



THE UNIVERSITY *of* EDINBURGH

This thesis has been submitted in fulfilment of the requirements for a postgraduate degree (e.g. PhD, MPhil, DClinPsychol) at the University of Edinburgh. Please note the following terms and conditions of use:

This work is protected by copyright and other intellectual property rights, which are retained by the thesis author, unless otherwise stated.

A copy can be downloaded for personal non-commercial research or study, without prior permission or charge.

This thesis cannot be reproduced or quoted extensively from without first obtaining permission in writing from the author.

The content must not be changed in any way or sold commercially in any format or medium without the formal permission of the author.

When referring to this work, full bibliographic details including the author, title, awarding institution and date of the thesis must be given.

Impact of insulin pump therapy and islet transplantation on progression of diabetic retinopathy in Type 1 diabetes.

Dr Laura Reid



Presented for the degree of MD (Doctor of Medicine)

University of Edinburgh

2021

Declaration

I declare that the content of this thesis is my own and that all contributions and collaborations have been explicitly acknowledged in the text.

The work published in this thesis has not been submitted for any other degree or professional qualification.

Where content has been submitted, or published in peer-review journals, this is clearly stated in the thesis in the relevant section

Laura J Reid
August 2021

Acknowledgements

I would firstly like to thank my primary supervisor Professor Shareen Forbes for her invaluable guidance and constant support throughout my MD time, both with the academic and clinical work, and in preparations for two periods of maternity leave. I would also like to thank Professor Bal Dhillon for welcoming and guiding me through what had been a relatively unknown world of ophthalmology. I have learnt a lot from them both.

I am also grateful to all those who facilitated the early study design, particularly Professor Helen Colhoun and Professor Sarah Wild, and Neville Lee at the Diabetic Retinopathy Screening Service for his assistance with data collection of retinal screening images.

This research would not have been possible without the support of the team at the Edinburgh Clinical Research Imaging Centre and image analysis. Particular thanks go to Dr Tom MacGillivray, Kirsty Hetherington, Dr Calum Gray, Ylenia Giarratano, Dr Tariq Farrah and Dr Emma Pead for their patience and support.

I owe a huge amount to the islet transplant teams, both in Edinburgh and in Edmonton. Thanks especially to Charlotte Ibbotson, Kirsty Duncan, Mr Andrew Sunderland and Mr John Casey for welcoming me as part of the Edinburgh islet transplant family, and to Dr Anna Lam and Dr Peter Senior for all their patience and support during my visit to the Edmonton islet transplant team.

I would also like to thank the Type 1 diabetes team and pump specialists, including Dr Fraser Gibb, Joan Grant and Lynn Gourley for their help in recruitment of study participants. I would especially like to thank Debbie Anderson and Alison Sudworth for imparting their diabetes technology and research knowledge, and for being such good company to work alongside each day. I would also like to thank all the participants who kindly agreed to participate in this study.

I am grateful to the funding bodies who have supported this research opportunity, particularly the Royal College of Surgeons (Royal Blind) and the Edinburgh and Lothians Health Foundation.

Finally, I would like to thank my family. Thanks to my mum for her love and encouragement, to my son Jack (aged 2) for giving me so much joy and for being an excellent sleeper when I was writing this thesis, to expected baby number two for hopefully not arriving before the viva, and thanks especially to my husband Paul, without whom I don't think I would ever have seen this MD through. His unwavering support, love and patience have kept me going throughout my research time and beyond.

Abstract

Diabetic retinopathy (DR) is one of the leading causes of blindness worldwide and affects almost all adults with Type 1 diabetes within 20 years of diagnosis. It is well established that optimising glycaemic control reduces DR risk, however little is known about the impact of individual Type 1 diabetes treatment modalities on the incidence and progression of DR.

This study examines the effects on DR progression of two treatments used in Type 1 diabetes; Continuous subcutaneous insulin infusion (CSII) therapy, and islet transplantation. Both treatments have been shown to improve glycaemic control when compared to multiple daily injections of insulin (MDI), but it has not been clearly established whether these glycaemic benefits correspond to microvascular benefits in terms of reduced DR risk. Reports of paradoxical early worsening of DR (EWDR) in people with rapid improvements in glycaemic control can complicate matters, as does the use of immunosuppressive medication in those undergoing islet transplantations.

In this thesis we test the hypothesis that rapid reduction of time in hyperglycaemia and hypoglycaemia post intervention with islet transplantation or CSII therapy will be associated with changes consistent with worsening DR in the short term (consistent with reports of EWDR), but reduced retinopathy risk in the long term. In addition, we assess the use of emerging retinal imaging techniques in DR, and propose that novel retinal biomarkers will be able to detect early retinopathy changes in individuals with diabetes following the introduction of these treatment interventions.

We conducted a retrospective analysis of graded DR screening data for people with Type 1 diabetes following commencement of CSII or islet transplantation, compared to continued MDI therapy. We show that in people with no or mild DR, CSII (n=204 people) is associated with significantly reduced risk of DR progression on multivariate Cox proportional hazard analysis (HR 0.56, p=0.02) over 2.3 years compared with those continuing on MDI (n=211 people). There was no significant difference in DR progression following islet transplantation compared to either CSII or MDI therapy. Furthermore, despite significant HbA1c reductions

in those commenced on CSII and islet transplantation versus those continued on MDI, there was no evidence of EWDR following the addition of these therapies.

Quantitative analyses of retrospectively collected DR screening images from CSII participants (n=78 at baseline) and islet transplanted participants (n= 29 at baseline) were also carried out using semi-automated software (Vessel Assessment and Measurement Platform for Images of the Retina (VAMPIRE)), which was designed in Scotland for the detection and quantification of retinal vascular geometry from fundus images. We assessed three retinal biomarkers which have been proposed as early indicators of DR incidence and severity; vessel calibre, vessel tortuosity and fractal dimensions (denoting vascular branching patterns). Increases in these markers have been associated with increased DR incidence and progression in previous fundus imaging studies. We found significant reductions in fractal dimensions ($p<0.001$) and vessel calibre measurements ($p<0.01$) over a mean 3.3 year period following the introduction of CSII therapy which may signify reduced burden of DR in participants post CSII, though further studies with suitable control participants are needed to confirm these preliminary findings. There were no significant changes in these retinal biomarkers over time in islet transplant participants, though study numbers were small leading to an underpowered analysis.

Finally, a prospective study was done to assess retinal biomarkers using other retinal imaging modalities; Optical Coherence Tomography (OCT), Optical Coherence Tomography Angiography (OCTA) and Ultrawide-field Scanning Laser Ophthalmoscopy (UWF-SLO), which are emerging as useful tools in DR imaging assessment. We looked at changes in vessel density, foveal avascular zone (FAZ) and acircularity index (AI) using OCTA, retinal and choroidal thickness using OCT, and vessel calibre using UWF-SLO. We found no significant change from baseline in any of these biomarkers over a 12 month follow up in 16 CSII participants, including no evidence of EWDR. However, comparison to previously collected healthy control data showed significant differences in these biomarkers in participants with diabetes compared to those without diabetes, with significant reductions in vessel density and choroidal thickness and significant increases in AI in individuals with diabetes. These findings support growing evidence of the potential value of these parameters as early markers of DR.

In conclusion, our study did not find any evidence for EWDR following the introduction of CSII therapy or islet transplantation in our cohort, despite early improvements in glycaemic control, but did find evidence for reduced DR progression in the longer term following CSII therapy, including reductions in DR biomarkers on fundus imaging. This indicates potential benefits of CSII in reducing the burden of microvascular complications due to DR in Type 1 diabetes. Though we didn't show long term DR risk reduction in islet transplant participants, we found stable DR up to 5 years post islet transplantation compared to CSII and MDI participants, despite the addition of potentially damaging immunosuppressive therapy. Our data also supports early reports for the growing utility of retinal biomarkers derived from novel imaging modalities in the early identification of DR, particularly markers of vessel density, choroidal thickness and AI. Further larger prospective studies will help characterise the full significance of these markers and their future potential in DR assessment and intervention.

Lay Summary

Diabetes is a condition where too much glucose (sugar) builds up in the bloodstream and other tissues in the body. The buildup of glucose over a long period of time causes damage to the body, particularly to small blood vessels in areas such as the eyes. As a result, diabetes is one of the main causes of blindness in the UK.

This study looks at whether certain treatments for diabetes help reduce the risk of developing diabetic eye disease. The first treatment, known as an insulin pump or continuous subcutaneous insulin infusion, replaces insulin, which is the substance no longer produced in Type 1 diabetes, using an infusion pump which the user keeps attached to the body. The second treatment, known as an islet transplant, involves transplanting the cells which make insulin (islet cells) back into the body to allow the affected individuals to make some of their own insulin again. Both treatments are known to help reduce the buildup of glucose in the body, but there is little evidence about whether they are better than standard treatment with regular injections of insulin at reducing diabetic eye disease. There have also been some concerns previously that these changes to treatment may cause worsening of eye disease in the short term due to rapid changes in blood glucose, or potentially due to other medications used to suppress the body's immune system following islet transplantation.

Early changes in diabetic eye disease usually go undetected by affected individuals, therefore annual screening is done to take photos of the back of the eye to assess for potentially damaging changes which can be treated before vision is affected. In addition to these standard eye photos, newer technology has recently been developed which enables us to analyse the 3D layers (using Optical Coherence Tomography (OCT)) and blood vessels (using Optical Coherence Tomography Angiography (OCTA)) at the back of the eye in more detail and get a wider look at the back of the eye (using Ultrawide-field Scanning Laser Ophthalmoscopy). These imaging modalities may provide more efficient ways to detect early changes in diabetic eye disease, and this was also assessed as part of this study.

We compared the degree of diabetic eye disease from standard eye photos, taken as part of the diabetes eye screening service for people with Type 1 diabetes, from people started on

insulin pump treatment, or after an islet transplant, to those who continued regular insulin injections. We showed that those on insulin pump treatment had reduced progression of diabetic eye disease compared to those on regular injections. People who received islet transplants had similar progression of diabetic eye disease to those on regular injections. There was no evidence of worsening eye disease compared to those on regular insulin injections, despite the rapid improvements in blood glucose levels and the introduction of immune modulating medications in those who received a transplant. Quantitative analysis of the eye photos, comparing vessel sizes and how twisted or branched individual vessels were, showed vessels were less wide with reduced complexity of branching patterns following insulin pump therapy, both of which have been proposed as early indicators of reduced diabetic eye disease, though more evidence is needed on this.

We also assessed people who had started treatment with insulin pump therapy using newer eye imaging technology. This didn't show any change in the 3D layers or blood vessels at the back of the eye in the year following treatment with insulin pump therapy. However, we did find that people with diabetes had reduced density of blood vessels across the back of the eye and a more irregular pattern (denoted by the acircularity index) of blood vessels at the central part of the eye which is responsible for the most focused vision compared to people without diabetes. We also showed people with diabetes had reduced thickness in one of the vascular layers known as the choroid layer which helps supply blood to the central part of the eye. This supports previous findings that these imaging modalities can be used to detect diabetic eye disease in new ways which we were not previously able to evaluate using eye photos alone.

In conclusion, we have shown that there is no worsening of diabetic eye disease following treatment with insulin pump therapy or islet transplantation, and that insulin pump therapy is associated with reduced progression of diabetic eye disease compared to regular insulin injections. We have also provided evidence that new imaging modalities provide other markers for diabetic eye disease that may help detect early eye changes in individuals with diabetes in the future.

Publications and Presentations

Publications

Continuous Subcutaneous Insulin Infusion Therapy Is Associated With Reduced Retinopathy Progression Compared With Multiple Daily Injections of Insulin But Is Not Associated With A Reduction In HbA1c. LJ Reid, F. Gibb, H. Colhoun, S. Wild, M. Strachan, K. Madill, B. Dhillon, S. Forbes. *Diabetologia* August 2021. 10.1007/s00125-021-05456-w

Effects of Islet Transplantation on Long Term Microvascular and Macrovascular Complications in Type 1 Diabetes. LJ Reid, F Baxter, S Forbes. *Diabetic Medicine* July 2021. e14570. 10.1111/dme.14570

Presentations

A framework for revealing retinal biomarkers in Optical Coherence Tomography Angiography (OCTA). Ylenia Giarratano, Alisa Pavel, Jie Lian, Rik Sarkar, Dan Pugh, Tariq Farrah, Neeraj Dhaun, Laura Reid, Shareen Forbes, Tom MacGillivray, Baljean Dhillon, Miguel O. Bernabeu. *Oral presentation at SINAPSE 2020 Annual Scientific Meeting*

A framework for the discovery of retinal biomarkers in Optical Coherence Tomography Angiography (OCTA). Ylenia Giarratano, Alisa Pavel, Jie Lian, Rayna Andreeva, Alessandro Fontanella, Rik Sarkar, Laura J Reid, Shareen Forbes, Dan Pugh, Tariq E. Farrah, Neeraj Dhaun, Baljean Dhillon, Tom MacGillivray, and Miguel O. Bernabeu. *Oral presentation at 7th MICCAI Workshop On Ophthalmic Medical Image Analysis 2020*

Reduced Progression Of Diabetic Retinopathy In Type 1 Diabetes Over Three Years With Clinical Islet Transplantation Or Continuous Subcutaneous Insulin Infusion Compared With Multiple Daily Insulin Injections. LJ Reid, A Lam, B Dhillon, K Duncan, C Ibbotson, A Sutherland, J Casey, A Koh, C Rudinsky, M Tennant, A Malcolm, AMJ Shapiro, P Senior, S Forbes. *Oral Presentation at 17th World Congress of International Pancreas and Islet Transplant Association 2019*

Insulin Pump Therapy Is Associated With Reduced Retinopathy Progression Compared With Multiple Daily Injections of Insulin. L. Reid, F. Gibb, H. Colhoun, S. Wild, M. Strachan, A. Ochs, K. Madill, B. Dhillon, S. Forbes. *Poster presentation at Diabetes UK March 2019.*

CGM Shows Islet Transplantation Prevents Hypoglycemia, Correcting Time in Range and Reducing Glycemic Variability, Despite Subnormal Beta-Cell Function. Shareen Forbes, Tolu Olutoyin Olateju, Anna Lam, John Casey, John Campbell, Laura Reid, Richard A. Oram, Andrew J. Malcolm, A.M. James Shapiro, Peter A. Senior. *Diabetes*. 2018; 67 (suppl 1): 143-OR. *Oral presentation at American Diabetes Association Conference June 2018.*

Donor HbA1c is inversely related to islet yield but does not impact on islet function at one year following islet transplantation in subjects with Type 1 diabetes. LJ Reid, L Irvine, N McGowan, D Mitchell, G Walker, K Duncan, C Ibbotson, A Sutherland, J Casey, S Forbes. *Diabetic Medicine*, Volume 35, Issue S1, P25, March 2018. *Poster presentation at Diabetes UK March 2018*

Contents

DECLARATION	I
ACKNOWLEDGEMENTS.....	II
ABSTRACT	IV
LAY SUMMARY	VII
PUBLICATIONS AND PRESENTATIONS.....	IX
CONTENTS.....	XI
LIST OF ABBREVIATIONS	XVI
1 INTRODUCTION	2
1.1 Background	2
1.2 Type 1 Diabetes Definition and Glycaemic Control	4
1.2.1 Definition and aetiology	4
1.2.2 Diabetes Pathology: Complications of hyperglycaemia.....	4
1.2.3 Measures of glycaemic control and variability	5
1.3 Therapies to improve glycaemic control in Type 1 Diabetes	7
1.3.1 Insulin Therapy in Type 1 Diabetes	7
1.3.2 Transplant therapy in Type 1 diabetes	10
1.4 Retinal Pathology in Diabetes	16
1.4.1 Retinal Anatomy and Blood Supply	16
1.4.2 Pathophysiology of diabetic retinopathy.....	17
1.4.3 Clinical Markers and Risk Factors for Diabetic Retinopathy	18
1.4.4 Early Worsening of Retinopathy	19
1.5 Retinal Imaging	21
1.5.1 Standard Fundus photography	21
1.5.1 Ultra-Widefield Scanning Laser Ophthalmoscopy	25
1.5.2 Optical Coherence Tomography	27
1.5.3 Fluorescein Angiography	28

1.5.4	Optical Coherence Tomography Angiography.....	28
1.5.5	Retinal Zones	30
1.6	Retinal Biomarkers	33
1.6.1	Vessel Density	33
1.6.2	Vessel Tortuosity	34
1.6.3	Vessel Calibre.....	36
1.6.4	Fractal Dimensions.....	36
1.6.5	Quantification of Foveal Avascular Zone Characteristics.....	38
1.6.6	Retinal and Choroidal Thickness	39
1.7	Project Aims And Hypothesis	43
1.7.1	Study Questions.....	43
1.7.2	Study Hypothesis	43
1.7.3	Project Aims.....	44
2	COMPARISON OF GRADED DIABETIC RETINOPATHY SCREENING OUTCOMES IN ISLET TRANSPLANTATION AND CONTINUOUS SUBCUTANEOUS INSULIN INFUSION VERSUS MULTIPLE DAILY INJECTIONS	45
2.1	Introduction	46
2.1.1	The Scottish Care Information (SCI-) Diabetes database.....	46
2.1.2	Scottish Diabetic Retinal Screening Programme	46
2.1.3	Retinal Evaluation at the Edmonton Islet Transplant Centre	48
2.1.4	CSII therapy and diabetic retinopathy	48
2.1.5	Islet cell transplantation and diabetic retinopathy.....	49
2.2	Methods	53
2.2.1	Study design.....	53
2.2.2	Patient groups.....	53
2.2.3	Data Collection.....	56
2.2.4	Statistical Analyses.....	58
2.3	Results Part A: Insulin Pump versus MDI	60
2.3.1	Study populations	60
2.3.2	HbA1c changes in CSII and MDI	63
2.3.3	Retinopathy events in CSII versus MDI	64
2.4	Discussion Part A	69
2.5	Results Part B: Islet Transplantation versus CSII and MDI	74
2.5.1	Study populations	74

2.5.2	HbA1c Changes at one year	76
2.5.3	Retinopathy Events in Islet Transplanted versus Non-Transplanted Participants.....	76
2.6	Discussion Part B	81
2.7	Conclusions	82
3	ASSESSMENT OF VASCULAR RETINAL BIOMARKERS ON FUNDUS IMAGES AFTER ISLET TRANSPLANTATION OR COMMENCEMENT OF CONTINUOUS SUBCUTANEOUS INSULIN INFUSION	83
3.1	Introduction	83
3.1.1	VAMPIRE software for fundus photo analysis	83
3.1.2	Role of retinal vascular geometry in diabetic retinopathy – current knowledge	85
3.2	Methods	90
3.2.1	Study design.....	90
3.2.2	Study Participants	90
3.2.3	Data collection	90
3.2.4	Image selection and assessment	91
3.2.5	VAMPIRE analysis.....	92
3.2.6	Statistical analysis	93
3.3	Results	95
3.3.1	Study Numbers and Baseline Characteristics	95
3.3.2	Changes in Retinal Biomarkers in CSII group over time.....	98
3.3.3	Changes in Retinal Biomarkers in islet transplanted group over time	103
3.3.4	Comparison of retinal biomarkers in CSII group versus islet transplantation group.....	107
3.3.5	Comparison of retinal biomarkers in diabetes patients with no diabetic retinopathy versus background diabetic retinopathy	108
3.3.6	Correlation between left and right eyes for all participants.....	109
3.4	Discussion	113
3.4.1	Summary of Key Findings.....	113
3.4.2	Vessel Calibre.....	115
3.4.3	Fractal Dimensions.....	116
3.4.4	Tortuosity.....	118
3.4.5	Study strengths and limitations	118
3.5	Conclusion	119

4	ANALYSIS OF RETINAL OUTCOMES FOLLOWING COMMENCEMENT OF CONTINUOUS SUBCUTANEOUS INSULIN INFUSION USING MULTIPLE RETINAL IMAGING TECHNIQUES	120
4.1	Introduction	120
4.1.1	Diabetic retinopathy changes in OCTA – FAZ and Vessel Density	120
4.1.2	Diabetic retinopathy changes in OCT	122
4.1.3	Diabetic retinopathy in UWF-SLO	125
4.2	Methods	126
4.2.1	Study Design and Participants	126
4.2.2	Study Visit Protocol.....	129
4.2.3	Retinal Assessment.....	132
4.2.4	Statistical Analysis.....	151
4.3	Results	152
4.3.1	Recruitment numbers and baseline data	152
4.3.2	Glycaemic Control and CGM Results across visits	155
4.3.3	Neuropathy and ACR	158
4.3.4	Retinal imaging results.....	159
4.4	Discussion	166
4.5	Conclusion	169
5	CONCLUSIONS	170
5.1	Hypothesis 1: Increased risk of EWDR post CSII or islet transplantation compared to continued MDI therapy – Evidence presented does not support this hypothesis in people with no or mild baseline retinopathy	170
5.2	Hypothesis 2: Reduced long term DR risk following CSII or islet transplantation compared to continued MDI therapy – Evidence presented supports this hypothesis	172
5.3	Hypothesis 3: Retinal biomarkers useful in early detection of DR – Evidence presented supports this hypothesis	174
5.4	Future Directions	175
5.5	Summary	176
6	REFERENCES	177
	APPENDIX 1: SUPPLEMENTARY INFORMATION FOR CHAPTER 2.....	189
	APPENDIX 2: SUPPLEMENTARY INFORMATION FOR CHAPTER 4.....	191

List of abbreviations

ACR	Albumin Creatinine Ratio
AI	Acircularity Index
ART	Automatic Retinal Tracking
AVR	Arteriovenous Ratio
BM	Bruch's membrane
BMI	Body mass index
BP	Blood pressure
CGM	Continuous Glucose Monitoring
CITR	Collaborative Islet Transplant Registry
CRAE	Central Retinal Artery Equivalent
CRVE	Central Retinal Vein Equivalent
CSII	Continuous Subcutaneous Insulin Infusion
CV	Co-efficient of variation
CVI	Choroidal Vascularity Index
DAFNE	Dose Adjustment for Normal Eating
DCCT	Diabetes Control and Complications Trial
DRS	Diabetic Retinopathy Screening
EDIC	Epidemiology of Diabetes Interventions
eGFR	Estimated Glomerular Filtration Rate
ETDRS	Early Treatment Diabetes Retinopathy Study
EWDR	Early Worsening of Diabetic Retinopathy
FA	Fluorescein Angiography
FAZ	Foveal Avascular Zone
FD	Fractal Dimensions
FOV	Field of View
GV	Glycaemic Variability
HbA1c	Glycosylated Haemoglobin
HR	Hazard Ratio
IAH	Impaired Awareness of Hypoglycaemia
IQR	Interquartile Range

MAGE	Mean Amplitude of Glucose Excursions
MDI	Multiple Daily Injections
MMF	Mycophenolate Mofetil
NPDR	Non-Proliferative Diabetic Retinopathy
OCT	Optical Coherence Tomography
OCTA	Optical Coherence Tomography Angiography
OR	Odds Ratio
PDR	Proliferative Retinopathy
RNFL	Retinal Nerve Fibre Layer
RPE	Retinal Pigment Epithelium
SCI	Scottish Care Information
SD	Standard Deviation
SDRG	Scottish Diabetic Retinopathy Grading
SIMD	Scottish Index of Multiple Deprivation
SPK	Simultaneous Pancreas and Kidney
TIR	Time in Range
UWF-SLO	Ultra-Widefield Scanning Laser Ophthalmoscopy
VAMPIRE	Vascular Assessment and Measurement Platform for Images of the Retina
VEGF	Vascular Endothelial Growth Factor

1 Introduction

1.1 Background

Diabetes is a chronic metabolic disorder, affecting an estimated 422 million adults worldwide (1). The costs of treatment are escalating, with a projected annual cost of £39.8 billion by 2035 (2). Complications affecting microvascular and macrovascular systems account for a large proportion of the disease burden, both in terms of healthcare costs, and the morbidity and mortality for those affected.

Diabetic retinopathy (DR) is one of the main microvascular complications of diabetes, affecting nearly all patients with Type 1 diabetes within 20 years of diagnosis (3). It is one of the leading causes of visual impairment and blindness in the UK (3) and worldwide (4). Early detection and treatment via national screening programmes has been effective in reducing sight threatening retinopathy (5).

Poor glycaemic control is one of the major modifiable risk factors for development of retinopathy (4). The landmark Diabetes Control and Complications trial (DCCT) showed that patients treated with intensive insulin to improve glycaemic control had a 76% reduction in risk of developing DR over six year follow up, compared with those receiving conventional treatment (6). As a result, the mainstay of current diabetes treatments aims to achieve near normal glycaemic control where possible. However, these targets can be limited by treatment related hypoglycaemia. Advancements in diabetes treatments have helped patients reach these desired glucose levels. The use of continuous subcutaneous insulin delivery via insulin pumps, and the development of islet transplant therapies aim to enable people with Type 1 diabetes to achieve improved glycaemic control, while minimising problematic hypoglycaemia.

While reductions in average glucose levels are beneficial in the longer term, the DCCT also showed that transient worsening of retinopathy was significantly more likely to occur short term, particularly in the first 12 months in intensively treated subjects (13.1%) versus the

conventional treatment group (7.6%) (7). This has raised concerns that rapid reductions in average glucose may paradoxically worsen retinopathy in the short term. This has been observed in early pregnancy, where women with poorer control at baseline and greater improvements in glycaemic control were at highest risk of retinopathy progression (8). Retinopathy screening frequency is increased during pregnancy as a result. Retinopathy progression and screening frequency for other scenarios where rapid improvements in glycaemic control occur are less well established.

During this MD, I assess the effect of changes to diabetes management and glycaemic control on DR changes, focussing on two treatments for Type 1 diabetes which can cause rapid improvements in glycaemic control; insulin pump therapy and islet transplantation.

1.2 Type 1 Diabetes Definition and Glycaemic Control

1.2.1 Definition and aetiology

Type 1 diabetes is an autoimmune metabolic disorder characterised by destruction of pancreatic beta cells leading to insulin deficiency and subsequent hyperglycaemia. The aetiology remains unclear, though models for pathogenesis suggest autoimmune processes stimulated by a combination of genetic predisposition and environmental triggers as a probable mechanism, leading to rapid loss of beta cell mass and decompensation due to insufficient insulin (9). This is distinct from the pathology in Type 2 diabetes where insulin continues to be produced but decompensation occurs due to insulin resistance at the target tissues. There is increasing recognition of the complexity of the spectrum of diabetes diagnoses, which include monogenic diabetes and gestational diabetes (10). This study will focus on Type 1 diabetes.

1.2.2 Diabetes Pathology: Complications of hyperglycaemia

High serum glucose levels result in accumulation of byproducts of glucose metabolism, including sorbitol and advanced glycation end products. In excess, these cause cellular injury due to osmotic and oxidative stress. This leads to microvascular damage and complications, primarily retinopathy, nephropathy and neuropathy. Atherosclerotic processes are also accelerated, resulting in macrovascular complications such as myocardial infarction and stroke (11).

Long term risk of developing these microvascular and macrovascular complications is lowered by reducing the burden of hyperglycaemia in people with Type 1 diabetes using intensive insulin therapy (12).

1.2.3 Measures of glycaemic control and variability

1.2.3.1 HbA1c

The gold standard measurement to monitor blood glucose control is glycosylated haemoglobin (HbA1c), which is a surrogate marker for average blood glucose levels over two to three months. HbA1c values greater than 48mmol/mol represent blood glucose levels in the diabetic range (13). Lower HbA1c values have been shown to correlate with a reduced risk of developing diabetes complications (14). Limitations of HbA1c include that it does not provide information about day to day fluctuations in glucose excursions, which have been shown to have potential effects on diabetes complications independently of HbA1c (15, 16), and it can be inaccurate in haemoglobin related pathology such as anaemia or haemoglobinopathies (17).

1.2.3.1 Self-Monitoring of Glucose Levels

Until recently, capillary blood glucose testing has been the mainstay for self-monitoring daily fluctuations in blood glucose levels. Frequent testing has been shown to correlate with lower HbA1c values (18) and, in those completing frequent daily tests, reduced microvascular complications are seen in those with more capillary test values that fall within the accepted target range (3.9-10mmol/L) (19). Current guidelines recommend a minimum of four capillary blood glucose tests per day for people with Type 1 diabetes, but in reality compliance with this is relatively poor (20).

The recent advent of continuous glucose monitoring (CGM) technology has provided an appealing alternative for self-monitoring glucose levels. It has the advantage of providing much more comprehensive information regarding daily glucose fluctuation than the snapshot picture offered by capillary testing, and can therefore provide greater insight into individuals with potentially different patterns of glycaemic variability (GV) despite the same average glucose (Figure 1-1) (21). CGM sensors measure interstitial glucose at regular intervals (typically logging glucose data every 1-5 minutes) to provide a near continuous trace of the glucose levels through the course of a day. The term encompasses both 'real-time' CGM

where users can be alerted to pre-defined changes in glucose levels, or 'intermittent' CGM ('flash' glucose monitoring) where users will only be aware of glucose levels on checking the device.

Measures of GV include the amount of time spent with blood glucose levels above, below, or within a specified target range¹, glucose standard deviation or the mean amplitude of glucose excursions (MAGE). Recommended clinical targets are now defined for many of these parameters to facilitate their use in clinical practice (22). Use of intermittent CGM, via Abbott's Libre device, has become widespread in many diabetes centres since becoming available on prescription in the UK for people with Type 1 diabetes, and has been associated with marked reductions in HbA1c (23).

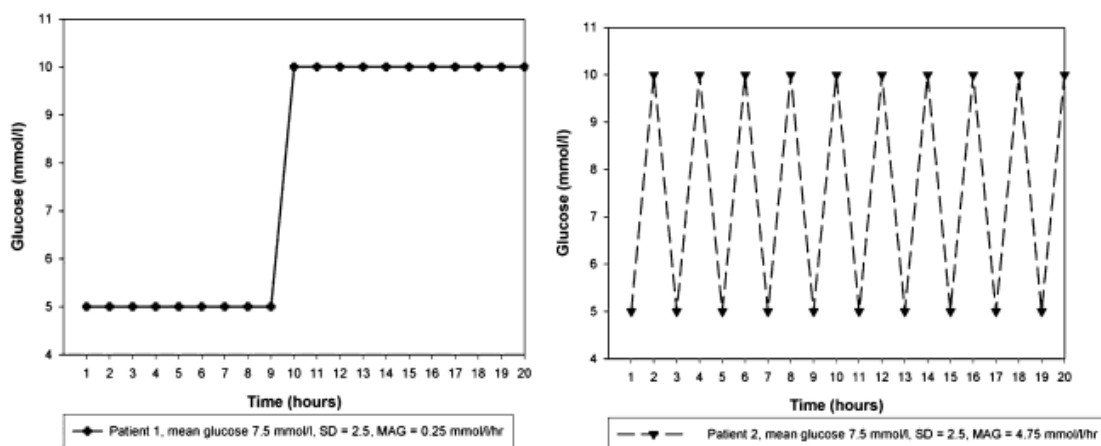


Figure 1-1 Example of two potential patients with identical mean and SD of glucose throughout a 20-h period, but markedly different glucose variability. Image from De Vries et al. (21)

¹ Glucose targets of 4-10mmol/l used for this study

1.3 Therapies to improve glycaemic control in Type 1 Diabetes

1.3.1 Insulin Therapy in Type 1 Diabetes

Insulin is a peptide hormone produced by the pancreatic beta cells. It regulates glucose metabolism and the use of glucose by tissues as an energy source. It also facilitates the uptake of glucose from the blood for storage in the liver and adipose tissue. The isolation of insulin for exogenous use was a life-saving discovery for those with the previously fatal diagnosis of Type 1 diabetes, who were unable to produce their own endogenous insulin (24). It continues to be the mainstay of treatment for Type 1 diabetes and is administered either via a pen device or via continuous subcutaneous infusion. Despite this, correctly predicting fluctuations in the body's insulin requirements remains difficult, leaving users vulnerable to the continued hyperglycaemia due to diabetes pathology, or to iatrogenic hypoglycaemia.

1.3.1.1 Multiple Daily Injections (MDI)

Frequent injections of animal, human or analogue insulin are administered using a pen device to mimic the typical physiological release of endogenous insulin. This is usually done using a basal bolus insulin regimen which typically involves four to five injections per day. Long-acting insulin is administered either once daily or twice daily to provide a constant slow release of basal insulin throughout the day. At mealtimes, when carbohydrate is consumed, a further fast acting insulin injection is administered to enable the body to metabolise the additional glucose appropriately.

1.3.1.2 Continuous subcutaneous insulin infusion

Continuous subcutaneous insulin infusion (CSII), also commonly referred to as insulin pump therapy, also aims to mimic physiological insulin release, but does so via the continuous infusion of small amounts of fast acting insulin via an insulin pump. At mealtimes the user can administer a larger dose of insulin via the pump, similar to the bolus injections used in MDI. CSII has the advantage of additional flexibility over MDI, as basal insulin rates can be adjusted throughout the day to account for changes in activity, heat, illness or other factors which may

influence glycaemic control. Indications for commencing CSII include failure to achieve target HbA1c levels or recurrent severe hypoglycaemia on MDI therapy (25). A Cochrane review assessing multiple studies showed a small but significant reduction of 0.3% (3.3mmol/mol) in HbA1c with CSII therapy compared with MDI (26). Though this is just a small difference in HbA1c, it is important to remember that the recurrent hypoglycaemia with MDI is one of the main indications for commencing CSII therapy, and this will contribute to lower HbA1c outcomes in MDI users despite suboptimal glycaemic control due to the presence of debilitating hypoglycaemic episodes (27).

Insulin pump technology can also be set up for coordinated use with CGM. Some CGM devices enable information about fluctuations in interstitial glucose to be fed back to the pump to suspend insulin delivery when hypoglycaemia occurs ('Low Glucose Suspend'), without intervention from the patient. This is recommended for clinical use in patients with reduced awareness of hypoglycaemia (28). 'Closed loop' systems, which can adjust insulin delivery based on the CGM independently of the patient have been developed, though their use is mainly limited to research settings. These are a focus for ongoing research, particularly with the aim to develop an affordable self-sufficient system for widespread use in Type 1 diabetes (29). Hybrid closed loop systems, which can adjust the delivery of basal insulin based on CGM feedback, but still require regular user input for appropriate bolus insulin at mealtimes, are in use in clinical practice, though the cost of both pump and CGM devices are a limiting factor in their widespread use.

1.3.1.3 The role of intensive insulin therapy: Diabetes Control and Complications Trial

The DCCT (12) was a landmark multicentre randomised clinical study which assessed the effect of intensive insulin therapy, designed to achieve blood glucose levels as close as possible to normal, using either MDI (minimum three injections daily) or CSII, on microvascular and macrovascular diabetes complications. Outcomes were compared to a control group on conventional insulin therapy consisting of one or two daily insulin injections. It was conducted between 1983 to 1993 and included 1441 people, aged 13 to 39, with Type 1 diabetes, recruited across 29 medical centres in USA and Canada. While the original study assessed outcomes over an average of 6.5 years, most of the original participants continued

to be followed up long-term as part of the Epidemiology of Diabetes Interventions and Complications study (EDIC), which is still ongoing.

At the end of the initial DCCT follow up period there was clear evidence of significant reductions in diabetes complications for those in the intensively treated insulin group, with a 76% reduction in incident retinopathy, 54% reduction in retinopathy progression, 39% reduction in the development of microalbuminuria and 60% reduction in incident neuropathy (12). Long term follow up of up to 30 years during EDIC has shown continued benefits for microvascular risk reductions (30-33) in the intensively treated group in addition to a reduction in macrovascular cardiovascular complications by 30% (7).

The main adverse outcome in the intensive group was a 2-3 fold increase in episodes of severe hypoglycaemia, where third party assistance is required to help manage a hypoglycaemic episode (34). In addition, although long term outcomes for retinopathy were improved in the intensively treated group, there was evidence of early retinopathy worsening within the first 6-12 months of intensive insulin treatment (7).

1.3.1.4 Impact of hypoglycaemia with insulin therapy

Hypoglycaemia has historically been the primary barrier to achieving tight glycaemic control using insulin therapy (35). Adults with type 1 diabetes report around two episodes of hypoglycaemia per week, with an annual prevalence of severe hypoglycaemia of 30-40% (36). Hypoglycaemia is associated with significant neurological and cardiovascular morbidity, reduced quality of life and mortality (37). Recurrent hypoglycaemia and longer duration of diabetes are associated with impaired awareness of hypoglycaemia (IAH), occurring in around 20% of people with type 1 diabetes (38). Increased incidence of hypoglycaemia is associated with high GV, which is itself thought to be a determinant of vascular damage in diabetes (39), though studies assessing effects of GV on long term microvascular complications are lacking (40). There is reasonable evidence suggesting that treatment using CSII can improve glycaemic control and reduce hypoglycaemia risk compared with MDI (41), though this is not always the case (42).

1.3.2 Transplant therapy in Type 1 diabetes

Please note that extracts from this section have also been published in a first author review by Reid *et al.* (43).

Transplantation of donor pancreatic tissue, either as a whole organ transplant, or as an infusion of pancreatic islet cells, is a treatment option considered for people with Type 1 diabetes who have ongoing problems with glycaemic control or severe diabetes complications, despite optimising insulin therapy. The treatment aims and target populations for each type of transplant differ and are outlined below, however both require the need for lifelong immunosuppression to prevent the body from rejecting the transplanted organ or cells. In addition, the transplant is only achieved through the use of deceased donor organs, which is a limited resource. As such transplantation use is limited to a small subset of individuals with Type 1 diabetes for whom the benefits of transplantation are felt to outweigh the risks associated. This study will focus on patients treated with islet transplantation rather than whole organ pancreas transplantation.

1.3.2.1 Pancreas Transplantation

Transplantation of a donor pancreas aims to restore the endogenous release of insulin in response to changes in glucose homeostasis. When successful, this can achieve normal or near normal blood glucose levels without the need for exogenous insulin, eliminating the risks of treatment related hypoglycaemia. These benefits come with substantial risk. For solitary pancreas transplantation five year patient survival is 82%, with five year graft survival of only 53% (44). As such, pancreas transplantation is typically only offered to people with end stage renal disease, who are already in need of a transplanted kidney and for whom a simultaneous pancreas and kidney (SPK) transplant can be performed. Outcomes for SPK transplantation are better than in pancreas alone, with five year patient survival of 89% and graft survival of 78% (44).

1.3.2.2 Islet Cell Transplantation

Islet cell transplantation offers a minimally invasive alternative to pancreas transplantation, to restore beta cell function and reduce glycaemic lability. Currently, islet transplantation in the UK is reserved for people with recurrent hypoglycaemia, with at least two episodes of severe hypoglycaemia in the two years prior to assessment, and IAH. Relatively low insulin requirements (typically less than 60 units per day) are also favoured to maximise treatment impact (28).

Islet transplant success can be defined in various ways. Insulin independence, reductions in insulin doses, improvement in glycaemic control, reduction of hypoglycaemia, return of hypoglycaemia awareness and c-peptide secretion are all used as markers of a successfully functioning islet graft. The International Pancreas and Islet Transplant Association have suggested that achieving a HbA1c below 53mmol/mol, clinically significant c-peptide production with more than 50% reduction in insulin requirements, and absence of severe hypoglycaemia indicates good islet graft function and should be the main aim of treatment, rather than insulin independence (45).

Composite scoring systems encompassing some or all of the above measures are often used to assess graft function. The most commonly used is the Beta score (46). This gives a categorical score ranging from 0 – 8 based on fasting plasma glucose, HbA1c, daily insulin dose or oral hypoglycaemic use, and stimulated c-peptide. The Beta2 score is a similar scoring system but uses a calculation to give a continuous score (47). Though this is less simple to calculate than the Beta score, it has the advantage of using a fasting c-peptide rather than a stimulated c-peptide (measured 90 minutes after a standard mixed meal challenge consisting of 360ml Ensure HP comprising 391kcal with 8.5g fat, 44g carbohydrate, 17g protein). As such it eliminates the need for a mixed meal challenge for assessment.

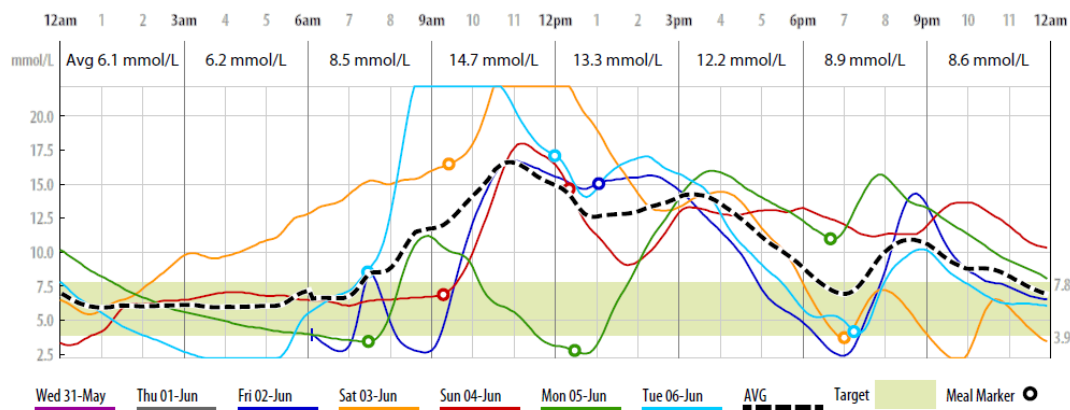
Islet transplantation is advantageous over whole organ pancreas transplantation in being a much simpler and safer procedure, involving infusion of cells into the portal vein of the liver under sedation. The cell preparation time typically takes around 24 hours, while the actual

procedure lasts around 2 hours. Most recipients can be discharged 48 hours after the procedure.

Infusions of more than 10,000 total islet cell numbers per recipient weight (IEQ/kg) are associated with better transplant outcomes (48). The number of islet cells yielded from an individual pancreas can vary, with most recipients requiring islet cells transfused from at least two donor pancreases to achieve this target. Occurrence of peri-procedural complications is low, with infusion related adverse events occurring in 4.2% of islet transplants from 2015-2018 (49).

The use of steroid free islet transplant protocols, established by the Edmonton group in 2000 (50), have seen improvements in islet transplant outcomes, with significant reductions in HbA1c from baseline (51). The Collaborative Islet Transplant Registry (CITR), have collated data from over 1000 subjects receiving islet transplants since 1999. Insulin independence rates have risen from 10% to 66% at one year post-transplant (51). Hypoglycaemic events have also been reduced, with elimination of severe hypoglycaemia in >90% of recipients five years post-transplant (51). Our own experience in the Scottish Islet Transplant Programme has shown diminished glycaemic variability post islet transplant (Figure 1-2).

A)



B)

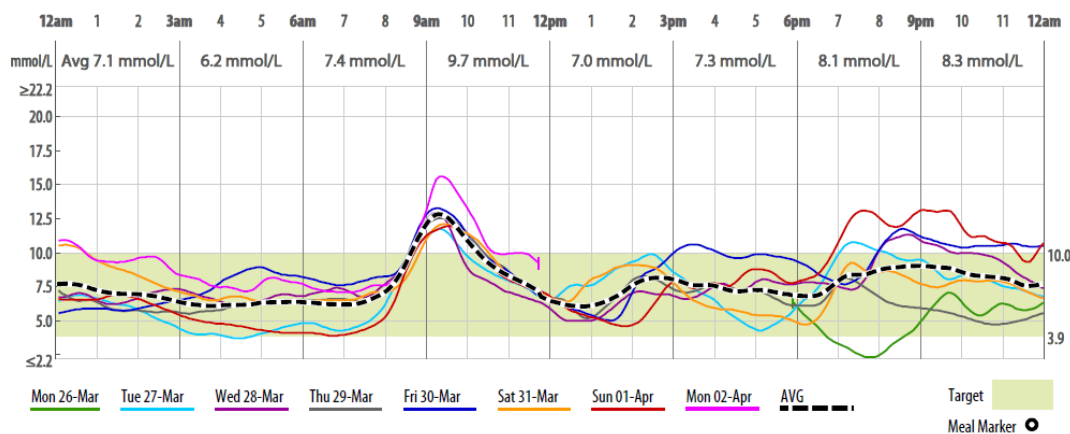


Figure 1-2 CGM from an Edinburgh islet transplant recipient pre transplant (A) and post first islet transplant (B). Estimated HbA1c reduced from 63mmol/mol (A) to 47mmol/mol (B). Average glucose reduced from 9.9mmol/l (A) to 7.7mmol/l (B). Standard deviation of glucose reduced from 4.9mmol/l in (A) to 2.2mmol/l in (B).

1.3.2.3 Immunosuppression in islet transplantation

To minimise alloimmune and autoimmune rejection of the donor cells, immunosuppressive drugs are used alongside islet transplantation. Steroid free immunosuppression has been used since the Edmonton trial with significant improvements in outcomes (50). A combination of an induction agent at the time of transplant is used with lifelong maintenance immunosuppression of at least two drugs.

Almetuzumab, a monoclonal antibody to CD52, is most commonly used as an induction agent. This has been shown to have better outcomes for insulin independence rates than earlier protocols using anti IL-2 receptor antibodies such as Daclizumab, particularly when combined

with anti-TNF alpha inhibitors such as Etanercept (52). Other induction agents include anti-thymocyte globulin: which are rabbit-derived antibodies against human T cells and basiliximab: a monoclonal antibody to the α chain (CD25) of the IL-2 receptor of T cells (43).

Tacrolimus is the main agent used for maintenance immunosuppression. Tacrolimus use is well established in whole organ transplantation, reducing rejection rates, and effectively inhibiting the immune response through the reduction of IL-2 upregulation via calcineurin inhibition. Unfortunately, side effects include nephrotoxicity, hypertension, hyperglycaemia, and hepatotoxicity (53). Levels are monitored carefully post-transplant to avoid these side effects and minimise graft loss.

Sirolimus was initially the main drug used in combination with tacrolimus. This inhibits IL2 via mTOR inhibition. It has the advantage of being less nephrotoxic than tacrolimus, though there are reports of declining renal function and proteinuria with its use (54, 55). It too is associated with side effects such as hypertension, diabetes like syndrome and hypercholesterolemia (53).

Mycophenolate Mofetil (MMF) is now typically favoured as a combination agent with tacrolimus, with associated improvements in insulin independence rates compared with sirolimus use (51). MMF inhibits inosine monophosphate dehydrogenase and has the advantage of not being nephrotoxic. However, the main side effects include gastrointestinal upset with nausea, vomiting and diarrhoea (53), which could leave subjects vulnerable to acute kidney injury in the post-operative period.

Though immunosuppression increases the risk of infection and malignancy, rates of these complications remain relatively low. Information from the CITR showed an overall incidence of malignancy of 0.01 events/person year follow up. Life threatening events, mainly related to infection and depleted granulocyte numbers, occurred in 5.4% of recipients between 2007 and 2018. Immunosuppression was thought to be linked with six of a total of 33 deaths reported post islet transplant since the registry began in 2001 (43, 49).

Immunosuppression has been shown to have adverse effects on renal function and is associated with higher rates of cardiovascular disease (53). This could be related to effects on blood pressure and lipids. This increased cardiovascular risk could have an indirect impact on DR risk though there is no clear evidence for this currently. There is limited evidence that the immunosuppressive drugs used in islet transplantation affect DR or neuropathy at all, though there have been case reports of people with peripheral neuropathy and optic neuropathy associated with Tacrolimus use (56, 57).

1.4 Retinal Pathology in Diabetes

1.4.1 Retinal Anatomy and Blood Supply

The retina is the light-sensitive layer at the back of the eye which is primarily responsible for visual perception. It is formed by the innermost neurosensory segment, composed mainly of photoreceptors, ganglion cells and bipolar cells, which relay the visual impulses generated by light, and an outermost retinal pigment epithelium (RPE) made up of metabolically active cells, responsible for maintaining nourishment and support to the neurosensory layer. On microscopic cross-sectional examination, ten distinct layers can be identified (Figure 1-3), formed by the uniform arrangement of the component retinal cell axons, nuclei and cell bodies, and other supporting structures. Advances in retinal imaging mean these layers can now be easily viewed in vivo using non-invasive technology (optical coherence tomography (OCT) - discussed later, see Chapter 1.5.2).

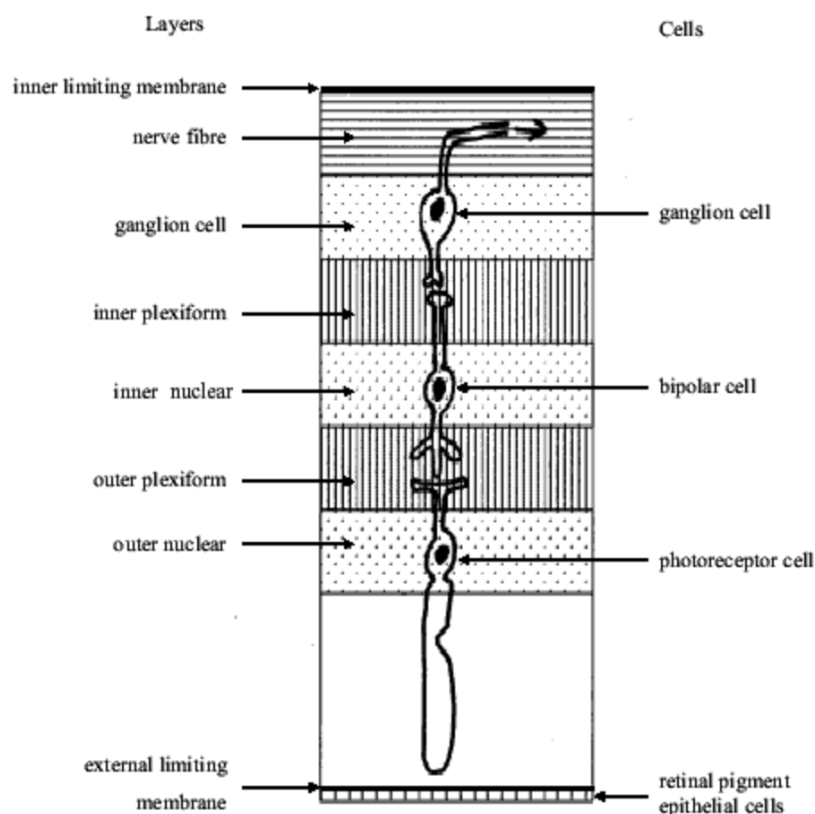


Figure 1-3 Retinal layers: Image from Scottish Diabetic Retinopathy Screening Collaborative (58)

The retina has a dual blood supply. The innermost layers, up to the inner nuclear layer, are supplied by a capillary network derived from the central retinal artery, which perfuse the inner two thirds of the retina. The outer third of the retina is supplied by the choroid vessels derived from the short posterior ciliary arteries of the ophthalmic artery and recurrent branches arising from the anterior ciliary arteries. The choroid is located below the RPE and is formed by four main layers; the innermost Bruch's membrane (BM), choriocapillaris (capillary layer), Sattler's layer (medium diameter vessels) and Haller's layer (large diameter vessels). Like the retina, the choroid can also be visualised using retinal imaging (OCT), but the individual layers are not typically so well defined.

Blood vessels from the central retinal artery enter onto the retinal surface via the optic disc. This is also the exit point for ganglion cell axons from the retinal nerve fibre layer (RNFL), which form the optic nerve. The optic disc is devoid of photoreceptor cells and forms a physiological blind spot.

The region responsible for the sharpest central vision is the fovea, located within the most central part of the retina known as the macula. To facilitate the uninterrupted passage of light to the photoreceptors posteriorly within the macula, the innermost layers of the neurosensory retina are displaced peripherally and there are no blood vessels overlying the fovea (forming the foveal avascular zone (FAZ)). As a result, the normal retina is much thinner at the fovea, with a thicker subfoveal choroid layer to ensure adequate blood supply to this region.

1.4.2 Pathophysiology of diabetic retinopathy

Diabetes affects the structure and cellular composition of the blood retinal barrier, which is made up of capillary basement membrane, pericytes and endothelial cells. Vascular pathology in DR is characterised by disruptions to vascular permeability, abnormal blood flow and closure or non-perfusion of capillaries.

Hyperglycaemia induced damage to the blood retinal barrier causes loss of endothelial cells and pericytes leading to dysfunctional acellular capillaries. The loss of the integrity of the

capillary wall contributes to microaneurysm formation, one of the early cardinal features of DR. These microaneurysms are prone to further damage due to haemorrhage. Extracellular fluid can also accumulate within the macula in early stages of the disease, causing macular oedema. This is associated with the presence of hard exudates on 2D imaging, formed by leakage of lipid rich plasma. Glycation of proteins and thickening within the basement membrane contributes to capillary dysfunction and hinders the distribution of oxygen and nutrients to the retina due to impaired blood flow. This is exacerbated by retinal leukostasis causing occlusion of the retinal microvasculature due to increased adherence of white cells in diabetes. Capillary occlusion leads to loss of functioning capillaries due to ischaemia and the presence of cotton wool spots due to retinal nerve fibre damage. It also results in the production of growth factors such as vascular endothelial growth factor (VEGF) to stimulate angiogenesis. New vessels are vulnerable to haemorrhage into the vitreous, destabilising the retina leading to detachment (59).

1.4.3 Clinical Markers and Risk Factors for Diabetic Retinopathy

Pathological features of DR are often evident on retinal imaging before any clinically significant visual changes are detected. They can be used to broadly classify DR into non proliferative diabetic retinopathy (NPDR), proliferative diabetic retinopathy (PDR) and maculopathy. NPDR is characterised by microaneurysms, localised haemorrhages within the retinal layers (dot, blot, and flame haemorrhages), hard exudates and venous changes. PDR refers to the presence of neovascularisation or vitreous haemorrhage. Maculopathy is reserved for retinopathy changes occurring in the macula (typically within one or two disc widths of the central fovea), though it is often used to describe macular oedema, which is a more specific form of maculopathy characterised by the presence of hard exudates on fundus imaging.

The production of reactive oxygen species, due to hyperglycaemia, is thought to be the main cause for the initial breakdown in the blood retinal barrier. The relationship between common modifiable atherosclerotic risk factors, including hypertension, hyperlipidaemia, and smoking, with increased DR risk is well established (4). A recent analysis assessing risk factors for PDR, clinically significant macular oedema, and ocular surgery from more than 30 years of

data from DCCT and EDIC has shown that mean HbA1c was the strongest risk factor for DR progression (60). Diabetes duration, age, mean diastolic BP and albumin excretion rate were also associated with increased DR risk in this study (60).

The impact of fluctuations in glucose levels, or GV, on the pathophysiology of DR is less well understood. A 2010 systematic review suggested that higher GV was associated with increased risk of diabetes complications for patients with Type 2 diabetes, but no effect was shown for Type 1 diabetes. Various methods were used to assess GV, including standard deviation (SD) of blood glucose, MAGE, mean of daily differences and the risk of severe hypoglycaemia. Of the five studies which looked at microvascular complications in Type 1 diabetes, only one used CGM to calculate GV, and this study did not assess GV effects on DR (15). A more recent study using CGM in 35 patients with Type 1 diabetes suggested that GV does have a role as a risk factor for retinopathy (16), though significance was lost in multivariate analysis, adjusting for hypertension and hypercholesterolemia.

1.4.4 Early Worsening of Retinopathy

Though hyperglycaemia is a well-established risk factor for DR, rapid improvements in glycaemic control are paradoxically associated with transient early worsening of diabetic retinopathy (EWDR) in the short term. Much of the evidence for this comes from early studies in the 1980s and 1990s which assessed diabetes outcomes following the introduction of intensive insulin treatment with MDI or CSII, versus what had been the conventional insulin treatment at the time, with only one or two daily insulin injections (61). The majority of these studies were small, including up to only 70 people. The DCCT provided large scale evidence for EWDR with intensive insulin treatment (7). EWDR was observed in 13% of those on intensive treatment versus 7.6% on conventional treatment over 12 months (OR 2.06, $p < 0.001$). Risk of EWDR was higher for both groups in those with higher baseline HbA1c (OR 1.61 intensive group, OR 1.32 conventional group for 1% increase in HbA1c) and longer duration of diabetes (OR 1.47 intensive group, OR 1.29 conventional group for 1yr increase in diabetes duration). Within the intensive group, larger reductions in HbA1c four to five months post study entry were also associated with increased EWDR risk (OR 1.60 for 1% decrease in HbA1c), though this may have been confounded by the fact that larger HbA1c reductions were

associated with higher baseline HbA1c. People with more severe retinopathy at baseline, particularly those with severe NPDR or early PDR, were also at the highest risk of EWDR (7).

The DCCT, and other early studies, predated national retinopathy screening programmes which have been shown to help reduce the burden of more severe diabetic retinal disease (62).

The use of intensive insulin is now the treatment standard for most people with type 1 diabetes, however many people with type 1 diabetes still struggle to achieve optimal glycaemic control despite the use of regular insulin boluses via MDI or CSII. Recent evidence as to whether EWDR is associated with rapid reductions of HbA1c following optimisation of intensive insulin regimens is lacking.

EWDR has been shown to develop following improved glycaemic control in pregnancy and following bariatric surgery, though other metabolic and hormonal factors may play a role in these special circumstances (8, 61, 63).

More recent observational studies assessing retinal changes in people with Type 1 diabetes with marked improvement in glycaemic control following the introduction of the libre flash glucose monitoring system showed the extent of glucose lowering was not associated with EWDR, though higher baseline HbA1c, longer diabetes duration and previous retinopathy were risk factors for retinopathy progression (64, 65).

Another study comparing retinal characteristics using OCT in people with type 1 diabetes one year following the introduction of CSII to those on MDI concluded there was no evidence of EWDR, despite significantly larger reductions in HbA1c in the CSII group versus MDI group (17.8 mmol/mol versus 3.1mmol/mol, $p<0.0001$) (66). They did report a small but significant increase in central retinal thickness but this was not associated with any clinical evidence of macular oedema (66).

1.5 Retinal Imaging

In many retinal pathologies, including diabetes, abnormal changes in retinal microvascular morphology precede a clinically detectable deterioration in visual function. Retinal imaging is therefore crucial to screen for at risk individuals with subtle subclinical anatomical change who may benefit from early treatment intervention.

1.5.1 Standard Fundus photography

Fundus photography has been the mainstay of DR imaging since 1968 when experts met at Airlie House in Virginia to establish a standard classification for the growing problem of DR (67). The classification required the use of seven standard field (30 degree field of view (FOV)) overlapping fundus images to visualise the retinal surface (Figure 1-4). Following some further modifications to the classification for the Early Treatment Diabetic Retinopathy Study (ETDRS), the resulting ETDRS grading has become the gold standard for fundus photograph DR assessment. A simplified version of the ETDRS grading, the International Clinical Disease Severity Score for diabetic retinopathy (68), was developed for more accessible clinical use, and derivatives are used for grading in UK retinopathy screening schemes (Table 1-1) (62, 69). The main features assessed include the presence or absence of microaneurysms, haemorrhage, soft and hard exudates, venous beading, intraretinal microvascular abnormality and neovascularisation, and the location of lesions relative to the macula.

Table 1-1 Approximate equivalence of currently used classification systems for UK diabetic retinopathy screening services and ETDRS gradings. Table derived from Royal College of Ophthalmologists Diabetic Retinopathy Guidelines 2012 (70).

ETDRS		National Screening Committee (England and Wales)	Scottish Diabetic Retinopathy Grading (SDRG) Scheme (Scotland)
10	None	R0 None	R0 None
20	Microaneurysms only	R1 Background	R1 Mild background
35	Mild NPDR		
43	Moderate NPDR	R2 Preproliferative	
47	Moderately severe NPDR		R2 Moderate observable DR
53	Severe NPDR		
61	Mild PDR	R3 Proliferative	R4 PDR
65	Moderate PDR		
71,75	High Risk PDR		
81,85	Advanced PDR		

For DR screening, one-, two- or three-field digital fundus imaging is typically employed rather than the more time consuming seven-field imaging (Figure 1-5) (71). A standard fundus camera will visualise a FOV of 30 degrees requiring x2.5 magnification of the retinal surface, though wide-angle cameras are available which can image a FOV of between 45 and 140 degrees, requiring less magnification (58). Non-mydratic photography is favoured, where possible, to minimise the risks associated with pharmacological pupil dilatation (71). In the UK, people with diabetes are screened annually from age 12 years.

The use of machine learning for the automated detection of retinal structures and retinal biomarker analysis is increasingly being used, with clear economic benefits (72-74). Since 2012 in Scotland, all screening images are initially reviewed via an autograder to identify images which are gradable and have no evidence of DR by identifying the presence of microaneurysm and dot haemorrhages, in addition to quality checks (72, 73). Only images which do not meet these criteria are passed on for manual analysis by a trained Diabetic Retinopathy Screening (DRS) grader. In 2018 just over half of 182,000 screening images were

identified to have met this criteria by the autograder (74). Assessment of other retinal characteristics including vessel calibre, tortuosity and fractal dimensions (see Chapter 1.6) can also be carried out using automated machine learning, and is an area of growing interest (75).

Standard fundus photography has the advantage of being a well validated screening tool in retinopathy assessment, which is quick to perform and relatively comfortable for those being imaged. As only 2D images are generated, assessment of macular oedema within the retinal layers is limited to the use of surrogate retinal markers, as 3D visualisation of the retinal layers is not possible. Image analysis still requires highly trained graders and can be a labour intensive process, however the development of automated analysis systems may reduce the workload involved in this process (72).

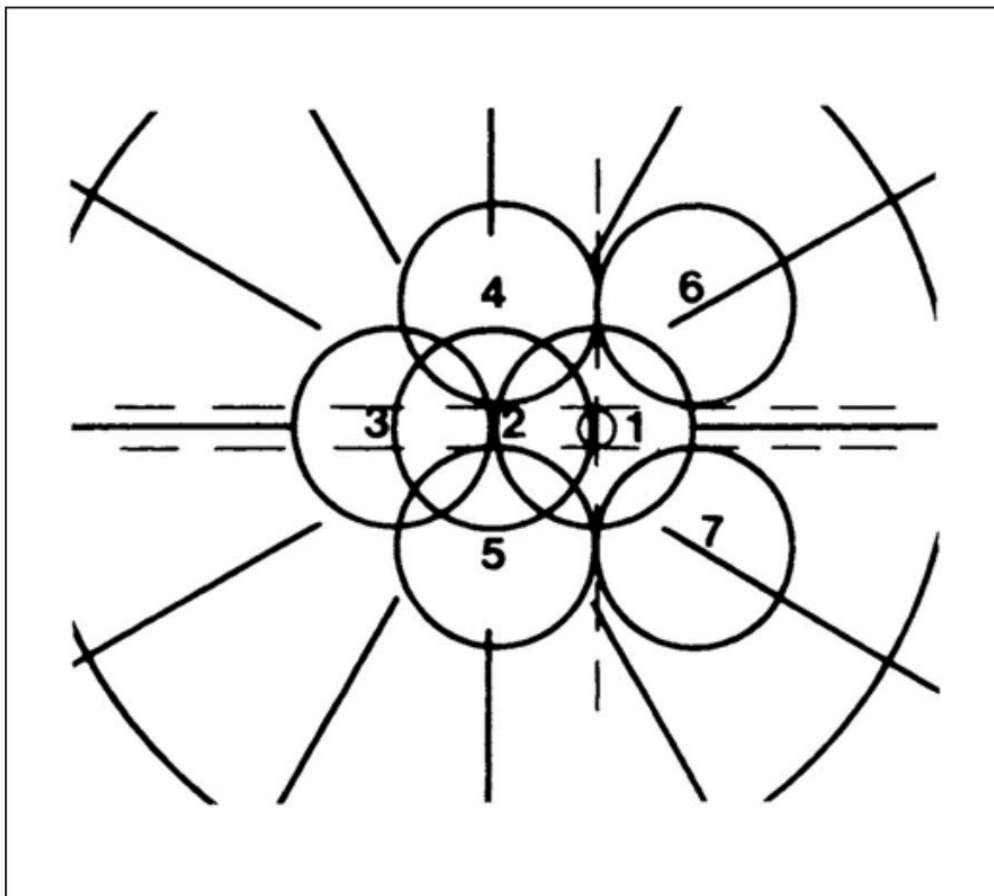


Figure 1-4 Standard seven field fundus imaging: Seven standard fields of the modified Airlie House classification of diabetic retinopathy. In this right eye, field 1 is centred on the optic nerve. field 2 is centred on the macula and field 3 is temporal to the macula. Image from Solomon 2019.



Figure 1-5 Example one field macula centred fundus photograph used in diabetic retinopathy screening showing 45 degree field of view. Image acquired as part of study

1.5.1 Ultra-Widefield Scanning Laser Ophthalmoscopy

Ultra-Widefield Scanning Laser Ophthalmoscopy (UWF-SLO) is a non-invasive imaging modality which captures 2D fundus images, similar to standard fundus photography, but visualises a much wider field of view with a single image (Figure 1-6). The term 'ultra-widefield' has been used to describe imaging which covers up to 80% of the retina (76). The process involves scanning the retina, point by point, using a focussed laser light beam. The reflected light is captured through a small aperture to provide a sharp high contrast image. By also using an ellipsoidal mirror to reflect the light, an ultra-wide image field of up to 200 degrees can be captured without the need for mydriasis.

UWF-SLO has been shown to be comparable to mydriatic seven standard field fundus photography in detecting DR in multiple studies (77-81), even without the use of pupil dilation (77, 78, 80), plus image acquisition can be done in less than half the time (80). The main problems with UWF-SLO are image artefact, including obstruction of part of the image due to eyelashes (78), or peripheral distortion due to the spherical nature of the eye (76). Despite this, the frequency of ungradable images has been shown to be reduced by up to 81% in over 8000 participants imaged with UWF-SLO compared to non-mydriatic multifield fundus photography (82). Comparisons of ungradable images with UWF-SLO versus mydriatic fundus photography is more variable, but UWF-SLO generally performs better (83). The ability of UWF-SLO to assess a larger retinal area in a shorter time offers potential for future widespread use in retinal screening programmes (82, 84).

(A)



(B)

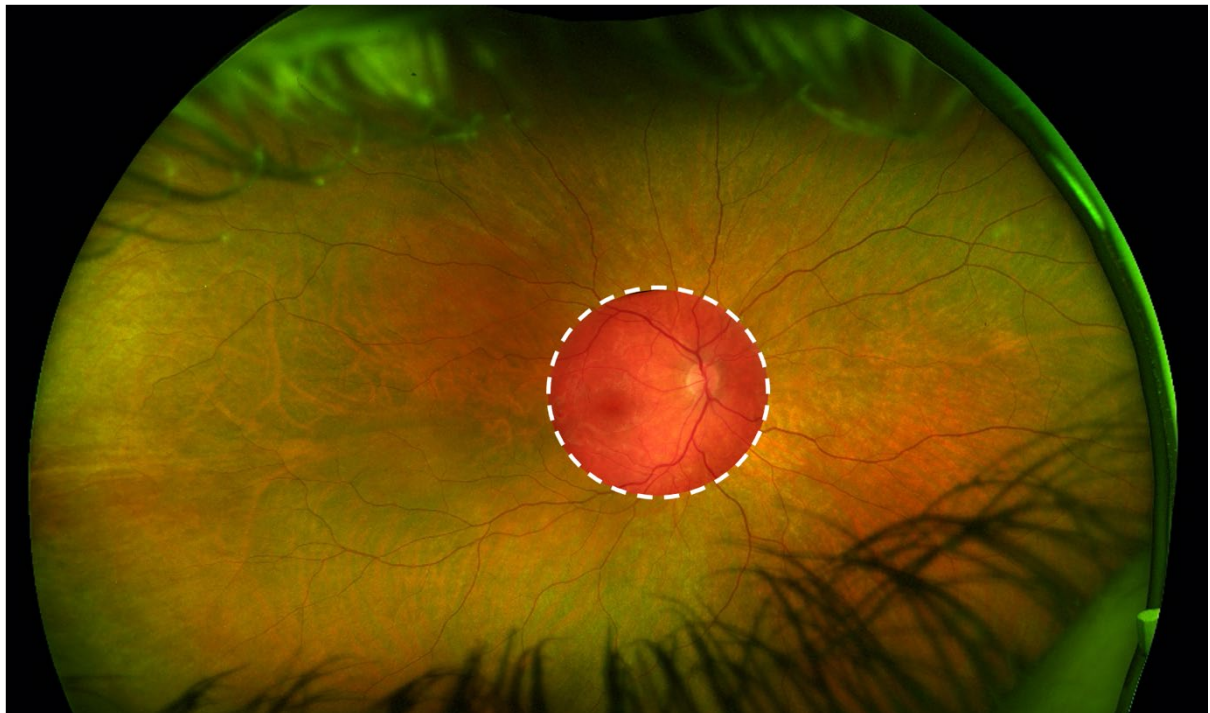


Figure 1-6 (A) Example Ultra-Widefield Scanning Laser Ophthalmoscopy image acquired as part of this study. (B) Same UWF-SLO image with superimposed fundus photograph from same participant (also acquired as part of study) highlighting additional field of view obtained in UWF-SLO compared to one field fundus photography (area contained within dashed white circle).

1.5.2 Optical Coherence Tomography

OCT is a non-invasive scan that provides high resolution cross-sectional imaging of the retina (Figure 1-7). The process is similar to that used in ultrasound, but rather than using the reflected echoes of pulsed sound waves, it detects the interference of reflected light sources using low coherence interferometry. Interferometry measures small displacements in interference patterns between two light sources, by processing differences in the light reflected. Scanning is repeated at multiple points along the retinal surface to form a one-dimensional line-profile of the tissue below. A-scans formed along the same vertical or horizontal axes are combined to create 2D cross-sectional 'B-scans', which can be used to build frontal 'C-scans' or "En-face" images (Figure 1-8). Together this builds a 3D picture of the separate retinal layers and underlying choroid layer. Advances in the light sources used and higher speeds of image acquisition using Spectral Domain OCT has allowed high quality images to be obtained due to reduced motion artefact and enhanced depth imaging. It has become a vital tool for the assessment of macular oedema in individuals with diabetes.

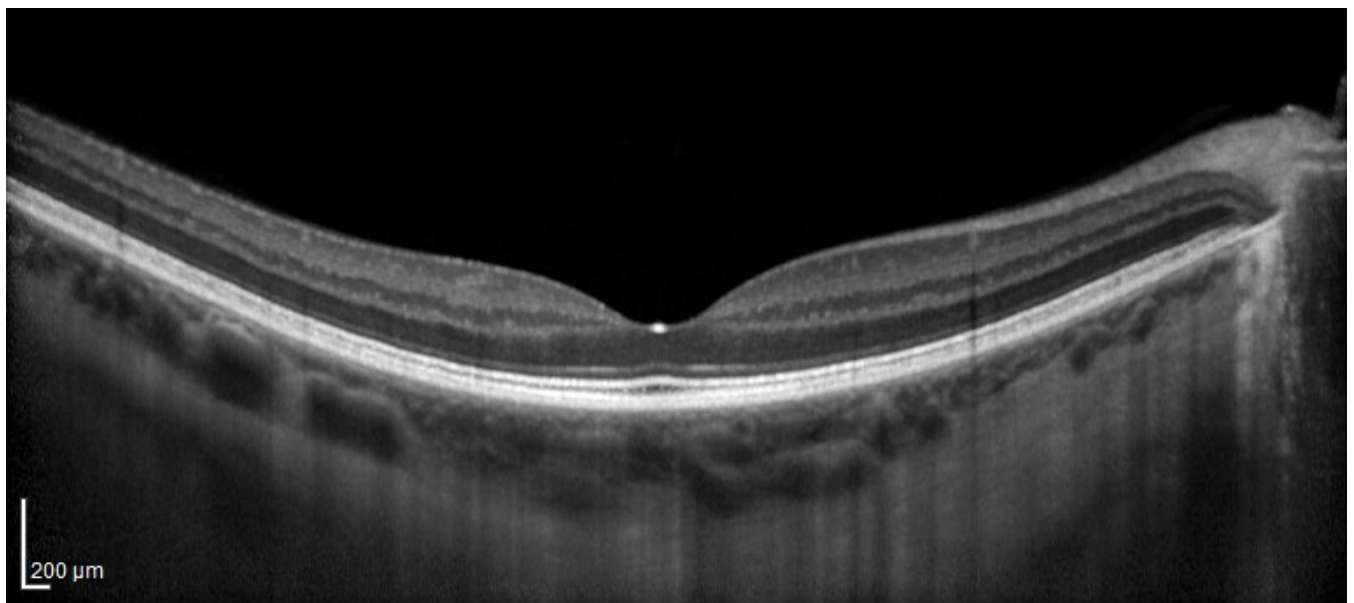


Figure 1-7 Example Optical Coherence Tomography. Image acquired as part of this study

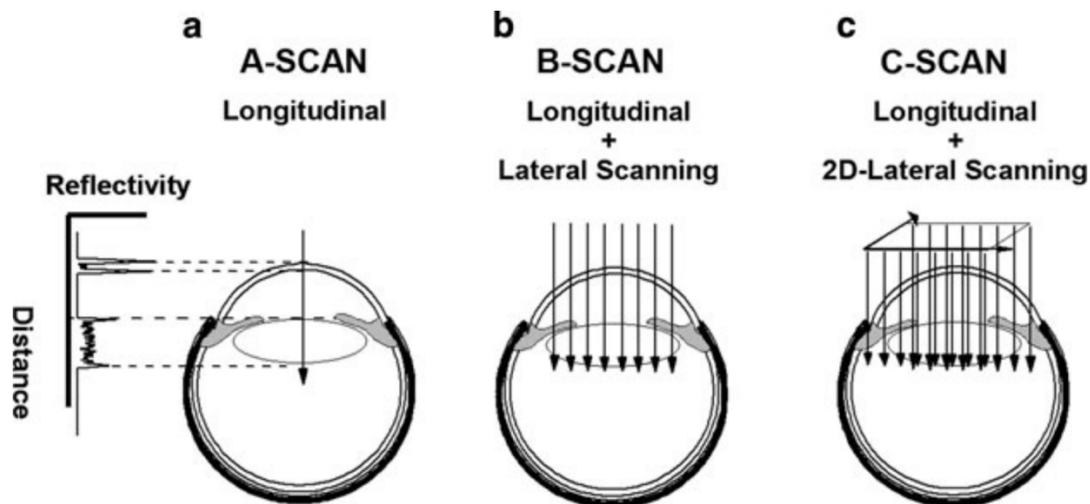


Figure 1-8 Composition of Optical Coherence Tomography images from A-scans, B-scans and C-scans. Image from Subhash et al Biomedical optical imaging technologies 2013 (85)

1.5.3 Fluorescein Angiography

Fluorescein angiography (FA) is the current gold standard imaging modality for DR assessment. It involves the injection of fluorescein dye to enable visualisation of the retinal vessels more directly. In addition to highlighting pathologies such as microaneurysms or neovascularisation, FA has the advantage of providing information regarding the integrity of the blood retinal barrier, represented by dye leakage. However, it is an invasive procedure where image capture is time sensitive following dye injection, making it difficult to image both eyes with one procedure. It also has the potential for severe side effects including anaphylaxis and nephrotoxicity, and is therefore not suitable for retinopathy assessment in many at risk diabetes groups including those with severe diabetic nephropathy or pregnant women.

1.5.4 Optical Coherence Tomography Angiography

Optical coherence tomography angiography (OCTA) is an emerging imaging technique that identifies vascular flow by detecting light reflected from the surface of moving red blood cells in high-speed OCT images. This generates high-resolution angiographic images similar to FA, but is non-invasive and does not require dye to be injected. This is particularly advantageous in patients who are not suitable for FA due to nephrotoxicity risks. OCTA can also be used to

segment deeper vascular layers for analysis, unlike FA which focusses on the superficial vascular layers. OCTA is not able to detect leaking red blood cells depicting a breakdown in the blood retinal barrier as in FA, however this can be advantageous for later follow up as surrounding vessels can be visualised, while in FA they may be obscured due to dye leak. Image acquisition is rapid, usually taking a few seconds, and repeated follow up scans can be obtained easily and safely. However, images are vulnerable to motion artefact in participants who are not able to maintain good fixation during the scan, which can limit its use in those with significant visual impairment due to macular pathology (86). It is currently used mainly in a research capacity, but there is growing interest in its role as an alternative to FA for clinical assessment (Figure 1-9).

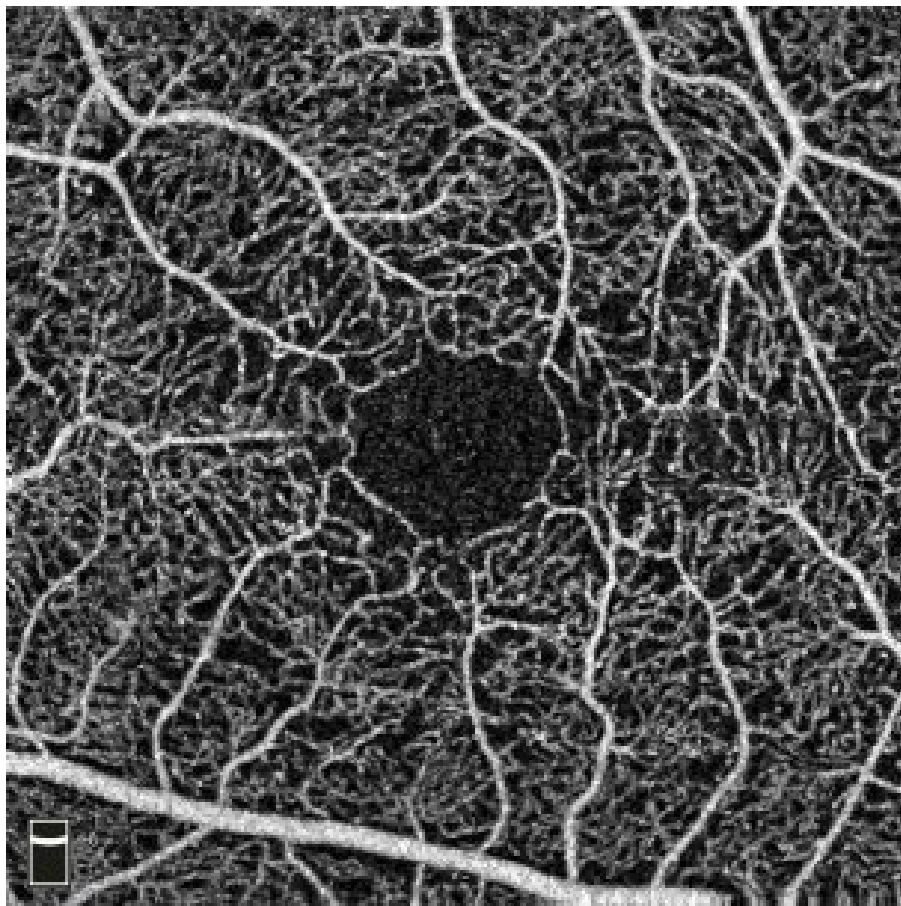


Figure 1-9 Example Optical Coherence Tomography Angiography acquired as part of this study – image depicts the superficial retinal layer showing vessels (in white) from the inner limiting membrane to the inner plexiform layer

1.5.5 Retinal Zones

The imaged retina is typically subdivided into zones to allow comparisons to be made between images for both clinical and research purposes. Standardised circular grid systems are used and are overlaid onto the image using well defined landmarks as a fixed centre point.

In fundus photography the optic disc forms the centre of one of the most commonly used grid systems (Figure 1-10). Concentric circles around the disc form the zones, with distances between the circles defined by the optic disc width. 'Zone A' forms the region immediately adjacent to the optic disc, formed by an annulus between 1 and 2 optic disc radii from the disc centre, 'Zone B' is formed outside that, measuring between 2 and 3 optic disc radii from the centre, 'Zone C' is between 2 and 5 optic disc radii of the centre and the 'Periphery' is any area outside of Zone C. Zone B is the region most frequently used for determining retinal measurements, as vessels on the immediate path from the optic disc centre within Zone A tend to be overlapping with frequent bifurcations, making uniform measurements difficult. Zone C, which incorporates Zone B but also a wider area of the retinal surface, is also commonly used, particularly for measurements where visualisation of longer segments of vessels may be needed. However, in images which are macula-centred rather than disc-centred, such as in DR screening, large portions of Zone C fall outside the imaged area, leading to limited analysis.

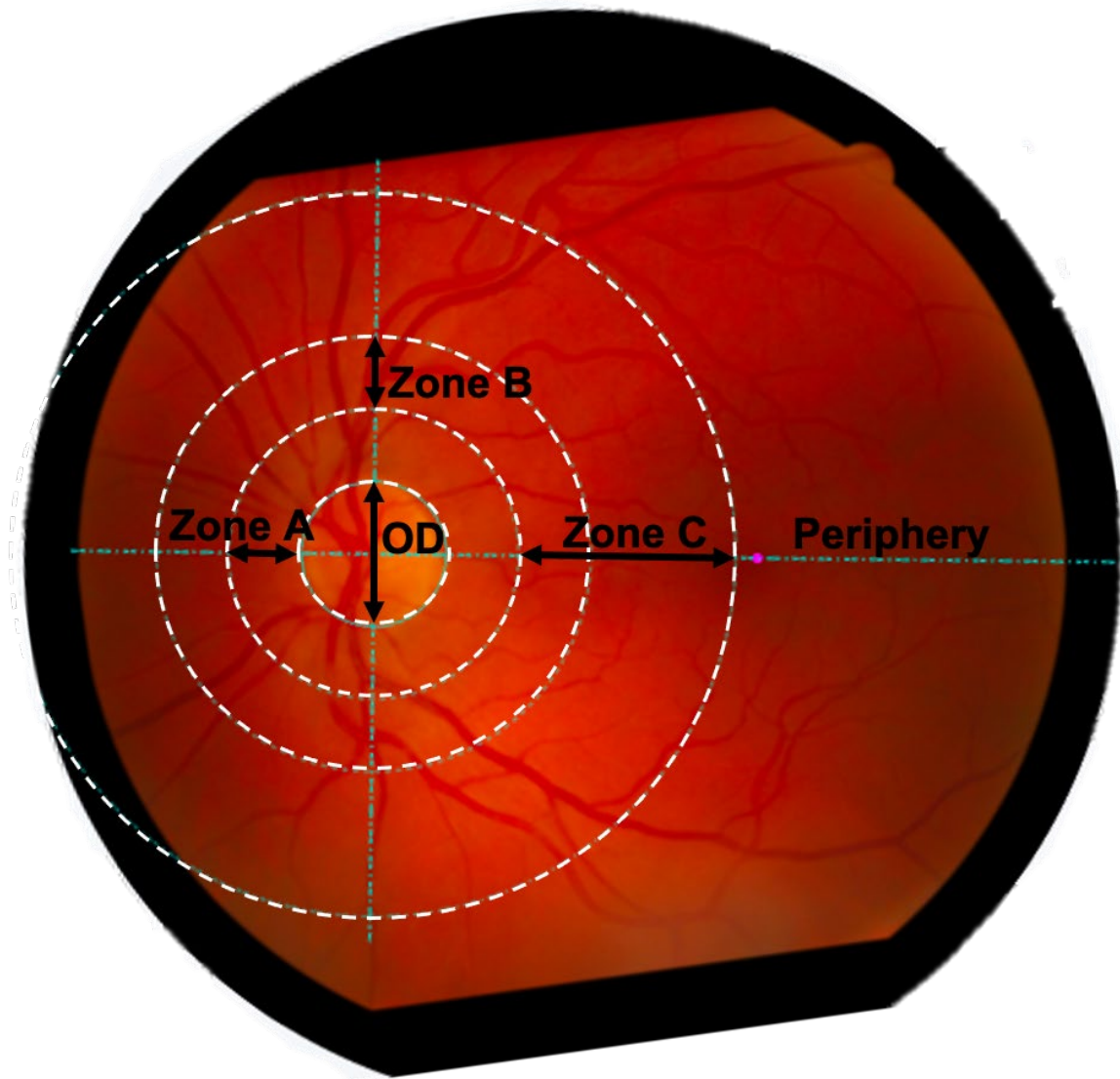


Figure 1-10 Retinal Grid with Image zones according to a reference system based on the optic disc (OD) centre, the optic disc radius, and the location of the fovea centre. Image taken as part of this study.

The ETDRS grid is another commonly used system for comparative retinal analysis (Figure 1-11). It is centred on the fovea and divides the retina into nine regions using three concentric rings: a central foveal ring with 1mm diameter, an inner macular ring (parafoveal) with 3mm diameter, and an outer macular ring (perifoveal) with 6mm diameter. The parafoveal and perifoveal rings are each further subdivided into four segments to form nasal, temporal, superior and inferior divisions. This system is frequently used in OCT to determine the thickness across different regions of the retina.

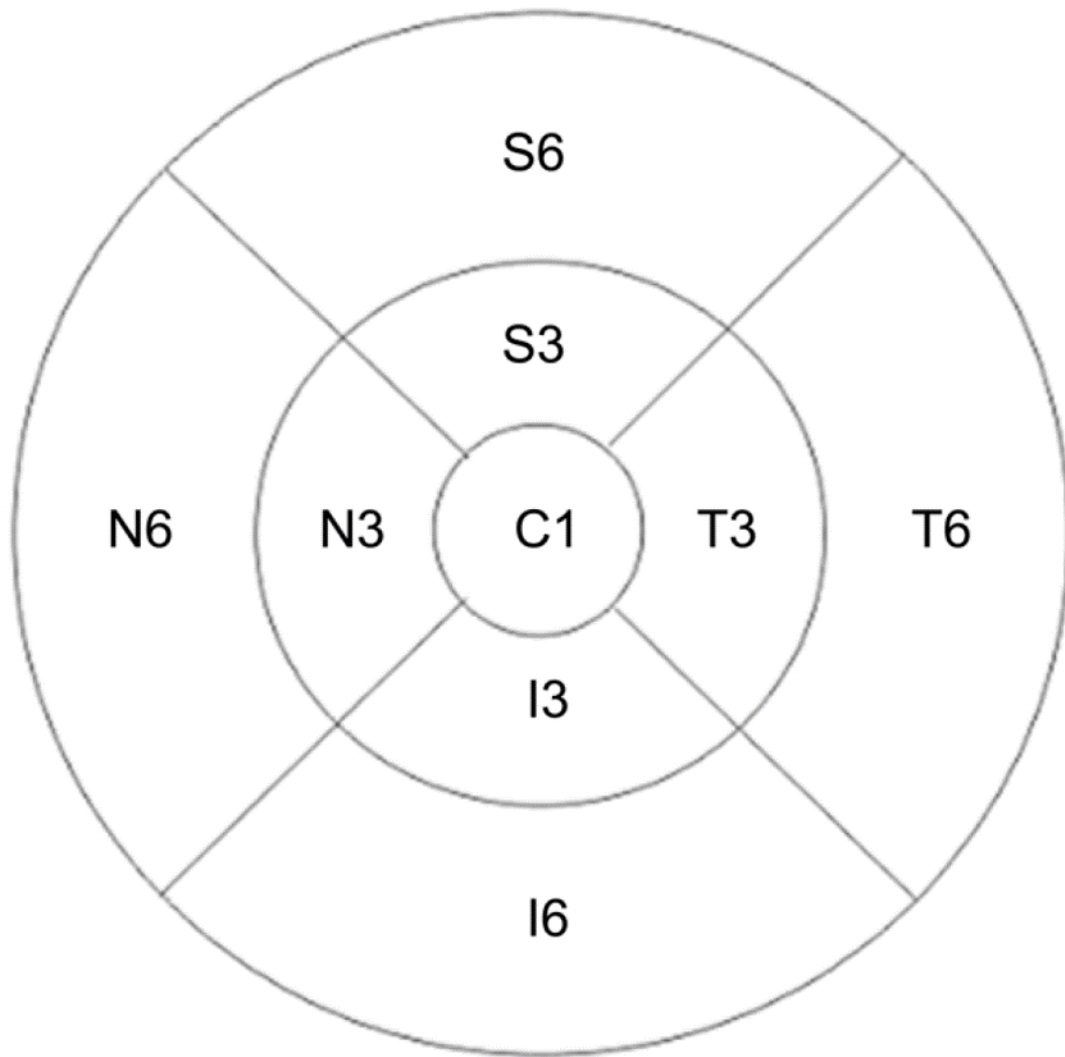


Figure 1-11 ETDRS grid Depicting the central foveal region (C1) and the superior (S), Temporal (T), Inferior (I) and Nasal (N) regions of the parafoveal (S3/T3/I3/N3) and Perifoveal (S6/T6/I6/N6) rings

1.6 Retinal Biomarkers

Retinal biomarkers are useful predictors for the development or presence of clinically significant retinopathy. In addition to the traditional pathological features of DR described above, newer areas of interest, including vessel density, vessel tortuosity, vessel calibre, fractal dimensions, FAZ characteristics, retinal thickness and choroidal thickness, are increasingly being developed for use in clinical assessment.

1.6.1 Vessel Density

Vessel density assesses the proportion of a defined retinal area occupied by vessels and is a useful way of quantifying microvascular changes from OCTA images. The standard approach calculates the vessel area density by creating a binary image of the area of interest from the original greyscale image to identify space occupied by vessels versus space that is not (Figure 1-12B) (87). From this, the percentage of pixel space occupied by vessels can be calculated. Larger wider vessels will therefore have more impact on the final density value than smaller narrower vessels. A second approach calculates the vessel length density, whereby large and small vessels are given the same weighting. For this, a skeletonised image is created (Figure 1-12C), uniformly outlining the central distribution of all vessels using a one pixel thick skeleton, regardless of their size (88). From this, the total length of all the skeleton vessels can be calculated as a proportion of the area of interest (88). This can be more useful in mapping changes in the distribution of vessels and areas of non-perfusion (88, 89).

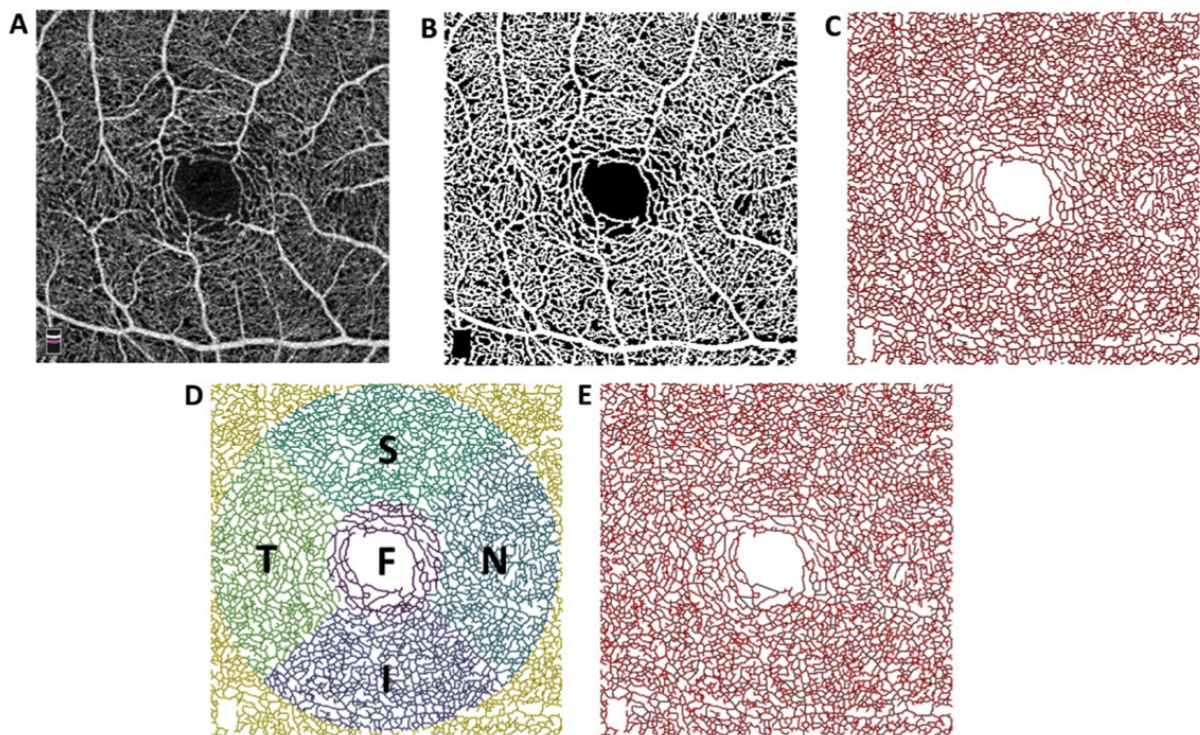


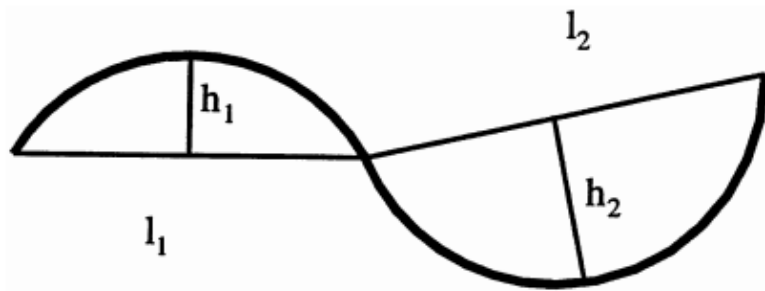
Figure 1-12 Example Binary and Skeleton OCTA images derived through work done by Ylenia Giarratano (Miguel Bernabeu research group) from OCTA images acquired in this study showing (A) Original superficial OCTA image. (B) Binary image. (C) Graph representation of the vascular network. (D) Regions of interest in the network: Foveal (F), Superior (S), Nasal (N), Inferior (I), Temporal (T). (E) Simplified Graph. (90).

1.6.2 Vessel Tortuosity

Tortuosity refers to the twisting nature and curvature of a line. Normal retinal vessels are straight with gentle curves while tortuous vessels are associated with retinal pathology (91). In its simplest terms, vessel tortuosity is essentially a quantification of how ‘wiggly’ the blood vessel is. For basic uniform curves, this can be achieved by considering the relationship between the length of a straight line joining two vessel points and the calculated length of the vessel arc between the same two points (Figure 1-13) (91). In reality, vessels form non uniform curves of variable width and length, making vessel tortuosity difficult to quantify. As such various methods have been described to achieve the best estimate for vessel tortuosity (75, 91). The favoured reported methods are simple and curvature tortuosity. Simple tortuosity is estimated as the actual path length of the vessel segment divided by the straight-line length. Curvature tortuosity is derived from the integral of the total squared curvature along the path of the vessel divided by the total length of the vessel arc. The latter takes into

account bowing and points of inflection (75, 91). These measures are limited in differentiating between a pathological tortuous retinal vessel typically displaying multiple twists and turns, and the normal smooth curved appearance of a healthy retinal vessel which may be due to the visualisation of the natural curvature of the globe on 2D imaging (92).

A



B



C

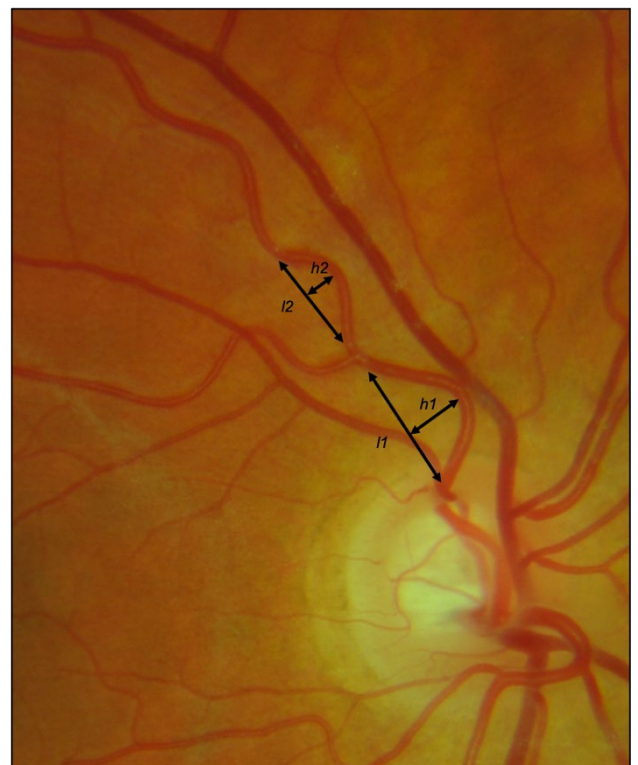


Figure 1-13 Illustration from Hart et al 1999 showing measurements used to calculate vessel tortuosity: This method involves the manual selection of points on fundus photographs that subdivide the tortuous vessel into a series of single arcs of varying chord length (L) and arrow height (H) from which the tortuosity can be quantified. (91). (B) Example of a tortuous vessel (indicated by black arrow) from fundus photograph taken as part of this study. (C) Magnified tortuous vessel from (B) displaying chord lengths (l) and arrow height (h) for tortuosity calculation

1.6.3 Vessel Calibre

Assessment of vessel calibre from retinal images is more accurately a measure of the internal vessel luminal width, as vessel walls tend to be poorly visualised on imaging. Three metrics, the central retinal artery equivalent (CRAE), the central retinal vein equivalent (CRVE) and the arteriovenous ratio (AVR), were developed to estimate vessel calibre through the work of Parr (93) and Hubbard (94). The CRAE and CRVE estimate the overall retinal arteriolar and venular calibre using averaged measurements from multiple vessels, with adjustments made to consider the impact of the vessel bifurcation pattern on the width of trunk vessels and their branches. As individual measurements, the CRAE and CRVE are vulnerable to distortion due to magnification effects in subjects with variable axial lengths. The AVR is a dimensionless ratio of CRAE versus CRVE which aims to minimise this problem. It can be flawed, in that changes of a similar magnitude in both CRAE and CRVE may not be detected. However, it is accepted that most pathologies preferentially affect arterioles over venules, making the AVR a useful tool in detecting changes in vessel calibre, particularly when information on the axial length for individual subjects is not available. Vessel measurements are usually taken from the six thickest arterioles or venules as they cross Zone B, based on modifications to the Parr-Hubbard calculation by Knudtson, who found that the number of vessels measured significantly impacts the AVR (95). Measurement of vessel calibre in Zone B, rather than Zone A, ensures that bifurcation of the source vessels has occurred. Variation in vessel calibre results from a number of biological factors, including advancing age, hypertension and diabetes (96). It can therefore be challenging to ascertain the impact of individual factors, particularly as many common determinants of vessel calibre have opposing effects (see Chapter 3.1.2.1 - typically vessel dilatation with diabetes and vessel narrowing with hypertension).

1.6.4 Fractal Dimensions

Fractal dimensions (FD) is a mathematical concept which characterises the complexity of a repeated pattern where the components (fractals) show self-similarity. In Euclidian geometry we think of shapes in whole integer dimensions, where a line would be one dimensional (length), a filled square two dimensional (length and height), and a filled cube 3 dimensional (length, height, and depth). However, this becomes more complex for irregular shapes where

the measurement of a dimension such as length can be affected by the scale of ruler used to measure it. This is illustrated by the coastal paradox where the measured length of a coastline increases as the size of the ruler used to measure it decreases (Figure 1-14). FD therefore attempts to quantify this by calculating a ratio comparing how the detail in a fractal pattern changes with the scale at which it is measured. Using this concept, an irregular 2D object can have a dimension which is not a full integer, but a fractional value between 1 and 2, while an irregular 3D object would have an FD of between 2 and 3.

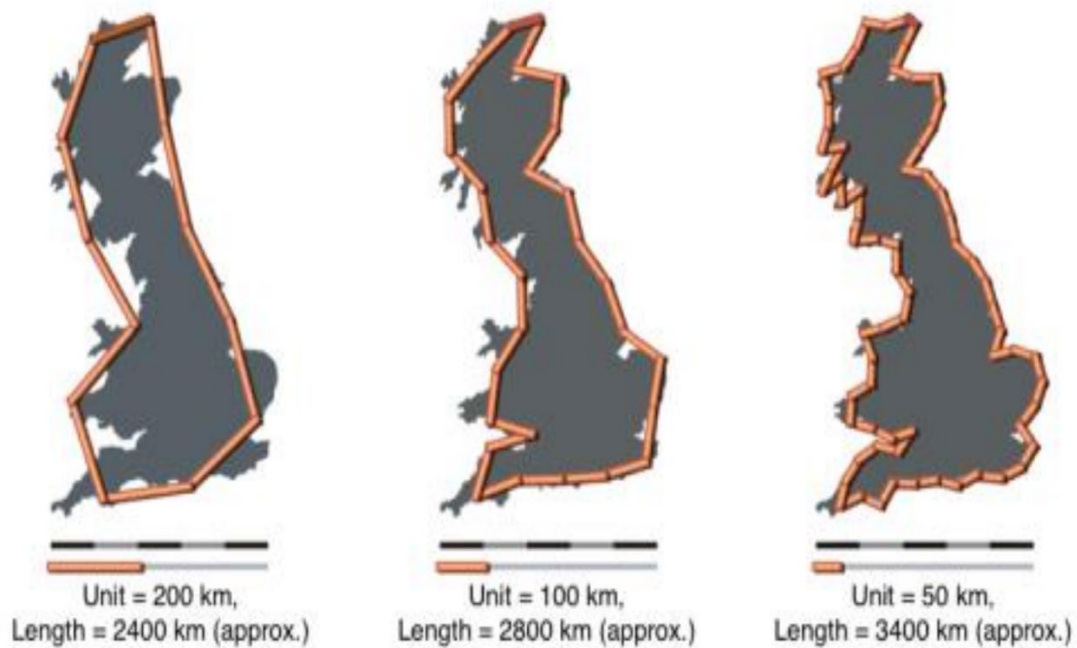


Figure 1-14 Coastal Paradox Illustration: The measured length of a coastline increases as the size of the ruler used to measure it decreases. Image from https://www.researchgate.net/publication/282183544_Spatio-Temporal_Analysis_of_Shoreline_Changes_along_Makran_Coast_Using_Remote_Sensing_and_Geographical_Information_System

In retinal terms, FD refers to the complexity of the retinal vascular network and vascular branching patterns. Lower FD values represent lower complexity of branching.

This is usually calculated from a skeletonised 2D retinal image using the box counting method, where a retinal image is segmented into smaller and smaller boxes for assessment and the presence or absence of vessels within these boxes is determined (97). An algorithm calculates the FD based on the changing number of boxes which are found to contain vessels as the scale of the assessment boxes changes (97).

1.6.5 Quantification of Foveal Avascular Zone Characteristics

As the region responsible for the clearest vision, quantifying changes to the fovea is important in assessing retinal pathology. Within the central fovea lies the FAZ (Figure 1-15). This region is typically free of capillaries in the normal retina to facilitate an unobstructed passage of light so the sharpest image can be obtained. Properties depicting the FAZ can be quantified for comparative analyses. To do this, the foveal region needs to be correctly identified. In normal physiology, the fovea can be characterised on OCT by a concave dip in the retina, the absence of the inner layers of the retina and a slight thickening of the ellipsoid zone (98), however it can become more challenging to identify in the presence of pathology where the expected appearance of the fovea may be altered. This process is usually automated, however manual corrections are important to ensure positioning is correct.

Once the central fovea is identified, the use of automated software can calculate the area and perimeter of the surrounding region of non-perfusion that will depict the FAZ. Where possible, analysis which involves consideration of the 3D nature of the capillary network is optimal, as bordering capillaries which are formed at a deeper retinal layers will not be visible on superficial 2D images (99).

FAZ shape has also been shown to be important in detecting pathology, with a circular or elliptical shape associated with healthy individuals, and acircularity seen in vascular pathology (99). This can be quantified using the acircularity index, defined as a ratio of the perimeter of the FAZ to the perimeter of a circle with equal area (100). The axis ratio can similarly quantify the FAZ shape by calculating the ratio of the widest and narrowest axis points (99). A perfectly circular FAZ will have an acircularity index and axis ratio of 1, with higher values indicating increased acircularity. Both measurements are dimensionless and therefore have the advantage of not requiring axial length measurements to adjust for magnification changes, similar to the use of AVR in vessel width assessment.

Accurately defining the FAZ borders, particularly in pathological states, can be challenging (see Chapter 4.2.3.1, figure 4-6) and adjacent regions are also often devoid of vasculature. As such FAZ measures which consider the broader central avascular region are proving useful.

The “adjacent area sum” measures capillary drop out surrounding the FAZ and has been shown to be a potentially useful alternative to assessment of FAZ area (101).

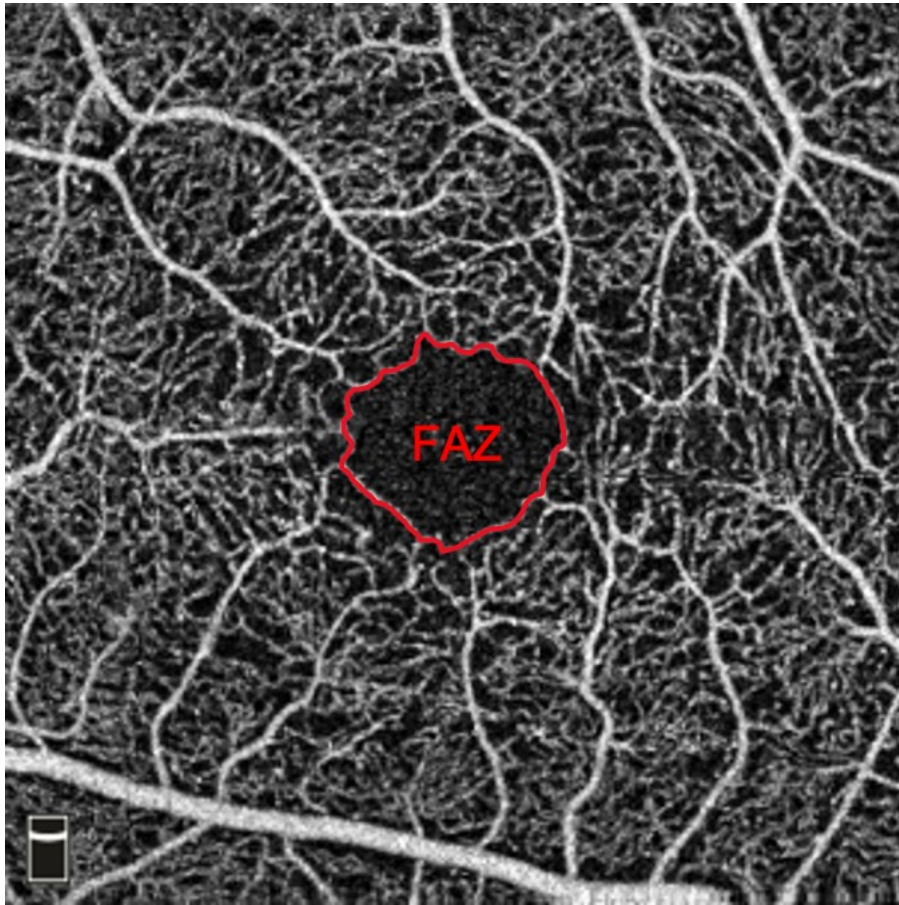


Figure 1-15 OCTA image from study (previously shown in Figure 1-9) now showing foveal avascular zone (FAZ) highlighted in red

1.6.6 Retinal and Choroidal Thickness

Retinal thickness refers to the distance between the Inner Limiting Membrane (ILM) and BM located immediately below the RPE (Figure 1-16). This can now be measured relatively easily on OCT using automated software which calculates the retinal thickness and volume across the nine sections of the ETDRS grid from multiple B-scans. The central retinal thickness, corresponding to the centre of the fovea, is the thinnest point in the normal retina (102). A summary value for the average macular volume across all nine sections of the ETDRS grid can also be obtained. Incorrect identification of the fovea centre due to pathology or poor fixation the person being imaged leads to inaccurate ETDRS grid placement and corresponding measurements (102). Manual checks are typically done to minimise this.

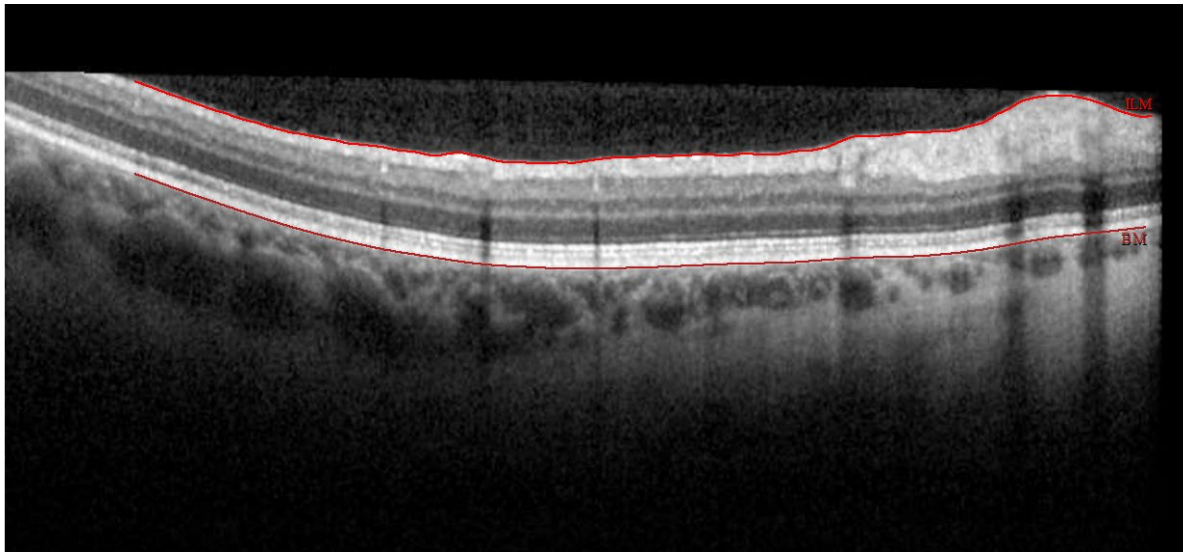


Figure 1-16 Example OCT image from this study showing the upper (ILM) and lower (BM) border for retinal thickness measurements

Developments in retinal imaging technology, particularly the widespread use of OCT with enhanced depth imaging capabilities, has enabled changes in choroid thickness to also be assessed. Unlike retinal thickness assessment, this process is typically not automated, instead relying on the user to identify the relevant borders for choroid thickness measurement. Measurement is taken perpendicularly from BM, located immediately below the RPE, to the choroid-sclera junction to calculate subfoveal choroid measurements (Figure 1-17), where the choroid is at its thickest (103), and nasal and temporal parafoveal measurements. Choroid layers are less well defined on OCT than retinal layers, therefore visualisation of the borders for choroid thickness measurement can be challenging (Figure 1-18). A study in Malay adults of 176 eyes found 60% had poor delineation of the choroid-sclera border, and a history of diabetes was associated with poor delineation (104). Despite this, inter- and intra-observer measurements have been shown to correlate well (105), with good correlation across devices in several studies (106).

While this study only considers choroidal thickness, newer measures are being developed which may provide a useful adjunct to the measurement of choroidal thickness alone. The choroidal vascularity index (CVI) has been developed over recent years as an alternative assessment of the choroid vasculature. It is defined as the ratio of vascular area to the total choroidal area, and is presented as a percentage. Unlike choroidal thickness measurements,

calculations are taken from a binarized image which may result in CVI being less prone to variations due to the challenging segmentation of choroidal borders required in choroidal thickness measurement (107). Lower CVI, corresponding to reduced choroidal vasculature and likely choroidal ischaemia, has been described in pathological states including age related macular degeneration and diabetes mellitus (107, 108).

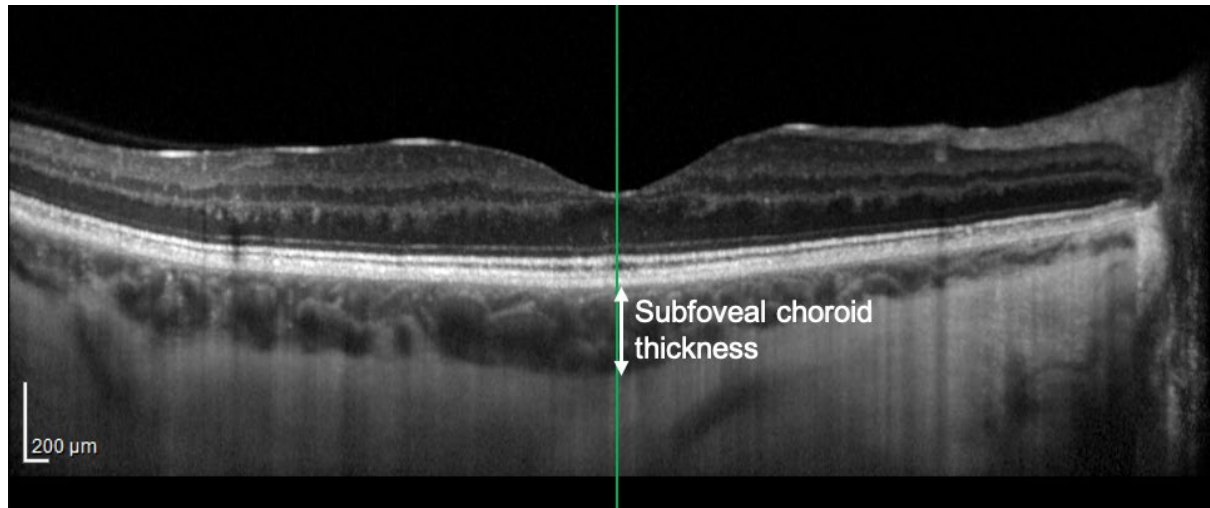


Figure 1-17 OCT image acquired from study showing subfoveal choroid thickness measurement

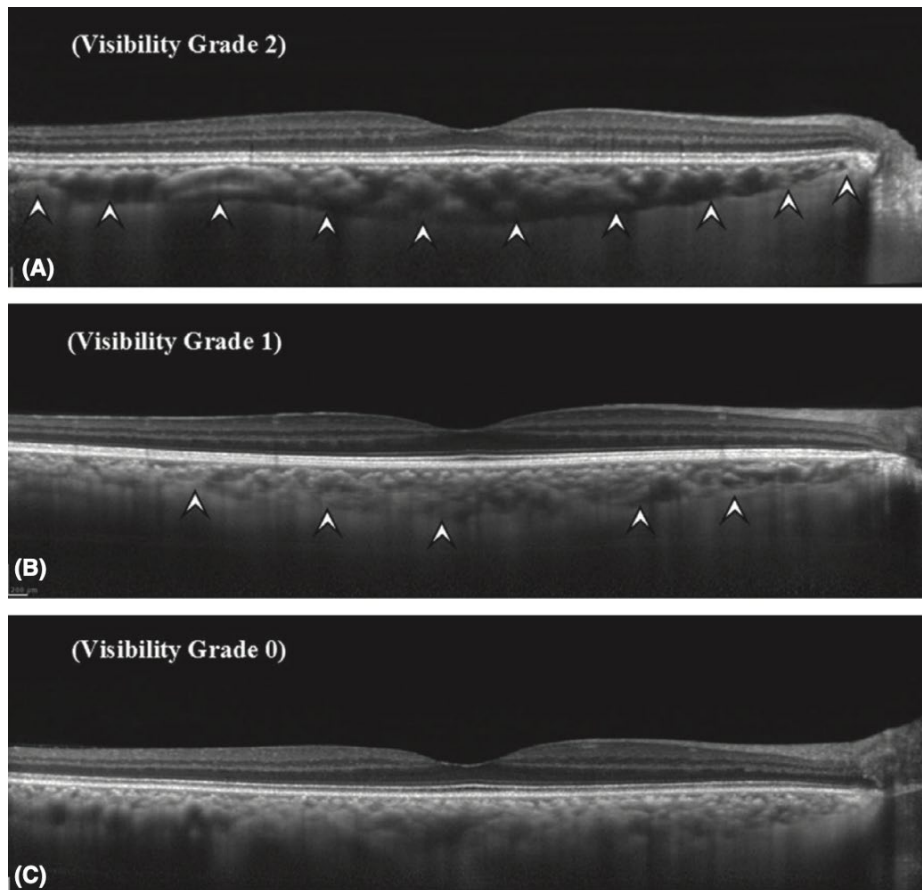


Figure 1-18 Image from Gupta et al 2016 showing variation in visibility of the choroid-scleral junction for choroid thickness measurement. Image (A) shows high visibility (Grade 2) depicting easily visible delineation versus Image (C) which show low visibility (Grade 0) depicting poor delineation (104).

1.7 Project Aims And Hypothesis

1.7.1 Study Questions

1. Are islet transplantation or CSII associated with early retinopathy worsening?
2. Do islet transplantation or CSII confer any long-term benefits for DR risk reduction over MDI therapy?
3. Are retinal biomarkers, assessed using fundus photography, UWF-SLO, OCT and OCTA, clinically useful tools for monitoring DR following the introduction of glucose lowering diabetes therapies?

1.7.2 Study Hypothesis

We hypothesise that rapid reduction of time in hyperglycaemia and hypoglycaemia post intervention with islet transplantation or CSII therapy will be associated with changes consistent with worsening DR in the short term (within 12-18 months post intervention) but reduced DR risk in the long term, when compared to those continued on treatment with MDI. We also propose that retinal biomarkers will be able to detect early retinopathy changes in individuals with diabetes following the introduction of these treatment interventions.

1.7.3 Project Aims

Aim 1: *Compare progression of DR in people with Type 1 diabetes following treatment with CSII and islet transplantation to those treated with MDI.*

The main focus will be on the first 12-36 months post CSII or islet transplantation treatment, particularly to establish if there is any EWDR following these therapies, using an MDI group as a comparator.

This will be completed using a retrospective analysis of graded retinopathy screening outcomes using retinal screening data from the national Scottish diabetes database and DRS.

Aim 2: *Assess quantitative changes in markers of DR in fundus photographs for individuals treated with CSII or islet transplantation using automated retinal detection software.*

The main focus will be on the first 12-36 months post CSII or islet transplantation treatment, assessing changes in vessel calibre, tortuosity and fractal dimensions over time, with the aim to establish if these markers are associated with DR severity or progression.

This will be completed using a retrospective analysis of information from the national Scottish diabetes database and screening photos obtained from the central DRS Service.

Aim 3: *Determine changes in DR markers using retinal imaging techniques following treatment with CSII or islet transplantation.*

The main focus will be on changes on OCTA and OCT imaging, particularly changes in vessel density, FAZ characteristics, retinal thickness and choroidal thickness in first 12 months post treatment.

This will be completed using prospectively collected data from recruited study participants.

Aim 4: *Determine effects of glycaemic control and variability on DR.*

This will be assessed across both retrospective and prospective studies using HbA1c, and CGM data where available.

2 Comparison of graded diabetic retinopathy screening outcomes in islet transplantation and continuous subcutaneous insulin infusion versus multiple daily injections

Please note that the sections of this chapter have been published in first author manuscripts.

Results Part A and Discussion Part A are part of a published first author original article by Reid *et al.* (109). Extracts from the Introduction and Methods are also published as part of the same publication (109), but were modified to include an additional study of islet transplantation participants which was carried out alongside the published study of CSII and MDI participants.

Extracts from the Introduction have also been published in a first author review by Reid *et al.* (43).

2.1 Introduction

The main aim of this study was to compare graded DR outcomes in people with type 1 diabetes following the introduction of CSII therapy or islet transplantation compared to people receiving continuing therapy with MDI. This involved examining data extracted from the Scottish Care Information Diabetes Database (SCI-Diabetes), Scottish DRS Programme and the Edmonton Islet Transplant programme.

2.1.1 The Scottish Care Information (SCI-) Diabetes database

The SCI-Diabetes database is an NHS funded integrated electronic patient record maintained by the Scottish Care Information Diabetes Collaboration based in Tayside. It was established as part of a Scottish Government initiative in 2002. It links clinical information for all registered patients with diabetes in Scotland from various electronic records within primary care, hospitals and pharmacies, providing access for all registered healthcare professionals who form part of the diabetes multidisciplinary team. Since 2006 this has included data from the Scottish DRS service, providing up to date information on retinal screening dates and outcomes. Access to laboratory data, including HbA1c, mode of current insulin therapy, prescribed medications, diabetes education history and demographic data are also collated.

2.1.2 Scottish Diabetic Retinal Screening Programme

All patients with diabetes are invited to participate in the national diabetic retinopathy screening programme. In Scotland, fundus photography is obtained every 12 months, or more frequently if necessary, and results graded according to the Scottish DRS Service Classification (69).

A macula centred image is taken for each eye by a trained screener and independently assessed by a trained grader (Figure 2-1). The grader assesses images for quality and provides a severity score for the level of retinopathy and maculopathy identified. Retinopathy severity is classed as follows: R0 - No DR, R1 - Mild background DR, R2 - Moderate observable DR, R3 - Referable DR, R4 - PDR. Currently, screening intervals are increased from every 12 months

to every 6 months for those with an R2 grading, with referral to ophthalmology services indicated for those graded R3 or R4 to confirm whether intervention is required.

Maculopathy severity is classed as follows: M0 – No maculopathy, M1 – Observable maculopathy, M2 – Referable maculopathy. Maculopathy typically refers to the presence or absence of macular oedema. As this cannot be fully assessed by a 2D retinal image, maculopathy in this context refers to whether surrogate markers for macular oedema such as haemorrhage or hard exudates, are present within the macular region. Ophthalmology referral is indicated for those with M2 grading, however 3D assessment, typically with OCT, is needed to confirm whether macular oedema is truly present. Large numbers of referrals are subsequently found to be false positives, with as few as 12-14% actually found to have macular oedema on follow up (110). Plans are in place to incorporate OCT into the Scottish retinopathy screening programme aiming to improve maculopathy assessment and reduce the numbers of false positive ophthalmology referrals.

Patients who require referral to ophthalmology for retinopathy or maculopathy are temporarily removed from the screening programme pending assessment and, where needed, treatment.

Fundus photographs obtained via screening, and the graded outcomes, are stored by the DRS Service in a central Scottish NHS database. Results can also be accessed locally via the SCI-Diabetes.

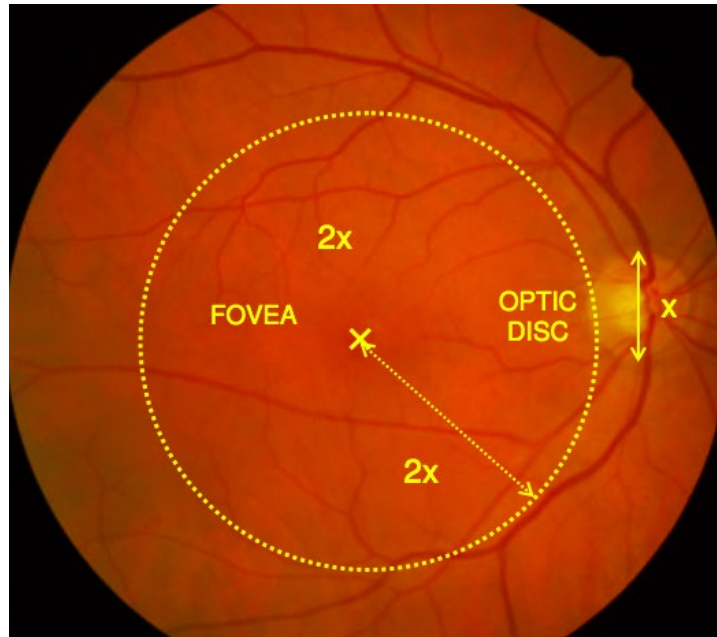


Figure 2-1 Single macular centred image visualising a region equivalent to at least twice optic discs widths from the fovea. Image from Scottish Diabetes Retinopathy Grading Scheme (69)

2.1.3 Retinal Evaluation at the Edmonton Islet Transplant Centre

The Islet Transplant department in Edmonton (Alberta, Canada) is the leading department in the world for islet transplantation, totalling over 300 islet transplants since the commencement of their program in the 1990s. They have paved the way for other islet transplant centres, and the protocol for islet transplantation and immunosuppression designed by the Edmonton team is used worldwide (50). Though there is no national diabetic retinopathy screening service established in Canada, retinal evaluation was routinely performed as part of the islet transplant preparation between 2000 and 2007 using seven field fundus photography taken as per the Airlie House protocol and independently graded by two blinded ophthalmologists using the ETDRS scheme.

2.1.4 CSII therapy and diabetic retinopathy

CSII therapy has been shown to reduce HbA1c compared to treatment with MDI (26) and was one of the interventions used in the DCCT to achieve intensive glycaemic control (12). As previously discussed, this group had reductions in risk of retinopathy long term, but higher

rates of DR in the first 12 months of treatment. Amongst both intensive and control groups, higher baseline HbA1c and larger reductions in HbA1c were risk factors for EWDR (7).

Early studies involving insulin pumps showed no improvement in DR following treatment with CSII despite reductions in HbA1c (111, 112). Deterioration in DR was also seen, particularly in patients with pre-proliferative changes prior to commencing on pump therapy. However, no control groups were used in these studies and patients were selected for CSII therapy due to poor glycaemic control on conventional treatment. As such it is unclear if retinopathy findings would have been the same in the absence of CSII.

More recent studies have shown stable retinopathy one year post CSII initiation, though small increases in central retinal thickness were observed compared to MDI controls (66). In adolescents, CSII is associated with lower rates of retinopathy than MDI controls (113).

Within NHS Lothian, people starting on CSII therapy are screened for DR before and three months after insulin pump initiation. There is no national consensus on additional retinal screening following CSII therapy at present.

2.1.5 Islet cell transplantation and diabetic retinopathy

Whole organ pancreas transplantation has been shown to stabilise retinopathy changes (114), with one study showing significant stabilisation or improvement in retinopathy post SPK transplantation in 90% of recipients, compared with 50% of controls. Effects were most beneficial for subjects with more severe retinopathy at baseline (114). There are relatively few long term clinical studies to identify if similar benefits are achieved with islet transplantation (Table 2-1).

An early report from the Edmonton group showed 4/47 subjects receiving islet transplants had a deterioration in eye disease requiring photocoagulation or vitrectomy within the first five months of transplant (115), though other small studies have shown stable disease in the early post-transplant period (116, 117). A meta-analysis from 2015 suggested that islet

transplantation may be more effective than medical therapy in reducing the progression of DR for people with longer durations of diabetes, though only three of the studies assessed included islet transplantation (118).

Venturini et al showed improved markers of DR changes following islet transplantation. They compared ten participants, one year after islet transplantation, with matched Type 1 diabetes controls on the islet transplant waiting list. They showed significant increase in blood flow velocities to the central retinal artery and central retinal vein on colour Doppler in transplant recipients, which was not seen in controls (119). Reduced retinal blood flow has been hypothesized as an early sign of retinopathy, though the clinical impact of this on recorded DR changes remains uncertain.

A group in Vancouver are the first to directly compare clinical retinopathy changes in islet transplant recipients with intensively treated medical controls (120, 121). Using a prospective crossover study, they compared DR progression in people receiving intensive insulin treatment on the islet transplant waiting list with the same people following receipt of their islet transplant. They reviewed a total of 45 subjects, with 29 crossing over to the treatment group post-transplant. Mean follow up time was 66 months in the islet group and 47 months in the intensive medical group. They showed DR progression was significantly more likely in the medical group than the islet transplant group, with no progression in 51 eyes from the islet transplant group, versus progression in 10 out of 82 eyes in the medical group. Participants with more advanced DR at baseline were more likely to progress to more advanced disease in the medical group.

Islet transplantation significantly reduces GV (122) and HbA1c (51) which may protect against retinopathy progression in the long term (6). The presence of c-peptide is also associated with reduced incidence of diabetes complications, including retinopathy (123). However possible adverse effects of abrupt tightening of glucose control on retinopathy progression remains a concern (7). At present, there is not currently any amended DR screening strategy for patients receiving islet transplants in Scotland.

Chapter 2: Comparison of graded retinopathy screening outcomes

Table 2-1 Summary of current evidence for effects of islet transplantation on retinopathy. HbA1c was significantly reduced post- transplant in all studies

Author and Year	Study Type	Number of ICT subjects included (and baseline DR grading)	Control Group	Follow Up Post transplant	Main Findings	Retinopathy Outcomes Post Islet Transplant
Short-term follow up						
Lee <i>et al</i> 2005 (117)	Cohort	8 included (3 no DR, 2 mild DR, 1 minimal NPDR, 2 PDR)	None	1-2 years	No progression in retinopathy compared to pre transplant baseline	Stable
Ryan <i>et al</i> 2005 (115)	Cohort	65 included (30 PDR, 18 NPDR) 47/65 completed procedure (defined as insulin independence for >4 weeks) and were assessed at follow up	None	5 years (retinal outcomes only reported for 5 months post-transplant)	Assessment within early post-transplant period showing progression in 4/47 patients, who required photocoagulation or vitrectomy within 5 months of transplant	Worsening
Venturini <i>et al</i> 2006 (119)	Prospective Case Control	10 included (2 no DR, 5 mild NPDR, 3 moderate NPDR)	10 medically treated subjects on islet transplant waiting list 3 no DR, 5 mild NPDR, 2 moderate NPDR)	1 year	Significantly increased retinal artery and vein blood flow on colour doppler in transplanted patients versus controls	Improved
Long-term follow up						
Thompson <i>et al</i> 2008 (120) and Thompson <i>et al</i> 2011 (124)	Prospective Crossover Cohort	27 included (51 eyes - 8 mild NPDR, 12 moderate DR, 3 severe NPDR, 28 PNPDR)	44 medically treated subjects on islet transplant waiting list - 27/44 later transplanted, 17/44 not transplanted (31 eyes – 8 mild NPDR, 7 moderate NPDR, 3	5.5 years (66 months)	Significantly reduced DR progression post islet transplant (0/51 eyes) versus controls (10/82 eyes), $p<0.01$. DR progression (controls only) highest in those with more severe DR baseline	Improved

Chapter 2: Comparison of graded retinopathy screening outcomes

			severe NPDR, 13 PDR)			
Tekin <i>et al</i> 2016 (116)	Cohort	9 included, 4/9 completed follow up (2 no PDR, 2 PDR) 5/9 withdrew early in study	None	7-8 years	No progression in retinopathy in 4 patients who completed study compared to pre transplant baseline	Stable

2.2 Methods

2.2.1 Study design

A retrospective cohort study was performed primarily at three diabetes centres within NHS Lothian in Edinburgh, Scotland using routine clinical data and diabetes retinal screening data. Data was extracted using records from the DRS and SCI-diabetes databases. Further data for islet transplanted participants was collected via collaboration with the world's leading islet transplantation centre in Edmonton. The primary outcome being assessed was a deterioration in DR grading during the follow up period from the baseline grading. The study protocol was approved by the Caldicott Guardian, the Scottish Diabetes Research Network, the DRS Collaborative Coordinator and Lead Clinician, and the Lothian Research Ethics Committee.

2.2.2 Patient groups

Three patient groups were assessed:

1. CSII intervention group
2. Islet transplantation intervention group
3. MDI control group

2.2.2.1 CSII Intervention Group

All participants were recruited from NHS Lothian. Diabetes records were reviewed for all participants with type 1 diabetes who were commenced on CSII therapy between 1st January 2013 and 1st December 2016 (n=293). Retinal data collected during annual retinopathy screening for these participants were reviewed from 1st January 2011 to 1st December 2018. Study entry was taken as the date of commencing CSII. Participants were identified using SCI-Diabetes, searching for all patients with Type 1 diabetes who were documented to be on CSII therapy in NHS Lothian. CSII start dates were verified using clinical records on SCI-Diabetes. The decision to commence CSII was not randomised, but instead based on clinical indication. Indications for commencing CSII therapy include failure to achieve target HbA1c and recurrent

hypoglycaemia despite optimising MDI therapy (25). It was recognised that the lack of randomisation could result in allocation bias in CSII participants, for example there was a risk that CSII participants may have had higher baseline HbA1c levels than MDI counterparts due to failure to achieve target HbA1c levels, or lower baseline HbA1c levels due to debilitating hypoglycaemia, either of which may have prompted their clinically indicated change in treatment from MDI. Attempts to limit this bias is considered further in the statistical analysis and discussion.

2.2.2.2 Islet transplant intervention group

Due to relatively small numbers of eligible participants at the Scottish Islet Transplant Centre in Edinburgh, participants were also assessed from the Edmonton islet transplant centre in Canada who collaborate with the Edinburgh centre.

All those who had received an islet transplant in Edinburgh since the Scottish Islet Transplant programme began in 2009 were reviewed for study inclusion. All those who had received an islet transplant in Edmonton between 2000 and 2007, when retinopathy data was being routinely collected as part of the Edmonton islet transplant programme, were also reviewed for inclusion. Study entry was taken as the date of first islet transplant. Eligible participants were identified using islet transplant records from the Scottish or Edmonton Islet Transplant Teams. Retinopathy data collection differed between the two sites. This is discussed further in Chapter 2.2.3.1.

2.2.2.3 MDI control group

All patients were recruited from NHS Lothian. When selecting an appropriate control group, consideration was made to the criteria for commencing CSII therapy within NHS Lothian, in order to find a subject group who would potentially be eligible for CSII but who had continued on MDI management.

Within NHS Lothian, people are recommended to have completed a structured diabetes education course on carbohydrate counting prior to commencing on insulin pump therapy.

This is usually in the form of a five day course known as DAFNE (Dose Adjustment for Normal Eating) and ensures patients have a good understanding of how to self-manage their diabetes, and are using an intensive basal bolus insulin regimen. As such, the MDI control group was selected from patients who had completed the DAFNE course, to ensure they had completed similar levels of diabetes education to CSII participants, but who had not proceeded to CSII therapy after the course.

DAFNE education leads to reductions in HbA1c (125). To minimise the effect of the DAFNE course on follow up HbA1c and retinopathy data, the average lag time between completing a DAFNE course and commencing on an insulin pump was calculated from data recorded on SCI-Diabetes. This was found to be 945 days. Study entry was therefore calculated using the date of DAFNE education plus 945 days. To attempt to match the control group study entry dates with the insulin pump study entry dates, all patients who were documented to have completed DAFNE between 1st January 2010 and 1st January 2015 were assessed, and those with a calculated study entry period between 1st January 2013 and 1st June 2017 were included in the study analysis. Those who had commenced CSII therapy following DAFNE were excluded from the control group.

2.2.2.4 Study Inclusions and Exclusions

People were included if they were aged >16 years, had documented type 1 diabetes, defined as a clinical diagnosis of type 1 diabetes with no evidence in the historical record of more than 6 months between diagnosis and insulin requirement, and no history of use of oral hypoglycaemic drug treatment other than adjuvant metformin. All participants were on insulin therapy at study entry and were participating in the Scottish DRS programme or Edmonton islet retinal imaging programme during the study period.

People were excluded if the date of commencing CSII therapy, islet transplantation or completion of diabetes education could not be verified, if baseline or follow up retinal images had not been taken, or those taken were deemed ungradable for both eyes, if they had been suspended from the retinal screening programme for assessment and potential treatment at

ophthalmology clinics, or if they had the maximum severity retinopathy grading (R4) at baseline and could not therefore 'progress' to higher DR severity grade.

2.2.3 Data Collection

2.2.3.1 Assessment of Retinopathy Grading

For Scottish participants (CSII intervention group, MDI control group, Edinburgh Islet Transplant intervention Group), retinopathy assessment was performed as part of the Scottish Diabetes Retinal Screening Programme and results extracted from the DRS Service. A single macula centred photograph was taken for each eye every 6 to 12 months and retinopathy grading assessed using the Scottish Diabetic Retinopathy Grading (SDRG) Scheme (69). Each image was given a severity score for retinopathy (R0 - R4) and maculopathy (M0 - M2). Statistical analysis focused on retinopathy outcomes rather than maculopathy outcomes, due to the high frequency of false positive referrals for suspected maculopathy from diabetes screening.

Where retinopathy gradings were available for both eyes, the more severe grading was used as the baseline grading.

The DR screening assessment immediately prior to the study entry date was taken as the baseline grading (median 71 (IQR 18 - 243) days) prior to study entry date).

For the Edmonton Islet transplant intervention group, retinopathy assessment was performed prospectively as part of their islet transplant data collection. Images were taken at baseline, 6 months, 1 year and 3 years post-transplant. Seven field fundus photography was performed at each retinal assessment and images graded using the Early Treatment Diabetic Retinopathy Study (ETDRS) grading. This was converted to an equivalent SDRGS grading as per Table 2-2.

Retinopathy progression was defined as a minimum one grade worsening in either eye from the baseline grading, using SDRGS gradings.

Chapter 2: Comparison of graded retinopathy screening outcomes

Table 2-2 Equivalent ETDRS to SDRGS retinal outcomes used for conversion of Edmonton islet transplant participants retinal results to equivalent retinal outcomes as Edinburgh participants

ETDRS Grading	Severity	SDRGS Grading
10	DR absent	R0
20	Microaneurysms only	R1
35 A-F	Mild NPDR	
43 A-B	Moderate NPDR	R2
47 A-D	Moderately severe NPDR	
53 A-E	Severe NPDR	R3
60	Treated PDR	R4
61	Mild PDR	
65 A-B	Moderate PDR	
71-75	High Risk PDR	
80	Advanced PDR	
90	Unable to grade	R6

2.2.3.2 Assessment of glycaemic control by HbA1c

For Scottish participants, HbA1c data were extracted from the SCI-diabetes database and timings of baseline and follow up HbA1c values relative to the time of study entry were variable. The HbA1c value immediately prior to the study entry date was taken as the baseline HbA1c (median 75 (IQR 27 - 172) days prior to study entry date). Subsequent follow up HbA1c readings for each individual were identified for each subsequent six-month period where available. Mean study HbA1c over three year follow up was estimated by calculating the mean HbA1c from the six-monthly values for each individual. Where multiple HbA1c readings had been taken within a six-month period, the HbA1c value closest to, but not exceeding, the six-month time point was used as the HbA1c value for that interval. The HbA1c at approximately one year post study entry was also recorded, with a range from 8 to 20 months from study entry accepted as the one-year HbA1c value in cases where 12-month values were not available. Study exit HbA1c was recorded as the last available HbA1c value recorded after 20 months from study entry. Change in HbA1c at one year and study exit was calculated for each individual by subtracting the baseline HbA1c from either the one-year or study exit HbA1c.

For Edmonton Islet transplant participants, HbA1c data was collected prospectively at baseline and one year post-transplant. Change in HbA1c at one year was calculated for each individual by subtracting the baseline HbA1c from the one-year HbA1c.

2.2.3.3 Assessment of other baseline variables

For the CSII and MDI groups, baseline data was extracted for the following variables from SCI-Diabetes: gender, weight, BMI, systolic BP, diastolic BP, cholesterol, creatinine, social deprivation index (assessed using quintile of Scottish Index of Multiple Deprivation (SIMD) score (126)), smoking status, age at diabetes diagnosis, diabetes duration, age at study entry.

Fewer baseline variables were available for the islet transplant group as participants were recruited from a larger geographical area covering multiple health boards and across the two sites. The following variables were assessed: gender, weight, BMI, diabetes duration, age at study entry.

2.2.4 Statistical Analyses

Baseline variables including age, sex, diabetes duration, baseline HbA1c, SIMD and diastolic BP were summarized as median with interquartile range (IQR) for continuous data and as percentages for categorical data. In the CSII versus MDI groups, baseline demographic and metabolic data were compared using the unpaired t-test for continuous normally distributed data and Chi-squared or Fisher's exact test for categorical data. Kaplan-Meier plots were used to assess time to an event corresponding to retinopathy progression or exit from the study for another reason (death, emigration, end of study period) in CSII and MDI groups, with calculation of statistical differences between groups using the log-rank test. As expected in a non randomised study, several baseline variables were significantly different between CSII and MDI groups. Attempts were made to match the groups retrospectively using propensity score matching, however this led to very reduced sample sizes which were not suitable for analysis. Instead univariate and multivariate Cox proportional hazards were performed using the following co-variables: CSII or MDI treatment Group, age, gender, diabetes duration,

baseline HbA1c, systolic BP, diastolic BP, cholesterol, creatinine, SIMD, smoking status and baseline DR grading.

To assess the impact of baseline, mean study, one year and study exit HbA1c in CSII and MDI groups, participants were stratified using the following HbA1c values, ($<58\text{mmol/mol}$ ($<7.5\%$)), $58 - 75\text{mmol/mol}$ ($7.5 - 9\%$) and $>75\text{mmol/mol}$ ($>9\%$)) and compared using Kaplan-Meier plots as above. Similarly, to assess the impact of the change in HbA1c at one year participants were stratified using the following values ($>5\text{mmol}$ ($>0.5\%$), -5 to 5mmol/mol (-0.5 to 0.5%) and $> -5\text{mmol/mol}$ (-0.5%) change) and compared using Kaplan-Meier plots as above.

For the islet transplant group only HbA1c values at baseline and one year were available. In addition, change in HbA1c was not able to be meaningfully evaluated within the islet transplant cohort as nearly all subjects ($55/66$, 83%) had a significant fall in HbA1c and were stratified to the ' $> -5\text{mmol/mol}$ change' group.

Statistical significance was assumed for $p < 0.05$. Statistical analyses were completed using R, version 3.4.1. As there was limited available data on the separate incidences of DR progression in MDI, CSII and islet subgroups, power analyses were not calculated prior to conducting this study.

2.3 Results Part A: Insulin Pump versus MDI

2.3.1 Study populations

Between 1st January 2013 and 1st December 2016, 293 people with type 1 diabetes who commenced CSII therapy were identified. Of these, 89 were excluded: 23 were aged <16 years at commencement of CSII, 23 had no available baseline and/or follow-up graded retinal screening results, 31 had been excluded from screening for further ophthalmology assessment or treatment, 3 had the maximum severity retinopathy grading at baseline and for a further 9 we were not able to verify if the dates for commencing CSII were correct. The remaining 204 were included in the analysis (Figure 2-2).

We identified 277 MDI controls who had completed structured diabetes education at a similar time to the CSII cohort but who did not proceed to CSII therapy largely due to patient preference. Of these, 66 were excluded: 34 had no available baseline and/or follow graded retinal screening results, 28 had been excluded from screening for further ophthalmology assessment or treatment, 1 had the maximum severity grading at baseline and for a further 3 we were not able to verify if the dates for diabetes education were correct. The remaining 211 were included in the analysis (Figure 2-2)

Baseline demographic and metabolic data is shown in Table 2-3. CSII participants were significantly younger, with earlier age of diabetes diagnosis, lower baseline HbA1c, higher diastolic BP and with lower proportions of people in more deprived socioeconomic quintiles. The majority of participants had either no DR or mild DR at baseline. There were no significant differences in baseline DR gradings between CSII versus MDI cohorts.

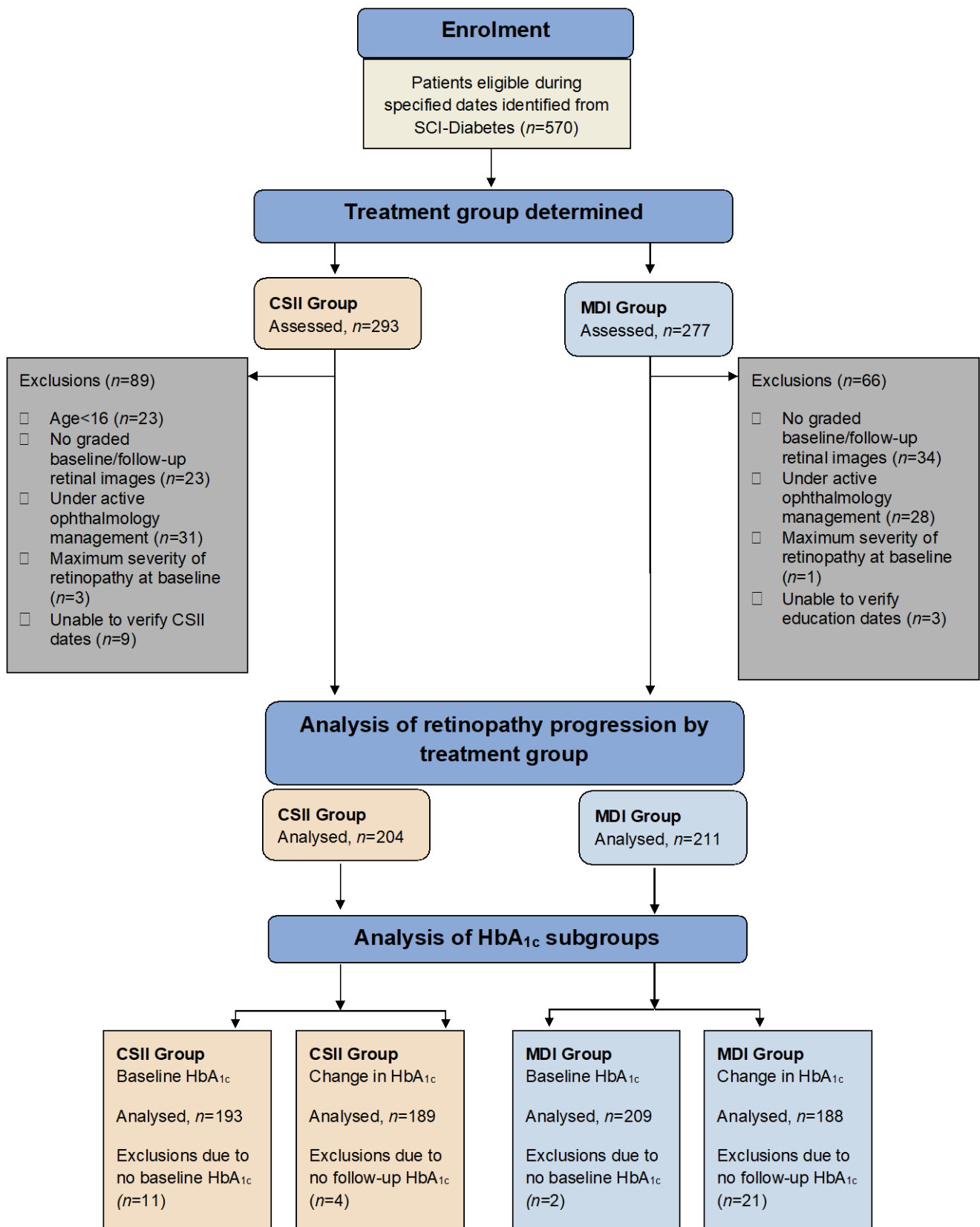


Figure 2-2 Flow chart showing number of people assessed and analysed for CSII and MDI groups and exclusions

Chapter 2: Comparison of graded retinopathy screening outcomes

Table 2-3 Baseline demographics and metabolic characteristics

Characteristic	CSII (n=204)	MDI (n=211)	p value
Age at Diagnosis (years)	15 (10 – 26)	21 (12 – 33)	<0.001
Age at Study Entry (years)	38 (29 – 48)	43 (31 – 53)	<0.001
Diabetes Duration (years)	18 (11 – 25)	16 (8 – 27)	0.63
Gender M:F (%M)	76:128 (37.3%)	90:121 (42.7%)	0.31
Weight (kg)	78.9 (67.6 – 90.3)	79.5 (67.6 – 91.5)	0.84
BMI (kg/m ²)	26.9 (24.0 – 31.2)	27.1 (24.2 – 30.4)	0.97
HbA1c (mmol/mol)	66 (58 – 74)	67 (60 – 80)	<0.05
HbA1c (%)	8.2 (7.5 – 8.9)	8.3 (7.6 – 9.5)	<0.05
SIMD	4 (3 – 5)	3 (2 – 5)	<0.05
Cholesterol (mmol/L)	4.7 (4.2 – 5.2)	4.8 (4.2 – 5.4)	0.14
Creatinine (µmol/L)	73 (67 – 83)	74 (68 – 84)	0.26
Systolic BP (mm/Hg)	128 (118 – 140)	128 (120 – 138)	0.95
Diastolic BP (mm/Hg)	78 (73 – 84)	77 (71 – 83)	<0.001
Smoking Status			0.20
<i>Non-Smoker</i>	141 (69%)	131 (62%)	
<i>Ex-Smoker</i>	48 (24%)	55 (26%)	
<i>Current Smoker</i>	15 (7%)	25 (12%)	
Baseline DR Grading			0.10
<i>R0</i>	93 (45.6%)	100 (47.4%)	
<i>R1</i>	109 (53.4%)	101 (47.9%)	
<i>R2</i>	0	2 (0.9%)	
<i>R3</i>	2 (1.0%)	8 (3.8%)	
<i>R4</i>	Excluded	Excluded	

2.3.2 HbA1c changes in CSII and MDI

The median number of six-monthly HbA1c values per person collected over a three-year study interval was 5 (IQR 4-6). There was a small but statistically significant difference in baseline HbA1c (CSII 66 mmol/mol (8.2%) versus MDI 67 mmol/mol (8.3%), $p<0.01$). In the CSII group, there was a significant reduction in mean study HbA1c (62 mmol/mol (7.8%)), one year HbA1c (59 mmol/mol (7.5%)) and study exit HbA1c (61 mmol/mol (7.7%)) from baseline (all $p<0.001$). In contrast, there were no significant changes in mean study HbA1c, one-year HbA1c or study exit HbA1c (all 67 mmol/mol (8.3%)), from baseline in the MDI group. At all follow up time points, CSII participants had a significantly lower HbA1c than MDI participants ($p<0.001$), and a greater HbA1c reduction (one year: -6 mmol/mol (-0.6%) versus -2 mmol/mol (-0.2%), $p<0.01$; study exit: -4 mmol/mol (-0.4%) versus -2 mmol/mol (-0.2%), $p=0.12$). In both groups, univariate regression analysis showed higher baseline HbA1c was significantly associated with greater reductions in HbA1c at one year ($p<0.0001$).

The distribution of HbA1c in the CSII and MDI groups is shown in Table 2-4. There was a higher proportion of MDI versus CSII participants in the highest baseline HbA1c group (>75 mmol/mol ($>9\%$)) but differences were not statistically significant (Table 2-4). Follow up HbA1c at one year increased by 5 mmol/mol ($>0.5\%$) in 11.1% versus 18.1% and decreased by more than 5 mmol/mol (0.5%) in 51.9% versus 29.3% ($p<0.001$; Table 2-4). Changes at study exit showed a similar pattern between MDI and CSII groups, though numbers of participants with available data for analysis was lower ($p=0.03$; Table 2-4).

Table 2-4 Numbers of all participants stratified to subgroups for baseline HbA1c and change in HbA1c at one year and study exit. P value comparing subgroups for all CSII and MDI or matched CSII and MDI cohorts calculated using Chi-squared.

	CSII (n=204)	MDI (n=211)	p value
Baseline HbA1c /total n with data	/193	/209	0.06
<58mmol/mol (<7.5%) n(%)	42 (21.8)	44 (21.1)	
58-75mmol/mol (7.5-9%) n(%)	107 (55.4)	96 (45.9)	
>75mmol/mol (>9%) n(%)	44 (22.8)	69 (33)	
Change in HbA1c at One Year /total n with data	/189	/188	<0.001
>-5mmol/mol (>-0.5%) n(%)	98 (51.9)	55 (29.3)	
-5 to 5 mmol/mol (-0.5 to 0.5%) n(%)	70 (37.0)	99 (52.7)	
>5mmol/mol (>0.5%) n(%)	21 (11.1)	34 (18.1)	
Change in HbA1c at Study Exit /total n with data	/142	/124	0.03
>-5mmol/mol (>-0.5%) n(%)	63 (44.4)	36 (29.0)	
-5 to 5 mmol/mol (-0.5 to 0.5%) n(%)	58 (40.8)	61 (49.2)	
>5mmol/mol (>0.5%) n(%)	21 (14.8)	27 (21.2)	

2.3.3 Retinopathy events in CSII versus MDI

Kaplan-Meier curves describing time to a DR progression event for comparator groups are shown in Figure 2-3(a-e). Mean follow up for the entire cohort was 839 days (2.3 years) with DR progression occurring in 38 participants (18.6%) in the CSII group versus 56 participants (26.5%) in the MDI group (Figure 2-3a, $p=0.0097$). Of these, unilateral retinal progression was identified in 21/58 (36.2%) people in the MDI group, versus 14/38 (36.8%) people in the CSII group, while the rest had bilateral retinal progression.

Pump participants had a reduced risk of DR progression on univariate (HR 0.58, $p=0.01$) and multivariate (HR 0.56, $p=0.02$) Cox proportional hazard analysis, adjusting for age, gender, diabetes duration, baseline HbA1c, BP, cholesterol, creatinine, SIMD, smoking status and baseline DR grading (Table 2-5).

Those with mild retinopathy at baseline were also at low risk of progression (HR 0.07), with the majority of events occurring in those with no baseline retinopathy (76/94, 80.9%). Older

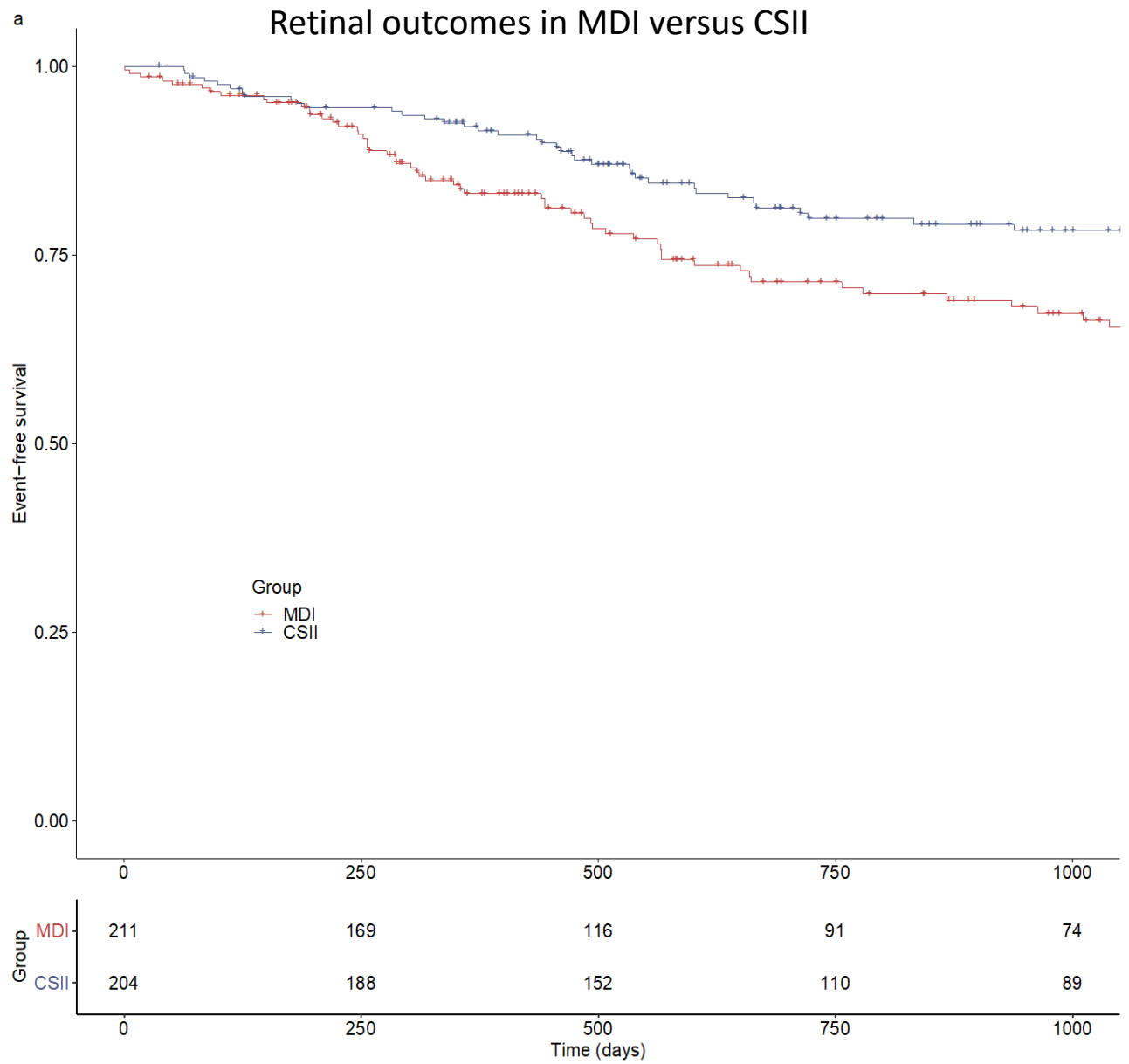
age was also associated with reduced risk of DR progression (HR 0.97), though longer diabetes duration (HR 1.03), increased creatinine (HR 1.01) and history of previous smoking (HR 1.89) were associated with increased DR progression risk on multivariate analysis.

Higher baseline HbA1c was associated with increased DR progression risk for the entire cohort on univariate and multivariate analyses (HR 1.03, $p < 0.001$, Table 2-5). However, this effect was driven by the MDI cohort, with baseline HbA1c having no significant impact on the frequency of DR progression occurring in the CSII cohort (Figure 2-3b, $p = 0.93$), while in the MDI cohort HbA1c > 75 mmol/mol ($> 9\%$) was associated with significantly higher proportion of participants with DR progression (28/69 participants, 40.6%) than those with HbA1c < 58 mmol/mol ($< 7.5\%$) (6/44 participants, 13.6%) (Figure 2-3c, $p = 0.0049$). The same outcomes were found when groups were stratified by mean study HbA1c, with no significant impact of high mean study HbA1c on DR progression noted in the CSII cohort ($p = 0.26$), while high mean study HbA1c was associated with increased DR progression in the MDI cohort ($p = 0.023$). HbA1c values at one year and study exit showed no significant impact on the frequency of DR progression in either cohort. Change in HbA1c at one year from baseline was not associated with DR progression in either cohort (CSII: Figure 2-3d ($p = 0.19$), MDI: Figure 2-3e ($p = 0.21$)).

Chapter 2: Comparison of graded retinopathy screening outcomes

Table 2-5 Hazard ratios for co-variables on univariate and multivariate cox proportional hazard analysis for CSII and MDI groups. Ref. denotes reference group.

Co-Variate	Univariate Analysis			Multivariate Analysis		
	HR	(95% CI)	p.value	HR	(95% CI)	p.value
Pump Group	0.58	0.39 – 0.88	0.01	0.56	0.34 - 0.91	0.02
Age at Study Entry	0.98	0.96 – 0.99	0.99	0.97	0.96 - 0.99	<0.01
Male Gender	0.84	0.55 – 1.29	0.43	0.69	0.40 - 1.19	0.18
Diabetes Duration	1.00	0.98 – 1.01	0.65	1.03	1.02 - 1.05	<0.001
Baseline HbA1c	1.03	1.01 – 1.03	<0.001	1.03	1.01 - 1.04	<0.001
Systolic	1.00	0.98 – 1.01	0.68	1.00	0.98 - 1.02	0.92
Diastolic	0.99	0.97 – 1.02	0.61	1.01	0.99 - 1.04	0.36
Cholesterol	0.97	0.78 – 1.22	0.82	0.94	0.73 - 1.21	0.62
Creatinine	1.00	0.99 – 1.01	0.87	1.01	1.00 - 1.02	0.01
SIMD	0.89	0.77 – 1.03	0.13	0.94	0.79 - 1.12	0.51
Smoking status						
Non-smoker	1.00 (Ref.)			1.00 (Ref.)		
Ex-smoker	1.54	0.98 – 2.41	0.06	1.89	1.13 - 3.17	0.02
Current smoker	1.60	0.84 – 3.08	0.15	0.94	0.43 - 2.07	0.88
Baseline DR Grading						
R0	1.00 (Ref.)			1.00 (Ref.)		
R1	0.11	0.06 – 0.20	<0.001	0.07	0.03 - 0.13	<0.001
R2	5.43	1.32 – 22.32	0.02	2.90	0.62 - 13.6	0.18
R3	1.04	0.38 – 2.84	0.94	0.28	0.09 - 0.86	0.03



Chapter 2: Comparison of graded retinopathy screening outcomes

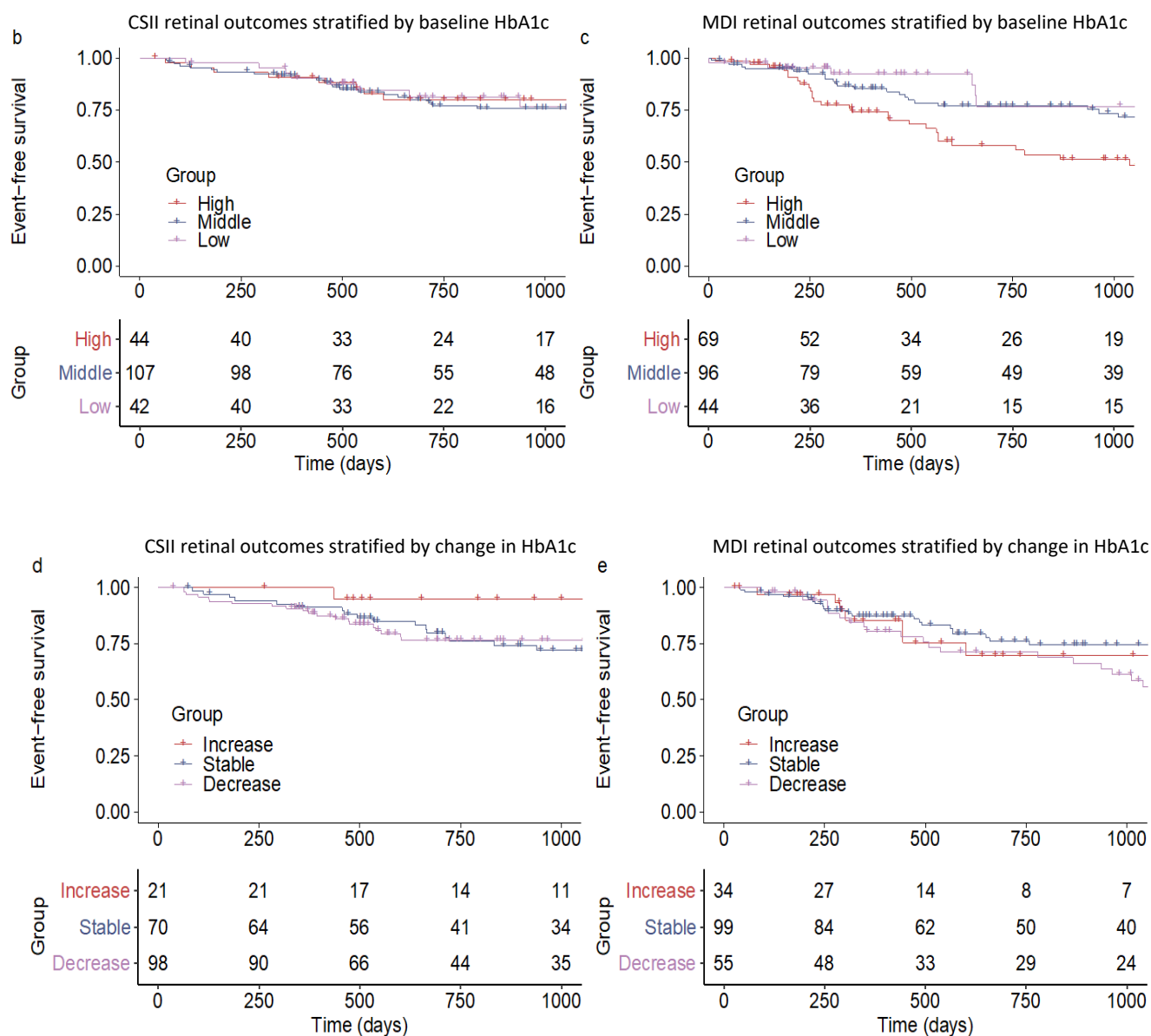


Figure 2-3 Kaplan–Meier survival plots compare event-free survival rates in (a) CSII/MDI treatment groups and (b–e) HbA_{1c} subgroups. An event corresponds to diabetic retinopathy progression and was defined as a minimum one-grade worsening in either eye from the baseline grading. Vertical dashes indicate participants who were censored due to incomplete 3 year follow-up. (a) Comparison of CSII (blue) and MDI (red) participants for the entire cohort. CSII was associated with significantly reduced retinopathy progression over 3 years compared with MDI ($p=0.0097$). (b, c) Comparison of participants with baseline HbA_{1c} <58mmol/mol (7.5%) (low: lilac), 58–75 mmol/mol (7.5–9%) (middle: blue) or >75mmol/mol (9%) (high: red) in the (b) CSII group and (c) MDI group. High baseline HbA_{1c} (>75mmol/mol (9%)) was associated with increased diabetic retinopathy progression in the MDI group ($p=0.0049$) but was not a determinant of diabetic retinopathy progression in the CSII group ($p=0.93$). (d, e) Comparison of unmatched participants with change in HbA_{1c} at 1 year of less than -5mmol/mol (0.5%) (decrease: lilac), -5 to 5 mmol/mol (0.5 to 0.5%) (stable: blue) or more than 5mmol/mol (0.5%) (increase: red) in the (d) CSII group and (e) MDI group. Change in HbA_{1c} at follow-up did not significantly impact diabetic retinopathy progression in either cohort.

2.4 Discussion Part A

In this real-world study, using a robust clinical database and a nationalized single DR scoring system we observed that CSII therapy was associated with significantly lower DR progression over a three year follow up period in adults with type 1 diabetes who had completed structured diabetes education than those on MDI therapy who had completed an equivalent structured diabetes education programme (18.6% in CSII vs 26.5% in MDI, $p=0.0097$). In addition, there was no evidence of EWDR in those using CSII compared to MDI over the first 18 months. This is despite significantly larger reductions in HbA1c in the CSII group (6mmol/mol (0.5%) reduction) versus MDI group (1mmol/mol (0.1%) reduction) at one year post study entry ($p<0.001$). Findings were confirmed on multivariate analysis adjusting for multiple potential confounders.

Those with longer duration of diabetes were found to be at higher risk of DR progression which was expected as this is a well-established risk factor for DR (4). Surprisingly older age was associated with a small but significant reduction in risk of DR progression in our cohort. This was driven by MDI participants, with no significant effect of age on DR progression when CSII participants were analysed in isolation. The addition of drug therapies such as statins and antihypertensives in older participants may have contributed to DR risk reduction, however we were unable to assess this in our cohort.

Studies of DR progression in adults are lacking in real world settings. However, our findings are consistent with a longitudinal study of adolescents (aged 12-20 years) treated with either CSII or MDI therapy over a period of 15 years (113). They showed a significantly lower risk of developing retinopathy in those treated with CSII versus MDI (OR 0.66), though the majority of assessments (79%) were from participants who were only reviewed once during the study period. Proportions of people developing retinopathy were slightly lower than in our cohort (17% in CSII, 22% in MDI), however this is likely to be consistent with a patient cohort of younger age and shorter diabetes duration than in our study. A further study assessed retinal changes in 31 adults following initiation of CSII using a range of imaging modalities, including OCT, and showed stable retinal characteristics over one year with no evidence of early DR worsening (66).

High baseline HbA1c (75mmol/mol (>9%)) was associated with increased DR progression in the MDI group. In contrast, DR progression in the CSII group was not found to be associated with baseline HbA1c. In the MDI group there were no significant reductions in follow up HbA1c values when compared to baseline levels. However, in the CSII group, follow up HbA1c values were significantly reduced from baseline, and were lower than HbA1c levels assessed at the same timepoints in the MDI group.

Absolute change in HbA1c at one year from baseline was not significantly associated with DR progression for either group. This is in keeping with recent findings for people with Type 1 diabetes who had marked HbA1c reductions following the introduction of flash glucose monitoring via the libre device. Results from these studies showed that there was no increased DR progression in 'responders' with a significant HbA1c reduction (median change -23mmol/mol) versus 'non responders' who did not have an overall reduction in HbA1c (median change +6mmol/mol) (65). However high baseline HbA1c >75mmol/mol was associated with increased DR progression (64).

We hypothesise that factors intrinsic to CSII therapy are protective against DR progression in those with high baseline HbA1c while those with high baseline HbA1c on MDI remain at increased DR risk. Many of the MDI participants did not achieve a substantial reduction in HbA1c on follow up and therefore had continued exposure to high glycaemic levels which may have contributed to increased DR risk. This is supported by the fact that high mean study HbA1c was significantly associated with DR progression in the MDI group, though this was not true in the CSII group.

While exposure to high glycaemic levels is a well-established risk factor for DR, recurrent disabling hypoglycaemia may also increase DR risk (127). Though recurrent disabling hypoglycaemia is a possible indication for consideration of CSII therapy, it was not possible for this to be accurately assessed within our cohorts and levels of hypoglycaemia within the groups may have differed. It is therefore possible that some of those perceived to have good glycaemic control, reflected by a lower baseline HbA1c, were exposed to higher levels of hypoglycaemia prior to the study period, which could have contributed to increased DR risk.

CSII therapy has been shown to reduce frequency of hypoglycaemia (128) which may also have conferred a benefit in terms of DR risk, even in those with no HbA1c reduction.

Other factors related to better control of diabetes (including GV, discussed further below) may have also contributed to the reduction in DR observed in the CSII group.

It is important to note that, within our retinopathy screening programme in Scotland, when people are commenced on CSII therapy recommendations are in place with regards to modifications to early HbA1c targets where appropriate, particularly for those with high baseline HbA1c levels, discussion with ophthalmology with regards to stabilising any known severe retinal disease prior to CSII start, and improved surveillance for retinopathy following the introduction of CSII (129). As such, the reductions in HbA1c, although significant in our study, may have been achieved more gradually in the CSII group, and some of these recommendations may have confounded retinal results.

Glycaemic control as measured by HbA1c has been shown to be important in reducing DR progression as evidenced from the DCCT study (130). However, other studies do not demonstrate an association between baseline HbA1c, or change in HbA1c, and DR progression following initiation of CSII therapy (131). Evidence from the DCCT also showed that HbA1c changes did not fully explain the risk of DR progression in type 1 diabetes, and that other features of glucose control, such as the extent of postprandial glucose excursions or counterregulatory responses to hypoglycaemia, which are not easily reflected by a summary measure such as HbA1c, may have an impact on the risk of developing complications (130). DCCT analyses assessing time in range (TIR) from seven point fingerstick data showed this had a strong association with the development of microvascular complications including retinopathy (19), though earlier DCCT analyses suggest within-day GV and MAGE were not predictive of DR (132).

More widespread availability of CGM systems in recent years has led to expanding interest in the role of glycaemic markers other than HbA1c, including TIR, GV and MAGE, with recommendations now in place for their use in routine diabetes management (22).

There is growing evidence from CGM that these markers are associated with DR in type 2 diabetes (15, 133, 134), however less is known about their role in type 1 diabetes (16). We and others have reported that islet transplantation in recipients with type 1 diabetes is associated with diminished GV and reductions in HbA1c in association with diminished progression of DR (121, 135, 136). Further studies assessing the impact of GV on DR progression are needed.

CSII is associated with higher initial costs due to the expense of the pump, necessary consumables, and pump education (137). However, longer-term cost benefit analyses studies indicate that such costs are offset by improved glycaemic control, enhanced quality of life markers and reductions in diabetes complications, and demonstrate that CSII is in fact a cost-effective treatment in Type 1 diabetes (137, 138). Many cost-benefit models highlight the reduced morbidity and mortality related costs secondary to reductions in problematic hypoglycaemia associated with CSII therapy (137), and projected cost savings related to diminished micro- and macrovascular complications are also evident with an 18.3% lifetime reduction in severe visual loss with CSII versus MDI therapy (138).

The strengths of this study are that it is a relatively large study providing real world data on DR progression in type 1 diabetes in an adult cohort following initiation of CSII therapy. Furthermore, DR assessment was performed within a single retinopathy screening programme. It provides reassurance that, in people with no DR or mild DR at baseline, there is no evidence of increased risk of DR progression following initiation of CSII, and there is long term benefit particularly in those with the highest HbA1c compared to continued MDI therapy.

The study has several limitations. As a retrospective real-world study, timings of retinal imaging and HbA1c collection were not uniform for participants over the study period. In addition, participants were not randomized to receive either CSII or MDI therapy resulting in potential confounding and allocation bias. Indeed, at baseline the CSII group was significantly younger with earlier age of diabetes onset, lower baseline HbA1c, higher diastolic BP and lived in less socio-economically deprived areas than the MDI group. Multivariate analyses were performed to minimise the impact of these differences. Study entry time for the MDI group,

who did not have any specific intervention, was based on the lag time from receiving structured education to commencing on CSII therapy. Although there was some variability in this lag time, when data were reassessed using an alternative study entry time based on the median lag time (841 days) rather than mean lag time (945 days) outcomes were unchanged, with results still showing significantly fewer retinal events in the CSII group versus the MDI group (see supplementary data in Appendix 1). Retinal data was sourced from the DRS programme, therefore people who had no retinal screening results could not be assessed (7.8% CSII, 12.3% MDI). This could represent a population of non-attenders who are not fully engaging with aspects of their diabetes management, and as such may be at higher risk of developing DR. In addition, few participants with advanced retinal disease, who are known to be at the highest interval risk of DR (139), were assessed as these people leave the screening programme to enter into a management pathway under the care of ophthalmologists, though numbers excluded due to receiving ophthalmology care were similar in both groups (10.6% CSII, 10.1% MDI). Reporting of retinopathy outcomes using SDRG assessment is not the gold standard, therefore we may have missed more subtle retinal changes that could have been picked up by using Early Treatment Diabetic Retinopathy Study (ETDRS) screening, however as SDRG is designed to detect clinically significant changes we feel findings are still clinically relevant. These problems relating to the use of screening data will have affected both groups, and therefore their impact on the study analysis was felt to be relatively small. Glycaemic analysis was limited to HbA1c data. Though this remains the gold standard for glycaemic assessment, further studies using CGM will help fully characterize the range of glycaemic factors involved in DR progression. Finally, this study was conducted in a predominantly white population across three diabetes centres from a single Scottish region, where a national DR screening service is offered for all people affected by diabetes. Therefore, results may not be generalizable to all people with type 1 diabetes from other ethnic groups, or where access to DR screening is limited.

2.5 Results Part B: Islet Transplantation versus CSII and MDI

2.5.1 Study populations

The number of islet transplants performed during the study periods at each site were 49 in Edinburgh and 107 in Edmonton. At the Edinburgh site there were 29 exclusions: 2 patients lived outside Scotland and were not participants of the Scottish Retinal Screening service, 25 had no baseline or follow graded retinal screening results and 2 had been excluded from screening for further ophthalmology assessment or treatment. At the Edmonton site there were 61 exclusions: 35 had no baseline or follow up retinal images and 26 had the maximum severity retinopathy grading at baseline. There were therefore 66 patients suitable for study inclusion (20 Edinburgh, 46 Edmonton) (Figure 2-4). HbA1c data at baseline and one year follow up was available for all included islet participants.

Comparisons were made to the NHS Lothian Pump and MDI groups which have been previously described in Chapter 2.3.1 (Figure 2-2).

Baseline demographic and metabolic data is shown in Table 2-6. Islet participants were significantly older, with longer diabetes duration and lower weight and BMI than CSII and MDI participants. Baseline HbA1c was also significantly lower in the MDI group compared to the islet group. The majority of participants in all groups had either no DR (R0) or mild DR (R1) at baseline, though there were significant differences in baseline gradings between islet and non-islet groups.

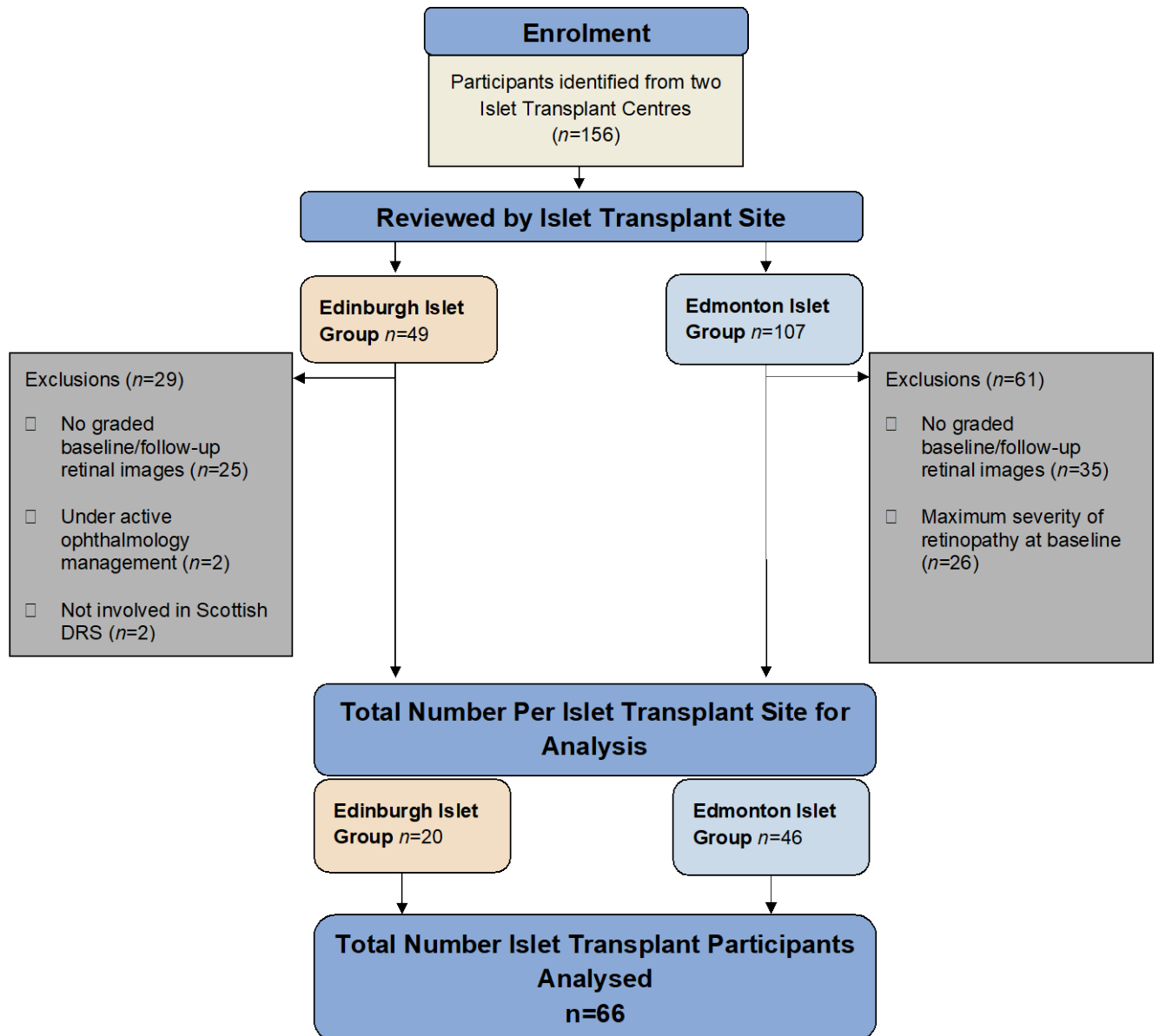


Figure 2-4 Flow chart of islet transplant study population numbers and exclusions

Chapter 2: Comparison of graded retinopathy screening outcomes

Table 2-6 Baseline demographic and metabolic data for all groups. Significant p values for differences between variables of either CSII or MDI groups in comparison to Islet groups denoted by *(p<0.05), **(p<0.01), ***(p<0.001)

Characteristic	Islet (n=66)	CSII (n=204)	MDI (n=211)
Age at Study Entry (years)	48 (40 – 53)	38 (29 – 48)***	43 (31 – 53)*
Diabetes Duration (years)	29 (19 – 37)	18 (11 – 25)***	16 (8 – 27)***
Gender M:F (%M)	23:43 (34.8%)	76:128 (37.3%)	90:121 (42.7%)
Weight (kg)	68.0 (60.8 – 77.9)	78.9 (67.6 – 90.3)***	79.5 (67.6 – 91.5)***
BMI (kg/m ²)	23.6 (22.0 – 26.9)	26.9 (24.0 – 31.2)***	27.1 (24.2 – 30.4)***
HbA1c (mmol/mol)	63 (54 – 73)	66 (58 – 74)	67 (60 – 80)**
Baseline DR Grading		***	***
R0	9 (13.6%)	93 (45.6%)	100 (47.4%)
R1	46 (69.7%)	109 (53.4%)	101 (47.9%)
R2	10 (15.1%)	0	2 (0.9%)
R3	1 (1.5%)	2 (1.0%)	8 (3.8%)
R4	Excluded	Excluded	Excluded

2.5.2 HbA1c Changes at one year

The mean reduction in HbA1c at one year for islet recipients was -18mmol/mol, which was significantly lower than HbA1c reductions seen in those commenced on CSII (-6mmol/mol reduction, p<0.0001) and those continued on MDI (-2mmol/mol reduction, p<0.0001).

2.5.3 Retinopathy Events in Islet Transplanted versus Non-Transplanted Participants

Kaplan-Meier curves describing time to DR progression for comparator groups are shown in Figure 2-5 and Figure 2-6 (p values calculated using log rank test). Participants were followed up to a maximum of 5 years with mean time to either a retinal event, or study exit for another reason, of 2.3 years. DR progression occurred in 14/66 participants (21.2%) in the islet group compared with 65/211 participants (30.1%) in the MDI group (

Figure 2-5, $p=0.039$) and 43/204 participants (21.1%) in the CSII group (Figure 2-6, $p=0.7$).

Univariate Cox proportional hazard analysis showed islet participants has a reduced risk of DR progression when compared to non-transplanted MDI participants (HR 0.55, $p=0.045$, Table 2-7), however on multivariate analysis adjusting for age, sex, diabetes duration, baseline HbA1c and baseline DR grading, significance was lost (HR 0.71, $p=0.38$, Table 2-7). There was no significant difference in islet and CSII treatment groups on univariate or multivariate analyses (Table 2-8).

Higher baseline HbA1c, longer diabetes duration and no retinopathy at baseline were significantly associated with increased DR progression in both comparator groups on multivariate analysis. Older age was associated with reduced DR risk in islet and MDI participants, but not CSII participants.

Chapter 2: Comparison of graded retinopathy screening outcomes

Table 2-7 Hazard ratios for co-variables on univariate and multivariate cox proportional hazard analysis for Islet and MDI groups. Ref. denotes reference group.

Co-Variate	Univariate Analysis			Multivariate Analysis		
	HR	(95% CI)	p.value	HR	(95% CI)	p.value
Islet Group	0.55	0.31 – 0.99	0.045	0.71	0.33 – 1.53	0.38
Age at Study Entry	0.96	0.95 – 0.98	<0.001	0.98	0.96 – 0.98	0.03
Male Gender	0.73	0.46 – 1.17	0.19	1.17	0.71 – 1.93	0.53
Diabetes Duration	0.98	0.97 – 1.002	0.09	1.03	1.01 – 1.04	<0.01
Baseline HbA1c	1.04	1.03 – 1.05	<0.0001	1.03	1.02 – 1.05	<0.0001
Baseline DR Grading						
R0	1.00 (Ref.)			1.00 (Ref.)		
R1	0.20	0.12 – 0.34	<0.0001	0.14	0.07 – 0.25	<0.0001
R2	0.56	0.22 – 1.42	0.22	0.59	0.20 – 1.78	0.35
R3	0.91	0.33 – 2.52	0.85	0.35	0.12 – 0.99	0.05

Table 2-8 Hazard ratios for co-variables on univariate and multivariate cox proportional hazard analysis for Islet and CSII groups. Ref. denotes reference group.

Co-Variate	Univariate Analysis			Multivariate Analysis		
	HR	(95% CI)	p.value	HR	(95% CI)	p.value
Islet Group	1.12	0.60 – 2.07	0.72	0.65	0.30 – 1.40	0.28
Age at Study Entry	0.98	0.96 – 1.00	0.11	0.98	0.96 – 1.00	0.17
Male Gender	1.15	0.68 – 1.96	0.60	1.6	0.91 – 2.82	0.10
Diabetes Duration	1.01	0.99 – 1.03	0.47	1.02	1.01 – 1.04	<0.01
Baseline HbA1c	1.02	1.00 – 1.04	0.02	1.04	1.02 – 1.06	<0.001
Baseline DR Grading						
R0	1.00 (Ref.)			1.00 (Ref.)		
R1	0.20	0.12 – 0.34	<0.001	0.10	0.05 – 0.21	<0.0001
R2	0.56	0.22 – 1.42	0.22	0.28	0.07 – 1.06	0.06
R3	0.91	0.32 – 2.52	0.85	0.60	0.08 – 4.49	0.62

Chapter 2: Comparison of graded retinopathy screening outcomes

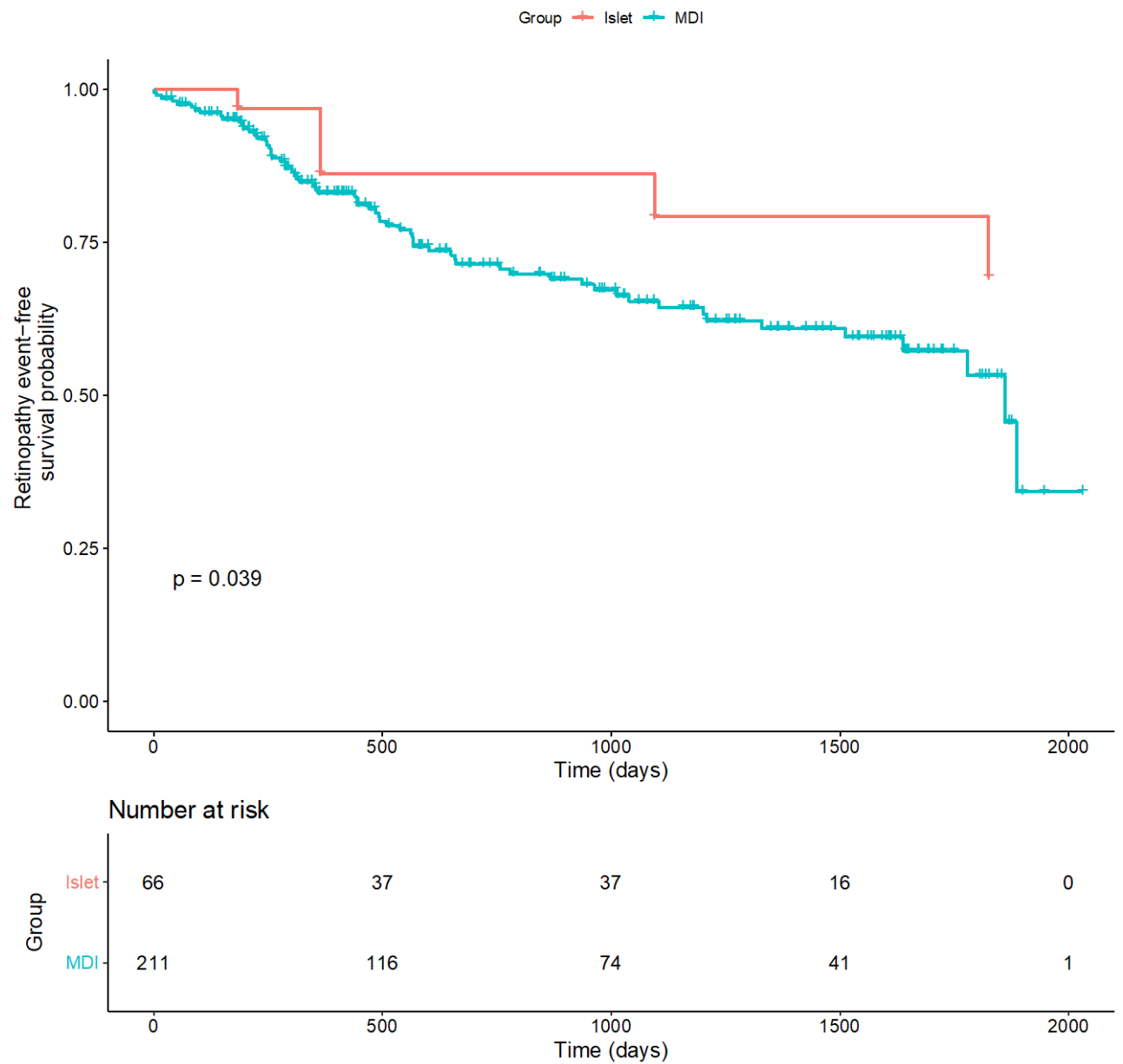


Figure 2-5 Kaplan Meier curve for DR progression in Islet vs MDI

Chapter 2: Comparison of graded retinopathy screening outcomes

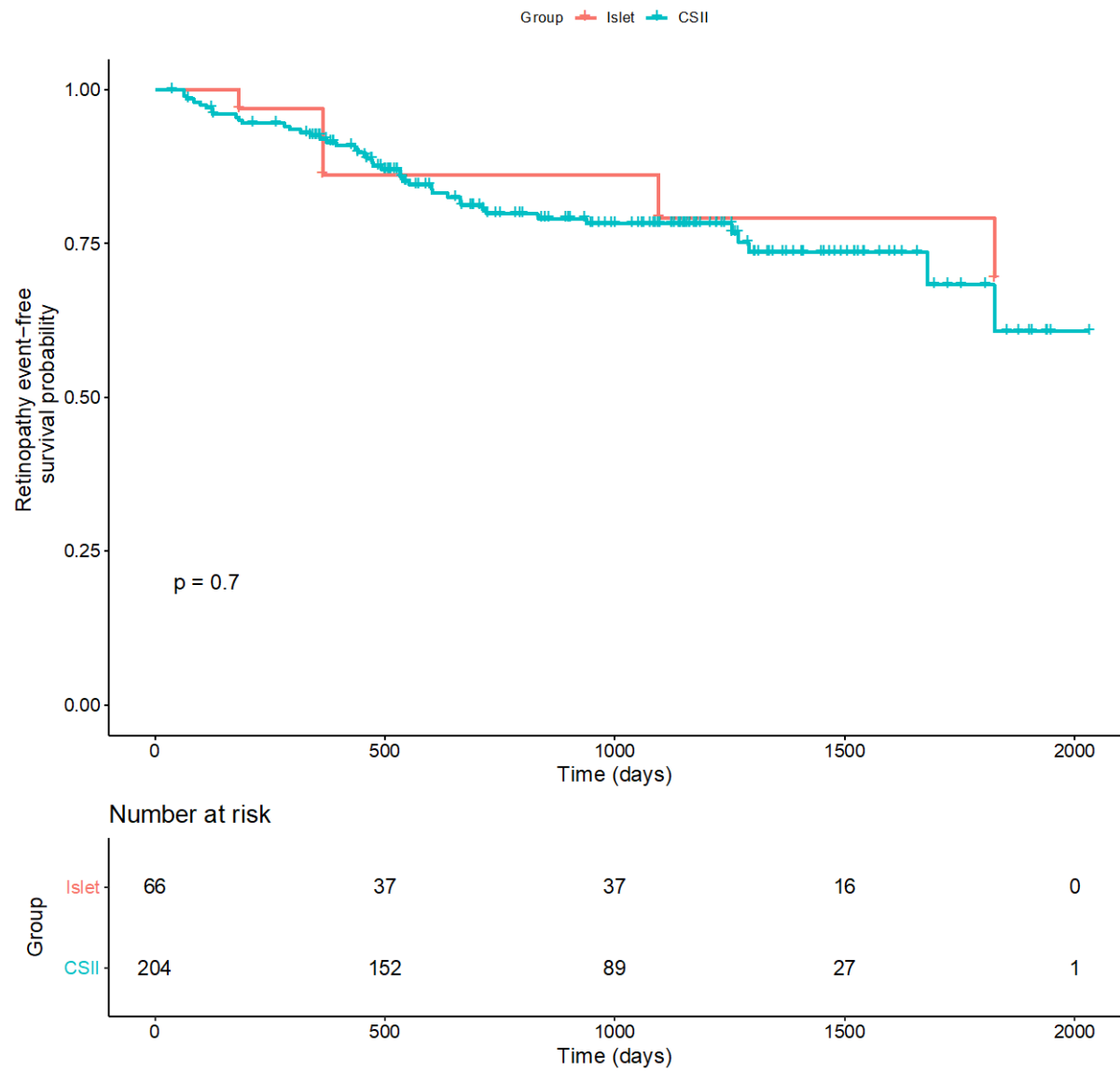


Figure 2-6 Kaplan Meier curve for DR progression in Islet vs CSII

2.6 Discussion Part B

We have shown no increased risk of DR progression associated with islet transplantation compared to people with type 1 diabetes who commence on CSII therapy or continue on MDI treatment. This includes no evidence of EWDR following islet transplantation despite significantly larger reductions in HbA1c in the first year post islet transplantation.

There are few studies assessing DR outcomes following islet transplantation. An early study from the Edmonton group suggested there may be early worsening of DR following islet transplantation, as DR progression was seen in 4/47 participants in the first 5 months post-transplant. However, 46% of participants had previous proliferative retinopathy at baseline and were therefore at high risk for further retinal complications. This study also lacked a control group.

A later study including 27 islet participants showed rates of DR progression post islet transplantation were reduced when compared to those receiving intensive medical therapy on the islet transplant waiting list (120). DR progression was significantly more likely in the intensively treated medical groups (progression in 10/82 eyes versus 0/51 eyes in the islet group) over three year follow up. This was a prospective crossover study where the same participants formed the intensively treated medical group prior to receiving an islet transplant, before crossing over to the islet group after transplantation.

Our study is one of the largest islet transplant studies assessing microvascular complications to date and provides reassuring real world evidence of stable retinopathy post islet transplantation, with DR progression rates which are on par with those seen in individuals receiving other treatment modalities for type 1 diabetes. This is despite this islet group being a high risk cohort with a longer diabetes duration burden at baseline.

There are several limitations to this study. islet and non-islet groups were significantly different at baseline, with islet participants being significantly older, with lower weight and BMI, and longer diabetes duration. There were also significantly larger proportions of people with mild DR at baseline than in the CSII and MDI groups. Multivariate analysis was used to

minimise the impact of these differences, however participants were not randomised to individual groups and there will likely be further bias that we have not been able to account for in our statistical analysis. Participants who either received an islet transplantation or who were commenced on CSII may also have had more interaction with medical services following a change to treatment, which may have facilitated improved retinal outcomes in these groups.

To maximise the total number of participants, islet transplanted individuals were assessed at two separate large islet transplant centres. Data collection differed across sites, including the use of different retinopathy assessment methods for islet participants. This could potentially have led to small differences in SDRG outcomes following conversion from the Edmonton ETDRS gradings. We feel the impact of this was minimal, given that the SDRG gradings rely on core retinopathy features which are easily defined using the ETDRS system. The latter is a more comprehensive grading system and may have picked up additional subtle retinal changes which may have been missed by using the SDRG assessment. This will have affected all groups, and as the SDRG is designed to detect clinically significant changes, we feel findings are still of clinical relevance.

The majority of participants had no or mild DR at baseline. Though this provides reassurance for those without known DR complications, findings may not be generalisable to all people with type 1 diabetes.

2.7 Conclusions

We have shown stable DR with no EWDR following the introduction of CSII and islet transplantation therapies compared with continued MDI, despite significant early reductions in glycaemia as measured by HbA1c. In addition, CSII therapy may be associated with significant reductions in DR progression compared to continued MDI therapy.

3 Assessment of vascular retinal biomarkers on fundus images after islet transplantation or commencement of continuous subcutaneous insulin infusion

3.1 Introduction

The main aim of this study was to quantify retinal vascular changes in patients following intervention with islet transplantation or CSII therapy using retinal morphometry. This involved evaluation of fundus images taken pre and post intervention via the DRS programme using a semi-automated analytic computer programme.

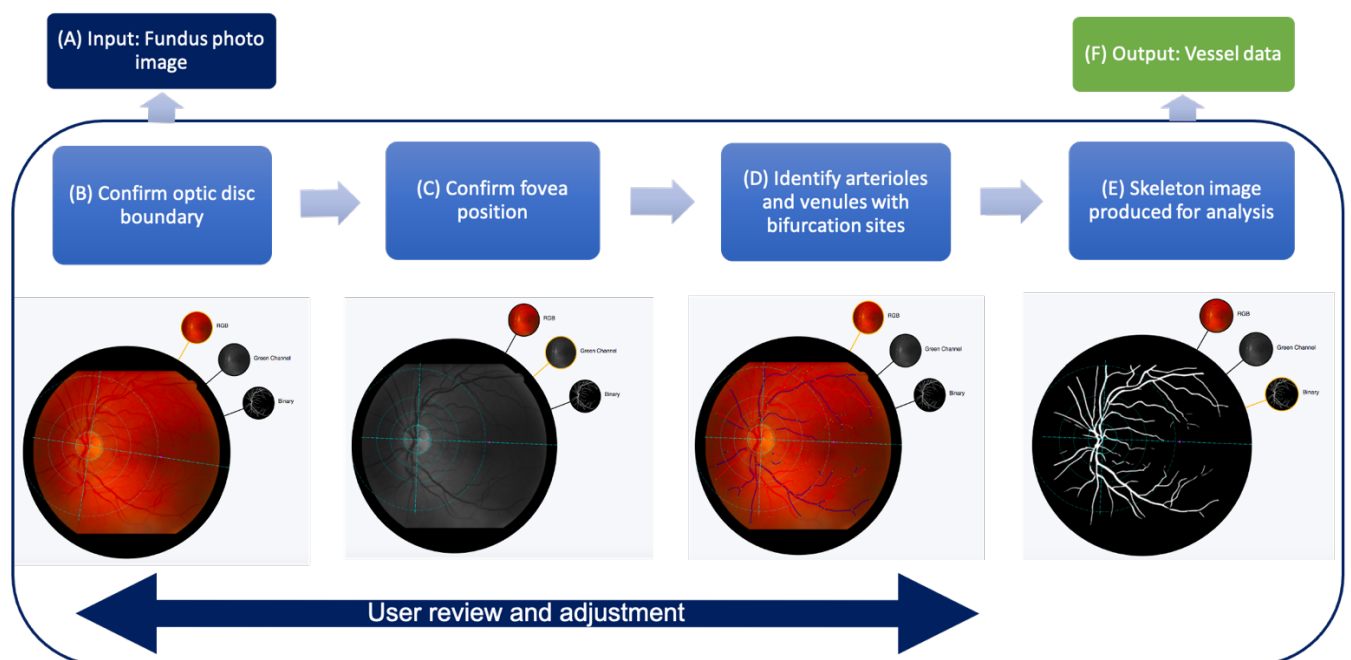
3.1.1 VAMPIRE software for fundus photo analysis

The Vessel Assessment and Measurement Platform for Images of the Retina (VAMPIRE) system is a software application developed in Scotland for semi-automated analysis and quantification of retinal vascular geometry from fundus images. The retinal biomarkers assessed by VAMPIRE focus on the retinal vasculature, and can be divided into three main areas: vessel calibre, vessel tortuosity and fractal dimensions (FD) (140).

The process firstly involves identification of key retinal landmarks, namely the optic disc and fovea, which can be manually checked by the user to ensure this has been appropriately placed. A retinal grid is assigned to the image based on the location of the optic disc to separate the image into zones for analysis (Figure 3-1). The software will then detect the retinal vasculature network to produce a vascular tree which can be represented as a binary skeleton image distinguishing the presence or absence of vessels (Figure 3-1). Classification of the vascular tree to identify arterioles versus venules is determined based on typical characteristics which are assessed using further algorithms. Again, the user can review this automated classification and adjust if needed. Once these vasculature landmarks have all been satisfactorily identified and reviewed, the image can be sent for processing and an output yielding the quantitative retinal biomarker information is produced (140).

Much of this processing relies on specially designed algorithms to process large volumes of data and is hidden to the user. A fully automated system is not currently available for this, and would be challenging to develop due to the use of colour images, however, adjustments can be made by the user as described, and the software has been validated using public datasets (141, 142).

VAMPIRE has previously been used to investigate microvascular retinal abnormalities in various diseases, including stroke, dementia, and renal impairment (143-145).



3.1.2 Role of retinal vascular geometry in diabetic retinopathy – current knowledge

To date, the VAMPIRE software has mainly been used in studies focussing on neurovascular disease, with limited evaluation of its role in assessing DR via the three main retinal biomarker groups. Similar automated software has shown these groups to have a potential role in predicting disease severity from fundus images in DR (Figure 3-2).

3.1.2.1 Vessel Calibre

Large population-based studies assessing individuals with both Type 1 and Type 2 diabetes have demonstrated that CRAE and CRVE measurements are significantly wider than those of healthy non-diabetic controls (146-148). Wider vessel calibres also reflect increased DR severity in several studies, where people with proliferative DR have higher CRAE and CRVE compared to those with diabetes without evidence of retinopathy (146, 149-151). Higher CRAE and CRVE measurements are also associated with increased incidence of DR in people with known diabetes (152-154). In one study an increase of 10 μm in CRVE (mean study CRVE 243.9 μm) was shown to be significant in increasing DR risk (OR 1.26) though this was not significant for CRAE (mean study CRAE 163.0 μm , OR 1.17) (152). In a separate study, comparison of individuals in the highest quartile for CRAE measurements ($>189 \mu\text{m}$) to those in lower quartiles ($<189 \mu\text{m}$) found those with higher CRAE values had a 46% increase in incident DR (11% vs 7%, OR 4.79) (153). Findings from small OCT studies have also found retinal vessel widening to be associated with DR incidence and severity (155, 156). Longer duration of diabetes and higher HbA1c are also shown to be associated with increased retinal vessel width (151, 154, 157).

It is hypothesised that retinal vessel widening may be an early sign of DR, due to compensatory vessel dilatation to increase blood flow in the context of retinal hypoxia. Inflammation and loss of autoregulation in the vessels due to damage from reactive oxygen species has also been thought to lead to widening, suggesting prolonged exposure of the vessels to hyperglycaemia is likely to be one of the mechanisms driving the process.

Changes in vein calibre are more apparent than changes in arterial calibre. While CRVE widening due to diabetes pathology was a consistent finding across studies, CRAE findings showed some variation. In a Danish population study of over 800 people with Type 1 diabetes, CRAE narrowing was found to be significantly associated with DR and other microvascular diabetes complications over 16 year follow up (158). This study had a longer follow up than others and is the only one showing a significant association between arteriolar width and DR in an exclusively Type 1 diabetes population. However, because baseline photographs were taken in 1995, images had to be converted from slides to a digitalized form for comparison to the digital follow up images. In addition, the protocol for retinal photographs differed at baseline and follow up, with two images taken at baseline, and seven images taken at follow up. It is possible that these differences impacted results. Arteriolar narrowing was also associated with incident Type 2 diabetes in an Australian population study over 5 year follow up.

Hypertension is well established to be related to arteriolar narrowing, with every 10mmHg rise in mean arterial BP causing an estimated 3.07 μm reduction in retinal arterial diameter on meta-analysis (159). This was adjusted for in the majority of studies but could still be a negative confounder given that it is a common comorbidity in people with diabetes. Impaired renal function has also been associated with reduced retinal vessel calibers

Across studies, absolute values for mean vessel widths in diabetic populations were varied, particularly for CRAE (range of means from fundus image studies: CRAE 146.1 - 193.5 μm , CRVE 210.31 - 222.6 μm). Comparisons of absolute CRAE and CRVE measurements between studies and establishing threshold values to represent increased DR risk is therefore a challenge.

3.1.2.2 Vessel Tortuosity

Increased tortuosity may be an early indicator of DR. In several large population-based studies of adults with diabetes, those with higher arteriolar tortuosity indices at baseline were found to have increased risk of DR progression in up to 10 year follow up (160-164). Even in the absence of detectable DR, one study has shown retinal vessels are more tortuous in people

with diabetes (n=224, median curvature tortuosity for arterioles 34.4×10^5 and venules 39.7×10^5) compared to healthy controls (n=103, median curvature tortuosity for arterioles 34.4×10^5 and venules 39.7×10^5) (165). Increased tortuosity is also associated with increased DR severity (165). When compared to individuals with diabetes without DR, tortuosity levels were significantly higher in mild (OR 1.53) and moderate (OR 1.67) non-proliferative DR, though not PDR (OR 0.91) (165).

Conversely, one prospective study assessing the role of tortuosity markers in PDR incidence found lower curvature arteriolar and venular tortuosity was significantly associated with increased incident PDR risk, though this was a small study (n=30 per group) in a relatively old population with Type 2 diabetes, where the potential effect of other significant comorbidities was not assessed (149). Evidence from more than 50,000 participants from the UK Biobank has shown that tortuosity is significantly increased due to advancing age (2.39% and 2.46% increase in arteriolar and venular tortuosity respectively per decade) and increased BP (1.15% and 0.63% increase in in arteriolar and venular tortuosity respectively per 10mmHg systolic BP) (166) so these factors should be considered when considering changes in tortuosity indices.

The use of tortuosity measures in diabetes retinal imaging is becoming increasingly widespread, however comparisons across studies remains a significant challenge. Different definitions are frequently used when referring to tortuosity, including simple versus curvature tortuosity as discussed in Chapter 1.6.2. Various semi-automated software devices are also used for subsequent calculations (167). As tortuosity values are numerically very small they are particularly prone to errors secondary to differences in calculation methodology (168). Findings are however generally consistent in concluding that increased tortuosity is associated with diabetes pathology.

3.1.2.3 Fractal Dimensions

The relationship between retinal vascular network branching patterns and DR is less well established, with some conflicting studies. Higher FD indices, indicating a more complex retinal branching pattern, were found in a Type 1 diabetes study of 729 adolescents with

established retinopathy compared to those with no evidence of retinopathy (OR 1.33 for each 0.01 increase in FD) (169). When FD values were stratified into quartiles, the group with the highest values (>1.47217) were found to be at increased risk of DR (OR 3.92) versus those with values in the lowest quartile (<1.45023).

Higher baseline FD measurements are also associated with increased risk of incident DR in a study of 102 participants with Type 1 diabetes (highest versus lowest tertile, OR 3.4) (154). Similar findings were seen in an Asian population with Type 2 diabetes where risk of incident DR decreased (OR 0.38) for each SD reduction in baseline FD (160). Though these studies suggest a role for increased FD as a biomarker for PDR, a long-term Danish study evaluating 181 people with type 1 diabetes over 16 years found no association. However, 43 of the 48 patients with PDR had received treatment with pan-retinal photocoagulation prior to assessment which may have affected FD measurements. The study did show that increased FD indices were associated with neuropathy and nephropathy, suggesting that it does play a role in predicting the risk of microvascular diabetes complications (170).

Conversely, one Type 2 diabetes study assessing fundus photographs in 718 people without retinopathy found lower FD values were associated with incident retinopathy at 10 year follow up (164).

Studies using OCTA suggest that reductions in FD are associated with increased DR risk. A small study of 13 eyes (8 participants) with known DR found mean FD in the superficial plexus was lower than in 56 eyes (29 participants) of healthy controls (FD 1.563 versus 1.638, $p = 4.513 \times 10^{-3}$) (171). This correlates with findings from another study assessing 84 eyes from 50 participants with DR where FD was lower in people with diabetes versus healthy controls, with lower FD values in those with increased DR severity (mean FD 1.695 in mild DR, 1.695 moderate DR, 1.665 PDR, 1.717 healthy controls) (172). (172). A larger study assessing 434 eyes (286 participants) with diabetes also found FD was significantly reduced with increasing DR severity (173). Reported FD values from OCTA are generally higher across all groups than those reported from fundus photography, and may reflect better vessel detection in the former. As with vessel tortuosity measurements, methods used to calculate FD varies across studies.

The mechanisms behind an increase in FD indices and potential increased microvascular disease are unclear. An increased branching pattern and complexity of the vascular network resulting from neovascularisation is a reasonable expectation and has been described previously (174, 175). However, increased fractal indices were also seen in studies without cases of neovascularisation (169). It has therefore been proposed that it is an early predictor of neovascularisation and may indicate arteriovenous differentiation in response to hypoxic cues (169).

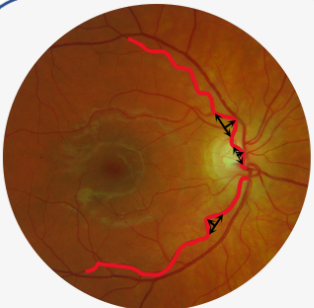
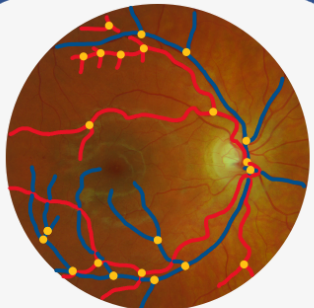
	 Vessel Calibre	 Vessel Tortuosity	 Fractal Dimension
Diabetes Versus Healthy Control	Increased vessel width in diabetes	Increased tortuosity in diabetes	Decreased FD in diabetes assessed by OCTA
Increased DR Severity	Increased width with increased severity	Increased tortuosity with increased severity	Increased FD in fundus photography studies Decreased FD in OCTA studies
DR Incidence and Progression	Increased width associated with increased DR incidence	Increased tortuosity associated with increased DR incidence	Mixed reports in fundus studies. Decreased FD associated with decreased incidence in OCTA studies

Figure 3-2 Illustrative summary of findings from current literature on retinal biomarkers in diabetes

3.2 Methods

3.2.1 Study design

A retrospective observational cohort study was conducted using DRS screening data for people with Type 1 diabetes who had been allocated to receive a change to their diabetes management through the introduction of either CSII therapy or islet transplantation. Longitudinal assessment of retinal images was performed to compare images pre and post intervention. The primary outcomes being assessed were changes in vessel calibre, vessel tortuosity and FD. The study protocol was approved by the Caldicott Guardian, the Scottish Diabetes Research Network, the DRS Collaborative Coordinator and Lead Clinician, and the Lothian Research Ethics Committee.

3.2.2 Study Participants

Images were analysed for two patient groups which have previously been described in Chapter 2.2.2:

1. CSII group
2. Edinburgh Islet transplant group

Inclusion criteria were the same as in Chapter 2.2.2.4 for islet transplant and CSII cohort. Due to large numbers of images for the entire CSII cohort, a sample of 80 participants from the CSII group were randomly selected for image analysis.

3.2.3 Data collection

Fundus photographs were obtained from the Scottish DRS Collaborative. Retinal data for people who had been identified as being suitable for either the CSII or islet group using the criteria described in Chapter 2.2.2.4 were reviewed via the DRS Collaborative, alongside corresponding intervention dates for commencing CSII therapy or receiving first islet transplantation. All images from two years pre intervention until the date of data extraction were obtained for analysis, in addition to the corresponding DRS grading which had been

assigned to the image. Image analysis using scanning laser ophthalmoscopy would also have ideally been used, however as this imaging did not form part of the screening protocol such images were not widely available for analysis. HbA1c values for the study period and baseline demographic data were extracted from the SCI diabetes database as per Chapter 2.2.3.

3.2.4 Image selection and assessment

Images were assessed at three timepoints: baseline, timepoint 1, taken at one year follow up, and timepoint 2, using the most recent available follow up image. Study entry was defined as the date of CSII start for the CSII group, and date of first islet transplantation for the islet group. The most recent image prior to study entry was taken as the baseline image for both groups. Images up to two years prior to study entry were accepted as the baseline image. Images as close to one year post study entry were taken as the timepoint 1 image, with images within 8 months to 18 months from study entry accepted. The last available image at the time of data extraction was taken as the image for timepoint 2. Only images taken more than two years from study entry were accepted for timepoint 2.

Images had been taken as part of the DRS service and were subject to a standard quality assessment as part of the screening assessment. Retinopathy grading was also independently assigned as part of the DRS service by an experienced grader using the SDRG Scheme. All images were macula centred fundus photographs. Images were taken across various DRS sites across Scotland, and as such a number of different fundus cameras were used. Information regarding the type of camera used for each image was not available.

Where more than one image had been taken for an eye on the same date, images were reviewed for further quality assessment and the image deemed to be of highest quality selected (assessment included position of optic disc, clarity of vessels, image brightness, presence of artefact). If there were no discernible quality differences between duplicate images, the first image taken on that date was selected for analysis.

3.2.5 VAMPIRE analysis

Baseline, timepoint 1 and timepoint 2 images for CSII and islet groups were uploaded to the VAMPIRE online system for analysis. Optic disc position, fovea position and vessel classification were manually checked for each image by the same operator. Images were then sent for further analysis using VAMPIRE.

The following data was validated for assessment by the automated VAMPIRE analysis:

1. Vessel calibre:

- Central Retinal Arteriole Equivalent (CRAE) in pixels
- Central Retinal Venular Equivalent (CRVE) in pixels
- Arteriole to Venule Ratio (AVR)

CRAE, CRVE and AVR were calculated using the 6 largest arterioles or venules crossing zone B based on the Parr-Hubbard-Knudtson method (See chapter 1.6.3) (95, 141).

2. Vessel Tortuosity:

- Number of straight arterioles and venules in Zone C
- Median, maximum and mean logarithm tortuosity for arterioles and venules

Tortuosity refers to curvature tortuosity (derived from the integral of the total squared curvature along the path of the vessel divided by the total length of the vessel arc) (see chapter 1.6.2) (176). As tortuosity is positivity skewed, the logarithm is used to normalise data for analysis and is reported in the results. All raw tortuosity values are less than 1 so all log transformed values are negative. Higher values (approaching 0) are more tortuous.

3. Fractal Dimensions (FD):

- FD (and standard deviation) within whole field of view (FOV) for arterioles, venules, and all combined vessels
- FD (and standard deviation) within Zone C for arterioles, venules, and all combined vessels

- FD (and standard deviation) within Zone C for main arterioles, venules, and all main combined vessels

FD measurements were calculated using skeleton images of the retinal vasculature generated from fundus photographs using the box counting technique (See chapter 1.6.4) (97).

Retinal zones for analysis

The retinal biomarkers above are calculated using a grid-based system to standardise the area of interest for comparison. This grid is centred on the optic disc to form zones A, B and C as previously described in chapter 1.5.5. All images for this study were derived from the DRS programme and were therefore macula centred images with a standard FOV of approximately 30 to 45 degrees. As such, vessels in the nasal segment of Zone C are not fully visualised. While this was true for all images, the extent of this varies depending on the specific position of the optic disc relative to the image centre. As such, this study has preferentially focussed on regions that do not primarily involve only Zone C for analysis where possible. This affected FD analysis, where only data from the whole FOV was used. Vessel tortuosity is calculated from vessels in Zone C, therefore no alternative region could be used for analysis. This is considered further in the discussion.

3.2.6 Statistical analysis

All analyses described below were performed using R Studio version 3.4.1. Results are expressed for continuous data as mean with SD or median with IQR, and as percentages for categorical data.

Baseline demographics and metabolic data for all comparator groups were assessed using unpaired t-test for continuous numerical data and Chi-squared or Fisher's exact test for categorical data. Linear regression analysis was carried out to assess univariate relationships between baseline variables (age, weight, BMI, gender, diabetes duration, baseline HbA1c, systolic BP, diastolic BP, creatinine and cholesterol) and baseline retinal biomarkers.

Retinal biomarker values at timepoint 1 and timepoint 2 were compared to baseline values for CSII and islet groups using unpaired t-test for all available patients, and paired t-test for participants with images at all three timepoints. All participants from both CSII and islet groups had at least one follow up image at timepoint 1 or timepoint 2.

Analysis of VAMPIRE data was primarily completed for right eye images for each group, as is convention, with the left eye analysis performed to check findings. Assessment of correlation between retinal biomarker data for right and left eyes at each timepoint was also calculated using Spearman correlation coefficient.

To evaluate differences in the magnitude of biomarker changes between CSII and islet groups at one year ('Delta 1' calculated as timepoint 1 biomarker value minus baseline biomarker value) and most recent follow up ('Delta 2' calculated as timepoint 2 biomarker value minus baseline biomarker value), deltas were compared using a multiple linear regression model, adjusting for age, blood pressure and baseline HbA1c.

VAMPIRE results for participants from either group that had been graded as either no retinopathy (R0) or mild retinopathy (R1) at baseline (right eye) were also compared. Numbers of participants with higher severity gradings (R2-R4) were very small and they were therefore not compared. Baseline demographics and retinal biomarkers were compared between R0 and R1 groups using unpaired t-test.

A p score of <0.05 was considered to be statistically significant for all calculations. It was acknowledged that, as multiple characteristics were being assessed, there was an increased risk of results showing statistical significance by chance and consideration was given to performing additional analyses in the future using the Bonferroni method to counteract this, though this was not carried out during this study.

As there was limited preliminary data regarding VAMPIRE retinal outcomes in people with diabetes, power analyses were not conducted prior to the study.

3.3 Results

3.3.1 Study Numbers and Baseline Characteristics

Our records identified 293 people who had commenced on CSII during the study period. Retinal images were obtained for 213/293 (23 were aged under 16 at time of CSII start, 26 had no baseline or follow up images, 31 were not in the screening programme due to active management under ophthalmology). Of these 80 were randomly selected for VAMPIRE analysis due to large numbers of images for processing in the entire cohort. Images from the right eye were primarily assessed. Two images were rejected for analysis by the Vampire software due to artefact. Of the remaining 78 participants analysed, 55 had images taken at all three of the timepoints being assessed. There were 63 people with images at baseline and timepoint 1, and 68 people with images at baseline and timepoint 2. Image numbers were similar for the left eye, with 79 participants analysed (one image rejected by Vampire), 59 with images at all three timepoints, 67 with baseline and timepoint 1 images, and 71 with baseline and timepoint 2 images. Mean follow up was 415 days (SD 83 days) for timepoint 1 images and 1210 days (SD 467 days) for timepoint 2 images.

There were 49 people who had received an islet transplant in Edinburgh during the study period. Retinal images were obtained for 30/49 (2 were not from Scotland and therefore not participating in the Scottish DRS programme, 17 had either no baseline or follow up images). For the right eye, one image was rejected by the VAMPIRE software leaving 29 with baseline images. Of these 11 had images at all three timepoints, with 20 people having had baseline and timepoint 1 images taken, and 11 having had baseline and timepoint 2 images taken. For the left eye no images were rejected by the Vampire software, leaving 30 people with baseline images, 12 people with images at all three timepoints, 18 people with images at baseline and timepoint 1, and 12 people with images at baseline and timepoint 2. Mean follow up was 408 days (SD 114 days) for timepoint 1 images and 1375 days (SD 665 days) for timepoint 2 images .

Baseline demographic and metabolic data for CSII and Islet groups are shown in Table 3-1. CSII participants were significantly younger with shorter duration of diabetes, higher weight and lower creatinine at baseline.

Univariate linear regression analysis revealed older age, longer duration of diabetes and higher serum creatinine were associated with lower arteriolar FD, CRAE and CRVE. Older age and higher serum creatinine were associated with reduced tortuosity, while higher systolic blood pressure was associated with increased tortuosity.

On multiple linear analysis adjusting for age, gender, diabetes duration, systolic BP and creatinine, only older age was found to be significantly associated with lower arteriolar FD and reduced tortuosity, while increased creatinine was associated with lower CRAE.

Baseline HbA1c showed no association with any retinal biomarkers on univariate or multivariate regression analysis.

Chapter 3: Assessment of vascular retinal biomarkers on fundus images

Table 3-1 Baseline Demographic and Metabolic Data for CSII and islet participants

Baseline Demographic and Metabolic Data				
Characteristic		CSII (n=78)	Islet (n=29)	p value
Age at Diagnosis (years)		16 (10 – 27)	15 (9 – 19)	0.08658
Age at Study Entry (years)		42 (35 – 53)	50 (46 – 54)	<0.001*
Diabetes Duration (years)		23 (13 – 31)	36 (28 – 41)	<0.001*
Gender M:F		26:52	8:20	
Weight (kg)		77.65 (67.50 – 89.72)	69.95 (61.80 – 82.30)	0.0432*
BMI (kg/m ²)		27.48 (23.91 – 30.98)	25.65 (22.63 – 28.65)	0.05668
Baseline HbA1c (mmol/mol)		64 (55 – 71)	57 (50 – 69)	0.2137
Systolic BP (mmHg)		128 (118 – 140)	136 (119 – 149)	0.6461
Diastolic BP (mmHg)		79 (75 – 84)	73 (66 – 82)	0.05362
Creatinine (μmol/L)		72 (67 – 80)	98 (80 – 119)	<0.001*
Cholesterol (mmol/L)		4.5 (4.1 – 5.1)	4.4 (3.5 – 4.9)	0.2026
HbA1c one year post treatment (mmol/mol)		60 (54 – 66)	53 (43 – 59)	0.05686
Change in HbA1c at one year (mmol/mol)		-3 (-8 – 2)	-7 (-14 – -1)	0.278
Baseline Retinopathy Grading	R0	48 (61.5%)	11 (39.3%)	
	R1	30 (38.5%)	16 (57.1%)	
	R2	0	0	
	R3	0	1 (0.04%)	

3.3.2 Changes in Retinal Biomarkers in CSII group over time

Changes in retinal biomarkers for CSII participants over time is summarised in Table 3-2. CSII participants had significantly lower FD for arterioles and venules at timepoint 1 (arterioles 1.27, $p=0.03$, venules 1.28, $p=0.02$) and timepoint 2 (arterioles 1.24 $p=1.8 \times 10^{-8}$, venules 1.26, $p=2.1 \times 10^{-6}$) follow up compared to baseline images (arterioles 1.29, venules 1.30) (Figure 3-3). CRAE and CRVE were also significantly reduced at timepoint 2 compared to right eye baseline images (CRAE 27.55 vs. 30.91, CRVE 38.58 vs 43.37, $p<0.01$) (Figure 3-4). No significant differences were found in follow up tortuosity values from baseline (Figure 3-5). Paired analysis ($n=55$) also yielded the same outcomes, with significant reductions in FD and vessel widths on follow up images.

Findings for analysis of left eye images were similar, though values for FD did not reach significance for timepoint 1 images compared to baseline.

The proportion of participants with detectable retinopathy using SDRG grading increased from 38.4% at baseline to 47.1% at >2 year follow up.

Chapter 3: Assessment of vascular retinal biomarkers on fundus images

Table 3-2 Changes in vessel calibre, fractal dimensions and mean tortuosity in CSII participants over time

Section	Analysis	Baseline (n=78)	Timepoint 1 (n=68)	Timepoint 2 (n=70)
Vessel Calibre (pixels)	CRAE	30.91 ± 7.19	30.07 ± 6.81	27.55 ± 7.07**
	CRVE	43.37 ± 10.67	41.71 ± 9.22	38.58 ± 10.45**
	AVR	0.72 ± 0.08	0.73 ± 0.07	0.72 ± 0.11
Fractals Whole FOV	Artery	1.29 ± 0.05	1.27 ± 0.06*	1.24 ± 0.06***
	Vein	1.30 ± 0.04	1.28 ± 0.05*	1.26 ± 0.06***
	Total	1.42 ± 0.03	1.40 ± 0.05**	1.38 ± 0.06***
Mean Tortuosity	Artery	-8.24 ± 0.38	-8.26 ± 0.37	-8.19 ± 0.52
	Vein	-8.39 ± 0.34	-8.32 ± 0.37	-8.36 ± 0.40

*p<0.05, **p<0.01, ***p<0.001 compared to baseline

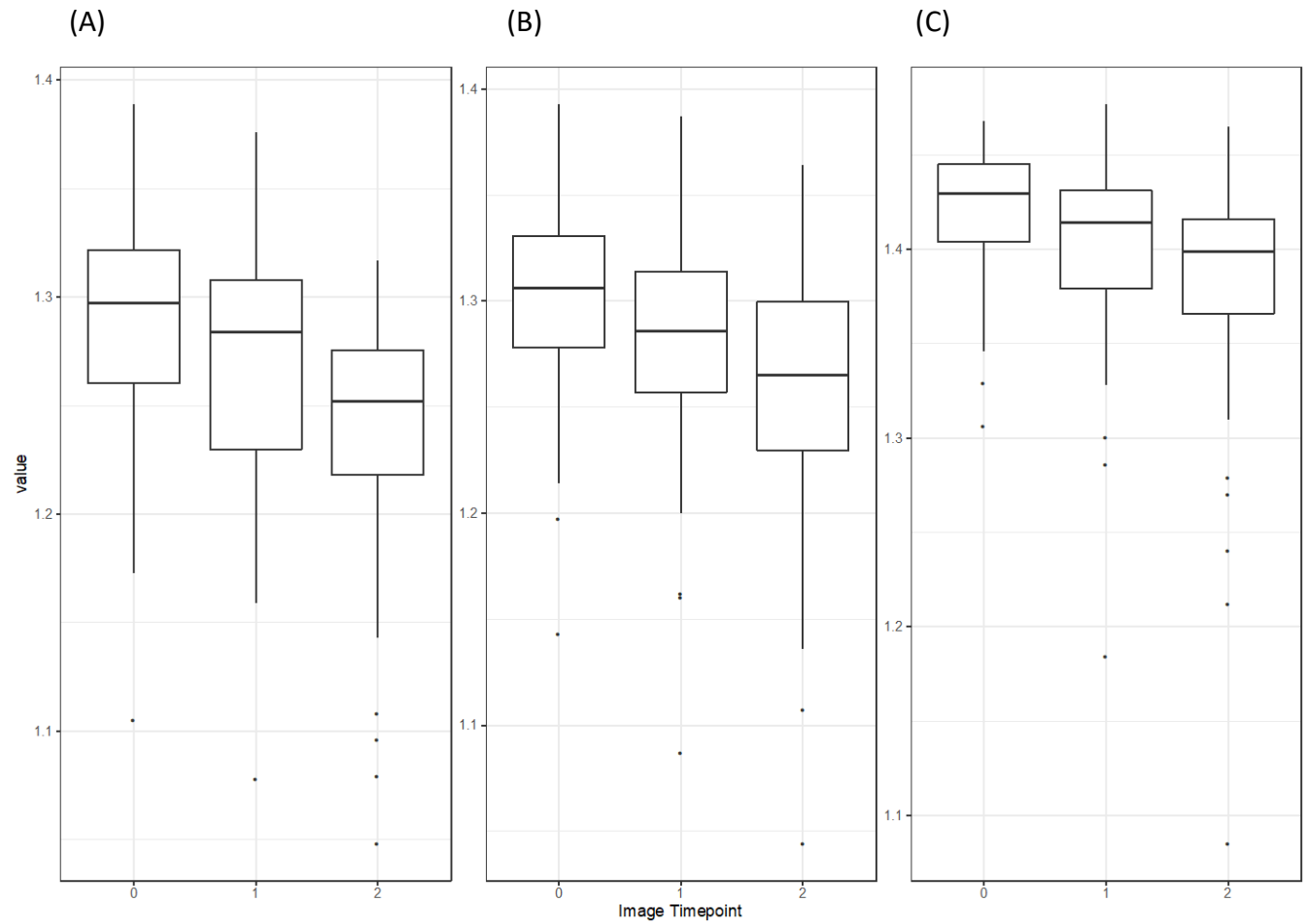


Figure 3-3 Boxplots showing changes in fractal dimensions in CSII participants over time for (A) arterioles, (B) venules and (C) all vessels. CSII participants had significantly lower FD at timepoint 1 and timepoint 2 compared to baseline.

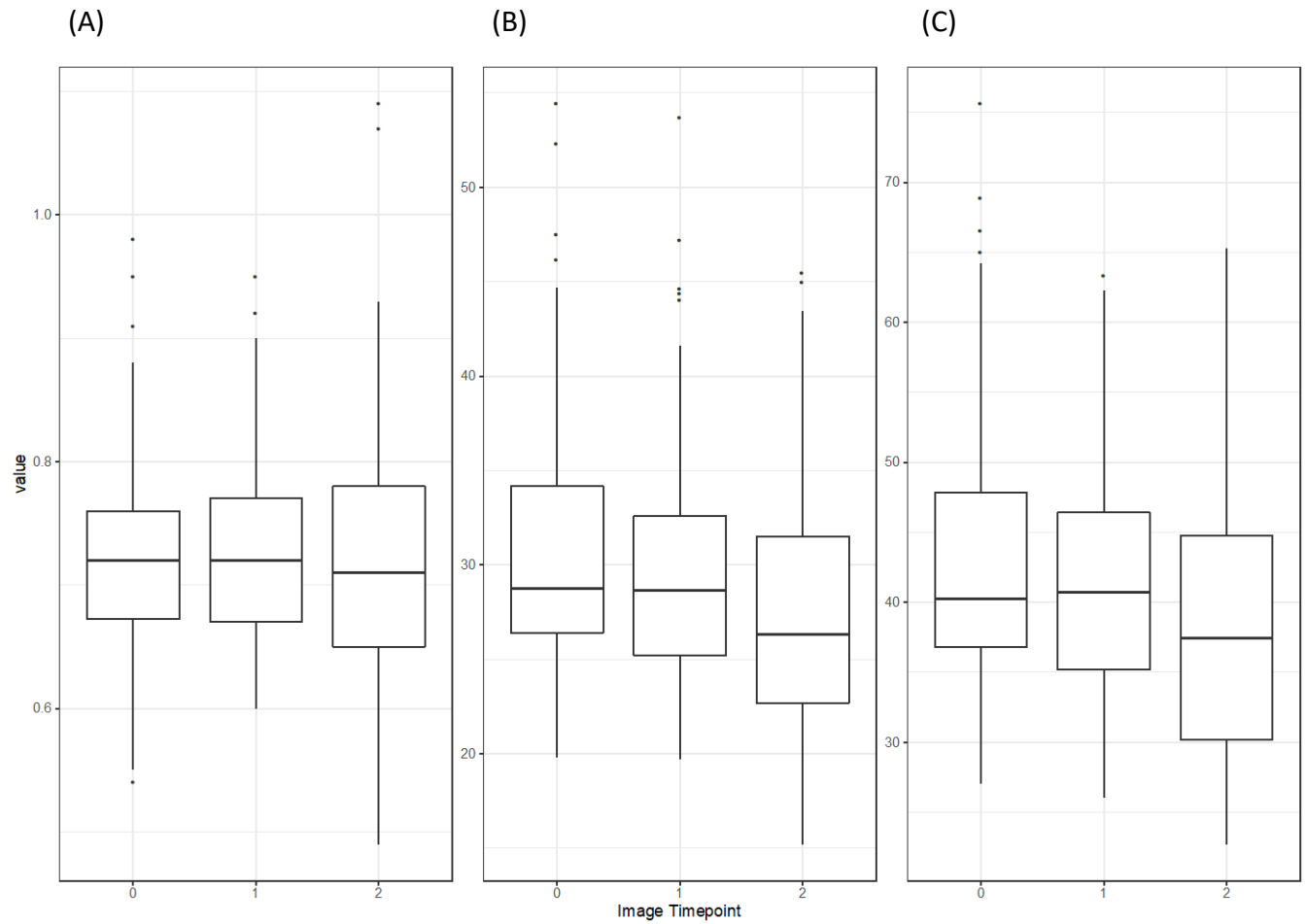


Figure 3-4 Boxplots showing changes in vessel calibre in CSII participants over time for (A) AVR, (B) CRAE and (C) CRVE. CSII participants had significantly lower CRAE and CRVE at timepoint 2 compared to baseline. There was no significant change in AVR across timepoints.

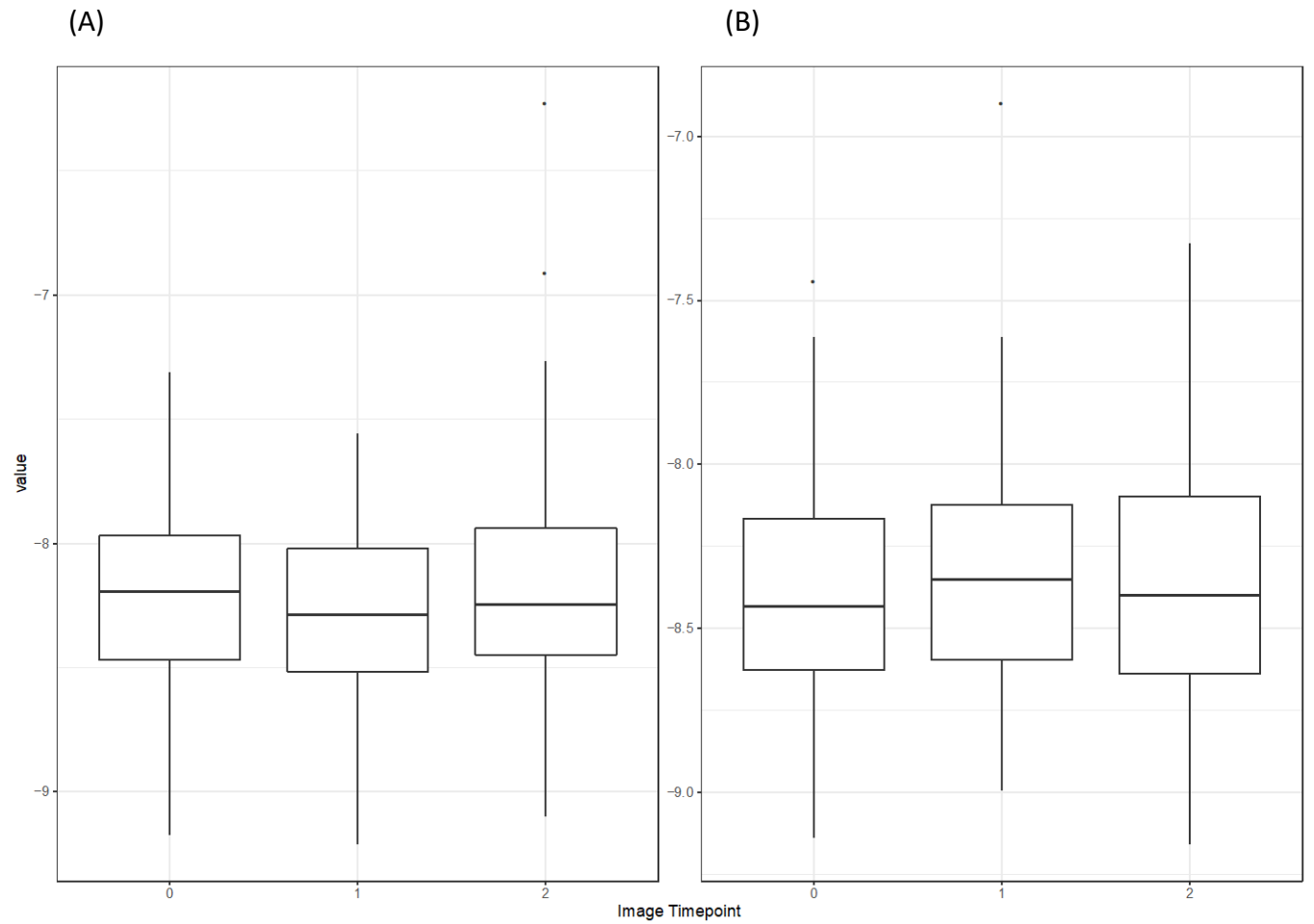


Figure 3-5 Boxplots showing changes in vessel tortuosity in CSII participants over time for (A) arterioles and (B) venules. No significant changes were seen in tortuosity across the timepoints

3.3.3 Changes in Retinal Biomarkers in islet transplanted group over time

Participant numbers for the islet group were small leading to an underpowered analysis for the whole cohort. There were no significant differences for retinal biomarkers seen at either timepoint 1 or 2 when compared to baseline images (Table 3-3, Figure 3-6, Figure 3-7, Figure 3-8). The proportion of participants with detectable retinopathy using SDRG grading decreased from 62.1% at baseline to 50.0% at timepoint 2.

Table 3-3 Changes in vessel calibre, fractal dimensions and mean tortuosity in islet participants over time

Section	Analysis	Baseline (n=29)	Timepoint 1 (n=23)	Timepoint 2 (n=12)
Vessel Width (pixels)	CRAE	24.34 ± 5.25	23.76 ± 5.12	22.78 ± 4.07
	CRVE	34.65 ± 7.59	34.29 ± 4.80	32.19 ± 5.60
	AVR	0.71 ± 0.12	0.70 ± 0.16	0.72 ± 0.15
Fractals Whole FOV	Artery	1.26 ± 0.07	1.22 ± 0.09	1.24 ± 0.06
	Vein	1.26 ± 0.05	1.25 ± 0.05	1.24 ± 0.10
	Total	1.40 ± 0.04	1.38 ± 0.05	1.36 ± 0.09
Mean Tortuosity	Artery	-8.41 ± 0.43	-8.25 ± 0.50	-8.38 ± 0.39
	Vein	-8.36 ± 0.37	-8.25 ± 0.41	-8.53 ± 0.20

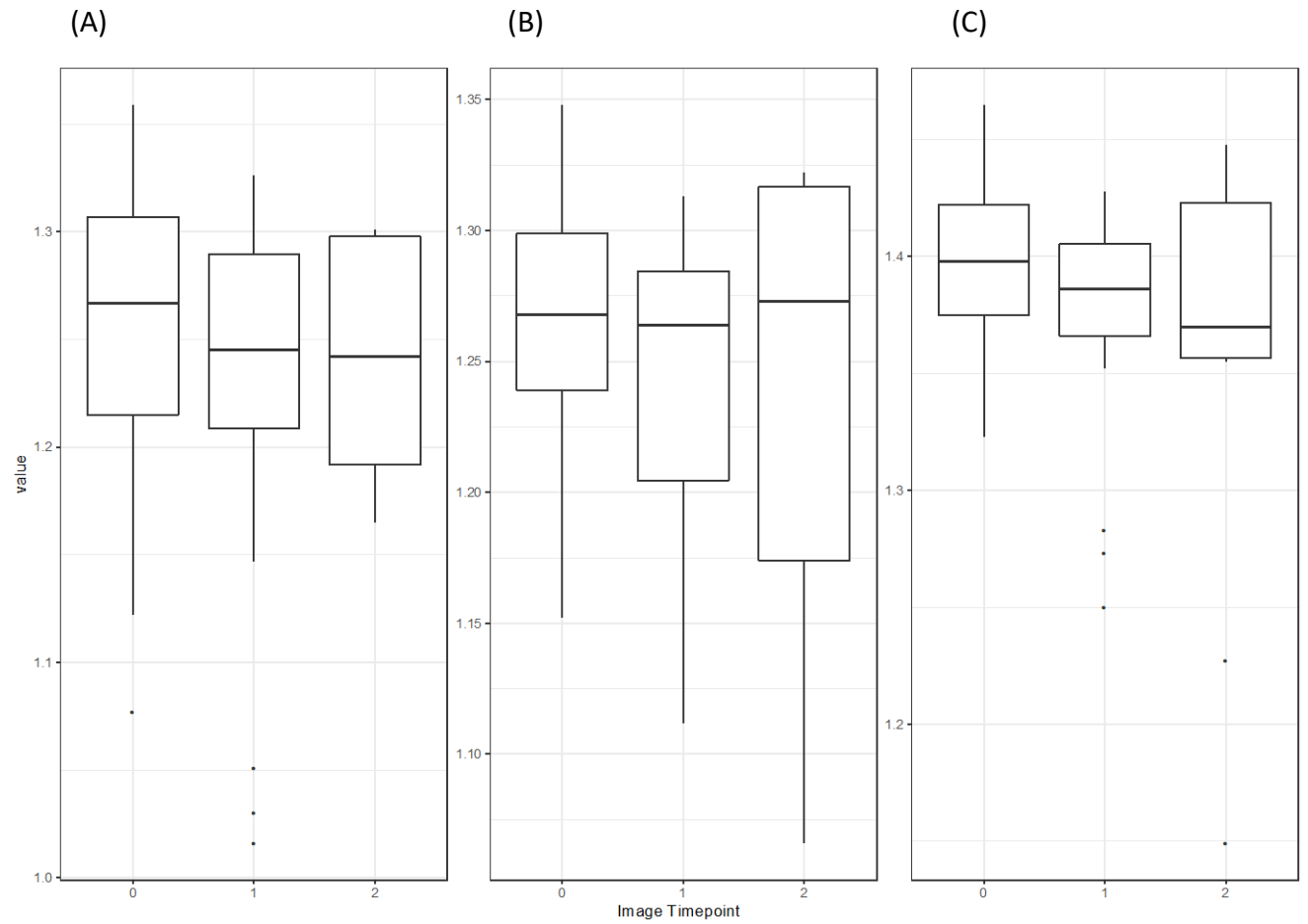


Figure 3-6 Boxplots showing changes in fractal dimensions in islet participants over time for (A) arterioles, (B) venules and (C) all vessels. No significant changes were seen across the timepoints.

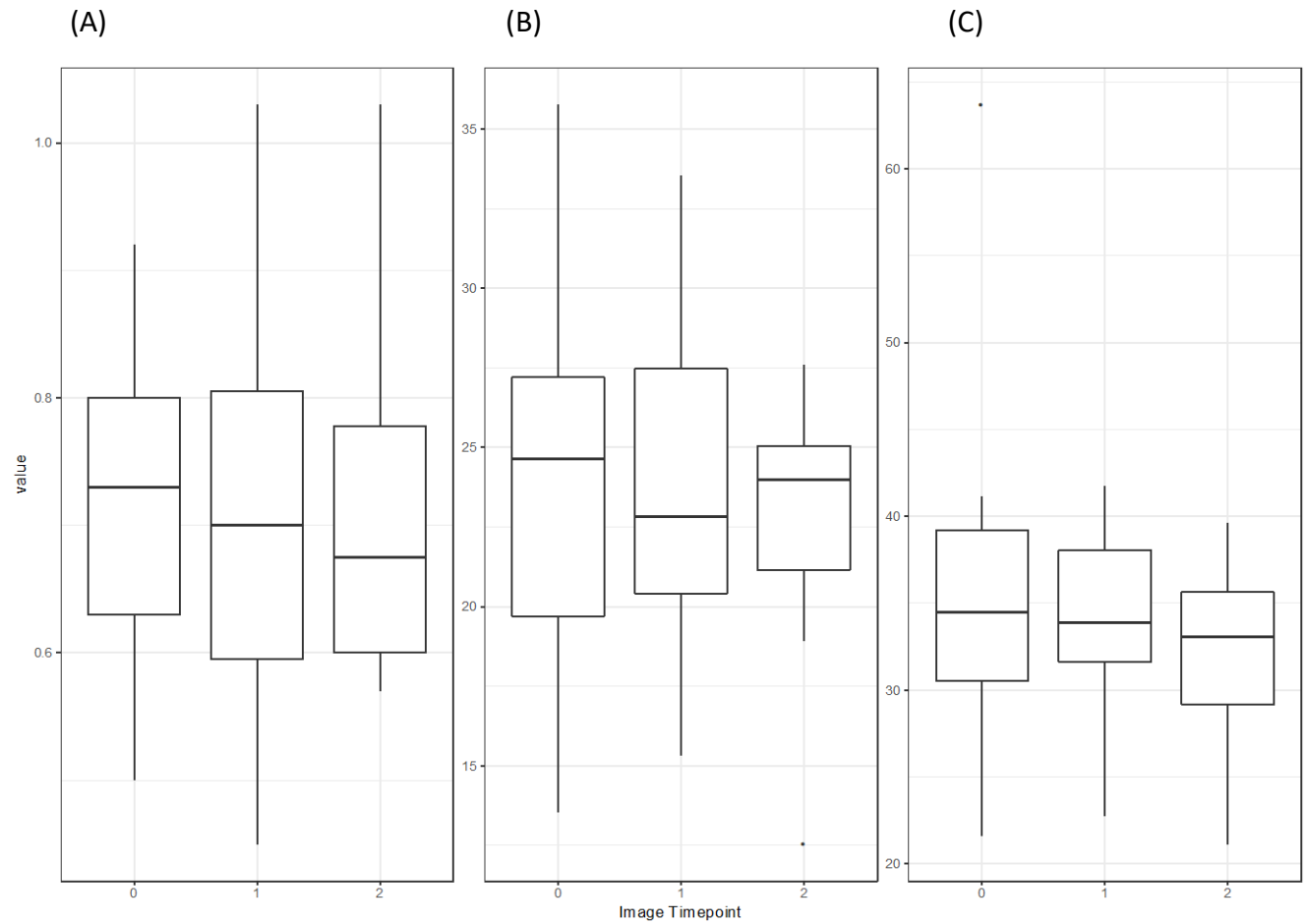


Figure 3-7 Boxplots showing changes in vessel calibre in islet participants over time for (A) AVR, (B) CRAE and (C) CRVE. No significant changes were seen across the timepoints.

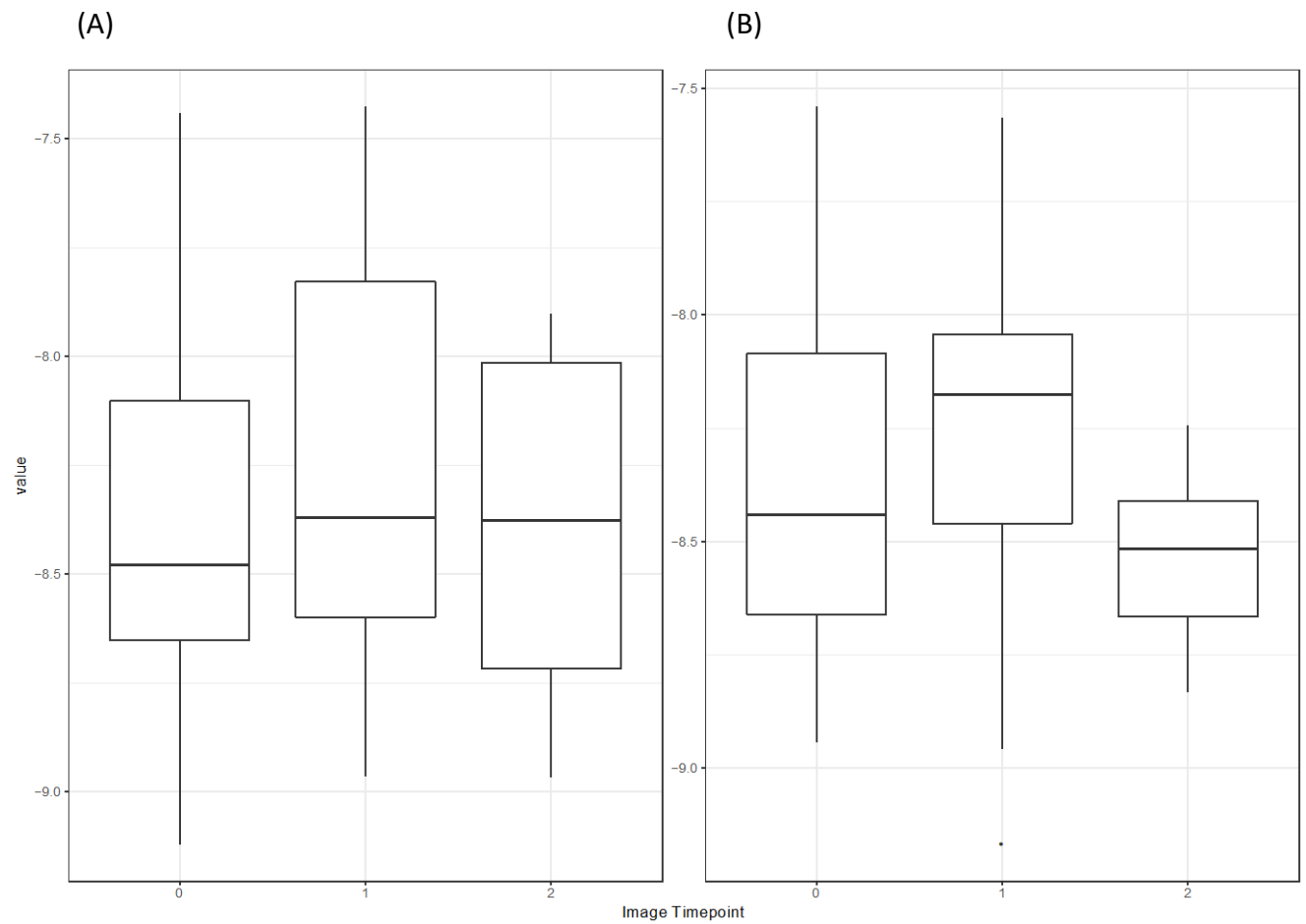


Figure 3-8 Boxplots showing changes in vessel tortuosity in islet participants over time for (A) arterioles and (B) venules. No significant changes were seen in tortuosity across the timepoints.

3.3.4 Comparison of retinal biomarkers in CSII group versus islet transplantation group

Comparisons of CSII versus islet groups at baseline found significantly narrower CRAE (24.3 versus 30.9 pixels, $p < 0.0001$) and CRVE (34.6 versus 43.4 pixels, $p < 0.0001$), and lower arteriolar FD (1.26 versus 1.29, $p = 0.02$) and venular FD (1.26 versus 1.30, $p = 0.002$) in the islet group (Table 3-4). There were no significant differences in rate of biomarker changes between islet and CSII groups at timepoint 1 (delta 1) or timepoint 2 (delta 2).

Table 3-4 Comparison of retinal biomarkers at baseline for CSII and islet participants. Islet participants had significantly narrower CRAE and CRVE, and lower arteriolar FD and venular FD when compared to CSII participants.

Section	Analysis	CSII baseline (n=78)	Islet baseline (n=29)
Vessel Width (pixels)	CRAE	30.91 ± 7.19	24.34 ± 5.25***
	CRVE	43.37 ± 10.67	34.65 ± 7.59***
	AVR	0.72 ± 0.08	0.71 ± 0.12
Fractals Whole FOV	Artery	1.29 ± 0.05	1.26 ± 0.07*
	Vein	1.30 ± 0.04	1.26 ± 0.05**
	Total	1.42 ± 0.03	1.40 ± 0.04
Mean tortuosity	Artery	-8.24 ± 0.38	-8.41 ± 0.43
	Vein	-8.39 ± 0.34	-8.36 ± 0.37

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ compared to pump baseline

3.3.5 Comparison of retinal biomarkers in diabetes patients with no diabetic retinopathy versus background diabetic retinopathy

There were 59 participants (48 CSII and 11 islet) with R0 grading and 47 participants (30 CSII and 17 islet) with R1 grading at baseline (right eyes only). Mean creatinine at baseline was significantly higher in R1 group versus R0 group (87 vs $77\mu\text{mol/L}$, $p=0.0432$). There were no other significant differences in baseline demographics or metabolic data. There were no significant differences between the baseline retinal biomarkers for R0 or R1 graded images (Table 3-5) or the magnitude of retinal biomarker changes at timepoint 1 (delta 1) or timepoint 2 (delta 2).

Table 3-5 Comparison of retinal biomarkers for all CSII and islet participants graded either R0 or R1 at baseline. There were no significant differences between the retinal biomarkers in those with R0 grading versus those with R1 grading at baseline.

Section	Analysis	R0 (n=59)	R1 (n=47)
Vessel Width (pixels)	CRAE	30.33 ± 7.85	27.66 ± 6.43
	CRVE	42.38 ± 10.77	39.31 ± 10.43
	AVR	0.72 ± 0.09	0.71 ± 0.09
Fractals Whole FOV	Artery	1.28 ± 0.06	1.27 ± 0.06
	Vein	1.30 ± 0.04	1.28 ± 0.05
	Total	1.42 ± 0.03	1.41 ± 0.04
Mean tortuosity	Artery	-8.26 ± 0.38	-8.30 ± 0.41
	Vein	-8.41 ± 0.31	-8.34 ± 0.39

3.3.6 Correlation between left and right eyes for all participants

Data for both eyes was available in 106 islet and CSII participants at baseline, 84 participants at one year and 78 participants at last available follow up. Positive correlation was present and significant for all biomarkers other than mean venular tortuosity (Table 3-6, Figure 3-9). Comparison of biomarker data for all islet and CSII participants with available data for both eyes revealed excellent correlation for CRAE and CRVE (R 0.76-0.89), moderate correlation for fractal dimension measurements (R 0.58- 0.78), and poor correlation for tortuosity measurements (R 0.03 – 0.40), particularly mean venular tortuosity (R 0.03-0.34). Findings were similar across all three timepoints (Table 3-7).

Table 3-6 Comparison of symmetry in right and left eyes for all islet and CSII participants at baseline

Correlation Coefficient comparing right and left eyes for islet and pump participants						
Retinal Biomarker		Mean (SD) Left Eye	Mean (SD) Right Eye	Mean difference (CI)	Spearman's Coefficient	Spearman's P value
Vessel Width (pixels)	CRAE	29.17 (7.19)	29.09 (7.34)	-0.10 (-0.56 - 0.36)	0.89	<0.0001
	CRVE	40.94 (10.12)	41.01 (10.68)	0.31 (-0.26 - 0.87)	0.88	<0.0001
	AVR	0.72 (0.08)	0.72 (0.09)	-0.01 (-0.03 - 0.001)	0.46	<0.0001
Fractals Whole FOV	Artery	1.28 (0.06)	1.28 (0.06)	0.0029 (-0.0026 - 0.0087)	0.70	<0.0001
	Vein	1.29 (0.05)	1.29 (0.05)	0.0021 (-0.0030 - 0.0072)	0.68	<0.0001
	Total	1.41 (0.04)	1.42 (0.09)	0.0008 (-0.0056 - 0.0039)	0.75	<0.0001
Mean Tortuosity	Artery	-8.26 (0.35)	-8.29 (0.39)	0.03 (-0.03 - 0.09)	0.4	<0.0001
	Vein	-8.36 (0.37)	-8.38 (0.35)	0.04 (-0.02 - 0.10)	0.03	0.76

Chapter 3: Assessment of vascular retinal biomarkers on fundus images

Table 3-7 Comparison of Spearman's coefficient assessing symmetry between right and left eyes for all islet and pump participants at all timepoints

Spearman's Correlation Coefficient comparing right and left eyes for islet and pump participants				
Section	Analysis	Baseline (n=106)	Timepoint 1 (n=84)	Timepoint 2 (n=78)
Vessel Width (pixels)	CRAE	0.89	0.83	0.76
	CRVE	0.88	0.81	0.84
	AVR	0.46	0.38	0.14
Fractals Whole FOV	Artery	0.70	0.75	0.58
	Vein	0.68	0.78	0.73
	Total	0.75	0.74	0.69
Mean Tortuosity	Artery	0.4	0.38	0.28
	Vein	0.03	0.18	0.34

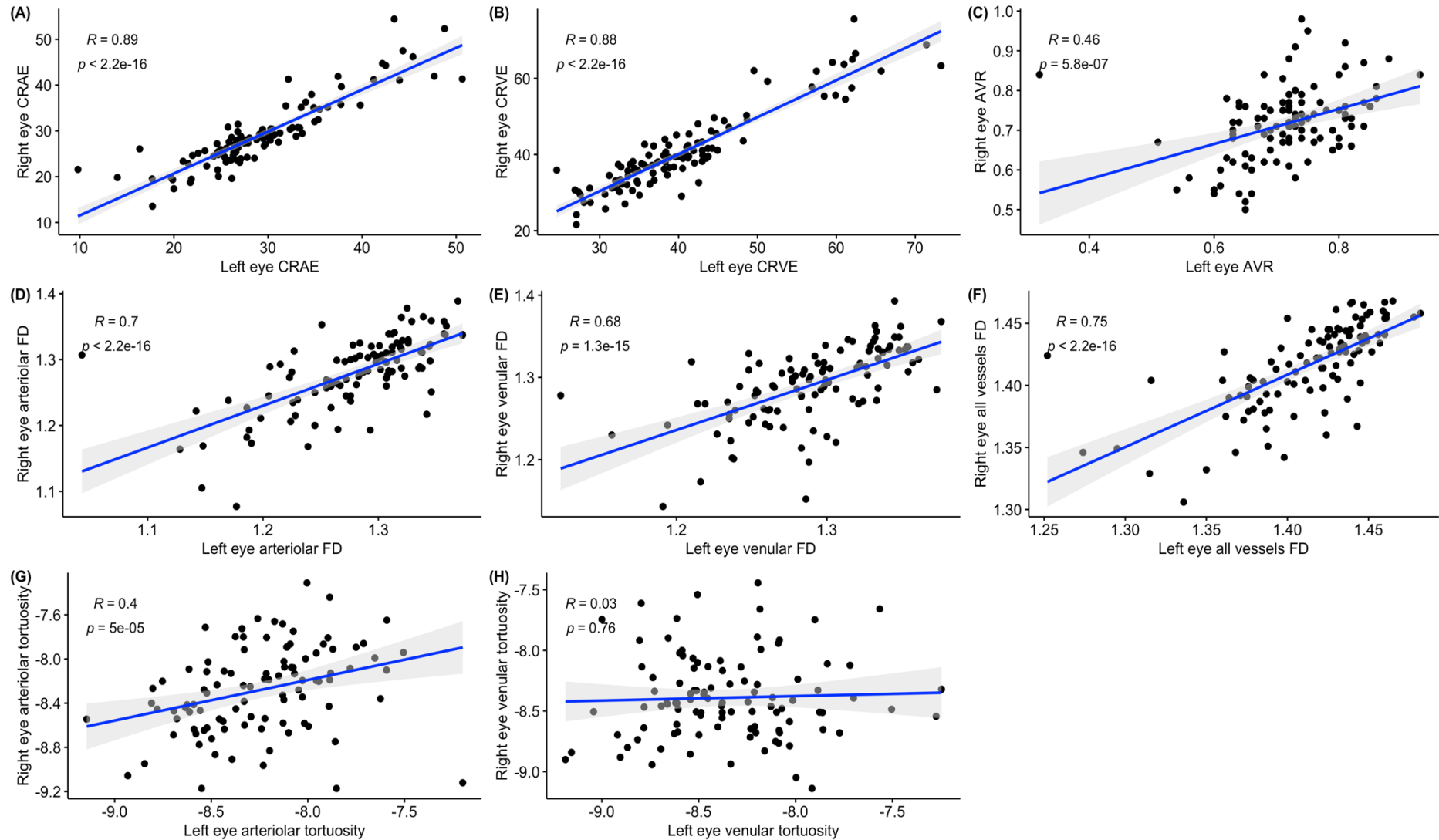


Figure 3-9 Scatter plots with regression line showing symmetry between right and left eyes for markers of vessel calibre: (A) CRAE, (B) CRVE, (C) AVR); fractal dimensions measures in (D) arterioles, (E)venules, (F) all vessels; and tortuosity values in (G) arterioles, (H) venules

3.4 Discussion

3.4.1 Summary of Key Findings

This study showed significant reductions in CRAE, CRVE and FD over time following the introduction of CSII therapy (Figure 3-10). There were no significant differences in the magnitude of retinal biomarker changes from baseline to follow up between CSII and islet transplant groups. Both groups had a significant reduction in HbA1c on follow up, however baseline HbA1c and change in HbA1c at follow up were not significantly associated with retinal biomarker changes.

There was no significant difference in retinal biomarkers at baseline or follow up in individuals with no retinopathy at baseline versus those with mild baseline retinopathy (Figure 3-10). Multiple regression analysis showed older age at baseline was significantly associated with lower arteriolar FD and vessel tortuosity, while higher baseline creatinine was associated with lower CRAE.

Positive correlation between biomarkers for right and left eyes was generally good, particularly for CRAE and CRVE markers, though no significant correlation was found for mean venular tortuosity.

	 Vessel Calibre	 Vessel Tortuosity	 Fractal Dimension
Changes with time following pump therapy	Significant decrease in vessel width over time	No significant change	Significant decrease in FD over time
Changes with time following islet therapy	No significant change	No significant change	No significant change
Comparison of R0 and R1 groups	No significant difference	No significant difference	No significant difference

Figure 3-10 Illustrative summary of key findings of effects of insulin pump therapy and islet transplantation on retinal biomarkers

3.4.2 Vessel Calibre

In the CSII group, CRAE and CRVE were significantly higher at baseline, prior to the introduction of CSII therapy, than at follow up. People with diabetes have previously been shown to have wider vessel width measurements than those of healthy controls, with higher CRAE and CRVE values seen in those with higher DR severity (146, 149-154). Reductions in CRAE and CRVE may therefore represent a reduced burden of retinal pathology due to stabilisation of blood glucose levels following CSII therapy.

CRAE and CRVE were found to be significantly lower in the islet cohort than the CSII cohort at baseline, however the groups had significant differences at baseline, including older age and higher creatinine in islet participants, therefore many factors may have contributed to these differences in baseline vessel calibre.

Follow up HbA1c was significantly reduced in both CSII and islet groups in our study, however, baseline HbA1c and change in HbA1c at follow up did not appear to impact vessel width, therefore the reduction in CRAE and CRVE may be due to other factors.

Increased blood pressure and older age are established to be associated with CRAE narrowing (151, 159). Blood pressure was recorded at baseline but follow up values were not assessed. There were no significant differences in blood pressure between any comparator groups at baseline, and no significant effect of baseline systolic or diastolic BP on vessel widths on regression analyses, therefore it was felt to be unlikely to account for significant differences in CRAE and CRVE at later follow up. Increased age was associated with reduction of CRAE and CRVE on univariate analysis, but not multivariate analysis adjusting for gender, creatinine, blood pressure and diabetes duration.

Higher serum creatinine was associated with reduced CRAE and CRVE on cross sectional analysis of baseline data. Creatinine was not assessed on follow up therefore it is possible that this impacted results. The association of vessel calibre with renal function has been reported previously in the literature in people without diabetes (177), where wider vessel

calibres was found in those with higher eGFR, however a separate study of individuals with type 2 diabetes found no link to renal function and CRAE or CRVE measurements (145).

Absolute vessel width measurements from the literature vary widely between studies, however, most report a percentage difference of between 2% and 6% in mean CRAE or CRVE values when comparing groups with severe diabetic retinal pathology to those without diabetic pathology. We report a larger percentage reduction within our cohort of approximately 11% at most recent follow up (timepoint 2) compared to baseline. Capturing images at different points in pulse cycle have been reported to lead to up to 3.9% or 4.8% change for major retinal arterioles or venules respectively and was not controlled for in this study (178). In addition, measurement of CRAE and CRVE using pixels, rather than μm is likely to have contributed to the larger percentage difference seen in our study. A 30 μm width is estimated to be represented by between 4 and 9 pixels using the highest resolution settings on standard fundus photograph cameras (179), resulting in a single pixel representing a width of between 3 and 7 μm (typically 5-6 μm per pixel reported). The specific resolution will be dependent on various factors relating to the image focus, axial length of the participant and optical properties of the camera (178). Images in this study were taken from different cameras with no available information regarding these variables. Results are therefore prone to overestimation of vessel width, as values will be rounded up if a vessel occupies part of a single pixel region.

There was no change in AVR on follow up in our study and no differences in AVR between groups. AVR is a dimensionless ratio and is therefore not as vulnerable to the magnification issues described above. However, it is impossible to evaluate whether this is due to no actual change in vessel width (and variation in CRAE and CRVE due to magnification and measurement issues) or whether it is because arteriolar and venular widths were significantly reduced but by a similar magnitude.

3.4.3 Fractal Dimensions

FD for the whole retinal FOV were significantly reduced following the introduction of CSII therapy. Reduced FD has been shown to be associated with decreased severity on fundus

photography, while more complex branching patterns corresponding to increased FD are considered to be formed in response to retinal hypoxia in more severe disease. This may therefore represent stabilisation or improvement in retinal disease due to reduced glycaemia, however, similar to vessel calibre analysis, HbA1c measurements did not appear to impact FD. In addition, comparisons of those with DR to those without DR revealed no significant differences in FD, though this was limited to those with only mild retinopathy. Older age was significantly associated with lower FD on cross-sectional analysis of baseline data, therefore reduced FD values on later follow up when participants are older may have been an expected finding. The use of macula centred images may also have affected fractal measurements due to limited visualisation of the nasal segment of Zone C (168). Fractal analyses using OCT images have yielded contradictory findings to those using fundus photography, suggesting that reduced fractals are associated with increased diabetes severity and higher retinopathy risk. As FD assessment is a relatively new area of interest in retinal imaging, it is difficult to draw clear conclusions on the significance of FD changes in these cohorts, particularly in the absence of a clear control group.

Evaluation using both disc centred and macula centred images, plus the addition of a healthy control group and larger numbers of participants with higher severity of retinopathy or a deterioration in retinopathy would have helped clarify whether the reduction in FD truly represents a positive impact of CSII and islet treatment on the retinal vasculature.

3.4.4 Tortuosity

This study did not demonstrate any significant changes in vessel tortuosity following the introduction of CSII or islet therapy. Increased tortuosity is established to be associated with increased DR severity therefore stable tortuosity measures over time are reassuring following the introduction of these therapies.

There was also no significant difference in tortuosity measures between participants with R0 or R1 grading at baseline. Had more participants had higher severity of DR at baseline we may have been more likely to observe higher tortuosity measures associated with increasing DR pathology.

3.4.5 Study strengths and limitations

This study was unique in quantifying retinal pathology following the introduction of CSII or islet transplant therapy in people with type 1 diabetes using vessel morphometry. Strengths included the longitudinal follow up, assessment of baseline metabolic characteristics including BP and renal function, and relatively large islet transplant cohort.

The main study limitation was the lack of control group, either of healthy non-diabetic individuals, or a cohort with type 1 diabetes with no treatment change and no significant HbA1c drop. This makes it difficult to judge what degree of change we would have expected due to age and increased diabetes duration. The main analysis focussed on the right eye which is convention in ophthalmic studies, though further analysis was also done using data from the left eye to confirm findings. Though correlation between biomarkers for left and right eye was strongly positive for almost all parameters, there was no significant correlation found for mean venular tortuosity. Almost all participants were graded to have either no DR or mild DR at baseline. The addition of participants with higher severity retinopathy gradings would also have been useful to fully evaluate the impact of DR severity on retinal vessel biomarkers. This was a retrospective study using retinal screening data, therefore we could not control for variation in image acquisition, including the use of different fundus cameras, how well the images were centred on the macula or the specific image magnification. This may have

particularly impacted vessel calibre results due to measurement in pixels. The availability of a disc centred images, in addition to the macula centred images, would have allowed more robust assessment of retinal Zone C and would have enhanced the analysis. The use of more advanced retinal imaging techniques, such as OCT, would also have been useful to fully characterise vessel morphology.

3.5 Conclusion

We have shown vascular markers of DR to be stable following the introduction of islet transplant or CSII therapy, with possible evidence of improvement in retinal characteristics associated with increased diabetic pathology.

4 Analysis of retinal outcomes following commencement of continuous subcutaneous insulin infusion using multiple retinal imaging techniques

4.1 Introduction

The main aim of this study was to quantify retinal changes in the first year following the introduction of CSII therapy or islet transplantation using multiple retinal imaging techniques to assess for evidence of EWDR and inform current knowledge and screening strategies. This involved prospective evaluation of participants using OCTA, OCT, UWF-SLO and fundus imaging.

4.1.1 Diabetic retinopathy changes in OCTA – FAZ and Vessel Density

Several studies emerging over the past few years have assessed changes in OCTA parameters in diabetic patients with varying severity of retinopathy and these have consistently shown that vessel density is reduced compared to healthy controls, (88, 89, 172, 180-184) with enlargement of the FAZ area in people with diabetes (89, 172, 173, 181, 183-188). Changes correlate with increasing DR severity, with lower vessel density (88, 89, 172, 173, 181-183) and larger FAZ areas (89, 172, 173, 181, 183, 184, 189) demonstrated in those with severe NPDR or PDR compared to those with no evidence of DR or mild disease. While there was some variation in the retinal layer being assessed (typically either the superficial or deep retinal layer) and scan size used (3x3mm or 6x6mm), findings were consistent across a variety of OCTA machines, and in patients of varying ethnicity. Where patients were assessed by both OCTA and FA, there was good correlation with vessel density and FAZ findings using both modalities (181, 190). The perifoveal region is generally regarded as the most sensitive area for detecting changes in vessel density, with one study favouring changes in the temporal perifoveal region as the best indicator for early DR changes (183).

Vessel density changes may precede FAZ enlargement. In two studies assessing people with no or mild DR compared to healthy controls, vessel density was reduced without any significant change in FAZ area (180, 181). Enlarged FAZ area correlates with reduced visual acuity (191) and therefore may indicate later retinopathy changes where vision can be affected. HbA1c and diabetes duration were found to have no correlation with vessel density or FAZ area in two studies (182, 185), though HbA1c did correlate with FAZ area (189) or vessel density (192) in other studies, therefore direct associations with glycaemia measures on these retinal biomarkers remain unclear. Only one study found no significant differences in vessel density and FAZ area between those with diabetes and those without, though this contained just 22 participants with diabetes, with relatively short mean duration of disease (<5years) (193).

While vessel density and FAZ area are the most common retinal markers to be assessed to date, the acircularity index may provide another useful indicator of DR risk. Krawitz et al looked at the acircularity index² in 73 people with type 2 diabetes and 27 age matched controls. They found acircularity index was significantly higher (indicating a more irregular, less circular FAZ perimeter) in NPDR and PDR groups compared to people with diabetes without retinopathy, and in healthy non-diabetic controls (99). Similar findings were found in a large study (286 subjects, 434 eyes) by Tang et al, who noted a correlation between increased FAZ perimeter irregularity³ with reduced visual acuity in addition to increasing DR severity (173).

There is currently limited evidence for the role of OCTA in assessing other retinal markers potentially associated with DR, such as vessel calibre, vessel tortuosity and FD, though a role for the use of these parameters in evaluating DR has been established using fundus photography (Chapter 3.1.2). A small number of studies suggest vessel calibre is increased in DR on OCTA (172, 173), in keeping with findings from fundus images. Vessel tortuosity was also increased in NPDR on OCTA in one study, though, as in fundus image studies, PDR was associated with a subsequent decrease in tortuosity (194). Unlike the majority of studies

² AI defined as ratio of the perimeter of the FAZ to the perimeter of a circle with equal area. A perfectly circular FAZ has an acircularity index equal to 1, with deviations from a circular shape leading to an increase in this metric (Tam et al., 2011)..

³ FAZ perimeter irregularity was calculated as the ratio of measured area of FAZ to the projected area of the circle, with a range of 0.0 to 1.0. A ratio closer to 0.0 indicates an irregular shape of FAZ, and a ratio closer to 1.0 indicates a circular shape of FAZ

assessing fractal dimensions indices in fundus imaging, branching complexity has been found to be reduced in DR on OCTA (171-173). Further studies of these additional biomarkers, including correlation with other retinal imaging modalities, may help clarify whether measurement from OCTA images will be of future use in assessing DR.

4.1.2 Diabetic retinopathy changes in OCT

4.1.2.1 Retinal thickness

OCT is now a standard imaging modality used to measure retinal thickness in the assessment of diabetic macular oedema. Macular oedema is caused by pathological disruption to the blood retinal barrier, leading to fluid leakage and accumulation within the retinal layers. This causes the layers to swell and leads to increased retinal thickness measurements on OCT in those with clinically significant macular oedema.

Diabetic changes in retinal thickness in the absence of clinically significant macular oedema are less well established. Increased retinal thickness has been observed with increased severity of retinopathy (195-200), irrespective of the presence of macular oedema (196). A recent Cochrane review concluded that retinal thickening on OCT improves the detection of subclinical macular oedema, where no evidence of clinical macular oedema is present (201). As such it is widely used in the assessment of patients with diabetes and is being incorporated into diabetic screening programmes.

However, some studies have conversely found retinal thinning in people with diabetes compared to healthy controls (202-207). All but one (205) of these studies assessed people with diabetes who had minimal or no established DR only. As such this may represent an early change in DR, with retinal thickening, due to the breakdown of the blood retinal barrier and fluid leak, occurring later. Retinal thinning has been shown to affect the inner nerve layers primarily (206) and may represent early neurodegenerative pathology due to diabetes related hyperglycaemia. Isolated measurement of the retinal nerve fibre layer has consistently shown this layer to be thinner in diabetic patients compared to healthy controls (197, 198, 200, 203,

207-209), even when central measurements of the retinal layer are found to be thickened (197, 198, 200).

Other factors may also account for differences in retinal thickness measurements between studies, including variation in OCT machines or study protocols used (210). The proportion of men and women included in the case or control groups may also have had an effect, with several studies showing that males have increased retinal thickening versus females (197, 210-212). Increasing age is also likely to impact outcomes (205). Differences in glycaemic control and diabetes duration of participants may play a role, though these effects have not been well established.

While specific OCT thresholds for defining macula oedema have not been fully established, central retinal thickness measures of $>300\ \mu\text{m}$ is thought to coincide with clinically significant macular oedema (201, 213), with central retinal thickness values of greater than $180\text{-}210\ \mu\text{m}$ proposed as an indication of at risk individuals with possible subclinical macular oedema (199, 212). Degenerative changes in the nerve fibre layers may account for thinning of the retina compared to non-diabetic controls, and is potentially an earlier sign of diabetes pathology.

4.1.2.2 Choroid thickness

The concept of diabetic choroidopathy, distinct from diabetic retinopathy, is an area of growing interest for the evaluation of diabetes related eye changes (85).

The majority of studies report thinning of the choroid in diabetes compared to healthy controls (214-224). This is thought to represent loss of functional choroidal blood vessels, leading to hypoxia in the outer retina and RPE, and subsequent DR. Thinner choroids in diabetes are also associated with the presence of microalbuminuria (215) which is known to be a good clinical indicator of early diabetic nephropathy. Only one study (225) found subfoveal choroidal thickness to be increased in people with diabetes versus healthy controls, though there was no significant difference in mean choroidal thickness and the majority of participants with diabetes did not have established DR. In those with established DR (23/246) no significant change in choroidal thickness was identified. Another study (226) specifically

assessed changes in subfoveal choroidal thickness in the presence of diabetic macular oedema and observed that this was significantly increased compared to healthy controls, though was not related to DR severity. Authors proposed that the choroid may therefore become oedematous due to similar processes which occur in the retinal layers in macular oedema, leading to the observed choroidal thickening.

While the presence of diabetes is generally associated with choroidal thinning, changes in choroidal thickness in relation to DR severity are less clear. Several studies report no association for increased DR severity and choroidal thickness (214, 216, 217, 220, 221) while others have shown increased choroid thinning with increased severity (218, 224, 227-229). Treatment with pan-retinal photocoagulation has also been shown to impact choroidal thickness, with thickening of the subfoveal choroidal region in the first 3 months post pan-retinal photocoagulation compared to baselines (230). This suggests that, while the measurement of choroid thickness is useful in distinguishing early diabetes changes from healthy controls, it may not correlate well with DR severity, or the presence of diabetic macular oedema and is therefore potentially less useful as a prognostic marker of disease severity. Other factors including age, axial length, intraocular pressure and BP, which were not controlled for in several analyses, may also impact choroidal thickness (226). Several studies also did not distinguish whether participants had type 1 or type 2 diabetes. Different OCT machines were used, and measurement of choroidal thickness varied. Subfoveal choroidal thickness was the primary measurement for most studies, however parafoveal thickness measurements were taken at varying intervals, typically up to 3000 μm either side of the fovea. The use of built-in automated software for part of the choroid thickness calculation versus manual measurements from one or more observer also varied across studies.

4.1.3 Diabetic retinopathy in UWF-SLO

UWF-SLO has the advantage over standard fundus photography of identifying peripheral DR lesions. Several studies report the use of UWF-SLO leads to additional cases of DR being identified in around 10% of those imaged, due to the identification of lesions which only occur outside of the standard seven-field fundus imaging (81, 231)

Visualisation of peripheral lesions can also alter the DR severity assessment. One study reported predominantly peripheral DR lesions in 308 of 751 eyes (41.0%) assessed, leading to increased DR severity in 83 eyes (26.9%) compared to seven field fundus imaging (79). Similarly, one large population study imaging more than 2000 people with established DR using UWF-SLO showed that predominantly peripheral retinopathy changes were seen in 21.3% of participants using UWF-SLO, leading to assignment of a more severe retinopathy grading in more than 9% when compared to grading by non-mydriatic fundus photography (232). A UK study assessing 781 people (over 1500 eyes) who had been referred following screening with two image fundus photographs, identified new vessel formation in 102 eyes using UWF-SLO (231). Of these, 30 eyes (29%) had new vessels outside the two-field area of screening and had been referred with a lesser degree of DR, and 12 eyes (11%) fell outside the region for standard seven field fundus photograph imaging (231).

Until recently there was no evidence that these peripheral lesions had any prognostic value for DR risk. A longitudinal study of 74 people with type 1 (49%) and type 2 (51%) diabetes over four years showed that eyes with lesions predominantly peripheral to the standard seven-field imaging had a 3.2 fold increased risk of two-step or more DR progression (ETDRS grading) and a 4.7 fold increased risk for progression to PDR. A greater extent of predominantly peripheral lesions was also associated with a higher risk of DR progression ($p < 0.004$) and onset of PDR ($p < 0.009$) (60).

4.2 Methods

4.2.1 Study Design and Participants

This was a prospective cohort study assessing patients with diabetes at regular intervals over one year, with comparison to a healthy non-diabetic population.

4.2.1.1 Study Groups

Recruitment was planned from four groups:

- **Group 1: CSII Group**

This included people with Type 1 diabetes who were starting on CSII therapy. Patients were identified from the NHS Lothian insulin pump waiting list and approached for participation in the study when a date for starting on CSII therapy had been assigned. Study visits were planned for prior to starting CSII, and at 1, 3, 6, 9 and 12 months from receiving CSII.

- **Group 2: Islet Transplant Group**

This included people with Type 1 diabetes awaiting islet transplants. Patients were identified from the Edinburgh islet transplant clinic list and approached for participation in the study when they became active on the islet transplant waiting list. Study visits were planned for pre-transplant and at 1, 3, 6, 9 and 12 months from receiving a first islet transplant.

- **Group 3: MDI Group**

This included age matched type 1 diabetes patients on standard MDI therapy. Patients on MDI were identified from the NHS Lothian Type 1 diabetes clinic and a written invitation to participate in the study was provided. Study visits were planned at 0, 3, 6, 9 and 12 months from recruitment.

- **Group 4: Non-Diabetic Healthy Control Group**

This was a control group of age matched non-diabetic healthy controls who were asked to attend for a one-off study visit.

4.2.1.2 Inclusion and Exclusion Criteria

Inclusion criteria for participants with diabetes (Groups 1-3)

- Age 25 – 70 years at study entry
- Known Type 1 Diabetes (>12 months since diagnosis)
- Able to understand and undertake the study procedures.
- Able to give signed informed consent.

Exclusion criteria for participants with diabetes (Groups 1-3)

- Impaired renal function (eGFR <60 at study entry)
- Active proliferative diabetic retinopathy

Inclusion criteria for participants without diabetes (Group 4)

- Aged 25 – 70 years at study entry
- No history of diabetes with HbA1c within normal range
- Able to understand and undertake the study procedures.
- Able to give signed informed consent.

Exclusion criteria for participants without diabetes (Group 4)

- History of significant co-morbidities including vascular disease, hypertension or hypercholesterolemia
- History of retinal disease
- Impaired renal function (eGFR <60)

4.2.1.3 Recruitment Process

Due to a lower than expected number of islet transplant procedures performed during the recruiting study period, and poor uptake from age matched controls with Type 1 diabetes on MDI, recruitment to Groups 2 and 3 was very small and not conducive to robust analysis. Data for these groups are therefore not discussed further in this chapter and are presented as supplementary data in Appendix 2. As such, participants with diabetes that have been

included for analysis in the results section of this chapter are from Group 1 only (CSII participants). Issues with recruitment are discussed further in the discussion.

Similarly, due to recruitment challenges to Group 4, retinal data for age and sex matched non-diabetic healthy controls was obtained with permission from another research group using the same retinal imaging devices and protocols described below on a 3:1 for control:case basis (via Dr Tariq Farrah and Dr Dan Pugh, Prof Neeraj Dhaun Renal Research Group).

4.2.2 Study Visit Protocol

The following information was obtained at each visit for all diabetes groups:

- Patient history including diabetes duration, smoking status, past medical history and medications.
- Weight
- Blood pressure (three BP measurements taken and the lowest reading recorded)
- Bloods for renal function, liver function, lipids and HbA1c.
- Urine sample for ACR
- Neuropathy assessment (see Section 4.2.2.1)
- Continuous Glucose Monitoring (See Section 4.2.2.2)
- Retinal assessment (See Section 4.2.3)

4.2.2.1 Neuropathy Assessment

Vibration perception thresholds were assessed using a neurothesiometer. The neurothesiometer consists of a vibratory probe which may be applied to the bony prominences of the feet. The voltage applied to the vibrator is adjusted and the subject signals when they perceive the vibration. Vibration perception thresholds were assessed at both the base of the first metatarsal-phalangeal joint and the medial malleolus. The participant is asked to remove their shoes and socks while seated comfortably, the meter is held against the base of the participant's first metatarsal-phalangeal joint and medial malleolus and then turned up gently until the patient feels the vibration. The voltage at which the subject perceives the signal is recorded (Range 0-25 volts). This is repeated three times at each point and an average value recorded. An average score of >15 volts is consistent with significant peripheral neuropathy.

4.2.2.2 Continuous Glucose Monitoring Assessment

During the study visit, a CGM device was placed subcutaneously on the participant's body to obtain further information about glycaemic control and variability during the week following

the visit. This was initially done solely using the blinded Medtronic ipro2 CGM, which is placed on the anterior abdominal wall. This records interstitial glucose readings every 3 minutes and is worn for up to 7 days. Calibration using regular capillary blood glucose monitoring is required for successful ipro2 download. Patients were asked to record at least four capillary blood glucose checks per day using their usual blood glucose testing meter, and return these values with the sensor to enable it to be downloaded and calibrated. Data was downloaded to a computer using Medtronic software. Downloads with less than 72 hours of data were deemed to be insufficient and excluded from analysis.

During the study period there was a change to standard blood glucose monitoring for people with Type 1 diabetes in NHS Lothian due to the approval of flash glucose monitoring (Freestyle Libre) on NHS prescription. The Freestyle Libre is an unblinded flash glucose monitoring system which is placed subcutaneously on the upper arm. This records interstitial glucose readings every minute and is worn for 14 days. It does not require calibration with capillary blood glucose monitoring. Glucose levels are checked by holding a reader up to the device. Continuous glucose data can also be downloaded later from the reader using Diasend or Libreview software.

All those who were already participating in the study switched from using standard capillary blood glucose testing as their primary method for monitoring glucose levels, to using the Freestyle Libre device. This had an impact on the study CGM assessment for two reasons. Firstly, participants were less willing to wear a second CGM device and therefore declined the blinded ipro2 device in favour of the Libre. Secondly, many participants who did accept the ipro2 sensor subsequently returned the sensors with little or no capillary blood glucose data for calibration, resulting in unsuccessful downloads which did not meet the criteria for inclusion in the analysis.

As a result, an amendment was made to the study protocol to allow Libre downloads to be used to obtain CGM data for those who were not willing to use the ipro2, or where the ipro2 download had failed.

Both the ipro2 and Libre downloads provided similar glycaemic data which included percentage TIR, percentage time below range, percentage time above range, mean glucose, estimated HbA1c and glucose SD for the duration of time the sensor was worn. The glucose target range was defined as 4-10mmol/l for both devices, rather than the established standard target range of 3.9-10mmol/l (19), as this was the default range reported on the device downloads. The co-efficient of variation (CV) glucose was calculated by dividing the glucose SD by mean glucose.

4.2.3 Retinal Assessment

Retinal assessment was carried out using four imaging modalities: OCTA, fundus photography, OCT and UWF-SLO. The most recent DR screening outcomes taken for each participant prior to study entry were also recorded by accessing the results from the SCI-Diabetes database.

4.2.3.1 OCTA

Imaging protocol

Images were obtained using the Optovue Avanti OCTA machine Version 2018.0.0.18. The same imaging protocol was used for each study visit and consisted of:

A 3D Widefield Motion Correction Technology (MCT) reference scan (Figure 4-1). This provides a 12x9mm reference retinal image over a 40 degree FOV and includes OCT imaging of the retinal layers. This outlines the position of key features, such as the fovea, to help align sequential images and reduce artefact due to eye motion during scanning

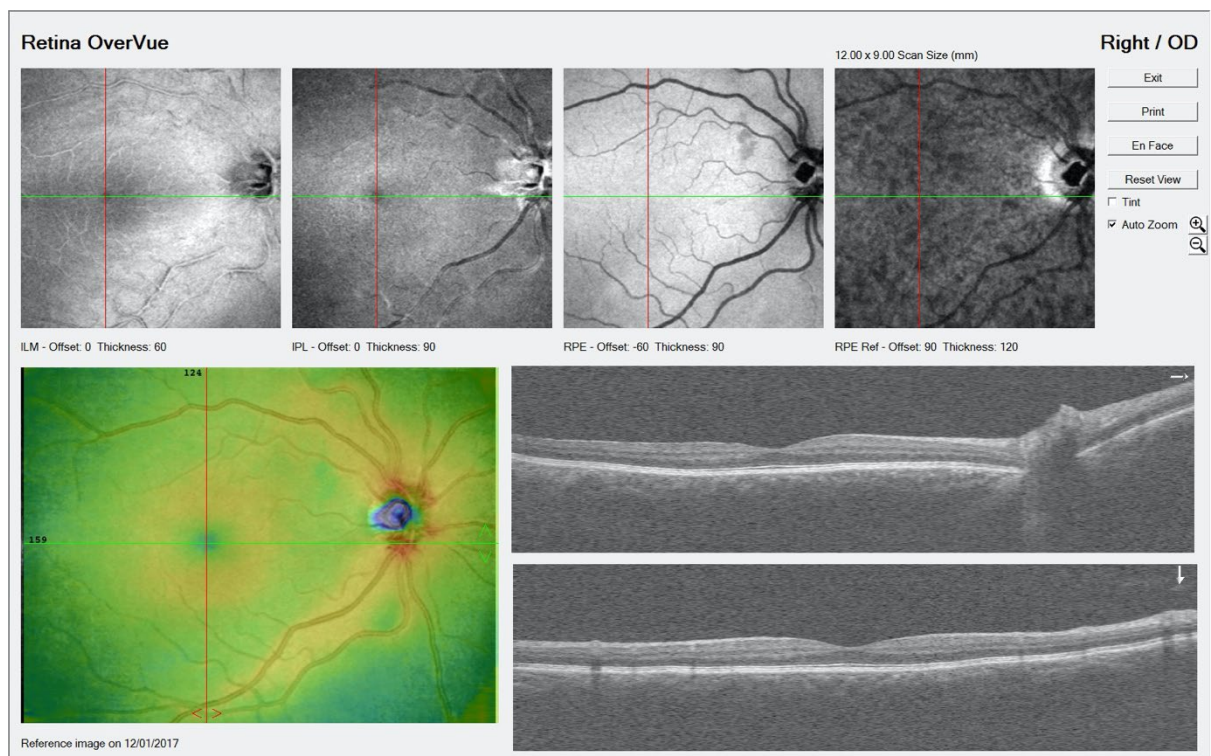
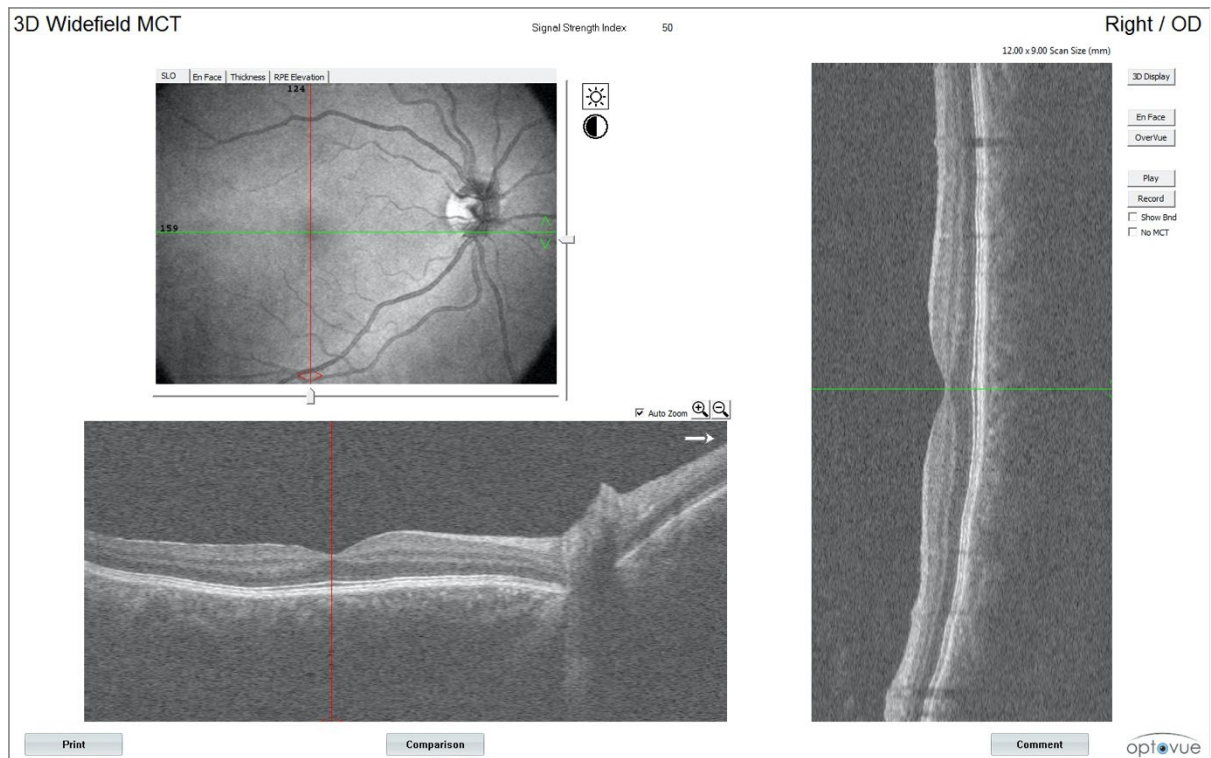
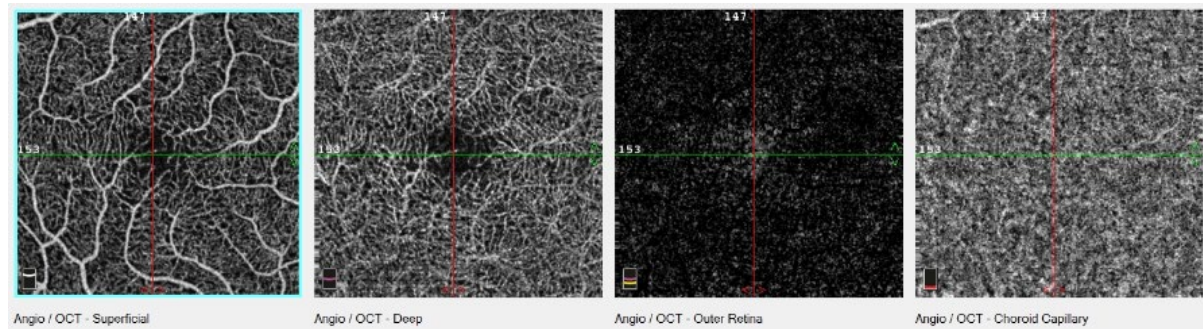


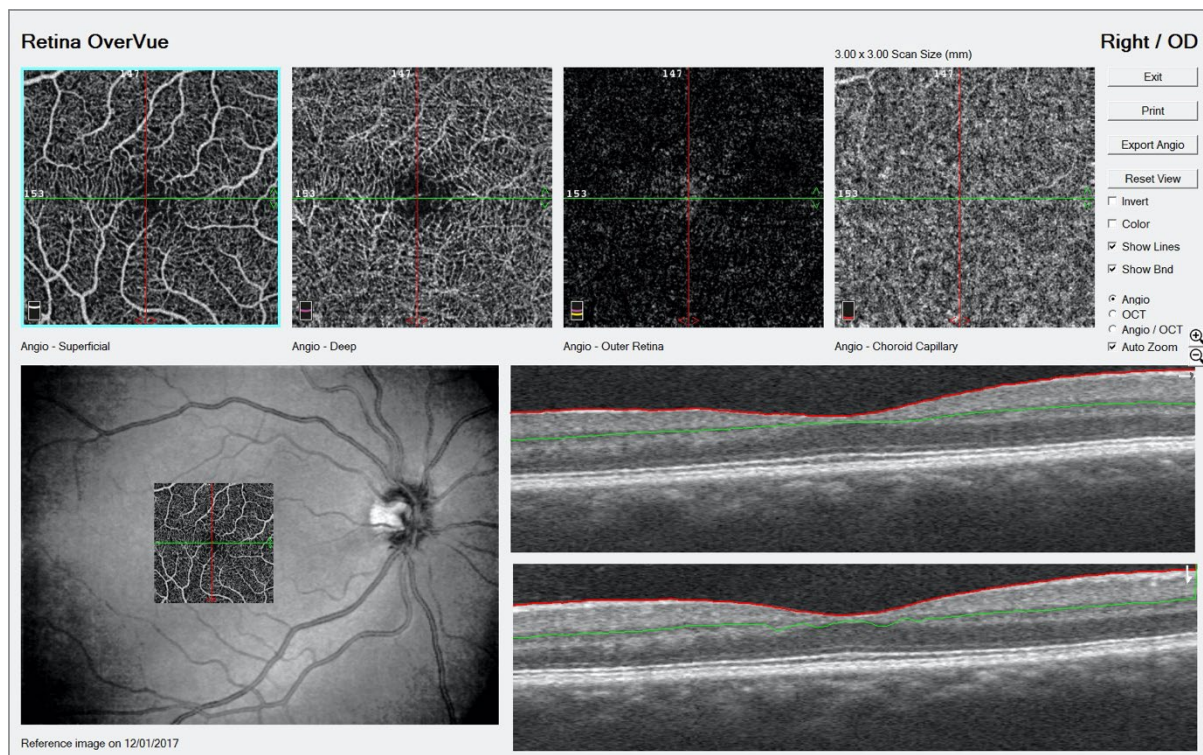
Figure 4-1 3D Widefield Motion Correction Technology (MCT) reference scan. Image taken as part of study.

1. Angio Retina scans (Figure 4-2). These Images identify vascular flow and provide the 3D outline of the retinal vasculature for a 3x3 mm² and 6x6 mm² area centred on the fovea. Images were segmented into four layers (segmentation settings discussed further below); superficial retinal layer, deep retinal layer, outer retinal layer and choroid capillaris layer.

(A)



(B)



(C)

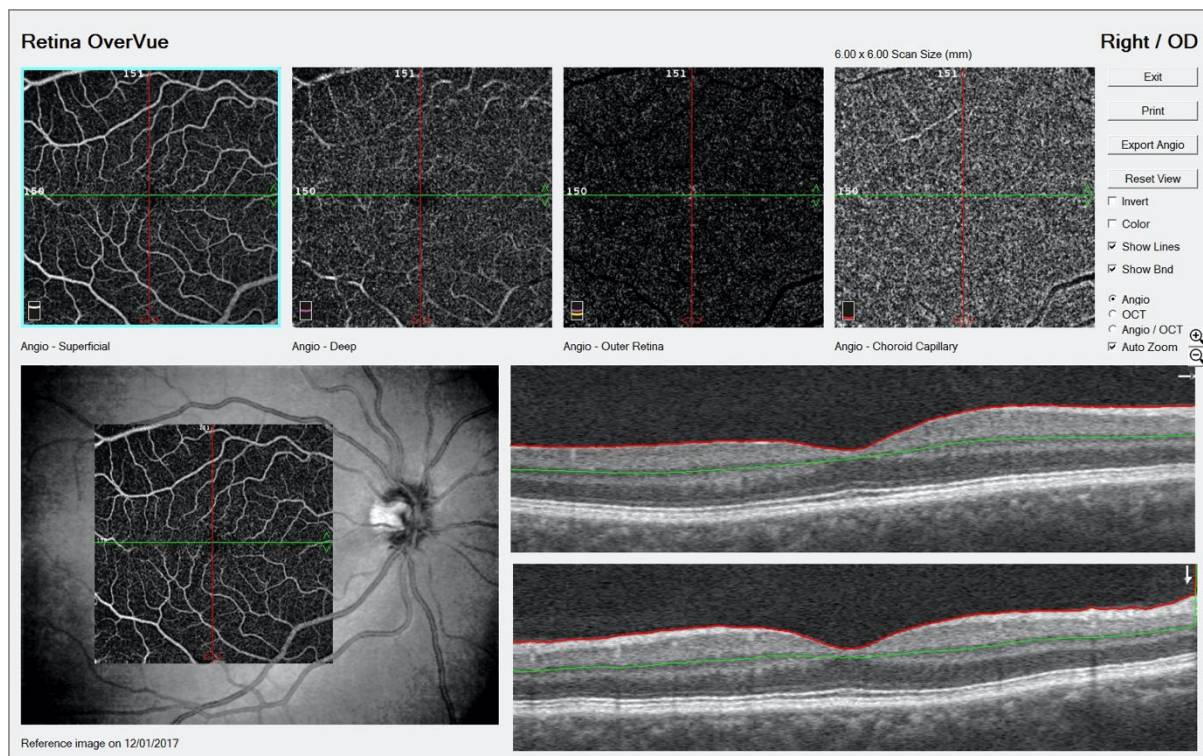


Figure 4-2 Angio Retina scans: (A) Four 2-dimensional images produced by OCTA fovea centred scan depicting retinal vessels in (left to right) superficial retinal layer, deep retinal layer, outer retinal layer and choroid capillaris layer. (B) Image output from Optovue OCTA for 3x3mm scan. (C) Image output from Optovue OCTA for 6x6mm scan. Images taken as part of this study.

2. An Angio Disc scan (Figure 4-3). This is similar to the Angio Retina scans but provided a 3x3 mm² area centred on the optic disc.

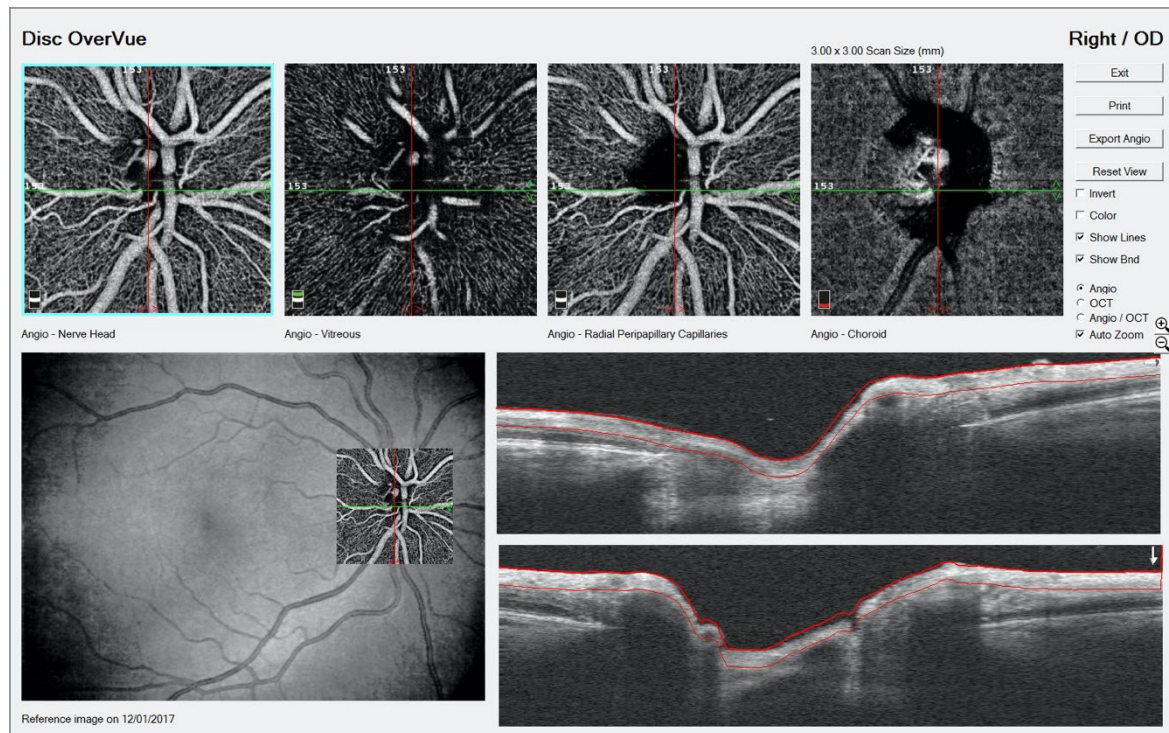


Figure 4-3 Image output from Optovue OCTA for angio disc scan. Images taken as part of this study.

Layer Segmentation

The four retinal layers for analysis were automatically segmented using built in Optovue software (Table 4-1), which determined the position of the anatomical retinal layers (namely the inner limiting membrane (ILM), inner plexiform layer (IPL), outer plexiform layer (OPL) and retinal pigment epithelium (RPE)) and segmented the OCTA images based on pre-defined measurements:

Table 4-1 Segmentation boundaries for four retinal layers for Optovue OCTA

	Upper border	Lower border
Superficial Layer	ILM	10 µm below the IPL
Deep Layer	10 µm below the IPL	10 µm above the OPL
Outer Retinal Layer	10 µm above the OPL	15 µm above the RPE
Choriocapillaris	15 µm above the RPE	59 µm below the RPE

Retinal Biomarker Measurements

Data for both the 3x3 mm² and 6x6 mm² Angio Retina scans was extracted using built in automated Optovue software for the following parameters:

1. Vessel density
2. Retinal thickness
3. FAZ Area
4. FAZ Perimeter
5. Acircularity Index

Vessel density and retinal thickness values were automatically calculated for the whole image, and for ETDRS regions based on the ETDRS grid (**Error! Reference source not found.**). The outermost perifoveal grid regions (S6/T6/I6/N6) were visualised on the larger 6x6 mm² image only, therefore values for these regions were not calculated from 3x3 mm² images, which included only the central foveal region (C1) and parafoveal regions (S3/T3/I3/N3). Analyses focused on data from the superficial and deep vascular layers.

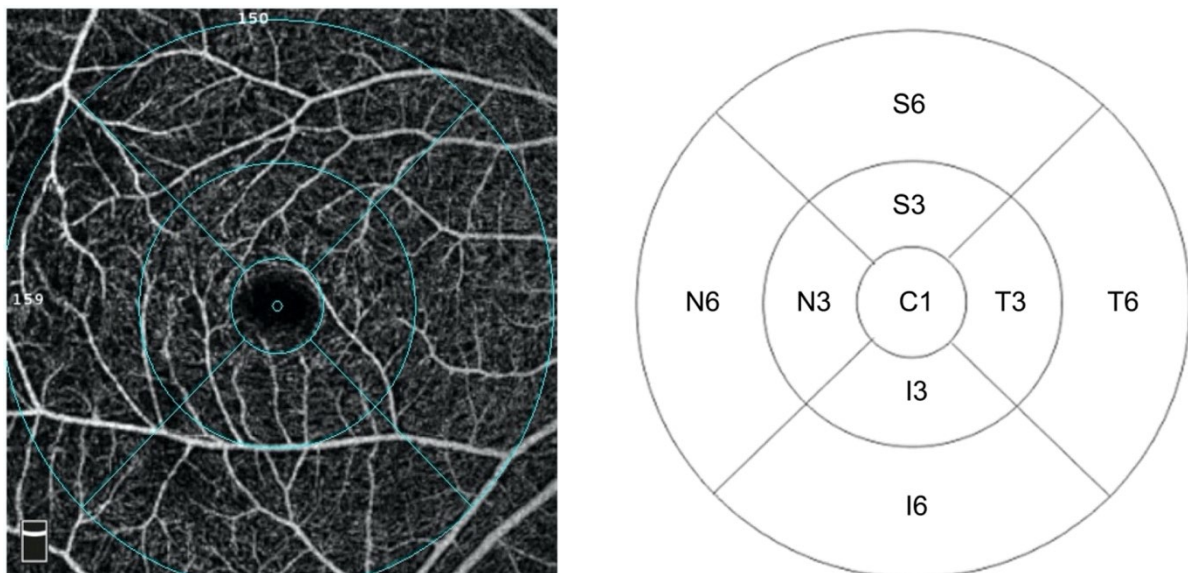


Figure 4-4 ETDRS grid on superficial 6x6mm OCTA image from this study (left) and ETDRS grid showing grid regions for analysis with central (C1), Superior (S), Temporal (T), Inferior (I) and Nasal (N) regions of the parafovea (S3, T3, I3, N3) and perifovea (S6, T6, I6, N6) (right).

The FAZ outline was fitted automatically using Optovue software for all images (Figure 4-5). While for most images the FAZ can be easily identified, for complex images, particularly where there is more extensive pathology, identifying the correct boundaries for the FAZ can be open to user interpretation (Figure 4-6). As such the FAZ outline which was automatically fitted by Optovue software was accepted for all images and not amended by the user.

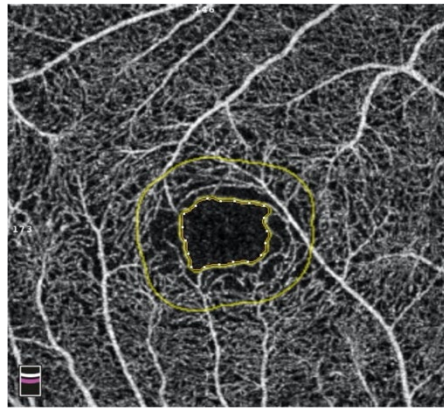


Figure 4-5 Automatic detection of FAZ (inner yellow circle) by Optovue software for FAZ area, perimeter and AI calculation. Image taken as part of this study.

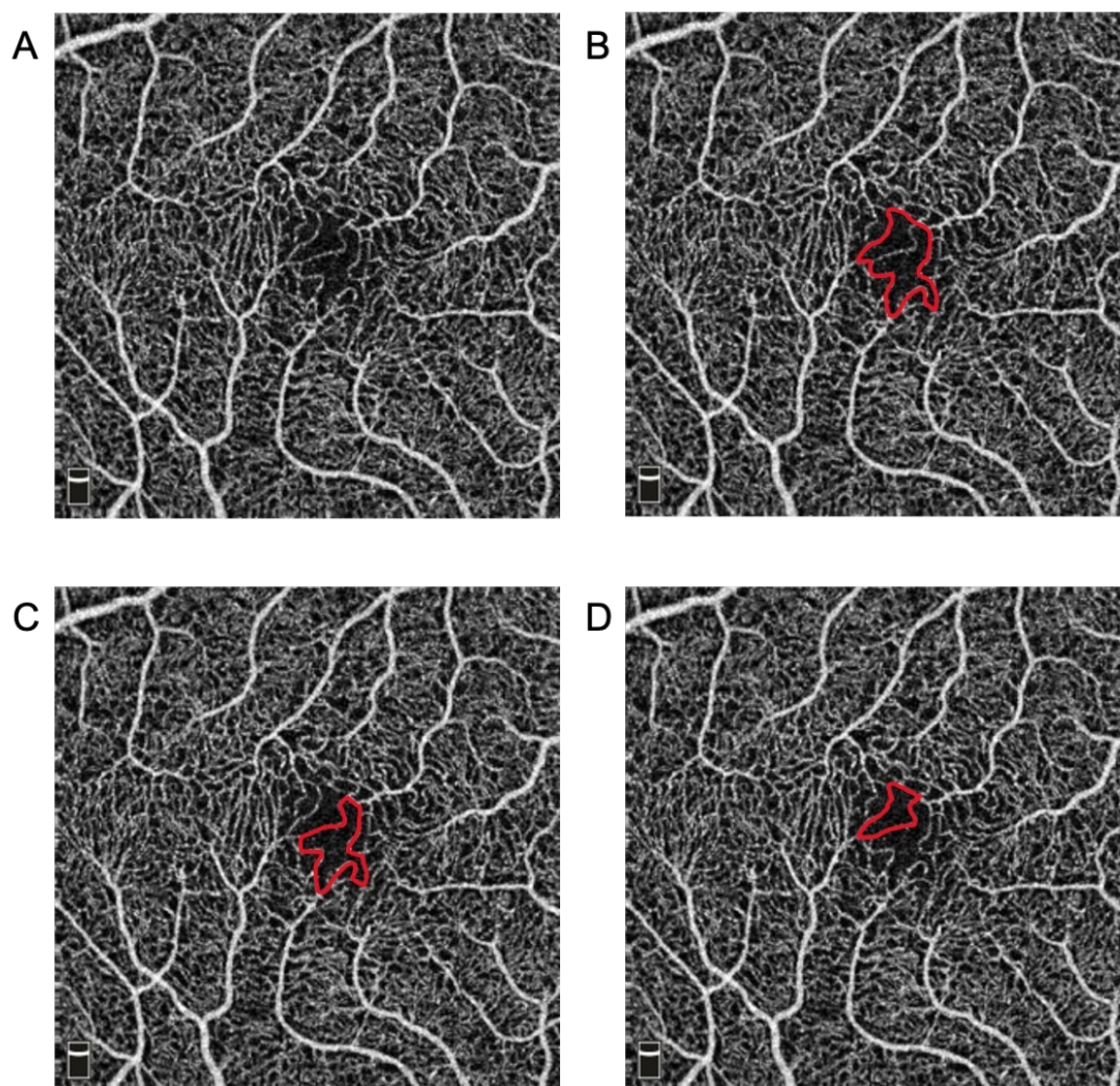


Figure 4-6 Challenges in identifying FAZ outline: (A) Original superficial 3x3 OCTA image from this study (B-D) Example interpretations of FAZ outline for OCTA image in (A).

Quality Assessment

All images were taken by the same imager using the protocol described above. Images were reviewed immediately after being obtained and repeat images taken if quality was deemed to be poor due to motion artefact (Figure 4-7) or poor positioning (Figure 4-8). An automated quality score (0-10) was also assigned to all images. Images scoring <5 were excluded from analysis.

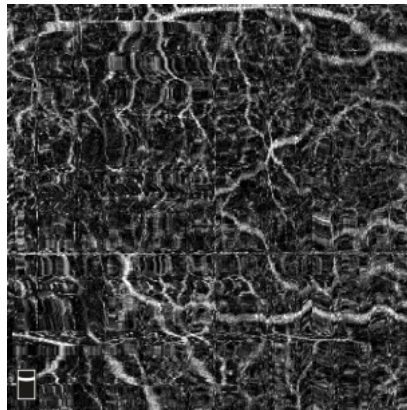


Figure 4-7 Motion artefact causing poor quality 3x3 OCTA image taken during this study. Image not included for analysis.

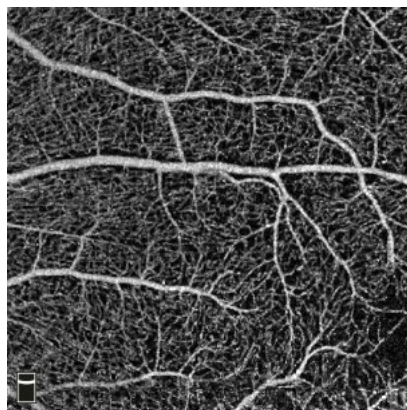


Figure 4-8 Poor positioning on 3x3 OCTA image taken during this study: Fixation on wrong point leading to image not centred on FAZ (part of FAZ visible bottom right corner). Image not included in analysis.

4.2.3.2 Fundus Photography

Fovea centred fundus images were obtained using the same format as the DRS programme. Quantitative analysis was performed using VAMPIRE software assessing vessel calibre, fractal dimensions and tortuosity. The details for this analysis are described further in Chapter 3.2.5.

4.2.3.3 Optical Coherence Tomography

Imaging Protocol

Images were obtained using the Heidelberg Spectralis Spectral-Domain OCT machine Version 1.9.14.0. The automatic retinal tracking (ART) mode was used for all scans. This ensures adequate B-scans are collected to capture the region of interest in the presence of small eye movements and helps minimise motion artefact. The same imaging protocol was used for each study visit using a previously established University of Edinburgh protocol (233) and consisted of:

1. A foveal centred horizontal line scan providing a cross section of retinal layers horizontally through the fovea (Figure 4-9). Enhanced depth imaging was enabled to better visualise the choroid layer
2. A foveal centred vertical line scan providing a cross section of retinal layers vertically through the fovea. Enhanced depth imaging was enabled to better visualise the choroid layer
3. A peripapillary circular line scan centred over the optic disc, with segmentation of the retinal nerve fibre layer (Figure 4-10)
4. A macular volume scan, consisting of 61 B scans taken horizontally at 120 μm intervals to enable a 3D reconstruction of the whole macula (Figure 4-11).

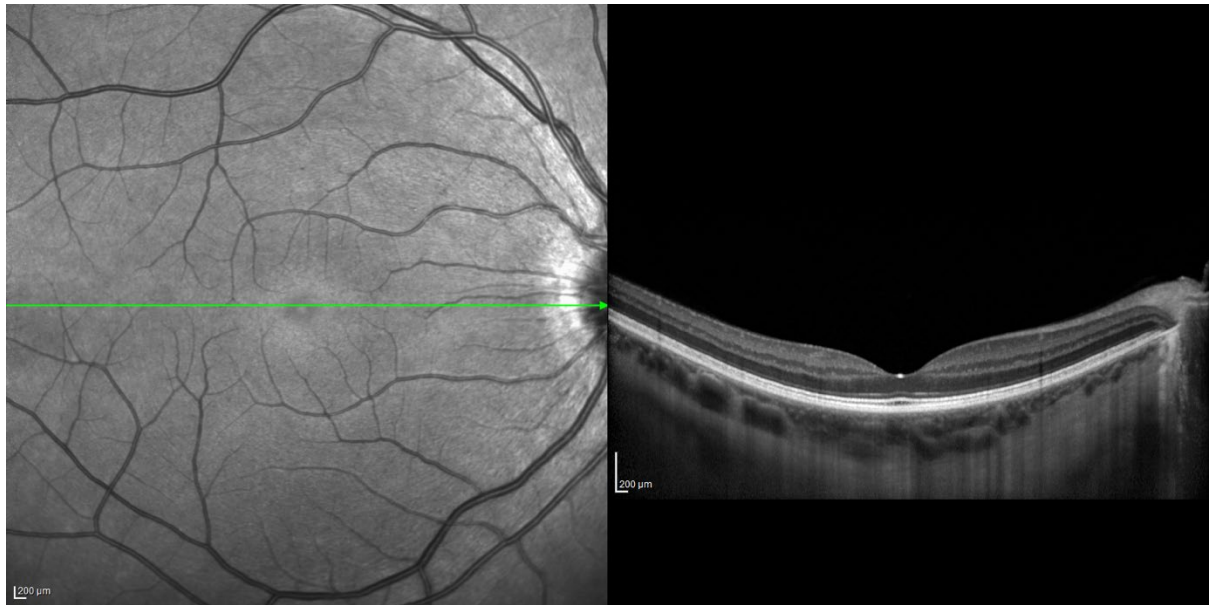


Figure 4-9 OCT Horizontal scan from this study

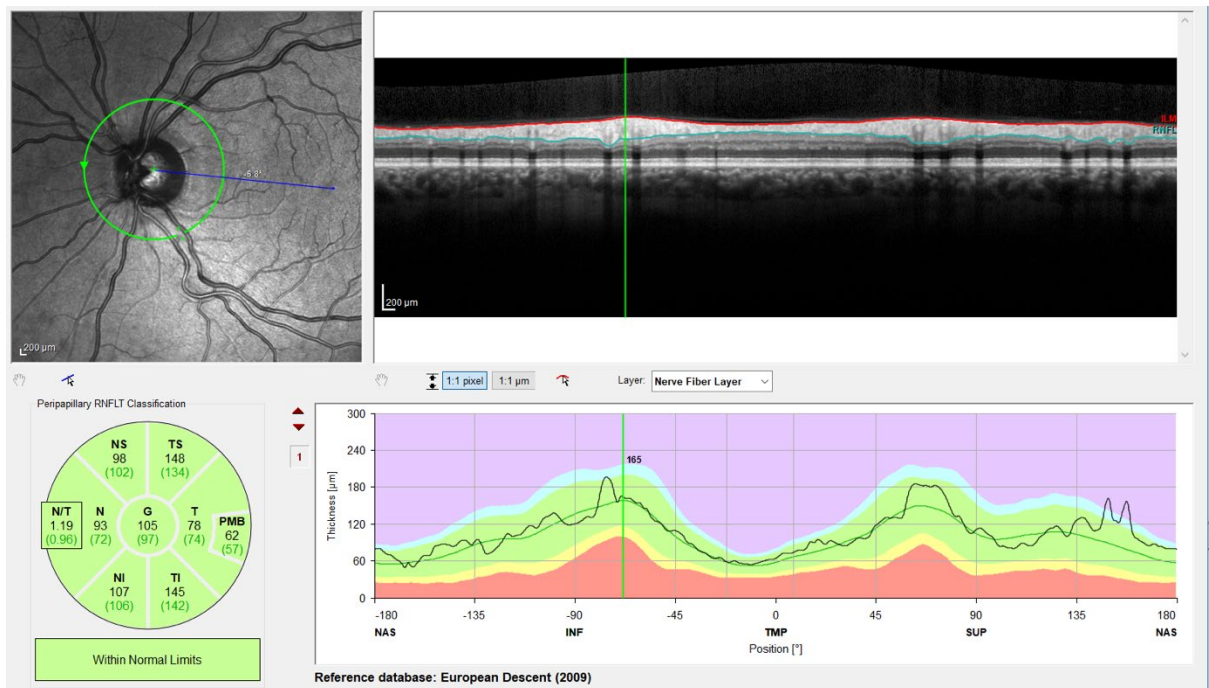


Figure 4-10 OCT disc RNFL scan from this study showing reference OCT images (top) and automated thickness calculations (bottom): papillo-macular bundle (PMB), temporal (T), temporal-superior (TS), nasal- superior (NS), nasal (N), nasal-temporal ratio (N/T), nasal-inferior (NI), temporal- inferior (TI), global (G)

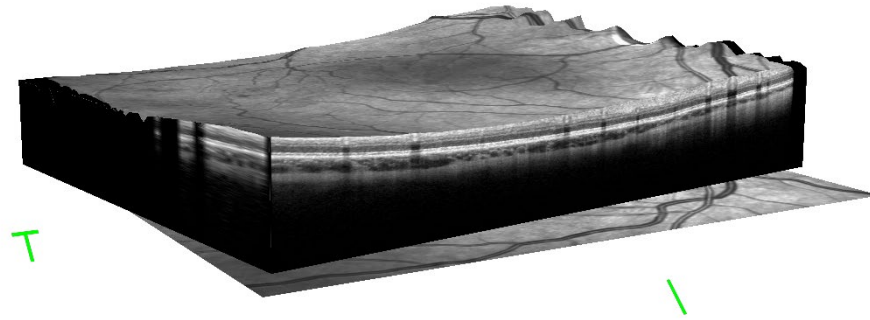


Figure 4-11 OCT 3D reconstruction of macula derived from images taken as part of this study

Retinal Biomarker Measurements

Using the scans above the following measurements were taken for analysis:

1. Retinal thickness measurements

These are derived from the macular volume scan using built in automated Optovue software. The software automatically segments the ILM and RPE layers on each B-scan for calculation of macular volume and retinal thickness across the entire macula. The user reviews the images to ensure the segmentation of these layers has been carried out correctly and can manually adjust the layer segmentation if necessary. A standard ETDRS grid is used to divide the image into the 9 ETDRS sections for analysis of retinal thickness in the various regions (Figure 4-12). This is automatically centred on the fovea. Again this is manually checked by the user to ensure adequate placement.

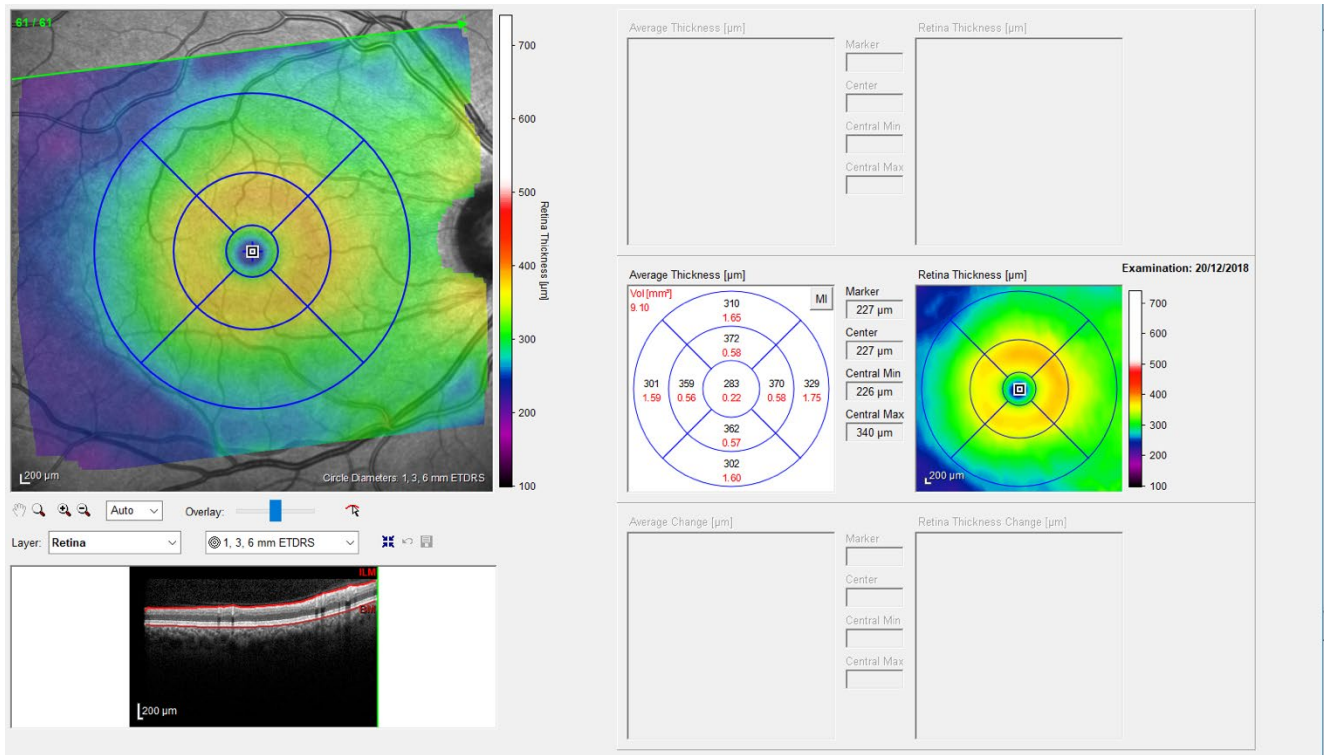


Figure 4-12 OCT Retinal thickness measurements from images taken in this study showing reference images (Left) and automated calculations (Right)

2. Choroidal thickness measurements

Subfoveal and parafoveal measurements were taken manually from the horizontal enhanced depth imaging line scan, using built in software calipers. Measurements were taken using a previously established University of Edinburgh Clinical Research Imaging Centre protocol in use by Prof Bal Dhillion and Prof Neeraj Dhaun research groups (234). The fovea is automatically identified and can be manually adjusted if necessary. Subfoveal choroid thickness was measured in a vertical line perpendicular to the horizontal retinal pigment epithelium, from the outer reflective line corresponding to the base of the RPE to the choroid scleral junction. Parafoveal choroid measurements were taken at 2000 μm ($\pm 2 \mu\text{m}$) either side of the fovea (nasally and temporally) parallel to the subfoveal thickness measurement (Figure 4-13).

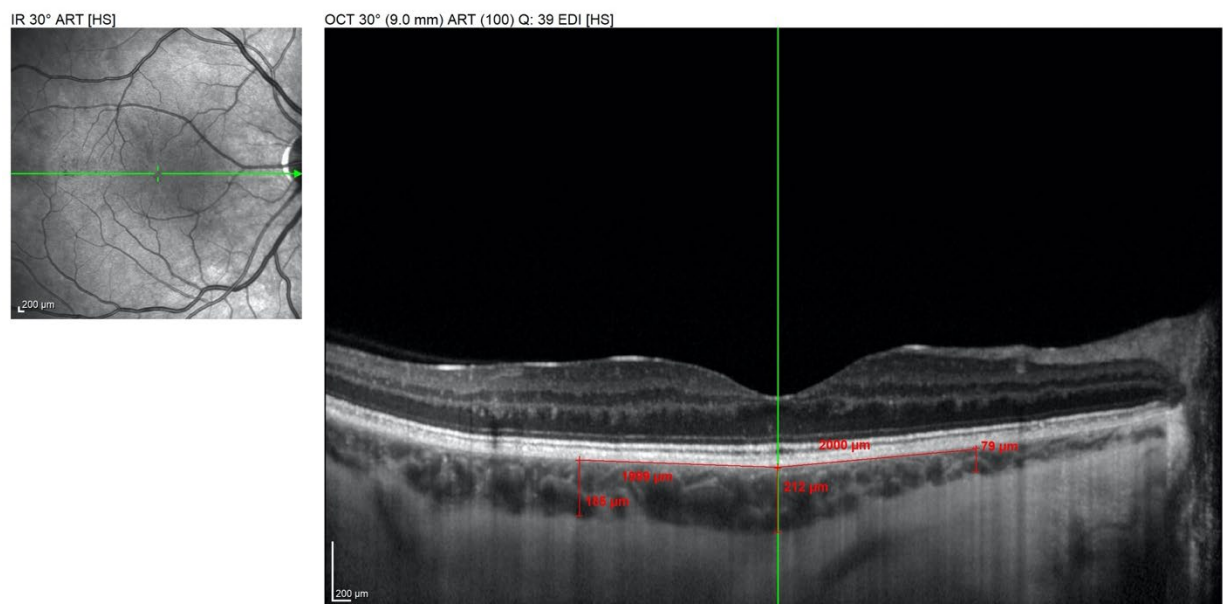


Figure 4-13 OCT Choroid thickness measurements: Subfoveal choroid measurement manually measured using vertical line perpendicular to horizontal retinal pigment epithelium (central red line), with parafoveal measurements take at 2000 μm ($\pm 2 \mu\text{m}$) either side of the fovea (nasally on right of image and temporally on left of image)

3. RNFL measurements

These are derived from the peripapillary circular line scan using built in automated Optovue software (Figure 4-10). Global (mean) RNFL thickness is automatically calculated,

in addition to thickness within ETDRS grid regions, with superior and inferior regions further divided into superior and inferior nasal and temporal sections. Thickness at the papillo-macular bundle, located within the temporal region and responsible for carrying information from the macula to the optic nerve, is also calculated, as well as the nasal temporal thickness ratio.

Quality Assessment

Images were reviewed at the time of scanning for adequate focus, image alignment, correct identification of key landmarks including the fovea and optic disc, and to ensure ART was activated with an adequate number of B scans captured (>90 accepted of maximum 100). A signal quality score was also given (0-40). Images were discarded if below the recommended quality score of 15 (235). Repeat images were taken if initial scans were deemed of inadequate quality. Repeat images were also taken if original images were poorly aligned (Figure 4-14). Grid placement for analysis was checked on all images and adjusted where necessary (Figure 4-15).



Figure 4-14 Example of poorly aligned OCT image taken during this study showing angled retinal layers (image not included for analysis)

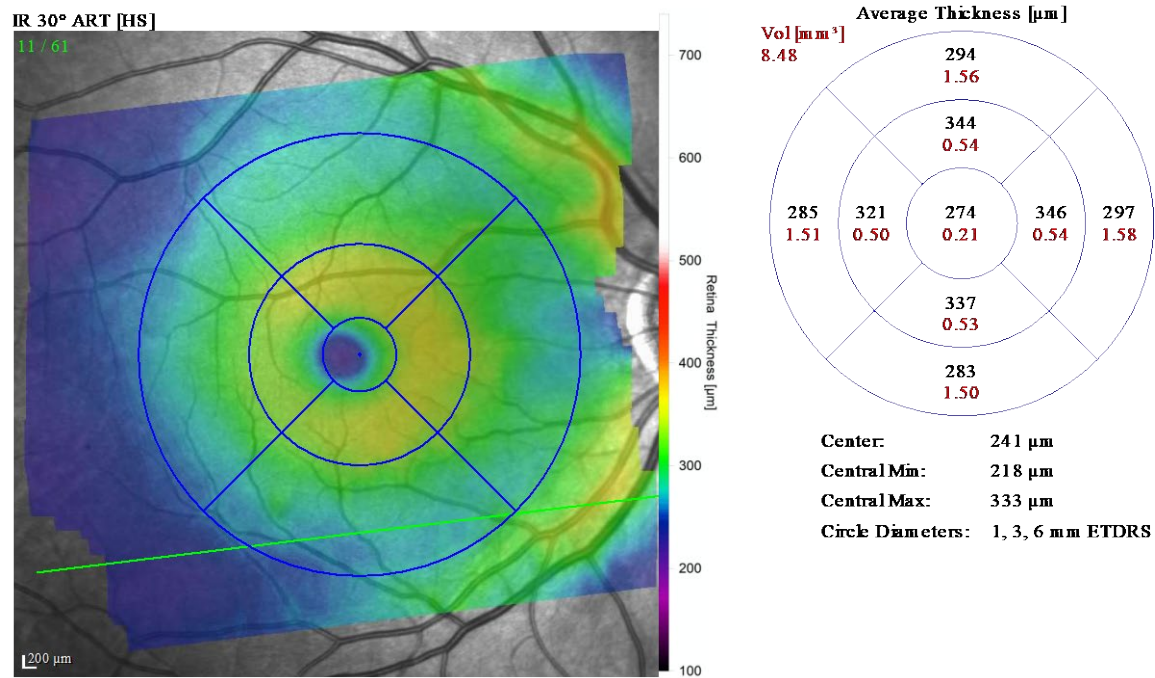


Figure 4-15 Example image from this study with poorly placed EDTRS grid which is not well centred on the fovea for retinal thickness analysis. In this example the EDTRS grid was manually adjusted for better positioning prior to data inclusion for analysis.

4.2.3.4 Ultrawide-field Scanning Laser Ophthalmoscopy

Imaging Protocol

Images were obtained using the Optos Advance California P200 system. Two non-mydratic fovea centred images were obtained for each eye, a red-green colour image (Figure 4-16) and a red-free image (Figure 4-17), encompassing a 200 degree FOV of the retina.



Figure 4-16 UWF-SLO Red Green Image from this study

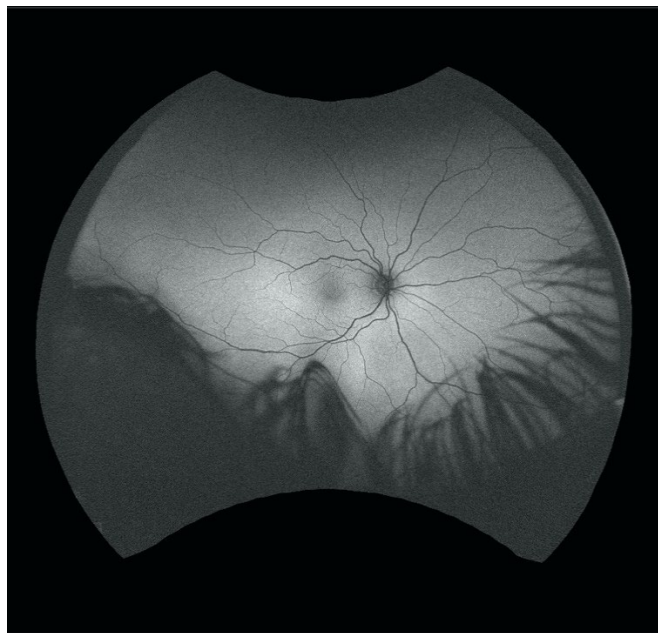


Figure 4-17 UWF-SLO Red-Free Image from this study

Retinal Biomarker Measurements

Vessel calibre was calculated at two regions formed by concentric bands around the optic disc using semi automated software (Figure 4-18). The inner band corresponds to retinal zone B; set to 2 to 3 optic disc radii from the optic disc centre. Vessel calibre is calculated for the three largest arteries and veins crossing at the inner and outer bands and a mean value calculated, along with the AVR.

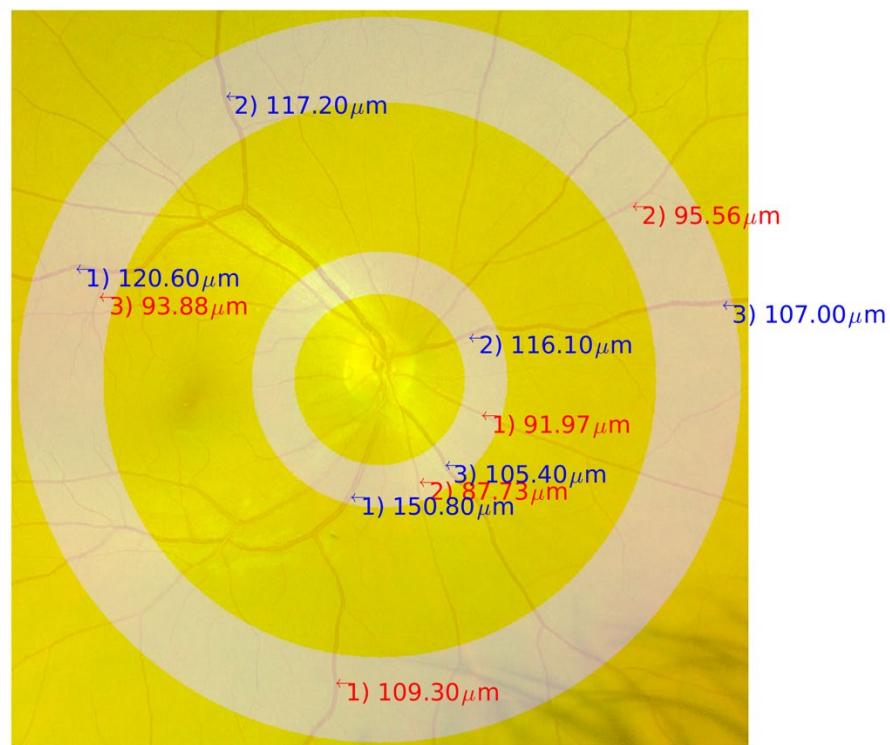


Figure 4-18 Vessel calibre calculation for UWF-SLO image from this study showing two concentric bands (paler yellow). Vessel calibre is calculated for the three largest arterioles (data output in red) and venules (data output in blue) crossing the inner and outer bands.

Quality Assessment

Images were automatically rejected for analysis if quality was poor or if the required parameters for vessel calibre calculation were not able to be visualised.

Images also inspected at time of image capture and repeat images taken if quality was felt to be inadequate, typically due to poor position, or obstruction of key features due to surrounding eye structures (eg. eyelashes) (Figure 4-19).

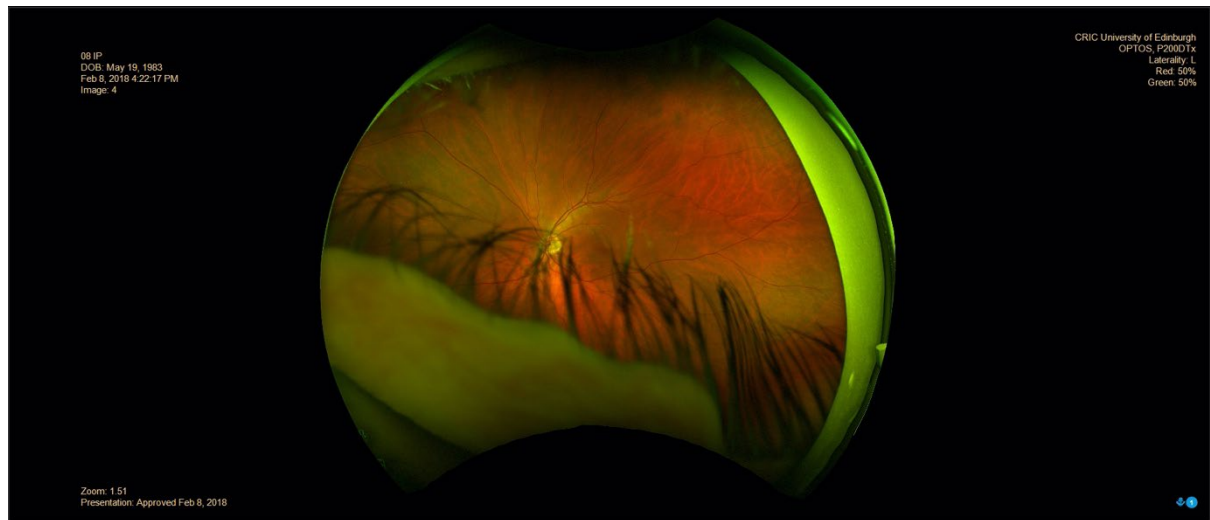


Figure 4-19 UWF-SLO image taken as part of this study with artefact due to eyelashes. Image not included for analysis.

4.2.4 Statistical Analysis

All analyses described below were performed using R Studio version 3.4.1. A p score of <0.05 was considered to be statistically significant. Results are expressed for continuous data as mean with SD or median with IQR, and as percentages for categorical data.

Results of retinal biomarkers for age and sex matched healthy controls were compared to results at baseline for CSII participants using unpaired t-test for continuous numerical data. Changes In glycaemic data, metabolic data and retinal biomarkers from OCTA and OCT imaging of CSII participants over one year (assessed at baseline, month 1, 3, 6 and 12) were assessed using one-way ANOVA test. Baseline fundus photographs and UWF-SLO photographs were compared to last available images (at 12 months for those completing full study period, at 6 months for those not completing full study period) using paired t tests.

Consistent preliminary data regarding retinal biomarker outcomes in people with diabetes was limited, particularly given the wide variation in methodologies for retinal biomarker calculation. As such power calculations were not performed prior to conducting the study.

4.3 Results

4.3.1 Recruitment numbers and baseline data

CSII initiation is carried out locally in groups of 12 participants. During the recruitment period there were four group starts. This is a lower number than predicted due to funding issues which were not foreseeable in the planning stage. Of the 48 people due to start on CSII therapy during the recruitment period, one decided not to proceed with the change to CSII therapy and one had an illness prior to the start date forcing them to dropout without a replacement. The remaining 46 people were invited to participate in the study during the preparation meeting and those interested were followed up to assess suitability. Of these, 16 eligible people agreed to participate. One person (IP10) moved out of Scotland after the initial visit and was not able to attend any follow up visits. Due to scheduling issues attendance at the month 9 visit was poor (9/16 unable to attend) and this was therefore not included in the analysis. Attendance is summarised in Figure 4-20. Baseline data is shown in Table 4-2.

Patient Code	Visit Month				
	0	1	3	6	12
IP01					
IP02					
IP03					
IP04					
IP05					
IP06					
IP07					
IP08					
IP09					
IP10					
IP11					
IP12					
IP13					
IP14					
IP15					
IP16					

Figure 4-20 Log of attended visits with green box indicating an attended visit and red box indicating no attendance

Table 4-2 Baseline data

Characteristic		CSII (n=16)
Age at Study Entry (years)		40 ± 9.2
Diabetes Duration (years)		6.4 ± 4.0
Gender M:F (%M)		7:9 (44)
Weight (kg)		81.6 ± 14.4
BMI (kg/m ²)		27.8 ± 4.9
HbA1c (mmol/mol)		63 ± 11
Cholesterol (mmol/L)		5.0 ± 0.8
Creatinine (μmol/L)		74 ± 10
Systolic BP (mm/Hg)		127 ± 12
Diastolic BP (mm/Hg)		82 ± 6
TDD Insulin		57 ± 31
Basal Insulin		28 ± 15
ACR		1.97 ± 1.88
Statin use		4/16
Antihypertensive use		3/16
Smoking Status	Non-	12
	Ex-	4
	Current	0

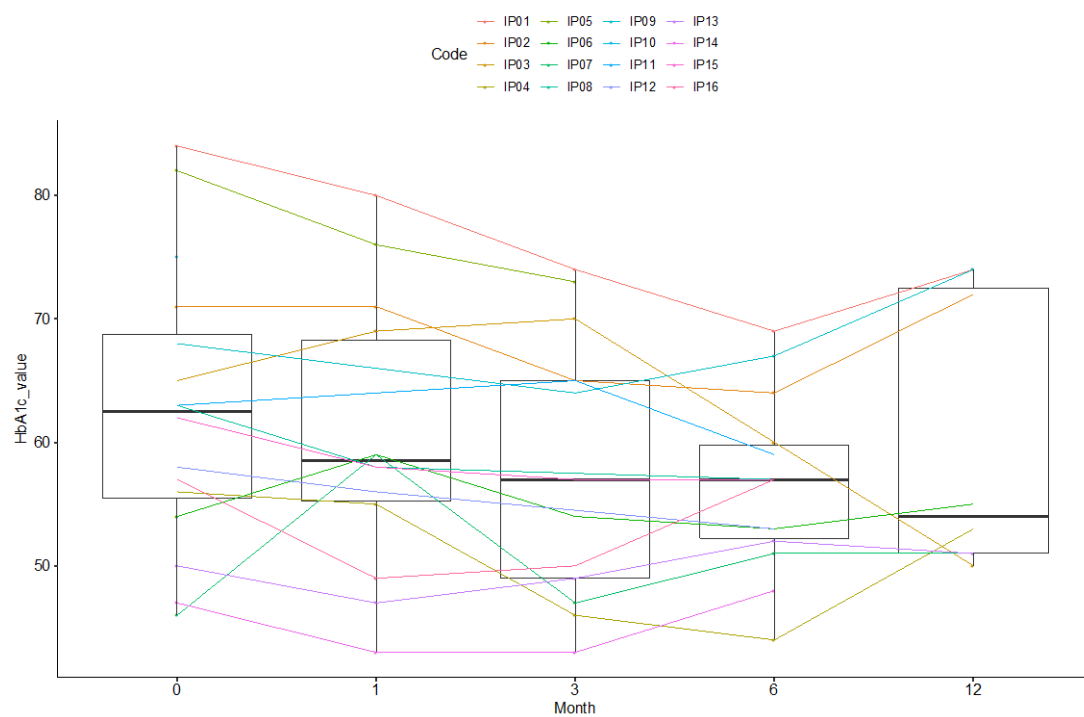
4.3.2 Glycaemic Control and CGM Results across visits

Changes in HbA1c and CGM data across the study visits is summarised in Table 4-3. There was a trend towards improved glycaemic control with reduced HbA1c and average glucose from baseline to month 6 but no statistically significant change in glycaemic markers across the whole study. Changes in HbA1c and percentage time in range (4-10mmol/L) across the study are shown in Figure 4-21.

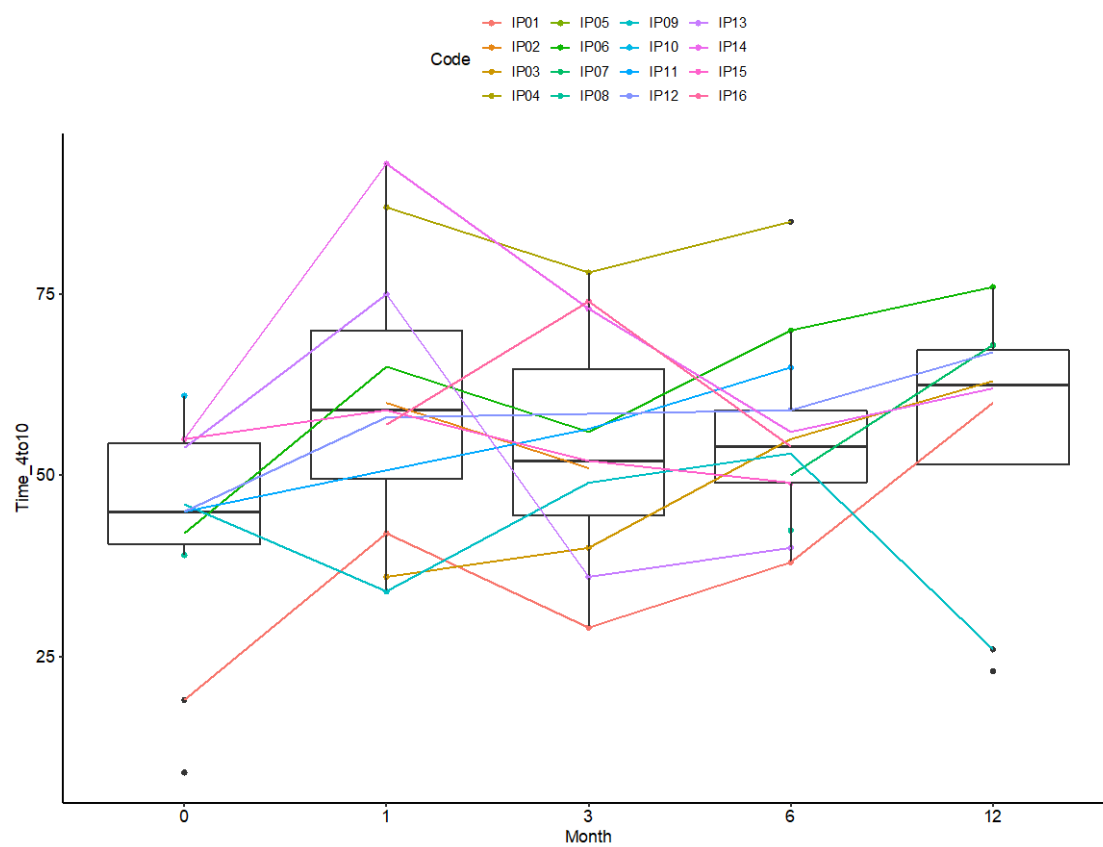
Table 4-3 Glycaemic control and CGM results across visits

HbA1c and CGM Results for Pump Participants (Mean $\pm$ SD)						
	Baseline	Month 1	Month 3	Month 6	Month 12	P Value
HbA1c (mmol/mol)	63 $\pm$ 11 N=16	60 $\pm$ 11 N=14	58 $\pm$ 11 N=13	57 $\pm$ 7 N=14	57 $\pm$ 11 N=11	0.50
No. completing adequate CGM	11/16	11/14	11/13	13/14	8/11	-
% Time >10mmol/L	54.9 $\pm$ 17.3	35.3 $\pm$ 20.7	41.2 $\pm$ 18.4	39.7 $\pm$ 14.4	38.4 $\pm$ 23.2	0.14
% Time 4-10mmol/L	42.7 $\pm$ 15.8	60.5 $\pm$ 19.1	54.0 $\pm$ 15.9	55.1 $\pm$ 12.8	55.6 $\pm$ 19.8	0.16
% Time <4mmol/L	2.4 $\pm$ 3.0	4.2 $\pm$ 4.7	4.8 $\pm$ 3.2	5.1 $\pm$ 5.2	6 $\pm$ 6.5	0.48
Average Glucose (mmol/l)	10.5 $\pm$ 1.5	9.1 $\pm$ 1.8	9.6 $\pm$ 1.6	9.3 $\pm$ 1.2	9.6 $\pm$ 2.5	0.39
SD Glucose	3.4 $\pm$ 0.6	3.1 $\pm$ 0.94	3.5 $\pm$ 0.5	3.2 $\pm$ 0.6	3.4 $\pm$ 0.8	0.73
CV Glucose	33.6 $\pm$ 8.4	34.2 $\pm$ 8.2	37.1 $\pm$ 4.4	35.7 $\pm$ 7.5	35.6 $\pm$ 6.2	0.82

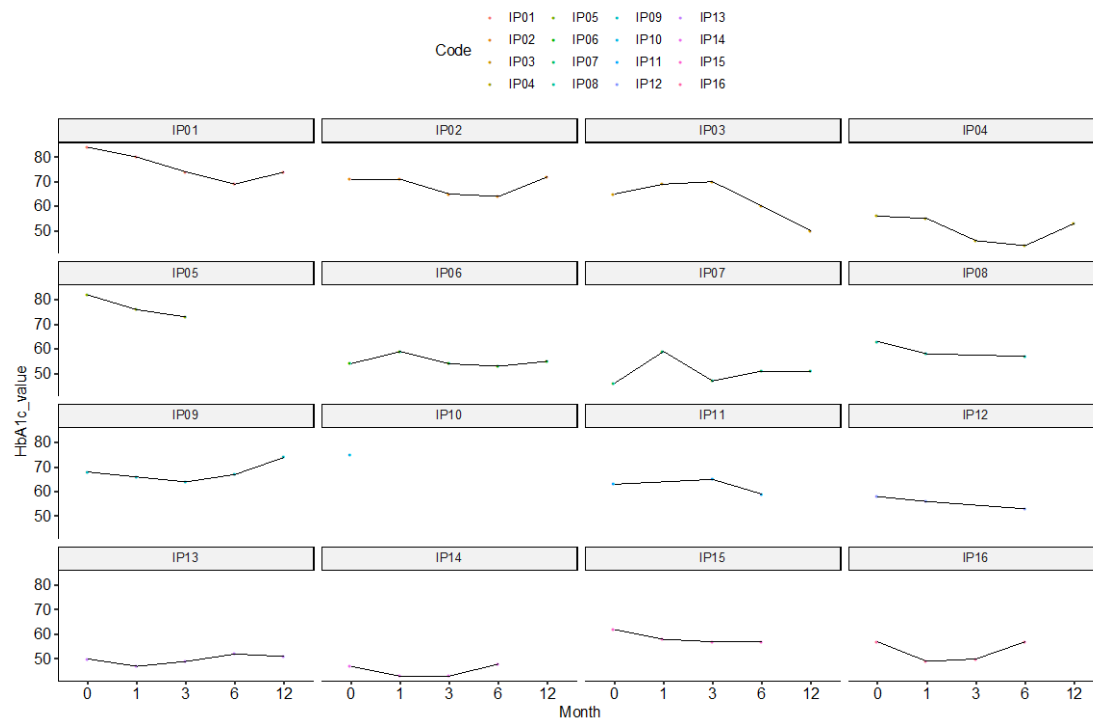
(A)



(B)



(C)



(D)

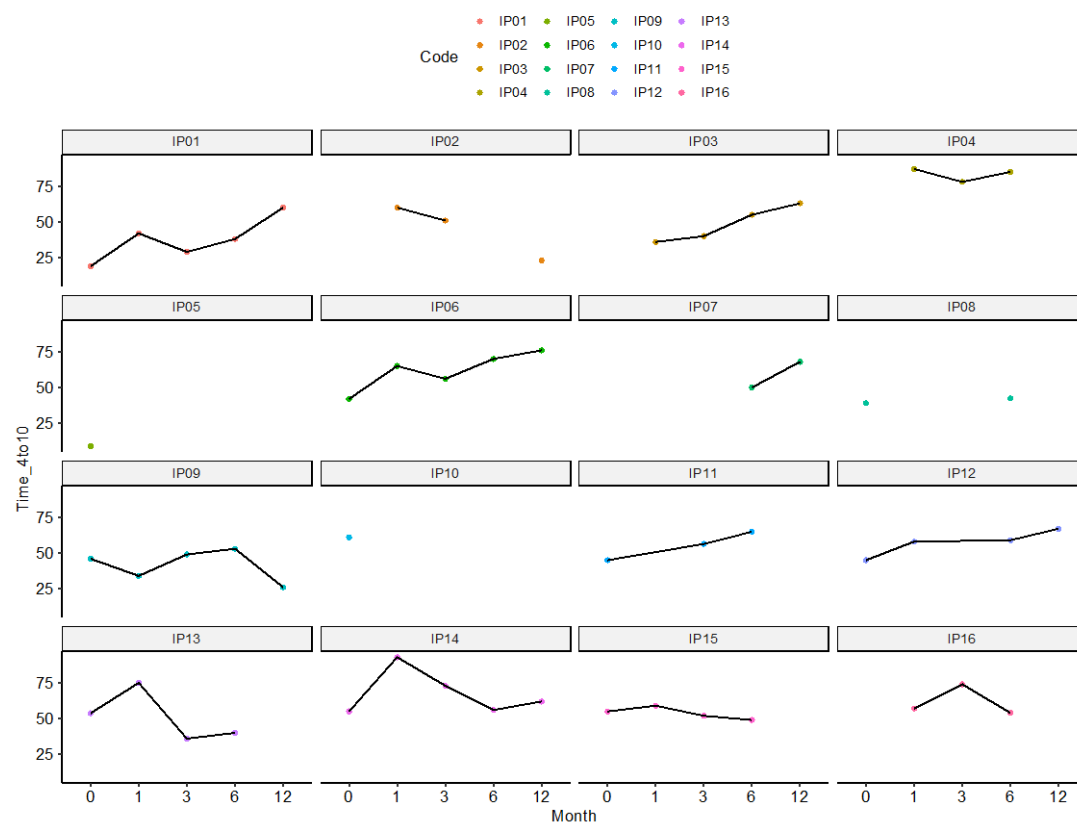


Figure 4-21 Box plots summarising (A) changes in HbA1c and (B) percentage time in target (4-10mmol/L) across visits. Changes for each participant are displayed as an overlying line graph on both (A) and (B). Changes for each

participant are also shown as individual line graphs for (C) changes in HbA1c and (B) percentage time in target (4-10mmol/L)

4.3.3 Neuropathy and ACR

At baseline one person had established peripheral neuropathy secondary to previous spinal pathology. All other participants had no history of peripheral neuropathy at baseline and neurothesiometer scores were within normal range. No participant developed new peripheral neuropathy, and there was no change to peripheral neuropathy in the individual with known peripheral neuropathy, during follow up.

There was no evidence of baseline nephropathy in any participants. During follow up there were no significant changes to ACR or creatinine measurements in any participants.

4.3.4 Retinal imaging results

4.3.4.1 OCTA: FAZ and Vessel Density Measurements

Baseline CSII and control OCTA results for FAZ area, FAZ perimeter, AI and vessel density measurements are shown in Table 4-4

Measurements from 3x3 images showed CSII participants had significantly lower vessel density at baseline compared to controls. The superficial parafoveal region was most sensitive to density changes, with significant differences seen in both the 3x3 images (CSII $48.25\% \pm 3.92\%$, Control $51.67\% \pm 1.76\%$, $p = 0.005$), and 6x6 images (CSII $50.28\% \pm 4.64\%$, $53.30 \pm 2.91\%$, $p = 0.03$).

There were no significant differences in FAZ area or perimeter between CSII and control groups, and no difference in vessel density at the fovea.

AI was significantly higher in CSII participants versus controls on 3x3 imaging (1.17 ± 0.06 Versus 1.11 ± 0.03 , $p = 0.004$) indicating increased irregularity (less circularity) in the FAZ shape in CSII participants.

There were no significant changes in FAZ characteristics or density measurements for any of the regions assessed in CSII participants over one year (Figure 4-22, Figure 4-23, Figure 4-24).

Chapter 4: Analysis using novel retinal imaging techniques

Table 4-4 FAZ and Vessel Density Measurements for CSII participants at baseline versus non-diabetic controls

Measure		3x3			6x6		
		CSII	Control	P Value	CSII	Control	P Value
FAZ Characteristics	Area (mm ²)	0.21 ± 0.09	0.24 ± 0.11	0.37	0.20 ± 0.08	0.23 ± 0.10	0.32
	AI	1.17 ± 0.06	1.11 ± 0.03	0.004	1.08 ± 0.04	1.07 ± 0.02	0.30
	Perimeter (mm)	1.85 ± 0.42	1.90 ± 0.45	0.72	1.67 ± 0.35	1.78 ± 0.40	0.40
Superficial Layer Density (%)	Whole ETDRS	45.66 ± 3.81	48.34 ± 1.81	0.02	49.30 ± 3.86	50.45 ± 3.02	0.36
	Fovea	25.16 ± 5.30	22.11 ± 6.74	0.17	29.75 ± 5.62	25.39 ± 6.44	0.05
	Parafovea	48.25 ± 3.92	51.67 ± 1.76	0.005	50.28 ± 4.64	53.30 ± 2.91	0.03
Deep Layer Density (%)	Whole ETDRS	50.04 ± 3.34	52.68 ± 2.25	0.01	52.76 ± 4.14	54.07 ± 6.75	0.52
	Fovea	35.93 ± 6.58	36.44 ± 7.57	0.83	41.98 ± 5.14	42.94 ± 7.70	0.68
	Parafovea	51.83 ± 3.40	54.74 ± 2.59	0.01	54.38 ± 3.80	56.56 ± 5.99	0.23

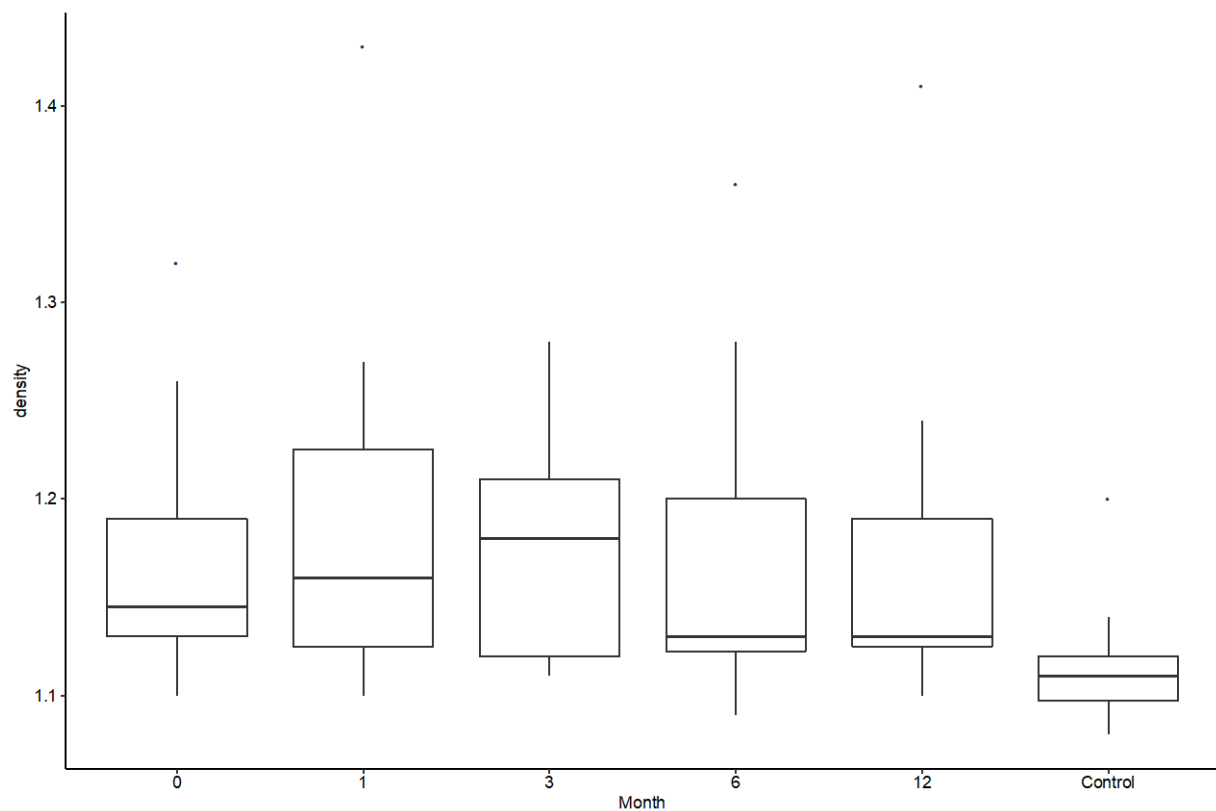


Figure 4-22 Changes in Acircularity Index assessed from 3x3mm OCTA images across the study period for CSII participants with diabetes, with comparison to control non-diabetic group

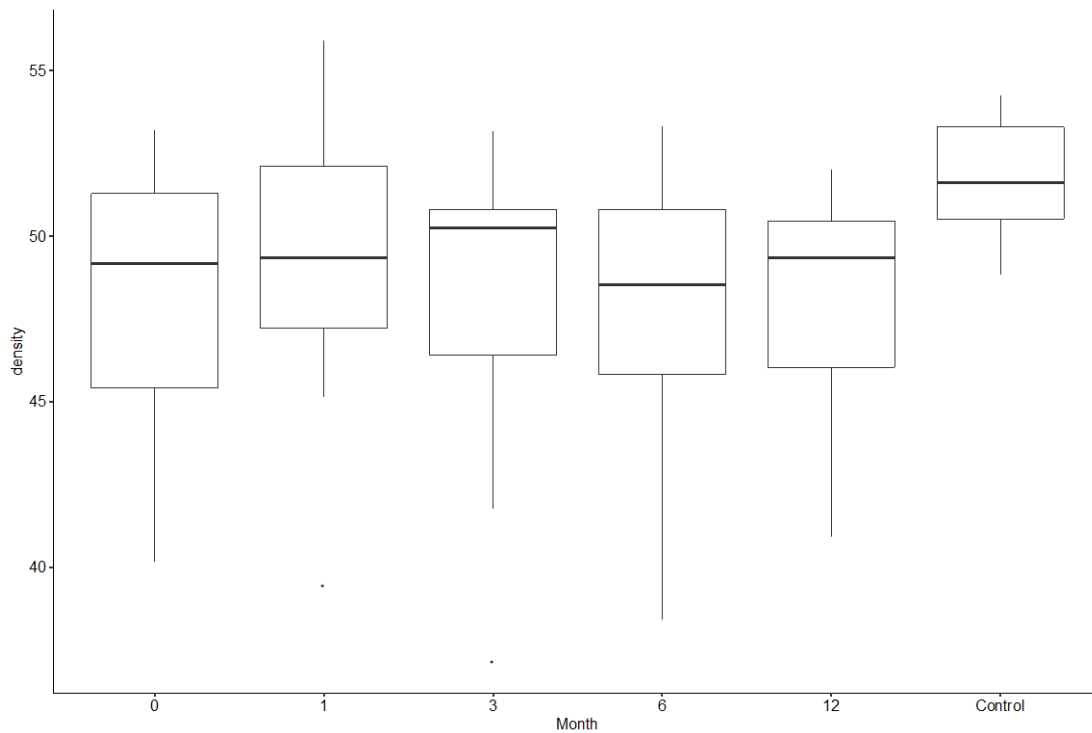


Figure 4-23 Changes in Parafoveal density assessed from 3x3mm superficial images across the study period for CSII participants with diabetes, with comparison to control non-diabetic group

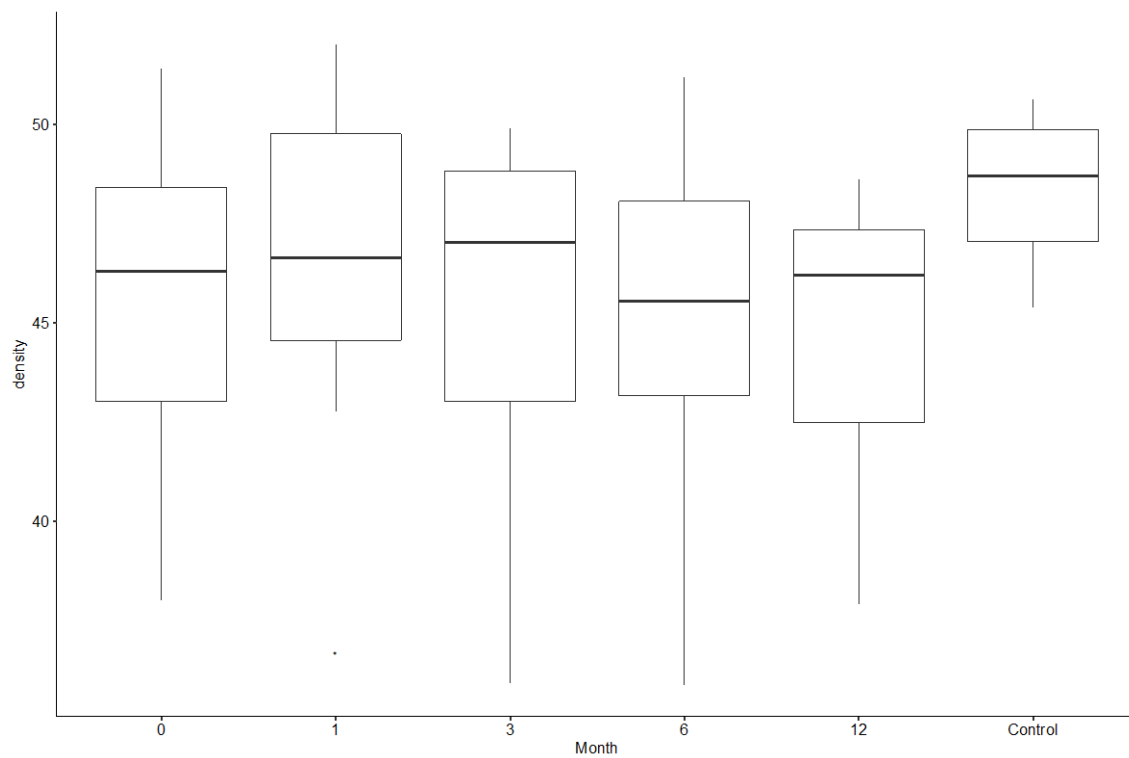


Figure 4-24 Changes in vessel density over whole ETDRS grid assessed from 3x3mm superficial images across the study period for CSII participants with diabetes, with comparison to control non-diabetic group

4.3.4.2 OCT: Retinal and Choroidal Thickness

Baseline OCT measurements for CSII participants and non-diabetic controls are summarised in Table 4-5.

There were no significant differences in retinal thickness, macular volume or RNFL thickness for CSII and control groups.

Choroid thickness was significantly thinner in CSII participants versus non-diabetic controls in the subfoveal (278.60 ± 80.53 versus 340.21 ± 74.77 , $p=0.04$) and nasal regions (205.70 ± 68.03 versus 293.29 ± 67.57 , $p=0.003$).

There were no significant changes in any OCT measurements for CSII participants over one year (Figure 4-25).

Table 4-5 OCT Retinal and Choroidal thickness in CSII participants at baseline versus non-diabetic controls

	CSII (Baseline)	Non-diabetic Controls	P Value
Central Retinal Thickness	281.80 ± 39.02	$283.61 \pm$	0.89
Retinal Macular volume	8.65 ± 0.37	8.87 ± 0.30	0.11
Subfoveal Choroid Thickness	278.60 ± 80.53	340.21 ± 74.77	0.04*
Nasal Choroid Thickness	205.70 ± 68.03	293.29 ± 67.57	0.003**
Temporal Choroid Thickness	241.40 ± 68.03	261.11 ± 71.09	0.44
Global RNFL Thickness	102.78 ± 15.21	99.26 ± 10.78	0.53

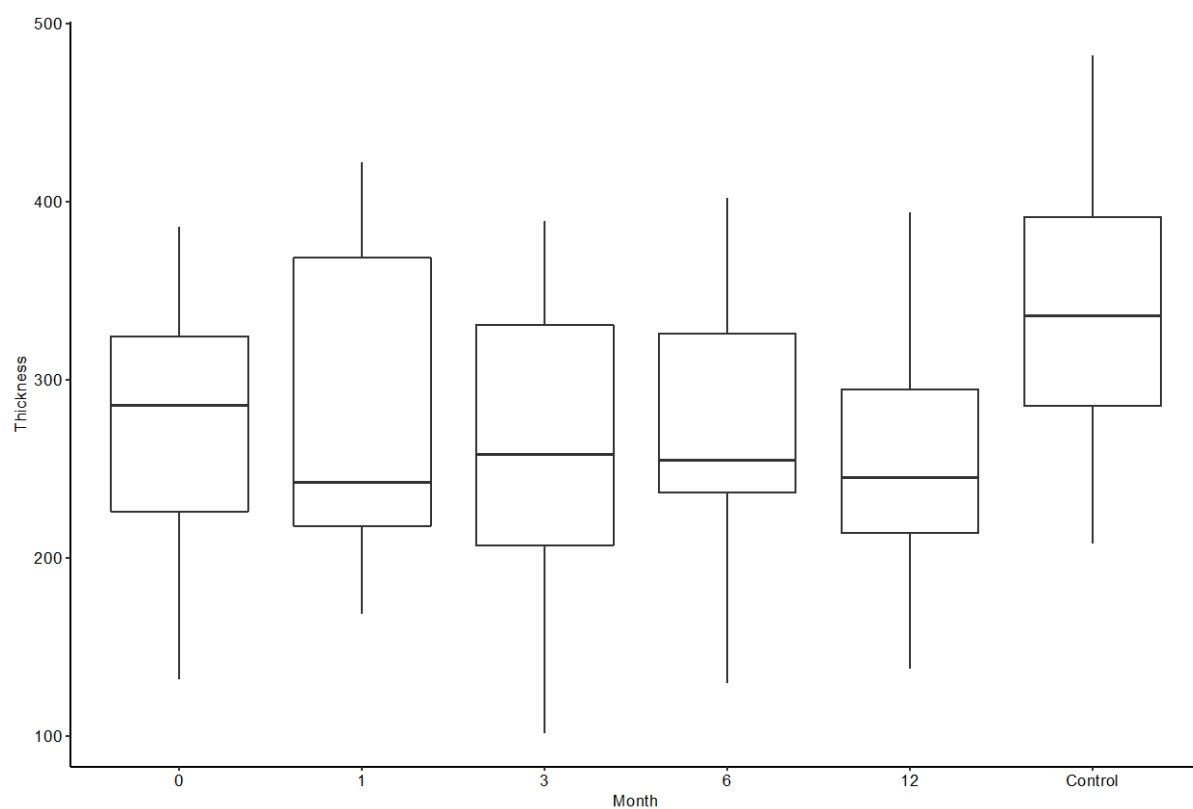


Figure 4-25 Changes in subfoveal choroidal thickness measurements assessed from OCT images across the study period for CSII participants, with comparison to control non-diabetic group

4.3.4.3 Fundus Imaging: VAMPIRE Analysis of Vessel Geometrics

Paired data were available for 14/16 participants (one excluded due to poor quality images, one excluded due to no follow up images after baseline visit). Results of VAMPIRE analysis from fundus images of CSII participants are shown in Table 4-6

There were no significant changes in any of the vessel geometrics assessed over the available study period.

Table 4-6 VAMPIRE Results CSII Baseline and One Year

VAMPIRE results				
Section	Analysis	Baseline image	Last available image	P Value
Vessel Width (pixels)	CRAE	28.63 ± 2.17	29.04 ± 1.80	0.42
	CRVE	38.59 ± 4.58	40.40 ± 3.83	0.10
	AVR	0.75 ± 0.07	0.72 ± 0.07	0.15
Fractals Whole FOV	Artery	1.32 ± 0.03	1.32 ± 0.03	0.96
	Vein	1.30 ± 0.04	1.31 ± 0.04	0.10
	Total	1.44 ± 0.02	1.44 ± 0.03	0.63
Tortuosity mean tortuosity log trans	Artery	-8.38 ± 0.32	-8.47 ± 0.27	0.20
	Vein	-8.31 ± 0.31	-8.36 ± 0.28	0.58

4.3.4.4 Optos UWF-SLO

Paired data for baseline and one year images was available for 14/16 participants (one excluded due to no follow up images after baseline visit, one excluded due to no suitable images of high enough quality for analysis at month 6 or 12). Results for analysis are shown in Table 4-7.

There were no significant changes in any of the vessel geometrics assessed over the available study period.

Table 4-7 Results of vessel calibre changes in inner and outer band for Optos UWF-SLO images

Optos UWF-SLO Results				
Section	Analysis	Baseline image	Last available image	P Value
Inner band	Mean arterial calibre	101.84 ± 12.69	98.20 ± 9.65	0.29
	Mean venular calibre	120.16 ± 11.04	113.57 ± 11.97	0.17
	AVR	0.85 ± 0.09	0.87 ± 0.13	0.41
Outer band	Mean arterial calibre	99.16 ± 8.25	96.38 ± 5.92	0.30
	Mean venular calibre	111.03 ± 10.28	109.13 ± 9.76	0.61
	AVR	0.89 ± 0.06	0.89 ± 0.06	0.77

4.4 Discussion

This study prospectively assessed a range of retinal biomarkers which have previously been associated with DR using four retinal imaging modalities and found no significant change in retinal characteristics in the 12 months following the introduction CSII therapy in people with established Type 1 diabetes. Importantly there was no evidence of EWDR despite a trend for improved glycaemic control in the first 6 months post CSII.

As previously discussed, data from the DCCT has established that improvements in glycaemic control are associated with significantly reduced DR risk (236), and CSII therapy has been shown to have small advantages over MDI therapy in improving markers of glycaemic control (26). It therefore follows that CSII may offer advantages with regards to microvascular risk reduction. However rapid improvements in glycaemic control has also been shown to cause EWDR (7) and current practice in our centre following the introduction of CSII therapy is for more frequent retinal screening intervals at baseline and 3 months post introduction of CSII therapy to detect any potentially clinically significant changes in diabetic eye disease. This study supports other findings from this thesis (See Chapter 2) that, in those with little or no DR at baseline, there is no evidence of early changes in keeping with DR progression (109).

There are very few studies which have previously assessed the early impact of CSII on microvascular retinal changes. Our study supports previous findings from Klefter *et al.* who similarly assessed retinal changes over one year post CSII therapy using OCT (66). While there was a small increase in central retinal thickness, there was no change in any other retinal markers assessed, and no clinically significant retinal changes. This study also assessed a control group who continued on MDI therapy, and found no significant difference in retinal markers compared to MDI counterparts, despite a significantly larger HbA1c reduction in the CSII group versus the MDI group (17.8mmol/mol versus 3.1mmol/mol). Participants were of similar age to those included in this study, though had a longer duration of diabetes, higher HbA1c and higher proportion of established retinopathy at baseline. Our study had the advantage of including a wider range of imaging modalities for assessment, including OCTA and UWF-SLO.

A more recent study assessed retinal characteristics using OCTA and OCT in people with Type 1 diabetes with no or mild DR who were already established on CSII therapy (mean duration of CSII therapy 5 years) compared to a similar cohort on MDI therapy (237). They showed no significant differences in retinal thickness on OCT, and no change in FAZ characteristics on OCTA between CSII and MDI groups, supporting our findings that changing from MDI to CSII is not associated with changes in these parameters in those with little or no established DR. They did show that superficial parafoveal density values were lower in MDI participants than CSII participants, suggesting that MDI is associated with increased microvascular damage compared to CSII. However, the CSII group had a significantly lower HbA1c than the MDI group, and though this may reflect improved glycaemic control as a direct result of CSII therapy, this cannot be directly established from the study and the lower HbA1c in the CSII group may therefore have confounded retinal outcomes.

Our study also compared findings in people with Type 1 diabetes commencing CSII therapy to healthy controls without diabetes. We have shown vessel density and choroidal thickness (214-224) are significantly reduced in participants with Type 1 diabetes versus those without diabetes, while acircularity index is significantly increased, indicating more a more irregular FAZ shape (99). No changes in retinal thickness, FAZ area or FAZ perimeter were identified.

These findings support growing evidence from the literature of the value of these parameters as early markers of DR. Reduced vessel density using OCTA in people with diabetes has now been established in multiple studies (88, 89, 172, 180-184) and may represent the loss of healthy retinal vasculature due to reduced perfusion from early microvascular damage. Vessel density changes are thought to precede changes in FAZ area (180, 181) and this may be why we did not see any reduction in FAZ area in our study, particularly as the majority of participants did not have any evidence of DR at baseline. We found the superficial parafoveal region to be the most sensitive to vessel density changes, contrary to other studies which identified early changes more readily in the perifoveal regions (183). Irregularity of the FAZ shape, denoted by an increased acircularity index is a relatively new retinal biomarker for DR. Our study suggests this could be a useful indicator of early changes associated with diabetes, though further studies with longer prospective follow up will help characterise whether this marker could be utilised for clinical retinal screening programmes. Changes in the choroid

vasculature is also an area of growing interest in diabetic eye disease, particularly with the increasing accessibility of OCT imaging, and our findings support previous studies assessing diabetic choroidopathy. We identified changes in both the subfoveal and nasal choroid regions. Pathology may be similar to that postulated for vessel density reduction in diabetes, with the loss of healthy choroidal vessels indicating early microvascular damage.

We did not identify any changes in retinal thickness in our study. Increased retinal thickness is associated with diabetic macular oedema and increased severity of DR. Participants in our study had no or mild DR at baseline and no evidence of clinically significant diabetic macular oedema, therefore this finding was not surprising.

There was also no change in assessment of other microvascular endpoints for sensory peripheral neuropathy and nephropathy, though this may have been due to the relatively short follow up period in a group without established diabetic neuropathy or nephropathy complications.

There was a trend towards improved glycaemic markers in the first 6 months following the introduction of CSII therapy, particularly for those with poorer glycaemic control at baseline, but this did not reach statistical significance.

This study provides a comprehensive prospective assessment of retinal disease following the introduction of CSII therapy using multiple retinal imaging modalities. It is strengthened by the inclusion of age and sex matched controls assessed using the same protocols for OCT and OCTA imaging.

This study has several limitations. Participants numbers were lower than anticipated due to funding issues for CSII therapy initiation at the time of recruitment which could not be foreseen prior to the study. This led to fewer eligible people being commenced on CSII therapy than expected. Had we been able to include more participants we may have been more likely to see significant changes in retinal markers post CSII therapy. Analysis of the relationship between glycaemic markers, particular CGM parameters, and retinal data were also affected by the small study numbers. There was also a lack of participants with higher

degrees of DR severity or clinically significant macular oedema, with all participants graded to have no or mild DR at baseline. Follow up time may have been too short, particularly given the lack of participants with high risk DR severity gradings. This study also lacked an adequate comparator group of participants on MDI therapy. Though multiple attempts were made to find a suitable MDI group for comparison, few volunteers came forward, and those who did were not well matched to the CSII therapy group, as they were an older highly motivated group with longer diabetes duration and excellent glycaemic control at baseline which made it difficult to establish any clear conclusions, particularly given the very small numbers which were not suitable for robust statistical analysis. Finally this study relied on automated software for extraction of the bulk of retinal data, particularly for OCTA analysis. While the use of automated data was consistent across participants, there may be unknown confounders which we have been unable to account for in our analysis.

Future work established during collaborations from this study aims to establish independent analysis of retinal data which may also identify retinal markers which would not be identified on automated analysis (90). Further studies assessing retinal changes post CSII therapy in those with higher severity DR at baseline are also needed to fully characterise the impact of CSII therapy on retinal disease, as well as larger studies with longer follow up periods. The addition of a suitable MDI comparator group would also have strengthened this study and more work comparing retinal changes in those who receive CSII therapy to those on continued MDI is needed.

4.5 Conclusion

In summary, we have shown stable retinal disease assessed by multiple retinal imaging modalities in people with Type 1 diabetes with minimal or mild DR in the first year following CSII therapy, with no evidence of EWDR, providing reassurance for physicians and those with Type 1 diabetes wishing to commence CSII therapy.

5 Conclusions

The early part of this thesis summarises what is already known with regards to DR changes relating to improved glycaemic control, including the potential risk of EWDR, and the limited evidence currently available in assessing DR changes associated with the introduction of CSII therapy and islet transplantation. The growing use of a wider range of retinal imaging modalities and their role in retinal pathology has also been discussed. The studies described in this thesis set out to address the following hypotheses:

1. Reduction of time in hyperglycaemia and hypoglycaemia post intervention with islet transplantation or CSII would result in changes consistent with EWDR when compared to those continued on MDI therapy.
2. Improved glycaemic control post intervention with islet transplantation or CSII would result in reduced risk of DR in the long term when compared to those continued on MDI therapy.
3. Retinal biomarkers will be able to detect early DR changes in individuals with diabetes following the introduction of these treatment interventions.

5.1 Hypothesis 1: Increased risk of EWDR post CSII or islet transplantation compared to continued MDI therapy – Evidence presented does not support this hypothesis in people with no or mild baseline retinopathy

Evidence from the three studies described in this thesis, including retrospective analysis of graded retinal screening outcomes (chapter 2), retrospective analysis of quantitative retinal biomarkers from retinal screening images (chapter 3), and prospectively collected retinal imaging data using multiple imaging sources including OCT, OCTA and UWF-SLO (chapter 4), did not support hypothesis 1. Specifically, these studies found no evidence of EWDR in the first 12 months post initiation of CSII therapy despite significant improvements in glycaemic markers. Similarly, there was no evidence of EWDR in the first 12 months post islet transplantation, though outcomes were assessed in one study only (chapter 2) due to small

study numbers which limited analysis in other studies. The studies included little or no participants with high severity of retinopathy at baseline, therefore these conclusions are limited to those with no or mild baseline retinopathy.

The original hypothesis was largely based on evidence from DCCT which showed EWDR in 13% of those on intensive insulin treatment versus 7.6% on conventional insulin treatment over 12 months (7). Mean baseline HbA1c of participants in DCCT was higher across groups than for participants in our studies (DCCT Mean HbA1c 72 - 74mmol/mol (8.8-9%) compared to a HbA1c of 63-67mmol/mol across our studies). As mean HbA1c is one of the strongest risk factors for DR (60) this will likely have contributed to higher rates of DR, including EWDR, seen in DCCT than in our studies. DCCT was also comparing the impact of conventional insulin therapy at the time, using administration of one or two daily injections of insulin, to intensive insulin with at least three daily insulin injections per day or insulin delivery by CSII. This intensive insulin regimen with either MDI or CSII is now considered standard care and all our participants across studies were managed with intensive insulin, either via MDI or CSII. As such, differences seen in DR progression between patient groups may have been less marked than in DCCT. The use of intensive insulin therapy in our cohort has likely contributed to the lower mean baseline HbA1c in our participants compared to those included in DCCT, in addition to other advancements in diabetes management such as improved insulin pen and pump delivery devices and the development of new insulins. DCCT also included more participants with higher severity of DR at baseline, who were more likely to be affected by EWDR, and DR in general. Early islet transplant studies where participants were found to have EWDR similarly included participants with higher HbA1c and higher severity of DR at baseline. Following the concerns highlighted by DCCT regarding EWDR, additional retinal monitoring has been put in place in many diabetes centres, including our own, for people who may be at risk of developing EWDR, to facilitate early ophthalmology input if needed. In our centres this includes an additional retinal screening assessment 3 months post introduction of CSII therapy, which may have helped protect our cohort from EWDR.

The lack of EWDR identified in the studies described in this thesis are in keeping with more recent studies which suggest that EWDR following tightening of glycaemic control is not as problematic as once thought. No EWDR was found on OCT assessment following the

introduction of CSII therapy in a study similar to that described in Chapter 4 (66). Another comparing OCTA and OCT changes in those established on CSII found no significant differences in retinal biomarkers between MDI and CSII participants (237). Rapid improvement in glycaemic control in Type 1 diabetes following other therapies, namely the introduction of flash glucose monitoring devices, has also not been found to increase risk of EWDR (65).

The inclusion of participants with higher severity of DR at baseline may have helped confirm whether there was any significant EWDR risk following CSII therapy or islet transplantation in the broader Type 1 diabetes population.

5.2 Hypothesis 2: Reduced long term DR risk following CSII or islet transplantation compared to continued MDI therapy – Evidence presented supports this hypothesis

Hypothesis 2 was addressed by reviewing real world data using images from the diabetes retinal screening service to assess retinal changes up to 3 years post intervention both in terms of graded retinal outcomes (chapter 2) and quantitative changes in retinal vasculature biomarkers (chapter 3).

Evidence from assessment of graded retinal imaging outcomes supports hypothesis 2 for CSII participants, showing reduced progression of DR in participants following the introduction of CSII compared to continued MDI therapy. Outcomes for islet transplanted participants showed reduced DR progression on univariate analysis of graded retinal outcomes compared to participants continued on MDI therapy, though significance was lost on multivariate analysis. DR progression in islet transplanted participants was comparable to those initiated on CSII therapy.

One of the main challenges in establishing the initial design for this study was identifying an appropriate control group and allocating an appropriate control study period in the absence of a specified intervention date for those not commenced on CSII or receiving islet transplantation. The control group chosen compared individuals who had received similar

diabetes education to those who received CSII therapy, were established on intensive insulin therapy via MDI (including carbohydrate counting) and who would have later been eligible for CSII therapy. This was primarily chosen as a suitable control group for the CSII participants rather than islet transplant recipients. Both the CSII and selected control MDI groups were subsequently also used as control groups for the islet transplant group, however, this was not an ideal comparator group for two main reasons. Firstly, the participants in the CSII and MDI group were not assessed for IAH, which is one of the main indications for islet transplantation and which would therefore be highly prevalent in the islet transplant participants. Secondly, the gold standard treatment for people with debilitating IAH who are being considered for islet transplantation would be a trial of sensor augmented CSII therapy prior to transplant, which would not have been in use for the majority of the comparator CSII participants. A more suitable control group would therefore likely have included people who were eligible for islet transplantation but who didn't proceed to islet transplantation, with consideration of a study period from the time of initial transplant referral or assessment. Alternative study designs for comparing CSII and MDI groups were also considered, including using the date of the diabetes education as time 0 and modelling the intervention group (CSII or MDI) using a time dependent model, but it was felt that the study design which was eventually used to compare CSII and MDI outcomes was clinically relevant and sufficient to address the study aims.

Assessment of the islet cohort was limited by smaller participant numbers resulting in the need to analyse graded retinal data collected from two distinct sites. Though this was not ideal as it involved converting DR grading scores for participants from one site, it has resulted in one of the largest analyses of DR in islet transplant recipients to date. The issue of small study cohorts is a recurring theme in islet studies and highlights one of the main challenges in studying a field with relatively few eligible participants at individual centres. This highlights how valuable collaboration between centres is in gathering adequate data to establish a sufficient evidence base.

Quantitative assessment of retinal biomarkers, using a sample of the same screening images reviewed for the graded retinal outcomes study in Chapter 2, was also carried out to investigate longer term retinal changes following CSII or islet transplant therapy. This showed significant reductions in CRAE, CRVE and FD over time following the introduction of CSII

therapy. This study was limited to participants involved with the diabetes retinal screening programme only and therefore study numbers for islet transplant participants were small, limiting analysis. This study also lacked a control MDI group who had not received an intervention. This limited the conclusions which could be drawn, particularly given that there is still uncertainty about the direction of changes in biomarkers such as FD associated with diabetes pathology. Acquiring more comprehensive information regarding the presence or absence of macular oedema, such as concurrent analysis of OCT images would also have helped consolidate conclusions from this study, and may be an area for future analysis as OCT becomes incorporated into the standard Scottish diabetes retinal screening programme.

Both of the studies assessing longer term DR outcomes were based on a retrospective study design. Study limitations associated with a retrospective assessment were therefore expected and have been discussed previously.

With the exception of the Edmonton islet participants, these studies included only those participants who were actively participating in the Scottish retinal screening programme, with the exclusion of those under active ophthalmology assessment. This was due to several reasons including challenges in obtaining and following up the relevant data from multiple sources, and additional complexities in establishing a robust study design to incorporate varied ophthalmic assessments in people with established pathology undergoing active retinal treatment. However, by excluding this group the studies are limited in their ability to assess DR progression in a higher risk population with more extensive baseline DR, and findings are therefore not generalisable to the entire Type 1 diabetes cohort. Further analyses of retinal data in people with T1DM with higher DR severity are needed to fully establish the extent of DR progression in a wider range of Type 1 diabetes participants and will be an area for potential future study.

5.3 Hypothesis 3: Retinal biomarkers useful in early detection of DR – Evidence presented supports this hypothesis

The utility of retinal biomarkers in DR detection were primarily assessed in the prospective study described in chapter 4 of this thesis. This study identified clear differences in several

biomarkers in people with type 1 diabetes compared to healthy controls, namely reduced vessel density and choroidal thickness and increased acircularity index, supporting the hypothesis that they could play a role in identifying early DR changes.

One of the aims of this study had been to assess changes in retinal biomarkers in the first year following intervention with CSII or islet transplantation. The study encountered challenges in participant recruitment due to various factors which have been discussed, resulting in a more limited analysis than was originally proposed. In particular, changes in DR markers post islet transplantation could not be assessed due to insufficient numbers. Results showed changes in retinal biomarkers post CSII treatment were stable, and support findings from the retrospective part of this thesis that there is no evidence of EWDR, but as a small pilot study the conclusions drawn will be limited.

5.4 Future Directions

The data presented in this thesis has focussed on DR changes directly relating to changes in the mode of insulin delivery from MDI to CSII, or following islet transplantation. While we have assessed glycaemic changes associated with treatment changes, this has largely been limited to changes in HbA1c. There is growing evidence that changes in GV and other glycaemic markers impact DR (15, 16, 133, 134) and this will be an important area for further study, particularly given the advent of widespread CGM and growing use of closed loop technology.

The population assessed in this thesis was mostly a Scottish Caucasian cohort therefore data will not be validated across a more diverse population. Further assessment of retinal changes in people from a broader range of ethnicities will ensure findings are generalisable to all those with Type 1 diabetes.

Similarly, the majority of data presented from our studies come from people with mild or no established DR who have been attending diabetes retinal screening services, and will not necessarily be representative of people with more severe DR or those who are not fully engaging with all aspects of their diabetes care. Analysis of retinal records for those with

established DR who have required further assessment or treatment under the ophthalmology team would be a useful next step in clarifying the impact of CSII and islet transplantation on those with more complicated retinal disease.

Data presented here has been strengthened through the inclusion of retinal outcomes from a range of imaging devices, however it is acknowledged that the cohorts assessed with novel retinal imaging devices such as OCTA were small. Larger prospective studies are needed to offer clarity on the role of novel retinal biomarkers in DR assessment.

5.5 Summary

In conclusion, the work presented in this thesis did not find any evidence for EWDR following the introduction of CSII therapy or islet transplantation in our cohort, despite early improvements in glycaemic control. There was evidence for reduced DR progression in the longer term following CSII therapy compared to continued MDI therapy, which could indicate potential benefits of CSII in reducing the burden of microvascular complications due to DR in Type 1 diabetes. This work also reports stable DR post islet transplantation compared to CSII and MDI participants, despite the addition of potentially damaging immunosuppressive therapy. The data supports early reports for the growing utility of retinal biomarkers derived from novel imaging modalities in the early identification of DR, particularly markers of vessel density, choroidal thickness and AI. Further larger prospective studies will help characterise the full significance of these markers and their future potential in DR assessment and intervention.

6 References

1. WHO | . WHO. 2017.
2. Hex N, Bartlett C, Wright D, Taylor M, Varley D. Estimating the current and future costs of Type 1 and Type 2 diabetes in the UK, including direct health costs and indirect societal and productivity costs. *Diabetic medicine : a journal of the British Diabetic Association*. 2012;29(7):855-62.
3. Mathur R, Bhaskaran K, Edwards E, Lee H, Chaturvedi N, Smeeth L, et al. Population trends in the 10-year incidence and prevalence of diabetic retinopathy in the UK: a cohort study in the Clinical Practice Research Datalink 2004-2014. *BMJ open*. 2017;7(2):e014444.
4. Yau JW, Rogers SL, Kawasaki R, Lamoureux EL, Kowalski JW, Bek T, et al. Global prevalence and major risk factors of diabetic retinopathy. *Diabetes care*. 2012;35(3):556-64.
5. Rohan TE, Frost CD, Wald NJ. Prevention of blindness by screening for diabetic retinopathy: a quantitative assessment. *BMJ*. 1989;299(6709):1198-201.
6. Nathan DM. The Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications Study at 30 Years: Overview. *Diabetes care*. 372014. p. 9-16.
7. Early worsening of diabetic retinopathy in the Diabetes Control and Complications Trial. *Archives of ophthalmology (Chicago, Ill : 1960)*. 1998;116(7):874-86.
8. Chew EY, Mills JL, Metzger BE, Remaley NA, Jovanovic-Peterson L, Knopp RH, et al. Metabolic control and progression of retinopathy. The Diabetes in Early Pregnancy Study. National Institute of Child Health and Human Development Diabetes in Early Pregnancy Study. *Diabetes care*. 1995;18(5):631-7.
9. Eisenbarth GS. Type I diabetes mellitus. A chronic autoimmune disease. *The New England journal of medicine*. 1986;314(21):1360-8.
10. American Diabetes A. Diagnosis and classification of diabetes mellitus. *Diabetes care*. 2004;27 Suppl 1:S5-S10.
11. Fowler MJ. Microvascular and Macrovascular Complications of Diabetes. *Clinical Diabetes*. 2008;26(2):77-82.
12. Diabetes C, Complications Trial Research G, Nathan DM, Genuth S, Lachin J, Cleary P, et al. The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. *The New England journal of medicine*. 1993;329(14):977-86.
13. Use of Glycated Haemoglobin (HbA1c) in the Diagnosis of Diabetes Mellitus: Abbreviated Report of a WHO Consultation. 2011.
14. Group TDCaCTR. The Relationship of Glycemic Exposure (HbA1c) to the Risk of Development and Progression of Retinopathy in the Diabetes Control and Complications Trial. *Diabetes*. 1995;44(8):968-83.
15. Nalysnyk L, Hernandez-Medina M, Krishnarajah G. Glycaemic variability and complications in patients with diabetes mellitus: evidence from a systematic review of the literature. *Diabetes, obesity & metabolism*. 2010;12(4):288-98.
16. Sartore G, Chilelli NC, Burlina S, Lapolla A. Association between glucose variability as assessed by continuous glucose monitoring (CGM) and diabetic retinopathy in type 1 and type 2 diabetes. *Acta diabetologica*. 2013;50(3):437-42.
17. Danne T, Nimri R, Battelino T, Bergenstal RM, Close KL, DeVries JH, et al. International Consensus on Use of Continuous Glucose Monitoring. *Diabetes care*. 2017;40(12):1631-40.
18. Miller KM, Beck RW, Bergenstal RM, Goland RS, Haller MJ, McGill JB, et al. Evidence of a strong association between frequency of self-monitoring of blood glucose and hemoglobin A1c levels in T1D exchange clinic registry participants. *Diabetes care*. 2013;36(7):2009-14.
19. Beck RW, Bergenstal RM, Riddlesworth TD, Kollman C, Li Z, Brown AS, et al. Validation of Time in Range as an Outcome Measure for Diabetes Clinical Trials. *Diabetes care*. 2019;42(3):400-5.
20. Moström P, Ahlén E, Imberg H, Hansson PO, Lind M. Adherence of self-monitoring of blood glucose in persons with type 1 diabetes in Sweden. *BMJ Open Diabetes Res Care*. 52017.
21. DeVries JH. Glucose variability: where it is important and how to measure it. *Diabetes*. 2013;62(5):1405-8.
22. Battelino T, Danne T, Bergenstal RM, Amiel SA, Beck R, Biester T, et al. Clinical Targets for Continuous Glucose Monitoring Data Interpretation: Recommendations From the International Consensus on Time in Range. *Diabetes care*. 2019;42(8):1593-603.

23. Tyndall V, Stimson RH, Zammitt NN, Ritchie SA, McKnight JA, Dover AR, et al. Marked improvement in HbA1c following commencement of flash glucose monitoring in people with type 1 diabetes. *Diabetologia*. 2019; p. 1349-56.
24. Banting FG, Best CH, Collip JB, Campbell WR, Fletcher AA. Pancreatic Extracts in the Treatment of Diabetes Mellitus. *Canadian Medical Association journal*. 1922;12(3):141-6.
25. (SIGN) SIGN. Management of Diabetes 2017 [Available from: <http://www.sign.ac.uk>.
26. Misso ML, Egberts KJ, Page M, O'Connor D, Shaw J. Continuous subcutaneous insulin infusion (CSII) versus multiple insulin injections for type 1 diabetes mellitus. *The Cochrane database of systematic reviews*. 2010(1):CD005103.
27. Pickup JC. Is insulin pump therapy effective in Type 1 diabetes? *Diabetic medicine : a journal of the British Diabetic Association*. 2019;36(3):269-78.
28. National Clinical Guideline C. National Institute for Health and Care Excellence: Clinical Guidelines. Type 1 Diabetes in Adults: Diagnosis and Management. National Institute for Health and Care Excellence: Clinical Guidelines. London: National Institute for Health and Care Excellence (UK) Copyright (c) 2015 National Clinical Guideline Centre.; 2015.
29. Bally L, Thabit H, Kojzar H, Mader JK, Qerimi-Hyseni J, Hartnell S, et al. Day-and-night glycaemic control with closed-loop insulin delivery versus conventional insulin pump therapy in free-living adults with well controlled type 1 diabetes: an open-label, randomised, crossover study. *The lancet Diabetes & endocrinology*. 2017;5(4):261-70.
30. de Boer IH, Group DER. Kidney disease and related findings in the diabetes control and complications trial/epidemiology of diabetes interventions and complications study. *Diabetes care*. 2014;37(1):24-30.
31. Martin CL, Albers JW, Pop-Busui R, Group DER. Neuropathy and related findings in the diabetes control and complications trial/epidemiology of diabetes interventions and complications study. *Diabetes care*. 2014;37(1):31-8.
32. Diabetes C, Complications Trial /Epidemiology of Diabetes I, Complications Research G, Lachin JM, White NH, Hainsworth DP, et al. Effect of intensive diabetes therapy on the progression of diabetic retinopathy in patients with type 1 diabetes: 18 years of follow-up in the DCCT/EDIC. *Diabetes*. 2015;64(2):631-42.
33. Group DER, Aiello LP, Sun W, Das A, Gangaputra S, Kiss S, et al. Intensive diabetes therapy and ocular surgery in type 1 diabetes. *The New England journal of medicine*. 2015;372(18):1722-33.
34. Hypoglycemia in the Diabetes Control and Complications Trial. The Diabetes Control and Complications Trial Research Group. *Diabetes*. 1997;46(2):271-86.
35. Cryer PE. Glycemic goals in diabetes: trade-off between glycemic control and iatrogenic hypoglycemia. *Diabetes*. 2014;63(7):2188-95.
36. Frier BM. Hypoglycaemia in diabetes mellitus: epidemiology and clinical implications. *Nature reviews Endocrinology*. 2014;10(12):711-22.
37. Seaquist ER, Anderson J, Childs B, Cryer P, Dagogo-Jack S, Fish L, et al. Hypoglycemia and diabetes: a report of a workgroup of the American Diabetes Association and the Endocrine Society. *Diabetes care*. 2013;36(5):1384-95.
38. Geddes J, Schopman JE, Zammitt NN, Frier BM. Prevalence of impaired awareness of hypoglycaemia in adults with Type 1 diabetes. *Diabetic medicine : a journal of the British Diabetic Association*. 2008;25(4):501-4.
39. Suh S, Kim JH. Glycemic Variability: How Do We Measure It and Why Is It Important? *Diabetes Metab J*. 2015;39(4):273-82.
40. Ceriello A, Monnier L, Owens D. Glycaemic variability in diabetes: clinical and therapeutic implications. *The lancet Diabetes & endocrinology*. 2019;7(3):221-30.
41. Pickup JC, Sutton AJ. Severe hypoglycaemia and glycaemic control in Type 1 diabetes: meta-analysis of multiple daily insulin injections compared with continuous subcutaneous insulin infusion. *Diabetic medicine : a journal of the British Diabetic Association*. 2008;25(7):765-74.
42. Beck RW, Riddlesworth TD, Ruedy KJ, Kollman C, Ahmann AJ, Bergenstal RM, et al. Effect of initiating use of an insulin pump in adults with type 1 diabetes using multiple daily insulin injections and continuous glucose monitoring (DIAMOND): a multicentre, randomised controlled trial. *The lancet Diabetes & endocrinology*. 2017;5(9):700-8.
43. Reid L, Baxter F, Forbes S. Effects of islet transplantation on microvascular and macrovascular complications in type 1 diabetes. *Diabetic medicine : a journal of the British Diabetic Association*. 2021;38(7):e14570.

44. Transport NBa. Annual Report on Pancreas and Islet Transplantation.
https://nhsbtdbe.blob.core.windows.net/umbraco-assets-corp/4609/pancreas_annual_report-2016-2017.pdf; 2017.
45. Rickels MR, Stock PG, de Koning EJP, Piemonti L, Pratschke J, Alejandro R, et al. Defining outcomes for beta-cell replacement therapy in the treatment of diabetes: a consensus report on the Igls criteria from the IPITA/EPITA opinion leaders workshop. *Transplant international : official journal of the European Society for Organ Transplantation*. 2018;31(4):343-52.
46. Ryan EA, Paty BW, Senior PA, Lakey JR, Bigam D, Shapiro AM. Beta-score: an assessment of beta-cell function after islet transplantation. *Diabetes care*. 2005;28(2):343-7.
47. Forbes S, Oram RA, Smith A, Lam A, Olateju T, Imes S, et al. Validation of the BETA-2 Score: An Improved Tool to Estimate Beta Cell Function After Clinical Islet Transplantation Using a Single Fasting Blood Sample. *American journal of transplantation : official journal of the American Society of Transplantation and the American Society of Transplant Surgeons*. 2016;16(9):2704-13.
48. Ryan EA, Lakey JR, Paty BW, Imes S, Korbitt GS, Kneteman NM, et al. Successful islet transplantation: continued insulin reserve provides long-term glycemic control. *Diabetes*. 2002;51(7):2148-57.
49. CITR. Collaborative Islet Transplant Registry Tenth Annual Report. 2017.
50. Shapiro AM, Lakey JR, Ryan EA, Korbitt GS, Toth E, Warnock GL, et al. Islet transplantation in seven patients with type 1 diabetes mellitus using a glucocorticoid-free immunosuppressive regimen. *The New England journal of medicine*. 2000;343(4):230-8.
51. Barton FB, Rickels MR, Alejandro R, Hering BJ, Wease S, Naziruddin B, et al. Improvement in outcomes of clinical islet transplantation: 1999-2010. *Diabetes care*. 2012;35(7):1436-45.
52. Bellin MD, Barton FB, Heitman A, Harmon JV, Kandaswamy R, Balamurugan AN, et al. Potent induction immunotherapy promotes long-term insulin independence after islet transplantation in type 1 diabetes. *American journal of transplantation : official journal of the American Society of Transplantation and the American Society of Transplant Surgeons*. 2012;12(6):1576-83.
53. Bamoulid J, Staeck O, Halleck F, Khadzynov D, Brakemeier S, Durr M, et al. The need for minimization strategies: current problems of immunosuppression. *Transplant international : official journal of the European Society for Organ Transplantation*. 2015;28(8):891-900.
54. Senior PA, Paty BW, Cockfield SM, Ryan EA, Shapiro AM. Proteinuria developing after clinical islet transplantation resolves with sirolimus withdrawal and increased tacrolimus dosing. *American journal of transplantation : official journal of the American Society of Transplantation and the American Society of Transplant Surgeons*. 2005;5(9):2318-23.
55. Leitao CB, Cure P, Messinger S, Pileggi A, Lenz O, Froud T, et al. Stable renal function after islet transplantation: importance of patient selection and aggressive clinical management. *Transplantation*. 2009;87(5):681-8.
56. Kessler L, Lucescu C, Pinget M, Charton MN, Mutschler V, Wolf P, et al. Tacrolimus-associated optic neuropathy after pancreatic islet transplantation using a sirolimus/tacrolimus immunosuppressive regimen. *Transplantation*. 81. United States 2006. p. 636-7.
57. Bhagavati S, Maccabee P, Muntean E, Sumrani NB. Chronic sensorimotor polyneuropathy associated with tacrolimus immunosuppression in renal transplant patients: case reports. *Transplantation proceedings*. 2007;39(10):3465-7.
58. Scottish Diabetic Retinopathy Screening Collaborative [Available from: <https://www.ndrs.scot.nhs.uk/>].
59. Ciulla TA, Amador AG, Zinman B. Diabetic retinopathy and diabetic macular edema: pathophysiology, screening, and novel therapies. *Diabetes care*. 2003;26(9):2653-64.
60. Hainsworth DP, Bebu I, Aiello LP, Sivitz W, Gubitosi-Klug R, Malone J, et al. Risk Factors for Retinopathy in Type 1 Diabetes: The DCCT/EDIC Study. *Diabetes care*. 2019;42(5):875-82.
61. Feldman-Billard S, Larger E, Massin P. Standards for screening and surveillance of ocular complications in people with diabetes SFDsg. Early worsening of diabetic retinopathy after rapid improvement of blood glucose control in patients with diabetes. *Diabetes & metabolism*. 2018;44(1):4-14.
62. Scanlon PH. The English National Screening Programme for diabetic retinopathy 2003-2016. *Acta diabetologica*. 2017;54(6):515-25.
63. Diabetes C, Complications Trial Research G. Effect of pregnancy on microvascular complications in the diabetes control and complications trial. The Diabetes Control and Complications Trial Research Group. *Diabetes care*. 2000;23(8):1084-91.

64. Al-Dalla Ali M, Stimson RH, Dover AR, Forbes S, Annoh R, Madill K, et al. HbA1c reduction following flash monitoring commencement is not independently associated with adverse diabetic eye disease outcomes in type 1 diabetes. *BMJ Open Diabetes Res Care*. 2020;8(2).
65. Linton K, Stimson RH, Dover AR, Forbes S, Madill K, Annoh R, et al. Substantial HbA1c Reduction Following Intermittent-Scanning Continuous Glucose Monitoring Was Not Associated With Early Worsening of Retinopathy in Type 1 Diabetes. *Journal of diabetes science and technology*. 2021;1932296821994091.
66. Klefter ON, Hommel E, Munch IC, Norgaard K, Madsbad S, Larsen M. Retinal characteristics during 1 year of insulin pump therapy in type 1 diabetes: a prospective, controlled, observational study. *Acta Ophthalmol*. 2016;94(6):540-7.
67. Wu L, Fernandez-Loaiza P, Sauma J, Hernandez-Bogantes E, Masis M. Classification of diabetic retinopathy and diabetic macular edema. *World J Diabetes*. 2013;4(6):290-4.
68. Wilkinson CP, Ferris FL, 3rd, Klein RE, Lee PP, Agardh CD, Davis M, et al. Proposed international clinical diabetic retinopathy and diabetic macular edema disease severity scales. *Ophthalmology*. 2003;110(9):1677-82.
69. Scottish Diabetic Retinopathy Grading Scheme 2007 v1.1 <https://www.ndrs.scot.nhs.uk/wp-content/uploads/2013/04/Grading-Scheme-2007-v1.1.pdf> [Available from: <https://www.ndrs.scot.nhs.uk/wp-content/uploads/2013/04/Grading-Scheme-2007-v1.1.pdf>].
70. Party DRGEW. The Royal College of Ophthalmologists Diabetic Retinopathy Guidelines 2012 [Available from: <https://www.rcophth.ac.uk/wp-content/uploads/2014/12/2013-SCI-301-FINAL-DR-GUIDELINES-DEC-2012-updated-July-2013.pdf>].
71. Goh JK, Cheung CY, Sim SS, Tan PC, Tan GS, Wong TY. Retinal Imaging Techniques for Diabetic Retinopathy Screening. *Journal of diabetes science and technology*. 2016;10(2):282-94.
72. Philip S, Fleming AD, Goatman KA, Fonseca S, McNamee P, Scotland GS, et al. The efficacy of automated "disease/no disease" grading for diabetic retinopathy in a systematic screening programme. *Br J Ophthalmol*. 2007;91(11):1512-7.
73. Fleming AD, Goatman KA, Philip S, Prescott GJ, Sharp PF, Olson JA. Automated grading for diabetic retinopathy: a large-scale audit using arbitration by clinical experts. *Br J Ophthalmol*. 2010;94(12):1606-10.
74. Styles CJ. Introducing automated diabetic retinopathy systems: it's not just about sensitivity and specificity. *Eye (Lond)*. 2019;33(9):1357-8.
75. Ikram MK, Cheung CY, Lorenzi M, Klein R, Jones TL, Wong TY, et al. Retinal vascular caliber as a biomarker for diabetes microvascular complications. *Diabetes care*. 2013;36(3):750-9.
76. Talks SJ, Bhatia D. The use of ultra-widefield imaging for the management of diabetic retinopathy. *Expert Review of Ophthalmology*. 2017;12(6):485-94.
77. Kernt M, Hadi I, Pinter F, Seidensticker F, Hirneiss C, Haritoglou C, et al. Assessment of diabetic retinopathy using nonmydriatic ultra-widefield scanning laser ophthalmoscopy (Optomap) compared with ETDRS 7-field stereo photography. *Diabetes care*. 2012;35(12):2459-63.
78. Rasmussen ML, Broe R, Frydkjaer-Olsen U, Olsen BS, Mortensen HB, Peto T, et al. Comparison between Early Treatment Diabetic Retinopathy Study 7-field retinal photos and non-mydriatic, mydriatic and mydriatic steered widefield scanning laser ophthalmoscopy for assessment of diabetic retinopathy. *J Diabetes Complications*. 2015;29(1):99-104.
79. Aiello LP, Odia I, Glassman AR, Melia M, Jampol LM, Bressler NM, et al. Comparison of Early Treatment Diabetic Retinopathy Study Standard 7-Field Imaging With Ultrawide-Field Imaging for Determining Severity of Diabetic Retinopathy. *JAMA ophthalmology*. 2019;137(1):65-73.
80. Silva PS, Cavallerano JD, Sun JK, Noble J, Aiello LM, Aiello LP. Nonmydriatic ultrawide field retinal imaging compared with dilated standard 7-field 35-mm photography and retinal specialist examination for evaluation of diabetic retinopathy. *American journal of ophthalmology*. 2012;154(3):549-59 e2.
81. Silva PS, Cavallerano JD, Sun JK, Soliman AZ, Aiello LM, Aiello LP. Peripheral lesions identified by mydriatic ultrawide field imaging: distribution and potential impact on diabetic retinopathy severity. *Ophthalmology*. 2013;120(12):2587-95.
82. Silva PS, Horton MB, Clary D, Lewis DG, Sun JK, Cavallerano JD, et al. Identification of Diabetic Retinopathy and Ungradable Image Rate with Ultrawide Field Imaging in a National Teleophthalmology Program. *Ophthalmology*. 2016;123(6):1360-7.
83. Singh RP, Hsueh J, Han MM, Kuriyan AE, Conti FF, Steinle N, et al. Protecting Vision in Patients With Diabetes With Ultra-Widefield Imaging: A Review of Current Literature. *Ophthalmic surgery, lasers & imaging retina*. 2019;50(10):639-48.

84. Wilson PJ, Ellis JD, MacEwen CJ, Ellingford A, Talbot J, Leese GP. Screening for diabetic retinopathy: a comparative trial of photography and scanning laser ophthalmoscopy. *Ophthalmologica Journal international d'ophtalmologie International journal of ophthalmology Zeitschrift fur Augenheilkunde*. 2010;224(4):251-7.
85. Molly Subhash H, Wang R. *Biomedical Optical Imaging Technologies*: Springer-Verlag Berlin Heidelberg; 2013.
86. Lee J, Rosen R. Optical Coherence Tomography Angiography in Diabetes. *Current diabetes reports*. 2016;16(12):123.
87. Jia Y, Bailey ST, Hwang TS, McClintic SM, Gao SS, Pennesi ME, et al. Quantitative optical coherence tomography angiography of vascular abnormalities in the living human eye. *Proceedings of the National Academy of Sciences of the United States of America*. 2015;112(18):E2395-402.
88. Agemy SA, Sripsema NK, Shah CM, Chui T, Garcia PM, Lee JG, et al. Retinal Vascular Perfusion Density Mapping Using Optical Coherence Tomography Angiography in Normals and Diabetic Retinopathy Patients. *Retina (Philadelphia, Pa)*. 2015;35(11):2353-63.
89. Samara WA, Shahlaee A, Adam MK, Khan MA, Chiang A, Maguire JJ, et al. Quantification of Diabetic Macular Ischemia Using Optical Coherence Tomography Angiography and Its Relationship with Visual Acuity. *Ophthalmology*. 2017;124(2):235-44.
90. Giarratano Y, Pavel A, Lian J, Andreeva R, Fontanella A, Sarkar R, et al. A Framework for the Discovery of Retinal Biomarkers in Optical Coherence Tomography Angiography (OCTA). *Ophthalmic Medical Image Analysis. Lecture Notes in Computer Science* 2020. p. 155-64.
91. Hart WE, Goldbaum M, Cote B, Kube P, Nelson MR. Measurement and classification of retinal vascular tortuosity. *International journal of medical informatics*. 1999;53(2-3):239-52.
92. Aslam T, Fleck B, Patton N, Trucco M, Azegrouz H. Digital image analysis of plus disease in retinopathy of prematurity. *Acta Ophthalmol*. 2009;87(4):368-77.
93. Parr JC, Spears GF. General caliber of the retinal arteries expressed as the equivalent width of the central retinal artery. *American journal of ophthalmology*. 1974;77(4):472-7.
94. Hubbard LD, Brothers RJ, King WN, Clegg LX, Klein R, Cooper LS, et al. Methods for evaluation of retinal microvascular abnormalities associated with hypertension/sclerosis in the Atherosclerosis Risk in Communities Study. *Ophthalmology*. 1999;106(12):2269-80.
95. Knudtson MD, Lee KE, Hubbard LD, Wong TY, Klein R, Klein BE. Revised formulas for summarizing retinal vessel diameters. *Curr Eye Res*. 2003;27(3):143-9.
96. Ikram MK, Ong YT, Cheung CY, Wong TY. Retinal vascular caliber measurements: clinical significance, current knowledge and future perspectives. *Ophthalmologica Journal international d'ophtalmologie International journal of ophthalmology Zeitschrift fur Augenheilkunde*. 2013;229(3):125-36.
97. Macgillivray TJ, Patton N, Doulal FN, Graham C, Wardlaw JM. Fractal analysis of the retinal vascular network in fundus images. *Annu Int Conf IEEE Eng Med Biol Soc*. 2007;2007:6456-9.
98. Liefers B, Venhuizen FG, Schreur V, van Ginneken B, Hoyng C, Fauser S, et al. Automatic detection of the foveal center in optical coherence tomography. *Biomed Opt Express*. 2017. p. 5160-78.
99. Krawitz BD, Mo S, Geyman LS, Agemy SA, Sripsema NK, Garcia PM, et al. Acircularity index and axis ratio of the foveal avascular zone in diabetic eyes and healthy controls measured by optical coherence tomography angiography. *Vision research*. 2017;139:177-86.
100. Tam J, Dhamdhere KP, Tiruveedhula P, Manzanera S, Barez S, Bearse MA, et al. Disruption of the Retinal Parafoveal Capillary Network in Type 2 Diabetes before the Onset of Diabetic Retinopathy. *Investigative ophthalmology & visual science*. 2011. p. 9257-66.
101. Aslam TM, Hoyle DC, Puri V, Bento G. Differentiation of Diabetic Status Using Statistical and Machine Learning Techniques on Optical Coherence Tomography Angiography Images. *Transl Vis Sci Technol*. 2020;9(4):2.
102. Chan A, Duker JS, Ko TH, Fujimoto JG, Schuman JS. Normal macular thickness measurements in healthy eyes using Stratus optical coherence tomography. *Archives of ophthalmology (Chicago, Ill : 1960)*. 2006;124(2):193-8.
103. Mrejen S, Spaide RF. Optical coherence tomography: imaging of the choroid and beyond. *Surv Ophthalmol*. 2013;58(5):387-429.
104. Gupta P, Cheng CY, Cheung CM, Htoon HM, Zheng Y, Lamoureux EL, et al. Relationship of ocular and systemic factors to the visibility of choroidal-scleral interface using spectral domain optical coherence tomography. *Acta Ophthalmol*. 2016;94(2):e142-9.
105. Shao L, Xu L, Chen CX, Yang LH, Du KF, Wang S, et al. Reproducibility of subfoveal choroidal thickness measurements with enhanced depth imaging by spectral-domain optical coherence tomography. *Investigative ophthalmology & visual science*. 2013;54(1):230-3.

106. Ferrara D, Waheed NK, Duker JS. Investigating the choriocapillaris and choroidal vasculature with new optical coherence tomography technologies. *Prog Retin Eye Res.* 2016;52:130-55.
107. Betzler BK, Ding J, Wei X, Lee JM, Grewal DS, Fekrat S, et al. Choroidal vascularity index: a step towards software as a medical device. *Br J Ophthalmol.* 2022;106(2):149-55.
108. Tan KA, Laude A, Yip V, Loo E, Wong EP, Agrawal R. Choroidal vascularity index - a novel optical coherence tomography parameter for disease monitoring in diabetes mellitus? *Acta Ophthalmol.* 2016;94(7):e612-e6.
109. Reid LJ, Gibb FW, Colhoun H, Wild SH, Strachan MWJ, Madill K, et al. Continuous subcutaneous insulin infusion therapy is associated with reduced retinopathy progression compared with multiple daily injections of insulin. *Diabetologia.* 2021.
110. Waugh N. Should automated grading of retinal photographs be used in the Scottish Diabetic Retinopathy Screening Service? : University of Aberdeen; 2009.
111. van Ballegooie E, Hooymans JM, Timmerman Z, Reitsma WD, Sluiter WJ, Schweitzer NM, et al. Rapid deterioration of diabetic retinopathy during treatment with continuous subcutaneous insulin infusion. *Diabetes care.* 1984;7(3):236-42.
112. Kelly TM, Sanborn GE, Haug PJ, Edwards CQ. Effect of insulin infusion pump use on diabetic retinopathy. *Archives of ophthalmology (Chicago, Ill : 1960).* 1984;102(8):1156-9.
113. Zabeen B, Craig ME, Virk SA, Pryke A, Chan AK, Cho YH, et al. Insulin Pump Therapy Is Associated with Lower Rates of Retinopathy and Peripheral Nerve Abnormality. *PloS one.* 2016;11(4):e0153033.
114. Giannarelli R, Coppelli A, Sartini M, Aragona M, Boggi U, Vistoli F, et al. Effects of pancreas-kidney transplantation on diabetic retinopathy. *Transplant international : official journal of the European Society for Organ Transplantation.* 2005;18(5):619-22.
115. Ryan EA, Paty BW, Senior PA, Bigam D, Alfadhli E, Kneteman NM, et al. Five-year follow-up after clinical islet transplantation. *Diabetes.* 2005;54(7):2060-9.
116. Tekin Z, Garfinkel MR, Chon WJ, Schenck L, Golab K, Savari O, et al. Outcomes of Pancreatic Islet Allotransplantation Using the Edmonton Protocol at the University of Chicago. *Transplantation direct.* 2016;2(10):e105.
117. Lee TC, Barshes NR, O'Mahony CA, Nguyen L, Brunicardi FC, Ricordi C, et al. The effect of pancreatic islet transplantation on progression of diabetic retinopathy and neuropathy. *Transplantation proceedings.* 2005;37(5):2263-5.
118. Virk SA, Donaghue KC, Wong TY, Craig ME. Interventions for Diabetic Retinopathy in Type 1 Diabetes: Systematic Review and Meta-Analysis. *American journal of ophthalmology.* 2015;160(5):1055-64.e4.
119. Venturini M, Fiorina P, Maffi P, Losio C, Vergani A, Secchi A, et al. Early increase of retinal arterial and venous blood flow velocities at color Doppler imaging in brittle type 1 diabetes after islet transplant alone. *Transplantation.* 2006;81(9):1274-7.
120. Thompson DM, Begg IS, Harris C, Ao Z, Fung MA, Meloche RM, et al. Reduced progression of diabetic retinopathy after islet cell transplantation compared with intensive medical therapy. *Transplantation.* 2008;85(10):1400-5.
121. Thompson DM, Meloche M, Ao Z, Paty B, Keown P, Shapiro RJ, et al. Reduced progression of diabetic microvascular complications with islet cell transplantation compared with intensive medical therapy. *Transplantation.* 2011;91(3):373-8.
122. Vantyghem MC, Raverdy V, Balavoine AS, Defrance F, Caiazzo R, Arnalsteen L, et al. Continuous glucose monitoring after islet transplantation in type 1 diabetes: an excellent graft function (beta-score greater than 7) Is required to abrogate hyperglycemia, whereas a minimal function is necessary to suppress severe hypoglycemia (beta-score greater than 3). *The Journal of clinical endocrinology and metabolism.* 2012;97(11):E2078-83.
123. Kuhlreier WM, Washer SL, Hsu E, Zhao M, Reinhold P, 3rd, Burger D, et al. Low levels of C-peptide have clinical significance for established Type 1 diabetes. *Diabetic medicine : a journal of the British Diabetic Association.* 2015;32(10):1346-53.
124. Thompson DM, Meloche M, Ao Z, Paty B, Keown P, Shapiro RJ, et al. Reduced progression of diabetic microvascular complications with islet cell transplantation compared with intensive medical therapy. *Transplantation.* 2011;91(3):373-8.
125. Walker GS, Chen JY, Hopkinson H, Sainsbury CAR, Jones GC. Structured education using Dose Adjustment for Normal Eating (DAFNE) reduces long-term HbA1c and HbA1c variability. *Diabetic medicine : a journal of the British Diabetic Association.* 2018;35(6):745-9.
126. Government S. Scottish Index of Multiple Deprivation 2016 [Available from: <https://www2.gov.scot/Topics/Statistics/SIMD/>].

127. Wright RJ, Frier BM. Vascular disease and diabetes: is hypoglycaemia an aggravating factor? *Diabetes/metabolism research and reviews*. 2008;24(5):353-63.
128. Quiros C, Gimenez M, Rios P, Careaga M, Roca D, Vidal M, et al. Long-term outcome of insulin pump therapy: reduction of hypoglycaemia and impact on glycaemic control. *Diabetic medicine : a journal of the British Diabetic Association*. 2016;33(10):1422-6.
129. The Royal College of Ophthalmologists Diabetic Retinopathy Guidelines December 2012 [Available from: <https://www.rcophth.ac.uk/wp-content/uploads/2014/12/2013-SCI-301-FINAL-DR-GUIDELINES-DEC-2012-updated-July-2013.pdf>.
130. Group TDCaCTR. The Relationship of Glycemic Exposure (HbA1c) to the Risk of Development and Progression of Retinopathy in the Diabetes Control and Complications Trial. 1995.
131. Marchand L, Kawasaki-Ogita Y, Place J, Fayolle C, Lauton D, Boulet F, et al. Long-Term Effects of Continuous Subcutaneous Insulin Infusion on Glucose Control and Microvascular Complications in Patients With Type 1 Diabetes. *Journal of diabetes science and technology*. 2017;11(5):924-9.
132. Kilpatrick ES, Rigby AS, Atkin SL. Effect of Glucose Variability on the Long-Term Risk of Microvascular Complications in Type 1 Diabetes. *Diabetes care*. 2009. p. 1901-3.
133. Lu J, Ma X, Zhou J, Zhang L, Mo Y, Ying L, et al. Association of Time in Range, as Assessed by Continuous Glucose Monitoring, With Diabetic Retinopathy in Type 2 Diabetes. *Diabetes care*. 2018;41(11):2370-6.
134. Hsu CR, Chen YT, Sheu WH. Glycemic variability and diabetes retinopathy: a missing link. *J Diabetes Complications*. 2015;29(2):302-6.
135. Reid L, Lam A, Dhillon B, Duncan K, Ibbotson C, Sutherland A, et al., editors. *Reduced Progression Of Diabetic Retinopathy In Type 1 Diabetes Over Three Years With Clinical Islet Transplantation Or Continuous Subcutaneous Insulin Infusion Compared With Multiple Daily Insulin Injections*. International Pancreas and Islet Transplantation Association; 2019; Lyon, France.
136. Forbes S, Senior PA, Shapiro AMJ. Islet transplantation in type 1 diabetes: moving forward. *The lancet Diabetes & endocrinology*. 2018;6(7):516-7.
137. Roze S, Smith-Palmer J, Valentine W, de Portu S, Norgaard K, Pickup JC. Cost-effectiveness of continuous subcutaneous insulin infusion versus multiple daily injections of insulin in Type 1 diabetes: a systematic review. *Diabetic medicine : a journal of the British Diabetic Association*. 2015;32(11):1415-24.
138. Roze S, Valentine WJ, Zakrzewska KE, Palmer AJ. Health-economic comparison of continuous subcutaneous insulin infusion with multiple daily injection for the treatment of Type 1 diabetes in the UK. *Diabetic medicine : a journal of the British Diabetic Association*. 2005;22(9):1239-45.
139. Ochs A, McGurnaghan S, Black MW, Leese GP, Philip S, Sattar N, et al. Use of personalised risk-based screening schedules to optimise workload and sojourn time in screening programmes for diabetic retinopathy: A retrospective cohort study. *PLoS Med*. 2019;16(10):e1002945.
140. Perez-Rovira A, MacGillivray T, Trucco E, Chin KS, Zutis K, Lupascu C, et al. VAMPIRE: Vessel assessment and measurement platform for images of the REtina. *Conf Proc IEEE Eng Med Biol Soc*. 2011;2011:3391-4.
141. E. Trucco AG, L. Ballerini, D. Relan, A. Cavinato, T. MacGillivray. **Morphometric Measurements of the Retinal Vasculature in Fundus Images with VAMPIRE** 2015 [
142. E. Trucco LB, D. Relan, A. Giachetti, T. MacGillivray, K. Zutis, C. Lupascu, D. Tegolo, E. Pellegrini, G. Robertson, P.J. Wilson, A. Doney and B. Dhillon. **Novel VAMPIRE algorithms for quantitative analysis of the retinal vasculature** 2013 [
143. Doubal FN, MacGillivray TJ, Patton N, Dhillon B, Dennis MS, Wardlaw JM. Fractal analysis of retinal vessels suggests that a distinct vasculopathy causes lacunar stroke. *Neurology*. 2010;74(14):1102-7.
144. McGrory S, Taylor AM, Kirin M, Corley J, Pattie A, Cox SR, et al. Retinal microvascular network geometry and cognitive abilities in community-dwelling older people: The Lothian Birth Cohort 1936 study. *Br J Ophthalmol*. 2017;101(7):993-8.
145. McKay GJ, Paterson EN, Maxwell AP, Cardwell CC, Wang R, Hogg S, et al. Retinal microvascular parameters are not associated with reduced renal function in a study of individuals with type 2 diabetes. *Scientific reports*. 2018;8(1):3931.
146. Kifley A, Wang JJ, Cugati S, Wong TY, Mitchell P. Retinal vascular caliber, diabetes, and retinopathy. *American journal of ophthalmology*. 2007;143(6):1024-6.
147. Tikellis G, Wang JJ, Tapp R, Simpson R, Mitchell P, Zimmet PZ, et al. The relationship of retinal vascular calibre to diabetes and retinopathy: the Australian Diabetes, Obesity and Lifestyle (AusDiab) study. *Diabetologia*. 2007;50(11):2263-71.

148. Nguyen TT, Wang JJ, Sharrett AR, Islam FM, Klein R, Klein BE, et al. Relationship of retinal vascular caliber with diabetes and retinopathy: the Multi-Ethnic Study of Atherosclerosis (MESA). *Diabetes care*. 2008;31(3):544-9.
149. Crosby-Nwaobi R, Heng LZ, Sivaprasad S. Retinal vascular calibre, geometry and progression of diabetic retinopathy in type 2 diabetes mellitus. *Ophthalmologica Journal international d'ophtalmologie International journal of ophthalmology Zeitschrift fur Augenheilkunde*. 2012;228(2):84-92.
150. Bek T. Diameter Changes of Retinal Vessels in Diabetic Retinopathy. *Current diabetes reports*. 2017;17(10):82.
151. Habib MS, Al-Diri B, Hunter A, Steel DH. The association between retinal vascular geometry changes and diabetic retinopathy and their role in prediction of progression--an exploratory study. *BMC Ophthalmol*. 2014;14:89.
152. Klein R, Myers CE, Lee KE, Gangnon R, Klein BE. Changes in retinal vessel diameter and incidence and progression of diabetic retinopathy. *Archives of ophthalmology (Chicago, Ill : 1960)*. 2012;130(6):749-55.
153. Rogers SL, Tikellis G, Cheung N, Tapp R, Shaw J, Zimmet PZ, et al. Retinal arteriolar caliber predicts incident retinopathy: the Australian Diabetes, Obesity and Lifestyle (AusDiab) study. *Diabetes care*. 2008;31(4):761-3.
154. Liew G, Benitez-Aguirre P, Craig ME, Jenkins AJ, Hodgson LAB, Kifley A, et al. Progressive Retinal Vasodilation in Patients With Type 1 Diabetes: A Longitudinal Study of Retinal Vascular Geometry. *Investigative ophthalmology & visual science*. 2017;58(5):2503-9.
155. Pedersen L, Jeppesen P, Knudsen ST, Poulsen PL, Bek T. Improvement of mild retinopathy in type 2 diabetic patients correlates with narrowing of retinal arterioles. A prospective observational study. *Graefes's archive for clinical and experimental ophthalmology = Albrecht von Graefes Archiv fur klinische und experimentelle Ophthalmologie*. 2014;252(10):1561-7.
156. Shao Q, Heussen FM, Ouyang Y, Hager A. Retinal vessel diameter changes in different severities of diabetic retinopathy by SD-OCT. *Eur J Ophthalmol*. 2016;26(4):342-6.
157. Velayutham V, Benitez-Aguirre PZ, Craig ME, Liew G, Wong TY, Jenkins AJ, et al. Innovative technology shows impact of glycaemic control on peripheral retinal vessels in adolescents with type 1 diabetes. *Diabetologia*. 2017;60(10):2103-10.
158. Broe R, Rasmussen ML, Frydkjaer-Olsen U, Olsen BS, Mortensen HB, Hodgson L, et al. Retinal vessel calibers predict long-term microvascular complications in type 1 diabetes: the Danish Cohort of Pediatric Diabetes 1987 (DCPD1987). *Diabetes*. 2014;63(11):3906-14.
159. Chew SK, Xie J, Wang JJ. Retinal arteriolar diameter and the prevalence and incidence of hypertension: a systematic review and meta-analysis of their association. *Curr Hypertens Rep*. 2012;14(2):144-51.
160. Lim LS, Chee ML, Cheung CY, Wong TY. Retinal Vessel Geometry and the Incidence and Progression of Diabetic Retinopathy. *Investigative ophthalmology & visual science*. 2017;58(6):Bio200-bio5.
161. Benitez-Aguirre P, Craig ME, Sasongko MB, Jenkins AJ, Wong TY, Wang JJ, et al. Retinal vascular geometry predicts incident retinopathy in young people with type 1 diabetes: a prospective cohort study from adolescence. *Diabetes care*. 2011;34(7):1622-7.
162. Cheung CY, Sabanayagam C, Law AK, Kumari N, Ting DS, Tan G, et al. Retinal vascular geometry and 6 year incidence and progression of diabetic retinopathy. *Diabetologia*. 2017;60(9):1770-81.
163. Klein R, Lee KE, Danforth L, Tsai MY, Gangnon RE, Meuer SE, et al. The Relationship of Retinal Vessel Geometric Characteristics to the Incidence and Progression of Diabetic Retinopathy. *Ophthalmology*. 2018;125(11):1784-92.
164. Forster RB, Garcia ES, Sluiman AJ, Grecian SM, McLachlan S, MacGillivray TJ, et al. Retinal venular tortuosity and fractal dimension predict incident retinopathy in adults with type 2 diabetes: the Edinburgh Type 2 Diabetes Study. *Diabetologia*. 2021.
165. Sasongko MB, Wong TY, Nguyen TT, Cheung CY, Shaw JE, Wang JJ. Retinal vascular tortuosity in persons with diabetes and diabetic retinopathy. *Diabetologia*. 2011;54(9):2409-16.
166. Tapp RJ, Owen CG, Barman SA, Welikala RA, Foster PJ, Whincup PH, et al. Associations of Retinal Microvascular Diameters and Tortuosity With Blood Pressure and Arterial Stiffness: United Kingdom Biobank. *Hypertension*. 2019;74(6):1383-90.
167. Kalitzeos AA, Lip GY, Heitmar R. Retinal vessel tortuosity measures and their applications. *Exp Eye Res*. 2013;106:40-6.
168. M. R. K. Mookiah SM, S. Hogg, J. Price, R. Forster, T. J. MacGillivray, E. Trucco. **Towards Standardization of Retinal Vascular Measurements: On the Effect of Image Centering** MICCAI OMIA2018 [

169. Cheung N, Donaghue KC, Liew G, Rogers SL, Wang JJ, Lim SW, et al. Quantitative assessment of early diabetic retinopathy using fractal analysis. *Diabetes care*. 2009;32(1):106-10.
170. Rasmussen ML, Broe R, Frydkjaer-Olsen U, Olsen BS, Mortensen HB, Peto T, et al. Retinal vascular geometry and its association to microvascular complications in patients with type 1 diabetes: the Danish Cohort of Pediatric Diabetes 1987 (DCPD1987). *Graefes archive for clinical and experimental ophthalmology = Albrecht von Graefes Archiv fur klinische und experimentelle Ophthalmologie*. 2017;255(2):293-9.
171. Zahid S, Dolz-Marco R, Freund KB, Balaratnasingam C, Dansingani K, Gilani F, et al. Fractal Dimensional Analysis of Optical Coherence Tomography Angiography in Eyes With Diabetic Retinopathy. *Investigative ophthalmology & visual science*. 2016;57(11):4940-7.
172. Kim AY, Chu Z, Shahidzadeh A, Wang RK, Puliafito CA, Kashani AH. Quantifying Microvascular Density and Morphology in Diabetic Retinopathy Using Spectral-Domain Optical Coherence Tomography Angiography. *Investigative ophthalmology & visual science*. 2016;57(9):OCT362-70.
173. Tang FY, Ng DS, Lam A, Luk F, Wong R, Chan C, et al. Determinants of Quantitative Optical Coherence Tomography Angiography Metrics in Patients with Diabetes. *Scientific reports*. 2017;7(1):2575.
174. Daxer A. Characterisation of the neovascularisation process in diabetic retinopathy by means of fractal geometry: diagnostic implications. *Graefes archive for clinical and experimental ophthalmology = Albrecht von Graefes Archiv fur klinische und experimentelle Ophthalmologie*. 1993;231(12):681-6.
175. Daxer A. The fractal geometry of proliferative diabetic retinopathy: implications for the diagnosis and the process of retinal vasculogenesis. *Curr Eye Res*. 1993;12(12):1103-9.
176. Lisowska A, Annunziata R, Loh GK, Karl D, Trucco E. An experimental assessment of five indices of retinal vessel tortuosity with the RET-TORT public dataset. *Conf Proc IEEE Eng Med Biol Soc*. 2014;2014:5414-7.
177. Daien V, Kawasaki R, Villain M, Ribstein J, Du Cailar G, Mimran A, et al. Retinal vascular caliber is associated with renal function in apparently healthy subjects. *Acta Ophthalmol*. 2013;91(4):e283-8.
178. Trucco E, Ruggeri A, Karnowski T, Giancardo L, Chaum E, Hubschman JP, et al. Validating retinal fundus image analysis algorithms: issues and a proposal. *Investigative ophthalmology & visual science*. 2013;54(5):3546-59.
179. Taylor DJ, Goatman KA, Gregory A, Histed M, Martin CN, Warburton TJ, et al. Image-quality standardization for diabetic retinopathy screening. *Expert Review of Ophthalmology*. 2014;4(5):469-76.
180. Simonett JM, Scarinci F, Picconi F, Giorno P, De Geronimo D, Di Renzo A, et al. Early microvascular retinal changes in optical coherence tomography angiography in patients with type 1 diabetes mellitus. *Acta Ophthalmol*. 2017;95(8):e751-e5.
181. Mastropasqua R, Toto L, Mastropasqua A, Aloia R, De Nicola C, Mattei PA, et al. Foveal avascular zone area and parafoveal vessel density measurements in different stages of diabetic retinopathy by optical coherence tomography angiography. *International journal of ophthalmology*. 2017;10(10):1545-51.
182. Durbin MK, An L, Shemonski ND, Soares M, Santos T, Lopes M, et al. Quantification of Retinal Microvascular Density in Optical Coherence Tomographic Angiography Images in Diabetic Retinopathy. *JAMA ophthalmology*. 2017;135(4):370-6.
183. Alam M, Zhang Y, Lim JJ, Chan RVP, Yang M, Yao X. Quantitative Optical Coherence Tomography Angiography Features for Objective Classification and Staging of Diabetic Retinopathy. *Retina (Philadelphia, Pa)*. 2020;40(2):322-32.
184. Nesper PL, Roberts PK, Onishi AC, Chai H, Liu L, Jampol LM, et al. Quantifying Microvascular Abnormalities With Increasing Severity of Diabetic Retinopathy Using Optical Coherence Tomography Angiography. *Investigative ophthalmology & visual science*. 2017;58(6):BIO307-BIO15.
185. Takase N, Nozaki M, Kato A, Ozeki H, Yoshida M, Ogura Y. Enlargement of Foveal Avascular Zone in Diabetic Eyes Evaluated by En Face Optical Coherence Tomography Angiography. *Retina (Philadelphia, Pa)*. 2015;35(11):2377-83.
186. de Carlo TE, Chin AT, Bonini Filho MA, Adhi M, Branchini L, Salz DA, et al. Detection of Microvascular Changes in Eyes of Patients with Diabetes but Not Clinical Diabetic Retinopathy Using Optical Coherence Tomography Angiography. *Retina (Philadelphia, Pa)*. 2015;35(11):2364-70.
187. Di G, Weihong Y, Xiao Z, Zhikun Y, Xuan Z, Yi Q, et al. A morphological study of the foveal avascular zone in patients with diabetes mellitus using optical coherence tomography angiography. *Graefes archive for clinical and experimental ophthalmology = Albrecht von Graefes Archiv fur klinische und experimentelle Ophthalmologie*. 2016;254(5):873-9.
188. Freiberg FJ, Pfau M, Wons J, Wirth MA, Becker MD, Michels S. Optical coherence tomography angiography of the foveal avascular zone in diabetic retinopathy. *Graefes archive for clinical and experimental ophthalmology = Albrecht von Graefes Archiv fur klinische und experimentelle Ophthalmologie*. 2016;254(6):1051-8.

189. Gozlan J, Ingrand P, Lichtwitz O, Cazet-Supervielle A, Benoudis L, Boissonnot M, et al. Retinal microvascular alterations related to diabetes assessed by optical coherence tomography angiography: A cross-sectional analysis. *Medicine (Baltimore)*. 2017;96(15):e6427.
190. Garcia JM, Lima TT, Louzada RN, Rassi AT, Isaac DL, Avila M. Diabetic Macular Ischemia Diagnosis: Comparison between Optical Coherence Tomography Angiography and Fluorescein Angiography. *Journal of ophthalmology*. 2016;2016:3989310.
191. Balaratnasingam C, Inoue M, Ahn S, McCann J, Dhrami-Gavazi E, Yannuzzi LA, et al. Visual Acuity Is Correlated with the Area of the Foveal Avascular Zone in Diabetic Retinopathy and Retinal Vein Occlusion. *Ophthalmology*. 2016;123(11):2352-67.
192. Golebiewska J, Olechowski A, Wysocka-Mincewicz M, Odrobina D, Baszynska-Wilk M, Groszek A, et al. Optical coherence tomography angiography vessel density in children with type 1 diabetes. *PloS one*. 2017;12(10):e0186479.
193. Goudot MM, Sikorav A, Semoun O, Miere A, Jung C, Courbebaisse B, et al. Parafoveal OCT Angiography Features in Diabetic Patients without Clinical Diabetic Retinopathy: A Qualitative and Quantitative Analysis. *Journal of ophthalmology*. 2017;2017:8676091.
194. Lee H, Lee M, Chung H, Kim HC. Quantification of Retinal Vessel Tortuosity in Diabetic Retinopathy Using Optical Coherence Tomography Angiography. *Retina (Philadelphia, Pa)*. 2018;38(5):976-85.
195. Cho HY, Lee DH, Chung SE, Kang SW. Diabetic retinopathy and peripapillary retinal thickness. *Korean J Ophthalmol*. 2010;24(1):16-22.
196. Goebel W, Kretzchmar-Gross T. Retinal thickness in diabetic retinopathy: a study using optical coherence tomography (OCT). *Retina (Philadelphia, Pa)*. 2002;22(6):759-67.
197. Oshitari T, Hanawa K, Adachi-Usami E. Changes of macular and RNFL thicknesses measured by Stratus OCT in patients with early stage diabetes. *Eye (Lond)*. 2009;23(4):884-9.
198. Park HY, Kim IT, Park CK. Early diabetic changes in the nerve fibre layer at the macula detected by spectral domain optical coherence tomography. *Br J Ophthalmol*. 2011;95(9):1223-8.
199. Sanchez-Tocino H, Alvarez-Vidal A, Maldonado MJ, Moreno-Montanes J, Garcia-Layana A. Retinal thickness study with optical coherence tomography in patients with diabetes. *Investigative ophthalmology & visual science*. 2002;43(5):1588-94.
200. Sugimoto M, Sasoh M, Ido M, Wakitani Y, Takahashi C, Uji Y. Detection of early diabetic change with optical coherence tomography in type 2 diabetes mellitus patients without retinopathy. *Ophthalmologica Journal international d'ophtalmologie International journal of ophthalmology Zeitschrift fur Augenheilkunde*. 2005;219(6):379-85.
201. Virgili G, Menchini F, Casazza G, Hogg R, Das RR, Wang X, et al. Optical coherence tomography (OCT) for detection of macular oedema in patients with diabetic retinopathy. *The Cochrane database of systematic reviews*. 2015;1:CD008081.
202. Biallostorski C, van Velthoven ME, Michels RP, Schlingemann RO, DeVries JH, Verbraak FD. Decreased optical coherence tomography-measured pericentral retinal thickness in patients with diabetes mellitus type 1 with minimal diabetic retinopathy. *Br J Ophthalmol*. 2007;91(9):1135-8.
203. Chen Y, Li J, Yan Y, Shen X. Diabetic macular morphology changes may occur in the early stage of diabetes. *BMC Ophthalmol*. 2016;16:12.
204. Nilsson M, von Wendt G, Wanger P, Martin L. Early detection of macular changes in patients with diabetes using Rarebit Fovea Test and optical coherence tomography. *Br J Ophthalmol*. 2007;91(12):1596-8.
205. Srinivasan S, Pritchard N, Sampson GP, Edwards K, Vagenas D, Russell AW, et al. Retinal thickness profile of individuals with diabetes. *Ophthalmic Physiol Opt*. 2016;36(2):158-66.
206. van Dijk HW, Kok PH, Garvin M, Sonka M, Devries JH, Michels RP, et al. Selective loss of inner retinal layer thickness in type 1 diabetic patients with minimal diabetic retinopathy. *Investigative ophthalmology & visual science*. 2009;50(7):3404-9.
207. Verma A, Raman R, Vaitheeswaran K, Pal SS, Laxmi G, Gupta M, et al. Does neuronal damage precede vascular damage in subjects with type 2 diabetes mellitus and having no clinical diabetic retinopathy? *Ophthalmic Res*. 2012;47(4):202-7.
208. Mehboob MA, Amin ZA, Islam QU. Comparison of retinal nerve fiber layer thickness between normal population and patients with diabetes mellitus using optical coherence tomography. *Pak J Med Sci*. 2019;35(1):29-33.
209. Peng PH, Lin HS, Lin S. Nerve fibre layer thinning in patients with preclinical retinopathy. *Can J Ophthalmol*. 2009;44(4):417-22.

210. Chalam KV, Bressler SB, Edwards AR, Berger BB, Bressler NM, Glassman AR, et al. Retinal thickness in people with diabetes and minimal or no diabetic retinopathy: Heidelberg Spectralis optical coherence tomography. *Investigative ophthalmology & visual science*. 2012;53(13):8154-61.
211. Bressler NM, Edwards AR, Antoszyk AN, Beck RW, Browning DJ, Ciardella AP, et al. Retinal thickness on Stratus optical coherence tomography in people with diabetes and minimal or no diabetic retinopathy. *American journal of ophthalmology*. 2008;145(5):894-901.
212. Massin P, Erginay A, Haouchine B, Mehidi AB, Paques M, Gaudric A. Retinal thickness in healthy and diabetic subjects measured using optical coherence tomography mapping software. *Eur J Ophthalmol*. 2002;12(2):102-8.
213. Panozzo G, Parolini B, Gusson E, Mercanti A, Pinackatt S, Bertoldo G, et al. Diabetic macular edema: an OCT-based classification. *Semin Ophthalmol*. 2004;19(1-2):13-20.
214. Esmaeelpour M, Brunner S, Ansari-Shahrezaei S, Nemetz S, Povazay B, Kajic V, et al. Choroidal thinning in diabetes type 1 detected by 3-dimensional 1060 nm optical coherence tomography. *Investigative ophthalmology & visual science*. 2012;53(11):6803-9.
215. Farias LB, Lavinsky D, Benfica CZ, da Silva MO, Lavinsky J, Canani LH. Changes in choroidal thickness and volume are related to urinary albumin excretion in type 2 diabetic patients without retinopathy. *Clin Ophthalmol*. 2018;12:1405-11.
216. Galgauskas S, Laurinaviciute G, Norvydaite D, Stech S, Asoklis R. Changes in choroidal thickness and corneal parameters in diabetic eyes. *Eur J Ophthalmol*. 2016;26(2):163-7.
217. Gerendas BS, Waldstein SM, Simader C, Deak G, Hajnajeab B, Zhang L, et al. Three-dimensional automated choroidal volume assessment on standard spectral-domain optical coherence tomography and correlation with the level of diabetic macular edema. *American journal of ophthalmology*. 2014;158(5):1039-48.
218. Do Jeong K, Park JY, Kim BN, Kim JS, Kang MJ, Hwang JH. Assessment of Choroidal Thickness Inside and Outside of Vascular Arcade in Diabetic Retinopathy Eyes Using Spectral-Domain Optical Coherence Tomography. *Scientific reports*. 2019;9(1):10780.
219. Kase S, Endo H, Takahashi M, Saito M, Yokoi M, Ito Y, et al. Alteration of choroidal vascular structure in diabetic retinopathy. *Br J Ophthalmol*. 2020;104(3):417-21.
220. Lee HK, Lim JW, Shin MC. Comparison of choroidal thickness in patients with diabetes by spectral-domain optical coherence tomography. *Korean J Ophthalmol*. 2013;27(6):433-9.
221. Querques G, Lattanzio R, Querques L, Del Turco C, Forte R, Pierro L, et al. Enhanced depth imaging optical coherence tomography in type 2 diabetes. *Investigative ophthalmology & visual science*. 2012;53(10):6017-24.
222. Shen ZJ, Yang XF, Xu J, She CY, Wei WW, Zhu WL, et al. Association of choroidal thickness with early stages of diabetic retinopathy in type 2 diabetes. *International journal of ophthalmology*. 2017. p. 613-8.
223. Wang XN, Li ST, Li W, Hua YJ, Wu Q. The thickness and volume of the choroid, outer retinal layers and retinal pigment epithelium layer changes in patients with diabetic retinopathy. *International journal of ophthalmology*. 2018;11(12):1957-62.
224. Lains I, Talcott KE, Santos AR, Marques JH, Gil P, Gil J, et al. Choroidal Thickness in Diabetic Retinopathy Assessed with Swept-Source Optical Coherence Tomography. *Retina (Philadelphia, Pa)*. 2018;38(1):173-82.
225. Xu J, Xu L, Du KF, Shao L, Chen CX, Zhou JQ, et al. Subfoveal choroidal thickness in diabetes and diabetic retinopathy. *Ophthalmology*. 2013;120(10):2023-8.
226. Gupta C, Tan R, Mishra C, Khandelwal N, Raman R, Kim R, et al. Choroidal structural analysis in eyes with diabetic retinopathy and diabetic macular edema-A novel OCT based imaging biomarker. *PloS one*. 2018;13(12):e0207435.
227. Regatieri CV, Branchini L, Carmody J, Fujimoto JG, Duker JS. Choroidal thickness in patients with diabetic retinopathy analyzed by spectral-domain optical coherence tomography. *Retina (Philadelphia, Pa)*. 2012;32(3):563-8.
228. Ünsal E, Eltutar K, Zirtiloğlu S, Dinçer N, Özdoğan Erkul S, Güngel H. Choroidal thickness in patients with diabetic retinopathy. *Clin Ophthalmol*. 2014. p. 637-42.
229. Vujosevic S, Martini F, Cavarzeran F, Pilotto E, Midena E. Macular and peripapillary choroidal thickness in diabetic patients. *Retina (Philadelphia, Pa)*. 2012;32(9):1781-90.
230. Zhu Y, Zhang T, Wang K, Xu G, Huang X. Changes in choroidal thickness after panretinal photocoagulation in patients with type 2 diabetes. *Retina (Philadelphia, Pa)*. 2015;35(4):695-703.

231. Talks SJ, Manjunath V, Steel DH, Peto T, Taylor R. New vessels detected on wide-field imaging compared to two-field and seven-field imaging: implications for diabetic retinopathy screening image analysis. *Br J Ophthalmol*. 2015;99(12):1606-9.
232. Silva PS, Cavallerano JD, Tolls D, Omar A, Thakore K, Patel B, et al. Potential efficiency benefits of nonmydriatic ultrawide field retinal imaging in an ocular telehealth diabetic retinopathy program. *Diabetes care*. 2014;37(1):50-5.
233. Cameron JR. The Eye as a Window to the Brain: investigating the clinical utility of retinal imaging derived biomarkers in the phenotyping of neurodegenerative disease.: Univeristy of Edinburgh; 2018.
234. Farrah TE, Dhillon B, Keane PA, Webb DJ, Dhaun N. The eye, the kidney, and cardiovascular disease: old concepts, better tools, and new horizons. *Kidney Int*. 2020;98(2):323-42.
235. Huang Y, Gangaputra S, Lee KE, Narkar AR, Klein R, Klein BE, et al. Signal quality assessment of retinal optical coherence tomography images. *Investigative ophthalmology & visual science*. 2012;53(4):2133-41.
236. Nathan DM. The Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications Study at 30 Years: Overview. *Diabetes care*2014. p. 9-16.
237. de Oliveira Loureiro T, Cardoso JN, Lopes C, Carreira AR, Rodrigues-Barros S, Vide-Escada A, et al. The effect of insulin pump therapy in retinal vasculature in type 1 diabetic patients. *Eur J Ophthalmol*. 2021:1120672121990576.

Appendix 1: Supplementary Information for Chapter 2

Additional analysis using alternative study entry time period for MDI group

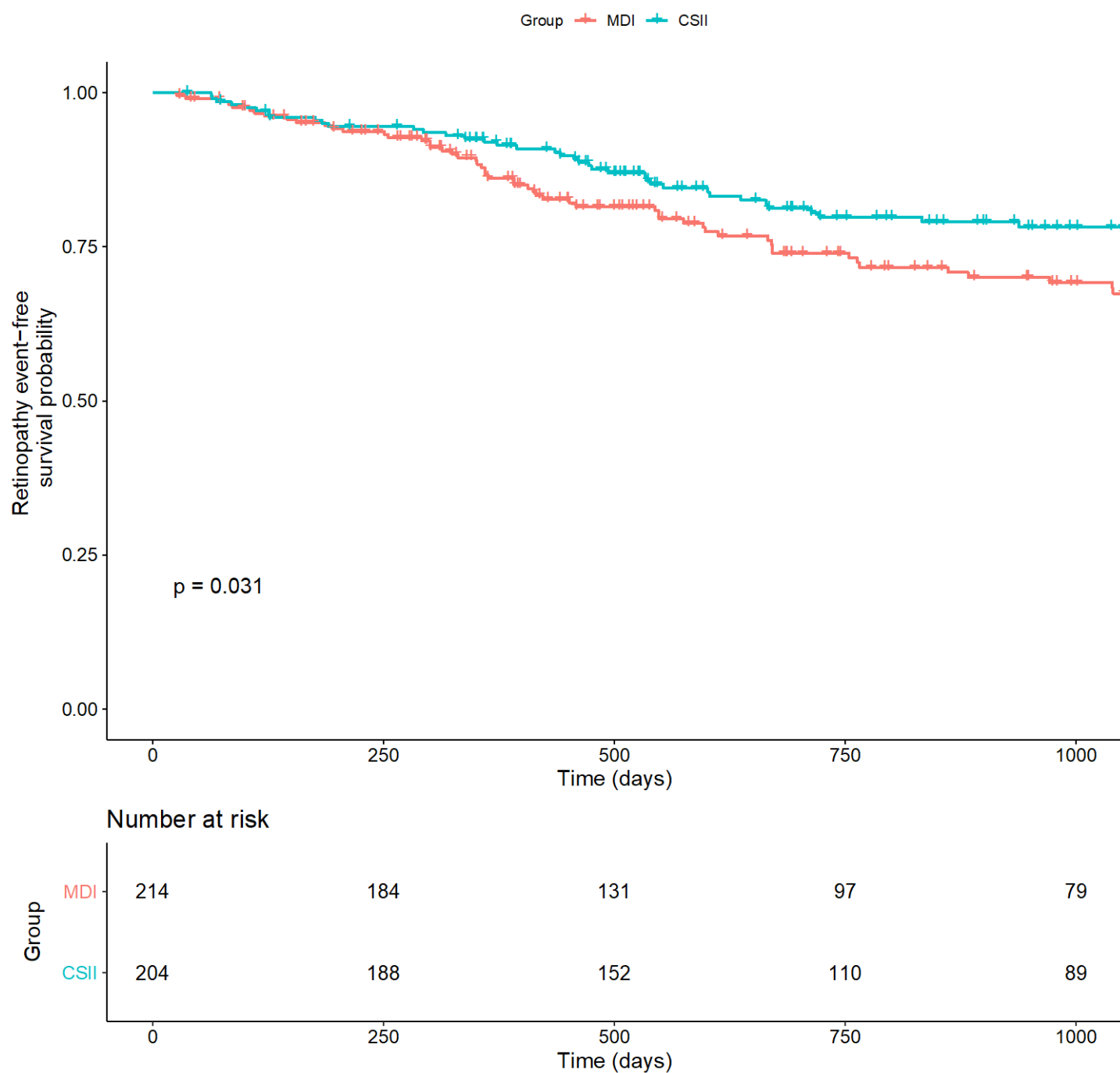
Methods for additional analysis

A secondary analysis was carried out using an alternative study entry for the MDI group. This was calculated using the median lag time from education to CSII commencement, rather than the mean lag time which was used for the main analysis. This was equivalent to 841 days from the date of completion of the diabetes education course. The remainder of the methodology was unchanged from the main analysis.

Results for additional analysis

Three people who were excluded in the primary analysis due to lack of follow up retinal data were able to be included following the shift in study entry time, bringing the total number of people included in the MDI group to 214.

The number of events in MDI group was 55, versus 38 events in CSII group which were unchanged. This remained significant on univariate ($p=0.03$) and multivariate ($p=0.045$) analyses, with no evidence of early DR worsening in the CSII group. The updated survival curve is also shown below (Appendix Figure 1.1).



Appendix Figure 1.1: Survival curve using study entry for the MDI group as 841 days from the structured education course, based on median lag time from the education to commencement of CSII therapy:

Appendix 2: Supplementary Information for Chapter 4

1. Recruitment Numbers for Group 2: Islet transplantation group

Five participants from the islet transplant waiting list were consented for inclusion in the study. Of these one withdrew prior to any follow up due to unforeseen medical issues unrelated to diabetes or islet transplantation (IT04). One received their islet transplant after the study period had ended (IT05). Of the remaining three, results were limited due to a combination of factors including equipment not being available at the time of islet transplant reviews and being unable to reschedule due to travel distance, failure of participants to manage to successfully complete the retinal imaging protocol due to frailty issues, or images which were obtained not meeting the quality criteria set out for study inclusion.

A summary of the suitable available images for each device are summarized in Appendix Table 2-1. Islet transplant recipient data for retinal imaging devices are therefore not presented due to very limited data with only completed data available from one participant for one device.

Appendix Table 2-1 Summary of available data for islet participants. Red: no data collected, green: data suitable for analysis, orange: data collected but unsuitable for analysis.

Patient Code	Imaging Device	Visit Month				
		0	1	3	6	12
IT01	OCT	Red	Red	Red	Red	Red
	OCTA	Green	Green	Green	Green	Green
	UWF-SLO	Red	Red	Red	Red	Red
IT02	OCT	Red	Red	Green	Red	Green
	OCTA	Orange	Orange	Orange	Orange	Green
	UWF-SLO	Orange	Green	Green	Green	Green
IT03	OCT	Red	Red	Red	Red	Red
	OCTA	Orange	Green	Green	Green	Orange
	UWF-SLO	Red	Red	Red	Red	Red

2. Recruitment Numbers for Group 3: MDI group

Five participants were consented for inclusion in the study. Some retinal imaging studies were not able to be completed at all visits due to equipment not being available at the time of reviews. Results data are presented below for months where data was available for all five participants (Months 0, 3 and 6 for UWF-SLO and OCTA, Months 3, 6 and 12 for OCT).

A summary of the suitable available images for each device are summarized in Appendix Table 2-2.

Appendix Table 2-2 Summary of available data for MDI participants. Red: no data collected, green: data suitable for analysis

Patient Code	Imaging Device	Visit Month			
		0	3	6	12
BB01	OCT	Green	Green	Green	Green
	OCTA	Green	Green	Green	Green
	UWF-SLO	Green	Green	Green	Green
BB02	OCT	Green	Green	Green	Green
	OCTA	Green	Green	Green	Red
	UWF-SLO	Green	Green	Green	Green
BB03	OCT	Red	Green	Green	Green
	OCTA	Green	Green	Green	Green
	UWF-SLO	Green	Green	Green	Red
BB04	OCT	Red	Green	Green	Green
	OCTA	Green	Green	Green	Green
	UWF-SLO	Green	Green	Green	Red
BB05	OCT	Red	Green	Green	Green
	OCTA	Green	Green	Green	Red
	UWF-SLO	Green	Green	Green	Red

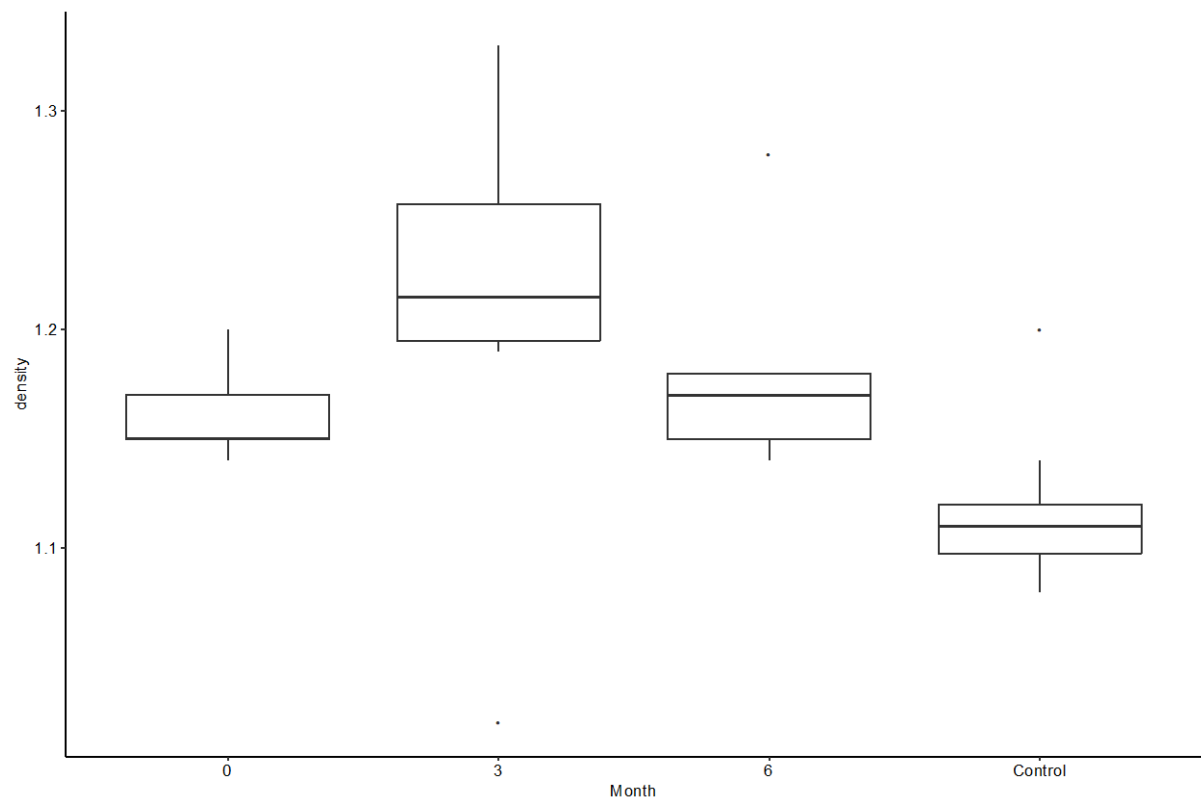
Retinal Imaging Results for Group 3: MDI Group

OCTA Results

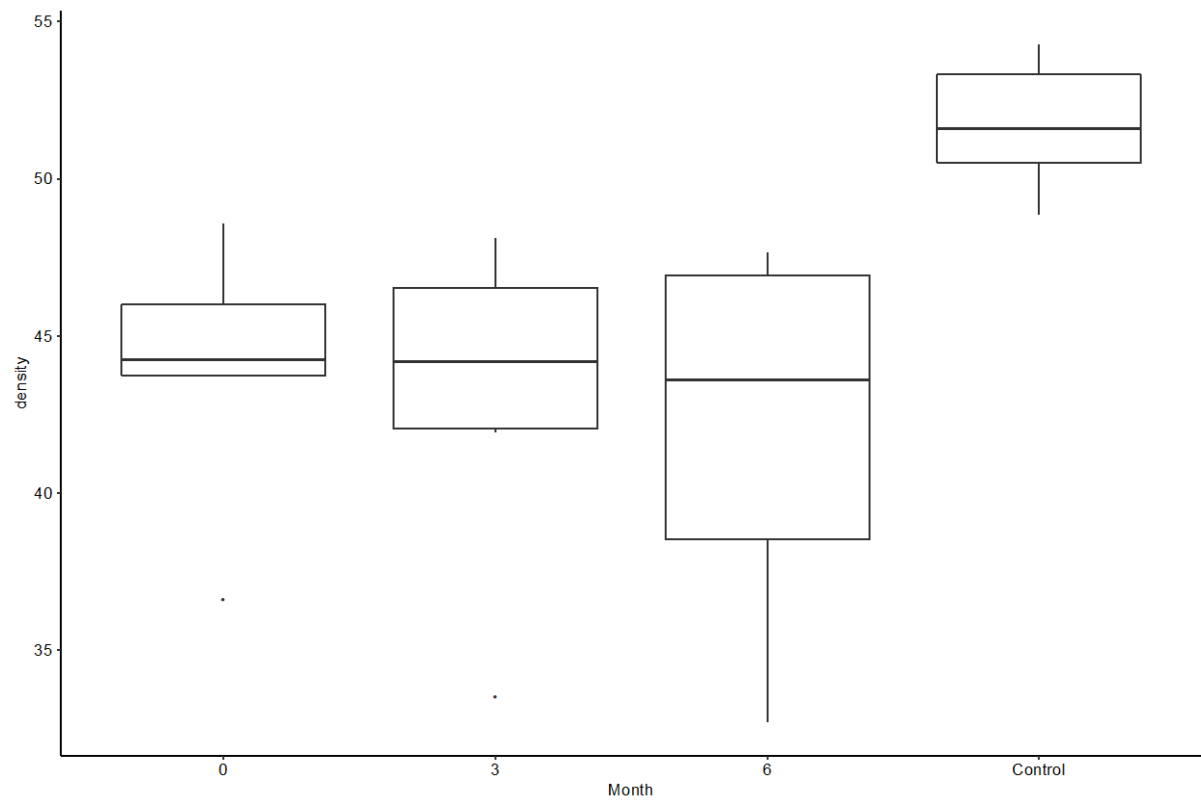
Data are shown displaying OCTA results for the five MDI participants who completed analysis at Months 0,3 and 6 of the study in Appendix Table 2-3 below. Boxplots are also shown for AI (Appendix Figure 2-1) and Superficial Layer Density results for the 'Whole ETDRS' section (Appendix Figure 2-2) and 'Parafovea' section (Appendix Figure 2-3) and include a comparison plot for the healthy controls described in Chapter 4.

Appendix Table 2-3 FAZ and Vessel density results for MDI participants at Months 0, 3 and 6 for 3x3mm and 6x6mm OCTA scans

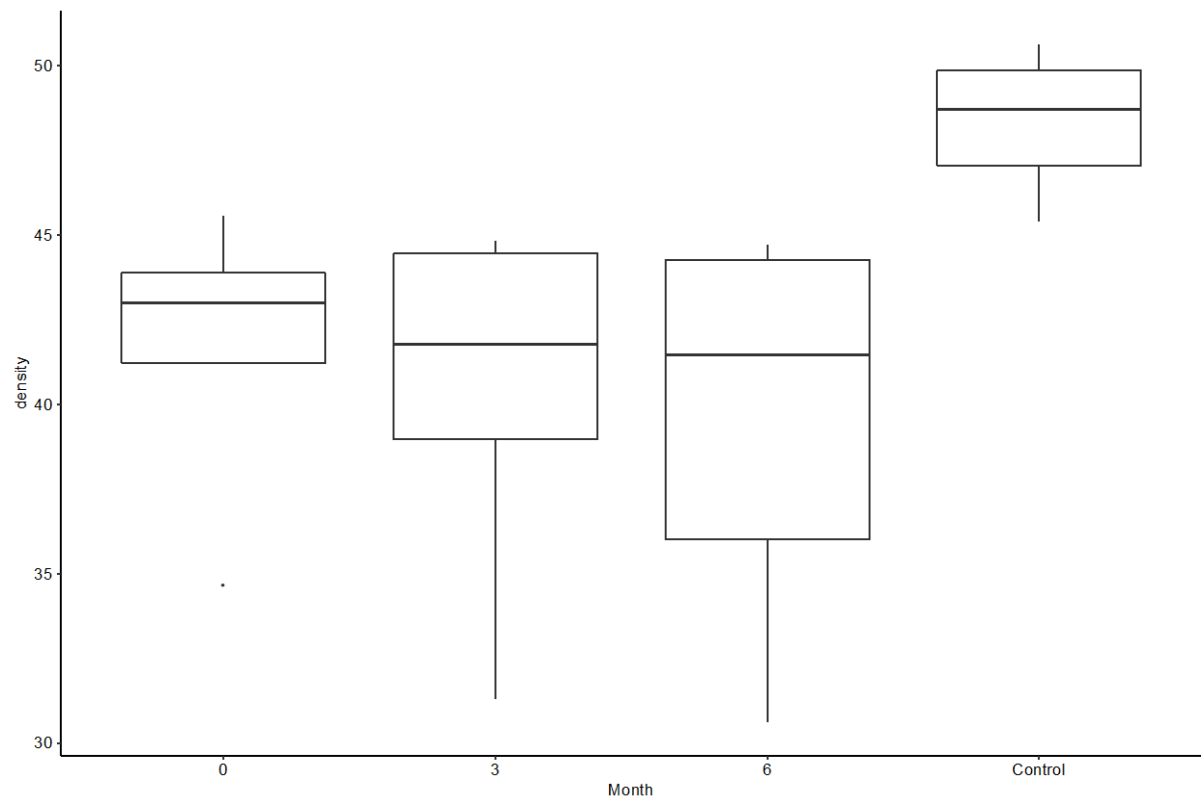
OCTA Results for MDI							
Measure		3x3			6x6		
		Month 0	Month 3	Month 6	Month 0	Month 3	Month 6
FAZ Characteristics	Area (mm ²)	0.23 ± 0.09	0.25 ± 0.13	0.24 ± 0.11	0.21 ± 0.08	0.25 ± 0.08	0.22 ± 0.11
	AI	1.16 ± 0.02	1.21 ± 0.10	1.18 ± 0.06	1.11 ± 0.04	1.11 ± 0.04	1.11 ± 0.03
	Perimeter (mm)	1.93 ± 0.41	2.07 ± 0.83	2.03 ± 0.57	1.76 ± 0.38	1.95 ± 0.36	1.80 ± 0.49
Superficial Layer Density (%)	Whole ETDRS	41.66 ± 4.23	40.52 ± 5.24	39.42 ± 6.02	45.02 ± 3.41	46.61 ± 1.93	46.80 ± 3.71
	Fovea	24.87 ± 5.84	20.28 ± 7.75	20.33 ± 5.64	29.57 ± 6.13	21.23 ± 6.32	25.01 ± 5.95
	Parafovea	43.83 ± 4.47	43.11 ± 5.30	41.89 ± 6.26	45.46 ± 3.85	46.87 ± 3.39	48.28 ± 5.22
Deep Layer Density (%)	Whole ETDRS	48.37 ± 1.48	48.88 ± 1.88	50.64 ± 2.51	48.53 ± 6.06	51.70 ± 4.29	51.41 ± 4.61
	Fovea	33.51 ± 7.71	32.25 ± 7.50	33.74 ± 9.03	39.23 ± 6.43	40.47 ± 10.97	39.31 ± 9.09
	Parafovea	50.29 ± 1.24	51.00 ± 2.69	52.82 ± 2.31	49.83 ± 5.27	53.06 ± 4.09	53.63 ± 3.91



Appendix Figure 2.1: Boxplot showing changes in Acircularity Index assessed from 3x3mm OCTA images across the available study period for MDI participants with diabetes, with comparison to control non-diabetic group



Appendix Figure 2.2: Boxplot showing changes in Parafoveal density assessed from 3x3mm superficial images across the available study period for MDI participants with diabetes, with comparison to control non-diabetic group



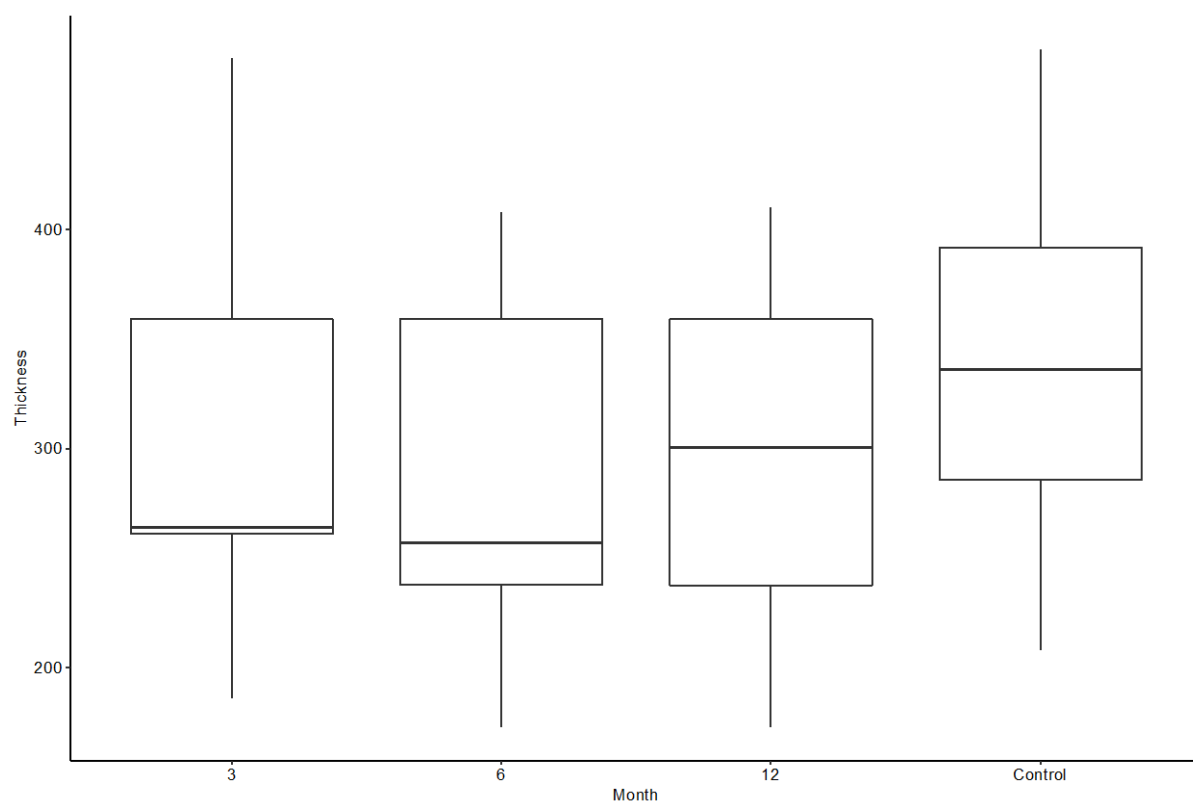
Appendix Figure 2.3: Boxplot showing changes in vessel density over the whole ETDRS grid assessed from 3x3mm superficial images across the available study period for MDI participants with diabetes, with comparison to control non-diabetic group

OCT Results

Data are shown for OCT results for the five MDI participants who completed analysis at Months 3, 6 and 12 of the study in Appendix Table 2-4 below. A boxplots is also shown for subfoveal thickness (Appendix Figure 2.4) and include a comparison plot for the healthy controls described in Chapter 4.

Appendix Table 2-4 Retinal, Choroidal and RNFL thickness results for MDI participants at Months 3, 6 and 12 for OCT scans

	Month 3	Month 6	Month 12
Central Retinal Thickness	280.00 ± 16.49	283.20 ± 25.38	282.75 ± 25.14
Retinal Macular volume	8.38 ± 0.60	8.41 ± 0.61	8.47 ± 0.71
Subfoveal Choroid Thickness	309.60 ± 112.38	287.00 ± 95.03	296.00 ± 102.65
Nasal Choroid Thickness	252.80 ± 90.27	237.80 ± 81.55	269.75 ± 84.76
Temporal Choroid Thickness	252.80 ± 98.98	269.20 ± 92.47	288.50 ± 108.92
Global RNFL Thickness	87.60 ± 12.80	87.00 ± 11.11	91.25 ± 9.22



Appendix Figure 2.4: Boxplot showing changes in subfoveal choroidal thickness across the available study period for MDI participants with diabetes, with comparison to control non-diabetic group

Optos UWF-SLO Results

Data are shown for Optos UWF-SLO results for the five MDI participants who completed analysis at Months 0, 3 and 6 of the study in Appendix Table 2-5.

Appendix Table 2-5 Mean arterial calibre, mean venular calibre and AVR results for MDI participants at Months 0, 3 and 6 for Optos UWF-SLO imaging

Section	Analysis	Month 0	Month 3	Month 6
Inner band	Mean arterial calibre	98.11 ± 15.76	102.12 ± 11.00	101.89 ± 6.40
	Mean venular calibre	113.02 ± 4.43	111.14 ± 6.37	104.02 ± 12.16
	AVR	0.87 ± 0.12	0.93 ± 0.14	0.99 ± 0.17
Outer band	Mean arterial calibre	94.62 ± 6.24	99.12 ± 7.06	92.59 ± 9.25
	Mean venular calibre	101.18 ± 8.77	105.80 ± 10.69	99.66 ± 18.27
	AVR	0.94 ± 0.07	0.94 ± 0.10	0.95 ± 0.11