM	349.8±15.0	349.8±15.1
IIM	344.6±15.2	344.1±14.4
TIM	333.9±14.5	332.9±13.9
SOM	311.5±16.5	301.8±13.0
NOM	320.0±16.6	317.0±15.2
IOM	299.2±16.5	289.0±13.6
TOM	286.3±13.9	284.0±13.2

Since Heidelberg auto-segmentation of the retinal layers, which will be described later (Section 4.10.3), became available, some studies have examined not only total retinal thickness but also the thickness of each individual layer in all ETDRS subfields. A study by Nieves-Moreno et al. (2017) involving 297 eyes of 297 healthy Caucasian participants (mean age 56 years, range 40.5-72 years, 179 females, 118 males) specifically examined the thicknesses of the inner retinal layers (NFL, GCL, IPL). The study used the Heidelberg Spectralis software for segmentation but no manual adjustments were made, which may lead to inaccuracies (Aojula et al., 2018). The mean CSF inner retinal layer thickness is shown in Table 4.4. The study found that retinal thickness was significantly higher in men compared to women in every subfield except for the temporal outer subfield.

Table 4.4. Mean CST and NFL, GCL and IPL thickness in the central subfield in 297 healthy Caucasian participants (Nieves-Moreno et al., 2017)

Subfield	Mean thickness (µm)
CST	278.2
Central subfield RNFL	12.61
Central subfield GCL	17.63
Central subfield IPL	22.02

Invernizzi et al. (2018) examined 200 eyes of 200 healthy Caucasian participants (mean age 39.9±13.9, range 20-74 years, 110 females) and provided the thickness of each retinal layer in all subfields as shown in Table 4.5. Invernizzi et al. (2018) found that total retinal thickness was greater in males compared to females across all subfields.

Table 4.5. Mean±SD retinal thickness (µm) in ETDRS subfields in each retinal layer in 200 healthy Caucasian participants (Invernizzi et al., 2018)

ETDRS subfield	RNFL Mean±SD	GCL Mean±SD	IPL Mean±SD	INL Mean±SD	OPL Mean±SD	ONL Mean±SD	RPE Mean±SD
CSF	12.8±1.8	17.2±3.9	22.5±3.2	19.4±4.5	26.0±6.0	92.2±9.3	17.4±1.9
SIM	25.0±2.3	53.7±4.3	42.2±2.9	40.2±3.4	34.4±7.2	70.1±10.0	15.5±1.7
NIM	22.7±2.2	54.0±4.5	43.6±3.1	40.7±3.5	33.5±8.1	73.1±10.04	15.5±1.6
IIM	27.7±3.3	54.0±4.0	41.9±3.0	40.5±3.4	33.5±7.3	66.8±10.6	14.6±1.5
TIM	17.5±1.1	49.2±4.5	42.0±3.1	37.5±3.2	30.9±4.1	74.1±7.8	14.9±1.5
SOM	39.1±5.0	35.1±3.0	28.4±2.4	32.1±2.3	26.4±2.4	61.9±6.3	13.6±1.4
NOM	53.8±7.2	37.3±3.7	28.7±2.9	33.9±2.6	28.3±3.4	57.3±7.0	13.6±1.4
IOM	43.3±6.9	32.5±3.1	26.3±2.6	31.0±2.5	26.2±2.9	52.8±6.5	13.0±2.6
TOM	18.8±1.4	36.7±4.0	32.3±2.5	33.3±2.4	26.7±2.1	58.7±6.1	12.9±1.4

Retinal thicknesses have been found to vary with different ethnicities. A study involving 86 eyes of 43 healthy Chinese participants (mean age 64 years, 28 females, 15 males) (Jiang et al., 2018) found thinner retina in the central and outer subfields compared to the studies by Grover et al. (2009) and Nieves-Moreno et al. (2017). The same study also examined the 86 eyes of 43 Chinese PWD and no DR, which is discussed in the next section. A study by Roh et al. (2013) found CST to be thinner in a Korean population (264.6±15.9µm) compared to Caucasian populations. A study of the Indian population found thinner CST (260.1±18.19µm) but thicker NFL in all subfields compared to Caucasian populations (Appukuttan et al., 2014).

4.7 ETDRS SUBFIELDS THICKNESS IN PWD AND NO OR MINIMAL DR

It is useful to compare the retinal thickness of healthy participants with the retinal thickness of PWD with no or minimal DR to determine if there are OCT changes that precede clinically visible changes in the retina on slit-lamp examination. There are limited studies on the retinal thicknesses of PWD with no or minimal DR (Chen et al., 2016, DRCR network., 2012, Jiang et al., 2018, Park et al., 2011) and even fewer studies that used

Heidelberg Spectralis (DRCR network., 2012, Jiang et al., 2018), which provide values for comparison with the results from this thesis.

One of these studies is by the Diabetic Retinopathy Clinical Research (DRCR) network that obtained mean ETDRS subfield thicknesses with Heidelberg Spectralis in 122 eyes of 122 participants (mean age 59 years, range 22-88, 67 females, 55 males, 70% Caucasian) with diabetes and no DR (ETDRS level 10, N=103) or minimal DR (ETDRS level 20, microaneurysms only, N=19) (DRCR network., 2012). This study provided the baseline ETDRS subfields measurements. The mean CPT was 277±25µm and the mean TMV was 8.4±0.4mm^3. The mean retinal thicknesses for all the subfields are shown in Table 4.6. The mean CPT, subfield thicknesses and TMV in males and females are shown in Table 4.7. The study showed that retinal thickness was significantly affected by gender but not age, duration of diabetes, type of diabetes, retinopathy severity or VA. These values are similar to those in healthy participants (Table 4.3) (DRCR network., 2012).

Another study that used Heidelberg Spectralis, involved 86 eyes from 43 Chinese participants (mean age 64 years, 28 females, 15 males) with diabetes and no DR (ETDRS level 10), found thinner retinal in all subfields compared to the DRCR network study (Table 4.6) (DRCR network., 2012, Jiang et al., 2018). It is assumed that Chinese eyes are more pigmented compared to Caucasian eyes and this is consistent with the proposal by Chauhan and Marshall (1999) that more pigmented individuals have thinner retina as previously discussed in Section 4.6.

Table 4.6. Retinal thicknesses in people with diabetes with no or minimal DR in studies by DRCR network. (2012) and Jiang et al. (2018)

ETDRS subfield	Retinal thickness (µm)	
	DRCR network. (2012) (N=122)	Jiang et al. (2018) (N=86)
CPT	227±25	215.8±18.9
CSF	270±24	258.4±23
SIM	335±18	332.9±18.6
NIM	338±18	334.3 ± 18.5
IIM	332±18	328.6 ± 16.4
TIM	324±17	319.9 ± 16.7
SOM	290±16	292.1 ± 17.5
NOM	305±19	307.4 ± 19.6
IOM	280±18	279.4 ± 17.2
TOM	279±15	276.4 ± 27.9

Table 4.7. Mean centre point thickness (µm), ETDRS subfield thickness (µm) and total macular volume (mm^3) in males and females in people with diabetes with no or minimal DR from the DRCR network. (2012) study (N=122)

ETDRS subfield	Retinal thickness or volume ±SD	
	Males (N=55)	Females (N=67)
CPT	233±27	222±21
CSF	278±23	262±22
SIM	340±15	330±20
NIM	343±15	333±19
IIM	338±15	327±19
TIM	329±13	319±19
SOM	292±15	289±17
NOM	309±19	301±19
IOM	281±16	279±19
TOM	281±13	276±17
Volume TMV	8.5±0.4	8.3±0.5

4.8 DEFINITIONS OF DMO BASED ON OCT

DMO has traditionally been determined by clinical examination and by the classical definition of CSMO as described in Chapter 2. Since the advent of OCT imaging, there have been several proposals to define DMO based on this imaging modality. Bressler et al. (2012) defined DMO that is not clinically visible on slit-lamp biomicroscopy but where the CPT has a thickness of ≥2 standard deviations taken from normative data for the specific OCT platform as subclinical DMO (Bressler et al., 2012, Pires et al., 2013). This thresholds' value to determine subclinical DMO would vary depending on the OCT used since each OCT platform has different normative retinal values for reasons described in Section 4.4.

Some studies have reported that the presence of subclinical macular oedema as defined by Bressler et al. (2012) is a good predictor of the progression of CIMO (Pires et al., 2013, Lobo et al., 2018). An issue with Bressler's definition is that CPT was used rather than CST. As previously described in Section 4.5 for the CSF on OCT, mean CST is regarded as a more consistent measure of thickness compared to CPT. Therefore, Bressler's definition is not widely used.

4.9 LIVERPOOL OCT DEFINITION OF DMO

Given the lack of a consensus definition of DMO, the St Paul's Eye Unit medical retinal team developed a local OCT-based definition at the start of the EDDMO study. This definition took into account both the retinal thickening and the presence of any intraretinal cyst greater than 50µm across the greatest diameter (Figure 4.4). Any intraretinal cyst less than 50µm may be due to scan artefact and therefore is not taken into consideration. Intraretinal cysts were included in this definition as an independent feature because the presence of intraretinal cysts indicated a breakdown of the BRB in DR (Xia and Rizzolo, 2017).

Retinal thickening in each subfield is considered as any mean retinal thickness ≥2 standard deviations above the mean retinal thickness in that subfield provided by the DRCR network. (2012) study (Figure 4.5). This is in recognition that retinal thickening may represent the accumulation of interstitial fluid that is undetected by current OCT technology and may precede the development of intraretinal cysts.

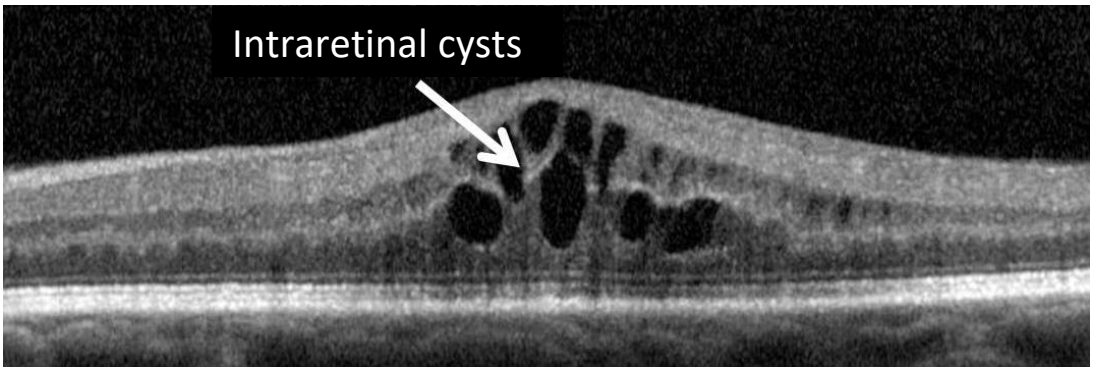


Figure 4.4. Example of intraretinal cysts on OCT. Images courtesy of D Parry.

The Liverpool OCT definition of DMO includes five grades: centre involving macular oedema (CIMO), centre threatening macular oedema (CTMO), non-centre threatening macular oedema (NCTMO), no macular oedema (NMO) and ungradeable. CIMO is defined as any intraretinal cyst and/or thickness ≥ 2SD (≥318µm) within the CSF in the EDDMO study (Figure 4.5). However, the DRCR network rounds this value up to ≥320µm to define DMO on OCT (DRCR network., 2012). Similarly, CTMO is defined as any intraretinal cyst and/or thickness ≥ 2SD within the inner subfields. NCTMO is defined as any intraretinal cyst and/or thickness ≥ 2SD within the outer subfields. NMO is defined as the absence of an intraretinal cyst or thickness <2SD in all subfields (Table 4.8, Figure 4.6).

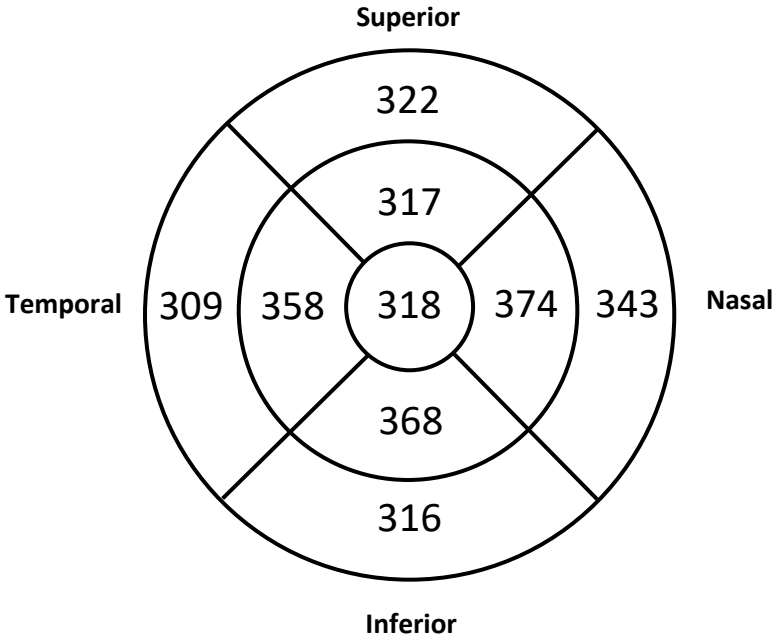


Figure 4.5. Retinal thickening is considered as ≥2SD above the mean retinal thickness for each subfield provided by the DRCR network. (2012) study. Retinal thickness threshold to be considered as thickening for each subfield is shown in the right eye.

Table 4.8. Liverpool OCT definition of diabetic macular oedema (DMO)

Definition	**Grades of DMO**
Central subfield intraretinal cyst and/or CST ≥ 2SD	Centre Involving Macular Oedema (CIMO)
Inner subfield intraretinal cyst and/or retinal thickness ≥ 2SD mean	Centre Threatening Macular Oedema (CTMO)
Outer subfield intraretinal cyst and/or retinal thickness ≥ 2SD mean	Non-Centre Threatening Macular Oedema (NCTMO)
No intraretinal cyst and retinal thickness <2SD in all subfields	No Macular Oedema (NMO)
Ungradeable	Ungradeable (U)

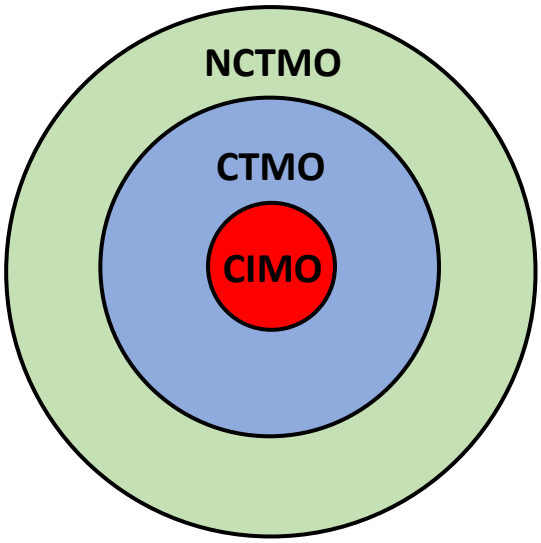


Figure 4.6. Liverpool OCT definition of diabetic macular oedema based on the presence of intraretinal cyst and or retinal thickening in the central subfield (Centre Involving Macular Oedema; CIMO), inner subfields (Centre Threatening Macular Oedema; CTMO), or outer subfields (Non-Centre Threatening Macular Oedema; NCTMO).

4.10 OCT METHODS

The following section describes the OCT acquisition and grading protocols used in this thesis. Spectral-domain OCT (Spectralis OCT; Heidelberg Engineering, Heidelberg eye explorer version 1.10.0.0, software version 6.8a) was used to perform all scans using a standardised scanning protocol. For each eye, a 31 line raster scan (30° x 25°) centred on the fovea was performed (with eye-tracking on) with automatic real-time tracking (ART) of 16-25 frames that was averaged to improve the image quality. ART is an image averaging function in the software that is used in conjunction with eye-tracking technology to take multiple images of the same area of the retina and average the images to improve image quality (Podkowinski et al., 2017). The higher the ART number, the higher the number of images that have been obtained and averaged to produce the final image. The EDI technique was used to obtain a single scan centred on the fovea with ART 100 for all participants. The OCT volume scan was not captured using EDI technology as the current Heidelberg software does not allow retinal layer auto-segmentation to be performed on EDI volume scans. OCT scans were obtained by trained technicians in the Royal Liverpool University Hospital. All grading of OCT images was performed by the PhD candidate (J Ku). Centring of the ETDRS grid on the fovea was essential to all ETDRS subfield measurements. Therefore, CST from a subset of the healthy participants and PWD were obtained by an experienced second-grader from the Royal Liverpool Hospital reading centre (Mr D Parry)

to examine inter-grader reliability. Inter-grader reliability analyses of the CST is performed in Sections 5.3.2 and 7.3.5.

4.10.1 GRADING OF THE QUALITY OF OCT IMAGES

OCT volume scans were graded as good, fair or ungradeable (Figure 4.7). Good quality scans have excellent or good discrimination of the retinal and subretinal layers particularly the external limiting membrane (ELM), ellipsoid zone and RPE/Bruch's complex throughout the majority of the scans acquired (Figure 4.7A). In fair quality scans, the discrimination of the retinal and subretinal layers (particularly the ELM, ellipsoid zone and RPE / Bruch's complex) may be less distinct than in a good quality scan but still of suitable quality for the extraction of reliable data. This should be the case throughout the majority of the scans acquired, especially in the subfoveal region (Figure 4.7B). In ungradeable scans, the discrimination of retinal and subretinal layers is not of suitable quality for the acquisition of reliable data (Figure 4.7C). The foveal depression can be graded as present, absent or ungradeable. The foveal depression must be visible for this to be graded as present (Figure 4.8). Vitreomacular traction (VMT) can be graded as absent, questionable evidence of VMT, definite evidence of VMT (Figure 4.9) and ungradeable. VMT with traction is graded as definite VMT (Figure 4.9A) while vitreomacular attachment (VMA) is considered a normal appearance and is graded as no evidence of VMT (Figure 4.9B). An epiretinal membrane (ERM) can be graded as absent, questionable evidence of ERM, definite evidence of ERM (Figure 4.10) and ungradeable. Macular holes can be graded as absent, questionable macular hole, partial-thickness macular hole (Figure 4.11), full-thickness macular hole or ungradeable.

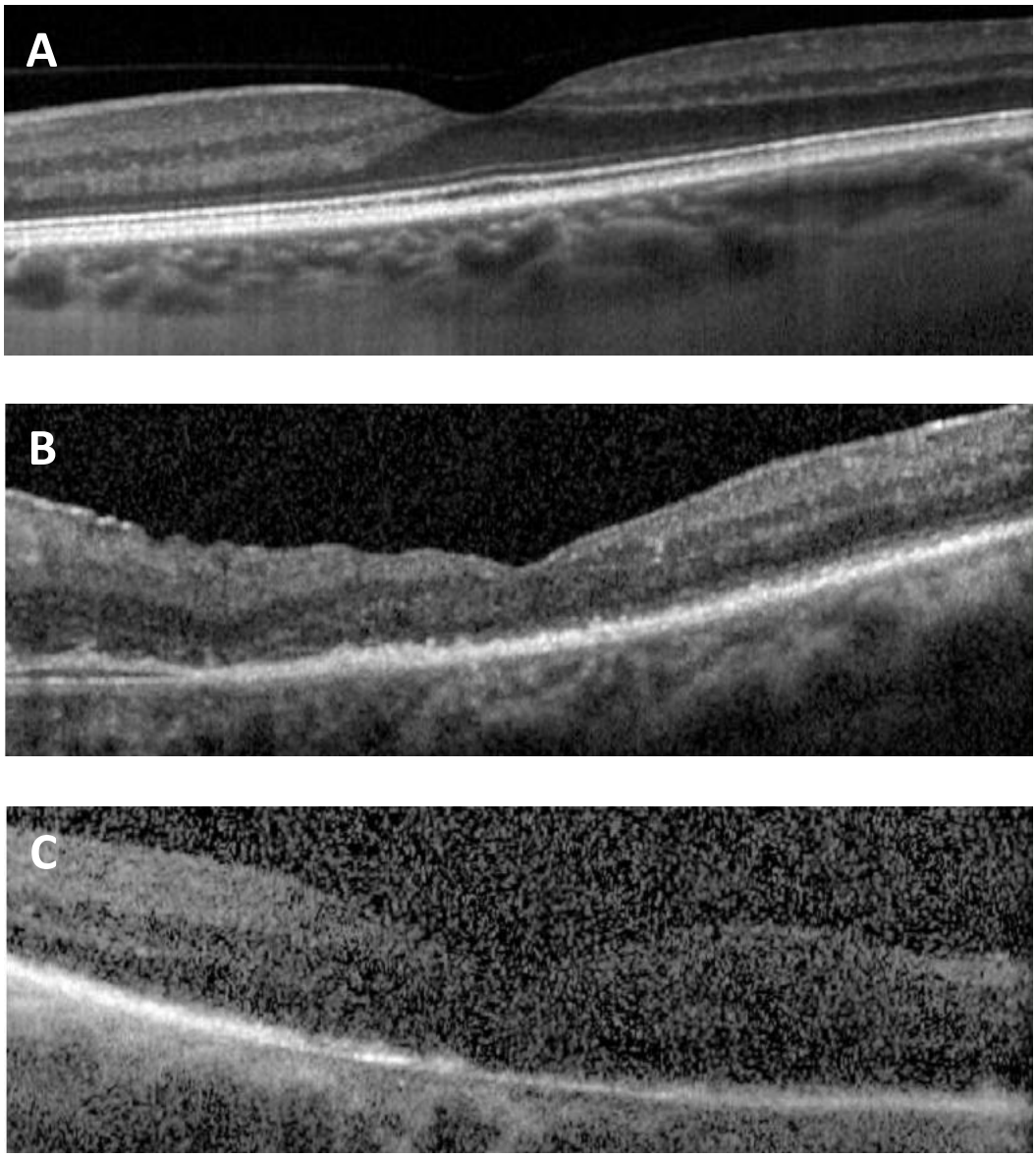


Figure 4.7. Reference OCT images of image quality. (A) Good quality scan (B) Fair quality scan (C) Ungradeable quality scan. Images courtesy of D Parry.

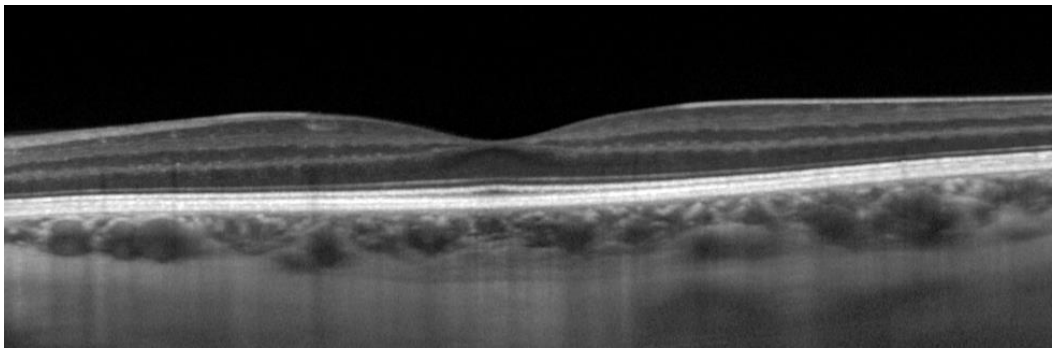


Figure 4.8. Example of normal foveal depression. Images courtesy of D Parry.

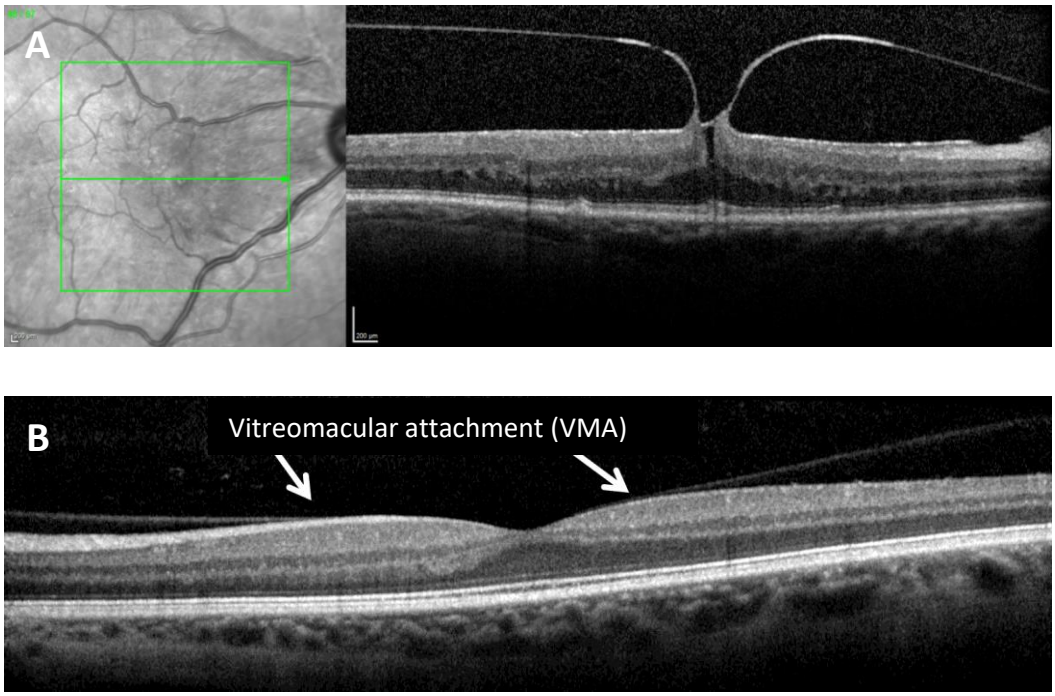


Figure 4.9. Reference images of vitreomacular interface (A) Vitreomacular traction (B) Vitreomacular attachment. Images courtesy of D Parry.

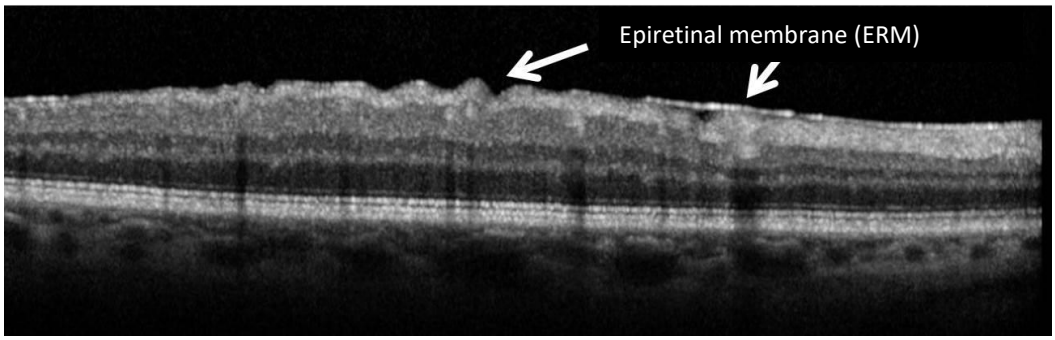


Figure 4.10. Example of an epiretinal membrane. Images courtesy of D Parry.

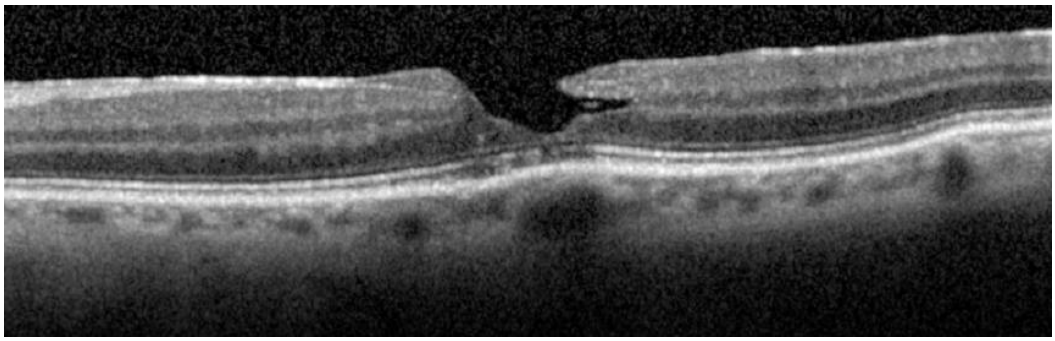


Figure 4.11. Example of partial-thickness macular hole. Images courtesy of D Parry.

4.10.2 MEASUREMENT OF CHOROIDAL THICKNESS

Choroidal thickness was measured using the standard Spectralis calliper tool aligned vertically from the centre of the fovea depression to the choroid sclera junction and perpendicular to the RPE. The distance from the choriocapillaris to the choroid sclera junction was taken as the choroidal thickness (Figure 4.12). The B-scan where the foveal depression was the deepest was used.

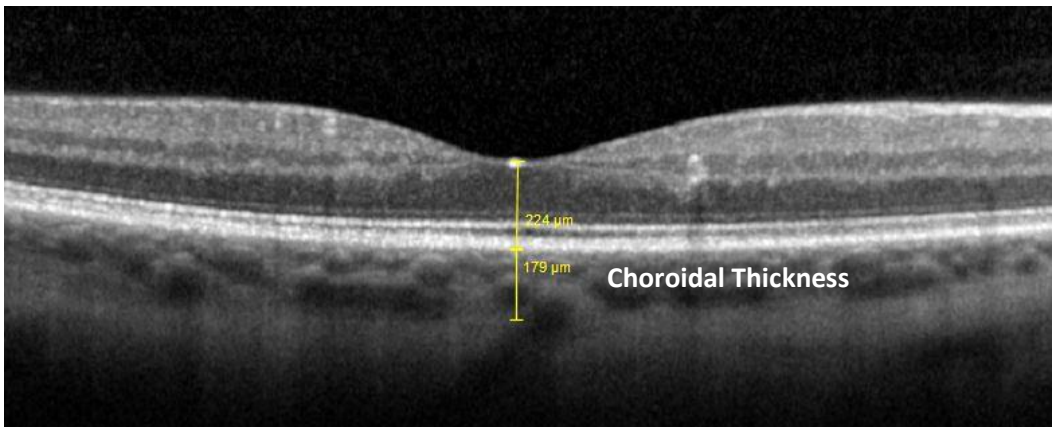


Figure 4.12. Example of centre foveal thickness measurement and central choroidal thickness measurement.

4.10.3 RETINAL LAYER SEGMENTATION

The Spectralis OCT software can perform auto-segmentation and provide retinal thickness measurements for the retinal layers shown in Table 4.9. Each layer was named using terminology as proposed by the international nomenclature for the OCT panel described in Section 4.2 (Staurenghi et al., 2014) (Figure 4.13). To obtain retinal layer thickness measurements, auto-segmentation was performed on all scans. All B-scans of each eye were examined to assess the quality of the auto-segmentation. Auto-segmentation was deemed acceptable if each of the layers was clearly demarcated and no manual adjustment was necessary (Figure 4.14A). Manual segmentation was deemed necessary if segmentation lines did not separate each layer clearly and needed to be manually adjusted (Figure 4.14B and 4.14C). Retinal segmentation was deemed as not possible (and the image ungradeable) if retinal layers were not sufficiently defined to allow for manual segmentation (Figure 4.14D).

Table 4.9. Retinal layers provided by the Heidelberg Spectralis Segmentation software

	Retinal layer
1	Total retinal thickness (ILM to the RPE/Bruch's complex)
2	Retinal nerve fibre layer (RNFL)
3	Ganglion cell layer (GCL)
4	Inner plexiform layer (IPL)
5	Inner nuclear layer (INL)
6	Outer plexiform layer (OPL)
7	Outer nuclear layer (ONL)
8	Retinal pigment epithelium (RPE)
9	Inner retinal layer (from the ILM to the ELM)
10	Outer retinal layer (from the ELM to the RPE/Bruch's complex)

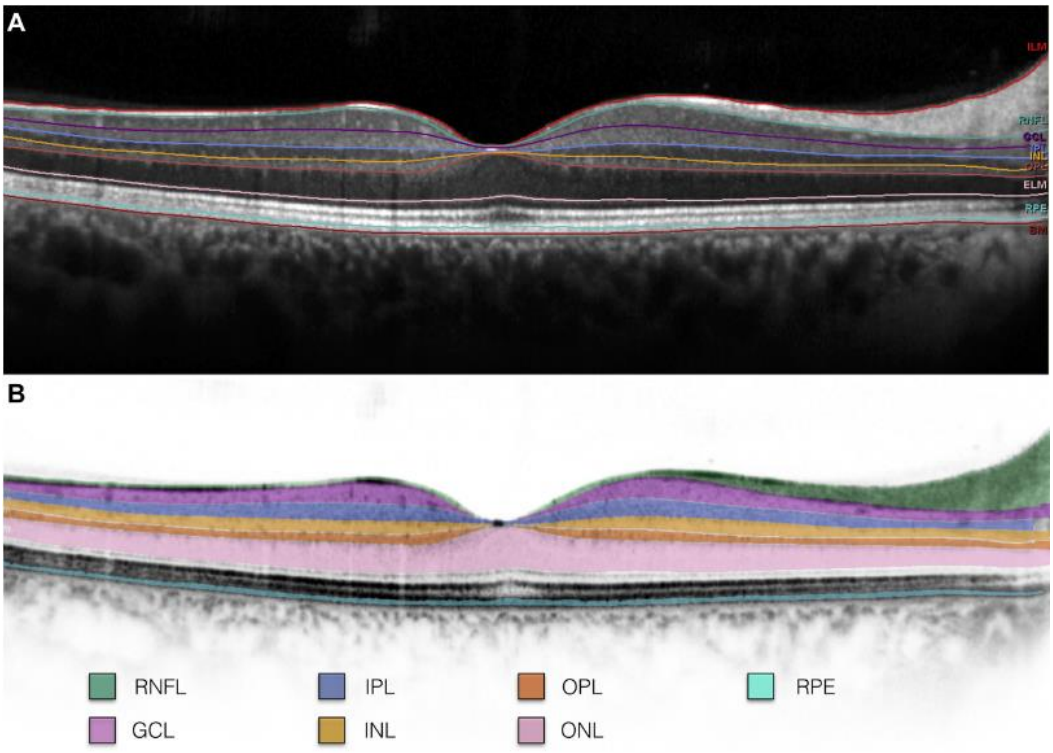


Figure 4.13. Segmentation of retinal layers performed using Heidelberg Spectralis software. (A) Boundaries of retinal layers (B) Colour representation of retinal layers. Retinal nerve fibre layer (RNFL), Ganglion cell layer (GCL), Inner plexiform layer (IPL), Inner nuclear layer (INL), Outer plexiform layer (OPL), Outer nuclear layer (ONL), Retinal pigment epithelium (RPE). Images from Invernizzi et al. (2018).

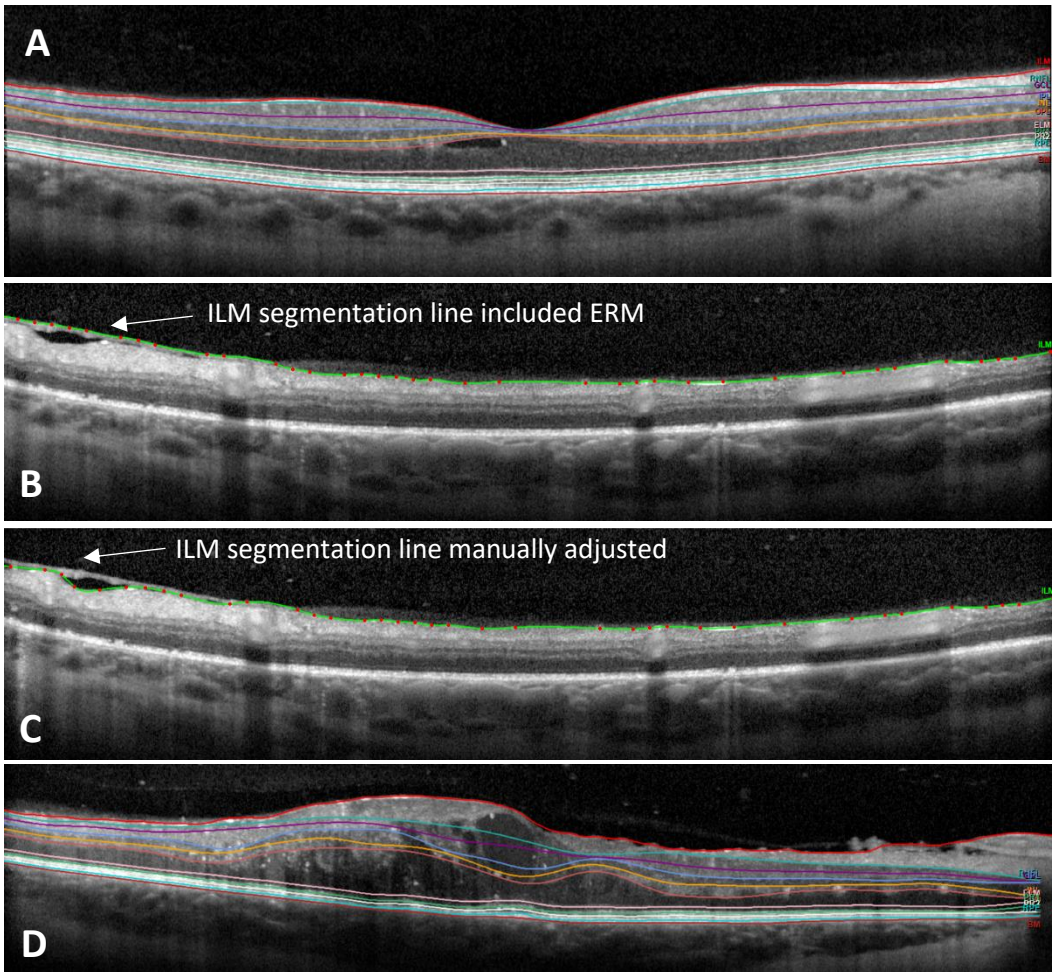


Figure 4.14. Segmentation of retinal layers. (A) Example of B-scan where all retinal layers were well defined with auto-segmentation. (B and C) Example of B-scan where manual adjustment of the ILM line was necessary to exclude the ERM. (D) Example of B-scan where retinal layers were not sufficiently defined to allow for auto-segmentation or manual adjustment (ungradeable).

4.11 CHAPTER CONCLUSION

This chapter covered a brief literature review on OCT relevant to the EDDMO study and retinal thickness measurements in healthy participants and PWD with no or minimal DR in some previous studies. This chapter also described the Liverpool OCT definition of DMO, which is used in the EDDMO study. The following chapter (Chapter 5) examines retinal thickness and volume in healthy participants and PWD with no or minimal DR in the EDDMO study and compares them with studies described in this chapter.

CHAPTER 5. OCT ANALYSIS OF HEALTHY PARTICIPANTS AND PEOPLE WITH NO DIABETES WITH NO OR MINIMAL DIABETIC RETINOPATHY

5.1 CHAPTER INTRODUCTION

Retinal thickness measurements are important for the diagnosis and monitoring of DMO (Amoaku et al., 2020). To evaluate the effect of DMO, a comparison of retinal thickness with normal eyes and eyes with no or minimal DR is needed. As described in Chapter 4, there are limited full retinal thickness and different layer thickness data in the literature using the Heidelberg Spectralis OCT in healthy participants and PWD with no or minimal DR (Invernizzi et al., 2018, Chopovska et al., 2011). There has also been a debate with regards to whether the axial length is related to retinal thickness (Ooto et al., 2011). This chapter adds to the existing literature by providing data on retinal thickness measurements in 50 healthy participants and examines the effects of age, gender and axial length on retinal thickness.

The DRCR network. (2012) study, described in Section 4.7 provided baseline ETDRS subfield measurements for PWD with no or minimal DR (ETDRS 10 and 20) to allow comparison of retinal thickness as DR progresses. However, the DRCR network. (2012) used the ETDRS DR classification while the EDDMO study used the LDESP classification which were described in Section 2.5. Therefore, this chapter uses the ETDRS classification to allow direct comparison with the DRCR network. (2012) study while subsequent chapters will use the LDESP classifications. Analyses performed in this chapter examines the effects of age, gender, type of diabetes, duration of diabetes and HbA_{1c} on retinal thickness in 112 PWD with no or minimal DR (ETDRS 10 and 20). There are further retinal thickness analyses on eyes with no or minimal DR according to their LDESP classification in Chapter 7. Therefore, there are no comparisons of the retinal thickness between healthy participants and PWD with no or minimal DR in this chapter as these comparisons will be made in Section 7.3.9.

5.2 METHODS OF DATA ANALYSES

General and OCT methods were covered in Chapters 3 and 4. Where data from both eyes were available, one eye was randomly selected for analysis. Bland-Altman analysis and intra-class correlation were used to assess inter-grader reliability in Section 5.3.2. Student's *t*-tests were used to examine differences between two groups (Sections 5.3.2, 5.3.3 and 5.3.4). Univariate linear regression was used to examine the effect of participant factors on

retinal thickness in Sections 5.3.3 and 5.3.4. Bonferroni corrections were made to adjust for multiple comparisons. All data analyses were performed using Excel (2016), GraphPad Prism (version 8) and SPSS (version 25).

5.3 RESULTS

5.3.1 ASSESSMENT OF OCT QUALITY

In the 50 healthy participants, 50 B-scans were available for grading and 42 (84%) of these were graded as being of good quality while 8 (16%) were of fair quality. There were no B-scans that were ungradeable. The foveal depression was present in all B-scans and there was no evidence of VMT, ERM or macular holes in any B-scans. Retinal auto-segmentation was possible in all layers in 31 (62%) B-scans while manual segmentation was required in 19 (38%). Retinal segmentation was not possible in all B-scans. Assessment of OCT quality in PWD is described in Section 7.3.4.

5.3.2 INTER-GRADER RELIABILITY

As discussed in Section 4.10, the positioning of the centre of the ETDRS grid is essential to the subsequent accuracy of obtaining retinal thicknesses and volumes in all subfields. Therefore, CST from 30 of the 50 healthy participants (60%) was obtained by the first grader (J Ku) and an experienced second grader (D Parry) from the Liverpool Ophthalmic Reading Centre to establish inter-grader reliability. There was no significant difference between CST obtained by the first and second graders (paired *t*-test, t=1.76, p=0.09). Bland-Altman analysis showed low bias (Figure 5.1). Similarly, a high degree of inter-grader reliability was found using intra-class correlation (ICC). The average measure ICC was 1.000 with a 95% confidence interval from 0.999 to 1.000 ($F_{29,29}$=3542,432; p<0.001).

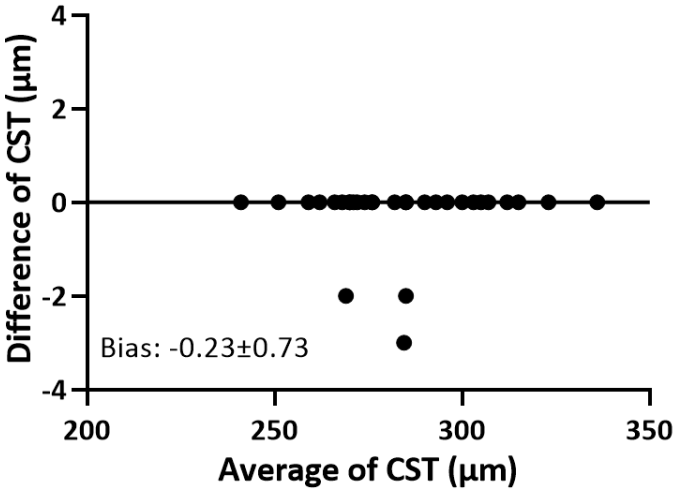


Figure 5.1. Bland-Altman plot showing the bias of central subfield thickness (CST) between grader 1 and 2 (N=30). The limits of agreement are not provided here due to all data agreeing except in three cases. Therefore, all differences are zero except in the three cases and the data are not normally distributed.

5.3.3 RETINAL THICKNESSES AND VOLUMES IN HEALTHY PARTICIPANTS

OCT imaging were obtained from 50 healthy participants (26 males, 24 females) with a mean±SD age of 55±14 years (range 22-85). Of the healthy participants, 48 were Caucasian, one Chinese and one other Asian. Due to the small number of non-Caucasian, it was not possible to compare ethnic differences. The participants' mean±SD near and distance VA were 0.06±0.16 logMAR and -0.08±0.12 logMAR respectively. The mean±SD CPT was 234.8±23.3 µm and the mean±SD mm^3 TMV was 8.67±0.33 mm^3. The mean±SD axial length was 23.63±1.2 mm (range 21.74 to 26.98 mm) and the mean±SD spherical equivalent was -0.44±2.1 dioptre sphere (DS) (range -6.5 to 3.63 DS). Their mean±SD µm full retinal thicknesses and volumes in all subfields are shown in Table 5.1 while the retinal thicknesses of individual retinal layers and subfields are shown in Table 5.2. The ETDRS subfield thicknesses and total macular volumes in males and females are shown in Table 5.3. Females had higher retinal thicknesses across most subfields compared to males except in the CST and NIM. Males had significantly higher CST (t=2.540, p=0.014) and lower IOM thicknesses compared to females (t=2.177, p=0.034).

Univariate linear regression was used to examine the effects of age, gender, axial length on different retinal thicknesses in healthy participants in the central, inner and outer subfields (Table 5.4). Adjustments for multiple comparisons were made with Bonferroni corrections. There was a significant correlation between the male gender and INL thickness in the CSF.

There were no significant correlations between retinal thickness and the other variables after the *p* value had been adjusted for multiple comparisons.

Table 5.1. Retinal thickness (μm) and volume (mm^3) in all ETDRS subfields in healthy participants

ETDRS subfield	Retinal thickness Mean±SD (μm)	Retinal volume Mean±SD (mm^3)
CSF	282.7±23.9	0.22±0.02
SIM	345.6 ±14.6	0.55±0.02
NIM	347.9±15.8	0.55±0.02
IIM	342.4±13.3	0.54±0.02
TIM	331.9±12.3	0.52±0.02
SOM	300.3±13.7	1.59±0.07
NOM	317.6±13.2	1.68±0.07
IOM	288.6±11.8	1.53±0.06
TOM	282.1±12.1	1.50±0.06

Table 5.2. Retinal thickness (μm) of each retinal layer in all ETDRS subfields in healthy participants

ETDRS subfield	RNFL Mean±SD	GCL Mean±SD	IPL Mean±SD	INL Mean±SD	OPL Mean±SD	ONL Mean±SD	RPE Mean±SD
CSF	13.3±2.0	16.3±4.6	22.4±4.1	19.8±6.3	26.5±5.6	94.1±9.7	18.0±2.1
SIM	25.3±2.8	52.7±4.6	42.2±2.9	39.4±3.3	33.4±6.8	70.7±10.3	16.7±1.5
NIM	21.9±2.4	52.0±4.9	41.9±3.4	40.0±3.9	35.5±9.9	72.0±13.8	16.7±1.7
IIM	26.1±2.7	52.3±4.6	41.8±3.2	40.5±3.4	34.4±6.4	67.2±10.1	16.1±1.7
TIM	18.1±1.3	48.1±4.4	41.9±3.1	39.6±3.5	30.3±2.9	73.8±7.2	15.8±1.4
SOM	38.3±5.6	34.4±3.4	28.0±2.7	31.1±2.1	26.7±1.9	61.2±7.1	14.6±1.4
NOM	50.4±7.0	37.4±3.7	29.1±2.9	34.1±2.3	30.1±3.9	57.4±8.0	14.0±1.4
IOM	44.5±6.3	32.5±3.0	26.3±2.3	30.4±2.3	27.0±2.4	53.1±6.0	13.8±1.3
TOM	19.1±1.6	34.6±4.2	31.6±2.7	32.5±2.0	26.8±1.8	58.9±5.5	13.8±1.2

Table 5.3. ETDRS subfield thickness and total macular volume in male and female healthy participants (N=50)

ETDRS subfield	Mean Retinal thickness (µm) or volume (mm^3)			
	Males (N=26) Mean±SD	**Females (N=24) Mean±SD**	***t*-test**	***P****
CSF	290.5±27.76	274.2±15.46	**2.540**	**0.014**
SIM	345.3±16.72	346±12.29	0.175	0.862
TIM	331.4±14.43	332.4±9.78	0.271	0.788
IIM	341.6±15.19	343.4±11.19	0.473	0.638
NIM	348.7±18.13	347.0±13.23	0.383	0.704
SOM	297.4±13.65	303.4±13.44	1.552	0.127
TOM	280.5±12.92	283.9±11.09	0.976	0.334
IOM	285.3±11.74	292.3±10.86	**2.177**	**0.034**
NOM	316.0±12.88	319.3±13.62	0.889	0.379
Volume TMV	8.63±0.35	8.72±0.30	1.052	0.298

*Statistically significant results are reported in bold

Table 5.4 Thickness values in the ETDRS central, inner and subfields and the effect of age, gender and axial length on these values in healthy participants

				Age (years)			Gender			Axial length		
Layer	**Subfield**	**Thickness (µm) Mean±SD**	**Intercept**	**Estimated coefficient**	***P***	**Adjusted *p***	**Estimated coefficient***	***P***	**Adjusted *p***	**Estimated coefficient**	***p***	**Adjusted *p***
Full retina	Centre	282.7±23.9	120.27	0.281	0.257	0.771	1.009	0.885	2.655	6.205	**0.023**	0.069
	IS	342.0±13.5	371.36	-0.149	0.313	0.939	-0.778	0.852	2.556	-0.882	0.578	1.734
	OS	279.2±11.9	381.31	-0.164	0.181	0.543	-0.947	0.782	2.346	-3.163	**0.019**	0.057
RNFL	Centre	13.3±2.0	14.22	0.024	0.255	0.765	1.060	0.081	0.243	-0.120	0.598	1.794
	IS	22.9±1.9	28.43	0.011	0.589	1.767	-0.148	0.798	2.394	-0.258	0.245	0.735
	OS	37.3±4.6	40.04	0.026	0.608	1.824	-1.907	0.181	0.543	-0.132	0.806	2.418
GCL	Centre	16.3±4.6	24.41	0.065	0.150	0.450	2.965	**0.023**	0.069	-0.560	0.249	0.747
	IS	51.3±4.4	45.27	-0.003	0.942	2.826	-0.412	0.762	2.286	0.272	0.601	1.803
	OS	34.7±3.2	23.35	0.014	0.672	2.016	-1.290	0.180	0.540	0.478	0.192	0.576
IPL	Centre	22.4±4.1	27.88	0.039	0.356	1.068	2.053	0.088	0.264	-0.369	0.415	1.245
	IS	42.4±3.0	36.34	0.012	0.721	2.163	-0.565	0.539	1.617	0.240	0.494	1.482
	OS	28.8±2.3	21.44	0.019	0.426	1.278	-1.219	0.079	0.237	0.293	0.264	0.792
INL	Centre	19.8±6.3	36.39	0.110	0.064	0.192	4.373	**0.011**	**0.033**	-1.054	0.098	0.294
	IS	39.1±3.1	42.03	0.003	0.941	2.823	0.323	0.736	2.208	-0.139	0.705	2.115
	OS	32.0±1.9	27.68	0.032	0.123	0.369	-1.024	0.079	0.237	0.133	0.541	1.623
OPL	Centre	26.5±5.6	33.06	0.008	0.896	2.688	2.953	0.080	0.240	-0.359	0.571	1.713
	IS	33.4±3.9	22.77	-0.046	0.277	0.831	1.301	0.278	0.834	0.527	0.249	0.747
	OS	27.7±1.8	19.65	-0.001	0.952	2.856	-0.249	0.654	1.962	0.348	0.105	0.315
ONL	Centre	94.1±9.7	89.20	0.083	0.435	1.305	0.111	0.970	2.910	0.012	0.992	2.976
	IS	70.9±8.4	41.78	0.109	0.230	0.690	-0.578	0.820	2.460	0.995	0.307	0.921
	OS	57.8±6.1	30.46	0.078	0.230	0.690	0.077	0.966	2.898	0.973	0.167	0.501
RPE	Centre	18.0±2.1	21.57	0.015	0.501	1.503	-0.485	0.448	1.344	-0.176	0.468	1.404
	IS	16.3±1.4	16.61	0.023	0.132	0.396	-0.337	0.434	1.302	-0.059	0.720	2.160
	OS	14.1 ±1.1	10.77	0.004	0.715	2.145	-0.067	0.842	2.526	0.131	0.310	0.930

RNFL (retinal nerve fibre layer), GCL (ganglion cell layer), IPL (inner plexiform layer), INL (inner nuclear layer), OPL (outer plexiform layer), ONL (outer nuclear layer), RPE (retinal pigment layer), centre (central subfield), IS (inner subfields), OS (outer subfields)

Statistically significant results are reported in bold and highlighted. Adjusted p values are adjusted with Bonferroni correction for multiple corrections

*The values for the estimated coefficient refer to the male gender

5.3.4 RETINAL THICKNESSES AND VOLUMES IN PWD WITH NO OR MINIMAL DR (ETDRS 10 AND 20)

There were a total of 112 PWD (68 males, 44 females) with no (ETDRS 10, N=29) or minimal DR (ETDRS 20, N=83). Their mean±SD age (range) was 56±15 years (20-86 years) and their mean±SD duration of diabetes (range) was 14.2±8.8 years (1-43 years). There were 26 participants with type 1 diabetes and 86 participants with type 2 diabetes. There were 93 Caucasian, 5 Indians, 4 Africans, 3 Other Asians, 2 Pakistani, 2 Chinese, 1 Gypsy Irish Traveller, 1 Mixed Caucasian and Black Caribbean and 1 participant with undisclosed ethnic background. Their mean±SD near and distance VA were 0.19±0.23 logMAR and 0.07±0.19 logMAR respectively. Their mean hRSD threshold was -0.64±0.21 logMAR. Their mean CPT± SD was 239.3±37.1 µm. Mean±SD ETDRS subfield thicknesses and volumes are shown in Tables 5.5 while the retinal thickness of each retinal layer and subfields are shown in Table 5.6. The ETDRS subfield thicknesses and total macular volumes between males and females are shown in Table 5.7. Males had significantly higher retinal thickness in the central, inner subfields, TOM and also TMV compared to females.

Univariate linear regression was used to examine the effects of age, gender, type of diabetes, duration of diabetes and HbA_{1c} on retinal thickness in PWD with no or minimal DR in the central, inner and outer subfields (Table 5.8). There was a significant positive correlation between age and INL and ONL in the CSF. Males had significantly higher full retinal thickness in the CSF and inner subfields. Males also had significantly higher GCL, IPL, INL and ONL in the CSF compared to females. PWD with type 2 diabetes had significantly higher ONL in the CSF. Interestingly, there was a significant negative correlation between the duration of diabetes and ONL in the CSF. There was a significant positive correlation between HbA_{1c} and both INL and OPL in the CSF. As reported in Section 3.5.2, not all participants had axial length measurements. Therefore, axial length was not included in this analysis due to a large amount of missing data (84 of 112, 74.3% missing).

Table 5.5. Retinal thickness and volume in ETDRS subfields in people with diabetes with no or minimal DR

ETDRS subfield	Retinal thickness Mean±SD (μm)	Retinal volume Mean±SD (mm^3)
CSF	284.5±30.8	0.22±0.02
SIM	339.1±19.4	0.53±0.03
NIM	341.6±20.7	0.54±0.03
IIM	334.7±19.5	0.53±0.03
TIM	328.4±18.7	0.52±0.03
SOM	294.1±16.6	1.56±0.09
NOM	308.5±19.2	1.63±0.10
IOM	282.4±16.5	1.50±0.09
TOM	278.5±14.6	1.48±0.08

Table 5.6. Retinal thickness of each retinal layer in ETDRS subfields in people with diabetes with no or minimal DR

ETDRS subfield	RNFL Mean±SD	GCL Mean±SD	IPL Mean±SD	INL Mean±SD	OPL Mean±SD	ONL Mean±SD	RPE Mean±SD
CSF	13.9±3.9	16.3±5.4	22.6±5.2	22.8±7.3	28.7±6.1	91.2±13.1	18.2±2.1
SIM	24.8±3.3	49.9±5.7	39.3±4.0	40.6±4.9	35.6±7.5	68.0±10.7	16.9±1.8
NIM	22.0±2.8	49.0±5.9	41.0±4.5	40.3±4.4	35.7±7.9	71.3±10.8	16.7±1.7
IIM	26.1±3.6	49.0±6.0	39.0±4.0	39.9±4.0	33.7±7.1	67.2±12.2	15.9±1.5
TIM	18.5±1.5	45.3±6.3	40.6±4.1	37.8±4.4	31.3±4.0	73.7±9.5	16.0±1.6
SOM	35.8±5.9	33.5±3.4	26.8±3.1	30.9±2.7	27.4±2.7	60.1±7.9	15.1±1.4
NOM	46.9±7.7	36.0±4.3	27.6±3.3	33.4±3.1	29.7±3.7	56.5±8.2	14.6±1.6
IOM	37.6±6.3	31.6±3.7	25.4±3.2	30.6±3.0	27.4±3.2	52.9±7.7	14.2±1.3
TOM	19.1±1.7	33.3±5.0	30.6±3.1	32.3±2.9	27.3±2.2	58.0±7.0	14.3±1.3

Table 5.7. ETDRS subfield thickness (μm) and total macular volume (TMV) (mm^3) in males and females in people with diabetes with no or minimal diabetic retinopathy (N=112)

ETDRS subfield	Retinal thickness (μm) or volume (mm^3) ±SD			
	Males (N=68) Mean±SD	**Females (N=44) Mean±SD**	***t*-test**	***P****
CSF	295.3±31.25	267.9±21.61	**5.080**	**<0.001**
SIM	343.5±19.67	332.4±17.12	**3.068**	**0.003**
NIM	346.0±21.52	334.8±17.64	**2.867**	**0.005**
IIM	338.7±20.93	328.5±15.41	**2.792**	**0.006**
TIM	333.9±18.72	319.8±15.35	**4.147**	**<0.001**
SOM	293.5±16.62	295.1±16.62	0.464	0.644
NOM	309.8±20.23	306.5±17.45	0.911	0.364
IOM	283.1±18.05	281.3±13.53	0.518	0.606
TOM	280.8±15.88	275.0±11.71	**2.088**	**0.039**
Volume TMV	8.38±0.60	8.00±0.98	**2.542**	**0.012**

*Statistically significant results are reported in bold

Table 5.8 Thickness values in the central subfield, inner ring subfields and outer ring subfields and the effects of age, gender, hRSD, type of diabetes, duration of diabetes and HbA_{1c} on these values in people with no or minimal diabetic retinopathy (ETDRS 10 and 20)

				Age (years)			Gender			Type of Diabetes			Duration of diabetes (years)			HbA_{1c}		
Layer	**Subfield**	**Thickness (µm) Mean±SD**	**Intercept**	**Estimated coefficient**	***p***	**Adjusted *p***	**Estimated coefficient***	***p***	**Adjusted *p***	**Estimated coefficient ****	***p***	**Adjusted *p***	**Estimated coefficient**	***p***	**Adjusted *p***	**Estimated coefficient**	***p***	**Adjusted *p***
Full retina	**Centre**	284.5±30.8	235.08	0.474	**0.028**	0.084	26.086	**0.000**	**0.000**	-12.296	0.172	0.516	-0.313	0.414	1.242	0.312	**0.045**	0.135
	IS	335.9±18.9	342.06	-0.165	0.232	0.696	10.885	**0.003**	**0.009**	-7.706	0.185	0.555	-0.311	0.209	0.627	0.103	0.300	0.900
	OS	291.3±16.1	310.72	-0.174	0.150	0.45	3.027	0.328	0.984	-9.898	0.052	0.156	-0.336	0.121	0.363	0.014	0.874	2.622
RNFL	**Centre**	13.9±3.9	8.24	0.050	0.092	0.276	1.808	**0.018**	0.054	-0.112	0.928	2.784	-0.021	0.684	2.052	0.032	0.136	0.408
	IS	22.8±2.3	21.00	0.015	0.407	1.221	0.674	0.145	0.435	-0.558	0.460	1.38	-0.009	0.773	2.319	0.017	0.192	0.576
	OS	34.7±4.9	34.83	0.014	0.726	2.178	-0.706	0.477	1.431	-1.560	0.339	1.017	-0.028	0.683	2.049	0.017	0.548	1.644
GCL	**Centre**	16.3±5.4	9.82	0.049	0.236	0.708	2.675	**0.013**	**0.039**	-0.987	0.569	1.707	-0.003	0.970	2.910	0.043	0.148	0.444
	IS	48.3±5.6	52.60	-0.097	**0.026**	0.078	0.929	0.397	1.191	-0.633	0.724	2.172	-0.080	0.298	0.894	0.033	0.292	0.876
	OS	33.7±4.0	40.10	-0.067	**0.026**	0.078	0.148	0.845	2.535	-1.242	0.321	0.963	-0.089	0.096	0.288	-0.008	0.720	2.160
IPL	**Centre**	22.6±5.2	13.90	0.054	0.163	0.489	2.918	**0.004**	**0.012**	-0.042	0.979	2.937	-0.004	0.953	2.859	0.059	**0.034**	0.102
	IS	40.0±3.8	42.07	-0.052	0.071	0.213	0.865	0.237	0.711	-0.788	0.510	1.53	-0.088	0.087	0.261	0.032	0.121	0.363
	OS	27.7±2.9	32.11	-0.037	0.096	0.288	0.131	0.817	2.451	-1.076	0.245	0.735	-0.091	**0.022**	0.066	-0.005	0.776	2.328
INL	**Centre**	22.8±7.3	7.24	0.164	**0.002**	**0.006**	4.975	**0.000**	**0.000**	-3.394	0.114	0.342	-0.049	0.591	1.773	0.099	**0.008**	**0.024**
	IS	39.6±3.7	38.86	-0.001	0.967	2.901	1.417	0.059	0.177	-1.024	0.403	1.209	-0.057	0.273	0.819	0.024	0.262	0.786
	OS	32.0±2.7	35.91	-0.019	0.372	1.116	-0.271	0.613	1.839	-1.607	0.070	0.21	-0.086	**0.024**	0.072	-0.004	0.794	2.382
OPL	**Centre**	28.7±6.1	17.03	0.049	0.293	0.879	1.022	0.388	1.164	0.893	0.645	1.935	0.090	0.278	0.834	0.095	**0.005**	**0.015**
	IS	34.1±4.4	30.42	0.013	0.703	2.109	1.513	0.088	0.264	0.047	0.974	2.922	-0.008	0.891	2.673	0.031	0.212	0.636
	OS	28.0±2.3	26.83	0.006	0.745	2.235	0.291	0.532	1.596	0.151	0.842	2.526	-0.022	0.494	1.482	0.013	0.331	0.993
ONL	**Centre**	91.2±13.1	78.57	0.347	**0.000**	**0.000**	8.857	**0.000**	**0.000**	-11.326	**0.004**	**0.012**	-0.409	**0.014**	**0.042**	0.034	0.608	1.824
	IS	70.0±8.6	71.49	0.063	0.347	1.041	3.111	0.071	0.213	-5.741	**0.043**	0.129	-0.139	0.248	0.744	-0.007	0.877	2.631
	OS	57.0±7.1	61.50	-0.029	0.598	1.794	1.711	0.225	0.675	-4.551	**0.050**	0.15	-0.060	0.540	1.620	0.006	0.880	2.640
RPE	**Centre**	18.2±2.1	19.10	-0.030	0.073	0.219	0.394	0.353	1.059	0.357	0.608	1.824	0.011	0.704	2.112	0.002	0.883	2.649
	IS	16.4±1.1	16.82	-0.014	0.128	0.384	0.155	0.493	1.479	0.196	0.596	1.788	0.011	0.467	1.401	-0.002	0.813	2.439
	OS	14.5±1.2	15.02	-0.009	9.335	28.005	0.150	0.539	1.617	-0.198	0.622	1.866	-0.004	0.822	2.466	0.002	0.755	2.265

RNFL (retinal nerve fibre layer), GCL (ganglion cell layer), IPL (inner plexiform layer), INL (inner nuclear layer), OPL (outer plexiform layer), ONL (outer nuclear layer), RPE (retinal pigment layer), centre (central subfield), IS (inner subfield), OS (outer subfield)

Statistically significant results are reported in bold and highlighted

Adjusted *p* values are adjusted with Bonferroni correction for multiple corrections

*The values for the estimated coefficient refer to the male gender

**The values for the estimated coefficient refer to type 2 diabetes

5.4 CHAPTER DISCUSSION

This chapter provides baseline OCT retinal thickness values in healthy participants performed with Heidelberg Spectralis based on the larger ETDRS grid which adds to the limited number of studies in this area (Chopovska et al., 2011, Invernizzi et al., 2018, Nieves-Moreno et al., 2017). Results from this chapter found similar full retinal thickness values across all ETDRS subfields compared to studies on Caucasian healthy participants by Chopovska et al. (2011) and Invernizzi et al. (2018) (Figure 5.2). The EDDMO study found similar CSF compared to other studies that only reported on CSF (Table 5.9). The EDDMO study reported similar mean retinal thickness in most subfields in different retinal layers in healthy participants compared to the study by Invernizzi et al. (2018); this is unsurprising as both studies mainly had Caucasian participants (Tables 4.5 and 5.2). Comparisons of RNFL thickness in the CSF is shown in Table 5.10

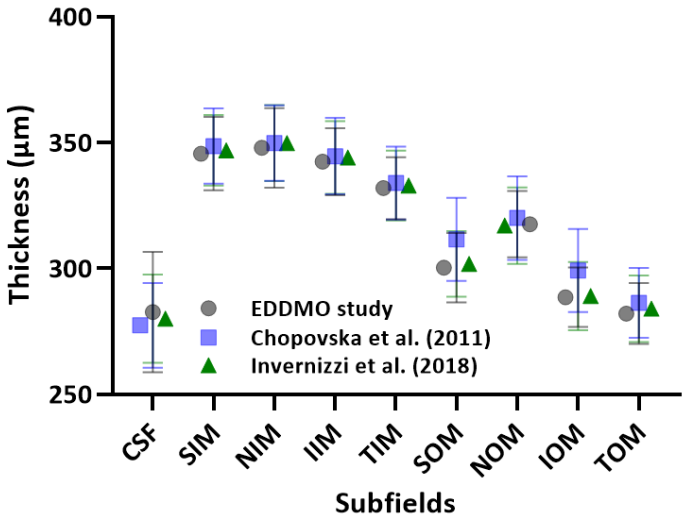


Figure 5.2 Comparison of mean±SD of the full retinal thickness (µm) in the EDDMO study and studies by Chopovska et al. (2011) and Invernizzi et al. (2018) in healthy participants. All studies used Heidelberg Spectralis OCT.

Table 5.9 Comparison of the full retinal thickness in the central subfield obtained from the EDDMO study with some previous studies in healthy participants. All studies used Heidelberg Spectralis OCT.

ETDRS subfield	Mean±SD central subfield thickness (µm)				
	EDDMO study, 50 eyes	Grover et al. (2009), 50 eyes	Chopovska et al. (2011), 34 eyes	Nieves-Moreno et al. (2017), 297 eyes	Invernizzi et al. (2018), 200 eyes
CSF	282.7±23.9	270.2±22.5	277.4±16.8	278.2*	280.1±17.5

*SD not available

Table 5.10 Comparison of the RNFL in the CSF obtained from the EDDMO study with previous studies in healthy participants. All studies used Heidelberg Spectralis OCT.

ETDRS subfield	Mean±SD retinal thickness±SD (µm)		
	EDDMO study, 50 eyes	Nieves-Moreno et al. (2017), 297 eyes	Invernizzi et al. (2018), 200 eyes
CSF	13.3±2.0	12.61*	12.8±1.8

*SD not available

In the healthy participants examined in the EDDMO study, there was little evidence of significant correlations between age and retinal layer thickness; this is in agreement with Invernizzi et al. (2018). However, it contrasts with the results of Alasil et al. (2013). They found thinning of the RNFL with ageing. This difference could be due to the age range of the participants and the OCT scan protocol used. The healthy participants examined in this chapter (mean age 55±14 years, range 22-85) and in Invernizzi et al. (2018) study included adult participants only while Alasil et al. (2013) examined 190 children and adult participants with a wider age range (mean age 53.7±16.3, range 9-86). In addition, Alasil et al. (2013) used an OCT scan protocol for glaucoma assessment that measured peripapillary RNFL (pRNFL) thickness centred on the optic disc; this scan protocol measured RNFL thickness from the whole retina (Lee et al., 2016). However, the study by Invernizzi et al. (2018) and the EDDMO study both used a macular OCT protocol centred on the fovea. In doing so, these studies would have evaluated a much smaller portion of retinal nerve fibres (Curcio and Allen, 1990).

Interestingly, there was no significant difference in full retinal thicknesses between male and female healthy participants except for the CSF and IOM (Table 5.3). This is in contrast to Invernizzi et al. (2018) where males had significantly higher retinal thickness in full retinal thicknesses in all subfields, IPL in the inner subfields, INL in the central and inner subfields, and ONL in all subfields. This difference could be due to the small sample size (N=50) for healthy participants in the EDDMO study compared to the study by Invernizzi et al. (2018) (N=200). Grover et al. (2009), who recruited a sample of 50 healthy participants, also did not find any statistically significant difference in retinal thicknesses between genders.

Similar to the study by Invernizzi et al. (2018), there was a significant correlation between axial length and full retinal thickness in the centre and outer ETDRS ring in healthy participants. However, Invernizzi et al. (2018) also found a significant correlation between axial length and GCL thickness in the CSF and outer subfields, IPL in the outer subfields and

OPL in the CSF. This difference could also be due to the larger sample size in Invernizzi et al. (2018) (N=200).

Macular volume measurements can provide another parameter in addition to thickness measurements to assess macular pathologies. Campbell et al. (2007b) examined 65 eyes with DR and suggested that central retinal volumes may be better than CPT in differentiating CSMO from non-CSMO. In another study, Campbell et al. (2007a) reported that retinal volume measurements may be less affected by scan artefacts caused by changes in fixation compared to CPT. Consequently, it may be of value to obtain full volume measurements in ETDRS subfields in healthy participants as a starting point for future comparisons. Results from the healthy participants in this chapter found that there were progressively increased retinal volumes from the CSF, to the inner subfields and outer subfields, which is similar to Murthy et al. (2015). In addition, the retinal volume was highest in the nasal outer subfield and lowest in the temporal inner subfield which is consistent with the normal macular anatomy and similar to Murthy et al. (2015) findings.

Full retinal thickness measurements in all ETDRS subfields from the DRCR network. (2012) study from 122 eyes of PWD with no or minimal DR (ETDRS 10 and 20) described in Section 4.7 provided values for comparison in other studies involving PWD. As discussed in Section 4.9, the DRCR network. (2012) study has been pivotal in providing a reference point to describe DMO using OCT because ≥2 standard deviations from mean CSF thickness derived from that study has been used in multiple studies to define DMO (Bandello et al., 2015, Bressler et al., 2014, Cunha-Vaz et al., 2016, Dodo et al., 2015, Mori et al., 2016, Mori et al., 2017, Ribeiro et al., 2015, Sun et al., 2014a, Sun et al., 2015, Vujosevic et al., 2016). Full retinal thickness values in PWD with no or minimal DR (ETDRS 10 and 20) from this chapter allowed for comparison with data from the DRCR network. (2012) study. The results from this chapter showed similar full-thickness measurements in all ETDRS subfields compared to the DRCR network. (2012) (Figure 5.3).

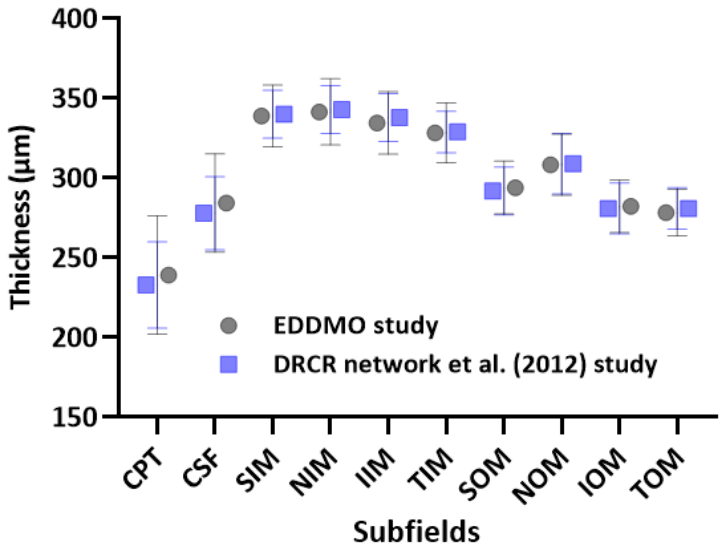


Figure 5.3 Comparison of mean±SD of the full retinal thickness (µm) in the EDDMO study and the DRCR network. (2012) study in PWD with no or minimal DR. Both studies used Heidelberg Spectralis OCT.

In the DRCR network. (2012) study, males had higher retinal thicknesses across all subfields. In the EDDMO study, PWD males had significantly higher retinal thickness across most subfields (CSF, SIM, NIM, IIM, TIM and TOM) compared to PWD females (Table 5.7). PWD males had significantly higher full retinal thicknesses in the CSF and inner subfields and the CSF of the GCL, IPL, INL and ONL compared to PWD females (Table 5.8). There were also significant correlations between age and INL and ONL in the CSF in PWD (Table 5.8)**.** This contrasted with the absence of a significant correlation between age and retinal thickness in the healthy participants (Table 5.4). These differences may be due to the larger sample size in the group of PWD (N=112) compared to the healthy participants (N=50). Some significant correlations between retinal thickness and type 2 diabetes, duration of diabetes and HbA_{1c} were reported in Section 5.3.4. As this analysis was performed on PWD with no or minimal DR, a similar analysis was repeated in all PWD, which will include PWD with more severe DR in Section 7.3.7.

In summary, this chapter provided OCT parameters for healthy participants and for PWD with no or minimal DR. Results from this chapter added to the existing literature and provide baseline values for PWD with more severe DR in subsequent chapters. Overall retinal thicknesses and layer thicknesses found in the EDDMO study in healthy participants and PWD with no or minimal DR are broadly comparable with previous studies. This is reassuring as it suggests that the methods used in the EDDMO study are reliable and reproducible.

CHAPTER 6. HRSD IN HEALTHY PARTICIPANTS AND PEOPLE WITH DIABETES WITH NO DIABETIC RETINOPATHY

6.1 CHAPTER INTRODUCTION

As with OCT thickness measurements, it is important to have an understanding of what constitutes normal healthy performance in vision tests, and in the case of this thesis, the normal hRSD threshold. This chapter provides information on the largest database on adult normative values for both 3AFC and 4AFC hRSD thresholds currently available. There are only two published studies that focus on hRSD thresholds in healthy participants (Wang et al., 2009a, Wang et al., 2013) and two studies where data is available from control groups in clinical studies (Bennett et al., 2016, Lott et al., 2021). The wide age range of participants available (16-90 years) in this chapter also allows for the assessment of the performance of the hRSD with ageing.

During the development of the hRSD test, various versions of the test were used. In the study by Wang et al. (2009b), there was no difference in the hRSD threshold between the 2AFC desktop and the 4AFC chart versions of the test. As discussed in Section 2.6.3, the 4AFC version of the hRSD test, available as an application on mobile devices were introduced in addition to the original 3AFC hRSD version. The advantage of this reduces the chance level of lucky guesses and decrease the likelihood of overestimating performance, without changing the measured threshold (Section 3.5.1). However, there has only been one conference abstract explicitly comparing the performance of the 3AFC and 4AFC tests, and this was potentially confounded because the 3AFC test was run on an iPod and 4AFC on an iPad (Bartlett et al., 2015). In the present study, the results of the 3AFC and 4AFC tests, presented on the same device (an iPod), will be compared. This is important as the differences between devices, such as screen size or device size, could affect performance. Given that some clinical studies have used the 3AFC version, it would be useful to have some assurance that data collected on patients in the present study can be directly compared with those earlier studies.

Another issue addressed in this chapter is the test-retest variability of the hRSD test in healthy participants; this has not been previously examined. Although there are relatively few published reports of hRSD threshold in healthy participants, it is valuable to know how consistently the test performs in a different setting with different participants, so a direct

comparison was made. One issue that is rarely addressed in the literature is how healthy participant groups that are recruited based on self-reporting are actually healthy. Often, VA and perhaps undilated ophthalmoscopy were the only information available (Ku et al., 2016, Wang et al., 2009b). In this study, macular OCT is available on the group of 50 healthy participants recruited to be age-matched controls for the PWD; these 50 participants are compared to 99 participants who had no OCT to address this issue. Lastly in this chapter, the hRSD threshold of healthy participants are compared with PWD who have no clinical evidence of DR (R0M0). The normative values established from this chapter are used for comparison with patient data in subsequent chapters.

6.2 METHODS OF DATA ANALYSES

Please refer to Chapters 3 and 4 for general and OCT methods. Bland-Altman analysis was used to assess test-retest reliability in Sections 6.3.2, 6.3.5. Student's *t*-tests were used to examine differences between the two groups (Sections 6.3.2, 6.3.4, 6.3.5, 6.3.7, 6.3.8, 6.3.10). Pearson correlation coefficient was used to examine the strength of correlation between two variables (Sections 6.3.1, 6.3.2, 6.3.3, 6.3.4, 6.3.5, 6.3.6, 6.3.7, 6.3.8, 6.3.10). All data analyses were performed using Excel (2016), GraphPad Prism (version 8) and SPSS (version 25).

6.3 RESULTS

6.3.1 3AFC RESULTS

The 3AFC version of the hRSD test has been available for longer than the 4AFC version and used in many older studies. For the 3AFC version of the hRSD test, data were obtained from 186 healthy adult participants (72 males, 114 females) who had a mean±SD age (range) of 42±17 years (16-90 years). Mean±SD hRSD threshold was -0.77±0.14 logMAR. Mean near VA (N=168) was -0.05±0.14 logMAR, distance VA (N=186) -0.03±0.12 and CS (N=170) was 1.72 ± 0.12 logCS units.

An analysis of the effects of age on the 3AFC hRSD threshold and near VA for the same eyes (Figure 6.1) generated a statistically significant correlation between age and hRSD threshold (Pearson r=0.35; p<0.001) with a slight increase in threshold (worse performance) with age. The slope of the least squares regression line was +0.0026. While this was similar to what was observed for near VA (r=0.51; p<0.001; regression slope: +0.0051), the difference between the two regression slopes was statistically significant ($F_{2,374}$=5.4; p=0.005) with

more decline in near VA with age compared to hRSD threshold. While the hRSD threshold across the sample correlated significantly with near VA (r=0.21, p=0.005), there was no significant correlation with CS (r=0.02, p=0.8; Figure 6.2).

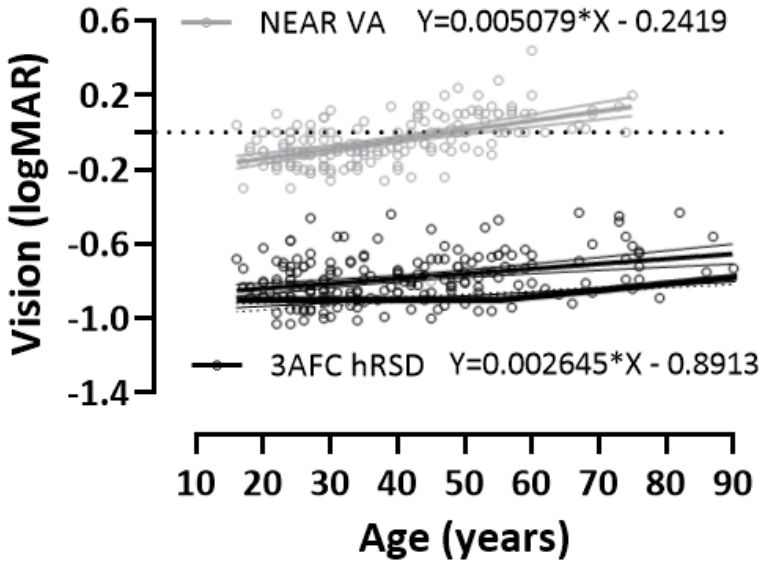


Figure 6.1. Effect of age on 3AFC hRSD threshold and near VA. A plot of 3AFC hRSD threshold (black circles) and near VA (grey circles; both logMAR) against age. For near VA, data were available for 168/186 participants. Least squares linear regression lines (±95% CI), with equations are shown. Solid black function at the bottom of the plot is taken from Wang et al. (2009b).

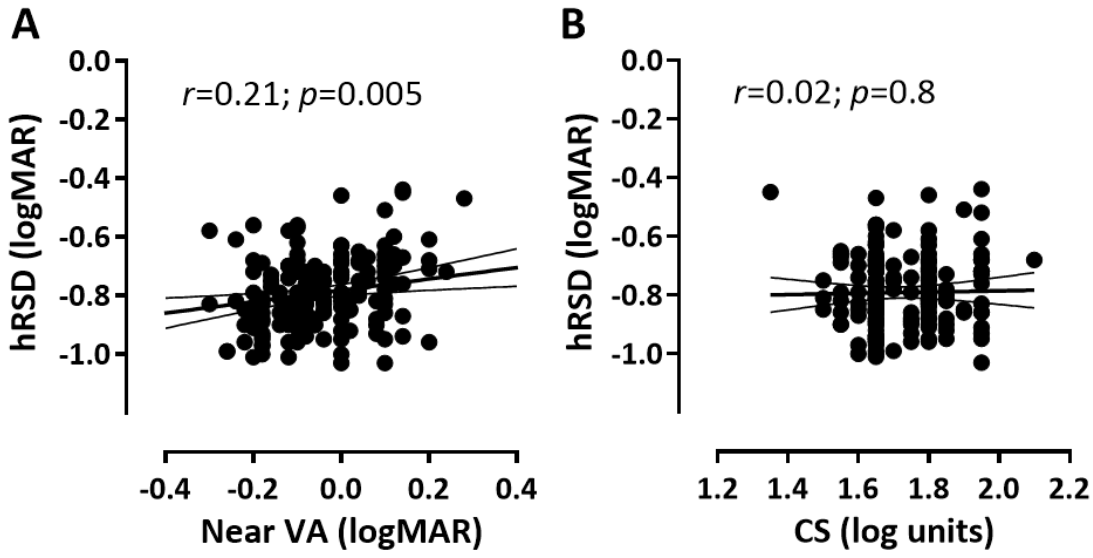


Figure 6.2. Relationship between 3AFC hRSD threshold (A) and near VA (B) in healthy participants. Solid line is the least-squares linear regression line (±95% CI). The correlation coefficient and its statistical significance are shown on each plot.

6.3.2 3AFC TEST-RETEST REPEATABILITY AND COMPARISON WITH DISTANCE AND NEAR VA

There are no published data on hRSD test-retest repeatability in healthy participants. Test-retest repeatability is important to assess the performance stability of the hRSD test. In this study, intrasessional, short-term and long-term repeatability is examined. The intrasessional test-retest repeatability of the 3AFC version was investigated in a subgroup of 74 participants (mean±SD age 43±16 years; range 16-80 years). The mean±SD thresholds for the first and second tests were -0.70±0.22 logMAR and -0.72±0.23 logMAR respectively; there was no statistically significant difference between these thresholds (paired *t*-test; t=1.722; p=0.089). The mean difference between the two tests was -0.02±0.12 logMAR. Data were available for 30 participants (mean±SD age 57±24 years, range 18-90 years) who were tested under identical conditions on two separate occasions, 64±42 days apart. Mean thresholds were -0.68±0.20 logMAR and -0.72±0.18 logMAR for the first and second tests respectively (t=0.997, p=0.327), and the mean difference between the two tests was 0.04±0.2 logMAR. Finally, 15 participants (mean±SD age 46±16 years, range 18-69 years) were tested on two occasions separated by a mean±SD of 39±0.9 months. Thresholds for tests 1 and 2 were -0.74±0.12 logMAR and -0.78±0.11 logMAR respectively (t=0.780, p=0.449), and the mean difference between the two tests was 0.04±0.18 logMAR.

Bland-Altman analyses of all three datasets (Figure 6.3), demonstrated the expected low mean biases. The 95% limits of agreement were wider when tests were conducted in different sessions separated by either several weeks (Figure 6.3B) or years (Figure 6.3C) compared to within the same session (Figure 6.3a). There was no obvious pattern to the scatter of the points on the plots. There were significant correlations between intrasessional (r=0.847, p<0.001) and short-term (r=0.407, p=0.026) hRSD thresholds but not long-term (r=-0.105, p=0.711) thresholds.

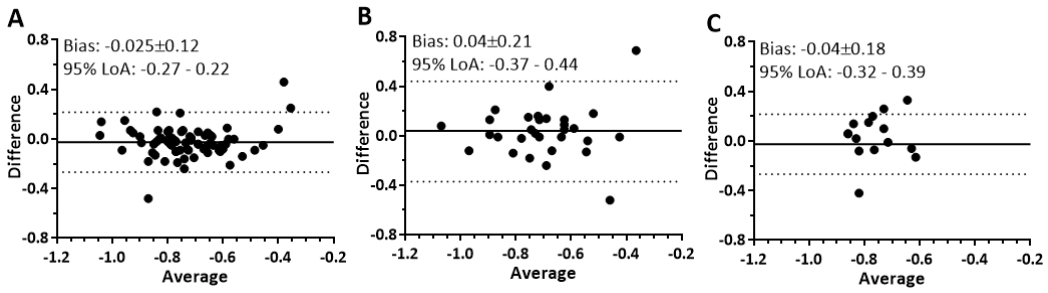


Figure 6.3. Bland-Altman plots for test-retest analysis of the 3AFC version of the hRSD test. Solid line is the mean bias, dotted lines are 95% limits of agreement. (A) Two tests run within a single session; N=74 (B) Two tests run 64±42 days apart; N=30 (C) Two tests run 39±0.9 months apart; N=15.

In the same participants who had short-term and long-term test-retest repeatability for the 3AFC hRSD, their distance and near VA test-retest repeatability were examined to compare them with their hRSD results. There were no intrasessional VA data available. Short-term test-retest repeatability data were available for 13 participants with distance VA and 17 participants with near VA. For their distance VA, the mean±SD thresholds for the first and second tests were -0.011±0.12 logMAR and -0.0015±0.09 logMAR respectively; there was no statistically significant difference between these thresholds (paired *t*-test; *t*=0.306; *p*=0.765). The mean difference between the two tests was -0.009±0.11 logMAR. For their near VA, the mean±SD thresholds for the first and second tests were -0.04±0.14 logMAR and -0.08±0.27 logMAR respectively; there was no statistically significant difference between these thresholds (paired *t*-test; *t*=0.511; *p*=0.617). The mean difference between the two tests was -0.034±0.28 logMAR. Bland-Altman analyses of the short-term VA of both distance and near VA showed low mean biases (Figure 6.4). The 95% limits of agreement were wider with near VA compared to distance VA.

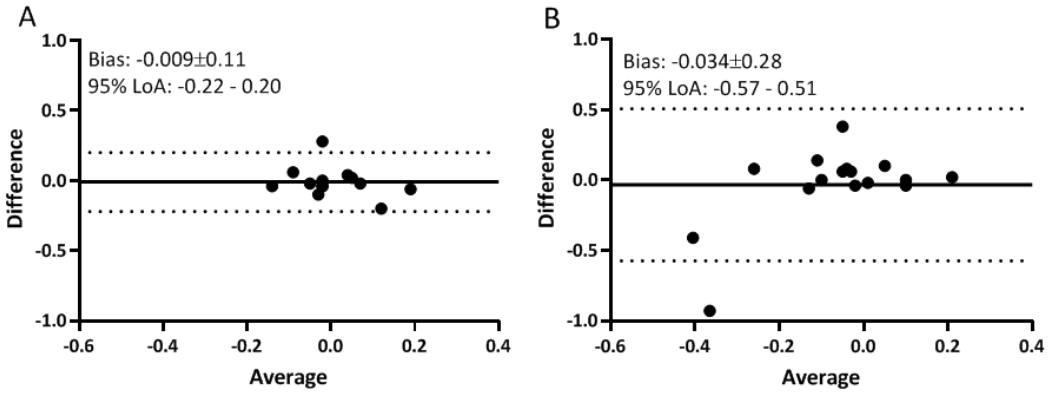


Figure 6.4. Bland-Altman plots for test-retest analysis of short-term VA. Solid line is the mean bias, and dotted lines are 95% limits of agreement. (A) Distance VA; N=13 (B) Near VA; N=17.

Long-term test-retest repeatability data were available for 15 participants with distance and near VA. For their distance VA, the mean±SD thresholds for the first and second tests were -0.03±0.15 logMAR and -0.03±0.15 logMAR respectively; there was no statistically significant difference between these thresholds (paired *t*-test; *t*=0.098; *p*=0.924). The mean difference between the two tests was 0.003±0.11 logMAR. For their near VA, the mean±SD thresholds for the first and second tests were 0.02±0.13 logMAR and 0.11±0.17 logMAR respectively; the difference between these thresholds was just statistically significant (paired *t*-test; *t*=2.157; *p*=0.049). The mean difference between the two tests was 0.08±0.15 logMAR. Bland-Altman analyses of the long-term VA of both distance and near VA showed low mean biases (Figure 6.5). Similar to short-term VA results, the 95% limits of agreement were wider with near VA compared to distance VA.

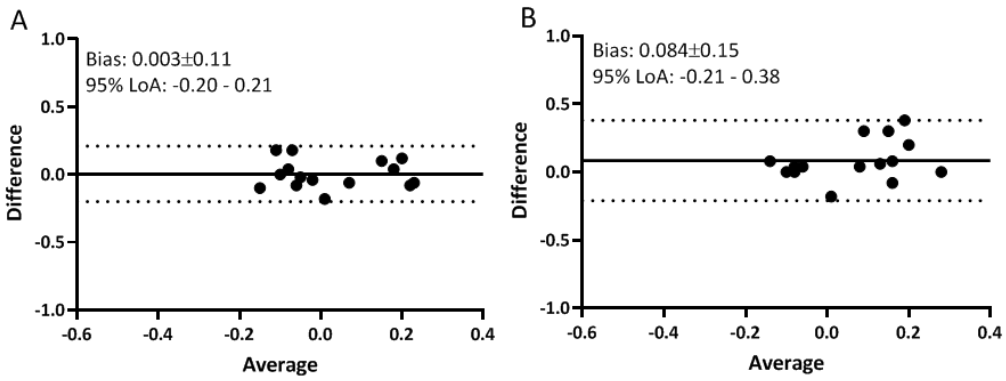


Figure 6.5. Bland-Altman plots for test-retest analysis of long-term VA. Solid line is the mean bias, and dotted lines are 95% limits of agreement. (A) Distance VA; N=15 (B) Near VA; N=15.

6.3.3 4AFC RESULTS

For the 4AFC version of the hRSD test, data were available for 149 healthy adult participants (64 males, 85 females) who had a mean±SD age (range) of 42±16 years (18-85 years). Mean±SD hRSD threshold was -0.79±0.12 logMAR. Mean near VA (N=149) was -0.01±0.15 logMAR, distance VA (N=149) was -0.06±0.12 and CS (N=106) was 1.68±0.10 logCS units.

Similar to the 3AFC threshold results, analysis of the effects of age on 4AFC hRSD threshold and near VA for the same eyes (Figure 6.6) generated a statistically significant correlation between age and 4AFC hRSD threshold (Pearson r=0.20; p<0.01) with a slight increase in threshold (worse performance) with age. The slope of the least squares regression line was +0.0015. While this was similar to what was observed for near VA (r=0.68; p<0.001; regression slope: +0.0062), the difference between the two regression slopes was statistically significant ($F_{1,294}$=33.98; p<0.001).

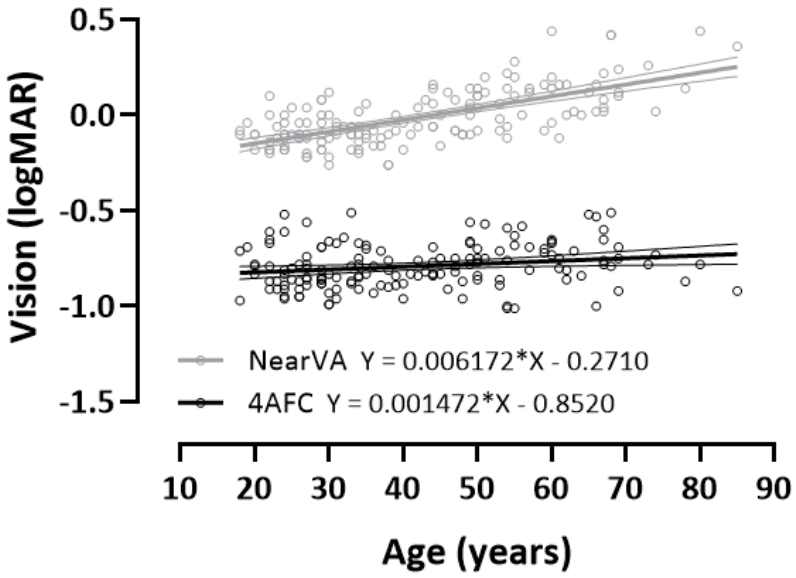


Figure 6.6. Effects of age on 4AFC hRSD threshold and near VA. A plot of 4AFC hRSD threshold (black circles) and near VA (grey circles; both logMAR) against age. Least squares linear regression lines (±95% CI) with equations, are shown.

6.3.4 4AFC TEST-RETEST REPEATABILITY

Test-retest repeatability of the 4AFC hRSD was available for only 7 participants (mean±SD age 37±13 years; range 18-50 years) tested 19±0.76 months apart (Figure 6.7). The mean±SD thresholds for the first and second tests were -0.83±0.11 logMAR and -0.83±0.12 logMAR respectively. There was no statistically significant difference between these thresholds (paired *t*-test; *t*=-0.096; *p*=0.926). There were no significant correlations between hRSD thresholds (*r*=0.490, *p*=0.265).

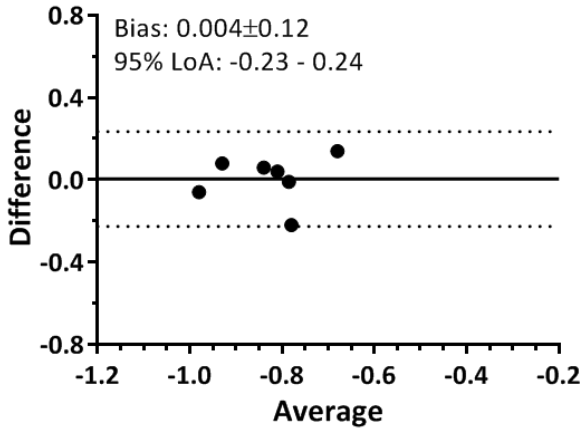


Figure 6.7. Bland-Altman plots for test-retest analysis of the 4AFC version of the hRSD test. Solid line is the mean bias, and dotted lines are 95% limits of agreement. Two tests run 19±0.76 months apart; N=7.

6.3.5 COMPARISON OF 3AFC AND 4AFC RESULTS

Comparison of the 3AFC and 4AFC versions of the hRSD were made in all participants who had performed the 3AFC and 4AFC versions of the hRSD test. A comparison was made in 106 participants who did both versions of the test in one session. 186 participants completed the 3AFC version, and 149 participants completed the 4AFC version. The mean±SD age (range) of the participants who undertook the 3AFC and 4AFC versions of the hRSD were 42±17 years (16-90 years) and 42±16 years (18-85 years) respectively (unpaired *t*; *t*=0.186; *p*=0.853). Other participant characteristics were described in sections 4.2 and 4.4. Mean 3AFC and 4AFC hRSD thresholds were -0.70±0.13 logMAR and -0.79±0.12 logMAR respectively (unpaired *t*; *t*=0.740; *p*=0.460).

106 participants (42 Males, 64 Females) completed the 3AFC and 4AFC versions of the hRSD test in a single session. The mean±SD age (range) of the participants was 37±13 years (18-72 years). Mean 3AFC and 4AFC hRSD thresholds were -0.82±0.12 logMAR and -0.81±0.11 logMAR respectively (paired *t*; *t*=1.334; *p*=0.185). Bland-Altman analysis (Figure 6.8) revealed a very low level of bias (-0.013±0.097 logMAR) and 95% limits of agreement of -0.20 to 0.18 logMAR.

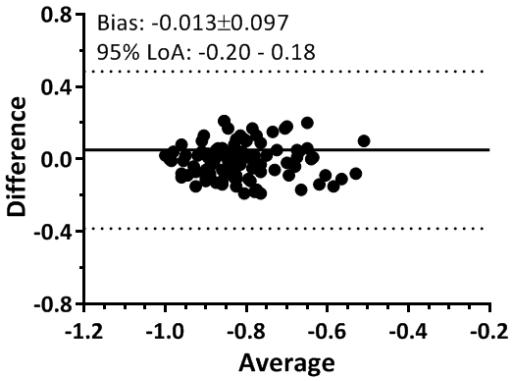


Figure 6.8. Bland-Altman plot showing mean bias (solid line) and 95% limits of agreement (dotted lines) for the 3AFC vs 4AFC version of the hRSD test.

Comparison of the effect of age on both the 3AFC and 4AFC hRSD thresholds and near VA was made (Figure 6.9), and there was a weak, though statistically significant correlation between age and both 3AFC (*r*=0.189, *p*=0.053) and 4AFC thresholds (*r*=0.219, *p*=0.024). There was a significant correlation between age and near VA (*r*=0.651, *p*<0.001).

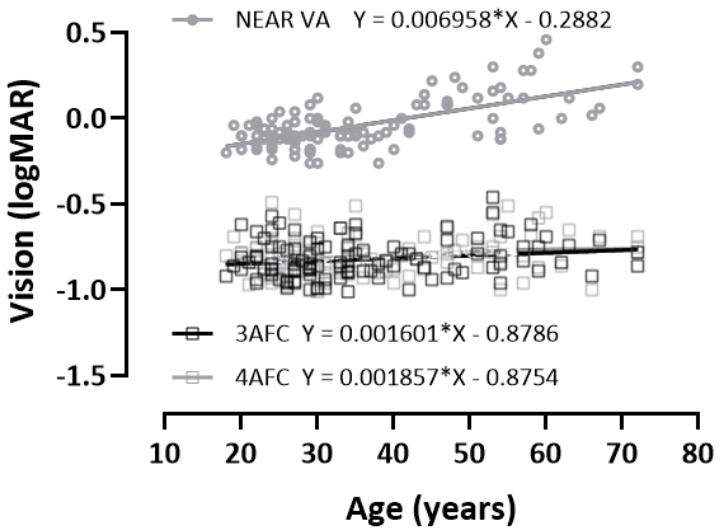


Figure 6.9. Influence of age on 3AFC and 4AFC threshold and on near VA for 106 participants. Least squares linear regression lines along with the functions for the lines are shown.

As there was no difference in 3AFC and 4AFC thresholds, these groups have been combined for comparisons with PWD in subsequent sections, unless otherwise stated. A description of the combined participants is as follows. All 4AFC thresholds (N=149) were combined with selected 3AFC thresholds in participants who only did the 3AFC version of the test (N=80) with a total of 229 participants (135 females, 94 males). This was done to ensure that results from participants who did both versions of the test did not overlap. The mean±SD age (range) of the combined participants were 44±18 years (16-90 years). Their mean±SD hRSD threshold was -0.77±0.13 logMAR. Their mean±SD distance and near VA was -0.04±0.12 logMAR and -0.02±0.15 logMAR respectively.

6.3.6 RELATIONSHIP BETWEEN AGE AND HRSD THRESHOLDS IN YOUNGER PARTICIPANTS (< 55 YEARS) AND OLDER PARTICIPANTS (≥55 YEARS)

As discussed in Section 2.6.3.5, Wang et al. (2009b) found that global hyperacuity reaches adult levels by 21 years of age and then and remains stable until 55 years old. Thereafter, global hyperacuity starts to deteriorate at the rate of 0.035 logMAR per decade. To determine if there is a deterioration of hRSD in healthy participants after 55 years old in the EDDMO study, the relationship between age and hRSD thresholds in younger participants (<55 years old) and older participants (≥55 years old) was examined in the 229 healthy participants previously described in Section 6.3.5 (Figure 6.10). There was no correlation between age and hRSD threshold in both younger participants (r=0.050, p=0.526) and older participants (r=0.119, p=0.354). The slopes of the least squares regression line were +0.0009 in the younger participants and +0.0019 in the older participants. The difference between the two regression slopes was not statistically significant ($F_{1,225}$=0.28, p=0.55).

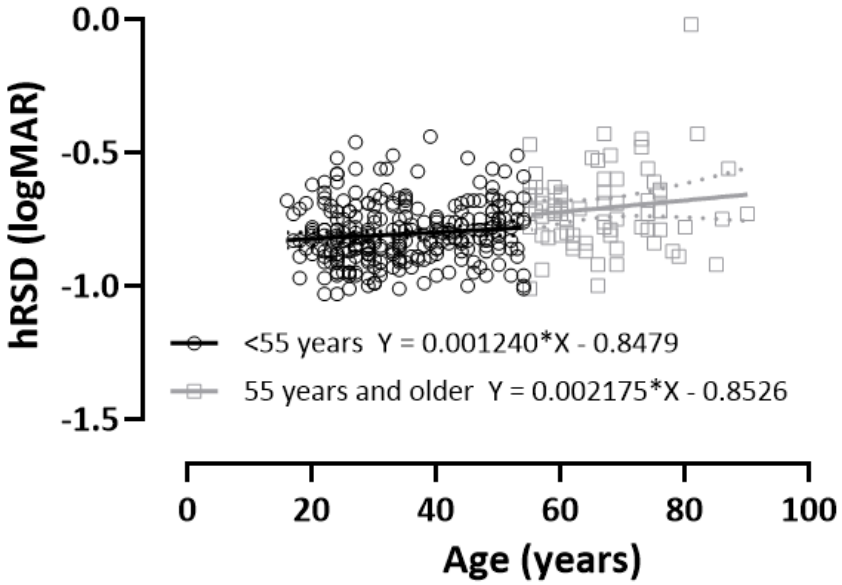


Figure 6.10. Relationship between age and hRSD thresholds in younger participants (<55y) and older participants (≥55y). Least squares linear regression lines (±95% CI), with equations are shown.

6.3.7 COMPARISON OF 4AFC HRSD THRESHOLD IN SELF-REPORTED VISUALLY HEALTHY PARTICIPANTS VS HEALTHY PARTICIPANTS WITH NORMAL MACULAR OCT

Often studies have control groups in which if VA is normal for age, and participants report no problems or ongoing treatment, then they are assumed to be healthy. However, in addition to the 99 self-reported visually healthy participants who undertook the 4AFC hRSD test, there were also 50 healthy participants who had normal macular OCT in the EDDMO study. All the healthy participants had normal anterior segment and undilated fundoscopy examinations on the slit-lamp. The mean±SD age (range) of the 99 participants who did not have any OCT (38 males, 61 females) and the 50 participants who had OCT (26 males, 24 females) were 36±13 years (18-69 years) and 55±14 years (22-85 years) respectively. Their mean 4AFC thresholds were -0.80±0.12 logMAR and -0.77±0.11 logMAR respectively (unpaired *t*-test; t=1.379, p=0.170). The mean±SD near VA for the participants who had no OCT and participants who had OCT were -0.05±0.12 logMAR and 0.06±0.16 logMAR respectively (unpaired *t*-test; t=4.830, p<0.001). The mean±SD distance VA for the participants who had no OCT and participants who had OCT were 0.06±0.12 logMAR and -0.08±0.12 logMAR respectively (unpaired *t*-test; t=0.933, p=0.352). As expected, there was no significant correlation between the CST of the participants who had OCT and their hRSD thresholds, distance or near VA (Figure 6.11).

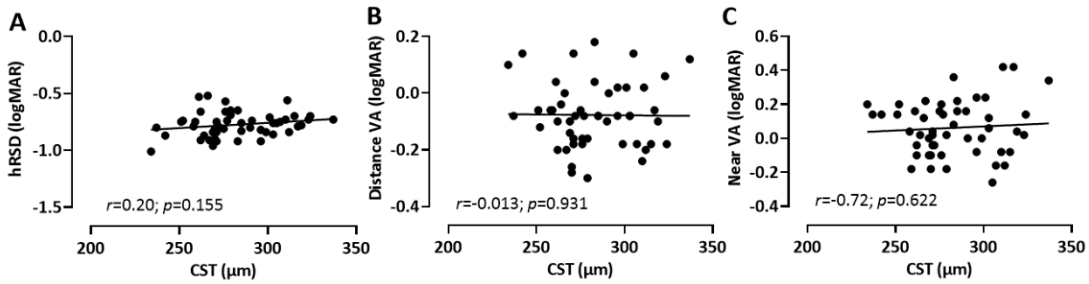


Figure 6.11. Relationship between central subfield thickness (CST) and hRSD (A), distance (B) and near VA (C) in healthy participants (N=50). Solid line is the least-regression line. The correlation coefficient and its statistical significance are shown on each plot.

6.3.8 HRSD TEST TIMES AND REQUIREMENTS FOR A THIRD TEST

For the 106 participants who completed both the 3AFC and 4AFC versions of the hRSD test, the mean±SD test times for the 3AFC and 4AFC versions were 193.3±56.6s and 194.2±54.9s respectively (paired *t*-test; *t*=-0.158, *p*=0.875). Test time results were available for 98 participants who undertook the 3AFC and 4AFC hRSD tests with both their right and left eyes. The mean test times for the 3AFC hRSD test for the right and left eyes were 195.6±61.4s and 182.6±50.9s respectively (paired *t*-test; *t*=2.11, *p*=0.037). The mean test times for the 4AFC test for the right and left eyes were 196.0±52.5s and 185.9±50.9s respectively (paired *t*-test; *t*=2.083, *p*=0.04). There were no significant correlations between age and test times for either the 3AFC (*r*=-0.095, *p*=0.332) or 4AFC (*r*=-0.075, *p*=0.446) versions of the test (Figure 6.12). The regression slopes for the 3AFC and 4ACF test times with age were very similar and not statistically significantly different ($F_{1,208}$=0.028, *p*=0.869). A third test was required when the within-session results of the hRSD test differed by 0.30 logMAR or more (Wang et al., 2013). In the 3AFC hRSD tests, a third test was required in 3.7% (7 of 190 eyes) while in the 4AFC hRSD tests, a third test was required in 2.5% (7 of 284 eyes).

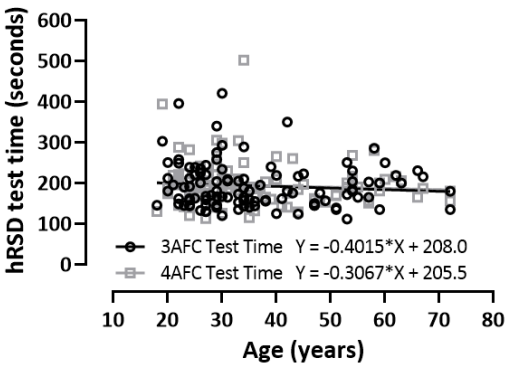


Figure 6.12. Effect of age on 3AFC and 4AFC test times. Least squares linear regression lines with the functions for the lines are shown.

6.3.9 RESULTS OF THE USABILITY QUESTIONNAIRE FOR HEALTHY PARTICIPANTS

149 healthy participants undertook a 4AFC hRSD usability questionnaire, which consisted of the 4 questions shown in Table 6.1. Most participants' agree or strongly agree that they understood how to use the test (question 1), found the test easy to use (question 2), found that the test did not take too long to do (question 3) and were able to use the test to test their vision (question 4). 106 of the participants undertook the 3AFC hRSD test in the same session and answered an additional question that compared the 3AFC and 4AFC versions of the test. While 42% of the participants expressed no preference between versions, 33% found the 3AFC version easier to use and 25% found the 4AFC version easier to use (Table 6.2).

Table 6.1. Usability questionnaire results from healthy participants (N=149). Participant responses to each statement shown as the % of participants rounded to whole numbers.

Questions	Strongly disagree	Disagree	Neutral	Agree	Strongly agree
1. I understood how to use the hRSD device.	1	0	1	17	81
2. The hRSD test was easy to use.	1	1	3	15	80
3. The hRSD test did not take too long to do.	1	2	3	32	62
4. I could use the hRSD device to test my own vision.	1	1	9	29	60

Table 6.2. Usability questionnaire results comparing the 4AFC vs 3AFC hRSD (N=106). Participant responses to each statement shown as % of participants rounded to whole numbers.

The 3AFC hRSD test was much easier to use	The 3AFC hRSD test was somewhat easier to use	There was no difference in the ease of using either test	The 4AFC hRSD test was somewhat easier to use	The 4AFC hRSD test was much easier to use
9	24	42	19	6

6.3.10 COMPARISON OF 4AFC HRSD THRESHOLD AND CENTRAL SUBFIELD THICKNESSES IN HEALTHY PARTICIPANTS VS PWD WITH NO DR (R0M0)

As discussed in Chapter 1, studies have found abnormalities in ERG, CS, colour vision, dark adaptation and microperimetry in PWD with no or minimal DR (Bearse et al., 2006, Hardy et al., 1992, Greenstein et al., 1993, Jackson et al., 2012). This section explores whether there are any differences in the hRSD thresholds between healthy participants and PWD with no DR. There were 29 PWD (17 Male, 12 Female) who had no DR (R0M0). Their mean±SD age (range) was 56±14 years (23-85 years), and their mean±SD duration of diabetes (range) was 7.3±3.9 years (1-16 years). Their mean±SD near and distance VA were 0.20±0.24 logMAR and 0.03±0.16 logMAR respectively. The hRSD thresholds of these participants were compared to the 229 healthy participants described in Section 6.3.5. The hRSD thresholds for the PWD and healthy participants were -0.68±0.18 logMAR and -0.77±0.13 logMAR respectively; these thresholds were statistically significantly different (unpaired *t*-test; t=3.12, p<0.002). The hRSD thresholds across the age range were then compared for both the PWD and healthy participants (Figure 6.13). There was an increase in the hRSD threshold (worse performance) in both healthy participants and PWD across the age range (Figure 6.13). However, the PWD performed consistently worse on the hRSD test across the age range by approximately 0.1 logMAR compared to the healthy participants. A comparison of the regression lines for the two groups demonstrated that while there was no significant difference in regression slopes ($F_{1,254}$=0.0017; p=0.97), the intercepts were significantly different ($F_{1,255}$=5.5; p=0.020) (Figure 6.13).

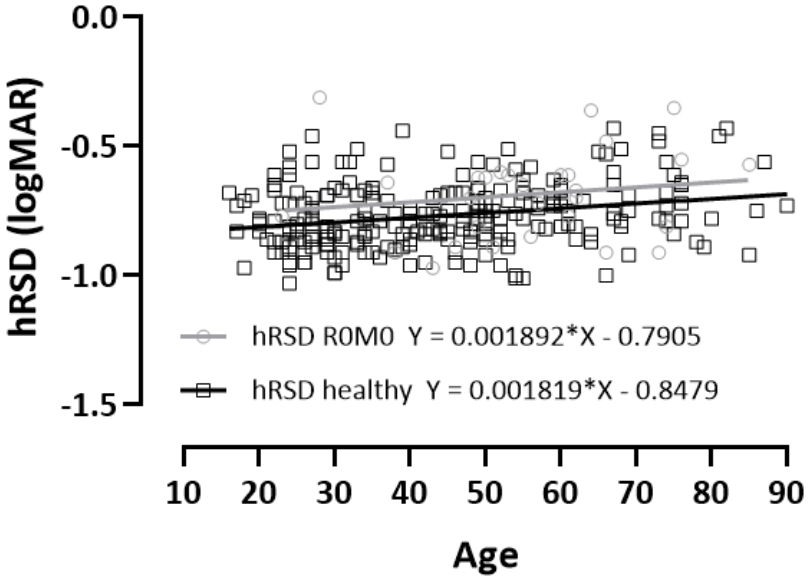


Figure 6.13. 4AFC hRSD threshold across the age range for people with diabetes with no diabetic retinopathy (R0M0; N=29; grey circles) and healthy participants (N=229; black squares). Least squares linear regression lines along with the functions for the lines are shown.

The CST of the PWD was compared with the CST of the 50 healthy participants who had OCT as described in Section 6.3.7. The mean CST of the PWD was thinner (279±23.9 µm) compared to the healthy participants (283±23.9 µm) but this difference was not statistically significant (unpaired *t*-test; t=0.61, p=0.54). There was no significant correlation between the CST of the PWD and their hRSD threshold, distance VA or near VA (Figure 6.14).

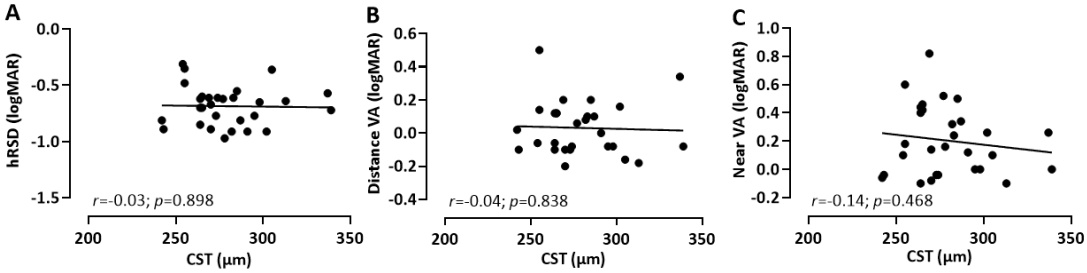


Figure 6.14. Relationship between central subfield thickness (CST) and hRSD (A), distance (B) and near VA (C) in people with diabetes with no diabetic retinopathy (R0M0). Solid line is the least-regression line. The correlation coefficient and its statistical significance are shown on each plot.

The mean±SD test times for the PWD (165±67s) were shorter compared to the healthy participants (194.2±54.9s), and this difference was statistically significant (unpaired *t*-test;

t=2.42, *p*=0.017). Similar to the healthy participants, the PWD reported high usability for the hRSD test (Table 6.3).

Table 6.3. Usability questionnaire results from people with diabetes and no diabetic retinopathy (R0M0) (N=29). Participant responses to each statement shown as the % of participants rounded to whole numbers.

Questions	Strongly disagree	Disagree	Neutral	Agree	Strongly agree
1. I understood how to use the hRSD device.	0	0	0	38	62
2. The hRSD test was easy to use.	0	0	0	41	59
3. The hRSD test did not take too long to do.	0	0	0	38	62
4. I could use the hRSD device to test my own vision.	0	0	7	38	55

6.3.11 SUMMARY OF RESULTS

6.3.11.1 Healthy participants (Sections 6.3.1 to 6.3.9)

There was a significant decline in both 3AFC and 4AFC hRSD thresholds and near VA with age but hRSD threshold was less affected by age compared to near VA (Sections 6.3.1, 6.3.3 and 6.3.5). 3AFC hRSD showed low intrasessional, short-term and long-term test-retest repeatability (Section 6.3.2). 4AFC hRSD showed low long-term test-retest repeatability (Section 6.3.4). There was no difference in the 3AFC and 4AFC hRSD thresholds (Section 6.3.5). There was no difference in the decline of hRSD threshold in younger participants <55 years old and older participants ≥55 years old (Section 6.3.6). There was no difference in hRSD threshold or distance VA in participants who had no OCT compared to participants who had OCT (Section 6.3.7). The right eye, which is tested first, had a longer hRSD test time compared to the left eye. There was no correlation between hRSD test time and age (Section 6.3.8). Participants reported good usability of the hRSD test (Section 6.3.9).

6.3.11.2 Healthy participants compared to PWD with no DR (Section 6.3.10)

hRSD threshold of PWD was significantly worse compared to healthy participants. PWD performed worse across the age range compared to PWD. PWD had a shorter hRSD test time compared to healthy participants. PWD reported good usability of the hRSD test.

6.4 CHAPTER DISCUSSION

The purpose of this chapter is to establish normative thresholds for the 3AFC and 4AFC versions of the hRSD test and to directly compare the two versions of the test on the same device. This is important to confirm whether the 3AFC results from older studies are comparable with more recent studies that have used the 4AFC hRSD test. The baseline test-retest variability of the hRSD test in normal participants was also investigated. This chapter offers macular OCT in addition to visual information (hRSD, distance and near VA) on the validity of self-reporting when recruiting healthy participants for studies. Several published studies have used normal VA and self-reported eye health (i.e. the absence of diagnosed conditions or ongoing treatment) to recruit participants (Wang et al., 2009b). The hRSD thresholds of healthy participants have also been compared with those of PWD who have no clinical evidence of DR (R0M0) to determine if there are any hRSD deficits in pre-clinical DR.

The 3AFC hRSD threshold obtained in this study (-0.77±0.14 logMAR) is lower (better performance) than thresholds from those reported in previous studies, but this may be due to the different mean age and age range of the participants involved (Figure 6.15). Wang et al. (2013) reported slightly poorer performance with the 3AFC iPod version of the task (-0.69 logMAR) for a control group of older healthy participants (N=27, mean age 69 years) in a clinical study. This corresponds well to a median of -0.69 logMAR recently reported for the same version of the task that was obtained from a group of healthy participants with a mean age of 74 years (N=32) (Lott et al., 2021). Broadly, it appears that in the hands of different researchers, and when used on different participants, the hRSD test performs consistently.

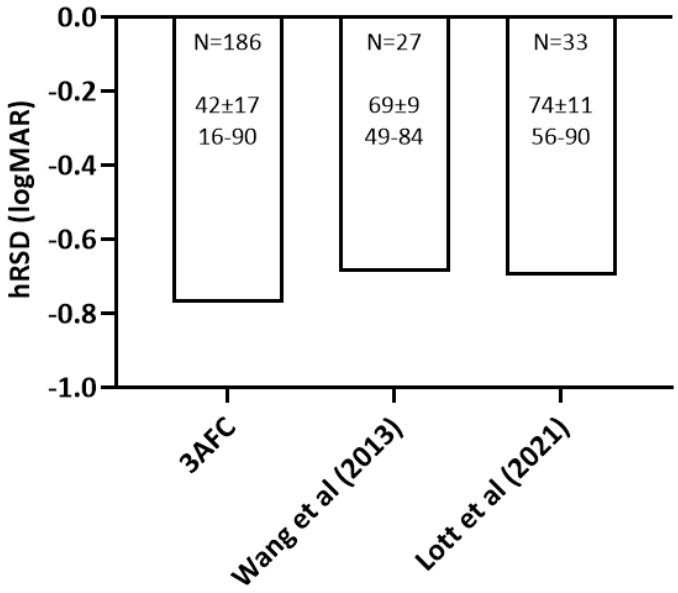


Figure 6.15. Comparison of mean 3AFC hRSD threshold compared with thresholds in other studies (Wang et al., 2013, Lott et al., 2021). The number of participants, mean±SD age (years) and age range in each study are shown.

The 4AFC (-0.79±0.12 logMAR) thresholds that we observed in this study were slightly poorer than that reported by Wang et al. (2009b) (Figure 6.16). They investigated both children and adults with the stimuli presented in two different formats, a 2AFC version presented on a computer monitor and a 4AFC version presented on charts. The thresholds returned from these two methods were in good agreement. For adults aged 22-78 years (N=97), they reported a threshold of -0.86 logMAR. There is one report on data from 10 relatively young control participants (mean age 36±6 years, range 16-66 years) from a clinical study in which the task was presented on an iPad that reported a threshold of -0.70±0.10 logMAR (Bennett et al., 2016). Given the differences in participant selection,

testing procedures and study settings, the values for 4AFC hRSD thresholds appear to be consistent between this study and previous studies.

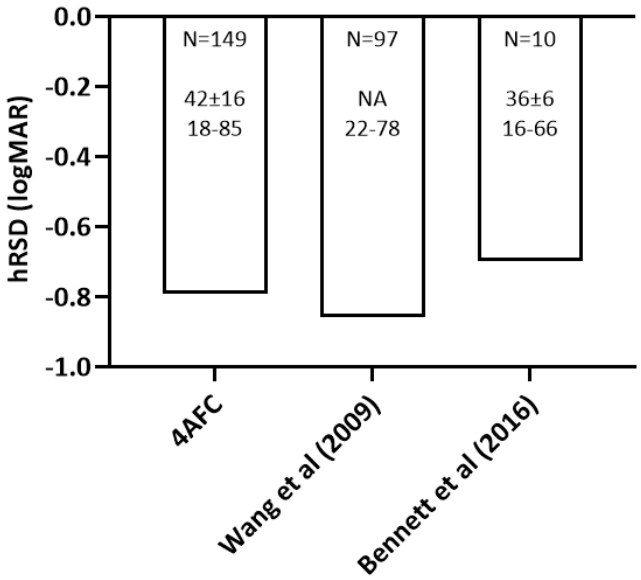


Figure 6.16. Comparison of mean 4AFC hRSD threshold compared with thresholds in other studies (Wang et al., 2009b, Bennett et al., 2016). Participants' number, mean±SD age (years) and age range in each study shown except for the mean±SD age from Wang et al. (2009b), which is not available (NA).

In the EDDMO study, healthy participants were recruited based on self-reporting and participants having a normal VA and slit-lamp examination, which is similar to most previous studies that provided normative hRSD thresholds (Wang et al., 2009b, Bennett et al., 2016, Lott et al., 2021). Only one study added having either a normal fundus examination or OCT as an inclusion criterion (Wang et al., 2013). Without OCT imaging, subtle macular pathology that may affect vision could be missed (Kowallick et al., 2018). Therefore, a comparison of participants who had no OCT (N=99) was made with participants who undertook additional macular OCT (N=50) to assess the quality of traditional methods of assuming normality vs additional OCT imaging. The mean±SD hRSD threshold for the participants who did not have any OCT (-0.80±0.12 logMAR) was better than the participants who had a normal macular OCT (-0.77±0.11 logMAR). The slight difference in hRSD thresholds may be due to the difference in the mean age of the participants, with the participants who did not have any OCT being younger (mean±SD age 36±13 years) compared to the participants who had the OCT (mean age 55±14 years). It was reassuring that there was no statistical significance between the hRSD thresholds.

The slight increase in the hRSD threshold with age observed in this chapter is consistent with previous reports (Wang et al., 2009b). While the correlation between age and hRSD threshold was statistically significant, the slope of the regression line implied an average decline of only 0.0026 logMAR per decade between the ages of 20 years and 80 years (Figures 6.1 and 6.6). Given that the decline in near VA over the same period proceeded at almost twice this rate (slope of regression=0.0051 logMAR), hRSD performance appears to be relatively resistant to the effects of normal ageing. Wang et al. (2009b) suggested that since hRSD measures the shape discrimination sensitivity with visual stimuli containing low spatial frequencies, it is much less subjected to the reduced optical quality of the ageing eye (Wang et al., 2009b). They also proposed that the rate of deterioration with ageing for hRSD is approximately 60% of that for VA (Wang et al., 2009b). Wang et al. (2009b) suggested that radial shape discrimination might be stable up to the fifth decade with a slight decline then occurring, even in the absence of any obvious pathology (the function represented by the solid black line in Figure 6.1 taken from Wang et al. (2009b). While we saw no evidence of this pattern in our data (Figure 6.10), it should be noted that our oldest participants were selected on the basis that they had no retinal pathology in their selected eye. As early AMD is a highly prevalent condition, our participants are probably unrepresentative of the general population at older ages into the seventh and eighth decades of life. Overall, while there is some decline in hRSD threshold with normal (non-pathological) ageing, this does indeed seem to be less marked compared to other aspects of visual function. This is a useful attribute for a test that might be used to detect age-related pathologies, such as AMD (Pitrelli Vazquez et al., 2018).

There has not been any previously published normative hRSD test-retest variability data available for the hRSD test. The mean difference observed between two hRSD tests within a single session and separated by several weeks or even many months was consistently close to zero and the limits of agreement relatively narrow. Even over an extended period, performance remained stable, although we were only able to assess this in a relatively small (and relatively young) group of participants. The Bland-Altman plots did not suggest any consistent relationship between average performance and variability. Low levels of test-retest variability are a desirable feature of a test that might be used longitudinally over extended periods of time to monitor for the development of disease (Wang et al., 2013). hRSD test-retest variability in PWD is explored in Chapter 9. The normative distance and near VA repeatability is approximately one line (5 letters) (Lovie-Kitchin and Brown, 2000). Lovie-Kitchin (1988) proposed that a reduction of 8 letters is an appropriate referral

criterion. An equivalent could be a deterioration in hRSD threshold of ≥0.25-0.3 logMAR as observed by Wang et al. (2013) when participants with early AMD or DR progressed to more advanced disease. The hRSD test-retest variability is comparable with distance and near test-retest variability. Interestingly, the hRSD and near VA showed more comparable limits of agreement while distance VA showed more narrow limits of agreement. This may be because the hRSD is a near task held at a reading distance.

There were no differences when comparing the 3AFC and 4AFC thresholds in participants who undertook both tests in a single session (paired *t*-test) or at different time points (unpaired *t*-test). The EDDMO study found no differences in age effects between the 3AFC and 4AFC versions of the test. Confirming that the two versions provide comparable thresholds is useful as it suggests that it is possible to make direct comparisons between older studies based on the 3AFC version and newer studies based on the 4AFC version.

There were no differences in the test times between the 3AFC and 4AFC versions of the test. Both versions took just over 3 minutes for each eye. Testing for the right eye took longer than the left eye for both versions of the test. As the right eye is routinely tested first, there may be a learning effect for the left eye to perform the test faster. Interestingly, PWD and no DR (R0M0) took less time to perform the task (165s). Wang et al. (2013) reported a test time of 92s in a combined group of participants who were visually normal (N=27), have AMD (N=37) and had DR (N=36). It is possible that participants with more visual pathology have less ability to proceed with the finer hRSD task and reach the endpoint faster. There may be an expectation that younger participants may perform the hRSD test faster but, there was no correlation between age and test times. The results of the usability questionnaire demonstrated that the majority of participants understood how to use the test, found it easy to use, felt it did not take too long and were confident using it (Tables 6.1 and 6.3).

Interestingly, a significant difference in the hRSD threshold between healthy participants and PWD who have no clinical evidence of DR was found (Section 6.3.10). This suggests that hRSD can detect deterioration in visual function before clinically visible DR. The PWD had a small (approximately 0.1 logMAR) but consistent deterioration of the hRSD threshold across the age range. This implies that the hRSD threshold for PWD needs to be set at a different range compared to healthy participants. In addition, due to the slight decline of hRSD with age, a change in hRSD threshold compared to baseline measurements may be a more suitable referral criterion instead of an absolute hRSD threshold cutoff (Kaiser et al., 2013).

However, these 29 participants may not be representative of patients with no DR. As part of the inclusion criterion for this study, PWD has an LDESP screening grade of M1 in one or more eyes. Of these 29 participants, only 3 were graded as R0M0 in both eyes while the other 26 had some DR in their fellow on clinical examination. This suggests that these participants may be part of the spectrum of patients with more severe diabetic changes compared to participants with R0M0 in both eyes in the community who have never been referred to the DEC.

Lastly, the EDDMO study found no difference in the usability of the two versions. Similar to previous results (Wang et al., 2013), all of the participants found the test easy to understand and execute. No participant failed to produce a result. Since this is a study of motivated healthy volunteers, comparisons are made with PWD in subsequent chapters.

In summary, this chapter established normative 3AFC and 4AFC hRSD thresholds, which is important for comparisons with PWD hRSD thresholds in subsequent chapters. This chapter showed that the 3AFC and 4AFC hRSD thresholds are equivalent, allowing comparisons with earlier studies. This chapter found that the hRSD test had desirable features to monitor visual function such as low test-retest variability, less decline with age compared to near VA and good usability. Interestingly, there was a decline in hRSD performance in PWD with no DR compared to HC, and further examination of hRSD performance in PWD is made in Chapter 8. The following chapter 7 examines retinal thickness as detected by OCT in PWD.

CHAPTER 7. CROSS-SECTIONAL ANALYSIS OF RETINAL THICKNESS FROM OCT IN PEOPLE WITH DIABETES

7.1 CHAPTER INTRODUCTION

In preceding chapters, OCT thickness measurements of healthy controls (HC) and PWD with no or minimal DR (ETDRS 10 and ETDRS 20) were been examined to allow comparisons with previous studies using ETDRS DR grades (Chapter 5). This chapter focuses on the cross-sectional analysis of PWD with a wide range of DR severity from mild to more severe disease.

DR screening was established to reduce the risk of visual morbidity from the ocular complications of diabetes. Since the introduction of DR screening in the UK, there has been much discussion on ways to improve its effectiveness and service provision (Harding et al., 2003, Olson et al., 2013). The current DR screening programme is based on the recognition of specific features from fundus photographs, as discussed in Chapter 2 (Section 2.5.4) (NHS Diabetic Eye Screening Programme, 2012). It is recognised that using these features is not as effective in detecting maculopathy compared to retinopathy. One study found that in a local screening service, 119 out of 311 PWD were screened as R1M1. In these PWD who were referred as maculopathy suspects, only 38.3% had OCT evidence of macular oedema. This means that most of the PWD in that study did not require a referral (Mackenzie et al., 2011).

This chapter examines the OCT data of PWD, with various levels of DR severity, to investigate baseline structural parameters to detect the presence, extent and severity of DMO. Specifically, the retinal thicknesses across the ETDRS subfields of OCTs collected in HC and PWD are examined according to their retinopathy and maculopathy grades based on the LDESP grading criteria described in Chapter 2, and Liverpool OCT criteria described in Chapter 4 and treatment outcomes. Both full retinal thickness and retinal thickness of different layers are examined. This chapter also specifically examines the full retinal thickness and thickness of different layers in PWD with early DR, which are graded according to their NDESP grades and Liverpool OCT grades (Section 7.3.9). This chapter begins with a descriptive analysis of the PWD. A descriptive analysis of the healthy controls (HC) can be found in Chapter 5. Given the length of this chapter, Figure 7.1 shows the organisation of the chapter.

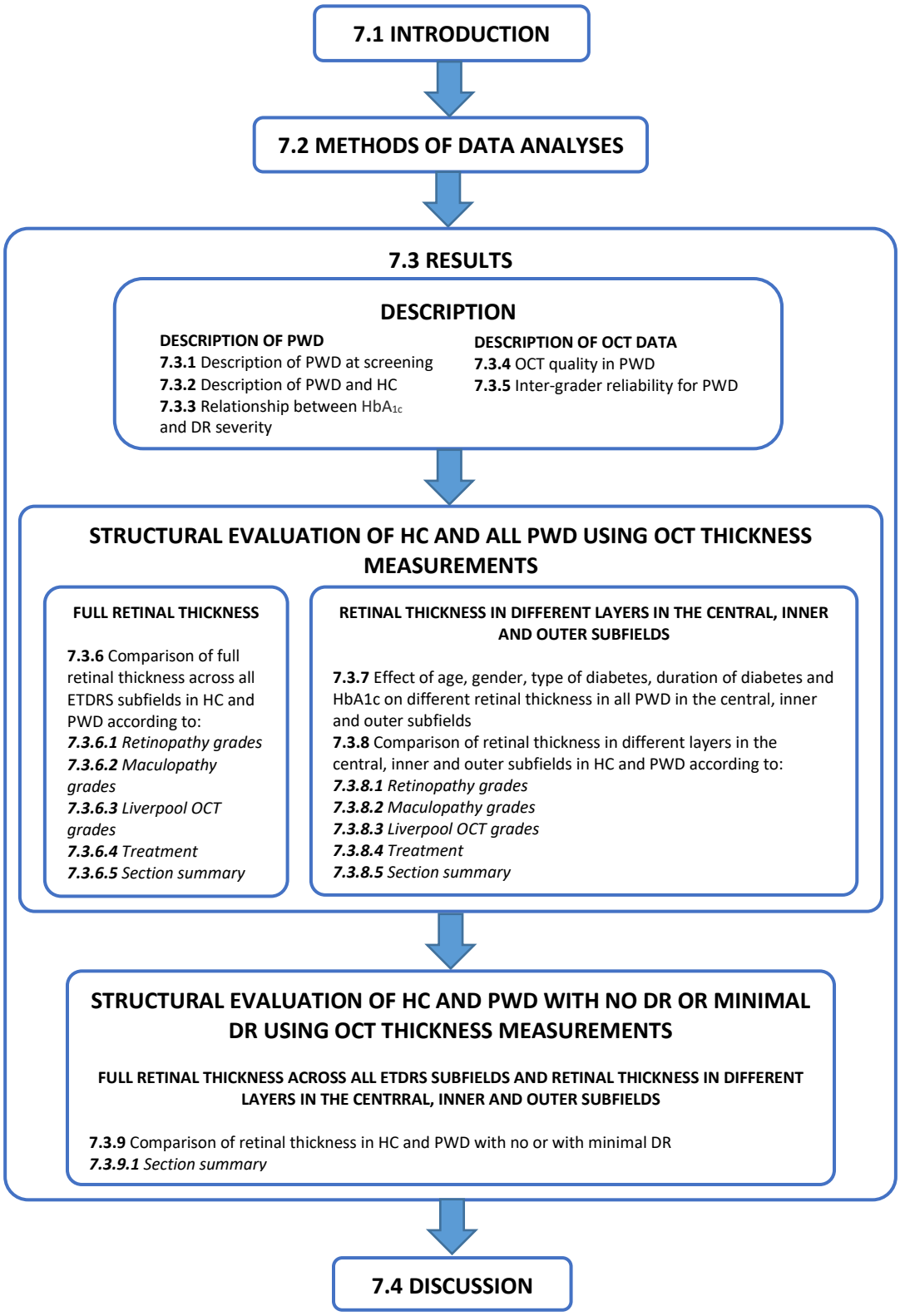


Figure 7.1 Organisation of Chapter 7.

7.2 METHODS OF DATA ANALYSES

General and OCT methods were covered in Chapters 3 and 4. Bland-Altman analysis was used to assess inter-grader reliability in Section 7.3.5. Student's *t*-tests were used to examine differences between two groups (Sections 7.3.3, 7.3.5, 7.3.6, 7.3.9). One-way ANOVA with Tukey post hoc tests was used to examine differences between groups (Sections 7.3.3, 7.3.6, 7.3.8, 7.3.9). Bonferroni corrections were made to adjust for multiple comparisons. Univariate linear regression was used to examine the effects of patient factors on retinal thickness in Section 7.3.7. Patient factors that could have affected retinal thickness such as age, gender, type of diabetes, duration of diabetes and HbA_{1c} were entered into the model as covariates. Pearson and Spearman's rank correlation coefficients were used to examine the strength of correlation between two variables (Sections 7.3.5, 7.3.7). Chi-square test was used to examine differences between categorical data and Fisher's exact test was used when there were values in these categories ≤5 (Section 7.3.9). All data analyses were performed using Excel (2016), GraphPad Prism (version 8) and SPSS (version 25).

7.3 RESULTS

7.3.1 DESCRIPTION OF PWD AT SCREENING

Participants comprised of PWD newly referred from the screening programme as being screen positive for R2+ retinopathy or M1 maculopathy in either eye. 310 PWD with M1 in one or both eyes, as determined by a grader in the LDESP and who attended a DEC in the HES, consented to participate in the study (Section 3.4). For all participants, each eye was graded by an ophthalmologist and given a retinopathy and maculopathy grade based on the LDESP grading criteria described in Chapter 2 (Section 2.5). Each eye was also graded according to the OCT definition of DMO developed in Liverpool (Section 4.9). Participant flow is shown in Figure 3.4. Eyes with concurrent macular pathology such as intermediate to severe atrophic AMD, nAMD, ERM, VMT, macular holes, post-operative cystoid macular oedema and amblyopia were excluded. Participants with cerebral pathologies resulting in visual impairment were excluded. After exclusion, data was available from 292 PWD for analysis. Where data was available from both eyes, one eye from each PWD was randomly selected for the study.

The mean±SD time from when participants had their screen event to their first visit in the DEC was 109±52 days. During this period of approximately 3 months, their DR screening grade could have changed, therefore, their DR screening grade from DEC and not their screening grade were used for analyses. 160 of the PWD attended for a follow-up visit. The mean±SD time elapsed between their first and second visits was 191±85 days. Longitudinal data from their second visit is presented in Chapter 9. Table 7.1 compares the grading at screening with that at the DEC.

This group of PWD comprised of patients who had been newly referred from LDESP to the DEC. Retinopathy and maculopathy grades are considered separately in this section. In LDESP, 23.3% (68/292 patients) was graded as having sight-threatening retinopathy (R2+). On the other hand, the majority (218/292 patients, 74.7%) had been referred from LDESP with maculopathy (M1) that could be potentially sight-threatening (Table 7.1). Of the 218 patients graded as M1 in LDESP, 142 (65.1%) were graded as M1 while 76 (34.9%) graded as M0 in DEC. In addition, of the 218 patients graded as M1 in LDESP, 78 (35.8%) were found to have NMO, 27 (12.4%) NCTMO, 35 (16.1%) CTMO and 78 (35.8%) CIMO in DEC. Notably, the proportion of eyes identified as M1 was higher at screening (74.7%) compared to at DEC (153/292, 52.4%) (Table 7.1). However, the proportion of eyes in each retinopathy grade (R0/R1/R2/R3) were quite similar between LDESP and DEC.

Table 7.1 Retinopathy and maculopathy grades of PWD at the LDESP and the DEC (N=292 eyes)

	Retinopathy grades				Maculopathy grades	
	R0	**R1**	**R2**	**R3**	**M0**	**M1**
LDESP	20 (6.8%)	204 (69.9%)	52 (17.8%)	16 (5.5%)	74 (25.3%)	218 (74.7%)
DEC	29 (9.9%)	202 (69.2%)	45 (15.4%)	16 (5.5%)	139 (47.6%)	153 (52.4%)

As described in Chapter 2, there are four criteria from the LDESP that grades an eye as M1. Table 7.2 shows the four criteria and the corresponding number of eyes graded under each criterion in the EDDMO study. The last criterion is not applicable as there were no stereoscopic photographs available. The majority of eyes graded as M1 had exudates within 1 disc diameter (DD) of the centre of the fovea. This first criterion is the simplest to identify among all of the criteria; if an eye is graded as M1 based on this criterion, the eye is screen positive and the LDESP grader does not need to consider the second or third criteria that

may also be present. Therefore, it is not a surprise that most of the eyes graded as M1 (181/292, 62%) were screen positive as a result of this first criterion.

Table 7.2 NDESP four definitions of maculopathy (M1) and the number of eyes graded under each of these categories and as M0 (no maculopathy) during screening in this study (N=292 eyes)

	NDESP definition of maculopathy	No. eyes
M0	Absence of M1 features.	74
M1	1. Exudate within 1 disc diameter (DD) of the centre of the fovea.	181
	2. Any microaneurysm or haemorrhage within 1DD of the centre of the fovea only if associated with a best VA of ≤ 6/12 (if no stereo).	19
	3. A group of exudates is an area of exudates that is greater than or equal to half the disc area and this area is all within the macular area.	18
	4. Retinal thickening within 1DD of the centre of the fovea (if stereoscopic photographs available).	Not applicable

7.3.2 DESCRIPTION OF PWD AND HC

Table 7.3 shows the descriptive analysis (number of eyes, age, gender, % of Caucasian in the group, distance VA, near VA, hRSD threshold) for both PWD and the 50 age and gender-matched HC. For the PWD, the type of diabetes, duration of diabetes, HbA_{1C} performed within the preceding 3 months of their first visit, % of PWD on insulin in the group and their BP is also shown. For the remainder of this results section, the retinopathy and maculopathy grades used were the ones given by the ophthalmologist who saw the patient during their consultation at the DEC clinic when the OCT data were also collected. Differences between PWD with different retinopathy (R0, R1, R2, R3), maculopathy (M0, M1), Liverpool OCT grades (NMO, NCTMO, CTMO, CIMO) and treatment outcomes were compared to provide additional background information to understand the demographics of these PWD, which were relevant for further analysis.

As expected, PWD tended to be of working age (mean±SD 54±14 years) but with a wide age range (20-86 years) (Table 7.3). The HC are well age-matched to the PWD (55±14 years, range 22-85 years). There were more male PWD (59.9%), not quite as well matched to the HC (52% males). The majority of the PWD were Caucasian (85%) with a long mean duration of diabetes (14.8±9.1 years) and elevated HbA_{1C} (72.9±21mmol/mol). Half of the PWD were on insulin. BP control was generally good (mean systolic 138mmHg, diastolic 82mmHg). The

majority had type 2 diabetes (70.9%) while 28.8% had type 1 and one PWD had maturity-onset diabetes of the young (MODY). The proportion of PWD with type 1 diabetes in the EDDMO study (28.8%) is higher than the UK prevalence of 8% (Diabetes UK, 2019) as the PWD with type 1 diabetes had a longer duration of diabetes (21.7±8.8 years) compared to PWD with type 2 diabetes (11.9±7.5 years) and they were likely to have more severe DR requiring referral to DEC.

For retinopathy, the majority of PWD were graded as R1 (69.2%). In generally, participants with worse retinopathy grades also had worse mean distance VA, hRSD threshold, longer duration of diabetes, worse HbA_{1C} and a higher proportion were on insulin. For the PWD graded based on maculopathy grades, there were slightly more participants graded as M1 (52.4%) compared to M0. In contrast to retinopathy, PWD graded as M0 and M1 had similar mean distance VA, near VA and hRSD threshold. M1 (76.2±21.8mmol/mol) had higher mean HbA_{1C} compared to M0 (69.2±19.5mmol/mol), which is unsurprising as an indicator of worse glycaemic control. For PWD graded based on their OCT classification, a high proportion were graded as NMO (43.8%), while 31.2% were graded as having CIMO. Interestingly, CTMO (80.8±20.7mmol/mol) and NCTMO (80.8±22.4mmol/mol) had the highest HbA_{1C} while NCTMO (16.0±7.4y) had the longest duration of diabetes. The relationship between DR severity and HbA_{1C} levels is further explored in Section 7.3.3.

Only 25 participants (8.6%) in this study required treatment after their first DEC visit. As expected, PWD who required treatment had worse distance VA (one indication for treatment), near VA, hRSD threshold, and higher HbA_{1C} levels compared to PWD who had no treatment. For the PWD who received treatment, their age, gender, distance VA, near VA, hRSD threshold, type and duration of diabetes, HbA_{1C}, NDESP grade and the treatments they received are shown in Table 7.4. Of the PWD who received treatment, 15 received PRP, 9 received macular laser and 7 commenced intravitreal aflibercept (Eylea). Of the 15 PWD who received PRP, most of them were graded as R3 (N=10) or R2 (N=4). However, a 36 years old male who was graded as R1M1 and CIMO in one eye also had high HbA_{1C} (96 mmol/mol); he was treated with PRP and macular laser. Unsurprisingly, all the PWD who received macular laser were maculopathy suspects and graded as M1. There was a 75 years old male who was graded as R1M0 and CIMO with a CST of 447µm in one eye; he received intravitreal aflibercept in that eye.

Table 7.3 Descriptive analysis of healthy controls (HC) and people with diabetes (PWD). One eye from each participant was randomly selected for analysis. Mean±SD shown for continuous variables.

	HC	All PWD	PWD retinopathy grades* (N=292)				PWD maculopathy grades* (N=292)		PWD OCT classification (N=292)				PWD treatment (N=292)	
			R0	R1	R2	R3	M0	M1	NMO	NCTMO	CTMO	CIMO	Not treated	Treated
No. eyes	50	292	29	202	45	16	139	153	128	31	42	91	267	25
Age (years) Mean±SD (range)	55±14 (22-85)	54±14 (20-86)	56±14 (23-85)	54±14 (20-86)	52±15 (22-82)	50±14 (28-84)	55±15 (20-86)	53±14 (21-86)	53±15 (20-86)	51±16 (22-86)	48±11 (20-67)	58±13 (28-85)	54±14 (20-86)	51±18 (22-84)
Gender (No.)														
M	26	175	17	122	28	8	84	91	70	16	26	63	157	18
F	24	117	12	80	17	8	55	62	58	15	16	28	110	7
Ethnicity Caucasian (%)	48 (96%)	248 (85%)	22 (76%)	174 (86%)	39 (87%)	14 (88%)	118 (85%)	130 (85%)	106 (83%)	26 (84%)	35 (83%)	81 (89%)	226 (85%)	22 (88%)
Distance VA (logMAR)	-0.08±0.12	0.06±0.19	0.03±0.16	0.06±0.17	0.08±0.25	0.11±0.28	0.07±0.19	0.06±0.19	0.03±0.14	0.03±0.21	0.03±0.15	0.14±0.24	0.05±0.17	0.17±0.33
Near VA (logMAR)	0.06±0.16	0.18±0.24	0.20±0.24	0.19±0.23	0.15±0.27	0.23±0.33	0.19±0.23	0.19±0.25	0.17±0.23	0.11±0.25	0.17±0.19	0.23±0.27	0.18±0.23	0.27±0.37
hRSD (logMAR)	-0.77±0.11	-0.61±0.24	-0.68±0.18	-0.63±0.23	-0.55±0.25	-0.47±0.29	-0.63±0.21	-0.60±0.26	-0.62±0.25	-0.67±0.17	-0.65±0.20	-0.57±0.24	-0.63±0.22	-0.44±0.33
Type Diabetes (No.)														
Type 1	NA	84	1	58	19	6	38	46	31	14	17	22	76	8
Type 2		207	28	144	26	9	101	106	97	17	25	68	191	16
Others**		1	0	0	0	1	0	1	0	0	0	1	0	1
Duration of Diabetes (years)	NA	14.8±9.1	7.3±3.9	15.1±8.7	15.7±10	20.9±10.6	14.7±8.8	14.8±9.3	14.4±8.8	16.0±7.4	14.9±9.8	14.8±9.7	14.8±9.1	14.8±8.4
HbA_{1C} (mmol/mol)	NA	72.9±21	58.4±13.9	72.5±20.1	79.0±22.5	87.1±23.7	69.2±19.5	76.2±21.8	69.9±20.6	80.8±22.4	80.8±20.7	70.7±20.0	71.2±19.6	91.4±27.5
On Insulin (%)	NA	145 (50%)	4 (14%)	101 (50%)	13 (29%)	9 (56%)	66 (47%)	79 (52%)	58 (45%)	19 (61%)	26 (62%)	38 (42%)	132 (49%)	13 (52%)
BP (mmHg)	NA													
Systolic		138±20	143±15	138±20	139±20	142±14	139±21	138±19	139±21	134±15	133±15	141±21	138±20	145±18
Diastolic		82±11	83±11	81±11	81±11	81±14	80.4±10.6	83±12	81±12	80±12	81±10	82±11	81±11	83±12

People with diabetes (PWD), no macular oedema (NMO), non-centre threatening macular oedema (NCTMO), centre threatening macular oedema (CTMO), centre involving macular oedema (CIMO), not applicable (NA). *All retinopathy and maculopathy grades have been given by ophthalmologists in the diabetic eye clinic and not from screening. **Maturity-onset diabetes of the young (MODY)

Table 7.4 Description of people with diabetes (PWD) who required treatment (N=25)

Age (y)	Gender	Distance VA (logMAR)	Near VA (logMAR)	hRSD (logMAR)	Type of diabetes	Duration of diabetes (y)	HbA_{1c} (mmol/mol)	LDESP grade**	Liverpool OCT grade	CST (µm)	Treatment received***
54	F	-0.04	0.14	-0.47	Type 1	24	83	R3AM1P1	CTMO	312	PRP
51	F	0.00	0.10	-0.60	Type 2	15	82	R3AM1	CIMO	290	PRP, macular laser
28	F	0.18	0.40	-0.50	Type 1	18	140	R3AM1	CIMO	351	PRP
31	F	0.10	0.02	-0.58	Others*	14	118	R3AM1P1	CIMO	331	PRP
52	M	-0.04	-0.06	-0.68	Type 2	12	101	R3AM1	CIMO	270	PRP, macular laser
84	M	1.08	1.32	0.38	Type 2	33	89	R3SM1P1	CIMO	446	Aflibercept
52	M	0.06	0.20	-0.89	Type 2	15	89	R3SM1	CTMO	303	PRP
43	M	0.30	0.32	-0.25	Type 1	23	67	R3AM0P1	CIMO	279	PRP
64	M	-0.08	0.16	-0.35	Type 2	20	116	R3AM0P1	NMO	251	PRP
40	M	-0.08	-0.08	-0.56	Type 1	27	73	R3AM0	NMO	256	PRP
32	M	0.04	0.12	-0.66	Type 1	24	76	R2M1	CIMO	341	Macular laser
74	F	1.00	0.82	0.19	Type 2	7	54	R2M1	CIMO	743	PRP, aflibercept, YAG capsulotomy[†]
74	M	0.32	0.64	-0.06	Type 2	2	42	R2M1	CIMO	640	Aflibercept
53	M	0.16	0.12	-0.72	Type 2	6	134	R1M1	CTMO	310	Macular laser
55	M	0.00	0.06	-0.76	Type 2	10	105	R3AM1	CIMO	286	PRP, macular laser
22	M	0.04	-0.02	-0.69	Type 1	7	131	R2M1	NCTMO	281	PRP, aflibercept
28	M	-0.06	0.00	-0.66	Type 1	24	117	R2M1	CTMO	281	Macular laser
60	M	0.02	0.16	-0.66	Type 2	15	119	R2M1	CIMO	380	Macular laser
82	F	0.84	1.18	0.20	Type 2	20	Not available	R2M1P1	CIMO	591	Aflibercept
32	M	-0.16	-0.04	-0.39	Type 2	10	56	R2M1	CTMO	312	PRP
48	F	0.16	0.24	-0.22	Type 1	18	79	R2M1	CIMO	425	Aflibercept
47	M	-0.04	0.22	-0.67	Type 2	1	Not available	R2M0	CTMO	279	PRP
36	M	0.06	0.36	-0.20	Type 2	1	96	R1M1	CIMO	289	PRP, Macular laser
50	M	0.02	-0.02	-0.77	Type 2	9	71	R1M1	CIMO	260	Macular laser
75	M	0.42	0.40	-0.45	Type 2	14	63	R1M0	CIMO	447	Aflibercept

*Maturity-onset diabetes of the young (MODY), **Liverpool Diabetic Eye Screening Programme, ***Peripheral retinal photocoagulation (PRP), [†]yttrium aluminium garnet

7.3.3 RELATIONSHIP BETWEEN HBA_{1C} AND DR SEVERITY

Mean HbA_{1c} results from Table 7.3 showed that HbA_{1c} levels were higher in PWD with more severe retinopathy grades, which are to be expected (Figure 7.2A). One-way ANOVA showed that there was a significant difference in HbA_{1c} between these groups ($F_{3,277}$=8.666, p<0.001). Tukey post hoc test revealed that HbA_{1c} was significantly higher in R3 (mean difference 28.70mmol/mol, p<0.001), R2 (mean difference 0.52mmol/mol, p<0.001) and R1 (mean difference 14.09 mmol/mol, p=0.004) compared to R0. In addition, R3 had significantly higher HbA_{1c} compared to R1 (mean difference 14.61 mmol/mol, p=0.03).

Again, as expected, PWD with maculopathy (M1; HbA_{1c} 76.2±21.8 mmol/mol) had significantly higher mean HbA_{1c} compared to PWD without maculopathy (M0; HbA_{1c} 69.2±19.5 mmol/mol, difference 7 mmol/mol, t=2.818, p=0.005; Figure 7.2B). There was a more complex relationship between HbA_{1c} and PWD with the various Liverpool OCT grades; patients with intermediate levels of disease severity (NCTMO and CTMO) had the highest mean HbA_{1c}. One-way ANOVA showed that the differences in HbA_{1c} between these groups were statistically significant ($F_{3,277}$=4.673, p=0.003). Tukey post hoc test demonstrated that CTMO (mean difference 10.86µm, p=0.021) and NCTMO (mean difference 10.94 µm, p=0.043) had significantly higher HbA_{1c} compared to NMO. There were no statistically significant differences between HbA_{1c} levels in CIMO and other groups. Unsurprisingly, patients requiring treatment (TT; 91.4±27.5 mmol/mol) had significantly higher HbA_{1c} levels compared to those who did not require treatment (NT; 71.2±19.6 mmol/mol, difference 20.2 mmol/mol, t=4.545, p<0.001; Figure 7.3D).

The relationship between HbA_{1c} and CST in PWD was also examined. The variability of HbA_{1c} levels compared to CST made it difficult to draw any firm conclusions. In addition, there were relatively few CST measurements over 400µm available for analysis. With the available data, no statistically significant correlation between HbA_{1c} and CST in PWD was detected (Spearman *rho*=-0.029, p=0.62) (Figure 7.3).

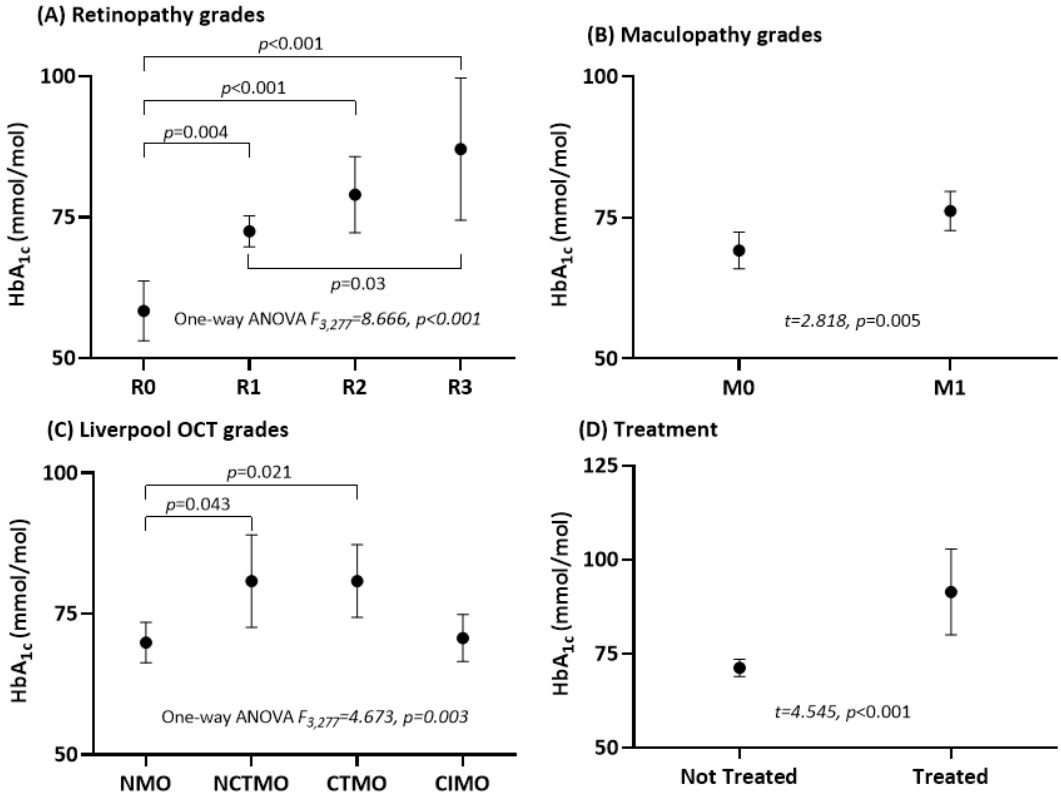


Figure 7.2. Mean (±95%CI) of HbA_{1c} of PWD with different retinopathy grades (A), maculopathy grades (B), Liverpool OCT grades (C) and treatment (D). One-way ANOVA and *t*-test results with *p* values shown. Only statistically significant pairwise comparisons are shown.

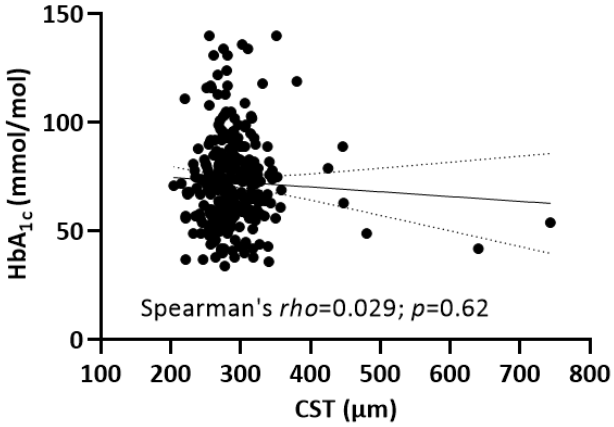


Figure 7.3 Relationship between HbA_{1c} and CST in PWD. The spearman rank correlation coefficient and its statistical significance are shown. Least squares linear regression line with 95% CI is also shown.

7.3.4 OCT QUALITY IN PWD

This section examines the quality of the OCT images obtained from the PWD. The grading protocol to assess the quality of OCT images was described in Chapter 4 as was the quality of the OCT images obtained from the HC. Scans from 292 eyes from the PWD were available for grading, of which 85 (29%) were graded as good quality while 207 (71%) were graded as fair quality. The foveal depression was detectable in the majority of the scans (N=278, 95%). There was no evidence of VMT in the majority of the scans (N=287, 98.3%); two had questionable evidence of VMT and three had definite evidence of VMT. There was no evidence of an ERM in the majority of the scans (N=290, 99%); one scan had questionable evidence of an ERM and one scan had definite evidence of ERM. None of the scans had evidence of macular holes. Automated retinal segmentation was possible in all layers in 68 (23.3%) scans while some manual segmentation was required in 218 (74.6%) scans. It was not possible to perform segmentation in 6 (2.1%) scans and only total retinal thickness is used for analyses in these scans.

7.3.5 INTER-GRADER RELIABILITY FOR PWD

To establish inter-grader reliability for OCT measurements, CST from 60 of the 292 PWD (20.5%) were measured independently by an experienced second-grader from the Liverpool Ophthalmic Reading Centre at the Royal Liverpool University Hospital. The 60 PWD were selected to match the proportions of the total group with regard to retinopathy, maculopathy, OCT grades and treatment outcome (Table 7.5). This was done because the PWD who have more severe pathology usually have increased distortion of their foveal anatomy that makes it more difficult to identify the foveal centre. It was normal practice for the first-grader to mark the ETDRS grid on the scan where the foveal depression was the deepest. The second-grader can then see the mark leading to a potential measurement bias (Smith and Noble, 2014).

There was no significant difference between CST obtained by the first (J Ku) and second grader (D Parry) (paired t=0.33, p=0.74). Bland-Altman analysis showed low bias (Figure 7.4) (Bland and Altman, 1986).

Table 7.5 Comparison of the proportion of people with diabetes (PWD) with the sample selected for inter-grader reliability in each retinopathy, maculopathy, OCT grade and treatment

	PWD retinopathy grades (N=292)				PWD maculopathy grades (N=292)		PWD OCT classification (N=292)				PWD treatment (N=292)	
	R0	R1	R2	R3	M0	M1	NMO	NCTMO	CTMO	CIMO	Not treated	Treated
EDDMO Study (N=292)	29 (9.9%)	202 (69.2%)	45 (15.4%)	16 (5.5%)	139 (47.6%)	153 (53.4%)	128 (43.8%)	31 (10.6%)	42 (14.4%)	91 (31.2%)	267 (91.4%)	25 (8.6%)
Sample for inter-grader reliability (N=60)	6 (10%)	42 (70%)	9 (15%)	3 (5%)	29 (48.3%)	31 (51.7%)	26 (43.3%)	6 (10%)	9 (15%)	19 (31.7%)	55 (91.7%)	5 (8.3%)

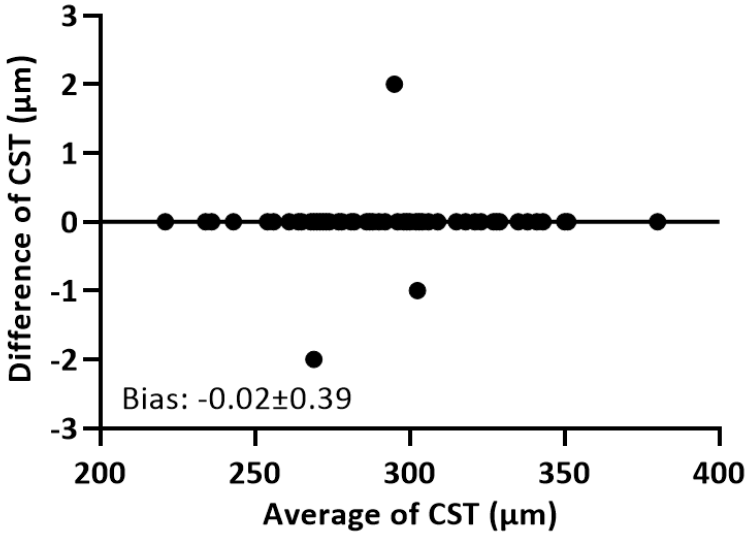


Figure 7.4. Bland-Altman plot showing bias of central subfield thickness (CST) between grader 1 and 2 (N=60). The limits of agreement are not provided here due to all data agreeing except in three cases. Therefore, all differences are zero except in the three cases and the data are not normally distributed.

7.3.6 COMPARISON OF FULL RETINAL THICKNESS ACROSS ALL ETDRS SUBFIELDS IN HC AND PWD

Full retinal thickness measurements across ETDRS subfields are often used in the assessment and monitoring of DR and DMO. Full retinal thickness is measured from the ILM to the posterior border of the RPE and Bruch's complex as described in Section 4.4. Therefore, comparisons of the full retinal thickness across all ETDRS subfields were made between HC and PWD, and the results are described in this section. A comparison of the full

retinal thickness (mean±SD) in HC and all PWD using *t*-statistics across all the ETDRS subfields found no significant differences between the two groups (Table 7.6). The full retinal thickness of the PWD is now be further examined according to their retinopathy grades (R0/R1/R2/R3), maculopathy grades (M0/M1), Liverpool OCT grades (NMO/NCTMO/CTMO/CIMO) and treatment outcomes, and comparisons are made with HC.

Table 7.6 Full retinal thickness (mean±SD, µm) in all ETDRS subfields in healthy controls (HC) and people with diabetes (PWD)

ETDRS Subfields	PWD (mean±SD, µm)	HC (mean±SD, µm)	*t*-test	*p*
CSF	291.3±51.5	282.7±23.9	1.16	0.25
SIM	345.8±35.9	345.6±14.6	0.04	0.97
NIM	349.1±42.4	347.9±15.8	0.19	0.85
IIM	341.3±35.3	342.4±13.3	0.23	0.82
TIM	333.8±28.8	331.9±12.3	0.45	0.65
SOM	303.2±35.5	300.3±13.7	0.57	0.57
NOM	315.5±30.4	317.6±13.2	0.48	0.63
IOM	289.2±26.5	288.6±11.8	0.15	0.88
TOM	285.7±26.1	282.1±12.1	0.95	0.34

7.3.6.1 Comparison of HC and PWD with different retinopathy grades

A comparison of the mean full retinal thickness across all ETDRS subfields in HC and PWD with different retinopathy grades is shown in Figure 7.5. The absolute difference (AD) is the difference between the mean retinal thicknesses of the two groups. The relative difference (RD) is the absolute difference (AD) in retinal thickness between two groups divided by the retinal thickness of HC or the group with the less severe DR grade expressed as a percentage. There are a few observations that can be made from Figure 7.5. Firstly, R0 had lower thickness compared to HC across all subfields. This varied in different subfields from -3.4µm (RD -1.2%) in the CSF to -10.7µm (RD -3.4%) in the NOM (Figure 7.5, Table 7.7).

R1 also had a lower retinal thickness compared to HC in all subfields except in the CSF and this varied from -0.2µm (RD -0.1%) in the TOM to -6.4µm (RD -2.0%) in the NOM (Figure

7.5B, Table 7.7). R2 had the highest retinal thickness across all subfields except in the IOM and TOM where R3 have the highest retinal thickness (Figure 7.5A). When PWD was further separated into R3S (N=6) and R3A (N=10), a more complex trend between the groups was revealed. While it might be expected that R3A would have the highest retinal thickness across all subfields, their CST of 288.8µm was similar to that of HC (282.7µm). R2 had the highest retinal thickness across most of the inner subfields except the TIM where the retinal thickness of R3A was slightly higher (TIM mean thickness R2 347.9 µm; R3A 348.7µm). R3A had the highest retinal thickness in all the outer subfields compared to HC and PWD with less severe retinopathy grades. CST was higher in R3S compared to R3A (CST: R3S 305.7µm, R3A 288.8µm) but still less than that of R2 (314.9µm). R3S also had a lower retinal thickness in the SIM compared to HC, which was not seen in the other subfields (SIM: R3S 340.5µm, HC 345.6µm) (Table 7.7).

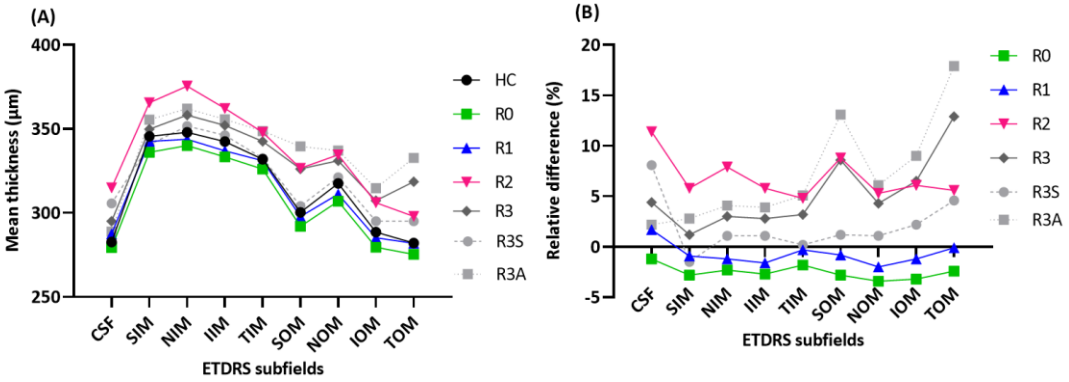


Figure 7.5 (A) Mean full retinal thickness (µm) across all ETDRS subfields of healthy controls (HC) and people with diabetes (PWD) with different retinopathy grades (R0/R1/R2/R3/R3A/R3S) (B) Relative difference (RD;%) of PWD with different retinopathy grades compared to HC in full retinal thickness across all ETDRS subfields. RD is the difference in retinal thickness between the different retinopathy groups and HC divided by the retinal thickness of the HC expressed as a percentage. Error bars have been omitted for clarity.

The differences discussed above were explored with One-way ANOVA. Bonferroni corrections were made to adjust for multiple comparisons. In Table 7.8, the retinal thickness in each subfield was compared in different retinopathy groups. Only statistically significant results are shown. The first observation is that there were more significant pairwise comparisons in the outer subfields compared to the CSF or inner subfields. In the CSF and inner subfields, there were three significant pairwise comparisons in each subfield. As previously described in Figure 7.5, R2 had the highest retinal thickness in the CSF and

inner subfields while R1, R0 and HC had the lowest. Significant pairwise comparisons in the CSF and inner subfields were between R2 and R1, R2 and R0 and R2 and HC as expected.

The retinal thickness of R2 and R3 were similar in the SOM (R2 326.6±73.2µm; R3 326.2±40.0µm), NOM (R2 334.5±57.3µm, R3 331.1±26.5µm) and IOM (R2 306.1±45.2µm, R3 307.3± 26.4µm). Therefore in SOM, NOM and IOM, significant pairwise comparisons were between R2 and R3 and less severe retinopathy grades and HC. The highest number of significant pairwise comparisons was in the TOM because R3 had a higher retinal thickness compared to all other groups (R3 318.6±53.3µm, R2 297.9±35.7µm, R1 281.9±17.9µm, R0 275.3±13.1µm, HC 282.1±12.1µm). In the TOM, there were also significant pairwise comparisons between R2 and R1, R2 and R0 and R2 and HC.

Table 7.7. Full retinal thickness in all ETDRS subfields in HC and in PWD with different retinopathy grades expressed as absolute difference (AD; calculated as the difference between the mean retinal thickness of different retinopathy groups and HC) and relative difference (RD; calculated as AD divided by the retinal thickness of HC expressed as a percentage)

	CSF			SIM			NIM			IIM			TIM			SOM			NOM			IOM			TOM		
Group	Mean± SD (μm)	AD (μm)	RD (%)	Mean ±SD (μm)	AD (μm)	RD (%)	Mean± SD (μm)	AD (μm)	RD (%)	Mean± SD (μm)	AD (μm)	RD (%)	Mean± SD (μm)	AD (μm)	RD (%)	Mean± SD (μm)	AD (μm)	RD (%)	Mean± SD (μm)	AD (μm)	RD (%)	Mean± SD (μm)	AD (μm)	RD (%)	Mean± SD (μm)	AD (μm)	RD (%)
HC (N=50)	282.7± 23.9	0	0	345.6±14.6	0	0	347.9± 15.8	0	0	342.4± 13.3	0	0	331.9± 12.3	0	0	300.3± 13.7	0	0	317.6± 13.2	0	0	288.6± 11.8	0	0	282.1± 12.1	0	0
R0 (N=29)	279.3± 23.9	-3.4	-1.2	336.0± 17.1	-9.6	-2.8	340.0± 20.5	-7.9	-2.3	333.3± 20.0	-9.1	-2.7	326.0± 18.8	-5.9	-1.8	291.9± 13.0	-8.4	-2.8	306.9± 16.8	-10.7	-3.4	279.5± 15.6	-9.1	-3.2	275.3± 13.1	-6.8	-2.4
R1 (N=202)	287.4± 33.6	4.7	1.7	342.5± 22.2	-3.1	-0.9	343.8± 22.5	-4.1	-1.2	336.9± 21.2	-5.5	-1.6	331.0± 21.3	-0.9	-0.3	297.8± 18.0	-2.5	-0.8	311.2± 20.0	-6.4	-2.0	285.2± 18.8	-3.4	-1.2	281.9± 17.9	-0.2	-0.1
R2 (N=45)	314.9± 101.6	32.2	11.4	365.6± 72.3	20.0	5.8	375.4± 89.9	27.5	7.9	362.2± 70.7	19.8	5.8	347.9± 48.7	16.0	4.8	326.6± 73.2	26.3	8.8	334.5± 57.3	16.9	5.3	306.1± 45.2	17.5	6.1	297.9± 35.7	15.8	5.6
R3 (N=16)	295.1± 53.6	12.4	4.4	349.8± 32.4	4.2	1.2	358.2± 30.6	10.3	3.0	352.1± 31.1	9.7	2.8	342.6± 38.7	10.7	3.2	326.2± 40.0	25.9	8.6	331.1± 26.5	13.5	4.3	307.3± 26.4	18.7	6.5	318.6± 53.3	36.5	12.9
R3S (N=6)	305.7± 80.2	23.0	8.1	340.5± 33.4	-5.1	-1.5	351.7± 34.9	3.8	1.1	346.2± 35.5	3.8	1.1	332.5± 41.1	0.6	0.2	304.0± 18.5	3.7	1.2	321.2± 14.5	3.6	1.1	295.0± 21.7	6.4	2.2	295.0± 36.3	12.9	4.6
R3A (N=10)	288.8± 33.1	6.1	2.2	355.4± 32.2	9.8	2.8	362.1± 28.9	14.2	4.1	355.7± 29.5	13.3	3.9	348.7± 38.0	16.8	5.1	339.5± 44.1	39.2	13.1	337.0± 30.8	19.4	6.1	314.7± 27.2	26.1	9.0	332.7± 58.4	50.6	17.9

Negative AD and RD indicate lower retinal thickness compared to HC while positive AD and RD indicate higher retinal thickness compared to HC.

Table 7.8. Summary of statistically significant ANOVA pairwise comparisons of full retinal thickness in HC and PWD with different retinopathy grades with absolute difference (AD; calculated as the difference between the mean retinal thickness of two groups), relative difference (RD; calculated as AD divided by the retinal thickness of the less severe group expressed as a percentage) and *p* value shown. Only statistically significant ANOVA pairwise comparisons are shown. Non-statistically significant ANOVA pairwise comparisons have been omitted for clarity. All *p* values were adjusted using Bonferroni corrections. The group with a more severe retinopathy grade is shown on the left column and the group with a less severe retinopathy grade is shown on the right column.

Subfield	Group/mean retinal thickness (μm)		Absolute difference between groups (μm)	Relative difference between groups (%)	*P*
CSF	R2/314.9	R1/287.4	27.5	9.6	0.005
	R2/314.9	R0/279.3	35.6	12.7	0.019
	R2/314.9	HC/282.7	32.2	11.4	0.011
SIM	R2/365.6	R1/342.5	23.1	6.7	<0.001
	R2/365.6	R0/336.0	29.6	8.8	0.002
	R2/365.6	HC/345.6	20.0	5.8	0.032
NIM	R2/375.4	R1/343.8	31.6	9.2	<0.001
	R2/375.4	R0/340.0	35.4	10.4	0.001
	R2/375.4	HC/347.9	27.5	7.9	0.005
IIM	R2/362.2	R1/336.9	25.3	7.5	<0.001
	R2/362.2	R0/333.3	28.9	8.7	0.002
	R2/362.2	HC/342.4	19.8	5.8	0.029
TIM	R2/347.9	R1/331.0	16.9	5.1	0.001
	R2/347.9	R0/326.0	21.9	6.7	0.006
	R2/347.9	HC/331.9	16.0	4.8	0.034
SOM	R3/326.2	R1/297.8	28.4	9.5	0.006
	R3/326.2	R0/291.9	34.3	11.8	0.006
	R3/326.2	HC/300.3	25.9	8.6	0.042
	R2/326.6	R1/297.8	28.8	9.7	<0.001
	R2/326.6	R0/291.9	34.7	11.9	<0.001
	R2/326.6	HC/300.3	26.3	8.8	0.001
NOM	R3/331.1	R0/306.9	24.2	7.9	0.048
	R2/334.5	R1/311.2	23.3	7.5	<0.001
	R2/334.5	R0/306.9	27.6	9.0	<0.001
	R2/334.5	HC/317.6	16.9	5.3	0.027
IOM	R3/307.3	R1/285.2	22.1	7.7	0.003
	R3/307.3	R0/279.5	27.8	9.9	0.002
	R2/306.1	R1/285.2	20.9	7.3	<0.001
	R2/306.1	R0/279.5	26.6	9.5	<0.001
	R2/306.1	HC/288.6	17.5	6.1	0.004
TOM	R3/318.6	R2/297.9	20.7	6.9	0.020
	R3/318.6	R1/281.9	36.7	13.0	<0.001
	R3/318.6	R0/275.3	43.3	15.7	<0.001
	R3/318.6	HC/282.1	36.5	12.9	<0.001
	R2/297.9	R1/281.9	16.0	5.7	<0.001
	R2/297.9	R0/275.3	22.6	8.2	<0.001
	R2/297.9	HC/282.1	15.8	5.6	0.009

7.3.6.2 Comparison of HC and PWD with different maculopathy grades

A comparison of the mean full retinal thickness of HC and PWD with different maculopathy grades is shown in Figure 7.6. It can be seen from Figure 7.6 that the CST of HC (282.7±23.9µm) was similar to that of M0 (282.6±30.9µm). On the other hand, M1 had a slightly higher CST (299.2±63.9µm) compared to both HC (282.7±23.9µm) and M0 (282.6±30.9 µm). From Table 7.9, it can be seen that M1 had a higher CST by 5.8% RD compared to HC. Figure 7.6A shows that all three groups (HC, M0 and M1) had higher retinal thicknesses in the inner subfields compared to the outer subfields, which is to be expected (Figure 7.6A). Figure 7.6B shows that M0 had a lower mean thickness across the inner and outer subfields compared to HC. This varied from -2.5µm (RD -0.9%) in the TOM to -7.6µm (RD -2.2%) in the IIM (Table 7.9). M1 had a higher retinal thickness across all subfields compared to HC as expected. This difference varied from 2.7µm in the NOM to 16.5µm in the CSF.

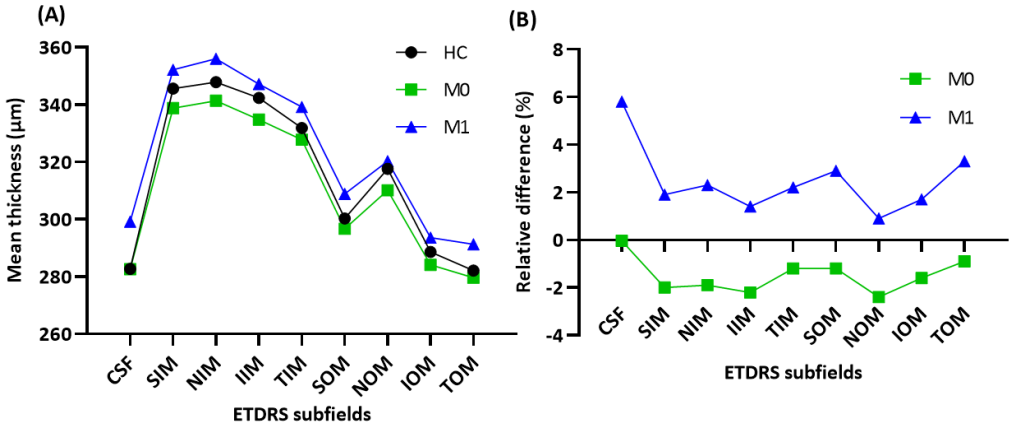


Figure 7.6 (A) Mean full retinal thickness (µm) across all ETDRS subfields of healthy controls (HC) and people with diabetes (PWD) with different maculopathy grades (M0/M1) (B) Relative difference (RD;%) of PWD with different maculopathy grades compared to HC in full retinal thickness across all ETDRS subfields. Error bars have been omitted for clarity.

One-way ANOVA was used to examine if the retinal thickness in any subfields were significantly different among the three groups (HC, M0 and M1). The results showed that there was a statistically significant difference in retinal thickness between M0 and M1 across all subfields (Table 7.10). There was no significant pairwise comparison between M1 and M0 compared to HC. It can be seen that when groups are defined by retinopathy or maculopathy grades, there was a small but consistent reduction in retinal thickness in PWD with no evidence of DR (R0 or M0) compared to the age-matched HC. When DR progressed or when there was a detectable maculopathy, retinal thickening was observed.

Table 7.9. Full retinal thickness in all ETDRS subfields in HC compared to PWD with different maculopathy grades expressed as absolute difference (AD; calculated as the difference between the mean retinal thickness of different retinopathy groups and HC) and relative difference (RD; calculated as AD divided by the retinal thickness of HC expressed as a percentage)

	CSF			SIM			NIM			IIM			TIM			SOM			NOM			IOM			TOM		
Group	Mean± SD (μm)	AD (μm)	RD (%)	Mean ±SD (μm)	AD (μm)	RD (%)	Mean± SD (μm)	AD (μm)	RD (%)	Mean± SD (μm)	AD (μm)	RD (%)	Mean± SD (μm)	AD (μm)	RD (%)	Mean± SD (μm)	AD (μm)	RD (%)	Mean± SD (μm)	AD (μm)	RD (%)	Mean± SD (μm)	AD (μm)	RD (%)	Mean± SD (μm)	AD (μm)	RD (%)
HC (N=50)	282.7± 23.9	0	0	345.6± 14.6	0	0	347.9± 15.8	0	0	342.4± 13.3	0	0	331.9± 12.3	0	0	300.3± 13.7	0	0	317.6± 13.2	0	0	288.6± 11.8	0	0	282.1± 12.1	0	0
M0 (N=139)	282.6± 30.9	-0.1	-0.04	338.8± 21.3	-6.8	-2.0	341.4± 22.3	-6.5	-1.9	334.8± 20.6	-7.6	-2.2	327.8± 20.7	-4.1	-1.2	296.7± 17.4	-3.6	-1.2	310.1± 19.6	-7.5	-2.4	284.1± 17.6	-4.5	-1.6	279.6± 15.6	-2.5	-0.9
M1 (N=153)	299.2± 63.9	16.5	5.8	352.2± 44.4	6.6	1.9	356.0± 53.8	8.1	2.3	347.2± 43.9	4.8	1.4	339.2± 33.7	7.3	2.2	308.9± 45.2	8.6	2.9	320.3± 37.0	2.7	0.9	293.6± 31.7	5.0	1.7	291.3± 31.9	9.2	3.3

Negative AD and RD indicate lower retinal thickness compared to HC while positive AD and RD indicate higher retinal thickness compared to HC.

Table 7.10. Summary of statistically significant ANOVA pairwise comparisons of full retinal thickness in HC and PWD with different maculopathy grades with absolute difference (AD; calculated as difference between the mean retinal thickness of two groups), relative difference (RD; calculated as AD divided by the retinal thickness of the less severe group expressed as a percentage) and *p* value shown. Only statistically significant ANOVA pairwise comparisons are shown. Non-statistically significant ANOVA pairwise comparisons have been omitted for clarity. All *p* values were adjusted using Bonferroni corrections. The group with more severe maculopathy grade is shown on the left column and the group with less severe maculopathy grade is shown on the right column.

Subfield	Group/mean retinal thickness (µm)		Absolute difference between groups (µm)	Relative difference between groups (%)	*P*
CSF	M1/299.2	M0/282.6	16.6	5.5	0.010
SIM	M1/352.2	M0/338.8	13.4	3.8	0.002
NIM	M1/356.0	M0/341.4	14.6	4.1	0.005
IIM	M1/347.2	M0/334.8	12.4	3.6	0.004
TIM	M1/339.2	M0/327.8	11.4	3.4	0.001
SOM	M1/308.9	M0/296.7	12.2	3.9	0.007
NOM	M1/320.3	M0/310.1	10.2	3.2	0.007
IOM	M1/293.6	M0/284.1	9.5	3.2	0.004
TOM	M1/291.3	M0/279.6	11.7	4.0	<0.001

7.3.6.3 Comparison of HC and PWD with different Liverpool OCT grades

Full retinal thickness across all ETDRS subfields of HC and PWD was analysed according to their Liverpool OCT grades. This OCT classification based on the presence and proximity of retinal thickening and intraretinal cyst to the fovea may be helpful to guide management decisions (Section 4.9).

As Figure 7.7 shows, NMO had a lower retinal thickness across all subfields compared to HC and other OCT grades consistent with the result reported and provided evidence of retinal thinning in PWD with no DR. This difference varied from -7.4µm (RD -2.6%) in the TOM to 15.6µm (RD -4.6%) in the IIM (Table 7.11). Pairwise comparisons showed that NMO had significantly lower thickness in the SIM (p=0.043), IIM (p=0.025) and TIM (p=0.027) compared to HC (Figure 7.7, Table 7.12).

The CST of HC and NCTMO were very similar (CST HC 282.7±23.9µm; NCTMO 282.9±16.9µm). NCTMO had higher retinal thickness across all inner and outer subfields compared to HC. This difference varied from 1.1µm (RD 0.3%) in the IIM to 8.7µm (RD 2.9%) in SOM (Table 7.11). CIMO had the highest CST with a wide SD among all participants

(325.5±75.8µm) as expected. It was also unsurprising that CIMO had the highest retinal thickness in the inner subfields and the SOM and NOM. However, it was interesting that CTMO had higher retinal thickness in the IOM and TOM among all groups surpassing that of CIMO (Figure 7.7, Table 7.11).

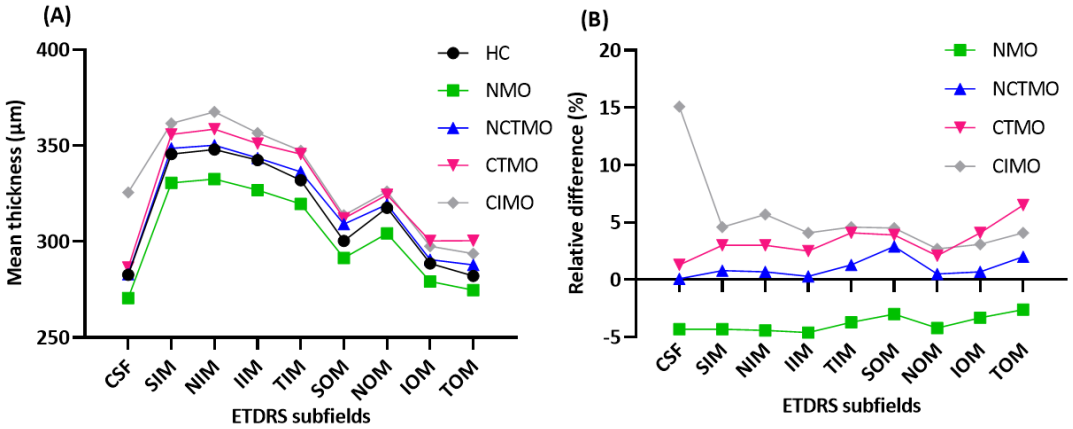


Figure 7.7 (A) Mean full retinal thickness (µm) across all ETDRS subfields of healthy controls (HC) and people with diabetes (PWD) with different Liverpool OCT grades (NMO/NCTMO/CTMO/CIMO) (B) Relative difference (RD;%) of PWD with different Liverpool OCT grades compared to HC in full retinal thickness across all ETDRS subfields. RD is the difference in retinal thickness between the different Liverpool OCT groups and HC divided by the retinal thickness of the HC expressed as a percentage. Error bars have been omitted for clarity.

ANOVA pairwise comparisons (Table 7.12) showed significant differences in the retinal thickness in the CSF in CIMO compared to all other groups, which are to be expected (CTMO $p<0.001$, NCTMO $p<0.001$, NMO $p<0.001$, HC $p<0.001$). In the inner subfields, CIMO had the highest retinal thickness while NOM had the lowest. Therefore, it was not surprising that there were significant differences in retinal thickness between CIMO and NMO in the inner subfields (all $p<0.001$). In the outer subfields, there were two significant sets of pairwise comparisons across all subfields (CIMO vs NOM and CTMO vs NOM). Subfields in the SIM, TIM and TOM shared four sets of significant pairwise comparisons (CIMO vs NMO, CIMO vs HC, CTMO vs NOM and NCTMO vs NMO).

Table 7.11. Full retinal thickness in all ETDRS subfields in HC compared to PWD with different Liverpool grades expressed as absolute difference (AD; calculated as difference between the mean retinal thickness of different retinopathy groups and HC) and relative difference (RD; calculated as AD divided by the retinal thickness of HC expressed as a percentage)

	CSF			SIM			NIM			IIM			TIM			SOM			NOM			IOM			TOM		
Group	Mean± SD (μm)	AD (μm)	RD (%)	Mean ±SD (μm)	AD (μm)	RD (%)	Mean± SD (μm)	AD (μm)	RD (%)	Mean± SD (μm)	AD (μm)	RD (%)	Mean± SD (μm)	AD (μm)	RD (%)	Mean± SD (μm)	AD (μm)	RD (%)	Mean± SD (μm)	AD (μm)	RD (%)	Mean± SD (μm)	AD (μm)	RD (%)	Mean± SD (μm)	AD (μm)	RD (%)
HC (N=50)	282.7± 23.9	0	0	345.6± 14.6	0	0	347.9± 15.8	0	0	342.4± 13.3	0	0	331.9± 12.3	0	0	300.3± 13.7	0	0	317.6± 13.2	0	0	288.6± 11.8	0	0	282.1± 12.1	0	0
NMO (N=128)	270.6± 22.9	-12.1	-4.3	330.6± 16.8	-15.0	-4.3	332.6± 18.4	-15.3	-4.4	326.8± 16.5	-15.6	-4.6	319.6± 16.2	-12.3	-3.7	291.4± 14.1	-8.9	-3.0	304.2± 17.1	-13.4	-4.2	279.2± 14.1	-9.4	-3.3	274.7± 11.9	-7.4	-2.6
NCTMO (N=31)	282.9± 16.9	0.2	0.1	348.5± 14.9	2.9	0.8	350.2± 13.9	2.3	0.7	343.5± 15.5	1.1	0.3	336.3± 13.0	4.4	1.3	309.0± 20.2	8.7	2.9	319.3± 17.7	1.7	0.5	290.5± 18.6	1.9	0.7	287.8± 14.9	5.7	2.0
CTMO (N=42)	286.4± 19.3	3.7	1.3	355.8± 21.6	10.2	3.0	358.5± 21.8	10.6	3.0	351.0± 22.6	8.6	2.5	345.6± 20.4	13.7	4.1	312.0± 19.8	11.7	3.9	324.3± 21.5	6.7	2.1	300.3± 23.8	11.7	4.1	300.4± 23.7	18.3	6.5
CIMO (N=91)	325.5± 75.8	42.8	15.1	361.6± 53.5	16.0	4.6	367.6± 65.5	19.7	5.7	356.5± 52.5	14.1	4.1	347.3± 39.1	15.4	4.6	313.7± 55.2	13.4	4.5	326.1± 43.9	8.5	2.7	297.5± 36.5	8.9	3.1	293.7± 36.5	11.6	4.1

Negative AD and RD indicate lower retinal thickness compared to HC while positive AD and RD indicate higher retinal thickness compared to HC.

Table 7.12. Summary of statistically significant ANOVA pairwise comparisons of full retinal thickness in HC and PWD with different Liverpool OCT grades with absolute difference (AD; calculated as difference between the mean retinal thickness of two groups), relative difference (RD; calculated as AD divided by the retinal thickness of the less severe group expressed as a percentage) and *p* value shown. Only statistically significant ANOVA pairwise comparisons are shown. Non-statistically significant ANOVA pairwise comparisons have been omitted for clarity. All *p* values were adjusted using Bonferroni corrections. The group with a more severe OCT grade is shown on the left column and the group with a less severe OCT grade shown on the right column.

Subfield	Group/mean retinal thickness (μm)		Absolute difference between groups (μm)	Relative difference between groups (%)	*P*
CSF	CIMO/325.5	CTMO/286.4	39.1	13.7	<0.001
	CIMO/325.5	NCTMO/282.9	42.6	15.1	<0.001
	CIMO/325.5	NMO/270.6	54.9	20.3	<0.001
	CIMO/325.5	HC/282.7	42.8	15.1	<0.001
SIM	CIMO/361.6	NMO/330.6	31.0	9.4	<0.001
	CIMO/361.6	HC/345.6	16.0	4.6	0.039
	CTMO/355.8	NMO/330.6	25.2	7.6	<0.001
	NCTMO/348.5	NMO/330.6	17.9	5.4	0.045
	NMO/330.6	HC/345.6	15.0	4.3	0.043
NIM	CIMO/367.6	NMO/332.6	35.0	10.5	<0.001
	CIMO/367.6	HC/347.9	19.7	5.7	0.029
	CTMO/358.5	NMO/332.6	25.9	7.8	0.001
IIM	CIMO/356.5	NMO/326.8	29.7	9.1	<0.001
	CTMO/351.0	NMO/326.8	24.2	7.4	<0.001
	NMO/326.8	HC/342.4	15.6	4.6	0.025
TIM	CIMO/347.3	NMO/319.6	27.7	8.7	<0.001
	CIMO/347.3	HC/331.9	15.4	4.6	0.004
	CTMO/345.6	NMO/319.6	26.0	8.1	<0.001
	NCTMO/336.3	NMO/319.6	16.7	5.2	0.007
	NMO/319.6	HC/331.9	12.3	3.7	0.027
SOM	CIMO/313.7	NMO/291.4	22.3	7.7	<0.001
	CTMO/312.0	NMO/291.4	20.6	7.1	0.009
NOM	CIMO/326.1	NMO/304.2	21.9	7.2	<0.001
	CTMO/324.3	NMO/304.2	20.1	6.7	<0.001
	NMO/304.2	HC/317.6	13.4	4.2	0.034
IOM	CIMO/297.5	NMO/279.2	18.3	6.6	<0.001
	CTMO/300.3	NMO/279.2	21.1	7.6	<0.001
TOM	CIMO/293.7	NMO/274.7	19.0	6.9	<0.001
	CIMO/293.7	HC/282.1	11.6	4.1	0.041
	CTMO/300.4	NMO/274.7	25.7	9.4	<0.001
	CTMO/300.4	HC/282.1	18.3	6.5	0.001
	NCTMO/287.8	NMO/274.7	13.1	4.8	0.043

7.3.6.4 Comparison of HC and PWD who were treated (TT) or not treated (NT)

The mean CST of HC (282.7±23.9µm) and PWD who were NT (285.0±31.2µm) were similar (Table 7.13). PWD who were treated had much higher retinal thickness across all subfields compared to HC or PWD who were NT (Figures 7.8A and 7.8B), as expected, as increased CST is an indication for macular treatment. The retinal thickness between HC (N=50) and PWD who were NT (N=267) was similar (Figures 7.8A and 7.8B), which implied that the retinal thickness of the small number of PWD who were treated (N=25) accounted for much of the differences between HC and PWD. PWD who were treated had a wider range of retinal thickness and therefore larger SD compared to PWD who were NT and HC (Table 7.13). There were two significant sets of ANOVA pairwise comparisons across all subfields between TT vs NT and TT vs HC as expected (Table 7.14).

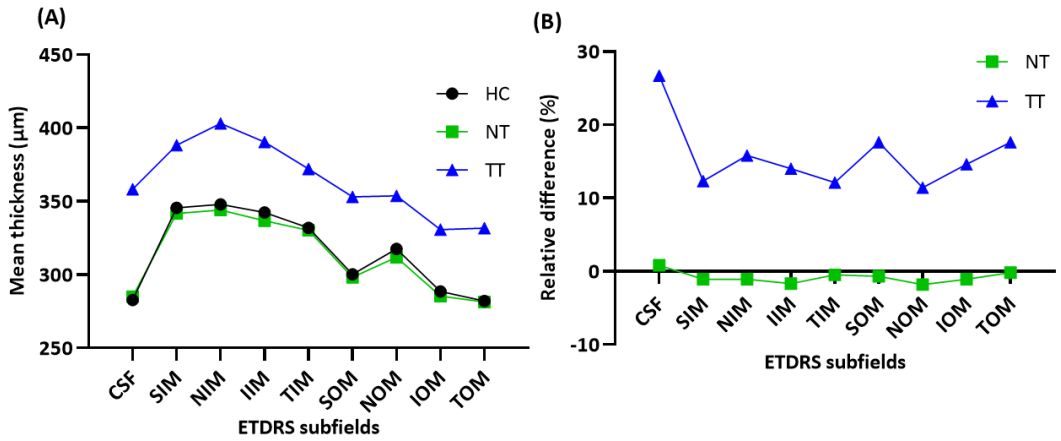


Figure 7.8 (A) Mean full retinal thickness (µm) across all ETDRS subfields of healthy controls (HC) and people with diabetes (PWD) who were not treatment (NT) or were treated (TT) (B) Relative difference (RD;%) of PWD with different treatments compared to HC. RD is the difference in retinal thickness between the different treatment groups and HC divided by the retinal thickness of the HC expressed as a percentage. Error bars have been omitted for clarity.

Table 7.13. Full retinal thickness in all ETDRS subfields in HC compared to PWD who were not treated (NT) and PWD who were treated (TT) expressed as absolute difference (AD; calculated as the difference between the mean retinal thickness of different retinopathy groups and HC) and relative difference (RD; calculated as AD divided by the retinal thickness of HC expressed as a percentage)

	CSF			SIM			NIM			IIM			TIM			SOM			NOM			IOM			TOM		
Group	Mean± SD (μm)	AD (μm)	RD (%)	Mean ±SD (μm)	AD (μm)	RD (%)	Mean± SD (μm)	AD (μm)	RD (%)	Mean± SD (μm)	AD (μm)	RD (%)	Mean± SD (μm)	AD (μm)	RD (%)	Mean± SD (μm)	AD (μm)	RD (%)	Mean± SD (μm)	AD (μm)	RD (%)	Mean± SD (μm)	AD (μm)	RD (%)	Mean± SD (μm)	AD (μm)	RD (%)
HC (N=50)	282.7± 23.9	0	0	345.6± 14.6	0	0	347.9± 15.8	0	0	342.4± 13.3	0	0	331.9± 12.3	0	0	300.3± 13.7	0	0	317.6± 13.2	0	0	288.6± 11.8	0	0	282.1± 12.1	0	0
NT (N=267)	285.0± 31.2	2.3	0.8	341.8± 22.3	-3.8	-1.1	344.0± 22.4	-3.9	-1.1	336.7± 21.3	-5.7	-1.7	330.2± 20.8	-1.7	-0.5	298.3± 18.1	-2.0	-0.7	311.9± 19.9	-5.7	-1.8	285.4± 18.3	-3.2	-1.1	281.4± 16.2	-0.7	-0.2
TT (N=25)	358.2± 127.7	75.5	26.7	388.2± 89.9	42.6	12.3	403.0± 111.7	55.1	15.8	390.4± 85.8	48.0	14.0	372.1± 60.1	40.2	12.1	353.1± 91.2	52.8	17.6	353.7± 71.7	36.1	11.4	330.8± 54.2	42.2	14.6	331.8± 54.2	49.7	17.6

Negative AD and RD indicate lower retinal thickness compared to HC while positive AD and RD indicate higher retinal thickness compared to HC.

Table 7.14. Summary of statistically significant ANOVA pairwise comparisons of full retinal thickness in HC and PWD who were not treated (NT) and PWD who were treated (TT) with absolute difference (AD; calculated as difference between the mean retinal thickness of two groups), relative difference (RD; calculated as AD divided by the retinal thickness of the less severe group expressed as a percentage) and *p* value shown. Only statistically significant ANOVA pairwise comparisons are shown. Non-statistically significant ANOVA pairwise comparisons have been omitted for clarity. All *p* values were adjusted using Bonferroni corrections. The group with a more severe grade is shown on the left column and the group with a less severe grade is shown on the right column.

Subfield	Group/mean retinal thickness (μm)		Absolute difference between groups (μm)	Relative difference between groups (%)	*P*
CSF	TT/358.2	NT/285.0	73.2	25.7	<0.001
	TT/358.2	HC/282.7	75.5	26.7	<0.001
SIM	TT/388.2	NT/341.8	46.4	13.6	<0.001
	TT/388.2	HC/345.6	42.6	12.3	<0.001
NIM	TT/403.0	NT/344.0	59.0	17.2	<0.001
	TT/403.0	HC/347.9	55.1	15.8	<0.001
IIM	TT/390.4	NT/336.7	53.7	15.9	<0.001
	TT/390.4	HC/342.4	48.0	14.0	<0.001
TIM	TT/372.1	NT/330.2	41.9	12.7	<0.001
	TT/372.1	HC/331.9	40.2	12.1	<0.001
SOM	TT/353.1	NT/298.3	54.8	18.4	<0.001
	TT/353.1	HC/300.3	52.8	17.6	<0.001
NOM	TT/353.7	NT/311.9	41.8	13.4	<0.001
	TT/353.7	HC/317.6	36.1	11.4	<0.001
IOM	TT/330.8	NT/285.4	45.4	15.9	<0.001
	TT/330.8	HC/288.6	42.2	14.6	<0.001
TOM	TT/331.8	NT/281.4	50.4	17.9	<0.001
	TT/331.8	HC/282.1	49.7	17.6	<0.001

7.3.6.5 Section summary

In PWD with no evidence of DR on clinical examination (R0 or M0) or on OCT (NMO), there was a small but consistent thinning in full retinal thickness across most subfields compared to HC (Sections 7.3.6.1-7.3.6.3). R2 had the highest retinal thickness across most inner subfields except TIM where R3A was slightly higher than all the other groups. R3 had the highest retinal thickness across the outer subfields (Section 7.3.6.1). M1 had higher retinal thickness in all subfields compared to HC and M0 (Section 7.3.6.2). CTMO had highest retinal thickness in the IOM and TOM among all groups surpassing that of CIMO (Section 7.3.6.3). Retinal thickness between HC and PWD who were NT were similar implied that the small number of PWD who were TT (N=25) accounted for much of the differences between HC and PWD (Section 7.3.6.4).

7.3.7 EFFECT OF AGE, GENDER, TYPE OF DIABETES, DURATION OF DIABETES, HBA_{1C}, ON DIFFERENT RETINAL LAYERS IN ALL PWD IN THE CENTRAL, INNER AND OUTER SUBFIELDS

Up to this point, only full retinal thickness in each ETDRS subfield has been examined. Therefore, in this section, the thickness of different retinal layers across the central, inner and outer ETDRS subfields of all PWD is analysed. The effect of various patient factors that may influence retinal thickness is also assessed. To do so, univariate linear regression was used to examine the effect of age, gender, type of diabetes, duration of diabetes and HbA_{1c} on retinal thickness in different retinal layers in the central, inner and outer subfields (Table 7.15). Adjustments for multiple comparisons were made with Bonferroni corrections. The results showed that there were both positive and negative correlations with age and retinal thickness across most retinal layers in the central, inner and outer ETDRS subfields. In particular, in the GCL and IPL, there were significant positive correlations between age and thickness in the CSF and significant negative correlations between age and retinal thickness in the inner and outer subfields. However, there were no consistent generalised positive or negative patterns of correlations that could be elucidated. Male gender appeared to be more positively correlated with increased retinal thickness in most layers but again, this was not consistent in all subfields.

Higher HbA_{1c} was correlated with increased retinal thickness in the INL and OPL in the central, inner subfields and outer subfields in PWD (Table 7.15). These correlations were significant even when adjusted for multiple comparisons except for the OPL in the inner subfields. Therefore, correlations of INL and OPL retinal thickness across all subfields (central, inner and outer subfields combined) with HbA_{1c} were further examined (Figure 7.9). The analysis showed that there was a significant correlation of an increase in INL thickness (Pearson r=0.24, p<0.001) with an increase in HbA_{1c} levels. The slope of the least squares regression line was +0.04397 and the equation indicated an increase of 0.043µm in INL thickness with every 1mmol increase of HbA_{1c}. Similarly, there was a significant correlation of an increase in OPL thickness (Pearson r=0.17, p=0.004) with an increase in HbA_{1c} levels. The slope of the least squares regression line was +0.02607 and the equation indicated an increase of 0.026µm in OPL thickness with every 1mmol increase of HbA_{1c}.

Table 7.15 Thickness values in the central subfield (CSF), inner subfields (IS) and outer subfields (OS) and the effects of age, gender, type of diabetes, duration of diabetes and HbA_{1c} on these values in all people with diabetes

				Age (y)			Gender			Type of Diabetes			Duration of diabetes (y)			HbA_{1c} (mmol/mol)		
Layer	**Subfield**	**Thickness (µm) Mean±SD**	**Intercept**	**Estimated coefficient**	***p***	**Adjusted *p***	**Estimated coefficient***	***p***	**Adjusted *p***	**Estimated coefficient ****	***p***	**Adjusted *p***	**Estimated coefficient**	***p***	**Adjusted *p***	**Estimated coefficient**	***p***	**Adjusted *p***
Full	**CSF**	291.3±51.5	241.86	0.947	**<0.001**	**<0.001**	13.504	**0.029**	0.087	-9.330	0.264	0.792	-0.260	0.503	1.509	0.012	0.937	2.811
	IS	343.5±34.8	342.86	-0.181	0.285	0.855	0.594	0.888	2.664	-7.255	0.205	0.615	0.172	0.518	1.554	0.175	0.083	0.249
	OS	298.6±26.4	305.07	-0.124	0.334	1.002	-2.128	0.509	1.527	-2.560	0.556	1.668	-0.390	0.054	0.162	0.125	0.101	0.303
RNFL	**CSF**	14.6±7.3	5.13	0.097	**0.007**	**0.021**	2.166	**0.016**	**0.048**	0.624	0.604	1.812	0.065	0.246	0.738	0.022	0.302	0.906
	IS	23.6±3.9	19.47	0.025	0.201	0.603	0.756	0.117	0.351	0.575	0.375	1.125	0.009	0.775	2.325	0.024	**0.032**	0.096
	OS	36.0±5.6	35.33	-0.014	0.608	1.824	-1.693	**0.014**	**0.042**	0.444	0.630	1.890	0.011	0.796	2.388	0.026	0.104	0.312
GCL	**CSF**	16.7±5.6	10.17	0.068	**0.014**	**0.042**	1.852	**0.007**	**0.021**	-0.753	0.416	1.248	0.056	0.190	0.570	0.020	0.216	0.648
	IS	48.7±6.0	52.45	-0.079	**0.007**	**0.021**	-0.263	0.721	2.163	0.056	0.955	2.865	-0.023	0.615	1.845	0.013	0.447	1.341
	OS	34.2±4.1	40.62	-0.091	**<0.001**	**<0.001**	-1.023	**0.031**	0.093	-0.648	0.311	0.933	-0.051	0.089	0.267	0.004	0.736	2.208
IPL	**CSF**	22.6±5.2	15.39	0.067	**0.008**	**0.024**	1.961	**0.002**	**0.006**	-0.122	0.886	2.658	0.047	0.230	0.690	0.025	0.096	0.288
	IS	40.1±4.0	41.73	-0.053	**0.007**	**0.021**	0.223	0.651	1.953	0.470	0.481	1.443	-0.026	0.397	1.191	0.016	0.179	0.537
	OS	28.2±3.2	31.41	-0.059	**<0.001**	**<0.001**	-0.574	0.128	0.384	-0.100	0.844	2.532	-0.040	0.092	0.276	0.013	0.134	0.402
INL	**CSF**	23.9±8.3	5.82	0.177	**<0.001**	**<0.001**	3.689	**<0.001**	**<0.001**	-0.386	0.767	2.301	0.086	0.159	0.477	0.073	**0.001**	**0.003**
	IS	40.4±4.7	35.88	-0.023	0.307	0.921	1.649	**0.004**	**0.012**	1.434	0.060	0.180	-0.006	0.874	2.622	0.053	**<0.001**	**<0.001**
	OS	32.7±3.2	33.24	-0.054	**<0.001**	**<0.001**	-0.234	0.538	1.614	0.874	0.089	0.267	-0.025	0.295	0.885	0.030	**0.001**	**0.003**
OPL	**CSF**	28.7±6.1	19.22	0.070	**0.018**	0.054	0.734	0.318	0.954	-0.042	0.966	2.898	0.111	**0.016**	**0.048**	0.051	**0.004**	**0.012**
	IS	34.5±4.5	30.08	0.016	0.478	1.434	0.046	0.934	2.802	0.696	0.352	1.056	0.061	0.082	0.246	0.030	**0.024**	0.072
	OS	28.3±2.6	25.82	0.017	0.167	0.501	-0.002	0.995	2.985	0.075	0.860	2.580	-0.019	0.327	0.981	0.025	**0.001**	**0.003**
ONL	**CSF**	92.3±15.6	85.46	0.209	**0.006**	**0.018**	5.592	**0.003**	**0.009**	-4.218	0.099	0.297	-0.204	0.086	0.258	-0.023	0.606	1.818
	IS	71.1±11.1	73.85	-0.019	0.726	2.178	3.700	**0.007**	**0.021**	-1.145	0.535	1.605	-0.144	0.093	0.279	-0.013	0.690	2.070
	OS	59.3±9.7	58.60	-0.082	0.081	0.243	2.261	0.053	0.159	-0.715	0.650	1.950	-0.088	0.229	0.687	0.076	**0.006**	**0.018**
RPE	**CSF**	18.0±3.0	19.560	-0.030	**0.001**	**0.003**	0.798	**0.001**	**0.003**	-0.007	0.983	2.949	-0.010	0.483	1.449	-0.003	0.625	1.875
	IS	16.4±1.5	17.449	-0.009	0.244	0.732	0.674	**<0.001**	**<0.001**	-0.192	0.438	1.314	-0.029	**0.013**	**0.039**	-0.006	0.141	0.423
	OS	14.5±1.3	15.737	-0.015	**0.016**	**0.048**	0.475	**0.002**	**0.006**	-0.176	0.398	1.194	-0.012	0.215	0.645	-0.006	0.083	0.249

RNFL (retinal nerve fibre layer), GCL (ganglion cell layer), IPL (inner plexiform layer), INL (inner nuclear layer), OPL (outer plexiform layer), ONL (outer nuclear layer), RPE (retinal pigment layer)

Univariate linear regression used

Statistically significant results are in bold

Adjusted p values are adjusted with Bonferroni correction for multiple comparisons

*The values for the estimated coefficient refer to the male gender

**The values for the estimated coefficient refer to type 2 diabetes

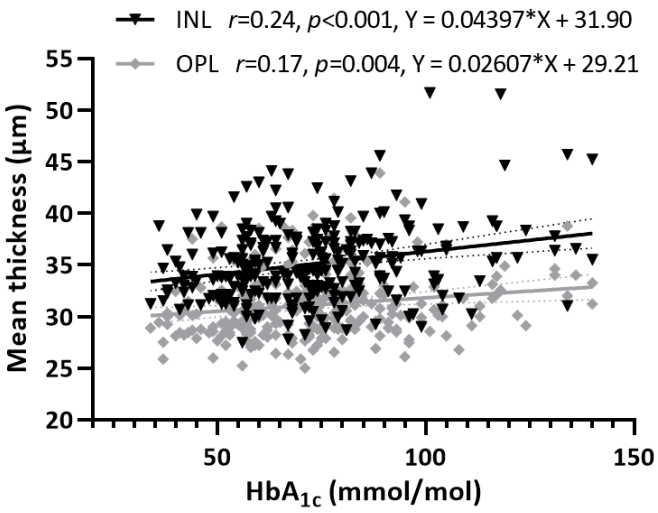


Figure 7.9. Correlation of INL and OPL thicknesses with HbA_{1c} levels. Pearson correlation coefficients and their statistical significance are shown. Least squares linear regression lines (±95% CI) with equations are also shown.

7.3.8 COMPARISON OF RETINAL THICKNESS IN DIFFERENT LAYERS IN THE CENTRAL, INNER AND OUTER SUBFIELDS IN HC AND PWD

In the previous section (Section 7.3.7), the retinal thickness of different retinal layers in all PWD was explored while considering various patient factors. However, the PWD were not separated by their retinopathy, maculopathy, Liverpool OCT classification or treatment outcome. A consistent pattern has emerged where there is full retinal thickness thinning in PWD with no evidence of DR on clinical examination or OCT (Section 7.3.6.5). However, this analysis leaves open the question of whether these overall thickness changes are a reflection of changes in all retinal layers or primarily driven by changes in particular retinal layers. Therefore, in this section, the retinal thicknesses of the different retinal layers were compared in HC and PWD using these classifications. Specifically, One-way ANOVA with Tukey post hoc tests was performed to reveal any significant differences in retinal thickness within these groups. Bonferroni corrections were made for multiple comparisons. Only statistically significant pairwise comparison results are shown for clarity.

7.3.8.1 Comparison of HC and PWD with different retinopathy grades

Figure 7.10 shows that the lines showing the retinal thicknesses of the different retinal layers appear quite close together in the central, inner and outer subfields (Figure 7.10 A, C and E, left-hand panel). Therefore, it is easier to visually discern the differences between the retinal thicknesses of the PWD with different retinopathy grades by examining the RD

graphs (Figures 7.10 B, D and F, right-hand panel). The RNFL will be examined first because it has been studied as part of DR being a neurodegenerative disease (Jonsson et al., 2016). In the CSF, R0 had the same retinal thickness as HC (R0 13.3±2.0µm, HC 13.3±2.4µm). In the inner and outer subfields, R0 had the lowest RNFL thickness compared to HC and other retinopathy grades (inner subfields 22.3±2.2µm; outer subfields 33.1±4.5µm). The RNFL thickness in R0 was lower than HC by 0.6µm (RD -2.6%) in the inner subfields and 4.2µm (RD -11.3%) in the outer subfields (Table 7.16). The difference in RNFL thickness between R0 and HC was statistically significant in the outer subfields (p=0.006; Table 7.17). R3 had the highest RNFL thickness across all subfields compared to other groups (CSF 19.4±20.6µm; inner subfields 26.6±3.7µm; outer subfields 42.1±5.3µm; Table 7.16).

The GCL and IPL layers had similar trends compared to the RNFL whereby R0 had a lower thickness compared to HC in all subfields (Table 7.16). Interestingly, all PWD in the inner subfields had thinner GCL and IPL compared to HC (RD: GCL: R0, -6.6%; R1, -4.9%; R2, -4.9%, R3, -4.3%; IPL: R0, -5.9%, R1 -5.4%, R2 -5.4%, R3 -4.2%).

Compared to HC, PWD in all retinopathy grades had thicker INL across all subfields except the outer subfields where R0 had the same mean INL thickness compared to HC (R0 32.0±3.0 µm, HC 32.0±1.9µm). Similarly, PWD in all retinopathy grades had thicker OPL across all subfields compared to HC.

R0 had a thinner ONL in all subfields (RD: CSF, -3.8%, inner subfields, -3.5% and outer subfields -4.5%) compared to HC. In contrast, R0 had a thicker RPE in all subfields (RD: CSF 1.7%, inner subfields 1.2%, outer subfields 4.3%) compared to HC (Table 7.16). Notably, there was the least number of significant pairwise comparisons in the CSF, more in the inner subfields and the most in the outer subfields (Table 7.17).

This section showed that R0, when compared to HC, had thinner RNFL in the inner and outer subfields, thinner GCL and IPL and ONL in all subfields; thicker INL in the CSF and inner subfields; thicker OPL and RPE in all subfields. However, only the difference in RNFL between R0 and HC in the outer subfields was statistically significant (p=0.006, Table 7.17).

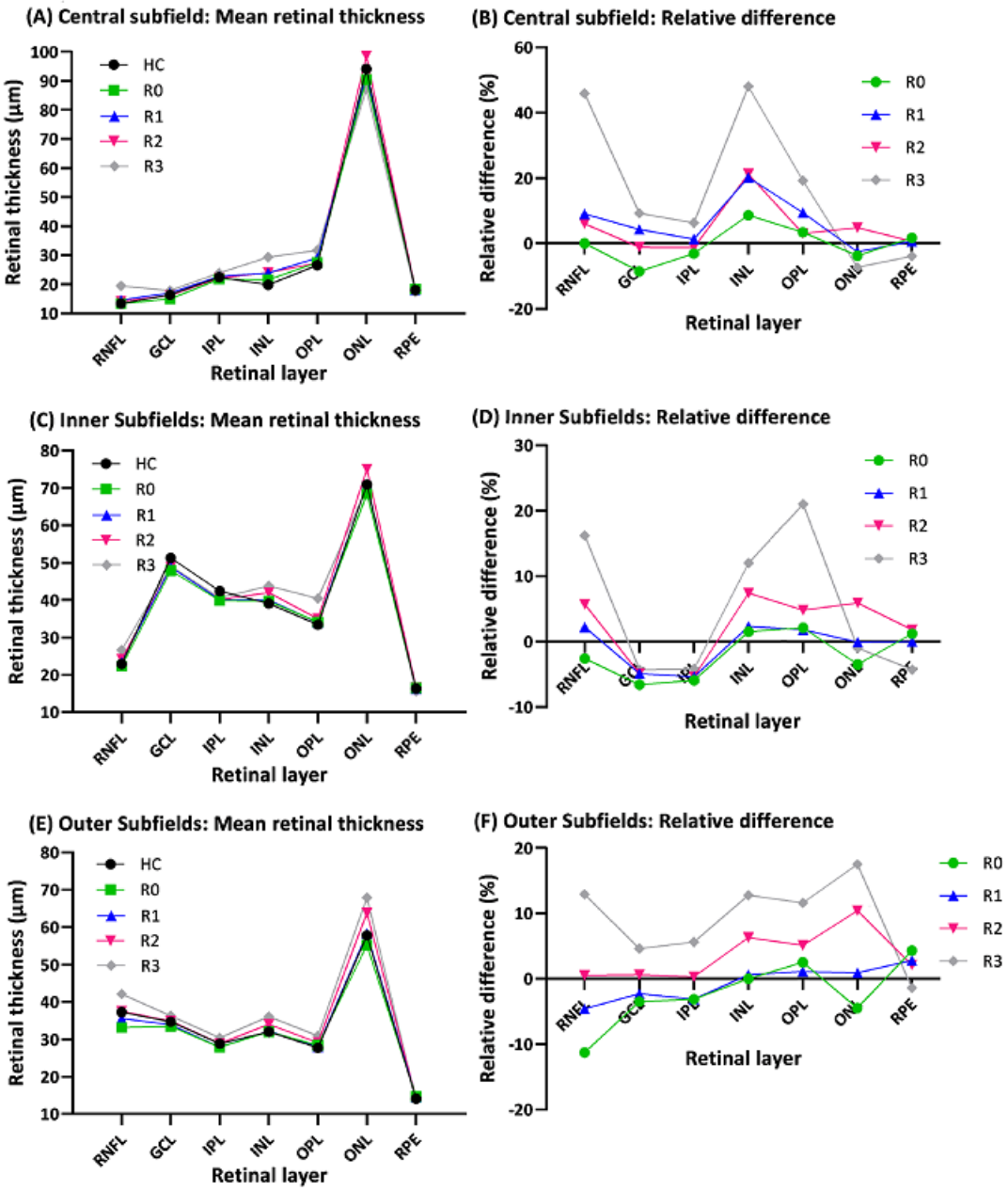


Figure 7.10 Mean retinal thickness (µm) and relative difference (RD; %) of different retinal layers in healthy controls (HC) and people with diabetes (PWD) with different retinopathy grades (R0/R1/R2/R3) in the central subfield (A, B), inner subfields (C, D) and outer subfields (E, F). RD is the difference in retinal thickness between the different retinopathy groups and HC divided by the retinal thickness of the HC expressed as a percentage. Error bars have been omitted for clarity.

Table 7.16. Retinal thickness of different retinal layers (μm) in the central, inner and outer subfields in HC and PWD with different retinopathy grades (R0, R1, R2, R3) expressed in absolute difference (AD; calculated as the difference between the mean retinal thickness of different retinopathy groups and HC) and relative difference (RD; calculated as AD divided by the retinal thickness of HC expressed as a percentage)

	Full			RNFL			GCL			IPL			INL			OPL			ONL			RPE		
Group	Mean±SD (μm)	AD (μm)	RD (%)	Mean±SD (μm)	AD (μm)	RD (%)	Mean±SD (μm)	AD (μm)	RD (%)	Mean±SD (μm)	AD (μm)	RD (%)	Mean±SD (μm)	AD (μm)	RD (%)	Mean±SD (μm)	AD (μm)	RD (%)	Mean±SD (μm)	AD (μm)	RD (%)	Mean±SD (μm)	AD (μm)	RD (%)
	Central Subfield																							
HC	282.7±23.9	0	0	13.3±2.0	0	0	16.3±4.6	0	0	22.4±4.1	0	0	19.8±6.3	0	0	26.5±5.6	0	0	94.1±9.7	0	0	18.0±2.1	0	0
R0	279.3±23.9	-3.4	-1.2	13.3±2.4	0	0	14.9±4.3	-1.4	-8.6	21.7±3.9	-0.7	-3.1	21.5±6.6	1.7	8.6	27.4±6.4	0.9	3.4	90.5±14.9	-3.6	-3.8	18.3±2.1	0.3	1.7
R1	287.4±33.6	4.7	1.7	14.5±6.4	1.2	9.0	17.0±5.6	0.7	4.3	22.7±4.9	0.3	1.3	23.8±8.1	4.0	20.2	29.0±6.1	2.5	9.4	91.7±13.7	-2.4	-2.6	18.1±2.0	0.1	0.6
R2	314.9±101.6	32.2	11.4	14.1±3.0	0.8	6.0	16.1±4.8	-0.2	-1.2	22.1±5.7	-0.3	-1.3	24.0±8.0	4.2	21.2	27.3±5.3	0.8	3.0	98.6±21.2	4.5	4.8	18.1±1.9	0.1	0.6
R3	295.1±53.6	12.4	4.4	19.4±20.6	6.1	45.9	17.8±9.0	1.5	9.2	23.8±8.8	1.4	6.3	29.3±12.8	9.5	48.0	31.6±6.9	5.1	19.2	87.2±19.2	-6.9	-7.3	17.3±1.7	-0.7	-3.9
	Inner Subfields																							
HC	342.0±13.5	0	0	22.9±1.9	0	0	51.3±4.4	0	0	42.4±3.0	0	0	39.1±3.1	0	0	33.4±3.9	0	0	70.9±8.4	0	0	16.3±1.4	0	0
R0	333.8±18.7	-8.2	-2.4	22.3±2.2	-0.6	-2.6	47.9±5.0	-3.4	-6.6	39.9±3.8	-2.5	-5.9	39.7±4.0	0.6	1.5	34.1±4.9	0.7	2.1	68.4±8.8	-2.5	-3.5	16.5±1.3	0.2	1.2
R1	338.6±20.9	-3.4	-1.0	23.4±3.9	0.5	2.2	48.8±6.3	-2.5	-4.9	40.1±4.1	-2.3	-5.4	40.0±4.1	0.9	2.3	34.0±4.0	0.6	1.8	70.8±10.1	-0.1	-0.1	16.3±1.4	0	0
R2	362.8±67.6	20.8	6.1	24.2±3.3	1.3	5.7	48.8±5.5	-2.5	-4.9	40.1±3.8	-2.3	-5.4	42.0±5.1	2.9	7.4	35.0±4.0	1.6	4.8	75.1±13.2	4.2	5.9	16.6±2.0	0.3	1.8
R3	350.7±32.3	8.7	2.5	26.6±3.7	3.7	16.2	49.1±6.0	-2.2	-4.3	40.6±4.4	-1.8	-4.2	43.8±9.4	4.7	12.0	40.4±6.4	7.0	21.0	70.2±18.6	-0.7	-1.0	15.6±1.5	-0.7	-4.3
	Outer Subfields																							
HC	297.2±11.9	0	0	37.3±4.6	0	0	34.7±3.2	0	0	28.8±2.3	0	0	32.0±1.9	0	0	27.7±1.8	0	0	57.8±6.1	0	0	14.1±1.1	0	0
R0	288.3±13.9	-8.9	-3.0	33.1±4.5	-4.2	-11.3	33.5±3.3	-1.2	-3.5	27.9±2.6	-0.9	-3.1	32.0±3.0	0	0	28.4±2.9	0.7	2.5	55.2±6.4	-2.6	-4.5	14.7±1.1	0.6	4.3
R1	294.5±17.6	-2.7	-0.9	35.6±5.2	-1.7	-4.6	33.9±4.1	-0.8	-2.3	27.9±3.1	-0.9	-3.1	32.2±2.7	0.2	0.6	28.0±2.3	0.3	1.1	58.3±8.0	0.5	0.9	14.5±1.3	0.4	2.8
R2	316.2±44.9	19.0	6.4	37.5±6.2	0.2	0.5	34.9±3.9	0.2	0.6	28.9±3.3	0.1	0.3	34.0±3.5	2.0	6.3	29.1±2.8	1.4	5.1	63.8±10.7	6.0	10.4	14.4±1.1	0.3	2.1
R3	320.8±33.7	23.6	7.9	42.1±5.3	4.8	12.9	36.3±4.7	1.6	4.6	30.4±3.7	1.6	5.6	36.1±5.3	4.1	12.8	30.9±3.4	3.2	11.6	67.9±18.9	10.1	17.5	13.9±1.7	-0.2	-1.4

Negative AD and RD indicate lower retinal thickness compared to HC while positive AD and RD indicate higher retinal thickness compared to HC.

Table 7.17. Summary of statistically significant ANOVA pairwise comparisons of different retinal layer thickness in the central (A), inner (B) and outer (C) subfields in HC and PWD with different retinopathy grades (R0, R1, R2, R3) with absolute difference (AD; calculated as difference between the mean retinal thickness of two groups), relative difference (RD; calculated as AD divided by the retinal thickness of the less severe group expressed as a percentage) and *p* value shown. Only statistically significant ANOVA pairwise comparisons are shown. Non-statistically significant ANOVA pairwise comparisons have been omitted for clarity. All *p* values were adjusted using Bonferroni corrections. The group with a more severe retinopathy grade is shown on the left column and the group with a less severe retinopathy grade is shown on the right column.

(A) Central Subfield

Retinal layer	Group/mean retinal thickness (μm)		Absolute difference between groups (μm)	Relative difference between groups (%)	*P*
Full	R2/314.9	R1/287.4	27.5	9.6	0.005
	R2/314.9	R0/279.3	35.6	12.7	0.019
	R2/314.9	HC/282.7	32.2	11.4	0.011
RNFL	R3/19.4	R0/13.3	6.1	45.9	0.045
	R3/19.4	HC/13.3	6.1	45.9	0.018
INL	R3/29.3	R0/21.5	7.8	36.3	0.019
	R3/29.3	HC/19.8	9.5	48.0	<0.001
	R1/23.8	HC/19.8	4.0	20.2	0.017
OPL	R3/31.6	HC/26.5	5.1	19.2	0.032

(B) Inner Subfields

Retinal layer	Group/mean retinal thickness (μm)		Absolute difference between groups (μm)	Relative difference between groups (%)	*P*
Full	R2/362.8	R1/338.6	24.2	7.1	<0.001
	R2/362.8	R0/333.8	29.0	8.7	0.001
	R2/362.8	HC/342.0	20.8	6.1	0.012
RNFL	R3/26.6	R1/23.4	3.2	13.7	<0.001
	R3/26.6	R0/22.3	4.3	19.3	0.002
	R3/26.6	HC/22.9	3.7	16.2	0.003
IPL	R1/40.1	HC/42.4	2.3	5.4	0.003
INL	R3/43.8	R1/40.0	3.8	9.5	0.010
	R3/43.8	R0/39.7	4.1	10.3	0.032
	R3/43.8	HC/39.1	4.7	12.0	0.002
	R2/42.0	HC/39.1	2.9	7.4	0.022
OPL	R3/40.4	R2/35.0	5.4	15.4	<0.001
	R3/40.4	R1/34.0	6.4	18.8	<0.001
	R3/40.4	R0/34.1	6.3	18.5	<0.001
	R3/40.4	HC/33.4	7.0	21.0	<0.001

(C) Outer Subfields

Retinal layer	Group/mean retinal thickness (µm)		Absolute difference between groups (µm)	Relative difference between groups (%)	*P*
Full	R3/320.8	R1/294.5	26.3	8.9	<0.001
	R3/320.8	R0/288.3	32.5	11.3	<0.001
	R3/320.8	HC/297.2	23.6	7.9	0.004
	R2/316.1	R1/294.5	21.6	7.3	<0.001
	R2/316.1	R0/288.3	27.8	9.6	<0.001
	R2/316.1	HC/297.2	18.9	6.4	0.001
RNFL	R3/42.1	R2/37.5	4.6	12.3	0.027
	R3/42.1	R1/35.6	6.5	18.3	<0.001
	R3/42.1	R0/33.1	9.0	27.2	<0.001
	R3/42.1	HC/37.3	4.8	12.9	0.014
	R0/33.1	HC/37.3	4.2	11.3	0.006
IPL	R3/30.4	R1/27.9	2.5	9.0	0.018
INL	R3/36.1	R1/32.2	3.9	12.1	<0.001
	R3/36.1	R0/32.0	4.1	12.8	<0.001
	R3/36.1	HC/32.0	4.1	12.8	<0.001
	R2/34.0	R1/32.2	1.8	5.6	0.006
	R2/34.0	HC/32.0	2.0	6.25	0.018
OPL	R3/30.9	R1/28.0	2.9	10.4	<0.001
	R3/30.9	R0/28.4	2.5	8.8	0.012
	R3/30.9	HC/27.7	3.2	11.6	<0.001
ONL	R3/67.9	R1/58.3	9.6	16.5	<0.001
	R3/67.9	R0/55.2	12.7	23.0	<0.001
	R3/67.9	HC/57.8	10.1	17.5	0.001
	R2/63.8	R1/58.2	5.6	9.6	0.003
	R2/63.8	R0/55.2	8.6	15.6	0.001
	R2/63.8	HC/57.8	6.0	10.4	0.012

7.3.8.2 Comparison of HC and PWD with different maculopathy grades

Retinal thickness of different retinal layers in HC and PWD with different maculopathy grades was compared. In the CSF and inner subfields, M0 and M1 had thicker RNFL compared to HC (Figures 7.11B and 7.11D, Table 7.18). In contrast, in the outer subfield, M0 and M1 had thinner RNFL compared to HC (Figure 7.11F, Table 7.18). However, these differences were not statistically significant (Table 7.19).

M0 and M1 had thinner GCL and IPL compared to HC in all subfields except for M1 who had thicker GCL and IPL in the CSF (GCL AD 0.9µm, RD 5.5%; IPL AD 0.5µm, RD 2.2%) compared to HC (Figure 7.11, Table 7.18). Pairwise comparisons showed that M0 had significantly thinner GCL in the inner subfields compared to HC (p=0.002, Table 7.19).

M0 and M1 had thicker INL and OPL compared to HC in all subfields compared to HC (Figure 7.11; Table 7.18). Pairwise comparisons showed that M1 had significantly thicker INL in the CSF (p<0.001) and inner subfields (p=0.014); M1 also had significantly thicker OPL in the CSF compared to HC (p=0.036, Table 7.19). M0 had a thinner ONL compared to HC while M1

had a thicker ONL compared to HC. M0 and M1 had thicker RPE compared to HC in all subfields except for M1 in the CSF (Figure 7.11, Table 7.18).

This section showed that M0 had thinner GCL, IPL and ONL and thicker INL, OPL and RPE compared to HC in all subfields (Figure 7.11, Table 7.18). Significant pairwise comparisons between M0 and HC were in the GCL (p=0.002) and IPL (p<0.001) in the inner subfields and RPE (p=0.041) in the outer subfields (Table 7.19).

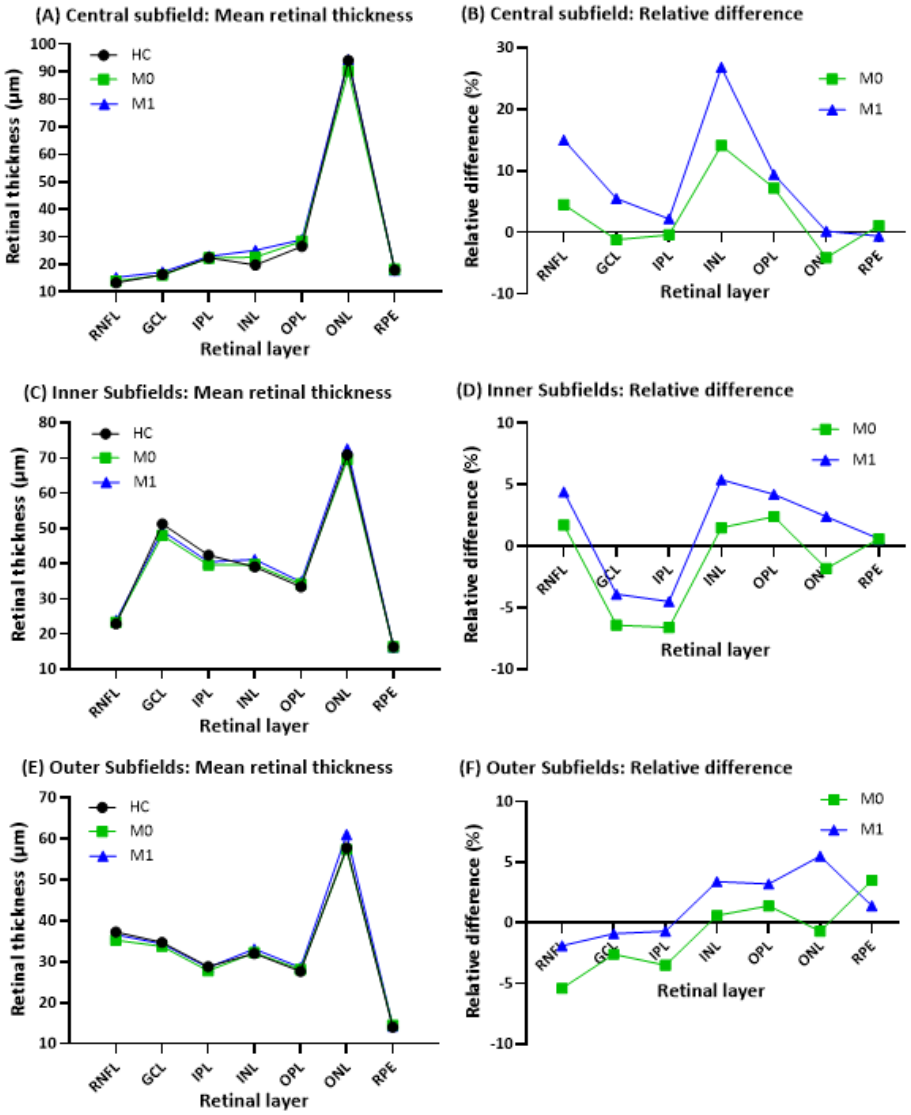


Figure 7.11 Mean retinal thickness (µm) and relative difference (RD; %) of different retinal layers in healthy controls (HC) and people with diabetes (PWD) with different maculopathy grades (M0/M1) in the central subfield (A, B), inner subfields (C, D) and outer subfields (E, F). RD is the difference in retinal thickness between the different maculopathy groups and HC divided by the retinal thickness of the HC expressed as a percentage. Error bars have been omitted for clarity.

Table 7.18. Retinal thicknesses of different retinal layers (μm) in the central, inner and outer subfields in HC and PWD with different maculopathy grades (M0, M1) expressed in absolute difference (AD; calculated as the difference between the mean retinal thickness of different maculopathy groups and HC) and relative difference (RD; calculated as AD divided by the retinal thickness of HC expressed as a percentage)

	Full			RNFL			GCL			IPL			INL			OPL			ONL			RPE		
Group	Mean±SD (μm)	AD (μm)	RD (%)	Mean±SD (μm)	AD (μm)	RD (%)	Mean±SD (μm)	AD (μm)	RD (%)	Mean±SD (μm)	AD (μm)	RD (%)	Mean±SD (μm)	AD (μm)	RD (%)	Mean±SD (μm)	AD (μm)	RD (%)	Mean±SD (μm)	AD (μm)	RD (%)	Mean±SD (μm)	AD (μm)	RD (%)
	Central Subfield																							
HC	282.7±23.9	0	0	13.3±2.0	0	0	16.3±4.6	0	0	22.4±4.1	0	0	19.8±6.3	0	0	26.5±5.6	0	0	94.1±9.7	0	0	18.0±2.1	0	0
M0	282.6±30.9	-0.1	-0.04	13.9±3.7	0.6	4.5	16.1±5.3	-0.2	-1.2	22.3±5.1	-0.1	-0.4	22.6±7.4	2.8	14.1	28.4±5.9	1.9	7.2	90.2±14.0	-3.9	-4.1	18.2±2.1	0.2	1.1
M1	299.2±63.9	16.5	5.8	15.3±9.5	2.0	15.0	17.2±5.9	0.9	5.5	22.9±5.3	0.5	2.2	25.1±9.0	5.3	26.8	29.0±6.3	2.5	9.4	94.3±16.7	0.2	0.2	17.9±1.9	-0.1	-0.6
	Inner Subfields																							
HC	342.0±13.5	0	0	22.9±1.9	0	0	51.3±4.4	0	0	42.4±3.0	0	0	39.1±3.1	0	0	33.4±3.9	0	0	70.9±8.4	0	0	16.3±1.4	0	0
M0	335.7±20.5	-6.3	-1.8	23.3±4.4	0.4	1.7	48.0±5.7	-3.3	-6.4	39.6±3.7	-2.8	-6.6	39.7±4.2	0.6	1.5	34.2±4.4	0.8	2.4	69.6±10.0	-1.3	-1.8	16.4±1.5	0.1	0.6
M1	348.7±42.1	6.7	2.0	23.9±3.4	1.0	4.4	49.3±6.2	-2.0	-3.9	40.5±4.3	-1.9	-4.5	41.2±5.1	2.1	5.4	34.8±4.6	1.4	4.2	72.6±11.9	1.7	2.4	16.4±1.6	0.1	0.6
	Outer Subfields																							
HC	297.2±11.9	0	0	37.3±4.6	0	0	34.7±3.2	0	0	28.8±2.3	0	0	32.0±1.9	0	0	27.7±1.8	0	0	57.8±6.1	0	0	14.1±1.1	0	0
M0	293.0±16.7	-4.2	-1.4	35.3±5.3	-2.0	-5.4	33.8±4.1	-0.9	-2.6	27.8±3.0	-1.0	-3.5	32.2±2.9	0.2	0.6	28.1±2.3	0.4	1.4	57.4±7.2	-0.4	-0.7	14.6±1.3	0.5	3.5
M1	303.7±31.9	6.5	2.2	36.6±5.7	-0.7	-1.9	34.4±4.1	-0.3	-0.9	28.6±3.3	-0.2	-0.7	33.1±3.4	1.1	3.4	28.6±2.8	0.9	3.2	61.0±11.2	3.2	5.5	14.3±1.3	0.2	1.4

Negative AD and RD indicate lower retinal thickness compared to HC while positive AD and RD indicate higher retinal thickness compared to HC.

Table 7.19. Summary of statistically significant ANOVA pairwise comparisons of different retinal layer thickness in the central, inner and outer subfields in HC and PWD with different maculopathy grades (M0, M1) with absolute difference (AD; calculated as difference between the mean retinal thickness of two groups), relative difference (RD; calculated as AD divided by the retinal thickness of the less severe group expressed as a percentage) and *p* value shown. Only statistically significant ANOVA pairwise comparisons are shown. Non-statistically significant ANOVA pairwise comparisons have been omitted for clarity. All *p* values were adjusted using Bonferroni corrections. The group with more severe grade maculopathy is shown on the left column and the group with less severe maculopathy grade is shown on the right column.

Retinal layer	Group/mean retinal thickness (µm)		Absolute difference between groups (µm)	Relative difference between groups (%)	*P*
Central Subfield					
Full	M1/299.2	M0/282.6	16.6	5.9	0.010
INL	M1/25.1	M0/22.6	2.5	11.1	0.030
	M1/25.1	HC/19.8	5.3	26.8	<0.001
OPL	M1/29.0	HC/26.5	2.5	9.4	0.036
Inner Subfields					
Full	M1/348.7	M0/335.7	13.0	3.9	0.002
GCL	M0/48.0	HC/51.3	3.3	6.4	0.002
IPL	M1/40.5	HC/42.4	1.9	4.5	0.011
	M0/39.6	HC/42.4	2.8	6.6	<0.001
INL	M1/41.2	M0/39.7	1.5	3.8	0.016
	M1/41.2	HC/39.1	2.1	5.4	0.014
Outer Subfields					
Full	M1/303.7	M0/293.0	10.7	3.7	0.001
INL	M1/33.1	M0/32.2	0.9	2.7	0.029
ONL	M1/61.0	M0/57.4	3.6	6.3	0.003
RPE	M0/14.6	HC/14.1	0.5	3.5	0.041

7.3.8.3 Comparison of HC and PWD with different Liverpool OCT grades

The Liverpool OCT grades were established based on full retinal thickness measurements and the presence of intraretinal cyst. However, this definition does not take into account the retinal thickness of different retinal layers. Therefore comparisons were also made between HC and PWD defined by their Liverpool OCT grades to explore if particular retinal layers accounted for changes in retinal thicknesses between groups.

NMO had lower retinal thickness compared to HC in the inner retinal layers (RNFL, GCL, IPL and INL) and ONL across all subfields except for INL in the CSF where NMO had higher retinal thickness compared to HC (NMO 20.3±5.4µm, HC 19.8±6.3µm; Figure 7.12, Table 7.20). Pairwise comparisons showed that NMO had significantly thinner ONL in the CSF

(p=0.001), GCL (p<0.001) and IPL (p<0.001) in the inner subfields and RNFL (p=0.004) and IPL (p=0.016) in the outer subfields compared to HC (Table 7.21).

As anticipated, PWD with the most severe Liverpool OCT grade of CIMO had the highest retinal thickness compared to other less severe Liverpool OCT grades (NMO, NCTMO and CTMO) and HC in the CSF across all layers except RPE (Figure 7.12, Table 7.20). Therefore, most of the significant pairwise comparisons in the CSF were between CIMO and other less severe OCT grades and HC (Table 7.21). However, unexpectedly, CTMO had the highest retinal thickness across most retinal layers except RPE in the outer subfields (Figure 7.12F). This is reflected in the significant pairwise comparisons in the outer subfields that were between CIMO and CTMO and other less severe OCT grades and HC (Table 7.21). It is interesting to note that all retinal layers had significant pairwise comparisons except for RPE (Table 7.21).

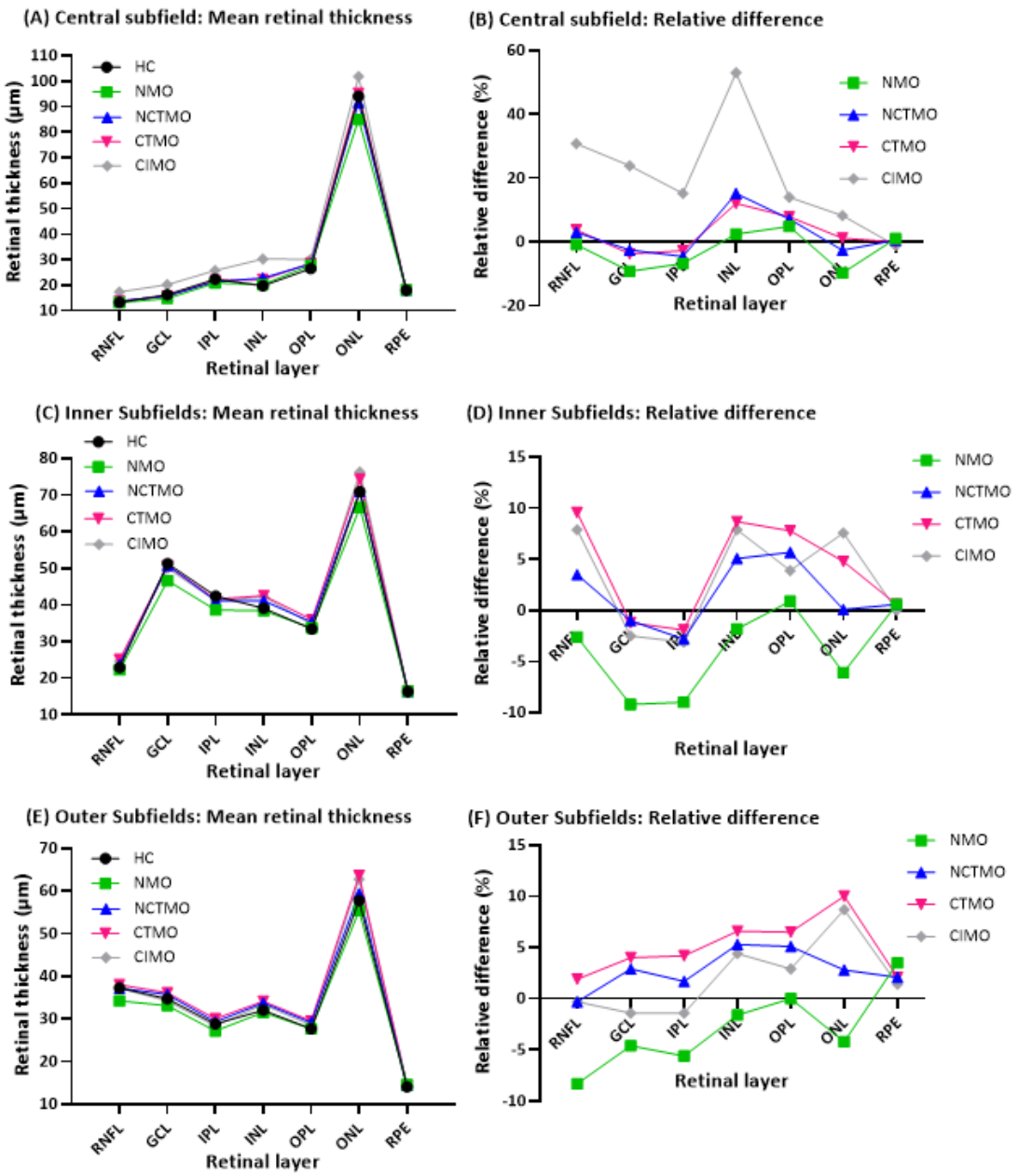


Figure 7.12 Mean retinal thickness (µm) and relative difference (RD; %) of various retinal layers in healthy controls (HC) and people with diabetes (PWD) with different Liverpool OCT grades (NMO/NCTMO/CTMO/CIMO) in the central subfield (A, B), inner subfields (C, D) and outer subfields (E, F). RD is the difference in retinal thickness between the different Liverpool OCT groups and HC divided by the retinal thickness of the HC expressed as a percentage. Error bars have been omitted for clarity.

Table 7.20. Retinal thicknesses of different retinal layers in the central, inner and outer subfields in HC and PWD with different Liverpool OCT grades (NMO, NCTMO, CTMO, CIMO) expressed in absolute difference (AD; calculated as the difference between the mean retinal thickness of different retinopathy groups and HC) and relative difference (RD; calculated as AD divided by the retinal thickness of HC expressed as a percentage)

	Full			RNFL			GCL			IPL			INL			OPL			ONL			RPE		
Group	Mean±SD (μm)	AD (μm)	RD (%)	Mean±SD (μm)	AD (μm)	RD (%)	Mean±SD (μm)	AD (μm)	RD (%)	Mean±SD (μm)	AD (μm)	RD (%)	Mean±SD (μm)	AD (μm)	RD (%)	Mean±SD (μm)	AD (μm)	RD (%)	Mean±SD (μm)	AD (μm)	RD (%)	Mean±SD (μm)	AD (μm)	RD (%)
	Central Subfield																							
HC	282.7±23.9	0	0	13.3±2.0	0	0	16.3±4.6	0	0	22.4±4.1	0	0	19.8±6.3	0	0	26.5±5.6	0	0	94.1±9.7	0	0	18.0±2.1	0	0
NMO	270.6±22.9	-12.1	-4.3	13.2±2.4	-0.1	-0.8	14.8±3.9	-1.5	-9.2	20.9±3.8	-1.5	-6.7	20.3±5.4	0.5	2.5	27.8±5.8	1.3	4.9	85.0±11.3	-9.1	-9.7	18.2±1.9	0.2	1.1
NCTMO	282.9±16.9	0.2	0.1	13.7±2.0	0.4	3.0	15.9±3.3	-0.4	-2.5	21.4±3.1	-1.0	-4.5	22.8±5.5	3.0	15.2	28.4±4.9	1.9	7.2	91.7±12.5	-2.4	-2.6	18.1±2.0	0.1	0.6
CTMO	286.4±19.3	3.7	1.3	13.8±2.5	0.5	3.8	15.7±3.9	-0.6	-3.7	21.8±3.3	-0.6	-2.7	22.2±4.3	2.4	12.1	28.6±6.5	2.1	7.9	95.2±11.2	1.1	1.2	18.0±1.7	0	0
CIMO	325.5±75.8	42.8	15.1	17.4±12.4	4.1	30.8	20.2±7.3	3.9	23.9	25.8±6.7	3.4	15.2	30.3±10.3	10.5	53.0	30.2±6.4	3.7	14.0	101.9±18.2	7.8	8.3	17.8±2.2	-0.2	-1.1
	Inner Subfields																							
HC	342.0±13.5	0	0	22.9±1.9	0	0	51.3±4.4	0	0	42.4±3.0	0	0	39.1±3.1	0	0	33.4±3.9	0	0	70.9±8.4	0	0	16.3±1.4	0	0
NMO	327.4±16.1	-14.6	-4.3	22.3±2.0	-0.6	-2.6	46.6±5.8	-4.7	-9.2	38.6±3.7	-3.8	-9.0	38.4±3.1	-0.7	-1.8	33.7±4.0	0.3	0.9	66.6±8.6	-4.3	-6.1	16.4±1.4	0.1	0.6
NCTMO	344.6±13.4	2.6	0.8	23.7±2.1	0.8	3.5	50.8±4.2	-0.5	-1.0	41.2±2.6	-1.2	-2.8	41.1±3.4	2.0	5.1	35.3±4.8	1.9	5.7	71.0±10.0	0.1	0.1	16.4±1.5	0.1	0.6
CTMO	352.7±20.9	10.7	3.1	25.1±6.9	2.2	9.6	50.7±6.5	-0.6	-1.2	41.6±4.6	-0.8	-1.9	42.5±5.5	3.4	8.7	36.0±5.6	2.6	7.8	74.3±10.8	3.4	4.8	16.4±1.5	0.1	0.6
CIMO	358.3±50.2	16.3	4.8	24.7±3.9	1.8	7.9	50.0±5.7	-1.3	-2.5	41.1±4.0	-1.3	-3.1	42.2±5.6	3.1	7.9	34.7±4.4	1.3	3.9	76.3±12.3	5.4	7.6	16.3±1.7	0	0
	Outer Subfields																							
HC	297.2±11.9	0	0	37.3±4.6	0	0	34.7±3.2	0	0	28.8±2.3	0	0	32.0±1.9	0	0	27.7±1.8	0	0	57.8±6.1	0	0	14.1±1.1	0	0
NMO	287.4±13.2	-9.8	-3.3	34.2±4.7	-3.1	-8.3	33.1±3.6	-1.6	-4.6	27.2±2.7	-1.6	-5.6	31.5±2.1	-0.5	-1.6	27.7±2.1	0	0	55.4±6.5	-2.4	-4.2	14.6±1.3	0.5	3.5
NCTMO	302.4±16.6	5.2	1.7	37.2±5.4	-0.1	-0.3	35.7±4.2	1.0	2.9	29.3±2.9	0.5	1.7	33.7±3.2	1.7	5.3	29.1±2.7	1.4	5.1	59.4±7.6	1.6	2.8	14.4±1.2	0.3	2.1
CTMO	310.0±20.2	12.8	4.3	38.0±5.4	0.7	1.9	36.1±4.3	1.4	4.0	30.0±3.5	1.2	4.2	34.1±3.8	2.1	6.6	29.5±3.1	1.8	6.5	63.6±8.8	5.8	10.0	14.4±1.1	0.3	2.1
CIMO	307.9±37.3	10.7	3.6	37.2±6.1	-0.1	-0.3	34.2±4.1	-0.5	-1.4	28.4±3.3	-0.4	-1.4	33.4±3.7	1.4	4.4	28.5±2.7	0.8	2.9	62.8±12.1	5.0	8.7	14.3±1.3	0.2	1.4

Negative AD and RD indicate lower retinal thickness compared to HC while positive AD and RD indicate higher retinal thickness compared to HC.

Table 7.21. Summary of statistically significant ANOVA pairwise comparisons of different retinal layer thickness in the central (A), inner (B) and outer (C) subfields in HC and PWD with different Liverpool OCT grades (NMO, NCTMO, CTMO, CIMO) with absolute difference (AD; calculated as difference between the mean retinal thickness of two groups), relative difference (RD; calculated as AD divided by the retinal thickness of the less severe group expressed as a percentage) and *p* value shown. Only statistically significant ANOVA pairwise comparisons are shown. Non-statistically significant ANOVA pairwise comparisons have been omitted for clarity. All *p* values were adjusted using Bonferroni corrections. The group with a more severe OCT grade is shown on the left column and the group with a less severe OCT grade is shown on the right column.

(A) Central Subfield

Retinal layer	Group/mean retinal thickness (μm)		Absolute difference between groups (μm)	Relative difference between groups (%)	*P*
Full	CIMO/325.5	CTMO/286.4	39.1	13.7	<0.001
	CIMO/325.5	NCTMO/282.9	42.6	15.1	<0.001
	CIMO/325.5	NMO/270.6	54.9	20.3	<0.001
	CIMO/325.5	HC/282.7	42.8	15.1	<0.001
RNFL	CIMO/17.4	CTMO/13.8	3.6	26.1	0.038
	CIMO/17.4	NMO/13.2	4.2	31.8	<0.001
	CIMO/17.4	HC/13.3	4.1	30.8	0.004
GCL	CIMO/20.2	CTMO/15.7	4.5	28.7	<0.001
	CIMO/20.2	NCTMO/15.9	4.3	27.0	0.001
	CIMO/20.2	NMO/14.8	5.4	36.5	<0.001
	CIMO/20.2	HC/16.3	3.9	23.9	<0.001
IPL	CIMO/25.8	CTMO/21.8	4.0	18.3	<0.001
	CIMO/25.8	NCTMO/21.4	4.4	20.6	<0.001
	CIMO/25.8	NMO/20.9	4.9	23.4	<0.001
	CIMO/25.8	HC/22.4	3.4	15.2	<0.001
INL	CIMO/30.3	CTMO/22.2	8.1	36.5	<0.001
	CIMO/30.3	NCTMO/22.8	7.5	32.9	<0.001
	CIMO/30.3	NMO/20.3	10.0	49.3	<0.001
	CIMO/30.3	HC/19.8	10.5	53.0	<0.001
OPL	CIMO/30.2	NMO/27.8	2.4	8.6	0.037
	CIMO/30.2	HC/26.5	3.7	14.0	0.006
ONL	CIMO/101.9	NCTMO/91.7	10.2	11.1	0.003
	CIMO/101.9	NMO/85.0	16.9	19.9	<0.001
	CIMO/101.9	HC/94.1	7.8	8.3	0.011
	CTMO/95.2	NMO/85.0	10.2	12.0	<0.001
	NMO/85.0	HC/94.1	9.1	9.7	0.001

(B) Inner Subfields

Retinal layer	Group/mean retinal thickness (μm)		Absolute difference between groups (μm)	Relative difference between groups (%)	*P**
Full	CIMO/358.3	NMO/327.4	30.9	9.4	<0.001
	CIMO/358.3	HC/342.0	16.3	4.8	0.018
	CTMO/352.7	NMO/327.4	25.3	7.7	<0.001
	NCTMO/344.6	NMO/327.4	17.2	5.3	0.037
	NMO/327.4	HC/342.0	14.6	4.3	0.032
RNFL	CIMO/24.7	NMO/22.3	2.4	10.8	<0.001
	CIMO/24.7	HC/22.9	1.8	7.9	0.032
	CTMO/25.1	NMO/22.3	2.8	12.6	<0.001
	CTMO/25.1	HC/22.9	2.2	9.6	0.030
GCL	CIMO/50.0	NMO/46.6	3.4	7.3	<0.001
	CTMO/50.7	NMO/46.6	4.1	8.8	0.001
	NCTMO/50.8	NMO/46.6	4.2	9.0	0.003
	NMO/46.6	HC/51.3	4.7	9.2	<0.001
IPL	CIMO/41.1	NMO/38.6	2.5	6.5	<0.001
	CTMO/41.6	NMO/38.6	3.0	7.8	<0.001
	NCTMO/41.2	NMO/38.6	2.6	6.7	0.006
	NMO/38.6	HC/42.4	3.8	9.0	<0.001
INL	CIMO/42.2	NMO/38.4	3.8	9.9	<0.001
	CIMO/42.2	HC/39.1	3.1	7.9	<0.001
	CTMO/42.5	NMO/38.4	4.1	10.7	<0.001
	CTMO/42.5	HC/39.1	3.4	8.7	0.001
	NCTMO/41.1	NMO/38.4	2.7	7.0	0.017
OPL	CTMO/36.0	NMO/33.7	2.3	6.8	0.037
ONL	CIMO/76.3	NMO/66.6	9.7	14.6	<0.001
	CIMO/76.3	HC/70.9	5.4	7.6	0.029
	CTMO/74.3	NMO/66.6	7.7	11.6	<0.001

(C) Outer Subfields

Retinal layer	Group/mean retinal thickness (μm)		Absolute difference between groups (μm)	Relative difference between groups (%)	*P**
Full	CIMO/307.9	NMO/287.4	20.5	7.1	<0.001
	CTMO/310.0	NMO/287.4	22.6	7.8	<0.001
	NCTMO/302.4	NMO/287.4	15.0	5.2	0.013
RNFL	CIMO/37.2	NMO/34.2	3.0	8.8	<0.001
	CTMO/38.0	NMO/34.2	3.8	11.1	<0.001
	NCTMO/37.2	NMO/34.2	3.0	8.8	0.047
	NMO/34.2	HC/37.3	3.1	8.3	0.004
GCL	CTMO/36.1	NMO/33.1	3.0	9.1	<0.001
	NCTMO/35.7	NMO/33.1	2.6	7.9	0.011
IPL	CIMO/28.4	CTMO/30.0	1.6	5.3	0.041
	CIMO/28.4	NMO/27.2	1.2	4.4	0.040
	CTMO/30.0	NMO/27.2	2.8	10.3	<0.001
	NCTMO/29.3	NMO/27.2	2.1	7.7	0.004
	NMO/27.2	HC/28.8	1.6	5.6	0.016
INL	CIMO/33.4	NMO/31.5	1.9	6.0	<0.001
	CTMO/34.1	NMO/31.5	2.6	8.3	<0.001
	CTMO/34.1	HC/32.0	2.1	6.6	0.009
	NCTMO/33.7	NMO/31.5	2.2	7.0	0.002
OPL	CTMO/29.5	NMO/27.7	1.8	6.5	<0.001
	CTMO/29.5	HC/27.7	1.8	6.5	0.004
	NCTMO/29.1	NMO/27.7	1.4	5.1	0.046
ONL	CIMO/62.8	NMO/55.4	7.4	13.4	<0.001
	CIMO/62.8	HC/57.8	5.0	8.7	0.010
	CTMO/63.6	NMO/55.4	8.2	14.8	<0.001
	CTMO/63.6	HC/57.8	5.8	10.0	0.013

7.3.8.4 Comparison of HC and PWD who were treated (TT) and not treated (NT)

When examining the retinal thicknesses of HC and PWD treatment outcomes, TT (N=25) had the highest retinal thickness compared to NT (N=267) or HC across most retinal layers in all subfields. Notable exceptions were GCL and IPL in the inner subfields where HC had the highest retinal thickness beyond that of TT and NT (Figure 7.13, Table 7.22). In addition, NT (14.5±1.3µm) had the thickest RPE in the outer subfields and not TT (TT 14.2±1.6µm, HC 14.0±1.1µm,). TT had a wider range of retinal thickness and therefore higher SD compared to those who had NT and HC (Table 7.22).

As expected, the most significant pairwise comparisons were between TT vs NT and TT vs HC. The significant pairwise comparisons between NT and HC were in the INL in the CSF (p=0.012) and GCL (p=0.010) and IPL (p<0.001) in the inner subfields. There were significant pairwise comparisons in all layers except RPE (Table 7.23).

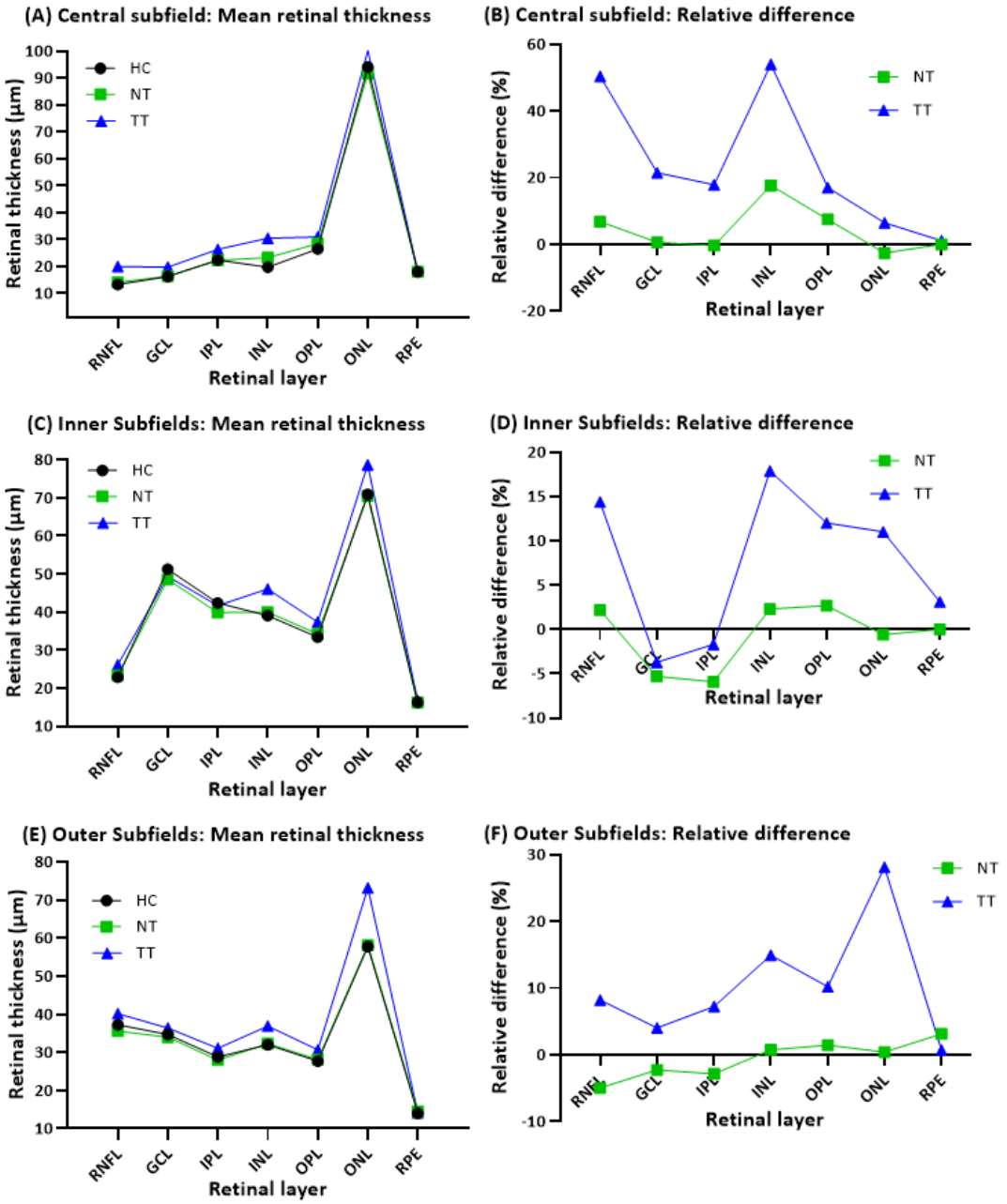


Figure 7.13 Mean retinal thickness (µm) and relative difference (RD; %) of various retinal layers in healthy controls (HC) and people with diabetes (PWD) who were not treated (NT) and PWD who were treated (TT) in the central subfield (A, B), inner subfields (C, D) and outer subfields (E, F). RD is the difference in retinal thickness between the different treatment groups and HC divided by the retinal thickness of the HC expressed as a percentage. Error bars have been omitted for clarity.

Table 7.22. Retinal thicknesses of different retinal layers in the central, inner and outer subfields in HC and PWD who were not treated (NT) or treated (TT) expressed in absolute difference (AD; calculated as the difference between the mean retinal thickness of different retinopathy groups and HC) and relative difference (RD; calculated as AD divided by the retinal thickness of HC expressed as a percentage)

	Full			RNFL			GCL			IPL			INL			OPL			ONL			RPE		
Group	Mean±SD (μm)	AD (μm)	RD (%)	Mean±SD (μm)	AD (μm)	RD (%)	Mean±SD (μm)	AD (μm)	RD (%)	Mean±SD (μm)	AD (μm)	RD (%)	Mean±SD (μm)	AD (μm)	RD (%)	Mean±SD (μm)	AD (μm)	RD (%)	Mean±SD (μm)	AD (μm)	RD (%)	Mean±SD (μm)	AD (μm)	RD (%)
	Central Subfield																							
HC	282.7±23.9	0	0	13.3±2.0	0	0	16.3±4.6	0	0	22.4±4.1	0	0	19.8±6.3	0	0	26.5±5.6	0	0	94.1±9.7	0	0	18.0±2.1	0	0
NT	285.0±31.20	2.3	0.8	14.2±5.4	0.9	6.8	16.4±4.9	0.1	0.6	22.3±4.3	-0.1	-0.4	23.3±7.7	3.5	17.7	28.5±6.0	2.0	7.5	91.7±14.7	-2.4	-2.6	18.0±1.9	0	0
TT	358.2±127.7	75.5	26.7	20.0±18.4	6.7	50.4	19.8±10.7	3.5	21.5	26.4±11.0	4.0	17.9	30.5±12.4	10.7	54.0	31.0±7.3	4.5	17.0	100.2±23.0	6.1	6.5	18.2±2.6	0.2	1.1
	Inner Subfields																							
HC	342.0±13.5	0	0	22.9±1.9	0	0	51.3±4.4	0	0	42.4±2.9	0	0	39.1±3.1	0	0	33.4±3.9	0	0	70.9±8.4	0	0	16.3±1.4	0	0
NT	338.2±21.1	-3.8	-1.1	23.4±3.7	0.5	2.2	48.6±6.1	-2.7	-5.3	39.9±4.1	-2.5	-5.9	40.0±4.1	0.9	2.3	34.3±4.3	0.9	2.7	70.5±10.4	-0.4	-0.6	16.3±1.4	0	0
TT	388.4±82.4	46.4	13.6	26.2±5.5	3.3	14.4	49.4±5.2	-1.9	-3.7	41.7±3.6	-0.7	-1.7	46.1±7.3	7.0	17.9	37.4±5.9	4.0	12.0	78.7±16.7	7.8	11.0	16.8±2.6	0.5	3.1
	Outer Subfields																							
HC	297.2±11.9	0	0	37.3±4.6	0	0	34.7±3.2	0	0	28.8±2.3	0	0	32.0±1.9	0	0	27.7±1.8	0	0	57.8±6.1	0	0	14.0±1.1	0	0
NT	294.5±17.1	-2.7	-0.9	35.6±5.4	-1.7	-4.6	34.0±4.0	-0.7	-2.0	28.0±3.0	-0.8	-2.8	32.3±2.8	0.3	0.9	28.1±2.4	0.4	1.4	58.1±7.7	0.3	0.5	14.5±1.3	0.5	3.6
TT	342.3±54.9	45.1	15.2	40.2±5.4	2.9	7.8	36.4±4.2	1.7	4.9	31.1±3.5	2.3	8.0	36.9±4.4	4.9	15.3	30.7±3.5	3.0	10.8	73.2±17.3	15.4	26.6	14.2±1.6	0.2	1.4

Negative AD and RD indicate lower retinal thickness compared to HC while positive AD and RD indicate higher retinal thickness compared to HC.

Table 7.23. Summary of statistically significant ANOVA pairwise comparisons of different retinal layer thickness in the central, inner and outer subfields in healthy controls (HC) and PWD who were not treated (NT) or were treated (TT) with absolute difference (AD; calculated as difference between the mean retinal thickness of two groups), relative difference (RD; calculated as AD divided by the retinal thickness of the less severe group expressed as a percentage) and *p* value shown. Only statistically significant ANOVA pairwise comparisons are shown. Non-statistically significant ANOVA pairwise comparisons have been omitted for clarity. All *p* values were adjusted using Bonferroni corrections. The group with a more severe grade is shown on the left column and the group with a less severe grade is shown on the right column.

Retinal layer	Group/mean retinal thickness (μm)		Absolute difference between groups (μm)	Relative difference between groups (%)	*P*
Central Subfield					
Full	TT/358.2	NT/285.0	73.2	25.7	<0.001
	TT/358.2	HC/282.7	75.5	26.7	<0.001
RNFL	TT/20.0	NT/14.2	5.8	43.7	<0.001
	TT/20.0	HC/13.3	6.7	53.4	<0.001
GCL	TT/19.9	NT/16.4	3.5	21.3	0.014
	TT/19.9	HC/16.3	3.6	22.1	0.031
IPL	TT/26.4	NT/22.3	4.1	18.4	0.001
	TT/26.4	HC/22.4	4.0	17.9	0.005
INL	TT/30.6	NT/23.3	7.3	33.1	<0.001
	TT/30.6	HC/19.8	10.8	54.5	<0.001
	NT/23.3	HC/19.8	3.5	17.7	0.012
OPL	TT/31.0	HC/26.5	4.5	17.0	0.013
ONL	TT/100.2	NT/91.7	8.5	9.3	0.030
Inner Subfields					
Full	TT/388.4	NT/338.2	50.2	14.8	<0.001
	TT/388.4	HC/342.0	46.4	13.6	<0.001
RNFL	TT/26.2	NT/23.4	2.8	14.2	0.001
	TT/26.2	HC/22.9	3.3	14.4	0.001
GCL	NT/48.6	HC/51.3	2.7	5.3	0.010
IPL	NT/39.9	HC/42.4	2.5	5.9	<0.001
INL	TT/46.1	NT/40.0	6.1	15.3	<0.001
	TT/46.1	HC/39.1	7.0	17.9	<0.001
OPL	TT/37.4	NT/34.3	3.1	9.0	0.005
	TT/37.4	HC/33.4	4.0	12.0	0.001
ONL	TT/78.7	NT/70.5	8.2	11.6	0.002
	TT/78.7	HC/70.9	7.8	11.0	0.013
Outer Subfields					
Full	TT/342.3	NT/294.5	47.8	16.2	<0.001
	TT/342.3	HC/297.2	45.1	15.2	<0.001
RNFL	TT/40.2	NT/35.6	4.6	12.9	<0.001
GCL	TT/36.4	NT/34.0	2.4	7.1	0.016
IPL	TT/31.1	NT/28.0	3.1	11.1	<0.001
	TT/31.1	HC/28.8	2.3	8.0	0.007
INL	TT/37.0	NT/32.3	4.7	14.6	<0.001
	TT/37.0	HC/32.0	5.0	15.6	<0.001
OPL	TT/30.7	NT/28.1	2.6	9.3	<0.001
	TT/30.7	HC/27.7	3.0	10.8	<0.001
ONL	TT/73.2	NT/58.1	15.1	26.0	<0.001
	TT/73.2	HC/57.8	15.4	26.4	<0.001

7.3.8.5 Section summary

This section shows that compared to HC, the reduction in the full retinal thickness in PWD with no clinical evidence of DR (R0M0, NMO) and were NT were mainly due to thinning of the inner retinal layers seen in the inner and outer subfields. In the CSF, there was thinning of the ONL (NMO) while in the inner subfields, there were thinning of the GCL and IPL (M0, NMO, NT groups). In the outer subfields, there were thinning of the RNFL (R0 and NMO groups) and IPL (NMO).

7.3.9 COMPARISON OF RETINAL THICKNESS IN HC (GROUP 1) AND PWD WITHOUT DR (GROUP 2) AND PWD WITH MINIMAL DR (GROUP 3)

Comparison of retinal thickness between HC and PWD in Sections 7.3.6 and 7.3.8 revealed that PWD who have been graded as R0 or R1 or M0 or NMO tended to have lower retinal thicknesses across most subfields compared to HC (Figure 7.14). This appeared to be due to thinning of the inner retinal layers (RNFL, GCL, IPL) and ONL across most subfields (Figure 7.15). However, in Sections 7.3.6 and 7.3.8, the classifications of each eye were based on retinopathy, maculopathy or Liverpool OCT grades independently of other classifications. Therefore, a participant graded as M0 could also have been graded as R2 in the same eye.

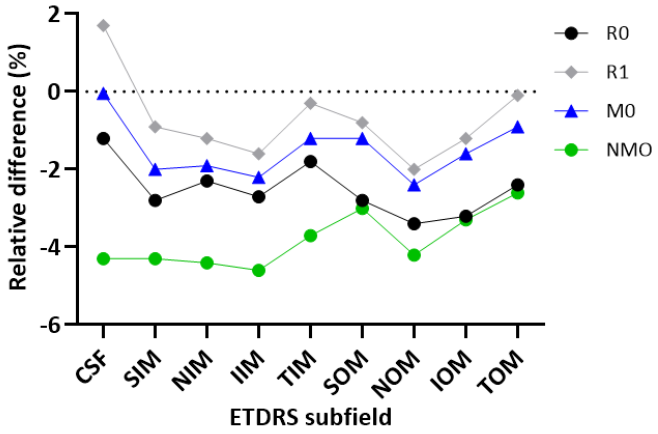


Figure 7.14 Relative difference (RD;%) of PWD with no retinopathy (R0; black line) or minimal retinopathy (R1; grey line), no maculopathy (M0; blue line) or no macular oedema (NMO; green line) compared to healthy controls (HC; dotted black line) in full retinal thickness across all ETDRS subfields. RD was calculated as the difference between mean retinal thicknesses of PWD graded as R0 or R1 or M0 or NMO and HC divided by the retinal thickness of HC expressed as a percentage. Error bars have been omitted for clarity.

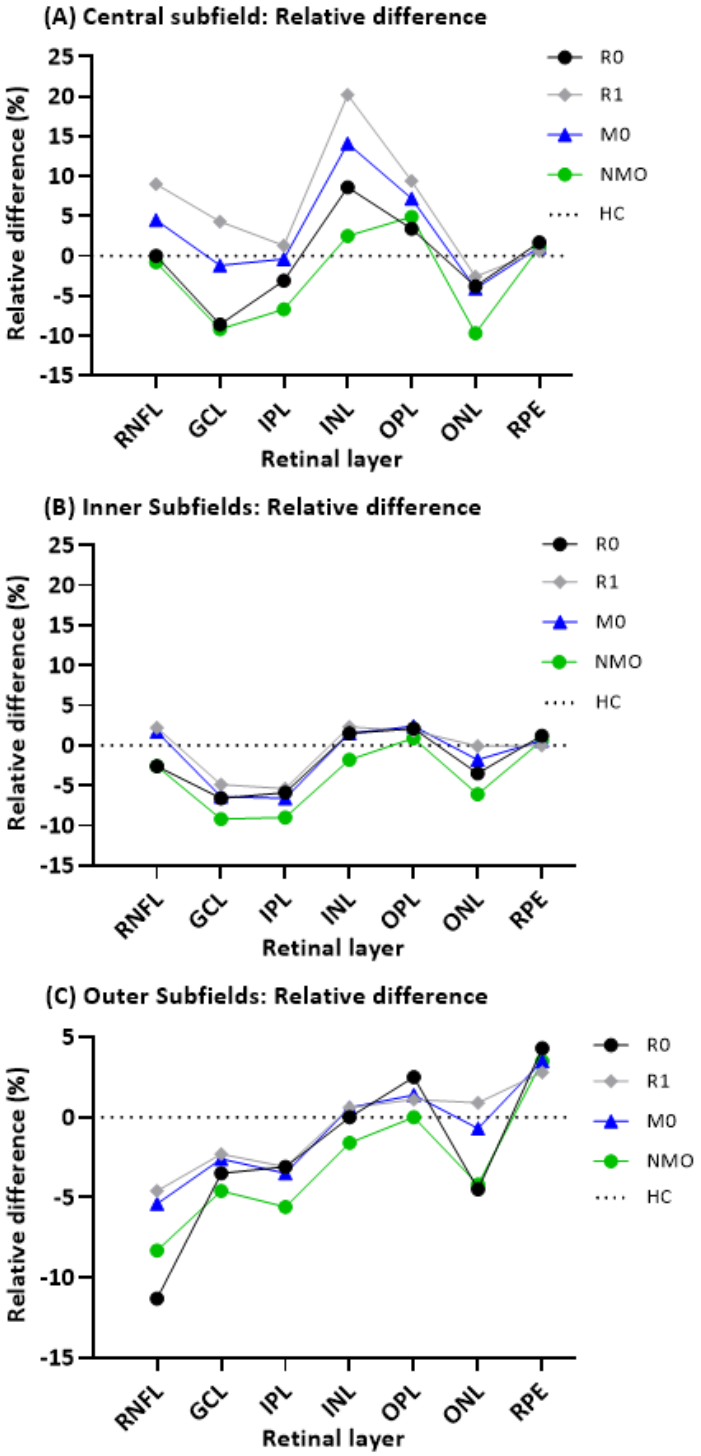


Figure 7.15 Relative difference (RD;%) of different retinal layers in healthy controls (HC; dotted black line) and people with diabetes (PWD) with no retinopathy (R0; black line) or minimal retinopathy (R1; grey line) or no maculopathy (M0; blue line) or no macular oedema (NMO; green line) in the central subfield (A), inner subfields (B) and outer subfields (C). RD was calculated as the difference between mean retinal thicknesses of PWD graded as R0 or R1 or M0 or NMO and HC divided by the retinal thickness of HC expressed as a percentage. Error bars have been omitted for clarity.

Based on the above observations, this section aims to explore if PWD had thinning of the retina in early DR (R0 and R1) compared to HC and if so, which of these layers are involved. All eyes graded as M1 or had macular oedema (CIMO, CTMO and NCTMO) were excluded. 50 eyes of HC (Group 1) were compared to 26 eyes with no DR and no macular oedema (Group 2; R0M0 and NMO) and 46 eyes with minimal DR and no macular oedema (Group 3; R1M0 and NMO).

The group of HC has been described in Section 7.3.2 (Table 7.3). The description of groups 2 and 3 are shown in Table 7.24. It can be seen that the three groups had a similar mean age (Group 1 55±14y, Group 2 55±14y, Group 3 57±17y; One-way ANOVA $F_{2,119}$=0.313, p=0.732) and gender distribution (χ^2=0.103, p=0.950) with no significant differences between the groups. The proportion of Caucasian (%) was significantly different between the groups (Group 1, 96%; Group 2, 77%; Group 3, 85%; p=0.032). It can be seen that Group 1 had the best hRSD threshold, distance and near VA among the groups. The hRSD threshold and distance VA were also progressively worse from Group 1 to 3. One-way ANOVA showed significant differences in hRSD threshold ($F_{2,119}$=9.475, p<0.001), distance ($F_{2,118}$=12.991, p<0.001) and near VA ($F_{2,119}$=6.896, p=0.001) between the groups. Further analyses of these differences are covered in the next chapter (Section 8.3.3), which examines the visual function of PWD compared to HC. There were also significant differences in the type of diabetes in groups 2 and 3 (p=0.024) with more type 1 diabetes in Group 3 (26%) compared to Group 2 (4%). As expected, Group 3 had a significantly longer duration of diabetes (Group 2, 7.3±4.1y; Group 3, 16.6±9.2y; t=4.886, p<0.001), higher HbA_{1C} levels (Group 2, 59.2±14.1mmol/mol; Group 3, 72.6±22.5; t=2.734, p=0.008) and a higher proportion of PWD on insulin (Group 2, 12%; Group 3, 50%; p=0.001) compared to Group 2. Both groups 2 and 3 had reasonably controlled BP with no significant differences in the systolic and diastolic BP between them (systolic t=1.135, p=0.261; diastolic t=1.213, p=0.230).

Table 7.24 Descriptive analyses of healthy controls (HC; Group 1) and people with diabetes (PWD) without diabetic retinopathy (DR) and no macular oedema (R0M0 and NMO; Group 2) and PWD with minimal DR and no macular oedema (R1M0 and NMO; Group 3)

Group (No. of eyes)	Group 1 (N=50)	Group 2 (N=26)	Group 3 (N=46)	Statistical differences between groups	
				Test	Results*
Age (y) Mean±SD (range)	55±14 (22-85)	55±14 (23-76)	57±17 (20-86)	One-way ANOVA with degrees of freedom and *p* value shown	$F_{2,119}$=0.313, *p*=0.732
Gender (No.) M F	26 24	14 12	23 23	Chi-square test	χ^2=0.103, *p*=0.950
Ethnicity Caucasian (%)	48 (96%)	20 (77%)	39 (85%)	Fisher's exact test	***p*=0.032**
hRSD (logMAR)	-0.77±0.11	-0.68±0.18	-0.61±0.25	One-way ANOVA with degrees of freedom and *p* value shown	$F_{2,119}$=9.475, ***p*<0.001**
Distance VA (logMAR)	-0.08±0.12	0.03±0.15	0.06±0.16		$F_{2,118}$=12.991, ***p*<0.001**
Near VA (logMAR)	0.06±0.16	0.21±0.25	0.19±0.22		$F_{2,119}$=6.896, ***p*=0.001**
Type Diabetes (No.) Type 1 Type 2	NA	1 (4%) 25 (96%)	12 (26%) 34 (73%)	Fisher's exact test	***p*=0.024**
Duration of Diabetes (y)	NA	7.3±4.1	16.6±9.2	T-statistics	*t*=4.886, ***p*<0.001**
HbA_{1c} (mmol/mol)	NA	59.2±14.1	72.6±22.5	T-statistics	*t*=2.734, ***p*=0.008**
On Insulin (%)	NA	3 (12%)	23 (50%)	Fisher's exact test	*p*=**0.001**
BP (mmHg) Systolic Diastolic	NA	144±15 84±11	137±24 79±12	T-statistics: Systolic Diastolic	*t*=1.135, *p*=0.261 *t*=1.213, *p*=0.230

*Statistically significant results shown in bold.
Not applicable (NA)

The mean±SD age of Group 2 was 55.1±13.8y (range 23-76y). There were 14 males and 12 females, and 20 (77%) were Caucasian. Mean± SD distance VA, near VA and hRSD threshold were 0.03±0.15, 0.21±0.25 and -0.68±0.18 logMAR respectively. One had type 1 diabetes while 25 had type 2 diabetes. Their mean duration of diabetes was 7.3±4.1y. Their mean±SD HbA_{1c} was 59.2±14.1mmol/mol and 3 (12%) were on insulin. Their mean± SD systolic and diastolic BP were 143.9±14.5mmHg and 83.6±11mmHg respectively.

The mean±SD age of Group 3 was 56.9±16.6y (range 20-86y). There were 23 males and 23 females, and 39 (85%) were Caucasian. Mean± SD distance VA, near VA and hRSD threshold were 0.06±0.16, 0.19±0.22 and -0.61±0.25 logMAR respectively. 12 had type 1 diabetes while 34 had type 2 diabetes. Their mean duration of diabetes was 16.6±9.2y. Their mean±SD HbA_{1c} was 72.6±22.5mmol/mol and 23 (50%) were on insulin. Their mean± SD

systolic and diastolic BP were 137.1±24.3mmHg and 78.8±11.5mmHg respectively. Compared to Group 2, Group 3 had more participants with type 1 diabetes and they also had a longer duration of diabetes, higher HbA_{1c} and more participants were on insulin.

Comparison of the full retinal thicknesses of the three groups revealed that Group 1 had the highest thickness across all subfields (Figure 7.16). This difference was statistically significant in the CSF between Groups 1 and 3 (p=0.047). In all the inner and outer subfields, Groups 2 and 3 had significantly lower full retinal thickness compared to Group 1 (Table 7.26).

Compared to Group 1 in the inner subfields, Group 2 had a -4.1% (p=0.001), -3.7% (p=0.009), -4.2% (p=0.001) and -3.2% (p=0.011) reductions in the SIM, NIM, IIM and TIM thicknesses respectively (Figure 7.16, Table 7.26). Similarly, in the outer subfields, Group 2 had reduced full retinal thicknesses compared to Group 1 (SOM RD -3.7%, p=0.008; NOM RD -4.5%, p=0.002; IOM RD -4.3%, p=0.001; TOM RD -3.5%, p=0.002).

Group 3 had significantly lower retinal thicknesses in the CSF, inner and outer subfields compared to Group 1. The difference in retinal thicknesses between Groups 1 and 3 ranged from -2.9% in the TOM (p=0.003) to -5.1% in the IIM (p<0.001) (Figure 7.16, Table 7.26).

There were also progressive reductions in the CSF and inner subfields and NOM with increasing DR severity from Group 1 to Group 2 and then to Group 3 (Table 7.25). For example, the mean CSF thickness of Group 1 was 282.7±23.9µm, which decreased to 275.2±18.6µm in Group 2 (RD -2.7%,) and this further decreased to 270.8±25.7µm in Group 3 (RD -4.2%). There were similar trends across the inner subfields and NOM (Figure 7.16, Table 7.25).

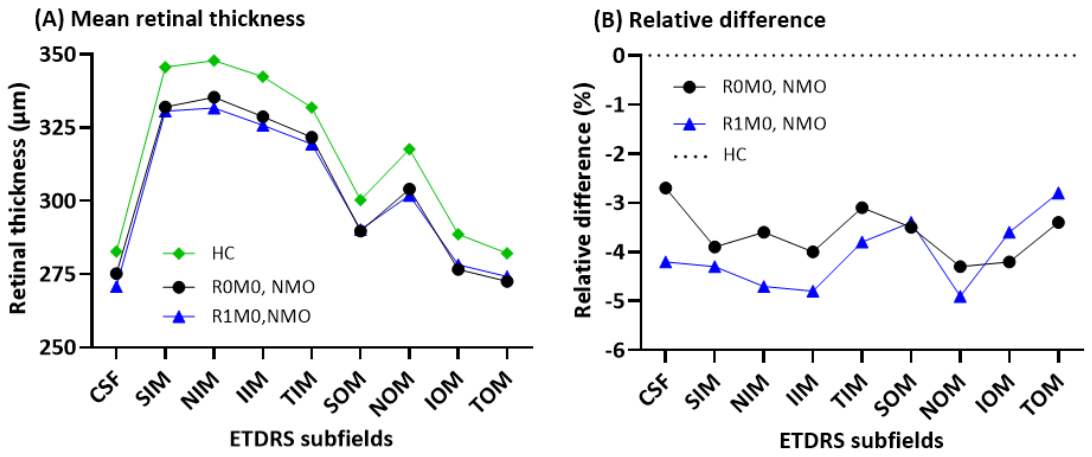


Figure 7.16 Mean full retinal thickness (µm) (A) and relative difference (RD;%) (B) in healthy controls (HC; green line in A, dotted black line in B), people with diabetes (PWD) who had no diabetic retinopathy and no macular oedema (R0M0 and NMO; black lines) and PWD with minimal diabetic retinopathy and no macular oedema (R1M0 and NMO; blue lines). RD was calculated as the difference between the mean retinal thickness of PWD and HC divided by the retinal thickness of the HC expressed as a percentage. Error bars have been omitted for clarity.

Table 7.25. Full retinal thicknesses (mean±SD, µm) in all ETDRS subfields in healthy controls (HC; Group 1), PWD with no diabetic retinopathy and no macular oedema (Group 2; R0M0 and NMO) and minimal diabetic retinopathy and no macular oedema (Group 3; R1M0 and NMO). Retinal thickness expressed in absolute difference (AD; µm) and relative difference (RD; %) compared to HC. RD was calculated as the difference between the mean retinal thickness of PWD and HC divided by the retinal thickness of the HC expressed as a percentage.

ETDRS Subfields	Group 1 (HC) (mean±SD, µm)	Group 2 (R0M0, NMO) (mean±SD, µm)	Group 3 (R1M0, NMO) (mean±SD, µm)	Group 1 and 2		Group 1 and 3	
				AD (µm)	RD (%)	AD (µm)	RD (%)
CSF	282.7±23.9	275.2±18.6	270.8±25.7	-7.5	-2.7	-11.9	-4.2
SIM	345.6±14.6	332.0±12.7	330.6±18.2	-13.6	-3.9	-15.0	-4.3
NIM	347.9±15.8	335.4±15.9	331.7±18.6	-12.5	-3.6	-16.2	-4.7
IIM	342.4±13.3	328.7±14.9	325.8±16.4	-13.7	-4.0	-16.6	-4.8
TIM	331.9±12.3	321.7±14.3	319.4±16.1	-10.2	-3.1	-12.5	-3.8
SOM	300.3±13.7	289.7±10.6	290.2±16.0	-10.6	-3.5	-10.1	-3.4
NOM	317.6±13.2	304.0±14.9	301.9±18.4	-13.6	-4.3	-15.7	-4.9
IOM	288.6±11.8	276.6±12.1	278.2±15.5	-12.0	-4.2	-10.4	-3.6
TOM	282.1±12.1	272.5±9.6	274.2±11.8	-9.6	-3.4	-7.9	-2.8

Table 7.26. Summary of statistically significant ANOVA pairwise comparisons of full retinal thicknesses in healthy controls (HC; Group 1), PWD with no diabetic retinopathy and no macular oedema (Group 2; R0M0 and NMO) and minimal diabetic retinopathy and no macular oedema (Group 3; R1M0 and NMO). Absolute difference (AD; calculated as difference between the mean retinal thickness of two groups), relative difference (RD; calculated as AD divided by the retinal thickness of the less severe group expressed as a percentage) and *p* value shown. Only statistically significant ANOVA pairwise comparisons are shown. Non-statistically significant ANOVA pairwise comparisons have been omitted for clarity. All *p* values were adjusted using Bonferroni corrections. The group with a more severe grade is shown on the left column and the group with a less severe grade is shown on the right column.

Subfield	Group/mean retinal thickness (μm)		Absolute difference between groups (μm)	Relative difference between groups (%)	*P*
CSF	Group 1/282.7	Group 3/270.8	11.9	4.4	0.047
SIM	Group 1/345.6	Group 2/332.0	13.6	4.1	0.001
	Group 1/345.6	Group 3/330.6	15.0	4.5	<0.001
NIM	Group 1/347.9	Group 2/335.4	12.5	3.7	0.009
	Group 1/347.9	Group 3/331.7	16.2	4.9	<0.001
IIM	Group 1/342.4	Group 2/328.7	13.7	4.2	0.001
	Group 1/342.4	Group 3/325.8	16.6	5.1	<0.001
TIM	Group 1/331.9	Group 2/321.7	10.2	3.2	0.011
	Group 1/331.9	Group 3/319.4	12.5	3.9	<0.001
SOM	Group 1/300.3	Group 2/289.7	10.6	3.7	0.008
	Group 1/300.3	Group 3/290.2	10.1	3.5	0.003
NOM	Group 1/317.6	Group 2/304.0	13.6	4.5	0.002
	Group 1/317.6	Group 3/301.9	15.7	5.2	<0.001
IOM	Group 1/288.6	Group 2/276.6	12.0	4.3	0.001
	Group 1/288.6	Group 3/278.2	10.4	3.7	0.001
TOM	Group 1/282.1	Group 2/272.5	9.6	3.5	0.002
	Group 1/282.1	Group 3/274.2	7.9	2.9	0.003

Analyses of the retinal thicknesses in the different retinal layers of the three groups showed that in CSF, Groups 2 and 3 had lower retinal thickness in the RNFL, GCL, IPL and ONL and higher retinal thickness in the INL, OPL and RPE compared to Group 1 (Figure 7.17, Table 7.27). In the inner and outer subfields, Groups 2 and 3 had lower retinal thicknesses in the RNFL, GCL, IPL, INL and ONL compared to Group 1.

OPL thickness was similar in all groups in the inner subfields (Group 1 33.4±3.9μm, Group 2 33.6±4.5μm, Group 3 33.8±3.3μm) and outer subfields (Group 1 27.7±1.8μm, Group 2 28.0±2.4μm, Group 3 27.6±1.7μm). Likewise, RPE thickness was similar in all groups in the inner subfields (Group 1 16.3±1.4μm, Group 2 16.6±1.3μm, Group 3 16.3±1.4μm) and outer subfields (Group 1 14.1±1.1μm, Group 2 14.8±1.0μm, Group 3 14.6±1.3μm) (Figure 7.17, Table 7.27).

Pairwise comparisons showed that in the CSF, Groups 2 and 3 had significantly lower ONL compared to Group 1 (Groups 1 and 2 p=0.041; Groups 1 and 3 p<0.001) (Table 7.28). In the inner subfields, Groups 2 and 3 had significantly lower GCL (Groups 1 and 2 p=0.003; Groups 1 and 3 p<0.001) and IPL (Groups 1 and 2 p<0.001; Groups 1 and 3 p<0.001) compared to Group 1. In the outer subfields, Groups 2 and 3 had significantly lower RNFL compared to Group 1 (Groups 1 and 2 p<0.001; Groups 1 and 3 p=0.015). In the outer subfields, Group 3 also had significantly lower GCL (p=0.015) and IPL (p=0.001) compared to Group 1.

In the CSF, Group 2 had lost -6.8% of ONL thickness (p=0.041) compared to Group 1 and this increased to -9.7% in Group 3 (p<0.001). In the inner subfields, Group 2 had lost -8.0% of GCL thickness (p=0.003) and this increased to -9.9% in Group 3 (p<0.001) compared to Group 1. Similarly, Group 2 had lost -7.4% of IPL thickness (p<0.001) and this increased to -9.0% in Group 3 (p<0.001) compared to Group 1 (Table 7.28).

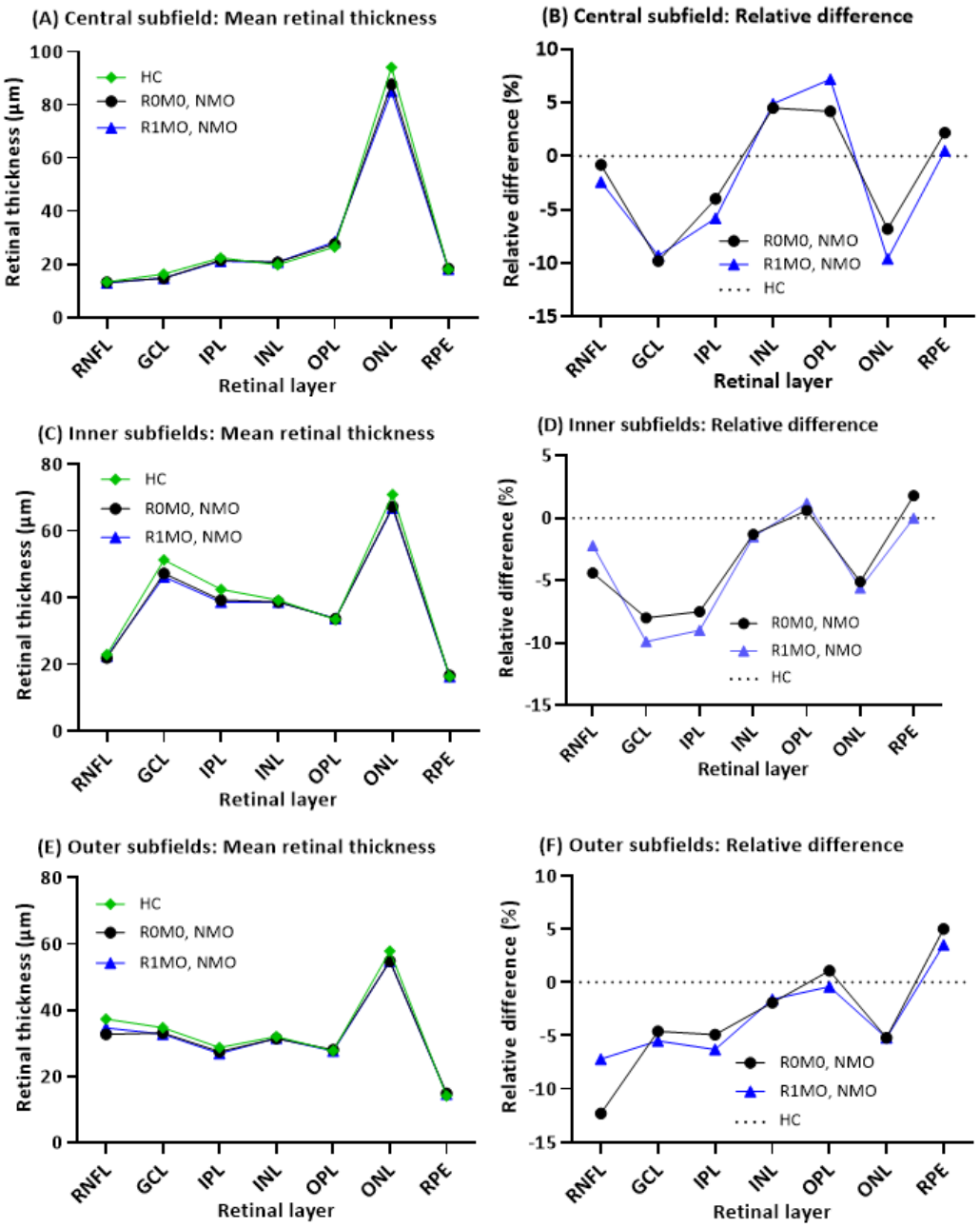


Figure 7.17 Mean retinal thickness (µm) and relative difference (%) of different retinal layers in healthy controls (HC; green lines in A, C and E, dotted black lines in B, D and F), people with diabetes (PWD) who had no diabetic retinopathy and no macular oedema (R0M0 and NMO; black lines) and PWD with minimal diabetic retinopathy and no macular oedema (R1M0 and NMO; blue lines) in the central subfield (A, B), inner subfields (C, D) and outer subfields (E, F). RD was calculated as the difference between the mean retinal thickness of PWD and HC divided by the retinal thickness of the HC expressed as a percentage. Error bars have been omitted for clarity.

Table 7.27. Retinal thicknesses of different retinal layers (µm) in the central, inner and outer subfields in HC (Group 1), PWD with no diabetic retinopathy and no macular oedema (Group 2; R0M0, NMO) and minimal diabetic retinopathy and no macular oedema (Group 3; R1M0, NMO) expressed in absolute difference (AD; calculated as the difference between mean retina thickness of PWD groups and HC) and relative difference (RD; calculated as AD divided by the retinal thickness of HC expressed as a percentage)

Subfield	Group 1 (HC) (mean±SD, µm)	Group 2 (R0M0, NMO) (mean±SD, µm)	Group 3 (R1M0, NMO) (mean±SD, µm)	Group 1 and 2		Group 1 and 3	
				AD (µm)	RD (%)	AD (µm)	RD (%)
Central Subfield							
Full	282.7±23.9	275.2±18.6	270.8±25.7	-7.5	-2.7	-11.9	-4.2
RNFL	13.3±2.0	13.2±2.3	13.0±2.6	-0.1	-0.8	-0.3	-2.4
GCL	16.3±4.6	14.7±4.0	14.8±3.9	-1.6	-9.8	-1.5	-9.3
IPL	22.4±4.1	21.5±3.9	21.1±4.0	-0.9	-4.0	-1.3	-5.8
INL	19.8±6.3	20.7±5.9	20.8±6.1	0.9	4.5	1.0	4.9
OPL	26.5±5.6	27.6±6.6	28.4±5.6	1.1	4.2	1.9	7.2
ONL	94.1±9.7	87.7±11.5	85.0±10.8	-6.4	-6.8	-9.1	-9.7
RPE	18.0±2.1	18.4±2.2	18.1±2.0	0.4	2.2	0.1	0.5
Inner Subfields							
Full	342.0±13.5	329.4±13.9	326.9±16.5	-12.6	-3.7	-15.1	-4.4
RNFL	22.9±1.9	21.9±2.0	22.4±1.8	-1.0	-4.4	-0.5	-2.2
GCL	51.3±4.4	47.2±4.8	46.2±5.6	-4.1	-8.0	-5.1	-9.9
IPL	42.4±3.0	39.2±3.2	38.6±3.6	-3.2	-7.5	-3.8	-9.0
INL	39.1±3.1	38.7±2.8	38.6±3.0	-0.5	-1.3	-0.6	-1.5
OPL	33.4±3.9	33.6±4.5	33.8±3.3	0.2	0.6	0.4	1.2
ONL	70.9±8.4	67.3±8.6	66.9±7.7	-3.6	-5.1	-4.0	-5.6
RPE	16.3±1.4	16.6±1.3	16.3±1.4	0.3	1.8	0	0
Outer Subfields							
Full	297.2±11.9	285.4±10.5	286.3±14.5	-11.8	-4.0	-10.9	-3.7
RNFL	37.3±4.6	32.7±4.3	34.6±4.7	-4.6	-12.3	-2.7	-7.2
GCL	34.7±3.2	33.1±2.8	32.8±3.7	-1.6	-4.6	-1.9	-5.5
IPL	28.8±2.3	27.4±1.9	26.9±2.6	-1.3	-4.9	-1.9	-6.6
INL	32.0±1.9	31.3±2.0	31.5±2.0	-0.6	-1.9	-0.5	-1.6
OPL	27.7±1.8	28.0±2.4	27.6±1.7	0.3	1.1	-0.1	-0.4
ONL	57.8±6.1	54.8±6.5	54.8±6.2	-3.0	-5.2	-3.0	-5.2
RPE	14.1±1.1	14.8±1.0	14.6±1.3	0.7	5.0	0.5	3.5

Table 7.28. Summary of statistically significant ANOVA pairwise comparisons of different retinal layer thicknesses in the central, inner and outer subfields in HC (Group 1) and PWD with no diabetic retinopathy and no macular oedema (Group 2; R0M0, NMO) and minimal diabetic retinopathy and no macular oedema (Group 3; R1M0, NMO) with absolute difference (AD; calculated as difference between the mean retinal thickness of two groups), relative difference (RD; calculated as AD divided by the retinal thickness of the less severe group expressed as a percentage) and *p* value shown. Only statistically significant ANOVA pairwise comparisons are shown. Non-statistically significant ANOVA pairwise comparisons have been omitted for clarity. All *p* values were adjusted using Bonferroni corrections. The group with a more severe grade is shown on the left column and the group with a less severe grade is shown on the right column.

Retinal layer	Group/mean retinal thickness (μm)		Absolute difference between groups (μm)	Relative difference between groups (%)	*P*
Central Subfield					
Full	Group 1/282.7	Group 3/270.8	-11.9	-4.2	0.047
ONL	Group 1/94.1	Group 2/87.7	-6.4	-6.8	0.041
	Group 1/94.1	Group 3/85.0	-9.1	-9.7	<0.001
Inner Subfields					
Full	Group 1/342.0	Group 2/329.4	-12.6	-3.7	0.002
	Group 1/342.0	Group 3/326.9	-15.1	-4.4	<0.001
GCL	Group 1/51.3	Group 2/47.2	-4.1	-8.0	0.003
	Group 1/51.3	Group 3/46.2	-5.1	-9.9	<0.001
IPL	Group 1/42.4	Group 2/39.2	-3.2	-7.5	<0.001
	Group 1/42.4	Group 3/38.6	-3.8	-9.0	<0.001
Outer Subfields					
Full	Group 1/297.2	Group 2/285.4	-11.8	-4.0	0.001
	Group 1/297.2	Group 3/286.3	-10.9	-3.7	<0.001
RNFL	Group 1/37.3	Group 2/32.7	-4.6	-12.3	<0.001
	Group 1/37.3	Group 3/34.6	-2.7	-7.2	0.015
GCL	Group 1/34.7	Group 3/32.8	-1.9	-5.5	0.015
IPL	Group 1/28.8	Group 3/26.9	-1.9	-6.6	0.001
RPE	Group 1/14.1	Group 2/14.8	0.7	5.0	0.044

7.3.9.1 Section summary

There was a progressive worsening of hRSD threshold, distance and near VA from Groups 1 to 3. There were thinning of the full retinal thickness in Group 2 in the inner and outer subfields and this progressed to involve all the subfields in Group 3. There were progressive reductions in retinal thickness in the CSF, inner subfields and NOM from Groups 1 to 3.

Reduction in full retinal thickness was mainly due to thinning of the inner retinal layers in the inner and outer subfields. In the CSF, Groups 2 and 3 had significantly lower ONL compared to Group 1. In the inner subfields, Groups 2 and 3 had significantly lower GCL and IPL compared to Group 1. In the outer subfields, Groups 2 and 3 had significantly lower

RNFL compared to Group 1 and Group 3 also had significantly lower GCL and IPL compared to Group 1. There were progressive loss of ONL in the CSF, GCL and IPL in the inner subfields from Groups 1 to 3.

7.4 CHAPTER DISCUSSION

This chapter examined the characteristics and OCT findings of a group of PWD who have been newly referred from the LDESP to the DEC during their first visit. This group of PWD is likely to be at a more severe end of the DR spectrum as they were screen positive in one or both eyes and they were referred to the DEC compared to the group of PWD who were screen negative and remained at LDESP.

The good inter-grader reliability results (Section 7.3.5) are reassuring as there were no significant differences in the CST found between the two graders. This indicates that subsequent measurements that were obtained by the first grader are valid and reliable. As discussed in Chapter 3, the centring of the ETDRS grid on the fovea used to obtain the CST during grading was crucial as all the measurements from each subfield and retinal layers were dependent on it.

Current DR screening is based on feature specific grading (NHS Diabetic Eye Screening Programme, 2012). Some of these features act as surrogate markers for DMO but it has been recognised that the current DR screening based on two-dimensional technology cannot detect DMO directly (Olson et al., 2013). Therefore, this screening system is considered to be less effective in detecting maculopathy compared to retinopathy (Olson et al., 2013). Early observations from the EDDMO study supports this idea (Section 7.3.1). The number of PWD in each category of retinopathy (R0, R1, R2, R3) were similar in both screening and at the DEC (Table 7.1). For the 292 PWD in this study, 74 (25.3%) were initially graded as M0 (no maculopathy) while 281 (74.7%) were graded as M1 (maculopathy) at screening. However, when they were seen in the DEC, there was a considerably smaller number graded as having maculopathy with 139 (47.6%) graded as M0 while 153 (52.4%) were graded as M1 (Table 7.1).

If it is accepted that an M1 referral from LDESP that is graded as M0 by DEC represents a false positive, then the relatively high number of these false positives currently represents an increased healthcare burden on the already stretched NHS. Furthermore, such referrals can cause anxiety for PWD who have received a notification to attend DEC for further

assessment (Clarke, 2014). In addition, PWD in the EDDMO study referred to DEC were commonly working-age adults (mean±SD age 54±14y; Table 7.3) and who may have needed to take time off from work or manage other responsibilities, thus reducing work productivity and increasing socioeconomic costs (International Diabetes Federation, 2015, Royal College of Ophthalmologists, 2017a). These issues are important given that the Royal College of Ophthalmologists has projected that the population with DR will increase between 20% and 80% in the next 20 years (Royal College of Ophthalmologists, 2017b). The Royal College of Ophthalmologists has also estimated that approximately 50% of referrals from the DR screening programme are at low risk of vision loss. This is consistent with findings from the EDDMO study that at the PWD's initial DEC visit, only 25 (8.6%) of the 292 PWD who were referred with some suspicion of DR required treatment (Tables 7.3 and 7.4) (Royal College of Ophthalmologists, 2017b).

The majority of the PWD in the EDDMO study were referred due to the presence of exudate within 1 disc diameter of the centre of the fovea (181 of 292 eyes; 62%; Table 7.2). The methods for determining a grade of M1 at LDESP using two-dimensional fundus photographs compared to DEC using three-dimensional slit lamp biomicroscopy by an ophthalmologist may account for some of the disparities in the proportion of M1. In addition, all PWD seen in the DEC received macular OCT making it easier for the ophthalmologist in DEC to diagnose DMO. This observation suggests that a number of PWD do not need be referred to DEC since M1 is referable while M0 is not. This finding highlights a need to change the screening grading criteria or add OCT imaging (or other means of effective testing) in LDESP to reduce the number of referrals to DEC (Mackenzie et al., 2011, Gale et al., 2017, MacEwen C et al., 2019).

Mackenzie et al. (2011) found that OCT imaging is a useful adjunct to colour fundus photography in screening for diabetic maculopathy. In a prospective audit of 311 patients referred from the diabetic eye screening programme in London to St George's Hospital with mild to moderate non-proliferative DR (R1) and maculopathy (M1) in either eye, the patients attended an OCT-guided surveillance clinic. The results showed that these cases had a 42.1% case of having no DMO on OCT imaging when graded by a retinal specialist (Mackenzie et al., 2011). The Gloucestershire Diabetic Eye Screening Programme includes a surveillance clinic with OCT. In an audit of 724 patients referred with background DR and maculopathy seen in the surveillance clinic with OCT, only 20% needed to be referred for further review by an ophthalmologist, thereby saving unnecessary hospital referral in 80%

of screen positive maculopathy patients (Gale et al., 2017). The Queen Alexandra Hospital in Portsmouth introduced a nurse-led OCT clinic and this caused 46% of patients with maculopathy being discharged back to community screening. The hospital estimated that this nurse-led OCT clinic saves 10 new appointment with an ophthalmologist weekly (MacEwen C et al., 2019). These studies indicate that a large proportion of maculopathy suspects (M1) does not need to be referred to DEC if OCT imaging was available alongside colour fundus photography.

PWD who had maculopathy (M1) had a 7 mmol/mol increase in HbA_{1c} compared to PWD without maculopathy (M0) while PWD who were TT had a 20.2 mmol/mol increase in HbA_{1c} compared to PWD who were NT (Section 7.3.3). Yun et al. (2016) found that for PWD who had diabetes for over 10 years, a 1% decrease in HbA_{1c} led to a 37% reduction in the risk of developing DR. Therefore, these HbA_{1c} differences between these groups of PWD are clinically relevant. Since glycaemic control is known to be an important factor in DR progression (Shamoon et al., 1993, Chew et al., 2010, Stratton et al., 2001), these values are unsurprising and confirm that PWD with maculopathy or were TT had worse glycaemic control. However, the HbA_{1c} data from this section only reflects the glycaemic control of the patients in the preceding two to three months before their first visit (Section 2.2.1), which may not reflect the disease severity over the previous years.

In DMO, fluid can initially accumulate in the inner nuclear layer (INL) and outer plexiform layer (OPL) due to a breakdown in the BRB and other factors. In later stages, fluid can accumulate in the IPL and RNFL until the entire thickness of the retina becomes oedematous (Murakami and Yoshimura, 2013, Das et al., 2015). Specifically, an increase in INL thickness has been reported to be due to leakage from the deep retinal plexus and Muller cell swelling (Vujosevic et al., 2016, Bandello et al., 2015). This chapter found that in PWD, higher HbA_{1c} correlated significantly with higher INL and OPL thicknesses across the central, inner and outer subfields (Section 7.3.7, Table 7.15, Figure 7.9). The EDDMO study found that in PWD with early DR (ETDRS 10 and 20, N=112), higher HbA_{1c} correlated significantly with thicker INL and OPL in the CSF (Section 5.3.4, Table 5.8). Since hyperglycaemia is known to cause metabolic dysregulation in diabetes, higher HbA_{1c} which indicates poorer glycaemic control can lead to increased thickness as more fluid accumulates in these layers (Stem and Gardner, 2013). Santos et al. (2019) found that macular oedema is mainly in the INL and OPL in the initial stages of DMO, which is consistent with results from the EDDMO study.

It has been traditionally thought that in PWD, retinal thickness starts from the normal range and then progressively increases with the severity of DR (Cho et al., 2010). However, the results from this chapter found that significant full retinal thinning occurs in PWD across the inner and outer subfields before DR becomes clinically visible on slit-lamp biomicroscopy or causes structural changes on OCT (Tables 7.2.4 and 7.2.5, Section 7.3.9). In PWD with minimal DR, there were significant full retinal thinning across all subfields (Tables 7.2.4 and 7.2.5, Section 7.3.9). Interestingly, there was progressively more reduction in full retinal thicknesses in the CSF, all inner subfields and NOM with increasing DR severity from HC to PWD without DR and then to PWD with minimal DR.

The reduction in retinal thickness was attributed to significant thinning in ONL in the CSF, GCL and IPL in the inner subfields and RNFL in the outer subfields in PWD without DR and in PWD with minimal DR compared to HC (PWD without DR vs HC; PWD with minimal DR vs HC; Figure 7.17, Table 7.28). Significant thinning of the GCL (p=0.015) and IPL (p=0.001) in the outer subfields also contributed to a reduction in retinal thickness in PWD with minimal DR compared to HC (Figure 7.17, Table 7.28).

To compare the results from this chapter with other studies, a review of the studies comparing mean full retinal thickness between HC and PWD without DR and PWD with minimal DR was made. As shown in Table 7.29, the EDDMO study has found significant thinning in the inner and outer subfields in PWD without DR compared to HC; this study has also found significant thinning in the CSF, inner and outer subfields in PWD with minimal DR compared to HC (Table 7.29). Bronson-Castain et al. (2009) and Verma et al. (2009) also found a significant decrease in full retinal thickness in PWD without DR compared to HC. In a later study, Bronson-Castain et al. (2012) found a significant decrease in full retinal thickness in PWD without DR with type 2 diabetes (p<0.03) but not type 1 diabetes (p>0.05) compared to HC. van Dijk et al. (2009) found significant thinning in the pericentral area between PWD with minimal DR compared to HC. In contrast, some groups have found an increase in retinal thickness in PWD without DR compared to HC: Araszkiewicz et al. (2012) in the perifoveal area (p=0.048), Sugimoto et al. (2005) in the superior macular area (p<0.05) and Lattanzio et al. (2002) in the macular area (p<0.001) (Table 7.29).

Table 7.29. Summary of studies of the full retinal thicknesses of healthy controls (HC; Group 1) and people with diabetes (PWD) with no retinopathy (Group 2) and PWD with minimal diabetic retinopathy (Group 3). Significant *p* values are in bold.

Study	Group (No. participants)	Retinal area	Retinal thickness, Mean±SD (μm)			Group 1 vs 2		Group 1 vs 3	
			Group 1	Group 2	Group 3	Thickness compared to Group 1	*P*	Thickness compared to Group 1	*P*
EDDMO study	Group 1: 50 Group 2: 26 Group 3: 46	Central subfield	282.7±23.9	275.2±18.6	270.8±25.7	↓	0.057	↓	**0.047**
		Inner subfields	342.0±13.5	329.4±13.9	326.9±16.5	↓	**0.002**	↓	**<0.001**
		Outer subfields	297. 2±11.9	285.4±10.5	286.3±14.5	↓	**0.001**	↓	**<0.001**
Rodrigues et al. (2015)	Group 1: 28 Group 2: 46 Group 3: 28	All subfields	284.1±13.4	279.0±14.3	271.5±26.2	↓	>0.05	↓	**0.032**
		Central subfield	260.6 ± 24.2	245.5 ± 24.4	254.7±46.9	↓	>0.05	↓	>0.05
Araszkiewicz et al. (2012)	Group 1: 31 Group 2: 54	Fovea	271	271	Not available	↔	0.22	Not available	
		Parafovea	328	335		↑	0.054		
		Perifovea	305	310		↑	**0.048**		
Bronson-Castain et al. (2012)	Group 1: 26 Group 2: 47	Central 20°	277.2±3.3	Type 1 diabetes: 270.7±3.2	Not available	↓	>0.05	Not available	
				Type 2 diabetes: 267.2±3.9		↓	**<0.03**		
Verma et al. (2012)	Group 1: 40 Group 2: 70	Fovea	169.8±14.3	169.4±18.5	Not available	↓	0.901	Not available	
		Central fundus	268.4±12.4	265.3±15.5		↓	0.270		
Park et al. (2011)	Group 1: 40 Group 2: 37 Group 3: 33	Macula	264.7±28.6	265.4±11.1	266.6±23.3	↑	0.512	↑	>0.05
		Superior macula	264.7±13.1	265.7±21.0	268.6±21.4	↑	0.776	↑	>0.05
		Temporal macula	256.1±12.2	258.0±13.1	258.8±18.3	↑	0.783	↑	>0.05
		Inferior macula	263.1±23.0	264.4±20.3	265.40±15.4	↑	0.751	↑	>0.05
		Nasal macula	269.1±12.2	271.3±14.5	273.7±13.7	↑	0.747	↑	>0.05
Cho et al. (2010)	Group 1: 50 Group 2: 20 Group 3: 20	Fovea	141.5±15.3	149.8±14.7	146.9±18.6	↑	>0.05	↑	>0.05
		Horizontal macula	219.2±16.0	207.9±17.8	205.4±17.5	↓	>0.05	↓	>0.05
		Vertical macula	220.1±18.8	209.3±19.3	206.6±15.3	↓	>0.05	↓	>0.05
Bronson-Castain et al. (2009)	Group 1: 26 Group 2: 15	Central 20°	253.7±2.5	243.4±3.7	Not available	↓	**0.02**	Not available	
van Dijk et al. (2009)	Group 1: 59 Group 2: 32 Group 3: 25	Fovea	208.1	211.7	202.0	↑	>0.05	↓	>0.05
		Pericentral	280.2	277.8	269.1	↓	>0.05	↓	**<0.05**
		Peripheral	243.0	241.0	238.7	↓	>0.05	↓	>0.05
Verma et al. (2009)	Group 1: 39 Group 2: 39	Fovea	177.7±14.6	168.6±16.5	Not available	↓	**0.012**	Not available	
Sugimoto et al. (2005)	Group 1: 48 Group 2: 32	Temporal	221.8±11.3	227.1±24.1	Not available	↑	>0.05	Not available	
		Superior	236.1±13.6	248.2±23.3		↑	**<0.05**		
		Nasal	228.7±11.1	237.2±23.2		↑	>0.05		
		Inferior	233.2±14.8	238.3±16.0		↑	>0.05		
Lattanzio et al. (2002)	Group 1: 50 Group 2: 46 Group 3: 66	Macula	161.9±12.9	211.0±37.6	370.8±159.6	↑	**<0.001**	↑	Not available
Massin et al. (2002)	Group 1: 60 Group 2: 30	Central	170±18	174±19	Not available	↑	>0.05	Not available	
		Mean of all subfields	150±19	152±16	Not available	↑	>0.05		

There is emerging evidence that DR is a neurodegenerative disease (Jonsson et al., 2016). As a result, there is interest in studying RNFL and GCL thicknesses in early DR. DR is further discussed as a neurodegenerative disease in Chapters 9 and 10. To compare the results from this chapter with other studies, a review of studies comparing RNFL in HC and PWD without DR and PWD with minimal DR was made and the results are summarised in Table 7.30. The EDDMO study found a decrease in RNFL in PWD without DR (p<0.001) and PWD with minimal DR (p=0.015) compared to HC in the outer subfields. Similar to the EDDMO study, Rodrigues et al. (2015) also found a decrease in RNFL in PWD without DR (p<0.001) and PWD with minimal DR (p<0.001) compared to HC. On the other hand, Vujosevic and Midena (2013), Verma et al. (2012), Park et al. (2011) and Sugimoto et al. (2005) all found a decrease in RNFL thickness in PWD without DR compared to HC in some areas. Bronson-Castain et al. (2012) also found similar results but only in PWD with type 2 diabetes but not type 1 diabetes. In contrast, Araszkiewicz et al. (2012) found a significant increase in RNFL thickness in PWD without DR compared to HC in the optic disc and inferior macular.

Similar to reviews of studies comparing full retinal thickness and RNFL thickness in HC and PWD with early DR, a review of studies comparing GCL thickness in HC and PWD without DR and PWD with minimal DR was also made and the results are summarised in Table 7.31. The EDDMO study found a decrease in GCL thickness in PWD without DR in the inner subfields (p=0.003) compared to HC; there was also a decrease in GCL thickness in PWD with minimal DR in the inner (p<0.001) and outer subfields (p=0.015) compared to HC. Rodrigues et al. (2015) found a decrease in GCL thickness in all subfields in PWD without DR (p=0.039) and PWD with minimal DR (p=0.003) compared to HC. van Dijk et al. (2009) found a decrease in the pericentral (p<0.05) and peripheral areas (p<0.05) in PWD with minimal DR compared to HC. In contrast to other studies, Araszkiewicz et al. (2012) found a significant increase in GCL (superior and inferior macula) and RNFL (optic disc and inferior macula) in PWD without DR compared to HC. Matlach et al. (2014) found that the RTVue OCT used in the study by Araszkiewicz et al. (2012) measured a thicker RNFL and RNFL-GCL-IPL thickness compared to the Cirrus OCT which may explain some of the differences in the results obtained by Araszkiewicz et al. (2012) compared to other studies.

Table 7.30. Summary of studies of mean retinal nerve fibre layer (RNFL) thickness of healthy controls (HC; Group 1) and people with diabetes (PWD) with no retinopathy (Group 2) and PWD with minimal diabetic retinopathy (Group 3). Significant *p* values are in bold.

Study	No. participants	Retinal area	Retinal thickness, Mean±SD (µm)			Group 1 vs 2		Group 1 vs 3	
			Group 1	Group 2	Group 3	Thickness compared to Group 1	*P*	Thickness compared to Group 1	*P*
EDDMO study	Group 1: 50 Group 2: 26 Group 3: 46	Central subfield	13.3±2.0	13.2±2.3	13.0±2.6	↓	1.000	↓	1.000
		Inner subfields	22.9±1.9	21.9±2.0	22.4±1.8	↓	0.126	↓	0.712
		Outer subfields	37.3±4.6	32.7±4.3	34.6±4.7	↓	**<0.001**	↓	**0.015**
Rodrigues et al. (2015)	Group 1: 28 Group 2: 46 Group 3: 28	All subfields	45.9 ± 24.6	30.4 ± 3.5	29.8±5.6	↓	**<0.001**	↓	**<0.001**
Vujosevic and Midena (2013)*	Group 1: 50 Group 2: 30	Superior inner	Not available	Not available	Not available	↓	**<0.001**	Not available	
		Superior outer				↓	**<0.001**		
		Inferior inner				↓	**0.01**		
		Inferior outer				↓	**<0.001**		
		Temporal outer				↓	**0.01**		
		Nasal outer				↓	**<0.001**		
Araszkiewicz et al. (2012)	Group 1: 31 Group 2: 54	Optic disc	105.9	115.2	Not available	↑	**<0.001**	Not available	
		Superior	132.0	133.7		↑	0.11		
		Inferior	135.7	162.5		↑	**<0.001**		
		Temporal	79.7	85.0		↑	0.25		
		Nasal	77.7	79.2		↑	0.69		
Bronson-Castain et al. (2012)	Group 1: 26 Group 2: 47	9 spots proximal to optic disc	35.1±0.8	Type 1 diabetes: 33.5±0.8	Not available	↓	>0.05	Not available	
				Type 2 diabetes: 31.8±0.8		↓	**0.01**		
Verma et al. (2012)	Group 1: 40 Group 2: 70	2.4mm around optic disc	33±0.01	27±0.01	Not available	↓	**0.018**	Not available	
Park et al. (2011)	Group 1: 40 Group 2: 37 Group 3: 33	Macula	39.9±9.3	39.4±8.4	39.8067.2	↓	0.710	↓	>0.05
		Superior macula	49.8±8.4	39.1±8.2	38.2±7.7	↓	**0.046**	↓	>0.05
		Temporal macula	35.7±7.0	32.6±7.2	34.4±6.5	↓	0.553	↓	>0.05
		Inferior macula	44.1±7.7	43.5±8.8	43.2±8.0	↓	0.647	↓	>0.05
		Nasal macula	40.1±6.9	39.7±6.5	40.0±7.2	↓	0.510	↓	>0.05
van Dijk et al. (2010)	Group 1: 40 Group 2: 19 Group 3: 20	Pericentral	23.6	23.6	22.3	↔	>0.05	↓	>0.05
		Peripheral	36.6	35.8	32.9	↓	>0.05	↓	**<0.05**
van Dijk et al. (2009)	Group 1: 59 Group 2: 32 Group 3: 25	Fovea	0.72	0.88	0.66	↑	>0.05	↓	>0.05
		Pericentral	16.7	16.6	15.2	↓	>0.05	↓	>0.05
		Peripheral	33.6	32.7	32.3	↓	>0.05	↓	>0.05
Sugimoto et al. (2005)	Group 1: 48 Group 2: 32	Temporal	109.5±16.2	109.0±14.9	Not available	↓	**<0.05**	Not available	
		Superior	148.7±18.0	141.4±18.6		↓	>0.05		
		Nasal	109.2±13.4	105.7±14.8		↓	**<0.05**		
		Inferior	142.5±14.4	142.0±20.3		↓	**<0.05**		

*Study measured ILM and RNFL thickness

Table 7.31. Summary of studies of mean ganglion cell layer (GCL) thickness of healthy controls (HC; Group 1) and people with diabetes (PWD) with no retinopathy (Group 2) and PWD with minimal diabetic retinopathy (Group 3). Significant *p* values are in bold.

Study	No. participants	Retinal area	Retinal thickness, Mean±SD (µm)			Group 1 vs 2		Group 1 vs 3	
			Group 1	Group 2	Group 3	Thickness compared to Group 1	*p*	Thickness compared to Group 1	*p*
EDDMO study	Group 1: 50 Group 2: 26 Group 3: 46	Central	16.3±4.6	14.7±4.0	14.8±3.9	↓	0.337	↓	0.254
		Inner subfields	51.3±4.4	47.2±4.8	46.2±5.6	↓	**0.003**	↓	**<0.001**
		Outer subfields	34.7±3.2	33.1±2.8	32.8±3.7	↓	0.135	↓	**0.015**
Rodrigues et al. (2015)*	Group 1: 28 Group 2: 46 Group 3: 28	All subfields	91.1±32.9	79.8±7.4	74.0±10.6	↓	**0.039**	↓	**0.003**
Vujosevic and Midena (2013)*	Group 1: 50 Group 2: 30	Central	Not available	Not available	Not available	↓	>0.05	Not available	
		Inner subfields				↓	>0.05		
		Superior outer				↓	>0.05		
		Nasal outer				↓	>0.05		
		Temporal outer				↓	>0.05		
Araszkiewicz et al. (2012)	Group 1: 31 Group 2: 54	Superior	99.1	102.5	Not available	↑	**0.007**	Not available	
		Inferior	100.0	104.8		↑	**0.003**		
van Dijk et al. (2010)	Group 1: 40 Group 2: 19 Group 3: 20	Pericentral	49.4	50.8	44.3	↑	>0.05	↓	>0.05
		Peripheral	29.9	30.5	28.7	↑	>0.05	↓	>0.05
van Dijk et al. (2009)*	Group 1: 59 Group 2: 32 Group 3: 25	Fovea	40.1	43.8	40.6	↑	>0.05	↑	>0.05
		Pericentral	106.1	105.9	100.6	↓	>0.05	↓	**<0.05**
		Peripheral	74.5	74.1	72.4	↓	>0.05	↓	**<0.05**

*Study measured GCL and IPL thickness

As part of the natural contour of the macula, the inner retinal layers gradually diminish and converge to disappear at the fovea centralis, which allows light to pass directly through to the photoreceptors (Provis et al., 2013). This anatomical feature may account for some of the differences seen in the results. In the CSF, the ONL, consisting of nuclei of photoreceptors is the thickest so small changes may be more readily detected (Figure 4.13) (Curcio et al., 1990). This may explain why thinning of the ONL in PWD with no or minimal DR compared to HC was only significant in the CSF (Table 7.28). However, the other retinal layers are thickest in the inner and outer subfields so any subtle changes would be more apparent in these subfields. Indeed, thinning of the inner nuclear layers (RNFL, GCL and IPL) in PWD with no or minimal DR were only significant in the inner or outer subfields (Table 7.28). These findings suggest that retinal thickness from the inner and outer subfields can provide valuable additional information on DR progression compared to CST alone. Using the EDDMO dataset, Zhu proposed a novel spatial statistical inference framework which correlates thickness measurements from all ETDRS subfields (Zhu W. et al., 2020a, Zhu W. et al., 2020b). This framework can add valuable information and aid clinicians in the early diagnosis and management of DMO.

It is important to note that OCT is a relatively new imaging technology which has seen rapid progress in scan acquisition and image quality. Unsurprisingly, the studies summarised in Tables 7.28, 7.29 and 7.30 have used various OCT machines with different scan protocols over the years. Heidelberg's auto-segmentation of different retinal layers used in the EDDMO study was a feature that was recently introduced. In addition to auto-segmentation provided by Heidelberg, this feature also allows the grader to manually adjust retinal layer boundary lines to accurately define each retinal layer. The combination of auto-segmentation with manual adjustment when necessary was not previously available and has greatly facilitated the evaluation of the thickness of the different retinal layers in the EDDMO study. It is possible that the retinal thickness measurements obtained in the EDDMO study are more accurate and therefore more revealing compared to some of the measurements in the literature. Figure 7.18 illustrates the improvement in OCT resolution from the EDDMO study using Heidelberg auto-segmentation of different retinal layers in a macular scan showing an eye with CIMO compared to an earlier study measuring full-thickness from ILM to RPE obtained using Zeiss OCT (Sugimoto et al., 2005).

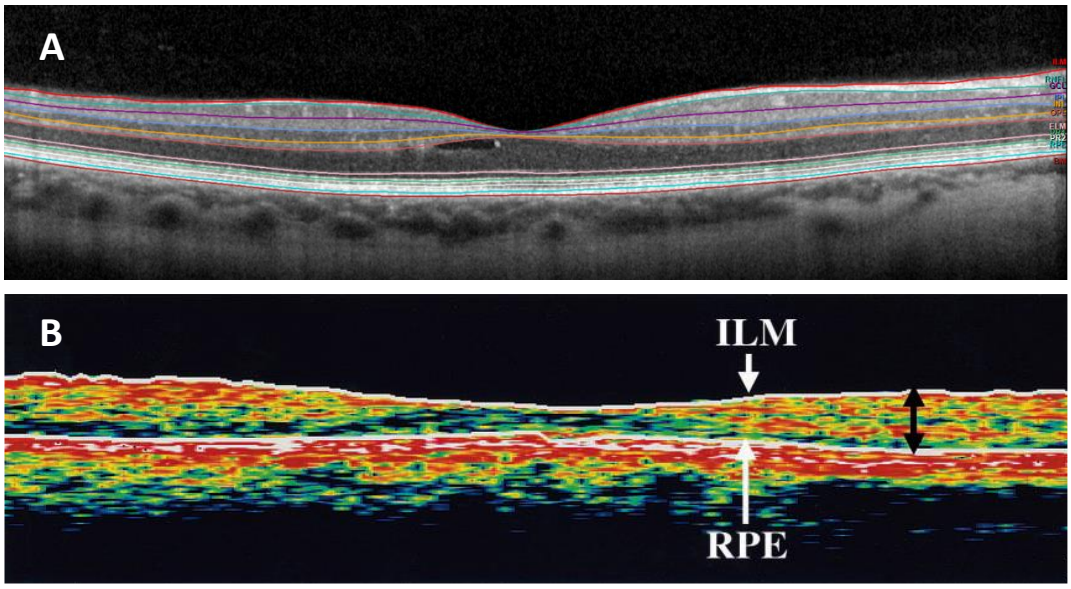


Figure 7.18. Heidelberg auto-segmentation of different retinal layers in a macular scan showing centre-involving diabetic macular oedema (CIMO) used in the EDDMO study (A) and full retinal thickness (black arrow) measured from the internal limiting membrane (ILM) to the retinal pigment epithelium (RPE) obtained using Zeiss OCT in a study by Sugimoto et al. (2005) (B).

Findings from this chapter suggest that there is initial thinning of the retina followed by progressive thickening in PWD with increasing DR severity. This non-linear pattern of retinal thickness fluctuation with the progression of DR illustrates some of the complexities in the analysis of OCT thickness information. Thinner retinal thickness does not indicate retinal health just as an underweight person who has type 1 diabetes would not be regarded as healthy. Instead, a low retinal thickness may indicate early DR and herald disease progression. Therefore, the retinal thickness of HC established in Chapter 5 (Section 5.3.3) is essential as a reference range.

In this chapter, both positive and negative correlations between age and retinal thickness in the different retinal layers in PWD were seen (Table 7.15). This is in contrast with the results from the HC data presented in Chapter 3 where no correlation between age and retinal thickness was found (Table 3.9). Other studies involving PWD of various levels of DR severity have found increased retinal thickness with age (Lattanzio et al., 2002) or no change in retinal thickness with age (Oshima et al., 1999, Massin et al., 2002). Both positive and negative correlations between gender and retinal thickness in PWD have also been found in this chapter (Table 7.15). Previous studies have found higher retinal thickness in men compared to women (Massin et al., 2002, DRCR network., 2012). Previous results on the effect of age and gender on retinal thickness in PWD with early DR (ETDRS 10 and 20,

N=112) found age to have little effect on retinal thickness while males have a higher full retinal thickness in the central and inner subfields compared to females (Section 3.3.4, Table 3.13). Similar to previous studies, results from this chapter have found no firm conclusions with regards to the correlation between retinal layer thickness, age and gender in PWD. These variations in retinal thickness values with age and gender may be confounded by the non-linear pattern of retinal thickness fluctuation with DR progression in this group of PWD who have non-homogenous DR severity.

In summary, results from this chapter have demonstrated a non-linear progression of retinal thickness change with DR severity. In PWD with no or minimal DR, the retinal thinning was mainly in the inner retinal layers in the inner and outer subfields. The following chapter will examine visual function in PWD compared with HC. The visual function of PWD with no or minimal DR is specifically examined in Section 8.3.4.

CHAPTER 8. CROSS-SECTIONAL ANALYSIS OF VISUAL FUNCTION IN PEOPLE WITH DIABETES COMPARED TO HEALTHY CONTROLS

8.1 CHAPTER INTRODUCTION

The previous chapter (Chapter 7) examined the structural changes in PWD as detected by OCT. This chapter will investigate the visual function (hRSD, distance and near VA) in PWD compared to HC. Current DR screening in England was reviewed in Chapter 2. Results from the previous chapter (Section 7.3.1) found that of the 218 eyes (74.7%, N=292) referred from LDESP as M1, only 153 eyes (52.4%, N=292) were subsequently found to have maculopathy (M1) on clinical examination with slit-lamp biomicroscopy by an ophthalmologist in DEC. DR screening is based on fundus photographs with distance VA and achieves a sensitivity of 82.8% (95%CI 78.0-87.6) and specificity of 92.9% (89.6-96.2) in detecting retinopathy (Scanlon et al., 2003). DR screening has a claimed sensitivity of 72.6% (65.6-78.7) and specificity of 66.8% (65.1-68.5) in detecting DMO confirmed on OCT (Prescott et al., 2014). DR screening based on fundus photography is better at detecting retinopathy compared to maculopathy, with current practice posing a challenge for the HES. Mackenzie et al. (2011) found that only 38.3% of PWD referred to hospital services as maculopathy suspects had OCT evidence of macular oedema.

An additional test that might be easily deployed before PWD are referred to the HES might be a useful means of decreasing the numbers being referred from screening, which will benefit both patients and the healthcare system. The hRSD test described in Chapter 2 (Section 2.6.3) is a relatively simple and inexpensive test and may have a role in detecting maculopathy in AMD (Pitrelli Vazquez et al., 2018). However, little is known about its ability to detect DMO (Pitrelli Vazquez et al., 2018, Wang et al., 2013). Having explored changes to the retinal structure in PWD in the previous chapter, this chapter concentrates on visual function assessed using hRSD testing, and distance and near VA. The performance of the hRSD test was compared to distance and near VA in detecting retinopathy and maculopathy, and analyses were done to assess if the addition of hRSD and or near VA to distance VA can improve the detection of retinopathy and maculopathy compared to distance VA alone. Other important properties of the hRSD test, such as testing time, and usability of the test from the patients' perspective were investigated. The structure of the chapter is shown in Figure 8.1.

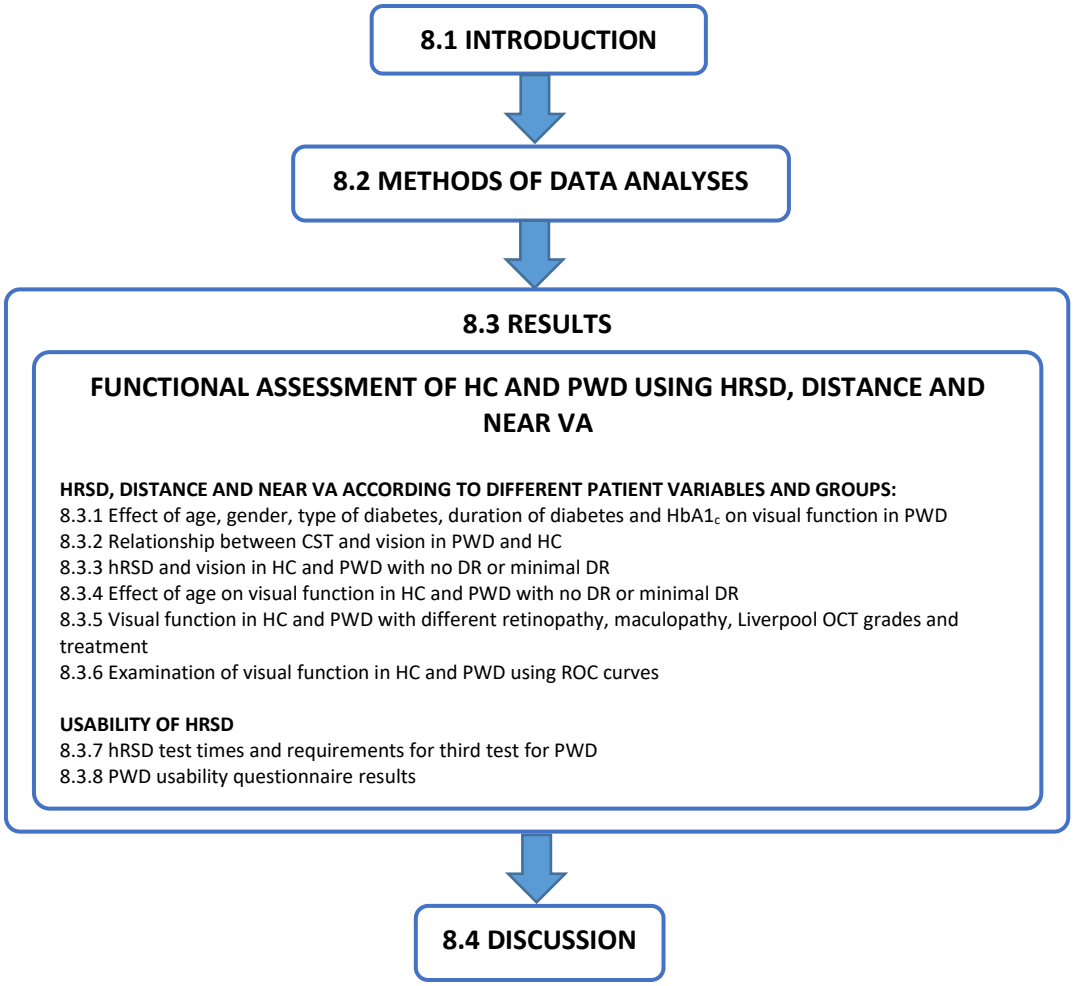


Figure 8.1 Organisation of Chapter 8.

8.2 METHODS OF DATA ANALYSES

General methods have been covered in Chapters 2 and 3. Univariate linear regression was used to examine the effect of patient factors on visual function in Section 8.3.1. Bonferroni corrections were made to adjust for multiple comparisons. Pearson and Spearman's rank correlation coefficient was used to examine the strength of correlation between variables (Sections 8.3.1, 8.3.2, 8.3.4, 8.3.7). One-way ANOVA with Tukey post hoc tests was used to assess visual function between HC and different PWD groups (Sections 8.3.3, 8.3.5). *t*-statistics and one-way ANOVA with Tukey post hoc tests were used to compare hRSD test time between different groups (Section 8.3.7).

Receiver operating characteristic (ROC) curves were used to determine the diagnostic ability of hRSD, distance and near VA to discriminate between different groups with various disease severities (Section 8.3.6) (Saunders et al., 2015). Since distance VA is routinely

obtained in clinical practice, distance VA was combined with hRSD (distance VA plus hRSD), near VA (distance VA plus near VA) or both (distance VA plus hRSD plus near VA) to determine if combining tests could improve the detection of DR or DMO. Logistic regression was used to obtain the predicted probabilities where distance VA and hRSD or near VA or both (hRSD and near VA) were considered as predictors. Then, the corresponding ROC curves were constructed using the predicted probabilities from the logistic regression. Optimal sensitivity and specificity is determined by the point closest to the top left-hand corner of the ROC curve (Perkins and Schisterman, 2006). The pROC package in R was used to compare the area under the curve (AUC) of two or more ROC curves using the Delong or bootstrap method (Section 8.3.7)(DeLong et al., 1988, Robin et al., 2011). The Delong method was used for comparison except for the comparison of PWD graded as M1 and M0 in Section 8.3.6 because the Delong method does not consider the direction of the curve. The bootstrap method was used as the ROC curve was very close to and crossed the diagonal line. These analyses were performed to determine if the AUC of one ROC curve was statistically significantly different from another. All data analyses were performed using Excel (2016), GraphPad Prism (version 8), SPSS (version 25) and R (version 4.0.2).

8.3 RESULTS

8.3.1 EFFECT OF AGE, GENDER, TYPE OF DIABETES, DURATION OF DIABETES AND HBA_{1C} ON VISUAL FUNCTION IN PWD

Changes in retinal thickness in PWD and comparisons with HC provide information about retinal pathology and change that might impact on visual function. Such changes can define different patient groups as in the Liverpool OCT criteria described in Chapter 4. To measure function, vision data was collected as described in Chapter 3. In this section, the results of VA and hRSD threshold measurements are reported. Univariate linear regression was used to examine the effect of age, gender, type of diabetes, duration of diabetes and HbA_{1c} on hRSD threshold, distance and near VA in all PWD (N=292; Table 8.1). The description of all PWD is covered in Section 7.3.2. Adjustments for multiple comparisons were made with Bonferroni corrections.

The results showed that increasing age correlated with a significant deterioration in hRSD threshold, distance and near VA, although this effect was slightly less marked for the hRSD threshold. Gender had a significant impact on distance VA alone; males had worse distance VA than females. Type and duration of diabetes did not appear to affect either the hRSD

threshold or VA. Increasing HbA_{1c} appeared to be correlated with a deterioration in hRSD threshold, distance and near VA, with the largest effect on hRSD threshold. This may suggest that poorer glycaemic control has more impact on hRSD threshold compared to distance or near VA. Therefore, the relationship between HbA_{1c} and vision was further examined. There were no correlations between HbA_{1c} and near VA (r=-0.02, p=0.795) or distance VA (r=0.04, p=0.482). Similarly, there was no correlation between HbA_{1c} and the hRSD threshold (r=0.09, p=0.136) (Table 8.1, Figure 8.2). Specific comparisons between HC and PWD with no or minimal DR are made in Section 8.3.3.

Table 8.1 Effect of age, gender, type of diabetes, duration of diabetes and HbA_{1c} on hRSD threshold, distance VA and near VA in all PWD

			Age (years)			Gender			Type of Diabetes			Duration of diabetes (years)			HbA_{1c}		
	Mean±SD (logMAR)	**Intercept**	**Estimated coefficient**	***p***	**Adjusted *p***	**Estimated coefficient***	***p***	**Adjusted *p***	**Estimated coefficient ****	***p***	**Adjusted *p***	**Estimated coefficient**	***p***	**Adjusted *p***	**Estimated coefficient**	***p***	**Adjusted *p***
hRSD threshold	-0.61±0.24	-0.958	0.004	**0.001**	**0.003**	-0.055	0.051	0.153	0.040	0.268	0.804	0.002	0.260	0.780	0.001	**0.024**	0.072
Distance VA	0.06±0.19	-0.252	0.006	**<0.001**	**<0.001**	-0.080	**<0.001**	**<0.001**	-0.012	0.686	2.058	<0.001	0.719	2.157	0.001	**0.041**	0.123
Near VA	0.18±0.24	-0.187	0.006	**<0.001**	**<0.001**	-0.048	0.083	0.249	0.029	0.433	1.299	0.001	0.660	1.980	0.001	**0.034**	0.102

Univariate linear regression used

Statistically significant results are in bold

Adjusted *p* values are adjusted with Bonferroni correction for multiple comparisons

*The values for the estimated coefficient refer to the male gender

**The values for the estimated coefficient refer to type 2 diabetes

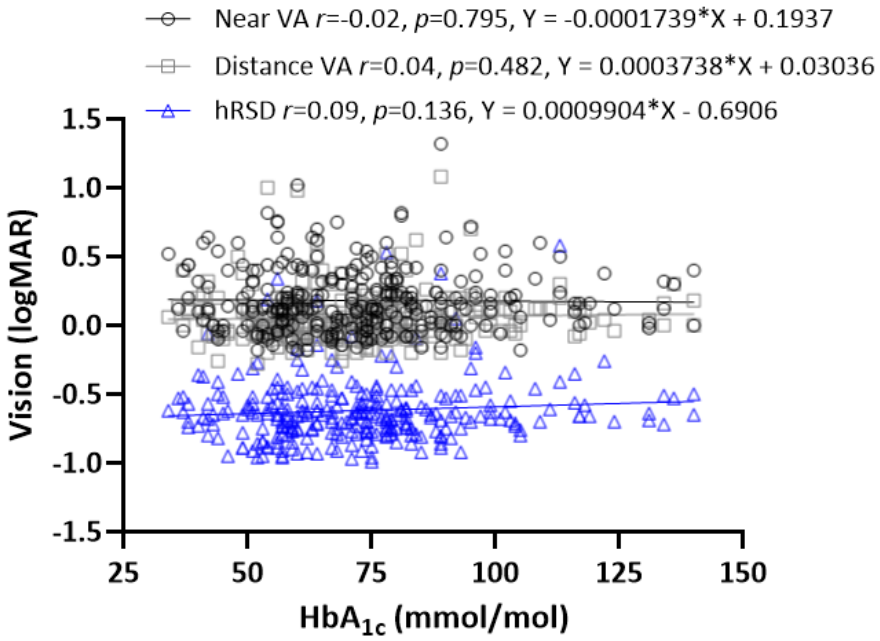


Figure 8.2. Correlation of HbA_{1c} with near VA, distance VA and hRSD threshold of PWD. Pearson correlation coefficients and their statistical significance are shown. Least squares linear regression lines, with equations, are also shown.

8.3.2 RELATIONSHIP BETWEEN CST AND VISION IN PWD AND HC

The relationships between CST and hRSD threshold, distance and near VA in HC (Section 4.8) and PWD with no evidence of DR (R0M0) were examined in Section 4.11. No significant correlation between CST and hRSD threshold, distance or near VA in HC and PWD with no evidence of DR (R0M0) was found. Similarly, when the results of all the PWD were examined in this section, no significant correlation between CST and hRSD threshold (Spearman *rho*=0.02, *p*=0.73), distance VA (Spearman *rho*=0.09, *p*=0.12) or near VA (Spearman *rho*=0.08, *p*=0.19) was found (Figure 8.3). As can be seen in Figure 8.3, this may be because most CST measurements fall between 200 to 400µm with only a few higher than 400µm that were available for analyses. However, for the few patients with CST >400µm, there was a trend towards worsening hRSD performance.

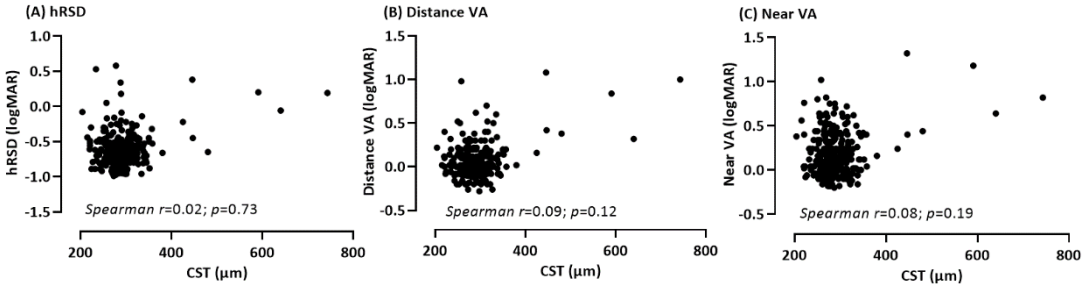


Figure 8.3. Relationship between central subfield thickness (CST) and hRSD (A), distance VA (B) and near VA (C) in people with diabetes. The Spearman correlation coefficient and its statistical significance are shown on each plot. Least-regression lines not shown due to outliers.

8.3.3 HRSD, DISTANCE AND NEAR VISION IN HC (GROUP 1) AND PWD WITHOUT DR (GROUP 2) AND MINIMAL DR (GROUP 3)

As discussed in Chapter 7 (Section 7.3.9), there has been considerable interest in determining the retinal thickness in PWD with early DR. In this section, a comparison of the hRSD threshold between HC (Group 1; N=50) and PWD without DR and NMO (R0M0 and NMO; Group 2; N=26) and PWD with minimal DR and NMO (R1M0 and NMO; Group 4, N=46) was made using ANOVA to determine the performance of the hRSD test in differentiating between these groups. Similar analyses were made with distance and near VA as a comparison. Bonferroni corrections were made for multiple comparisons. Although the descriptive analysis of these groups is covered in Section 7.3.9 (Table 7.24), it is important to note that compared to group 2, group 3 had significantly more participants with type 1 diabetes, a longer duration of diabetes, higher HbA_{1c} and a higher proportion of PWD were on insulin; hRSD and distance VA were also progressively worse from group 1 to group 3.

One-way ANOVA showed generally worse performance in hRSD ($F_{2,119}$=9.475, p<0.001, distance ($F_{2,118}$=12.991, p<0.001) and near VA ($F_{2,119}$=6.896, p=0.001) with increasing disease severity between the three groups (Table 8.3). Pairwise comparisons showed significant differences in hRSD between groups 1 and 3 (p<0.001); distance VA between groups 1 and 2 (p=0.006) and groups 1 and 3 (p<0.001), and near VA between groups 1 and 2 (p=0.008) and 1 and 3 (p=0.006) (Table 8.2). The results from this section suggest a decline in visual function in PWD compared to HC before any referable DR.

Table 8.2. Summary of ANOVA and pairwise comparisons of hRSD, distance and near VA in healthy controls (HC; Group 1), PWD with no diabetic retinopathy (Group 2) and minimal diabetic retinopathy (Group 3)

Test	Vision in groups (logMAR)	One-way ANOVA with degrees of freedom and *p* value shown	Pairwise comparison between groups		Mean difference logMAR (A-B)	Standard Error	*P**
			A	B			
hRSD	Group 1: -0.77±0.11	$F_{2,119}$=9.475, ***p*<0.001**	Group 1	Group 2	-0.094	0.450	0.114
	Group 2: -0.68±0.18		Group 1	Group 3	-0.165	0.038	**<0.001**
	Group 3: -0.61±0.25		Group 2	Group 3	-0.070	0.046	0.376
Distance VA	Group 1: -0.08±0.12	$F_{2,118}$=12.991, ***p*<0.001**	Group 1	Group 2	-0.110	0.035	**0.006**
	Group 2: 0.03±0.15		Group 1	Group 3	-0.142	0.029	**<0.001**
	Group 3: 0.06±0.16		Group 2	Group 3	-0.032	0.035	1.000
Near VA	Group 1: 0.06±0.16	$F_{2,119}$=6.896, ***p*=0.001**	Group 1	Group 2	-0.150	0.049	**0.008**
	Group 2: 0.21±0.25		Group 1	Group 3	-0.132	0.042	**0.006**
	Group 3: 0.19±0.22		Group 2	Group 3	0.018	0.050	1.000

* *p* value adjusted for Bonferroni corrections. Statistically significant results shown in bold.

8.3.4 EFFECT OF AGE ON VISUAL FUNCTION IN HC (GROUP 1) AND PWD WITHOUT DR (GROUP 2) AND MINIMAL DR (GROUP 3)

The effect of age on hRSD threshold, distance and near VA in Groups 1, 2 and 3 were examined. The characteristics of these groups have been described in Section 8.3.3. Although there was an overall deterioration in hRSD threshold and distance VA in all three groups with advancing age, there were no significant correlations between age and hRSD in all three groups (Group 1 r=0.13, p=0.351; Group 2 r=0.09, p=0.662; Group 3 r=0.23, p=0.123). The slopes of the least-squares regression lines for groups 1, 2 and 3 were +0.001037, +0.001171 and +0.003435 respectively. The difference between the regression slopes was not statistically significant ($F_{2,116}$=0.54; p=0.584) (Figure 8.4A).

A similar analysis of the effect of age on distance VA was performed. There were significant correlations between age and distance VA in all three groups (Group 1 r=0.44, p=0.001; Group 2 r=0.417, p=0.038; Group 3 r=0.373, p=0.011). The slopes of the least-squares regression lines for groups 1, 2 and 3 were +0.003691, +0.004552 and +0.003590 respectively. The difference between the regression slopes was not statistically significant ($F_{2,115}$=0.10; p=0.909) (Figure 8.4B).

For near VA, groups 1 and 2 showed a deterioration with age (Group 1 r=0.13, p=0.412; Group 2 r=0.003, p=0.988) while Group 3 showed an improvement with age (Group 3 r=-0.17, p=0.265) but none of these correlations were significant. The slopes of the least-squares regression lines for groups 1, 2 and 3 were +0.001537, +5.004e-005 and +-0.002222 respectively. The difference between the regression slopes was not statistically significant ($F_{2,111}$=0.81; p=0.447) (Figure 8.4C).

Interestingly, Figure 8.4 suggests a subtle trend whereby Groups 2 and 3 had worse visual function (hRSD, distance and near VA) across all age groups compared to HC.

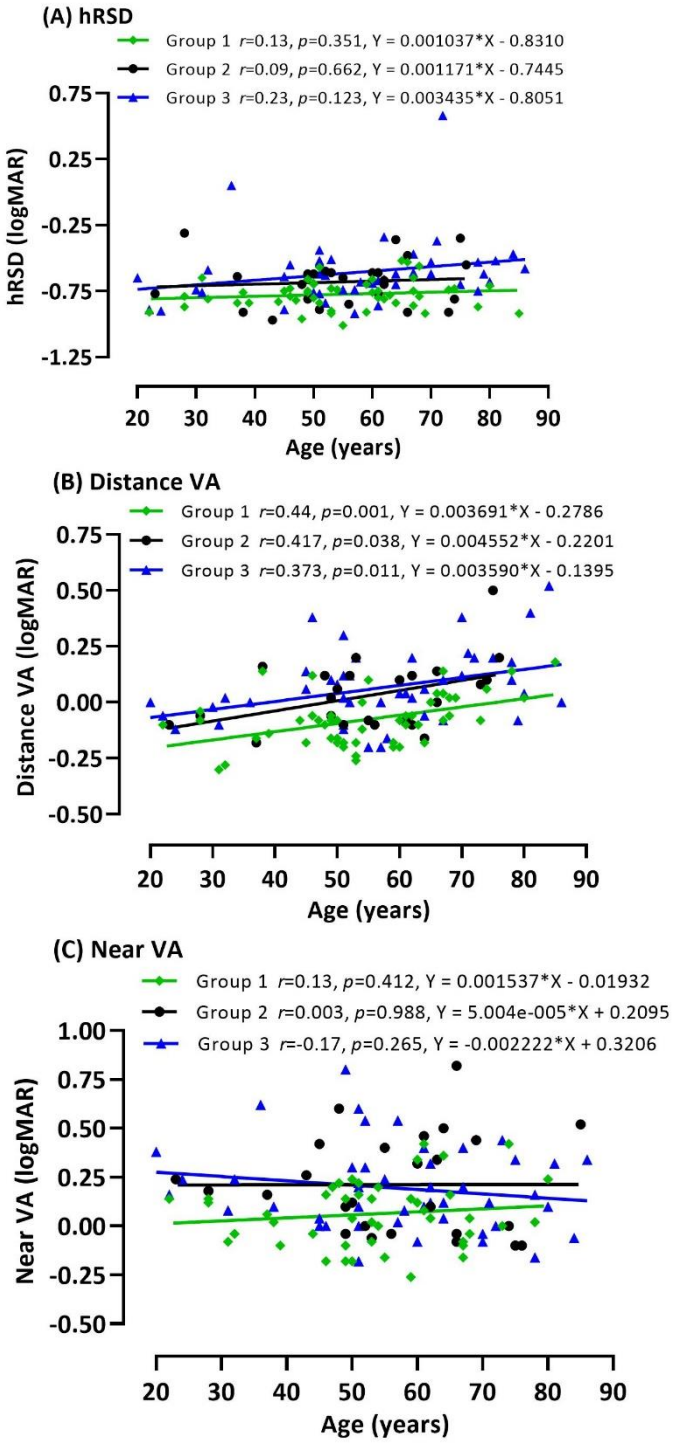


Figure 8.4 Effect of age on hRSD threshold (A), distance (B) and near VA (C) in healthy controls (Group 1), PWD without DR (Group 2) and PWD with minimal DR (Group 3). Pearson correlation coefficients and their statistical significance are shown. Least squares linear regression lines, with equations, are also shown. ±95% CI lines are omitted to improve clarity.

8.3.5 VISUAL FUNCTION IN HC AND PWD WITH DIFFERENT RETINOPATHY, MACULOPATHY, LIVERPOOL OCT GRADES AND TREATMENT

The preceding sections (Section 8.3.3 and 8.3.4) concentrated on PWD prior to the establishment of clear diabetic eye disease and its progression. In this section, a comparison of the mean hRSD threshold, distance VA and near VA in HC and PWD based on retinopathy, maculopathy and Liverpool OCT grading and treatment is made (Figure 8.5). Results from this chapter were based on PWD clinical examinations at their initial visit by an ophthalmologist in DEC. Therefore treatment in this chapter refers to whether PWD required treatment after their initial visit in DEC. The longitudinal data of PWD at their follow-up visit, after any treatment, if needed, is examined in the next chapter (Chapter 9). A description of each group and their mean values is shown in Table 7.3. (Section 7.3.2). It was generally seen that hRSD worsened with DR disease severity defined by retinopathy grades while distance and near VA remained relatively unchanged (Figure 8.5A). When examining maculopathy grades, hRSD generally deteriorated with maculopathy severity but there was less difference between M0 and M1; similar to retinopathy grades, distance and near VA remained relatively unchanged across maculopathy severity (Figure 8.5B). In contrast, there was a less obvious association with hRSD, distance and near VA with severity based on OCT grading (Figure 8.5C). When examining treatment, there was a general deterioration in hRSD, distance and near VA from HC to PWD who were not treated (NT) and PWD who were treated (TT) (Figure 8.5D). Based on these observations, pairwise comparisons of hRSD performance of DR defined by retinopathy grades, maculopathy grades, Liverpool OCT grades and treatment were performed with ANOVA using SPSS to examine if the mean hRSD thresholds were significantly worse with disease severity. Bonferroni corrections were made for multiple comparisons.

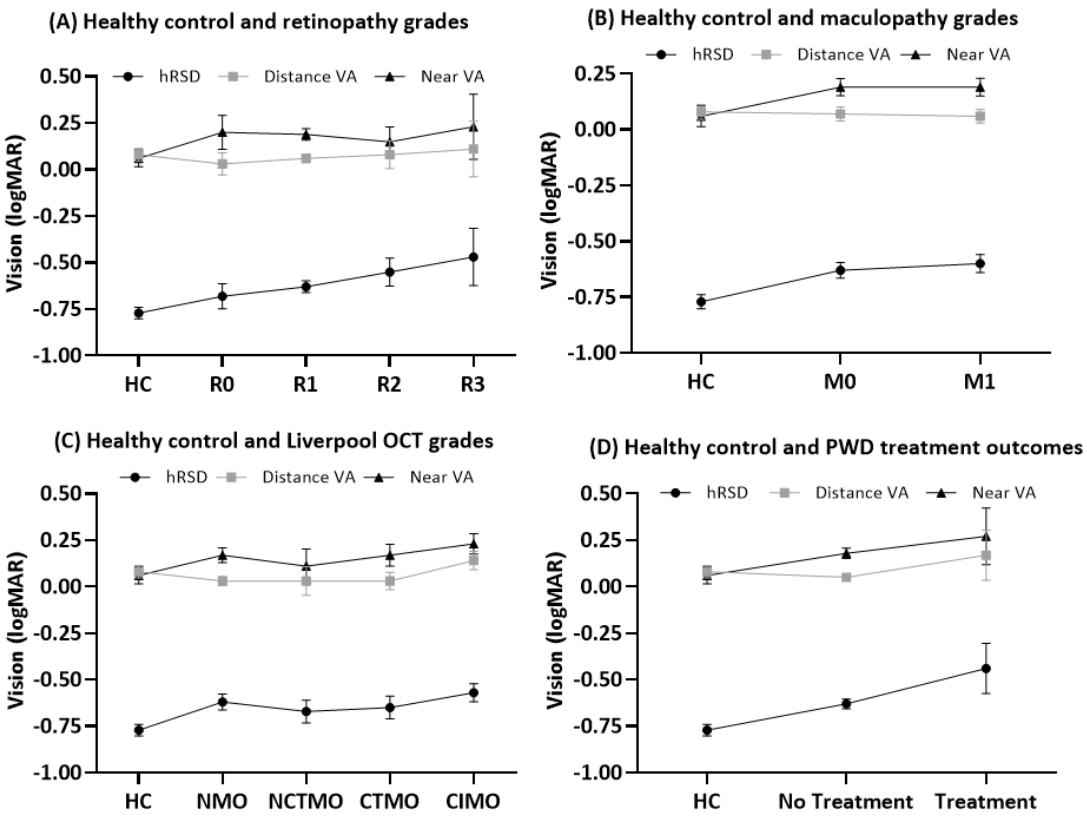


Figure 8.5. Mean (±95%CI) of hRSD threshold, distance and near VA of HC and PWD with different retinopathy grades (A), maculopathy grades (B), Liverpool OCT grades (C) and treatment (D).

ANOVA results showed significant differences in the hRSD threshold between retinopathy groups ($F_{4,337}$=9.608, p<0.001) (Table 8.3). Pairwise comparisons showed that R3 had a significantly worse hRSD threshold compared to HC (mean difference 0.308 logMAR, p<0.001), R0 (mean difference 0.218 logMAR, p=0.013) and R1 (mean difference 0.164 logMAR, p=0.034). R2 (mean difference 0.225 logMAR, p<0.001) and R1 (mean difference 0.114 logMAR, p<0.001) also had significantly worse hRSD threshold compared to HC (Table 8.3).

Table 8.3 Pairwise comparisons of hRSD threshold (logMAR) between HC and PWD with different retinopathy grades (R0, R1, R2 and R3)

Group A	Group B	Mean difference (Group A- Group B)	Standard Error	*P**
HC	R0	-0.090	0.051	0.400
	R1	-0.144	0.034	**<0.001**
	R2	-0.225	0.045	**<0.001**
	R3	-0.308	0.063	**<0.001**
R0	HC	0.090	0.051	0.400
	R1	-0.055	0.043	0.717
	R2	-0.136	0.052	0.070
	R3	-0.218	0.068	**0.013**
R1	HC	0.144	0.034	**<0.001**
	R0	0.055	0.043	0.717
	R2	-0.081	0.036	0.161
	R3	-0.164	0.057	**0.034**
R2	HC	0.225	0.045	**<0.001**
	R0	0.136	0.052	0.070
	R1	0.081	0.036	0.161
	R3	-0.082	0.064	0.695
R3	HC	0.308	0.063	**<0.001**
	R0	0.218	0.068	**0.013**
	R1	0.164	0.057	**0.034**
	R2	0.082	0.064	0.695

* *p* value adjusted for Bonferroni corrections. Statistically significant results shown in bold.

ANOVA showed significant differences in the hRSD threshold between HC and PWD with different maculopathy grades ($F_{2,339}$=11.797, p<0.001). Pairwise comparisons showed that M0 (mean difference -0.144 logMAR, p<0.001) and M1 (mean difference -0.175 logMAR, p<0.001) had significantly worse hRSD threshold compared to HC. However, there was no significant difference in the hRSD thresholds between the M0 and M1 groups (Table 8.4).

Table 8.4 Pairwise comparisons of hRSD threshold (logMAR) between HC and PWD with different maculopathy grades (M0 and M1)

Group A	Group B	Mean difference (Group A- Group B)	Standard Error	*p*
HC	M0	-0.144	0.037	**<0.001**
	M1	-0.175	0.036	**<0.001**
M0	HC	0.144	0.037	**<0.001**
	M1	-0.031	0.026	0.717
M1	HC	0.175	0.036	**<0.001**
	M0	0.031	0.026	0.717

* *p* value adjusted for Bonferroni corrections. Statistically significant results shown in bold.

Similar to previous hRSD results, ANOVA showed a significant difference in the hRSD threshold between HC and PWD with various OCT grades ($F_{4,337}$=7.592, p<0.001). Pairwise comparisons showed that NMO (Mean difference -0.151 logMAR, p=0.001) and CIMO (mean difference -0.209 logMAR, p<0.001) had significantly worse hRSD threshold compared to HC (Table 8.5).

Table 8.5 Pairwise comparisons of hRSD threshold (logMAR) between HC and PWD with various OCT grades (NMO, NCTMO, CTMO and CIMO)

Group A	Group B	Mean difference (Group A- Group B)	Standard Error	*p*
HC	**NMO**	-0.151	0.037	**0.001**
	NCTMO	-0.102	0.050	0.436
	CTMO	-0.126	0.046	0.069
	CIMO	-0.209	0.039	**<0.001**
NMO	**HC**	0.151	0.037	**0.001**
	NCTMO	0.049	0.044	1.000
	CTMO	0.025	0.039	1.000
	CIMO	-0.058	0.030	0.548
NCTMO	**HC**	0.102	0.050	0.436
	NMO	-0.049	0.044	1.000
	CTMO	-0.023	0.052	1.000
	CIMO	-0.107	0.046	0.204
CTMO	**HC**	0.126	0.046	0.069
	NMO	-0.025	0.039	1.000
	NCTMO	0.023	0.052	1.000
	CIMO	-0.083	0.041	0.434
CIMO	**HC**	0.209	0.039	**<0.001**
	NMO	0.058	0.030	0.548
	NCTMO	0.107	0.046	0.204
	CTMO	0.083	0.041	0.434

* *p* value adjusted for Bonferroni corrections. Statistically significant results shown in bold.

ANOVA also showed a significant difference in the hRSD threshold between HC and PWD who were NT and TT ($F_{2,339}$=20.366, p<0.001). The hRSD threshold of PWD who were NT (mean difference -0.144 logMAR, p<0.001) and PWD who were TT (mean difference -0.334 logMAR, p<0.001) were significantly worse than HC (Table 8.6). As expected, the hRSD threshold was also significantly worse in PWD who were TT compared to PWD who were NT (mean difference -0.190 logMAR, p<0.001).

Table 8.6 Pairwise comparisons of hRSD threshold (logMAR) between HC and in PWD who were not treated (NT) and PWD who were treated (TT)

Group A	Group B	Mean difference (Group A- Group B)	Standard Error	*p*
HC	**NT**	-0.144	0.033	**<0.001**
	TT	-0.334	0.053	**<0.001**
NT	**HC**	0.144	0.033	**<0.001**
	TT	-0.190	0.045	**<0.001**
TT	**HC**	0.334	0.053	**<0.001**
	NT	0.190	0.045	**<0.001**

* *p* value adjusted for Bonferroni corrections. Statistically significant results shown in bold.

In summary, this section found that hRSD seemed to be sensitive to the progression of retinopathy and PWD who were treated. However, hRSD did not distinguish between different grades of maculopathy or DMO as detected by OCT particularly well.

8.3.6 EXAMINATION OF VISUAL FUNCTION IN HC AND PWD USING RECEIVER OPERATING CHARACTERISTIC (ROC) CURVES

ROC curves provide a graphical method of examining the ability of a test to discriminate between defined groups, which are cases and controls in the EDDMO study. A ROC curve is created by plotting the true positive rate (TPR) against the false positive rate (FPR) across thresholds, to allow the identification of the threshold providing optimum sensitivity and specificity of a test in detecting a condition (Saunders et al., 2015). Statistical methods of ROC analysis were covered in Section 8.2. Test performance (hRSD, distance and near VA) was summarised using the AUC of the ROC curves with its *p*-value to evaluate the ability of the test to discriminate between cases and controls at 0.5 (better than chance; Saunders et al. (2015).

This section investigates the performance of hRSD, distance and near VA using ROC analysis to distinguish between eyes in the following four groups: 1. HC vs PWD, 2. PWD with CIMO vs other Liverpool OCT grades, 3. PWD graded as M0 vs M1, 4. PWD who were TT compared to those who were NT. The OCT definitions of PWD used in the second group were described in Chapter 4 while the LDESP definition of M0 and M1 used in the third group were described in Chapter 2.

8.3.6.1 Healthy controls (HC) vs people with diabetes (PWD)

HC (N= 50) were compared to all PWD (N=292). The descriptive statistics for these groups can be found in Section 7.3.2 (Table 7.3). The results showed that the area under the curve (AUC, 95% CI) for hRSD was 0.75 (0.69-0.81, p<0.001) (Figure 8.6). The optimum hRSD threshold that maximised sensitivity and specificity was -0.73 logMAR. At this hRSD threshold, the sensitivity was 68% and specificity was 76% as shown by the dotted lines in Figure 8.6. By comparison, the AUC (95% CI) for distance and near VA were 0.74 (0.67-0.82, p<0.001; optimum VA -0.05 logMAR for maximal sensitivity 71% and specificity 70%) and 0.64 (0.56-0.72, p=0.002; optimum VA 0.09 logMAR for maximal sensitivity 64%, specificity 56%) respectively (Figures 8.6). These results suggest that both hRSD and distance VA distinguished between HC and PWD moderately well.

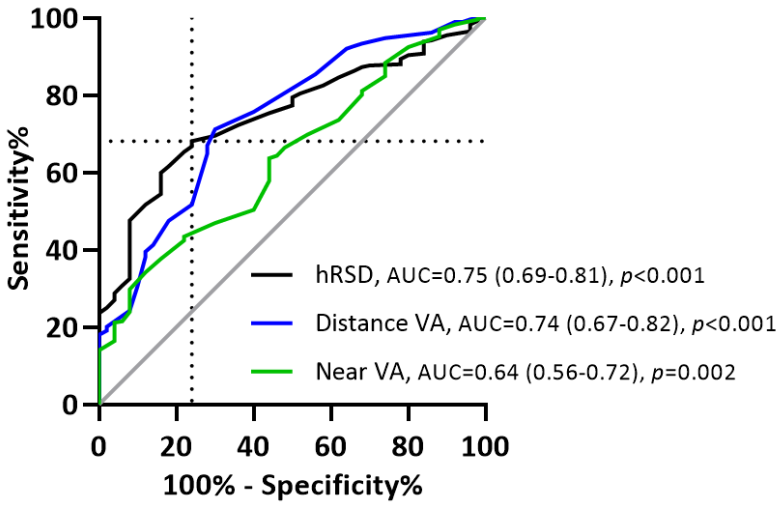


Figure 8.6. Receiver operating characteristic (ROC) curve for the performance of hRSD (black line), distance VA (blue line) and near VA (green line) in distinguishing between HC and PWD. The area under the curve (AUC; 95% confidence interval) and accompanying *p* value are shown. Dotted lines indicate a sensitivity of 68% and specificity of 76% at an hRSD threshold of -0.73 logMAR.

As distance VA is routinely collected in clinical practice when assessing PWD, an analysis was performed to examine the effect of combining hRSD and near VA to distance VA on AUC. When hRSD was added to distance VA, the discrimination between the two groups improved; AUC improved to 0.81 (0.75-0.87, p<0.001) with a sensitivity of 80% and sensitivity of 74% (Figure 8.7). The AUC for hRSD plus near VA was 0.76 (0.69-0.82, p<0.001; sensitivity 64%, specificity 80%) while the AUC for hRSD plus distance VA plus near VA was 0.81 (0.75-0.87, p<0.001; sensitivity 77%, specificity 76%) (Figure 8.7). Delong's test for two

correlated ROC curves was used to test the extent to which these differences were statistically significant. There was no significant difference between the curves for hRSD alone and distance VA alone (p=0.806). hRSD plus distance VA performed significantly better than distance VA alone (p=0.006). However, hRSD plus distance VA was not significantly different from the combination of hRSD plus distance plus near VA (p=0.839).

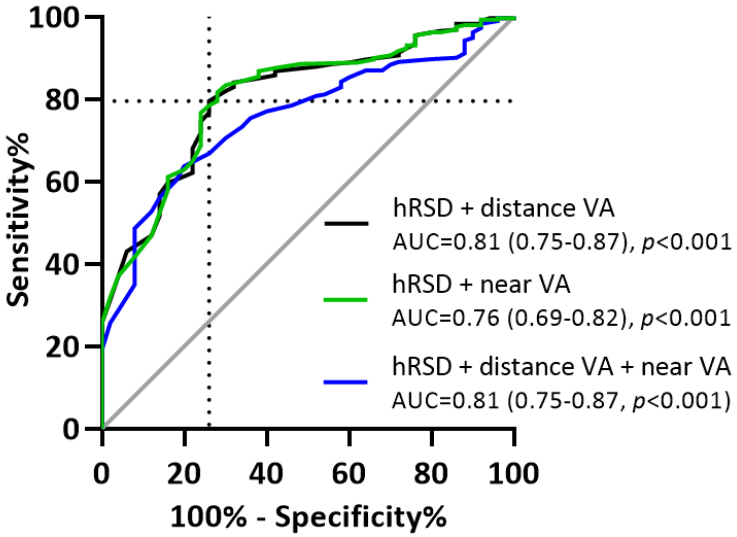


Figure 8.7. Receiver operating characteristic (ROC) curve for the performance of hRSD plus distance VA (black line), hRSD plus near VA (blue line) and hRSD plus distance VA plus near VA (green line) in distinguishing between HC and PWD. The area under the curve (AUC; 95% confidence interval) and accompanying *p* value are shown. Dotted lines indicate a sensitivity of 80% and specificity of 74% for hRSD plus distance VA.

8.3.6.2 PWD with CIMO compared to other Liverpool OCT grades

A key question that remains is not the ability of vision tests to discriminate between HC and PWD, but whether they can discriminate between different groups of PWD. It would be useful to discriminate between referred PWD with CIMO and other Liverpool OCT grades, between those graded as M0 and M1 and between PWD who might require treatment and those who do not. Therefore these were the groups selected for further analysis.

ROC analysis in PWD with CIMO compared to other Liverpool OCT grades (NMO, NCTMO and CTMO) showed that the AUC (95% CI) for hRSD, distance and near VA were 0.58 (0.51-0.65, p=0.021, sensitivity 44%, specificity 67%), 0.66 (0.59-0.72, p<0.001, sensitivity 64%, specificity 54%) and 0.58 (0.51-0.65, p=0.025, sensitivity 58%, specificity 58%) respectively (Figure 8.8). The results indicated that distance VA had the best performance in detecting CIMO compared to hRSD and near VA.

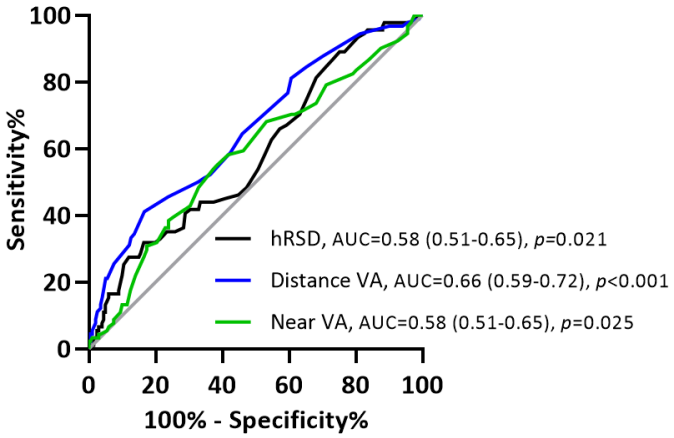


Figure 8.8. Receiver operating characteristic (ROC) curve for the performance of hRSD (black line), distance VA (blue line) and near VA (green line) in distinguishing between PWD graded as CIMO and PWD graded as NMO, NCTMO and CTMO. The area under the curve (AUC; 95% confidence interval) and accompanying *p* value are shown.

The combination of hRSD plus distance VA did not improve the AUC (0.66, 0.59-0.72, $p<0.001$; sensitivity 64%, specificity 54%) compared to distance VA alone (Figure 8.9). The AUC for hRSD plus near VA was 0.60 (0.53-0.67, $p=0.007$; sensitivity 70%, specificity 50%) while the AUC for hRSD plus distance plus near VA was 0.67 (0.60-0.73, $p<0.001$; sensitivity 60%, specificity 64%) (Figures 8.9). Delong's test for two correlated ROC curves showed that there was no significant difference between distance VA and hRSD ($p=0.076$), distance VA and hRSD plus distance VA ($p=0.442$). There was also no significant difference between distance VA and hRSD plus distance VA plus near VA ($p=0.485$).

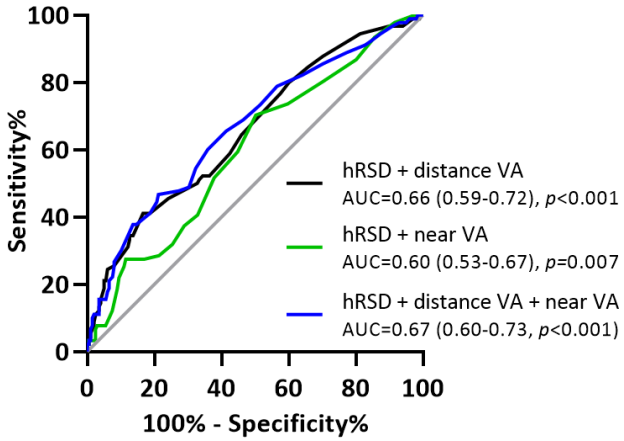


Figure 8.9. Receiver operating characteristic (ROC) curve for the performance of hRSD plus distance VA (black line), hRSD plus near VA (green line) and hRSD plus distance VA plus near VA (blue line) in distinguishing between PWD graded as CIMO and PWD graded as NMO, NCTMO and CTMO. The area under the curve (AUC; 95% confidence interval) and accompanying *p* value are shown.

8.3.6.3 PWD graded as M0 compared to M1

ROC analysis in PWD graded as M0 compared to M1 showed that the AUC (95% CI) for hRSD, distance and near VA were 0.52 (0.45-0.58, *p*=0.592; sensitivity 55%, specificity 46%), 0.52 (0.45-0.59, *p*=0.606; sensitivity 46%, specificity 66%) and 0.51 (0.44-0.58, *p*=0.764; sensitivity 56%, specificity 50%) respectively (Figure 8.10). These results were not significantly different from a chance level of AUC 0.5.

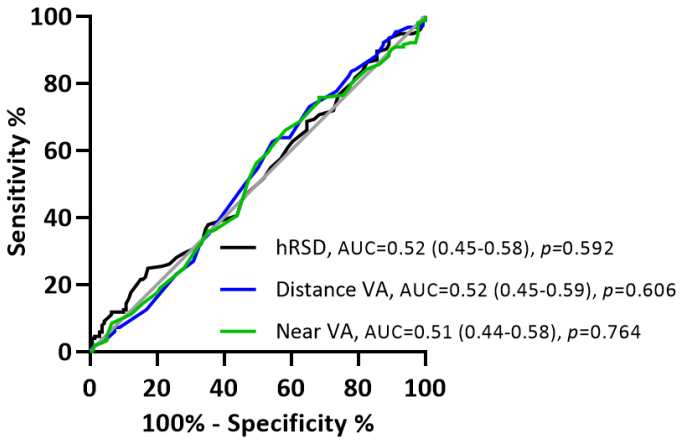


Figure 8.10. Receiver operating characteristic (ROC) curve for the performance of hRSD (A), distance VA (B) and near VA (C) in distinguishing between PWD graded as M0 and M1. The area under the curve (AUC; 95% confidence interval) and accompanying *p* value are shown.

The addition of hRSD to distance VA only improved the AUC to 0.55 (0.49-0.62, *p*=0.109; sensitivity 56%, specificity 55%) (Figure 8.11). The AUC for hRSD plus near VA was 0.56 (0.49-0.62, *p*=0.092; sensitivity 63%, specificity 48%). The AUC for hRSD plus distance VA plus near VA was 0.55 (0.49-0.62, *p*=0.114; sensitivity 67%, specificity 44%) respectively (Figures 8.11). These results were not statistically significant. The bootstrap test for two correlated ROC curves showed no significant difference between distance VA and hRSD (*p*=0.970), distance VA and hRSD plus distance VA (*p*=0.227), and distance VA and hRSD plus distance VA plus near VA (*p*=0.328).

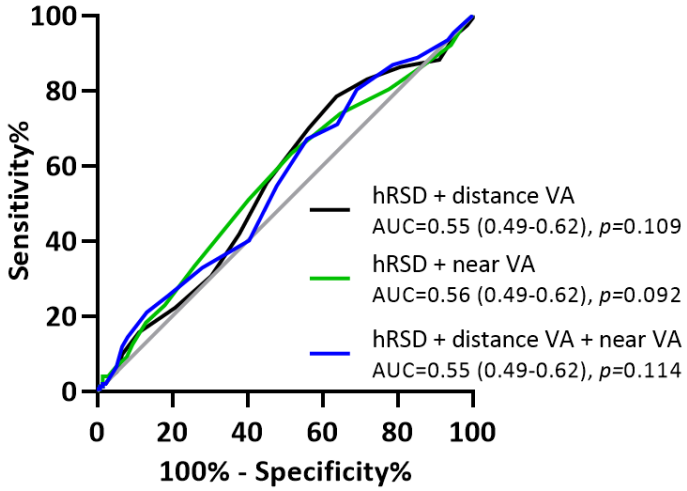


Figure 8.11. Receiver operating characteristic (ROC) curve for the performance of hRSD plus distance VA (A), hRSD plus near VA (B) and hRSD plus distance VA plus near VA (C) in distinguishing between PWD graded as M0 and M1. The area under the curve (AUC; 95% confidence interval) and accompanying *p* value are shown.

8.3.6.4 PWD who were treated (TT) vs were not treated (NT)

ROC analysis in PWD who were TT vs NT showed that the AUC (95% CI) for hRSD, distance and near VA were 0.68 (0.56-0.79, *p*=0.004; sensitivity 60%, specificity 66%), 0.59 (0.48-0.71, *p*=0.121 sensitivity 64%, specificity 49%) and 0.55 (0.43-0.55, *p*=0.432 sensitivity 56%, specificity 50%) respectively (Figure 8.12). These results showed that hRSD performed better in distinguishing PWD who were treated from those who were not, compared to distance and near VA.

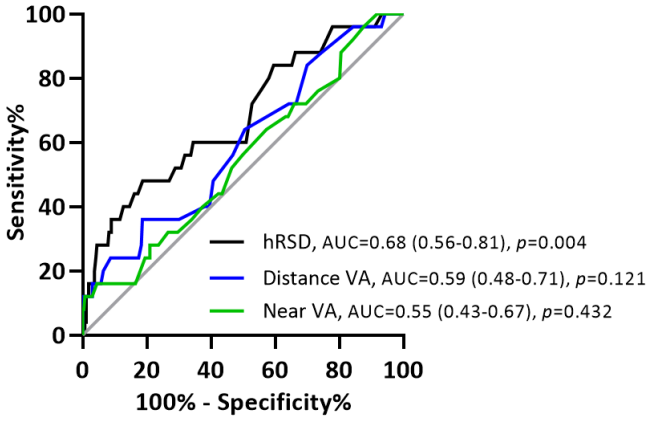


Figure 8.12. Receiver operating characteristic (ROC) curve for the performance of hRSD (black line), distance VA (blue line) and near VA (green line) in distinguishing between PWD who were treated (TT) and those who were not treated (NT). The area under the curve (AUC; 95% confidence interval) and accompanying *p* value are shown.

The addition of hRSD to distance VA improved the AUC to 0.66 (0.55-0.77, *p*=0.007; sensitivity 50%, specificity 72%) (Figure 8.13). The AUC for hRSD plus near VA was 0.69 (0.58-0.81, *p*=0.001; sensitivity 50%, specificity 82%). The addition of hRSD plus distance VA plus near VA further improved the AUC to 0.71 (0.60-0.81, *p*<0.001; sensitivity 65%, specificity 59%) (Figure 8.13). (Figure 8.13). Delong's test for two correlated ROC curves showed that there was no statistical difference between distance VA and hRSD (*p*=0.238), distance VA and hRSD plus distance VA (*p*=0.357) and distance VA and hRSD plus distance VA plus near VA (*p*=0.086).

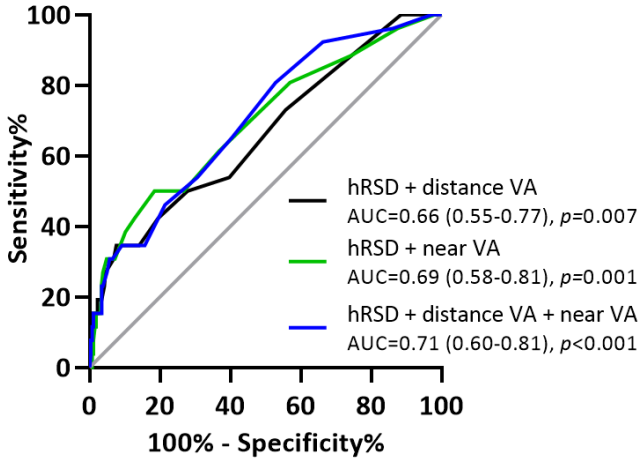


Figure 8.13. Receiver operating characteristic (ROC) curve for the performance of hRSD plus distance VA (black line), hRSD plus near VA (green line), hRSD plus distance VA plus near VA (blue line) in distinguishing between PWD who were treated and those who were not treated. The area under the curve (AUC; 95% confidence interval) and accompanying *p* value are shown.

8.3.7 HRSD TEST TIMES AND REQUIREMENTS FOR A THIRD TEST FOR PWD

This section examines the hRSD test time in PWD as this is an important consideration when introducing a test in clinical practice. The mean±SD hRSD test time for one eye for all the PWD was 161.6±61.2s. The right eye was routinely tested prior to the left eye. Therefore, as expected, the mean test time for the right eyes (177.1±71.2s) was significantly longer than the left eyes (143.8±53.1s) (paired *t*-test; *t*=6.06, *p*<0.001) indicating a learning effect. There was a significant correlation between age and hRSD test times with the older participants taking longer to perform the test than younger ones (*r*=-0.27, *p*<0.001), with a marked increase in variability among older participants (Figure 8.14). However, there was no statistically significant correlation between hRSD thresholds and test times (*r*=-0.10, *p*=0.09) (Figure 8.15). A third test was required when the within-session

results of the hRSD test differed by 0.30 logMAR or more (Wang et al., 2013). A third test was required in only 13.4% (39 of 292 eyes).

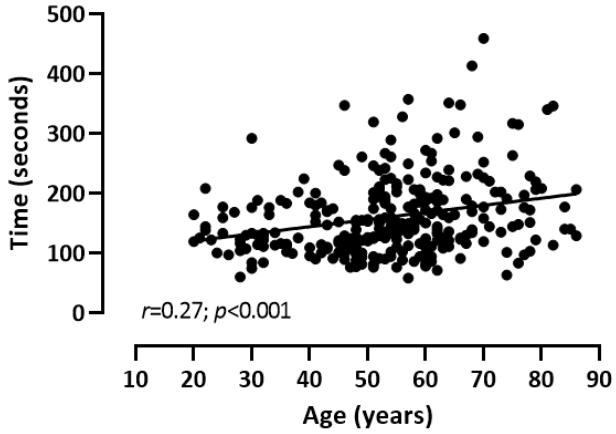


Figure 8.14. Relationship between hRSD test times and age in PWD. Least squares linear regression lines are shown.

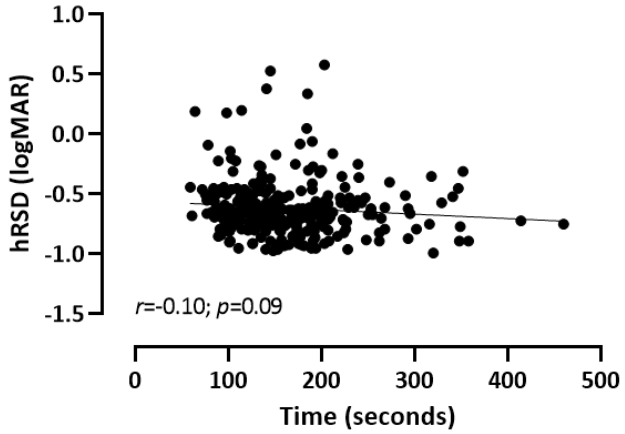


Figure 8.15. Relationship between hRSD threshold and test times in PWD. Least squares linear regression lines are shown.

Mean±SD hRSD test time between PWD with different OCT grades were specifically examined (NMO 160.1±67.1s; NCTMO 149.9±52.5s; CTMO 162.2±60.9s; 167.4±65.3s) (Figure 8.16). One-way ANOVA showed that there was no statistically significant difference in hRSD test time between these groups ($F_{3,288}$=0.612, p=0.608).

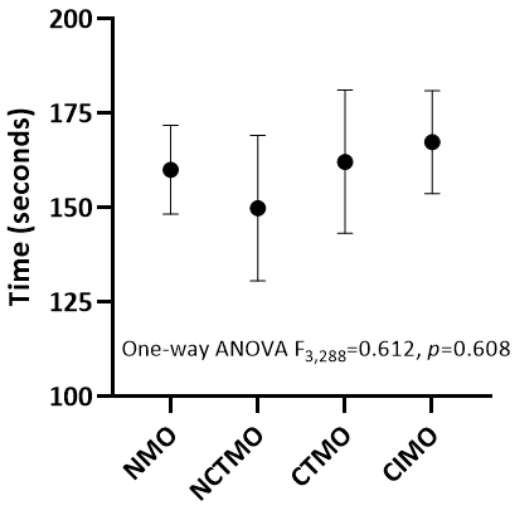


Figure 8.16. Mean (±95%CI) of hRSD test times of PWD with different OCT grades (NMO, NCTMO, CTMO, CIMO). One-way ANOVA results with *p* value shown.

A comparison of PWD hRSD test times was made with those of HC (Table 8.8). HC took significantly longer to perform the hRSD test compared to PWD (t=4.648, p<0.001). This is likely due to HC having a better mean±SD hRSD threshold (-0.77±0.11 logMAR) compared to PWD (-0.61±0.24) and therefore taking longer to reach the threshold (Table 7.3, Section 7.3.2). PWD hRSD test times were also significantly longer in both right and left eyes compared to HC (Right eye t=4.404, p 0.017; Left eye t=6.777, p<0.001). Similar to PWD, HC had longer test times in the right eyes compared to the left eyes also indicating a learning effect (Table 8.7).

Table 8.7. Comparison of hRSD test times (seconds) between healthy controls (HC; 4AFC) and people with diabetes (PWD)

Eyes	Number of eyes		Test time (seconds)		*t*-test	*p*
	HC	PWD	HC	PWD		
All eyes	106	292	194.2 ± 54.9	161.6±61.2	4.648	<0.001
Right eyes	98	265	196.0±52.5	177.1±71.2	2.404	0.017
Left eyes	98	259	185.9±50.9	143.8±53.1	6.777	<0.001

8.3.8 PWD USABILITY QUESTIONNAIRE RESULTS

Similar to the HC (Section 6.10), all the PWD undertook a 4AFC hRSD usability questionnaire which consisted of 4 questions and their responses are shown in Table 8.8. Most participants understood how to use the hRSD test, found it easy to use, did not think the

test took too long and could use the test independently. Two PWD (1%) strongly disagreed with being able to use the hRSD to test their vision (question 4); one had mild dementia while the other participant moved to the UK 6 months ago and had language barriers.

Table 8.8. Usability questionnaire results from all PWD (N=292). Participant responses to each statement shown as % of participants rounded to whole numbers.

Questions	Strongly disagree	Disagree	Neutral	Agree	Strongly agree
1. I understood how to use the hRSD device	0	0	1	30	69
2. The hRSD test was easy to use	0	1	2	28	69
3. The hRSD test did not take too long to do	0	0	2	32	66
4. I could use the hRSD device to test my own vision	1	2	4	29	64

8.3.9 SUMMARY OF RESULTS

In all PWD, increasing age correlated with a significant deterioration in hRSD threshold and distance and near VA. This effect was slightly less marked for the hRSD threshold compared to distance and near VA (Section 8.3.1). hRSD worsened with DR disease severity defined by retinopathy and maculopathy grades while distance and near VA remained relatively unchanged (Section 8.3.5).

In PWD with no or minimal DR, there was no significant correlation between age and hRSD threshold but there was a significant correlation between age and distance and near VA (Section 8.3.4). There was a decline in hRSD threshold, distance and near VA in PWD with no or minimal DR compared to HC (Section 8.3.3).

The results of Section 8.3.6 has been summarised in Tables 8.9 and 8.10. Both hRSD (AUC 0.75) and distance VA (AUC 0.74) distinguished between HC and PWD moderately well; hRSD plus distance VA improved the AUC to 0.81 compared to distance VA alone (p=0.006).

Distance VA (AUC 0.66) had the best performance in detecting CIMO compared to hRSD (AUC 0.58) and near VA (AUC 0.58); hRSD plus distance VA plus near VA improved AUC to 0.67 but these differences were not statistically significant.

hRSD (AUC 0.52), distance (AUC 0.52) and near VA (AUC 0.51) performed poorly in distinguishing PWD graded as M0 and M1; the addition of hRSD or near VA to distance VA did not improve AUC significantly.

hRSD (AUC 0.68) performed better in distinguishing PWD who were treated from those who were not in comparison to distance (AUC 0.59) and near VA (AUC 0.55); the addition of hRSD plus near VA to distance VA improved AUC to 0.71.

The hRSD test time for each eye was approximately 2.5 minutes (161.6±61.2s) (Section 8.3.7). HC had a better hRSD threshold compared to PWD and therefore took longer to perform to reach the threshold (t=4.648, p<0.001). PWD found good usability with the hRSD test (Section 8.3.8).

Table 8.9 Area under the receiver operating curve (AUC of ROC) of hRSD, distance and near VA in different groups with *p*-value indicating AUC above chance (0.5; Saunders et al. (2015) and the optimum visual threshold (logMAR) with maximal sensitivity (%) and specificity (%)

Groups	AUC (95%CI), *p*	Optimum threshold (logMAR)	Sensitivity (%)	Specificity (%)
HC vs PWD				
hRSD	0.75 (0.69-0.81), *p*<0.001	-0.73	68	76
Distance VA	0.74 (0.67-0.82), *p*<0.001	-0.05	71	70
Near VA	0.64 (0.56-0.72), *p*=0.002	0.09	64	56
hRSD + distance VA	0.81 (0.75-0.87), *p*<0.001	0.80*	80	74
hRSD + near VA	0.76 (0.69-0.82), *p*<0.001	0.86*	64	80
hRSD + distance + near VA	0.81 (0.75-0.87), *p*<0.001	0.82*	77	76
Non-CIMO vs CIMO				
hRSD	0.58 (0.51-0.65), *p*=0.021	-0.61	44	67
Distance VA	0.66 (0.59-0.72), *p*<0.001	0.01	64	54
Near VA	0.58 (0.51-0.65), *p*=0.025	0.15	58	58
hRSD + distance VA	0.66 (0.59-0.72), *p*<0.001	0.28*	64	54
hRSD + near VA	0.60 (0.53-0.67), *p*=0.007	0.29*	70	50
hRSD + distance + near VA	0.67 (0.60-0.73), *p*<0.001	0.30*	60	64
M0 vs M1				
hRSD	0.52 (0.45-0.58), *p*=0.592	-0.67	55	46
Distance VA	0.52 (0.45-0.59), *p*=0.606	0.05	46	66
Near VA	0.51 (0.44-0.58), *p*=0.764	0.15	56	50
hRSD + distance VA	0.55 (0.49-0.62), *p*=0.109	0.53*	56	55
hRSD + near VA	0.56 (0.49-0.62), *p*=0.092	0.52*	63	48
hRSD + distance + near VA	0.55 (0.49-0.62), *p*=0.114	0.52*	67	44
NT vs TT				
hRSD	0.68 (0.56-0.79), *p*=0.004	-0.61	60	66
Distance VA	0.59 (0.48-0.71), *p*=0.121	0.01	64	49
Near VA	0.55 (0.43-0.67), *p*=0.432	0.13	56	50
hRSD + distance VA	0.66 (0.55-0.77), *p*=0.007	0.09*	50	72
hRSD + near VA	0.69 (0.58-0.81), *p*=0.001	0.10*	50	82
hRSD + distance + near VA	0.71 (0.60-0.81), *p*<0.001	0.08*	65	59

*predicted probabilities from logistic regression of two or three predictors

Table 8.10 Comparison of receiver operating curve (ROC) of distance VA and other visual function tests in different groups with an area under the curve (AUC) and 95% CI shown

ROC 1 (AUC, 95%CI)	ROC 2 (AUC, 95%CI)	*p* value
HC vs PWD		
Distance VA (0.75, 0.67-0.82)	hRSD (0.75, 0.69-0.81)	0.806
	hRSD + distance VA (0.81, 0.75-0.87)	0.006
	hRSD + distance + near VA (0.81, 0.75-0.87)	0.839
Non CIMO vs CIMO		
Distance VA (0.66, 0.59-0.72)	hRSD (0.58, 0.51-0.65)	0.076
	hRSD + distance VA (0.66, 0.59-0.72)	0.442
	hRSD + distance + near VA (0.67, 0.60-0.73)	0.485
M0 vs M1		
Distance VA (0.52, 0.45-0.59)	hRSD (0.52, 0.45-0.58)	0.970
	hRSD + distance VA (0.55, 0.49-0.62)	0.227
	hRSD + distance + near VA (0.55, 0.49-0.62)	0.328
NT vs TT		
Distance VA (0.59, 0.48-0.71)	hRSD (0.68, 0.56-0.79)	0.238
	hRSD + distance VA (0.66, 0.55-0.77)	0.357
	hRSD + distance + near VA (0.71, 0.60-0.81)	0.086

8.4 CHAPTER DISCUSSION

The previous chapter (Chapter 7) examined the structural variables of PWD who had been newly referred from the LDESP to the DEC. Data was obtained during their first visit to the DEC. In this chapter, the visual function of these patients is explored. In clinical practice, distance VA is typically measured. Although near VA is also measured, it is more affected by presbyopia and dependent on patients wearing their habitual corrections. Therefore, distance VA is measured more often compared to near VA. In the EDDMO study, hRSD and near VA testing were performed with the participants' habitual optical correction or age-appropriate near correction if they had forgotten to bring their glasses (Section 3.5.1, Table 3.1). Although VA is simple to obtain and non-invasive, there is now a greater understanding that it only measures one component of visual function. Other common assessments of visual function include CS, visual field, dark adaptation and colour vision (Silveira, 2019). As discussed in Chapter 2, the hRSD test has shown some promise in detecting macular pathology in AMD (Pitrelli Vazquez et al., 2018, Lott et al., 2021), but there are limited studies on the ability of the hRSD test in detecting DR and DMO (Wang et al., 2013).

The EDDMO study found progressive deterioration of mean hRSD threshold with worsening retinopathy (Figure 8.17B) and significant differences between PWD with different retinopathy grades ($F_{4,337}$=9.608, p<0.001) (Table 8.3, Section 8.3.5). Similarly, Wang et al. (2013) compared hRSD threshold in HC (N=27) and PWD with various levels of severity of DR (N=36); there were 11 PWD with mild to moderate NPDR, 12 with severe to very severe NPDR or pre-PDR and 13 with PDR or NPDR affecting the fovea. In comparison, there were both more HC (N=50) and PWD (N=292) in the EDDMO study. Wang et al. (2013) found that there was a progressive deterioration of the hRSD threshold with increasing DR severity (Figure 8.17A). One-way ANOVA showed that the mean hRSD threshold between the four groups was significantly different (p<0.001); pairwise comparisons showed that PWD with severe to very severe NPDR or pre-PDR and PWD with PDR or NPDR affecting the fovea had a significantly worse hRSD threshold than PWD with mild to moderate NPDR (p<0.001). As expected, the pairwise comparisons summarized in Table 8.3 showed that PWD with more severe retinopathy grades had a worse hRSD threshold compared to PWD with less severe retinopathy grades or HC. Interestingly, Wang et al. (2013) found progressive deterioration of distance VA with increasing severity of DR (Figure 8.17A) but this was not seen in the EDDMO study (Figure 8.17B). It can be seen from Figures 8.17A and 8.17B that the PWD from the study by Wang et al. (2013) had worse distance and hRSD thresholds compared to the EDDMO study. The smaller groups of PWD in the study by Wang et al. (2013) likely represented participants with more severe disease with more extreme macular pathology and a wider range of hRSD thresholds compared to PWD in the EDDMO study. For example, in the EDDMO study, PWD with R0 and R3 had hRSD thresholds of -0.68±0.18 logMAR and -0.47±0.29 logMAR respectively (Figure 8.17B). On the other hand, in the study by Wang et al. (2013), PWD with mild to moderate NPDR and PWD with proliferative DR or non-proliferative DR affecting the fovea had hRSD thresholds of -0.48 logMAR and -0.03 logMAR respectively (Figure 8.17A).

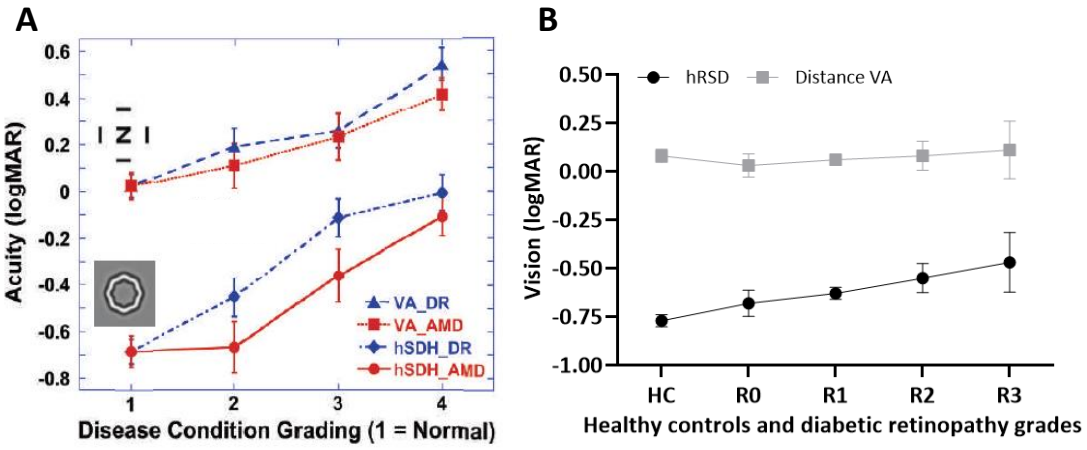


Figure 8.17 A. Mean±95%CI of distance VA and hRSD threshold of PWD with various severities of diabetic retinopathy (DR, blue lines, N=36; 1, healthy controls, HC, N=27; 2, mild to moderate non-proliferative DR, N=11; 3, severe to very severe non-proliferative DR, N=12; 4, proliferative DR or non-proliferative DR affecting the fovea, N=13) and age-related macular degeneration (AMD, red lines, N=37; 1, HC, N=27; 2, early AMD, N=10; 3, high-risk intermediate AMD, N=11; 4, advanced AMD, N=16) from the study by Wang et al. (2013). B. Mean±95%CI distance visual acuity (grey line) and hRSD threshold (black line) of PWD with various severities of DR (N=292; HC, HC, N=50; R0, no DR, N=29; R1, background DR, N=202; R2, pre-proliferative DR, N=45; R3, proliferative DR, N=16) from the EDDMO study.

In the same study, Wang et al. (2013) examined the relationship between the hRSD threshold and CST of these 36 PWD combined with 37 participants with various severities of AMD. In the EDDMO study, we found no significant correlation between the hRSD threshold and CST because we only had DR data and not AMD (Section 8.3.2). Wang et al. (2013) found that the hRSD threshold deteriorated significantly with increasing CST (r=0.58, p<0.001). However, the CST of PWD and participants with AMD were not examined separately in the study by Wang et al. (2013).

In a conference abstract, Wang et al. (2015) reported on the hRSD threshold of 33 PWD with DMO receiving monthly to bi-monthly anti-VEGF injection treatment. For the PWD who completed the 3-month visit (N=28), the mean±SD hRSD threshold in their study eyes prior to treatment was -0.22±0.21. In the EDDMO study, for the 25 eyes which required treatment, their hRSD threshold was -0.44±0.33 logMAR prior to treatment which again suggests that PWD in the study by Wang et al. (2015) had more severe disease (Table 7.3). A more thorough comparison of the study by Wang et al. (2015) is made with data from the

EDDMO study in the next chapter (Chapter 9) that examines longitudinal data of PWD including treatment effects. Several other studies published as conference abstracts have examined the hRSD threshold in PWD but their data analyses were combined with patients with various forms of maculopathy and the groups of PWD were not studied separately (Bartlett et al., 2015, He et al., 2013, Wang et al., 2010).

The hRSD threshold of PWD and HC was examined and compared with distance and near VA. When assessing the effectiveness of any test, it is important to differentiate between the effect of normal ageing and the effect of pathology on the test results. This chapter found that in HC who had OCT imaging (N=50), there was no significant correlation between age and hRSD (r=0.13, p=0.351) or between age and near VA (r=0.13, p=0.412). However, there was a significant correlation between age and distance VA (r=0.44, p=0.001) (Section 8.3.4, Figure 8.4). Comparison of the effect of age on the 106 HC who completed both the 3AFC and 4AFC hRSD tests in a previous chapter (Section 6.3.5, Figure 6.9) found a weak but statistically significant correlation between age and both 3AFC (r=0.19, p=0.05) and 4AFC thresholds (r=0.22, p=0.02). However, in the same group of HC, there was a significant, and more marked, correlation between age and near VA (r=0.65, p<0.001). When the relationship between age and hRSD thresholds was examined in younger participants (<55 years) and older participants (≥55 years) in 229 HC (all HC who completed 4AFC hRSD and HC who only did 3AFC hRSD; Section 4.2.6, Figure 4.10), there were no correlations between age and hRSD threshold in both younger participants (r=0.05, p=0.53) and older participants (r=0.12, p=0.35). The overall results suggest that in the absence of pathology, there is a correlation between age and hRSD performance, but that the hRSD test is more resistant to the general effects of ageing compared to distance or near VA. This would be a useful feature of the hRSD test to discriminate between normal ageing and pathology compared to distance or near VA.

It was found in Chapter 6 (Section 6.2.10) that the hRSD threshold of PWD with no evidence of DR (R0M0) was worse compared to HC. Similarly, this chapter showed that in PWD with no DR (R0M0 and NMO) or minimal DR (R1M0 and NMO), there was a deterioration in hRSD threshold, distance and near VA compared to HC (Table 8.2). One-way ANOVA showed that PWD with minimal DR had a significantly worse hRSD threshold compared to HC (p<0.001); PWD with no DR and PWD with minimal DR also had significantly worse distance and near VA compared to HC (Section 8.3.3, Table 8.3). These results agree with findings from Chapter 7 and support the notion that there are both structural and functional changes in eyes of PWD prior to clinically visible changes.

One of the objectives of this chapter was to compare the relative performances of hRSD, distance and near VA in detecting DMO. From these results, it can be seen that hRSD performance does not exceed the performance of distance VA in differentiating HC from PWD or in detecting CIMO; this group is important because they are more likely to need treatment and close monitoring (Section 8.3.6, Table 8.9). Another objective of this chapter was to determine if the addition of hRSD to distance VA, which is routinely collected in DR screening, can improve the detection of DR. The effect of combining hRSD plus near VA plus distance VA were also studied. It can be seen in Table 8.9 that for the detection of PWD compared to HC, the addition of hRSD to distance VA (AUC 0.74, 95%CI 0.67-0.82, p<0.001) improved the AUC to 0.81 (95%CI 0.75-0.87, p<0.001). A comparison of the distance VA and hRSD and distance VA ROC curves (Table 8.10) showed that this improvement was statistically significant (p=0.006). The British Diabetic Association suggest that a screening test for DR should have a sensitivity of 80% and a specificity of 95% (Scanlon, 2017a). The hRSD test, even when combined with distance or near VA does not reach these standards in detecting DR (Table 8.9).

For the detection of PWD who were treated, hRSD plus distance VA increased AUC to 0.66 (95%CI 0.55-0.77, p=0.007) while the combination of hRSD plus near VA plus distance VA increased AUC further to 0.71 (95%CI 0.60-0.81, p<0.001) (Table 8.9). Although these findings suggest that PWD who will require treatment might be better detected using the hRSD test in addition to distance VA, these slight improvements do not justify the time and cost of introducing the hRSD test to the LDESP to make a practical difference in the real world. In addition, a comparison of the ROC curves of PWD who were not treated with those who were treated showed that these differences were not statistically significant (Table 8.10).

Similar to the HC, PWD found the hRSD to have good usability (Section 8.3.8, Table 8.8). However, the hRSD test times suggest that each test would take approximately 5min 20sec for both eyes, which need to be considered if introduced to DR screening (Section 8.3.8). As previously mentioned above, there are limited studies on the ability of the hRSD test in detecting DR and DMO. Therefore, it is challenging to compare the results of the EDDMO study with other studies (Wang et al., 2015, Wang et al., 2013, Wang et al., 2010, He et al., 2013, Bartlett et al., 2015).

There is evidence that the current surrogate markers for defining the presence of maculopathy (M1) at screening are not reliable at detecting DMO. The Grampian Screening Programme found that only 12% of patients with surrogate markers referred to an

ophthalmologist had indications of DMO when examined by slit-lamp biomicroscopy while the Liverpool Screening Programme found this number to be around 14% (Olson et al., 2013). Since there are inherent issues with using the existing definition of M1 to detect DMO, this may account for the poor apparent performance of the hRSD test (AUC 0.52, 95%CI 0.45-0.58, *p*=0.592), distance VA (AUC 0.52, 95%CI 0.45-0.59, *p*=0.606) and near VA (AUC 0.51, 95%CI 0.44-0.58, *p*=0.764) in distinguishing PWD graded as M0 from M1 (Table 8.9). Similarly, although the hRSD threshold was worse in PWD with M1 (-0.60±0.26 logMAR) compared to M0 (-0.63±0.21 logMAR), this difference was not statistically significant (*p*=0.717) (Table 8.4). In addition to the definitional issues of M1, the EDDMO study was limited by several factors. Firstly, there were only a small number of PWD with CST >400µm which makes the detection of DMO using the hRSD test challenging (Figure 8.3). Secondly, PWD retinopathy and maculopathy groups in Sections 8.3.5 and 8.3.6 were not analysed separately. Therefore, PWD with a severe retinopathy grade (e.g. R3) could have no maculopathy (M0) and vice versa, confounding some of the results.

In summary, results from this chapter have shown that there are functional changes defined by hRSD threshold, distance and near VA in eyes of PWD prior to clinically visible changes. These findings are consistent with the structural changes seen on OCT in eyes of PWD with no clinical or OCT evidence of DR. The hRSD test appears more resistant to the effects of ageing compared to distance or near VA. The hRSD test does not meet the standard of the British Diabetic Association as a screening test for DR. However, the results from this chapter have been limited by the inherent definitional issues of using M1 as a surrogate marker for DMO, the lack of a consensus for an OCT definition of DMO and the small number of PWD with DMO (CST >400µm) (Scanlon, 2017a). The following chapter (Chapter 9), will examine the longitudinal data of PWD who attended a second visit.

CHAPTER 9. ANALYSIS OF LONGITUDINAL DATA IN PEOPLE WITH DIABETES

9.1 CHAPTER INTRODUCTION

Previous chapters presented an analysis of cross-sectional retinal structural information obtained from OCT imaging (Chapter 7) and visual functional data collected from PWD (Chapter 8). In this chapter, the longitudinal data for PWD is examined to explore the performance of the hRSD test in assessing both change and stability in the retina of PWD compared to distance and near VA. Parameters also examined for longitudinal change are CST as measured by macular OCT, the commencement of treatment and change in HbA_{1c} levels. There were 159 PWD who attended for a consecutive second visit after mean±SD 191±86 days. As the EDDMO study was an observational study, the follow-up interval was determined by the ophthalmologist who assessed the PWD during their first visit in DEC.

Macular OCT has been widely used to diagnose and monitor DMO. There has been some discussion in the literature about using specific features seen on macular OCT as longitudinal biomarkers of disease progression and response to treatment (Browning et al., 2008, Browning et al., 2007, DRCR network., 2006). In longitudinal studies of PWD with a mildly thickened maculae, the DRCR Network has recommended monitoring CST on OCT measurements to define change (Browning et al., 2008). CST was chosen because of its higher reproducibility and correlations with other measurements of the central macula (Browning et al., 2008). The DRCR Network defined change as the difference in the thickness between two measurements made at different times (Browning et al., 2008). In a DRCR Network study on the diurnal variation in retinal thickness in 156 eyes with centre-involving DMO, a threshold of 50µm change in CST was considered to be clinically meaningful as it exceeded the upper 95% confidence interval for the detection of change of 40µm (based on reproducibility data from 1147 eyes) (DRCR network., 2006). As the EDDMO study has a large proportion of eyes with no or mild macular thickening, the DRCR Network definition as described above was applied to define a change in macular thickness in the EDDMO study.

HbA_{1c} has been used in the diagnosis and management of diabetes as it is a biomarker of glycaemic control (Inzucchi, 2012) (Section 2.2.1). Therefore, HbA_{1c} levels will be examined in this chapter to determine if there is any correlation between changes in HbA_{1c} and vision.

Most physicians agree that 0.5% (5.5 mmol/mol) would be considered a clinically relevant change (American Diabetes Association, 2014, Lenters-Westra et al., 2014, Campbell et al., 2019). Therefore, the EDDMO study uses a threshold of greater than or equal to 5.5 mmol/mol to define a change in HbA_{1c} between visits.

The description of PWD who were NT (N=143 eyes) and PWD who were TT (N=16 eyes) is covered in Section 9.3.1. PWD who were NT will be studied separately (Sections 9.3.3 to 9.3.6) from PWD who were TT. In PWD who were TT, CST, vision and HbA_{1c} are examined to evaluate treatment effect (Section 9.3.7). In patients who were NT and were stable (defined as ±49um change in CST), this chapter will also investigate the test-retest variability of hRSD in PWD (Section 9.3.8).

In Chapter 7, a comparison of retinal thickness between HC and PWD without DR and PWD with minimal DR during their first visit found thinning of the RNFL, GCL and IPL, thus supporting the concept of neurodegeneration being important in DR (Section 7.3.9). The ganglion cell complex consists of the ganglion cell axons, cell bodies and dendrites, which reside in the RNFL, GCL and IPL respectively (Scuderi et al., 2020). Similar to DR, glaucoma may also be thought of as a neurodegenerative condition; it has been described as a group of optic neuropathies characterised by progressive degeneration of retinal ganglion cells and their axons resulting in visual field loss (Scuderi et al., 2020).

Ganglion cell loss detected by OCT along with visual field tests has been used to diagnose and monitor glaucoma (Kim and Park, 2018). Although different OCT manufacturers have various protocols to measure glaucoma changes, these protocols either measure ganglion cell axons around the optic nerve head (peripapillary RNFL, pRNFL) or measure the thickness of the RNFL, GCL and IPL at the macula. The monitoring of glaucoma using pRNFL protocols has been well established but the macular ganglion cell complex protocols are a more recent addition (Kim and Park, 2018). The macular contains more than 50% of ganglion cells and the sizes of the ganglion cell bodies in that area are up to 20 times larger than the diameter of axons, thus making them easier to measure (Curcio and Allen, 1990, Wassle et al., 1989). There is evidence that structural changes in ganglion cells precede functional changes detected on visual field tests in glaucoma (Medeiros et al., 2013, Zhang et al., 2016, Mohammadzadeh et al., 2020). Therefore, macular ganglion cell complex thickness has been used as a reliable biomarker in detecting pre-perimetric glaucomatous damage (Scuderi et al., 2020). Since macular GCL and IPL thickness is strongly correlated

with macular ganglion cell counts (Zhang et al., 2014), GCL and IPL loss also reflect central visual field deficits better than pRNFL (Shin et al., 2014).

There are limited studies with longitudinal data in DRN and these studies are examined in the discussion of this chapter (Section 9.4) (Sohn et al., 2016, Kim et al., 2018, Lim et al., 2019). However, there is substantial longitudinal data using OCT to diagnose and monitor glaucoma (Mohammadzadeh et al., 2020, Zhang et al., 2016, Medeiros et al., 2013, Hammel et al., 2017). Therefore, the ganglion cell complex thickness (RNFL, GCL and IPL) used to diagnose and monitor glaucoma is specifically examined in PWD with no or minimal DR who attended for a second visit in Section 9.3.9 to explore if longitudinal thickness changes of these layers would be potentially suitable as biomarkers for DRN as well. In addition, in Section 7.3.9 loss of ONL in the CSF was described. Therefore, longitudinal change in the other layers (INL, OPL, ONL and RPE) will also be examined for completeness.

9.2 METHODS OF DATA ANALYSES

General and OCT methods have been covered in Chapters 3 and 4. All the measurements of interest used in this chapter are continuous variables. One eye from each participant was randomly selected for analysis except in Section 9.3.1 where the eye with the more severe DR or OCT grade was selected to assess follow-up interval and place of follow-up; where there was only one eye available for analysis, the DR and OCT grade of that eye was used. In this chapter, paired *t*-test and Wilcoxon signed-rank test (Sections 9.3.4, 9.3.5, 9.3.6, 9.3.7, 9.3.8, 9.3.9) were used to examine differences between the means of two groups. Pearson and Spearman's rank correlation coefficients were used to examine the strength of correlation between two variables as appropriate (Sections 9.3.4, 9.3.6, 9.3.8). Kruskal-Wallis test with Dunn's multiple comparisons test was used to examine change in CST, vision and HbA_{1c} where there were more than two groups and assumptions for one-way ANOVA were violated (Sections 9.3.5, 9.3.6). Bland-Altman analysis and intra-class correlation were used to assess test-retest reliability in Section 9.3.8. All data analyses were performed using Excel (2016), GraphPad Prism (version 8) and SPSS (version 25).

9.3 RESULTS

9.3.1 DESCRIPTION OF PWD AT THE SECOND VISIT

There were 292 PWD who were referred from the LDESP to the DEC; 159 of these patients attended for a follow-up visit (Figure 3.2). The mean±SD time between their first and

second visits was 191±86 days (Table 9.1). Description of the 292 PWD during visit 1 has been covered in Section 7.3.2 (Table 7.3). Of the 292 PWD who attended the DEC, 267 (91.4%) did not require treatment at the time of their first visit. 143 of the 292 PWD (48.9%) attended for a follow-up visit. The mean±SD time between these first and second visits was 192±84 days. 25 (8.6%) of the 292 PWD in the study population were TT and of these 25, 16 attended for a follow-up visit within the period of this study. For this TT group, treatment was delivered between the two visits. The mean±SD time between their first and second visits was 178±100 days. The description of the group of 159 PWD during visits 1 and 2 are shown in Table 9.1. Further description of the TT group is covered in Section 9.3.7 which will examine treatment effects. Note that throughout the rest of this chapter, all PWD means the group of 159 who attended two visits.

It can be seen from Table 9.1 that generally, the PWD who were NT and PWD were TT had a similar mean age (NT 54±14years; TT 53±20years) and duration of diabetes (NT 15±10; TT 15±10years). There were more males in both NT and TT groups but this gender gap was wider in the TT group (NT M 59%, F 41%; TT M 75%, F 25%). In both NT and TT groups, most PWD were Caucasian (NT 90%; TT 88%) and had type 2 diabetes (NT 67%; TT 62%).

Unsurprisingly, the TT group had higher CST, worse distance VA, near VA, hRSD threshold, higher HbA_{1c} levels and worse BP control compared to the NT group (Table 9.1). In the NT group, their CST (visit 1 287.3±35.2µm, visit 2 289.2±41.8µm), distance VA (visit 1 0.04±0.16 logMAR, visit 2 0.04±0.17 logMAR), near VA (visit 1 0.16±0.23 logMAR, visit 2 0.13±0.21 logMAR) and hRSD threshold (visit 1 -0.64±0.20 logMAR, visit 2 -0.63±0.21 logMAR) were generally similar in visits 1 and 2. In the TT group, their CST (visit 1 390.8±149.4µm, visit 2 319.3±72.2µm) improved by visit 2 and there were also small improvements in their distance (visit 1 0.26±0.39 logMAR, visit 2 0.21±0.36 logMAR) and near VA (visit 1 0.35±0.43 logMAR, visit 2 0.32±0.35 logMAR) but not hRSD threshold (visit 1 -0.33±0.36 logMAR, visit 2 -0.31±0.31 logMAR). In both NT and TT groups, their HbA_{1c} improved and a smaller proportion of PWD was on insulin during their second visit compared to their first visit (Table 9.1).

Table 9.1 Descriptive statistics for participants who attended twice (Total N=159)

	All (N=159)		Not treated (NT) (N=143)		Treated (TT) (N=16)	
	Visit 1	**Visit 2**	**Visit 1**	**Visit 2**	**Visit 1**	**Visit 2**
Days between visits	191±86		192±84		178±100	
Age at first visit (years) Mean±SD (range)	54±15 (20-84)		54±14 (20-84)		53±20 (22-84)	
Gender **M (%)** **F (%)**	 97 (61%) 62 (39%)		 85 (59%) 58 (41%)		 12 (75%) 4 (25%)	
Race **Caucasian (%)**	 144 (90%)		 130 (90%)		 14 (88%)	
Type Diabetes (No.) **Type 1** **Type 2**	 53 (33%) 106 (67%)		 47 (33%) 96 (67%)		 6 (38%) 10 (62%)	
Duration of Diabetes at first visit (years)	15±10		15±10		15±10	
CST (µm)	297.7±64.9	292.3±46.3	287.3±35.2	289.2±41.8	390.8±149.4	319.3±72.2
Distance VA (logMAR)	0.07±0.21	0.06±0.20	0.04±0.16	0.04±0.17	0.26±0.39	0.21±0.36
Near VA (logMAR)	0.18±0.26	0.15±0.23	0.16±0.23	0.13±0.21	0.35±0.43	0.32±0.35
hRSD (logMAR)	-0.61±0.24	-0.60±0.24	-0.64±0.20	-0.63±0.21	-0.33±0.36	-0.31±0.31
HbA_{1c} (mmol/mol)	71.6±20.5	70.8±18.8	70.2±18.9	69.8±18.0	84.9±28.9	78.7±23.9
On Insulin (%)	82 (52%)	72 (45%)	73 (51%)	65 (45%)	9 (56%)	7 (44%)
BP (mmHg) **Systolic** **Diastolic**	 139±19 82±12	 140±25 82±12	 138±19 82±12	 139±26 82±12	 146±18 82±12	 150±18 83±11

The above descriptive statistics are based on randomly selecting one eye from each participant for analysis. However, ophthalmologists in DEC decide the follow-up interval for each patient based on both eyes and it is assumed that the follow-up interval would be determined by the most severe eye which needs an earlier follow-up. Therefore, the following section will examine follow-up intervals based on the most severe DR or OCT grade for all PWD from visit 1 where there were two eyes available for analysis. Where there was only one eye available for analysis, the DR or OCT grade of that eye was used. Please note that these follow-up intervals were when patients attended for their second visit and not necessarily the follow-up interval recommended by the ophthalmologist; patients who had cancelled or not attended their initial appointment would be seen at a later date than initially intended.

Table 9.2 shows the duration of follow-up according to the eye with the more severe DR grade and OCT grade from visit 1 in all PWD. There was one PWD who was graded as R0M0 who returned after 63 days for a review. Please note that the other eye of the same patient

was graded as R1M0 and had CSMO. However, the eye graded as R1M0 was amblyopic and therefore excluded from the analysis. It can be generally seen that, as might be expected, eyes with less severe DR grades (R1M0, 216±94 days) had longer follow-up intervals compared to eyes with more severe DR grades (R3AM1, 90 days). Similarly, eyes with less severe OCT grades (NMO 210±90 days) had longer follow-up intervals compared to eyes with more severe OCT grades (CIMO 170±78 days).

Table 9.2 Days between visits in all PWD (N=159) according to the eye with the most severe diabetic retinopathy (DR) and OCT grades where data from both eyes were available. Where there were only data from one eye available, the DR and OCT grades of that eye was used.

Grade	No. eyes	Mean±SD duration of follow up (days)*
Diabetic Retinopathy		
R0M0*	1	63
R1M0	43	216±94
R1M1	74	189±77
R2M0	11	177±73
R2M1	24	178±97
R3SM0*	2	178, 182
R3AM0*	2	133, 90
R3SM1*	1	141
R3AM1*	1	90
OCT		
NMO	58	210±91
NCTMO	19	206±99
CTMO	21	183±71
CIMO	47	170±78

*Individual duration of follow-up listed where there were very few eyes in the group

Local guidelines at the Royal Liverpool University Hospital allow PWD with R1M1 in their worse eye to have a follow-up at digital surveillance (DS) clinics instead of DEC (Chapter 2). Of the 159 PWD who attended for a follow-up visit, 134 attended DEC while 25 attended DS (Table 9.3). Table 9.3 shows that for the 134 PWD seen in DEC, there were 32 PWD (23.9%) graded as R1M0 that could have been discharged to RDS and 60 PWD (44.8%) graded as R1M1 that could have been followed-up at DS. For the 25 PWD seen in DS, there were 11 (44%) graded as R1M0 that could have been discharged back to RDS.

Table 9.3 Diabetic retinopathy (DR) grades of PWD who had a follow-up in the diabetic eye clinic (DEC; N=134) and digital surveillance (DS; N=25). The eye with the more severe DR was used where data from both eyes were available. Where there were only data from one eye available, the DR grade of that eye was used.

Follow-up clinic	Diabetic Retinopathy Grade	No. eyes
Diabetic eye clinic	R0M0	1
	R1M0	32
	R1M1	60
	R2M0	11
	R2M1	24
	R3SM0	2
	R3AM0	2
	R3SM1	1
	R3AM1	1
Digital surveillance	R1M0	11
	R1M1	14

9.3.2 OCT QUALITY IN PWD AT THE SECOND VISIT

This section examines the quality of the OCT images obtained from the PWD during their second visit to investigate whether the quality of these images is equivalent to images from their first visit described in Section 7.3.4. The grading protocol to assess the quality of OCT images was described in Chapter 4. Scans from 159 eyes (one eye per participant, selected at random) were available for grading, of which 56 (35%) were graded as good quality while 103 (65%) were graded as fair quality. The foveal depression was present in the majority of the scans (N=149, 94%). There was no evidence of VMT in the majority of the scans (N=155, 97.5%), two had questionable evidence of VMT and two had definite evidence of VMT. There was no evidence of an ERM in the majority of the scans (N=157, 99%), two scans had questionable evidence of an ERM. None of the scans had evidence of macular holes. Automated retinal segmentation was possible in all layers in 63 (39.6%) scans while some manual segmentation was required in 91 (57.2%) scans. It was not possible to have retinal segmentation performed in 5 (3.1%) scans and only total retinal thickness was used for analysis in these scans. Similar to the OCT image quality assessment from visit 1 (Section 7.3.4), the OCT quality assessment from this section reassures that image quality remained high for PWD during their second visit.

9.3.3 CHANGE IN RETINOPATHY, MACULOPATHY AND OCT GRADES IN ALL PWD AND PWD WHO WERE NOT TREATED (NT) AND PWD WHO WERE TREATED (TT) IN VISITS 1 AND 2

This section examines the proportion (%) of eyes in all PWD, NT and TT groups according to their retinopathy, maculopathy and OCT grades in visits 1 and 2. The pattern of change in individual eyes will be examined in Section 9.3.5. Table 9.4 shows the retinopathy, maculopathy and OCT grades of all PWD (N=159), PWD who were NT (N=143) and PWD who were TT (N=16) during their first and second visits.

Table 9.4 shows that there was a shift in the proportion of eyes with more severe retinopathy and maculopathy grades to less severe grades in the NT group during visit 2 compared to visit 1. There were 106 (74.1%) R1 eyes during visit 1 and this increased to 120 (83.9%) in visit 2. Meantime, the proportion of R2 eyes decreased by more than half during visit 2 (26 eyes; 18.2%) compared to visit 1 (14 eyes; 9.8%). Similarly, the number of M0 eyes increased from 66 (46.2%) in visit 1 to 77 (53.8%) in visit 2 while the number of M1 eyes decreased from 77 (53.8%) in visit 1 to 66 (46.2%) in visit 2. Although there was less change in their OCT grades between visits, there was still a trend towards an improvement with more NMO eyes (visit 1 62 eyes, 43.4%; visit 2 71 eyes, 49.7%) and fewer CIMO eyes (visit 1 46 eyes, 32.2%; visit 2 43 eyes, 30.1%) in visit 2 compared to visit 1. Therefore was a general improvement in the NT group between visit 1 and visit 2.

In PWD who were TT (N=16), the trends in their retinopathy, maculopathy and OCT grades between visits were more difficult to interpret due to the small number of eyes (Table 9.4) that had treatment between visits. There was an increase in the number of R1 (visit 1, 4 eyes, 25%; visit 2, 6 eyes (37.5%) and R3 (visit 1, 4 eyes, 25%; visit 2, 6 eyes, 37.5%) eyes between visits. Meantime, the proportion of R2 eyes had decreased by half between visits (visit 1, 8 eyes, 50%; visit 2, 4 eyes, 25%). There was a trend towards an improvement of their maculopathy grades with an increase in M0 eyes (visit 1, 3 eyes, 18.8%; visit 2, 6 eyes, 37.5%) and a decrease in M1 eyes (visit 1, 13 eyes, 81.3%; visit 2, 10 eyes, 62.5%) between visits. Likewise, there was a general increase in the portion of eyes with less severe OCT grades (NMO and NCTMO) while the number of CTMO eyes had decreased (visit 1, 4 eyes, 25%; visit 2 0 eyes, 0%) and the number of CIMO eyes remained similar (visit 1, 10 eyes, 62.5%; visit 2, 11 eyes, 68.8%) between visits.

Table 9.4 Retinopathy, maculopathy and OCT grades of eyes in people with diabetes (PWD) who had a second visit. The number of eyes in each category (all PWD, not treated, treated) during visit 1 or 2 is shown with the corresponding percentage of the total number of eyes in each category displayed in adjacent brackets.

	All PWD N=159 (%)		PWD not treated (NT) N=143 (%)		PWD treated (TT) N=16 (%)	
	Visit 1	**Visit 2**	**Visit 1**	**Visit 2**	**Visit 1**	**Visit 2**
Retinopathy						
R0	9 (5.7)	7 (4.4)	9 (6.3)	7 (4.9)	0 (0)	0 (0)
R1	110 (69.2)	126 (79.2)	106 (74.1)	120 (83.9)	4 (25)	6 (37.5)
R2	34 (21.4)	18 (11.3)	26 (18.2)	14 (9.8)	8 (50)	4 (25)
R3 **R3A** **R3S**	6 (3.8) 3 (1.9) 3 (1.9)	8 (5.0) 5 (3.1) 3 (1.9)	2 (1.4) 2 (1.4) 0 (0)	2 (1.4) 0 (0) 2 (1.4)	4 (25) 3 (18.8) 1 (6.3)	6 (37.5) 5 (50) 1 (6.3)
Maculopathy						
M0	69 (43.4)	83 (52.2)	66 (46.2)	77 (53.8)	3 (18.8)	6 (37.5)
M1	90 (56.6)	76 (47.8)	77 (53.8)	66 (46.2)	13 (81.3)	10 (62.5)
OCT						
NMO	63 (39.6)	73 (45.9)	62 (43.4)	71 (49.7)	1 (6.3)	2 (12.5)
NCTMO	18 (11.3)	14 (8.8)	17 (11.9)	11 (7.7)	1 (6.3)	3 (18.8)
CTMO	22 (13.8)	18 (11.3)	18 (12.6)	18 (12.6)	4 (25)	0 (0)
CIMO	56 (35.2)	54 (34.0)	46 (32.2)	43 (30.1)	10 (62.5)	11 (68.8)

9.3.4 RELATIONSHIP BETWEEN CHANGE IN CST AND CHANGE IN VISION IN PWD WHO WERE NOT TREATED (NT)

In this section, change in CST was examined in eyes (N=143) that were NT. Eyes (N=16) that were TT will be discussed separately in Section 9.3.7 to examine treatment effects. Using the OCT definitions of change in CST in Section 9.1, there were only 2 eyes that deteriorated, 141 that remained stable and no eyes improved (Table 9.5). Of the eyes which deteriorated, the CST in one eye increased from 341µm to 419µm and its distance VA deteriorated from 0.04 logMAR to 0.2 logMAR while its hRSD threshold (visit 1 -0.66 logMAR, visit 2 -0.65 logMAR) and near VA (visit 1 0.12 logMAR, visit 2 0.12 logMAR) showed minimal or no change. In the second eye which deteriorated, its CST increased from 480µm to 574µm; hRSD threshold (visit 1 -0.65 logMAR, visit 2 -0.29 logMAR), distance (visit 1 0.38 logMAR, visit 2 0.5 logMAR) and near VA (visit 1 0.44 logMAR, visit 2 0.76 logMAR) all deteriorated. Of the eyes which remained stable, there was a minimal increase in CST (0.60±7.9 µm) while hRSD (increase 0.01±0.13 logMAR), distance (decrease

0.003±0.11 logMAR) and near VA (decrease 0.003±0.11 logMAR) essentially remained the same (Table 9.5).

Table 9.5 Mean±SD change in hRSD, distance and near VA (logMAR) in eyes in which central subfield thickness (CST) remained stable between visits 1 and 2. Positive numbers represent a deterioration while negative numbers represent an improvement.

	No. eyes	Change in CST (µm)	Change in hRSD (logMAR)	Change in distance VA (logMAR)	Change in near VA (logMAR)
Stable	141	0.60±7.9	0.01±0.13	-0.003±0.11	-0.003±0.11

Although there was little change in mean CST, hRSD, distance and near VA in eyes that did not receive treatment between visits 1 and 2, the relationship between CST and the visual parameters was examined using correlation analysis. Unsurprisingly, there were no statistically significant correlations between change in CST and change in hRSD (*rho*=0.007, *p*=0.936), distance (*rho*=0.019, *p*=0.817) and near VA (*rho*=0.047, *p*=0.581), with no obvious pattern seen. (Figure 9.1).

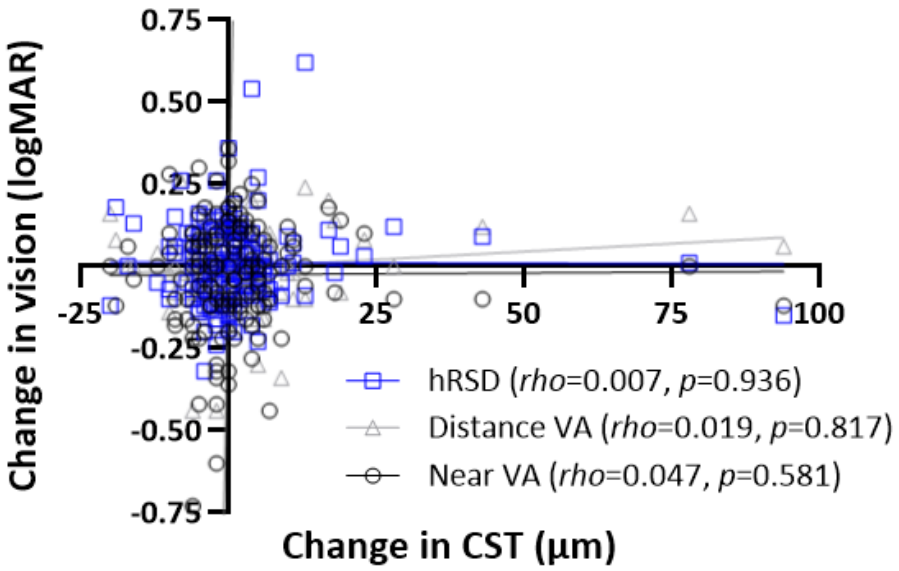


Figure 9.1 Relationship between change in central subfield thickness (CST; µm) and change in hRSD, distance and near VA (logMAR). Spearman *rho* and *p* value shown.

9.3.5 RELATIONSHIP BETWEEN CHANGE IN RETINOPATHY, MACULOPATHY AND OCT GRADES AND CHANGE IN CST AND VISION IN PWD WHO WERE NOT TREATED (NT) BETWEEN VISITS

This section explores the relationship between the change in retinopathy, maculopathy and OCT grades with change in CST and vision in eyes of PWD who were NT between visits. The data for eyes that received treatment is examined separately in Section 9.3.7. When eyes were examined according to their retinopathy grades, that the majority had no change in their retinopathy grade (stable; N=121), 6 eyes had a worse grade (deteriorated) while 16 eyes had a better grade (improved) (Table 9.6). Likewise, when eyes were examined according to their maculopathy grades, the majority were stable (N=99), 16 eyes had a worse grade (deteriorated) while 28 eyes had a better grade (improved) (Table 9.6). Similar to findings from retinopathy and maculopathy grades, the majority of eyes were stable according to their OCT grades (N=105), 15 eyes deteriorated while 23 eyes improved (Table 9.6).

The mean CST (µm), hRSD threshold, distance and near VA (logMAR) of eyes which deteriorated remained stable or improved according to their retinopathy, maculopathy and OCT grades were compared between visits 1 and 2 using Student's *t*-test (Table 9.6). There were no significant changes in CST, hRSD, distance or near VA in most groups between visits. However, near VA significantly improved in eyes that had stable maculopathy grades (visit 1 0.17±0.24 logMAR, visit 2 0.14±0.21 logMAR, t=2.031, p=0.045) and in eyes that had improved OCT grades (visit 1 0.23±0.22 logMAR, visit 2 0.12±0.17 logMAR, t=2.385, p=0.026) (Table 9.6).

Table 9.7 breaks down the change in CST (µm), hRSD threshold, distance and near VA (logMAR) in eyes which deteriorated, remained stable or improved according to their retinopathy, maculopathy and OCT grades for eyes at visit 2 compared to visit 1. When eyes were examined according to their retinopathy grades, there was little difference in their CST between visits; stable eyes had an increase of 2.10±13.40µm, eyes which deteriorated had an increase of 0.50±5.86µm while eyes which improved had an increase of 1.00±8.33µm. Similarly, there was little change in their hRSD threshold, distance or near VA between visits. Unsurprisingly, the Kruskal-Wallis test showed that there was no significant difference in CST, hRSD threshold, distance and near VA in eyes which have deteriorated, remained stable or improved according to their retinopathy grades (Table 9.8).

When eyes were examined according to their maculopathy grades, there was little change in their CST, hRSD threshold, distance or near VA (Table 9.7). Kruskal-Wallis test showed that there was no significant difference in CST, hRSD threshold, distance and near VA in eyes which have deteriorated, remained stable or improved according to their maculopathy grades (Table 9.8).

Given the results above, when eyes were examined according to their OCT grades, there was little change in their CST, hRSD threshold or distance VA (Table 9.7). However, in eyes which had improved OCT grades, their near VA improved by -0.11±0.22 logMAR. Kruskal-Wallis test showed no significant difference in CST, hRSD threshold, distance and near VA in eyes which have deteriorated, remained stable or improved (Table 9.8).

Table 9.6 Comparison of mean±SD CST (µm), hRSD, distance and near VA (logMAR) in eyes of people with diabetes (PWD) who were not treated (NT) which have deteriorated, remained stable or improved according to their retinopathy, maculopathy and OCT grades in visits 1 and 2

	No. eyes	CST (µm)		Paired *t*-test		hRSD (logMAR)		Paired *t*-test		Distance VA (logMAR)		Paired *t*-test		Near VA (logMAR)		Paired *t*-test	
		Visit 1	Visit 2	*t*	*p*	Visit 1	Visit 2	*t*	*p*	Visit 1	Visit 2	*t*	*p*	Visit 1	Visit 2	*t*	*p**
Retinopathy grades																	
Deterioration	6	282.50 ±30.59	283.00 ±29.05	0.209	0.843	-0.60±0.19	-0.56±0.23	0.512	0.631	0.02±0.12	0.08±0.15	1.008	0.360	0.11±0.22	0.07±0.17	0.865	0.427
Stable	121	288.70 ±35.41	290.80 ±43.01	1.723	0.087	-0.66±0.20	-0.64±0.21	0.954	0.342	0.04±0.15	0.04±0.18	0.173	0.863	0.17±0.22	0.13±0.21	1.965	0.052
Improvement	16	278.60 ±35.58	279.60 ±36.34	0.480	0.638	-0.56±0.18	-0.55±0.22	0.266	0.794	0.10±0.23	0.08±0.16	0.694	0.499	0.12±0.25	0.15±0.21	0.600	0.558
Maculopathy grades																	
Deterioration	16	292.30 ±39.02	295.00 ±42.92	1.509	0.152	-0.60±0.20	-0.61±0.18	0.224	0.826	0.08±0.17	0.08±0.21	0.133	0.896	0.14±0.19	0.15±0.20	0.527	0.606
Stable	99	286.4± 35.31	288.1± 43.70	1.145	0.255	-0.64±0.21	-0.63±0.22	1.145	0.255	0.04±0.16	0.03±0.16	0.877	0.383	0.17±0.24	0.14±0.21	2.031	**0.045**
Improvement	28	287.60 ±33.46	290.00 ±34.74	1.430	0.164	-0.67±0.17	-0.66±0.20	0.397	0.695	0.03±0.19	0.05±0.21	1.623	0.117	0.12±0.21	0.11±0.20	0.067	0.947
OCT grades																	
Deterioration	15	285.70 ±22.98	287.30 ±26.46	0.845	0.412	-0.63±0.15	-0.60±0.28	0.726	0.480	-0.03±0.13	-0.004±0.19	0.523	0.609	0.06±0.15	0.03±0.16	1.042	0.315
Stable	105	288.00 ±39.31	290.50 ±47.07	1.849	0.067	-0.64±0.21	-0.64±0.20	0.080	0.937	0.05±0.17	0.05±0.18	0.116	0.908	0.16±0.23	0.15±0.22	0.393	0.695
Improvement	23	285.40 ±18.41	284.50 ±17.96	0.850	0.405	-0.66±0.16	-0.61±0.20	1.642	0.115	0.07±0.11	0.05±0.14	0.858	0.400	0.23±0.22	0.12±0.17	2.385	**0.026**

*Statistically significant results are in bold

Table 9.7 Mean±SD change in CST (µm), hRSD, distance and near VA (logMAR) in eyes of people with diabetes (PWD) who were not treated (NT) which have deteriorated, remained stable or improved according to their retinopathy, maculopathy and OCT grades in visit 2 compared to visit 1. Positive numbers represent a deterioration while negative numbers represent an improvement.

	No. eyes	Change in CST (µm)	Change in hRSD (logMAR)	Change in distance VA (logMAR)	Change in near VA (logMAR)
Retinopathy grades					
Deterioration	6	0.50±5.86	0.03±0.16	0.06±0.14	-0.03±0.09
Stable	121	2.10±13.40	0.01±0.13	-0.001±0.11	-0.03±0.17
Improvement	16	1.00±8.33	0.01±0.12	-0.03±0.15	0.02±0.16
Maculopathy grades					
Deterioration	16	2.7±7.1	-0.006±0.10	-0.004±0.11	0.02±0.12
Stable	99	1.64±14.2	0.01±0.13	-0.009±0.12	-0.04±0.19
Improvement	28	2.43±9.0	0.01±0.15	0.03±0.10	-0.001±0.11
OCT grades					
Deterioration	15	1.53±7.0	0.04±0.19	0.02±0.17	-0.03±0.12
Stable	105	2.6±14.3	0.00±0.11	-0.001±0.10	-0.006±0.16
Improvement	23	-0.91±5.15	0.05±0.16	-0.02±0.12	-0.11±0.22

Table 9.8 Kruskal-Wallis test results of eyes of people with diabetes (PWD) who were not treated (NT) which have deteriorated, remained stable or improved according to their retinopathy, maculopathy and OCT grades between visits

Kruskal-Wallis test	Change in CST (µm)	Change in hRSD (logMAR)	Change in distance VA (logMAR)	Change in near VA (logMAR)
Retinopathy grades	χ^2=0.766, *p*=0.682	χ^2= 0.414, *p*=0.813	χ^2= 1.223, *p*=0.543	χ^2= 0.632, *p*=0.729
Maculopathy grades	χ^2= 4.554, *p*=0.103	χ^2= 0.780, *p*=0.677	χ^2= 1.638, *p*=0.441	χ^2= 1.566, *p*=0.457
OCT grades	χ^2= 3.023, *p*=0.221	χ^2= 1.659, *p*=0.436	χ^2=1.864, *p*=0.394	χ^2=3.554, *p*=0.169

9.3.6 RELATIONSHIP BETWEEN CHANGE IN HBA_{1C} AND CHANGE IN CST AND VISION IN PWD WHO WERE NOT TREATED (NT) BETWEEN VISITS

HbA_{1c} is routinely used to monitor glycaemic control in PWD. Here a threshold of greater than or equal to 5.5 mmol/mol is used to define a change in HbA_{1c} between visits as previously described (Section 9.1) (American Diabetes Association, 2014, Lenters-Westra et al., 2014, Campbell et al., 2019). Of the 143 PWD who had NT, there were 94 PWD for whom had HbA_{1c} were recorded at visits 1 and 2 and these results were used for analysis in this section. There were 20 PWD whose HbA_{1c} had deteriorated (greater than or equal to 5.5 mmol/mol increase), 42 who had remained stable (within 5.4 mmol/mol) while 32 improved (greater than or equal to 5.5 mmol/mol decrease) between visits.

Table 9.9 compares the mean±SD CST, hRSD, distance and near VA in eyes of PWD in these three groups. As expected, there was a significant increase in HbA_{1c} levels in the group that deteriorated (t=9.882, p<0.001), a significant decrease in HbA_{1c} levels in the group that improved (t=7.904, p<0.001) and no significant change in the HbA_{1c} levels in the group that remained stable (t=0.379, p=0.707). Interestingly, CST significantly increased in the group that remained stable (visit 1 291.8±41.99 µm, visit 2 298.5±57.45 µm, t=2.173, p=0.036) while there was no significant change in CST in groups that deteriorated or improved. However, note that the increase in CST was much less than the 50 µm limit defined in Section 9.1. Near VA significantly improved in both the groups whose HbA_{1c} deteriorated (visit 1 0.20±0.21 logMAR, visit 2 0.12±0.18 logMAR, t=2.447, p=0.024) and improved (visit 1 0.16±0.20 logMAR, visit 2 0.11±0.19 logMAR, t=2.490, p=0.018). However, there were no changes in the hRSD threshold or distance VA between visits in any of the groups.

Table 9.9 Comparison of mean±SD CST (µm), hRSD, distance and near VA (logMAR) in eyes of people with diabetes (PWD) who were not treated which have deteriorated, remained stable or improved according to change in their HbA_{1c} (deterioration ≥5.5 mmol/mol; stable ±5.4 mmol/mol; improvement ≤5.5 mmol/mol) in visits 1 and 2

Change in HbA_{1c}	No. PWD	HbA_{1c} (mmol/mol)		Paired *t*-test		CST (µm)		Paired *t*-test		hRSD (logMAR)		Paired *t*-test		Distance VA (logMAR)		Paired *t*-test		Near VA (logMAR)		Paired *t*-test	
		Visit 1	Visit 2	*t*	*p*	Visit 1	Visit 2	*t*	*p*	Visit 1	Visit 2	*t*	*p*	Visit 1	Visit 2	*t*	*p*	Visit 1	Visit 2	*t*	*p**
Deterioration	20	69.70± 17.56	83.45± 20.77	9.882	**<0.001**	285.0± 29.58	284.8± 29.50	0.119	0.907	-0.56± 0.22	-0.56± 0.19	0.019	0.985	0.04± 0.14	0.05± 0.13	0.444	0.662	0.20± 0.21	0.12± 0.18	2.447	**0.024**
Stable	42	66.69± 13.84	66.52± 14.45	0.379	0.707	291.8± 41.99	298.5± 57.45	2.173	**0.036**	-0.66± 0.18	-0.64± 0.20	0.526	0.602	0.03± 0.15	0.05± 0.18	0.703	0.486	0.12± 0.21	0.14± 0.22	0.866	0.391
Improvement	32	81.97± 22.81	66.00± 17.07	7.904	**<0.001**	284.9± 32.11	285.6± 34.21	0.593	0.557	-0.67± 0.14	-0.67± 0.16	0.325	0.747	0.05± 0.15	0.04± 0.15	0.564	0.577	0.16± 0.20	0.11± 0.19	2.490	**0.018**

*Statistically significant results are in bold

Their mean change in HbA_{1c}, CST and vision are shown in Table 9.10. Kruskal-Wallis test showed a significant difference in HbA_{1c} levels of PWD who have deteriorated, remained stable and improved (χ^2=80.33, p<0.001). Dunn's multiple comparisons test revealed that the group that had deteriorated was significantly worse compared to those who have remained stable (mean rank difference 31.00 mmol/mol, p<0.001) or improved (mean rank difference 68.00 mmol/mol, p<0.001). The group that improved was also significantly better than those who have remained stable (mean rank difference 37.00 mmol/mol, p<0.001).

Kruskal-Wallis test was used to examine if there was any significant difference in the change in CST and vision in the PWD whose HbA_{1c} had deteriorated, remained stable or improved (Table 9.11). The results showed a significant difference in their change in near VA (χ^2=7.787, p=0.020) but not in their CST, hRSD or distance VA (Table 9.11). However, Dunn's multiple comparisons test showed no difference in the near VA in the groups which deteriorated, remained stable or improved.

Table 9.10 Mean±SD change in CST (µm), hRSD (logMAR), distance and near VA (logMAR) in PWD who were not treated (NT) whose HbA_{1c} had deteriorated, remained stable or improved between visits. Positive numbers represent a deterioration while negative numbers represent an improvement.

	No. of PWD	Change in HbA_{1c} (mmol/mol)	Change in CST (µm)	Change in hRSD (logMAR)	Change in distance VA (logMAR)	Change in near VA (logMAR)
Deterioration	20	13.75±6.22	-0.20±7.52	0.00±0.12	0.01±0.08	-0.08±0.14
Stable	42	-0.17±2.85	6.74±20.10	0.01±0.15	0.02±0.14	0.02±0.15
Improvement	32	-15.97±11.43	0.63±5.96	0.01±0.11	-0.01±0.10	-0.05±0.11

Table 9.11 Kruskal-Wallis test results of PWD who were not treated (NT) whose HbA_{1c} have deteriorated, remained stable or improved according to their change in CST and vision between visits

Kruskal-Wallis test	Change in CST (µm)	Change in hRSD (logMAR)	Change in distance VA (logMAR)	Change in near VA (logMAR)
HbA_{1c}	χ^2=1.882, p=0.390	χ^2=0.050, p=0.975	χ^2=3.348, p=0.188	χ^2=7.787, ***p*=0.020***

*Statistically significant results are in bold

There was no significant correlation between change in HbA_{1c} and change in CST (Figure 9.2; r=-0.030, p=0.771). There was also no significant correlation between change in HbA_{1c} and change in hRSD (r=-0.005, p=0.960), distance (r=0.073, p=0.485) and near VA (r=0.013, p=0.905) Figure 9.3).

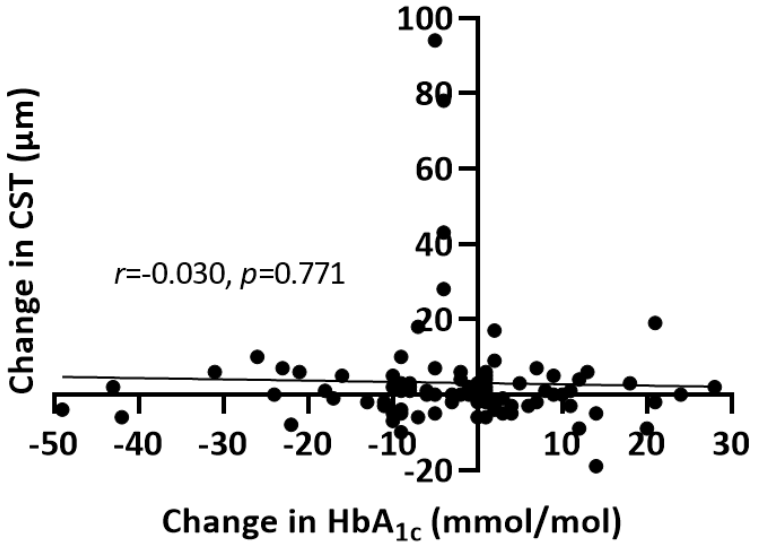


Figure 9.2 Relationship between change in HbA_{1c} (mmol/mol) and change in central subfield thickness (CST; µm) in people with diabetes who were not treated (NT). Pearson *r* and *p* value shown.

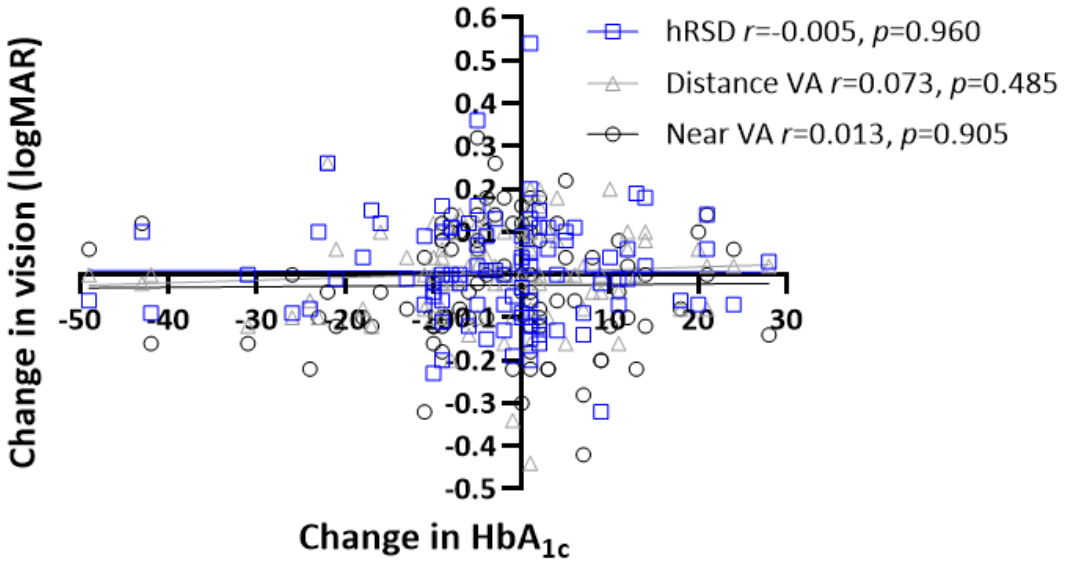


Figure 9.3 Relationship between change in HbA_{1c} (mmol/mol) and change in hRSD, distance and near VA (logMAR) in people with diabetes who were not treated (NT). Pearson *r* and *p* value shown.

9.3.7 EFFECT OF TREATMENT ON CST, VISION AND HBA_{1C}

There was a small group of 16 eyes which received treatment prior to their second visit. The general description of these eyes is shown in Table 9.1 while their retinopathy, maculopathy and OCT grades in visits 1 and 2 are shown in Table 9.4. The description of each eye and the treatment received is shown in Table 9.12, five eyes received intravitreal aflibercept, four received PRP laser, four received macular laser while three eyes received a combination of treatment. After treatment, the CST of these eyes decreased from 390.8±149.4µm to 319.3±72.2µm but this did not reach statistical significance (Z=-1.941, *p*=0.052) (Table 9.13). There were no significant differences between the hRSD threshold, distance and near VA and HbA_{1c} of PWD who were TT between visits (Table 9.13). One of the objectives of the EDDMO study is to examine the ability of the hRSD test to monitor DMO. Therefore, a separate analysis was performed for the 7 eyes which had received intravitreal aflibercept to treat macular oedema (Table 9.14) (Virgili et al., 2017). There was a significant improvement in the CST (visit 1 510.4±155.7µm, visit 2 346.0±99.3µm; *t*=3.456, *p*=0.014) and the near VA of these eyes (visit 1 0.65±0.49 logMAR, visit 2 0.53±0.40 logMAR; *t*=2.948, *p*=0.026) but there was no significant change in their hRSD threshold or distance VA. HbA_{1c} in these participants was not statistically different between visits (visit 1 76.3±31.7 mmol/mol, visit 2 60.8±7.3 mmol/mol, *t*=1.715, *p*=0.185).

Table 9.12 Description of people with diabetes (PWD; N=16) who were treated (TT) and attended for a follow-up visit

Age (years)	Gender	Type of diabetes	Duration of diabetes (years)	CST (µm)		Distance VA (logMAR)		Near VA (logMAR)		hRSD (logMAR)		HbA_{1c}* (mmol/mol)		NDESP grade**		Treatment received***
				Visit 1	Visit 2	Visit 1	Visit 2	Visit 1	Visit 2	Visit 1	Visit 2	Visit 1	Visit 2	Visit 1	Visit 2	
54	F	Type 1	24	312	346	-0.04	-0.06	0.14	0.04	-0.47	-0.44	83	NA	R3AM1P1	R3AM1P1	PRP
84	M	Type 2	33	446	310	1.08	0.84	1.32	1.14	0.38	0.14	89	61	R3SM1P1	R3AM0P1	Aflibercept
43	M	Type 1	23	279	271	0.3	0.32	0.32	0.34	-0.25	-0.34	67	76	R3AM0P1	R3SM0P1	PRP
40	M	Type 1	27	256	265	-0.08	-0.06	-0.08	0.26	-0.56	-0.4	73	77	R3AM0	R3AM0P1	PRP
74	F	Type 2	7	743	420	1.00	0.88	0.82	0.64	0.19	0.13	54	NA	R2M1	R2M1P1	PRP, aflibercept, YAG capsulotomy†
74	M	Type 2	2	640	536	0.32	0.4	0.64	0.54	-0.06	-0.08	42	NA	R2M1	R1M1	Aflibercept
53	M	Type 2	6	310	309	0.16	0.14	0.12	0.12	-0.72	-0.69	134	131	R1M1	R1M0P1	Macular laser
22	M	Type 1	7	281	285	0.04	0.14	-0.02	0.04	-0.69	-0.35	131	55	R2M1	R3AM0P1	PRP, aflibercept
28	M	Type 1	24	281	280	-0.06	-0.08	0	0.12	-0.66	-0.55	117	96	R2M1	R1M1P1	Macular laser
60	M	Type 2	15	380	353	0.02	-0.14	0.16	0.26	-0.66	-0.7	119	107	R2M1	R2M1P1	Macular laser
82	F	Type 2	20	591	245	0.84	0.86	1.18	0.9	0.2	0.33	NA	NA	R2M1P1	R2M1P1	Aflibercept
32	M	Type 2	10	312	320	-0.16	-0.1	-0.04	-0.1	-0.39	-0.46	56	51	R2M1	R3AM1P1	PRP
48	F	Type 1	18	425	321	0.16	0	0.24	0.14	-0.22	-0.42	79	56	R2M1	R2M1	Aflibercept
36	M	Type 2	1	289	290	0.06	0.14	0.36	0.48	-0.2	-0.08	96	92	R1M1	R1M1P1	PRP, macular laser
50	M	Type 2	9	260	253	0.02	-0.1	-0.02	-0.1	-0.77	-0.69	71	71	R1M1	R1M1P1	Macular laser
75	M	Type 2	14	447	305	0.42	0.1	0.4	0.34	-0.45	-0.4	63	71	R1M0	R1M0	Aflibercept

*Not available (NA) **NHS Diabetic Eye Screening Programme, ***Peripheral retinal photocoagulation (PRP), †yttrium aluminium garnet (YAG)

Table 9.13 Comparison of CST, vision and HbA_{1c} of people with diabetes (PWD) who were treated (TT) and a follow-up visit (N=16). Paired *t*-test or Wilcoxon signed-rank test is chosen depending on whether the data is normally distributed.

	Visit 1 Mean±SD	Visit 2 Mean±SD	Test	Test results
CST (µm)	390.8±149.4	319.3±72.2	Wilcoxon signed-rank test	Z=-1.941, *p*=0.052
hRSD (logMAR)	-0.33±0.36	-0.31±0.31	Paired *t*-test	*t*=0.173, *p*=0.864
Distance VA (logMAR)	0.26±0.39	0.21±0.36	Wilcoxon signed-rank test	Z=-1.223, *p*=0.221
Near VA (logMAR)	0.35±0.43	0.32±0.35	Paired *t*-test	*t*=0.171, *p*=0.865
HbA_{1c} (mmol/mol)	84.9±28.9	78.7±23.9	Paired *t*-test	*t*=0.603, *p*=0.552

Table 9.14 Comparison of CST, vision and HbA_{1c} of PWD who had intravitreal aflibercept and a follow-up visit (N=7). Paired *t*-tests were used for comparison.

	Visit 1 Mean±SD	Visit 2 Mean±SD	Paired *t*-test	*P**
CST (µm)	510.4±155.7	346.0±99.3	3.456	**0.014**
hRSD (logMAR)	-0.09±0.39	-0.09±0.30	0.000	0.999
Distance VA (logMAR)	0.55±0.42	0.46±0.39	1.489	0.187
Near VA (logMAR)	0.65±0.49	0.53±0.40	2.948	**0.026**
HbA_{1c} (mmol/mol)	76.3±31.7	60.8±7.3	1.715	0.185

*Statistically significant results are in bold

9.3.8 TEST-RETEST VARIABILITY OF HRSD, DISTANCE AND NEAR VA IN PWD WHICH WERE NOT TREATED (NT) AND HAD STABLE CST

The hRSD test-retest variability in HC was examined in Chapter 6. Test-retest repeatability is important to assess the performance stability of the hRSD test. However, there is no published data on the hRSD test-retest variability in PWD. Given that the analysis above demonstrated that 141 eyes that were NT remained stable as defined by their change in CST between visits (±49um), analysis of these eyes provided an opportunity to assess test-retest characteristics of the hRSD test in this clinical population (Section 9.1). The tests were performed 194±84 days apart. The performance of the hRSD test was compared with those of distance and near VA using paired *t*-test. There were no significant difference between hRSD threshold (*t*=0.494, *p*=0.622), distance (*t*=0.091, *p*=0.927) and near VA (*t*=0.984, *p*=0.326) of these eyes between visits (Table 9.15). Pearson correlation showed

significant correlation between hRSD (r=0.797, p<0.001), distance (r=0.777, p<0.001) and near VA (r=0.692, p<0.001) in visits 1 and 2 (Figure 9.4).

Table 9.15 Comparison of vision of people with diabetes (PWD) who were not treated (NT) and had stable central subfield thickness (CST) between visits. Paired *t*-tests were used for comparison.

	Visit 1 Mean±SD	Visit 2 Mean±SD	Paired *t*-test	*p*
hRSD (logMAR)	-0.64±0.20	-0.63±0.21	0.494	0.622
Distance VA (logMAR)	0.04±0.16	0.04±0.17	0.091	0.927
Near VA (logMAR)	0.16±0.23	0.13±0.21	0.984	0.326

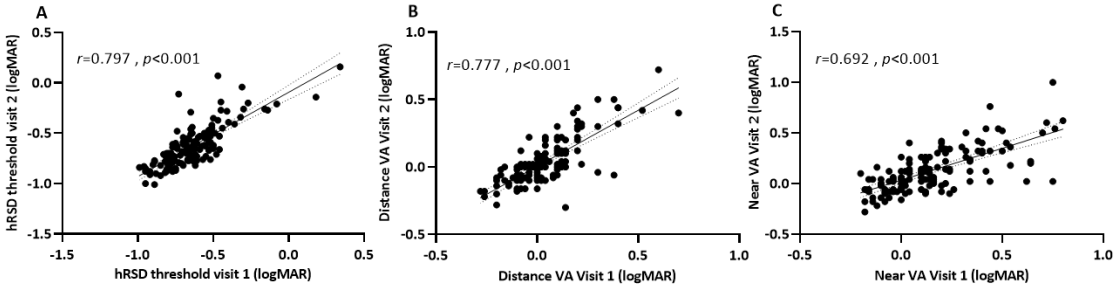


Figure 9.4 Relationship between hRSD (A), distance VA (B) and near VA (C) in visits 1 and 2 in people with diabetes who were not treated (NT) and stable central subfield thickness (±49µm) between visits. Pearson correlation coefficients and their statistical significance are shown. Least squares linear regression lines (±95% CI) are also shown.

Bland-Altman plots for hRSD, distance and near VA showed low biases and narrow limits of agreement across all three tests (Figure 9.5). However, the biases and limits of agreement in near VA (bias -0.025±0.17, LoA -0.36-0.31) were wider compared to hRSD (bias 0.012±0.13, LoA -0.24-0.27) and distance VA (bias -0.002±0.11, LoA -0.22-0.22).

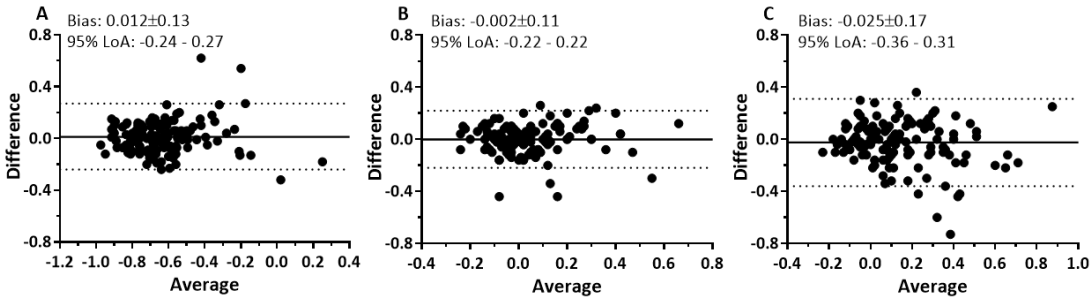


Figure 9.5 Bland-Altman plots for test-retest analysis of the hRSD test (A), distance VA (B) and near VA (C) in eyes with stable CST (N=141). Solid line is the mean bias (mean±SD also shown); dotted lines are 95% limits of agreement.

Similarly, a high degree of test-retest reliability for hRSD, distance and near VA was found using ICC. The average measure ICC for hRSD was 0.886 with a 95% confidence interval from 0.842 to 0.918 ($F_{142,142}$=8.797; p<0.001), for distance VA was 0.874 with a 95% confidence interval from 0.824 to 0.910 ($F_{140,140}$=7.883; p<0.001) and for near VA was 0.814 with a 95% confidence interval from 0.741 to 0.866 ($F_{142,142}$=5.434; p<0.001).

The individual patients' variation in hRSD, distance and near VA between visits are shown in Figure 9.6. The mean±SD individual variation in hRSD, distance VA and near VA between visits were 0.009±0.13 logMAR, -0.004±0.11 logMAR and -0.028±0.17 logMAR respectively.

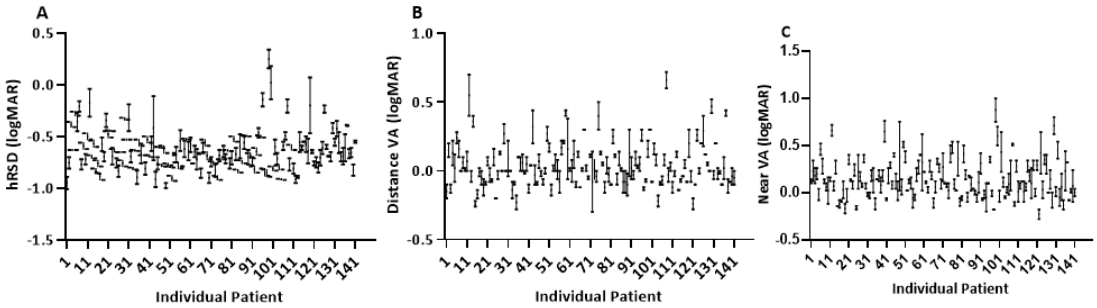


Figure 9.6 The individual variability of the hRSD test (A), distance VA (B) and near VA (C) in eyes with stable CST (N=141) between visits. Each symbol shows the mean and range of vision between visits 1 and 2.

9.3.9 CHANGE IN DIFFERENT RETINAL LAYER THICKNESS IN PWD WITH NO OR MINIMAL DR BETWEEN VISITS

In Chapter 7 (Section 7.3.9), a comparison of retinal thickness between HC, PWD without DR (R0M0 and NMO; N=26) and PWD with minimal DR (R1M0 and NMO; N=46) during their first visit was made. Of the 72 PWD described in Section 7.3.9, there were 23 PWD who remained as either having no DR (R0M0 and NMO; N=8) or minimal DR (R1M0 and NMO;

N=15) during their second visit available for longitudinal analysis. PWD in these two groups were combined for analysis in this section due to the small number of participants in each group. Retinal thickness from all subfields was combined for analysis to allow comparison with other studies. This section will examine the change in thickness in different retinal layers in PWD with no and minimal DR between visits. There were no longitudinal data in HC available for comparison.

The mean±SD age of this group of PWD was 56±17y (range 20-81y). The mean±SD time between visits was 205±109 days. There were 7 males and 16 females, 22 (96%) were Caucasian (Table 9.16), 6 had type 1 diabetes while 17 had type 2 diabetes. Their mean duration of diabetes during their first visit was 15±10y. During their first visit, there were 11 participants on insulin and during the second visit, there were 10 participants on insulin. Their mean± SD distance VA, near VA and hRSD threshold remained stable between visits (distance VA, visit 1 0.07±0.15 logMAR, visit 2 0.05±0.15 logMAR, paired *t*-test 1.352, *p*=0.191; near VA, visit 1 0.17±0.21 logMAR, visit 2 0.11±0.16 logMAR, *t*=1.725, *p*=0.100; hRSD visit 1 -0.67±0.14 logMAR, visit 2 -0.68±0.13 logMAR, *t*=0.151, *p*=0.881). The mean±SD HbA_{1c} at the time of their first visit was 70.2±26.1 mmol/mol and this decreased to 63.7±16.1 mmol/mol at the time of their second visit (*t*=2.321, *p*=0.037). Their mean±SD systolic and diastolic BP both increased slightly at their second visit but only the increase in their systolic BP was significantly different (systolic BP visit 1 141±17mmHg, visit 2 148±20mmHg, *t*=2.470, *p*=0.031; diastolic BP visit 1 79±11 mmHg, visit 2 87±10 mmHg, *t*=1.660, *p*=0.125).

Table 9.16 Descriptive analysis of people with diabetes (PWD) without diabetic retinopathy (DR) or minimal DR in visits 1 and 2

	PWD (N=23)		Statistical differences between groups	
	Visit 1	Visit 2	*t*-test	*p*
Days between visits	205±109		Not available	
Age (y) Mean±SD (range)	56±17 (20-81)		Not available	
Gender (No.) **M** **F**	 7 16		Not available	
Ethnicity **Caucasian (%)**	22 (96%)		Not available	
Type Diabetes (No.) **Type 1** **Type 2**	 6 (26%) 17 (74%)		Not available	
Duration of Diabetes (y)	15±10		Not available	
On Insulin (%)	11 (47.8%)	10 (44.5%)	Not available	
BP (mmHg) **Systolic** **Diastolic**	 141±17 79±11	 148±20 87±10	 2.470 1.660	 **0.031** 0.125
HbA_{1C} (mmol/mol)	70.2±26.1	63.7±16.1	2.321	**0.037**
hRSD (logMAR)	-0.67±0.14	-0.68±0.13	0.151	0.881
Distance VA (logMAR)	0.07±0.15	0.05±0.15	1.352	0.191
Near VA (logMAR)	0.17±0.21	0.11±0.16	1.725	0.100

*Statistically significant results shown in bold.

There was a significant decrease in GCL (visit 1 37.73±3.56µm, visit 2 37.27±3.84µm, *t*=2.523, *p*=0.020), IPL (visit 1 31.98±2.48µm, visit 2 31.61±2.69µm, *t*=2.517, *p*=0.020) and INL (visit 1 33.89±1.92µm, visit 2 32.96±1.11µm, *t*=3.129, *p*=0.005) between visits (Table 9.17). There was no significant change in RNFL, OPL, ONL and RPE thickness between visits.

Table 9.17 Comparison of retinal thickness in different layers in all ETDRS subfields in people with diabetes (PWD) without diabetic retinopathy (DR) or minimal DR in visits 1 and 2

Thickness	Visit 1 (µm) Mean±SD	Visit 2 (µm) Mean±SD	Paired *t*-test	*p**
RNFL	26.83±2.64	26.89±2.80	0.150	0.882
GCL	37.73±3.56	37.27±3.84	2.523	**0.020**
IPL	31.98±2.48	31.61±2.69	2.517	**0.020**
INL	33.89±1.92	32.96±1.11	3.129	**0.005**
OPL	29.90±2.38	29.31±1.95	1.923	0.068
ONL	63.96±6.80	64.37±6.69	0.780	0.444
RPE	15.98±1.23	16.29±1.26	1.776	0.090

*Statistically significant results shown in bold.

To determine the rate of change in retinal thickness between visits, estimated absolute annual change and estimated relative annual change were calculated (Table 9.18). Estimated absolute annual change was calculated as the difference in retinal thickness of each participant between visits divided by the number of days between their visits and multiplied by 365. The values shown in Table 9.18 are the mean±SD of all the participants. On the other hand, the estimated relative annual change was calculated by dividing the estimated absolute annual change by the retinal thickness of each PWD in visit 1 expressed as a percentage. Table 9.18 shows the mean±SD estimated absolute annual change and the estimated relative annual change in retinal thickness in RNFL, GCL, IPL, INL, OPL and ONL and RPE. In all the layers, the SDs were quite wide (Table 9.18).

Table 9.18 Estimated annual change in retinal thickness in different layers in people with diabetes (PWD) without diabetic retinopathy (DR) or minimal DR. Estimated absolute annual change was calculated as the difference in retinal thickness of each participant (N=23) between visits divided by the number of days between their visits and multiplied by 365 (Mean±SD shown). Estimated relative annual change was calculated by dividing the estimated absolute annual change by the retinal thickness in visit 1 expressed as a percentage.

Retinal layer	Estimated absolute annual change in retinal thickness (µm) Mean±SD	Estimated relative annual change in retinal thickness (%) Mean±SD
RNFL	-1.18±5.28	-4.19±20.46
GCL	-0.66±2.42	-1.86±6.39
IPL	-0.60±1.83	-1.89±5.69
INL	-1.49±3.06	-4.24±8.93
OPL	-2.45±7.89	-7.58±25.22
ONL	3.89±18.24	7.59±35.66
RPE	0.59±2.41	4.01±15.48

9.4 CHAPTER DISCUSSION

In this chapter, data from a large group of participants who attended two visits was explored. Of the total of 292 participants in the EDDMO study, these longitudinal data were available for over half (55%; 159 of 292). In addition, of the 25 eyes which were referred for treatment after the first visit, data from a follow-up visit were available from 16 (64%) eyes. Therefore, the findings from this chapter fills a gap in the literature on the performance of the hRSD test in monitoring DR and DMO. The original aim was to investigate how well the hRSD test (and other vision tests) could track deterioration or improvement. However, as is apparent from the data presented in this chapter, most eyes were in effect stable over six months (192±84 days, Section 9.3.1). Given no or little change in retinal structure, the data do allow an examination of the test-retest stability of the various tests of interest. Further discussion on the follow-up of patients will be made in Chapter 10.

In the EDDMO study, it was unsurprising that there was a minimal difference between hRSD thresholds in visit 1 (-0.64±0.20 logMAR) and visit 2 (-0.63±0.21 logMAR) in the NT group (Table 9.1) given the structural stability observed. This is the minimum that would be expected for an effective test. In the NT group, there was also a trend towards an improvement of their retinopathy and maculopathy grades (Table 9.4) along with a slight improvement (0.4 mmol/mol) in their HbA_{1c} and a decreased in the proportion of PWD on insulin (Table 9.1), that suggests that the health promotion messages received during their first visit may have been effective.

There are limited studies on the performance of the hRSD test in patients with DR and DMO (Wang et al., 2013, Bartlett et al., 2015, He et al., 2013, Wang et al., 2010) to compare with results from the EDDMO study. There is only one conference abstract with longitudinal data on hRSD test performance in DMO in eyes receiving bi-monthly anti-VEGF injections (Table 9.19). In that study, Wang et al. (2015) found a significant improvement in the hRSD threshold (visit 1 -0.22±0.21 logMAR, visit 2 -0.33±0.21 logMAR, p<0.002) but not distance VA (visit 1 0.30±0.21 logMAR, visit 2 0.31±0.22 logMAR) and CST (visit 1 358±100 µm, visit 2 349±114µm) at the 90 days visit (N=28). At the 180 days visit (N=23), they found significant improvement in hRSD threshold (visit 1 -0.21±0.20 logMAR, visit 2 -0.33±0.22 logMAR, p<0.001) and CST (visit 1 355±89µm, visit 2 316±78µm, p<0.007) but not distance VA. It is likely that the PWD in the EDDMO study had more severe DMO compared to Wang et al. (2015) as the participants in the EDDMO study had worse CST, hRSD and distance VA (Table

9.19). However, findings from the EDDMO study was limited by the small number of PWD who received anti-VEGF therapy (N=7).

Table 9.19 Comparison of hRSD threshold (logMAR), distance VA (logMAR) and central subfield (CST;µm) in eyes which have received anti-VEGF therapy in Wang et al. (2015) and the EDDMO study

Study	**Wang et al. (2015) (90 days, N=28 eyes; 180 days, N=23 eyes)**						**EDDMO study (N=7 eyes)**		
Follow-up interval	**Baseline**	**90 days**	***P****	**Baseline**	**180 days**	***P****	**Baseline**	**178±100 days**	***P****
hRSD (logMAR)	-0.22±0.21	-0.33±0.21	**<0.002**	-0.21±0.20	-0.33±0.22	**<0.001**	-0.09±0.39	-0.09±0.30	0.999
Distance VA (logMAR)	0.30±0.21	0.31±0.22	>0.05	0.33±0.21	0.29±0.21	>0.05	0.55±0.42	0.46±0.39	0.187
CST (µm)	358±100	349±114	>0.05	355±89	316±78	**<0.007**	510.4±155.7	346.0±99.3	**0.014**

*Statistically significant results are in bold

As shown in Table 9.19, longitudinal results from the eyes which had received intravitreal anti-VEGF therapy showed that a change in CST thickness does not necessarily correlate with a change in vision. There is emerging evidence that CST is only part of the story in predicting vision in eyes with DMO. Browning et al. (2007) found that central retinal thickness only modestly correlated with VA after treatment in eyes with DMO. There may be several explanations for this observation. Sun et al. (2015) reported that disorganisation of the retinal inner layers (DRIL) seen on OCT was a more reliable biomarker to predict VA in eyes with current or previous DMO compared to CST. DRIL is assessed by examining the integrity of two boundaries, the first one between the GCL and IPL complex and INL and the second one between INL and OPL (Sun et al., 2015). These boundaries were selected because the fluid in DMO is initially located in the INL and OPL and over time, it may also involve the IPL and RNFL until the entire thickness of the retina becomes oedematous (Salmon and Bowling, 2015). DRIL is thought to represent disorganisation or destruction of cells within the inner retinal layers, including bipolar, amacrine or horizontal cells and a disruption of pathways that transmit visual information from the photoreceptors to the ganglion cells (Sun et al., 2014a). It is postulated that these delicate visual pathways may not return to normal functioning even after the resolution of DMO because cell axons may snap after their elasticity limit has been reached due to DMO (Sun et al., 2014a, Pelosini et al., 2011). There is also evidence that DR can cause capillary non-perfusion in the outer retinal layers leading to photoreceptor compromise and decreased vision (Scarinci et al.,

2015, Scarinci et al., 2016). This ultimately leads to DMI, which is known to be an important predictor of poor visual outcome in eyes with DR (Sim et al., 2013). In the EDDMO study and the study by Wang et al. (2015), no assessments of DRIL and DMI were made. Therefore, it is possible that in the eyes which have received intravitreal aflibercept, there was underlying DRIL and DMI that could account for a lack of change in vision even in the face of the significant change in CST.

In this chapter, the criterion used to define a change in CST was 50µm between visits 1 and 2 based on the DRCR Network criteria (DRCR network., 2006), this difference is quite small (Section 9.1). One aspect of the reasoning for this 50µm criterion is that any intraretinal cyst that was greater than 50µm was considered significant as any intraretinal cyst less than or equal to 50 µm could be due to scan artefact (Section 3.1.8). Although there could be a potential discrepancy between structural stability and functional stability for various reasons previously discussed, the vast majority of PWD in this chapter remained functionally and structurally stable even with this tight definition of CST thickness. Therefore, although there was a significant increase in CST (visit 1 291.8±41.99 µm, visit 2 298.5±57.45 µm, t=2.173, p=0.036) in the group of PWD who were NT between visits, this difference was still far less than 50 µm and would not be regarded as clinically significant (Section 9.3.6).

Since the majority of the PWD eyes for which longitudinal data were available remained stable, the data provided an opportunity to assess the test-retest variability of the hRSD test in PWD. Comparisons of the test-retest variability of the hRSD test were made with distance and near VA, which are commonly used in clinical practice. Stable test-retest variability is desirable in a test so that if there is a change in test results, the assessor can be confident that the change is due to a change in the disease and not due to test-retest variability. Lovie-Kitchin and Brown (2000) found the criterion for judging change on commonly used clinical vision tests such as the Bailey-Lovie distance VA chart in HC to be one line (5 letters; 0.1 logMAR). Agardh et al. (2011) studied 53 eyes of PWD under routine care to establish the variability of distance VA measured on four different occasions within a month. The PWD had a range of DR severity ranging from no DR to PDR with reasonably well-controlled diabetes. Agardh et al. (2011) found that the mean variability in distance VA was 0.08 logMAR. In the EDDMO study, the mean individual variability in hRSD, distance and near VA between visits in eyes which were NT (Figure 9.6) was minimal (hRSD 0.009±0.13; logMAR: distance VA -0.004±0.11 logMAR, near VA -0.028±0.17 logMAR). This

may be due to the narrow definition of change in CST of ±49µm used in the EDDMO study. In addition, follow-up data were available for 159 PWD (55%) during the follow-up period; the PWD who attended follow-up may have been more compliant compared to PWD who did not attend for follow-up.

In eyes which had stable CST as defined earlier, the test-retest variability of the hRSD (bias 0.012±0.13, LoA -0.24-0.27) was comparable with that of distance (bias -0.002±0.11, LoA -0.22-0.22) and near VA (bias -0.025±0.17, LoA -0.36-0.31) (Figure 9.4). A comparison of the Bland-Altman analysis of test-retest variability of HC from Chapter 6, PWD from this section and a study by Lovie-Kitchin and Brown (2000) was made (Table 9.20). Results from Chapter 6 also found that the 3AFC and 4AFC hRSD test provides the same threshold and can be considered equivalent. Table 9.16 shows that PWD had low bias and narrow 95% limits of agreement comparable to data from HC. Reassuringly, the hRSD test-retest variability had lower bias and narrower limits of agreement in PWD who had done the test 194±84 days compared to HC who had done the 3AFC hRSD test 64±42 days apart (bias 0.04±0.21, LoA -0.37-0.44). The hRSD test-retest variability in PWD was comparable to the 4AFC hRSD test in HC done 19±0.76 months apart (bias 0.004±0.12, LoA -0.23-0.44). An ICC above 0.8 is regarded as a sign of good reliability and ICCs of this order were found for hRSD (ICC 0.886), distance (0.874) and near VA (0.814) (Section 9.3.8) (Liljequist et al., 2019). The absence of increased test-retest variability in the hRSD test in a group of patients with known or likely retinal pathology is useful given that there other conditions such as glaucoma where increased test-retest variability in patients complicates the detection of disease progression (Guimarães et al., 2019).

The hRSD test currently employed in the USA has a threshold of a deterioration of 0.3 logMAR to alert the treating clinician to review the patient earlier (Wang et al., 2013). Given the test-retest variability with a bias of -0.24 to 0.27, a change of 0.3 logMAR can be considered an appropriate threshold to detect deterioration. Lovie-Kitchin and Brown (2000) studied the test-retest variability of distance and near VA in 93 HC. In the study, they used the Bailey-Lovie distance VA chart which was also used to assess distance VA in the EDDMO study. Lovie-Kitchin and Brown (2000) found narrower LoA for both distance and near VA compared to those observed for the hRSD test here. However, in their study, the time between tests was only 2-3 days apart (Table 9.20).

Table 9.20 Comparison of Bland-Altman analysis in the assessment of test-retest variability of healthy controls (HC) and people with diabetes (PWD) who were not treated and stable central subfield thickness (CST)

Study	Participants	Version of hRSD test	No. of eyes	Time between tests	Bias	95% limits of agreement
EDDMO Study	**HC**	3AFC	74	Intrassesional	-0.025±0.12	-0.27-0.22
			30	64±42 days	0.04±0.21	-0.37-0.44
			15	39±0.9 months	-0.04±0.18	-0.32-0.39
		4AFC	7	19±0.76 months	0.004±0.12	-0.23-0.24
	PWD	4AFC	141	194±84 days	0.012±0.13	-0.24-0.27
		Bailey-Lovie (Distance)	141	194±84 days	-0.002±0.11	-0.22-0.22
		ETDRS (Near)	141	194±84 days	-0.025±0.17	-0.36-0.31
Lovie-Kitchin and Brown (2000)	**HC**	Bailey-Lovie *(Distance)	93	2-3 days	Not available	±0.105
		Bailey-Lovie (Near)	93	2-3 days	Not available	±0.114

*Bailey-Lovie high contrast letter chart

Some studies use correlation analysis of the same test repeated over a time period to assess test-retest variability. Therefore, a comparison of the correlation analysis of hRSD threshold of HC from Chapter 6, hRSD, distance and near vision from PWD from this chapter and some published studies were made (Table 9.21). Interestingly, only the intrasessional 3AFC hRSD in HC had a significant correlation (r=0.847, p<0.001) but not the short (64±42 days) or long term (39±0.9 months) hRSD. However, 4AFC hRSD (r=0.797, p<0.001), distance (r=0.777, p<0.001) and near VA (r=0.692, p<0.001) in PWD, which had a longer time between tests (194±84 days) compared to the short or long term 3AFC hRSD in HC all showed significant correlation. This may be due to the higher number of eyes available for correlation analysis in the intrasessional 3AFC in HC (N=74) and PWD (N=141). Lovie-Kitchin (1988) found a significant correlation in intrasessional distance VA measured using the Bailey-Lovie VA chart (r=0.98, p<0.01). Arditi and Cagenello (1993) also found a high correlation in distance VA measured using ETDRS charts several weeks apart (r=0.829, p not available).

Table 9.21 Comparison of correlation analysis in the assessment of test-retest variability of healthy controls (HC) and people with diabetes (PWD) who were not treated and stable central subfield thickness (CST)

Study	Participants	Vision Test	No. of eyes	Time between tests	*r*	*p*
EDDMO study	**HC**	3AFC	74	Intrassesional	0.847	<0.001
			30	64±42 days	0.407	0.026
			15	39±0.9 months	-0.105	0.711
		4AFC	7	19±0.76 months	0.490	0.265
	PWD	4AFC	141	194±84 days	0.797	<0.001
		Bailey-Lovie (Distance)	141	194±84 days	0.777	<0.001
		ETDRS (Near)	141	194±84 days	0.692	<0.001
Lovie-Kitchin (1988)	**HC**	Bailey-Lovie (Distance)	115	Intrassesional	0.98	<0.01
Arditi and Cagenello (1993)	**HC**	ETDRS (Distance)	5	Several weeks	0.829	Not available

It is also noteworthy that there are some results in this chapter which, although are statistically significant, are unlike to have any clinical relevance. An example was the significant improvement in the near VA of both groups whose HbA_{1c} had deteriorated (visit 1 0.20±0.21 logMAR, visit 2 0.12±0.18 logMAR, t=2.447, p=0.024) and improved (visit 1 0.16±0.20 logMAR, visit 2 0.11±0.19 logMAR, t=2.490, p=0.018) (Section 9.3.6). These changes in near VA between visits are still within the accepted normal variability of 0.1 logMAR (Lovie-Kitchin and Brown, 2000).

As previously mentioned in the introduction to this chapter, there are limited longitudinal studies on DRN. These studies demonstrated progressive RNFL, GCL and IPL loss consistent with findings from this chapter (Section 9.3.9) (Sohn et al., 2016, Kim et al., 2018, Lim et al., 2019). Sohn et al. (2016) studied DRN in human participants, in mouse models and deceased donor eyes. In the human study, they examined 45 participants with type 1 diabetes with no or minimal DR over a median period of 73 months (range 37-79 months). They found RNFL loss of 0.25µm/year and GCL and IPL combined loss of 0.29µm/year. These values exceeded normal age-related RNFL loss of 0.133µm/year and GCL and IPL loss

of 0.149µm/year measured using similar OCT protocols (Demirkaya et al., 2013). The OCT protocols used by Sohn et al. (2016) and Demirkaya et al. (2013) are similar to the protocol used in the EDDMO study, which is based on the ETDRS grid. Therefore, the values from these studies can be compared with values from the EDDMO study. In another study by the same research group, they found a high correlation between perimetric functional loss detected using the Rarebit visual field test and GCL loss in PWD with no or minimal DR (van Dijk et al., 2011). Using data from Bogunovic et al. (2014) which showed the difference in mean thickness between patients with early glaucoma to those with severe glaucoma to be 5-8µm for the RNFL and 1-8µm for the GCL, Sohn et al. (2016) estimated that after 10-20 years, the ganglion cell complex loss caused by DRN would be equivalent to severe glaucoma. However, van Dijk et al. (2011) utilised the Rarebit visual field test which uses a suprathreshold (highly discriminable) stimuli that allow for the detection of tiny distributed areas of absolute scotoma within otherwise normal areas of vision (Tachyla et al., 2019). Therefore, the visual field loss seen in DRN may be more diffuse compared to glaucoma (Sohn et al., 2016). In the mouse model of the study, Sohn et al. (2016) found progressive inner retinal thinning with increasing duration of diabetes. In addition, they found ganglion cell loss but no difference in pericyte density or acellular capillaries in diabetic mice compared to the control group suggesting that DRN precedes microvasculopathy. Similarly in the donor's eyes, they found significantly thinner RNFL compared to age-matched donors but no difference in the retinal capillary density.

Lim et al. (2019) studied pRNFL in 101 PWD with type 2 diabetes with no or mild to moderate NPDR and 63 HC for three years. They found that the estimated mean pRNFL loss was -0.92 µm/year in the no DR group and -1.16 µm/year in the NPDR group, which was 2.9 fold and 3.3 fold greater, respectively, compared to HC. Kim et al. (2018) examined 87 eyes of PWD with type 2 diabetes with no DR or mild NPDR over 4 years and 40 HC. They found that PWD who had a deterioration of their DR had greater macular GCL and IPL loss (0.39±0.33 µm/year) compared to PWD who remained stable (0.22±0.30 µm/year) and the HC (0.20±0.21 µm/year). The retinal thickness values from the studies by Lim et al. (2019) and Kim et al. (2018) are not directly comparable with values from the EDDMO study due to different OCT scan protocols.

Table 9.22 shows the annual change in RNFL, GCL and IPL in HC (Demirkaya et al., 2013) and PWD with no and minimal DR in the EDDMO study compared to the study by Sohn et al. (2016). Data from the EDDMO study showed that PWD with no or minimal DR had greater

than expected age-related retinal thickness loss in the RNFL, GCL and IPL compared to HC (Demirkaya et al., 2013). However, results from the EDDMO study also had a greater annual loss of these layers compared to the study by Sohn et al. (2016). The difference may be due to a few factors. Firstly, the group of PWD in the EDDMO study (56±17 years) were older compared to the PWD in Sohn et al. (2016) (31±10 years). Secondly, the EDDMO study was limited by a small number of PWD (N=23) with a short duration of follow-up (205±109 days) and the results may not be as precise as indicated by the wide SD. The COVID-19 pandemic interrupted the longitudinal component of the EDDMO study and only 54.5% (159 of 292) of PWD had a follow-up visit. In addition, since the EDDMO study was an observational study, most PWD with no or minimal DR would have been discharged after their first visit. Therefore, it is likely that these 23 PWD with no or minimal DR had a more severe disease in their fellow eye which required them to have a follow-up visit. Stratton et al. (2013) found that PWD with no DR in one eye and mild NPDR in the other eye were more likely to progress to develop sight-threatening DR compared to PWD with no DR in both eyes. Therefore, the group of PWD studied in Section 9.3.9 may be more likely to have DR progression and loss of RNFL, GCL and IPL compared to the study by (Sohn et al., 2016).

Table 9.22. Annual change in RNFL, GCL and IPL in the EDDMO study compared to studies by Demirkaya et al. (2013) and Sohn et al. (2016)

Retinal layer	**Annual change in retinal thickness**		
Study	**Demirkaya et al. (2013), N=45, Mean±SD age 47±17, cross-sectional study, healthy participants**	**EDDMO study, N=23, Mean±SD age 56±17 years, Follow-up 205±109 days, no or minimal DR**	**Sohn et al. (2016), N=120, Mean±SD age 31±10 years, Follow-up 23 months, no or minimal DR**
RNFL Mean±SD (µm)	-0.133	-1.18±5.28	-0.25
GCL Mean±SD (µm)	0.149*	-0.66±2.42	-0.29*
IPL Mean±SD (µm)		-0.60±1.83	

* Combined GCL and IPL measurements

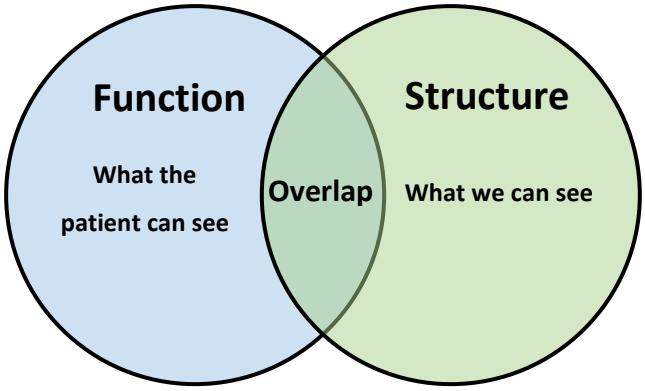
Interestingly, the EDDMO study found a statistically significant loss of INL between visits (visit 1 33.89±1.92, visit 2 32.96±1.11, t=3.129, p=0.005) (Section 9.3.9). The estimated absolute annual change in INL was -1.49±3.06µm per year. The loss of INL in PWD with no or minimal DR was also seen in a study by van Dijk et al. (2011). Since ganglion cells, amacrine cells, bipolar cells, horizontal cells and photoreceptors are all involved in the

transmission of visual signals to the brain and INL contains the cell bodies of bipolar cells (Masland, 2001), it is possible that the ganglion cell complex thinning is a proxy for more general neuronal tissue loss (van Dijk et al., 2011).

In clinical practice, there is usually an expected overlap between a patient's visual function and structural changes on clinical examination (Figure 9.7A). However, data from this chapter shows that structural changes (CST) do not necessarily correlate with a change in vision (Table 9.19). This chapter has also reported progressive thinning of the GCL, IPL and INL in PWD with no or minimal DR consistent with findings from other studies (Sohn et al., 2016, Kim et al., 2018, Lim et al., 2019, van Dijk et al., 2011). Thinning of these layers can result in functional visual impairment prior to any detectable changes on clinical examination (Section 6.3.10). Therefore, when there is a gap between a patient's reduced visual function and no obvious structural changes are seen on clinical examination (Figure 9.7B), clinicians need to consider causes such as DRIL, DMI and DRN. DRIL and DMI can be detected by multimodal imaging but DRN can be undetected and yet have significant visual morbidity with diffuse visual field loss (Sohn et al., 2016). These findings may represent a paradigm shift in clinicians in the assessment and management of PWD who have a mismatch between visual function and structural assessment; clinicians should consider visual deficits due to DRN.

The next chapter (Chapter 10) will discuss a number of issues of interest to this thesis including DR screening, definitions of using M1 as a surrogate marker for DMO, the lack of consensus on the OCT definition of DMO, the role of the hRSD test in DR screening, and the role of using retinal thickness measured by OCT as surrogate markers in DRN.

A. Expected overlap between patient's visual function and structure



B. Gap between patient's visual function and structure

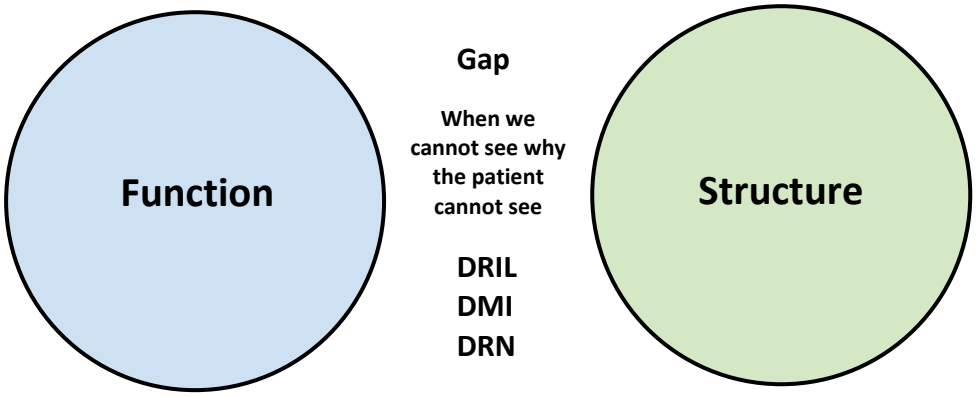


Figure 9.7. The expected overlap between a patient's visual function (what the patient can see) and structural changes (what we, the clinicians can see) on clinical examination (A). When there is a gap between the patient's visual function and structure in PWD (when we cannot see why the patient cannot see), clinicians need to consider causes such as disorganisation of the retinal inner layers (DRIL), diabetic macular ischaemia (DMI) and diabetic retinal neuropathy (DRN).

CHAPTER 10. THESIS DISCUSSION

10.1 CHAPTER INTRODUCTION

This thesis has explored both functional and structural effects on the macula in PWD by using hRSD and distance and near VA as functional assessments and OCT features of structural integrity. This chapter starts with a general discussion about screening in the UK with a focus on the issues around using biomarkers for DMO to determine referral threshold and the OCT definition of DMO of importance in the treatment pathway. These are issues that have played a key role in some of the results in this thesis. Then there will be a discussion on the role of hRSD in DR screening and the role of using retinal thickness as detected by OCT as a potential biomarker for DRN.

10.2 SCREENING AND DIABETIC MACULOPATHY

Diabetes affects over 4.9 million people in the UK and this number is predicted to increase to 5.5 million by 2030 (Diabetes UK, 2019). Approximately one-third of PWD develop DR (Yau et al., 2012). A national DR screening programme was started in the UK in 2003 and current NDESP guidelines recommend annual DR screening for all PWD age 12 and over (Scanlon et al., 2015). However, given the rapid increase in PWD and increasingly tight NHS budgets, annual DR screening in its current format may be unsustainable long-term. In several countries, a move to longer intervals has been proposed and implemented in some countries (Scanlon, 2017b).

One of the issues highlighted in the EDDMO study was the use of biomarkers within the definition of M1 for risk for progression to maculopathy requiring treatment. The majority of PWD (74.7%; 218 of 292) who were referred from LDESP to DEC were maculopathy suspects (M1) and 34.9% (76 of 218) were subsequently graded as M0 while 35.8% (78 of 218) were found to have no macular oedema on OCT when seen in DEC (Sections 7.3.1 and 7.3.2). It is known that with photographic screening, there is an over-referral of maculopathy suspects to the relatively expensive and under-pressure HES (Olson et al., 2013). As this is likely to be system-wide, the situation would be improved by approaches that improved the identification of DMO at an early stage.

As discussed in Chapter 1, the initial capital costs, including workforce training, have prevented the use of OCT routinely in DR screening in the UK (Olson et al., 2013). As a

result, some DR screening services in the UK selectively use OCT to screen M1 patients prior to being referred to DEC or as part of DS (Mackenzie et al., 2011, Gale et al., 2017, MacEwen C et al., 2019).

Another approach to reducing NHS costs could be to change follow-up intervals in DEC and screening intervals in RDS. In the EDDMO study, the majority of PWD who were not treated had no change in their retinopathy (84.6%), maculopathy (69.2%) or OCT grades (73.4%) after 6 months (191±86 days) (Sections 9.3.1 and 9.3.5). This suggests that most PWD were stable and their follow-up interval could be extended.

10.3 SCREENING PATHWAYS AND FOLLOW-UP

In Liverpool, ophthalmologists in DEC determined whether patients required treatment or follow-up based on various factors including clinical examination, OCT findings and glycaemic control. Those patients deemed to require follow-up were seen again at a time interval deemed appropriate by the treating ophthalmologist to monitor for any progression in the patients' DR or DMO (Royal College of Ophthalmologists, 2012). Several factors are likely to inform an ophthalmologists' view of what is an appropriate follow-up interval. Firstly, it is known that patients with a more severe baseline DR have a higher risk of progression to PDR or macular oedema and therefore require more frequent follow-ups (Klein et al., 1998). The ETDRS research group found that the proportion of eyes that progressed to PDR within one year was 5% in eyes with mild NPDR, 12-27% of eyes with moderate NPDR and 52% of eyes with severe NPDR (ETDRS, 1991e, ETDRS, 1991d, Aiello, 2003). The International Council of Ophthalmology (ICO) recommends that patients with mild, moderate and severe NPDR be followed up at 6-12 months, 3-6 months and under 3 months respectively (Wong et al., 2018). However, the ICO screening intervals appears too frequent and further discussion regarding screening intervals is made below.

The conventional screening interval in the English NDESP (ENDESP) for eyes without DR is 12 months (Broadbent et al., 2021). However, some studies examining DR screening intervals have found that screening intervals can be extended (Day et al., 2014, Echouffo-Tcheugui et al., 2013, DCCT, 2017, Scanlon et al., 2015, Taylor-Phillips et al., 2016). Day et al. (2014) and Echouffo-Tcheugui et al. (2013) found that for PWD without DR, screening intervals can be safely extended from 1 to 2 years. Scanlon et al. (2015) found that screening intervals can be extended from 1 to 3 years to increase cost-effectiveness in the NHS. However, when patients' personalised risk factors (age, duration of diabetes, HbA_{1c},

renal function, smoking status, diabetes type, and body mass index) were taken into consideration, screening high-risk groups every 2 years and low-risk groups every 5 years further improved cost-effectiveness (Scanlon et al., 2015).

Despite the evidence, conventional DR screening intervals have not been extended and the reasons are likely to be multifactorial. Some factors which have prevented extension are inertia to change and fear of disengagement by PWD. Another factor may be that the ETDRS research group studies, which established the risk of DR progression, were performed 30 years ago and the options for glycaemic, BP and lipid control were limited (ETDRS, 1991e, ETDRS, 1991d, Klein et al., 2008). The risk of progression to PDR has been impacted by the improvement in the medical management of diabetes and this can be seen in the WESDR study in patients with type 1 diabetes (Klein et al., 2008). The WESDR study started recruitment in 1979 and its report on the 25-year progression of DR showed that participants who were diagnosed more recently had a lower prevalence of PDR compared to other participants who were diagnosed earlier at a given duration of diabetes (Figure 10.1) Besides improved medical management, the authors concluded that greater exposure to health professionals as a result of participation in the study helped participants improve diabetes control (Klein et al., 2008).

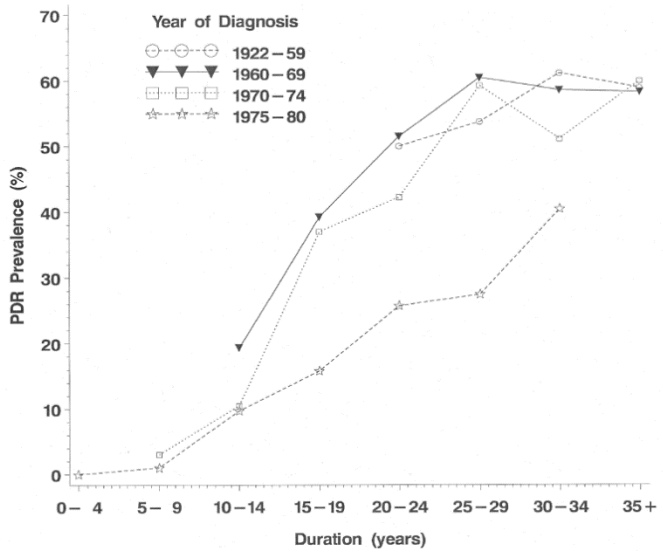


Figure 10.1 Relationship between the prevalence of proliferative diabetic retinopathy (PDR) according to the duration of diabetes in each of the four periods of diabetes diagnosis in the Wisconsin epidemiologic study of diabetic retinopathy (WESDR) study (Klein et al., 2008).

The DR screening programme in Liverpool first started as a research project in 1991 and was one of the earliest systematic screening centres to be established in the UK (Harding et al., 1995). Broadbent et al. (2021) recently published results from the Individualised Screening for Diabetic Retinopathy (ISDR) study, which is the largest randomised-control trial in DR screening to date. The ISDR study had a purpose-built dynamic data warehouse linking primary and secondary care patients' information that fed into a risk-calculation engine to determine each participant's risk of becoming screen positive (R2+, M1). Broadbent et al. (2021) found that including personalised risk factors (age, duration of diabetes, HbA_{1c}, systolic BP and total cholesterol) to determine individualised screening intervals (6, 12 or 24 months for high, medium and low-risk groups respectively) is safe with substantial improvement in cost-effectiveness for the NHS, which is consistent with previously mentioned studies (Day et al., 2014, Echouffo-Tcheugui et al., 2013, Scanlon et al., 2015).

The LDESP has had good engagement with primary and secondary care providers over the decades that helped the ISDR study. The Royal Liverpool University Hospital where the EDDMO study was conducted also has a diabetes centre with a one-stop multi-disciplinary care service. Education provided to patients regarding diabetes self-management can significantly improve glycaemic control (Chrvala et al., 2016). The EDDMO study found an improvement in the mean HbA_{1c} of PWD who were TT although this change did not reach statistical significance (visit 1 84.9±28.9mmol/mol, visit 2 78.7±23.9 mmol/mol, t=0.603, p=0.552) (Table 9.15). PWD who had NT also had a small decrease in HbA_{1c} between visits (visit 1 70.2±18.9 mmol/mol, visit 2 69.8±18.0 mmol/mol, Table 9.3). Considering that a 1% reduction in HbA_{1c} decreases the progression of retinopathy by 37%, this observation is encouraging as this suggests that the health education provided during their first visit to optimise their diabetes control may have been effective (Stratton et al., 2000). However, the HbA_{1c} target for most individuals with type 1 and type 2 diabetes is 6.5% (48mmol/mol) and for patients with type 2 diabetes on medications associated with hypoglycaemia, the target is set at 7.0% (53mmol/mol) (NICE, 2015c, NICE, 2015b). Therefore, the glycaemic control for the EDDMO participants was still far from ideal. Improved communication and effective monitoring clearly have a role to play in improving patient outlook. This is even more important if the interval between actual clinical interactions with services were to be increased.

In this study, most PWD had stable retinopathy, maculopathy and OCT grades between visits (Table 9.8). For the PWD who had a change in grades, there was a trend towards an improvement (Table 9.6). Health education provided during their first visit may account for a shift in the proportion of PWD with more severe retinopathy and maculopathy grades to less severe grades in the NT group during visit 2 compared to visit 1 (Table 9.6). It is also possible that the DR grading performed by ophthalmologists using slit-lamp biomicroscopy was inaccurate as misclassification to a more advanced stage (false positive) is more common than misclassification to a less advanced stage (false negative) (Scanlon et al., 2015). However, it not possible to verify the accuracy of the DR grading as there were no concurrent digital photographs taken in the EDDMO study during visits 1 or 2.

Another vital point to note is that the EDDMO study population were recruited from hospital eye services while the other study populations were from RDS in the community (Day et al., 2014, Echouffo-Tcheugui et al., 2013, Scanlon et al., 2015). By the nature of being screen positive, patients in DEC would be at a higher risk of developing PDR than patients in RDS and each DEC visit would cost more than an RDS visit. Results from Chapter 9 (Section 9.3.1) suggest that current follow-up intervals in DEC are too conservative. Therefore, it may be more beneficial for the patients in DEC to have personalised risk factors calculations to determine follow-up intervals in DEC. Further studies on the safety of extending follow-up intervals in DEC with the application of personalised risk factors would be useful.

Besides extending follow-up intervals, another option to reduce the burden on HES is to refer more patients directly into the DS pathway from RDS instead of referring PWD to DEC. DS serves as an intermediate screening pathway between DEC and RDS (Public Health England, 2020). In the Royal Liverpool University Hospital, all PWD attending DS have the benefit of receiving macular OCT as well as colour fundus photographs. OCT is currently not a routine part of DS pathways across the UK (Public Health England, 2020). In the EDDMO study, most of the PWD who returned for a second visit had follow-up visits in DEC (84%; 134 of 159) instead of DS (16%; 25 of 159) (Section 9.3.1). For 134 PWD seen in DEC, there were 32 PWD (23.9%) graded as R1M0 that could have been discharged to RDS and 60 PWD graded as R1M1 (44.8%) that could have been seen in DS (Table 9.5). For the 25 PWD seen in DS, there were 11 (44%) graded as R1M0 that could have been discharged back to RDS. While it is recognised that not all patients can have a follow-up in RDS or DS (e.g. because of other conditions such as media opacities that would make them unsuitable), results from

Chapter 9 (Section 9.3.1) showed that more PWD can be discharged to RDS and that the DS pathway is underutilised. Further research is needed to investigate whether the patterns observed here are consistent with practice elsewhere.

10.4 CLASSIFICATION OF MACULOPATHY USING OCT

One of the challenges within the EDDMO study was that although several OCT classification schemes for DMO have been proposed, there is currently no widely accepted system (Panozzo et al., 2020, Parodi Battaglia et al., 2018, Panozzo et al., 2004, Kang et al., 2004, Kim et al., 2006, Koleva-Georgieva and Sivkova, 2008, Helmy and Atta Allah, 2013, Bolz et al., 2014, Reznicek et al., 2016). As mentioned above, the success of the feature-specific grading system based on fundus photographs is its ability to predict the development of PDR and DMO. One of the barriers to the widespread acceptance of any of the existing DMO OCT classifications is that these systems are yet to demonstrate prognostic ability to determine DMO progression or visual outcome. Each system examines different factors. An example of this is a classification recently proposed by the European School for Advanced Studies in Ophthalmology with 8 variables known by the mnemonic TCED-HFV including foveal thickness (T), intraretinal cysts (C), ellipsoid zone and or external limiting membrane status (E), disorganisation of the inner retinal layers (D), hyperreflective foci (H), subfoveal fluid (F) and vitreoretinal relationship (V); this latest system remains complicated and time-consuming to apply (Panozzo et al., 2020).

With no consensus on a DMO OCT classification, a local classification system was developed by the Royal Liverpool University Hospital St Paul's Eye Unit for the EDDMO study as described in Chapter 4. The Liverpool OCT classification only evaluates two factors across all ETDRS subfields, the presence of intraretinal cyst ≥50µm and retinal thickness ≥2SD compared to normative values. However, this system can be time-consuming as it requires the comparison of retinal thickness across all 9 subfields in both eyes, thereby examining 18 values. A simplification of this system is therefore proposed to evaluate the presence of intraretinal cysts with a diameter of ≥50µm across all the ETDRS subfields but to only examine retinal thickness in the CSF (Table 10.1). Therefore, clinicians would only need to remember one number in the CSF (≥ 318 µm) instead of 9 numbers. If this new system were to be used, the OCT grade of the majority of PWD, which were NT (82.4%, 220 of 267) or TT (88%, 22 of 25) in visit 1, would remain the same (Table 10.2). The three TT PWD which had a change in their OCT grade were screen positive based on their retinopathy grade (R2+) would have been referred to DEC regardless (Table 10.2). However, as in most PWD, CST

remained stable over two visits in the EDDMO study (Section 9.3.4), it was not possible to determine the prognostic ability of the current or new proposed Liverpool DMO OCT classification to predict DMO progression or visual outcome. It would be useful to determine the prognostic ability of the new proposed Liverpool DMO definition on a group of PWD with a longer follow-up.

Table 10.1. New proposed Liverpool OCT definition of diabetic macular oedema

Definition	**Grades of DMO**
Central subfield intraretinal cyst* and or CST ≥ 318 µm	Centre involving macular oedema (CIMO)
Inner subfield intraretinal cyst*	Centre threatening macular oedema (CTMO)
Outer subfield intraretinal cyst*	Non-centre threatening macular oedema (NCTMO)
No intraretinal cyst and retinal thickness <2SD in all subfields	No macular oedema (NMO)
Ungradeable	Ungradeable (U)

*Intraretinal cyst ≥50µm

Table 10.2 Change in the proportion of PWD classification from visit 1 with the new proposed Liverpool OCT definition of diabetic macular oedema

	No change in OCT grade	**Change in OCT grade**
No treatment (N=267)	220 (82.4%)	47 (17.6%)
Had treatment (N=25)	22 (88%)	3 (12%)

10.5 FUNCTIONAL TESTS IN DETECTING DMO

Although OCT is the reference standard test in detecting DMO, the absence of a widely agreed OCT DMO definition makes it more challenging to compare the performance of the hRSD test in detecting DMO with other macular diseases. For detecting nAMD, FA has been the standard test while OCT aids diagnosis and follow-up (Usman et al., 2019). Besides the methods of diagnosis, another difference is the management of nAMD compared to DMO. Upon the diagnosis of nAMD, timely anti-VEGF therapy is given (Khanna et al., 2019). In contrast, in DMO, there is an emphasis on glycaemic control, management of systemic risk factors and anti-VEGF is typically offered in CIMO with CST ≥400µm and reduced vision (Amoaku et al., 2020).

In the EDDMO study, the AUC for the hRSD test in detecting CIMO from non-CIMO using the Liverpool OCT definition was 0.58 (95% CI 0.51-0.65) (Table 8.9). At an hRSD threshold of -0.61 logMAR, the sensitivity and specificity of the hRSD test to detect CIMO vs non-CIMO was 43.96% and 66.67% respectively (Table 8.9). In comparison, the AUC for the hRSD test in distinguishing nAMD from intermediate AMD was 0.69 (95% CI 0.58-0.80) (Pitrelli Vazquez et al., 2018). At a similar hRSD threshold of -0.60 logMAR, the sensitivity and specificity of the hRSD test to detect nAMD compared to intermediate AMD was 79% and 54% respectively. The findings that the hRSD test had better AUC and sensitivity in detecting nAMD compared to CIMO was likely related to Pitrelli Vazquez et al. (2018) using FA to diagnose nAMD whereby participants had clear evidence of severe macular pathology. nAMD and DMO have different disease presentation which may explain the differences in hRSD threshold. nAMD leads to a disruption and damage to the retinal tissue architecture early in the disease course whereas DMO causes tissue oedema which leads to damage over a longer timescale. As discussed above, the EDDMO study used the presence of intraretinal cyst ≥50μm and retinal thickness ≥2SD compared to normative values to define CIMO, and some PWD classified as CIMO may have relatively well-preserved macular structure and function. Indeed, only a minority of CIMO (17.6%; 16 of 91 PWD with CIMO; Section 7.3.2) patients were treated at this relatively early stage.

The results from Chapter 8 showed that the performance of hRSD was not significantly superior to distance VA in discriminating between different severity groups (HC vs PWD, Non-CIMO vs CIMO, M0 vs M1, NT vs TT; Table 8.10). Moreover, the addition of hRSD to distance VA does not significantly improve the detection of CIMO, M1 or TT PWD compared to distance VA alone (Table 8.10). Since distance VA is an established part of RDS and worse VA (≥0.3 logMAR) has been associated with a fivefold increase in prevalence in DMO, there is no role in using hRSD to screen for DMO within RDS on the basis of this evidence (Olson et al., 2013). However, the interpretation of these results is limited by the relatively small number of participants with CST >400 (Section 8.3.2) and PWD who needed treatment (N=25) (Section 7.3.2). However, hRSD may be useful for remote monitoring of patients with DMO, which is discussed below.

To reduce the burden on the NHS, it would be ideal if some of the low-risk PWD (e.g. R1M1) could be monitored remotely at home for the development of DMO, instead of being monitored in DEC or DS. Self-monitoring can lead to prompt detection of disease deterioration and enable prompt intervention. The process of self-monitoring can lead to

patients taking more responsibility for their condition and PWD are often accustomed to self-monitoring of their glucose level or BP (Vas et al., 2017).

If the hRSD test were to be used as a remote monitoring tool for DMO then thresholds for normal or disease states would need to be established. Specifically, an hRSD threshold cut-off value whereby there is a high index of suspicion for centre involving macular disease or a change in the hRSD threshold that is beyond normal variability would be useful. This raises a number of issues as to what constitutes normal. The EDDMO study found no differences in the hRSD threshold, distance or near VA in participants who self-reported as being healthy and participants who had additional normal OCT (Section 6.3.7). These findings are reassuring as it suggests that studies that rely on groups of self-reported healthy participants are probably recruiting a suitably normal sample. Results suggested hRSD normative values to be -0.77 logMAR (Sections 6.3.1 and 6.3.3) and this is broadly consistent with other published data particularly when age is taken into account (Chapters 2 and 6). However clear evidence has been presented that the hRSD threshold for PWD, even in the absence of any DR, was 0.9 logMAR worse compared to HC (-0.68 logMAR; Sections 6.3.10 and 8.3.3). Notably, the hRSD threshold for PWD who have progressed to minimal DR (-0.61 logMAR, Section 8.3.3) is slightly worse again and is similar to the hRSD threshold of participants with intermediate AMD (-0.60 logMAR) (Pitrelli Vazquez et al. (2018).

In the EDDMO study, there was a decline in the hRSD threshold of PWD graded as R3, and this observation is likely because this group of participants with proliferative retinopathy also had severe maculopathy. The hRSD threshold for PWD graded as R3 was -0.47±0.29 logMAR and the threshold for PWD who required treatment was -0.44±0.33 logMAR (Table 7.3). These thresholds are similar to the hRSD threshold (-0.47 logMAR) of 19 eyes which developed nAMD in a study by Pitrelli Vazquez et al. (2018). This suggests that at an hRSD threshold of -0.47 logMAR, there is substantial macular pathology affecting visual function that would require treatment. Therefore an hRSD threshold of -0.47 logMAR or worse, would be suitable as a cut-off that could prompt a referral for further assessment if hRSD were to be used as a remote monitoring tool. With limits of agreement of -0.27 to 0.22 logMAR (intrasessional; Section 6.3.2) in normal participants and -0.24 to 0.27 logMAR (194±84 days apart) in PWD (Section 9.3.8), a deterioration in the hRSD threshold of 0.3 logMAR or more as proposed by Wang et al. (2013) should also activate a referral for

further assessment. Therefore, hRSD can be used as a home monitoring tool to prompt a re-presentation of patients to eye services when positive.

There are several other functional tests that could have a role in improving the detection of DMO. Although Amsler grid testing is commonly provided to patients for home monitoring of macular conditions, it is recognised that the test has poor validity in detecting macular diseases (Achard et al., 1995, Schuchard, 1993). The PHP test has shown promise in the home monitoring of patients with AMD (Chew et al., 2014, AREDS Home Study Research Group, 2014, Thomas et al., 2015). However, there is only one study published in Portuguese that examined the PHP test in detecting DMO in 60 eyes of 33 PWD (Matos et al., 2012); the study found the sensitivity and specificity of the PHP in detecting DMO to be 70.6% and 11.5% respectively. Data from Chapter 9 only found two PWD, who had deteriorated according to their CST, and no PWD improved (Section 9.3.4). With so few PWD showing clear evidence of change, it is not possible to draw conclusions about the ability of the hRSD test to track change in the same eyes over time and its suitability as a home monitoring tool for DMO. However, prospectively collected longitudinal data is rarely available for new tests and diagnostics.

Wang et al. (2013) proposed that since hRSD is a hyperacuity test, it is less affected by the deterioration of the eye's optical system such as media opacities compared to VA (Wang et al., 2013). The EDDMO study found that the hRSD test was less affected by ageing compared to near VA and it had good usability (Chapters 6 and 8). As discussed in Chapter 2, these features of the hRSD test may provide some advantages over other tests as a home monitoring tool for DMO.

Besides PHP and hRSD, a more recent vernier hyperacuity alignment task (Alleye) performed on mobile devices has been approved by the FDA for home monitoring; it has shown modest performance in discriminating between eyes with dry and nAMD (AUC 0.66, 95% CI 0.52-0.80) but there are no published data on its performance in detecting DMO (Schmid et al., 2018, Schmid et al., 2019).

Meantime, the Monitoring for nAMD Degeneration Reactivation at Home (MONARCH) study is evaluating the ability of three home monitoring tests (hRSD, KeepSight journal, MultiBit) to detect nAMD reactivation but the results are yet to be published (Ward et al., 2021).

10.6 RETINAL THICKNESS IN PWD

One of the major themes to emerge in this thesis are the patterns of retinal thickness changes observed in PWD. One of the interesting results is that PWD with no or minimal DR had retinal thinning mainly in the inner retinal layers (Section 7.3.9) along with worse visual function compared to HC as mentioned above (Sections 6.3.10 and 8.3.3). These findings are linked to the concept of DRN. Therefore, this section will discuss the possible pathogenesis of DRN and the time course of DRN with regards to microvasculopathy to understand the role of retinal thinning as measured by OCT as a biomarker for DRN.

There is still much uncertainty regarding the mechanism of DRN. There is evidence that hyperglycaemia disrupts the metabolic environment of the retina and reduces signals from insulin receptors that are crucial for neuronal development, growth and survival, which leads to neural apoptosis (Simo et al., 2014). In addition, cellular factors such as increased oxidative stress, inflammation, loss of neuroprotective factors and glutamate excitotoxicity all contribute to the process of neurodegeneration and glial cell dysfunction (Simo et al., 2014, Stem and Gardner, 2013, Barber, 2003). Glial cells play an essential role in supporting retinal neurons and maintaining the BRB. Therefore, reactive gliosis, in addition to neural apoptosis can cause increased vascular permeability and leakage of intraretinal fluid. These processes could all thereby lead to an initial thinning of the retina in early DR followed by retinal thickening when intraretinal fluid starts to accumulate (Jonsson et al., 2016, Sugimoto et al., 2005).

The concept of retinal thinning prior to the onset of microvasculopathy in early DR is supported by experimental studies in mouse models and clinical studies (Sohn et al., 2016, Reis et al., 2014). In mice that were rendered type 1 diabetic with streptozotocin, there was a decrease in combined RNFL and GCL thickness of 17.5% after 6 weeks, and 39.2% after 20 weeks of diabetes induction (Sohn et al., 2016). However, the eyes of these mice showed no microvascular changes even 20 weeks after diabetes induction. Reis et al. (2014) found that PWD without DR could have evidence of DRN detected by multifocal ERG (mfERG) before the onset of microvasculopathy as demonstrated by an intact BRB confirmed by vitreous fluorometry. Therefore, the timeframe between when DRN (detected by retinal thinning or by other means) takes place and the onset of DMO and PDR when retinal thickening occurs, can provide a window of opportunity for neuroprotective agents to work. This concept is illustrated in Figure 10.2. However, the identification and monitoring of DRN require suitable biomarkers, which is further discussed next.

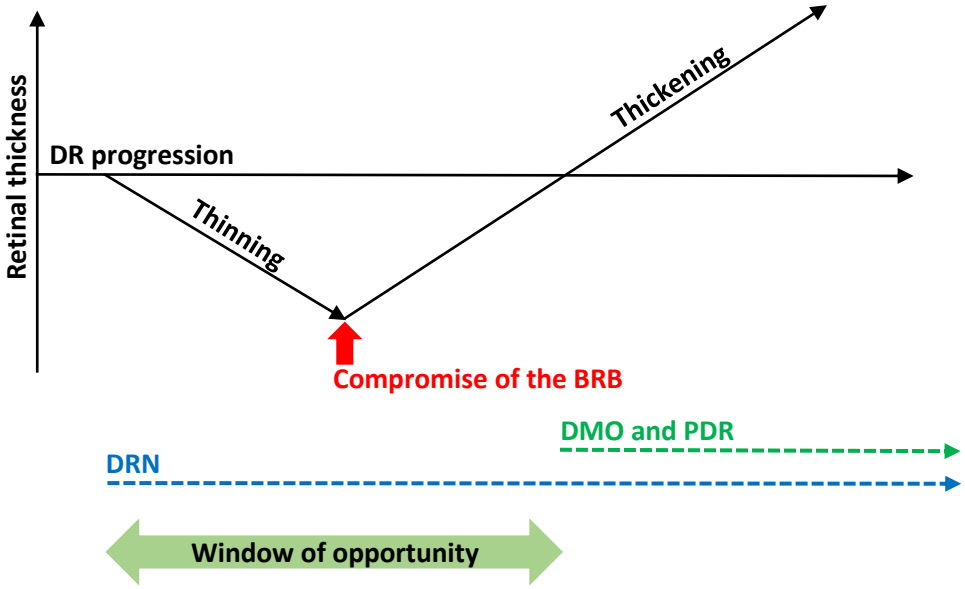


Figure 10.2 As diabetic retinopathy (DR) progresses, retinal thinning as detected by OCT can occur in diabetic retinal neuropathy (DRN) followed by the compromise of the blood-retinal barrier (BRB). This in turn can cause diabetic macular oedema (DMO) and proliferative diabetic retinopathy (PDR) and lead to retinal thickening. The window of opportunity for neuroprotective agents to work in DRN should be prior to the onset of DMO and PDR.

The EDDMO study found that PWD with no or minimal DR had significant thinning of the RNFL, GCL, IPL and ONL compared to HC (Figure 10.3, Section 7.3.9). Significant thinning of the GCL, IPL and INL in PWD with no or early DR between visits 1 and 2 (Section 9.3.6) was also found. These data add to the evidence that DRN can affect early DR and precede visible vasculopathy (Jonsson et al., 2016).

PWD with no or minimal DR had 7.2% to 12.3% RNFL thinning compared to HC (Figure 10.3). It is estimated that in glaucoma, by the time a visual field defect is detected on standard automated perimetry, at least 25% to 35% of ganglion cells have been irreversibly lost (Kerrigan-Baumrind et al., 2000) and 17% RNFL thinning (Wollstein et al., 2012) has occurred. Therefore, while the PWD with no or minimal DR had some degree of RNFL loss, it is not at the level whereby visual field defects on standard automated perimetry would be expected.

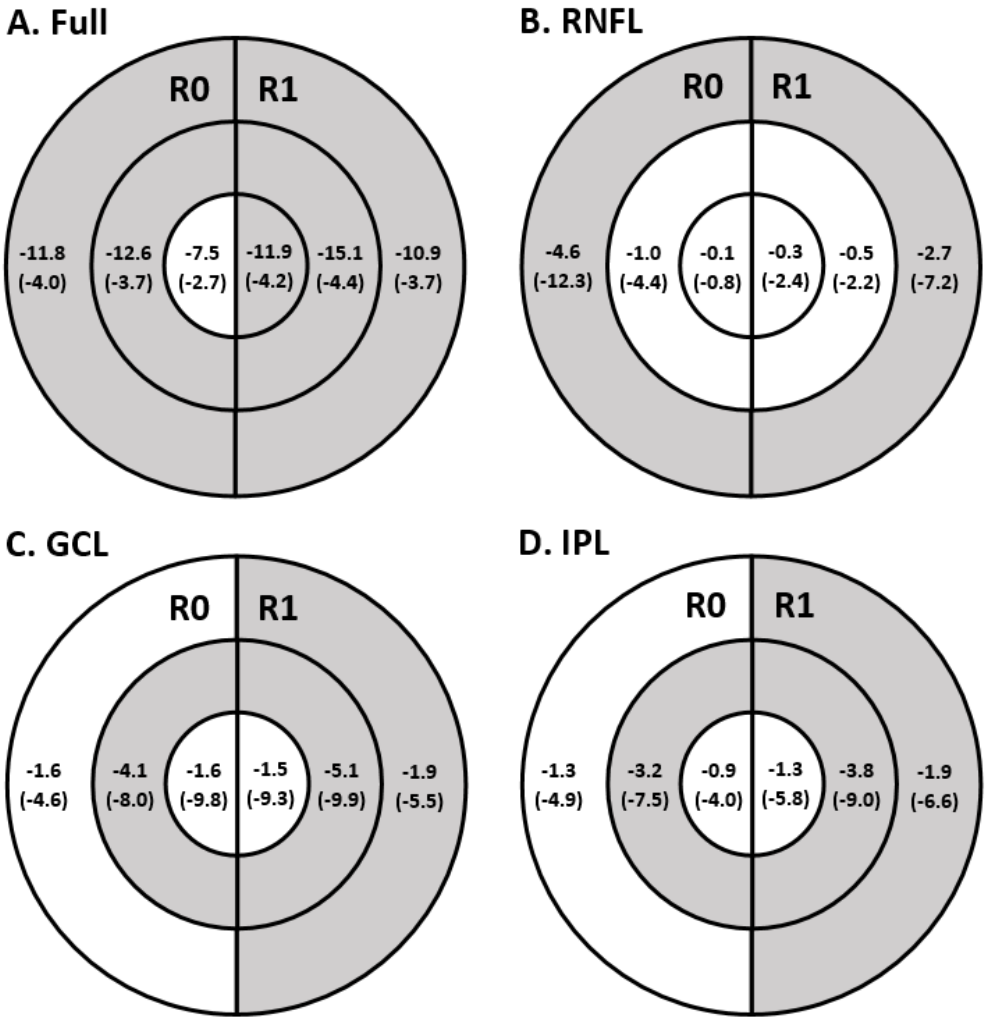


Figure 10.3. Retinal thinning of the full retinal layer (A), RNFL (B), GCL (C) and IPL (D) in PWD with no DR (R0M0; shown on the left) and PWD with minimal DR (R1M0; shown on the right) in all ETDRS subfields from the EDDMO study (Section 7.3.9). Absolute difference (µm) with the relative difference (%) in brackets between each group and healthy controls (HC) shown. Subfields in grey indicate a statistically significant difference <0.05 in retinal thickness of each group compared to HC.

The EDDMO study found full retinal (-7.5 to -15.1µm), RNFL (-0.1 to -4.6µm), GCL (-1.5 to -5.1µm) and IPL (-0.9 to -3.8µm) thinning in PWD with no or minimal DR (Figure 10.3). Montesano et al. (2021) studied 134 PWD with no DR using Heidelberg Spectralis OCT and they found significant full retinal (-3.47µm), GCL (-1.04µm) and IPL (-1.89µm) thinning across the ETDRS subfields compared to HC. However, they found no significant difference in RNFL thickness between PWD and HC. Montesano et al. (2021) also found significantly reduced BCVA, Pelli-Robson CS and FDT perimetry in PWD compared to HC. In the study by Montesano et al. (2021), PWD had a shorter duration of diabetes (2 months) compared to

the PWD with no DR in the EDDMO study (7.3±4.1 years), which may account for the more extensive inner retinal thinning seen in the EDDMO study.

Similar to the study by Montesano et al. (2021), the EDDMO study found functional deficits (measured using the hRSD test) with thinning of the inner retinal layers that have not been previously demonstrated. PWD with no clinical evidence of DR has a worse hRSD threshold of approximately 0.1 logMAR across the age range compared to HC (Section 6.3.10). This trend was similarly observed in distance and near VA (Section 8.3.4). PWD with no DR had a worse mean hRSD threshold of 0.9 logMAR compared to HC and this difference increased to 0.16 logMAR between PWD with minimal DR and HC (Section 8.3.3). Although these small differences are unlikely to be practically significant at these early stages, their consistency is marked.

It is known that functionally, DRN can manifest as ERG, CS, colour vision, dark adaptation or microperimetry abnormalities (Bearse et al., 2006, Hardy et al., 1992, Greenstein et al., 1993, Jackson et al., 2012). There is emerging evidence from the EDDMO (Section 9.3.9) and other studies (Sohn et al., 2016, Demirkaya et al., 2013) that there is a progressive loss of the inner retinal layers in DRN in PWD with no or minimal DR. In addition, due to the complexities in accurately measuring retinal layers in more severe DR, which will be discussed below, it is unknown if retinal thinning is accelerated with more severe disease. Therefore, if these small but consistent changes are progressive, then their compounded effect is likely to become clinically meaningful over time. However, more longitudinal studies on DRN are needed.

Retinal thickness as measured by OCT has been used in many studies of DRN (Sohn et al., 2016, Simo et al., 2019, Santos et al., 2019). However, these previous studies have mainly concentrated on the retinal layers which comprise of the ganglion cell complex (RNFL, GCL and IPL), while the other layers remained unexplored (Sohn et al., 2016, Santos et al., 2017). This approach may have been because OCT protocols used to study glaucoma, which is another neurodegenerative disease, have mainly used ganglion cell complex thinning to diagnose and monitor the condition (Kim and Park, 2018). Therefore, these OCT protocols have been applied to study DRN.

In PWD with no or minimal DR, the EDDMO study found significant thinning of the ONL (cross-sectional data, Section 7.3.9) and INL (longitudinal data, Section 9.3.9), which are not part of the ganglion cell complex. The ONL is comprised of photoreceptors nuclei while the

INL is comprised of the nuclei of the bipolar, amacrine, horizontal and Müller cells (Curcio et al., 1990). Since DRN and glaucoma differ in their pathogenesis and based on the changes seen in the retinal layers that are not part of the ganglion cell complex in this study, it is reasonable to infer that DRN could affect other layers that are not involved in glaucoma. However, it is not currently known if thinning of the ganglion cell complex is sufficient as a proxy for more general neuronal tissue loss or if changes in the other retinal layers can add value as biomarkers for DRN (van Dijk et al., 2011). Therefore, further studies on exploring the other retinal layers and possibly the relationship between changes in different layers would be useful.

As mentioned above, an additional complexity in using retinal thickness as a biomarker for detecting DRN is the possibility that vascular leakage can result in increased retinal thickness and act as a confounding factor that masks retinal thinning in more severe DR (Santos et al., 2017). In the EDDMO study, thinning of the inner retinal layers in PWD with no or minimal DR became more apparent when these groups were analysed separately (Chapter 7). Bandello et al. (2015) studied 194 PWD with NPDR and they found thinning of the ganglion cell complex but this thinning was masked by the presence of retinal oedema resulting from leakage from the retinal vessels and an increase in the extracellular space.

As previously discussed, the window of opportunity for neuroprotective agents to work is likely between the onset of DRN and the development of DMO and PDR (Figure 10.2). The EDDMO study has found that even in young PWD with no DR, there is a decline in visual function compared to HC (Sections 6.3.10, 8.3.4). In this group of PWD, neuroprotective agents may be the most beneficial since they will likely have a longer duration of diabetes and possibly more severe DR over time. The FA study assessed the effects of two topical neuroprotective drugs (brimonidine and somatostatin) on the prevention of DRN using mfERG and found that in PWD with pre-existing neurodysfunction, there was a deterioration in the implicit time in the mfERG in the placebo group but not the groups who received the drugs (Simo et al., 2019, Santos et al., 2017). However, mfERG reflects cone photoreceptor and bipolar cell function and may not directly measure inner retinal dysfunction seen in DRN (Hood et al., 2003). In addition, mfERG is a time-consuming investigation that requires trained personnel, specialised equipment and analysis; currently, it may be best reserved for clinical trials instead of routine clinical use (Hernández et al., 2020). There are further studies to investigate other potential topical agents such as

glucagon-like peptide 1 (GLP-1) to prevent DRN but translation into clinical trials are needed (Hernandez et al., 2016).

10.7 CHAPTER CONCLUSION

Historically, treatment for DR has been targeted at the more advanced stages when the BRB has been compromised and significant hypoperfusion has occurred, and when DMO and PDR have occurred. Current DR treatment includes laser photocoagulation, intravitreal injections and surgery, which are invasive, expensive and have many potential complications (Stitt et al., 2016). These treatment issues highlight a need for DR care to move from disease treatment towards disease prevention and health promotion (Figure 10.4).

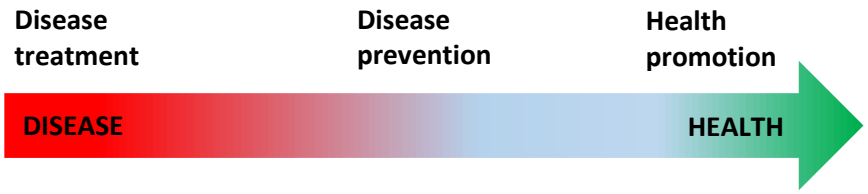


Figure 10.4 Need for diabetic retinopathy (DR) care to move from disease treatment towards disease prevention and health promotion.

There is a 5% annual increase in diabetes and DR remains a major cause of vision loss in working-age adults (Scanlon et al., 2015). The increasing pressure on the NHS coupled with a wider acceptance of remote monitoring of conditions triggered by the COVID-19 pandemic has stimulated a search for suitable home monitoring tools for DMO and other macular conditions and this search continues. In this thesis, both functional and structural data provide evidence of early changes consistent with early DRN. The detection and monitoring of DRN using suitable biomarkers and the development of effective neuroprotective agents are essential. However, diabetes is a systemic condition and although ophthalmologists can play a role in managing DR the overall management of diabetes and its complications will continue to require a multi-disciplinary and multi-dimensional approach.

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APPENDICES

APPENDIX 1. HEALTHY PARTICIPANT INFORMATION SHEET

UNIVERSITY OF LIVERPOOL

The Royal Liverpool and Broadgreen University Hospitals NHS Trust

Early Detection of Diabetic Macular Oedema (EDDMO)

Healthy Participant Information Sheet **Version: 4 Date: 19/10/16**

Chief Investigator: **Dr Paul Knox**
Eye & Vision Sciences, IACD
Tel: 0151 7949042 Email: pcknox@liverpool.ac.uk

You are being invited to participate in a non-invasive research study. Before you decide whether to participate, it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and feel free to ask us if you would like more information or if there is anything that you do not understand. Please also feel free to discuss this with friends or relatives if you wish. You do not have to accept this invitation and should only agree to take part if you want to.

Thank you for reading this.

What is the purpose of the study?

To study a new test for detecting diabetic macular oedema (swelling at the back of the eye because of diabetes).

Why have I been chosen to take part?

We have invited you to take part because we need to take measurements in healthy people who have normal vision as comparison with people suffering from diabetes.

Do I have to take part?

Taking part is entirely voluntary and even if you decide to take part you are free to withdraw at anytime without explanation and without incurring any disadvantage.

What will happen if I take part?

You will be invited to the Royal Liverpool University Hospital eye clinic where you will complete the new vision test - this will take approximately 40 minutes. The test runs on an iPod Touch, a device about the size of a mobile phone. On the device screen you will see some large circles. One of these will be slightly distorted with "bumps" appearing round its edge. You will simply touch the screen of the device to indicate which of the four circles is distorted. Over a number of attempts the size of the distortions will get smaller, until you have to guess which circle is distorted. When the device has worked out how big the distortions have to be for you to see them, it will calculate a score which we will record. Each eye is done in turn with the other eye patched during the test. After the iPod test, you will have some scans to measure and take pictures of the back of the eyes. This involves shining a light into your eye, and is not uncomfortable. You will also have your vision tested on standard vision charts and we will examine the back of your eyes for you.

Expenses and / or payments

There are no expenses or payments available for taking part.

Are there any risks in taking part?

There are no risks posed to you by doing the new test.

Are there any benefits in taking part?

There are not likely to be any direct benefits to you from taking part but your involvement may help other people with diabetes in the future.

What if I am unhappy or if there is a problem?

- Please contact the investigator, Dr Ku (0151 7949312, jku@liverpool.ac.uk) and she will try to help.
- If you remain unhappy or have a complaint which you feel you cannot deal with in this way, please contact the University of Liverpool Research Governance Officer on 0151 794 8290 (ethics@liverpool.ac.uk).
- When contacting the Research Governance Officer, please provide details of the name or description of the study (so that it can be identified), the researcher(s) involved, and the details of the complaint you wish to make.

Will my participation be kept confidential?

The results of the tests will be kept on secure, password protected computers. The anonymised data (without any personal identifying information) will be kept for up to 10 years, and will be used in further analysis. Only anonymised data, that cannot be related to you personally, will be released or discussed publicly at scientific meetings or in research publications.

Will my taking part be covered by an insurance scheme?

This research is covered by both the University of Liverpool and NHS insurance policies.

What will happen to the results of the study?

Eventually the results of the study will be published in the form of abstracts at scientific meetings and research papers. If we find any abnormalities we will refer you for appropriate treatment.

What will happen if I want to stop taking part?

You can withdraw from this research study at anytime, without explanation. If data has already been collected, we will still include it.

Who has reviewed the study?

This study has been reviewed by the North West – Preston Research Ethics Committee.

Who can I contact if I have further questions?

Investigator: Dr Jae Ku, 0151 7949312, jku@liverpool.ac.uk

APPENDIX 2. DIABETIC PARTICIPANT INFORMATION SHEET

UNIVERSITY OF LIVERPOOL

The Royal Liverpool and Broadgreen University Hospitals NHS Trust

Early Detection of Diabetic Macular Oedema (EDDMO)

Diabetic Participant Information Sheet **Version: 4 Date: 19/10/16**

Chief Investigator: **Dr Paul Knox**
Eye & Vision Science, IACD
Tel: 0151 7949042 Email: pcknox@liverpool.ac.uk

We would like you to consider participating in a non-invasive research study, currently running in the clinic to which you have been referred. This study is not part of your clinic appointment. Please attend your clinic appointment even if you do not wish to take part in the study. Before you decide, it is important for you to understand why the research is being done and what it will involve. Please read the following information carefully; feel free to ask us questions if you would like more information or if there is anything that you do not understand. Please also feel free to discuss this with friends or relatives if you wish. You do not have to accept this invitation and should only agree to take part if you want to. ***Thank you for reading this.***

What is the purpose of the study?

To study a new test for detecting diabetic macular oedema (swelling at the back of the eye because of diabetes).

Why have I been chosen to take part?

We have invited you to take part because we need to make measurements in people with diabetes who have been referred from the diabetic screening programme for further assessment.

Do I have to take part?

Taking part is entirely voluntary and even if you decide to take part you are free to withdraw at any time without explanation and without affecting any of your care that you would receive.

What will happen if I take part?

During your clinic visit in the Royal Liverpool University Hospital eye clinic, you will complete a new vision test. The test runs on an iPod Touch, a device about the size of a mobile phone. On the device screen you will see some large circles, one of which will be distorted with "bumps" around its edge. You will simply touch the screen to indicate which of the circles is distorted. Over a number of attempts the size of the distortions will get smaller, until you have to guess which circle is distorted. When the device has worked out how big the distortions have to be for you to see them, it will calculate the score which we record. Each eye is done in turn with the other eye patched during the test. After the iPod test, you will have some quick scans to measure the length and refractive power of your eyes. This involves shining a light into your eye, and is not uncomfortable. These tests will take no longer than 20 minutes and will often be quicker. Once the tests are completed, you will continue as normal with your other assessments, under the care of your normal doctor. If you are invited to a follow-up appointment, the iPod test will be repeated at the next clinic appointment.

Expenses and / or payments

There are no expenses or payments available for taking part.

Are there any risks in taking part?

There are no risks posed to you by doing the new test.

Are there any benefits in taking part?

There are not likely to be any direct benefits to you from taking part but your involvement may help other people with diabetes in the future.

What if I am unhappy or if there is a problem?

- Please contact the investigator, Dr Ku (0151 7949312, jku@liverpool.ac.uk) and she will try to help.
- If you remain unhappy or have a complaint which you feel you cannot deal with in this way, please contact the University of Liverpool Research Governance Officer on 0151 794 8290 (ethics@liverpool.ac.uk).
- When contacting the Research Governance Officer, please provide details of the name of the study (so that it can be identified), the researcher(s) involved, and the details of the complaint you wish to make.

Will my participation be kept confidential?

The results of the tests will be kept on secure, password protected computers. The anonymised data (without any personal identifying information) will be kept for up to 10 years, and will be used in further analysis. Only anonymised data, that cannot be related to you personally, will be released or discussed publicly at scientific meetings or in research publications.

Will my taking part be covered by an insurance scheme?

This research is covered by both the University of Liverpool and NHS insurance policies.

What will happen to the results of the study?

Eventually the results of the study will be published in the form of abstracts at scientific meetings and research papers. The results will not be available to you or to your care team and will have no effect on your medical care.

What will happen if I want to stop taking part?

You can withdraw from this research study at anytime, without explanation. If data has already been collected, we will still include it.

Who has reviewed the study?

This study has been reviewed by the North West – Preston Research Ethics Committee.

Who can I contact if I have further questions?

Investigator: Dr Jae Ku, 0151 7949312, jku@liverpool.ac.uk

APPENDIX 3. HEALTHY PARTICIPANT CONSENT FORM

UNIVERSITY OF LIVERPOOL

The Royal Liverpool and Broadgreen University Hospitals NHS Trust

CONSENT FORM (Healthy participant)
Version: 4 Date 19/10/16

Title of Research Project:	Early Detection of Diabetic Macular Oedema (EDDMO)
Researcher(s):	**Chief Investigator: Dr Paul Knox** **Dr Jae Ku, Prof Simon Harding, Dr Deborah Broadbent, Dr Ticiana Criddle, Dr Amira Stylianides**

	Please initial box
1. I confirm that I have read and have understood the information sheet EDDMO (healthy participants) dated 19/10/16, for the above study. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily.	☐
2. I understand that my participation is voluntary and that I am free to withdraw at any time without giving any reason, without my rights being affected.	☐
3. I understand that data collected from the study may be looked at by regulatory authorities or by persons from the Trust where it is relevant to my taking part in this research. I give permission for these persons to have access to this information.	☐
4. I understand that, under the Data Protection Act, I can at any time ask for access to the information I provide and I can also request the destruction of that information if I wish.	☐
5. I agree to have non-invasive scans to take pictures and measurements from my eyes	☐
6. I agree to take part in the above study.	☐

Participant Name	Date	Signature
Name of Person taking consent	Date	Signature
Researcher	Date	Signature

The contact details of the Chief Investigator are:
Dr Paul Knox
Eye & Vision Science (IACD)
William Duncan Building
6 West Derby St
Liverpool L7 8TX
Tel: 0151 7949042
Email: pcknox@liverpool.ac.uk

Participant Number:

APPENDIX 4. DIABETIC PARTICIPANT CONSENT FORM

UNIVERSITY OF LIVERPOOL

The Royal Liverpool and Broadgreen University Hospitals NHS Trust

CONSENT FORM (Diabetic Participant)

Version 4 Date 19/10/16

Title of Research Project: Early Detection of Diabetic Macular Oedema (EDDMO)

Researcher(s): **Chief Investigator: Dr Paul Knox**
Dr Jae Ku, Prof Simon Harding, Dr Deborah Broadbent, Dr Ticiana Criddle, Dr Amira Stylianides

		Please initial box
1.	I confirm that I have read and have understood the information sheet EDDMO (diabetic participants) dated 19/10/16, for the above study. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily.	☐
2.	I understand that my participation is voluntary and that I am free to withdraw at any time without giving any reason, without my medical care being affected.	☐
3.	I understand that the research team may need access to my medical records for the purposes of this study.	☐
4.	I understand that my medical notes and data collected from the study may be looked at by regulatory authorities or by persons from the Trust where it is relevant to my taking part in this study. I give permission for these persons to have access to this information.	☐
5.	I understand that, under the Data Protection Act, I can at any time ask for access to the information I provide and I can also request the destruction of that information if I wish.	☐
6.	I agree to have non-invasive scans to take measurements from my eyes.	☐
7.	I agree to take part in the above study.	☐

Participant Name	Date	Signature
Name of Person taking consent	Date	Signature
Researcher	Date	Signature

The contact details of the Chief Investigator are:
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Participant Number: ☐