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Retinal vessel traits and their association with diabetic retinopathy and cognitive decline in a population with type 2 diabetes

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Abbreviations

ACR: albumin-creatinine ratio
AD: Alzheimer's disease
AGEs: advanced glycation end products
AIBL: Australian Imaging, Biomarkers and Lifestyle Flagship Study of Aging
AIC: Akaike information criterion
ARIC: Atherosclerosis Risk in Communities study
AusDiab: Australian Diabetes, Obesity and Lifestyle Study
AVR: arteriovenous ratio
BMI: body mass index
c-statistic: concordance statistic
CASI-S: Cognitive Abilities Screening Instrument-Short form
CASP: Critical Appraisal Skills Programme
CI: confidence interval
CIND: cognitive impairment with no dementia
CRAE: central retinal arterial equivalent
CRIC Chronic Renal Insufficiency Cohort
CRVE: central retinal venular equivalent
DCPD1987: Danish Cohort of Pediatric Diabetes 1987
DR: diabetic retinopathy
DRUID: Darwin Region Urban Indigenous Diabetes Study
DST: Digit Symbol Test
Dunedin Study: Dunedin Multidisciplinary Health and Development Study
EDC: Pittsburgh Epidemiology of Diabetes Complications Study
EDIS: Epidemiology of Dementia in Singapore
ET2DS: Edinburgh Type 2 Diabetes Study
ETDRS: Early Treatment of Diabetic Retinopathy Study
Faces: Faces and Family Pictures subtest
FD: fractal dimension
HbA1c: glycated haemoglobin
HDL: high density lipoprotein
HR: hazard ratio
ISD: Information and Services Division
IVAN: Interactive Vessel Analysis

LALES: Los Angeles Latino Eye Study

LDR: Lothian Diabetes Register

LM: Logical Memory subtest

LNS: Letter-Number Sequencing test

MCI: mild cognitive impairment

MESA: Multi-Ethnic Study of Atherosclerosis

MHVS: Mill Hill Vocabulary Scale

MI: myocardial infarction

MMSE: Mini-Mental State Exam

MR: Matrix Reasoning test

NHS: National Health Service

NPDR: non-proliferative diabetic retinopathy

OR: odds ratio

PCA: principle components analysis

PDR: proliferative diabetic retinopathy

QUATRZ: QUantitative Analysis of Retinal vessel Topology and size

RVTs: retinal vessel traits

SCI-DC: Scottish Care Information-Diabetes Collaboration

Scottish DRS: Scottish Diabetic Retinal Screening Collaborative

SD: standard deviation

SIMD: Scottish Index of Multiple Deprivation

SiMES: Singapore Malay Eye Study

SINDI: Singapore Indian Eye Study

SIVA: Singapore “I” Vessel Assessment

SOUL-D: South London Diabetes Study

TMTB: Trail Making Test Part B

UKPDS: United Kingdom Prospective Diabetes Study

VAMPIRE: Vascular Assessment and Measurement Platform for Images of the Retina

VEGF: vascular endothelia growth factor

VFT: Borkowski Verbal Fluency Test

WAIS: Wechsler Adult Intelligence Scale

WESDR: Wisconsin Epidemiologic Study of Diabetic Retinopathy

WHO: World Health Organization

WTCRF: Western General Hospital’s Wellcome Trust Clinical Research Facility

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Declaration

I declare that this thesis is of my own composition. The work presented has not been submitted for any other degree or professional qualification.

The data used in this thesis was from the Edinburgh Type 2 diabetes Study, which began in 2006 and had data collection time points at baseline, years 1, 4 and data linkage in year 8. Data used from these time points were collected, cleaned, and at times manipulated by previous research team members. I took part in the data collection for the study at year 10 and performed primary collection, data cleaning and data entry as well as derivation of certain variables.

Signed:

Date: June 10th, 2020

Abstract

Background People with diabetes are at an increased risk of developing vascular disease, which is the leading cause of morbidity and mortality in this population. The retina is one of the few places in the body that offers non-invasive visualisation of the vascular system and thus provides a rich platform to evaluate local and systemic vascular disease. Recent advancements in retinal image analysis tools allow us to evaluate the retinal microvasculature in a more efficient and unbiased way compared to manual methods. Local retinal changes may provide insight into vascular disease prior to overt pathological changes.

Aim The aim of this thesis was to explore and evaluate retinal vessel traits in relation to various manifestations of vascular disease, specifically diabetic retinopathy and cognitive decline, using prospectively collected data. In addition to undertaking this research, this PhD project also aimed to contribute to the collection of primary data from in ongoing longitudinal cohort in order to provide data not only for this project, but for many other future and ongoing projects.

Methods Edinburgh Type 2 Diabetes Study is a cohort of 1,066 adults aged 60-75 years with type 2 diabetes living in the Lothian region of Scotland. Data were collected through research clinics as well as record linkage. Diabetic retinopathy status was obtained from the national screening programme and to evaluate cognitive decline, dementia diagnosis was obtained from a combination of medical records, death records and self-report. Cognitive decline was also evaluated using cognitive status derived from a battery of cognitive tests administered at baseline and then again after 10 years. Retinal images were analysed using VAMPIRE software for central retinal arteriolar equivalent (CRAE), central retinal venular equivalent (CRVE), arteriolar and venular tortuosity, fractal dimension and density.

Results A total of 83 participants (11.6%) developed retinopathy over 10 years. After controlling for a wide number of cardiometabolic, diabetic and vascular risk factors, there was evidence of an association between

increased venular tortuosity and incident retinopathy (odds ratio (OR) 1.51, 95% confidence interval (CI) 1.15 to 1.98, p = 0.003), as well as decreased standardised fractal dimension and incident retinopathy (OR 0.75, 0.58 to 0.96, p = 0.025).

Of the total 1066, 106 (9.9%) were determined to have a dementia diagnosis after 10 years of follow-up. Cognitive decline, as measured by cognitive testing after 10 years, controlling for baseline cognitive status, was measured in the 581 returning participants. There were no independent associations between the retinal vessel traits and cognitive decline, using either dementia or the general intelligence factor, after controlling for various covariates. There was, however, evidence of age-related decreases in fractal dimension and density over the course of the study.

Conclusions This thesis has provided evidence from the ET2DS that venular tortuosity and fractal dimension are independently associated with diabetic retinopathy. The independent associations were modest and need to be contextualised within the heterogeneity that exists within the supporting literature as well as replicated in other studies, but they provide exciting support for the use of the retinal vessel traits in future risk prediction modelling for diabetic retinopathy. There was no evidence of an association between the reported retinal vessel traits and cognitive decline. Novel findings regarding age-related decreases in fractal dimension and density are important as more information is coming to light regarding the vessel traits and their associations with vascular disease.

Lay Summary

People with diabetes are at a higher risk of damage to the blood vessels, which is the leading cause of death and disease in people with diabetes. The retina, which is the layer of tissue in the very back of the eye, is one of the few places in the body that blood vessels can be richly visualised using non-invasive methods, so it offers a unique platform to evaluate the health of the blood vessels in the eye as well as providing a surrogate for health of blood vessels throughout the body. Recent advancements in image analysis tools for the retina allows for more efficient and detailed evaluation of these tiny blood vessels. It is the hope that small changes in the blood vessels of the eye provide insight into damage throughout the body as an early marker before major disease is established.

The aim of this thesis was to explore and evaluate retinal blood vessel measurements in relation to various manifestations of vessel damage, specifically diabetic retinopathy (vessel disease in the eye) and cognitive decline. Measurements evaluated in this thesis include blood vessel widths, how curved and twisted the vessels are, called tortuosity, and how complex the vessel system is in the image, called fractal dimension. Another important aim of this PhD project was to contribute to the data collection for the ongoing study that this project was a part of, in order to contribute a substantial amount of data for not only this project but many others.

Data from the Edinburgh Type 2 Diabetes Study were used, which includes 1,066 adults aged 60-75 years with type 2 diabetes living in the Lothian region of Scotland. Data were collected at baseline and after 10 years through research clinics as well as linking with medical and other records. Diabetic retinopathy status was obtained from the national screening programme records and to evaluate cognitive decline, dementia diagnosis was obtained from a combination of medical records, death records and self-report. Cognitive decline was also evaluated using cognitive status derived from cognitive tests administered at baseline and then again after 10 years.

A total of 83 participants developed retinopathy over 10 years. After taking in to account a wide number of other risk factors, there was evidence that increased tortuosity of the retinal veins was associated with a participant developing retinopathy after 10 years. Also, decreased fractal dimension was associated with developing retinopathy. The analysis suggested tortuosity may be a good candidate marker to help predict those who will develop retinopathy.

Of the total 1066, 106 were determined to have a dementia diagnosis after 10 years. Cognitive decline, as measured by cognitive testing was measured in the 581 returning participants. There were no withstanding associations between the retinal blood vessel measurements and cognitive decline. There was, however, evidence of age-related decreases in vessel network complexity over the course of the study.

The findings from this thesis provide evidence that in this population with type 2 diabetes, tortuosity of the retinal veins and fractal dimension are associated with developing diabetic retinopathy, above and beyond other known risk factors. These findings are promising for the role of retinal vessel traits in the development of risk prediction and stratification for diabetic retinopathy, but require further validation in other similar studies as well as contextualisation within the field regarding heterogeneity of the methods and findings. There was no evidence of an association between the retinal vessel measurements and cognitive decline.

1 Introduction

1.1 Diabetes

Diabetes mellitus is a metabolic condition of multiple aetiologies characterised by chronic hyperglycaemia (American Diabetes Association, 2014). Hyperglycaemia is caused by a reduced ability of the pancreas to produce insulin, impaired insulin action, or a combination of both. Sustained hyperglycaemia can cause damage to the vascular system of the body and is associated with increased risk of heart disease, stroke, kidney disease and death (Fowler, 2011).

Insulin is a hormone secreted by the pancreas which is a key factor in the uptake of blood glucose into the cells of the body. β-cells of the pancreas are able to detect nutrient levels in the body through changes in glycolysis, a metabolic pathway responsible for converting glucose into usable energy, and respond by altering the secretion of insulin. In other cells of the body the end product of glycolysis, pyruvate, is mainly converted to lactate, but due to low expression of lactic acid dehydrogenase in the β-cells, pyruvate will enter the higher energy yield metabolic processes in the mitochondria. This rapid increase in cellular energy initiates insulin secretion from the β-cells (Chen, Cruzat and Newsholme, 2016).

β-cell dysfunction is a leading factor in the onset of diabetes, both type 1 and type 2. Type 1 diabetes is caused by an autoimmune attack on the β-cells, resulting in their reduced ability to produce insulin. Onset of type 2 diabetes is more complex and can begin with decreased insulin action (insulin resistance) due to obesity and physical inactivity, leading to hyperglycaemia and an up regulation of insulin production (Stumvoll, Goldstein and Haeften, 2005). Hyperinsulinaemia is a result of, and contributing factor to, insulin resistance in target tissues and a build-up of glucose in the blood, which also increases insulin resistance. Hyperglycaemia will cause the β-cells to produce even more insulin and can lead to their dysfunction and death, which

can occur relatively early in the disease process (Reaven, 1988; Weyer *et al.*, 1999).

Symptoms associated with diabetes include polyuria and polydipsia (increased urination and thirst), as well as blurred vision and unexplained weight loss. Diagnosis can be confirmed by several types of glucose tests. These include (1) a non-fasting plasma glucose concentration ≥ 11.1 mmol/l, (2) a fasting plasma glucose concentration of ≥ 7.0 mmol/l, or (3) a plasma glucose concentration ≥ 11.1 mmol/l two hours after commencing an oral glucose tolerance test where the patient would be fasting and then consume a glucose drink (World Health Organization, 2006). Another method of measuring glucose levels is by glycated haemoglobin (HbA1c) which is an indicator of glucose levels over the previous 10 to 12 weeks, but this method is relied on less and is not recommended for certain patients (World Health Organization, 2011). An HbA1c of > 6.5% is used to indicate the presence of diabetes. Any one of the glucose tests can confirm a diagnosis of diabetes if the patient is also presenting with polyuria and polydipsia, but if these symptoms are not present then repeat glucose measures should be taken for confirmation.

Classifications of diabetes falls mainly into two types: type 1 and type 2, although there are other forms including gestational diabetes, and monogenetic causes such as in maturity onset diabetes of the young (MODY). Type 1 diabetes is associated with genetic susceptibility as well as environmental triggers of an autoimmune response and is often associated with a younger age of onset. The aetiology of type 2 diabetes is complicated and involves common factors such as obesity, age, and lifestyle, but also includes genetic causes, which is thought to contribute to the wide variations in incidence based on geographical and ethnic differences (Adeghate et al., 2006). Type 2, the more prevalent type, has historically been associated with an older age of onset but has been increasingly diagnosed in children (Dabelea *et al.*, 2014).

Treatment for type 1 diabetes includes regular subcutaneous insulin injections and monitoring. Type 2 can be treated with a continuum of interventions that include dietary restrictions, weight reduction, exercise, oral glucose lowering agents, as well as insulin injections (American Diabetes Association, 2018). Medication choices for treatment of type 2 diabetes depends on the specific HbA1c target, comorbidities and contraindications of the individual. Metformin remains the most widely preferred anti-hyperglycaemic medication, and can be intensified with dual or triple treatment with other medications such as sulphonylurea, dipeptidyl peptidase-4 inhibitors (DPP-4), thiazolidinedione and sodium-glucose cotransporter 2 (SGLT2) inhibitor (National Institute for Health and Care Excellence, 2015; American Diabetes Association, 2018). There are newer non-insulin injectables, such as glucagon-like peptide-1 (GLP-1) receptor agonists, used to treat type 2 diabetes for patients that are struggling to control their glucose levels with oral agents alone (Meier, 2012). Insulin is initiated in type 2 diabetes if oral agents or non-insulin injectables are contraindicated or not controlling blood glucose levels sufficiently.

While there are effective treatments for diabetes, there are still many barriers to people with the disease living healthy lives. One such barrier is perceived stigma, which can be social or even from healthcare providers (Browne *et al.*, 2013). It is a common misperception that diabetes, especially type 2, is the fault of the patient and the disease comes solely from a person's own poor eating and exercise habits. A high proportion of people with both type 1 and type 2 diabetes report feeling this type of stigma (N. F. Liu *et al.*, 2017). Stigma can create a barrier to seeking medical treatment and to proper self-management, which are both necessary in managing the primary disease as well as the associated complications.

1.1.1 Burden of disease

The most recent report by the World Health Organization (WHO) estimated the prevalence of diabetes at 422 million worldwide in 2014, and in 2017, the International Diabetes Federation (IDF) estimated 451 million to have

diabetes, half of whom remain undiagnosed (Roglic, 2016; Cho *et al.*, 2018). In the United Kingdom, 3.8 million people have been diagnosed with diabetes and roughly another million have diabetes that is undiagnosed (Diabetes UK, 2018, 2019). The WHO estimates that the incidence of diabetes has quadrupled since 1980, largely due to increased rates of type 2 diabetes, and the age standardised prevalence in adults has increased from 4.7% to 8.5% in the same time period (Roglic, 2016). In an analysis in the US, with data from 2000-2011, it is estimated that the population from age 20 has an increased lifetime risk of diabetes that has gone up 20% for men and 13% for women when compared with data from 1985-1989 (Gregg *et al.*, 2014).

Roughly 90% of cases of diabetes are attributed to type 2 diabetes, 8% type 1 diabetes and the remaining 2% are more rare types (Diabetes UK, 2019). Diabetes constitutes a large burden on human health as well as the health care system, and currently costs the National Health Service (NHS) £10 billion annually, or about 10% of the budget, with 80% of that cost due to complications and comorbidities (Hex *et al.*, 2012). Obesity is increasing worldwide as unhealthy eating patterns combined with reduced physical activity are rapidly rising, putting a strain on health care globally (Zheng, Ley and Hu, 2018). If past trends continue, the global economic burden is set to increase from $1.3 trillion (US dollars) in 2015 to $2.5 trillion in 2030, or as much as 2.2% of the global gross domestic product (GDP) (Bommer *et al.*, 2018).

1.1.2 Morbidity and mortality

Diabetes carries an increased risk of morbidity and mortality so much so that it is estimated a 50-year-old person with diabetes will die an average of six years earlier than a person without diabetes of the same age (Roger et al., 2012). People with diabetes have an increased risk of vascular damage, which is the main cause of morbidity and mortality. Sustained hyperglycaemia promotes vascular damage through increased inflammatory mechanisms, oxidative stress, formation of advanced glycation end products (AGEs), which are the product of glycation of plasma proteins in the

presence of sugar, endothelial dysfunction and other modes, and the vasculature's counteractive measures to these damaging effects are suppressed (Rask-madsen and King, 2013; Singh *et al.*, 2014; Carol Yimlui Cheung *et al.*, 2015).

This combination of increased damage and reduced ability of the cells to repair themselves leads to vascular complications that are broadly categorized as macrovascular and microvascular complications. Macrovascular disease, often used interchangeably with cardiovascular disease, refers to the larger blood vessels in the body and manifests as coronary artery disease, such as myocardial infarction, cerebrovascular disease, which includes stroke, as well as peripheral vascular disease (Fowler, 2011). Microvascular disease refers to damage in the small vessels and in people with diabetes usually refers to retinopathy, nephropathy and neuropathy. It has been estimated that as many as 50% of people with diabetes have evidence of microvascular disease and roughly a quarter have macrovascular disease (Litwak *et al.*, 2013).

Complications from macrovascular disease account for the largest burden of disease and largest economic burden in people with diabetes. Heart disease and stroke are the leading cause of morbidity and mortality among people with type 2 diabetes (Fowler, 2011). Death from cardiovascular disease is two to four times more likely in adults with diabetes compared to adults without (CDC and USD HHS, 2012). Diabetic retinopathy, a significant manifestation of microvascular disease in the eyes, is the leading cause of blindness in adults under 75 years (Fong *et al.*, 2004), and diabetes is the leading cause of kidney failure (Atkins, 2005; Levey and Coresh, 2012).

Diabetes is not just related to the incidence of vascular disease, but recent studies have found an increased risk of death from cancer, although it's not fully understood what the mechanism for the increased risk is (Emerging Risk Factors Collaboration *et al.*, 2011). Also, type 2 diabetes is related to increased risk of cognitive impairment and dementia (Biessels *et al.*, 2014; Biessels and Despa, 2018). Again, the mechanism for the role of diabetes in

dementia is not yet clear, and some evidence suggests that lower early age cognition predicts type 2 diabetes (Altschul, Starr and Deary, 2018). As people with diabetes are living to more advanced ages this relationship is becoming more apparent and the importance for understanding their interrelatedness and possibility for intervention is growing. Cognitive decline and the association with the retina in diabetes is a central focus of this thesis and the relationship will be discussed further in this chapter.

1.2 Diabetic Retinopathy

Diabetic retinopathy (DR) is a common microvascular complication in people who have diabetes. A study from 2010 estimated the prevalence of DR to be 28.5%, and vision threatening retinopathy to be 4.4% of US adults with diabetes (Zhang *et al.*, 2010). However, this estimate is conservative, with some studies estimating prevalence of DR as high as 55% in their population and vision threatening retinopathy as high as 7.1% (Cheung, Mitchell and Wong, 2010; Ting, Cheung and Wong, 2016). Diabetic retinopathy is the leading cause of vision loss in middle-aged people, throughout the world, and from 1990 to 2015, all other common causes of blindness and vision impairment saw a reduced prevalence, except for diabetic retinopathy, which increased during this time (Klein, 2007; Flaxman *et al.*, 2017).

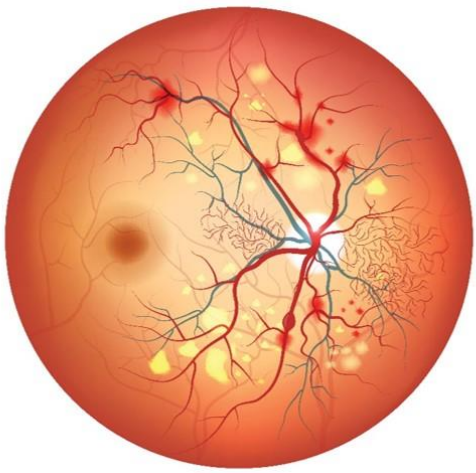

Figure 1. Cartoon graphic of retina with different features of diabetic retinopathy- photo credit: TefiM

1.2.1 Clinical features of progression of diabetic retinopathy and pathophysiology

Diabetic retinopathy, is categorised into different severity stages, which may differ depending on the specific scale you are using, but the main feature being a difference between non-proliferative (NPDR) and proliferative DR (PDR). Early forms of the disease are often asymptomatic, with vision impairment not being present until late stages in the disease, after significant tissue damage.

In general, very mild non-proliferative DR is characterised as the presence of microaneurysms only, progressing to mild NPDR with the addition of hard exudates, cotton-wool spots and mild retinal haemorrhages (Meyerle, Chew and Ferris III, 2008; Tarr *et al.*, 2012). As the disease progresses from moderate to severe NPDR, these features will be found more widespread throughout the retina, the haemorrhages becoming more severe, and the additional presence of venous beading. The most severe stage of the disease is the onset of PDR, which is defined by the presence of newly formed, finely looping, blood vessels, termed neovascularisation (Danis and Davis, 2008). Some of these features can be seen in **Figure 1**.

Diabetic maculopathy is a category of DR, related both to NPDR and PDR, and is the most common cause of vision loss in people with type 2 diabetes (Ciulla et al. 2003). Maculopathy is the result of macular oedema, characterised by retinal thickening in the area of the macula from fluid and plasma constituents that accumulate as a result of vascular damage leading to increased permeability and leaky vessels (Meyerle, Chew and Ferris III, 2008). This increased permeability causes a breakdown in the inner blood-retinal barrier, which serves to protect the outer neural retinal tissues from the harmful molecules in the circulatory system of the inner retinal vessels (Klaassen, Van Noorden and Schlingemann, 2013) This fluid accumulation results in hard exudates and can lead to vision impairment and loss.

The retina is one of the earliest tissues in the body to show diabetic damage as the capillary endothelial cells in the retina are particularly susceptible to

the negative effects of the various cascades that occur due to the presence of hyperglycaemia (Brownlee, 2005; Cheung, Mitchell and Wong, 2010). Changes in the retinal endothelium in the presence of hyperglycaemia begin to cause damage to capillaries in the forms described in the previous paragraph, namely microaneurysm, cotton wool spots and hard exudates, which lead to the loss of capillaries. This results in areas of low and non-perfusion. Overtime, with the accumulation of vascular damage, can lead to retinal ischaemia and hypoxia and increased vascular permeability. There is also evidence that inflammatory mechanisms contribute to retinal vascular damage (Meyerle, Chew and Ferris III, 2008). Hypoxia stimulates a release (VEGF), leading to proliferation of new vessels, neovascularization which is the marker of advanced PDR. These new, delicate vessels can leak and rupture easily, leading to fluid build-up behind the retina which can cause vitreous detachment, haemorrhage and loss of sight (Tarr *et al.*, 2012).

1.2.2 Treatment of diabetic retinopathy

Diabetic retinopathy often begins asymptomatically but can progress to blurred vision, reduced visual acuity and eventual blindness. The current treatment mainstay for DR is controlling hyperglycaemia and blood pressure, which are part of the direct cause of retinal vascular damage in order to prevent progression of the disease (Mohamed, Gillies and Wong, 2007). More advanced retinopathy can be treated with laser photocoagulation, however, this does not treat the underlying cause of DR and can damage vision, leading to night blindness, and so new treatments are currently being sought. Anti-VEGF intraocular injections are currently being introduced, as VEGF plays an important role in new vessel formation when stimulated by hypoxia (Do *et al.*, 2008). Other novel treatments being investigated include protein kinase C inhibitors, steroid injections and somatostatin receptor agonists (Duh, 2008). Other pathways and molecules of interest as possible treatment targets include erythropoietin as a suppressor of retinal neovascularisation and carbonic anhydrase to reduce vascular permeability (Cheung, Mitchell and Wong, 2010).

1.2.3 Diabetic retinopathy screening and grading

Retinopathy is the most common complication in diabetes, and roughly all people with type 2 diabetes will show evidence within 20 years of diagnosis (Fowler, 2008). More than 60% of all people with type 2 diabetes will have some form of retinopathy (Fong *et al.*, 2004). High prevalence of DR has led to many health systems adopting regular diabetic screening programs to help diagnose and monitor the disease to reduce to the risk of progression. The Scottish Diabetic Retinal Screening Collaborative (Scottish DRS) is one such programme that exists to promote equitable care to all people in Scotland with diabetes (Zachariah, Wykes and Yorston, 2015). The Scottish DRS aspires to monitor all its patients on a yearly basis by taking retinal images, where possible, and screen for retinopathy and maculopathy to help advise appropriate care and treatment. The Scottish DRS scheme uses their own retinopathy grading criteria which goes from R0 (no observable retinopathy) to R4, which is used when there is evidence of PDR.

There are other grading scales used for diabetic retinopathy and the most commonly used is the Early Treatment of Diabetic Retinopathy Study (ETDRS) grading system, often referred to as the modified Airlie House classification system, referring to the scale from which it was developed (Early Treatment Diabetic Retinopathy Study Research Group, 1991). The ETDRS scale has been used as the gold standard of retinopathy diagnosis for some time and uses 7-field stereoscopic images for review. The classifications span 13 categories from 10, indicating no retinopathy, up to 85, indicating severe vitreous haemorrhage or retinal detachment. The ETDRS scale is commonly used in research settings but is less commonly used clinically due its complexities and image requirements.

1.2.4 Diabetic retinopathy risk factors

Risk factors for DR reported by epidemiological studies in people with type 2 diabetes vary widely, but the most commonly reported are lifestyle and cardiometabolic factors including systolic blood pressure, cholesterol, obesity, diabetes factors including hyperglycaemia and duration of diabetes

(Stratton *et al.*, 2001; Tam *et al.*, 2009; Romero-Aroca *et al.*, 2010, 2011; Zhang *et al.*, 2010; Pugliese *et al.*, 2012; Bertelsen *et al.*, 2013; Salinero-Fort *et al.*, 2013; Xu *et al.*, 2014; Y. Liu *et al.*, 2017). Risk of vascular disease, including heart disease and stroke are increased in people with DR (Cheung, Rogers, *et al.*, 2007; Cheung, Wang, *et al.*, 2007). Ethnicity has been shown to be an important risk factor for retinopathy, with higher incidences in South Asian and African American populations (Wong *et al.*, 2006; Raymond *et al.*, 2008). However, as the ET2DS population is made of up 98% ethnically white participants, differences in ethnicity are not evaluated in this thesis.

Demographic, lifestyle and cardiometabolic risk factors

Age has been linked with diabetic retinopathy, but the association has been shown to be complicated. In the United Kingdom Prospective Diabetes Study (UKPDS) there was an association between older age and progression of established retinopathy but not retinopathy incidence (Stratton *et al.*, 2001). Participants with DR in the US based National Health and Nutrition Examination Survey (NHANES) 2005-2006 were slightly older than those without retinopathy (Zhang *et al.*, 2010). A cohort in England showed that people with NPDR were slightly older than those with no retinopathy, but participants with PDR were younger than those with NPDR (Jones *et al.*, 2012). In two cross-sectional studies, younger age was associated with increased risk of any retinopathy and more advanced retinopathy (Pugliese *et al.*, 2012; Y. Liu *et al.*, 2017). Although these findings are contrary to other studies, it is not wholly unexpected as earlier-onset diabetes, before the age of 40, confers a higher risk of advanced retinopathy (Song and Gray, 2011).

Gender has been found to be a risk factor in several studies, such as the cross-sectional NHANES study, which found evidence that male sex was independently associated with prevalent DR (Zhang *et al.*, 2010). However, another study, which collected data prospectively, found a possible increased risk of incident retinopathy in women, after four years (Salinero-Fort *et al.*, 2013), but the vast majority of epidemiological studies have not found evidence of sex as an independent risk factor.

Increased blood pressure is thought to be an important modifiable risk factor for diabetic retinopathy as the mechanical stretching and damage of the endothelium from increased blood flow can lead to the release of VEGF (Cheung, Mitchell and Wong, 2010). Historically, epidemiological studies often did not find an association between systolic blood pressure and DR, but clinical trials of strict glycaemic control noted systolic blood pressure as a modifiable risk factor (Stratton *et al.*, 2001; Mohamed, Gillies and Wong, 2007). More recent epidemiological studies have found an association between elevated systolic blood pressure or presence of hypertension and DR, indicating it is a strongly related risk factor (Romero-Aroca *et al.*, 2010; Zhang *et al.*, 2010; Jones *et al.*, 2012; Xu *et al.*, 2014; Y. Liu *et al.*, 2017).

Obesity has been established as a consistent risk factor for vascular disease, with the increase of fatty acids activating adipocytokines, such as proinflammatory markers and procoagulant factors, that can act unfavourably on other organs and lead to cardiovascular dysfunction (Calabro *et al.*, 2013). Obesity is generally considered as a body mass index (BMI) over 30, but this definition can differ depending on the population. In relation to DR, there have been a plethora of epidemiological studies showing a relationship with obesity (usually defined using BMI, but also with waist-to-hip ratio (WHR)), but the findings are inconsistent and often not strong enough to indicate a direct causal association (van Leiden *et al.*, 2002; Cheung and Wong, 2007). Currently there is a lack of clarity of the role obesity plays in the development of DR, but it is still an important factor to consider because it has a clear impact on the vascular system.

In general, smoking is a risk factor for vascular damage due to its negative effect on vascular endothelial cells as well as increasing damaging inflammatory cascades and instigating oxidative stress to the vasculature, all of which are factors in the development of DR (Powell, 1998; Tsiara, Elisaf and Mikhailidis, 2003). However, few studies have identified smoking as an independent risk factor of DR. Conversely, two studies have reported an inverse relationship between smoking and retinopathy (Stratton *et al.*, 2001; Pugliese *et al.*, 2012). In both cases, study authors were unable to determine

an exact reason for this surprising result but commented the findings could be limited by not knowing the number of cigarettes that were smoked, publication bias and the confounding nature of smokers possibly being less likely to adhere to strict medical treatment.

The association between blood lipids and DR varies between studies. Total cholesterol was associated with minimal NPDR in a Dutch study, but not more advanced retinopathy (van Leiden *et al.*, 2002). In a study of type 1 diabetes, retinopathy was associated with low density lipoprotein (LDL) and inversely associated with high density lipoprotein (HDL) concentrations, in men (Lyons *et al.*, 2004). Two other studies also identified increased LDL cholesterol as a risk factor for retinopathy (Salinero-Fort *et al.*, 2013; Y. Liu *et al.*, 2017). In another study, total cholesterol to HDL ratio was associated with diabetic macular oedema (Romero-Aroca *et al.*, 2010). This variance in association may indicate a role of dyslipidaemia in retinopathy, but probably not a causal pathway.

Diabetes related risk factors

Sustained hyperglycaemia is believed to be one of the initiating causes of the many factors contributing to vascular endothelial dysfunction and vascular permeability that are a part of establishing DR, which include inflammation, oxidative stress, formation of AGEs and protein kinase C (Cheung, Mitchell and Wong, 2010; Singh *et al.*, 2014). Therefore, it is unsurprising that many epidemiological studies have identified increased and uncontrolled HbA1c as the most common risk factor for diabetic retinopathy (Stratton *et al.*, 2001; Tam *et al.*, 2009; Romero-Aroca *et al.*, 2010; Zhang *et al.*, 2010; Jones *et al.*, 2012; Pugliese *et al.*, 2012; Salinero-Fort *et al.*, 2013; Xu *et al.*, 2014; Y. Liu *et al.*, 2017).

In many studies, a longer duration of diabetes has been found to be associated with increased risk of diabetic retinopathy and is often used clinically as an important risk factor (Romero-Aroca *et al.*, 2010; Zhang *et al.*, 2010; Jones *et al.*, 2012; Pugliese *et al.*, 2012; Salinero-Fort *et al.*, 2013; Xu *et al.*, 2014; Y. Liu *et al.*, 2017). The longer a person’s vascular system is

exposed to sustained or intermittent hyperglycaemia, the more damage will be inflicted. This is also related to the association between treatment of diabetes and risk or progression of DR, specifically those that take insulin for type 2 diabetes, as well as some studies showing taking oral treatments versus no medications being at an increased risk (Stratton *et al.*, 2001; Romero-Aroca *et al.*, 2010; Zhang *et al.*, 2010; Jones *et al.*, 2012; Pugliese *et al.*, 2012; Y. Liu *et al.*, 2017). There is a common need to increase intensity of medication dosage and type the longer a person has diabetes in order to control hyperglycaemia.

Diabetic retinopathy and other forms of vascular disease

Data from the Framingham Study suggested a link between DR and cardiovascular disease and in a paper from 2012 Pugliese et al also showed evidence of a cross-sectional association (Hiller *et al.*, 1988; Pugliese *et al.*, 2012). One study found diabetic retinopathy to be a risk factor for cardiovascular mortality, specifically in women (Juutilainen *et al.*, 2007). Prospective data from the Atherosclerosis Risk in Communities study (ARIC) demonstrated that diabetic retinopathy is a risk factor for incident ischaemic stroke during the nearly eight year follow-up (Wong *et al.*, 2002; Cheung, Rogers, *et al.*, 2007). There is a debate between whether this association is due to microvascular damage of the retina simply being a precursor to more overt damage of the larger vessels, or if there is a specific mechanistic link between retinopathy and macrovascular disease.

Diabetic retinopathy is itself a type of microvascular disease, so it is often considered to be associated with other forms of microvascular disease, such as nephropathy. Microalbuminuria indicates that there are small amounts of protein entering the urine due to pathologically increased permeability of the kidneys and is an early marker of nephropathy. The optimal diagnosis of microalbuminuria is with a timed urinary excretion, but often this is not feasible, so an untimed albumin-creatinine ratio (ACR) is used, with >3 mg/mmol indicating presence of microalbuminuria, although different ranges are cited especially regarding variations within sex and age (Kidney Disease

Improving Global Outcomes, 2013). There is strong evidence of an association between microalbuminuria and DR, as evidenced in many studies (Romero-Aroca *et al.*, 2010; Pugliese *et al.*, 2012; Xu *et al.*, 2012; Bertelsen *et al.*, 2013). One study, that used a cut-off of >3.4 mg/mmol ACR to indicated microalbuminuria, demonstrated an association between increased risk of retinopathy and levels of microalbuminuria currently less than the standard cut-off, indicating that a lower threshold, as low as 1.16 mg/mmol, may be clinically relevant (Bertelsen *et al.*, 2013).

Genetic risk factors of diabetic retinopathy

The largest know GWAS study evaluating DR, to date, that also incorporated duration of diabetes and glycaemic control, did not identify any withstanding genetic variants in the total, multi-ethnic study population, but did find a network of genes in the African American subgroup related to PDR (Pollack *et al.*, 2019). Previous replication studies have also been unable to replicate findings regarding DR related genetic variants (Grassi *et al.*, 2012; Hosseini *et al.*, 2015). There may indeed be a heritability to diabetic retinopathy, but it is likely to be modest and significantly outweighed by non-genetic risk factors. This thesis does not evaluate the genetics of DR as GWAS was not performed in the utilised cohort, so this is just a cursory description of the subject.

1.3 Cognitive Decline

1.3.1 Cognitive ageing and impairment

The study of cognitive ageing and impairment is an area of research interested in how the function of memory changes throughout a person's life, and specifically, mechanisms of decline in later years. Encompassed in the normal, non-pathological process of ageing, it is believed that various memory functions will naturally decrease with age, including working memory, spatial memory and memory for faces (Schaie, 2015). Other cognitive deficits are in relation to verbal abilities and include word-recall and language production, which may manifest as hesitation and repetitions when speaking (Schaie, 2015). Cognitive ageing greatly depends on different

factors including cognitive ability in younger years, education level, socio-economic status and health and physical fitness (Smith, 2016).

When cognitive ability is below what is normally expected for a person's age and education this is termed 'cognitive impairment', and the term 'cognitive decline' describes accelerated cognitive aging, which can span from mild to severely debilitating (Harada, Natelson Love and Triebel, 2013). Mild cognitive impairment (MCI) is a syndrome used to describe people that exhibit reduced cognitive ability for what would be expected, but the impairment does not overtly interfere with daily life (Gauthier *et al.*, 2006). When cognitive impairment is present to a degree at which daily functioning, mood and behaviour of the person are affected, a dementia diagnosis may be considered (Ritchie and Lovestone, 2002). However, not all people that exhibit symptoms of cognitive impairment will progress to develop frank dementia.

Mild cognitive impairment

Mild cognitive impairment is present in 10%-20% of people over 65 years and is often thought of as an intermediate step between normal cognition and dementia as between 11% and 33% of people with MCI will develop dementia within two years, most commonly Alzheimer's disease, and many of the diagnostic criteria for MCI overlap with dementia (Gauthier *et al.*, 2006; Petersen, 2011; Petersen *et al.*, 2014). However, the progression is heterogeneous, and a proportion of people with MCI will return to a normal cognitive range, although with increased risk of later cognitive impairment (Koepsell and Monsell, 2012; Sachdev *et al.*, 2013). This is because MCI can be caused by a varied number of stimuli, such as depression, anxiety, drug use or other comorbidities and when these are appropriately treated a person may regain cognitive function (Petersen *et al.*, 2014). If a person is diagnosed with MCI the most promising course of treatment may be to reduce risk factors for dementia which include controlling systolic hypertension (Gauthier *et al.*, 2006).

Dementia

Dementia is prevalent in about 5% of the population over 60 years and as the population is becoming older the absolute numbers are growing rapidly worldwide (Prince *et al.*, 2015). Dementia is not a singular disease but has various aetiologies and may present and progress in a variety of ways. Primary dementias include, Alzheimer's dementia, vascular dementia, dementia with Lewy bodies and frontotemporal dementia, to name a few (Ritchie and Lovestone, 2002).

Alzheimer's dementia is a neurogenerative disease and is the most common type of dementia. There are two types of diagnostic lesions in the brain associated with Alzheimer's which are neuritic plaques resulting from amyloid β-protein deposits building up to pathological levels, and neurofibrillary tangles composed of hyperphosphorylated tau proteins (Selkoe, 2001). The build-up of these plaques and tangles lead to a cascade which includes inflammatory responses and oxidative stress that results in progressive cellular dysfunction in the brain and ultimately dementia.

Vascular dementia, the second most common cause of age-related dementia, is defined by evidence of cognitive impairment across multiple domains that is associated with clinical evidence of cerebrovascular disease (often using imaging such as MRI and computed tomography scanning and/or patient history of vascular disease such as stroke) (Gorelick *et al.*, 2011). The energy requirements for the brain to function optimally are vast. Any alterations in the cerebral vascular system that supplies vital nutrients and maintains necessary conditions for normal functioning, such as those seen with cerebrovascular disease, can have an important impact the brain, resulting in cognitive impairment, although this is not fully understand at this time (Iadecola, 2013).

These two types of dementia, vascular and Alzheimer's, do not always exist independently and it is not uncommon to find a mixed aetiology of dementia with signs of cerebrovascular disease in persons that also have neurodegenerative pathology reflecting Alzheimer's dementia (Knopman *et*

al., 2003; Gorelick *et al.*, 2011; Iadecola, 2013; Toledo *et al.*, 2013). There is still a lot unknown about the exact causes and progression of dementia and how the burden of different pathologies, independently and together, drive cognitive impairment, but it is most likely a combination of many complex factors that can differ person-to-person.

It is important to understand the underlying cause of dementia in a person, as best as possible, in order to take appropriate action aimed at reducing progression and preventing associated co-morbidities. There is no cure for dementia and treatment is limited but often involves treatment of vascular risk factors such as hypertension, hypercholesterolemia and smoking, as well as symptoms of depression (O'Brien and Thomas, 2015). Also, if a person has diabetes, it may be important to target insulin resistance, such as through diet, exercise and medication (Cholerton *et al.*, 2016).

Certain classes of drugs can be used to help reduce the progression of Alzheimer's dementia such as acetylcholinesterase inhibitors and memantine hydrochloride, but there is no effective treatment in reversing the effects of dementia (Qaseem *et al.*, 2008). There are currently no medications used to treat vascular dementia, however, it has been suggested that there may be some benefit of treating patients with a vascular dementia diagnosis in the same manner as Alzheimer's, not least because Alzheimer's disease cannot reliably be ruled out in elderly patients with cognitive decline (Knopman, 2006).

1.3.2 Diabetes and cognitive decline

There is an increasingly well-established association between diabetes and cognitive impairment, with diabetes acting as a risk factor for accelerated cognitive decline (Luchsinger and Mayeux, 2004; Tilvis *et al.*, 2004; Biessels *et al.*, 2014; Zhang *et al.*, 2017; Biessels and Despa, 2018). Cognitive impairment in diabetes has a variety of phenotypes and is likely to be multi[factorial in aetiology (Biessels and Despa, 2018). While the precise pathophysiological mechanisms underlying the links are unknown, proposed theories rooted in evidence suggest the role of peripheral metabolic

alterations due to insulin resistance. The latter may indirectly cause damage in the brain, vascular injury as a consequence of hyperglycaemia and disruption of insulin to perform its normal functions in the brain (Cholerton *et al.*, 2016). However, it is most likely a combination as there are multiple risk factors mediating the relationship between diabetes and cognitive decline (Feinkohl *et al.*, 2015).

Insulin is known to be an important factor in the brain for memory and cognitive processes. Peripheral insulin is transported across the blood-brain barrier to the central nervous system, which is rich with insulin receptors (Banks, Jaspan and Kastin, 1997). Insulin has a neuroprotective effect in the brain, working against cell death, oxidative stress, neurotoxic effects of amyloid β-protein build-up and ischemia, which all help support learning and memory functions (Gasparini *et al.*, 2001; Ghasemi *et al.*, 2013). However, prolonged elevated circulating insulin, combined with insulin insensitivity, as seen in diabetes, can have an opposing effect (Cholerton *et al.*, 2016).

Underscoring this important link between insulin and cognition, a recent population based study in Finland found insulin resistance to be an independent predictor of cognitive decline (Ekblad *et al.*, 2017). In a Chinese population, researchers found that high fasting glucose concentrations, yet not in the range for a diabetes diagnosis, was associated with dementia independently of vascular risk factors and magnetic resonance imaging findings on vascular damage (Mortimer *et al.*, 2010). This indicates that blood glucose and insulin resistance, independently of other risk factors, may play a causal role in increased incidence of poor cognition in diabetes.

In addition to links with accelerated cognitive decline, a recent meta-analysis has shown that diabetes is associated with an increased risk of any type of dementia, but in particular with vascular dementia (127% increased risk) and also a 56% increased risk of Alzheimer's dementia (Gudala *et al.*, 2013). Studies have identified reductions in brain volume alongside cognitive impairments in people with type 2 diabetes, in comparison to no diabetes, which do not correspond with vascular lesions, leading to the conclusion that

the association is not mediated only through vascular damage from diabetes (Moran *et al.*, 2013, 2016; Brundel, Kappelle and Biessels, 2014).

Insulin deficiency and insulin resistance have been identified as important links to the neurodegeneration that is seen in Alzheimer's dementia. Raised peripheral insulin levels, as a result of diabetes, leads to lower brain insulin levels and dysfunction of insulin receptors in the brain, and reduced clearance of amyloid β-protein and increased hyperphosphorylation of tau proteins (Ahmed, Mahmood and Zahid, 2015). There is also evidence that diabetes is associated with Alzheimer's neurodegeneration independently of amyloid β and tau proteins, as oxidative stress caused by hyperglycaemia increases programmed cell death in the central nervous system (Vincent, Brownlee and Russell, 2002). It is possible that vascular risk factors associated with both diabetes and Alzheimer's disease have a direct effect on Alzheimer's disease not mediated directly through infarcts related to vascular dementia, but through concomitant cerebrovascular disease (Knopman, 2006). This helps to support a view that dementia (Alzheimer's, vascular and mixed aetiology) can be considered forms of vascular disease in people with diabetes. Also, that defining and diagnosing each separately is highly complicated.

1.3.3 Risk factors for cognitive decline

With the increased prevalence of dementia in people with diabetes it is important to understand the other risk factors for dementia to evaluate if there are modifiable means of reducing the disease before it is established. Despite many epidemiological studies demonstrating associations between various risk factors and dementia, there is still very little evidence of any strongly predictive risk factors, aside from age. Because dementia is somewhat of a rare outcome, as opposed to something like diabetic retinopathy, and the ethical concerns with including people with dementia in a study, the study of dementia and its risk factors is often underpowered. Also, much of this type of research has been performed in the general population, so may not be wholly applicable to people with diabetes.

A longitudinal study with 20 years follow-up aimed to develop a risk prediction tool for late-life dementia risk in a middle-aged population. This study found that older age, higher blood pressure, higher total cholesterol and obesity to be greatly associated with incident dementia (Kivipelto *et al.*, 2006). Another prospective study with a long follow-up of 27 years, also found middle age obesity to be an independent risk factor for dementia (Whitmer, Gunderson, *et al.*, 2005). A very large analysis that combined four prospective studies from several European countries, with 528 incident cases of dementia, identified age, female sex and smoking to be associated with increased risk of dementia (Launer *et al.*, 1999). Due to the relationship between dementia and cardiovascular disease, a retrospective study that included 8845 participants investigated markers of cardiovascular health in midlife and their associations with later development of dementia and identified high blood pressure, high cholesterol, smoking and diabetes at midlife to be associated with dementia, individually and as a composite score (Whitmer, Selby, *et al.*, 2005).

Cerebrovascular disease is an accepted risk factor for dementia, especially vascular dementia. Ischaemic stroke is known to be largely associated with dementia, and both have similar risk factors (Gorelick, 1997). In the Framingham study there was an estimated doubling in risk of dementia after stroke (Ivan *et al.*, 2004). Another prospective study interested in dementia and stroke found an association between the two, as well as other markers of small vessel disease, which included white matter hyperintensities, ventricular atrophy and cerebral infarcts (Kuller *et al.*, 2005). Currently, research into white matter hyperintensities, which can be seen using magnetic resonance imaging, is very intriguing as a possible biomarker for dementia and stroke as their presence greatly increases the risk of both diseases (Wardlaw, Valdés Hernández and Muñoz-Maniega, 2015).

Epidemiological studies have had mixed findings regarding the association between myocardial infarction (MI) and dementia, but findings from the Rotterdam study identified unrecognised MI, diagnosed by electrocardiogram but not self-reported or identified in medical notes, to be associated with

dementia in men, but not associated with recognised MI (Ikram *et al.*, 2008). The prospective Cardiovascular Health Cognition Study found that participants that went on to develop dementia after the nearly 6 years of follow-up were more likely to have previously been diagnosed with coronary artery disease and had a bypass, after adjustments for age and sex (Kuller *et al.*, 2005).

Along with age, hypertension is probably the most consistently cited risk factor for dementia and because it is modifiable through medication and lifestyle, poses a possibility for risk reduction. A 15 year longitudinal study found higher blood pressure at age 70 to be associated with development of dementia at age 79-85, although they also found blood pressure to have a greater decrease in people with dementia closer to the time of diagnosis (Skoog *et al.*, 1996). Studying the causal relationship of hypertension on dementia using observational data in elderly people, especially in a population with diabetes, is exceedingly difficult as many would be receiving treatment for hypertension, confounding the association.

With consistent findings that blood pressure is associated with dementia, research groups have set out to determine if the use of antihypertensives could reduce the risk of dementia. The findings of such studies have been mixed. An observational study which found a modest reduction in dementia and cognitive decline in men that received antihypertensive treatment compared with those that had evidence of hypertension but did not receive treatment, and this risk reduction increased with length of time on the antihypertensive medication (Peila *et al.*, 2006). However, in a randomised controlled trial in elderly people given an antihypertensive or placebo, there was no reduction in incident dementia, but this study was ended early due to a reduced risk of mortality, stroke and heart failure in the treatment group (Peters *et al.*, 2008). In a more recent promising randomised controlled trial, researchers evaluated the effects of risk factor reduction through diet, exercise and cognitive training on cognitive decline in a slightly at-risk elderly population, and found, when compared to participants that received normal risk factor reduction information, a modest reduction in cognitive decline

(Ngandu *et al.*, 2015), indicating that there is possibly more benefit in reducing the underlying causes of hypertension and other risk factors.

Excessive alcohol consumption can have a detrimental effect on the brain and cognition (Joyce, 1994). However, current epidemiological evidence suggests that moderate alcohol intake may be beneficial for dementia risk most likely due to alcohol's effect on reduced platelet adhesion, increasing HDL cholesterol and improving endothelial function (Ruitenberg *et al.*, 2002; Mukamal *et al.*, 2003; Anstey, Mack and Cherbuin, 2009). But not all alcohol intake is beneficial as high intake of alcohol is generally believed to increase risk of dementia (Luchsinger and Mayeux, 2004). So, while alcohol intake is associated with dementia risk, the amount of alcohol matters to whether that association is positive or negative.

Severe hypoglycaemic episodes are characterised by low blood glucose concentrations that require assistance for recovery and are generally a consequence of insulin treatment of diabetes. Cellular metabolism in the brain is nearly exclusively dependent on glucose to function normally, so it has been hypothesized that these acute episodes of low blood glucose can cause dysfunction in the brain, that can accumulate to cognitive impairment. This was shown in a study from 1991, where recurrent episodes of severe hypoglycaemia were associated with cognitive impairment in people treated with insulin (Langan *et al.*, 1991). Similar findings were identified in the Edinburgh Type 2 Diabetes Study (ET2DS), which demonstrated an association between a history of hypoglycaemia and lower cognitive ability after four years and a steeper cognitive decline, as well as incident hypoglycaemic episodes associated with increased cognitive decline (Feinkohl *et al.*, 2014). Similar findings were reported from the ARIC cohort study, which demonstrated a prospective association between a history of hypoglycaemia and incident dementia (Lee *et al.*, 2018). There is, however, an uncertainty if hypoglycaemia is the cause of the cognitive decline or if reduced cognitive function, and ability to perceive oncoming hypoglycaemia, is a risk factor for hypoglycaemic episodes (Bruce *et al.*, 2009; Feinkohl *et al.*, 2014). Understanding this relationship more thoroughly, especially if

hypoglycaemia is a risk factor for cognitive decline, could offer an opportunity to identify modifiable risk factors for cognitive decline (Biessels *et al.*, 2014)

Apolipoprotein E (APOE) is a plasma protein that is involved in cholesterol transport and the presence of the e4 variant, as opposed to alleles e2 and e3, to be largely increased in those with Alzheimer's dementia (Farrer *et al.*, 1997; Slooter *et al.*, 2004). Due to its role in lipid transport, the APOE e4 genetic variant has also been investigated and found to be associated with cardiovascular disease mortality (Rosvall *et al.*, 2009).

1.3.4 Measuring cognitive decline

The term cognitive ability simply refers to the skills and competencies a person has in order to complete mental based activities and includes areas such as memory, language, motor skills, and visual-spatial processing. Cognitive impairment is a pathologic reduction in these abilities based on some criteria, often in research this is based on a single or multitude of tests, or a portion of the population somewhere below the average. Cognitive decline is a reduction in cognitive ability over time at a greater trajectory than what is expected for the age and education level of the person. In research, cognitive decline often requires measuring cognitive ability at more than one time point and comparing between them. Alternatively, many studies of cognitive decline use a dementia diagnosis as a binary outcome of cognitive decline, indicating the person's cognitive ability has declined so much it has reached a pathological state.

While dementia is a good marker of cognitive decline, diagnosis of dementia is not infallible, and dementia does not manifest the same in all people. A dementia diagnosis requires a robust diagnostic process and is only common in the elderly. There are many people with probable dementia that are not diagnosed or do not meet the exact criteria and there are people with a dementia diagnosis that may have come well after the disease was established.

Cognitive decline is a complex health condition that cannot be easily measured. The research field of cognitive epidemiology provides a method to test cognitive ability, separate from a dementia diagnosis, as a risk factor and outcome for health and lifestyle parameters and generally is undertaken using a battery of validated cognitive tests (Deary and Batty, 2007). It is a difficult task to measure and describe cognitive ability and decline using clinical based tests that often need to be undertaken relatively quickly and with simple instructions. Different tests and combinations of tests have been created over the years and include the well-known Wechsler Adult Intelligence Scale (WAIS) and Stanford-Binet test (Thorndike, Hagen and Sattler, 1986; Wechsler, 1997). The general intelligence factor, *g*, is now commonly used in cognitive epidemiology, which was first described by Spearman in 1904. Spearman believed that people possess a general intelligence that underpins cognitive abilities and that performance in one cognitive domain is related to performance in other domains. Therefore, *g* is a measure that correlates with and explains a large portion of variance between different cognitive tests and represents cognitive ability over a range of domains.

In order to measure and report general intelligence, measurement of different cognitive domains needs to be undertaken using specifically designed and validated cognitive subtests, like what are available in the WAIS and Stanford-Binet tests. Domains that need to be considered include nonverbal reasoning, memory (verbal memory, nonverbal memory and working memory), executive function and processing speed. Scores of tests that measure these various domains are combined using reductive statistical methods such as principle components analysis or factor analysis that provide a single value that incorporates the values for the various tests.

There are many limitations in the study of cognitive ageing and change, including the fact that many participants in studies on ageing tend to be more intelligent and healthier than the general population, making it difficult to extrapolate the findings. Another problem is that if one is studying ageing in an older population there is an expectation of increased mortality and

morbidity related dropouts, so how should these samples be analysed in order to avoid severe survival bias? Rabbitt et al. investigated a longitudinal cohort that had significant dropout and death rates over the course of their 20-year study and determined that those that dropped out and died would have contributed significantly to the results and by excluding them the effects of ageing were underestimated (Rabbitt, Lunn and Wong, 2008). By excluding those that have dropped out or died you will be left with a healthier or more mildly unhealthy population which will inevitably alter your findings. How missing data is handled, as well as statistical models chosen for analysis are important in managing these issues.

It should also be taken in to consideration, when undertaking the measurement of cognition with measures such as *g*, that the historical context of such measures is rooted deeply in the eugenics movement of the early 20th century (Pilgrim, 2008). Specifically, the belief that intelligence was a heritable trait was of interest to the field of eugenics and how its measurement could be applied to the movement. Spearman was widely praised by popular eugenicists for his work on the discovery of general intelligence (McDougall, 1914). However, current psychological research aims to use measures of intelligence to study cognitive status and investigate pathology in order to improve the lives of people and not as a determinant of worthiness.

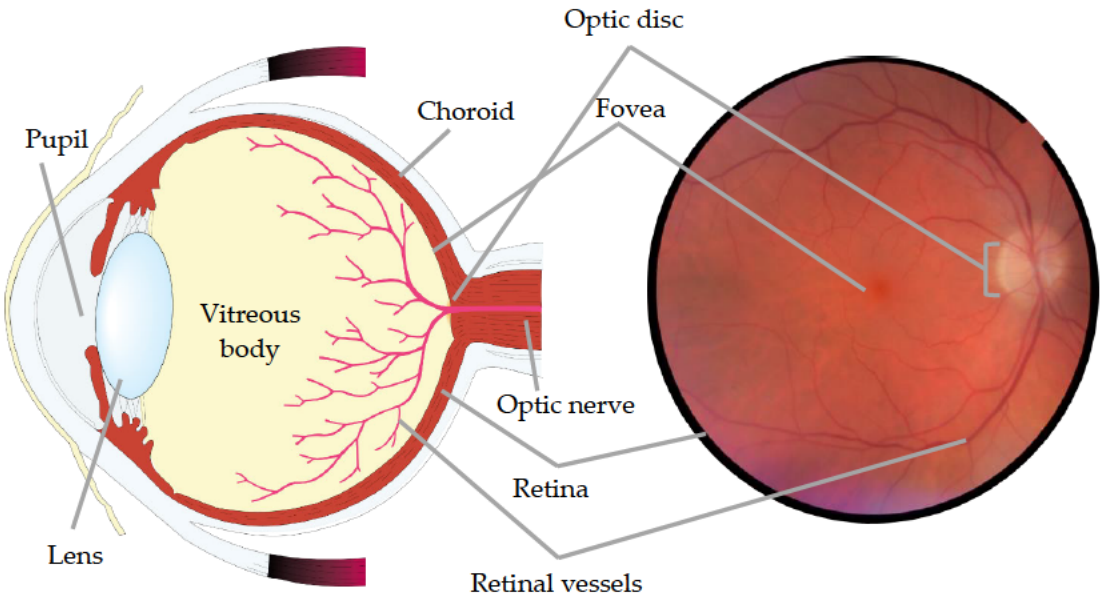

Figure 2. Human eye and retinal anatomical features

1.4 Retinal Vessel Traits

1.4.1 The retina

The retina is the light-sensitive, inner-most membrane of the eye, forming a barrier between the outer choroid and the inner vitreous body (**Figure 2**). The retina measures in thickness between 0.1mm to 0.56mm and forms a continuous structure extending from the optic nerve at the back of the eye up to the ciliary body and iris nearer the front.

There is a neural layer of the retina which contains the photoreceptor cells known as rods and cones, which process light and colour and transmit signals via chemical transduction to the brain (**Figure 3**). Beneath this layer, closer to the choroid is the retinal pigmented epithelium. Blood supply to the retina comes from two sources, the choroidal capillaries and the central retinal artery. The tight-junctions in the pigmented epithelium play an important role in maintaining the blood-retinal barrier between the vascular system of the choroid and that of the retina (Snell and Lemp, 2013).

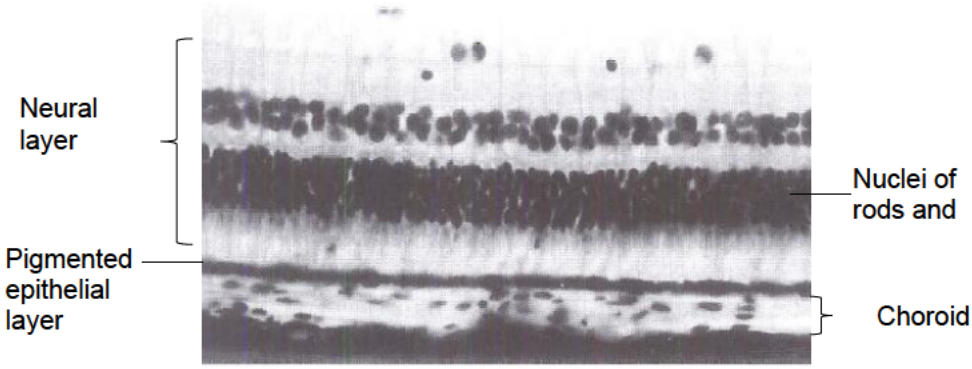

Figure 3. Photomicrograph section of the retina showing the different layers (Image reproduced with permission from Snell & Lemp 2013)

1.4.2 Retinal imaging and analysis

The fundus is a term used to describe the back of the eye and capturing photographic images of the fundus has been of interest to researchers and clinicians for hundreds of years. The first iteration of the fundus camera, which uses reflected light to capture a two-dimensional image of the fundus tissues was developed in 1910 by Gullstrand (Abràmoff and Kay, 2013).These digital images usually include the optic disc, the macula as well as the main retinal vessels which enter from the optic disc and they offer the ability for immediate evaluation and sufficient detail to diagnose and stage diabetic retinopathy which is why it is regularly used in research studies evaluating diabetic retinopathy (Patton and Constable, 2006; Baumal and Duker, 2018). Fundus imaging has the capability of obtaining a resolution of 7 to 20 µm, providing exquisite detail of the important features (MacGillivray *et al.*, 2014).

Other methods for imaging the vascular structures of the retina include optical coherence tomography (OCT), which is a newer technology that can provide non-invasive images of the retinal structures with a resolution up to 1 µm offering a vast amount of detail of the microstructures of the retina and is the standard for diagnosis and monitoring of diabetic macular oedema (Baumal and Duker, 2018). OCT angiography (OCT-A) and fluorescein angiography (FA) are two imaging techniques that offer evaluation of blood flow in the retinal vessels. Fluorescein angiography is an older method that

involves injecting fluorescent dye and imaging the retina guided by the dye and OCT-A involves repeated, rapid imaging that can be knitted together to show dynamic flow and is arguably superior to the older FA method (Spaide, Klancnik and Cooney, 2015).

Retinal vasculature formation and maturation, including patterning, is guided by VEGF, various axon guidance molecules, as well as the transforming growth factor beta (TGFβ), and blood flow (Fruttiger, 2007). The pattern of the retinal vasculature seeks to optimise the efficiency of blood flow and any changes in the efficiency can lead to alterations in the geometric characteristics and these deviations may lead to impaired microcirculatory transport (MacGillivray *et al.*, 2014). In diabetes, the vascular alterations and damage caused by the disease increase sheer stress of the vasculature and decrease efficiency of blood flow (Tooke, 1995). Understanding the differences and changes within the retina and their relationship to disease manifestation and progression as a possible mechanism to stratify risk, is key in using the retina as a source of biomarkers for local and systemic vascular disease.

The retina offers a unique platform for research on the vascular system as it is one of the only locations in the body to easily visualise the vascular system non-invasively. Within the past decade, retinal-imaging and analysis technology has improved. A limiting factor of digital retinal images used to be a lack of reliable software to conduct efficient analysis; generally, analysis would have to be carried out on a manual basis, reducing the number of images that could be included within a study to a manageable level of only several hundred. This subsequently led to the development of several software packages that are currently being used by research groups around the world. As the analysis of retinal images is becoming ever more automated, the potential for quick and easy, non-invasive, testing for pre-symptomatic stages of cardiovascular and microvascular disease is rapidly becoming a focus for researchers and clinicians. In diabetes, the retina, as a very early marker for vascular disease is especially important as it is altered

very early on in the disease, due to hyperglycaemia, inflammation and endothelial dysfunction (Carol Yimlui Cheung *et al.*, 2015).

Retinal traits that can be analysed using this software commonly include vessel widths, bifurcation angles and coefficients, fractal dimensions and vessel tortuosity, although numerous novel measurements are sometimes reported. Vessel widths, as measured by a summary index reflecting true vessel widths, can be broken down into three different measurements: central retinal artery equivalent (CRAE), central retinal vein equivalent (CRVE) and the ratio of the two, arteriovenous ratio (ARV). Vessel widths are currently the most researched retinal trait. Vessel tortuosity describes the degree of curvature of a vessel, and bifurcation angles describes the angles of the branching vessels. Fractal dimension (FD) describes how a pattern fills a two-dimensional space, and in the application of retinal imaging, measures the branching complexity of the retinal vasculature (Perez-Rovira *et al.*, 2011).

1.4.3 VAMPIRE Software

Vascular Assessment and Measurement Platform for Images of the Retina (VAMPIRE) software offers researchers the ability to quantitatively analyse retinal images on a semi-automatic basis (Perez-Rovira *et al.*, 2011). This state-of-the-art software, developed in an international collaboration based at the Universities of Edinburgh and Dundee, has a wide range of applications, and is expected to be a useful tool in identifying biomarkers for the diagnosis and progression of chronic disease (MacGillivray et al. 2014; Patton & Constable 2006). In 2018, the online VAMPIRE version was launched and includes an improved user-interface compared with the previous desktop versions.

For vessel detection, the software utilises a two-dimensional Gabor wavelet algorithm as a pre-processing step to segment the retinal vessels within fundus camera images (Soares *et al.*, 2006; Perez-Rovira *et al.*, 2011). The basis of VAMPIRE image processing is to identify which pixels in the image are vessels and which are not. This relies on the retinal vessels being linear

in shape, they generally take on a Gaussian profile to their cross-sectional intensity distribution, and all originate from a single focal region, the optic disc (Patton and Constable, 2006). All algorithms in VAMPIRE were implemented in MATLAB (r2017a, Mathworks, USA) .

When using VAMPIRE, the user uploads an image, upon which the software detects the boundary of the optic disc and the position of the fovea. The user can adjust these features as necessary. The software then generates a map of the vasculature and attempts to classify vessels as arterioles or venules, which can be edited by the user if necessary. A final analysis step is undertaken to generate the various quantitative traits. After a user is trained, VAMPIRE software is easy to use in order to generate a vast amount of retinal feature data that are reproducible and easily accessible.

There are other software platforms that offer similar capabilities such as the Singapore “I” Vessel Assessment (SIVA) software, which was developed in collaboration with National University of Singapore and Singapore Eye Research Institute (Singapore Eye Research Institute, 2011), open source Interactive Vessel ANalaysis (IVAN) software produced by the University of Wisconsin, and Quantitative Analysis of Retinal Vessel Topology and siZe (QUARTZ) (Fraz *et al.*, 2015), to name a few. While there are many similarities between the software types, there are also differences in algorithm implementation, automaticity and interface options.

1.4.4 Retinal vessel trait associations

Early changes in the microvasculature of the retina are theorised to be a response to, and product of, the accumulation of damage caused by vascular risk factors, such as hypertension (MacGillivray *et al.*, 2014). It is believed that insult caused by high blood pressure, inflammation and other mechanisms, would affect changes in the smallest and most delicate vessels of the microvasculature and local alterations reflect widespread microvascular disease. These changes include widening or narrowing of the vessels, changes in the twisted nature, reflected as tortuosity and the overall complexity of the vascular network, or fractal dimension, to name a few.

In recent years, there have been many studies that have investigated these retinal trait changes and their association with chronic disease, especially diseases and outcomes involving the macro- and microvasculature. A study which utilised VAMPIRE software investigated fractal measures of retinal vasculature and their association with cognitive ageing (Taylor *et al.*, 2015). This study from the Lothian Birth Cohort evaluated a single retinal image in later life compared with intelligence quotient (IQ) measured at a young age and found no association between fractal measures of the retina and cognitive ageing. Researchers have investigated retinal microvascular changes as an indicator of diabetic nephropathy, as well as stroke risk, although not all studies found evidence of an association (Ding *et al.*, 2012; Kawasaki *et al.*, 2012; McKay *et al.*, 2018). A population based cohort study from China found an association between retinal vascular widths and markers of chronic kidney disease, especially in people with diabetes (Bao *et al.*, 2015).

In the review produced by Sun et al. (2009), the authors discuss retinal vascular structure and the links that have been researched with risk factors such as cardiovascular health, stroke, blood pressure and diabetes, as well as many others. There is substantial evidence suggesting that smaller arteriolar and venular width is associated with higher current blood pressure and older age, while larger vascular width is associated with diabetes and/or higher blood glucose and cigarette smoking. Obesity is associated with smaller arterioles and larger venules. Mechanistic theories for pathophysiological changes in retinal vasculature include sustained hypertension, ageing, atherosclerosis, inflammation and endothelial dysfunction, as well as blood flow parameters, including oxygenation and shear stress (Sun *et al.*, 2009). Specifically in people with diabetes, the hypothesis is that arteriolar and venular dilation may reflect hyperperfusion resulting from hyperglycaemia and lactic acidosis from retinal hypoxia (Grunwald, DuPont and Riva, 1996; Sun *et al.*, 2009)**.**

Retinal vascular measurements and diabetic retinopathy

Diabetic Retinopathy is one of the earliest complications of diabetes which is reflected in the high prevalence and can lead to visual impairment and vision loss, but with early detection, the disease can be treated and slowed reducing the burden on the patients and health care system. Clinical evidence of retinopathy may actually be a late indicator of microvascular damage to the retina and the blood-retinal barrier and being able to stratify people by their risk of developing retinopathy before these overt pathological pathways have begun is important (Carol Yimlui Cheung *et al.*, 2015). Utilising regularly obtained retinal images together with automated or semi-automated analysis software is of great interest in the medical field to reduce burden of disease.

Recent studies have provided some evidence for the usefulness of new software in helping to predict and detect diabetic retinopathy before serious pathology occurs, although substantial heterogeneity in such studies makes firm interpretations difficult**.** There has also been evidence of retinal vascular traits providing information on other forms of retinopathy, for example, a study evaluating retinopathy of prematurity found increased tortuosity in eyes effected by the disease when compared with healthy eyes (Wilson *et al.*, 2012). This association in retinopathy of prematurity may provide an analogy between retinal tortuosity and the development of diabetic retinopathy. **Chapter 2 (page 37)** of this thesis is a systematic literature review that explores the current evidence around the association between retinal vessel traits and diabetic retinopathy.

Retinal vascular measurements and cognition

As the retina functions to process light and colour for sight and relay these messages to the brain the retina is considered an extension of the brain and nervous system. During early embryological development, the retina is derived from the ectoderm, which is derived from the neural tube, also known as the neuroectoderm (Snell and Lemp, 2013). The ectoderm also gives rise to the optic nerve and other structures that connect the eye to the brain. It is

hypothesised that these structural and developmental connections persist and are reflected in co-development of disease. In a population-based study in Iceland, researchers found evidence of the co-existence of microvascular lesions in both the retina and brain, especially in those with diabetes (Qiu et al. 2008). These findings indicated an interrelatedness between the pathology of the retina and brain, although it should be noted this analysis was not conducted prospectively, so any temporal relationship cannot be determined.

Utilising the retinal vasculature as an indicator of the role of small vessel health in the brain in relation to cognitive state and decline is gaining a great deal of research interest (Patton *et al.*, 2005; Umemura, Kawamura and Hotta, 2017; McGrory, Ballerini, Doubal, *et al.*, 2019). It has the potential to illuminate subclinical small vessel changes before overt pathology and allow for early detection and treatment of cognitive impairment and dementia, which are experiencing rapidly increasing incidence with aging populations around the world. The large Atherosclerosis Risk in Communities (ARIC) study found, in a cross-sectional analysis, that retinal microvascular abnormalities, which included focal narrowing, haemorrhages and microaneurysms were associated with subclinical cerebral infarcts, independently of stroke risk factors (Cooper *et al.*, 2006). **Chapter 3 (page 79)** of this thesis is a systematic literature review exploring the relationship between retinal vessel traits and cognitive decline. All current research pertaining to that association was compiled and evaluated in order to provide context for the findings of this thesis.

There have been other systematic reviews similar to that conducted in **Chapter 3**, including a recent one on the use of fundus photography to research retinal changes in dementia, which found some evidence for the use of measures including vessel widths and fractal dimension (McGrory et al. 2017). While offering hope for the use of retinal biomarkers in diagnosis and prognosis of patients, the review called for further prospective studies that utilise standardised protocols for retinal imaging and analysis, as well as combination with other clinical biomarkers such as cerebrospinal fluid

sampling and neuroimaging and paying attention to differences in dementia type.

Another systematic review also found evidence that retinal vascular abnormalities may be associated with markers of cognitive decline and dementia, although the associations were tenuous, and more robust in cross-sectional studies compared with longitudinal studies (Heringa et al. 2013). In this review, the authors evaluated any dementia, and not by specific type, specifically Alzheimer's, vascular dementia or mixed, so we cannot determine distinct differences by aetiology. These systematic reviews reflect the wide-ranging findings of recent studies that have evaluated retinal vascular markers in relation to cognition and that further research is required to determine true associations and what subgroups of patients on the spectrum of cognitive decline would benefit from novel biomarkers derived from retinal imaging. The review conducted in this thesis was compared with these other recent reviews for methodological similarities and differences, as well as results.

1.4.5 Limitations in measuring retinal vessel traits

Although moving from manual measurement methods to semi-automated software has enabled more objective measurements of retinal vascular parameters to be obtained, there are limitations. One such issue is standardising optic disc size to counteract camera magnification and obtain reliable measurements across image sets. It is now widely accepted that the vertical optic disc varies between people but is on average around 1,800 microns in diameter so this is the size generally utilised for standardisation, but this is still debated (Quigley *et al.*, 1990; McGrory *et al.*, 2018).

Racial differences have also been shown to have an effect on retinal vascular parameters, making relationships with risk factors more difficult to parse (Li *et al.*, 2013). One factor is iris pigment colour variability, as a surrogate for retinal pigmentation. This can be linked with ethnicity and might contribute to errors in measurements or could account for some genetic differences between study populations due to differences in contrast and pixel density of

retinal images that were both stronger in Caucasian, blue-eyed children, compared with brown-eyed East Asian children (Rochtchina *et al.*, 2008). Further research is needed to fully understand how these factors affect the ability to measure and track changes in the retinal vasculature and compare people with different ethnic and genetic backgrounds.

Poor retinal image quality, due to either technical or anatomical issues, can hamper a study causing some participant data to be unusable if the software cannot accurately identify key features such as the vessels or optic disc boundary. Other problems might be inappropriate comparison of measurements from different software or measures produced by different graders (McGrory *et al.*, 2018).

Retinal vascular widths are the most commonly evaluated parameter, but these measures suffer from certain issues. The most common descriptors for vessel widths are the summary indices CRAE and CRVE, which reflect outer vessel width. These measures are not able to capture the internal luminal widths, which may show greater changes from vascular damage (Carol Yimlui Cheung *et al.*, 2015). Also, there is a confounding effect of associated venular and arteriolar sizes, but simply controlling for the other, which is the standard statistical approach, can create concerns of multicollinearity. Also, the utilisation of AVR, which is calculated as a ratio of CRAE and CRVE, may mask important differences in the two vascular measures.

Another concern is the symmetry of retinal vascular changes between the right and left eye, or rather the asymmetry. While there are many symmetrical properties observed in the retinal vasculature there is evidence of pathology affecting the retina asymmetrically, which complicates the theory that the changes in the retinal vasculature is a representation of the wider micro- and macrovascular system of the body. Choosing to analyse a single eye or both eyes and average the results requires consideration so as not to mask a true association or to select a significant finding that is only present in a single eye (Cameron *et al.*, 2017).

1.5 Chapter Summary

People with diabetes are at an increased risk of developing vascular related complications such as diabetic retinopathy and increased cognitive decline. There is currently very limited ability to accurately predict those that will go on to develop these quality of life-limiting conditions. The retina, being one of the few places in the body where the vasculature can be visualised non-invasively, offers a unique place to study local and systemic vascular disease, and possibly add novel predictive capability to already established vascular risk factors. Using recently developed semi-automated software, the retina can be intricately studied as a high-impact vascular disease prediction tool.

2 Association between retinal vessel traits and retinopathy: a systematic literature review

2.1 Introduction

Diabetic Retinopathy has a high prevalence amongst people with diabetes and can lead to visual impairment and vision loss, but with early detection the disease can be treated and progression slowed, reducing the burden on the patient's quality of life as well as the health care system (Fendrick, Javitt and Chiang, 1992; Mazhar *et al.*, 2011). Utilising regularly obtained retinal images together with automated or semi-automated analysis software to measure retinal traits for the prediction of diabetic retinopathy is of great interest in the medical field to reduce burden of disease.

Recent studies indicate that there is evidence of the usefulness of new software in determining an association between retinal vessel traits and retinopathy, possibly before serious pathology (S.L. Rogers *et al.*, 2008; Roy, Klein and Janal, 2011; Klein *et al.*, 2018). The purpose of this systematic literature review was to provide comprehensive, evidence-based, information on the current epidemiological research that has been conducted to better understand the association between retinal vessel traits and diabetic retinopathy. I aimed to include all relevant studies and have presented their findings, as well as the details of how the studies were conducted, including population characteristics, measures of the exposure and outcomes, and discussed their methodological quality. This review serves to support the study design and considerations for this thesis, as well as provide a platform of analysis and comparison for the findings.

2.2 Objectives

The objective of this systematic review was to identify and collate all studies that have evaluated the association between retinal vessel traits (RVTs) and diabetic retinopathy (DR) in people with diabetes, using fundus images and a digital platform for semi-automatic or automatic analysis.

2.3 Methods

2.3.1 Types of studies

I included non-randomised observational epidemiological design studies (i.e. cross-sectional, case-control, cohort). Experimental studies, such as randomised controlled trials, were considered if the population fit the inclusion criteria and the intervention did not confound the results and make the inclusion inappropriate. An example of this could be a randomised controlled trial evaluating an intervention used for something other than treating diabetic retinopathy that may also include retinal vessel trait measurements, for the entire study or a subset, and diabetic retinopathy as an outcome.

2.3.2 Types of participants

People with diabetes mellitus (type 1 or type 2) or general population studies with a subset of people with diabetes that stratified outcomes by diabetes status that could be analysed separate from those without diabetes were included.

2.3.3 Types of retinal vessel traits

I included studies that evaluated any RVT identified using fundus imaging and assisted by automated or semi-automated computer software designed for measuring retinal features. Studies were excluded if they used manual methods of measuring RVTs, even if digitally assisted. I also excluded studies if they were using the traits to diagnose existing diabetic retinopathy.

2.3.4 Types of outcome measures

Outcomes of interest were limited to any measure of DR. I included studies if they considered DR as a dichotomous outcome or if they classified DR according to severity measure, such as mild, moderate and proliferative.

2.3.5 Search methods for identification of studies

Using the OVID® platform, I searched the MEDLINE and EMBASE databases for all relevant studies, using a sensitive search strategy. The full

search strategy can be found in **Appendix 1**. I also undertook hand-searching of the references of relevant studies and reviews. The electronic search was limited to English language studies conducted in humans. The search was undertaken on February 14th, 2019 and included the time period from 1997 to the search date.

2.3.6 Data collection

Any studies were considered for inclusion if they met the inclusion criteria for study type, participant type as well as outcome and vessel analysis. Studies were excluded if they included less than 100 participants, due to low power to detect an association. Also, studies that only had data published in abstracts or conference proceedings, and not a fully peer-reviewed published reference, were excluded due to a paucity of information being provided in such abbreviated texts. Studies were excluded if their methodology was wholly different from the other studies, so much so that comparison would be inappropriate.

A data extraction form was prepared to identify key information and findings from the included studies. Data extracted included the type of study, study aim, location, follow-up time frame, number of participants included, type and duration of diabetes, age and gender of participants, RVTs and how they were analysed, DR grading scheme used, outcomes, statistical models used and covariates controlled for. I collected the outcome data for any analyses evaluating the association between RVTs and DR. If a study reported data on other outcomes, such as other retinal pathology, this type of data was not collected as it was out with the scope of this review.

2.3.7 Assessment of study quality

Using a modified version of the Critical Appraisal Skills Programme (CASP) appraisal tool I evaluated the quality of each study (CASP UK, 2018). The CASP tool includes separate but similar tools for case-control studies and cohort studies, making easy comparison of quality between the study types. CASP is designed as a check-list style resource to help organise and evaluate the quality of studies using an evidence-based approach. I

evaluated and reported on eight domains of quality for each study: (1) Did the study address a clearly focused issue? (2) Was the study recruited in an acceptable way; in case-control studies, were cases and controls recruited appropriately? (3) Was the exposure and outcome accurately measured to minimise bias? (4) Have the authors identified all important confounding factors and included them in the analysis? (5) Was the follow up of the subjects complete enough and long enough? (6) How precise are the results and do you believe the results? (7) Do the results fit with other available evidence? (8) Are sources of funding and/or conflicts of interest reported? See **Appendix 2** for further details on the criteria I was looking for to fulfil each domain.

To present the findings of the quality assessment I generated tables with a row for each study and a column for each domain. I either reported the methods of the study or in some cases stated 'Yes' if the criteria were met or the specific reasons the requirements were not fully met. I added a colour to each cell to help give a quick visual assessment: green indicates that all criteria were met; yellow indicates that there are some concerns with the study in relation to that domain or that not all the criteria were met; red indicates serious concerns with the domain.

2.3.8 Measures of effect

I planned to undertake meta-analysis using fixed-effects methods if the data extracted from the included studies was deemed to be satisfactorily homogenous in terms of exposures, outcomes and modelling methods. However, after inclusion and extraction, it was clear that the studies and their data varied too greatly for meta-analysis. I therefore undertook thorough descriptions of the included studies' methods and results, using tables and figures to present the results of the individual studies.

2.4 **Results**

2.4.1 Results of the search

A total of 8087 references were identified in the EMBASE and MEDLINE database search and after de-duplication 6131 references remained. An

additional 43 duplicates were identified during the title and abstract review. After titles and abstracts were reviewed, full articles were retrieved for 76 references. After full references were reviewed, a total of 36 references from 14 studies were included in this systematic literature review. See **Appendix 3** for the full study selection PRISMA flow diagram.

Twelve references were excluded because of low sample size (<100 participants) (Kristinsson, Gottfredsdottir and Stefansson, 1997; Kunicki *et al.*, 2009; Bucca *et al.*, 2012; Crosby-Nwaobi and Heng, 2012; Talu, 2013; Habib *et al.*, 2014, 2016; Muraoka *et al.*, 2014; Leontidis *et al.*, 2015; Leontidis, Al-Diri and Wigdahl, 2015; Popovic *et al.*, 2018; Kostic *et al.*, 2018). Three references were excluded because the relevant analyses were only presented in conference abstracts (Sandoval *et al.*, 2016; Forster *et al.*, 2018; Li *et al.*, 2018). The study by Lim et al. was excluded due to serious inconsistencies between the methods and results as well as statistical modelling concerns (Lim *et al.*, 2017). A further study was excluded due to methods of analysis being inconsistent with the remainder of the included studies, and the aim of the study was to compare different methods of fractal dimension analysis for optimal predictive ability for proliferative diabetic retinopathy (PDR) (Orlando *et al.*, 2017).

2.4.2 Included Studies

See **Table 1**, **Table 2 and Table 3 (on pages 51, 52 and 53, respectively)** for general characteristics of included studies by study type. For this chapter, as well as **Chapters 3, 5-7**, all tables and figures are at the end of their respective chapter. This was done for clarity as many of the tables and figures are long and impede the ability to read the text and it was difficult to place them near the relevant text.

Fourteen studies were included in this review. They include, in alphabetical order, the **Australian Diabetes, Obesity and Lifestyle Study (AusDiab)** (Tikellis *et al.*, 2007), the **Blue Mountains Eye Study** (Kifley *et al.*, 2007), **Danish Cohort of Pediatric Diabetes 1987 (DCPD1987)** (Broe et al. 2014), **Desheng Diabetic Eye Study** (Yang *et al.*, 2016), **Darwin Region Urban**

Indigenous Diabetes Study (DRUID) (Dirani *et al.*, 2010), **Grauslund 2010** (Grauslund *et al.*, 2010), **Inter99 Eye Study** (Drobnjak *et al.*, 2017), **New Jersey 725 Study** (Roy, Klein and Janal, 2011), **Multi-Ethnic Study of Atherosclerosis (MESA)** (Nguyen *et al.*, 2008), **Sasongko 2011** (Sasongko *et al.*, 2011), **Singapore Indian Eye Study (SINDI)** (Tsai *et al.*, 2011), **Singapore Malay Eye Study (SiMES)** (Cheung *et al.*, 2012), **Sydney Paediatric Diabetes Study** (Alibrahim *et al.*, 2006) and the **Wisconsin Epidemiologic Study of Diabetic Retinopathy (WESDR)** (Klein *et al.*, 2012). Many of the included studies include multiple references so I have included the main reference in text and in the references section for this literature review I included the other references under the study heading. Supporting references and excluded studies for this chapter that are not specifically an included study are in the general reference section of the thesis.

Eight of the included studies performed cross-sectional analyses of the data (Blue Mountains Eye Study, Desheng Diabetic Eye Study, DRUID, Grauslund 2010, Inter99 Eye Study, MESA, Sasongko 2011 and SINDI). Four studies reported both cross-sectional and longitudinal data (AusDiab, DCPD1987, SiMES and Sydney Paediatric Diabetes Eye Study). Two studies included only longitudinal data (New Jersey 725 and WESDR).

The number of participants ranged from 110 up to 2366. In the longitudinal studies the follow-up times ranged from 2.5 years to 16 years. Three studies were undertaken in participants with only type 2 diabetes, four studies in participants with type 1 diabetes and seven studies that included mixed aetiology, although most of the mixed studies had a far larger proportion of those with type 2 diabetes. Five studies were conducted in Australia, three in Denmark, three in the United States, two in Singapore and one in China.

Twelve of the included studies reported on vessel widths, five reported on tortuosity and five reported on fractal dimension (FD). Other vessel measurements that were reported included length-distance ratio (LDR), branching angles, branching coefficients between parent and daughter

vessels, amongst several other branching geometry measures. For this review I chose to only report data on widths (central retinal arteriolar equivalent (CRAE) and central retinal venular equivalent (CRVE), tortuosity and FD, as these are the traits described in the primary analysis of this thesis. For the diagnosis and classification of DR, thirteen studies reported using the Early Treatment of Diabetic Retinopathy Study (ETDRS) classification system or another modified Airlie House Classification of DR system. One study reported DR using a simplified version of the Wisconsin grading system (AusDiab). See **Table 4 (on page 54)** for information on DR and RVTs for the individual studies. **Appendix 4** contains further information on the retinal images used in each study.

2.4.3 Findings of included studies

Due to the heterogeneity of retinal vessel analysis, outcome reporting, statistical modelling and other factors, meta-analysis was not considered an appropriate method for comparing the included studies. I therefore have undertaken a descriptive approach of the findings of the individual studies using visual association figures that indicate if the study found evidence of an association using their specific statistical modelling methods. There was no pooling of raw data to create the figures using methods such as meta-analysis. For studies that did not find a statistically significant association between the RVT and DR, an arrow was used to indicate the direction of the point estimate, see **Figure 4 (page 62)** as an example. In meta-analysis, the logic is to combine results of several, usually small, studies, which themselves may have not had power to reach statistical significance, but when combined, the evidence may be statistically significant, indicating a possible true effect. Therefore, using arrows to indicate direction of a non-significant association allows readers to see any trends that are important, and possibly would have shown statistical significance if meta-analysis was employed. For the figures I reported on the outcome ‘any DR’, where available, if not available I included the study reported comparison such as PDR or varying severity levels of DR and specified this within the plot.

Appendix 5 contains further information on the statistical models used and covariates controlled for. **Table 5 (page 56)** contains the extracted results from the individual studies and is located at the end of this chapter. Some of the included studies had multiple reports of the same RVTs in different references with different results, with it not always being clear why there were differences. I chose to include results from the reports with the greatest number of participants and/or the longest follow-up, where possible.

Central Retinal Arterial Equivalent (CRAE)

Twelve studies reported on CRAE, but two studies reported both cross-sectional and longitudinal data, so these two studies are included twice on the association plot. Of the eight studies reporting cross-sectional data, only one found a statistically significant association between wider CRAE and any DR. For the seven studies that did not find a statistically significant association, there was a mixture of point estimates favouring both increased and decreased CRAE. In the six studies that reported longitudinal data, four studies found an association between wider CRAE and DR, three of which were reporting any DR and one on PDR. The other two studies with no statistically significant association both favoured wider CRAE, with one study reporting on progression to PDR and the other on any DR. See **Figure 4 (page 62)**.

It should be noted that for several of the studies that did not show a statistically significant association within their population, MESA, SINDI and Desheng, there was a pattern of wider CRAE for minimal and/or mild DR, but then narrower CRAE for moderate or severe DR.

Central Retinal Venular Equivalent (CRVE)

As with CRAE, 12 studies reported on CRVE, and two reported both cross-sectional and longitudinal data so they appear twice on the plot. For the cross-sectional data, seven of the eight studies reported a positive association between wider CRVE and DR and the one study with a non-significant association also favoured wider CRVE. Four of those studies

reported on any DR, and three reported varying levels of DR severity compared with no DR.

For the longitudinal data, four of the six studies reported evidence that wider CRVE is associated with incident DR or progression of DR. Two of these studies reported on any DR compared with no DR, and the other two reported specifically on PDR. For the remaining two studies, one reported an association between narrower CRVE, and DR and one study reported no association, but the point estimate also favoured narrower CRVE(**Figure 5, page 63)**.

Arterial tortuosity

Five studies reported on arterial tortuosity, four of which reported cross-sectional data, of which one also reported longitudinal data, and one that only reported longitudinal data. Of the cross-sectional reports, two of the four found evidence of a positive association between increased arterial tortuosity and DR. Of the two showing an association, one compared mild or moderate DR to no DR and one compared any DR to no DR. For both longitudinal reports, there was evidence of an association between increased arterial tortuosity and any DR. The two studies that did not find a statistically significant association reported point estimates also favouring increased arterial tortuosity and any DR. See **Figure 6 (page 63).**

Venular tortuosity

The same five studies that reported on arterial tortuosity also reported on venular tortuosity, with four studies reporting cross-sectional data, and two reporting longitudinal data. Only a single study, reporting longitudinal data, demonstrated a statistically significant association between increased venular tortuosity and any DR, but the remaining four studies, although not statistically significant, also had point estimates supporting this association. See **Figure 7 (page 64)**.

Fractal Dimension (FD)

Fractal dimension was reported as an outcome in five studies, two of which provided both cross-sectional and longitudinal data, one that reported cross-sectional data only and two that reported longitudinal data only. Of the three studies reporting cross-sectional data one showed an association between decreased FD and PDR, one showed no association, but had a point estimate favouring increased FD and any DR, and one demonstrated a positive association between increased FD and any DR. For the longitudinal data, three reported no association, two of which had point estimates favouring decreased FD, the third did not provide the numeric data. One longitudinal report showed evidence of decreased FD being associated with PDR. See **Figure 8 (page 64)**.

2.4.4 Quality assessment of included studies

See **Table 6 (page 65)** at the end of this chapter for full details on quality assessment for the individual studies. The first, seventh and eighth domains have been omitted from the table as all studies met the requirements and there was limited space for the table.

Did the study address a clearly focused issue?

All the included studies set out to address clearly focused research questions, with no major concerns.

Was the study recruited in an acceptable way?

Seven of the fourteen studies were deemed to have recruited their study populations in an acceptable way to reduce bias (Blue Mountains Eye Study, Grauslund 2010, Desheng Diabetic Eye study, DCPD1987, SiMES, AusDiab and New Jersey 725 Study).

The following six studies utilised mostly sufficient methods to reduce bias, but there were some concerns regarding recruitment. The DRUID study experienced difficulties in recruiting participants due to practical and sensitivity issues in recruiting indigenous people in Australia. They used several methods to try to identify and recruit participants, but they still

encountered issues in recruiting sufficient participants. The Inter99 study is a large Danish population-based study and for the smaller eye study, a portion of the larger cohort were invited. Standard World Health Organisation (WHO) definitions of diabetes were utilised to select participants, but the investigators also allowed for self-reported cases of diabetes, which could lead to bias. The Sasongko 2011 study recruited consecutively from two separate eye clinics, but they provided no information on the non-attenders or how generalizable their study population is. The MESA study was not fully powered to evaluate people with diabetes which reduces their applicability to this analysis. The SINDI study used robust methods of identifying participants that included age-stratified, random sampling from computer-generated lists provided by the Singapore Ministry of Home Affairs, but there was evidence that those excluded were at a higher risk of poor health as they were older and had higher blood pressure and HbA1c. However, those excluded from the SINDI study were also more likely to be female and non-smokers. The WESDR study is at possible risk of misclassification bias of their participants with diabetes as they used the age of onset to categorise type 1 and type 2 diabetes, which can be unreliable.

I noted strong concerns regarding the recruitment of the Sydney Paediatric Diabetes Study. I identified multiple references that fit the inclusion criteria for the review that all used this study population for different analyses but there is no continuity between the studies regarding how they chose their study populations from the larger cohort. It is concerning that there is no mention that the individual studies are using the same population as another similar analysis. Also, there is no information provided in any of the studies regarding the non-participating invitees. I have chosen to include the references as one study, so I do not include the same participants multiple times in the review.

Was the exposure and outcome accurately measured to minimise bias?
Aside from the Desheng Eye Study, which met all the criteria, each of the included studies were missing some level of description of how the exposure

and outcomes were measured that would minimise bias. For most studies, they did not describe who, and if they were trained or blinded, was measuring the retinal vessels or grading for DR. Also, several studies did not provide information on if intra/intergrader reliability measures were undertaken. In one case, the name of the software used for analysis was not provided.

Have the authors identified all important confounding factors and included them in the analysis?

All of the included studies controlled for certain participant characteristics in their analysis. Five of the included studies controlled for all the characteristics identified as being important. The remaining 12 studies were missing one or more characteristics in their analyses, most notably, several studies that evaluated vessel widths did not control for venular width when analysing arterial width, or vice versa. This is important as the two are highly correlated, but also controversial due to concerns with multicollinearity. Also, several studies did not control for duration of diabetes, which is an important factor for development of diabetic retinopathy.

Was the follow-up of subjects complete enough and long enough?

For seven of the studies, this field was not relevant as they only conducted cross-sectional analyses. For the remaining six studies that did provide follow-up data, four were deemed to have sufficient numbers of participants and follow-up was appropriately long (DCPD1987, SiMES New Jersey 725, WESDR). For the AusDiab study, the follow-up was only five years, which may be too short for the outcome and also there was no clear description of attrition. There were major concerns with the follow-up data presented for the Sydney Paediatric Diabetes Study as follow-up was only on average 2.9 years, which is considered too short for diabetic retinopathy.

How precise are the results? Do you believe the results?

Most of the included studies were not well powered towards participants with diabetes as they generally made up a smaller subset of a larger cohort. Also, quite a few studies did not include confidence intervals or other measures that help to understand the magnitude of the effect, making it difficult to

interpret the results. However, even with underpowered studies, several were still able to detect statistically significantly differences. The Sydney Paediatric Diabetes Study used the same base population for several different analyses, and at times with different conclusions. Because limited information was provided on how these analyses, and the population they were based on, differed or were the same, there is doubt from opposing findings from the same population.

Do the results fit with other available evidence?
Overall, the findings from each study were determined to fit within the wider literature. Currently there is a paucity of evidence regarding the association between retinal vessel traits and diabetic retinopathy.

Are sources of funding and/or conflicts of interest reported?
All included studies provided sources of funding and financial support.

2.5 Discussion

2.5.1 Summary of the main results

Fourteen studies were included in this review. Due to major differences in study design, analysis methods and reporting of results, meta-analysis was not possible as a method for combining data from the individual studies. Several of the longitudinal analyses suggest there may be an association between wider CRAE and risk of diabetic retinopathy, but this association is not as evident in cross-sectional studies. Many of the included studies that evaluated CRVE demonstrated an association between wider CRVE and risk of diabetic retinopathy, in both cross-section and longitudinal studies. Only five studies evaluated tortuosity, and there is some evidence that increased arterial tortuosity may be associated with risk of diabetic retinopathy. Although the majority of the studies that evaluated venular tortuosity did not find any statistically significant associations, all had point estimates that favoured increased venular tortuosity and an association with retinopathy, which, if meta-analysis was performed, may have provided evidence of an association. The results of the five studies that looked at the relationship

between fractal dimension and risk of diabetic retinopathy were mixed, but with a possibility that lower fractal dimension is associated with retinopathy. Further discussion of these findings, put in the context of this thesis, will be continued in the general discussion chapter.

2.5.2 Quality and completeness of data

Overall, the quality of the included studies was moderate, with only minor concerns that were often unavoidable, or due to insufficient description within the report. There were some concerns with how participants were selected from large study populations, and measurement of the RVTs and DR was not always made clear. Most of the included studies had low numbers of people with diabetes, which reduces the power, making findings difficult to interpret. I only had major concerns with the findings from one study, the Sydney Paediatric Diabetes Study, as the same population was used in multiple analyses, but there was no explanation of how the individual analyses chose the participants they used, and the inclusion numbers, and at times the conclusions varied between each report. This same study also had a limited follow-up.

2.5.3 Conclusions

There is evidence to suggest that certain retinal vessel traits are associated with diabetic retinopathy. These associations need to be further explored in large, longitudinal cohorts that have sufficient numbers of people with diabetes to conduct well-powered analyses. Determining if there is a prospective relationship between vessel traits and retinopathy, above and beyond current known risk factors, will help to establish if retinal vessel traits could be used as an early detection method for diabetic retinopathy.

Table 1. Cross-sectional studies general characteristics

Study name	N	Diabetes Type	Mean age, years (SD)	Sex, Male %	Duration of diabetes, years (SD)	DR prevalence/incidence (%)
DRUID	n = 110	Unclear/mixed	50.8 (11.1)	77.20%	Not reported	n = 30 (27.3%) prevalent DR
Blue Mountains Eye Study	n = 255 with diabetes	Type 2	Not reported, ≥49 years	Not reported	Not reported	n = 74 (29%) prevalent DR
Inter99 Eye Study	n = 199 with diabetes	Type 2 (1.5% had type 1)	51.3 (6.7) DR; 51 (7.2) no DR	72% DR; 56% no DR	Not reported	n = 42 (21%) prevalent DR
Grauslund 2010	n = 208	Type 1	57.8 (12.6)	62.40%	42.0 (10.0)	n = 79 (38.0%) prevalent PDR
Sasongko 2011	n = 244 with diabetes	Mixed	59.0 (15.0)	40.90%	15.0 (12.0)	n = 114 (44%) prevalent DR
MESA	n = 892	Mixed	65.2 (9.2)	52.10%	5.3 (7.7)	n = 165 (18.5%) prevalent DR
SINDI	n = 980	Mixed	61.2 (9.8) DR; 60.1 (10.0) no DR	55.4% DR; 51.5% no DR	Not reported	n = 327 (33.4%) prevalent DR
Desheng Diabetic Eye Study	n = 1340	Type 2	64.0 (8.2)	39.8%	11.7 (7.5) any retinopathy; 7.4 (5.8) no retinopathy	n = 472 (35.2%) prevalent DR

DR = diabetic retinopathy; SD = standard deviation

Table 2. Studies providing cross-sectional and longitudinal data general characteristics

Study name	N	Diabetes Type	Mean age, years (SD)	Sex, Male %	Duration of diabetes, years (SD)	DR prevalence/incidence (%)	Follow-up years (SD)
DCPD1987 *	Cross-sectional n = 181; Longitudinal n = 185	Type 1	21.0 (3.3)	49.70%	13.5 (3.3)	Cross-sectional n = 48 (26.5%) PDR; Longitudinal n = 50 (27.0%) incident PDR	16 years
SiMES	Cross-sectional n = 594; Longitudinal n = 434	Mixed, "mostly type 2 diabetes"	61.5 (9.3)	42.90%	8.5 (8.4)	Longitudinal: n = 59 (13.6%) incident DR	6 years
AusDiab	Cross-sectional n = 657; Longitudinal n = 250	Mixed	60.9 (13.0) new diabetes; 62.8 (11.6) known diabetes	48% new diabetes; 55% known diabetes	Not reported	Cross-sectional: n = 83 (12.6%) prevalent DR; Longitudinal: n = 20 (8.0%) incident DR	5 years
Sydney Paediatric Diabetes Study*	Cross-sectional: n = 352 and n = 590; Longitudinal: n = 645, n = 729, n = 736, n = 944	Type 1	Median age reported from 13.5 to 14.4	44.20% to 47.1%	Ranged from 4.7 to 7.2	Cross-sectional n = 85 to 137 (9.0% to 18.8%) prevalent DR; Longitudinal n = 262 to 287 (39.0% to 44.4%) incident DR	Two cross-sectional analyses and five longitudinal, ranging from 2.5 to 3.8 years

*Sydney Paediatric Diabetes Study includes six references reporting on the same population for different analyses of various retinal vessel traits, methods and participant numbers; reported results were from the largest and/or with the longest follow-up
DR = diabetic retinopathy; PDR = proliferative diabetic retinopathy; SD = standard deviation

Table 3. Longitudinal studies general characteristics

Study name	N	Diabetes Type	Mean age, years (SD)	Sex, Male %	Duration of diabetes, years (SD)	DR prevalence/incidence (%)	Follow-up years (SD)
New Jersey 725	n = 468	Type 1	27.2 (10.7)	41.0%	10.1 (8.5)	Incident DR not provided; n = 274 (58.5%) prevalent DR at baseline	6.1 (0.5)
WESDR*	n = 2366 (n = 996 Type1; n = 1370 Type 2)	Both/mixed	Type 1 27.9 (12.3); Type 2 64.5 (11.0); combined 44.9 (18.1)	Type 1 50.3%; Type 2 45.2%; combined 47.7%	Type 1 13.3 (9.7); Type 2 11.1 (7.9)	56% incident DR	10

*Some analyses for WESDR were reported separately for Type 1 diabetes and Type 2, and some were reported together; WESDR reported follow-up for 14 years, but data at years 4, 6 and 10 were reported; these are the eligible participants from baseline, not necessarily the number included in the analyses; in the 2018 WESDR paper, authors used 3846 person-intervals which covered 4-6 year intervals, some participants were included in multiple intervals

DR = diabetic retinopathy; SD = standard deviation

Table 4. Retinopathy grading and retinal vessel measurement specifications

Study name	Retinopathy grading system	Retinal vessel measurements	Software	Region measured (disc diameters from disc margin; centred on)
DRUID	Simplified ETDRS	CRAE, CRVE, AVR	Not specified	0.5 to 1.0; OD centred
Blue Mountains Eye Study	Shortened modified Airlie House system	CRAE, CRVE	Retinal Analysis	0.5 to 1.0; OD centred
Inter99 Eye Study	ETDRS	CRAE, CRVE	IVAN and custom-developed Danish software	0.5 to 1.0; OD and macula centred, not specified which used
Grauslund 2010	ETDRS adaptation of the modified Airlie House classification system	FD	IRIS-Fractal	3.5 OD radii surrounding centre of OD
Sasongko 2011	Modified Airlie House Classification system	Tortuosity	SIVA	0.5 to 2.0; OD centred
MESA	Modified Airlie House Classification system	CRAE, CRVE	Retinal Analysis software	0.5 to 1.0; OD or fovea centred, not specified which used
SINDI	Modified Airlie House Classification system	CRAE, CRVE	Not specified	OD centred
Desheng Diabetic Eye Study	ETDRS	CRAE, CRVE, AVR	IVAN	0.5 to 1.0; OD centred
DCPD1987*	modified ETDRS	Longitudinal: CRAE, CRVE, FD; Cross-sectional: tortuosity, branching angles, LDR;	IVAN (CRAE, CRVE, FD) and SIVA (tortuosity, branching angles, LDR)	0.5 to 1.0 for IVAN; 0.5 to 2.0 for SIVA; OD centred
SiMES	modified Airlie House Classification system	CRAE, CRVE, tortuosity, branching angle, FD	SIVA 1.0	0.5 to 2.0; OD or macula centred, not specified which used

Study name	Retinopathy grading system	Retinal vessel measurements	Software	Region measured (disc diameters from disc margin; centred on)
AusDiab	simplified version of Wisconsin grading system	CRAE, CRVE	Retinal Analysis	0.5 to 1.0; OD or macula centred, not specified which used
Sydney Paediatric Diabetes Study	ETDRS	CRAE, CRVE, LDR, tortuosity, FD, branching angle and coefficients	One study used IRIS-Fractal, three used SIVA, three references did not report the specific software	One study evaluated 0.5 to 1.0; the study using IRIS-Fractal used 3.5 optic disc radii and the three studies using SIVA used 0.5 to 2.0; four studies reported using OD centred images
New Jersey 725	ETDRS	CRAE, CRVE	Retinal Analysis	0.5 to 1.0; OD
WESDR	ETDRS	CRAE, CRVE, LDR, tortuosity, branching angle and coefficient, asymmetry factor, junctional exponent FD	IVAN (CRAE, CRVE); SIVA 3.2 (remaining traits)	0.5 to 1.0; OD

AVR = arterio/venular ratio; CRAE = central retinal arteriolar equivalent; CRVE = central retinal venular equivalent; ETDRS = Early treatment of diabetic retinopathy study; FD = fractal dimension; IRIS-Fractal = International Retinal Imaging Software-Fractal ; IVAN = Integrative Vessel Analysis; LDR = length to distance ratio; OD = optic disc; SIVA = Singapore "I" Vessel Assessment

*DCPD1987 used different photography and analyses tools for assessment of retinal traits; both are listed; the 'cross-sectional' analyses reported by this study was performed on the follow-up data

Table 5. Included study results

Study name	Comparisons	CRAE	CRVE	Tortuosity- arteriolar	Tortuosity- venular	Fractal Dimension
DRUID	DR vs Per 1 SD decrease CRAE; Per 1 SD increase CRVE	OR 0.92 (95% CI 0.54 to 1.54); p = 0.74	OR 1.62 (95% CI 0.94, 2.80); p = 0.08			
Blue Mountains Eye Study	Any DR and moderate-severe NPDR; Per 1 SD increase CRAE; Per 1 SD decrease CRVE	Any retinopathy OR 1.2 (95% CI 0.9 to 1.8; Moderate-severe NPDR OR 1.5 (95% CI 0.8 to 2.9)	Any retinopathy OR 1.1 (95% CI 0.8 to 1.6); Moderate-severe NPDR OR 2.5 (95% CI 1.2 to 4.9)			
Inter99 Eye	DR; mean difference	6.3µm (95% CI 1.0 to 11.6) p = 0.02	7.9µm (95% CI 0.7 to 15.2) p = 0.03			
Grauslund 2010	PDR; per 1 SD decrease					OR 1.45 (95% CI 1.04 to 2.03)
Sasongko 2011	per 1 SD increase vs no DR			Mild DR OR 1.53 (95% CI 1.03 to 2.05) p = 0.014; mod DR OR 1.67 (95% CI 1.10 to 2.55) p = 0.016; vision threatening DR OR 0.91 (95% CI 0.54 to 1.54) p = 0.72	Mild DR OR 1.10 (95% CI 0.79–1.55) p = 0.56; mod DR OR 1.27 (95% CI 0.95–1.70) p = 0.10; vision threatening DR OR 0.96 (95% CI 0.69 to 1.33) p = 0.80	

Study name	Comparisons	CRAE	CRVE	Tortuosity- arteriolar	Tortuosity- venular	Fractal Dimension
MESA	mean µm ±SE for no DR, minimal DR and early-severe DR	No DR 145.8 ± 0.74, minimal DR 146.4 ± 1.44, early-severe DR 144.2 ± 1.69, p for trend 0.34	No DR 220.6 ± 1.20, minimal DR 221.9 ± 2.34, early-severe DR 228.6 ± 2.72, p for trend 0.003			
SINDI	mean µm ±SE for no DR, minimum DR, Mild DR, severe DR	no DR 144.37 ± 0.68, minimum DR 145.52 ± 1.23, mild DR 145.29 ± 1.22, moderate DR 142.31 ± 1.97, severe DR 142.66 ± 2.31, p for trend 0.487	no DR 210.52 ± 0.99, minimum DR 208.47 ± 1.81, mild DR 212.27 ± 1.79, moderate DR 216.14 ± 2.88, severe DR 216.91 ± 3.39, p for trend 0.044- moderate and severe DR statistically larger than no DR			
Desheng Diabetic Eye Study	OR (95% CI) for any DR (4th quartile compared with first); mean µm ±SE for no DR, mild DR, moderate DR and severe DR	OR 0.96 (95% CI 0.64 to 1.41); no DR 152.28µm ± 2.85, mild DR 153.83µm ± 2.92, moderate DR 149.29µm ± 4.11, severe DR 144.35 µm ± 2.75, p for trend = 0.75	OR 2.00 (95% CI 1.36 to 2.95); no DR 227.14µm ± 1.38, mild DR 233.94µm ± 1.63, moderate DR 243.55µm ± 4.52, severe DR 236.40µm ± 3.87, p for trend < 0.0001			
DCPD1987 cross-sectional	OR (95% CI) per 1 SD increase in PDR			OR 1.12 (95% CI 0.75 to 1.69) p = 0.573	OR 1.17 (95% CI 0.75 to 1.83) p = 0.485	

Study name	Comparisons	CRAE	CRVE	Tortuosity- arteriolar	Tortuosity- venular	Fractal Dimension
DCPD1987 longitudinal	OR (95% CI) per -10um change (CRAE) or +10µm change (CRVE) in PDR; OR (95% CI) per -0.01 change in FD in PDR	OR 1.56 (95% CI 1.07 to 2.27) p = 0.03- narrower	OR 1.36 (95% CI 1.05 to 1.76) p = 0.02- wider			OR 1.22 (95% CI 1.09 to 1.37) p <0.01- reduced
SiMES* cross-sectional data	mean (95% CI)	any DR 136.7µm (95%CI 134.2 to 139.1) p for difference with no DR = 0.391, no DR 135.5 (95% CI 133.8 to 137.3); p for trend comparing no DR, minimal DR, mild DR and mod to severe DR = 0.051	any DR 311.3µm (95%CI 207.6 to 214.9) p for difference with no DR = 0.001, no DR 204.9 (95% CI 202.3 to 207.4); p for trend comparing no DR, minimal DR, mild DR and mod to severe DR <0.001	any DR 2.64 (x10^4) (95% CI 2.43 to 2.86), no DR 2.54 (x10^4) (95% CI 2.40 to 2.69) p = 0.408; p for trend comparing no DR, minimal DR, mild DR and mod to severe DR 0.879	any DR 4.53 (x10^4) (95% CI 4.17 to 4.92), no DR 4.20 (x10^4) (95% CI 3.96 to 4.46) p = 0.098; p for trend comparing no DR, minimal DR, mild DR and mod to severe DR 0.742	any DR 1.406 (95%CI 1.397 to 1.415), no DR 1.400 (95% CI 1.394 to 1.407) p = 0.227; p for trend comparing no DR, minimal DR, mild DR and mod to severe DR 0.092
SiMES* longitudinal data	any DR, per SD increase	any DR RR 1.56 (95% CI 1.17-2.09)- wider	any DR RR 0.68 (95% CI 0.52-0.89)- narrower	any DR RR 1.31 (95% CI 1.08 to 1.60)- increased arteriolar tortuosity	any DR RR 1.27 (95% CI 1.06-1.53)- increased venular tortuosity	figures not reported
AusDiab** cross-sectional data	any DR, per SD increase	OR 1.20 (95% CI 0.74 to 1.93) p = 0.46	OR 2.24 (95% CI 1.33 to 3.77) p = 0.002			
AusDiab** longitudinal data	Any DR, widest 25% compared with narrowest 75% (reference)	OR 5.21 (95% CI 1.24 to 21.88) p = 0.024	OR 0.47 (95% CI 0.09 to 2.40) p = 0.364			

Study name	Comparisons	CRAE	CRVE	Tortuosity- arteriolar	Tortuosity- venular	Fractal Dimension
Sydney Paediatric Diabetes Study*** cross-sectional data	Tortuosity outcome: highest quartile versus remaining 3 and per SD increase; FD outcome: 2nd, 3rd and 4th quartiles compared with 1st and per 0.01 increase			4th quartile vs 1-3 OR 2.05 (95% CI 1.18 to 3.55) p = .011; per SD increase OR 1.42 (95% CI 1.11 to 1.83) p = .005	4th quartile vs 1-3 OR 1.20 (95% CI 0.67 to 2.14) p = .546; per SD increase OR 1.08 (95% CI 0.84 to 1.39) p = .554	2nd quartile compared with 1st OR 2.07 (95% CI 1.03 to 4.18) p = 0.041; 3rd quartile compared with 1st OR 3.01 (95% CI 1.53 to 5.94) p = 0.001; 4th quartile compared with 1st OR 3.92 (95% CI 2.02 to 7.61) p <0.001; Per 0.01 increase OR 1.37 (95% CI 1.21 to 1.56) p < 0.001
Sydney Paediatric Diabetes Study*** longitudinal data	width outcomes: 4th quartile compared with 1st and per SD increase; FD outcome: 2nd, 3rd and 4th quartiles compared with 1st;	4th quartile compared with 1st HR 3.44 (95% CI 2.08 to 5.66) p < 0.001; per SD increase HR 1.46 (1.22 to 1.74) p < 0.001	4th quartile compared with 1st HR 0.78 (95% CI 0.50 to 1.23) p = 0.281; per SD increase HR 0.82 (0.69 to 0.98) p = 0.028			2nd quartile compared with 1st HR 0.82 (95% CI 0.53 to 1.26) p = 0.36; 3rd quartile compared with 1st HR 0.97 (95% CI 0.63 to 1.49) p = 0.88; 4th quartile compared with 1st HR 0.93 (95% CI 0.60 to 1.45) p = 0.75

Study name	Comparisons	CRAE	CRVE	Tortuosity- arteriolar	Tortuosity- venular	Fractal Dimension
New Jersey 725	progression to PDR, smallest quartile compared with largest (CRAE), largest quartile compared with smallest (CRVE)	OR 1.99 (95% CI 0.87 to 4.55) p = 0.4; no association with maculopathy	OR 4.63 (95% CI 2.02 to 10.63) p = 0.003; no association with maculopathy			
WESDR	change in CRAE/CRVE, per 10µm for incidence of DR, progression of DR, incidence of macular oedema and incidence of PDR; remaining RVTs used standardised measures so per SD increase	Incidence of DR OR 1.17 (95% CI 0.97 to 1.40) p = 0.1; Progression of DR OR 1.11 (95% CI 0.99 to 1.24) p = 0.08; Incidence of macular oedema OR 1.05 (95% CI 0.89 to 1.23) p = 0.56; Incidence of PDR OR 0.90 (95% CI 0.78 to 1.03) p = 0.13	Incidence of DR OR 1.26 (95% CI 1.10 to 1.43) p < 0.001; Progression of DR OR 1.21 (95% CI 1.12 to 1.30) p < 0.001; Incidence of macular oedema OR 1.16 (95% CI 1.03 to 1.31) p = 0.004; Incidence of PDR OR 1.19 (95% CI 1.07 to 1.32) p < 0.001	Incidence of DR OR 1.17 (95% CI 1.01 to 1.35) p = 0.04; Progression of DR OR 1.08 (95% CI 0.95 to 1.22) p = 0.24; Incidence of PDR OR 1.04 (95% CI 0.87 to 1.23) p = 0.70	Incidence of DR OR 1.14 (95% CI 0.95 to 1.35) p = 0.15; Progression of DR OR 1.03 (95% CI 0.91 to 1.16) p = 0.68; Incidence of PDR OR 1.03 (95% CI 0.90 to 1.18) p = 0.67	Incidence of DR OR 0.97 (95% CI 0.76 to 1.22) p = 0.77 arteriolar FD; OR 0.92 (95% CI 0.76 to 1.12) p = 0.42 venular; Progression of DR OR 0.98 (95% CI 0.83 to 1.17) p = 0.85 arteriolar FD; OR 1.05 (95% CI 0.91 to 1.22) p = 0.49 venular; Incidence of PDR OR 0.90 (95% CI 0.71 to 1.14) p = 0.67 arteriolar; OR 0.87 (95% CI 0.72 to 1.06) p = 0.17 venular

Study name	Comparisons	CRAE	CRVE	Tortuosity- arteriolar	Tortuosity- venular	Fractal Dimension

*SiMES- two reports, using different software programmes, reported on CRAE and CRVE, I reported results from the Cheung 2012 paper using SIVA; there is an abstract, Cheung 2015, with 6-year follow-up results, these results can be found below in the longitudinal analyses section
**AusDiab study reporting cross-sectional data reported for those with diabetes by different strata, I reported those with known diabetes >5 years as this is more representative of most other studies with established diabetes cohorts
***Sydney Paediatric Diabetes Study includes six references reporting on the same population for different analyses of various retinal vessel traits, methods and participant numbers
Items in red indicate statistical significance as determined by the study authors
CI = confidence interval; CRAE = central retinal arteriolar equivalent; CRVE = central retinal venular equivalent; DR = diabetic retinopathy; FD = fractal dimension; HR = hazard ratio; NPDR = nonproliferative diabetic retinopathy; OR = odds ratio; PDR = proliferative diabetic retinopathy; RR = risk ratio; RVT = retinal vessel trait; SD = standard deviation; SE = standard error

Figure 4. CRAE and risk of diabetic retinopathy

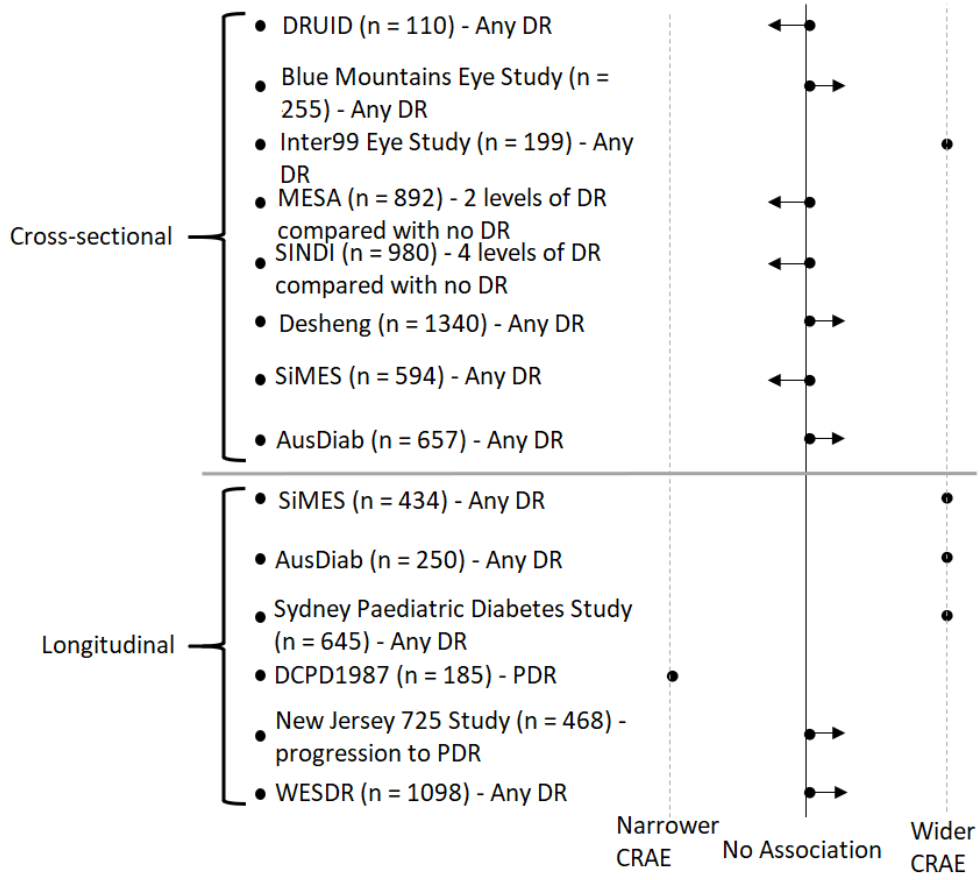

Figure 5. CRVE and risk of diabetic retinopathy

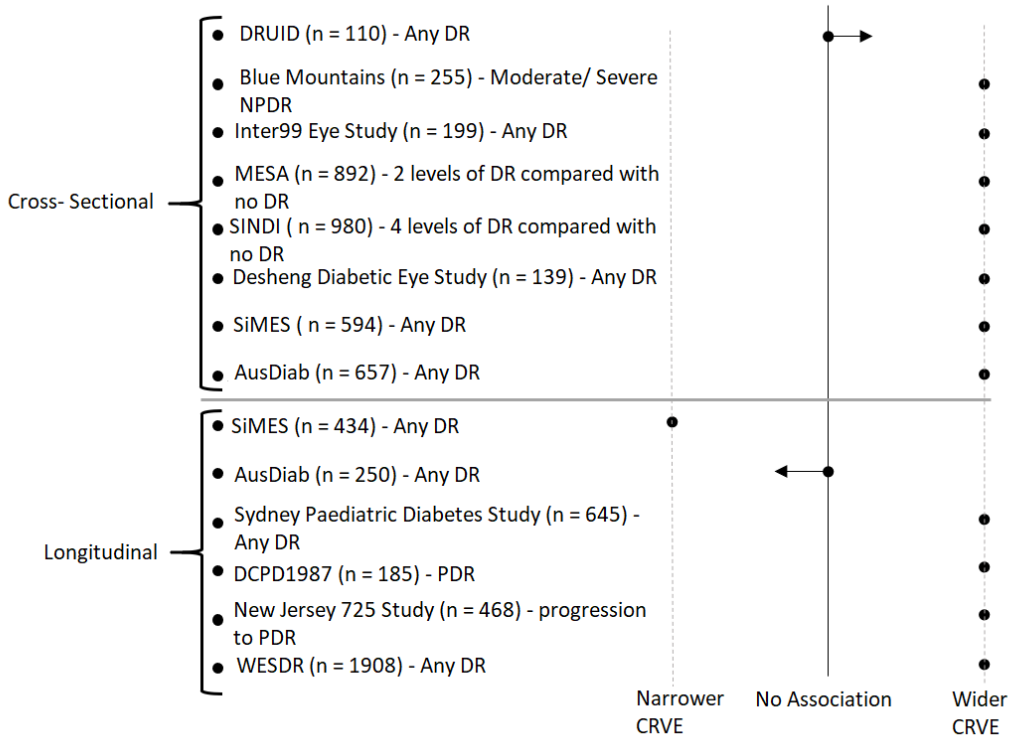

Figure 6. Arterial tortuosity and risk of diabetic retinopathy

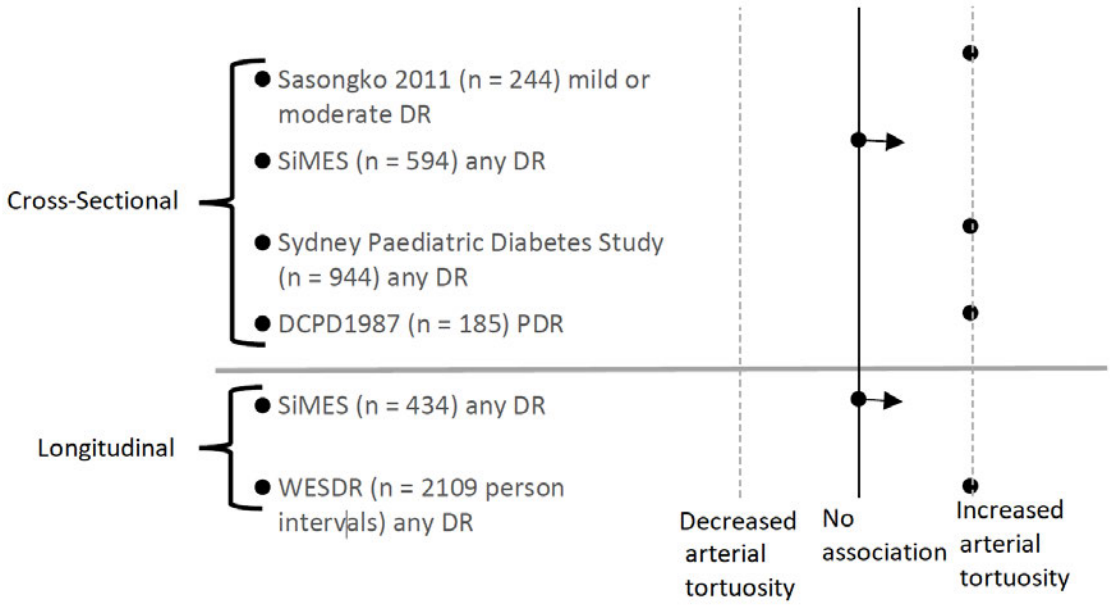

Figure 7. Venular tortuosity and risk of diabetic retinopathy

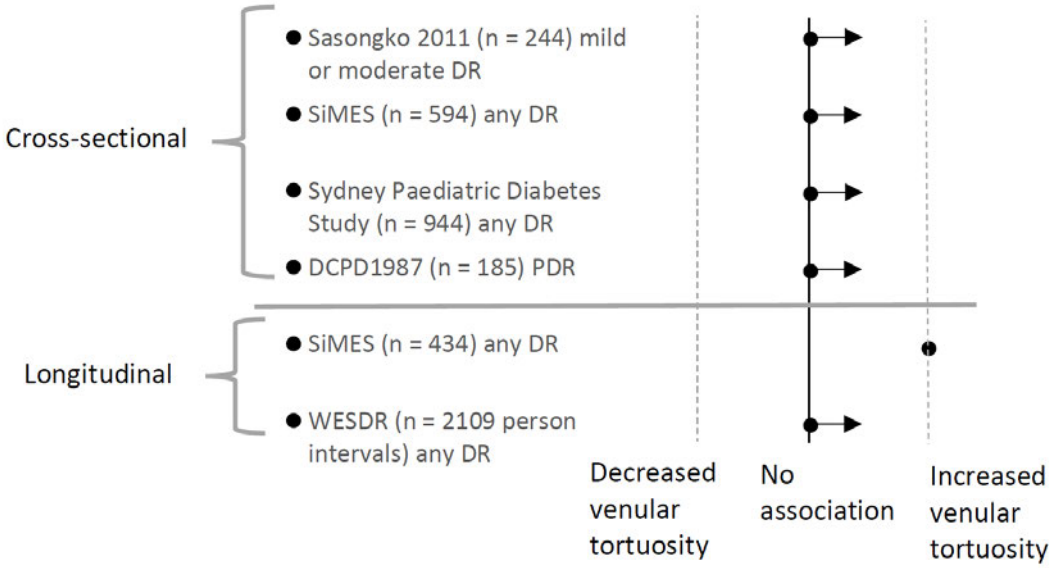

Figure 8. Fractal dimension and risk of diabetic retinopathy

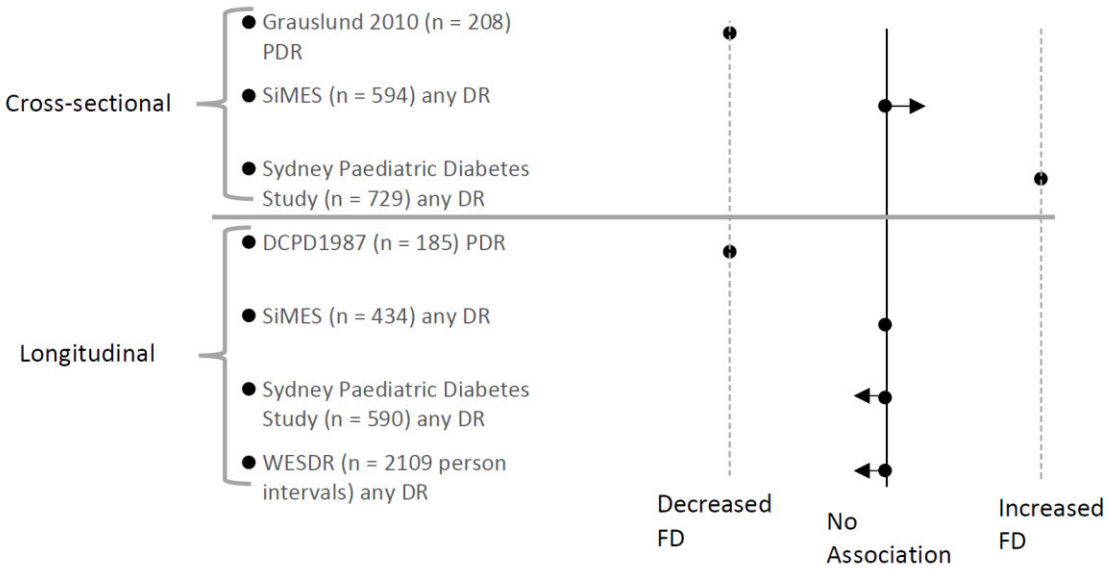

Table 6. Included study quality using modified CASP tool

Study	Study recruited in an acceptable way?	Exposure and outcome accurately measured?	Confounding factors included in the analysis?	Follow up complete and long enough?	Precision of results/ believability of results?
DRUID	Unable to identify participants in traditionally used methods due to sensitivity issues of researching Indigenous people; used three main methods to identify participants: (1) recruiting through local Aboriginal Health Service, (2) using community and family networks, (3) contacting people through the Norther Territory Department of Health and Community Services; study experienced difficulty in recruitment due to regional and population limitations	Did not specify software used for vessel analysis or if the grader was trained, masked or used a protocol; Outcome measured by an ophthalmologist at an outside centre according to simplified ETDRS severity scale	Did not control for CRAE/CRVE	n/a	Finding were modestly precise for such a small study
Blue Mountains Eye Study	All community dwelling, permanent residents in 2 postcode area of west Sydney, Australia, aged ≥49 years; 11.3% (501) refused to participate	Used Retinal Analysis software using the ARIC study methods and grading protocol, reported acceptable intra/intergrader reliability, no indication of masking or training; Outcome evaluated using modified Airlie House classification, but no indication on training or masking of grader	Did not control for duration of diabetes, blood glucose or CRAE/CRVE	n/a	Did not include CIs but the participant with diabetes were few

Study	Study recruited in an acceptable way?	Exposure and outcome accurately measured?	Confounding factors included in the analysis?	Follow up complete and long enough?	Precision of results/ believability of results?
Inter99 Eye	Inter99 study population comprised an age and sex-stratified sample of n = 13,016 participants residing in 11 suburban municipalities in south-western Copenhagen County from the randomised Danish Civil Registration System, comprising all participants permanently residing in Denmark; Inter99 Eye study invited n = 1,437 from larger study and n = 970 participated (67.5% invited and 7.5% total study); used standard WHO definition of diabetes, but also used self-reported cases of diabetes	For the exposure, used IVAN software a custom-developed Danish software, masked graders, using a protocol and reported acceptable intra/inter-grader reliability; Retinopathy defined using ETDRS grading system, but did not specify training or blinding of those that graded images	Did not control for duration of diabetes	n/a	Not well powered for those with diabetes, wide Cis

Study	Study recruited in an acceptable way?	Exposure and outcome accurately measured?	Confounding factors included in the analysis?	Follow up complete and long enough?	Precision of results/ believability of results?
Grauslund 2010	Participants identified from Fyn County, Denmark based on insulin prescription in 1973, all type 1 diabetes patients from Fyn County, Denmark with an onset before the age of 30 years identified (n = 727)	Exposure measurement done with IRIS-Fractal software by trained grader, but no mention of blinding, and used a second grader to assess intergrader reliability, with acceptable findings; To measure the outcome they used ETDRS from modified Airlie classification but no indication of who performed analysis and if trained and blinded	Yes	n/a	CIs were quite wide due to low numbers
Sasongko 2011	Consecutively recruited from the diabetes eye clinics at the International Diabetes Institute, Melbourne Australia (diabetic patients) and from the general eye clinics at the Royal Victorian Eye and Ear Hospital (non-diabetic), but no information on non-attenders or how generalizable the population is	For the exposure, used a standard protocol, trained, masked graders using SIVA software, but no intra/intergrader reliability reported; For the outcome, they used masked graders using Airlie House Classification	Yes	n/a	Very low powered but still found significant associations

Study	Study recruited in an acceptable way?	Exposure and outcome accurately measured?	Confounding factors included in the analysis?	Follow up complete and long enough?	Precision of results/ believability of results?
MESA	Recruited equal men and women, aged 45-84; however not fully powered for diabetes; used random digit dialling in some areas, information brochure mailed to targeted areas, and referral of elderly people	For the exposure they used a detailed protocol for measurement using Retinal Analysis software by trained graders masked to participant characteristics, with acceptable intra/intergrader reliability; for the outcome, the modified Airlie House classification system was used but it was not indicated if assessors were blinded	Yes	n/a	Modestly precise results but no CIs provided
SINDI	Age-stratified by 10-year age group from random sampling method from computer-generated list provided by Singapore Ministry of Home Affairs; participants excluded from total study were older, higher BP, glucose and HbA1c, likely to be females and non-smokers	Used 'semi-automated computer-assisted program' and did not report on training, masking or use of a protocol, but did report acceptable intra/inter-grader reliability; For the outcome, a modified Airlie House Classification System was utilised but no information on who performed the grading and their training level	Did not control for duration of diabetes	n/a	Study was very low powered for people with diabetes and no CIs were provided; At risk of chance findings as included many outcomes and covariates

Study	Study recruited in an acceptable way?	Exposure and outcome accurately measured?	Confounding factors included in the analysis?	Follow up complete and long enough?	Precision of results/ believability of results?
Desheng Diabetic Eye Study	Participants were identified from an age-related eye disease screen programme in Desheng community of urban Beijing by using posters, pamphlets and phone calls and utilised a pre-approved study protocol	Yes, used IVAN software with standardised protocol and single masked grader with acceptable intragrader reliability; For the outcome, a trained ophthalmologist used ETDRS grading standards, with grading reproducibility assessed in 5% of eyes	Did not adjust for CRAE/CRVE	n/a	Moderately precise CIs
DCPD1987	Participants identified from a nationwide population-based cohort of Danish children with type 1 diabetes, initially studied in 1987-1989 n = 720, n = 339 included in baseline of this study in 1995 and eligible for 16-year follow-up in 2011	The different reports on the study used IVAN, Fractal Analyser and SIVA software, reported using protocol, but only one reported graders as blinded and none reported intra/intergrader reliability; Outcome measured by a single trained grader using modified ETDRS scale but no information on blinding	Did not control for CRAE/CRVE	Had a sufficient number at follow-up and clearly explained attrition; follow-up after 16 years	Relatively small numbers so results were only moderately precise

Study	Study recruited in an acceptable way?	Exposure and outcome accurately measured?	Confounding factors included in the analysis?	Follow up complete and long enough?	Precision of results/ believability of results?
SiMES	Age-stratified by 10-year age group from random sampling method from computer-generated list provided by Singapore Ministry of Home Affairs - n = 4148 eligible, n = 3280 (78.7% participated); fair representation of Singapore population in terms of age, housing type, socioeconomic status	Trained graders masked to participant characteristics used SIVA software and a standardized protocol; poor intra/inter-grader reliability; For the outcome the ETDRS scale was used, but training and blinding of graders was not mentioned	Did not adjust for CRAE/CRVE	Sufficient number at follow-up at 6 years	The study is not powered for people with diabetes and they included many outcomes and covariates, leading to a large risk of chance
AusDiab	Total cohort included a nationally representative sample of 11,247 adults from 42 randomly selected urban and rural areas	Trained, masked graders used standard protocol and Retinal Analysis software and reported acceptable intra/intergrader reliability; Outcome measured by a single, masked grader according to simplified Wisconsin grading system, but not information on training	Yes	Had a sufficient number at follow-up but did not fully explain attrition; follow-up after 5 years	Not very precise as they only presented sensitivity analysis which had few participants

Study	Study recruited in an acceptable way?	Exposure and outcome accurately measured?	Confounding factors included in the analysis?	Follow up complete and long enough?	Precision of results/ believability of results?
Sydney Paediatric Diabetes Study	No information on non-participating invitees; multiple references reporting on the same group of participants but with different n values and no continuity of how their respective studies were formulated	Three studies did not report the software used to measure the exposure, but three studies used SIVA and one used IRIS-Fractal; acceptable intra/intergrader reliability reported; For the outcome, standardised protocol used and ETDRS modified Airlie House classification, ophthalmologist graded, masked to participant characteristics using	Yes	Follow-up was only maximum 2.9 years	One report did provide a power calculation and at least one report had relatively precise CIs and strong effects; There are concerns regarding the believability of the findings as different analyses from this population found opposing results without discussion.
New Jersey 725	Identified from a random review of n = 13,615 medical records, included patients diagnosed with diabetes, treated with insulin before age 30, currently taking insulin, and excluded those with type 2 diabetes	Graded at external location using standard protocol and Retinal Analysis software with graders masked to participant characteristics but did not report intra/inter-grader reliability for this population; Outcome evaluated by masked graders at an external centre specialised in fundus photography reading using modified ETDRS Airlie House classification	Did not control for duration of diabetes or lipid markers; included multiple eyes for each participant but did control for this	70% participated in follow-up at six years, which is a modestly robust follow-up timeframe	Wide CIs and not well powered

Study	Study recruited in an acceptable way?	Exposure and outcome accurately measured?	Confounding factors included in the analysis?	Follow up complete and long enough?	Precision of results/ believability of results?
WESDR	Sample selected from 10135 (identified by medical charts) diabetic patients who received primary care in an 11-county area in southern Wisconsin from 1979 to 1980; possible misclassification bias of diabetes type as they used age of onset to categorise	For measuring the exposure, utilised masked graders, and reported acceptable intra/intergrader reliability, but no information given on if a protocol was used; For the outcome, ETDRS Airlie House classification system used; trained graders were blinded to the measurements at previous visits	Did not control for CRAE/CRVE	Yes, data reported for 4, 6- and 10-year follow-up	Moderately precise

ARIC = Atherosclerosis Risk in Communities Study; BP = blood pressure; CI = confidence interval; CRAE = central retinal arterial equivalent; CRVE = central retinal venular equivalent; ETDRS = Early Treatment of Diabetic Retinopathy Study; IRIS-Fractal = International Retinal Imaging Software-Fractal ; IVAN = Integrative Vessel Analysis; n/a = not applicable; SIVA = Singapore "I" Vessel Assessment; WHO = World Health Organisation

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3 Association between retinal vessel traits and cognitive decline: a systematic literature review

3.1 Introduction

The retina is considered an extension of the cerebrovascular system, both of which share developmental and physiologic characteristics, as well as pathological markers (Cooper *et al.*, 2006; Snell and Lemp, 2013). These links make the retina a plausible candidate for early detection of pathology in the cerebrovasculature, including dementia and cognitive decline. In recent years, there have been many studies that have investigated retinal traits and their association with chronic disease including cognitive impairment and decline, this is due to the fact that the retina is a such an easily visualised part of the vascular system.

As previously described in **Section 1.4**, a study from the Lothian Birth Cohort, which utilised VAMPIRE software, investigated fractal measures of retinal vasculature and their association with cognitive ageing by evaluating a single retinal image in later life compared with IQ measured at a young age and found now association (Taylor *et al.*, 2015). The large Multi-Ethnic Study of Atherosclerosis (MESA) study observed larger arteriolar width measures over 8 years of follow-up that were associated with decreased cognitive measures in their large, prospective longitudinal cohort (Hughes et al. 2016). There have been several other studies evaluating these traits that used different methods of describing cognitive decline and measuring retinal traits, with varying results.

The purpose of this systematic literature review was to provide comprehensive, evidence-based information on the current epidemiological research that has been conducted to better understand the association between retinal vessel traits and cognition. I aimed to include all relevant studies and present their findings, as well as the details of how the studies were conducted, including population characteristics, measures of the exposure and outcomes, and to discuss their methodological quality. The

review serves to support the study design and considerations for this thesis, as well as provide a platform of analysis and comparison of the primary results. The methods in this chapter are very similar to those in **Chapter 2**, but methodological descriptions are included so that if a person is reading this chapter out of order still receives the full contextualisation.

3.2 Objectives

The objective of this systematic review was to identify and collate all studies that have evaluated the association between retinal vessel traits (RVTs) and cognitive ability or cognitive decline, using fundus images and a digital platform for semi-automatic or automatic analysis.

3.3 Methods

3.3.1 Types of studies

I included non-randomised, observational epidemiological study designs (i.e. cross-sectional, case-control, cohort). Experimental studies, such as randomised controlled trials, were considered if the population fit the inclusion criteria and the intervention did not confound the results and make the inclusion inappropriate. For instance, if a randomised controlled trial was evaluating an intervention for something other than the treatment of cognitive decline, and had measured retinal vessel traits for the study, or a subset of the study, and also had cognitive decline or dementia as an outcome, the study may be considered for inclusion.

3.3.2 Types of participants

There were no restrictions to the type of participant considered for inclusion, based on features such as age or comorbidities.

3.3.3 Types of RVTs

I included studies that evaluated any RVT identified using fundus imaging and assisted by automated or semi-automated computer software designed for measuring retinal features. Studies were excluded if they used manual methods of measuring RVTs, even if digitally assisted. The most common

RVTs to be studied included widths (central retinal arteriolar equivalent (CRAE) and central retinal venular equivalent (CRVE), tortuosity and fractal dimension (FD).

3.3.4 Types of outcome measures

Outcome measures were limited to any measure of cognitive ability or change, such as tests or a battery of tests, specifically designed to measure a single or multiple cognitive domain. I also included studies with participants based on a cognitive diagnosis, such as dementia or cognitive impairment. Studies were excluded if they did not specify how cognitive status was measured.

3.3.5 Search methods for identification of studies

Using the OVID® platform, I searched the MEDLINE, EMBASE and PsychINFO databases for all relevant studies, using a sensitive search strategy. The full search strategy can be found in **Appendix 6.** I also undertook hand-searching of the references of relevant studies and reviews. The electronic search was limited to English language studies conducted in humans. The search date was undertaken on February 14th, 2019 and included the time period from 2007 to the present date. The year 2007 was chosen as a previous PhD student conducted a similar systematic review for their thesis project, which the search strategy of this review was based on, and was subsequently published (Ding *et al.*, 2008)**.**

3.3.6 Data collection

Any study that met the inclusion criteria for study type, participant type as well as outcome and vessel analysis, were considered for inclusion. Studies were excluded if they included less than 100 participants, due to low power to detect an association. Also, studies that only had data published in abstracts or conference proceedings, and not a fully peer-reviewed published reference, were excluded due to a paucity of information being provided in such abbreviated texts.

A data extraction spreadsheet was prepared to identify key information and findings from the included studies. Data extracted included the type of study, location, follow-up time frame, number of participants included, age and gender of participants, retinal vessel measurements and how they were analysed, how cognitive status or decline was measured, including criteria for pathophysiological diagnosis, outcomes, statistical models used and covariates controlled for. I collected the outcome data for any analyses evaluating the association between RVTs and cognition. If a study reported data on other outcomes, such as brain volume or imaging, this type of data was not collected as it was out with the scope of the review.

3.3.7 Assessment of study quality

Using a modified version of the Critical Appraisal Skills Programme (CASP) quality appraisal tool I evaluated the quality of each study (CASP UK, 2018). The CASP tool includes separate but similar tools for case-control studies and cohort studies, making easy comparison of quality between the different study types. CASP is designed as a check-list style resource to help organise and evaluate the quality of studies using an evidence-based approach. This tool does not generate a single value or score, but rather provides a narrative for each of the included domains. I evaluated and reported on eight areas of quality for each study: (1) Did the study address a clearly focused issue? (2) Was the study recruited in an acceptable way; in case-control studies, were cases and controls recruited appropriately? (3) Was the exposure and outcome accurately measured to minimise bias? (4) Have the authors identified all important confounding factors and included them in the analysis? (5) Was the follow up of the subjects complete enough and long enough? (6) How precise are the results? Do you believe the results? (7) Do the results fit with other available evidence? (8) Are sources of funding reported? See **Appendix 2** for further information I used to evaluate each domain.

To present the findings of the quality assessment I generated a table with a row for each study and column for each domain. I either reported the methods of the study or in some cases stated ‘Yes’ if the criteria were met, or

the specific reasons the requirements were not fully met. I added a colour to each cell to help give a quick visual assessment: green indicates that all criteria were satisfactorily met; yellow indicates that there were some concerns with the study in relation to that domain; red indicates serious concerns with the domain.

3.3.8 Measures of effect

I planned to undertake meta-analysis using fixed-effects methods if the data extracted from the included studies were deemed to be satisfactorily homogenous in terms of exposures, outcomes and modelling methods. However, after inclusion and extraction, it was clear that the studies and their data varied too greatly for meta-analysis. Narrative synthesis methods, along with tables and figures, were undertaken to describe and present the data from the individual studies. I chose to report on the most commonly reported RVTs, which included vessel widths (CRAE and CRVE), as well as tortuosity, which is separated into arteriolar and venular tortuosity, and finally on fractal dimension (FD), which includes a total FD measure and also separate FD measures for the arteriolar and venular vessels. A few of the studies reported on other vessel features, which are noted in the study descriptions, but not reported on in this review.

3.4 **Results**

3.4.1 Results of the search

A total of 872 citations were identified in the search plus an additional three from hand searching, for a total of 875 citations. Seven-hundred and nineteen of which were non-duplicates. After reviewing titles and abstracts, 644 were deemed not relevant. Seventy-five full articles were retrieved. Thirty-eight were excluded after full text screening and a further six were excluded during data extraction. A total of 31 references from 15 studies were included. See **Appendix 7** for the full study selection flow diagram (PRISMA).

3.4.2 Included Studies

See **Table 7, Table 8, and Table 9 (pages 100, 101 and 102, respectively)** for general information about the included studies. Studies are arranged by study type (case-control, longitudinal and cross-sectional) and by ascending order of participants included. As with **Chapter 2**, all tables and figures are at the end of the chapter.

Fifteen studies were included in this review. They include, in alphabetical order, the **Australian Imaging, Biomarkers and Lifestyle Flagship Study of Aging (AIBL)** (Frost *et al.*, 2013), **The Blue Mountains Eye Study** (Liew *et al.*, 2009), **Cheung 2014** (Cheung et al., 2014a), **Chronic Renal Insufficiency Cohort (CRIC)** (Yaffe *et al.*, 2013), **Dunedin Multidisciplinary Health and Development Study (Dunedin Study)** (Shalev *et al.*, 2013), **Epidemiology of Dementia in Singapore (EDIS)** (Ong *et al.*, 2014), **Edinburgh Type 2 Diabetes Study (ET2DS)** (Ding *et al.*, 2011), **Los Angeles Latino Eye Study (LALES)** (Gatto *et al.*, 2012), **Lothian Birth Cohort 1936 (LBC1936)** (McGrory *et al.*, 2016), **Pittsburgh Epidemiology of Diabetes Complications Study (EDC)** (Nunley *et al.*, 2018)**,** the **Rotterdam Study** (de Jong *et al.*, 2011)**, Singapore Malay Eye Study (SiMES)** (Cheung et al., 2014b)**, South London Diabetes Study (SOUL-D)** (Naidu *et al.*, 2016)**, Wisconsin Epidemiologic Study of Diabetic Retinopathy (WESDR)** (Ryan *et al.*, 2016), and **Williams 2015** (Williams *et al.*, 2015)**.**

Four studies used case-control methods (AIBL, Cheung 2014, SOUL-D and Williams 2015), nine were cross-sectional studies (Blue Mountains Eye Study, CRIC, Dunedin Study, EDIS, ET2DS, LALES, LBC1936, SiMES and WESDR) and two studies provided longitudinal data (EDC and the Rotterdam Study).

The number of participants included in each study ranged from 119 to 5553. For the longitudinal analyses the mean follow-up times were 11.6 years in the Blue Mountains Eye Study and 17.8 years for EDC. The majority of studies were conducted in a general population, ten studies total, although one study

was specific to the elderly Latino population. Four studies were conducted in people with diabetes (two type 2 and two type 1 diabetes). One study was conducted in a group of people with chronic renal insufficiency.

Fourteen of the 15 included studies evaluated the width measures of CRAE and CRVE, which was the most commonly reported vessel trait. Of the 14, three also reported on AVR. Seven studies reported on both tortuosity and FD. Tortuosity was consistently separated by vessel types: arteriolar and venular. For FD, some studies reported total FD and several reported separate analyses based on arteriolar or venular vessels. See **Table 7.** Case-control studies general characteristics

Study name	N	Population type	Mean age, years (SD)	Sex, Male %	How cognition/dementia was measured
SOUL-D	n = 137 (n = 69 cases; n = 68 controls)	Type 2 diabetes	55.63 (11.07)	64.7% cases; 44.9% controls	Cases chosen as lowest decile of TICSM scores
AIBL	n = 148 (n = 25 AD; n = 123 controls)	General	72.4 (7.5) AD; 71.6 (5.6) controls	48% AD; 45% controls	AD using NINCDS-ADRDA criteria
Cheung 2014	n = 426 (n = 136 AD; n = 290 controls)	General	74.8 (5.7) AD; 73.9 (4.6) controls	47% AD ; 53% controls	Clinically diagnosed dementia (clinical neurologic and neuropsychiatric assessment- CT or MRI reviewed as part of diagnostic process); Diagnosis using DSM-IV criteria, and AD diagnosis followed the NINDS-ADRDA criteria
Williams 2015	n = 507 (n = 213 AD; n = 294 controls)	General	79.6 (7.8) cases; 76.3 (6.6) controls	36% cases; 40% controls	Diagnosis of AD made by a senior clinician using the NINCDS-ADRDA criteria

AD = Alzheimer's disease; AIBL = Australian Imaging, Biomarkers and Lifestyle Flagship study of aging; CT = computerised tomography; DSM = Diagnostic and statistical manual of mental disorders; MRI = magnetic resonance imaging; NINCDS-ADRDA = National Institute of Neurological and Communicative Disorders and Stroke – Alzheimer's Disease and Related Disorders Association; SOUL-D = South London Diabetes Study; TICSM = modified Telephone Interview of Cognitive Status

Table 8. Cross-sectional studies general characteristics

Study name	N	Population type	Mean age, years (SD)	Sex, Male %	How cognition/dementia was measured
WESDR	n = 244	Type 1 diabetes	55.2 (8.3)	53.0%	Mental efficiency and executive function (Trail making Test A and B; Stroop Word and Interference Test; Grooved Pegboard; DSST; Verbal Fluency) nonverbal memory (Rey Complex Figure Test and DSST), verbal memory (Immediate and Delayed recall)
EDIS	n = 300	General	67.3 (4.8) NCI; 71.1 (6.3) CIND-mild ; 74.1 (5.4) CIND-moderate	56.2% NCI; 46.2% CIND-mild; 31.9% CIND-moderate	Consensus meetings held between study clinicians, neuropsychologists and research staff; reviewed clinical and blood assessments as well as neuropsychological tests and MRI scans to determine status: no cognitive impairment, cognitive impairment no dementia
CRIC	n = 588	Chronic renal insufficiency	65.3 (5.6)	52.6%	Six test battery: modified MMSE (3MS); Trail-Making Test A and B; Category fluency; Buschke Selective Reminding Test; Boston Naming Test
LBC1936	n = 683	General	72.5 (0.7)	51.5%	General intelligence factor *g* measured using domains of processing speed, visuospatial ability, memory and crystallised ability; derived from principle component analysis
LALES	n = 809	Latino	70.3 (6.9)	40.5%	CASI-S and SENAS; low CASI-S or low SENAS if their total CASI-S or SENAS score was <10th percentile for their age in 5 year categories

Study name	N	Population type	Mean age, years (SD)	Sex, Male %	How cognition/dementia was measured
Dunedin study	n = 922	General	38	52.0%	WAIS-IV: (Trail making test, Mental Control and Verbal Paired Associates test, Rey Auditory Verbal Learning test, grooved-pegboard, one-legged balance and grip-strength tests of motor function and three tasks from Cambridge Neuropsychological Tests Automated Battery); 'informant reports' at age 38 using mailed questionnaires to a nominated person
ET2DS	n = 954	Type 2 diabetes	67.2 (4.2)	50.9%	7 cognitive function tests: Faces and Family Pictures subtest; Logical Memory from WMS-III; Matrix Reasoning; Letter–Number Sequencing, DSST from WAIS-III; Borkowski Verbal Fluency Test; Trail Making Test B; MHVS used to estimate pre-morbid cognitive ability
SiMES	n = 1202	General	68.5 (5.4)	50.7%	AMT score ≤6/10 in participants with 0-6 years of formal education and an AMT score ≤8/10 in those with more than 6 years of formal education
Blue Mountain Eye Study	n = 1988	General	75.1 (9.1) cognitive impairment, 68.6 (8.2) no cognitive impairment	47.1% cognitive impairment; 42.6% no cognitive impairment	MMSE ≤23 defined as cognitive impairment

AMT = Abbreviated Mental Test; CASI-S = Cognitive Abilities Screening Instrument- Short form; CIND = cognitive impairment no dementia; CRIC = Chronic Renal Insufficiency Cohort; DSST = Digit symbol substitution test; EDIS = Epidemiology of Dementia in Singapore; ET2DS = Edinburgh Type 2 Diabetes Study; LALES = Los Angeles Latino Eye Study; LBC1936 = Lothian Birth Cohort 1936; MHVS = Mill Hill Vocabulary Scale; MMSE = mini-Mental State Exam; SENAS = Spanish English Neuropsychological Assessment Scales; SiMES = Singapore Malay Eye Study; WAIS = Wechsler Adult Intelligence Scale; WMS = Wechsler Memory Scale; WESDR = Wisconsin Epidemiologic Study of Diabetic Retinopathy

Table 9. Longitudinal studies general characteristics

Study name	N	Population type	Mean age, years (SD)	Follow-up	Sex, Male %	How cognition/dementia was measured

				years (SD)		
EDC	n = 119	Type 1 diabetes	Time of cognitive assessment: 49.6 (7.0)	17.6 (8.0)	52.1%	DSST, Purdue Pegboard Test, Stroop colour word task, verbal fluency test, Trail Making Test B, animals verbal fluency task and the Rey-Osterrieth Complex Figure copy task; Participants with a single test score 1.5 standard deviations or worse were classified as having mild cognitive impairment and two or more test scores 1.5 standard deviations or worse were classed as clinically relevant cognitive impairment
Rotterdam Study	n = 5553	General	67.7 (8.0)	11.6 (4.4)	41.4%	First measured MMSE and GMS, if MMSE <26 and GMS >0 participant given Cambridge Examination of Mental Disorders in the Elderly and an informant interview; Participants suspected of having dementia further examined by a neurologist, a neuropsychologist, and if possible, had an MRI; also used direct linkage with medical records from GP; Diagnosis of dementia and subtype made in accordance with DSM-III-R

DSM = Diagnostic and statistical manual of mental disorders; DSST = Digit Symbol Substitution Test; GMS = Geriatric Mental State Exam; GP = general practitioner; MMSE = mini-Mental State Exam; MRI = magnetic resonance imaging

Table 10 **(page 103)** for details on retinal vessels assessed in each study and the methods used and **Appendix 8** for further details on eye analysed and image specifications.

As the measurement of cognition can be difficult and often subjective, it is not surprising that there were many different methods used to measure and report cognitive status or cognitive decline. Three of the four case-control studies utilised a diagnosis of Alzheimer's disease (AD) as their case definition of cognitive decline. Four studies utilised a single scoring system to identify cognitive impairment or function: one study utilised the modified Telephone Interview of Cognitive Status (TICSM) tool, another used the Cognitive Abilities Screening Instrument-Short form (CASI-S) and/or Spanish English Neuropsychological Assessment Scales (SENAS) tools. A third study considered a lower score of the Abbreviated Mental Test (AMT), to indicate lower cognitive function and a fourth used the Mini-Mental State Exam (MMSE) to generate their cognitive outcome. Eight studies used a combination of tests and criteria to either determine a single measure of cognition or to investigate different cognitive domains separately.

3.4.3 Findings of included studies on selected RVTs

See **Appendix 9** for a description of the statistical models used and covariates included and see **Table 11 and Table 12 (page 105 and 108, respectively)** for full results from each included study.

Vessel widths

I identified 14 studies that reported on CRAE and CRVE. Four studies used a case-control design, eight studies were cross-sectional and two studies were longitudinal.

Central Retinal Arteriolar Equivalent (CRAE)

Only three of the 14 studies identified an association between CRAE at baseline and cognitive decline and all three found that a narrower CRAE was associated with increased risk of cognitive decline. The three studies included one case-control study that evaluated AD (AIBL) and two cross-sectional studies that evaluated cognitive ability by different tests (WESDR and LALES). AIBL estimated a mean and standard deviation (SD) of 129.1µm (SD 10.3) in the control group and 122.9µm (SD 12.4) in the group

with AD and p value of 0.027 after adjusting using a false discovery rate method. It is assumed they were reporting units in µm, but that was not specified. WESDR reported a beta-coefficient (95% confidence interval (CI)) for CRAE per 10µm change of 0.140 (95% CI 0.062 to 0.219), $p < 0.001$ for mental efficiency, but there was no difference in verbal memory. The LALES study used the lowest decile scores of the Cognitive Abilities Screening Instrument-Short form (CASI-S) to indicate cognitive dysfunction and determined that decreased CRAE was associated with cognitive dysfunction (odds ratio (OR) 2.17, 95% CI 1.19 to 3.97). Of the studies that reported findings that were not statistically significant, one had a point estimate favouring narrower CRAE and two wider CRAE. Overall, there was little consensus between the 14 studies regarding the relationship between baseline CRAE and cognitive decline. See **Figure 9 (page 110).**

One of the longitudinal studies, EDC, also evaluated change in CRAE over time and found evidence that all participants showed a decrease in CRAE but there was faster narrowing of CRAE (slope) in participants that had clinically relevant cognitive decline, compared with no cognitive decline or mild cognitive decline. These results are not included in **Figure 9** because none of the other studies evaluated change in CRAE, and would therefore be inappropriate to compare.

Central Retinal Venular Equivalent (CRVE)

Of the 14 studies, two found an association between narrower CRVE and cognitive decline (AIBL and Cheung 2014) and four found an association between wider CRVE and cognitive decline (WESDR, Dunedin Study, Blue Mountain Eye Study and the Rotterdam Study). Two of these studies were case-control, three cross-sectional and one longitudinal. AIBL reported CRVE of 182.7µm (SD 15.8) in the control group and 169.7µm (SD 15.3) in the group with AD and a $p = 0.0049$, again, I assumed the unit is in µm. Cheung 2014 reported an OR of 2.01 (95% CI 1.27 to 3.19) when comparing AD cases and controls for CRVE, and the report stated that the association was of narrower CRVE and AD. WESDR again reported on mental efficiency and

reported a beta of -0.127 (95% CI -0.207 to -0.047), p = 0.002, indicating that wider CRVE was associated with reduced mental efficiency. The Dunedin Study used 16 different cognitive assessment tests looking at multiple domains and found associations between wider CRVE and many of the tests and domains. Please see **Table 11 (page 105)** for the full list of results from the Dunedin Study. The Blue Mountain Eye Study used a cut-off point of ≤23 on the Mini-Mental State Exam (MMSE) to determine cognitive impairment and found the widest CRVE quartile to be associated (OR 1.8, 95% CI 1.0 to 3.2), p = 0.03. The only longitudinal study, the Rotterdam Study, used multiple screening exams and examination by multiple medical professionals trained at identifying dementia, to determine dementia status and when comparing this status with CRVE (per SD increase) they found a hazard ratio (HR) of 1.11 (95% CI 1.00 to 1.22), which indicates a possible association. Of the studies who's findings were not statistically significant but reported their numeric findings, three reported a point estimate favouring narrower CRVE and one reported favouring wider CRVE. See **Figure 10 (page 111)**.

The EDC study also evaluated change in CRVE over the study period and found that on average all participants demonstrated a decrease in CRVE over time, but there was no difference between participants with cognitive impairment or not.

Tortuosity

A total of seven studies reported tortuosity as an outcome. Four were case-control studies and three cross-sectional. No longitudinal studies evaluated tortuosity.

Tortuosity-arterial

One study found an association between increased arterial tortuosity and cognitive decline (Cheung 2014), and one study found an association between decreased arterial tortuosity and cognitive decline (Williams 2015), both of which were case-control studies. Cheung 2014 reported an OR of 1.80 (95% CI 1.40 to 2.31) per SD increase in tortuosity. Also reporting per SD increase, Williams 2015 reported an OR of 0.78 (95% CI 0.63 to 0.97) p =

0.027. Of the non-significant findings, one study favoured decreased arterial tortuosity and one favoured increased, both of which were cross-sectional. See **Figure 11 (page 112)**.

Tortuosity-venular

Two case-control studies reported an association between increased venular tortuosity and cognitive decline (SOUL-D and Cheung 2014) and a single case-control study found an association between decreased venular tortuosity and cognitive decline (AIBL). SOUL-D reported the difference in venular tortuosity as a beta coefficient of 0.13 (95% CI 0.002 to 0.250) with higher venular tortuosity in the cases, as determined by the lowest 10% of scorers using the Modified Telephone Interview for Cognitive Status (TICSM). AIBL reported a venular tortuosity of 7.660 (SD 1.554) in the controls group and 6.952 (SD 2.601) in the cases, p = 0.042. There are generally no units assigned to tortuosity. Cheung 2014 reported an OR of 1.94 (95% CI 1.48 to 2.53) associating increased venular tortuosity with AD. See **Figure 12 (page 112)**.

Fractal Dimension (FD)

Seven studies evaluated FD. Four studies evaluated total FD and five studies evaluated FD in arterial and venular vessels separately. Two studies evaluated total FD as well as FD split by vessel type.

FD-total

In the four studies that evaluated total FD, two found an association between decreased FD and cognitive decline (Cheung 2014 and SiMES) and two studies found no association, but one demonstrated a point estimate also favouring decreased FD. One study that found an association was a case-control and one a cross-sectional study. Cheung 2014 reported an OR of 1.54 (95% CI 1.23 to 1.93), supporting an association between decreased FD and AD. As with tortuosity, FD has no associated unit. SiMES compared the 1st quintile to the 5th of FD measurements and reported an OR of 1.71 (95% CI 1.03 to 2.82), p = 0.036, with lower FD being associated with cognitive

impairment as determined by a low score on the Abbreviated Mental Test (AMT). See **Figure 13 (page 113)**.

FD-arterial (FD-a)

Three of the five studies that evaluated FD-a found an association between decreased FD and cognitive decline, two case-control studies (AIBL and Cheung 2014) and one cross-sectional (EDIS). AIBL reported arterial FD to be 1.235 (SD 0.052) in the controls and 1.201 (SD 0.061) in the AD cases, p = 0.021. Cheung 2014 reported decreased arterial FD in those with AD with an OR of 1.35 (95% CI 1.08 to 1.68). EDIS, which evaluated people with cognitive impairment with no dementia (CIND) as determined by clinical criteria, found decreased arterial FD to be associated with CIND with an OR of 1.86 (95% CI 1.20 to 2.88). Both studies that did not find a statistically significant result also reported point estimates that favoured decreased FD. See **Figure 14 (page 113)**.

FD-venular (FD-v)

Four of the five studies that reported on FD-v found an association between decreased FD and cognitive decline, four of which were case-control studies and one cross-sectional (AIBL, Cheung 2014, Williams 2015 and EDIS), and the fifth study, while not statistically significant, also found a point estimate favouring decreased FD. AIBL reported venular FD of 1.21 (SD 0.05) in the controls and 1.17 (SD 0.048) in the AD cases, p = 0.003. Cheung 2014 reported an OR of 1.47 (95% CI 1.17 to 1.84) indicating decreased venular FD in the AD cases. Williams 2015 also reported decreased venular FD associated with AD cases with an OR of 0.77 (95% CI 0.062 to 0.97) p = 0.025. EDIS, using per SD change, found an OR of 2.09 (95% CI 1.35 to 3.22) indicating decreased venular FD to be associated with moderate cognitive impairment. See **Figure 15 (page 114).**

The findings from the studies reporting on fractal dimension, total, arterial and venular, demonstrate evidence of an association between decreased fractal dimension and cognitive decline.

3.4.4 Quality assessment of included studies

Please see **Table 13 (page 115)** for full descriptions of study quality on the predefined categories using the modified CASP tool. Note, I have not included columns for domains 1, 7 and 8 because the criteria was met for each study and there were few concerns regarding these domains.

Did the study address a clearly focused issue?

All included studies appropriately stated their research questions and used appropriate methods to answer the question. The EDIS study did not state in their methods which specific vessel measurements they would be evaluating, which increases the risk of bias as they may have chosen the measurements after analysis. The three studies that evaluated Alzheimer's disease as their outcome utilised case-control methods as Alzheimer's is relatively rare in a general population of elderly people.

Was the study recruited in an acceptable way?

Of the four case-control studies, there was concern in three with how their cases and controls were identified. For the SOUL-D study, the authors used the lowest 10% of the cognitive test as the cases, but the n = 69 is far less than 10% of n = 1084, the size of the total cohort. The AIBL study does not explain how their cases of AD were chosen from the larger cohort study. Williams 2015 used an opportunistic recruitment strategy that included non-systematically identifying participants from clinical records using NINCDS-ADRDA criteria. The way they identified controls was also subject to bias, asking carers, friends and family of the cases, who were not matched on any important criteria to the cases.

For the cross-sectional studies, one study out of nine had recruitment that appeared to be at risk of introducing bias. The EDIS study included participants from the larger Singapore Chinese Eye Study (SCES) but only about 9% of those from SCES were enrolled in EDIS and it was not clear why such a small number was used. EDC, one of the two longitudinal studies did not include sufficient information to determine adequate participant selection or if the study was representative of the wider population. The other

longitudinal study, Rotterdam Study, used appropriate methods for identifying participants.

Was the exposure and outcome accurately measured to minimise bias?
Nearly all of the included studies were missing some criteria for accurate exposure and outcome measurement. Most commonly, for evaluation of the retinal vessel traits, there was no report of intra- or intergrader reliability, or in two cases, there was evidence of high variation between graders. In several studies there was no mention of blinding or masking of those that were measuring the retinal traits, and less commonly there was no indication of the use of a protocol or training of the assessors. There were also several studies that, when describing how cognition was measured, failed to indicate who would be performing the cognitive tests and if they received training.

Have the authors identified all important confounding factors and included them in the analysis?
Seven of the included studies did include all important confounding variables in their analyses. For the remaining studies that only had minor concerns, most commonly lipids were not controlled for in the statistical models used. Three studies did not include prevalent cardiovascular markers and two studies did not control for CRAE or CRVE in the respective analyses. It is important to control for these variables as CRAE and CRVE are highly correlated. One study did not control for blood pressure and a further study did not control for stroke, which is an important variable when evaluating cognition. The Dunedin Study was noted in red, indicating high risk of concern as they only controlled for gender and CRAE/CRVE in their analyses. The EDC study was also noted in red, as in the methods they describe controlling for several variables in their models, but most of the presented data was uncontrolled or was unclear how the variables were controlled for.

Was the follow-up of subjects complete enough and long enough?
There were only two longitudinal studies included in this review, EDC and the Rotterdam Study, and it was determined they both had enough participants

at follow-up and 17.6 years 11.6 years, respectively, was sufficient follow-up time.

How precise are the results? Do you believe the results?
Four of the studies provided credible, precise results. For the CRIC study, I have indicated this study as high concern as the authors did not provide any numeric data and only described the results. For the remaining studies, the most common concerns were low numbers and/or imprecise estimates, which are often linked. Another major concern was risk of chance findings as many of the studies included many outcomes and subgroups, but did not control for, or even discuss, the possibility of type I error. It is commonly accepted that out of 20 analyses, one association will be statistically significant due to chance, so studies that provide more than 20 analyses without controlling for multiple testing could be at risk of spurious findings.

Do the results fit with other available evidence?
This question can still be quite subjective as there has not been extensive research in this field to establish an answer. However, the AIBL study reported decreased tortuosity associated with AD, which is inconsistent with other studies. This difference could be suggestive of misinterpretation of the results and not necessarily opposing findings. Also, this could also be a sign of publication bias, and more studies that had evidence of an opposing association may have chosen not to publish their findings.

Are sources of funding reported?
All studies reported where the funding for the study came from. However, the LALES study only reported the specific grant numbers for the funding, and not the funding bodies themselves.

3.5 **Discussion**

3.5.1 Summary of main results

Overall, there appears to be a relationship between decreased fractal dimension and increased risk of cognitive decline, which was especially

apparent when arterial and venular FD were evaluated separately. However, there were still only a few studies that evaluated this vessel trait. Venular width, as described by CRVE appeared to have some evidence to be wider in those more at risk of cognitive decline. There is currently little or no evidence supporting an association between retinal vessel tortuosity and cognitive decline. The findings from this systematic literature review will be further discussed in the general discussion chapter of this thesis, in context of the findings from the primary analysis **(Chapter 8)**

3.5.2 Quality completeness of data

Overall quality of the included studies was good. There were only a few cases of major concern, which included two studies that used either inferior recruitment methods or did not explain their recruitment methods, one study that did not adequately describe or control for other risk factors in their model, one study that did not provide any results data, only briefly described the results, and a final study that used improper statistical analysis methods. There were other, concerns with recruitment, as well as exposure and outcome measurement and not controlling for all appropriate covariates, but these were minor and more likely a product of a lack of reporting rather than study design.

3.5.3 Agreements and disagreements with similar reviews

It is important to compare the findings of this systematic review to other similar reviews in order to understand the relevance and accuracy of the findings. As there is currently a great amount of interest in understanding if RVTs can help to understand the pathogenesis and possibly predict cognitive decline there have been several other systematic reviews investigating a similar research question as this one. In this section I present the findings of four such systematic reviews and compare their methodology and findings to this systematic review (Ding *et al.*, 2008; Heringa *et al.*, 2013; Mcgrory *et al.*, 2017).

The structure of this systematic review was partly based on a previous review conducted by Ding et al. (Ding *et al.*, 2008). This review used a similar definition of cognitive decline, however the retinal signs included in the Ding review were more inclusive and included signs of retinopathy and other markers such as arteriovenous nicking. Ding included in the search all studies up until July 2007, when the search was conducted, and included a total of six studies, three of which evaluated the kinds of RVTs also included in this review. Meta-analysis was not conducted due to heterogeneity. In these three studies there was no evidence of an association between CRAE or CRVE and cognitive decline, which is in agreement with this review.

A review published in 2013 by Heringa et al. included studies with a any measure or retinal vasculature, like Ding *et al.*, 2008 (Heringa *et al.*, 2013). Their outcome included dementia and cogntivie functioning, similar to this review, but also included brain imaging measures. There was more inclusion and exclusion criteria applied within the Heringa et al. review, which included excluding studies in people with type 1 diabetes, studies in people <50 years, studies that did not control for age and population based studies with ≤250 people or patient-based studies with ≤50 people. The search was conducted between 1990 and 2013 and 16 studies were included, five of which were also included in this review. Meta-analysis was not conducted due to heterogeneity. This review concluded that in cross-sectional studies, while there was some association between both narrower CRAE and wider CRVE in those with dementia or decrease cognitive functioning the associations were inconsistent in direction and general not significant. In the longitudinal studies there was no association with CRAE and the associations with CRVE were mixed. Overall, these findings fit with the findings of this systematic review.

A recent review by McGrory et al., included any retinal vascular sign, including retinopathy and age-related macular degeneration, as well as the RVTs included in this review, and focused on studies with participants with a diagnosis of dementia (Mcgrory *et al.*, 2017). The review by McGrory et al. included 10 studies, five of which were included in this review. Because this

review focused solely on dementia as an outcome, they had more homogenous data and were able to conduct meta-analyses. For cross-sectional studies, there were mixed results regarding both CRAE and CRVE, but two of the three studies in the meta-analyses showed evidence of lower CRAE and CRVE in people with Alzheimer's dementia, but the third study showed no difference to controls, so overall there was no statistically significant difference. There was evidence of serious heterogeneity in each meta-analyses. The same three cross-sectional studies were included in a meta-analysis of fractal dimension, and there was evidence of reduced fractal dimension, or a sparser vascular network, in the cases with Alzheimer's compared to those without. In the single longitudinal study that reported on CRAE and CRVE there was no association dementia and CRAE, but there was evidence of wider CRVE in those with dementia, which was mostly driven by those with vascular dementia. The findings of the McGrory et al. review were similar this those of this review, including possible evidence of reduced fractal dimension in those with cognitive decline. Also, there is currently very little longitudinal evidence to support the association between RVTs and cognitive decline.

3.5.4 Conclusions

There is evidence to support an association between retinal vessel characteristics, specifically venular width and fractal dimension, and cognitive decline. These associations should be explored further, specifically with high powered longitudinal studies. Further discussion will be conducted in **Chapter 8**, specifically to help put the primary findings of this thesis in context.

Table 7. Case-control studies general characteristics

Study name	N	Population type	Mean age, years (SD)	Sex, Male %	How cognition/dementia was measured
SOUL-D	n = 137 (n = 69 cases; n = 68 controls)	Type 2 diabetes	55.63 (11.07)	64.7% cases; 44.9% controls	Cases chosen as lowest decile of TICSM scores
AIBL	n = 148 (n = 25 AD; n = 123 controls)	General	72.4 (7.5) AD; 71.6 (5.6) controls	48% AD; 45% controls	AD using NINCDS-ADRDA criteria
Cheung 2014	n = 426 (n = 136 AD; n = 290 controls)	General	74.8 (5.7) AD; 73.9 (4.6) controls	47% AD ; 53% controls	Clinically diagnosed dementia (clinical neurologic and neuropsychiatric assessment- CT or MRI reviewed as part of diagnostic process); Diagnosis using DSM-IV criteria, and AD diagnosis followed the NINDS-ADRDA criteria
Williams 2015	n = 507 (n = 213 AD; n = 294 controls)	General	79.6 (7.8) cases; 76.3 (6.6) controls	36% cases; 40% controls	Diagnosis of AD made by a senior clinician using the NINCDS-ADRDA criteria

AD = Alzheimer's disease; AIBL = Australian Imaging, Biomarkers and Lifestyle Flagship study of aging; CT = computerised tomography; DSM = Diagnostic and statistical manual of mental disorders; MRI = magnetic resonance imaging; NINCDS-ADRDA = National Institute of Neurological and Communicative Disorders and Stroke – Alzheimer's Disease and Related Disorders Association; SOUL-D = South London Diabetes Study; TICSM = modified Telephone Interview of Cognitive Status

Table 8. Cross-sectional studies general characteristics

Study name	N	Population type	Mean age, years (SD)	Sex, Male %	How cognition/dementia was measured
WESDR	n = 244	Type 1 diabetes	55.2 (8.3)	53.0%	Mental efficiency and executive function (Trail making Test A and B; Stroop Word and Interference Test; Grooved Pegboard; DSST; Verbal Fluency) nonverbal memory (Rey Complex Figure Test and DSST), verbal memory (Immediate and Delayed recall)
EDIS	n = 300	General	67.3 (4.8) NCI; 71.1 (6.3) CIND-mild ; 74.1 (5.4) CIND-moderate	56.2% NCI; 46.2% CIND-mild; 31.9% CIND-moderate	Consensus meetings held between study clinicians, neuropsychologists and research staff; reviewed clinical and blood assessments as well as neuropsychological tests and MRI scans to determine status: no cognitive impairment, cognitive impairment no dementia
CRIC	n = 588	Chronic renal insufficiency	65.3 (5.6)	52.6%	Six test battery: modified MMSE (3MS); Trail-Making Test A and B; Category fluency; Buschke Selective Reminding Test; Boston Naming Test
LBC1936	n = 683	General	72.5 (0.7)	51.5%	General intelligence factor *g* measured using domains of processing speed, visuospatial ability, memory and crystallised ability; derived from principle component analysis
LALES	n = 809	Latino	70.3 (6.9)	40.5%	CASI-S and SENAS; low CASI-S or low SENAS if their total CASI-S or SENAS score was <10th percentile for their age in 5 year categories
Dunedin study	n = 922	General	38	52.0%	WAIS-IV: (Trail making test, Mental Control and Verbal Paired Associates test, Rey Auditory Verbal Learning test, grooved-pegboard, one-legged balance and grip-strength tests of motor function and three tasks from Cambridge Neuropsychological Tests Automated Battery); 'informant reports' at age 38 using mailed questionnaires to a nominated person
ET2DS	n = 954	Type 2 diabetes	67.2 (4.2)	50.9%	7 cognitive function tests: Faces and Family Pictures subtest; Logical Memory from WMS-III; Matrix Reasoning; Letter–Number Sequencing, DSST from WAIS-III; Borkowski Verbal Fluency Test; Trail Making Test B; MHVS used to estimate pre-morbid cognitive ability
SiMES	n = 1202	General	68.5 (5.4)	50.7%	AMT score ≤6/10 in participants with 0-6 years of formal education and an AMT score ≤8/10 in those with more than 6 years of formal education

Study name	N	Population type	Mean age, years (SD)	Sex, Male %	How cognition/dementia was measured
Blue Mountain Eye Study	n = 1988	General	75.1 (9.1) cognitive impairment, 68.6 (8.2) no cognitive impairment	47.1% cognitive impairment; 42.6% no cognitive impairment	MMSE ≤23 defined as cognitive impairment

AMT = Abbreviated Mental Test; CASI-S = Cognitive Abilities Screening Instrument- Short form; CIND = cognitive impairment no dementia; CRIC = Chronic Renal Insufficiency Cohort; DSST = Digit symbol substitution test; EDIS = Epidemiology of Dementia in Singapore; ET2DS = Edinburgh Type 2 Diabetes Study; LALES = Los Angeles Latino Eye Study; LBC1936 = Lothian Birth Cohort 1936; MHVS = Mill Hill Vocabulary Scale; MMSE = mini-Mental State Exam; SENAS = Spanish English Neuropsychological Assessment Scales; SiMES = Singapore Malay Eye Study; WAIS = Wechsler Adult Intelligence Scale; WMS = Wechsler Memory Scale; WESDR = Wisconsin Epidemiologic Study of Diabetic Retinopathy

Table 9. Longitudinal studies general characteristics

Study name	N	Population type	Mean age, years (SD)	Follow-up years (SD)	Sex, Male %	How cognition/dementia was measured
EDC	n = 119	Type 1 diabetes	Time of cognitive assessment: 49.6 (7.0)	17.6 (8.0)	52.1%	DSST, Purdue Pegboard Test, Stroop colour word task, verbal fluency test, Trail Making Test B, animals verbal fluency task and the Rey-Osterrieth Complex Figure copy task; Participants with a single test score 1.5 standard deviations or worse were classified as having mild cognitive impairment and two or more test scores 1.5 standard deviations or worse were classed as clinically relevant cognitive impairment
Rotterdam Study	n = 5553	General	67.7 (8.0)	11.6 (4.4)	41.4%	First measured MMSE and GMS, if MMSE <26 and GMS >0 participant given Cambridge Examination of Mental Disorders in the Elderly and an informant interview; Participants suspected of having dementia further examined by a neurologist, a neuropsychologist, and if possible, had an MRI; also used direct linkage with medical records from GP; Diagnosis of dementia and subtype made in accordance with DSM-III-R

DSM = Diagnostic and statistical manual of mental disorders; DSST = Digit Symbol Substitution Test; GMS = Geriatric Mental State Exam; GP = general practitioner; MMSE = mini-Mental State Exam; MRI = magnetic resonance imaging

Table 10. Retinal Vessel Trait Assessment

Study name	Retinal vessel traits assessed	Software	Region measured
SOUL-D	CRAE, CRVE, AVR, tortuosity, FD	SIVA	0.5 to 2.0 disc diameters from disc margin; OD or macula centred, not specified
AIBL	CRAE, CRVE, AVR, tortuosity, FD, branching measures	SIVA	0.5 to 2.0 disc diameters from disc margin; OD centred
Cheung 2014	CRAE, CRVE, FD, tortuosity, and bifurcation angles	SIVA 3.0	0.5 to 2.0 disc diameters away from disc margin; OD or macula centred, not specified
Williams 2015	CRAE, CRVE, tortuosity, FD, branching angle	SIVA 3.0	0.5 to 2.0 disc diameters away from disc margin; OD or macula centred not specified
WESDR	CRAE, CRVE	Not specified	Not specified
EDIS	CRAE, CRVE, FD, tortuosity	SIVA 3.0	0.5 to 2.0 disc diameters away from disc margin; OD centred
CRIC	CRAE, CRVE	IVAN	0.5 to 1.0 disc diameters from disc margin; OD or macula centred, not specified
LBC1936	CRAE, CRVE, AVR, LDR, branching patterns, FD, tortuosity	SIVA V3	OD centred
LALES	CRAE , CRVE	Not specified	temporal edge of OD centred
Dunedin Study	CRAE, CRVE	SIVA 3.0	0.5 to 2.0 disc diameters from disc margin; OD centred
ET2DS	CRAE, CRVE	Semi-automatic software in MATLAB	0.5 to 1.0 disc diameters from disc margin; OD centred
SiMES	Tortuosity, FD, branching angle	SIVA 1.0	0.5 to 2.0 disc diameters from disc margin; OD or macula centred, not specified
Blue Mountains Eye Sudy	CRAE, CRVE	Retinal Analysis	0.5 to 1.0 disc diameters from disc margin; OD centred

Study name	Retinal vessel traits assessed	Software	Region measured
EDC	CRAE, CRVE (baseline, follow-up, change over time calculated as the slope using least absolute value median regression models, cumulative average)	IVAN	0.5 to 1.0 disc diameters from disc margin; OD centred
Rotterdam	CRAE, CRVE	Retinal Analysis	OD centred

AVR = arterial/venular ratio; CRAE = central retinal arterial equivalent; CRVE = central retinal venular equivalent; FD = fractal dimension; IVAN = Integrative Vessel Analysis; LDR = length to diameter ratio; SIVA = Singapore "I" Vessel Assessment

Table 11. Study results: vessel widths

Study name	Comparisons	CRAE	CRVE
SOUL-D	Lowest decile cognitive scores compared between cases and controls	No association	No association
AIBL	Compared vessel traits between AD cases and controls, mean (SD), FDR adjusted p-value	Controls 129.1 (SD 10.3); AD 122.9 (SD 12.4), p = 0.027	controls 182.7 (SD 15.8); AD 169.7 (SD 15.3), p = 0.0049
Cheung 2014	Per SD decrease (width and FD) or increase (tortuosity) in vessel traits between AD cases and controls	OR 1.22 (95% CI 0.78 to 1.91)	OR 2.01 (95% CI 1.27 to 3.19)- narrower in AD
Williams 2015	Per SD increase in vessel traits between AD cases and controls	OR 1.11 (95% CI 0.83 to 1.47) p = 0.481	OR 0.99 (95% CI 0.75 to 1.32) p = 0.960
WESDR	CRAE per 10um change for domains mental efficiency and verbal memory; CRVE per 15um change for domain mental efficiency; (beta (95% CI))	Mental efficiency 0.140 (95% CI 0.062 to 0.219) p <0.001; verbal memory −0.062 (95% CI −0.126 to 0.003) 0.06	Mental efficiency−0.127 (95% CI −0.207, −0.047) 0.002
EDIS	Moderate CIND compared with no cognitive impairment, per SD increase or decrease of vessel trait	No association when controlling for age and sex	No association when controlling for age and sex

Study name	Comparisons	CRAE	CRVE
CRIC	Mean test scores compared with CRAE and CRVE quartiles	No association	No association
LBC1936	general intelligence factor compared with 1 SD change in vessel trait	beta −0.001, p = 0.97	beta 0.010, p = 0.74
LALES	low CASI-S (lowest 10%) compared with CRAE lowest quartile and CRVE highest quartile	OR 2.17 (95% CI 1.19 to 3.97)- narrower CRAE	OR 1.08 (95% CI 0.59 to 1.96)
Dunedin Study	16 cognitive tests compared with vessel width	no association- data not reported	WAIS-IV (verbal comprehension beta -0.181 (95% CI -0.269 to -0.095) p < 0.001; perceptual reasoning beta -0.124 (95% CI -0.211 to -0.037) p = 0.005; working memory beta -0.147 (95% CI -0.234 to -0.061) p = 0.001; processing speed beta -0.141 (95% CI -0.224 to -0.055) p = 0.001); Executive function (TMTB beta 0.114 (95% CI 0.027 to 0.201) p = 0.010; WMS-III mental control beta -0.096 (95% CI -0.180 to -0.010) p = 0.028; CANTAB Rapid Visual Information Processing beta -0.126 (95% CI -0.215 to -0.039) p = 0.005) Memory (Rey Auditory Verbal learning: total recall beta -0.113 (95% CI -0.198 to -0.029) p = 0.008; Rey Auditory Verbal Learning: delayed recall beta -0.087 (-0.171 to -0.003) p = 0.043; WMS-III Paired Associates: total recall beta -0.068 (95% CI -0.171 to -0.003) p = 0.126; WMS-III Paired Associates: delayed recall beta 0.040 (95% CI -0.048 to 0.128) p = 0.372; CANTAB Visual Paired Associates Learning: total errors beta 0.080 (95% CI -0.007 to 0.164) p = 0.072) Motor Function (Grooved pegboard beta 0.113 (95% CI 0.028 to 0.198) p = 0.009; One-legged balance beta −0.152 (95% CI −0.240 to −0.065) p = 0.001; Grip strength beta 0.025 (95% CI −0.034 to 0.083) p = 0.408; CANTAB Reaction Time: 5-choice reaction time beta 0.007 (95% CI −0.079 to 0.094) p = 0.872

Study name	Comparisons	CRAE	CRVE
ET2DS	cognitive ability scores compared with vessel traits	VFT beta −0.025±0.038; Faces beta 0.027±0.039; TMTB beta 0.001±0.043; DST beta −0.065±0.038; MR beta −0.005±0.04; LM beta −0.066±0.039; LNS beta −0.012±0.048	VFT beta 0.030±0.042; Faces beta 0.052±0.043; TMTB beta 0.003±0.01; DST beta 0.057±0.037; MR beta 0.022±0.042; LM beta −0.029±0.027; LNS beta −0.008±0.024
Blue Mountains Eye Study	Cognitive impairment status compared with CRAE (narrowest quartile compared with widest) and CRVE (widest quartile compared with narrowest)	No difference	OR 1.8 (95 % CI 1.0 to 3.2) p = 0.03
EDC	Cognitive impairment status compared with baseline CRAE and CRVE	No cognitive impairment 179.9 um (SD 19.9); mild cognitive impairment 179.8 um (SD 16.8); clinically relevant cognitive impairment 178.5 um (SD 16.8); test of trend beta -0.18 (p = 0.69)	No cognitive impairment 266.5 um (SD 38.3); mild cognitive impairment 266.0 um (SD 35.6); clinically relevant cognitive impairment 264.1 um (SD 40.4); test of trend beta -0.94 (p = 0.29)
Rotterdam Study	Dementia status compared with width per SD decrease (CRAE) or increase (CRVE)	HR 1.05 (95% CI 0.96 to 1.16)	HR 1.11 (95% CI 1.00 to 1.22)

Study name	Comparisons	CRAE	CRVE

Data in RED indicates a statistically significant finding as specified by the study – for data that is not obvious for the direction of vessel change, I have made a note

AD = Alzheimer's disease; CANTAB = The Cambridge Neuropsychological Test Automated Battery; CASI-S = Cognitive Abilities Screening Instrument- Short form; CI = confidence interval; CIND = cognitive impairment no dementia; CRAE = central retinal arterial equivalent; CRVE = central retinal venular equivalent; FD = fractal dimension; HR = hazard ratio; LNS = letter-number sequence; MR = matrix reasoning; OR = odds ratio; SD = standard deviation; TMTB = trail making test B; VFT = verbal fluency test; WAIS = Wechsler Adult Intelligence Scale; WMS = Wechsler Memory Scale

Table 12. Study results: tortuosity and fractal dimension

Study name	Comparisons	Tort- a	Tort-v	FD total	FD-a	FD-v
SOUL-D	Lowest decile cognitive scores compared between cases and controls	No association	Difference in beta 0.013 (95% CI 0.002 to 0.25)- higher in cases compared with controls	No association		
AIBL	Compared vessel traits between AD cases and controls, mean (SD), FDR adjusted p-value	No association	Controls 7.660 (SD 1.554), AD 6.952 (SD 2.601), p = 0.042		Controls 1.235 (SD 0.052), AD 1.201 (SD 0.061), p = 0.021	Controls 1.210 (SD 0.05), AD 1.171 (SD 0.048), p = 0.003
Cheung 2014	Per SD decrease (width and FD) or increase (tortuosity) in vessel traits between AD cases and controls	OR 1.80 (95% CI 1.40 to 2.31)- increased in AD	OR 1.94 (95% CI 1.48 to 2.53)- increased in AD	OR 1.54 (95% CI 1.23 to 1.93)- decreased in AD	OR 1.35 (95% CI 1.08 to 1.68) - decreased in AD	OR 1.47 (95% CI 1.17 to 1.84)- decreased in AD

Study name	Comparisons	Tort- a	Tort-v	FD total	FD-a	FD-v
Williams 2015	Per SD increase in vessel traits between AD cases and controls	OR 0.78 (95% CI 0.63 to 0.97) p = .027- decreased in AD	OR 1.01 (95% CI 0.82 to 1.24) p = 0.952	OR 0.85 (95% CI 0.68 to 1.06) p = 0.141	OR 0.92 (95% CI 0.74 to 1.14) p = 0.436	OR 0.77 (95% CI 0.62 to 0.97) p = 0.025- decreased in AD
EDIS	Moderate CIND compared with no cognitive impairment, per SD increase or decrease of vessel trait	0.82 (0.53 – 1.25)	No association when controlling for age and sex		OR 1.86 (95% CI 1.20 to 2.88)- decreased FD	OR 2.09 (95% CI 1.35 to 3.22)- decreased FD
LBC1936	general intelligence factor compared with 1 SD change in vessel trait	beta 0.014, p = 0.64	beta 0.002, p = 0.96		beta 0.014, p = 0.65	beta 0.016, p = 0.61
SiMES	Cognitive impairment status compared with quintiles of vessel traits	no association	no association	quintile 1 compared with quintile 5 OR 1.71 (95% CI 1.03 to 2.82) p = 0.036		

Data in RED indicates a statistically significant finding as specified by the study – for data that is not obvious for the direction of vessel change, I have made a note

AD = Alzheimer's disease; CI = confidence interval; CIND = cognitive impairment no dementia; CRAE = central retinal arterial equivalent; FD = fractal dimension; HR = hazard ratio; OR = odds ratio; SD = standard deviation

Figure 9. CRAE and risk of cognitive decline

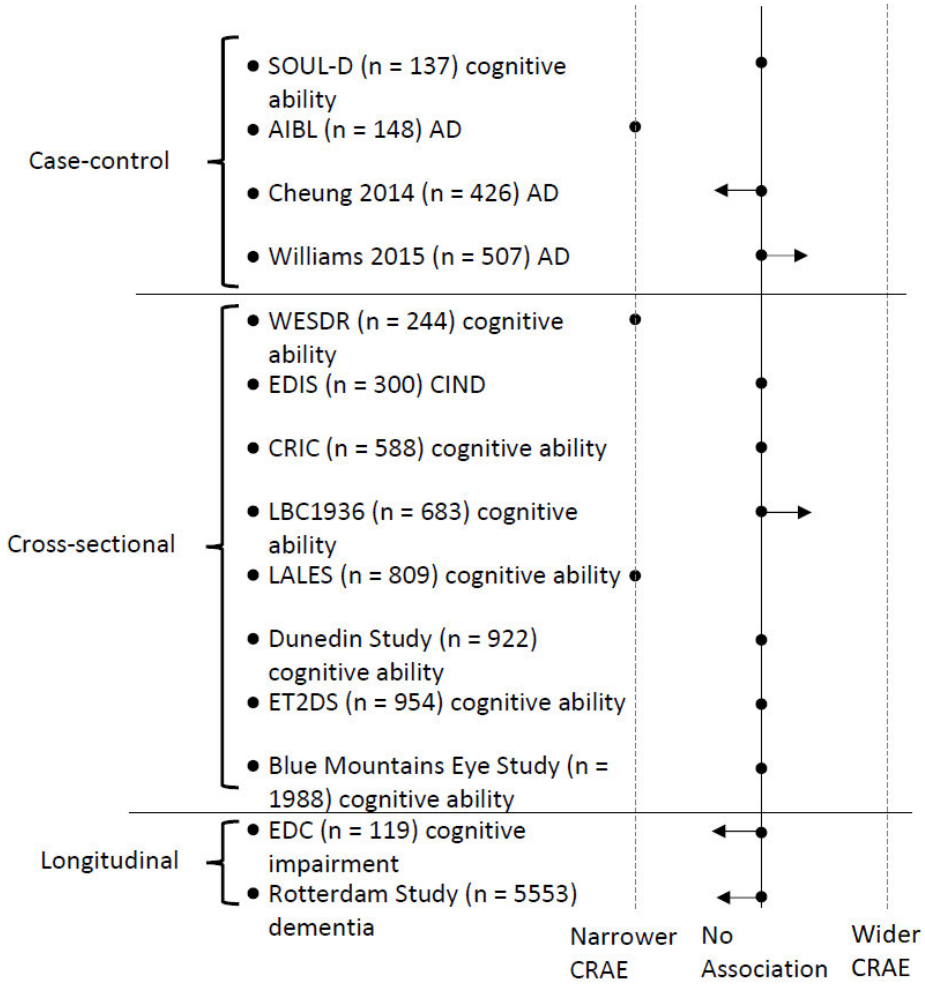

Figure 10. CRVE and risk of cognitive decline

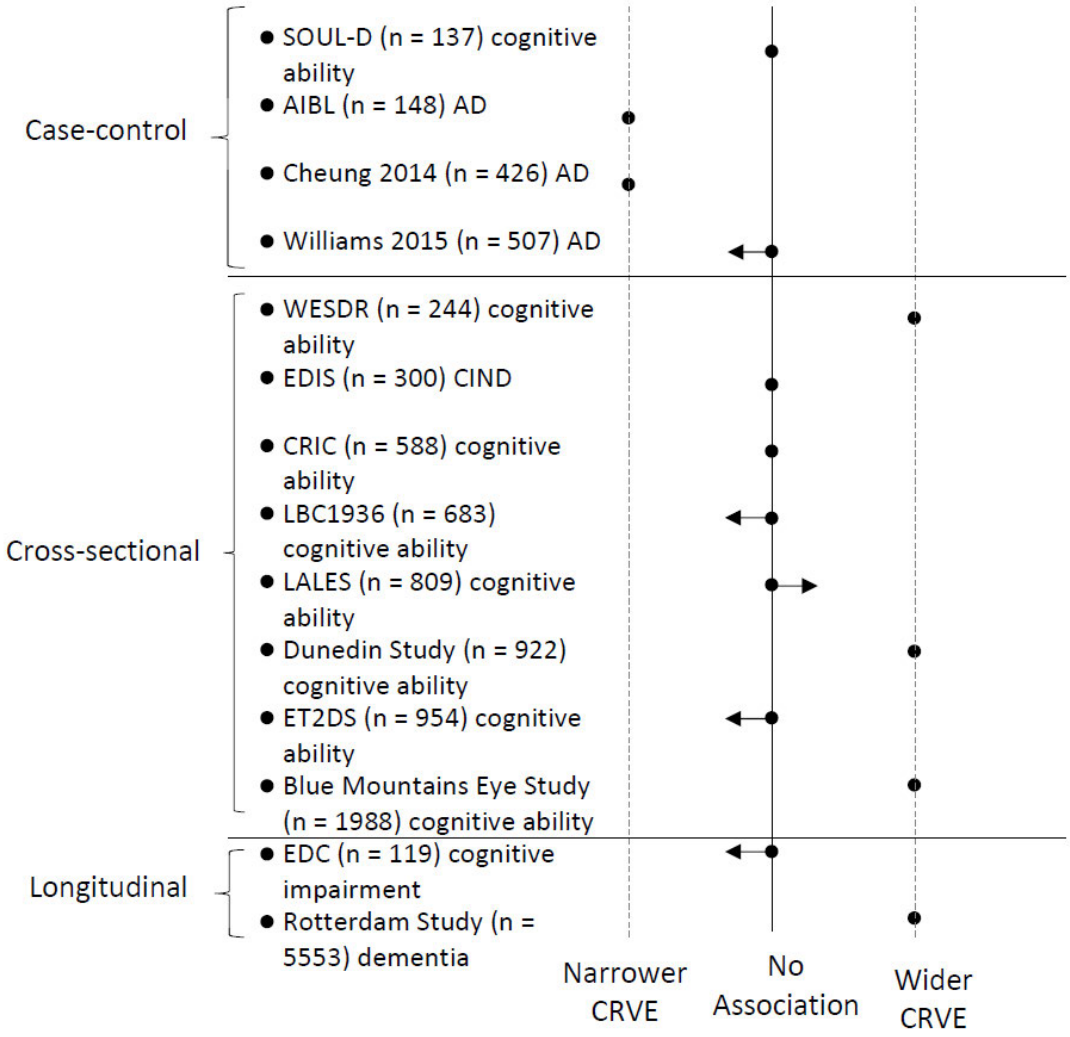

Figure 11. Arterial tortuosity and risk of cognitive decline

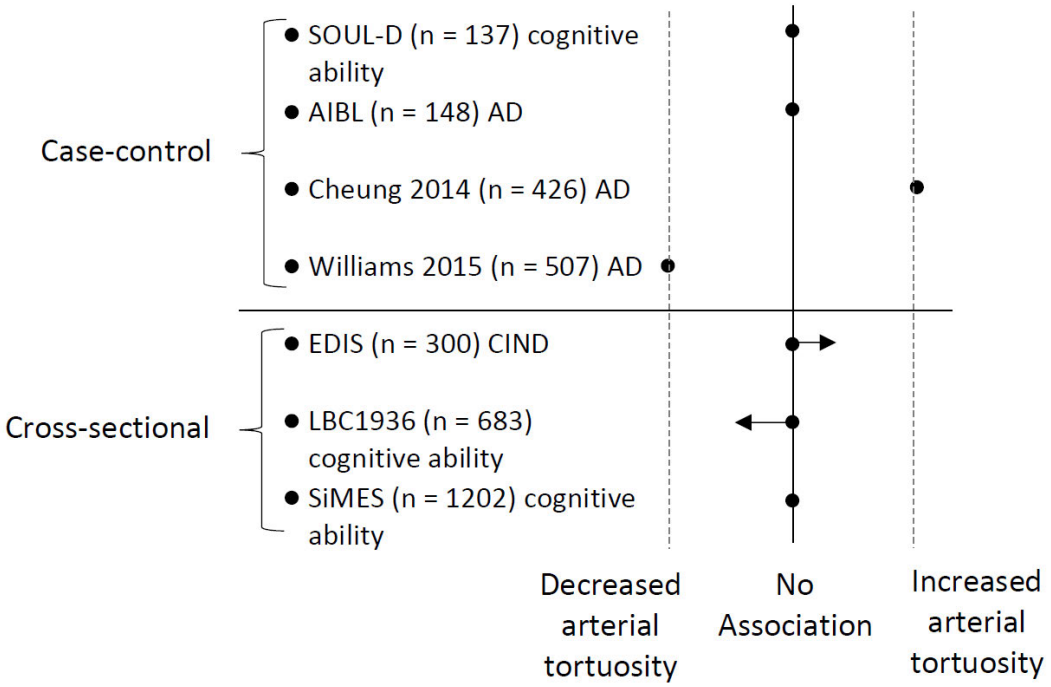

Figure 12. Venular tortuosity and risk of cognitive decline

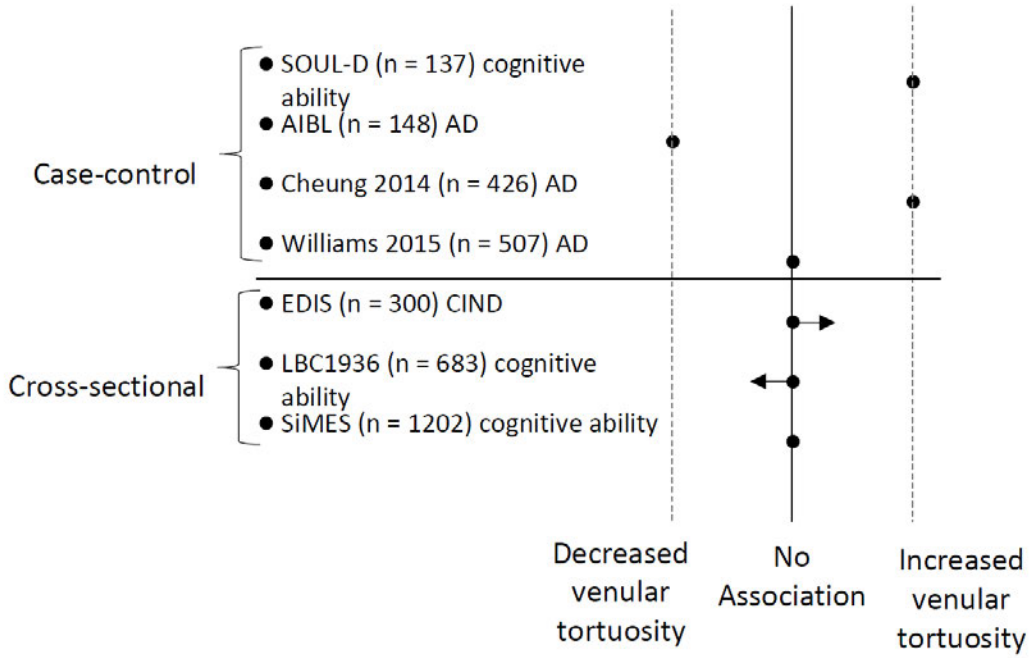

Figure 13. Total fractal dimension and risk of cognitive decline

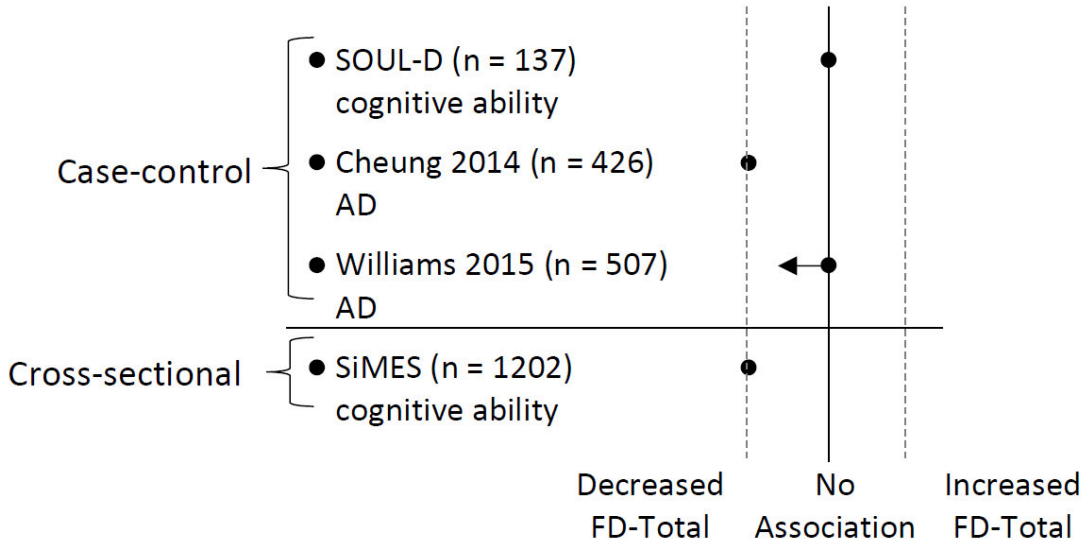

Figure 14. Arteriolar fractal dimension and risk of cognitive decline

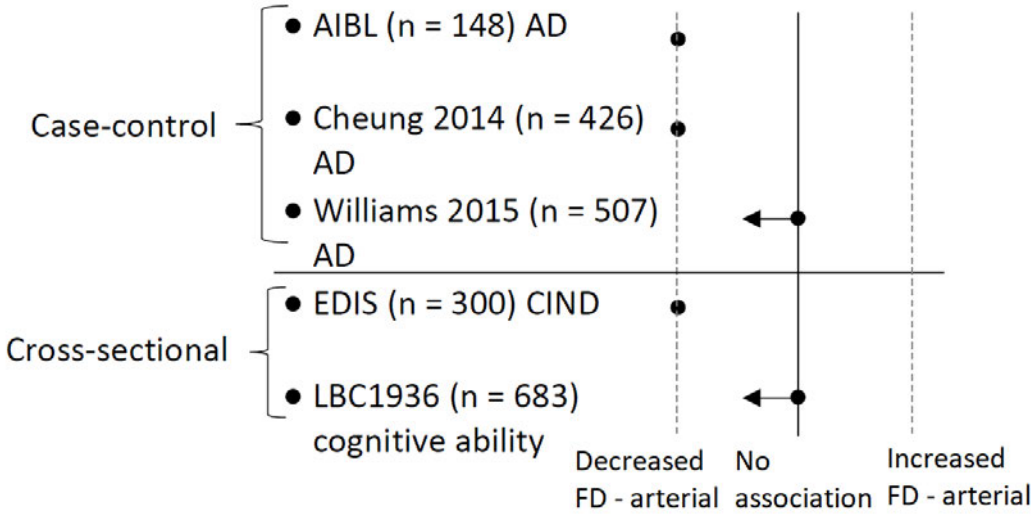

Figure 15. Venular fractal dimension and risk of cognitive decline

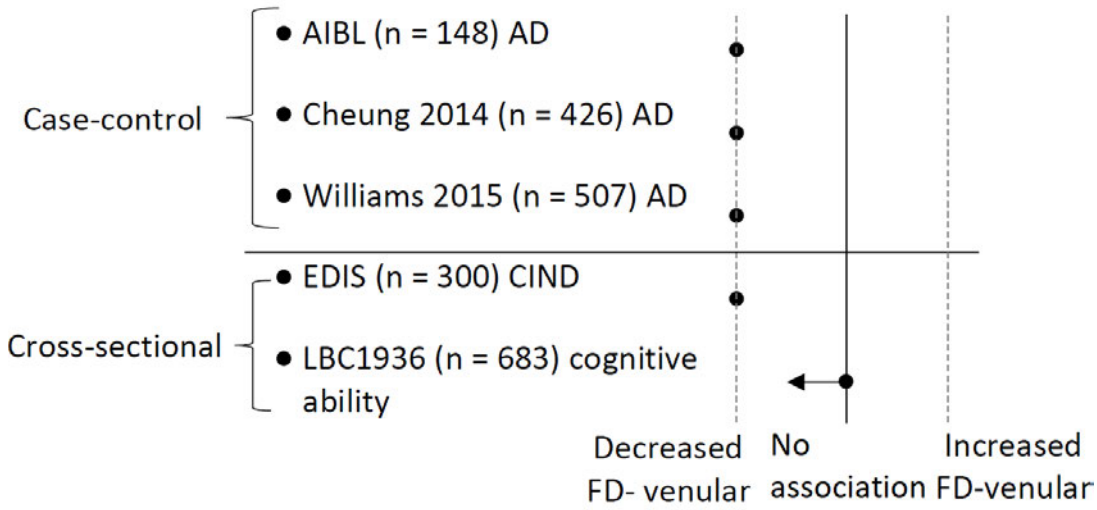

Table 13. Quality Assessment of Included Studies using a modified CASP Tool

Study	Study recruited in an acceptable way?	Exposure and outcome accurately measured?	Confounding factors included in the analysis?	Follow complete and long enough?	How precise are the results?
SOUL-D	Cases were identified as the lowest 10% scoring of TICSM, but 69 is lower than 10% of the available population (n = 1084), should be n = 108; Number of controls low, and did not indicate if they age/sex matched controls to cases, only that they were 'randomly' selected	Used trained grader using SIVA software and a specified protocol, but did not specify blinding; reported acceptable intra/intergrader reliability	Did not include lipid markers or prevalent CVD	n/a	Used small numbers of cases and controls so results were not very precise
AIBL	Overall, the study does not specifically explain their methodology for choosing participants in the retinal study from the larger cohort; They had a larger number of those with an AD diagnosis than enrolled; No indication of how the controls were matched and if they are of a good match	Exposure measured with SIVA software, with trained, masked graders, and used standardised protocol but no intra/intergrader reliability measures reported	Did not include lipid markers or prevalent CVD	n/a	Did not provide CI's but the p-values were quite strong after false discovery rate controlling methods

Study	Study recruited in an acceptable way?	Exposure and outcome accurately measured?	Confounding factors included in the analysis?	Follow complete and long enough?	How precise are the results?
Cheung 2014	Cases enrolled consecutively from one of three tertiary hospitals in Singapore; unclear if any exclusion criteria was applied; Controls, with no history of stroke or cognitive impairment, were selected from the Singapore Epidemiology of Eye Disease (SEED) program and matched by race, gender and age, controls had higher blood pressure and less likely to have diabetes, compared with cases	Trained, masked graders using SIVA software and standardised protocol; study reported inter and intra-grader reliability (high variation) and states in discussion that there were measurement errors related to these measures that could lead to misclassification; unclear if retinal imaging were taken at different times and places compared with controls	Yes	n/a	Relatively precise; did not correct for multiple testing and included many outcomes

Study	Study recruited in an acceptable way?	Exposure and outcome accurately measured?	Confounding factors included in the analysis?	Follow complete and long enough?	How precise are the results?
Williams 2015	Utilised an opportunistic recruitment strategy: participants identified, non-systematically, in clinic and files from the population of those with a diagnosis of AD, made by a senior clinician using the NINCDS-ADRDA criteria within a hospital memory clinic; those with mixed or vascular dementia not included; Controls recruited from (1) carers of patients attending any out-patient clinic in the study hospital, (2) using a university press release, (3) cases asked friends to participate, (4) a series of talks given to AD patient support groups in the region led to volunteers; controls selected as 'cognitively normal' and they were not matched on important criteria to cases	Utilised SIVA software and a standard protocol; analysis performed by a blinded, single assessor, and reported good intragrader reliability	Yes	n/a	The participant numbers were very low and the authors did not account for multiple testing, but the CIs were not too wide
WESDR	Sample selected from 10135 (identified by medical charts) diabetic patients who received primary care in an 11-county area in southern Wisconsin from 1979 to 1980	For the exposure, only described as 'computer-assisted'; For the outcome, no information was provided on number of testers or any training received	Did not control blood pressure, lipids or CVD in to their	n/a	Results were not precise and they did not correct for multiple testing

Study	Study recruited in an acceptable way?	Exposure and outcome accurately measured?	Confounding factors included in the analysis?	Follow complete and long enough?	How precise are the results?
EDIS	Study population derived from larger Singapore Chinese Eye Study (SCES) which had n = 4604 that were randomly selected from the community and invited for an interview; but only very small number in SCES (~9%) enrolled in EDIS	As mentioned, the specific vessel traits that were to be evaluated as exposure variables were not specified (aside from FD) in the methods; utilised SIVA software and trained, masked graders using a protocol from SCES, but did not report intra/inter-grader reliability; outcome evaluated by trained research psychologists who administered cognitive tests in habitual language and MRIs were graded blindly	Yes	n/a	The study included very small numbers in some analyses leading to weak associations and wide Cis
CRIC	Identified from seven clinical centres around the US, which will have renal insufficiency enrichment within specialised clinics, which each to enrol 430 to 500 in a 33 month period; most centres utilise an central large database to identify participants	Did not indicate if graders were trained/masked or intra/inter-grader reliability, but use a protocol and IVAN software; did not state who conducted cognitive tests	Did not control for lipids or CRAE/CRVE and hypertension was only included as a dichotomous variable	n/a	No data was actually provided, only descriptive
LBC1936	Participants identified from the Scottish Mental Survey 1947 and invited to participate	Exposure measured with SIVA software and trained graders; no intra/inter-grader correlations available for these study images but the same grader, using the same software on a different pop, had acceptable reliability measures; Measured outcome accurately and utilised a generalised intelligence factor	Yes	n/a	No associations survived after false discover rate methods

Study	Study recruited in an acceptable way?	Exposure and outcome accurately measured?	Confounding factors included in the analysis?	Follow complete and long enough?	How precise are the results?
LALES	Door-to-door census of all residents living within six census tracts in La Puente, California, conducted between 2000-2004 to identify eligible individuals; of 7789 eligible, 6357 (81.6%) completed clinical exam	To measure exposure, used semi-automated software (did not specify) and also did not describe blinding, training or use of a protocol and did not report on inter/intra grader comparisons; to measure the outcome, utilised appropriate tests, but did not discuss training, or how many testers there were	Did not include stroke or lipids	n/a	Had quite wide CIs, reducing precision
Dunedin Study	Complete birth cohort that included 91% of eligible births from April 1972 to March 1973 (n = 1037) in Dunedin, New Zealand	Utilised trained, masked graders using SIVA software and a protocol, but did not report intra/inter grader reliability; The outcome was measured accurately with validated tests but no information on who performed the tests	Only controlled for gender and CRAE/CRVE	n/a	Results at high risk of multiple testing and did not provide data for CRAE
ET2DS	Participants were identified from the Lothian Diabetes Register and shown to be representative	The exposure was evaluated by trained, blinded single grader using 'custom-written validated image analysis program', and reported an acceptable intragrader reliability; For the outcome, a seven cognitive test battery was utilised, given by specially trained research assistants, trained and observed for validation by one expert	Yes	n/a	Moderately precise but likelihood of chance findings

Study	Study recruited in an acceptable way?	Exposure and outcome accurately measured?	Confounding factors included in the analysis?	Follow complete and long enough?	How precise are the results?
SiMES	Age-stratified by 10 year age group from random sampling method from computer-generated list provided by Singapore Ministry of Home Affairs - 4148 eligible, 3280 (78.7% participated); fair representation of Singapore population in terms of age distribution, housing type, socioeconomic status; not specified how they got to n = 1202 for this study	For the exposure, the study utilised SIVA software with trained, masked graders utilising a standard protocol; study reported inter/intragrader reliability (high variation) and states in discussion that there were measurement errors related to these measures that could lead to misclassification; For the outcome, the study used a validated, modified test but no description of training or who performed the tests	Did not control for CRVE/CRAE and used blood pressure and lipids as dichotomous variables and not continuous	n/a	For full analysis the CIs were relatively narrow but for subgroup/stratified analysis the n values were very small which resulted in quite imprecise results
Blue Mountains Eye Study	Invited all community dwelling, permanent residents in two postcode area of west Sydney, Australia, aged ≥49 years; 11.3% (501) refused to participate, 353 of them (8%) agreed to a brief interview	Used masked graders and Retinal Analysis software and reported satisfactory inter/intragrader reliability; Outcome derived using MMSE, which was given by trained medical practitioner; single use of MMSE as a cognitive ability score may lead to misclassification bias, although used a low cut-point	Yes	n/a	Quite wide CI's that include 1.0 when they report p < 0.05

Study	Study recruited in an acceptable way?	Exposure and outcome accurately measured?	Confounding factors included in the analysis?	Follow complete and long enough?	How precise are the results?
EDC	Participants in the EDC study (n = 658) were diagnosed with childhood-onset type 1 diabetes between 1950 and 1980 and were seen within 1 year of diagnosis at the Children's Hospital of Pittsburgh; no further discussion regarding representativeness of study; participants for this analysis (n = 119) included participants with at least 3 retinal images during the 28 years of follow-up; the analysis sample was described as being younger and with less duration of diabetes and the larger cohort	The exposure was measured using IVAN software with validated methods, but no information was provided on who graded the images and if there was any measures of inter/intragrader reliability;The outcome was measured accurately with validated tests but no information on who performed the tests	Study authors described controlling for various parameters but it was not at all clear if the covariates were all combined in a single model or if they just evaluated each variable individually with the outcome; Also, only presented some analyses as uncontrolled	Yes, 17.6 years	Authors did not present 95% confidence intervals; standard deviations of individual measures were relatively large indicating a wide variation
Rotterdam	All inhabitants aged 55 years and over in a district of Rotterdam, Netherlands, n = 10274 eligible subjects, n = 7983 participated in baseline	Trained, masked graders, using Retinal Analysis software, with good intragrader and intergrader correlation; Outcome measured with highly specified diagnosis criteria for dementia and it's subtypes by neurologists and neuropsychologists	Yes	Yes, 11.6 years	Yes

AD = Alzheimer's dementia; CI = confidence interval; CRAE = central retinal arterial equivalent; CRVE = central retinal venular equivalent; CVD = cardiovascular disease; FD = fractal dimension; IVAN = Integrative Vessel Analysis; ; NINCDS-ADRDA = National Institute of Neurological and Communicative Disorders and Stroke – Alzheimer's Disease and Related Disorders Association; SIVA = Singapore "I" Vessel Assessment; TICSM = modified Telephone Interview of Cognitive Status

3.6 References- included studies

Australian Imaging, Biomarkers and Lifestyle Flagship study of aging (AIBL)

Ellis, K.A. et al., 2009. The Australian Imaging, Biomarkers and Lifestyle (AIBL) study of aging: Methodology and baseline characteristics of 1112 individuals recruited for a longitudinal study of Alzheimer's disease. *International Psychogeriatrics*, 21(4), pp.672–687.

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Frost, S. et al., 2013. Retinal vascular biomarkers for early detection and monitoring of Alzheimer's disease. *Translational psychiatry*, 3, p.e233.

The Blue Mountains Eye Study (BMES)

Liew, G. et al., 2009. Retinal microvascular signs and cognitive impairment. *Journal of the American Geriatrics Society*, 57(10), pp.1892–1896.

Cheung 2014

Cheung, C. et al., 2013. Evidence of microvascular network alterations in the retinas of people with Alzheimer's disease. *Alzheimer's and Dementia*, 9(4 SUPPL. 1), p.P743.

Cheung, C.Y.L. et al., 2014. Microvascular network alterations in the retina of patients with Alzheimer's disease. *Alzheimer's and Dementia*, 10(2), pp.135–142.

Ong, Y.-T. et al., 2013. Assessment of retinal microvasculature mayaid dementia subtype differentiation. *Alzheimer's and Dementia*, 9(4), pp.P331–P332.

Chronic Renal Insufficiency Cohort (CRIC)

Grunwald, J.E. et al., 2012. Association between retinopathy and cardiovascular disease in patients with chronic kidney disease (from the Chronic Renal Insufficiency Cohort [CRIC] Study). *American Journal of Cardiology*, 110(2), pp.246–253.

Yaffe, K. et al., 2013. Retinopathy and cognitive impairment in adults with CKD. *American Journal of Kidney Diseases*, 61(2), pp.219–227.

Dunedin Multidisciplinary Health and Development Study (DMHDS)

Shalev, I. et al., 2013. Retinal Vessel Caliber and Lifelong Neuropsychological Functioning: Retinal Imaging as an Investigative Tool for Cognitive Epidemiology. *Psychological Science*, 24(7), pp.1198–1207.

Epidemiology of Dementia in Singapore (EDIS)

Hilal, S. et al., 2013. Prevalence of cognitive impairment in Chinese: Epidemiology of Dementia in Singapore study. *Journal of Neurology, Neurosurgery and Psychiatry*, 84(6), pp.686–692.

Ikram, M.K. et al., 2012. Retinal microvascular geometric parameters and cognitive impairment. *Journal of Hypertension*, 30, p.e119.

Ong, Y.-T. et al., 2014. Retinal Vascular Fractals and Cognitive Impairment. *Dementia and Geriatric Cognitive Disorders Extra*, 4(2), pp.305–313.

Edinburgh Type 2 Diabetes Study (ET2DS)

Ding, J. et al., 2011. Association of retinal arteriolar dilatation with lower verbal memory: The Edinburgh Type 2 Diabetes Study. *Diabetologia*, 54(7), pp.1653–1662.

Ding, J. et al., 2011a. Retinal arteriolar dilatation is associated with impaired memory in men: the Edinburgh Type 2 Diabetes Study. *Diabetic Medicine*, 28, pp.42–43.

Los Angeles Latino Eye Study (LALES)

Gatto, N.M. et al., 2011. Retinal microvascular abnormalities and cognitive function in latino adults in los angeles. *American Journal of Epidemiology*, 173, p.684.

Gatto, N.M. et al., 2012. Retinal microvascular abnormalities and cognitive function in latino adults in Los Angeles. *Ophthalmic Epidemiology*, 19(3), pp.127–136.

Lothian Birth Cohort 1936 (LBC1936)

McGrory, S. et al., 2016. Retinal microvascular network geometry and cognitive abilities in community-dwelling older people: The Lothian Birth Cohort 1936 study. *British Journal of Ophthalmology*, p.993-998.

Patton, N. et al., 2007. The association between retinal vascular network geometry and cognitive ability in an elderly population. *Investigative Ophthalmology and Visual Science*, 48(5), pp.1995–2000.

Taylor, A.M. et al., 2015. Retinal vascular fractal dimension, childhood IQ, and cognitive ability in old age: the Lothian Birth Cohort Study 1936. *PloS one*, 10(3), p.e0121119.

Pittsburgh Epidemiology of Diabetes Complications Study (EDC)

Nunley, K.A. et al., 2015. Early development of proliferative retinopathy increases risk of cognitive dysfunction in middle-aged adults with type 1 diabetes. *Neurology,* 84(3), p. 223-32

Nunley, K.A. et al., 2018. Long-term changes in retinal vascular diameter and cognitive impairment in type I diabetes. *Diabetes & Vascular Disease Research,* 15(3), p. 223-32

The Rotterdam Study

de Jong, F.J. et al., 2011. Retinal vascular caliber and risk of dementia: The Rotterdam Study. *Neurology*, 76(9), pp.816–821.

Singapore Malay Eye Study (SiMES)

Cheung, C.Y. et al., 2011. Quantitative and qualitative retinal microvascular characteristics and blood pressure. *Journal of Hypertension*, 29(7), pp.1380–1391.

Cheung, C.Y.-L. et al., 2014. Retinal vascular fractal dimension is associated with cognitive dysfunction. *Journal of Stroke and Cerebrovascular Diseases*, 23(1), pp.43–50.

SOUL-D

Naidu, V. et al., 2015. Associations between retinal markers of microvascular disease and cognitive impairment in newly diagnosed Type 2 diabetes. *Diabetic Medicine*, 32 (Suppl., pp.42, P43.

Naidu, V. et al., 2016. Associations between Retinal Markers of Microvascular Disease and Cognitive Impairment in Newly Diagnosed Type 2 Diabetes Mellitus: A Case Control Study. *PloS one*, 11(1), p.e0147160.

Wisconsin Epidemiologic Study of Diabetic Retinopathy (WESDR)

Ryan, C.M. et al., 2016. Associations between recent severe hypoglycemia, retinal vessel diameters, and cognition in adults with type 1 diabetes. *Journal of Diabetes and its Complications*, 30(8), pp.1513–1518.

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Williams 2015

Williams, M.A. et al., 2014. The prevalence of age-related macular degeneration in Alzheimer's disease. *Journal of Alzheimer's Disease*, 42(3), pp.909–914.

Williams, M.A. et al., 2015. Retinal microvascular network attenuation in Alzheimer's disease. *Alzheimer's and Dementia: Diagnosis, Assessment and Disease Monitoring*, 1(2), pp.229–235.

4 Methods

The purpose of the methods chapter is to lay out the research questions I intend to answer in this thesis, provide details on the data and analysis methods used in this thesis. I will describe the design of the Edinburgh Type 2 Diabetes Study (ET2DS), which is the cohort of people used in the analysis. Data collection methods for the cohort at baseline and year-10 follow-up will be clarified, and analysis techniques for each research question described. I was actively involved in the primary data collection for the year-10 follow-up of ET2DS, which included interviews in a clinical or home setting, as well as linkage with routinely collected data. Previously collected data on general health and demographics, as well as retinopathy and cognitive data from the baseline of ET2DS have been reproduced and modified for the specific needs of this thesis. The protocol for the baseline phase of the ET2DS has been previously published as well as information on baseline retinopathy and cognition (Price *et al.*, 2008; Ding *et al.*, 2010; Marioni *et al.*, 2010). I used the checklist set out in the Strengthening the Reporting of Observational studies in Epidemiology (STROBE) statement for reporting methods and findings throughout this thesis (Elm *et al.*, 2008).

4.1 Research questions

For this thesis, I aim to evaluate and answer the following research questions:

1. Is there an association between baseline retinal vessel traits and incident diabetic retinopathy after 10 years of follow-up?
2. Is there an association between baseline retinal vessel traits and incident cognitive decline after 10 years of follow-up?
3. Is there a change in retinal vessel traits over 10 years and are these changes associated with cognitive decline?

4.2 Edinburgh Type 2 Diabetes Study

To answer these research questions, I used data from ET2DS, which is a population-based prospective cohort established in 2006-2007. The participants are made up of 1066 men and women aged between 60 and 75 years at baseline, all with established type 2 diabetes and living in the Lothian region of central Scotland. The study was established in order to investigate cognitive and vascular complications of type 2 diabetes and the potential risk factors and pathways that are associated.

4.2.1 Study population recruitment and follow-up

Participants of ET2DS were chosen from the Lothian Diabetes Register (LDR), now a part of the Scottish Care Information-Diabetes Collaboration (SCI-DC), which contains clinical information on nearly all people with diabetes living in Lothian. At the time of the study baseline, all people within the LDR had a diagnosis of diabetes according to the World Health Organisation (WHO) criteria, described in **Section 1.1**, but in order to be enrolled in ET2DS participants had to meet certain criteria to ensure a robust diagnosis of type 2 diabetes. These criteria included:

- participants had to be taking oral anti-diabetic medications and/or insulin;
- or, if participants managed their diabetes through diet control methods alone, had to have a glycated haemoglobin (HbA1c) measure of >6.5% at the baseline clinic.

If a person was only using dietary methods to control their diabetes and had an HbA1c measurement below 6.5%, their clinical records were reviewed and the diabetes diagnosis confirmed by a consultant diabetologist. In order to make sure enrolled participants did not have type 1 diabetes, records were carefully evaluated and potential participants excluded if the person started insulin within one year of diagnosis, or if there was evidence of pancreatic disease at the baseline clinic, or if they were treated with insulin and under 35 years of age at diagnosis. Other reasons for exclusion were:

- a potential participant's diagnosis could not be confirmed and their clinical and/or GP records could not be reviewed;
- they were non-English speakers;
- had poor eyesight (corrected visual acuity was worse than 6.36 for distance vision or unable to read large print text);
- they were unable or unwilling to provide consent for their participation;
- those who were physically unable to complete all elements of the assessment.

The exclusions based on English language and eyesight were made because they prohibited participants from adequately undertaking certain cognitive tests.

ET2DS Sample size

A sample size of 1000 participants was calculated as the number needed to achieve 90% power (at the 5%, two-tailed significance level) to detect the difference between a continuous outcome measure and predictor variable with a Pearson correlation coefficient of ≥0.10. This sample size was calculated to be sufficient to detect, at the same power and significance level, any risk factor that contributed 1% or more to the variance in the outcome. Considering this study was designed as a prospective cohort, it was determined that retention of 800 participants at follow-up would be enough to achieve 90% power at the same significance level with a Pearson correlation coefficient ≥0.12.

ET2DS Baseline Recruitment

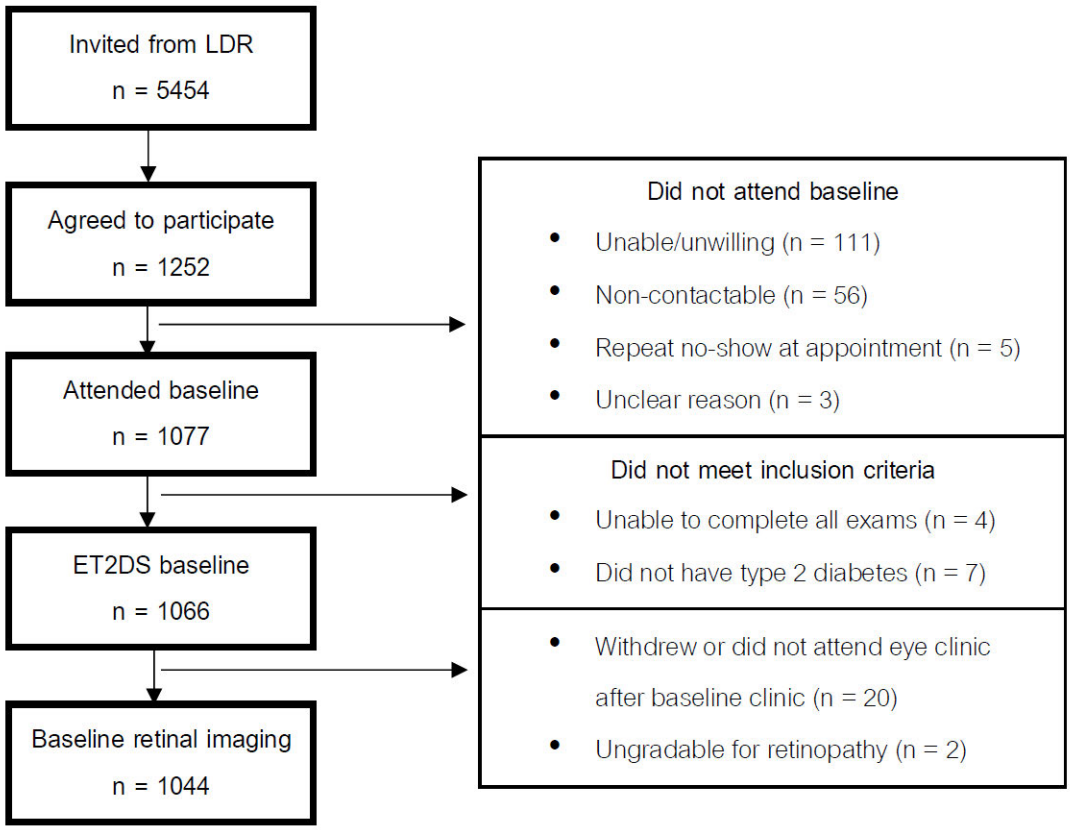
Recruitment of participants to the ET2DS commenced August 1, 2006, at which time a randomly selected sample of men and women by five-year age bands were selected from the eligible people in the LDR. A total of 5454 eligible participants were invited and 1252 agreed to participate. From the 1252, 1077 attended the baseline clinic. Eleven participants were excluded after or during attendance: four participants were not able to complete all the examinations for physical or emotional reasons, and seven did not meet the

ET2DS criteria for type 2 diabetes after full review of their clinical records. A total of 1066 participants were enrolled (Marioni *et al.*, 2010).

In order to obtain retinal fundus images that were used to evaluate retinopathy and retinal vessel traits, participants were asked to return for an appointment with a trained ophthalmologist. Fundus imaging took place within 2-3 weeks after the baseline clinic. Of the 1066 enrolled participants, 1044 returned for fundus imaging (Ding *et al.*, 2010). More information on the study retinal images can be found below in section 4.3.2. See **Figure 16** for a full description of the baseline participant flow.

Data collection clinics took place at one year and four years after the initial baseline clinic. These time points are not discussed within this thesis as data were not used from these time points for any of the analyses.

Figure 16. ET2DS baseline participant attendance



Year-10 follow-up

Throughout the first year of my PhD programme I became actively involved in the primary data collection for the year-10 follow-up for ET2DS. This included data collection with participants in both the clinical and home setting, as well as arranging appointments and transportation for participants and liaising with the research staff where the clinical appointments took place and samples processed. Prior to sending appointment invitations for this phase of ET2DS, a newsletter was sent out to all participants that were still enrolled within the study and not known to be deceased. The newsletter, created by another ET2DS team member/PhD student (Anniek Sluiman, AS) with contributions from the study team, included information on findings from ET2DS as well as other diabetes related information. This was done in order to remind participants of their continuing enrolment in the study prior to receiving the clinic attendance letter, as it had been several years since their last invitation. If newsletters were returned by mail, the participant's information was looked up within the NHS Lothian electronic health records system, known as TRAK, for any changes to the participant's contact details or if they were deceased.

Clinic invitations were sent out two weeks prior to the intended date, with a proposed date and time of an appointment and included a return envelope and tear-off page where the participant could indicate if they could make the appointment. Participants that returned the slip stating that they could not attend or participants that we did not hear back from were contacted by phone to arrange a suitable time. If a participant could not be contacted, they were added to a list to check if their contact details had changed or if they were deceased. Participants that could still not be reached were added to a list to be contacted at a later date. Participants that did not want to, or could not, attend a clinic visit were offered a home visit appointment. If this was unacceptable, we requested the reason they did not want to attend for the purposes of evaluating non-attendance.

Clinic appointments for the year-10 follow-up began on August 1, 2016. One researcher, AS, began undertaking appointments at the Western General

Hospital's Wellcome Trust Clinical Research Facility (WTCRF) and I joined on October 1, 2016. Primary data collection was completed for this phase in the end October 2017.

Ethical approval

At all data collection time points full consent was obtained from all participants at the onset of the appointment. At baseline, the study received full ethical permission from the Lothian Medical Research Ethics committee to carry out data collection. All data linkage that took place throughout the study period was also done under full ethical permission.

New and updated requisite research and development (R&D) and ethical approvals from the NHS were obtained for the year-10 follow-up prior to initiation of data collection. The application for R&D and ethical approval was submitted using the Integrated Research Application System (IRAS), NHS R&D Form Version 5.3.0, by AS, along with the study's principle investigator, Jackie Price. The Lothian NHS Board gave a favourable ethical opinion for the application on May 23, 2016 and R&D approval was granted on June 17, 2016. The favourable response letters can be found in **Appendix 10 and Appendix 11**. Most of the data collection took place at the WTCRF, which provides specialist support for undertaking clinical research, including clinical facilities, research nursing staff support and medical laboratory for sample processing. Along with the R&D approval, I was granted a three-year NHS Honorary Research Contract after lodging a Research Passport application, Version 3, which allowed me to carry out research activities detailed in the R&D approved application for the study.

4.2.2 Data collection

Baseline clinic examinations

At the baseline clinic, a wide number of physiological and cognitive assessments were undertaken by members of the ET2DS team working on the study at that time. Also, medical and other health related questionnaires were collected in order to get a full medical profile for each participant. All

assessments were performed by trained researchers who adhered to standard protocols and used a specifically designed data collection form. Inter- and intra-observer variability was evaluated for quality control (Price *et al.*, 2008).

Fasting venous blood samples were obtained and used to measure factors such as total serum cholesterol, HbA1c and inflammatory markers. Early morning urine samples were collected to measure creatinine and albumin output. Height was measured, without shoes, using a wall mounted measuring tape and estimated to the nearest 0.1 cm. Weight was measured without shoes or bulky clothing and recorded to the nearest 0.1 kg. Hip and waist measurements were taken to the nearest 0.1 cm. Systolic and diastolic blood pressure measurements were taken in the right arm using a standard stethoscope and aneroid dial sphygmomanometer after 10 minutes of rest. Systolic pressures were measured in the right and left brachial, posterior tibial and dorsalis pedis, using a Doppler probe and aneroid sphygmomanometer. A 12-lead electrocardiogram (ECG) was taken using a GE Marquette MAC 1200 ECG machine while the participant was lying still and relaxed. Self-administered questionnaires were provided that covered demographics, diabetes history and treatment, educational attainment, smoking and alcohol habits, current medications, occupation and employment, stress, satisfaction with life, subjective social status, personality, and history of cardiovascular and cerebrovascular disease including the WHO chest pain and Edinburgh Claudication questionnaires. Digital retinal photography was taken at a separate appointment roughly 2-3 weeks after the initial appointment (**sections 4.3.1** and **4.4.4** give further details on retinal imaging and grading).

Cognitive testing assessed major domains of cognitive ability, including delayed non-verbal memory, verbal declarative memory, logical memory, non-verbal reasoning, working memory, information processing speed, executive functioning, mental flexibility and crystallised intelligence. The cognitive tests that were administered, only if the participant presented with a blood glucose of ≥4.0 mmol/l and demonstrated adequate visual acuity,

included the combined Junior and Senior Mill Hill Vocabulary Scale (MHVS) (Raven, Raven and Court, 1998) and Wechsler memory Scale-III UK Faces and Family Pictures subtest (Faces) and Logical Memory subtest (LM) (Wechsler, 1987). From the WAIS, 3rd Edition (WAIS-III UK) participants undertook the Matrix Reasoning test (MR), the Letter-Number Sequencing test (LNS) and Digit Symbol Test (DST) (Wechsler, 1998). Trail Making Test Part B (TMTB) (Spreen & Strauss 1991) and Borkowski Verbal Fluency Test (VFT) (Lezak, 1995) were also administered. Other tests undertaken to screen for possible dementia and depressive mood tendencies were the Mini-mental State Exam (MMSE) (Folstein and Folstein, 1975), and Hospital Anxiety and Depression score (HAD) (Zigmond and Snaith, 1983), respectively. Baseline cognitive testing methods for the ET2DS have been published (Ding *et al.*, 2011).

Year-10 follow-up examinations

Physiological examinations undertaken at year-10 were nearly the same as baseline and the same standard operating procedures (SOPs) were followed, including instrumentation, in order to ensure continuity between data collection points. Examinations that were undertaken by myself and one other ET2DS team member (AS) included height and weight, hip and waist measurements, systolic and diastolic blood pressure, as well as systolic blood pressures taken in the right and left brachial, posterior tibial and dorsalis pedis using a Doppler probe. Early morning urine was collected as well as a non-fasting blood sample.

A questionnaire was administered to provide updated information regarding diabetes treatment and progression, any cardiovascular, cerebrovascular, liver disease or any other reasons for seeking medical attention since the previous data collection, including the WHO chest pain and Edinburgh Claudication questionnaires. The questionnaire also covered smoking and alcohol habits. We interviewed participants to fill out a validated standardized physical activity questionnaire called the International Physical Activity Questionnaire (IPAQ) (Craig *et al.*, 2003), which was not performed at any

previous data collection point, but included several questions to help understand how much a participant's physical activity would have changed over the course of the study.

Cognitive testing was also undertaken at the 10-year follow-up and included all previously mentioned tests from baseline. In addition, the Diery-Liewald reaction time test (DLRT) (Deary, Liewald and Nissan, 2011) test was administered, which was not previously undertaken by the participants. Cognitive testing was administered by two researchers, myself and AS. We received training in cognitive test administration from the Human Testing Development Officer at the Centre for Cognitive Ageing and Cognitive Epidemiology (CCACE) at the University of Edinburgh. Throughout the data collection, we regularly performed quality control, which included sitting in on each other's appointments to make sure we were following the same protocol and confident we were conducting the interviews and tasks in the same manner. Scoring of the tests was undertaken by the tester, but to check for errors, 10% of the participants' cognitive tests were re-graded by the other tester. Any concerns with grading were discussed and consensus reached.

As our cohort is made of elderly participants, it was common that they were unable or unwilling to travel to the research centre but were still interested in participating in the study. For these participants we conducted home visits. If a participant required a home visit, a suitable date and time was arranged over the phone and a new confirmation letter was sent out. All physiological and cognitive tests and measures that were carried out in the clinic setting were also able to be performed in a participant's home. At all times, two researchers were present for each home visit for safety, continuity and time efficiency reasons.

For home visits, we used the same type and brand of stethoscope, sphygmomanometer and Doppler probe to measure blood pressure. The scale used to measure weight was regularly checked against the scale used in the clinic setting and found to be consistently providing equivalent readings. Urine and blood samples were also obtained in the same manner

as in the clinic, and samples were kept in a cool box and brought to the medical laboratory at the end of the day. Time between draw, delivery to the laboratory and processing were noted. All cognitive testing materials and protocols were identical between the clinic and home setting.

Data linkage and secondary data collection

At baseline, routinely collected data were obtained from the Information and Services Division (ISD) of the NHS Health Services Scotland on all medical and surgical discharges from Scottish hospitals since 1981 (Scottish Morbidity Record [SMR01] scheme) and any International Statistical Classification of Diseases and Related Health Problems, 10th revision (ICD-10) codes which indicated cardiovascular or cerebrovascular disease. This data linkage was also carried out in 2010 and 2015 to cover the periods after the initial linkage. At the 10-year follow-up, data for all 1066 participants were gathered from SCI-DC, which included HbA1c, systolic and diastolic blood pressure, total and high density lipoprotein (HDL) cholesterol, triglycerides and laser photocoagulation treatment. Retinopathy and maculopathy gradings, along with the retinal fundus images used to evaluate retinal vessel traits at the year-10 follow-up, were linked from the Scottish Diabetic Retinopathy Screening Collaborative (Scottish DRS), which are further described in **section 4.4.4**.

In addition, at the year-10 follow-up, TRAK records were evaluated for all participants by one member of the research team who is a Specialist Registrar in Endocrinology and Diabetes, Dr Sheila Grecian (SG), to identify cases of liver disease, cardiovascular and cerebrovascular disease and dementia. Death records were identified for cause of death for participants that had died since the previous data collection. Death records were gathered from the National Records of Scotland, Birth, Death and Marriage Records. Questionnaires that resembled the medical questionnaire provided in clinic were sent to the GPs of the invited participants that did not come to the year-10 follow-up and were not known to be deceased.

4.3 Retinal images and vessel traits

4.3.1 Retinal images

Baseline

Data on baseline RVTs were generated by a software operator using images taken at an appointment approximately two to three weeks after the participant's ET2DS baseline appointment. A total of 1066 participants attended their baseline appointment, and 1046 returned for the eye clinic and retinal imaging and 1044 had successful imaging for retinopathy grading (20 individuals did not attend eye clinic, and 2 had ungradable images from poor quality). Fundus photography was undertaken within the NHS Princess Alexandria Eye Pavilion in Edinburgh. Pupillary dilation, mydriasis, was achieved using 1% tropocamide drops in both eyes and inspected after 20 minutes for dilation. Dilation was not undertaken if the participant had ocular opacities, such as cataracts, or the participant had a false eye. Standard 7-field non-stereoscopic colour photographs were taken of both eyes at 35° using a TOPCON TRC-50FX system (Topcon Optical Company, Tokyo, Japan). The images were taken by a single, specially trained medical photographer. All images were saved in non-compressed JPEG or TIFF format.

Year-10 follow-up used to measure change in retinal vessel traits

I requested retinal images from the Scottish DRS programme in order to evaluate change in RVTs over the 10 years of the study. Within the DRS programme, annual retinal images were taken after pupil dilation, using 1% tropicamide, with a TOPCON NW8 fundus system with Nikon D700 digital backs at a 45°-50° angle. Photography was performed by specially trained and accredited medical photographers. I was provided with all images within the Scottish DRS database from 2005 through 2017 of ET2DS participants who had consented.

I performed follow-up analysis as a 1:1 matched nested case-control study to evaluate the association between changes in RVTs and cognitive decline.

This method was employed to avoid concerns of survival bias that would enter the data due to the age of the cohort and the fact that assessing change in RVTs requires a follow-up image, which would only be available for participants that were alive and well enough to have attended an imaging appointment. Using a nested case-control design, as opposed to a traditional case-control study, reduces concerns of bias as the control population is drawn from the same cohort as the cases. To reduce possible bias relating to the use of different imaging methods and software versions at baseline compared with follow-up, an image from the DRS taken close in time to baseline ET2DS attendance was analysed as well as a follow-up image (instead of using the previously analysed baseline ET2DS image).

Cases of overt cognitive decline were chosen as participants with dementia and/or in the lowest decile of *g* at year 10. Age, within 5-year bands, and sex matched controls were chosen from those in the top 50% of year-10 *g*. The participants chosen for the cases and controls had to have attended the year-10 clinic and had at least two retinal images that were a minimum five years apart, but the longest duration between images was chosen. Reasons that participants did not have a full 10 years of follow-up were generally that the latest available images were of poor quality and would not yield measurements for a reliable comparison or that participants did not return annually for their intended screening appointments. Poor image quality in older people is not uncommon due to other existing eye conditions, drooping eyelids, inability to hold their head still and eye-lashes obstructing the image.

4.3.2 Retinal vessel traits measurement

The values used for baseline retinal vessel traits (RVTs) were produced by a previous ET2DS team member, Emmanuel Sandoval Garcia (ESG). I prepared the baseline RVT data from ESG for use in my analyses evaluating baseline traits and follow-up outcomes. Separately, I produced data for the change in RVTs, evaluating changes in the traits from retinal images near baseline through the follow-up of the study. Along with the next two sections,

Figure 17 and **Table 14** provide a description of the different retinal images used in the analyses and various relevant features.

Baseline retinal vessel traits

Retinal vessel data at baseline were measured using VAMPIRE software version 3.1.0 by ESG. The right eye was used unless it was not suitable for analysis, in which case the left eye was chosen, if appropriate. For this thesis, baseline vessel traits that were evaluated were width measures, central retinal arterial equivalent (CRAE) and central retinal venular equivalent (CRVE), as well as median arterial and venular tortuosity, and fractal dimension of the vascular network detected in an image.

Year-10 follow-up – change in retinal vessel traits

I performed the measurement of RVTs on the follow-up images using VAMPIRE WEB. This version is very similar to version 3.1.0 but not exactly the same in terms of the measurement processes. It would therefore be inappropriate to directly compare from images analysed using version 3.1.0 to VAMPIRE WEB. As with the baseline traits, the right eye was used unless it was not suitable for analysis, in which case the left eye was chosen if appropriate. The software provides data on the optic disc location and size, fovea position, CRAE, CRVE and arteriovenous ratio (a ratio of the CRAE and CRVE width measurements) the number of straight vessels, tortuosity (separated by venular and arterial vessels, separately) as median and max raw figures, as well as a mean of the log transformed measurements. Data were also provided on the density of vessels as both pixels and percentage for Zone B and Zone C, separately. Finally, fractal dimension is reported, separately for venular and arteriolar vessels, for the whole field of view, Zone C or Zone C main vessels. Visual description of the different zones is provided in **section 4.3.3**.

For this thesis, I evaluated CRAE and CRVE (both reported in units of pixels), the mean log transformed arterial and venular tortuosity, Zone B density in pixels and whole field of view fractal dimension. Zone B and whole field of view were chosen for density and fractal dimension, respectively,

because it was determined, from visual assessment, that many of the images taken from the Scottish DRS did not fully incorporate Zone C. Zone B was nearly always completely intact in the images, so this zone offers more standardised information. It did not seem appropriate to use a measure that focused primarily on Zone C when other options were available, as the values might vary depending on how much of Zone C was available in the image. When evaluating the change in RVTs, I used a simple difference between baseline and follow-up.

For both baseline and follow-up RVT measurements, graders followed a pre-specified protocol and were blinded to outcome status of the participant.

Figure 17. Image timeline for retinal vessel trait analysis

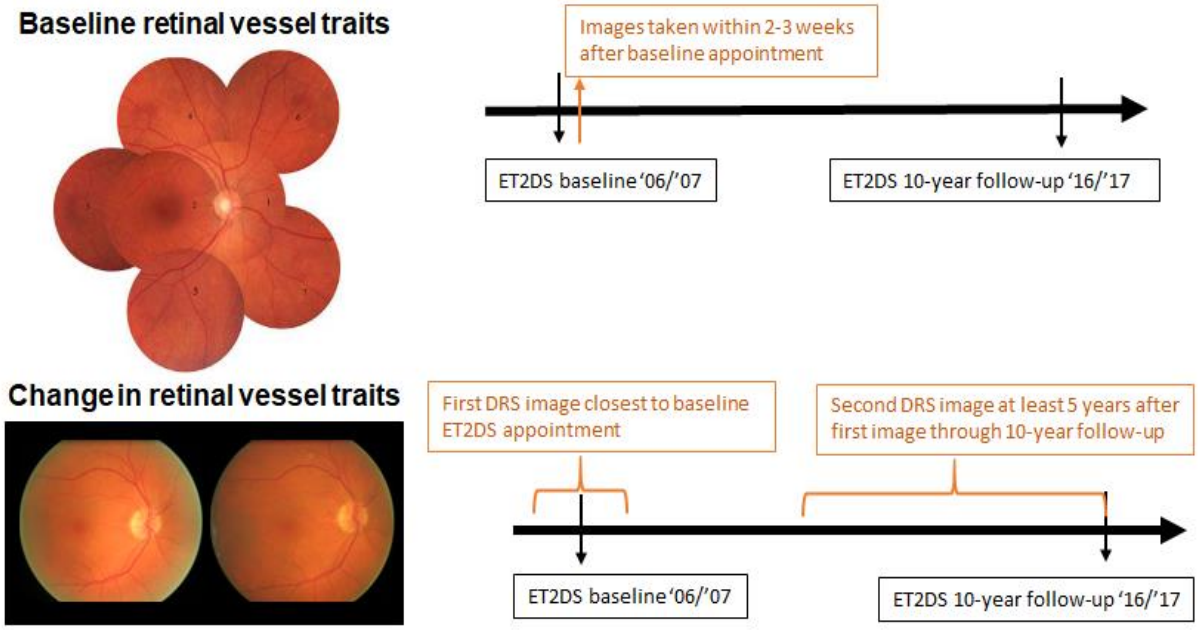


Table 14. Comparison of images used for retinal vessel trait analysis

Description	Baseline retinal vessel traits	Change in retinal vessel traits over 10 years, nested case-control
Research questions	Used for research questions 1 and 2	Used for research question 3
Image source	ET2DS study	Scottish DRS
Image timeline	Two to three weeks after baseline ET2DS appointment	Baseline images were closest to original ET2DS baseline appointment and follow-up images were closest to 10-year follow-up appointment or latest usable image
Image details	7-field, non-steroscopic colour digitial fundus images at 35° using a TOPCON TRC-50FX system (OD centred image used for analysis)	Single-field, non-stereoscopic, fovea centred, colour digital fundus images at a 45°-50° angle using a TOPCON NW8 fundus system with Nikon D700 digital backs
Grading technician	Emmanuel Sandoval Garcia	Rachel Bedenis Forster
Traits evaluated	CRAE, CRVE, medial arterial and venular tortuosity, fractal dimension	CRAE, CRVE, mean log arterial and venular tortuosity, arterial, venular and total density (zone B pixels), and arterial, venular and total fractal dimension (whole field view)
VAMPIRE version	3.1.0	VAMPIRE WEB

All analysis was done on the right eye, unless the quality was unsuitable for the software, in which case the left was used if available; CRAE = central retinal arterial equivalent, CRVE = central retinal venular equivalent; ET2DS = Edinburgh Type 2 Diabetes Study; Scottish DRS = Scottish Diabetic Retinopathy Screening Collaborative; VAMPIRE = Vascular Assessment and Measurement Platform for Images of the Retina

4.3.3 VAMPIRE Software

Figure 18 shows the features and zones detected and labelled in the VAMPIRE software. For measuring vessel widths a summary index variable is used to approximate vessel widths of the central artery and venule and are called CRAE and CRVE. The software calculates these parameters from the six widest arteriole and venule vessels that cross the inner doughnut of zone B (Knudtson *et al.*, 2003). Tortuosity, which is a measure of how vessels twist and turn, with a smaller value indicating a straighter vessel, is calculated using a curvature-based algorithm using the six widest arteriole and venule

vessels crossing between the optic disc boundary to the outer circle of zone C (Annunziata *et al.*, 2014; Lisowska *et al.*, 2014). Fractal dimension describes the complexity or density of the retinal vascular network and is measured by VAMPIRE using a method known as multifractal analysis using the generalised sand box method which calculates fractal dimension over several “scales” (Stosic and Stosic, 2006).

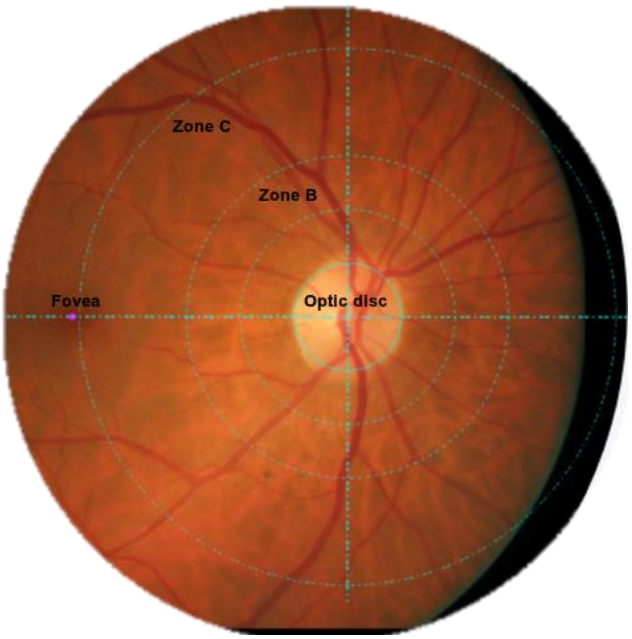


Figure 18. VAMPIRE measurement zones

For context, **Figure 19,** below, provides a visual comparison for the different types of vessel traits and what one-unit change looks like. As a note, arteries in a fundus camera image are lighter in colour than veins which tend to be a darker red/orange colour. For vessel widths and tortuosity there is little obvious difference detectable by visual inspection of the images, but a difference can be detected using software analysis. One standard deviation change in fractal dimension does appear visually different.

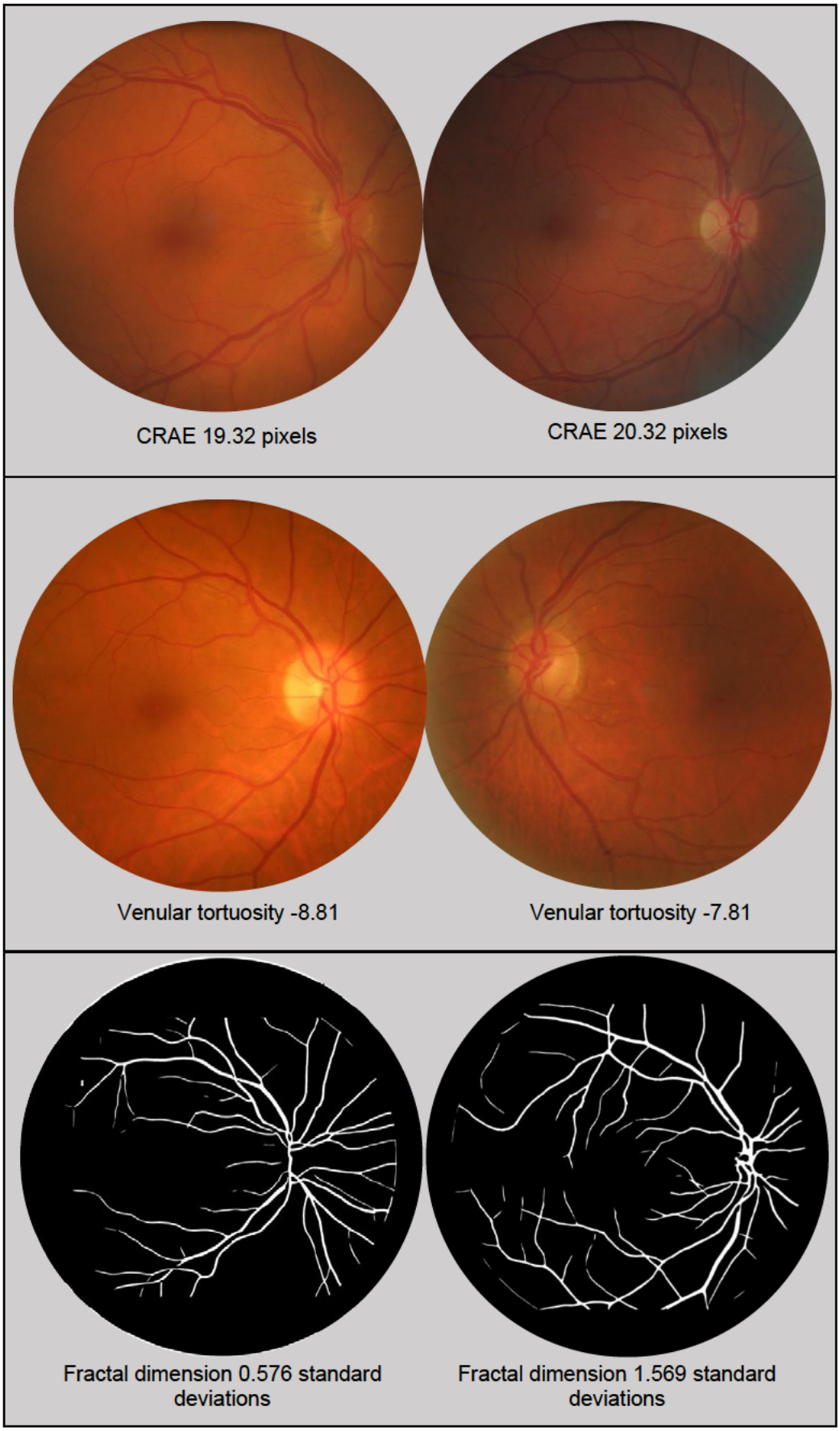


Figure 19. Comparison of one-unit change for retinal vessel traits

4.3.4 RVT measurement reliability and agreement

It is important for RVT data generated using VAMPIRE to be reliable between graders and within graders, meaning no matter who graded the images, the values produced would be similar, and that values are independent of the grader and time of analysis.

Interclass correlation coefficient (ICC) measures of inter and intra-rater reliability represent the proportion of total variability in the outcome attributable to the difference in graders or time and are commonly used for continuous data. I used the average, fixed rater ICC method to evaluate the reliability between and within graders for the various RVTs reported in this thesis using a two-way mixed effects design to evaluate the mean of two graders or two measurements for consistency (Koo and Li, 2016). For baseline measurements, 3%, 30 participants, were used for the analysis of inter- and 3%, 31 participants, for intra-grader reliability. For the follow-up change in RVT analysis, 10%, 35 participants, were randomly selected for analysis of intra-grader agreement. Interclass correlation coefficients are a relative measure and therefore dependent on the variability in the sample.

In addition to calculating ICCs, I created Bland-Altman plots for baseline inter- and intra-rater and change analysis intra-rater RVTs reported in the thesis which offered a graphical representation of the agreement of measures within and between graders (Bland & Altman 1986). This method helps to visualise by how much a measure varies and applies 95% confidence interval limits to evaluate agreement.

4.4 **Variable measurements and definitions**

This section describes the measurement of individual variables that were used in the description of the study population and the multivariable statistical analyses relating to my research questions. This includes baseline data variables on demography, cardiovascular disease/risk factors, and variables relating to the main outcomes of cognition and diabetic retinopathy. Measurement of retinal vessel traits (my main predictor variables) have already been described in the preceding section.

4.4.1 Demographics

Demographic data were used to describe the full ET2DS at baseline, the population that returned for follow-up and as covariates within several statistical models. Variables included age (in years), sex, body mass index (BMI) in kg/m^2, systolic blood pressure in mmHg, total and HDL cholesterol, both in mmol/l. Data on alcohol habits and smoking were also reported. For alcohol, participants indicated how many units per week they generally consumed. To evaluate the effects of alcohol, participants were categorised as consuming no alcohol, consuming between 1 and 13 units per week and consuming above 14 units per week, as 14 units is the current NHS recommended weekly consumption upper limit based on recent research findings (Gillum *et al.*, 2018). For smoking, participants were categorised as an ex-smoker, never smoker or current smoker and within models, current and ex-smokers were combined as 'ever-smokers'.

To better understand the study population in the context of wider Scotland in terms of deprivation, the Scottish Index of Multiple Deprivation (SIMD) was identified for each participant based on their post code at baseline. The SIMD incorporates data from seven domains (current income, employment, health, education, housing, geographic access and crime) in order to describe the deprivation level of residents. At the time of the baseline phase of the ET2DS, the 2009 SIMD methodology was being used. This categorised people with a code of 1 through 5, with 1 indicating the most deprived areas and 5 as the least deprived areas (Scottish Government, 2009).

4.4.2 Diabetes related variables

Variables relating to diabetes included glycated haemoglobin, also known as HbA1c, reported as a percent (%). Duration of diabetes was reported in years and treatment of diabetes at baseline included options for 'diet controlled', oral anti-diabetic 'tablets' or 'insulin'. Participants could be included in both the tablets and insulin categories if they received both and for the purposes of this thesis, these participants were classed solely in the insulin category in order to avoid double counting. During the follow-up time, non-insulin

injectable medications have become more common, so for the 10-year follow-up, medication lists were carefully evaluated for presence of these medications and a separate category was made, but this information was not evaluated within this thesis. In the medical questionnaire, participants were asked if they ever experienced a hypoglycaemic episode where they needed assistance. If a participant answered ‘yes’ to this question they were considered to have experienced a severe hypoglycaemic episode.

4.4.3 Macrovascular events

Macrovascular events comprise cases of myocardial infarction (MI), angina, coronary intervention, stroke and transient ischaemic attack (TIA). At baseline and follow-up, macrovascular events were identified by a combination of patient reporting and medical records. Strict criteria with a defined protocol were used in order to ensure events truly reflected the medical history of the participant. For all events, participants had to have indication of the event in their medical records as well as patient reporting of the event. Patient reporting could be direct reporting of the event with date and hospital they were seen in, medications indicating the condition (specifically for angina), or a positive WHO chest pain questionnaire for MI or angina. If the participant did not report the event, their case and the medical evidence were discussed between the research team and a consensus agreed upon. If the patient reported an event, but there was no medical records confirming it, the case was reviewed by the research team and in most cases it was decided the participant did not meet the minimum threshold. However, on occasion, specifically if the condition occurred and was treated overseas, the event was accepted.

For the purposes of this thesis, baseline macrovascular events were used as a descriptive variable as well as a covariate in several of the statistical models. For the retinopathy outcome analyses, I included a combined ‘history of macrovascular disease’ variable that included MI, angina, stroke, TIA and coronary intervention. For the cognition outcome analyses, nearly the same

combined variable was used, but stroke was removed and was evaluated separately as it is strongly related to cognitive decline.

4.4.4 Retinopathy

Baseline retinopathy

Using the digital retinal images from baseline of ET2DS (see **section 4.3.4**) retinopathy grading was undertaken by two trained optometrists, working independently, using all 7-fields. A predefined protocol was developed prior to grading using Early Treatment Diabetic Retinopathy Study (ETDRS) criteria (Early Treatment Diabetic Retinopathy Study Research Group, 1991). Diabetic retinopathy was considered to be present if microaneurysms alone, or in combination with other characteristic lesions, which include haemorrhages, cotton wool spots, intraretinal microvascular abnormalities, hard exudates, venous beading, venous loops and/or reduplication, fibrous proliferations, pre-retinal haemorrhage, vitreous haemorrhage and new vessels. A grade of 10 (diabetic retinopathy not present) up to 81 (advanced proliferative retinopathy) was given for each eye. See **Table 15** for severity based on grade levels. To generate a binary retinopathy outcome for the purposes of this thesis, the grading of the worst eye of each participant was used and any grade 20 or above was considered as present diabetic retinopathy.

Incident retinopathy at year-10 follow-up

At the 10-year follow-up phase of the ET2DS, I requested diabetic retinopathy grading from the Scottish DRS programme at the time that I obtained digital retinal images. As for the retinal images, the grades provided were also from 2005 through 2017 for each consented participant within ET2DS. In the retinopathy screening programme, images are first processed through automatic software (Zachariah, Wykes and Yorston, 2015). This software is designed to detect any possible abnormalities, which will then be flagged and push the image to be reviewed by specially trained staff. Images that are not flagged are given a grading of R0. The Scottish DRS uses a specific grading scheme that goes from R0 (no diabetic retinopathy) to R4

(proliferative diabetic retinopathy) (Scottish Diabetic Retinopathy Screening Collaborative, 2007). Further information on the DRS grading scheme is given in **Table 16**.

Table 15. Retinopathy severity scale from ETDRS modified for use in ET2DS (adopted from Retinopathy grading protocol for ET2DS, 2007)

Grade level	Severity
10	Diabetic retinopathy absent
14*	Diabetic retinopathy questionable
15*	Diabetic retinopathy questionable
20	Microaneurysms only
35**	Mild NPDR
43	Moderate NPDR
47	Moderately severe NPDR
53	Severe NPDR
61	Mild PDR
65	Moderate PDR
71	High risk PDR (1)
75	High risk PDR (2)
81	Advanced PDR

*levels 14 and 15 are not considered separate steps of the scale but are pooled 10 or 20; **NPDR levels 35 and above require presence of microaneurysms; NPDR = non proliferative diabetic retinopathy, PDR = proliferative diabetic retinopathy

I defined incident retinopathy as any grade R1 or above during the follow-up period (after 2007 or the participant's baseline appointment). It is not uncommon for a person to receive a grade of R1 to then go back to R0 at another grading, which can be due to artefacts in the imaging, healing of microaneurysms without further progression of retinopathy, or other various reasons. Because of this, if a participant received a grade of R1 and never any higher, they needed to have a grade of R1 at a minimum of two consecutive screenings to be considered to have incident retinopathy. A participant was also considered to have incident retinopathy if there were laser photocoagulation scars present, as this is generally an indication of treatment of diabetic retinopathy.

Table 16. Scottish diabetic retinopathy grading scheme (adapted from Scottish Diabetic Retinopathy Screening Collaborative, 2007)

Grade level	Severity
R0	No diabetic retinopathy anywhere
R1	Background diabetic retinopathy- mild
R2	Background diabetic retinopathy- observable
R3	Background diabetic retinopathy- referable
R4	Proliferative diabetic retinopathy
R6	Not adequately visualised

4.4.5 Cognitive decline and dementia

Dementia

In order to generate a robust diagnosis of dementia, data from multiple sources were collected and then dementia was defined according to pre-determined criteria. As described in a previous section of this chapter (**Data linkage and secondary data collection** on **page 134**), one member of the ET2DS research team, SG, reviewed the medical records (TRAK, hospital discharge, or death records) of all participants in ET2DS for evidence of a clinical dementia diagnosis. Another team member, AS, combined this information with data collected at the year 10 follow-up research clinics and linked records to identify individual cases of dementia according to the following criteria:

1) Indication of clinical dementia diagnosis from medical records (TRAK, hospital discharge, or death certificate)
2) AND at least one of the following:
    a. Self/carer report of dementia
    b. GP report of dementia
    c. Medications for dementia
    d. Mini-mental State Exam (MMSE) <24 at year 10 or missing MMSE at year 10 (indicating non-attendance)

A consensus panel was convened to arbitrate each case of dementia and a final list was generated. Information on subtype of dementia was collected,

where possible, specifically if the dementia diagnosis was Alzheimer's dementia, vascular or mixed.

General intelligence factor g

Using results from the cognitive tests undertaken in the ET2DS, I generated a single factor, the general intelligence factor *g*, to discuss and evaluate cognitive decline. The general intelligence factor *g* was calculated using the data reduction technique known as principle components analysis (PCA), which generates a single factor that correlates with and explains a large portion of variance between the different cognitive tests and represents cognitive ability over a range of domains (Spearman, 1904). This technique is commonly used in social sciences to help analyse latent variables, which are variables that cannot be directly measured. Different aspects of cognition were measured using the various cognitive tests and then PCA used to generate a single measure to discuss cognition as a whole. Cognitive decline was used as an outcome in the analyses models by including the year-10 *g* as the outcome and baseline *g*' as a covariate, which is a common method used in psychology research to evaluate cognitive change (Gow *et al.*, 2008). Production of *g* for this thesis is further discussed in **section 4.5.3**.

Factor analysis is a similar method to PCA used for variable reduction when evaluating latent variables in the social sciences. PCA is an arguably less complex technique that uses the original data to construct a single set of linear variates and can evaluate how the original variables contribute. Factor analysis creates a mathematical model from which the factors are estimated (Field, Miles and Field, 2012b). PCA was chosen at the baseline of the study as the data reduction method to generate a single intelligence factor, so it is what was continued for analysis. Also, PCA allows for the evaluation of the individual cognitive tests within statistical modelling means without increasing the risk of multiple testing. When using factor analysis, the factors generated are wholly separate from the underlying tests it is made up from and therefore to evaluate tests individually, one would be drastically increasing the analysis dimensions (Dunteman, 1989).

4.5 Data preparation and analysis

4.5.1 Data entry and cleaning

At baseline and year-10 follow-up, members of the study team manually entered data into a Microsoft Access database. At baseline, double data-entry was undertaken for all participants, and yielded an error rate of 0.02%. At the year-10 follow-up, 10% of entries were re-entered and an error rate of 0.018% resulted. These errors were corrected by consulting the original paper data entry form and discussion between the research team, if needed.

Impossible and outlier values

In addition to correcting the identified errors, the range of values for all variables was evaluated for impossible and miscoded data. Individual participant files and data sources were inspected to determine if values in the database were correct. For continuous variables, outliers were evaluated using a cut-off of ±2.5 standard deviations from the mean. If the outlier value was physiological plausible it was left but noted for later model evaluation analysis. Also, individual tests were considered for impossible or implausible values and checked against the original data collection form and corrected if necessary.

4.5.1 Missing data

All data were evaluated for missing values. During the data cleaning process, variables that had contained zeros were inspected to make sure these were true zeros and not missing data, or if there were missing data, that they were not meant to be a true zero value. Any true missing data were coded as ‘NA’. Analysis with regression only uses complete-case analysis, so it is important to understand how the participants with missing data can alter the interpretation of the results. This was done by comparing important variables between the whole population and those with complete-cases only and no missing data. This helps to understand if the complete cases were similar to the whole population or if the analysis results need to be tempered due to missing data.

It is common in epidemiological research that authors do not report or fully consider the effect of missing data which can have a great impact on the findings (Eekhout *et al.*, 2012). There are techniques that can be employed when data is missing that could alter the final analysis, and generally these techniques require the assumption of missing completely at random or missing at random (Baraldi and Enders, 2010). Multiple imputation was used to calculate the *g* variable when some test scores were missing for an individual, but such methods are unlikely to be necessary or appropriate for other variables used in this thesis. More is discussed on imputation regarding *g* in the following sections.

Albumin to creatinine ratio (ACR)

The baseline albumin to creatinine ratio (ACR) variable, as originally derived, had a large amount of missing data, n = 622 missing (58.3%). This is due to many participants having an albumin measurement below the threshold of detection. For the purposes of this thesis, I chose to take the upper limit of 6.0 mg/l for the ratio. This allowed me to obtain an ACR for nearly all participants. Due to this method of imputation, individual ACR values may be uninformative.

4.5.2 Variable distributions and transformations

Continuous variables used for analysis were visually evaluated for normality using histogram plots. Variables that were found to be skewed, or non-normal, were transformed for analyses. See **Appendix 12** for variable histograms.

Variables that were found to be non-normal were duration of diabetes, ACR, the TMTB cognitive test, and the retinal vessel traits of tortuosity, both arterial and venular, as well as fractal dimension. All variables, aside from fractal dimension, were log transformed using the natural log. Fractal dimension was only slightly skewed so rank transformation was used. This method was used for previous research of the same group, so methods were maintained for continuity.

4.5.3 Variable derivations

Fractal dimension

Fractal dimension is a unit-less value that lies between 1 and 2. Due to this feature, when used in logistic regression, the resulting odds ratio and 95% confidence interval can be difficult to interpret because they are usually in relation to a one point increase or decrease. For this reason, a standardised fractal dimension value was used in the logistic regression models. The standardised values were created by subtracting the mean and dividing by the standard deviation for each participant's fractal dimension value. This produces a scaled variable with a mean of 0 and standard deviation of 1, but the values maintain the same relationship to one another as the unscaled variable. When used in logistic regression, the odds ratio can be interpreted as the odds given an increase of one standard deviation.

General intelligence factor g

Of the cognitive tests described in **section 4.2.2**, tests incorporated into the *g* calculation included the LM and Faces tests, MR, LNS, DST, TMTB and VFT. For both the LM and Faces tests, the immediate and delayed tests were highly correlated, so a sum was used. The TMTB variable, at both baseline and year-10, had a strong right-tail skew, so a natural log of this test outcome was always used.

At baseline and follow-up, there were times when a participant physically could not or refused to complete all seven cognitive tasks. If a participant completed at least four of the seven cognitive tests, statistical imputation methods were used to incorporate these participants into the *g* calculation. A multiple imputation method was used, which is a method of imputation that generates several models of the imputed data using features of non-missing data and then combining the imputed models to account for the uncertainty of the missing data (Sterne *et al.*, 2009). This method was chosen, as opposed to a simpler method of using the population mean, because multiple imputation better accounts for the variations in participants and missing data. After performing imputation, I compared the results with and without the

imputed figures and found no overall change in the variables, meaning these participants were not unduly affecting the results. Imputation is not an ideal method within analyses, but the appropriate and predefined methods used allowed for greater power of the *g* variable within the analyses.

PCA was conducted on age-adjusted cognitive test scores. All scores from the baseline and year-10 collection were combined into one data row so PCA could be carried out on a single column. The resulting regression scores of the first component were re-organised based on the time point they were collected and were considered the *g* score for that participant. Using this method of PCA allows comparison of the means of *g* between the time points. If PCA was conducted separately for baseline and year-10, you could not directly compare them as the mean and standard deviation for each would be 0 and 1, respectively. Performing the calculation in this way allows the baseline *g* mean to be centred on 0, with a standard deviation of 1 and the follow-up *g* scores to be evaluated in relation to baseline. Using a scree plot of a PCA, which plots each eigenvalue against the factor, provides a criterion for factor selection. For our PCA, the scree plot and eigenvalues <1 suggest that 1 component adequately describes the data, with 48.06% of the variation accounted for. The un-rotated factor loadings, which represent the correlation between the cognitive tests and the resulting *g* can be found in **Table 17**. **Table 18** demonstrates the strong correlation between the resulting *g* variable and the individual cognitive tests that are incorporated.

Table 17. Factor loadings from *g* principal component analysis

Cognitive test	Factor loading
Faces test	0.489
Logical Memory subtest (LM)	0.603
Borkowski Verbal Fluency Test (VFT)	0.644
Matrix Reasoning (MR)	0.691
Letter-Number Sequencing (LNS)	0.749
Digit Symbol Test (DST)	0.790
Trail Making Test Part B (TMTB)	-0.827

Table 18. Correlation between cognitive status and individual cognitive tests

	year 10 *g*	LM	TMTB	Faces	MR	DST	BVFT	LNS
year 10 *g*	-	0.657***	-0.846***	0.581***	0.719***	0.821***	0.647***	0.776***
LM		-	-0.306***	0.354***	0.382***	0.452***	0.292***	0.439***
TMTB			-	-0.254***	-0.522***	-0.741***	-0.401***	-0.572***
Faces				-	0.333***	0.414***	0.327***	0.313***
MR					-	0.487***	0.358***	0.518***
DST						-	0.451***	0.515***
BVFT							-	0.464
LNS								-

Values: Pearson correlation coefficient; *p<0.05 **p<0.01 ***p<0.001; BVFT = Borkowski Verbal Fluency Test, DST = Digit Symbol Test; LM = Logical Memory, LNS = Letter Number Sequencing, MR = Matrix Reasoning, TMTB = Trail-Making Test-B

4.5.4 Univariate statistical analysis

Univariate analysis was carried out to evaluate the association between the different variables within the thesis. When comparing two groups, for continuous variables Welch's two-sample t-test was used and categorical variables using Pearson's chi-squared test with Yate's continuity correction. Correlations between continuous variables within a single group was done using Pearson's correlation coefficient. For univariate analysis within a single group between a continuous and categorical variable (of no more than two levels), the point-biserial correlation was used.

4.5.5 Multivariable statistical analysis

To answer the three research questions, multivariable regression models were used. For the regression analyses, I constructed the models by adding in related groups of covariates in a step-wise fashion to better understand the association between the predictor and outcome variables in relation to the covariates, building up to the fully inclusive models. If the variables were all added at once, it would be difficult to understand if certain categories of variables were more strongly associated than others. The blocks of covariates included in the regression were:

1. the unadjusted model with just the RVT exposure variable and outcome;
2. addition of age and sex;
3. then cardio-metabolic risk factors (BMI, smoking status, systolic blood pressure, total cholesterol:HDL ratio);
4. diabetic risk factors (HbA1c, duration of diabetes and diabetic treatment type);
5. finally, vascular disease risk factors were added (a composite history of macrovascular disease as well as ACR).

If there was no evidence of an association at the unadjusted or age and sex adjusted stages, the analysis was halted in order to avoid the risk of findings through multiple testing.

Retinal vessel traits and incident diabetic retinopathy

The provide an answer to the first research question, binomial logistic regression was employed to evaluate the relationship between baseline retinal vessel traits and incident diabetic retinopathy. Originally, cox proportional hazards regression was planned, but during the analysis, it was determined to not be an appropriate model due to outlier residuals creating high leverage within model, therefore producing an unreliable model that would not be likely be generalisable to other populations . More about model evaluation is described in **section 4.5.6**. The baseline RVTs were produced using the ET2DS study retinal images described in **Section 4.3.** The incident diabetic retinopathy outcome was derived using data from the Scottish DRS, as described in **Section 4.4.4.**

Evaluating biomarker model improvement

If any RVTs were found to be associated with incident retinopathy I planned to evaluate the addition of the RVT using Harrell's concordance statistic, or c-statistic, to see if the model was improved. The c-statistic is used to evaluate the discriminative ability of the logistic regression model and is a unit-less index denoting the probability that a randomly selected subject who experienced the outcome will have a higher predicted probability of having the outcome occur compared to a randomly selected subject who did not experience the event. This is equivalent to the area under the receiver operating curve and describes the goodness of fit of a logistic regression model. I also planned to compare the two models, with and without the RVT, using the likelihood ratio test, which compares the observed frequencies of the model to the predicted frequencies.

Normally when evaluating the usefulness of a new biomarker, you would include the biomarker in an established risk prediction model and use the c-statistic to determine the effect the new biomarker has. Unfortunately, there is currently no risk prediction model for diabetic retinopathy in general use (Sabanayagam *et al.*, 2016). There have been several publications referring to the development of a prediction tool, but none have been reliably

replicated and implemented in clinical practice (Skevofilakas *et al.*, 2010; Stratton *et al.*, 2014). I used the findings of large cross-sectional and prospective studies that estimated the impact of common factors associated with diabetic retinopathy to inform which variables would be most important in a risk prediction model and then compare the RVT with this model. The risk factors to be used in the proposed model would have to be routinely collected and available. From the evidence from other studies, I chose to include HbA1c, systolic blood pressure and ACR as the 'base prediction model' (Stratton *et al.*, 2001; Henricsson *et al.*, 2003; Tam *et al.*, 2009; Zhang *et al.*, 2010; Romero-Aroca *et al.*, 2010, 2011; Pugliese *et al.*, 2012; Bertelsen *et al.*, 2013; Salinero-Fort *et al.*, 2013; Xu *et al.*, 2014; Y. Liu *et al.*, 2017).

Retinal vessel traits and cognitive decline

To answer research question 2: 'What is the association between baseline retinal vessel traits and incident cognitive decline?', two different analyses were conducted, both using the same baseline RVTs used to answer research question 1, described in in **Section 4.3**. One analysis used a dementia diagnosis as an outcome, as described in **Section 4.4.5** and logistic regression. For the second analysis, multiple linear regression was used to evaluate the relationship between the baseline RVTs and cognitive decline by the general intelligence factor *g*, also described in **Section 4.4.5**. If there was evidence of an association, linear regression was used to evaluate the RVTs and their association with the individual cognitive tests.

Also, I evaluated the relationship between baseline retinopathy and dementia combined with the lowest decile *g* using age and sex adjusted logistic regression. For sensitivity analysis, incident dementia was combined with the lowest decile of *g* at year-10.

Retinal vessel trait changes and cognitive decline

Unadjusted logistic regression was used to evaluate the third research question regarding the association between change in retinal vessel traits and cognitive decline. The RVTs used in this analysis were derived from the retinal fundus images gathered from the Scottish DRS and described as

‘change in retinal vessel traits’, discussed further in **Section 4.3.**This analysis was done using a nested case-control design, further description of the cases and controls are described in **Section 4.3.1**.

4.5.6 Model evaluation and diagnostics

The purpose of model evaluation and diagnostics is to better understand if the model produced is robust and fits the observed data well and that the model is not overly influenced by a small number of cases. Evaluation may also help to better know if the model can be generalised to other samples.

Firstly, model specific assumptions were evaluated to make sure they were not violated, which included the assumptions of linearity and multicollinearity. Next, I checked for evidence of interaction between predictor variables. Then, I evaluated how the predictor variables within the models improved the overall model by evaluating the change in the Akaike information criterion (AIC), the c-statistic, and also looking at the goodness of fit through the Hosmer-Lemeshow goodness of fit test. For the Hosmer-Lemeshow test, a non-significant p-value does not necessarily mean the model is a good fit, but rather that there is not any evidence of a poor fit. Finally, residuals were inspected for evidence of outliers and influential cases. An absolute value > 3 was cause for further investigation, if more than 1% of sample cases had standardised residuals over 2.5, this indicated evidence of unacceptable error and was investigated. If more than 5% of cases had standardised residuals of above 2, then it was possible the model was a poor representation of the actual data and should be investigated.

4.5.7 Sensitivity and subgroup analysis

A strict definition of incident retinopathy was used in order to make sure only those truly with retinopathy were included in the analysis. This definition required that if a participant had a retinopathy grading of R1, they were only classed as having retinopathy if they had an R1 grading at a minimum of two consecutive screenings. Sensitivity analysis was undertaken to evaluate if there was a difference in findings if this definition was relaxed to include all participants that received an R1 grading.

Subgroup analysis was planned to evaluate the different severities of retinopathy gradings. Grading groups would include mild retinopathy (R1), moderate retinopathy (R2 and R3) and proliferative retinopathy (R4).

Dementia diagnosis and lower *g* values are related and both indicate a level of cognitive decline. However, not all people with a lower *g* value have been diagnosed with dementia. Sensitivity analysis was planned to combine participants with a dementia diagnosis with those that were in the lowest 10% of follow-up *g*, as it can be argued these participants are all experiencing a level of cognitive decline.

As described in the introduction chapter of the thesis, dementia is not a single pathology and the term is used to described various underlying aetiologies, which include Alzheimer's disease and vascular dementia, among others. I planned to perform subgroup analysis looking at the different underlying pathologies of a dementia diagnosis, but unfortunately this level of detail was not available in a standardised way that would avoid bias, so no such subgroup analysis was performed.

When evaluating cognition, there are several lifestyle and health factors that can be related to cognitive decline, which include alcohol consumption and the experience of hypoglycaemic episodes. Subgroup analysis was undertaken to see if there was differential association between RVTs and cognitive decline by differences in these characteristics. Participants were divided by alcohol consumption with a no alcohol group, low to moderate consumption group of 14 or less units of alcohol per week and a high consumption group over 14 units per week. At baseline, participants indicated if they ever experienced a hypoglycaemic episode that required assistance. Participants that responded 'yes' to this question were compared to participants that replied 'no'. Age and sex adjusted models were used to evaluate these subgroups.

A two-sided p value ≤ 0.05 was used to indicate evidence of statistical significance. Logistic regression results were presented as odds ratios (OR) and 95% confidence intervals (CI). Linear regression results were presented

as standardised beta coefficients (β), R^2 values. Aside from calculating *g*, all statistical analyses were performed using R version 3.5.1. The intelligence factor *g* was calculated using SPSS software version 21.

4.6 Chapter conclusion

This chapter sought to lay out the research questions of the thesis, explain how the data used within this thesis were generated and analysed. Methods of the ET2DS were laid out including the recruitment and data collection procedures. Variable definitions were provided and clear explanation of analysis methods were reported based on the outcomes of incident retinopathy and cognitive decline. Logistic regression and linear regression methods were described to evaluate the relationships between the RVTs and outcomes of retinopathy and cognitive decline.

5 Results I: ET2DS and descriptive statistics

This chapter provides descriptive details of the Edinburgh Type 2 Diabetes Study (ET2DS) total population at baseline and presents relevant information regarding the participants that returned for the year-10 follow-up. The ET2DS participants are compared to those that did not attend at baseline, but were invited, to assess how representative participants are of people with type 2 diabetes living in Lothian. Similarly, reasons for non-attendance at year-10 are given and demographic details are provided to compare non-attenders to attenders at year 10, and a comparison of those that died during the follow-up period with those that were still alive is presented. Description of the retinal vessel traits evaluated in this thesis and their association with covariates are provided. Finally, missing data is presented and summarised. For this chapter, and the following two results chapters, tables and figures are provided at the end of the chapter.

5.1 ET2DS baseline characteristics and representativeness

At the baseline of ET2DS in 2006/2007, a total of 1066 participants attended the study clinic and were included in the cohort. The average age was 67.9 (standard deviation (SD) 4.2) years and 51.3% of the population was male. **Table 19 (page 171)** describes the demographic characteristics of the baseline participants, as well as invited potential participants that did not participate in the study, for comparison. Overall, at recruitment the ET2DS cohort provided a good representation of older people in Lothian with type 2 diabetes for important characteristics such as age, cholesterol, HbA1c and duration of diabetes and male and female were nearly perfectly 50% each. However, non-responders were more likely to be female, have higher systolic blood pressure and be from more deprived areas, compared with attenders, but these differences were small and unlikely to have a clinical impact. **Figure 20 (page 172)** depicts the participant flow of ET2DS from baseline through the 10 year follow-up.

5.2 ET2DS 10-year follow-up

5.2.1 Clinic and home visit attendance and reasons for non-attendance

The 10-year follow-up data collection phase for the ET2DS began in 2016/2017, after all ethical and institutional approval was granted. To include as many participants as possible, all ET2DS participants were contacted if they were not known to be dead and had not explicitly withdrawn from the study previously. A total of 845 participants (79% of original 1066) were contacted and 582 participants attended for a clinic or home visit. However, one participant that attended the clinic was determined to be incapable of providing consent, so the appointment was aborted, and this person's data was not included in the follow-up data. The final attendance was 581 for year-10 follow-up (69% of those contacted and 54% of total cohort).
After data collection, there were 264 participants that were invited for this round of data collection but did not attend a clinic or home visit (31% of invited). The reasons for non-attendance are listed in **Table 20 (page 172)**.

The most common reason for non-attendance amongst invitees was death which was unknown to the research team at the time of sending invites (n = 84). Chronic physical health conditions were the next most common reason (n = 49), which included conditions such as cancer, pulmonary conditions, diabetes related problems and long-term hospitalisation. Closely following were participants that were no longer interested in participating in the study but did not give a health or other related reason (n = 44).

Mental health conditions, such as dementia, depression, anxiety and other cognitive problems, were the fourth most common reason given for non-attendance (n = 30), followed by a total of 24 participants who the team were never able to make contact with. For these participants, multiple efforts were made to get in touch, including checking for new contact details, but no communication was established.

Acute physical health conditions were the next main reason for non-attendance (n = 15), including conditions such as myocardial infarction,

stroke and infection. If a participant missed their first appointment due to an acute condition, I or another team member would always ask if it would be possible to see them after some time had passed or offer a home visit. Often it was eventually possible to see participants that suffered an acute illness during the follow-up period, unless time did not allow, or mitigating complications made it inappropriate. A total of 10 participants had full-time carer roles for spouses or other family members and were not able to attend the clinic or receive a home visits. Eight participants had moved an unreasonable distance for a clinic or home visit.

Total attrition through the 10-year study period was 485 (45.5%). The main reason for study attrition was death, n = 308 (63.5% of total attrition). A total of 53 participants declined attendance after baseline (10.9% of total attrition). At the latest data collection, 94 participants had a health concern preventing them from attending an appointment (19.4% of total attrition) and 30 participants were lost to follow up at the latest data collection (6.2% of total attrition) (**Table 21 (page 173))**.

5.2.2 Subgroup comparisons

This section compares differences in baseline demographic and cardiometabolic risk factors in the study sub-populations to better understand how they may impact the data and conclusions drawn from it. Firstly, I looked at differences in study participants that attended the 10-year follow-up versus those that did not return. I also looked at those that were deceased after 10-years versus those still alive. Finally, I looked at differences in baseline variables in returning participants that had a clinic visit versus a home visit. It would be expected that participants receiving a home visit would have poorer health at the follow-up, as this was a primary reason for not attending the clinic, but looking at the evidence of the baseline variable to determine the differences in these populations is important. Also, within this section I evaluate the breakdown of cardiovascular events from baseline and follow-up.

Comparison between year-10 attenders and non-attenders

Evaluating baseline variables, those that attended the year-10 clinic or a home visit were of an overall healthier profile than those that did not attend. As presented in **Table 22 (page 173)**, the year-10 attenders were younger (67.3 vs 68.6 years), had a lower body mass index (BMI) (31.1 vs 31.9) and systolic blood pressure (132.1 vs 134.8 mmHg), had a shorter median duration of diabetes (6 vs 7 years), were more likely to be treating their diabetes using diet compared with tablets or insulin, had less history of stroke (4.1% vs 7.8%) and cardiovascular disease (CVD) (27.5% vs 38.6%) and lower median albumin/creatinine ratio (ACR) (1.1 vs 1.3).

Comparison between deceased at year-10 and those alive

Comparing baseline variables of deceased participants to those still alive at the time of the 10-year follow-up, living participants were healthier at baseline than those who died. **Table 23 (page 174)** provides relevant baseline characteristics comparing those deceased and alive at the 10-year follow-up and shows those alive were younger (67.4 vs 69.1 years), had a lower systolic blood pressure (132.6 vs 134.9 mmHg), less duration of diabetes (7.5 vs 9.4 years), more likely to be controlling their diabetes through and tablets than insulin, less stroke (3.7% vs 10.7%) and CVD (27.0% vs 45.5%), and lower ACR (2.2 vs 6.2 mg/mmol).

Comparison between participants that received clinic visits and home visits

There were some minor, and probably not clinically relevant, differences at baseline between those that attended a clinic or home visit at year-10. This included more men, proportionally, having a home visit than clinic visit, a higher HbA1c in those that had a home visit, home visit participants were more likely to be on tablet medications and less likely to be diet controlled for diabetes treatment and home visit participants were more likely to have had a stroke at baseline. See **Table 24 (page 175)** for further data on these differences. **Table 25 (page 175)** shows that those that had a home visit had a far lower average general intelligence *g* and more likely to have had dementia, and not including these participants in the analysis would have

biased the data. There was no evidence to suggest a difference in incident diabetic retinopathy between clinic versus home participants.

Prevalent and incident cardiovascular events

To better understand the breakdown of the combined CVD events, **Table 26 (page 176)** presents data on the number of participants that experienced a macrovascular event prior to baseline and then during the follow-up period. At baseline, 150 participants had experienced a myocardial infarction which was 14.1% of the total 1066 ET2DS population. Angina had been diagnosed in 298 participants (28.0%), 62 experienced a stroke (5.8%), 31 a transient ischemic attack (2.9%) and 110 had had a coronary intervention (10.3%). Through the 10-year follow-up 108 participants experienced an incident myocardial infarction (10.1%), an additional 67 were diagnosed with angina (8.7%), 88 participants experienced a stroke (8.3%), 41 experienced a transient ischaemic attack (3.8%) and 65 had a coronary intervention (6.1%). Proportions in **Table 26** are determined for the entire ET2DS population (n = 1066), except for angina, for which the proportion of incident angina is only for the 768 participants that did not have prevalent angina at baseline.

5.3 Retinopathy at baseline and year-10 follow-up

At baseline, there were 340 cases of retinopathy, most of which (293) were background retinopathy (R1). There were 28 cases of R2 retinopathy, four of R3 and 15 participants had proliferative retinopathy (R4). At follow-up, of the 718 participants that did not have evidence of retinopathy at baseline, a total of 82 had evidence of incident retinopathy, by the strict definition of any R2-R4 grading, or if they only had an R1 grading and no higher, they had to have been given an R1 at a minimum of two consecutive screenings. Seventy-seven cases were R1, one case of R2, R3 and R4 each had two cases. See **Table 27 (page 176)** for details of retinopathy grades at baseline and year-10 follow-up. If the definition of incident retinopathy was relaxed to include any grade over R0 there would be 253 incident cases of retinopathy. This larger figure was used for sensitivity analysis.

Missing retinopathy data at baseline was because a participant did not come to the ET2DS screening appointment and did not have a DRS screening around the time of ET2DS baseline. There were only eight participants at baseline with no retinopathy screening information. At follow-up there were a total of 20 participants with no follow-up retinopathy screening data. These cases were because a participant had not been to any DRS screening after the baseline of ET2DS (2005-2007). Of the 20 participants that had no follow-up retinopathy screening 16 died during the study follow-up. Two died in 2007, one in 2008, four in 2009, one in 2010, three in 2011, one in 2012 and 2013, two in 2014 and one in 2017.

5.4 Dementia and cognition at baseline and year-10 follow-up

At baseline, none of the ET2DS participants had dementia, which was part of the exclusion criteria for the study. But during the 10 years of follow-up, we determined a total of 106 participants to have incident dementia. Evaluating cognitive decline over the 10 years of the study required participants to come back for in-depth cognitive testing, so it is important to understand how the data is impacted by those that did not attend and/or were deceased, at the time we undertook the follow-up appointments. See **Table 28 (page 176)** for baseline *g* and dementia status by attenders versus non-attenders and those deceased or alive at the year-10 follow-up of ET2DS. Participants that attended the year-10 clinics or home visits had a higher baseline *g* compared to those that did not re-attend (0.21 (SD 0.9) vs -0.26 (SD 1.0), respectively) and were less likely to be diagnosed with dementia (6.0% vs 14.6%, respectively). This same pattern was found comparing baseline *g* for those that died over the follow-up versus those that were alive (-0.3 (SD 1.0) vs 0.1 (SD 1.0), respectively) and incident dementia was also higher in those that died over the follow-up period (13.5% vs 8.4%). These findings demonstrate that the population for whom we have follow-up cognitive scores for are also those that had higher cognitive ability at baseline and were less likely to be diagnosed with incident dementia.

5.5 Retinal vessel traits

5.5.1 Retinal vessel trait distributions and missing data

1046 participants had baseline retinal images available for vessel trait analysis. The missing 20 participants did not attend the eye clinic appointment. Eighteen participants did not have images suitable for central retinal arteriolar equivalent (CRAE), central retinal venular equivalent (CRVE) and tortuosity measurements and 31 participants did not have images suitable for fractal dimension measurements. Images that were unsuitable were either due to them being too out of focus for the software to accurately capture the vessels, or there were too few suitable vessels to generate a robust analysis. In total, there were 1028 participants with CRAE, CRVE and tortuosity measures and 1015 with fractal dimension measurements, or 96% and 95% of the total population, respectively.

Mean CRAE was 33.03 (SD 3.87) pixels and mean CRVE was 44.67 (SD 5.01) pixels. Tortuosity is unit-less and reported as a mean value after log transformation of the measured values. The mean arterial tortuosity value was -10.07 (SD 1.15) and mean venular tortuosity was -9.95 (SD 0.89). The mean rank fractal dimension was 1.75 (SD 0.07) and is a unitless value that falls between 1 and 2. These values are presented in **Table 29 page (177)** and **Appendix 12** presents histograms to demonstrate the distribution of the RVTs within the study population, along with other baseline characteristics.

5.5.2 Repeatability of retinal vessel traits

Retinal vessel traits that were interrogated at baseline and used in this thesis include CRAE, CRVE, log transformed median arterial tortuosity and venular tortuosity, and total fractal dimension. For the evaluation of change in RVTs, the same traits were evaluated, plus both arterial and venular fractal dimension, separately and the addition of a density measurement (total, arterial and venular, separately).

Table 30 (page 177) presents the repeatability of the RVT measures using the average, fixed rater method. For baseline inter-and intra-grader

repeatability measures, evaluating the inter-class correlation (ICC) and 95% confidence intervals, values represent moderate to good reliability. Change analysis was only carried out by a single grader so only the intra-grader ICC values were measured, and from the ICC values and 95% CIs, there was moderate to good reliability. Bland-Altman plots were also created for the baseline inter-grader and change intra-grader repeatability measures to demonstrate level of agreement. **Appendix 13** presents Bland-Altman plots for baseline inter-grader agreement and **Appendix 14** for the follow-up intra-grader agreement.

5.5.3 Association between retinal vessel traits and baseline characteristics

It is important to understand relationships between the RVTs and the other variables of interest that are associated with the outcomes, diabetic retinopathy and cognitive decline. **Table 31 (page 178)** shows the correlations between the five RVTs and the important demographic, cardiometabolic, diabetic and vascular variables.

In the ET2DS population, decreased CRVE and decreased arterial tortuosity, were associated with increased age (r -0.08 (p = 0.015) and -0.07 (p = 0.020), respectively). Increased CRAE and CRVE were associated with being female (correlation coefficient (r) 0.14 (p < 0.001) and 0.07 (p = 0.017), respectively) and increased arterial tortuosity was associated with being male (r -0.017 (p <0.001). Increased CRVE and increased venular tortuosity were both associated with increased BMI (r 0.08 (p = 0.012) and 0.10 (p <0.001), respectively). Increased arterial tortuosity was associated with increased total cholesterol to high density lipoprotein (HDL) ratio (r 0.08 (p = 0.016)), while increased fractal dimension was associated with a decreased total cholesterol to HDL ratio (r -0.07 (p = 0.022). Increased venular tortuosity was associated with increased systolic blood pressure (r 0.08 (p = 0.009) while history of stroke and cardiovascular disease were both associated with a decreased venular tortuosity (r -0.08 (p = 0.011) and -0.06 (p = 0.048).

5.6 Missing data

Describing missing data and understanding the role it may play is a crucial part of any epidemiological analysis. **Table 32 (page 179)** presents the populations of participants used in the analyses undertaken in this thesis, based on the entire population and the population for which no data is missing. For the types of regression analyses that will be used, only participants with no missing data are included, so it is important to see how this population may or may not be different from the whole population. From visual inspection of **Table 32**, there do not appear to be any differences of concern between the total population and the populations with no missing data for the variables that will be used in the regression analyses. A total of 1008 cases of 1066 had no missing data (n = 58 (5.4%) with missing data) in regard to the models that would be used for evaluating dementia. For the evaluation of incident retinopathy, of the 718 eligible participants, 689 had no missing data (n = 29 (4.0%) had missing data). Year-10 data, which was used to evaluate cognitive decline using change *g* in, had full case data for 558 of the 581 attending participants (n = 23 (4.0%) with missing data). See **Appendix 15** and **Appendix 16** for graphical representations of the individual descriptive variables based on whole cases and cases with missing data for the whole ET2DS population. Throughout the following results chapters, n values for the variables are presented when describing the data in order to demonstrate where missing data are present.

5.7 Chapter summary

This chapter summarised the data from the ET2DS collected at baseline and at the year-10 follow-up, which are relevant for this thesis. At baseline, the 1066 participants in the ET2DS were shown to be largely representative of older people with type 2 diabetes living in the Lothian region of Scotland. A total of 581 participants returned for the year-10 follow-up, and these participants were found to have a healthier profile based on baseline cardiometabolic variables, compared with non-attenders. After 10 years, participants were found to have less cumulative diabetic retinopathy than

those diagnosed at baseline and poorer cognition. Also, participants that had died by year-10 were found to be unhealthier at baseline, when compared to those that had survived to the year-10 follow-up.

Outcome variables used in subsequent chapters of this thesis include 82 cases of incident retinopathy, 106 cases of incident dementia and the general intelligence factor *g*.

Description of RVT measurements and their repeatability was evaluated and there was moderate to good repeatability for the different graders and time points, which helps to validate the use of the RVTs in analysis. There was very little missing data within the dataset and it was demonstrated that there was little difference between the total population and whole case datasets, making the use of whole-case analysis appropriate in further statistical analysis.

Table 19. Baseline characteristics of ET2DS study population compared with non-responders (data on non-responders from Marioni et al. 2010)

	ET2DS (n = 1066)	Non-responders (n = 4386*)
Age - years	67.9 (4.20)	67.9 (4.35)
Sex - male	547 (51.3%)	1839 (41.9%)
Systolic blood pressure - mmHg	133.3 (16.44)	137.2 (18.15)
Total cholesterol - mmol/l	4.3 (0.90)	4.2 (0.96)
HbA1c - %Hb	7.4 (1.13)	7.4 (1.36)
Diabetes Treatment- insulin	186 (17.4%)	704 (16.1%)
Duration of diabetes - years		
Up to 5 years	516 (48.4%)	2135 (48.7%)
5 years or more	550 (51.6%)	2251 (51.3%)
SIMD quintile		
1 (most deprived)	127 (11.9%)	736 (16.8%)
2	208 (19.5%)	1134 (25.9%)
3	188 (17.6%)	820 (18.7%)
4	194 (18.2%)	782 (17.8%)
5 (least deprived)	349 (32.7%)	897 (20.5%)

Values are mean (SD) or n (%); *n = 4388 actual number of non-responders but two subjects from the Lothian Diabetes Register did not have any data so were discarded from analyses; HbA1c = glycated haemoglobin, mmHg = milligrams of mercury, mmol/l = millimoles per litre, SIMD = Scottish Index of Multiple Deprivation

Figure 20. ET2DS follow-up participant attendance

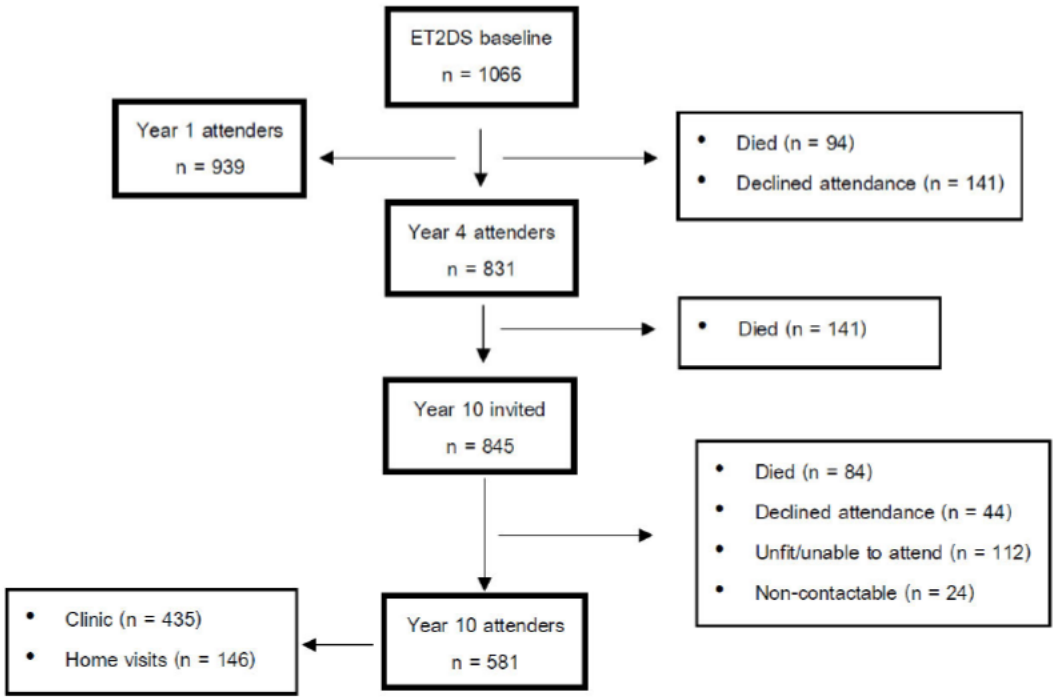


Table 20. Attrition and reason for non-attendance at year-10 ET2DS clinic or home visit

	Year-10 non-attenders (n = 264)
Deceased	84 (9.9%)
Chronic physical health condition	49 (5.8%)
Declined attendance	44 (5.2%)
Mental health condition	30 (3.6%)
Non-contactable	24 (2.8%)
Acute physical health condition	15 (1.8%)
Full time carer	10 (1.2%)
Relocation	8 (1.0%)
Values are n (% of total invited)	

Table 21. ET2DS attrition through follow-up (total n = 485)

Collection time-point	Attenders	Dead	Health	Declined attendance	Loss to follow up
Baseline	1066 (100%)	0 (0%)			
Year-4	831 (78.0%)	88 (8.3%)	26 (2.4%)	9 (0.8%)	14 (1.3%)
Year-10	581 (54.5%)	220 (20.6%)	94 (8.8%)	44 (4.1%)	30 (2.8%)
Cumulative	-	308 (28.9%)	-	53 (5.0%)	-
Values are n (%)					

Table 22. Baseline characteristics of ET2DS year-10 attenders compared with non-attenders

	Year 10 attenders (n = 581)	Non-attenders (n = 485)	p-value
Age - years	**67.3 (4.2)**	**68.6 (4.2)**	**<.001**
Sex - male	296 (50.1%)	251 (51.8%)	.793
Ever Smoker	316 (54.4%)	252 (52.0%)	.428
BMI – kg/m^2	**31.1 (5.5)**	**31.9 (5.9)**	**.024**
Systolic blood pressure - mmHg	**132.1 (14.7)**	**134.8 (18.2)**	**.008**
Total Cholesterol – mmol/l	4.3 (0.9)	4.3 (0.9)	.464
Hba1c - %Hb	7.4 (1.1)	7.5 (1.2)	.260
Duration of diabetes - years	**6.0 (3.0 – 10.0)**	**7.0 (4.0 – 12.0)**	**.006**
Diabetes Treatment			**.004**
Diet controlled	127 (21.9%)	71 (14.6%)	
Tablets	365 (62.8%)	316 (65.2%)	
Insulin	89 (15.3%)	97 (20.0%)	
History of stroke	**24 (4.1%)**	**38 (7.8%)**	**.010**
History of CVD	**160 (27.5%)**	**187 (38.6%)**	**<.001**
ACR – mg/mmol	**1.1 (0.7 – 1.7)**	**1.3 (0.8 – 2.4)**	**.002**

Values are mean (SD), median (IQR) or n (%); ACR = albumin/creatinine ratio, CVD = cardiovascular disease, HbA1c = glycated haemoglobin, IQR = interquartile range, kg/m^2 = kilograms per metre squared, mmHg = milligrams of mercury, mg/mmol = miligram/milimole, mmol/l = millimoles per litre

Table 23. Baseline characteristics of ET2DS population alive at year-10 follow-up compared with those that had died

	Dead at year-10 (n = 310)	Alive at year-10 (n = 756)	p-value
Age - years	**69.1 (4.1)**	**67.4 (4.2)**	**<.001**
Sex - male	176 (55.2%)	371 (49.7%)	.099
Ever Smoker	156 (48.9%)	412 (55.2%)	.061
BMI – kg/m^2	31.8 (5.9)	31.3 (5.6)	.189
Systolic blood pressure - mmHg	**134.9 (18.5)**	**132.6 (15.5)**	**.048**
Total Cholesterol – mmol/l	4.3 (1.0)	4.3 (0.9)	.532
Hba1c - %Hb	7.5 (1.1)	7.4 (1.1)	.245
Duration of diabetes - years	**7.0 (4.0 – 13.0)**	**6.0 (3.0 – 10.0)**	**<.001**
Diabetes Treatment			**<.001**
Diet controlled	46 (14.4%)	152 (20.3%)	
Tablets	194 (60.8%)	487 (65.2%)	
Insulin	78 (24.5%)	108 (14.5%)	
History of stroke	**34 (10.7%)**	**28 (3.7%)**	**<.001**
History of CVD	**145 (45.5%)**	**202 (27.0%)**	**<.001**
ACR - mg/mmol	**1.5 (0.9 – 3.4)**	**1.1 (0.7 – 1.7)**	**.002**

Values are mean (SD), median (IQR) or n (%); ACR = albumin/creatinine ratio, CVD = cardiovascular disease, HbA1c = glycated haemoglobin, IQR = interquartile range, kg/m^2 = kilograms per metre squared, mmHg = milligrams of mercury, mg/mmol = miligram/milimole, mmol/l = millimoles per litre

Table 24. Baseline characteristic between year-10 clinic and home visits

	Clinic visit year-10 (n = 435)	Home visit year-10 (n = 146)	p-value
Age - years	67.2 (4.1)	67.7 (4.4)	.245
Sex - male	**237 (54.5%)**	**87 (60.6%)**	**.003**
Ever smoker	243 (55.9%)	73 (50.0%)	.219
BMI – kg/m^2	30.8 (5.5)	31.8 (5.4)	.076
Systolic blood pressure - mmHg	132.0 (14.5)	132.1 (15.3)	.966
Total Cholesterol – mmol/l	4.3 (0.9)	4.3 (0.9)	.954
Hba1c - %Hb	**7.3 (1.0)**	**7.6 (1.2)**	**.006**
Duration of diabetes - years	7.3 (3.0 – 10.0)	8.4 (3.0 – 12.0)	.164
Diabetes Treatment			
Diet controlled	**107 (24.6%)**	**20 (13.7%)**	**.006**
Tablets	**305 (70.1%)**	**115 (78.8%)**	**.047**
Insulin	62 (14.3%)	27 (18.5%)	.243
History of stroke	**13 (3.0%)**	**11 (7.5%)**	**.017**
History of CVD	111 (25.5%)	49 (33.7%)	.060
ACR – mg/mmol	2.0 (0.7 – 1.6)	2.4 (0.8 – 1.8)	.523

Values are mean (SD), median (IQR) or n (%); ACR = albumin/creatinine ratio, CVD = cardiovascular disease, HbA1c = glycated haemoglobin, IQR = interquartile range, kg/m^2 = kilograms per metre squared, mmHg = milligrams of mercury, mg/mmol = miligram/milimole; mmol/l = millimoles per litre

Table 25. Year-10 outcomes based on clinic versus home visits

	Clinic visits (n = 435)	Home visits (n = 146)	p-value
Year-10 *g*	-0.08 (0.9)	-0.95 (1.0)	**<.001**
Dementia	17 (3.9%)	18 (12.3%)	**<.001**
Incident retinopathy	42 (9.7%)	13 (8.9%)	.885

Values are mean (SD) or n (%)

Table 26. Participants that experienced a macrovascular event at baseline and during 10-year follow-up

	Baseline prevalence	Incident at year-10
Myocardial infarction	150 (14.1%)	108 (10.1%)
Angina	298 (28.0%)	67 (8.7%)*
Stroke	62 (5.8%)	88 (8.3%)
Transient ischaemic attack	31 (2.9%)	41 (3.8%)
Coronary intervention	110 (10.3%)	65 (6.1%)

Values are n (%); N = 1066; *incident angina was only evaluated in those without angina at baseline

Table 27. Retinopathy grading at baseline and incident retinopathy at follow-up using Scottish DRS grading scheme

Retinopathy grade	Baseline (n = 1066)	Year-10 follow-up (n = 718)
R0	718 (67.4%)	616 (85.8%)
R1	293 (27.5%)	77 (10.7%)
R2	28 (2.6%)	1 (0.1%)
R3	4 (0.4%)	2 (0.3%)
R4	15 (1.4%)	2 (0.3%)
Missing	8 (0.8%)	20 (2.8%)

Values are n (%)

Table 28. Baseline *g* and dementia status of year-10 attenders and deceased

	Attenders (n = 581)	Non-attenders (n = 485)	p-value	Deceased year-10 (n = 319)	Alive year-10 (n = 747)	p-value
Baseline *g*	0.21 (0.9)	-0.26 (1.0)	**<.001**	-0.3 (1.0)	0.1 (1.0)	**<.001**
Dementia	35 (6.0%)	71 (14.6%)	**<.001**	43 (13.5%)	63 (8.4%)	**.012**

Values are mean (SD) or n (%)

Table 29. Baseline retinal vessel traits

	n	Total population (n = 1066)
CRAE - pixels	1028	33.03 (3.87)
CRVE - pixels	1028	44.67 (5.01)
Arterial tortuosity-log	1028	-10.07 (1.15)
Venular tortuosity-log	1028	-9.95 (0.89)
Fractal dimension-rank	1015	1.75 (0.07)

Values are mean (SD); CRAE = central retinal arterial equivalent, CRVE = central retinal venular equivalent

Table 30. Inter-class correlation (ICC) evaluating repeatability for ET2DS RVTs

Retinal vessel traits	Baseline inter-grader ICC (95% CI) n = 30	Baseline intra-grader ICC (95% CI) n = 31	RVT Change intra-grader ICC (95% CI) n = 35
CRAE	0.86 (0.70 to 0.93)	0.98 (0.96 to 0.99)	0.98 (0.97 to 0.99)
CRVE	0.88 (0.75 to 0.94)	0.95 (0.90 to 0.98)	0.97 (0.95 to 0.99)
Arterial tortuosity - median	0.91 (0.81 to 0.96)	0.96 (0.92 to 0.98)	0.86 (0.71 to 0.93)
Venular tortuosity - median	0.87 (0.72 to 0.94)	0.98 (0.95 to 0.99)	0.88 (0.76 to 0.94)
Total fractal dimension	0.81 (0.61 to 0.91)	0.98 (0.97 to 0.99)	1.00 (0.99 to 1.00)
Arterial fractal dimension	-	-	0.98 (0.97 to 0.99)
Venular fractal dimension	-	-	0.98 (0.97 to 0.99)
Total density	-	-	0.99 (0.99 to 1.00)
Arterial density	-	-	0.99 (0.97 to 0.99)
Venular density	-	-	0.98 (0.97 to 0.99)

CI = confidence interval, CRAE = central retinal arterial equivalent, CRVE = central retinal venular equivalent, ICC = interclass correlation

Table 31. Relationship between baseline retinal vessel traits and baseline variables

	CRAE	CRVE	Arterial tortuosity	Venular tortuosity	Fractal dimension
Age - years	-0.02(.489)	**-0.08(.015)**	**-0.07(.020)**	-0.03(.372)	-0.04(.262)
Sex - male	**0.14(<.001)**	**0.07(.017)**	**-0.17(<0.001)**	-0.05(.136)	0.04(.169)
Ever Smoker	-0.01(.751)	0.04(.245)	0.00(.971)	-0.05(.141)	-0.04(.196)
BMI – kg/m^2	0.03(.273)	**0.08(.012)**	0.03(.295)	**0.10(<.001)**	0.01(.743)
Systolic blood pressure - mmHg	-0.04(.260)	-0.02(.565)	0.01(.645)	**0.08(.009)**	-0.03(.397)
Total cholestero:HDL ratio	-0.06(.064)	0.02(.625)	**0.08(.016)**	0.01(.708)	**-0.07(.022)**
Hba1c - %Hb	-0.02(.436)	-0.02(.539)	-0.00(.954)	0.04(.189)	-0.01(.741)
Duration of diabetes - years	0.03(.423)	-0.01(.741)	-0.02(.494)	-0.01(.746)	0.02(.479)
Diabetes Treatment					
Diet controlled	0.03(.300)	-0.02(.447)	-0.01(.802)	0.05(.135)	-0.00(.935)
Tablets	-0.02(.533)	0.04(.220)	-0.01(.817)	-0.-5(.086)	0.02(.518)
Insulin	-0.01(.761)	-0.02(.547)	0.00(.911)	-0.01(.759)	-0.01(.834)
History of stroke	0.02(.599)	-0.01(.862)	-0.03(.413)	**-0.08(.011)**	0.01(.650)
History of CVD	-0.06(.066)	0.00(.990)	0.02(.451)	**-0.06(.048)**	-0.03(.411)
ACR – mg/mmol	0.00(.875)	-0.01(.678)	-0.01(.755)	0.03(.299)	0.00(.884)

Values are Pearson's correlation coefficient (p-value); ACR = albumin/creatinine ratio, CVD = cardiovascular disease, HbA1c = glycated haemoglobin, kg/m^2 = kilograms per metre squared, mmHg = milligrams of mercury, mg/mmol = miligrams/milimoles, mmol/l = millimoles per litre

Table 32. Baseline characteristics used in analyses comparing total population versus those with no missing data

	Total ET2DS (n = 1066)	ET2DS no missing data (n = 1008)	Total population without prevalent retinopathy (n = 718)	Population without prevalent retinopathy & no missing data (n = 689)	Total year 10 attenders (n = 581)	Year 10 attenders & no missing data (n = 558)
Age - years	67.9 (4.2)	67.8 (4.2)	67.9 (4.2)	67.8 (4.2)	67.3 (4.2)	67.3 (4.1)
Sex - male	547 (51.3%)	515 (51.1%)	353 (49.2%)	337 (48.9%)	296 (50.9%)	284 (50.9%)
Ever Smoker	568 (53.3%)	536 (53.2%)	381 (53.1%)	364 (52.8%)	316 (54.4%)	301 (53.9%)
BMI – kg/m^2	31.4 (5.7)	31.4 (5.6)	31.5 (5.6)	31.4 (5.6)	31.1 (5.5)	31.1 (5.4)
Systolic blood pressure - mmHg	133.3 (16.4)	133.4 (16.4)	133.2 (15.8)	133.3 (15.9)	132.1 (14.71)	132.1 (14.6)
Total cholesterol:HDL ratio	3.5 (1.1)	3.5 (1.1)	3.5 (1.1)	3.5 (1.1)	3.5 (1.1)	3.5 (1.1)
Hba1c - %Hb	7.4 (1.1)	7.4 (1.1)	7.3 (1.1)	7.3 (1.0)	7.4 (1.1)	7.4 (1.1)
Duration of diabetes - years	8.1 (6.5)	8.1 (6.4)	6.5 (5.0)	6.6 (5)	7.6 (6.2)	7.6 (6.1)
Diabetes Treatment						
Diet controlled (%)	198 (18.6%)	185 (18.4%)	169 (23.5%)	163 (23.7%)	127 (21.9%)	118 (21.1%)
Tablets (%)	681 (63.9%)	648 (64.3%)	474 (66.0%)	455 (66.0%)	420 (72.2%)	354 (63.4%)
Insulin (%)	186 (17.4%)	175 (17.4%)	74 (10.3%)	71 (10.3%)	89 (15.3%)	86 (15.4%)
History of stroke	62 (5.8%)	55 (5.5%)	30 (4.2%)	28 (4.1%)	24 (4.1%)	22 (3.9%)
History of CVD	347 (32.6%)	321 (31.8%)	224 (31.2%)	212 (30.8%)	160 (27.5%)	151 (27.1%)
ACR – mg/mmol	3.4 (13.4)	3.4 (13.6)	2.6 (10.3)	2.7 (10.5)	2.1 (5.7)	2.1 (5.8)
Diabetic retinopathy	340 (32.0%)	319	-	-	173 (29.8%)	166 (29.7%)

Values are mean (SD) or n (%); ACR = albumin/creatinine ratio, CVD = cardiovascular disease, HbA1c = glycated haemoglobin, HDL = high density lipoprotein, kg/m^2 = kilograms per metre squared, mmHg = milligrams of mercury, mg/mmol = miligrams/milimoles, mmol/l = millimoles per litre

6 Results II: Retinal vascular traits and incident retinopathy

This chapter presents the results of analyses on incident retinopathy as an outcome. The purpose of these analyses were to determine the association between retinal vessel traits (RVTs) measured at the baseline of the Edinburgh Type 2 Diabetes Study (ET2DS) and incident retinopathy over the 10 years of the study. This chapter presents baseline characteristics of the study population (including RVTs) by incident retinopathy status. The results of logistic regression used to evaluate the association between RVTs and incident retinopathy are then presented. The aim was to help determine if RVT measures might be useful as a novel biomarker for the development of diabetic retinopathy. Information regarding model evaluation is presented to help understand the fit and generalisability of the models.

6.1 Baseline characteristics by retinopathy status

At the baseline of the ET2DS, of the 1066 participants, 340 were determined to have prevalent diabetic retinopathy and eight did not have sufficient data to determine their retinopathy status (they did not attend the baseline ET2DS eye screening and did not attend a DRS screening around the time of ET2DS baseline). These 348 people were excluded from the following analysis as I was interested in understanding the temporal relationship between measured RVTs and retinopathy, so those that already have evidence of the disease do not provide appropriate evidence. Therefore, I have evaluated the remaining population of 718 study participants for this analysis.

Table 33 (page 189) presents baseline demographics information based on incident retinopathy status. Overall, those that went on to develop incident retinopathy were similar to those that did not for the variables evaluated, aside from those with retinopathy having increased HbA1c (7.6 vs 7.2 %Hb, standard deviation (SD) for both 1.0). Continuous variables are displayed using box plots based on incident retinopathy status in **Figure 21 (page 190).** Although there was evidence of increased HbA1c in those with incident retinopathy, the box plots show the differences were not large and most likely

not clinically relevant. **Figure 22 (page 191)** provides a visual representation using bar graphs of the differences in the categorical variables based on retinopathy status. There was little difference between those with diabetic retinopathy and those without. Although there were no statistically significant differences there was a trend towards more diet controlled diabetes at baseline in those that did not go on to develop incident retinopathy, which can be visualised in **Figure 22**.

6.2 Retinal vascular traits and incident retinopathy status

6.2.1 Univariate analysis of retinal vascular traits by retinopathy status

When evaluated by retinopathy status, there was evidence that those that went on to develop retinopathy had a narrower central retinal arteriolar equivalent (CRAE) (32.03 (SD 3.71) vs 33.03 (SD 3.78) pixels, p = 0.018), which represents narrower arterioles. This pattern was also observed with venules, where those with incident retinopathy had narrower central retinal venular equivalent (CRVE) compared with those with no retinopathy (43.54 (SD 4.36) vs 44.53 (SD 5.04) pixels, p = 0.044). There was no evidence of a difference in arterial tortuosity based on retinopathy status with the mean population arterial tortuosity of -10.11 for both groups (SD 1.26 with retinopathy and 1.11 without retinopathy). However, there was evidence of increased venular tortuosity in those with incident retinopathy (-9.71 (SD 1.06) vs – 10.01 (SD 0.87), respectively, p = 0.016. There was also reduced fractal dimension, or vascular complexity, in those with incident retinopathy. Those without retinopathy had a fractal dimension of 1.75 (SD 0.08) and those with retinopathy, 1.74 (SD 0.07), p = 0.048. The values can be found in **Table 34 (page 191).**

6.2.2 Linearity of retinal vascular traits and retinopathy

To assess linearity of the relationship between baseline RVTs and incident retinopathy, I created quartiles of each RVT and evaluated the risk of

retinopathy in each quartile. This method was chosen to provide reasonably sized groups in order to create meaningful crude incidence values to evaluate linearity. This was done in order to understand the direction of any associations that may have been found between the independent and dependent variables. Formal linearity testing for model evaluation is discussed later in the chapter after the logistic regression is described.

Table 35 (page 192) gives the risk of incident retinopathy for each RVT by quartile and **Figure 23 (page 193)** presents these findings graphically. There was a reduced risk of retinopathy with decreasing CRAE. CRVE was similar, but with slightly lower risk in the first quartile, compared with the second, and then a reduction of risk in the third and fourth quartiles. Arterial tortuosity displayed a possible J-shaped relationship, with an increased risk in the first and fourth quartiles, but a reduced risk in the second and third. This could be interpreted as very low or very high arterial tortuosity associated with incident retinopathy and may be a case for not using this RVT strictly as a continuous variable. Both venular tortuosity and fractal dimension show similar, but opposite patterns, with increased risk clearly in one quartile, the fourth for venular tortuosity and first for fractal dimension, and then reduced risk in the remaining three quartiles. These could both be cases for combining the three lower risk quartiles and comparing them to the high-risk quartile.

There are different methods of analysis of RVTs other than using them as the continuous variables directly extracted from the software. One method is to evaluate them as categorical values. This can be done by creating subgroups, such as quartiles, and analysing them as such. Taking this further, if there appears to be higher risk of the outcome at the upper and lower tails, which might indicate a J-shaped risk association, one could combine the upper and lower tail as a high risk group and compare it with the middle, lower risk group. Since I currently have very little information on the true association and these cut-offs are still very arbitrary, I chose to continue using the RVTs as continuous variables within the models.

6.3 Association between retinal vascular traits and incident retinopathy – Logistic regression

See **Table 36** for full logistic regression results.

6.3.1 CRAE

In the unadjusted model, decreased CRAE was associated with incident retinopathy with an odds ratio (OR) of 0.93 (95% confidence interval (CI) 0.87 to 0.99) and a p value of 0.028. This relationship was maintained after multivariable adjustment for age and sex (OR 0.93, 95% CI 0.87 to 0.99, p = 0.023), as well as after adding cardiometabolic (OR 0.93, 95% CI 0.87 to 0.99, p = 0.030) and diabetes related risk factors (OR 0.93, 95% CI 0.87 to 1.00, p = 0.038), with very little change in the point estimate at each step. However, the relationship lost statistical significance after vascular disease history and CRVE were incorporated into the model (OR 0.95, 95% CI 0.87 to 1.03, p = 0.212). This indicates a relationship between decreased CRAE and incident retinopathy, but not beyond other vascular markers of pathology and venular width.

6.3.2 CRVE

In the unadjusted model there was no evidence of an association between CRVE and incident retinopathy (OR 0.95, 95% CI 0.91 to 1.00, p = 0.058). After adjusting for age and sex and then cardiometabolic risk factors, the point estimate and confidence interval did not change, but there was evidence of an association between decreased CRVE and incident retinopathy, although very small (OR 0.95, 95% CI 0.91 to 1.00, p = 0.048). The association was lost after adding in diabetes related risk factors, although again there was no change to the point estimate (p = 0.051). However, when vascular disease risk factors and arterial width were accounted for, there was no evidence of an association (OR 1.00, 95% CI 0.58 to 1.75, p = 0.999).

6.3.3 Arterial tortuosity

There was no evidence of an association between arterial tortuosity and incident retinopathy in the unadjusted model (OR 0.99, 95% CI 0.81 to 1.22; p = 0.946). Analysis was ended after the age and sex adjusted model did not show any indication of an association (OR 0.99, 95% CI 0.80 to 1.22, p = 0.923).

6.3.4 Venular tortuosity

From the logistic regression, there was evidence of a strong association between increased venular tortuosity and incident retinopathy. This relationship was evident in the unadjusted stage, OR 1.43 (95% CI 1.11 to 1.84; p = 0.005) and was maintained after each block of covariates was added. After all covariates were added the OR was 1.51 (95% CI 1.15 to 1.98) with a p value of 0.003. Above all commonly cited risk factors and potential biomarkers for retinopathy, increased venular tortuosity was independently associated with increased incident retinopathy.

6.3.5 Fractal dimension

In the unadjusted logistic regression model there was no evidence of an association between fractal dimension and incident retinopathy, but after age and sex adjustment there was possible evidence of an association (OR 0.79, 95% CI 0.62 to 1.00) with p = 0.05, so further analysis was carried out. After cardiometabolic and diabetes risk factors were added there was an association between fractal dimension and retinopathy (OR 0.76, CI 0.60 to 0.98) and p = 0.033. And after all covariates were added the OR was 0.75 (95% CI 0.58 to 0.96) and p value was 0.017. Because a standardised variable was used for fractal dimension, the findings can be interpreted that with each standard deviation increase in fractal dimension, the odds of incident retinopathy decreases by 0.25.

6.3.6 Model evaluation

Model evaluation was performed on the most complete model analysed. So, for arterial tortuosity that would have been the age and sex adjusted model but for the remaining RVTs that would have been the fully inclusive Model 5.

Model assumptions

Linearity between continuous variables and the outcome is a necessary assumption to meet when conducting regression analysis. In section 6.2.2, a crude measure of linearity was explored with the retinopathy outcome in order to influence how the results of the logistic regression were interpreted. In order to satisfy the linearity assumption for model evaluation, formal plots were generated and visually inspected to evaluate linearity between the continuous predictor variables and the logit of the outcome. There was no visual evidence of non-linearity for CRAE, arterial tortuosity and fractal dimension. There was a possible increase in the mid-range CRVE values, but this may have been due to outliers. Venular tortuosity also showed possible increased risk at both the very high and very low-end measures. All continuous covariates were then inspected by adding an interaction term of the log of itself within the model. All covariates met the linearity assumption, except for the CRVE model. For this reason, the extreme outlier values within the CRVE model were removed. There were no differences in the findings of the model after these values were removed, but the linearity assumption was no longer violated. Values presented in **Table 36** represent the re-fitted model. There was no evidence of multicollinearity within any of the models.

Model Fit

Model fit was evaluated by comparing the c-statistic and AIC between the unadjusted model (Model 1) and the fully adjusted model (Model 5). Also, the Hosmer-Lemeshow goodness of fit test was used to look for evidence of poor model fit. All models demonstrated good fit, by c-statistic and AIC, and did not show evidence of poor fit. See **Appendix 17** for values. When evaluating residuals, there was no evidence of strong outliers or influential cases

6.3.7 Sensitivity analysis

Incident retinopathy was defined using a very strict definition in order to only include those that had established diabetic retinopathy. This definition required that if a person only had a retinopathy grading of R1, they must have this grading on at least two consecutive screenings in order to eliminate those with an image artefact, or possibly with a true microaneurysm that healed without progression. If the definition of incident retinopathy was loosened to include all those that ever had an R1 grading after baseline, the number with incident retinopathy would be 239. When the full logistic regression models included these 239 participants as having the outcome, there was no association found between any of the RVTs and incident retinopathy. See **Appendix 18.** As there were very few cases of retinopathy at the R2, R3 or R4 level, sensitivity analysis was not undertaken by separating out the severity of retinopathy.

6.4 Evaluation of RVTs as biomarkers for incident retinopathy

From the fully controlled logistic regression models, it was clear that there was an association between increased venular tortuosity and incident retinopathy, as well as decreased fractal dimension and retinopathy. From this evidence, the next step would be to add the trait to an existing risk model for retinopathy. A reliable and clinically validated risk prediction model does not currently exist for retinopathy. Therefore, I created a basic model, using evidence from many studies that looked at risk factors for retinopathy, and then added in the RVTs of interest to see if the model was improved. Comparing a basic model of HbA1c, ACR and systolic blood pressure, to the same model with venular tortuosity added, the c-statistic, or discriminative ability of the model, improved from 0.624 to 0.640 and was confirmed by the Likelihood ratio test, p = 0.013. Also the AIC measure improved from 487.65 to 483.43, which decreases with improvement. However, when fractal dimension was added to the basic model the c-statistic decreased from 0.625 to 0.621 and a p value of 0.048, indicating the model does not improve. These values can be found in **Table 37 (page 194)**. While these values do

not necessarily indicate a good model, as general convention suggests a c-statistic over 0.7 to be good, it is demonstrating the clinical relevance of venular tortuosity, which should be considered in future risk prediction model generation.

6.5 Chapter summary

This chapter presented my results on the association of baseline retinal vessel traits with incident retinopathy over the 10 years of follow-up. In the ET2DS, 82 participants went on to develop evidence of diabetic retinopathy, out of the 718 that had no retinopathy at baseline. These 82 participants were similar to those without retinopathy for many baseline characteristics, except that they had higher HbA1c. From univariate analysis, narrower arterioles and venules, as wells as increased venular tortuosity and decreased fractal dimension were found in participants that went on to develop retinopathy. After applying multivariable logistic regression, controlling for a large number of metabolic, diabetic and vascular risk factors, increased venular tortuosity and decreased fractal dimension were associated with incident retinopathy. Venular tortuosity, in particular, showed potential as a novel biomarker for diabetic retinopathy in an analysis comparing the RVT to the most regularly cited risk factors for diabetic retinopathy.

Table 33. Baseline demographic parameters by retinopathy status

	n	Incident retinopathy (n = 82)	No incident retinopathy (n = 616)	p-value
Age - years	718	67.4 (4.1)	67.9 (4.2)	0.222
Sex - male	718	40 (48.2%)	300 (48.7%)	0.920
Ever Smoker - yes	718	40 (48.2%)	335 (54.4%)	0.337
BMI - kg/m^2	718	31.0 (5.9)	31.5 (5.6)	0.720
Systolic blood pressure - mmHg	716	134.7 (14.8)	132.9 (15.9)	0.286
Cholesterol:HDL ratio	714	3.6 (1.2)	3.5 (1.1)	0.564
Hba1c - %Hb	712	**7.6 (1.0)**	**7.2 (1.0)**	**0.006**
Duration of diabetes - years	712	5.0 (3.0 – 9.0)	5.0 (3.0 – 9.0)	0.515
Diabetes Treatment	717			0.186
Diet controlled (%)		13 (15.7%)	152 (24.7%)	
Tablets (%)		60 (72.3%)	399 (64.8%)	
Insulin (%)		10 (12.0%)	63 (10.2%)	
Macrovascular events - yes	718	25 (30.1%)	206 (33.4%)	0.625
ACR – mg/mmol	713	1.2 (0.9 – 2.1)	1.1 (0.7 – 1.7)	0.054

Values are mean (SD), median (IQR) or n (%); ACR = albumin/creatinine ratio, CVD = cardiovascular disease, HbA1c = glycated haemoglobin, IQR = interquartile range, kg/m^2 = kilograms per metre squared, mmHg = milligrams of mercury, mg/mmol = miligram/millimole, mmol/l = millimoles per litre

Figure 21. Incident retinopathy and continuous variable

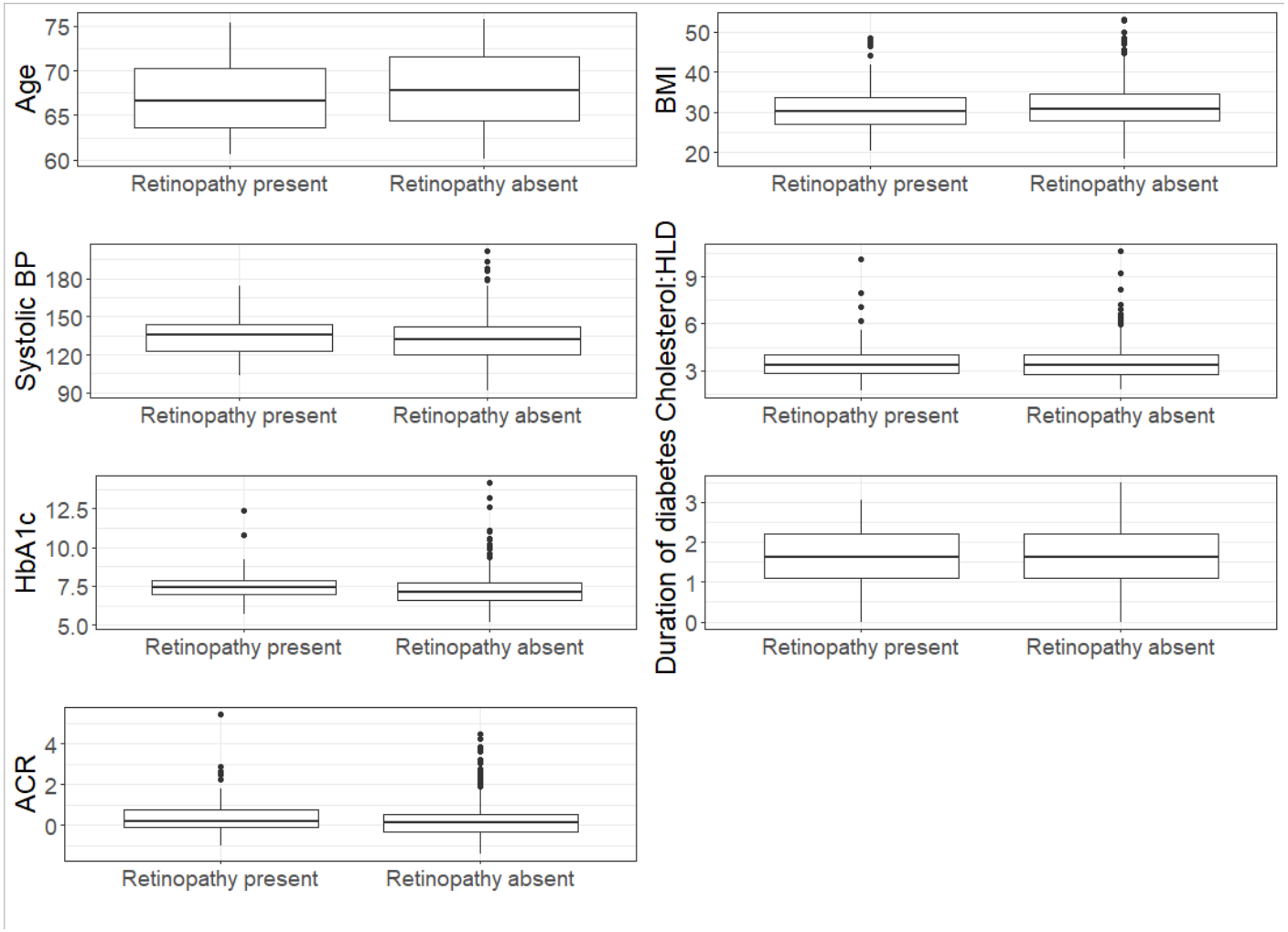


Figure 22. Incident retinopathy and categorical variables

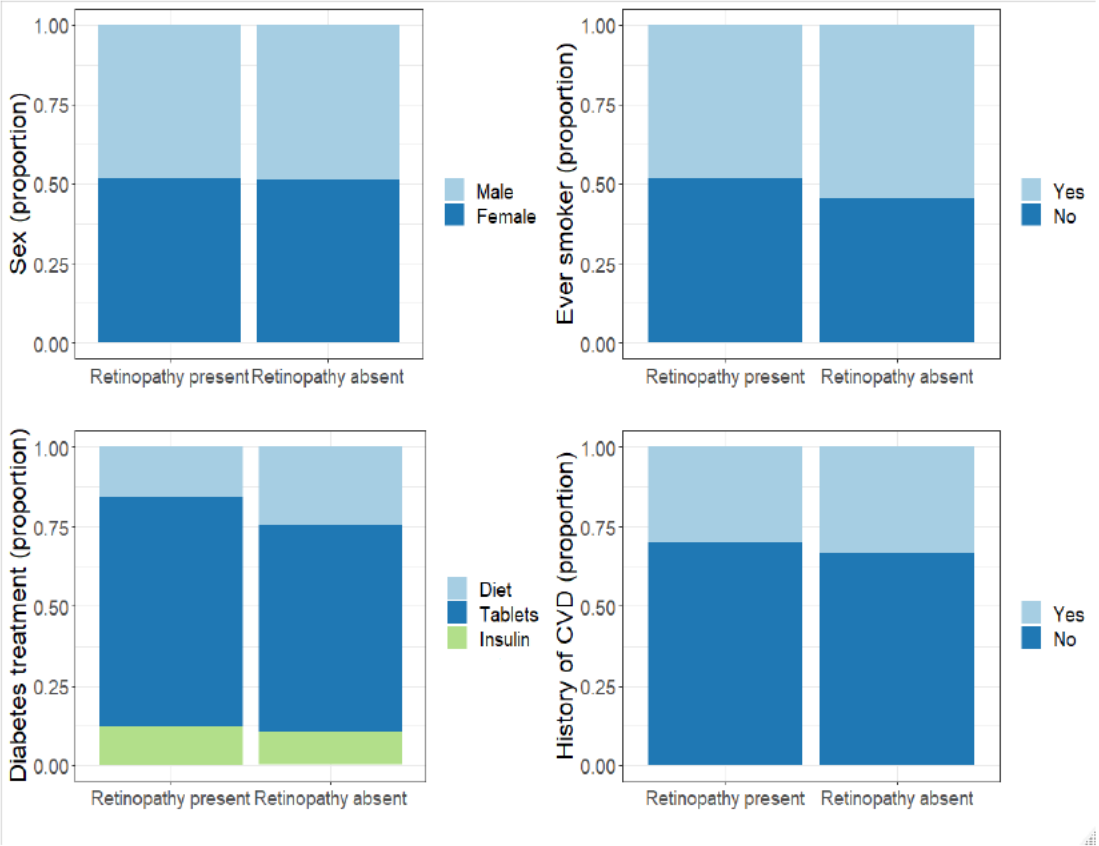


Table 34. Incident diabetic retinopathy and baseline retinal vessel traits

	n	Incident retinopathy (n = 82)	No incident retinopathy (n = 616)	p-value
CRAE - pixels	694	32.03 (3.71)	33.03 (3.78)	**.018**
CRVE - pixels	694	43.54 (4.36)	44.53 (5.04)	**.044**
Arterial tortuosity-log	694	-10.11 (1.26)	-10.11 (1.11)	.914
Venular tortuosity-log	694	-9.71 (1.06)	-10.01 (0.87)	**.016**
Fractal dimension-rank	686	1.74 (0.08)	1.75 (0.07)	**.048**

Values are mean (SD); CRAE = central retinal arterial equivalent, CRVE = central retinal venular equivalent

Table 35. Retinopathy risk per quartile RVT

Quartile	CRAE (n = 675)	CRVE (n = 675)	Arterial tortuosity (n = 675)	Venular tortuosity (n = 675)	Fractal dimension (n = 666)
1	25 (17.4%)	23 (13.5%)	24 (14.5%)	16 (9.5%)	29 (17.2%)
2	23 (13.7%)	25 (15.0%)	19 (11.3%)	17 (10.2%)	15 (9.1%)
3	18 (10.6%)	19 (11.2%)	14 (8.2%)	17 (10.0%)	18 10.8%)
4	14 (8.3%)	13 (7.7%)	23 (13.5%)	30 (17.6%)	18 (10.8%)

Values are n (%); CRAE = central retinal arterial equivalent; CRVE = central retinal venular equivalent

Figure 23. Retinopathy risk per quartile of retinal vessel trait

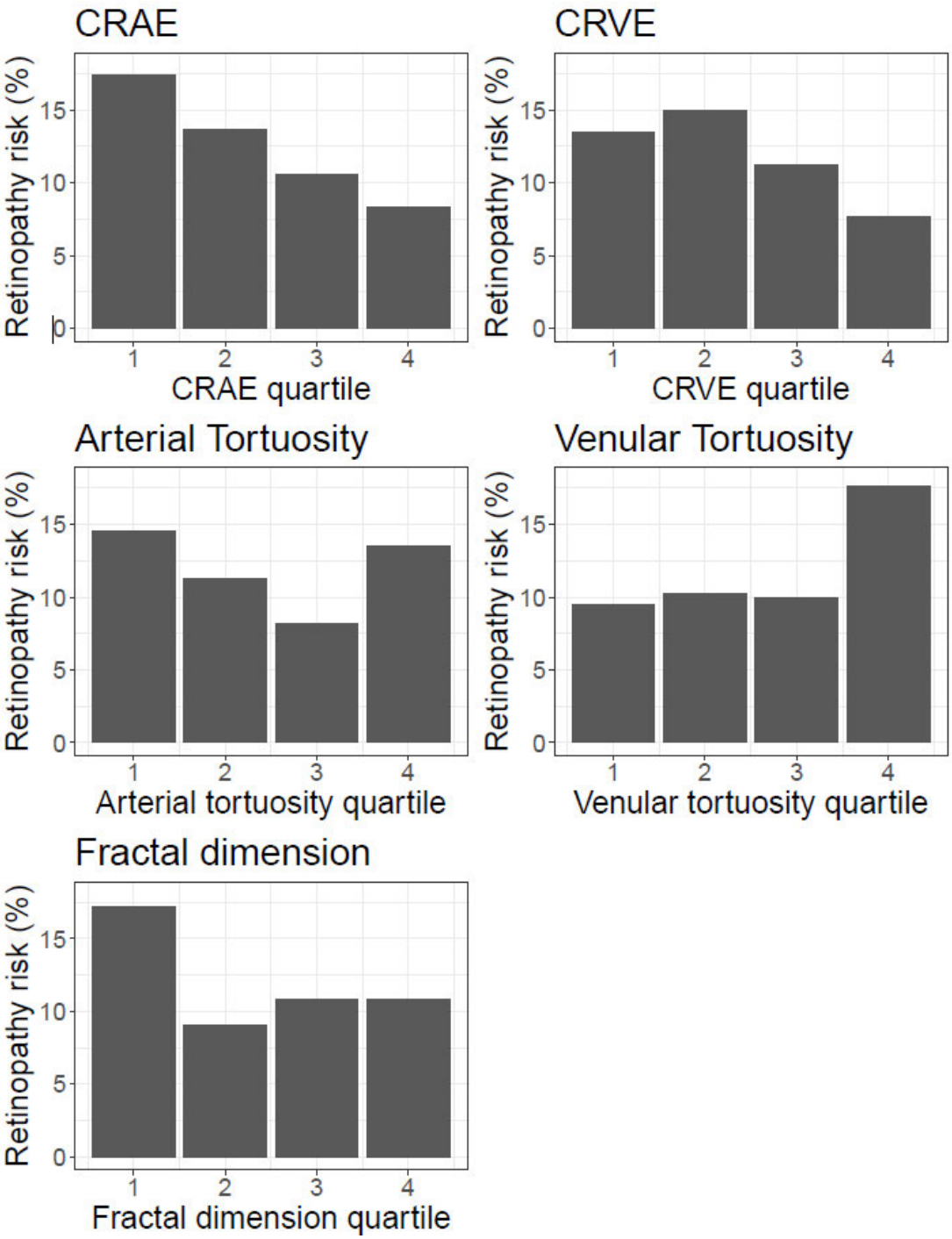


Table 36. Logistic regression: RVTs and incident retinopathy

	CRAE		CRVE		Arterial tortuosity		Venular tortuosity		Fractal dimension-standardised	
Model	OR (95% CI)	p	OR (95% CI)	p	OR (95% CI)	p	OR (95% CI)	p	OR (95% CI)	p
1	0.93 (0.87 to 0.99)	**.028**	0.95 (0.91 to 1.00)	.058	0.99 (0.81 to 1.22)	.946	1.43 (1.11 to 1.84)	**.005**	0.80 (0.63 to 1.01)	.059
2	0.93 (0.87 to 0.99)	**.023**	0.95 (0.91 to 1.00)	**.047**	0.99 (0.80 to 1.22)	.923	1.44 (1.12 to 1.86)	**.004**	0.79 (0.62 to 1.00)	**.050**
3	0.93 (0.87 to 0.99)	**.030**	0.95 (0.91 to 1.00)	**.048**			1.49 (1.16 to 1.93)	**.002**	0.80 (0.63 to 1.02)	.073
4	0.93 (0.87 to 1.00)	**.038**	0.95 (0.91 to 1.00)	.051			1.57 (1.21 to 2.04)	**.001**	0.76 (0.60 to 0.98)	**.033**
5	0.95 (0.87 to 1.03)	.212	1.00 (0.58 to 1.75)	.999			1.51 (1.15 to 1.98)	**.003**	0.75 (0.58 to 0.96)	**.025**

Model 1 – unadjusted, Model 2 – age and sex adjusted, Model 3 – Model 2 + cardiometabolic risk factors (BMI, smoking status, systolic blood pressure, total cholesterol:HDL ratio), Model 4 – Model 3 + diabetes related risk factors (HbA1c, duration of diabetes, diabetic treatment type), Model 5 – Model 4 + vascular disease (composite CVD, ACR) + CRAE or CRVE when analysing CRAE or CRVE; ACR = albumin/creatinine ratio; BMI = body mass index; CI = confidence interval; CRAE = central retinal arterial equivalent; CRVE = central retinal venular equivalent; HbA1c = glycated haemoglobin; HDL = high density lipoprotein; Model 5 CRAE and CRVE n = 655 (n = 63 removed due to missing data); Model 5 tortuosity n = 659 (n = 59 observations removed due to missing data); Model 5 fractal dimension n = 641 (n = 77 observations removed due to missing data)

Table 37. Comparing discrimination of models with addition of RVT

	Venular tortuosity			Fractal dimension-standardised		
	c-statistic	AIC	Likelihood ratio test p	c-statistic	AIC	Likelihood ratio test p
Base model	0.624	487.65	**.013**	0.625	485.22	**.048**
Base model + RVT	0.640	483.43		0.621	483.32	

Base model is made up of HbA1c, systolic blood pressure and ACR; AIC = Akaike information criterion; ACR = albumin to creatinine ration; RVT = retinal vessel trait

7 Results III: Retinal vascular traits and cognitive decline

This results chapter focuses on cognition as an outcome, specifically cognitive decline. For this thesis I have used two outcomes to describe cognitive decline: (1) dementia diagnosis at follow-up as a binary outcome and (2) change in the continuous variable, *g* (general intelligence factor), made from a composite of seven cognitive tests that seek to measure different cognitive domains. This chapter describes the Edinburgh Type 2 Diabetes Study (ET2DS) population that went on to have dementia and describes how *g* relates to other variables of interest. To evaluate the relationship between retinal vessel traits and cognitive decline I used logistic regression when evaluating dementia as an outcome and linear regression when evaluating change in *g* as an outcome. The results of a novel case-control analysis assessing whether change in retinal vessel traits (RVTs), and not just baseline values, were associated with cognitive decline, are also presented. As with the previous chapter, model evaluation is reported in order to better understand how the models fit the data and how generalizable these findings may be to other similar populations.

7.1 Incidence of dementia and relationship with *g*

I used both dementia and change in the derived general intelligence factor *g* to describe cognitive decline. For analyses on dementia, I used the entire ET2DS cohort of 1066 as dementia was identified partly through medical records. Using medical records for dementia diagnosis allows us to evaluate all participants within the cohort and not just those living at follow-up and available for dementia screening. Medical records, or in some cases, death records, for all 1066 participants were reviewed for evidence of dementia diagnosis. Because the study has such records for all participants, there was essentially no missing data for dementia. However, participants that have dementia but have not received testing or a formal diagnosis of dementia would still not be included as having dementia.

At baseline, none of the ET2DS participants had dementia, while incidence of dementia over the 10-year follow-up period included 106 participants, or 9.9%. For year-10 *g*, participants had to undergo follow-up clinical testing, so the number used in this analysis was 581. As expected, these two outcome variables were related to each other. Of the 106 participants in the whole study population diagnosed with dementia at year 10, 35 (33%) attended the year-10 follow-up clinic (6% of follow-up population) and of these, 17 (49%) were in the lowest decile of year-10 *g*. Of the 58 participants in the lowest 10% of follow-up *g*, 17 (29%) also had dementia. Participants with dementia at follow-up had a statistically significant lower mean *g* score (-1.83 (SD 0.90)), compared to those without dementia (-0.20 (SD 1.01), $p < 0.001$) (**Table 38 (page 207)**). **Figure 24 (page 207)** demonstrates the relationship between year 10 *g* and dementia diagnosis.

7.2 Longitudinal association between baseline RVTs and incident dementia

7.2.1 Demographics by dementia status

Table 39 (page 208) as well as **Figures 25 and 26 (page 209 and 210, respectively)** give baseline demographic information by dementia status. Participants that went on to develop dementia were older (69.4 years compared to 67.8 years, $p < 0.001$) had lower body mass index (BMI) (30.0 vs 31.6, $p = 0.005$) and were more likely to have retinopathy at baseline (40.6% vs 30.9%, $p = 0.045$). People that went on to develop dementia were more likely to be men and have a self-reported history of ever smoking, however these differences were not statistically significant, and a larger study population would be required to more definitively draw these conclusions. At baseline, those who went on to develop dementia had, on average, diabetes for a year longer, more likely to have had a stroke or other macrovascular disease history, but again, these differences were not statistically significant. Systolic blood pressure, total cholesterol:high density lipoprotein (HDL) cholesterol ratio, HbA1c, diabetes treatment and albumin/creatinine ratio (ACR) were all similar between those with and without dementia at follow-up.

7.2.2 Retinal vessel traits and dementia

Retinal vessel traits were compared by dementia status. Overall, there was no evidence of a difference between the two groups. Mean central retinal arteriolar equivalent (CRAE) did not differ based on dementia status (33.00 (standard deviation (SD) 4.30) vs 33.03 (SD 3.82)). Similarly, mean central retinal venular equivalent (CRVE) was very similar between the groups (43.77 (SD 5.42) for those with dementia and 44.77 (4.96) in those without dementia). For both arterial tortuosity and venular tortuosity, the participants with no dementia had slightly lower tortuosity values compared to those with dementia: -10.08 (SD 1.14) vs -10.04 (SD 1.24) and -9.96 (SD 0.88) vs -9.89 (SD 0.96), respectively, but no statistical differences were identified. Mean fractal dimension was 1.75 (SD 0.07) in both those with and without dementia. See **Table 40 (page 211).**

Linearity of retinal vessel traits and dementia

As with the retinopathy outcome, it is important to evaluate the relationship between the RVTs and cognition and whether there is a clear linear relationship. Quintiles were created for each RVT and the risk of dementia within each quintile was calculated **(Table 41 (page 211)).** These relationships are visualised in the bar charts in **Figure 27 (page 212)**. CRAE demonstrated a possible J-shaped relationship with dementia, with increased risk in the first and fourth quintiles. CRVE demonstrated a more straightforward relationship, with increased risk in the first quintile and similar risk in the second through fifth quintiles. Both arterial and venular tortuosity had risk patterns that were difficult to interpret, with risk varying between the quintiles. This was also true of fractal dimension. The participant numbers with dementia per quintile were often quite low, so this basic analysis was not adequately powered to fully demonstrate any true association, only to view possible direction. In model evaluation, I used statistical methods to determine if the assumption of

7.2.3 Association between retinal vessel traits and dementia- logistic regression

See **Table 42 (page 213)** for full logistic regression results for all RVTs. None of the evaluated RVTs showed any evidence of an association with dementia, using logistic regression. There were no statistically significant results from the unadjusted or age and sex adjusted models, so model building was not continued. For CRAE, the age and sex adjusted odds ratio (OR) was 0.99 (95% confidence interval (CI) 0.94 to 1.05) and 1.03 (95% CI 0.99 to 1.07) for CRVE. The confidence intervals were quite narrow, indicating a precise result using these data. Arterial and venular tortuosity measures had ORs of 0.91 (95% CI 0.80 to 1.16) and 0.92 (95% CI 0.73 to 1.16), respectively. The standardised fractal dimension showed no evidence of an association with dementia (OR 1.05, 95% CI 0.85 to 1.29).

7.2.4 Model evaluation

Model assumptions

When evaluating linearity between continuous independent variables and the logit function the assumption of linearity was upheld for all age and sex adjusted models presented that evaluated the association between the retinal vessel traits and dementia. There was also no evidence of non-linearity based on visual inspection.

For the age and sex adjusted models evaluating the association between RVTs and dementia there was no evidence of multicollinearity between the variables included in the models. This was demonstrated by variance inflation factors (VIFs) that were well below 10 and an average VIF no greater than one.

Model fit

All age and sex adjusted models demonstrated good fit, by c-statistic and AIC and did not show evidence of poor fit. See **Appendix 19** for values. When evaluating residuals, there was no evidence of strong outliers or influential cases.

7.2.5 Sensitivity and subgroup analysis

For sensitivity analysis, I increased the dementia outcome definition to include participants that fell in the lowest 10% for *g* at year-10. These participants may not have been diagnosed with dementia formally, but they did display diminished cognitive capabilities when compared with the whole cohort. This increased the number of people with the outcome from 106 to 147. There were no major changes to the findings. There was evidence of an association between increased CRVE and dementia and/or lowest decile *g*, OR 1.04 (95% CI 1.01 to 1.06, p = 0.020) in the unadjusted model, but this association was no longer evident after adjusting for age and sex. See **Appendix 20** for full results of this sensitivity analysis.

Subgroup analysis was undertaken to evaluate any variable effects based on important characteristics in an age and sex adjusted model with dementia and/or lowest decile *g* as the outcome. Specifically, I looked at if there were any differential associations in those that consumed no alcohol, moderate alcohol (1 to 14 units per week) or high alcohol (15 or more units per week). There was some evidence that in those that did not drink alcohol, increased venular tortuosity was associated with dementia and/or lowest decile *g* (OR 1.45, 95% CI 1.07 to 1.98, p <0.05), but none of the other alcohol categories had a similar association. In participants that drank 15 or more units of alcohol per week, decreased standardised fractal dimension was associated with dementia and/or lowest decile *g* (0.59, 95% CI 0.36 to 0.95, p <0.05).

I also evaluated if there was a difference between those that had experienced a hypoglycaemic episode at baseline or not. There was an association between increased arterial tortuosity and the outcome, dementia and/or lowest decile *g*, OR 1.74 (95% CI 1.06 to 3.00, p <0.05), but the confidence interval was wide. This finding would need to be investigated in a larger cohort with more documented episodes of hypoglycaemic events. There were no differences between the subgroups for any of the other RVTs. See **Table 43 (page 214)** for full results from the subgroup analysis.

7.3 Longitudinal association between baseline retinal vessel traits and cognitive decline

7.3.1 Cognitive decline and risk factor variables

In the population that returned for the year-10 follow-up, the mean *g* was found to be -0.29 (SD 1.07). This unit-less variable can be compared to the baseline *g* variable that has a mean of 0 and standard deviation of 1. This value indicates that average cognition decreased from baseline to follow-up. In those that returned for year-10 data collection, their baseline *g* was 0.21 (SD 0.95), so compared with the baseline mean of 0 for the whole population, those that returned had a higher cognition at baseline (see **section 5.4**).

In an unadjusted, univariate analysis, the general intelligence factor *g* at year-10 was negatively associated with age, which can be interpreted that with increased age, *g* is found to be decreased. There was also evidence of decreased *g* values in men, compared with women. After controlling for age and sex there was a slight, but significant association between lower *g* scores and increased BMI (-0.104), increased HbA1c (-0.119) and increased ACR (-0.159). Participants taking oral tablets or insulin at baseline were more likely to have a lower *g* at year-10, compared to those with diabetes management through diet alone (-0.124). Having a history of stroke or other macrovascular disease at baseline was also associated with lower *g* (0.146 and 0.128, respectively). Also, lower *g* at baseline was associated with lower *g* at follow-up. There was no correlation between follow-up *g* and smoking history, systolic blood pressure, total cholesterol:HLD ratio, duration of diabetes or retinopathy, at baseline. See **Table 44 (page 215)** for correlations between follow-up *g* and baseline risk factors as well as **Figure 28 and Figure 29 (page 216 and 217, respectively)** for graphical representations.

7.3.2 Cognition and retinal vessel traits

In the participants that returned for the year-10 data collection, their baseline CRAE and CRVE means were 33.02 (SD 3.88) and 44.75 (SD 4.97) pixels, respectively. The log arterial tortuosity mean was -10.04 (SD 1.15) and log

venular tortuosity was -9.96 (SD 0.88). Mean fractal dimension was 1.75 (SD 0.07). See **Table 45 (page 217).**

Evaluating the age and sex adjusted univariate relationship between the baseline RVTs and cognition at year-10, there were no obvious correlations between cognition and CRAE, CRVE or fractal dimension. There was evidence of a correlation between increased arterial and venular tortuosity and lower *g* (correlation coefficients -0.117 and -0.103, respectively). See **Table 46 (page 218)** and **Figure 30 (page 218)** for full correlations and a visual representation of the associations between the RVTs and follow-up *g*.

7.3.3 Association between retinal vessel traits and cognitive decline- linear regression

Table 47 (page 219) presents the full linear regression results evaluating the relationship between baseline RVTs and cognitive decline. There was no evidence of an association between any of the measured RVTs and cognitive decline in the unadjusted or age and sex adjusted models.

7.3.4 Sensitivity and Subgroup analysis

For the models in **Table 47**, year-10 *g* was used as the outcome, controlling for baseline *g* in order to evaluate cognitive decline, and not just cognitive status. If baseline *g* was removed, there was an association between increased arterial and venular tortuosity at baseline and lower *g* at year-10, in an age and sex adjusted linear regression model (standardised betas -0.127, p = 0.002; -0.087, p = 0.033, respectively), but these associations were lost after further controlling for cardiometabolic risk factors.

Subgroup analysis was evaluated by looking at participants by their alcohol consumption (no alcohol, moderate alcohol, high alcohol) and by separately looking at participants that experienced a hypoglycaemic event at baseline. For participants that drank moderate alcohol (1-14 units per week), there was a strong association between increased arterial tortuosity and lower year-10 *g*. The standardised beta was -0.217 and the p value was less than 0.001. There was no evidence of any other associations between RVTs and

cognitive decline in any of the subgroups in an age and sex adjusted linear regression (**Table 48 (page 219)**).

7.3.5 Model evaluation

Model assumptions

There were no concerns with multi-collinearity for any of the models when evaluating the variance inflation factor (VIF). There was also no evidence of heteroscedasticity when visually evaluating the standardised residual plots. The residuals vs fitted plots did not indicate any issues of non-linearity. The Durbin-Watson test was used to evaluate independent errors, which showed no concerns with autocorrelation.

Model fit

Using standardised residual plots, and Cook's distance, there was no evidence of highly influential outliers in any of the age and sex adjusted models evaluating RVTs and cognitive decline. Q-Q plots showed general normality for all models.

7.4 Association between baseline retinopathy and cognitive decline

In addition to understanding the association between RVTs at baseline and cognitive decline, it is also of interest to evaluate if there is a relationship between retinopathy at baseline and cognitive decline at follow-up. Using logistic regression, there was evidence of a possible association between retinopathy and dementia and/or lowest decile *g* (OR 1.44, 95% CI 1.00 to 2.06, p = 0.047) but this association was no longer significant after adjustment for age and sex. There was also no association between baseline retinopathy and year-10 *g*, using linear regression.

7.5 Change in retinal vessel traits and cognitive decline, a nested case-control study

7.5.1 Demographics based on cognitive decline case status

A total of 170 participants were included in this nested case-control study, 85 cases and 85 controls. Cases and controls had to have attended the year-10 data collection for full cognitive testing and have a minimum of 5 years between their baseline and follow-up retinal imaging. Cases were participants that had diagnosed dementia and/or were in the lowest 10% for follow-up *g*. Controls were randomly selected, age and sex matched from the top 50% of follow-up *g*.

When comparing the baseline demographic profiles of the cases and controls, controls were more likely to have smoked (41.1% cases vs 56.5% controls, $p = 0.046$), cases had a slightly higher HbA1C (7.5% cases vs 7.1% controls, $p = 0.026$) and cases were more likely to have had a stroke (4.7% cases vs 0.0% controls, $p = 0.043$). All other reported demographics were similar between the groups. See **Table 49 (page 220)** for the full comparison.

At the year-10 follow-up, cases had a higher rate of stroke (12.9% cases vs 0.0% controls, $p < 0.001$), as was the case with baseline stroke. HbA1c, CVD prevalence and diabetic retinopathy prevalence were all roughly the same between cases and controls. There was a longer follow-up time between retinal images for the controls, compared with cases (8.6 years cases vs 9.2 years controls, $p = 0.017$) See **Table 47 (page 220)** for full details.

7.5.2 Change in RVTs through study follow-up

Before comparing cases and controls, it is of interest to know if there were detectable changes in RVTs over the period between the baseline visit and 10-year follow-up. Cases and controls were evaluated separately as it is statistically inappropriate to treat them as one group. There was no evidence of any changes in CRAE, CRVE or arterial and venular tortuosity in either the cases or controls. There was, however, a decrease in fractal dimension

(total, arterial and venular) as well as a decrease in density (total, arterial and venular) for both the cases and controls (**Table 51 (page 221)).**

When comparing cases and controls, there was a greater decrease in the controls for venular fractal dimension, total vessel density and arterial density. For venular fractal dimension, there was a decrease of 0.03 in the cases vs 0.07 in the controls (p = 0.049). For total density, there was a decrease of 722 pixels in the cases and 989 pixels in the controls (p = 0.033) and for arterial density, 327 pixels in the cases and 416 pixels in the controls (p = 0.027). See **Table 52 (page 221)** for full differences in RVTs between cases and controls. **Appendix 21** provides a graphical representation of the change in RVTs using spaghetti plots.

7.5.3 Association between change in retinal vessel traits and cognitive decline – logistic regression

When the association between change in RVTs and cognitive decline was evaluated using univariate logistic regression, there were no differences between cases and controls for CRAE, CRVE, tortuosity (arterial and venular), fractal dimension (total, arterial and venular) or venular density. There was an association indicating that greater decreased total density and arterial density were associated with the control status (OR 0.96, 95% CI 0.92 to 0.99, p = 0.036 for total density and OR 0.92, 95% CI 0.84 to 0.99, p = 0.029 for arterial density). See **Table 53 (page 223)** for full univariate logistic regression analyses.

As noted previously, there was a longer follow-up time for the controls by about seven months, when compared with the cases, so it is possible the greater decrease in fractal dimension and density in the controls was merely due to longer progression of time. Logistic regression was repeated for total and arterial density, controlling for the length of follow-up time between the retinal images. There was no longer an association between the changes in the RVTs and cognitive decline (**Table 54 (page 223)**). This was not a pre-planned analysis but was important to see if the difference, although small, in follow-up time variable was having an effect on the association.

7.5.4 Model evaluation

Model assumptions

Linearity was assessed visually and there was no evidence of non-linearity for the models for the RVTs CRAE, CRVE, tortuosity (arterial and venular), arterial fractal dimension, venular fractal dimension, total and venular density. There was possible non-linearity in the total fractal dimension so the highest outliers were removed (only three cases over two standard deviations), which improved the linearity but did not alter the logistic regression outcome. Arterial density also showed some visual evidence of non-linearity, and when extreme outliers were removed the plot appeared more normal and the logistic regression results were not altered. There was no evidence of non-linearity for any of the models when tested for using interaction terms of the log of the continuous variable.

Model fit

Because the main models for this analysis were univariate, there was no need to evaluate the c-statistic or AIC values. Using Hosmer-Lemeshow goodness of fit test to evaluate if there was evidence of poor model fit, there was no evidence of poor model fit when evaluated CRAE, tortuosity (arterial and venular), fractal dimension (total, arterial and venular) or density (total, arterial and venular). The model evaluating change in CRVE did show evidence of a poorly fit model. A new model was created evaluating the log of the absolute change in CRVE. This model did not show any evidence of a poor fit. This model showed evidence of an association between increased log absolute change in CRVE and cognitive decline (OR 1.08, 95% CI 1.02 to 1.16, p = 0.009). This can be interpreted as more extreme changes in CRVE, either direction, were associated with cognitive decline. This analysis was not pre-planned, but offers a possible alternative to the poorly fit model using a simple change in CRVE.

There was no evidence of serious outliers or influential cases for any of the models when evaluating residuals.

7.6 Chapter summary

This chapter presented results from the evaluation of dementia and cognitive decline in the ET2DS population at follow-up. Dementia was diagnosed in 106 of the 1066 (9.9%) participants, and the general intelligence factor *g* was evaluated in the 581 participants that returned for follow-up analysis. There was no evidence of an association between RVTs and cognitive decline, using either outcome. When evaluating the relationship between change in RVTs during the study follow-up and cognitive decline, using a nested case-control study design, there was evidence of an association between less of a decrease in total and atrial density in the cases, but this association was most likely due to the longer follow-up in the controls than differences in cognitive status.

Table 38. Difference between year-10 *g* values for participants with and without dementia

	Dementia (n = 35)	No dementia (n = 546)	p-value
Year 10 *g*	-1.83 (0.90)	-0.20 (1.01)	**<0.001**

Values are mean year-10 *g* (SD); SD = standard deviation

Figure 24. Dementia and year-10 *g*

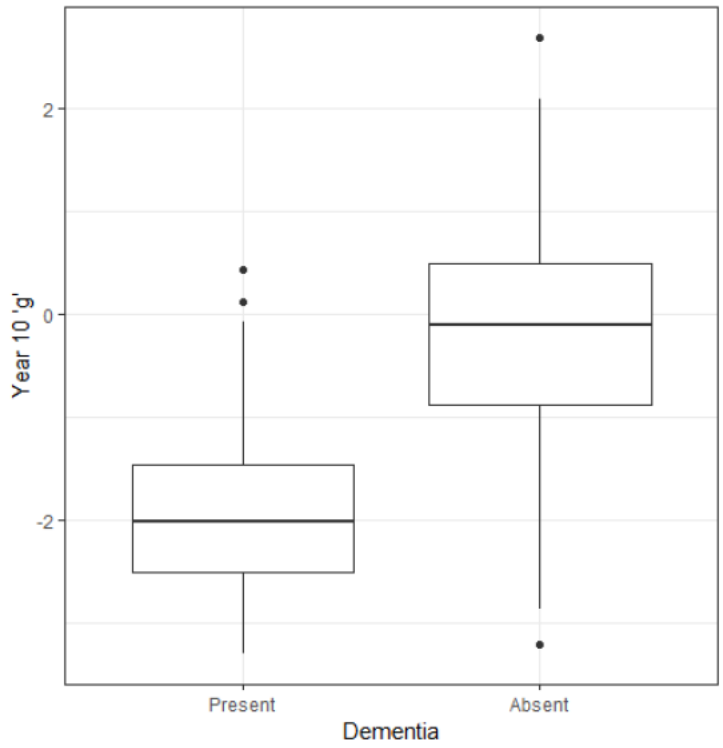


Table 39. Baseline demographic parameters by dementia status at follow-up

	n	Dementia (n = 106)	No dementia (n = 960)	p-value
Age - years	1066	**69.4 (4.0)**	**67.8 (4.2)**	**<.001**
Sex - male	1066	64 (60.4%)	483 (50.3%)	.062
Ever Smoker - yes	1066	47 (44.3%)	521 (54.3%)	.065
BMI – kg/m^2	1065	**30.0 (5.2)**	**31.6 (5.7)**	**.005**
Systolic blood pressure - mmHg	1064	133.9 (15.7)	133.2 (16.5)	.696
Total cholesterol:HDL ratio	1057	3.6 (1.3)	3.5 (1.1)	.760
Hba1c - %Hb	1057	7.5 (1.5)	7.4 (1.1)	.232
Duration of diabetes - years	1041	7.0 (3.0 – 12.0)	6.0 (3.0 – 11.0)	.197
Diabetes Treatment	1065			.623
Diet controlled		18 (17.0%)	180 (18.8%)	
Tablets		66 (62.3%)	615 (64.1%)	
Insulin		22 (20.8%)	164 (17.1%)	
Stroke - yes	1066	7 (6.6%)	55 (5.7%)	.884
Macrovascular events - yes	1066	43 (40.6%)	304 (31.7%)	.081
ACR – mg/mmol	1056	1.3 (0.8 – 2.1)	1.2 (0.8 – 2.0)	.330
Diabetic retinopathy - Yes	1058	**43 (40.6%)**	**297 (30.9%)**	**.045**

Values are mean (SD), median (IQR) or n (%); ACR = albumin/creatinine ratio, HbA1c = glycated haemoglobin, kg/m^2 = kilograms per metre squared, mg/mmol = milligram/millimole, mmHg = milligrams of mercury, mmol/l = millimoles per litre

Figure 25. Dementia status and continuous covariates

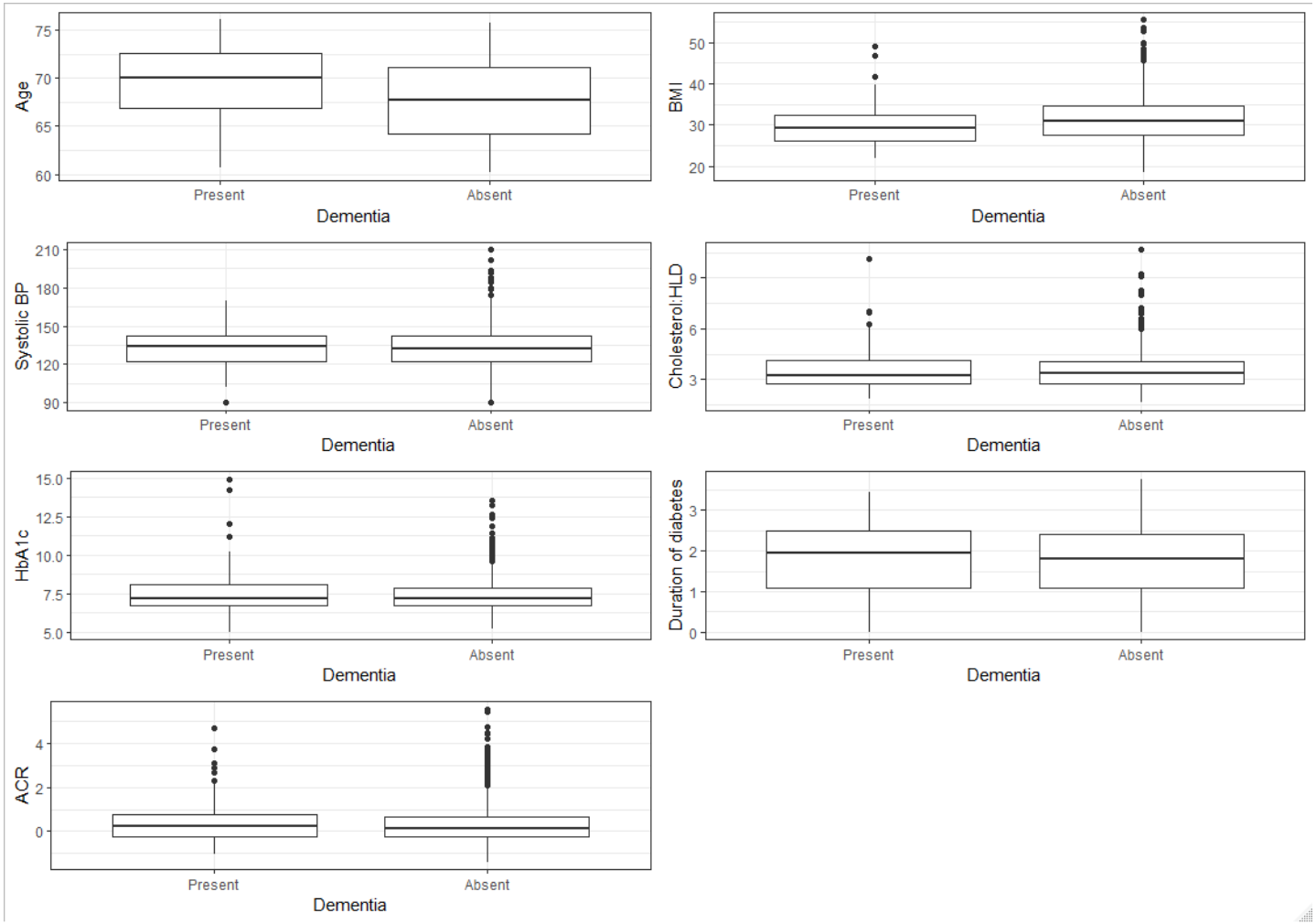


Figure 26. Dementia status and categorical covariates

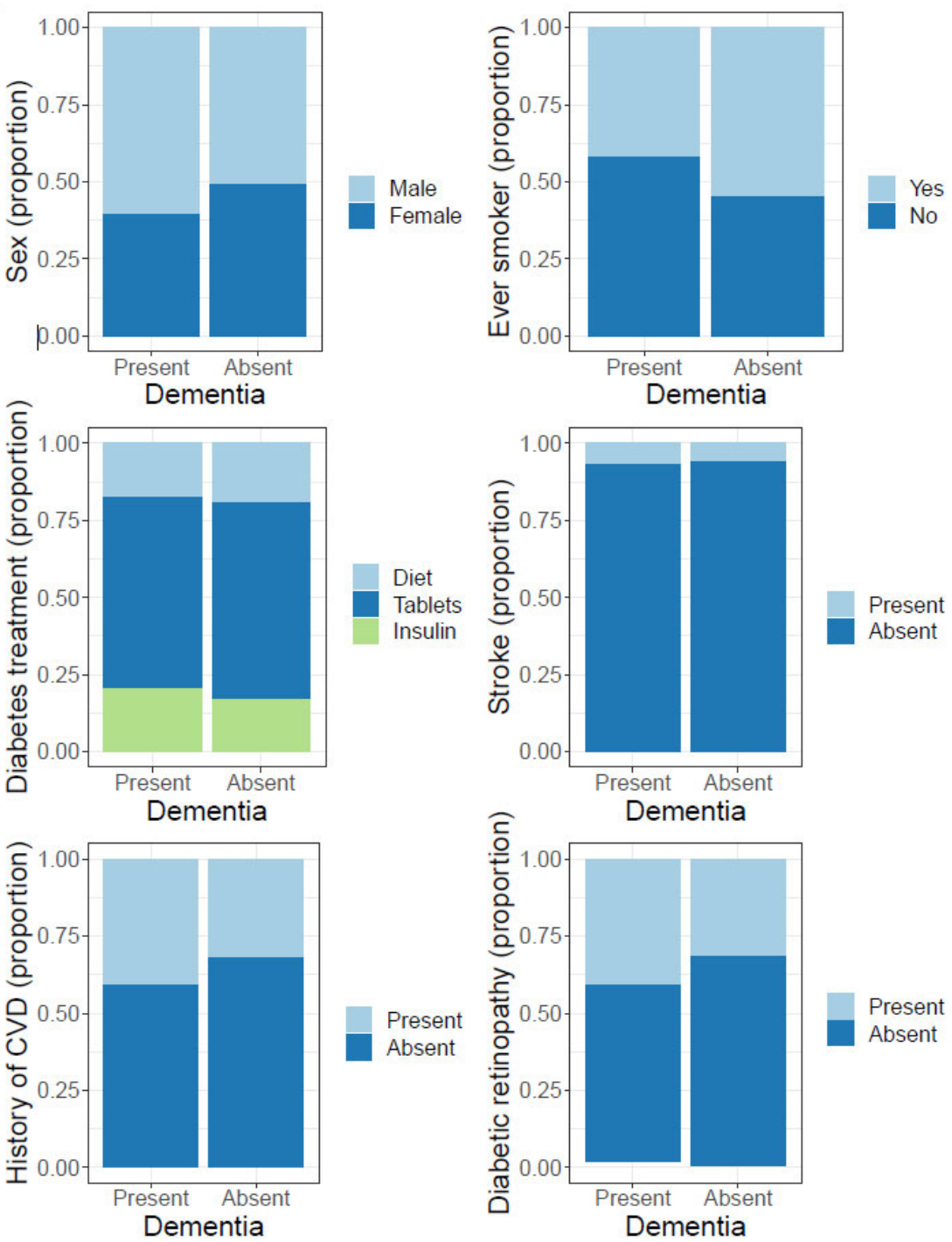


Table 40. Retinal vessel traits by dementia status

	n	Dementia (n = 106)	No dementia (n = 960)	p-value
CRAE - pixels	1028	33.00 (4.30)	33.03 (3.82)	0.948
CRVE - pixels	1028	43.77 (5.42)	44.77 (4.96)	0.06
Arterial tortuosity-log	1028	-10.04 (1.24)	-10.08 (1.14)	0.738
Venular tortuosity-log	1028	-9.89 (0.96)	-9.96 (0.88)	0.476
Fractal dimension-rank	1015	1.75 (0.07)	1.75 (0.07)	0.552

Values are mean (SD); CRAE = central retinal arterial equivalent, CRVE = central retinal venular equivalent

Table 41. Dementia risk per quintile of RVT

Quintile	CRAE (n = 1028)	CRVE (n = 1028)	Arterial tortuosity (n = 1028)	Venular tortuosity (n = 1028)	Fractal dimension (n = 1015)
1	25 (12.1%)	25 (12.1%)	24 (11.5%)	20 (9.7%)	20 (9.9%)
2	14 (6.8%)	21 (10.2%)	15 (7.4%)	26 (12.6%)	21 (10.3%)
3	20 (9.7%)	19 (9.3%)	22 (12.0%)	15 (7.3%)	23 (11.3%)
4	18 (8.8%)	19 (9.2%)	15 (7.3%)	15 (7.3%)	14 (6.7%)
5	23 (11.3%)	19 (9.2%)	24 (11.7%)	24 (11.7%)	20 (9.9%)

Values are n (%); CRAE = central retinal arterial equivalent; CRVE = central retinal venular equivalent

Figure 27. Evaluating dementia risk within RVT quintiles for evidence of linearity

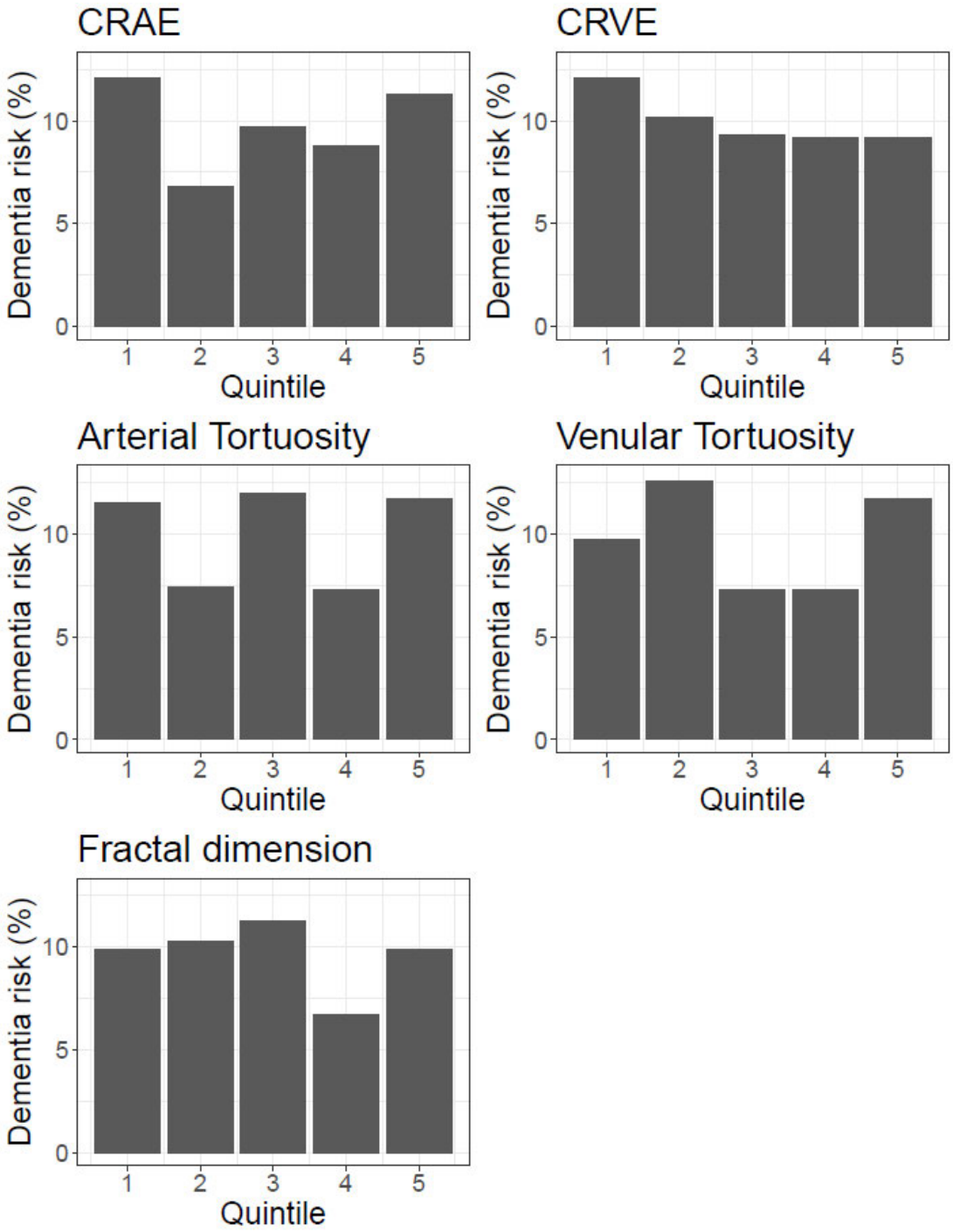


Table 42. Association between incident dementia and retinal vessel traits - logistic regression

	CRAE		CRVE		Arterial tortuosity		Venular tortuosity		Fractal dimension-standardised	
	OR (95% CI)	p	OR (95% CI)	p	OR (95% CI)	p	OR (95% CI)	p	OR (95% CI)	p
Model 1	1.00 (0.95 to 1.06)	.948	1.04 (1.00 to 1.08)	.061	0.97 (0.81 to 1.16)	.737	0.92 (0.73 to 1.16)	.475	1.07 (0.86 to 1.31)	.552
Model 2	0.99 (0.94 to 1.05)	.801	1.03 (0.99 to 1.07)	.143	0.96 (0.80 to 1.16)	.683	0.92 (0.73 to 1.16)	.475	1.05 (0.85 to 1.29)	.673
Model 3	-	-	-	-	-	-	-	-		
Model 4	-	-	-	-	-	-	-	-		
Model 5	-	-	-	-	-	-	-	-		

Model 1 – unadjusted, Model 2 – age and sex adjusted, Model 3 – Model 2 + cardiometabolic risk factors (BMI, smoking status, systolic blood pressure, total cholesterol:HDL ratio), Model 4 – Model 3 + diabetes related risk factors (HbA1c, duration of diabetes, diabetic treatment type), Model 5 – Model 4 + vascular disease (stroke, composite CVD, ACR, retinopathy) + CRAE or CRVE when analysing CRAE or CRVE; n = 1028 for CRAE, CRVE and tortuosity (28 cases with missing data) and n = 1015 for fractal dimension (51 cases with missing data); ACR = albumin/creatinine ratio; BMI = body mass index; CI = confidence interval; CRAE = central retinal arterial equivalent; CRVE = central retinal venular equivalent; HbA1c = glycated haemoglobin; HDL = high density lipoprotein; OR = odds ratio

Table 43. Association between RVTs and dementia plus lowest decile year 10 *g* by subgroup analysis

	No alcohol (n = 459)	**Moderate alcohol consumption (n = 394)**	**High alcohol consumption (n = 213)**	**Experienced a hypo episode (n = 113)**	**No hypo episode (n = 948)**
	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)
CRAE	1.02 (0.96 to 1.09)	0.98 (0.90 to 1.06)	0.90 (0.79 to 1.03)	0.96 (0.83 to 1.11)	1.02 (0.97 to 1.07)
CRVE	0.99 (0.94 to 1.05)	0.95 (0.89 to 1.00)	0.95 (0.87 to 1.04)	0.99 (0.88 to 1.10)	1.04 (1.00 to 1.08)
Arterial tortuosity	1.05 (0.84 to 1.31)	1.07 (0.80 to 1.28)	1.28 (0.85 to 1.94)	**1.74 (1.06 to 3.00)***	0.85 (0.71 to 1.00)
Venular tortuosity	**1.45 (1.07 to 1.98)***	0.98 (0.71 to 1.36)	0.81 (0.45 to 1.45)	0.73 (0.39 to 1.36)	0.88 (0.71 to 1.10)
Fractal dimension-standardised	0.98 (0.75 to 1.27)	1.01 (0.74 to 1.38)	**0.59 (0.36 to 0.95)***	0.91 (0.51 to 1.64)	1.08 (0.86 to 1.36)

Age + sex adjusted models; *p<0.05 **p<0.01 ***p<0.001; CI = confidence interval; CRAE = central retinal arterial equivalent; CRVE = central retinal venular equivalent; OR = odds ratio

Table 44. Correlation between Year-10 g and risk factor variables

	Unadjusted	Age and sex adjusted
	Year-10 *g*	**Year-10 *g***
Age - years	**-0.265*** **	-
Sex - male	**0.088***	-
Ever Smoker - yes	-0.085	-0.072
BMI – kg/m^2	-0.021	**-0.104***
Systolic blood pressure - mmHg	-0.052418	-0.024
Cholesterol:HDL ratio	-0.016	-0.035
HbA1c- mmol/mol	**-0.087****	**-0.119****
Duration of diabetes - log	0.067	-0.078
Diabetes Treatment	-0.079	**-0.124****
History of stroke	**0.149*****	**0.146*****
History of CVD	**0.140*****	**0.128****
ACR-log – mg/mmol	**-0.158*****	**-0.159*****
Diabetic retinopathy	0.028	0.027
Baseline *g*	**0.774*****	**0.767*****

Values are Pearson's correlation coefficient; *p<0.05 **p<0.01 ***P<0.001; ACR = albumin:creatinine ration; BMI = body mass index; CVD = cardiovascular disease; HbA1c = glycated haemoglobin; kg/m^2 = kilograms per metre squared, mmHg = milligrams of mercury, mg/mmol = miligram/millimole, mmol/l = millimoles per litre

Figure 28. Correlation between year-10 *g* and continuous risk factors

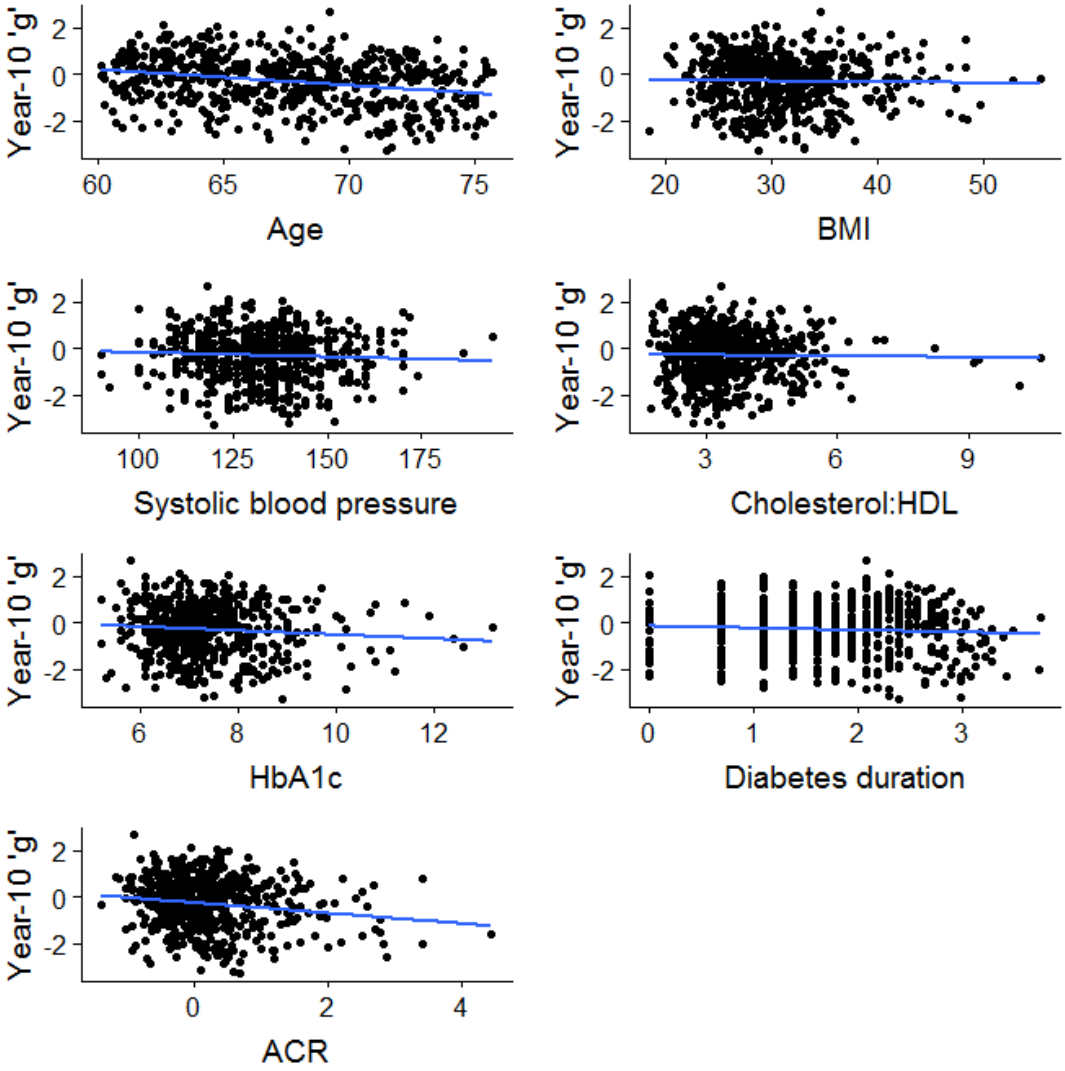


Figure 29. Correlation between Year-10 *g* and categorical risk factor variables

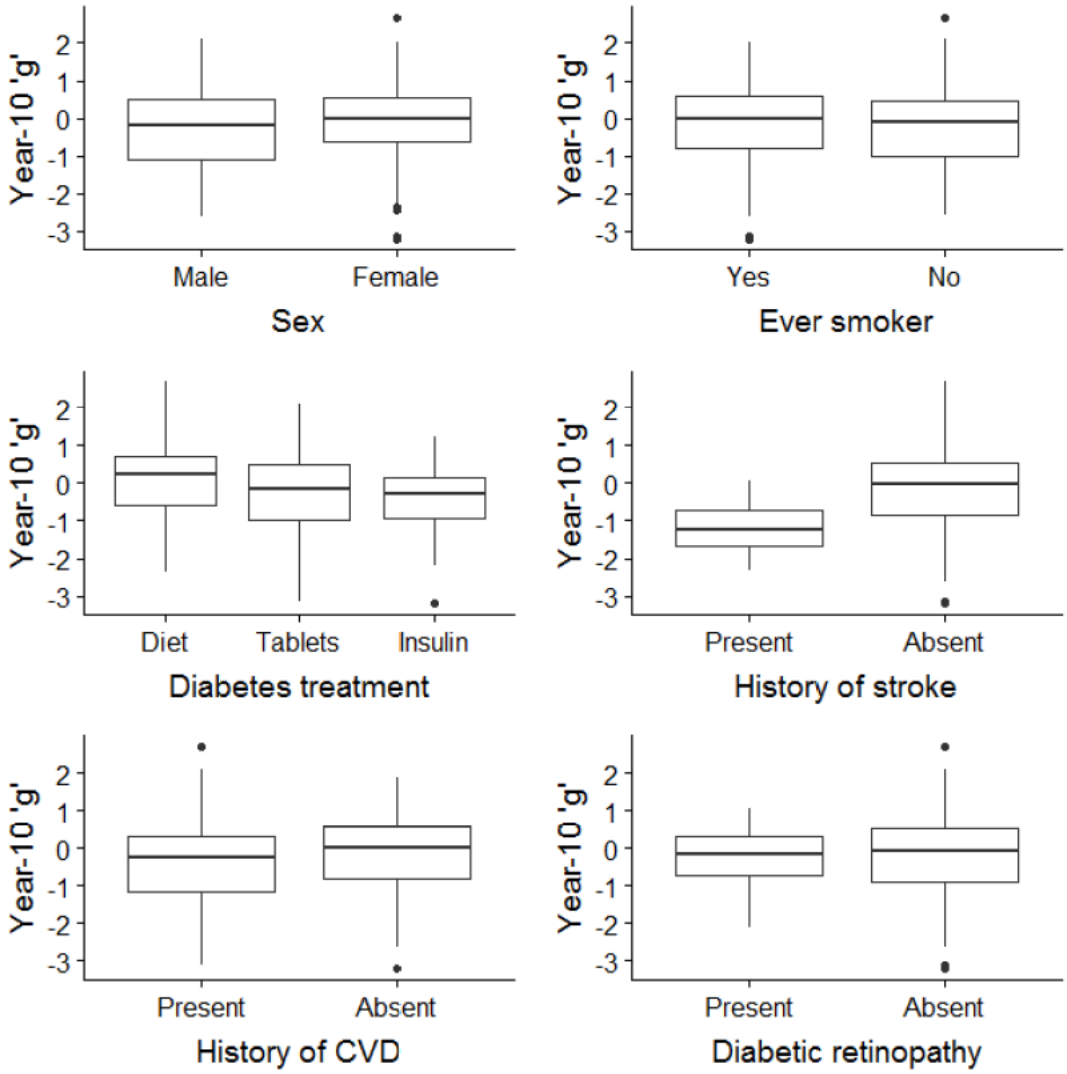


Table 45. *g* variables and baseline retinal vessel traits for ET2DS year 10 attenders

	n	Mean (SD)
CRAE- pixels	565	33.02 (3.88)
CRVE- pixels	565	44.75 (4.97)
Arterial tortuosity- log	565	-10.04 (1.15)
Venular tortuosity- log	565	-9.96 (0.88)
Fractal dimension- rank	558	1.75 (0.07)

CRAE = central retinal arterial equivalent; CRVE = central retinal venular equivalent; SD = standard deviation

Table 46. Correlation between Year-10 *g* and retinal vessel traits

	Unadjusted	Age+Sex adjusted
	Year-10 *g*	**Year-10 *g***
CRAE	-0.010	-0.030
CRVE	0.029	-0.007
Arterial tortuosity-log	**-0.122****	**-0.117****
Venular tortuosity-log	**-0.084***	**-0.103***
Fractal dimension-rank	0.052	0.047

Values are Pearson's correlation coefficient; *p<0.05 **p<0.01 ***p<0.001; CRAE = central retinal arterial equivalent, CRVE = central retinal venular equivalent

Figure 30. Correlation between retinal vessel traits and Year-10 *g*

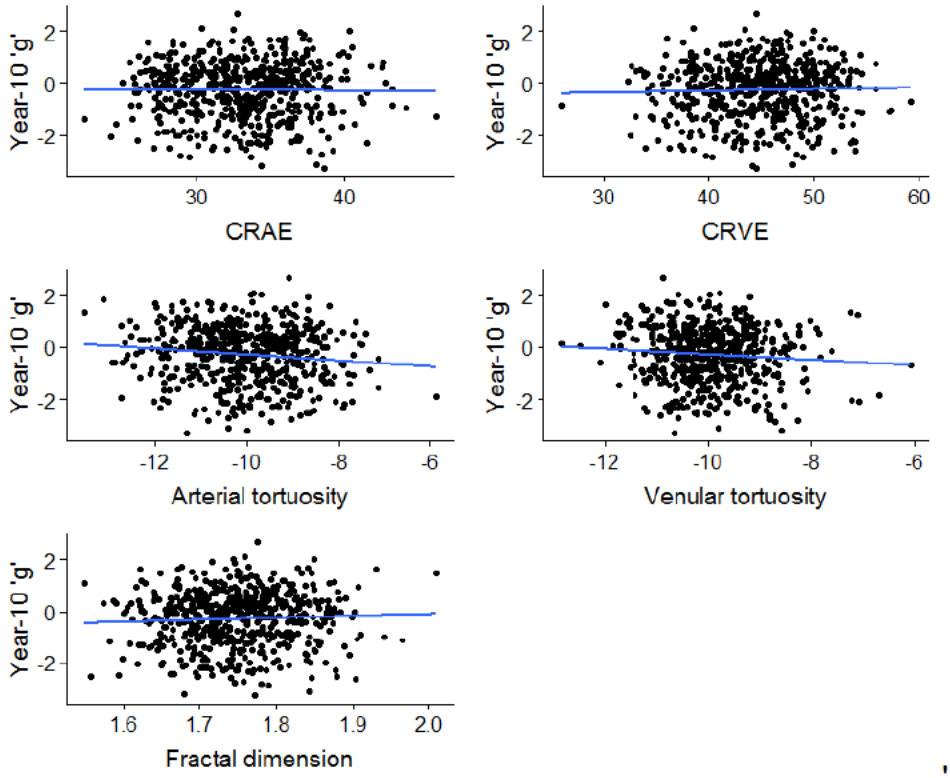


Table 47. Longitudinal association between cognitive decline and retinal vessel traits - linear regression

	CRAE			CRVE			Arterial tortuosity			Venular tortuosity			Fractal dimension		
	R^2	stand. β	p	R^2	stand. β	p	R^2	stand. β	p	R^2	stand. β	p	R^2	stand. β	p
Model 1	.60	-0.011	.671	.60	-0.027	.317	.60	-0.033	.226	.60	-0.038	.152	.60	-0.006	.818
Model 2	.62	-0.015	.577	.62	-0.034	.200	.62	-0.040	.133	.62	-0.042	.109	.62	-0.006	.812
Model 3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Model 4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Model 5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Model 1 – unadjusted, Model 2 – age and sex adjusted, Model 3 – Model 2 + cardiometabolic risk factors (BMI, smoking status, systolic blood pressure, total cholesterol:HDL ratio), Model 4 – Model 3 + diabetes related risk factors (HbA1c, duration of diabetes, diabetic treatment type), Model 5 – Model 4 + vascular disease (stroke, composite CVD, ACR, retinopathy) + CRAE or CRVE when analysing CRAE or CRVE; n = 560 for CRAE, CRVE and tortuosity (21 cases with missing data) and n = 554 for fractal dimension (27 cases with missing data); ACR = albumin/creatinine ratio; BMI = body mass index; CI = confidence interval; CRAE = central retinal arterial equivalent; CRVE = central retinal venular equivalent; HbA1c = glycated haemoglobin; HDL = high density lipoprotein

Table 48. Retinal vessel traits and cognitive decline- linear regression subgroup analysis- age and sex adjusted

	No alcohol (n = 224)	Moderate alcohol (n = 226)	High alcohol (n = 131)	Experienced hypo episode (n = 48)	No hypo episode (n = 530)
CRAE	**-0.135***	0.068	0.017	<-0.001	-0.016
CRVE	-0.082	0.093	-0.052	-0.106	-0.029
Arterial tortuosity	-0.024	**-0.217*****	-0.147	-0.065	-0.037
Venular tortuosity	-0.118	-0.51	-0.043	-0.217	-0.021
Fractal dimension	0.003	0.019	0.151	-0.051	-0.001

Values are standardised beta coefficients; *p<0.05 **p<0.01 ***p<0.001; CRAE = central retinal arterial equivalent; CRVE = central retinal venular equivalent

Table 49. Baseline demographics by cognitive decline case status

	n	Cases (n =85)	Controls (n =85)	p-value
Age - years	170	68.6 (4.2)	68.4 (4.1)	.705
Sex - male	170	47 (55.3%)	47 (55.3%)	1
Ever Smoker - yes	170	**35 (41.1%)**	**48 (56.5%)**	**.046**
BMI- kg/m^2	170	30.6 (5.3)	29.9 (5.5)	.396
Systolic blood pressure - mmHg	169	131.4 (13.1)	132.9 (13.9)	.480
Total cholesterol - mmol/l	168	4.2 (0.8)	4.2 (0.9)	.844
Hba1c - %Hb	169	**7.5 (1.3)**	**7.1 (0.97)**	**.026**
Duration of diabetes- years	168	8.7 (6.8)	7.4 (5.2)	.186
Diabetes Treatment				
Diet controlled	170	16 (18.8%)	25 (29.4%)	.107
Tablets	170	63 (74.1%)	59 (69.4%)	.496
Insulin	168	14 (16.7%)	8 (9.5%)	.170
Stroke - Yes	170	**4 (4.7%)**	**0 (0.0%)**	**.043**
CVD - Yes	170	26 (30.6%)	17 (20.0%)	.112
ACR – mg/mmol	169	3.0 (12.0)	1.8 (3.4)	.376
Diabetic retinopathy - Yes	168	30 (34.5%)	28 (33.3%)	.871

Values are mean (SD) or n (%); ACR = albumin/creatinine ratio, CVD = cardiovascular disease, HbA1c = glycated haemoglobin, IQR = interquartile range, kg/m^2 = kilograms per metre squared, mg/mmol = milligram/millimole, mmHg = milligrams of mercury, mmol/l = millimoles per litre

Table 50. Follow-up comorbidity profile of cases and controls

	n	Cases (n =85)	Controls (n =85)	p
Follow-up time- years	170	8.6 (1.7)	9.2 (1.5)	**.017**
Hba1c - %Hb	143	7.5 (1.4)	7.3 (1.1)	.300
Stroke - Yes	170	11 (12.9%)	0 (0%)	**<.001**
CVD - Yes	170	20 (23.5%)	12 (14.1%)	.117
Diabetic retinopathy - Yes	114	10 (17.5%)	9 (15.8%)	.802

Values are mean (SD) or n (%); CVD = cardiovascular disease, HbA1c = glycated haemoglobin

Table 51. Difference between baseline and follow-up RVTs

Change in RVTs	Cases (n = 85)	p	Controls (n = 85)	p
CRAE - pixels	1.05 (-0.50 to 2.61)	.180	-0.03 (-1.69 to 1.62)	.968
CRVE - pixels	1.25 (-0.21 to 2.72)	.092	1.68 (-0.33 to 3.70)	.100
Arterial tortuosity	-0.08 (-0.18 to 0.03)	.135	<0.01 (-0.11 to 0.11)	.985
Venular tortuosity	-0.08 (-0.18 to 0.03)	.139	-0.04 (-0.15 to 0.07)	.503
Total fractal dimension	-0.04 (-0.05 to -0.02)	<.001	-0.05 (-0.06 to -0.06)	**<.001**
Arterial fractal dimension	-0.06 (-0.07 to -0.04)	<.001	-0.07 (-0.09 to 0.06)	**<.001**
Venular fractal dimension	-0.03 (-0.05 to -0.02)	<.001	-0.05 (-0.06 to -0.04)	**<.001**
Total density	-722 (-909 to -534)	<.001	-989 (-1150 to -828)	**<.001**
Arterial density	-327(-417 to -236)	<.001	-461 (-540 to -383)	**<.001**
Venular density	-392 (-493 to -292)	<.001	-523 (-611 to -435)	**<.001**

Values are mean difference (95% CI); CI = confidence interval; CRAE = central retinal arterial equivalent; CRVE = central retinal venular equivalent

Table 52. Change in RVTs by cognitive decline case status

	n	Cases (n =85)	Controls (n =85)	p-value
Change in CRAE - pixels	170	1.05 (7.19)	-0.03 (7.68)	.342
Change in CRVE - pixels	170	1.25 (6.79)	1.68 (9.34)	.734
Change in Arterial tortuosity	146	-0.08 (0.46)	0.00 (0.48)	.300
Change in Venular tortuosity	166	-0.08 (0.46)	-0.04 (0.50)	.597
Change in total fractal dimension	170	-0.04 (0.06)	-0.05 (0.05)	.161
Change in arterial fractal dimension	170	-0.06 (0.07)	-0.07 (0.07)	.075
Change in venular fractal dimension	170	-0.03 (0.06)	-0.05 (0.05)	**.049**
Change in total density	170	-722 (869)	-989 (147)	**.033**
Change in arterial density	170	-327 (419)	-416 (364)	**.027**
Change in venular density	170	-393 (466)	-523 (406)	.053

Values are mean (SD); CRAE = central retinal arterial equivalent; CRVE = central retinal venular equivalent, SD = standard deviation

Table 53. Association between cognitive decline and change in RVTs – Univariate Logistic regression

	Change in CRAE		Change in CRVE		Change in Arterial tortuosity		Change in Venular tortuosity	
	OR (95% CI)	p	OR (95% CI)	p	OR (95% CI)	p	OR (95% CI)	p
Logistic regression	0.98 (0.94 to 1.02)	.341	1.01 (0.97 to 1.05)	.732	1.05 (0.49 to 2.26)	.905	1.16 (0.61 to 2.20)	.654

	Change in total fractal dimension		Change in arterial fractal dimension		Change in venular fractal dimension	
	OR (95% CI)	p	OR (95% CI)	p	OR (95% CI)	p
Logistic regression	0.02 (<0.01 to 5.04)	.162	0.02 (<0.01 to 1.44)	.078	0.01 (<0.01 to 0.95)	.052

	Change in total density		Change in arterial density		Change in venular density	
	OR (95% CI)	p	OR (95% CI)	p	OR (95% CI)	p
Logistic regression	**0.96 (0.92 to 0.99)**	**.036***	**0.92 (0.84 to 0.99)**	**.029***	0.93 (0.87 to 1.00)	.056

CI = confidence interval; CRAE = central retinal arterial equivalent; CRVE = central retinal venular equivalent; OR = odds ratio; n = 170 for CRAE and CRVE; n = 124 for arterial tortuosity; n = 162 for venular tortuosity; n = 170 for fractal dimension and density

Table 54. Controlling for follow-up time- change in density logistic regression

	Change in total density		Change in arterial density	
	OR (95% CI)	p	OR (95% CI)	p
Logistic regression	0.99 (0.99 to 1.00)	.165	0.99 (0.99 to 1.00)	.143

CI = confidence interval; OR = odds ratio; n = 170

8 Discussion

8.1 Summary of key findings

The Edinburgh Type 2 Diabetes Study (ET2DS) is a prospective cohort made up of 1066 elderly men and women with type 2 diabetes from the Lothian region of Scotland. At baseline, the cohort was found to be generally representative of the wider Lothian population with diabetes. After 10 years, 581 participants returned for data collection at either a clinic appointment or home visit. The participants that returned for data collection were generally healthier, with higher cognitive ability, at baseline.

8.1.1 Association between retinal vessel traits and incident retinopathy

There was a total of 82 participants with incident diabetic retinopathy after 10 years. After adjusting for a wide range of demographic, cardiometabolic, diabetic and vascular risk factors, there was an association between increased venular tortuosity and incident retinopathy (odds ratio (OR) 1.51, 95% confidence interval (CI) 1.15 to 1.98; p = 0.003). For every one-point increase in venular tortuosity, which is very close to one standard deviation (SD for total population is 0.89), the odds of incident retinopathy increased 1.51 times. The confidence interval indicates a strong association, with relatively good precision, that is statistically significant. Venular tortuosity was found to improve the discriminative ability of the logistic regression model, when compared with a basic model of conventional risk factors for retinopathy. These findings require further validation in a similar population but venular tortuosity should potentially be considered in future risk prediction model generation for diabetic retinopathy.

There was also an association between decreased total fractal dimension and incident retinopathy (OR 0.75, 95% CI 0.58 to 0.96; p = 0.025). Due to the nature of this variable, a standardised fractal dimension variable was used, so the logistic regression findings can be interpreted as every standard deviation increase in fractal dimension decreased the odds of incident

retinopathy by 0.25. There were no independent associations between the remaining retinal vessel traits (RVTs) and incident retinopathy.

8.1.2 Association between retinal vessel traits and cognition

Overall, there were no associations between any of the reported RVTs and cognitive decline, measured by incident dementia or using cognitive testing and derivation of the general intelligence factor *g*. When evaluating cognitive decline, the baseline *g* value was controlled for in the model, otherwise the outcome would have been cognitive status at follow-up. When this baseline value was removed, there was an association between increased tortuosity, both arterial and venular, and lower cognitive status at year-10, when adjusting for age and sex. This association was no longer present after further controlling for cardiometabolic risk factors.

In the evaluation of change in the RVTs and cognitive decline, as reported in the nested case-control study, there was evidence of an overall reduction in fractal dimension and density measures over the study period in both the cases and controls. When evaluating these associations using simple logistic regression there initially appeared to be an association between reduced change in total and arterial density in the cases, compared with the controls, but when the duration of follow-up was controlled for, this association was no longer statistically significant. These novel findings suggest that fractal dimension and density show a general, age-related decrease in elderly people with type 2 diabetes, but this decrease was not associated with cognitive decline.

8.1.3 Implications for future prediction models

Diabetic retinopathy is highly prevalent in people with diabetes, especially as the duration of diabetes increases. There is a need to understand who will continue with underlying, asymptomatic disease and who will develop severe, sight-threatening proliferative disease. Installation of retinopathy screening programmes has provided the opportunity to image and grade for retinopathy on a regular basis as well as monitor for progression, but and there is yet to

be a reliable prediction model to assist in helping to determine who is most at risk of progression, prior to irreversible pathophysiology (Sabanayagam *et al.*, 2016).

The evidence from this thesis provides a piece to the puzzle to help determine the usefulness of retinal venular tortuosity for inclusion in a prediction model, along with other reliable risk factors, such as blood pressure, HbA1c and microalbuminuria. Findings from the systematic review in **Chapter 2,** regarding venular tortuosity, provide further evidence. Another longitudinal cohort also found increased venular tortuosity to be associated with incident retinopathy (C.Y. Cheung *et al.*, 2015). This study similarly looked at any diabetic retinopathy as an outcome. The five other studies in the systematic review that evaluated venular tortuosity did not find a statistically significant association with diabetic retinopathy, but four of them were cross-sectional, so could not offer evidence of temporality. Interestingly, all had a direction of association that did supported an association between venular tortuosity and retinopathy, so it is possible they were under powered to find an association, and do not necessarily provide evidence against this association.

More evidence is needed to definitively determine if venular tortuosity could be useful in a prediction model, in the form of replication of the findings from this thesis and also looking more specifically at prediction of severe forms of the disease. If venular tortuosity is able to help predict progression to vision-threating retinopathy, it could easily be implemented as retinal imaging is already acquired annually in Scotland for people with diabetes and in many other countries. If at risk people could be identified, they could be targeted for more intensive risk factor reduction and increased monitoring. This could help reduce related health care costs as limiting retinopathy to non-proliferative disease is far cheaper to treat, and people who are not at increased risk may not need as frequent of screening, freeing up related resources and costs (Schmier *et al.*, 2009).

8.2 Contextualising the findings

8.2.1 Strengths of using ET2DS

The ET2DS database contains a wide number of variables that have been collected using standard methods that ensure highly accurate data. Primary data collection was conducted at several time points (mainly at baseline, year 1, year 4 and year 10) adhering to prespecified detailed standard operating procedures in order to ensure accuracy between participants and between time points. Physiological and cognitive testing was performed by trained researchers, who regularly underwent quality control measures in order to limit observer bias. The same instruments and measurement tools were used by all researchers and kept as similar as possible between data collection at different time points. In addition, Information Services Division (ISD) Scotland, which manages the national database of NHS records, provided a good portion of the linked health data. ISD Scotland is world renowned as one of the best sources for reliable, high quality data, which is due to the high level of quality assurance of the data (Ireland, 2017).

The baseline sample size of ET2DS was calculated to be able to determine a statistically significant association at 90% power between a predictor variable and outcome that had a correlation coefficient ≥0.10, as well as detect risk factors. The sample size was also calculated to account for dropouts at follow-up and still maintain the same power and significance with a correlation coefficient ≥0.12.

Concerns of misclassification bias were limited through rigorous diagnostic definitions and criteria for all outcomes and events. This includes, but not limited to, dementia, cardiovascular and cerebrovascular events and retinopathy. Data entry was performed manually and included backstop methods to ensure as few errors as possible, which included double data entry and evaluating the database for impossible values, outliers and missing data.

ET2DS currently has a prospective data collection period of 10 years, which is exceedingly rare in an elderly population with diabetes. The main reason

for attrition through the data collection period was death. Other reasons for not returning to the 10-year research clinic were chronic or acute health concerns, full-time carer responsibilities and not being able to contact a participant. Attrition bias is always a concern in cohort studies, but every effort to limit non-death related attrition was made, which resulted in low numbers and less concerns for this type of bias affecting the representativeness of the population (Brilleman, Pachana and Dobson, 2010). In addition, data were collected through data linkage and routine data sources for the entire study population, reducing the impact of non-attendance.

8.2.2 Limitations of ET2DS

Limitations of low events and subgroups

Although ET2DS is a large cohort, evaluating certain subgroups, which reduces the population size, or rare outcomes, may produce underpowered analyses. This could result in type II error: a negative finding may not necessarily be indicative of evidence of no association, but rather an absence of evidence to prove an association (Altman and Bland, 1995). The sample size calculation for the cohort recommended 1,000 participants at baseline and 800 at follow-up to adhere to the specified power and significance levels to limit type I and type II error.

Unfortunately, the cognitive decline outcome as measured with *g*, could only be analysed in participants that returned for cognitive testing at follow-up. Thus, the analysis was only carried out in 581 participants, which was lower than the original power calculation covered (Price *et al.*, 2008). However, there would still be 90% power to detect a difference in correlation ≥0.15 in the population size that returned for cognitive testing, as opposed to the originally calculated 0.12 for follow-up. Although the incident retinopathy logistic regression only included 82 cases out of 718 participants, there was still a statistically significant relationship, with a moderate effect size, with increased venular tortuosity and decreased fractal dimension, indicating that

the sample size was sufficient to minimise type I and type II error in this analysis.

The lack of power to evaluate certain groups was especially noted regarding disease severity and subtypes related to my specific outcomes. For diabetic retinopathy, I could not look at the association of the RVTs by severity, most notably non-proliferative versus proliferative diabetic retinopathy because our incident cases of proliferative disease were so few. These disease stages are markedly very different, especially on a microvascular level, and evaluating the RVTs could differ between them.

With the cognitive decline outcome, specifically dementia, I was not able to evaluate the association of RVTs with different dementia aetiology: Alzheimer's, vascular or mixed dementia. In **Chapter 1**, I highlighted differences between these forms of dementia, and these differences, especially regarding neurodegenerative versus cerebrovascular disease could be reflected in different pathological associations between RVTs and dementia development. However, given the difficulties inherent in diagnosing the different forms of dementia, the likely substantial overlapping pathology and the likelihood of mis-diagnosis (at least until post-mortem) (Knopman *et al.*, 2003), even had I had full medical records and diagnoses available for my analysis, this may not have helped address the underlying question.

Misclassification bias

As previously mentioned, misclassification bias was limited in ET2DS using multiple, rigorous data sources. However, there is still concern for this type of bias for certain outcomes, such as retinopathy and dementia.

Dementia diagnosis within the NHS is not achieved with a single diagnostic test, but requires a multitude of factors and a progressive pathway to identify and diagnose the possible condition (National Institute for Health and Care Excellence (NICE), 2018). For this reason, and several others, dementia remains underdiagnosed by possibly as much as half of those who have it (Connolly *et al.*, 2011). All people with underlying dementia-related cognitive

pathology will not be identified as having frank dementia, which introduces bias into epidemiological research.

Within this thesis, using *g* to more directly measure cognition through specially designed cognitive tests, rather than only relying on a clinical dementia diagnosis, allowed me to evaluate cognitive decline more specifically. However, this method relies on participants attending a research clinic and completing the tasks, which often leads to reduced numbers for analysis when comparing to dementia diagnoses that can be ascertained from medical records. Also, evaluating cognition using these methods may be describing different phenotypes of cognitive decline. While this does not necessarily pose a problem for this thesis, because I am interested in a predictive marker and not aetiology of cerebral disease, this should still be kept in mind when interpreting the results.

Retinopathy diagnosis requires a person to attend annual retinopathy screening and be physically able to undergo imaging. Physical limitations common within an elderly cohort with diabetes would limit both the attendance and ability to obtain a clear image for retinopathy. Therefore, there may be a number of participants within the cohort that have clinical diabetic retinopathy but have not been identified within the screening programme. However, the Scottish Diabetic Retinopathy Screening Collaborative (Scottish DRS), has a very high participation rate, with 84% of eligible participants having been to a screening appointment in the previous 15 months (Scottish Diabetes Monitoring Group, 2016).

Healthy returning volunteer bias

It is a common phenomenon in longitudinal research that participants that return for later follow-up are generally healthier. There are some obvious reasons for such an occurrence, such as participants that died during the follow-up would most likely have had a less healthy profile at baseline than those that survived, and very unhealthy participants may not physically be able to attend an appointment. I tried to minimise the latter concern by

holding home visits, in order to incorporate as many participants that were willing but unable to attend the clinic.

I acknowledge that testing in a home setting could provide variable results to those obtained in the clinic setting. As a study team, we chose to perform home visits as the risk of varying results was outweighed by the potential loss of data that would occur given the age and declining health of our study population. The same researchers were conducting the clinic visits and the home visits, which helped to ensure continuity between research settings. To better understand the extent of potential bias from using both clinical and home visit data, analysis was performed comparing these two populations of participants on important health and demographic variables. Home visit participants did have a more unhealthy profile at baseline, but this serves to strengthen their inclusion as without them, the study would be more biased towards healthier participants.

Participants that attended the year-10 clinic, when comparing baseline characteristics with non-attenders, were younger, had a reduced body mass index (BMI) and systolic blood pressure, had diabetes for less time, were more likely to be controlling their diabetes with diet rather than with tablets or insulin, less likely to have had a stroke or other macrovascular event and had a reduced albumin-creatinine ratio (ACR). It was also clear that returning participants had higher cognitive function at baseline, compared to those that did not return. This type of survival bias is more likely to obscure any findings as the returning population will be less likely to suffer from negative health outcomes and have an overall healthier profile of covariates.

Although concerns with survival bias leading to a healthier returning population should be considered when interpreting the results, it should be noted that death accounted for a large portion of the attrition, and these death rates would not differ by much in the target population, making the findings of the study more generalisable to the wider population. This was demonstrated in a previous longitudinal study, which evaluated representativeness of their cohort of elderly participants during the follow-up,

based on routinely collected data and compared with an external group of people not enrolled in a study. They found that there were no major concerns of attrition bias, indicating these concerns may not have a large impact on the outcomes and generalisability (Lacey, Jordan and Croft, 2013).

Retinopathy and duration of diabetes

In the analysis assessing incident diabetic retinopathy (DR) as an outcome, it was imperative to remove participants that already had established retinopathy in order to investigate the temporal relationship between RVTs and incidence of retinopathy. Other studies have shown that people with diabetes that do not develop signs of progressive retinopathy earlier in their disease trajectory are possibly less likely to develop any clinically meaningful retinopathy during further follow-up (Jones *et al.*, 2012). This trend is also supported from an early analysis of the Wisconsin Epidemiology Study of Diabetic Retinopathy (WESDR) cohort, where those diagnosed with retinopathy earlier in the study were more likely to have progressive disease compared with participants that did not have retinopathy at baseline but developed it later on, who were more likely to have more mild retinopathy (Klein *et al.*, 1989). When developing a risk prediction tool for DR that would evaluate venular tortuosity for inclusion, it is recommended that a cohort with very newly diagnosed diabetes be used, before retinopathy can be established, and therefore capturing more people that will go on to have moderate and severe DR, and not just mild forms of the disease.

Representativeness and changing demographics

Representation of the wider population in a study population is not static. Even if a study population is found to be generalisable on certain key matters at the start of the study, years later, this same study population may no longer represent the population they are meant to. For example, the vast majority of participants in ET2DS are ethnically white, which was representative of the elderly population in Lothian at baseline. However, this demographic has most likely changed over time. Ethnicity is a known contributing risk factor for cardiovascular multimorbidity so changes in this

demographic may reduce the generalisability of the findings of the study (Mathur *et al.*, 2011).

Changing patterns of deprivation profiles by the Scottish Index of Multiple Deprivation (SIMD) may also affect the representativeness of the study findings over time. SIMD has been updated twice since the 2009 version used for ET2DS baseline was implemented. The designations for the data zones have changed in some areas, which can be seen in the interactive maps available online (Scottish Government, 2019). These concerns will be common among longitudinal studies, as the target population will often change by certain demographics during the follow-up period, and good study design, with measurement of a wide number of variables, can help to combat these issues. There is also a more general concern with SIMD in that it may be poor at describing individuals as the data zones are a generalisation and often the populations within them are quite heterogenous in terms of resources (Fischbacher, 2014). For this reason, SIMD was not used as a covariate within the models, but only to describe the representativeness of the study population at baseline in comparison to the target population.

8.2.3 Strengths and weaknesses of analysis

VAMPIRE

Utilising the Vascular Assessment and Measurement Platform for Images of the Retina (VAMPIRE) software allowed for efficient analysis of the fundus images from participants in the ET2DS. This software was developed through an international collaboration and is constantly being improved upon in order to keep up with the growing demand for retinal analysis. In the results of this thesis, using a validated tool limited the measurement bias of the RVTs which was reflected in the acceptable intra- and intergrader reliability assessments. VAMPIRE software has been used successfully for other similar applications, such as identifying an association between fractal dimension and small vessel disease in two separate groups of participants (McGrory et al. 2018). A recent study, the first to use this type of software in felines, found VAMPIRE software to be a consistent diagnostic tool for

systemic hypertension in cats (Cirla *et al.*, 2019). Using the Genetics of Diabetes Audit and Research- Tayside Scotland (GoDARTS) cohort in Dundee, the results from VAMPIRE software were used in combination with genetic and routine clinical information to create a robust prediction model for major cardiovascular events (Fetit *et al.*, 2019).

Producing and reporting of RVTs requires many choices, such as variations of a trait (maximum, minimum or median tortuosity), measurement zones (zone B, C or whole field), and very importantly, the mathematical algorithms incorporated in the software, all of which add heterogeneity when comparing to other datasets or studies. There is a need for structured debates on areas of standardisation of RVTs, which has been demonstrated by Mookiah et al, when they performed a pilot analysis using VAMPIRE software to analyse retinal images that were centred in different locations, fovea centred versus macula centred, and found noticeable differences in the output (Mookiah *et al.*, 2018).

Other areas where more evaluation of standardisation is needed is with differing algorithms, which has been demonstrated regarding tortuosity measurements by Lisowska et al. They showed varying levels of correlation when using five different algorithms to measure tortuosity (Lisowska *et al.*, 2014). They compared types of algorithms that were relatively simplistic, but failed to incorporate local changes, as well as more complex measures, specifically the curvature integral measures. The curvature integral measures were found to perform the best but required the presence of vessel centrelines and also were most sensitive to sample variation, indicating that algorithm selection needs to be considered alongside the study sample it will be used with. The tortuosity algorithm used in the version of VAMPIRE 3.1.0 software to measure the ET2DS baseline RVTs utilises a curvature based algorithm (Annunziata *et al.*, 2014).

There are also different theories and methods for fractal analysis that alter the way the fractal dimension of a retina is measured and analysed. Fractal analysis is often done using segmented vessels and a method known as box-

counting, but one group of researchers, Azemin et al, chose to use a Fourier Fractal Dimension (FFD) method that did not rely on vessel segmentation, but rather greyscale images, which they claim reduces manual input (MacGillivray *et al.*, 2007; Azemin *et al.*, 2011). Another complex choice for fractal analysis of the retina is the use of a singular fractal dimension or multifractal analysis, which is used when a single fractal descriptor does not fully capture the pattern (Stosic and Stosic, 2006). Multifractal analysis, which is what is incorporated in VAMPIRE software, calculates fractal dimension over several scales. How the output is then used is subject to the users and can vary.

Retinal vessel analysis is currently of great interest to many researchers who study vascular disease and there are several other popular platforms for analysis, which include Singapore “I” Vessel Assessment (SIVA; National University of Singapore, Singapore), Quantitative Analysis of Retinal Vessel Topology (QUARTZ), Interactive Vessel Analysis (IVAN; University of Wisconsin Madison, WI) and Retinal Analysis (RA; Department of Ophthalmology and Visual Science, University of Wisconsin), to name a few. Although similar methods are reported between the software packages in terms of calculation of vessel measures, there will of course be algorithm differences and other variations that can make comparison between the software findings difficult.

There is some indication that measurements between software platforms are similar, despite the differences mentioned in previous paragraphs, such as the findings presented by Downie et al in their conference abstract showing good agreement between VAMPIRE and IVAN software (Downie et al., 2015). However, a recent study carried out a direct comparison of a wide number of vessel measurements between VAMPIRE and SIVA and found poor agreement between the software platforms (McGrory et al. 2018). Another study, which compared results for CRAE and CRVE between SIVA, IVAN and RA platforms, also found poor agreement (Yip et al., 2016). However, associations with systemic factors, including age, blood pressure and cholesterol, were similar between applications. Yip et al indicated that

use of a conversion algorithm between the platforms could help overcome differences. Caution should be taken when comparing results between studies that use different software to analyse vessels and future work needs to go into standardisation if the use of vessel traits in risk prediction becomes established.

Measurement and analysis of retinal vessel traits

In this thesis, all RVTs were evaluated and analysed as continuous variables. Other studies have created categorical variables for the different RVTs, generally with quartiles and then compare the largest quartile to the lower three (Cheung *et al.*, 2008; Sophie Louise Rogers *et al.*, 2008; Roy, Klein and Janal, 2011; Sasongko *et al.*, 2012; Yang *et al.*, 2016). I chose to use the variables as continuous because transforming continuous variables into discrete variables generally loses power for the analyses (Altman and Royston, 2006). Also, there is still relatively little known about these traits regarding normal health. There are currently no accepted cut-points and the arbitrary cut-points generated in one study may be very different from another. Because of these concerns, using these measures as discrete variables can be quite subjective and uninformative as there is evidence lacking regarding useful comparison groups. Furthermore, there is evidence that creating a discrete variable from a continuous one can increase the risk of type I error (Austin and Brunner, 2004). However, there may be a case for handling data in this manner, for example to combine the highest and lowest quartiles of a variable, or remove the higher or lower end of the values, if there is evidence that there is a j-shaped association with a certain outcome and not a simple linear association. But further investigation is warranted into this in order to make an informed decision about analysis.

Within this thesis, there were possibly non-linear results between arterial tortuosity and retinopathy, **Figure 23**, where there appeared to be an increased risk in the first and fourth quartiles. However, categorisation of arterial tortuosity was not undertaken as it was not a part of the a priori analysis plan, and after careful consideration, would possibly be more likely

to lead to spurious results through arbitrary cut-points and multiple testing. I feel this area of research deserves greater evaluation, not only within ET2DS, but in other similar studies as well, in order to make an informed decision of how best to categorise these variables.

There is evidence that the microvasculature of the retina pulses slightly during different phases of the cardiac cycle, with a decrease in width in systole and minor increase at the start of diastole, equalling a 4.8% change in width (Chen *et al.*, 1994). These findings indicate that fundus images should be taken synchronised with electrocardiogram (ECG) readings to limit bias due to the cardiac cycle. However, a study evaluating inter- and intra-observer bias between different software packages did not find a difference when using ECG-synced versus non-synced images (Wei *et al.*, 2016). It is also debatable whether the software packages are sensitive enough to even pick up these very slight changes in width as the resolution of a fundus camera is roughly 7 microns and pulsatile changes are likely to be sub micron in size (MacGillivray *et al.*, 2014).

Very few studies have evaluated quantitative changes in the retinal vessels over time, so this thesis represents a novel application for this type of analysis. For analysing change in retinal vessels, routinely collected fundus images were used from the national retinopathy screening programme in Scotland, the Scottish Diabetic Retinal Screening Collaborative (Scottish DRS). This allowed me access to many high-quality retinal photos to choose from for each participant during the follow-up period. I was able to utilise the expertise and resources of images already collected and significantly reduce the time participants would spend in the research clinic in order to gather retinal images specifically for research purposes. This reduced research costs and recycled resources that would have otherwise not been used after the retinopathy screening was performed. This is one of the first studies to use images from the Scottish DRS with VAMPIRE software and there is great potential for future collaboration with this vast resource of fundus images.

Negatives of using images from the Scottish DRS include the requirement that study participants attended their annual NHS retinopathy screening appointment. Other limitations of using images taken for Scottish DRS purposes, rather than specifically for research purposes, included potential difficulties meeting the requirements for accurate use of retinal analysis software. For example, the use of different cameras or camera systems may influence certain image components. VAMPIRE, nor other similar software, have the capacity to standardise measurements between images from different camera systems, which could affect the results.

Specific to this thesis, having an older population may have resulted in poorer image quality, and this problem may have been accentuated as participants aged during follow-up. It is common for retinal images to be more difficult to take in elderly people for many reasons, but mainly due to difficulty keeping the head still and eyes open enough, eye lashes creating artefacts as well as cataracts or other eye pathologies. I found that the software was still able to analyse vessels in images that were slightly blurry, but it is possible that the decrease in density and fractal dimension over time, seen in both the cases and controls, was due to poorer quality of follow-up images. In depth study is needed to better understand the capacity of the software and the appropriateness of comparing images of different quality. A suggestion would be to create a grading system of image quality and only comparing images of similar quality, although this would most likely severely reduce the number of usable images per person. Such automated image quality grading has been implemented in other analysis software (Welikala *et al.*, 2016).

When measuring change in the RVTs, there were concerns with survival bias, as participants available for follow-up retinal imaging would most likely be of a healthier profile than those that did not attend retinal imaging at least five years after their baseline image. This would most likely introduce selection bias and to overcome these concerns, a nested case-control study was used in order to create a more balanced comparison. Using a nested case-control design reduces common bias concerns in choosing the controls

compared with standard case-control studies because the controls are chosen from the same population as the cases.

Statistical analysis

The statistical analyses completed throughout this thesis were prepared and conducted using a detailed statistical analysis plan. Covariates were carefully chosen based on evidence of their association, or potential association, with the predictor and/or outcome variables. As I was interested in possible future use of RVTs within prediction models, covariates were chosen as known risk factors that are commonly measured and available through routine health data.

Although not all of the variables used as covariates in the regression models were statistically significantly associated with the vessel traits, their addition to the models were still important as not all confounding can be evaluated simply through hypothesis testing, and also, thinking ahead to future meta-analysis, addition of clinically relevant variables is important. In the literature, these variables have been previously associated with retinal vessel traits and with the outcomes.

It is a generally accepted rule of thumb that one would want to have 10 cases per predictor variable within a logistic regression model to avoid issues of separation that can arise from a small population with one or more strongly predictive covariates (Albert and Anderson, 1984). Within some of the logistic regression models presented in this thesis this suggested ratio was violated. However, the models did not encounter problems with convergence and there was no evidence of separation or of any highly predictive risk factors. If separation was a major issue, there are techniques to overcome the problem, including combining covariates into a single factor using principle components analysis (PCA). However, this would reduce any ability to understand which factors are most associated with the outcome. Another method of overcoming such problems would be to use Firth's method of producing finite parameters through penalised maximum likelihood estimations (Firth, 1993; Heinze and Schemper, 2002).

Methods for controlling for multiple testing were considered, but not employed within this analysis. A p value of 0.05 was used to indicate statistical significance throughout, but it is common convention that with this alpha level, type I error (rejecting the null hypothesis incorrectly) can occur once in every 20 comparisons. Bonferroni adjustment is a commonly used method that seeks to reduce the p value threshold in relationship to the number of test comparisons undertaken. However, controlling for multiple testing is a widely debated topic, with some arguing that correction is arbitrary (do you correct for number of tests in a single paper, an entire study, the duration of a researcher's career?), and that type I is understood to be part of epidemiological research and controlling for it will only introduce more type II error (Perneger, 1998; Feise, 2002; Morgan, 2007).

To combat concerns of type I error, a specific statistical analysis plan was created prior to any analysis in this thesis and adhered to strictly. There were few times where non-*a priori* analyses were carried out, and these analyses were explicitly noted. Evaluation of the effect size of the analysis, the confidence interval or beta values, and number of participants in the analyses were all taken into account when assessing a result, rather than just evaluating if a specific p value fell below the 0.05 threshold.

Replication of the findings of this thesis in an independent dataset are crucial to understand if venular tortuosity or fractal dimension could be a useful biomarker for retinopathy. It is not uncommon to find associations between potential risk factors and disease outcomes in a study, to later find out the risk factor does not aid in prediction (Wald and Morris, 2011). Unfortunately, the ET2DS was not considered large enough to split the cohort in to two populations to develop the models in one group and then a separate group to validate the findings. Finding a similar study group that have measured the necessary covariates would be ideal to validating the results of this thesis.

When thinking about and describing model fit, it is useful to retain the words of George Box in mind and keep perspective: "All models are wrong, but some are useful" (Box and Draper, 1987). Developing well-fitting models (ie

no serious outliers or influential cases) allows you to draw sound conclusions using the evidence for the studied population, but if the assumptions of the model type are violated, then you are limited in the generalisation of the model to other data sets (Field, Miles and Field, 2012a).

Overall, the models generated in this thesis did not violate the assumptions of the model type and did not have evidence of poor fit. In cases where there were concerns, the reasons for poor fit or assumption violation were explored and remedied where possible, often with little or no change to the overall findings. There were problems with a poorly fit model for the density measures in the change in RVT and cognitive decline analysis, specifically with regards to linearity. Because this measure is relatively new, there is no evidence around what would constitute normality. It is therefore difficult to determine what would be a physiological outlier. Although there are possible issues with linearity, evaluating the spaghetti plots in **Appendix 21**, it is clear to see the linear relationship of decreased density over time.

8.2.4 Comparisons with other similar studies

Overall, there was a significant amount of heterogeneity amongst other similar studies, which include ethnicity, age and diabetes type of the study population, measurement and reporting of the outcome, software and algorithms used to produce the RVTs and how the RVTs were used in the analysis, to name a few of the most important factors. I have touched upon all of these topics previously in this chapter but want to emphasize how these differences can accumulate to make it quite difficult to compare between studies that appear on the outside to be measuring the same things. However, it is still important to look for overall association similarities that can help lead us to make robust conclusions regarding the use of RVTs.

Collaborative effort in the form of providing raw data for re-analysis or meta-analysis and future study development is needed if these differences are to be overcome. In the following sections, I provide rudimentary comparisons between the results of this thesis and those of the studies included in the systematic review chapters (**Chapters 2 and 3**). It should be kept in mind

when reading these cursory descriptions, the possible sources of heterogeneity that limit a direct comparison. Details of the individual studies are provided in the tables within the systematic review chapters and appendices.

Diabetic retinopathy

In this section I compare the findings of this thesis to those of the systematic review in **Chapter 2**, which attempted to collate the most relevant studies for comparison.

The findings of the logistic regression evaluating the relationship between CRAE and incident retinopathy identified a borderline statistically significant association between narrower CRAE and DR, but this association was lost after controlling for markers of macrovascular disease. These findings indicate there to be evidence of an association, but possibly not a good predictive marker since the association is not independent of other known risk factors. However, of the longitudinal studies in the systematic review in **Chapter 2**, the majority identified wider CRAE to be associated with diabetic retinopathy. Three of these studies (AusDiab, Sydney Paediatric Diabetes Study and New Jersey 725) compared the largest quartile of CRAE with the smallest three quartiles. A single study, DCPD1987, which was conducted in younger people with type 1 diabetes, found narrower CRAE to be associated with proliferative diabetic retinopathy (PDR).

Regarding venular width, most of the studies, both cross-sectional and longitudinal, found an association between wider CRVE and retinopathy, using their various methods of evaluating the outcome. Contrary to this, the findings from my analysis found a very borderline statistically significant association between decreased CRVE and incident retinopathy when controlling for age and sex, cardiometabolic and diabetes risk factors, but was no longer associated when controlling for vascular risk factors. Only a single longitudinal study (SiMES) also found evidence of narrower CRVE being associated with incident retinopathy. The discrepancy between ET2DS,

SiMES and the wider literature may be an issue with statistical model differences or linearity and further investigation into this issue is warranted.

When I evaluated the relationship between arterial tortuosity and incident retinopathy in the ET2DS population, I found no association after 10 years. Of the other similar studies, three out of four cross-sectional studies identified a relationship between increased arterial tortuosity and retinopathy and one of two longitudinal studies found the same relationship for incident retinopathy. The remaining two studies did not find any statistically significant associations, but their point estimates favoured the same relationship. As previously discussed, it may not be completely appropriate to treat all the RVTs as continuous variables and instead create a discrete variable using specific cut-points. My own basic analysis of risk of diabetic retinopathy by quartile of arterial tortuosity indicated a possibility that both the more extreme lower and higher values may be indicative of higher risk and combining these categories could be informative as a measure of deviation from normality. As with CRAE, the differences could also be due to different statistical modelling choices, especially regarding covariates.

The results from this thesis identified increased venular tortuosity as being associated with incident retinopathy, even after controlling for a wide number of covariates. As previously referred to in section **8.1.3**, the findings from the systematic literature review are in modest agreement, as only a single longitudinal study (SiMES) that followed-up with participants for six years, also found increased venular tortuosity to be associated with diabetic retinopathy. SiMES was a smaller study than ET2DS, with a total of 434 participants, and 59 cases of incident retinopathy, most with type 2 diabetes, but they also had participants with type 1 diabetes. The remaining studies, four cross-sectional and one longitudinal, did not find a statistically significant association, but all presented point estimates that favoured this relationship. The ET2DS analysis was the largest study and followed-up participants longer than any of the other studies evaluating venular tortuosity. It is very possible that with further follow-up, larger sample sizes, or more similar covariates, these other studies would find similar results.

Fractal dimension is a dynamic variable, incorporating considerable information about the vascular geometric structure and there is great interest in understanding the relationship with retinal pathology. In my analysis, I found evidence that decreased fractal dimension, and therefore decreased vascular complexity, was associated with incident diabetic retinopathy. This association was only corroborated in one cross-sectional and one longitudinal study, out of seven analyses, both of which evaluated proliferative diabetic retinopathy (PDR) as the outcome. The remaining three longitudinal studies did not find a statistically significant association, although two did have point estimates favouring decreased fractal dimension. In studies that evaluated PDR as an outcome, it would not be surprising to find a higher fractal dimension due to the presence of neovascularisation (Orlando *et al.*, 2017).

This brings up an interesting aspect of studying diabetic retinopathy using fractal dimension, in that during the course of the disease, there are cumulative vascular changes that may create opposing findings in fractal dimension. During the early phase of the disease, when microaneurysm are the main pathology, it is unclear if there would be any effect on fractal dimension, but as the disease progresses to more moderate or severe NPDR, there may actually be a reduction in vessel complexity because cumulative vascular damage has resulted in reduced number of vessels. In contrast, when a person has PDR, characterised by newly formed vessels, you may expect to see an increase in fractal dimension as a result. It is still unclear at this stage how the disease course of DR alters fractal dimension, if at all, but as more studies such as the data presented in this thesis, suggest that there may be, disease stage and severity need to be incorporated and considered. Unfortunately, in this thesis there were not enough people with incident PDR to undertake robust analysis.

The authors of another study, SiMES, also attempted to evaluate the use of RVTs as a potential predictive biomarker for retinopathy when compared with traditional risk factors (Cheung *et al.*, 2017). In this study the authors used PCA to combine the 12 traits provided by SIVA software. They combined the

PCA results with a model including established risk factors (age, sex, blood pressure, BMI, duration of diabetes, HbA1c, total cholesterol, HDL and estimated glomerular filtration rate (eGFR)) and evaluated the concordance statistic (c-statistic) and used Hosmer-Lemeshow to evaluate goodness of fit. They found an improvement of the c-statistic from 0.733 to 0.793 ($p = 0.031$) with the addition of the components. This agrees with my finding of the addition of venular tortuosity to a basic model of known risk factors, although I only used HbA1c, systolic blood pressure and albumin-creatinine ratio as the base model.

Cognitive decline

In the findings from the literature review in **Chapter 3**, from 14 studies that evaluated the relationship between retinal vessel widths and cognitive decline, there was little evidence of an association with dementia or lower cognition. For both CRAE and CRVE there were a few studies that did find an association, but the directions were mixed and the majority of studies found no association. The results from the primary analysis in this thesis also found no association between vessel widths and dementia or cognitive decline after 10 years, after adjusting for age and sex.

For tortuosity, the results from this thesis did not find an association between either arterial or venular tortuosity and dementia or cognitive decline. These findings are in congress with the findings of the literature review, which found most studies to show no association and those that did find an association had mixed results. In this thesis, it is worth noting that for the analysis of cognitive decline, when baseline *g* was removed and the analysis was then only describing year 10 cognitive status and not decline, there was an association with increases in both arterial and venular tortuosity after age and sex adjustment. This association was lost after further adjustment for cardiometabolic risk factors. This should be considered when comparing with other studies evaluating this relationship as they may control for different covariates.

Findings from the few previous studies that evaluated fractal dimension, indicate there may be an association between decreased fractal dimension and decreased cognitive ability. However, this conclusion was only drawn from case-control and cross-sectional data and not from any longitudinal studies. It is therefore difficult to compare the results from the analysis of ET2DS, which did not find an association between fractal dimension and cognitive decline, measured either by dementia as an outcome or by *g*, after age and sex adjustment. Also, most of the studies in the literature review that evaluated fractal dimension looked specifically at Alzheimer's dementia as their outcome, whereas my study had a mixed aetiology of dementia. However, when looking at the unadjusted correlation between year 10 *g* and fractal dimension, there is a relationship supporting the conclusions of the literature review, but not statistically significant. This analysis was only able to be done in the 581 participants that returned for the follow-up of the study, and possibly with a larger cohort the analysis would have reached statistical significance.

One study, the Lothian Birth Cohort 1936 (LBC1936), which was included in the literature review in **Chapter 3**, published a new report of the findings from their study after the literature review search, so was not specifically included (McGrory, Ballerini, Okely, *et al.*, 2019). This newer analysis of the cohort used growth-curve analysis of cognitive testing from three data collection time points after the RVTs were collected. Their previous reports evaluated only one time point of cognitive testing and compared it with childhood IQ to evaluate cognitive decline. This paper did not find any meaningful associations between the RVTs and cognitive decline, which corroborate the findings of this thesis, although it should be noted that LBC1936 is conducted in a general cohort of elderly people, and not specific to people with diabetes.

Very few studies have evaluated change in RVTs over time and the effect of age and other factors. This study is unique in the length of follow-up time and the findings showing a clear reduction in fractal dimension. In a study in adolescents with type 1 diabetes in Australia, there was an increase in arteriolar and venular width, as well as venular tortuosity during the 2.6 year

follow up (Liew *et al.*, 2017). It is not surprising there was an increase in vessel widths due to the age of the participants as it is expected the vessels would increase in size in early adolescents. This study did not find an association in fractal dimension. In contrast to our study, the WESDR study provided evidence of narrowing of arterials (CRAE) and widening of the venules (CRVE) after 6 years (Klein *et al.*, 2012). WESDR did not report evaluating fractal dimension or change in other vessel traits.

8.3 Mechanistic explanation of changes in the retinal microvasculature

A study published in 1986 demonstrated in three participants that increased brachial artery blood pressure from exercise, after increasing over a certain threshold, also leads to an increase in retinal vessel blood pressure that rose incrementally with brachial artery blood pressure (Robinson *et al.*, 1986). In this study, the authors did not find any statistically significant changes in the width of the larger retinal vessels and concluded any changes due to vascular resistance would likely have occurred in smaller vessels that could not be captured by the imaging techniques used. This study demonstrated that there is a normal process of blood pressure autoregulation in the retina and with even a short duration of increased systemic blood pressure there were effects on the retinal blood flow.

A more recent study with 51 participants and similar methods did find that with exercise there was a natural narrowing of the retinal arteries attributed to autoregulation, but this response was not found in older participants (above 40 years) (Jeppesen, Gregersen and Bek, 2004). Retinal blood flow alterations have also been shown to be associated with diabetes (Yoshida *et al.*, 1983; Palkovits *et al.*, 2013). It is therefore probable that with sustained blood flow changes not only from chronically increased blood pressure, but also age and damage caused by hyperglycaemia, it is likely the smaller vessels in the retina undergo cumulative alterations in response.

These evident changes lead to the question of whether alterations in the retinal microvasculature are a normal response to the blood flow changes,

due to pathological processes or possibly a mixture? The exact mechanistic pathways are currently unknown for those with diabetes or in the wider population (Carol Yimlui Cheung *et al.*, 2015). Other theories have centred around common pathophysiological changes associated with sustained hypertension, ageing, atherosclerosis, inflammation and endothelial dysfunction, as well as blood flow parameters, including oxygenation and shear stress (Sun *et al.*, 2009). Changes in the microvasculature in diabetes include increased sheer stress and reduced blood flow efficiency that put the vasculature at greater risk of damage (Tooke, 1995).

In depth in vitro studies are needed to fully elucidate mechanistic pathways for changes in the retinal vasculature. While imaging of the retina is readily available and relatively simple, direct measurement and manipulation of the retinal microvasculature's functional aspects, such as blood flow, is extremely difficult and invasive and researchers often resort to surrogate measures. There are promising results using flickering light to induce a vasodilatory response and study the endothelial function, as well as Doppler technology to study retinal blood flow and retinal oximetry to study oxygen saturation (Carol Yimlui Cheung *et al.*, 2015). Further research and development in technology is needed in this area.

8.4 Future role of retinal vessel traits as a biomarker for vascular disease and recommendations for future research

There is great excitement around the development of RVTs as biomarkers for vascular disease as many researchers have consistently found associations with risk factors and other factors associated with vascular pathology as well as vascular disease itself. However, there are still many questions left unresolved regarding the predictive capacity of RVTs and the heterogeneity between studies and software tools used to produce RVTs. As described previously, there are concerns with comparability of research findings that use different software platforms for RVT analysis and retinal differences may be quite different across different populations, such as by age, ethnicity and

comorbidities. These concerns only become more apparent as the list of vessel traits grows, with little understanding which, if any, play an important role in disease progression.

One way to incorporate information from multiple traits is using reductive statistical methods such as PCA, which was briefly touched on in Section **8.3.4** in a study evaluating retinopathy (Cheung *et al.*, 2017). A recently published study evaluating RVTs and cardiovascular risk chose, instead of evaluating each of the 54 vessel traits alone, to use PCA to combine traits into three 'vascular patterns' to describe the participants and look for associations between the patterns and cardiovascular risk (Arnould *et al.*, 2018). The pattern described as 'sparse vascular network', comprising lower vascular complexity and increased width variability, was found to be associated with greater cardiovascular risk. This type of analysis was briefly described in a paper on the WESDR study, where the authors used PCA to combined eleven RVTs, along with a wide number of covariates, to evaluate prediction of incident or progression DR, or incident proliferative diabetic retinopathy (Klein *et al.*, 2018).This paper reported no significant improvements in the models with the addition of the PCA vessel trait variable. Using methods such a PCA may help to better understand which factors may be most relevant in risk prediction and help to best utilise studies that do not have the power to evaluate each trait separately.

The findings from this thesis, regarding the predictive capabilities of certain RVTs for retinopathy are very exciting. However, they require further validation. This is likewise for the novel findings regarding age-related change in fractal dimension and density. This would include validation using very similar methods and software as well as using other software or algorithms to determine how such differences would affect the outcomes and conclusions. Unfortunately, there are very few other cohorts specific to type 2 diabetes with the same power and follow-up time as ET2DS. Also, the high-quality data collection regarding risk factors, phenotypes and data linkage is generally missing in other cohorts, especially regarding retinal imaging. One such study that could be a good candidate for validation would the Genetics

of Diabetes Audit and Research Tayside and Scotland (GoDARTS) study based at the University of Dundee. This study also involves a series of routine linkages of a population of people with type 2 diabetes in Scotland, focused mainly in the Tayside region (Hébert *et al.*, 2018). This study has already obtained fundus retinal images, as well as diabetic retinopathy gradings and dementia diagnoses. Due to the nature of the study, there would not be cognitive testing available that would match what has been done in ET2DS.

Use of the UK Biobank, which contains data on over half a million people, has been considered as a comparative option. Although over 80,000 people in UK Biobank have retinal images, some of the images are unsuitable for use with software such as VAMPIRE due to quality (MacGillivray *et al.*, 2015). However, another research group working with QUARTZ software has been working to create a support vector machine classification tool to automatically classify the quality of images in the UK Biobank (Welikala *et al.*, 2016). Determining image suitability in an automatic way reduces bias and allows for quick processing, as opposed to manual assessment. Such tools are necessary when evaluating large numbers of image, which would be a necessity to validate the findings of this thesis.

It is necessary to determine if the vessel traits should be used in prediction models as continuous variables or if categorical variables, larger versus smaller or grouping the largest with the smallest, would be more appropriate and useful. These types of categorisations may be outcome specific. This may become easier to determine as more information is gathered on what makes up a ‘healthy’ retinal geometric structure.

8.4.1 Future research recommendations

- Increased collaborative effort to evaluate and surmount heterogeneity between RVT measurement software;
- Determine if using reductive statistical methods, such as principle components analysis, are a suitable way to describe the retinal traits and their associations with vascular disease

- Replication of findings that venular tortuosity and fractal dimension are independently associated with incident diabetic retinopathy;
- Further exploration of venular tortuosity as a suitable biomarker for diabetic retinopathy with increased focus on disease severity;
- Further evaluation of changes in retinal vessel traits over time, and replication of age-related decreased fractal dimension and density
- Research into normal value ranges for the RVTs in a healthy population, which will help to best understand if the traits should be used continuously or as categorical variables.

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Appendices

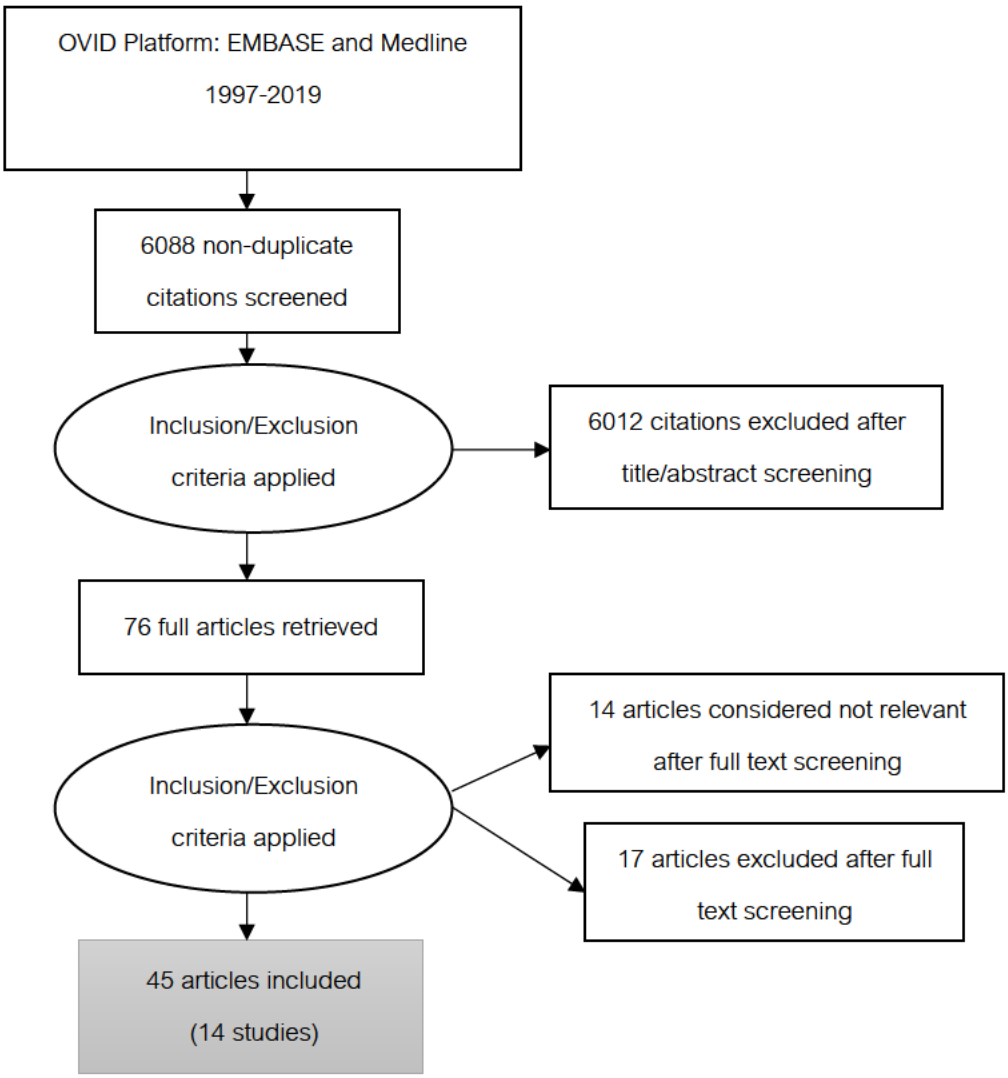
Appendix 1. Retinopathy literature review: OVID Platform electronic search strategy of EMBASE and MEDLINE databases

1. retina*.mp.
2.microvascu*.mp.
3. arteriol*.mp.
4. venul*.mp.
5. vascu*.mp.
6. 2 or 3 or 4 or 5
7. 1 and 6
8. retinopathy/ or retinopath*.mp.
9. maculopathy.mp. or retina maculopathy/
10. 8 or 9
11. diabetes mellitus/ or diabet*.mp.
12. 7 and 10 and 11
13. limit 12 to english language
14. limit 13 to human
15. limit 14 to yr="1997 -Current"
16. remove duplicates from 15

Appendix 2. CASP Quality appraisal domain criteria

Domain	Criteria
1) Did the study address a clearly focused issue?	Did the authors clearly explain methods that minimised bias?
2) Was the study recruited in an acceptable way; in case-control studies, were cases and controls recruited appropriately?	Did the authors clearly explain methods that minimised bias?
3) Was the exposure and outcome accurately measured to minimise bias?	Did the authors identify software used for vessel trait analysis, were graders trained and masked? Did graders use a protocol and did they report intra/inter-grader reliability? How was retinopathy determined/cognition measured and were pre-specified criteria identified? Were trained, blinded graders used to diagnose retinopathy/measure cognition?
4) Have the authors identified all important confounding factors and included them in the analysis?	Common covariates for retinopathy: age, sex, smoking, blood pressure, lipid measures, duration of diabetes, blood glucose. Additional for cognition: history of stroke. Were these variables controlled for or was there discussion around the variables controlled for were used or not used? Did authors control for CRAE and CRVE in the opposing width analyses? Inclusion of these covariates is just a guideline to assist the reader, but not necessary if the study authors are clear why they chose the covariates they used.
5) Was the follow up of the subjects complete enough and long enough?	Follow-up for at least six years was considered acceptable, three to five years was of possible concern and less than three years was considered insufficient follow-up time. Was there a large, unexplained proportion of the baseline participants missing from the follow-up?
6) How precise are the results and do you believe the results?	Evaluation of this domain was based on several features including population size, point estimates, confidence intervals, multiple testing and attrition.
7) Do the results fit with other available evidence?	Any gross deviations from similar studies?
8) Are sources of funding and/or conflicts of interest reported?	Did the study authors report the sources of any funding?

Appendix 3. Retinopathy literature review: study selection PRISMA flow diagram



Appendix 4. Retinopathy literature review: additional image features

Study name	Predominant eye used	Type of fundus image
DRUID	Both	Non-mydriatic retinal camera, Topcon TRC-NW5S; half underwent dilation
Blue Mountains Eye Study	Right	Stereoscopic 35° retinal photographs using Zeiss FF3, digitised using CanoScan FS2710; 6 field
Inter99 Eye Study	Both	60° colour fundus photographs on transparency film
Grauslund 2010	Right	45° fundus photograph, Topcon TRC-NW6S; pupil dilation; 9-field
Sasongko 2011	Not specified	Not specified
MESA	Right	Non-mydriatic, 45° retinal camera
SINDI	Right	Non-mydriatic, 45° digital retinal camera, Canon CR-Dgi 20D; pupil dilation
Desheng Diabetic Eye Study	Right	Stereoscopic, 30° digital fundus photography, Zeiss Visucam Pro; pupil dilation; 7 field
DCPD1987	Right	**CRAE, CRVE, FD:** mydriatic, 40°-60° retinal cameras, digitalised using DigitDia 5000 Filmscanner, pupil dilation; **Tortuosity, branching angles, LDR:** mydriatic, 45° retinal images using 3D OCT-2000 Spectral-Domain Optical Coherence Tomography System; 7 field
SiMES	Right	45° digital retinal camera, Canon CR-Dgi 10D; pupil dilation
AusDiab	Left	Non-mydriatic, digital retinal camera, Canon CR6-45NM
Sydney Paediatric Diabetes Study	Three studies used right eyes, two studies used both and one study did not specify	Stereoscopic retinal photograph, Topcon TRC 50-VT, digitised using CanoScan FS2710; pupil dilation; 7 field
New Jersey 725	Both	Stereoscopic retinal images based on Diabetic Retinopathy Study protocol; digitised using high-resolution scanner; 7 field
WESDR	Right or combined into mean	stereoscopic, 30° fundus photographs; 7 field

CRAE = central retinal arteriolar equivalent; CRVE = central retinal venular equivalent; FD = fractal dimension; LDR = length to diameter ratio

Appendix 5. Retinopathy literature review: statistical models and covariates

Study name	Statistical model	Variables controlled for
DRUID	Generalised estimating equation modelling (OR 95% CI)	Age, sex, smoking, mean arterial BP, C-reactive protein, BMI, history of laser treatment, HbA1c, generalised estimating equations accounted 'for right and left eye data together'
Blue Mountains Eye Study	Logistic regression (OR 95% CI)	Age, sex, blood pressure, smoking, haemoglobin, fibrinogen, white cell count, BMI, alcohol intake and lipids (triglycerides, HDL), CRAE/CRVE
Inter99 Eye	Linear regression (coefficient, 95% CI)	Age, sex, HbA1c, systolic BP, smoking, total cholesterol, HDL cholesterol
Grauslund 2010	Logistic regression (OR 95% CI)	Age, sex, duration of diabetes, HbA1c, systolic blood pressure, smoking
Sasongko 2011	Logistic regression (OR 95% CI)	Age, sex, duration of diabetes, HbA1c, systolic BP, cholesterol, BMI, use of insulin and other medications, CRAE/CRVE
MESA	Linear regression and ANCOVA (mean SE)	Age, sex, race/ethnicity, exam centre, systolic BP, BMI, total cholesterol, triglycerides, current smoking, C-reactive protein, CRVE/CRAE, HbA1c, duration of diabetes; also performed subgroup analysis based on different race/ethnic groups
SINDI	Multiple linear regression and ANCOVA (mean SE)	Age, sex, systolic BP, BMI, total cholesterol, current smoking status, CRAE/CRVE
Desheng Diabetic Eye	Linear regression and ANCOVA (mean SE)	Age, sex, duration of diabetes, smoking status, systolic BP, diastolic BP, HbA1c, creatinine, total cholesterol, HDL, C-reactive protein, use of insulin and albuminuria, BMI; those with laser treatment analysed in separate multivariate linear analysis
DCPD1987	Logistic regression (OR 95% CI)	sex, age, diabetes duration, HbA1c, blood pressure, BMI, VPT, mean AER, level of retinopathy, CRAE.CRVE
SiMES	Linear regression and ANCOVA (mean 95% CI)	age, sex, BP, BMI, total cholesterol, smoking status, HbA1c, diabetes duration
AusDiab	Logistic regression (OR 95% CI)	age, sex, systolic BP, BMI, alcohol consumption, smoking, HDL, triacylglycerol, CRVE/CRAE, Hba1c, diabetes duration

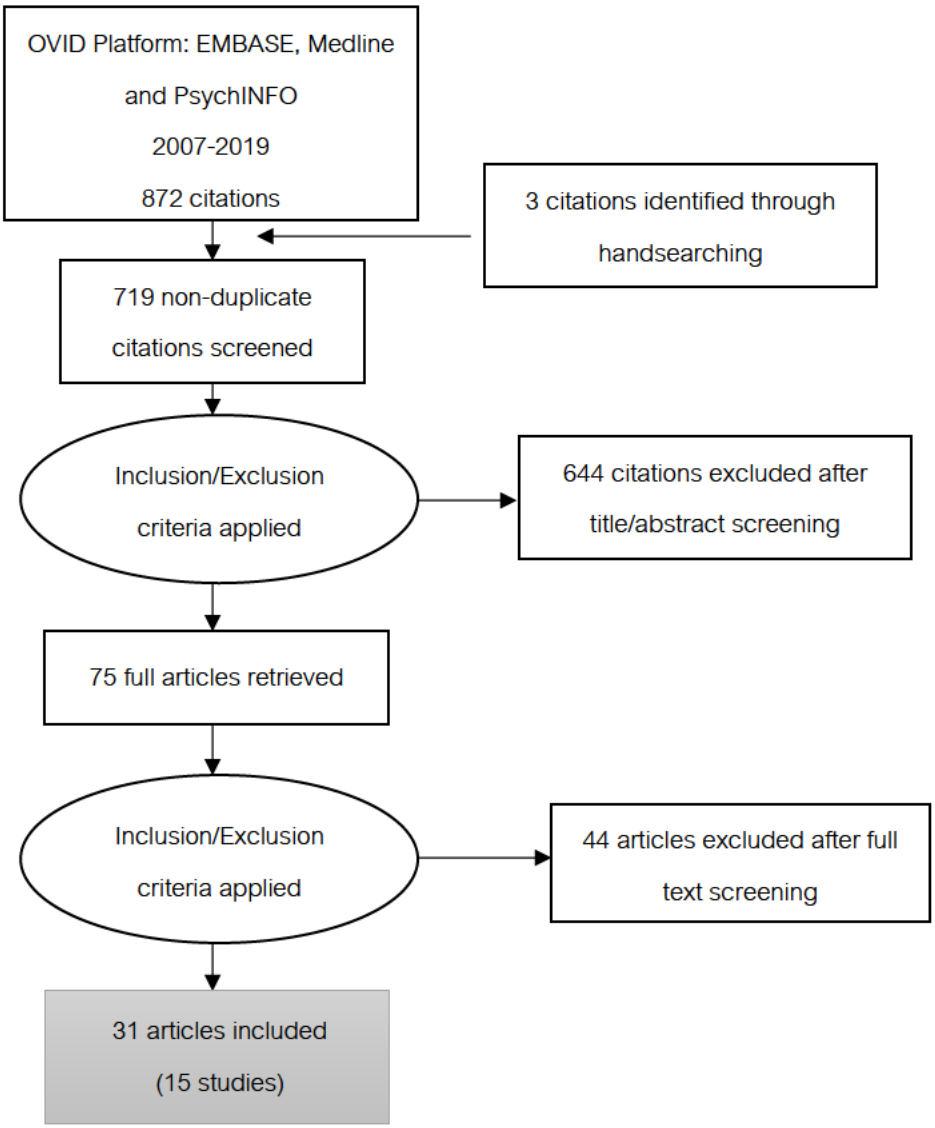
Study name	Statistical model	Variables controlled for
Sydney Paediatric Diabetes Study	Two studies used Cox proportional hazards regression models, four used logistic regression models and one used generalised estimating equations with ANOVA	All six controlled for age, sex; 5 studies also controlled for diabetes duration, HbA1c, BP, BMI; four controlled for cholesterol; two controlled for pubertal stage; three controlled for CRAE/CRVE; one controlled for ACR and one for 'diabetic retinopathy risk factors'
New Jersey 725	Generalized estimating equations	CRAE/CRVE, age, socioeconomic status, sex, BMI, HbA1c, proteinuria, ocular perfusion pressure and refractive error
WESDR	Logistic regression and discrete linear logistic regression (OR/HR/RR 95% CI)	Sex, duration of diabetes, glycosylated haemoglobin, mean arterial BP, antihypertensive medications, severity of DR at baseline

ACR = albumin/creatinine ratio; AER = albumin excretion rate; ANCOVA = analysis of covariance; ANOVA = analysis of variance; BMI = body mass index; BP = blood pressure; CI = confidence interval; CRAE = central retinal arterial equivalent; CRVE = central retinal venular equivalent; DR = diabetic retinopathy; HbA1c = glycated haemoglobin; HDL = high density lipoprotein; HR = hazard ratio; OR = odds ratio; RR = risk ratio; VPT = vibration perception threshold

Appendix 6. Cognition literature review: OVID Platform electronic search strategy of EMBASE, MEDLINE, and PsychINFO databases

1. retina*.mp.
2. microvascu*.mp.
3. arteriol*.mp.
4. venul*.mp.
5. vascu*.mp.
6. 2 or 3 or 4 or 5
7. 1 and 6
8. cognit*.mp.
9. Alzheim*.mp.
10. dementi*.mp.
11. memor*.mp.
12. neuropsycholog*.mp.
13. 8 or 9 or 10 or 11 or 12
14. 7 and 13
15. limit 14 to yr="2007 -Current"
16. limit 15 to human
17. remove duplicates from 16

Appendix 7. Cognition literature review: study selection Prisma flow diagram



Appendix 8. Cognition literature review: additional image features

Study name	R/L eye used	Type of fundus image	Region measured
SOUL-D	Best quality	Topcon TRC 50VT; pupil dilation	0.5 to 2.0 disc diameters from disc margin; OD or macula centred, not specified
AIBL	Not specified	Non-mydriatic, 45°, digital retinal camera Canon CR-1	0.5 to 2.0 disc diameters from disc margin; OD centred
Cheung 2014	Not specified, both eyes imaged	45° digital retinal camera, Canon CR-DGI 10D or Canon CR-1 40D; pupil dilation	0.5 to 2.0 disc diameters away from disc margin; OD or macula centred, not specified
Williams 2015	Right	500 Canon CR-DGI, dilation	0.5 to 2.0 disc diameters away from disc margin; OD or macula centred not specified
WESDR	Not specified, both eyes imaged	Stereoscopic colour fundus photos of both eye; pupil dilation; 7-field	Not specified
EDIS	Random	Non-mydriatic digital, dilation	0.5 to 2.0 disc diameters away from disc margin; OD centred
CRIC	Mean value of both eyes	Non-mydriatic 45° digital retinal camera, Canon CR-DGI; nonpharmacological dilation of pupils	0.5 to 1.0 disc diameters from disc margin; OD or macula centred, not specified
LBC1936	Right	Non-mydriatic 45° digital retinal camera, Canon CR-DGI	OD centred
LALES	Not specified, both eyes imaged	Stereoscopic fundus photos using Topcon TRC50EK retinal camera, digitised using Nikon Scanner	temporal edge of OD centred
Dunedin Study	Mean value of both eyes	Digital retinal camera Canon NMR-45 20	0.5 to 2.0 disc diameters from disc margin; OD centred
ET2DS	Random	35° digital retinal camera, non-stereoscopic; pupil dilation; 7-field	0.5 to 1.0 disc diameters from disc margin; OD centred

Study name	R/L eye used	Type of fundus image	Region measured
SiMES	Not specified, both eyes imaged	45° digital retinal camera, Canon CR-DGI 10D; pupil dilation	0.5 to 2.0 disc diameters from disc margin; OD or macula centred, not specified
Blue Mountains Eye Study	Either eye (mainly right)	Stereoscopic 35° retinal photographs using Zeiss FF3, digitised using CanoScan FS2710; 6 field	0.5 to 1.0 disc diameters from disc margin; OD centred
EDC	Right	Stereoscopic photographs	0.5 to 1.0 disc diameters from disc margin; OD centred
Rotterdam	Best quality image	20° field mydriatic fundus colour transparencies digitised using Nikon LS-4000	OD centred

OD = optic disc

Appendix 9. Cognition literature review: statistical models and covariates

Study Name	Statistical model	Variables controlled for
SOUL-D	Linear regression (beta coefficient, 95% CI)	Age, sex, ethnicity, BMI, resting systolic and diastolic BP, receipt of antihypertensive medications, retinopathy, neuropathy, HbA1c
AIBL	ANCOVA analysis (mean, SD)	Age, sex, hypertension, diabetes, smoking status, APOE ε4 carrier, Benjamini and Hochber false discovery rate method
Cheung 2014	Logistic regression (OR, 95% CI)	Age, gender, ethnicity, smoking, hypertension, diabetes, hypercholesterolemia, and history of MI, CRAE/CRVE (for respective analyses)
Williams 2015	Logistic regression (OR, 95% CI)	Sex, smoking, diabetes, CVD diagnosis, hypercholesterolemia diagnosis, mean arterial BP, medication use
WESDR	Multivariate model (beta coefficient, 95% CI)	Age, sex duration of diabetes, education, visual impairment
EDIS	Logistic regression (OR, 95% CI)	Age, sex, education level, socioeconomic status, smoking, mean arterial BP, random blood glucose, total cholesterol, presence of stroke
CRIC	Logistic regression (OR, 95% CI)	Age, sex, race, education, coronary artery disease, hypertension, diabetes and stroke
LBC1936	Linear regression (standardised beta coefficients, p-value)	Age, sex, hypertension, diabetes, CVD, stroke, smoking status, APOE status, current depression symptoms, visual acuity of both eyes, SES, education and age 11 IQ
LALES	Logistic regression (OR 95% CI)	Age, sex, highest education level, language of exam administration, smoking, retinopathy for diabetes
Dunedin Study	Linear regression (beta coefficient, 95% CI)	Sex, CRAE/CRVE (for respective analyses)
ET2DS	Linear regression (beta coefficient, SE)	Age, sex, MHVS, education level, CVD risk factors (smoking status, systolic BP, presence of macrovascular disease) and depression symptoms, CRAE/CRVE (respective analysis)
SiMES	Logistic regression (95% CI)	Age, sex, low income, low education, hypertension, smoking, hyperlipidaemias and CKD
Blue Mountain Eye Study	Logistic regression (OR 95% CI)	Age sex, blood pressure, diabetes mellitus, smoking, systolic BP, history of CVD, education

Study Name	Statistical model	Variables controlled for
EDC	Median regression (beta coefficient)	Baseline retinal traits presented in unadjusted model; change in traits (slope) controlled for a measure of hyperglycaemia, diabetic retinopathy, white matter hyperintensities, diabetes duration, alcohol consumption, smoking status, CRAE/CRVE
Rotterdam Study	Cox proportional hazard (HR, 95%CI)	Age, sex, CRAE, CRVE, systolic BP, hypertensive meds, total cholesterol, serum C-reactive protein, smoking, diabetes, CVD, cardiovascular risk, stroke; stratified by APOE ε4 status

APOE ε4 = apolipoprotein E ε4 variant; BMI = body mass index; BP = blood pressure; CKD = chronic kidney disease; CI = confidence interval; CRAE = central retinal arterial equivalent; CRVE = central retinal venular equivalent; CVD = cardiovascular disease; HbA1c = glycated haemoglobin; MHVS = Mill Hill Vocabulary Scale; SD = standard deviation; SES = socioeconomic status

Appendix 10. ET2DS year-10 Ethics favourable letter

Lothian NHS Board

South East Scotland Research Ethics Committee 01

Waverley Gate
2-4 Waterloo Place
Edinburgh
EH1 3EG
Telephone 0131 536 9000

www.nhslothian.scot.nhs.uk

NHS Lothian

Date 23 May 2016 (reissued 06 July 2016 to correct document dates)
Your Ref
Our Ref

Enquiries to: Sandra Wyllie
Extension: 35473
Direct Line: 0131 465 5473
Email: Sandra.Wyllie@nhslothian.scot.nhs.uk

23 May 2016

Professor Jackie Price
Professor of Molecular Epidemiology and
Honorary Consultant in Public Health
The University of Edinburgh
Centre for Population Health Sciences
Medical School, Teviot Place
Edinburgh
EH8 9AG

Dear Professor Price

Study title:	**Risk factors for cognitive decline in people with type 2 diabetes; the Edinburgh Type 2 Diabetes Study 10 year follow-up (Version 1)**
REC reference:	**16/SS/0098**
Protocol number:	**AC16046**
IRAS project ID:	**194995**

Thank you for your letter of 18 May 2016, responding to the Committee's request for further information on the above research and submitting revised documentation.

The further information has been considered on behalf of the Committee by the Chair and Committee member, George Howat

We plan to publish your research summary wording for the above study on the HRA website, together with your contact details. Publication will be no earlier than three months from the date of this opinion letter. Should you wish to provide a substitute contact point, require further information, or wish to make a request to postpone publication, please contact the REC Manager, Mrs Sandra Wyllie, sandra.wyllie@nhslothian.scot.nhs.uk.

Confirmation of ethical opinion

On behalf of the Committee, I am pleased to confirm a favourable ethical opinion for the above research on the basis described in the application form, protocol and supporting documentation as revised, subject to the conditions specified below.

Conditions of the favourable opinion

The REC favourable opinion is subject to the following conditions being met prior to the start of the study.

Management permission must be obtained from each host organisation prior to the start of the study at the site concerned.

Management permission should be sought from all NHS organisations involved in the study in accordance with NHS research governance arrangements. Each NHS organisation must confirm through the signing of agreements and/or other documents that it has given permission for the research to proceed (except where explicitly specified otherwise).

Guidance on applying for NHS permission for research is available in the Integrated Research Application System, www.hra.nhs.uk or at http://www.rdforum.nhs.uk.

Where a NHS organisation's role in the study is limited to identifying and referring potential participants to research sites ("participant identification centre"), guidance should be sought from the R&D office on the information it requires to give permission for this activity.

For non-NHS sites, site management permission should be obtained in accordance with the procedures of the relevant host organisation.

Sponsors are not required to notify the Committee of management permissions from host organisations

Registration of Clinical Trials

All clinical trials (defined as the first four categories on the IRAS filter page) must be registered on a publically accessible database within 6 weeks of recruitment of the first participant (for medical device studies, within the timeline determined by the current registration and publication trees).

There is no requirement to separately notify the REC but you should do so at the earliest opportunity e.g. when submitting an amendment. We will audit the registration details as part of the annual progress reporting process.

To ensure transparency in research, we strongly recommend that all research is registered but for non-clinical trials this is not currently mandatory.

If a sponsor wishes to contest the need for registration they should contact Catherine Blewett (catherineblewett@nhs.net), the HRA does not, however, expect exceptions to be made. Guidance on where to register is provided within IRAS.

It is the responsibility of the sponsor to ensure that all the conditions are complied with before the start of the study or its initiation at a particular site (as applicable).

Ethical review of research sites

NHS sites

The favourable opinion applies to all NHS sites taking part in the study, subject to management permission being obtained from the NHS/HSC R&D office prior to the start of the study (see "Conditions of the favourable opinion" below).

Approved documents

The final list of documents reviewed and approved by the Committee is as follows:

Document	*Version*	*Date*
Covering letter on headed paper		18 May 2016
GP/consultant information sheets or letters		
Letters of invitation to participant	1	17 May 2016
Non-validated questionnaire [ET2DS Participant Questionnaire]	v1.0	28 March 2016
Other [RAPA Questionnaire]	v1.0	28 March 2016
Participant information sheet (PIS) including Consent Form	1.1	17 May 2016
REC Application Form [REC_Form_20042016]		20 April 2016
Research protocol or project proposal [ET2DS Protocol 2016]		
Summary CV for Chief Investigator (CI) [CV Jackie Price]		
Validated questionnaire [IPAQ Interview Questionnaire]	v1.0	28 March 2016

Statement of compliance

The Committee is constituted in accordance with the Governance Arrangements for Research Ethics Committees and complies fully with the Standard Operating Procedures for Research Ethics Committees in the UK.

After ethical review

Reporting requirements

The attached document *"After ethical review – guidance for researchers"* gives detailed guidance on reporting requirements for studies with a favourable opinion, including:

- Notifying substantial amendments
- Adding new sites and investigators
- Notification of serious breaches of the protocol
- Progress and safety reports
- Notifying the end of the study

The HRA website also provides guidance on these topics, which is updated in the light of changes in reporting requirements or procedures.

User Feedback

The Health Research Authority is continually striving to provide a high quality service to all applicants and sponsors. You are invited to give your view of the service you have received and the application procedure. If you wish to make your views known please use the feedback form available on the HRA website: http://www.hra.nhs.uk/about-the-hra/governance/quality-assurance/

HRA Training

We are pleased to welcome researchers and R&D staff at our training days – see details at http://www.hra.nhs.uk/hra-training/

16/SS/0098	Please quote this number on all correspondence

With the Committee's best wishes for the success of this project.

Yours sincerely

Dr Janet Andrews
Chair

Email:sandra.wyllie@nhslothian.scot.nhs.uk

Enclosures: "After ethical review – guidance for researchers"

Copy to: *Dr Fiach O'Mahony*
Mr Gavin Robertson, NHS Lothian Research & Development Office

Appendix 11. ET2DS year-10 Research & Development favourable letter

University Hospitals Division

NHS Lothian

Queen's Medical Research Institute
47 Little France Crescent, Edinburgh, EH16 4TJ

FM/GM/Approval

17th June 2016

Research & Development
Room E1.12
Tel: 0131 242 3330

Email:
R&DOffice@nhslothian.scot.nhs.uk

Director: Professor David E Newby

Dr Jackie F Price
University of Edinburgh Medical School
Teviot Place
Edinburgh
EH8 9AG

Dear Dr Price,

Lothian R&D Project No: 2016/0132

Title of Research: Risk factors for cognitive decline in people with type 2 diabetes; the Edinburgh Type 2 Diabetes Study 10 year follow-up (Version 1)

REC No: 16/SS/0098

Participant Information Sheet:
Version 1.1 Dated 17th May 2016

Consent Form:
Version 1.1 Dated 17th May 2016

Protocol: Version 1.0 Dated 2016

I am pleased to inform you that this study has been approved for NHS Lothian and you may proceed with your research, subject to the conditions below. This letter provides Site Specific approval for NHS Lothian.

We note that this project includes researchers who will require an Honorary Research Contract from NHS Lothian. The individuals concerned Anniek Sluiman and Rachel Bedenis should contact our offices with a view to applying for the necessary documentation. Please note all final paperwork will have to be signed and returned to our R&D offices before the researchers can commence work on the project.
Please note that the NHS Lothian R&D Office must be informed if there are any changes to the study such as amendments to the protocol, recruitment, funding, personnel or resource input required of NHS Lothian.

Substantial amendments to the protocol will require approval from the ethics committee which approved your study and the MHRA where applicable.

Please inform this office when recruitment has closed and when the study has been completed.

I wish you every success with your study.

Yours sincerely

Ms Fiona McArdle
Deputy R&D Director

STAB 14

Appendix 12. Variable distributions

Figure 31. Descriptive variable distributions- normal

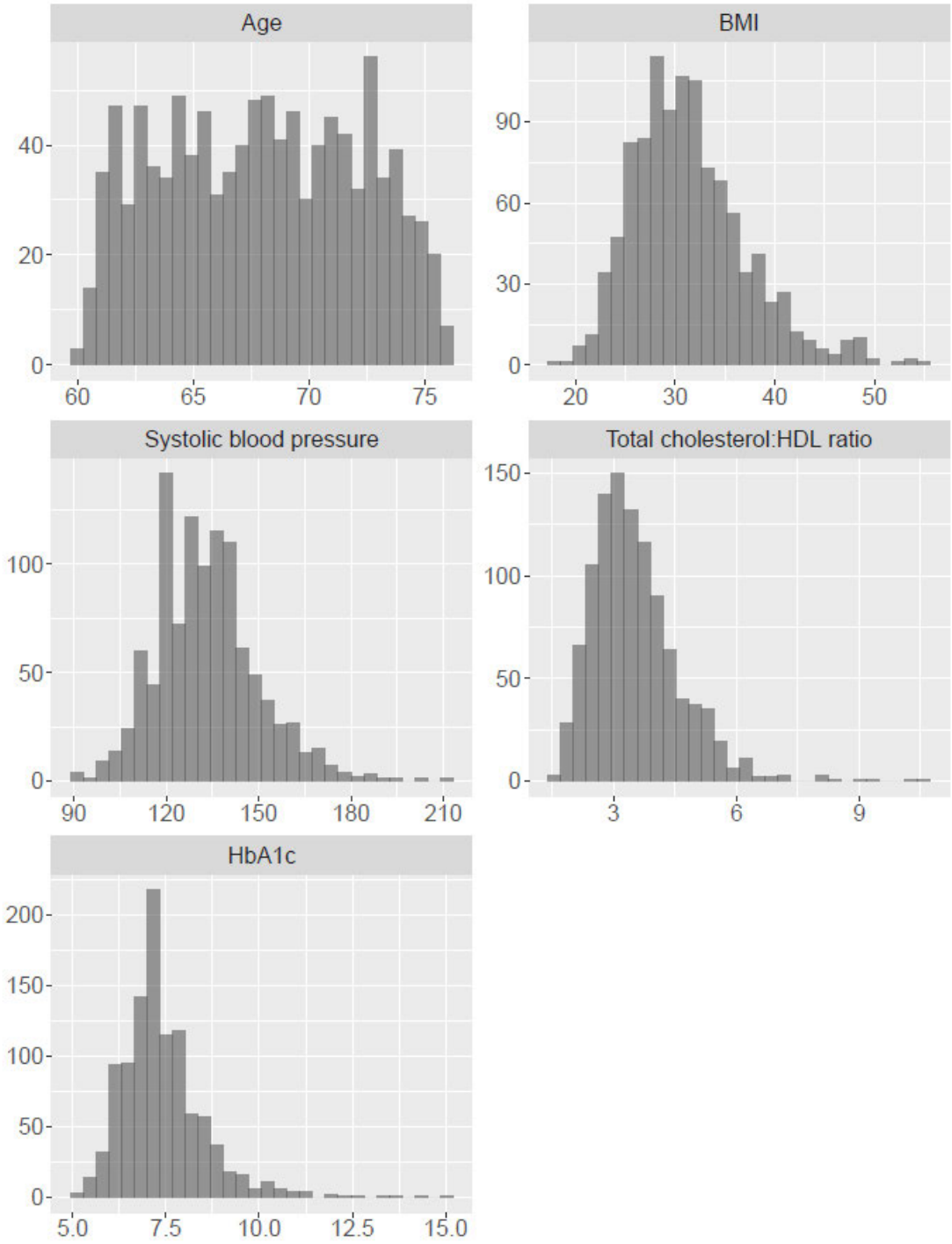


Figure 32. Descriptive variable distributions- non-normal and transformed

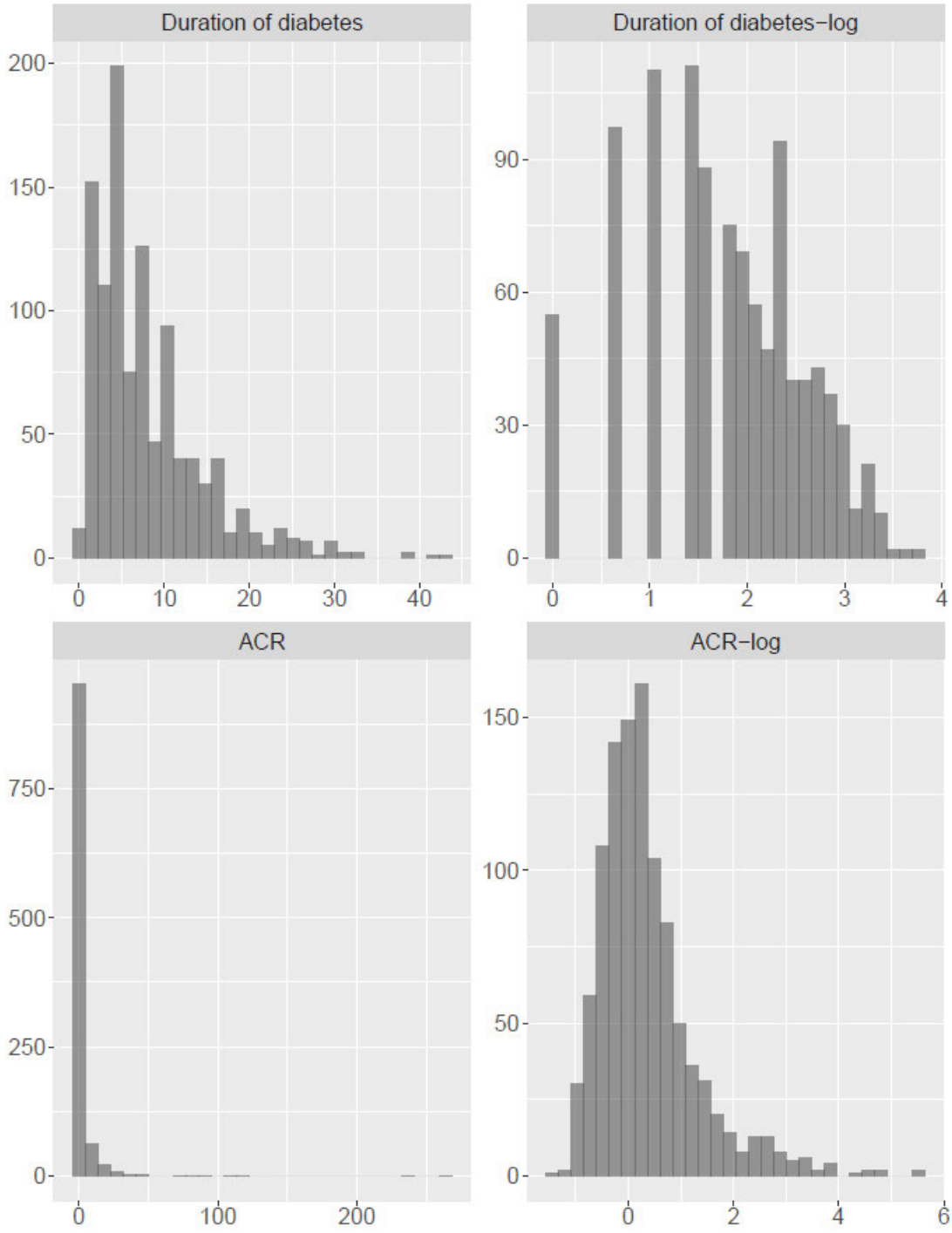


Figure 33. Retinal vessel trait distributions- normal

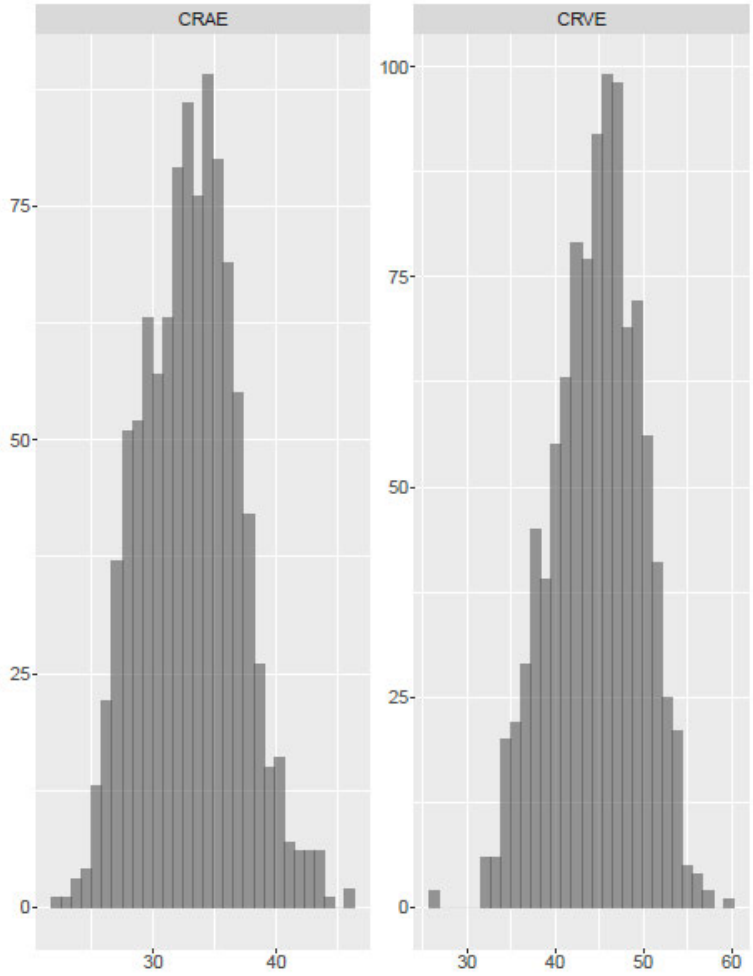


Figure 34. Retinal vessel trait distributions- non-normal and transformed

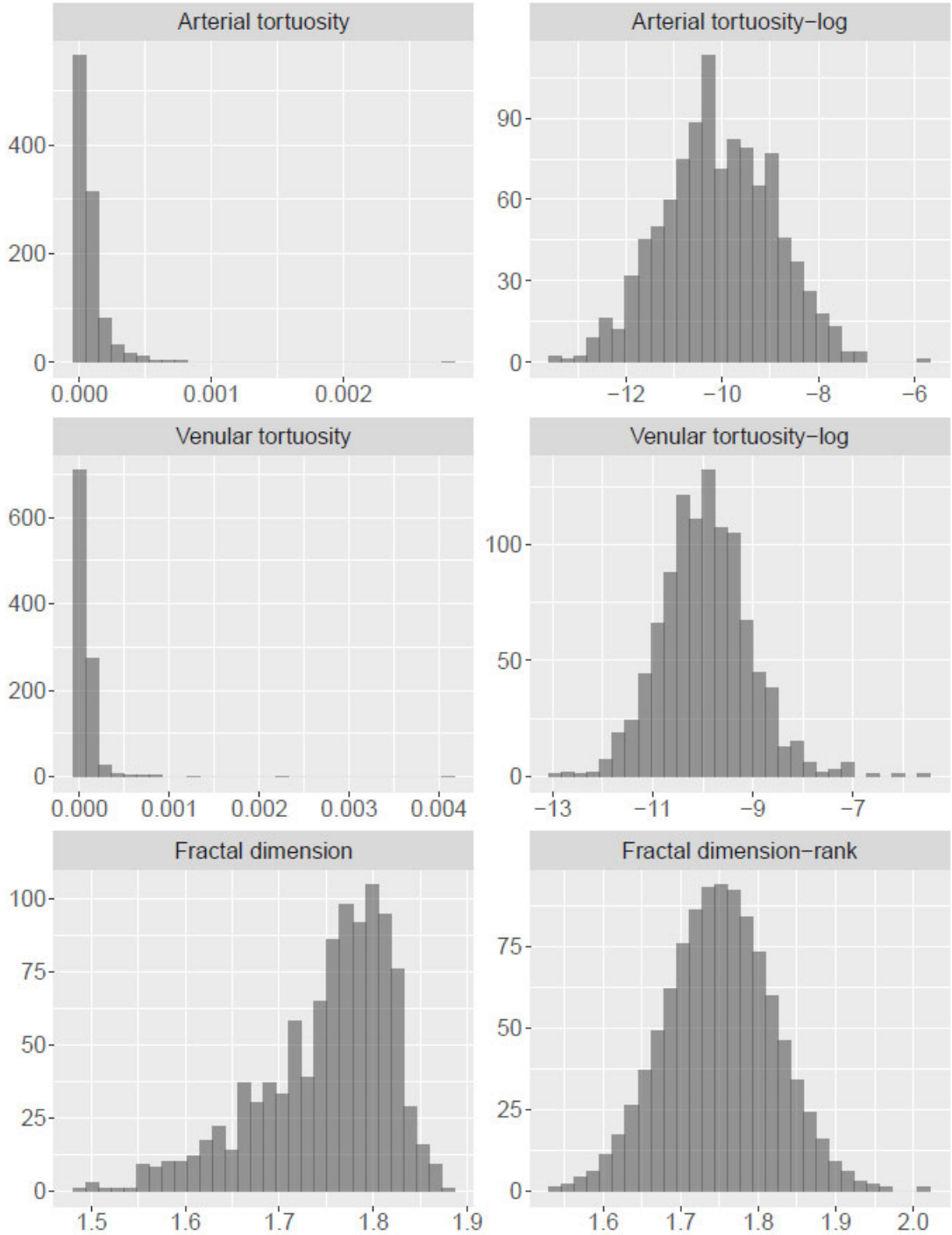


Figure 35. Cognitive tests and *g* distributions- normal

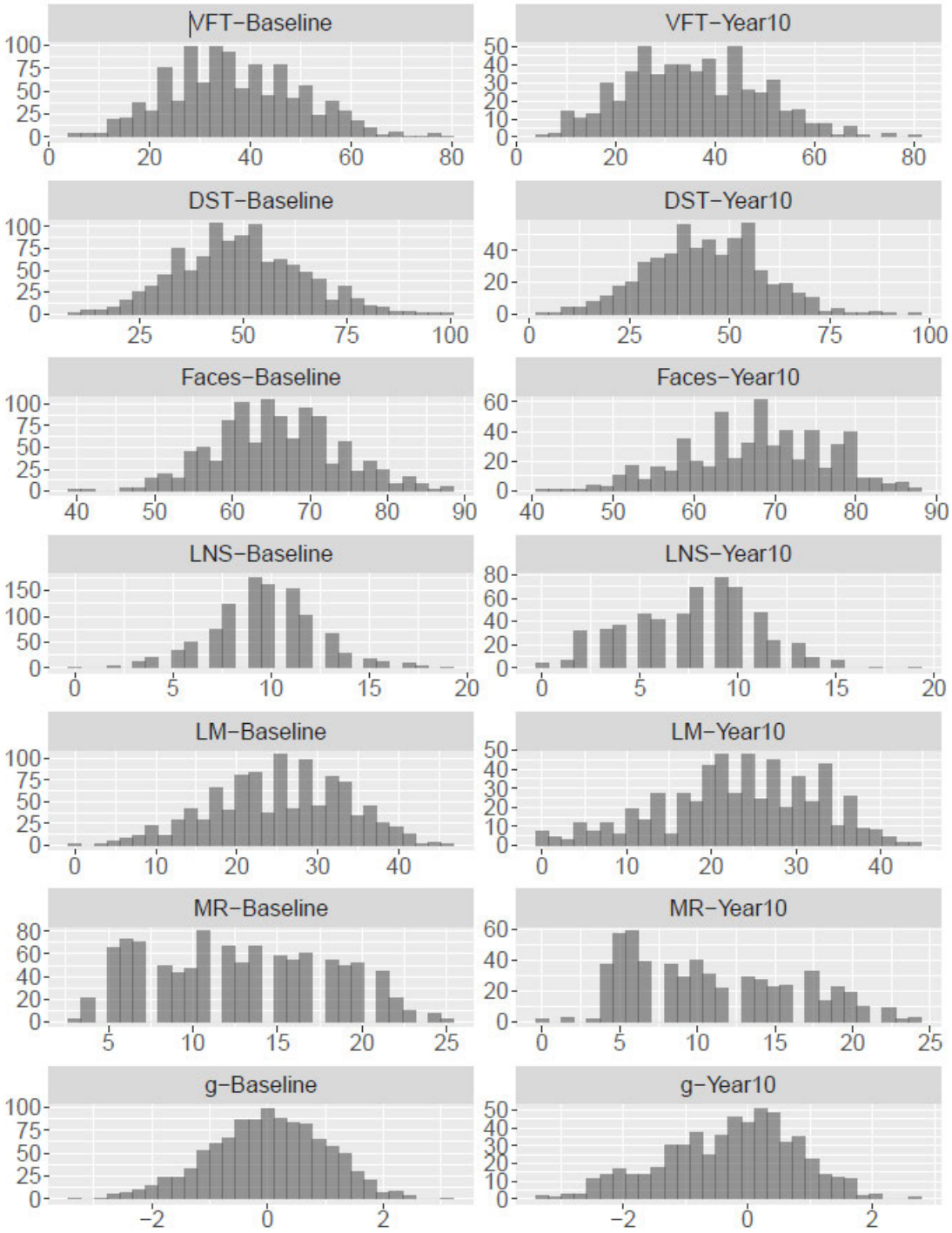
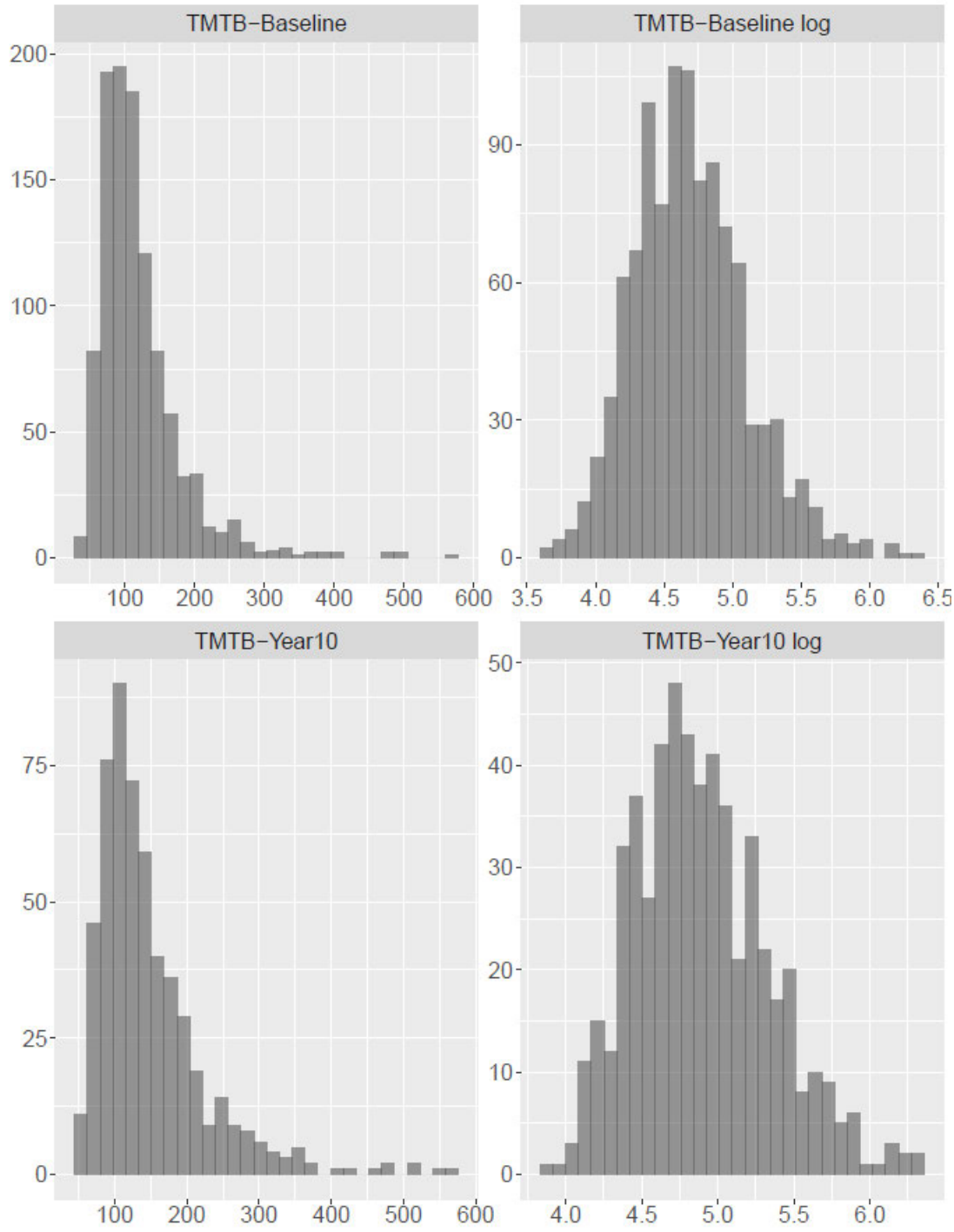
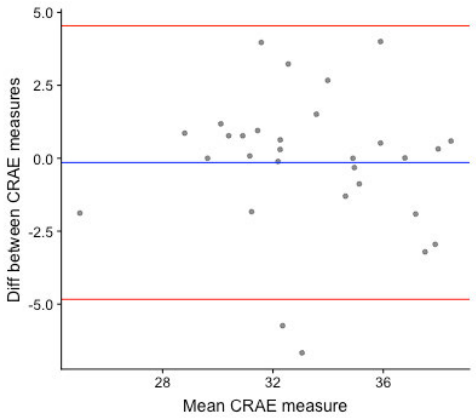
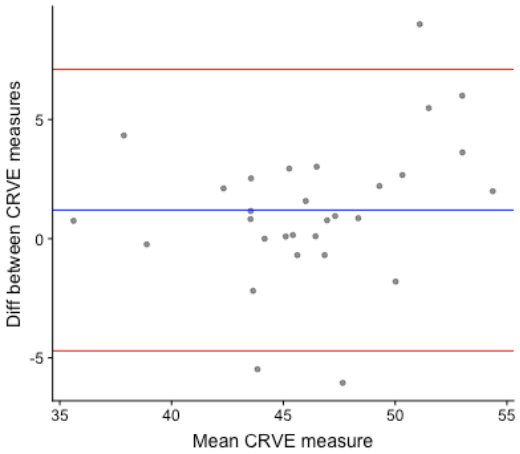


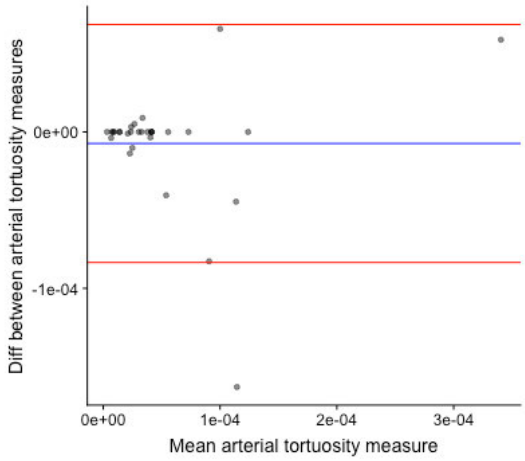
Figure 36. Cognitive test distributions- non-normal and transformed

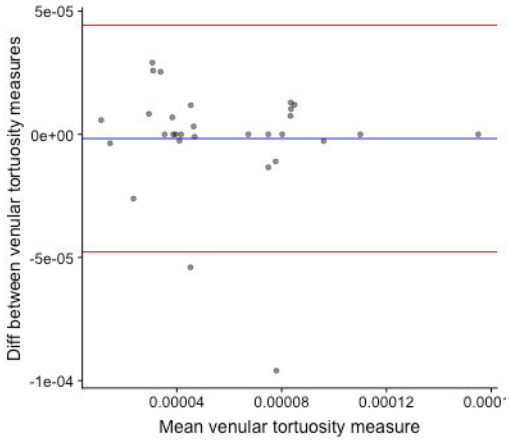


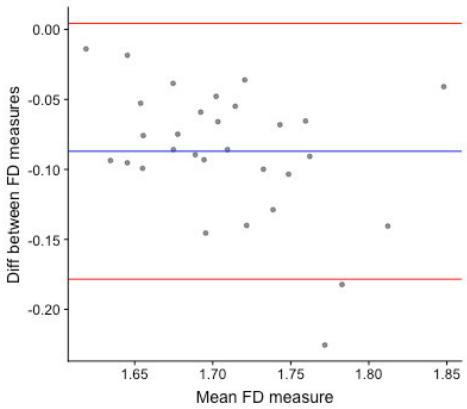
Appendix 13. Bland-Altman plots of baseline intergrader agreement


Diff between CRAE measures
5.0
2.5
0.0
-2.5
-5.0
28
32
36
Mean CRAE measure

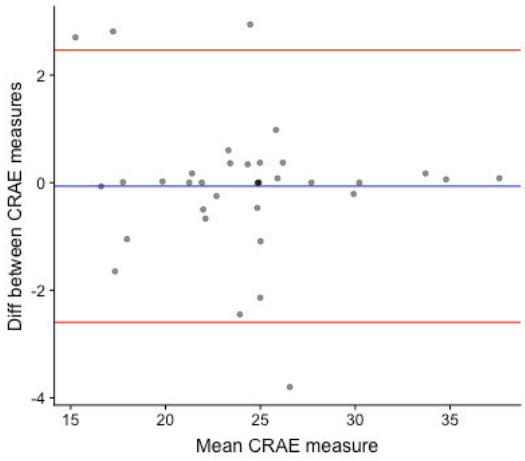

Diff between CRVE measures
5
0
-5
35
40
45
50
55
Mean CRVE measure

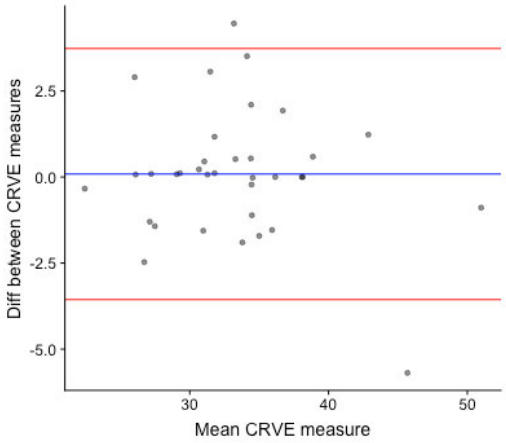

Diff between arterial tortuosity measures
0e+00
-1e-04
0e+00
1e-04
2e-04
3e-04
Mean arterial tortuosity measure

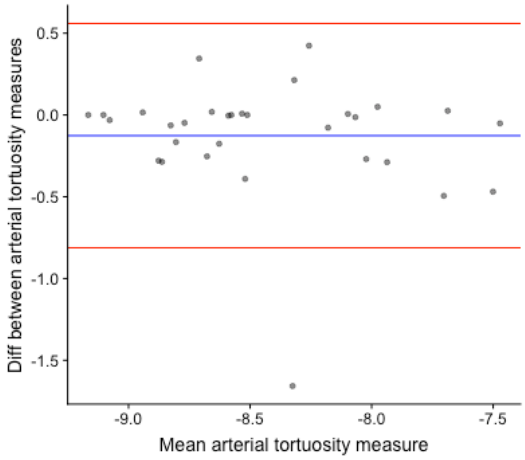

Diff between venular tortuosity measures
5e-05
0e+00
-5e-05
-1e-04
0.00004
0.00008
0.00012
Mean venular tortuosity measure

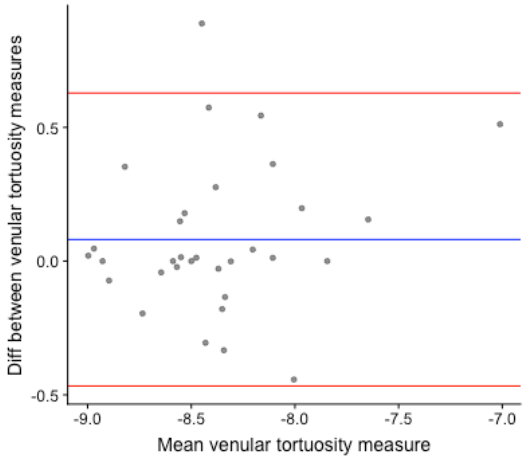

Diff between FD measures
0.00
-0.05
-0.10
-0.15
-0.20
1.65
1.70
1.75
1.80
1.85
Mean FD measure

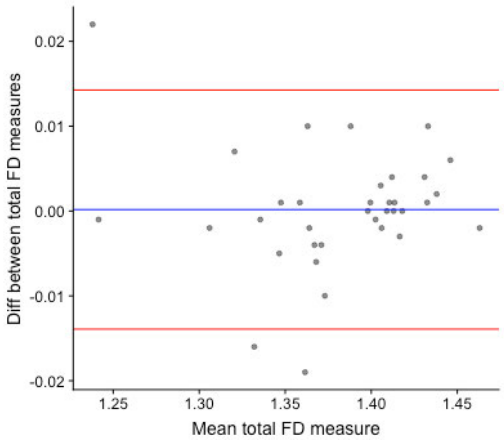
Appendix 14. Bland-Altman plots of follow-up intragrader agreement

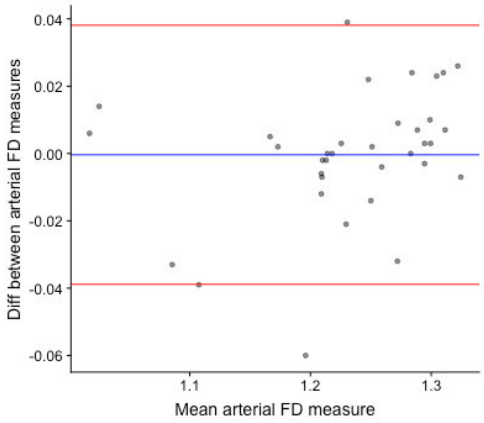


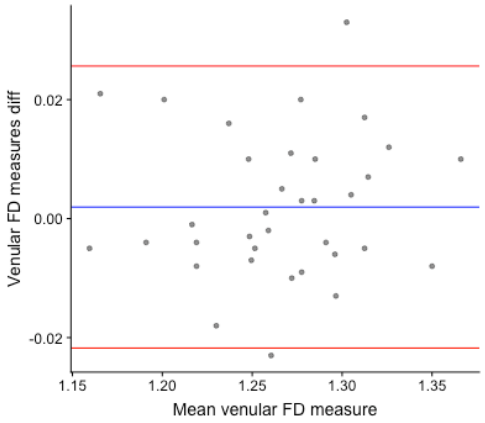


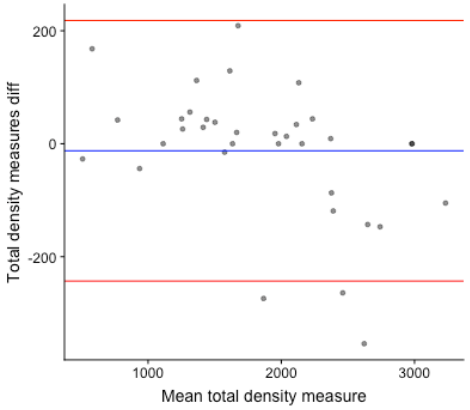


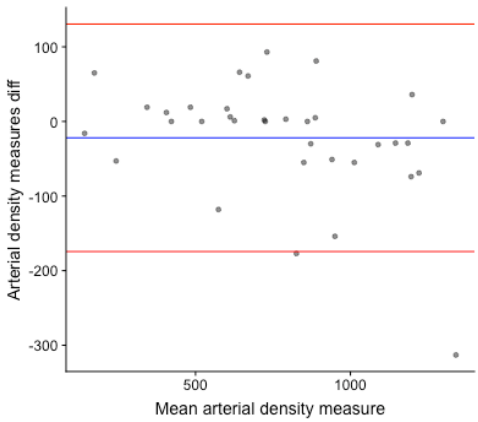


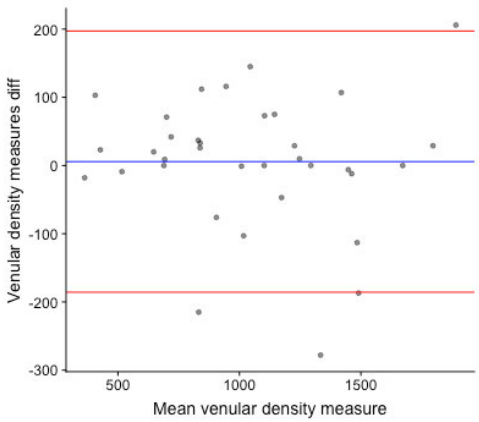



Diff between arterial FD measures
0.04
0.02
0.00
-0.02
-0.04
-0.06
1.1
1.2
1.3
Mean arterial FD measure

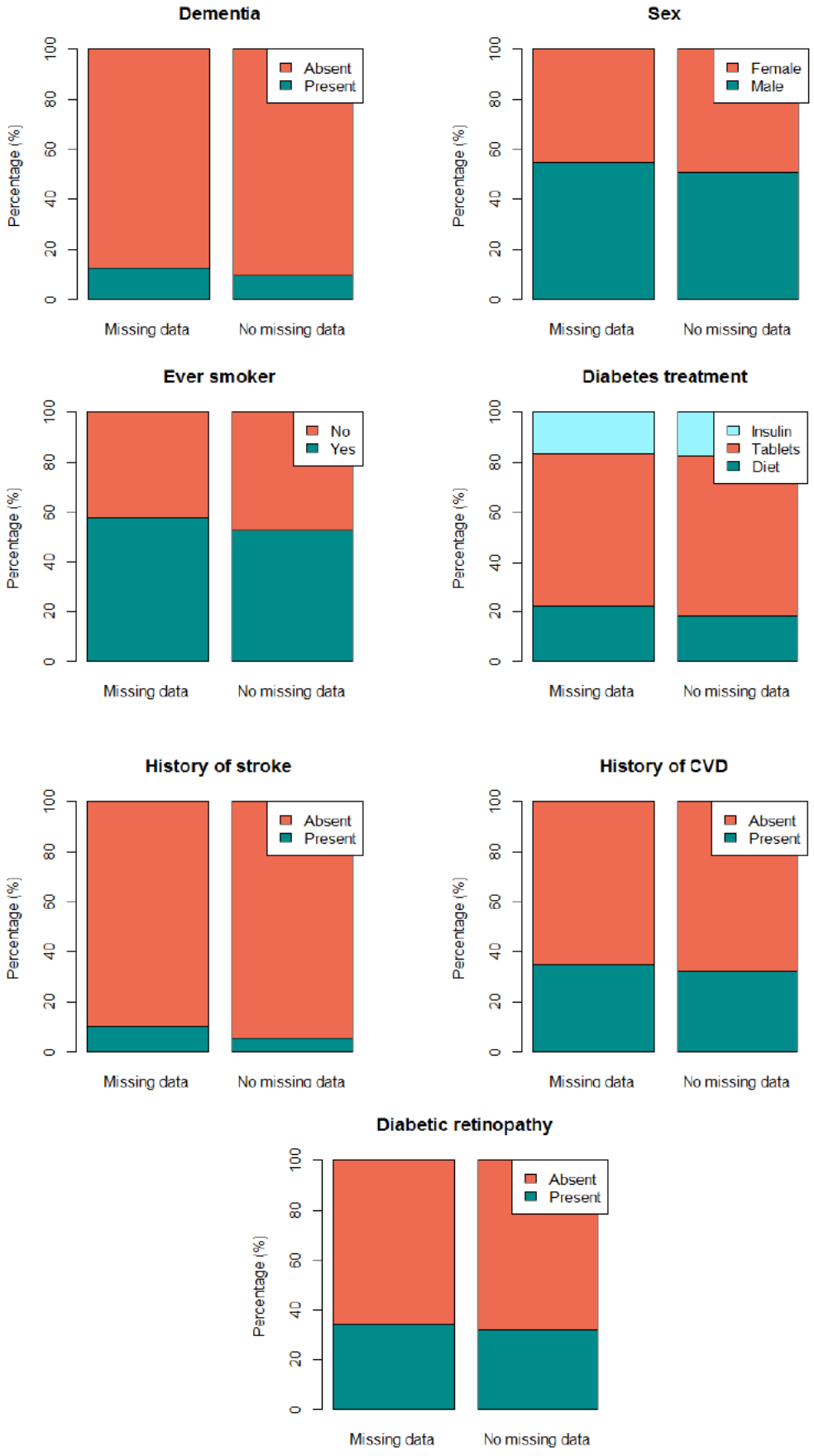

Venular FD measures diff
0.02
0.00
-0.02
1.15
1.20
1.25
1.30
1.35
Mean venular FD measure


Total density measures diff
200
0
-200
1000
2000
3000
Mean total density measure

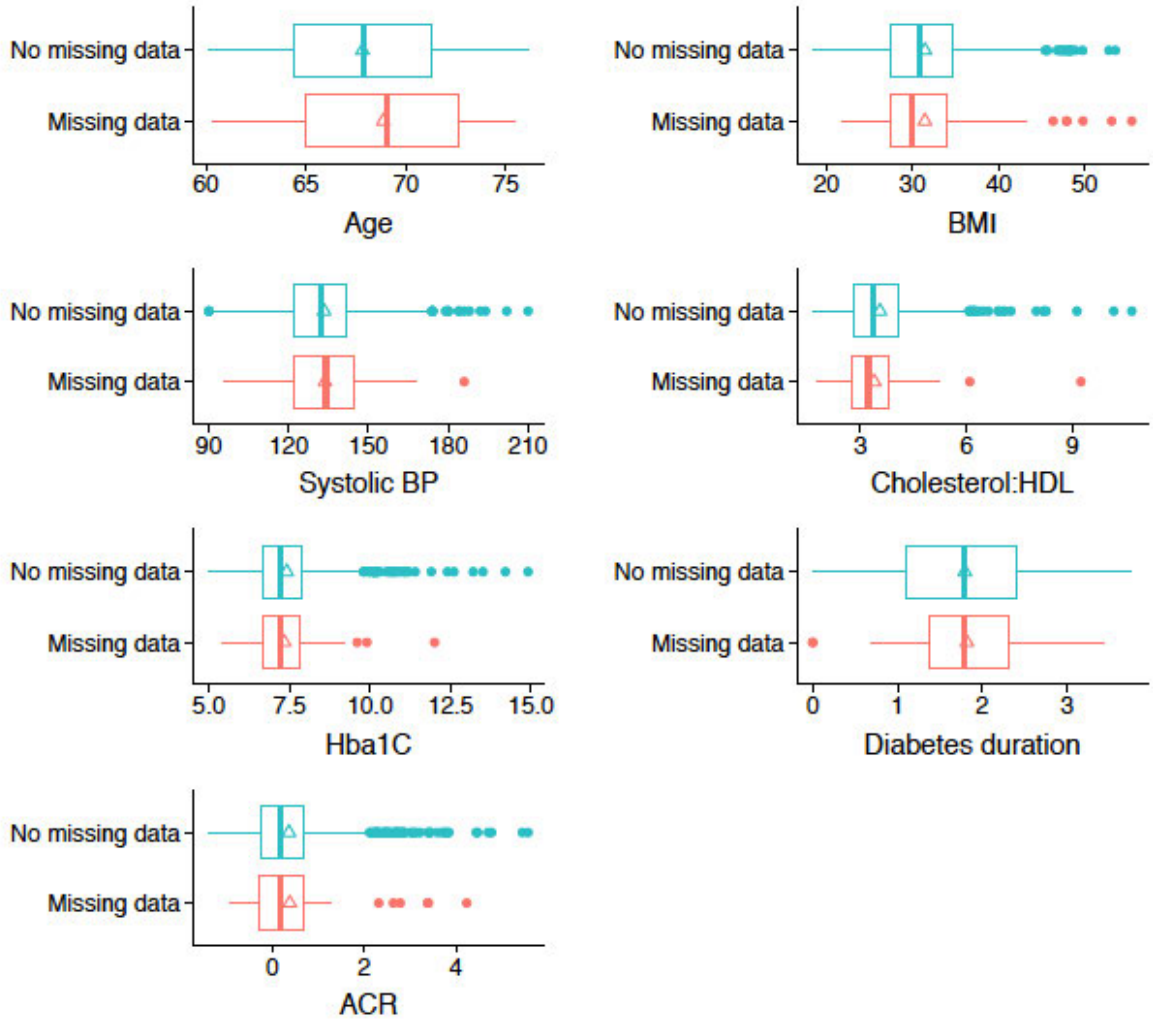

Arterial density measures diff
100
0
-100
-200
-300
500
1000
Mean arterial density measure


Venular density measures diff
200
100
0
-100
-200
-300
500
1000
1500
Mean venular density measure

Appendix 15. Categorical variable comparison between whole cases and cases with missing data



Appendix 16. Continuous variable comparison between whole cases and cases with missing data



Appendix 17. Model fit- logistic regression, RVTs and incident retinopathy

	CRAE			CRVE			Arterial tortuosity			Venular tortuosity			Fractal dimension-standardised		
	c	AIC	H-L	c	AIC	H-L	c	AIC	H-L	c	AIC	H-L	c	AIC	H-L
Unadjusted	0.573	490.4		0.569	490.7		0.512	495.3		0.583	487.6		0.557	489.1	
Fully adjusted	0.647	485.9	.588	0.657)	485.9	.588	0.631	490.6	.970	0.652	481.9	.370	0.649	.483.0	.896

AIC = Akaike information criterion; c = concordance (c) statistic; CRAE = central retinal arterial equivalent; CRVE = central retinal venular equivalent; H-L = Hosmer-Lemeshow p value

Appendix 18. Sensitivity analysis- relaxed definition of incident retinopathy (n = 239)

	CRAE		CRVE		Arterial tortuosity		Venular tortuosity		Fractal dimension-standardised	
	OR (95% CI)	p	OR (95% CI)	p	OR (95% CI)	p	OR (95% CI)	p	OR (95% CI)	p
Model 5	1.00 (0.96 to 1.04)	.988	1.00 (0.97 to 1.03)	.996	0.96 (0.85 to 1.09)	.531	1.08 (0.93 to 1.25)	.296	1.08 (0.92 to 1.28)	.335

*p<0.05 **p<0.01 ***p<0.001

Model 1 – unadjusted, Model 2 – age and sex adjusted, Model 3 – Model 2 + cardiometabolic risk factors (BMI, smoking status, systolic blood pressure, total cholesterol:HDL ratio), Model 4 – Model 3 + diabetes related risk factors (HbA1c, duration of diabetes, diabetic treatment type), Model 5 – Model 4 + vascular disease (composite CVD, ACR) + CRAE or CRVE when analysing CRAE or CRVE; ACR = albumin/creatinine ratio; BMI = body mass index; CI = confidence interval; CRAE = central retinal arterial equivalent; CRVE = central retinal venular equivalent; HbA1c = glycated haemoglobin; HDL = high density lipoprotein; HR = hazard ratio; OR = odds ratio

Appendix 19. Model fit- logistic regression, RVTs and dementia

	CRAE			CRVE			Arterial tortuosity			Venular tortuosity			Fractal dimension-standardised		
	c	AIC	H-L	c	AIC	H-L	c	AIC	H-L	c	AIC	H-L	c	AIC	H-L
Unadjusted	0.500	660		0.553	656.5		0.507	659.9		0.501	659.9		0.520	648.1	
Age and sex adjusted	0.625	647.4	.648	0.630	645.3	.871	0.624	647.3	.403	0.625	646.9	.266	0.618	637.7	.516

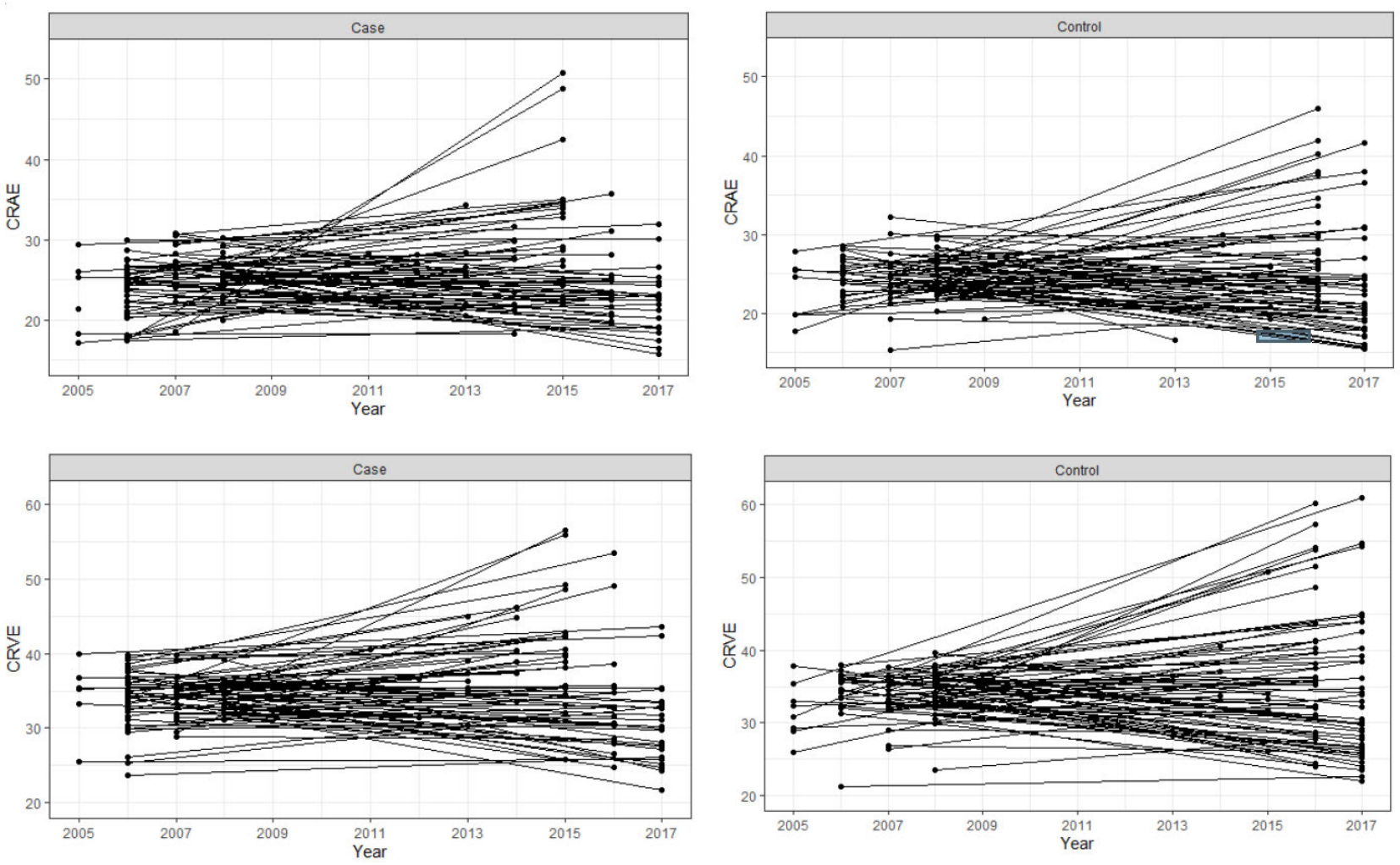
AIC = Akaike information criterion; c = concordance (c) statistic; CRAE = central retinal arterial equivalent; CRVE = central retinal venular equivalent; H-L = Hosmer-Lemeshow p value

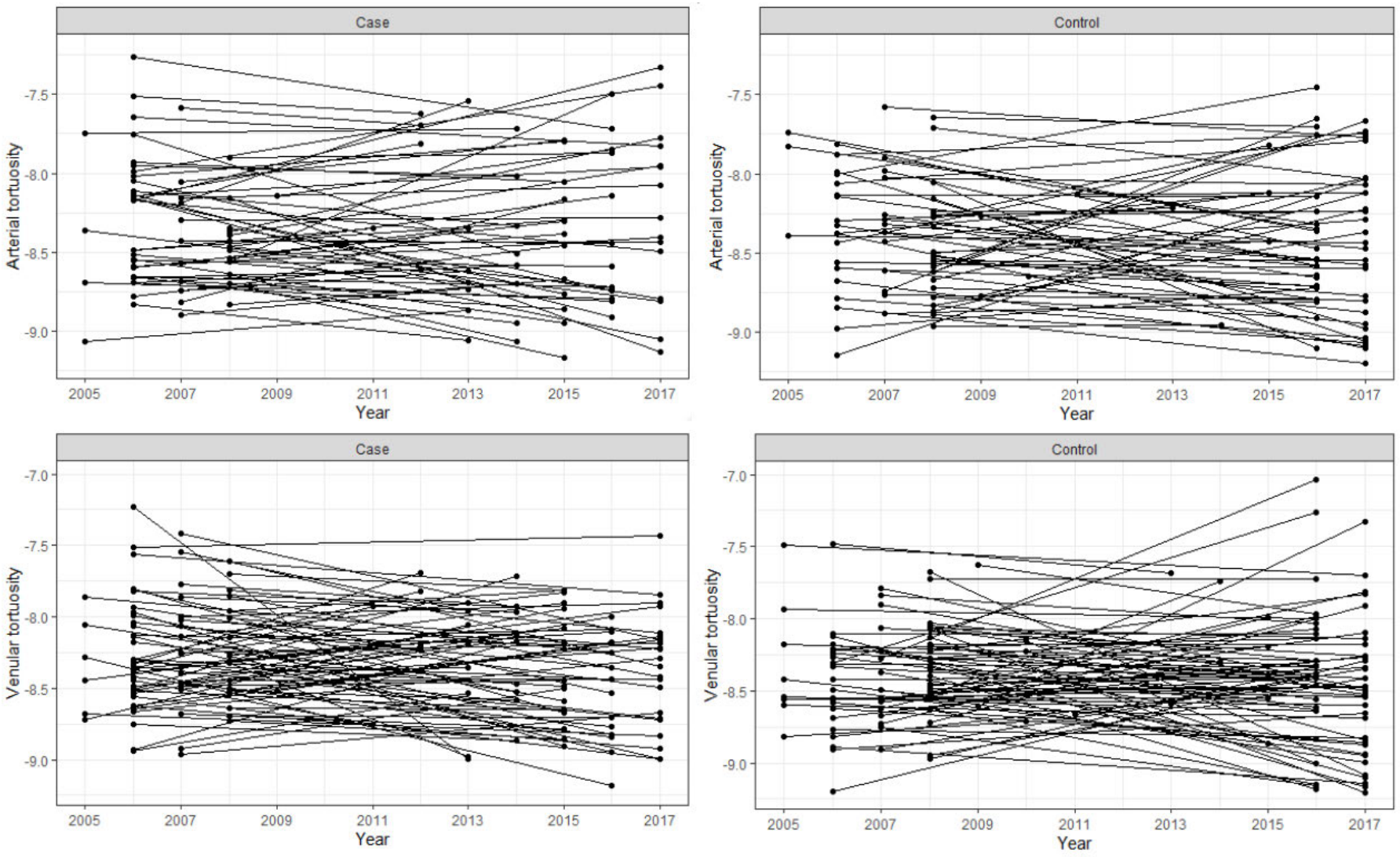
Appendix 20. Association between RVTs and dementia and/or lowest decile g- sensitivity analysis

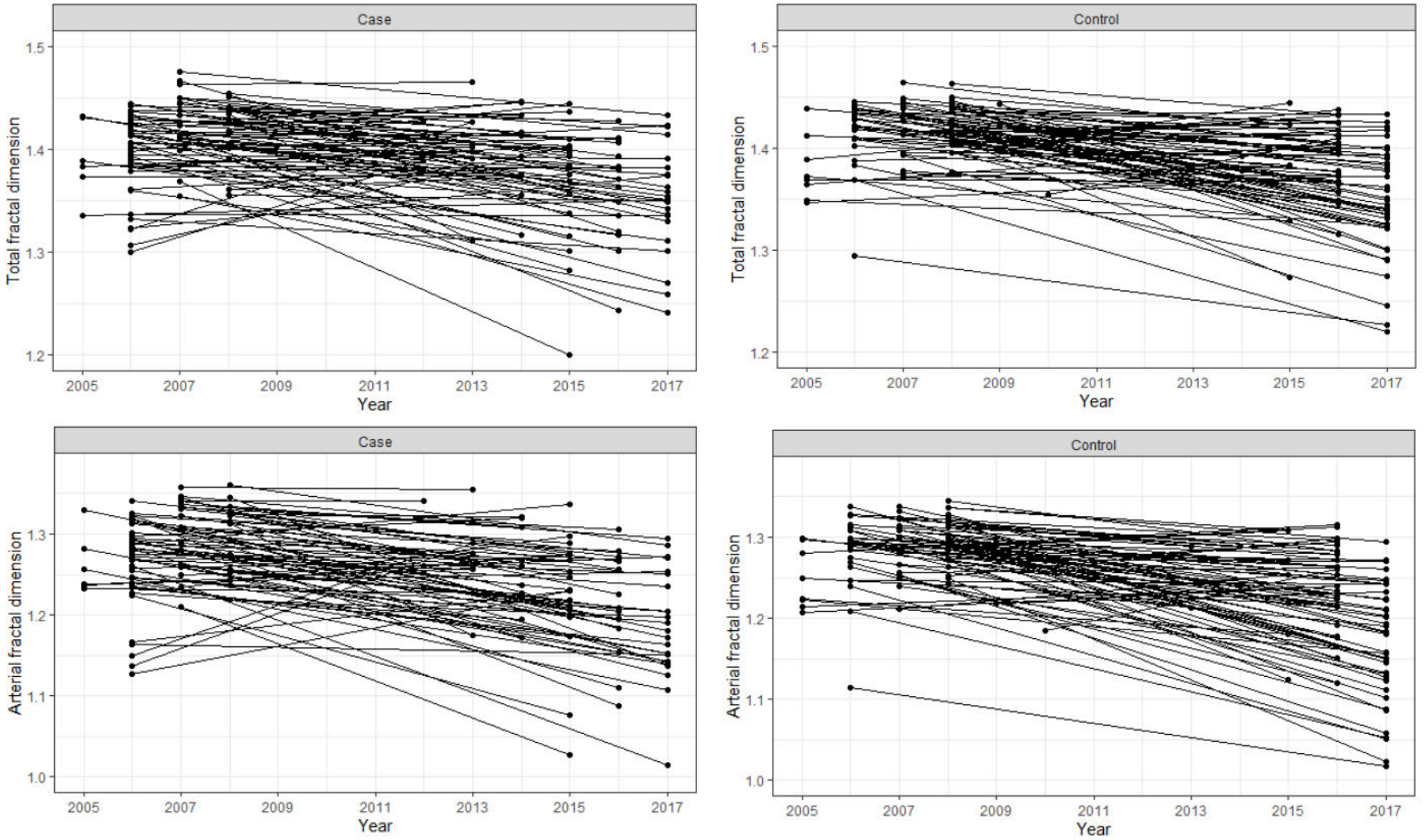
	CRAE		**CRVE**		**Arterial tortuosity**		**Venular tortuosity**		**Fractal dimension-standardised**	
	OR (95% CI)	p-value	OR (95% CI)	p-value	OR (95% CI)	p-value	OR (95% CI)	p-value	OR (95% CI)	p-value
Model 1	1.02 (0.97 to 1.07)	.469	**1.04 (1.01 to 1.08)**	**.020***	0.92 (0.79 to 1.08)	.321	0.87 (0.71 to 1.06)	.167	1.12 (0.94 to 1.35)	.209
Model 2	1.01 (0.96 to 1.06)	.755	1.03 (1.00 to 1.07)	.077	0.91 (0.78 to 1.07)	.256	0.86 (0.71 to 1.06)	.161	1.10 (0.92 to 1.32)	.306
Model 3	-	-	-	-	-	-	-	-		
Model 4	-	-	-	-	-	-	-	-		
Model 5	-	-	-	-	-	-	-	-		

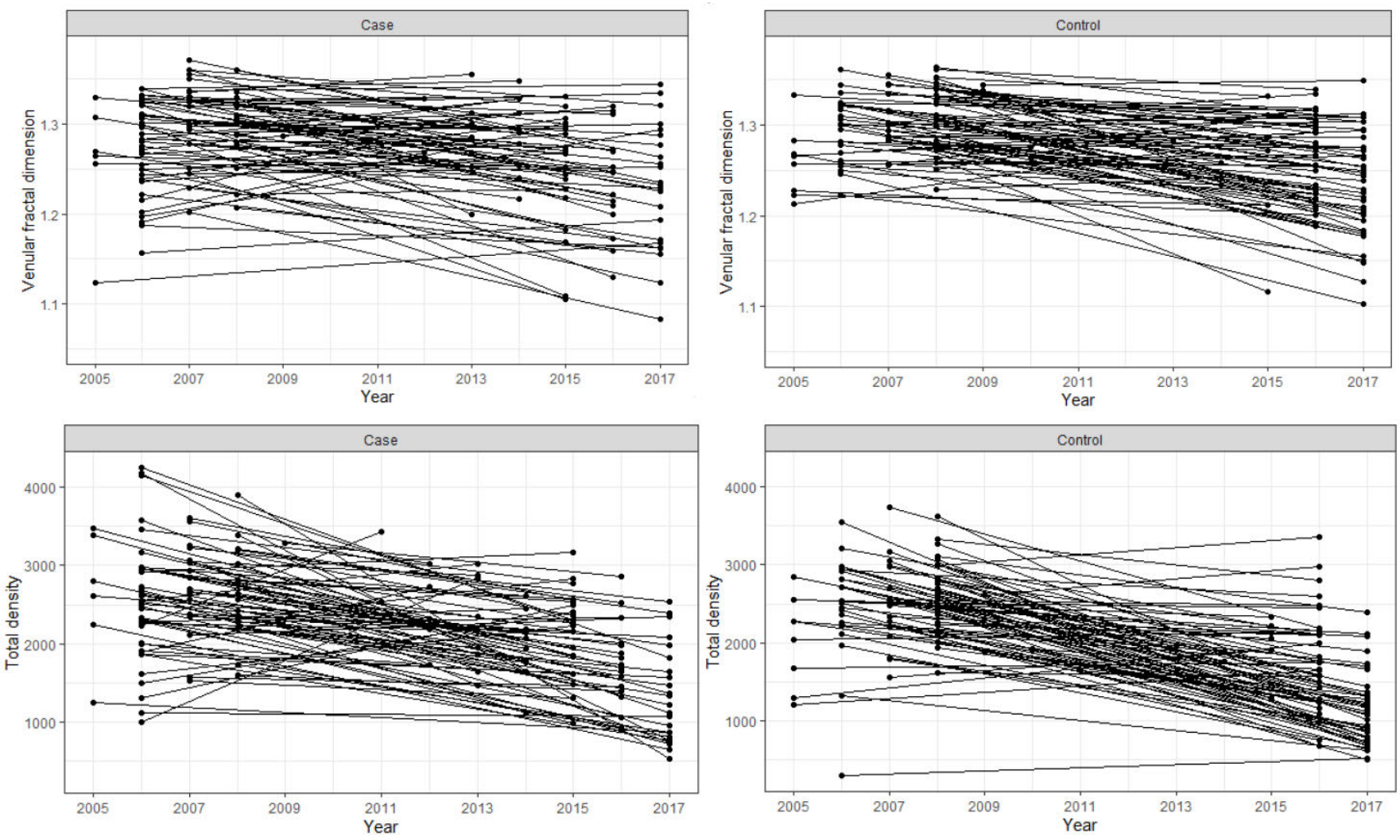
Model 1 – unadjusted, Model 2 – age and sex adjusted, Model 3 – Model 2 + cardiometabolic risk factors (BMI, smoking status, systolic blood pressure, total cholesterol:HDL ratio), Model 4 – Model 3 + diabetes related risk factors (HbA1c, duration of diabetes, diabetic treatment type), Model 5 – Model 4 + vascular disease (stroke, composite CVD, ACR, retinopathy) + CRAE or CRVE when analysing CRAE or CRVE; ACR = albumin/creatinine ratio; BMI = body mass index; CI = confidence interval; CRAE = central retinal arterial equivalent; CRVE = central retinal venular equivalent; HbA1c = glycated haemoglobin; HDL = high density lipoprotein; OR = odds ratio

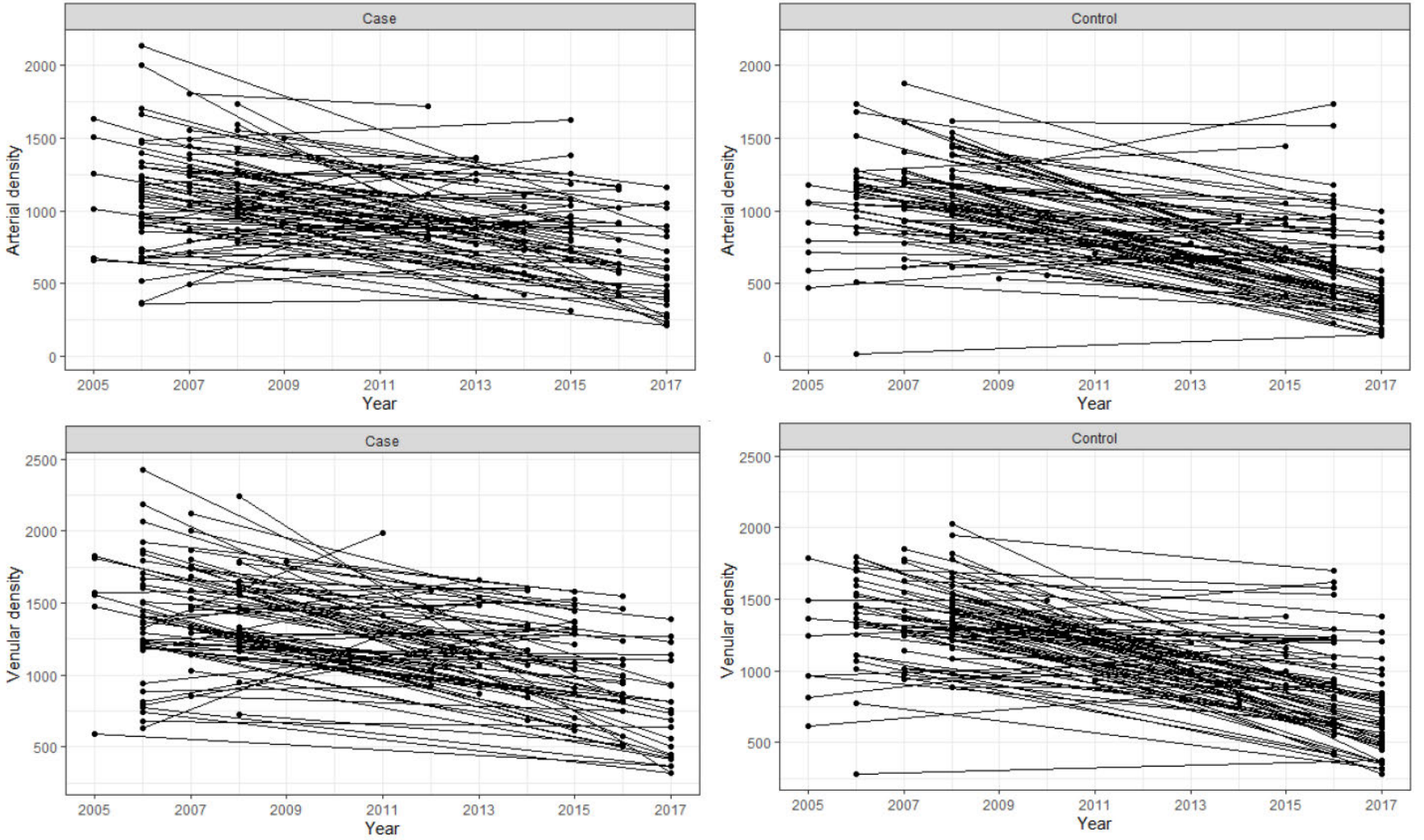
Appendix 21. Change in retinal vessel traits- spaghetti plots




Case
Control
Arterial tortuosity
Venular tortuosity
-7.0
-7.5
-8.0
-8.5
-9.0
2005
2007
2009
2011
2013
2015
2017
Year


Case
Control
Case
Control
Total fractal dimension
Arterial fractal dimension
Year
1.5
1.4
1.3
1.2
1.1
1.0
2005
2007
2009
2011
2013
2015
2017


Case
Control
Venular fractal dimension
1.1
1.2
1.3
Total density
1000
2000
3000
4000
2005
2007
2009
2011
2013
2015
2017
Year


Case
Control
Arterial density
Venular density
Year
2005
2007
2009
2011
2013
2015
2017
0
500
1000
1500
2000
2500