THE UNIVERSITY *of* EDINBURGH

Retinal Microvascular Abnormalities and Cognitive Function in Older People with Type 2 Diabetes

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Declaration

I, Jie Ding, declare that this thesis is my own composition.

I performed a systematic review on the association between retinal microvascular abnormalities and cognitive dysfunction. I retrieved digital retinal images from the Edinburgh Type 2 Diabetes Study participants in 2006/7, and conducted computer-assisted measurements to quantify parameters of retinal vascular network geometry. I have prepared and analysed the data pertaining to those measurements. I also carried out further analysis on additional diabetic retinopathy data collected by others. Both parts of the analysis are included in the thesis. The Edinburgh Type 2 Diabetes Study was already in progress when my project commenced. Therefore the clinical and cognitive information, employed widely in the present thesis, was collected through the effort of other colleagues.

The systematic review shown in Chapter 2 was published during the second year of my project (Ding et al., 2008). The paper is included in the appendices with permission from BMJ Publishing Group.

No portion of the work referred to in the thesis has been submitted for any other degree or professional qualification.

Signed……

Date……

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Abstract

The deleterious effects of Type 2 diabetes on the brain have been shown to result in a greater prevalence of age-associated cognitive impairment and an enhanced risk of age-related cognitive decline in older diabetic populations. Type 2 diabetes is a complex metabolic disorder. Apart from the negative impact of abnormalities intrinsic to diabetes, diabetes-associated cerebral microvascular disease may contribute to this accelerated cognitive ageing.

Direct in vivo evaluation of the cerebral microcirculation is difficult in humans and the vessels themselves are too small to permit detailed visualisation with current neuroimaging methods. The microvasculature of the retina may offer a window into such vascular status of the brain as there is considerable homology between the retina and cerebral microcirculations. Moreover, the retinal vasculature is known to be affected by a wide range of systemic pathologies and is unique in that it is the only vasculature that can be directly visualised and photographed.

Retinal microvascular abnormalities (RMAs) have been understudied risk factors in cognitive ageing epidemiological research. Few reports have comprehensively examined cognitive function in relation to diabetic retinopathy. Also the relationship between cognitive function and quantitative aspects of retinal vascular network geometry has not been investigated in people with Type 2 diabetes. The results of a systematic review reported in this thesis showed inconsistent findings on the importance of the association between retinal microvascular abnormalities and cognitive dysfunction in predominantly non-diabetic populations. This may have reflected substantial differences between studies regarding the choice of population under study, the methods applied for measuring and defining RMAs, the types of neuropsychological tests administered for assessing cognitive function, and the approach taken in data analysis.

The principal aim of the original research described in this thesis was to examine the associations of cognitive test performance with severity of diabetic retinopathy and quantitative parameters of retinal vascular network in a population-based sample of older people with Type 2 diabetes. Objective, reproducible and computerized retinal image analysis was used to quantify retinal vessel calibres and arteriolar bifurcation geometry in order to detect subtle changes in retinal vascular network. A valid estimation of peak prior cognitive ability allowed the further exploration of the impact of retinal microvascular abnormalities on imputed cognitive decline from best-ever levels of cognitive function to that measured in old age.

The analysis was based on a cohort of 547 men and 519 women aged 60-75

years with Type 2 diabetes, randomly sampled from the Lothian Diabetes Register, Scotland, in 2006/2007 (the Edinburgh Type 2 Diabetes Study). A battery of seven cognitive tests was administered and standard 7-field binocular digital retinal photography undertaken. The Mill Hill Vocabulary Scale was used to estimate pre-morbid cognitive ability. Diabetic retinopathy was evaluated independently by two optometrists using a standardised grading protocol (a modification of the Early Treatment of Diabetic Retinopathy Scale). Quantitative retinal vascular parameters were measured by myself from a digital image of field 1 using semi-automated, computer-based methods. Retinal vessel calibres were summarised as the central retinal arteriolar and venular equivalents (CRAE and CRVE, respectively) and arterio-venous ratio (AVR). Retinal arteriolar bifurcation geometry was expressed as arteriolar bifurcation angles (BA), arterial branching coefficient (BC), and sub-optimality (degree of deviation from optimality) of the retinal arteriolar angles. The statistical analyses were based on the 1,044 study participants who had both gradable retinal images and cognitive testing.

Both general cognition, as indexed by a general cognitive factor reflecting the variance common to all the cognitive tests used, and most of the individual cognitive tests were negatively affected in participants with diabetic retinopathy relative to those without. These cognitive measures also showed a significant relationship with increasing severity of diabetic retinopathy (none, mild, and moderate-severe). Those with moderate-severe diabetic retinopathy had worst performances on general cognitive function, executive function, information processing speed, non-verbal memory and mental flexibility. When lifetime decline was estimated from peak, prior cognitive level, severity of diabetic retinopathy was significantly associated with a greater decline in information processing speed, non-verbal memory and mental flexibility and, in men for general cognition and executive function. The associations of severity of diabetic retinopathy with general cognition, executive function and information processing speed were independent of socio-demographic characteristics, cardiovascular risk factors, macrovascular disease, mood and hyperglycaemia. The associations with estimated decline in specific cognitive measures resulted principally from the impact of diabetic retinopathy on general cognitive ability.

The study also showed that larger retinal arteriolar and venular calibres were both significantly associated with lower test scores on verbal memory in men. Multiple linear regression analyses demonstrated larger retinal arteriolar calibre was associated with a significantly greater decline in verbal memory after possible confounding by retinal venular calibre and vascular risk factors and disease was taken into account. In contrast, the study did not support an independent association between retinal venular calibre and cognitive decline in men or in women with Type 2 diabetes. Parameters of retinal arteriolar bifurcation geometry were not associated with cognitive outcome.

Overall, these findings support the hypothesis that cerebral microvascular disease associated with Type 2 diabetes, reflected by the presence and severity of diabetic retinopathy, may exacerbate the effects of ageing on cognitive function. In particular, alterations in the blood-brain barrier may be an important pathophysiological mechanism in the occurrence of cognitive dysfunction in diabetic patients. They further may be added to the knowledge that gained from previous pathologic and brain imaging investigations demonstrating a relationship between markers of cerebral microvascular disease and cognitive dysfunction in diabetes. The role of quantitative parameters of retinal vascular network geometry in diabetes-related cognitive impairment is less clear. Prospective studies are required to clarify the temporal sequence of these associations and the eventual clinical significance of these small, early cognitive function changes. Such a follow-up project involving the present study population is underway. From a clinical perspective, if the above findings are substantiated, diabetes-associated cognitive dysfunction may be amenable to treatment and preventive strategies specifically targeted at protecting the cerebral microvasculature and reducing the risk of developing even mild microvascular disease in an ageing diabetic population.

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Abbreviations

AVR	Arterioles to venules ratio
BBB	Blood brain barrier
BA	Branching angles
BC	Branching coefficient
BMI	body mass index
CRAE	Central Retinal Artery Equivalent
CRVE	Central Retinal Venule Equivalent
CWS	Cotton wool spots
CVD	cardiovascular disease
DBP	diastolic blood pressure
DR	diabetic retinopathy
DST	Digit Symbol Test
ET2DS	Edinburgh Type 2 Diabetes Study
GCF	general cognitive factor
HADS	Hospital Anxiety and Depression Scale
HE	Hard Exudates
HbA1c	haemoglobin A1c
HDL	high-density lipoprotein
ICC	intraclass correlation coefficient
IQR	interquartile range
IRMAs	intraretinal microvascular abnormalities
LDL	low-density lipoprotein
LMT	Logical Memory Test
MAs	microaneurysms
MI	myocardial infarction
MMSE	Mini-Mental State Examination
MRI	magnetic resonance imaging
MHVS	Mill Hill Vocabulary Test
NPDR	Non-proliferative diabetic retinopathy
PAD	peripheral arterial disease
PDR	Proliferative diabetic retinopathy
SBP	systolic blood pressure
SD	standard deviation
SE	standard error
TC	total cholesterol
TIA	transient ischaemic attacks
T2DM	Type 2 diabetes mellitus
TMTB	Trail Making Test part B
VFT	Verbal Fluency Test
WHR	Waist to hip ratio

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Chapter 1

General Background to Type 2 Diabetes, Cognitive Dysfunction and Retinal Microvascular Abnormalities

1.1. Introduction

Ongoing demographic changes resulting in global ageing of the general population mean that cognitive impairment and dementia are becoming increasingly important contributors to morbidity and mortality in elderly people. According to the 2003 World Health Report Global Burden of disease estimates, dementia accounted for 11.2% of all years lived with disability among people aged 60 years and over, which was greater than stroke (9.5%) or cardiovascular disease (5.0%) (WHO, 2003). Currently in the UK, dementia affects nearly 700,000 people over the age of 65 years old and is expected to increase more than 100% in incidence by the year 2051 (Knapp et al., 2007). Even in individuals without frank dementia, cognitive impairment, which manifests itself as mild to severe dysfunctions across different cognitive domains, limits people's quality of life and survival, and constitutes a major determinant of long-term institutionalization and dependency in old age (Waldstein and Elias, 2003). Furthermore, people with cognitive impairment are at greater risk of developing dementia over time. Owing to their increasing prevalence and devastating impact on ageing populations, cognitive impairment and dementia are placing a large burden of support on carers and society more widely. A major public health challenge is therefore to identify people at high risk of cognitive impairment so that they might benefit from preventive measures and therapeutic interventions (Price et al., 2006).

When the brain undergoes neurodegenerative changes associated with ageing, marked variation between individuals indicates that clinically relevant

cognitive decrements may occur due to specific pathologies, for example vascular disease. One of the most important risk factors for vascular disease is Type 2 diabetes, which has been associated with an accelerated rate of cognitive decline in addition to a higher incidence of stroke and dementia. As early as 1976, diabetes mellitus was considered 'a special kind of accelerated aging' because it increases an individual's susceptibility to degenerative disease (Kent, 1976). More recently, diabetes has been shown to confer a two-fold or greater increase in risk of vascular dementia as well as a 1.2 to 2.3 times greater risk of Alzheimer's disease (Cukierman et al., 2005; Biessels et al., 2006). Despite the probable role of diabetes in accelerated brain ageing, there is no clear evidence to date that diabetes management affects the rate or nature of cognitive dysfunction (Cukierman et al., 2005).

Worldwide estimates suggest that Type 2 diabetes will increase in prevalence to 366 million by the year 2030 (Wild et al., 2004). With increased early diagnosis and improved glycaemic control, the mortality rate resulting from diabetes-related complications has decreased. However, as people with diabetes live longer, the incidence of diabetes-related cognitive impairment in elderly people will rise and become increasingly evident, underscoring the importance of furthering our knowledge of the mechanisms underlying these disruptions. Potential risk factors, if appropriately identified and modified, may pave the way for delaying the onset and reducing the burden of cognitive impairment attributable to Type 2 diabetes.

As a progressive, life-long condition, Type 2 diabetes is a complex metabolic disorder and is associated with the development of both macro and microvascular diseases. Current interventions aimed at reducing the impact of macrovascular disease in non-diabetic populations (reduction of blood pressure, lipid-lowering therapy, use of aspirin and anti-oxidant vitamins) have had little effect on cognitive decline, except by preventing major strokes (PROGRESS Collaborative

Group, 2001; Heart Protection Study Collaborative Group, 2002a,b; Kang et al., 2007).

Microvascular disease is the hallmark of chronic hyperglycaemia which is characterised by structural changes in the capillary walls, occurring in eyes, kidney and peripheral nervous system. Based on findings from neuropathological and neuroimaging studies, microvascular disease in the brain may also play a role in cognitive dysfunction. However, investigation of potential links between microvascular diasease and cognitive impairment in Type 2 diabetes has been restricted to small and clinic-based studies because direct in vivo evaluation of the cerebral microcirculation is difficult in humans and requires sophisticated neuroimaging techniques (Strachan et al., 2008a). The microvasculature of the retina therefore provides a possible and more accessible window into the status of the small vessels of the brain given that the two vasculatures are developmentally related, of similar size and share physiologic characteristics (Patton et al., 2005). Changes in the retinal vasculature may reflect the analogous changes in the cerebral vasculature and the retinal vasculature can be directly visualised and photographed.

This thesis explores the association between measures of retinal microvasculature and cognitive function in older people with Type 2 diabetes. Figure 1 illustrates a hypothesised role of retinal microvascular abnormalities as proxy indicators of cerebral microvascular disease in the aetiology of cognitive dysfunction in Type 2 diabetes. The first, introductory chapter, provides general background information on the epidemiology of Type 2 diabetes and cognitive impairment, following which is a review of studies investigating the relationships between Type 2 diabetes, diabetes-related risk factors, and cognitive dysfunction in older people. An overview of retinal microvascular abnormalities and their associations with major vascular risk factors, cerebro- and cardio-vascular disease is also provided and

methods for evaluation of retinal microvascular abnormalities associated with diabetes are summarised.

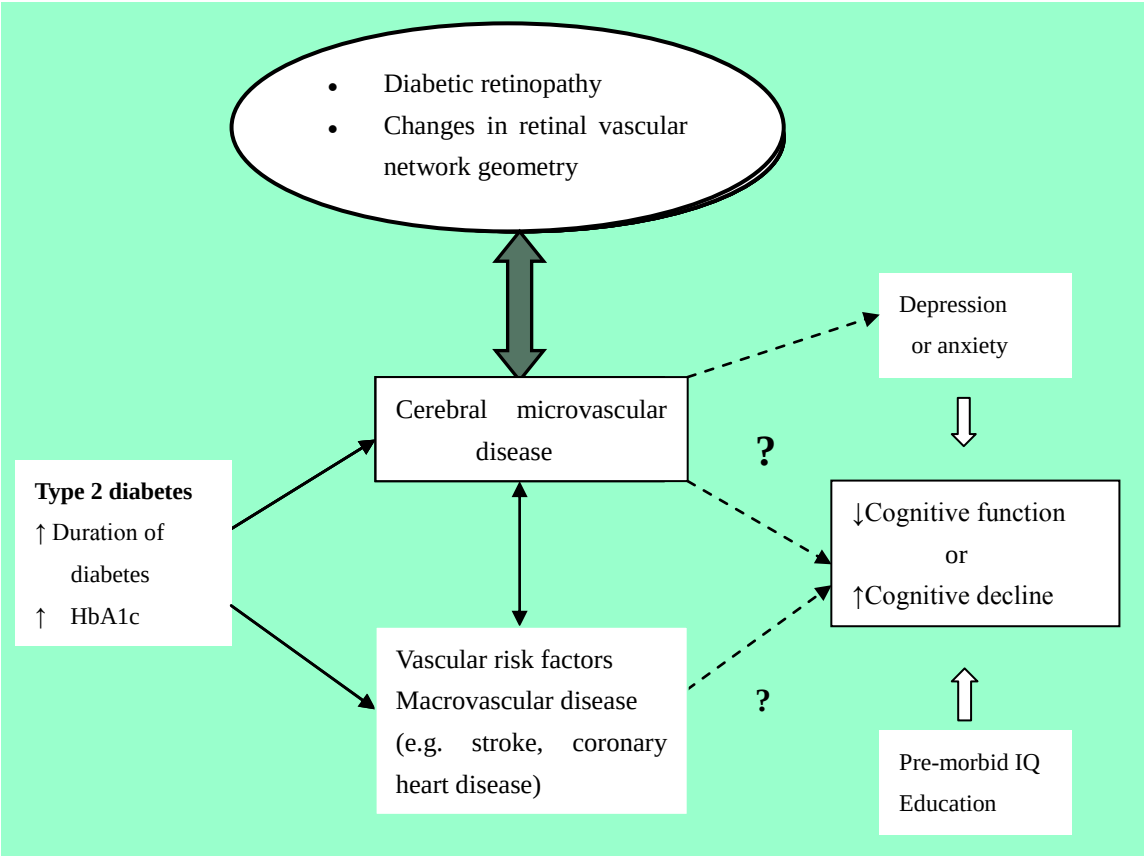


Figure 1: Diagram of potential relationships between retinal microvascular abnormalities as 'surrogate' markers of cerebral microvascular disease and cognitive dysfunction in Type 2 diabetes

Chapter 2 focuses on retinal microvascular abnormalities that may serve as surrogate markers of cerebral microvascular disease and provides a systematic review on the association between these abnormalities and cognitive dysfunction. Chapter 3 describes the background and objectives of the present thesis. The methodology of the original research project and current study is described in Chapter 4, and the results in Chapter 5. Chapter 6 is a discussion of the main findings and methodological issues arising in the study. In Chapter 7, the conclusions of the study are summarised, followed by the recommendations for future research.

1.2. Type 2 diabetes

1.2.1. Definition

The concept ‘Diabetes Mellitus’ is used as a general term for describing a group of metabolic diseases, characterised by chronic hyperglycaemia, due to either insulin deficiency or to the impaired effectiveness of insulin action, or a combination of both. Hyperglycaemia in turn causes damage to many organs, in particular blood vessels and nerves. Type 2 diabetes (formerly known as ‘non-insulin-dependent’) is the most common form of diabetes caused by varying degrees of defects in both insulin secretion and action, accounting for as much as 90 to 95% of all cases of diabetes. The term ‘non-insulin-dependent’ diabetes has been replaced in favor of an aetiological classification rather than by treatment modality. Other former terms ‘adult-onset’, and ‘maturity-onset’ diabetes have less specificity (since Type 2 diabetes may also occur in children and adolescents) and are therefore no longer in use. This thesis focuses on Type 2 diabetes in older adults.

1.2.2. Pathophysiology

Normal glucose homeostasis

In healthy people, blood glucose levels are normally maintained within a narrow range by the harmonization between glucose absorption, uptake and metabolism by peripheral tissues and hepatic glucose production (Saltiel and Kahn, 2001). Insulin is the main hormone for the regulation of blood glucose levels. It is secreted by pancreatic β -cells in response to elevated blood glucose, and exerts its major biological effects by binding to and activating a specific plasma membrane receptor in peripheral target tissues. Insulin stimulates uptake in muscle and fat cells by translocating glucose transporter-4 from intracellular sites to the cell surface. Thereafter, insulin promotes glycolysis as well as storage of glucose and other

substances by stimulating glycogen, lipid and protein synthesis. In hepatocytes, insulin stimulates lipid and glycogen synthesis and inhibits the production and release of glucose by suppressing glycogenolysis and gluconeogenesis (Alberti and Zimmet, 1998; Kahn, 2003; Lyssenko et al., 2005). .

Insulin resistance and β-cell dysfunction

An array of metabolic alterations have been identified to explain the gradual deterioration of glucose homeostasis leading to Type 2 diabetes, including peripheral insulin resistance, overproduction of glucose in the liver, and progressive abnormalities in pancreatic β-cells function (Kahn 2003; Katsilambros and Tentolouris, 2003; Lyssenko et al. 2005). These defects could be attributable to the interplay of environmental risk factors (e.g. obesity) with a genetic susceptibility and are exacerbated by hyperglycaemia itself which can damage the β-cell ('glucotoxicity') (Katsilambros and Tentolouris, 2003).

Insulin resistance

Insulin resistance refers to a metabolic condition in which physiological concentrations of insulin fail to produce their biological effects occurring in all insulin-dependent tissues, primarily skeletal muscles, adipose tissue and the liver. Specifically, in skeletal muscle, which accounts for more than 80% of insulin-stimulated glucose use, insulin resistance is manifested by decreased glucose uptake and impaired glucose use by non-oxidative glucose disposal (e.g. glycogen formation). In the liver, insulin resistance is characterised by failure to suppress glucose output, leading to an overproduction of glucose. In adipose tissue, rates of lipolysis and production of circulating non-esterified fatty acids (NEFAs) are not inhibited due to impaired ability of insulin, which further stimulates gluconeogenesis, triglyceride synthesis and glucose production in the liver as well as impairing glucose utilization by skeletal muscle (McGarry, 2002; Katsilambros and Tentolouris,

2003). Figure 2 shows the impact of insulin resistance on glucose homeostasis. Several possible mechanisms could be involved in the loss of insulin sensitivity. These include a decrease in glucose transport due to a defect in the insulin-signaling pathway in target tissues, or to an excessive release of NEFAs and an over-secretion of inflammatory cytokines and adipokines from the adipose tissue (Virally et al., 2007). In addition to active fatty acid oxidation in liver, an over-secretion of glucagon may also contribute to increased gluconeogenesis. There are a number of factors associated with insulin sensitivity, such as age, ethnicity, other genetic and environmental factors as well as the interaction between them (Kahn, 2003). Among these, lifestyle changes could, at least in part, explain insulin resistance, including overall or intra-abdominal obesity, smoking, physical inactivity and some dietary factors (Kahn, 2003; Parillo and Riccardi, 2004).

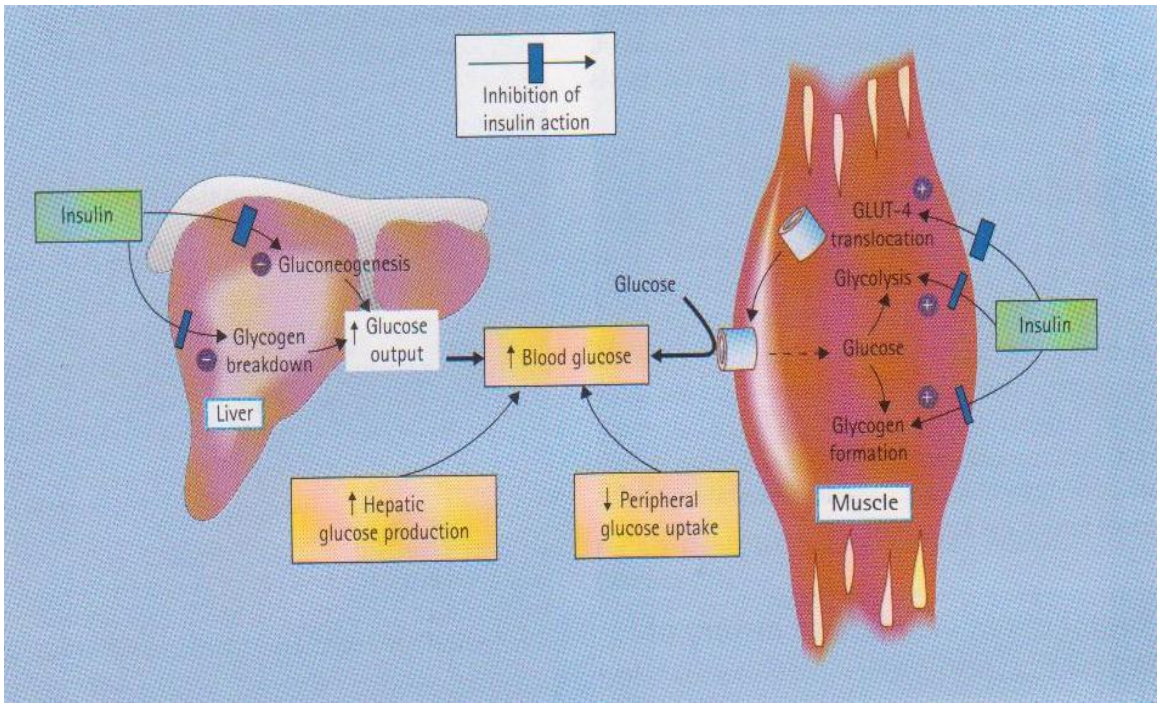


Figure 2: Impact of insulin resistance on glucose homeostasis. Blood glucose tends to rise because of both increased hepatic glucose production and decreased insulin-mediated uptake and use of glucose by peripheral tissues (Reproduced from Katsilambros and Tentolouris, 2003)

In addition to impaired glucose tolerance, insulin resistance is often accompanied by a cluster of other metabolic abnormalities, such as central obesity

(overweight with fat deposits mainly around the waist), dyslipidaemia, hypertension, hypercoagulability and microalbuminuria (Reaven, 1988). This combination of risk factors is referred to as 'metabolic syndrome', 'insulin resistance syndrome' or 'syndrome X' (Isomaa, 2003).

β-cell dysfunction

β-cell dysfunction refers to a decrease in the sensitivity of β-cells in response to glycaemic stimuli and/or a reduction of insulin secretion, including abnormalities in pulsatile insulin release and in insulin secretion kinetics, quantitative and qualitative abnormalities of insulin, β-cell loss and its progression (Virally et al., 2007). As a consequence, insulin responses during an oral glucose tolerance test are delayed and reduced (Alberti and Zimmet, 1998; Kahn, 2003). Possible mechanisms have been proposed to explain the progressive reduction in insulin response, including genetic defects in β-cell mass or function, abnormalities in the secretory process due to prolonged exposure to increased levels of glucose (glucotoxicity) or NEFAs (lipotoxicity), or both, and amyloid deposition in the islets (El-Assaad et al., 2003; Stumvoll et al., 2005).

Given the complexity as well as the long-term nature of Type 2 diabetes, the metabolic pathways and the sequence of underlying defects leading to the condition have remained elusive. The prevailing view is that insulin resistance is compensated for by the adaptive capacity of the β-cells to increase insulin concentrations, but if insulin resistance worsens, a progressively elevated insulin demand results in β-cell exhaustion in susceptible individuals, leading to a subclinical stage of glucose intolerance (Kahn, 2003; Lyssenko et al., 2005). However, abnormalities of insulin secretion without impairment of insulin sensitivity have been demonstrated in first-degree relatives of individuals with Type 2 diabetes (Pimenta et al., 1995). As well as during the course of the disease, the relative contributions of insulin resistance and β-cell dysfunction in the pathophysiology of T2DM may also differ by

ethnic background (Alberti and Zimmet, 1998; Kahn 2003).

1.2.3. Clinical manifestations

As a direct result of high glucose concentrations, diabetes may present with characteristic symptoms such as thirst and polyuria. However, Type 2 diabetes starts for many individuals early in life and remains asymptomatic for a long period of time. It may be noted only after certain medical complications appear. The long term complications include progressive development of disease of the capillaries of the kidney and retina, damage to the peripheral nerves and excessive atherosclerosis (Ekoé et al., 2001). The clinical manifestations of these complications therefore include nephropathy that may lead to renal failure, retinopathy with potential blindness, neuropathy with risk of foot ulcers, amputation and automatic dysfunction. These individuals have a greatly increased risk of cardiovascular, peripheral vascular and cerebrovascular disease but rarely experience ketoacidosis following an episode of extreme hyperglycaemia. In contrast to Type 1 diabetes due to an absolute lack of insulin, many patients with Type 2 diabetes can control their blood glucose levels by losing weight, modifying diet, or taking oral anti-diabetic agents that stimulate insulin secretion or enhance insulin action in muscle and the liver. Daily exogenous insulin injections are usually required only when metabolic control is extremely poor or diet and oral medication regimens have failed (Ryan, 2001).

1.2.4. Diagnostic criteria

Diagnosis of diabetes is based on blood glucose levels from either a fasting or random plasma glucose test and/or an oral glucose tolerance test. The fasting plasma glucose (FPG) test is based on the blood glucose concentration after an overnight fast (no caloric intake for at least 8 hours), in contrast to a random plasma glucose test where fasting is not required. The oral glucose tolerance test (OGTT) is

based on the intake of 75-g glucose in 250-300mL water by a fasting subject, with measurement of blood glucose both after fasting and 2 hours after the glucose intake. The revised World Health Organization (WHO) diagnostic criteria posed in 1999 are currently used in the diagnosis of DM (Figure 3). In these updated criteria (compared with the old WHO 1980, 1985 criteria) diabetes is clinically defined as a FPG level ≥7.0 mmol/L on two separate occasions or following the OGTT, a 2 hour post-prandial glucose level (2hPPG) ≥11.0 mmol/L (WHO, 1999; WHO, 2006). These diagnostic thresholds have been shown to have high sensitivity and specificity for identifying people with and without microvascular complications (e.g. retinopathy and nephropathy) of diabetes associated with chronic hyperglycaemia (McCane et al., 1994; Engelgau et al., 1997; Expert Committee on the Diagnosis and Classification of Diabetes Mellitus, 1997).

<u>One abnormal laboratory value is diagnostic in symptomatic patients; two values are required in asymptomatic subjects.</u>		
Fasting Plasma Glucose (FPG)	≥7.0 mmol/L (126mg/dL)	or
Random Plasma Glucose (RPG)	≥11.0 mmol/L (200mg/dL)	
<u>The Glucose Tolerance Test is only required for borderline cases using the above tests</u>		
Fasting Plasma Glucose (FPG)	≥7.0 mmol/L (126mg/dL)	and/or
2hr Oral Glucose Tolerance Test (OGTT)	≥11.0 mmol/L (200mg/dL)	

Figure 3: World Health Organization (WHO) diagnostic criteria 1999 for diabetes mellitus (Reproduced from Cichowska, 2004).

For epidemiological studies, the WHO recommends the use of the 2-h glucose criteria alone as the best method largely due to the concerns about ensuring the fasting status of subjects (WHO 1985; WHO 1999). Although similar to the WHO diagnostic criteria, the American Diabetes Association recommends in epidemiological studies, using the fasting glucose testing only in the interest of standardisation and facilitation of field work as well as less costs (Expert Committee

on the diagnosis and classification of diabetes mellitus, 1997). Recent studies have shown that if the FPG test alone is used, up to 31% of the diabetic subjects are not being diagnosed compared with the practice of using both the FPG test and the OGTT (DECODE 1999; DECODE 2003). Questions have been raised though whether these recommendations are valid as studies have also shown that fasting and 2-h glucose criteria do not identify the same groups of individuals (DECODE 2003). For example, young and obese subjects are more likely to have diagnostic FPG values than diagnostic 2-h glucose values, whereas women and elderly subjects are more likely to have raised isolated OGTT levels (Shaw et al., 1999; DECODE 1999; DECODE 2003). The differences between subjects may reflect the fact that the two tests represent physically different aspects of glucose metabolism and are therefore likely to be influenced differently by the ageing process and other factors (Anderson and Gale, 2002). Using only one of the criteria could probably introduce bias to studies and therefore impact on the estimated prevalence of diabetes.

1.2.5. Prevalence and incidence

Type 2 diabetes (T2DM) is a relatively common disease affecting many adults and has reached epidemic proportions worldwide (Zimmet et al., 2001). This increase in prevalence of T2DM has accelerated due to a rise in incidence which is mainly driven by increasing obesity rates globally and ageing population structures in developed countries, increased migration of susceptible populations accompanied by shifts in lifestyle and improved survival in people with diabetes (Lipscombe and Hux, 2007).

The concept of ‘diabetes of the elderly’ groups together aging long-term diabetic patients and older diabetic patients in whom diabetes has recently been identified. Different sources of data can provide population-based estimates of the prevalence and incidence of T2DM in elderly populations, including national

statistics and population surveys. Overall, the estimated prevalence based on self-report or doctor-diagnosed disease from interviews/questionnaires or registers are underestimates of the total prevalence of diabetes, which includes individuals with both known and undiagnosed T2DM. The prevalence of undiagnosed DM can only be estimated from biochemical testing of blood samples and is highly dependent on the actual measurements used, such as a fasting blood glucose or oral glucose tolerance test, and the type of blood specimen taken. In general the prevalence of T2DM varies due to these methodological differences and the diagnostic criteria used to define the disease as well as the risk factor profile of the cohort (e.g. obesity, physical activity, deprivation and genetic differences) and by geographical regions or countries. An overview of population studies on the total prevalence and incidence of both diagnosed and undiagnosed T2DM, in particular in people aged 60 years and over is given below.

In the general population people aged 60 years and older, prevalence of T2DM was higher than in any other age category. Patients within this age range accounted for more than half (54%) of the total Type 2 diabetes population (The International Diabetes Federation, 2006). Table 1 lists the large scale studies that have estimated the prevalence of T2DM. The prevalence of diagnosed T2DM assessed by recall of a previous doctor's diagnosis of DM on questionnaire varied from 6.6% to 24.7% in men aged 60 years or over and from 5.1% to 22.4% in women of the same age. The estimated total T2DM prevalence (diagnosed and undiagnosed) ranged from 13.0% to 41.0% in men and from 10.6% to 34.6% in women, with Asian Indians having the highest prevalences in both sexes. The proportion of undiagnosed T2DM in these aged populations was as high as 60%. For example, in the combined cohort of 8,538 individuals aged 60-79 years from the British Regional Heart study and the British Women's Heart and Health study, the prevalence of undiagnosed T2DM using the fasting glucose criteria alone was 5.4% and the total prevalence was 11.8% in mixed subjects (Thomas et al., 2005). The use

of the 2-h glucose criteria has resulted in higher prevalence rates of undiagnosed T2DM than those observed by using fasting glucose criteria. In the KORA survey (Rathmann et al., 2003), for example, age-specific prevalence rates for men were as follows: 8.1% in men aged 60-64 years, 8.9% in those aged between 65 and 69 years, and 13.3% in men 70 years and older. For women, the proportions with undiagnosed DM were 7.3%, 8.2% and 7.1%, respectively. As Table 1 shows, although in subjects older than 60 years of age there is some evidence that total prevalence rates are slightly higher in females than in males, gender differences have not been observed in other studies.

Incidence may not be a particularly useful concept in T2DM given that the disease remains asymptomatic for a considerable time and the time at which the disease process commenced is unknown for almost all the cases. There are far fewer reports on the incidence of T2DM than on prevalence. In the Hoorn Study, the incidence of T2DM was based on both fasting and 2-h glucose measurements in 610 men and 732 women aged 50-75 years. The 6-year cumulative incidence was estimated at 10.5% for men and 9.4% for women (de Vegt et al., 2001). In the Atherosclerosis Risk in Communities (ARIC) Study of 12,107 subjects aged 45-64 years, after 9 years, the incidence per 1000 person-years was 23.4 in men and 25.1 in women among African Americans, and 15.9 in men and 10.4 in women among white adults (Brancati et al., 2000). These results demonstrate that as with prevalence the incidence in populations varies depending on age structure and characteristics of the populations as well as the diagnostic methods used. However, most studies show that the incidence rates of T2DM are higher in older people than in younger people.

Table 1: Prevalence of diagnosed and undiagnosed T2DM defined by 2hPG and/or FPG criteria in older people from different population studies

Reference	Location	Population & N (age in years)	Age group (≥60yrs)	Undiagnosed T2DM (%)			Diagnosed T2DM (%)	Total T2DM(%)
				FPG ≥7.0 and/or 2h PG≥11.1 (mmol/l)	FPG≥7.0 (mmol/l)	Criteria		
Dunstan et al. (2002)	Australia	Total in study 11,247 both sexes				WHO		
		(25+) M/F	65-74	7.9/9.0		1999	12.8/13.9	20.7/23.5
			75+	9.6/13.9			6.6/8.8	16.2/22.7
		Both	65-74/75+	8.5/12.1			9.4/10.9	17.9/23.0
Rathmann et al.	Southern	Total in study 1653 both sexes				WHO		
(2003)	Germany	(55-74) M/F	60-64	8.1/7.3		1999	7.2/9.7	15.3/17.0
			65-69	8.9/8.2			13.3/8.2	22.2/16.4
			70-74	13.3/7.1			9.8/9.9	23.1/17.0
DECODE Study	13 European	Total in study 15,606 both sexes				WHO		
Group (2003)	Cohorts	(30-89) M/F	60-69	7.1/6.8		1999	8.5/9.3	15.5/16.1
			70-79	10.4/11.8			12.9/15.4	23.4/27.3
			80-89	7.1/20.9			12.4/22.4	19.5/43.3
DECODA Study	11 Asian	Total in study 24,335 both sexes				WHO		
Group (2003)	Cohorts	(30-89) M/F	60-69	8.1/7		1999	6.7/7.1	14.9/14.2
		(Chinese & Japanese)	70-79	6.3/7.9			9.2/8.1	15.5/16.0
			80-89	7.2/9.1			10.7/16.9	17.9/26.0
		M/F	60-69	16.4/13.9			24.7/20.7	41.0/34.6
		(Indian)	70-79	12.7/14.4			19.9/18.6	32.6/33.0
			80-89	14.4/10.7			9.8/7.1	24.2/17.7
Thomas et al. (2005)	UK	4252 M, 4286 F (60-79) M/F	60-79		5.7/5.2	ADA	7.6/5.1	13.0/10.6
Cowie et al.	U.S.	Total in study 10,291 both sexes	≥60		7.0/4.9	ADA	16.0/14.4	23.0/19.3

NHANES 1999-2002 (2006)	(20+)	M/F	

FPG: Fasting Plasma Glucose; M: Male, F: Female; N: Number; ADA: American Diabetes Association; WHO: World Health Organization; DECODE: Diabetes Epidemiology: Collaborative analysis of Diagnostic Criteria in Europe; DECODA: Diabetes Epidemiology: Collaborative analysis of Diagnostic Criteria in Asia; NHANES: National Health and Nutrition Examination Survey

1.3. Cognitive dysfunction

1.3.1. Cognitive function and neuropsychological assessment

Cognition or cognitive function is defined as conscious mental activity that is not principally sensory or emotional, comprising a large range of higher brain functions related to the selection, acquisition, storage, manipulation and organization of information (Deary and Batty, 2007). Different aspects of mental function can be conceptualized, measured, and sometimes correlated with underlying neuroanatomical systems (Lezak, 1983). The pragmatic approach taken by most people when measuring cognitive function is to use validated neuropsychological tests (also referred to as cognitive or mental tests), which have been derived by using comparisons between patients with circumscribed brain lesions and normal controls (Woodruff-Pak, 1997). In epidemiological studies, neuropsychological assessments have been applied to determine cognitive aspects that are disproportionately affected either by normal ageing or other pathological processes. Individual neuropsychological tests have been proven to be sufficiently sensitive and specific to diagnose more severe cognitive deficits such as dementia (Lezak, 1995). However, these individual tests are normally designed to assess a single major aspect or 'domain' of intellectual function and some tests are not sensitive enough to detect the more subtle changes in cognitive function due to a limited range of scores. Therefore, a comprehensive cognitive examination with multiple neuropsychological tests is often required in studies to address a wide range of cognitive function and domains.

Though each individual may have strengths in specific areas, consistent empirical findings from large, representative population samples reveal that all cognitive tests tend to correlate positively with one another. People who perform well on one mental task are more likely to do well on most others, despite large variations in the tests' contents (Deary, 2000). Overall cognitive abilities can be summarized by a common factor resting on the correlations among test scores, namely general intelligence or *g*. This was discovered by Spearman in 1904, who invented the factor analytic method which enabled the determination of the degree to which each of the cognitive measures correlated with the general cognitive factor

(Spearman, 1904). A general factor may then be defined as the first unrotated principal component (or factor) from a correlation matrix of a battery of cognitive tests administered to a sample of population (Deary and Batty, 2007).

With the subsequent addition of cognitive test data as well as introduction of more sophisticated statistical methods, the central role of the general intelligence factor has not changed but only been reinforced (Detterman, 2006). In particular, there is a growing consensus on a hierarchical model of cognitive ability with g at the pinnacle and group factors of ability (which describe major correlated domains of cognitive ability, such as memory, verbal and visuospatial) at the next lower level (Carroll, 1993). The g-factor was considered to be general in the sense that it was likely to be present to some degree in nearly all measures of cognitive ability (Caroll, 1993). More recently, there is empirical evidence that the g factor from very different test batteries is almost perfectly correlated (Johnson et al., 2008), and it is the g factor that carries most of the predictive power of mental tests (Deary et al., 2010), lending further support on the consistency and accuracy of its measurement. Further detailed description of the 'hierarchical model' of cognitive abilities led to the proposal of two yet substantially correlated aspects of general intelligence, fluid and crystallised abilities (Horn, 1994).

1.3.2. Age-associated cognitive decline

Cognitive impairment is generally taken to mean either decreased ability relative to the population norm (e.g. their age cohort) at a point in time, or a decline in the cognitive abilities of an individual comparing their current with their prior ability. While some individuals are able to maintain high levels of cognitive function into extreme old age, it is more common for older people to experience cognitive decline. The shift from mild age-associated changes, to a subclinical state of cognitive impairment, to more severe form of clinical dementia, is a continuous spectrum of cognitive abilities without defined boundaries.

The term age-associated cognitive decline or normal (non-pathological) cognitive ageing is used to describe the commonly noted changes in cognitive ability

that are inherent to the ageing process. Several other terms, which are thought to be a variant of normal ageing rather than a distinct pathological entity have also been developed, including ‘Benign Senescent Forgetfulness’, ‘Age-Associated Memory Impairment’ (AAMI) and ‘Age-Consistent Memory Impairment’ (ACMI) (Ritchie et al., 2001; Bischkopf et al., 2002; Petersen, 2004).

In general, these cognitive changes may present in four major patterns. First, there is little age-associated decline in some mental functions (crystallised intelligence), such as general knowledge, vocabulary, and arithmetic ability, which may remain stable or even increase with age through the influences of education and cultural experience. However, other aspects of mental capabilities begin to decline during adulthood, with some further acceleration of decline in very old age (Hedden and Gabrieli, 2004; Park and Reuter-Lorenz, 2009). These cognitive processes, collectively called ‘fluid’ abilities, include memory, reasoning, processing speed and executive functions which may be particularly vulnerable to biological ageing as well as neurological damage (Anstey and Christensen, 2000; Salthouse, 2001). Furthermore, when one fluid mental domain declines others are likely to do so also, and some of the age-related declines could be underpinned by a decline in the general cognitive factors (Wilson et al., 2002; Deary et al., 2009). Secondly, in all affected cognitive domains, slowed information processing speed appears to contribute largely to age-associated decline and the slowing has begun by the early adulthood (Der and Deary, 2006). Thirdly, rather than presenting an abrupt shift from stable to declining cognitive performance, many of these cognitive abilities that decline with age appear to do so in a gradual and linear trend or more complex manner (Salthouse, 1991). Also, the cognitive ageing process manifests as significant inter-individual differences in both the level of cognitive ability and rate of decline, in which age explained less than one sixth of the total variance (Salthouse, 1991). The individual differences in cognitive ability in old age may reflect not only differences in the degree to which change (typically deterioration) has taken place but also differences in prior cognitive ability (Deary et al., 2009). The latter is an enduring feature of mental life throughout adulthood. Indeed, recent empirical data have shown that childhood intelligence accounts for at least 50% of the variance in

cognitive ability in old age in people without dementia, which in turn is largely determined by genetic factors (Deary et al., 2009).

1.3.3. Neurobiological mechanisms of age-associated cognitive decline

Both hereditary and environmental factors could contribute to normal cognitive ageing. Although there is a putative link between age-associated changes in brain structure and cognitive ageing, the exact neurobiological mechanism underlying age-associated cognitive decline is unknown. Several possible explanations have been proposed, in particular highlighting individual differences in normal cognitive ageing.

Overall structural changes in brain ageing involve a steady decrease in the weight and volume of the brain, and the latter is balanced by an increase in ventricular spaces and cerebrospinal fluid. The manners in which the shrinkage occurs are less clear but may reflect a reduction in neuronal size and number and a decrease in dendritic synapses or loss of synaptic plasticity (i.e. connections with other neurons) (Raz et al, 2005). Compared with the brain on the whole, age-associated shrinkage is much smaller but does not occur to the same extent in the cerebral parenchyma. For example, the prefrontal cortex is most affected and the occipital least, showing an anterior–posterior gradient (Raz and Rodrigue, 2006). Also brain atrophy is steadier in grey matter (the neuron cell bodies) and cortical thickness than in white matter (the nerve fibres connecting different brain areas) (Deary et al., 2009). Furthermore, cerebral dopamine receptor density declines with age, which relates to neurotransmitter levels and plays a central role in regulating attention and in modulating response to contextual stimuli (Hedden and Gabrieli, 2004). These normative age-associated changes in brain structure could be attributable to the effects of oxidative stress, recurrent inflammation reactions, age-related vascular and microvascular changes and stress-related corticosteroid levels (Whalley et al., 2004; Raz and Rodrigue, 2006).

The loss of white matter integrity particularly in the prefrontal region has

also been proposed to play a crucial role in normal cognitive ageing, since it leads to impaired information transfer between different cortical areas, from a loss of transfer speed in the case of demyelination to complete disconnection when axonal disruptions occur (Deary et al., 2009). Interactions between distant cortical areas are considered as crucial for the emergence of higher cognitive functions (Sullivan and Prefferbaum, 2006). Recent structural and functional neuroimaging studies supported the effect of white matter lesions in cognitive ageing (Frisoni et al., 2007).

Two major assumptions pertaining to cognitive reserve have been widely used in an attempt to explain how, in the face of normative age-associated structural brain changes, individuals vary greatly in the process of cognitive ageing (Stern, 2009). The first is neural reserve, defined as the brain's capacity to buffer the effects of insults, and which may differ between people. The second assumption, namely neural compensation, is that the ageing brain may compensate structural losses in functional areas by recruiting previously unrelated parts of the brain to take over cognitive function roles (Hedden and Gabrieli, 2004; Sullivan and Prefferbaum, 2006). The main determinants of cognitive reserve are early life cognitive ability, educational attainment and occupational status, with the former being a strong predictor of the latter two (Whalley et al., 2004; Salthouse, 2009).

1.3.4. Dementia

Relative to normal levels of intellectual function, at the opposite end of the cognitive continuum in old age are the severe cognitive deficits associated with dementia. Rather than a single biological condition or disease, dementia represents a constellation of symptoms, characterised by a chronic or progressive decline in cortical and/or subcortical functions including memory, reasoning and communication skills. As the condition progresses, complex cognitive impairment occurs, such as a gradual loss in ability to carry out daily activities (Ritchie and Lovestone, 2002; van der Flier and Scheltens, 2005). Moreover, these dementia symptoms are usually accompanied by disturbance of mood, behavior and personality. Different types of dementia have been identified and the most common forms are Alzheimer's disease (AD) and vascular dementia (VaD).

AD is a degenerative disorder and typically begins with difficulty recalling recently learned material only, worsening over time to further loss of memory and decrements in other cognitive and functional abilities. Pathologically, AD is characterised by the presence of neuritic amyloid plaques and neurofibrillary tangles of hyperphosphorylated tau protein, seen upon post-mortem examination of the brain. Although a definitive diagnosis can only be made with evidence of neuropathologic features at autopsy, the accuracy (positive predictive value) of different clinical criteria for AD is found to be reasonably high (above 80%) when compared with a neuropathologic diagnosis (Graves, 2004). Currently, standardised diagnostic criteria for AD involve the development of memory impairment, accompanied by impairment of at least one of the other cognitive domains, including aphasia, apraxia, agnosia or a disturbance of executive functioning, which cannot be explained by other neurologic or psychiatric disorders (American Psychiatric Association, 2000)

Vascular dementia is a heterogeneous term for a wide variety of vascular lesions including large -and small-vessel disease of extra –and intra-cranial arteries that result in dementia because of cerebral infarction, hypoperfusion or haemorrhage (Román, 2002; van der Flier and Scheltens, 2005). Large vessel VaD is usually thrombo-embolic in nature and may result in cerebral damage either through multiple discrete cerebral infarcts (multi-infarct dementia) leading to a stepwise deterioration in frontal lobe executive function, or a single major infarct in a critical area of the brain (strategic infarct dementia). The most common form is a consequence of cerebral small vessel disease (subcortical ischaemic VaD) and is associated with the presence of lacunar infarcts and white matter lesions (Román et al., 2002; Chui, 2007). Because of these different vascular pathologies underlying VaD, no single set of clinical criteria have become universally accepted for its diagnosis (Graves, 2004). Currently, the clinical diagnosis of VaD requires that patients fulfill criteria for ‘dementia’ and present evidence of cerebrovascular disease based on clinical information reflecting vascular risk profile and/or neuroimaging. Given that the criteria for AD formed the basis of criteria for all dementia, this approach to the diagnosis of VaD is likely to be overly biased towards AD, emphasizing memory

loss and progressive, irreversible decline as the primary symptoms rather than the pattern of motor slowing and executive deficits typically associated with VaD (Rockwood, 2002; Selnes and Vinters, 2006). Moreover, the severity and type of lesions on neuroimaging required for a diagnosis of VaD remain controversial (Selnes and Vinters, 2006). These obstacles prevent a clear separation between AD and VaD in epidemiological investigations. Another major complication to the study of specific dementia subtypes is the increasing recognition of mixed dementia. Vascular dementia frequently co-exists with AD in the same patient and the two conditions possibly share underlying risk factors and neuropathological features (Ritchie and Lovestone, 2002).

1.3.5. Subclinical cognitive impairment

Assuming a continuous spectrum between normal cognitive function and dementia, another category of 'mild cognitive impairment (MCI)' has been introduced in an attempt to characterise an immediate or transitional state of cognitive impairment i.e. one which falls outside the limits of normal ageing but is insufficient for the diagnosis of dementia. Other alternative terms have also been proposed, including mild cognitive decline, 'cognitive impairment, no dementia' (CIND) and Mild Cognitive Disorder (MCD), but MCI is widely favoured as the diagnostic entity of subclinical cognitive impairment (Davis and Rockwood, 2004). Clinically, MCI is a syndrome characterised by decline in one or more cognitive domains (usually memory) greater than expected for an individual's age and education level but to a degree that does not interfere notably with activities of daily living (Petersen, 2004). Based on the type of neuropsychological profile, the clinical subsets of MCI include non-amnestic and amnestic MCI and the latter is further categorized as single or multiple domains (Petersen, 2004; Portet et al., 2006). Amnestic MCI has greater rates of progression to dementia, particularly to AD and could constitute a prodromal stage of this disorder (Fisher et al., 2007; Gauthier et al., 2006; Petersen, 2004).

However, the suitability of the concept of MCI in large population-based studies has been questioned. The consensus criteria for the diagnosis of MCI

incorporate clinical judgment as shown in Figure 4 and are not derived just from neuropsychological test cut points. Thus, the concept of MCI may be more reliable within a clinical context as most of the work attempting to characterise it has been carried out in the memory clinics, where participants would already be experiencing sufficient memory challenges to require consultation with a clinician (McDowell, 2004). While subjects in clinic samples typically show uniform progression of cognitive decline, in four large population studies, MCI failed to demonstrate temporal stability as a large proportion (from 26 to 32%) of the participants initially identified as having MCI were subsequently classified as having no cognitive impairment at follow-up (from 1 to 5 years) (Ritchie et al. 2001; Larrieu et al., 2002; Fisk et al., 2003; Fisk and Rockwood, 2005). Furthermore, MCI has been shown to be a relatively poorer predictor for the onset of dementia in the general population (Ritchie et al., 2001).

1. Cognitive complaint (usually memory), preferably corroborated by an informant
2. Cognitive impairment (usually memory) for age and education
3. Essentially normal general cognitive function
4. Largely preserved activities of daily living
5. Not demented

Figure 4: Clinical criteria for mild cognitive impairment (Reproduced from Kelley and Petersen, 2007).

More recently, the term 'Vascular cognitive impairment' (VCI) has received increasing attention. VCI was proposed to define the whole spectrum of mild to severe cognitive impairment that shares presumed cerebrovascular morbidity (O'Brien et al., 2003). The cognitive profile of VCI was also suggested to be specific and predominantly subcortical, with relatively preserved memory but impaired psychomotor and executive functions (Selnes and Vinters, 2006). However, VCI is an umbrella term comprising heterogeneous groups, and developing a single, uniform set of diagnostic criteria that apply to all subtypes of VCI has been challenging (Selnes and Vinters, 2006; Moorhouse and Rockwood, 2008).

1.3.6. Prevalence and incidence of cognitive impairment and

dementia

The difficulties in distinguishing between normative and non-normative cognitive ageing, and the inconsistencies in defining cognitive impairment are a severe challenge to measuring prevalence and incidence. In particular, prevalence estimates of subclinical cognitive impairment vary considerably depending upon which definitional classification is used as well as the population sampling and assessment procedures. For example, subtle changes to the 'stringent diagnostic criteria' such as removing the stipulation of a subjective memory complaint or normal activities of daily living, have been shown to significantly increase the prevalence of MCI (Fisk et al., 2003). Estimates of MCI have ranged from 3% to 19% in those aged 65 years and older because of various assessment criteria applied (Gauthier et al., 2006). The prevalence estimates for age-associated cognitive decline (AACD) have ranged from 21% in those aged 60 and older (Ritchie et al., 2001), to 27% in those aged 65 and older (Hanninen et al., 1996). In general subclinical cognitive impairment is common in the older population and can increase in prevalence with the steadily ageing population. However, due to conflicting findings, it is not clear whether prevalence varies by sex. Similarly, the incidence rates of MCI have ranged greatly from 8 to 58 per 1000 per year in the general elderly population (Gauthier et al., 2006), depending on diagnostic criteria, sample structure, follow-up and cognitive measures. Furthermore, annual conversion rates from MCI to dementia have been found to be high in clinic samples ranging from 10% to 15% but are often substantially lower in population-based studies (i.e., 3.8%-6.3%) (Farias et al., 2009).

Despite the use of different methodologies for case identification and definition between studies, increases in the prevalence and incidence of dementia with age have been consistently reported (Graves, 2004). For example, in an analysis of data from the UK, the prevalence of dementia from all causes ranged from 1.3% to 2.9% among those 65-74 years of age, between 5.9% and 12.2% among those 75-84 years of age, and from 20.3% to 32.5% in those aged 85 years or above (Knapp et al., 2007). An age-related trend in dementia prevalence was observed for both sexes. Further analyses demonstrated the AD was the dominant type of dementia, accounting for 62% of all cases (and was more common in women), while VaD and

mixed dementia, accounting for nearly one third (27%) of all cases (and was more common in men). Similarly, among participants in the Cardiovascular Health Study, a steady increase in the incidence of dementia with age was noted in both men and women (Fitzpatrick et al., 2004). Whereas further analyses of subtypes showed a steep increase in the incidence of both AD and VaD with age, the incidence rates did not vary by sex or race.

1.3.7. Estimated lifetime cognitive change

Cognitive ability is a measurement of cognitive function at a specific point in time. In older people, performance at any single point in time on age-sensitive (fluid) cognitive tests reflects both a person's prior cognitive state together with the extent to which their cognitive state has declined with age or age-associated pathologies. The detection and quantification of cognitive impairment simply by comparing an individual's current test performance with test norms relative to their peers cannot therefore give an accurate measure of an individual's degree of cognitive decline during ageing. Rather, measurement of age-related cognitive decline requires a comparison of an individual's cognitive ability in old age with their prior level of cognitive ability from a period preceding the point at which the decline is thought to commence (Crawford, 1992). However, such prior cognitive data especially while individuals are still in good health are rarely available (Deary et al., 2004a). As a result, prior or premorbid mental ability is commonly estimated by using vocabulary and knowledge-based tests, including the Wechsler Test of Adult Reading (WTAR) test (Wechsler, 2001), the Mill Hill Vocabulary Scale (MHVS) (Raven et al., 1998) and the National Adult Reading Test (NART) (Nelson and Willison, 1991), with the latter two being used more frequently. The MHVS requires the subject to underline which of six words is closest in meaning to a target word. The NART requires the subject to read aloud 50 words, none of which follow normal English rules of grapheme-phoneme correspondence and/or stress (Deary and Batty, 2007). As measures of crystallised intelligence, these types of test scores vary little with age, and are relatively resistant to the effects of neurological or psychiatric morbidity (Crawford, 1992; Crawford, 2003; Salthouse, 2004). Their use as indicators of peak prior cognitive ability has been validated. For example, the NART

remained stable before and after identification of mild dementia and showed high inter-rater and test-retest reliability (Crawford, 1992; McGurn et al, 2004). Moreover, the score in old age correlated highly with childhood IQ (Crawford et al, 2001). Good retrospective validity in healthy old people (Deary et al., 2004a) means that the estimation of lifetime best cognitive ability from these test scores can be compared with the results on fluid intelligence tests to assess the difference and degree of potentially clinically significant cognitive decline. For instance, by using linear regression, adjusting scores on a fluid mental test for NART has been shown to be a valid, instantaneous measure of relative lifetime cognitive change or cognitive decline (Deary et al., 2004a).

1.4. Cognitive dysfunction associated with Type 2 diabetes

This section provides an overview of empirical studies investigating the relationship between Type 2 diabetes and cognitive function. The potential mechanisms leading to cognitive impairment in people with Type 2 diabetes are described first followed by a description of the extent and the pattern of cognitive impairment in people with Type 2 diabetes in late adulthood. In recognition of the probable importance of the complications and comorbidities associated with Type 2 diabetes, including vascular disease, in diabete-related cognitive impairment, a further review is presented of studies examining the influence of these and specific characteristics on cognitive function.

The aforementioned literature review included only epidemiological studies published in the English language and identified primarily by searching MEDLINE. Searches used combinations of key words relating to Type 2 diabetes, diabetes-related risk factors and diseases of interest (e.g. hypertension, hyperlipidemia, cardiovascular disease) and to cognitive function (e.g. cognitive decline, cognitive impairment or cognitive performance). References cited in the articles identified through the MEDLINE search were also examined.

1.4.1. Possible pathophysiological mechanisms of cognitive dysfunction in people with Type 2 diabetes

Given that Type 2 diabetes is a complex disorder, it is likely that multiple different, synergistic processes intrinsic or related to diabetes may interact to initiate and promote cognitive decrements (Strachan et al., 2008b). Apart from genetic predisposition (e.g. through variation in the apolipoprotein E gene), a number of pathophysiological mechanisms have been proposed to explain the association between diabetes and accelerated cognitive decline in older adults.

Ischaemic cerebrovascular disease

Macroangiopathy

Type 2 diabetes is known to contribute to macroangiopathy in the intra and extracranial arteries, including formation of atherosclerotic plaques, ulceration of the intima, thickening of the arterial media and calcifications (Mazzone et al., 2008). The large-vessel atherosclerosis may lead to cerebral hypoperfusion, infarction and commonly results in artery-to-artery embolic stroke (Manschot et al., 2007). Ischaemic stroke (embolic or non-embolic) in the brain may be confined to the cerebral cortex, the underlying white matter, or both. Thus depending on the area affected within the nervous system, focal or lateralized symptoms of cognitive dysfunction might occur due to ischaemic injuries to the territory supplied by a given cerebral artery. This stroke-related cognitive impairment might not only be attributable to diabetes, but also to diabetes associated risk factors for vascular comorbidity (Mankovsky and Ziegler 2004; Almdal et al., 2004). Type 2 diabetes typically develops in the context of a cluster of vascular and metabolic risk factors, including obesity, insulin resistance, atherogenic dyslipidaemia, hypertension, and prothrombotic and proinflammatory states. Several risk factors from the metabolic syndrome might be predictors of cerebrovascular disease, ischaemic stroke and accelerated cognitive decline and dementia (Kalmijn et al., 1996; Kuusisto et al., 1997; Yaffe et al., 2004a; Whitmer et al., 2005). These risk factors in the metabolic syndrome in combination with Type 2 diabetes itself might reinforce these effects.

Microangiopathy

At the level of the microvasculature, Type 2 diabetes is associated with pathological changes in its principal components including endothelial cells and the capillary basement membrane (Lorenzi and Cagliero, 1991). While much is known about diabetes-induced microangiopathy in kidney, retinal and peripheral nerves, the contribution of diabetes to cerebral microvascular pathogenesis and cognitive dysfunction is less clear. Functional properties of the blood-brain barrier (BBB) decline with age (Farkas and Luiten, 2001). Recently, using magnetic resonance imaging on patients with Type 2 diabetes, increased BBB permeability has been suggested to play a role in the increased levels of cognitive impairment in diabetes (Starr et al., 2003). Additionally, chronic exposure to hyperglycaemia in diabetes has been shown to disturb vascular homeostasis and result in abnormalities of cerebral capillaries, such as basement membrane thickening (Gispen and Biessels, 2000; Biessels et al., 2002). These microvascular changes might lead to chronic and insidious ischaemia of the brain (Bissels et al., 2006). Unlike causing circumscribed vascular lesions, diabetes might evoke more generalized and widespread microvascular disease in the brain, causing microinfarcts, probably leading to brain atrophy, lacunar infarcts and white matter lesions that have been demonstrated in neuroimaging studies (Bissels et al., 2006; van Harten et al., 2006).

Glucose toxicity

Similar to the role of aberrant glucose metabolism in other long-term complications of diabetes, ‘toxic’ effects of hyperglycaemia have also been suggested in slowly progressive functional and structural abnormalities in the brain (Gispen and Biessels, 2000). Chronic hyperglycaemia ‘toxicity’ could thus be one of the determinants of cognitive changes in people with diabetes. Animal studies have demonstrated that induced hyperglycaemia via administration of streptozotocin to impair production and secretion of insulin from beta cells in the pancreas, is associated with cognitive impairment and abnormalities in synaptic plasticity and neuronal density (Biessels et al., 2002). Several mechanisms have been suggested through which high glucose concentrations might mediate this effect, including an increased glucose flux through the polyol and hexosamine pathways, disturbances of

intracellular neurotransmitter second messenger pathways, an imbalance in the generation and scavenging of reactive oxygen species, and an accumulation of advanced glycation end products (AGEs) or proteins (Brownlee, 2001). These processes can affect brain tissue directly, as well as leading to microvascular disease. Given that some of these mechanisms, such as oxidative stress, increased formation of AGEs and microvascular pathology have also been implicated in the ageing process of the brain, the glucose-mediated effects on brain structure and cognition are likely to accelerate brain ageing and could certainly reduce the threshold for cognitive impairment and dementia in combination with other pathological alterations (Bissels et al., 2006).

Insulin changes and amyloid metabolism

Insulin resistance, at least in the early stages of Type 2 diabetes contributes to compensatory hyperinsulinaemia and the latter has been identified as a risk factor for accelerated cognitive decline and dementia (Kalmijn et al., 1995; Kuusisto et al., 1997; Luchsinger et al., 2004). Part of this association between high insulin concentrations and increased risk of cognitive dysfunction is likely to be mediated through vasoactive effects of insulin on vascular disease (Baron, 1994). In addition, insulin might have direct effects on the brain. Insulin is transported directly across the blood-brain barrier and can be produced locally in the brain. Insulin receptors are distributed widely within the brain, in particular with dense amounts in the hippocampus and the cortex. Insulin modulates homoeostasis of food intake and energy in the brain and could also play a role in learning and memory (Zhao and Alkon, 2001). Ageing is associated with changes in insulin and its receptors in the brain and activation of the insulin receptors has been found to be impaired in autopsy samples of patients with Alzheimer's disease (Frölich et al., 1998).

Similar to the toxic effects of hyperglycaemia on amyloid metabolism by the formation of AGEs, alternations in insulin homoeostasis could affect the metabolism of amyloid-β peptide (Aβ) by changes of insulin and its receptors in the brain (Craft and Watson, 2004). Aβ is derived from the amyloid precursor and forms neuritic senile plaques with other proteins. Excessive Aβ can be cleared through

processes involving insulin-degrading enzyme (Farris et al., 2003). Hyperinuslinaemia in Type 2 diabetes has been suggested to stimulate Aβ secretion and inhibit the extracellular degradation of Aβ by competition for insulin-degrading enzyme (Gasparini and Xu, 2003).

1.4.2. Frequency and patterns of cognitive impairment in people with Type 2 diabetes

There have been a number of published reviews describing and analysing the impact of Type 2 diabetes on cognitive functions. Strachan (Strachan et al., 1997) published in 1997 a critical analysis of the literature before the majority of the longitudinal studies were finished, which included mainly cross-sectional studies using clinic-based samples. Stewart (Stewart and Liolitsa, 1999) later on in 1999 added more evidence to the link explaining all the possible mechanisms of this association. Awad (Awad et al., 2004) revisited the studies included in these reviews as well as those more recently published, and estimated effect sizes showing poorer domain-specific cognitive performance of Type 2 diabetic groups compared with performance of control groups. Cukierman (Cukierman et al., 2005) systematically reviewed and summarized twenty-five prospective studies relating Type 2 diabetes status to decline in cognitive function over time. Overall, these studies clearly showed that Type 2 diabetes was associated with moderate reductions in cognitive performance and with accelerated cognitive decline

The majority of cross-sectional studies have demonstrated that older adults with Type 2 diabetes perform more poorly than controls without diabetes on a variety of cognitive function tests. In particular, cognitive measures assessing psychomotor efficiency, frontal/executive function, and learning and memory skills are most often affected (Stewart and Liolitsa, 1999; Awad et al., 2004; van Harten et al., 2006). The cognitive performance of patients with Type 2 diabetes relative to controls is often reported using effect size estimates, which show between-group differences in standard deviation units. Moderate to large effect sizes on affected domains are observed in people with diabetes older than 65 years. These range from 0.4SD to 1.0SD, corresponding to the 34^{th} to 15^{th} percentile of reference values (e.g. reduced

performance with an effect size of 1SD places the mean performance of the diabetic group around the 15th percentile of the control group) (Stewart and Liolitsa, 1999; Awad et al., 2004). Smaller effect sizes (<0.5SD) are found in relatively younger adults (<60 years old) with Type 2 diabetes (Stewart and Liolitsa, 1999; Awad et al., 2004). This cognitive profile is suggestive of accelerated ageing and may reflect difficulty in processing unstructured information, which would particularly affect performance in cognitively demanding situations that require rapid information processing or divided attention (Brands et al., 2007; Biessels et al., 2007).

The longitudinal, population-based studies included in the systematic review by Cukierman demonstrating that Type 2 diabetes was a risk factor for accelerated age-related cognitive decline (Cukierman et al., 2005), used cognitive screening instruments for assessing global cognitive function or batteries of more comprehensive neuropsychological tests (mostly including the Digit Symbol Substitution test, DSST). DSST is a primary measure of psychomotor speed and a secondary measure of working memory function (Joy et al., 2004), given that processing speed includes encoding and retrieval speed, the speed with which information is processed in working memory, as well as response speed, the speed of executing a motor response (Kyllonen and Christal, 1990). As a pooled estimate, compared with people without diabetes, people with Type 2 diabetes were 1.2 times more likely to experience cognitive decline as measured by a global functioning test such as the Mini-Mental State Exam (95%CI 1.1, 1.4) and 1.7 times more likely to develop cognitive decline as measured by the DSS (95%CI 1.3, 2.3). Taken together, all available data indicate that the rate of cognitive decline due to ageing is increased 1.5-fold to 2.0-fold in older individuals with Type 2 diabetes (Cukierman et al., 2005). However, a recent study examining the effect of Type 2 diabetes on cognition in the oldest old (age at study entry 85 years) did not find an association between diabetes and accelerated cognitive decline (van den Berg et al., 2006). Whether this was an artifact due to survivor effect or another phenomenon is unknown. Studies evaluating clinically significant cognitive impairment and diabetes have also shown that individuals with Type 2 diabetes are more likely to develop mild cognitive impairment (odds ratio 1.4 to 1.8) independent of age and co-morbid diseases (Yaffe

et al., 2004b; Luchsinger et al., 2007).

Although there is compelling evidence that cognitive impairment and/or decline are potential complications of diabetes, the risk factors for cognitive decrements in older individuals with Type 2 diabetes are still uncertain. Above the age of 60 gradually progressive cognitive decline in people with diabetes mostly occurs in the context of neurodegenerative processes of the brain and develops over many years (Biessels et al., 2007). Diabetes-specific risk factors affecting cognitive ability before the clinical onset of frank dementia need to be identified. Most of the currently available studies addressing this issue are cross sectional, relating cognition to concurrent vascular and metabolic risk factors and often suffer from methodological limitations, including small sample sizes, bias in recruitment of subjects and failure to adjust for confounding factors. Even in large-scale, prospective studies, a very narrow range of diabetes-specific information is available. It is important to note that many of the potential risk factors for age-related cognitive decline may affect cognitive ageing through mediating effects on each other, and that the two conditions may share common risk factors. Although there are mechanistic studies that provide pathophysiological leads, it remains unclear which of these are clinically relevant.

1.4.3. Diabetes-related risk factors, comorbidities and cognitive function

There are various characteristics of people with Type 2 diabetes, such as hyperglycaemia, macro- and micro vascular disease which could be essential in explaining their increased risk of cognitve impairment. These risk factors are also potentially amenable to treatment or prevention. However, there are few epidemiological studies addressing whether or not such risk factors are actually important. Most studies were not specifically designed to assess the effects of diabetes on the risk of cognitive impairment, but rather aimed to identify risk factors for cognitive impairment in older people in a broader sense.

This section provides an overview of current epidemiological evidence for

the effects of diabetes-related risk factors and co-morbid conditions on cognitive functioning in people with Type 2 diabetes as well as relevant evidence from general population samples.

Non-modifiable risk factors

Age

Although normal ageing has a global effect on cognitive changes, it disproportionately influences certain aspects of cognitive function. Crystallised functions seem quite resistant to the ageing process, whereas fluid intelligence acquired as a result of genetic factors may be particularly susceptible (Deary and Batty, 2007). These patterns are supported by population-based epidemiological studies on the relation between chronological age and cognitive function in older cohorts. In cross-sectional and longitudinal studies, older age (at baseline) has been associated with a downward shift in the distribution of global cognitive ability scores, such as the MMSE and with an increased risk of progressive decline on the MMSE over time (Brayne et al., 1998; Comijs et al., 2004). Administration of a cognitive test battery in the Massachusetts Male Ageing Study showed an inverse, linear association between chronological age and performance on measures of working memory, speed and attention and spatial relations in 981 middle-aged to older men (Fonda et al., 2005). Similarly, in cross-sectional data from the Seattle Longitudinal Study, notable age differences from age 25 to age 81 were observed on a number of different cognitive tasks (Schaie, 1996). Older subjects tended to perform relatively poorly on the measures of perceptual speed, verbal memory, spatial orientation and reasoning. In 7-year longitudinal comparisons, linear age-related declines were observed from age 60 to 88. Conversely, in both occasions, older subjects up to their late 70s obtained equivalent or higher scores on verbal ability compared with younger subjects (Schaie, 1996; Hultsch et al., 1998; Zelinski and Burnight, 1997).

It is matter of debate as to whether the observed patterns of cognitive decline during ageing occur as a result of ageing itself, due to the effects of concomitant age-associated pathologies, or both (Hedden and Gabrieli, 2004). Another possible explanation is that age differences in cognition, especially detected

in a cross-sectional data set, are inextricably confounded with birth cohort effects in which individuals from different birth cohorts would have variations in cognitive function even if they are of the same age. This is due to different past exposures to unmeasured risk factors. Failure to consider the age-associated pathologies could lead to an underestimation of their impact and lead to the false assumption that ageing per se is the underlying cause of the observed cognitive decline. Indeed, the effects of Type 2 diabetes on the brain and cognitive function are most marked in the elderly and mimic the effects of ageing. This may pose a major challenge in distinguishing the effects of diabetes from those of 'normal ageing'. In a study of 113 people with Type 2 diabetes and 51 control subjects, age was inversely associated with performance on the measures of attention and executive function, information processing speed and memory within the Type 2 diabetic group, and there was a significant interaction between age and group for the memory domain (Manschot et al., 2006). This pointed to an interaction between Type 2 diabetes and cognitive ageing, and therefore the latter may be accelerated by diabetes. Furthermore, it has been observed in longitudinal studies of older adults that Type 2 diabetes is cognitively equivalent to aging by three years in men and women (Okereke et al, 2008).

Sex

Findings from population-based epidemiological studies on differences in cognition between men and women are mixed and somewhat contradictory. Some cross-sectional analyses have suggested that women may outperform men on memory and vocabulary tasks (Barrett-Connor and Kritz-Silverstein, 1999; Aartsen et al., 2004). Conversely, men scored better on measures of visuospatial speed and visuoconstructive performance in another Finnish study (Portin et al., 1995). Other studies failed to find a sex difference in general ability as indexed by the sum of a cognitive test battery (Portin et al., 1995; Cullum et al., 2000). Results from longitudinal analyses showed no sex differences in 4-year risk of cognitive decline on any of the eight CAMCOG subscales in the CC75C study (Cullum et al., 2000) or in the rate of decline over six years on several measures of speed tasks in the Longitudinal Aging Study Amsterdam (Aartsen et al. 2004). Where sex differences

have been observed, several hypotheses have been proposed in an attempt to account for these, including male-female differences in brain reserve and differential susceptibility to normal or pathological age-associated changes (van Exel et al., 2001). However, differences in cognitive function between men and women remain unclear based on current evidence.

Similarly very little is known about the effect of sex on cognitive function in people with Type 2 diabetes. There are no available data directly comparing older diabetic men and women and only limited data comparing diabetes as a risk factor for cognitive dysfunction in men versus women. It has been hypothesized that diabetes may be a more-potent risk factor for cognitive dysfunction in women than in men, given their higher risk of diabetes-related cardiovascular disease (subclinical and clinical vascular disease) and early loss of possible protective effects of estrogen due to earlier menopause on cognitive functioning (Coker and Shumaker, 2003). However, what evidence does exist generally supports a similar relationship between Type 2 diabetes and cognition in men and women. A cross-sectional analysis of the Framingham Study cohort found no evidence of interactions between diabetes status and sex on multiple measures of cognitive test scores in 551 men (mean age=65.7 years) and 872 women (mean age=67.2 years) (Elias et al., 2005). In a larger sample of approximately 6,000 older community–dwelling men from the Physicians' Health Study II and more than 6,000 similar women from the Women's Health Study, there was no evidence of interactions between sex and Type 2 diabetes status on 2-year cognitive decline for general cognition and verbal memory (Okereke et al., 2008).

Duration of diabetes

Another variable, closely related to age, which may affect cognition and has been investigated in a number of recent epidemiological studies is the period of time an individual has had diabetes. Such duration of diabetes is normally calculated by subtracting age at diagnosis from current age. Because people with diabetes may have the disease many years before diagnosis, the true duration of diabetes is often difficult to estimate precisely and may therefore be underestimated. Furthermore it can be challenging to determine whether duration of disease per se or age of onset of

diabetes has the more effect on cognitive changes. Despite these difficulties, there is increasing evidence from recent studies suggesting longer duration of diabetes may be an important risk factor for cognitive dysfunction in older people with Type 2 diabetes.

In an early observational study of the Framingham Study cohort, Elias (Elias et al., 1997) examined the development and duration of Type 2 diabetes at biennial visits over a period of 30 years in 1, 811 men and women (187 subjects with Type 2 diabetes and 1,624 nondiabetic subjects). Administering a battery of eight cognitive tests at the end of follow-up (aged 55-88 years), the investigators showed that duration of diabetes was associated with an increased odds of impairment (standardised Z scores below the 25^{th} percentile) on tests of verbal memory and abstract reasoning (similarities of WAIS). In a more recent study, compared with normoglycaemics, people with Type 2 diabetes more that 15 years had significantly slower processing speed and poorer executive performance. These cognitive abilities were intermediate in the group with diabetes of less than 15 years (Saczynski et al., 2008). In cross-sectional analyses, increasing duration of diabetes has been associated with poorer scores on measures of verbal memory, information processing speed and motor speed (Cosway et al., 2001; Manschot et al., 2006; van Harten et al., 2007) in people with Type 2 diabetes. In large, longitudinal cohorts of older adults, longer duration of diabetes was associated with greater 2-year cognitive decline in general cognition and verbal memory in men and women after multivariate adjustment (Okereke et al., 2008).

Duration of diabetes represents a composite measure of the physiological insult of hyperglycaemia and other diabetes comorbidities, such as hypertension, macro- and microvascular disease. Thus, association between duration of diabetes and diminished cognitive function may reflect either direct and/or indirect effects (eg. through a mediating role of clinical and subclinical cardiovascular disease) of diabetes on cognition. Whether a clustering of these diabetes-associated risk factors or shared underlying pathophysiological mechanisms contribute to the increased risk of cognitive dysfunction needs to be further clarified.

Major modifiable risk factors

Glycaemic control

In people with diabetes, the aim of treatment is to maintain normal glucose levels and utilization in order to prevent vascular and other complications that result from hyperglycaemia. Glycaemic control is the ability to keep glucose levels in the normal range. Glycated haemoglobin (GHb) percentage is a measure of integrated glycaemic control over the past 2-3 months, with extra weighting for the past one month. This percentage reflects the proportion of haemoglobin that has become glycosylated because glucose is attached to it by a slow non-enzymatic process. Because blood cells live for about 2-3 months, GHb levels reveal the average levels of glucose during that period. In clinical practice, HbA1c is one of the GHb species most often measured, which is present in the largest amounts and results from the attachment of glucose to the N-terminal amino acid valine of the β-chain of haemoglobin. Higher HbA1c has been found to be associated with an increased risk of micro- and macrovascular complication in diabetes (DCCT 1993; UKPDS34, 1998). Normal levels of HbA1c are 4-6% whereas HbA1c levels suggesting poor glycaemic control are between 8-11.9% (American Diabetes Association, 2002). Although HbA1c has been widely used as an index of long-term glycaemic control, its use as a diagnostic tool for Type 2 diabetes has been limited due to lack of the standardised measurements between laboratories, which makes results difficult to compare.

Current literature on the relationship between HbA1c levels and neuropsychological functions is limited and results are inconsistent. An analysis based on nearly 2,000 postmenopausal women observed an increased risk (OR= 1.4; 95% CI 1.0, 1.9) of mild cognitive impairment over four years for every 1% increase in HbA1c at baseline after adjusting for age, education, race, depression, alcohol use and medication. Those with HbA1c of more than 7% had a nearly 4-fold increase in developing MCI (Yaffe et al., 2006). Another investigation, involving 5,632 elderly men and women from the Italian Longitudinal Study on Aging, found higher HbA1c levels were associated with risk of 8-year cognitive decline, particularly in memory (Maggi et al., 2009). In some cross-sectional studies of people with Type 2 diabetes,

an inverse relationship between HbA1c and global cognition (van Harten et al., 2007), working memory (Pelmuter et al., 1990; Munshi et al., 2006), executive functioning (Munshi et al., 2006), learning (Reaven et al., 1990) and psychomotor performance (Reaven et al., 1990; Ryan and Geckle, 2000; Manschot et al., 2006) has been shown. By contrast, other studies failed to demonstrate a relationship whether in people with diabetes (Cosway et al., 2001; Manschot et al., 2007; Umegaki et al., 2008; Ruis et al., 2009) or in a general population (van den Berg et al., 2006; Saczynski et al., 2008). It has been argued that the lack of association in these studies may reflect relatively well-controlled diabetes in the study sample, as evident from the participants' HbA1c level, or a negative effect of HbA1c on cognition after longer diabetes duration.

Results from clinical trials lend further support to a negative association between HbA1c and intellectual functioning. Early trials, which were limited by small size and short duration of follow-up (<7 months) reported that improved glycaemic control caused improved cognitive test performance mainly in the areas of attention, learning, and complex psychomotor function (Gradman et al., 1993; Meneilly et al., 1993; Naor et al., 1997). More recently, Ryan et al. (Ryan et al., 2006) conducted a multi-centre randomized double-blind clinical trial of rosiglitazone vs. glyburide therapy in a larger sample of 145 older adults with Type 2 diabetes, and found that better glycaemic control may improve working memory function over a 6-month period.

Despite the inconsistency in findings from epidemiological studies, several mechanisms have been proposed whereby HbA1c may affect cognition. For example, chronic hyperglycaemia per se could cause cognitive impairment by direct neuronal damage possibly caused by advanced glycosylated endproducts (AGEs), leading to its degeneration as reflected in global and hippocampal atrophy as well as neuropathologic markers of Alzheimer's disease. It is also possible that hyperglycaemia has an indirect impact on neuronal damage via cerebral microvascular and macrovascular atherosclerotic disease.

Hypertension/blood pressure

One of the most studied risk factors for cognitive decline in later life is high blood pressure, given that blood pressure is easily measured, potentially modifiable and a major risk factor for cerebrovascular disease. However, so far results from epidemiological studies in general populations have been mixed—some studies reported a negative effect of high blood pressure on cognition and others reported no association. Variation in findings may be partially explained by age and timing of blood pressure assessment. Also nowadays a substantial proportion of older individuals have been taking anti-hypertensive medication, and it is unclear how accurately the measured blood pressure values actually reflect long-term cumulative exposure levels (Herbert et al., 2004). These issues have been addressed in a series of recent large-scale longitudinal studies which have shown that higher blood pressure levels during middle-age, especially if not treated effectively predict poorer cognitive outcomes in old age. In these studies, cognitive tests were administered to untreated hypertensive patients and test scores were linked to blood pressure levels determined in mid-life (Elias et al., 1993; Launer et al., 1995; Kilander et al., 1998; Qiu et al., 2005). For example, in the Framingham study, measurements of blood pressure were obtained at five biennial examinations during the period 1956-1964 when few hypertensive participants received treatment (Elias et al., 1993). Blood pressure values were based on the average of five measurements. With cognitive function assessed 20 years later, the results for the total sample showed a negative, linear relationship between both SBP and DBP and performance on a global composite score and several subtests of WAIS (the Logical Memory immediate and delayed recall, the Visual Reproductions, and the Digits Backward). Each 10mmHg increase in blood pressure was associated with a decline of 0.04 to 0.07 standard deviations in the composite score. Similar results were found in sub-analyses of participants who were untreated at the time of blood pressure measurement or throughout the entire study period.

There is less evidence that the same negative effect of hypertension on cognition is present in later life and in particular, three studies have reported a U-shaped relation between SBP and cognitive function (Guo et al., 1997; Glynn et al., 1999; Bohannon et al., 2002). Findings suggest that midlife levels of hypertension

may contribute to individual differences in cognitive function in late adulthood, whereas blood pressure in old age may bear a relatively weaker relation to cognitive impairment or decline over and above that of other morbidity (Swan et al., 1998). Waldstein suggested that non-significant associations between hypertension and cognitive function in old age might reflect selective attrition (Waldstein, 1995).

Recent analyses of data from a sub-sample of 1,811 subjects (mean age=67 years) participating in the Framingham Heart Study, demonstrated significant synergistic interactive effects of hypertension and Type 2 diabetes. In the total sample, each 10-mmHg increase in blood pressure was significantly associated with higher risk of performing below the 25th percentile for verbal memory (OR: 1.29 95%CI 1.10, 1.52). Within the diabetic group (n=187), each 10-mmHg increase in blood pressure was associated with 'highest' levels of risk of poor performance on a composite cognitive test score (OR: 2.03 95%CI 1.12, 3.71) as well as on visuospatial measures (OR: 1.68 95%CI 1.04, 2.69). The highest risk for poor performance were also associated with a diagnosis of Type 2 diabetes in the presence of hypertension (n=576) (i.e. OR=1.97 and 1.82 for the composite and visuo-spatial scores respectively (Elias et al., 1997). The comorbid effect of diabetes and hypertension on cognitive function has been reported in other studies (Kuusisto et al., 1993; Posner et al., 2002; Hassing et al. 2004; Manschot et al., 2007). For example, an analysis based on 258 older adults (mean age=83 years) in the OCTO-Twin Study observed a steeper 6-year decline (by an additional 0.29 points per 2-year interval) in global cognitive function related to Type 2 diabetes at baseline but not related to hypertension. However, there was a significant interaction between diabetes and hypertension in rate of change in MMSE such that those with both diseases had greatest decline (additionally by 0.42 points per 2-year interval) compared with those free from both (Hassing et al., 2004). In contrast, another two population-based studies failed to find the modulating effect of hypertension on cognitive function in Type 2 diabetes (Kanaya et al., 2004; Fontbonne et al., 2001).

Dyslipidaemia/serum lipids

Given that the brain has a very high lipid content, there is considerable

biological plausibility for a positive association between serum lipids and intellectual functioning. Low circulating lipid levels, perhaps resulting from chronic morbidity and/or poor nutrition intake or absorption in old age, may directly affect neuronal metabolism, neurotransmitter systems, and cognitive processes. However, the current literature of the relationship between serum lipid levels and cognitive functioning in late-life has been inconsistent as to whether high or low serum cholesterol concentrations are associated with cognition or indeed at all.

A recent systematic review examined population-based longitudinal data on the relation of serum lipids with cognitive decline or impairment (Anstey et al., 2008). Three of the seven studies included found that higher total serum cholesterol (TC) in older individuals was associated with a decreased risk of both cognitive decline and cognitive impairment (Wada et al., 1997; Hyman et al., 1996; Solfrizzi et al., 2004). However, no relationship was observed between TC levels and cognitive performance in another three studies, including one with a large sample size (n=1,147) (Kalmijn et al., 1996; Karlamangla et al., 2004; Reitz et al., 2005). In addition, neither high density lipoprotein (HDL) nor LDL-cholesterol levels were associated with the cognitive outcome in these studies.

However, several other studies have demonstrated a negative association between blood lipid levels and cognition. One large-scale study included in Anstey's systematic review (Kivipelto et al., 2001), involving 1,352 elderly Finnish men and women, found that higher mid-life (mean age=50 years) TC increased the risk of mild cognitive impairment (OR1.9 95%CI 1.2, 3.0) over a 21 year follow-up period. Moreover, in other investigations, beneficial effects of satin use or lipid-lowering drugs were noted either on IQ change from childhood to old age in dementia-free older survivors of the Scottish Mental Health Survey (Starr et al., 2004), or on estimated lifetime cognitive decline in older patients with Type 2 diabetes (Manschot et al., 2007). In elderly people with diabetes, hypertriglyceridemia was also related to poorer performance on the measures of verbal fluency, reaction times and backward digit span (Perlmuter et al., 1990; Helkala et al., 1995). It is possible that the inverse relationship of blood lipid levels with cognitive function may be partly or fully mediated through atherosclerosis-based co-morbidity, such as large-vessel stroke.

Obesity

Obesity is not only associated with metabolic disturbances such as hypertension, Type 2 diabetes and dyslipidaemia, but also is an independent risk factor for cardiovascular disease and mortality, including stroke. In this context, a relationship may also exist between obesity and cognition. Also, obesity could have a more direct effect on cognitive dysfunction through its subclinical inflammatory potential (Jeong et al., 2005), eventually leading to structural changes in the brain (Jagust et al., 2005). Despite the biological plausibility of such an association, current literature relating obesity to cognition has been limited to relatively few reports in older adults.

A sex-specific analysis based on data from the Framingham Heart Study showed worse cognitive ability in obese individuals (Body Mass Index (BMI) $\geq$ 30 kg/m^2) (Elias et al., 2005). After adjusting for socio-demographic and cardiovascular risk factors, obese men performed relatively worse compared with those non-obese men on a global composite score, and on individual tests of visual reproductions and digit span forward. In another population-based study, obesity was assessed at mid-life time (mean age 53 years) and cognitive function was measured 8 years later. Highest baseline quartile of waist to hip ratio, but not BMI, was associated with worse cognitive performance on visual memory, executive function and visual motor skills (Wolf et al., 2007). Contrary to these findings, two studies involving large number of older individuals either failed to establish an association between waist circumference (abdominal obesity) and cognition or found an inverted U-shaped relationship between BMI and cognition (Kuo et al., 2006; Dik et al., 2007). A more recent study based on older patients with Type 2 diabetes did not find an association between BMI and estimated lifetime cognitive change (Ruis et al., 2009).

Smoking

Smoking is a major modifiable risk factor for impaired pulmonary function and vascular disease. Smoking may also affect cognition either being mediated via an effect on vascular or pulmonary pathologies or through a more direct effect on the central nervous system, such as disturbing blood flow in the brain. Results from early

epidemiological studies on the relationship between smoking and cognition were inconclusive. Many of them were cross-sectional, with smoking assessed by self-report which is more prone to recall bias and which may be influenced by cognitive status at the time of assessment, especially in elderly people (Woodward, 2002). These limitations have been at least partially addressed in a number of more recent studies.

In a longitudinal analysis based on 9, 209 participants aged 65 years and older, both male and female current smokers declined at greater rate on MMSE scores compared with those who had never smoked over a mean period of 2.3 years. Moreover, a higher number of pack-years of smoking, reflecting higher lifetime exposure levels, were weakly but significantly associated with the greatest rate of cognitive decline (Ott et al., 2004). Another investigation based on middle-aged men and women from the British 1946 birth cohort study, fully controlled for people's prior mental ability (at age 15 years) in addition to a range of possible confounders. There was a significant trend between the number of cigarettes smoked at age 43 and the rate of 10-year decline in verbal memory (Richards et al., 2003).

A beneficial effect of smoking cessation on reduced age-related cognitive decline has also been noted in some studies (Richards et al., 2003). In the Honolulu-Asia Aging study, the likelihood of scoring poorly on the CASI instrument was inversely related to the time since giving up smoking in elderly Japanese-American men (Galanis et al., 1997). In another analysis based on older people with evidence of asymptomatic atherosclerosis, former female smokers had better performance on the age-sensitive Auditory Verbal Learning and the Trail Making tests compared with current smokers (Stewart et al., 2006).

Despite these findings in general populations, very few investigations have linked smoking status to cognition in large numbers of people with Type 2 diabetes (Arvanitakis et al., 2006; Umegaki et al., 2008; Ruis et al., 2009) and results are also conflicting. Whether smoking status could modify the relation of Type 2 diabetes to cognitive function (people with Type 2 diabetes who smoked performed worse than those with diabetes who did not smoke), needs to be explored.

Other modifiable risk factors

Alcohol intake

Despite chronic alcohol abuse causing progressive neurodegenerative disease, recent epidemiological studies have paid more attention to the long term effects of regular light to moderate alcohol intake on cognitive function. Moderate alcohol consumption has been found to be associated with lower risk for coronary heart disease and stroke (Pearson, 1996; Mukamal et al, 2006; Elkind et al., 2006) in the general populations. Also, moderate drinking in older adults was reported to stimulate appetite and improve mood. Given the beneficial effects on physical health, it is plausible that moderate alcohol intake could be protective against impaired cognitive function. However, findings from epidemiological studies have so far been mixed. In a recent meta-analysis (Peters et al., 2008), the pooled estimates from seven population-based prospective studies (aged ≥65 years) failed to demonstrate an association between moderate amounts of alcohol consumption and risk of cognitive decline (RR: 0.89 95%CI: 0.67 to 1.17). As there was significant heterogeneity between the available studies, the authors of the review stated that the results should be interpreted with caution. Indeed, the included studies varied in the populations examined, methods of assessing exposure to alcohol, classification of alcohol use, follow-up periods, neuropsychological tests and controlling for potential confounding factors. A J-shaped curve between the level of alcohol consumed and cognitive performance was seen in the majority of the studies although with wide confidence intervals and lack of statistical significance (Peters et al., 2008). In particular, there was a lack of standardisation regarding the operational definition of moderate amount of alcohol intake, which varied widely between studies. Also some studies used ‘non-drinkers’ as the reference category for comparison, including both lifetime abstainers as well as quitters, whereas others either preferred the lowest intake category excluding abstainers or chose infrequent/less than one drink per month as their reference level.

It has also been speculated that there may be a sex difference in the relationship of alcohol with cognitive function, given that patterns of alcohol use and

alcohol metabolism are likely to differ for men and women (Dufouil et al., 1997; Elias et al., 1999; Kalmijn et al., 2002; McGuire et al., 2007). However, a recent study found both male and female current drinkers performed better on a task of global cognitive ability relative to abstainers and former drinkers (Bond et al., 2004).

The effects of alcohol intake have also been investigated in people with Type 2 diabetes (although the same methodological limitations apply). Among adults with Type 2 diabetes, moderate alcohol consumption has been associated with a reduced risk of cardiovascular disease, metabolic syndrome and levels of inflammation and endothelial dysfunction markers (Wakabayashi wt al., 2002; Freiberg et al., 2004; Howard et al., 2004; Shai et al., 2004). Consistent with these findings, in a cross-sectional study of 119 men with Type 2 diabetes, Fan et al. (2008) found that moderate alcohol drinkers who had at least 1 drink per month to no more than 2 drinks per day, scored significantly better on Digit Span Backward, Digit Symbol and Trail Making B tests, compared with abstainers after controlling for various diabetes-related, psycho-social and vascular factors. Heavy drinkers (more than 3 drinks a day) were excluded from the study. More recently, the Nurses' Health Study examined 1,698 women aged 71-80 years with Type 2 diabetes and found that at baseline, moderate alcohol drinkers (those consuming 1.0-9.9 g of alcohol or about 1 drink per day) had higher mean scores on a general cognition test compared with abstainers (Townsend et al., 2009). The association was unchanged after excluding women who decreased their alcohol drink after the diagnosis of diabetes. However, moderate alcohol consumption was not associated with 4-year cognitive decline. Higher alcohol consumption (10-30 g of alcohol per day) was not associated with either initial cognition or cognitive decline.

Depression

Depression is a common mental disorder in the elderly. Unlike the normal day-to-day fluctuations of moods, clinical depression or major depressive disorder is a persistent and profound syndrome that is characterised by symptoms of depressed mood, loss of interest or enjoyment, and physical symptoms such as fatigue, loss of energy and sleeping difficulties. The specific diagnostic criteria for clinical dementia are shown in Figure 5.

A. Five (or more) of the following symptoms have been present nearly everyday during the same 2-week period and represent a change from previous functioning; at least one of the symptoms is either depressed mood or loss of interest or pleasure.
(1) depressed mood
(2) markedly diminished interest or pleasure in all, or almost all, activities
(3) significant weight loss when not dieting or weight gain, or decrease or increase in appetite
(4) insomnia or hypersomnia
(5) psychomotor agitation or retardation
(6) fatigue or loss of energy
(7) feelings of worthlessness or excessive or inappropriate guilt (which may be delusional)
(8) diminished ability to think or concentrate, or indecisiveness
(9) recurrent thoughts of death (not just fear of dying), recurrent suicidal ideation without a specific plan, or a suicide attempt or a specific plan for committing suicide

B. The symptoms cause clinically significant distress or impairment in social, occupational, or other important areas of functioning.

C. The symptoms are not due to the direct physiological effects of a substance or a general medical condition or mood-incongruent hallucinations.

D. The symptoms are not better accounted for by bereavement.

Figure 5: Clinical diagnostic criteria for Major Depressive Disorder (American Psychiatric Association, 1994)

There is growing evidence in the literature suggesting depression may also be an important and treatable risk factor for cognitive dysfunction in the elderly. Despite the different subtypes of depression, individuals with depression are likely to share the main symptoms of depressed mood, including the difficulty in initiating responses, maintaining effort in attention or concentration, which may be particularly pertinent to cognitive task performance in information processing speed, learning, memory and executive function (Herrmann et al., 2007). A negative cross-sectional association between depression and cognitive function has been consistently observed in large-population based studies (Fuhrer et al., 1992; Baune et al., 2006). It is possible that depression may initiate or accelerate the cognitive ageing process. Given that depression and cognitive impairment often co-exist, it is also possible that impaired cognitive function may present during a depressive episode because of diminished attention and motivation, and may be directly related to the disease itself and its neuropathological aspects. Alternatively, depressive symptoms could be simply a psychological reaction to perceived deterioration in cognitive function. However, the observed association often persists after clinical recovery of depression (Butters et al., 2000; Butters et al., 2004).

Reverse causation (depression caused as a result of cognitive impairment) is also unlikely to account for recent findings from longitudinal studies relating depression to late-life cognitive decline in time (Yaffe et al., 1999; Paterniti et al., 2002; Wilson et al., 2004). For example, Wilson and associates (2004) found that increased self-reported depressive symptoms at baseline predicted annual rate of cognitive decline in a composite score independently of baseline cognitive function. In addition, results were unchanged after excluding those with cognitive impairment at baseline. In an earlier study involving elderly women without dementia, a significant dose-related association between the number of self-reported depressive symptoms and the rate of 4-year decline in performance on three cognitive tasks (the MMSE, the WAIS Digit Symbol and the Trail-Making test B) was reported, after controlling for a number of possible confounders including baseline cognitive ability (Yaffe et al., 1999).

Although the precise basis of the association between cognitive impairment and depression in late-life remains unclear, several neurobiological mechanisms have been proposed. A shared nervous system pathology underlying both depression and cognitive dysfunction has been suggested, in particular involving the impact of cerebral microvascular disease, such as white matter hyper-intense lesions shown on magnetic resonance imaging (Thomas and O'Brien, 2008). Another possibility is that both cognitive deficits and depression may be associated with higher levels of cortisol and dysregulation of the hypothalamic-pituitary-adrenal axis (HPA). In particular, elevated glucocorticoid levels may have deleterious effects on the structure and function of the hippocampus, a key locus for cognitive function including learning and memory. The hippocampus is also a core region in the limbic system and has widespread connections to diverse cortical areas which are known to constitute the neuroanatomical network of mood regulations (Drevets, 2000).

The prevalence of depressive symptoms is higher in older adults with Type 2 diabetes relative to the general population, and the presence of depression and diabetes has been noted to worsen the course of both, particularly through diabetic complications, poorer glycaemic control and a great number of depressive relapses.

Thus depression may also increase vulnerability to and/or exacerbate existing cognitive deficits. So far only a few studies have evaluated the effect of depression on cognition in people with Type 2 diabetes (Lowe et al., 1994; Gregg et al., 2000; Watari et al., 2006). Although results are mixed, these studies suggest that depression does not account for all of the cognitive decrements associated with diabetes.

Premorbid cognitive ability

As mentioned earlier, the concept of 'cognitive reserve' maintains that there are aspects of brain structure and function (e.g. neural plasticity and efficiency of neural networks) that buffer the effects of neuropathology such that the greater the reserve, the more severe the pathology must be to produce functional impairment (Richards and Deary, 2005). Within the context of the reserve hypothesis, there is individual variation in the development of clinical impairment of cognitive function, and some individuals may have some physical brain structures and/or cognitive strategies that serve as protective reserves against clinical level of deterioration. There is a wide range of putative indices of cognitive reserve, chief among which is pre-morbid (early life) cognitive ability (Whalley et al., 2004; Stern et al., 2006). It has been noted that the level of cognitive ability in old age is substantially (about 50%) determined by childhood mental ability.

It is possible that a higher premorbid or childhood ability indicates greater cognitive reserve, providing greater resistance to the effects of diverse pathological insults. A number of studies have demonstrated a relation of child or early life cognitive ability, as estimated from mental test scores in the first two decades of life, with diverse health outcomes in late life. For example, higher ability levels are related to lower rates of total and cardiovascular disease mortality and morbidity (Hart et al., 2004; Deary et al., 2004b; Batty et al., 2005; Batty et al., 2007), lower frailty (Deary et al., 2004b), higher quality of life (Bain et al., 2003), and reduced rates of major psychiatric disease (Walker et al., 2002; Zammit et al., 2004; Batty et al., 2005). Moreover, higher ability is associated with less cognitive decline or improved performance in later adulthood and old age (Snowdon et al., 1996; Richards et al., 2004; Bourne et al., 2007). For instance, a follow-up study of the UK

1946 birth cohort examined the relation between cognitive ability at age 15 years and cognitive decline from 43 to 53 years in men and women (Richards et al., 2004). Participants with higher childhood ability experienced less cognitive decline on tasks of memory and search speed. More importantly, these results were observed after accounting for later-life educational attainment, social class and a range of health indicators.

In turn, pre-morbid ability is also influenced by diverse biological influences including a genetic component as well as early exposure to social and material environmental factors. For example, advantageous socio-economic conditions in childhood may directly affect cognitive development, possibly through a greater exposure to cognitive stimuli (Everson-Rose et al., 2003).

As an alternative to the 'cognitive reserve' hypothesis, the 'use it or lose it' hypothesis has been proposed to explain the relationship between premorbid ability and cognitive function in later life, suggesting that use-dependent processes afford cognitive protection. Those with high childhood mental ability may have received more education and worked at more intellectually demanding tasks, which further enable them to acquire a repertoire of life skills that support a mentally stimulating and socially engaged life style, thus 'exercising' their mental faculties more throughout the life-course than those who were initially less cognitively able (Christensen and Henderson., 1991; Staff et al., 2004; Bourne et al., 2007).

Education level

There is now a large body of literature on the relationship between early-age educational attainment and cognitive function in later life. In population-based studies, a strong positive association has consistently been observed between years of formal education and better cognitive performance, but the findings on the association with cognitive decline have been mixed (Wight et al., 2002; Anstey et al., 2003; Lee et al., 2003; Seeman et al., 2005). It has been argued that even with statistical adjustments, change in cognitive function between two time points is hard to distinguish from level of function at either time point, especially when the predictor of interest such as education, is highly correlated with the cognitive

outcome. Thus multiple cognitive assessments in time are needed to separate initial level of cognition from rate of change. However, fewer such studies are available and some of them have failed to find an association between education level earlier in life and cognitive decline associated with ageing (Christensen et al., 2001; Gerstorf et al., 2006; Van Dijk et al., 2008).

In addition, the specific pattern of what aspects of cognition may be involved and protected is yet not clear and possibly due to difference in study populations, cognitive tasks and frequency of consecutive assessments. For example, in an analysis of 865 elderly men and women from the MacArthur Studies of Successful Aging with three phases of cognitive assessment, formal educational attainment was highly positively associated with performance on all five measures of both crystallised and fluid abilities over a 7-year follow-up period (Seeman et al., 2005). However, data from the Australia Longitudinal Study of Ageing spanning an 8-year period with three phases of cognitive assessment showed an inverse association between level of education and cognitive decline in cognitive processing speed but not in verbal ability and memory (Anstey et al., 2003). In another study covering 6 years with 5 cognitive assessments showed slower decline on a measure of general cognitive mental status for higher level of education but not on domain-specific cognitive tests (Alley et al., 2007).

Proposed mechanisms for a protective effect of education on subsequent cognitive decline in older age include confounding with other education-related factors. It is speculated that education may act as a marker for general socioeconomic conditions such as general health status and health-related lifestyles. However, in most studies, education has been shown to be significantly and independently associated with cognition after statistical control of factors such as health and life-style related variables. A second hypothesis (consistent with ‘use it or lose it’ proposal) is that education could lead to occupations, social environment or leisure activities that entail greater ongoing mental cognitive stimulation over the lifetime. This theory has been supported by findings from some studies in which individuals who engage in leisure activities and continued education have better cognitive ability

in mid to late life, although the benefits of such activities may be most evident in those with least formal education (Wight et al., 2002; Rafnsson., 2006). Finally, it is possible that education may have more direct effects on the brain and cognitive processes by promoting brain development resulting in greater dentritic branching and eventually leading to greater 'brain reserve' capacity.

Macrovascular disease

Atherosclerosis is a progressive disease of large and medium-sized arteries characterised by the accumulation of lipids and fibrous elements in the arterial wall (Ross, 1999; Lusis, 2000). Atherosclerotic lesions begin as fatty streaks and may eventually progress into more advanced lesions of atherosclerotic plaques. Plaques often become gradually more complex with calcification, ulceration and haemorrhage. Although these lesions can grow large and block blood flow, the most important and common complication is the rupture of the atherosclerotic plaque and the formation of thrombus (Lusis, 2000).

Atherosclerosis can affect several arterial beds. The term 'macrovascular disease' collectively refers to atherosclerotic disease that obstructs the blood supply to the heart (e.g. coronary heart disease), brain (e.g. cerebrovascular disease) and the lower limbs (e.g. peripheral arterial disease). As highlighted earlier, large-vessel atherosclerosis of intra and extracranial arteries may lead to cerebral hypoperfusion and commonly results in artery-to-artery embolic stroke. On the other hand, cerebral blood flow is autoregulated by intracranial mechanisms of altering blood vessel diameters in response to cerebral hypofusion. However, if systemic blood pressure levels fall below the autoregulative window, cerebral blood flow follows perfusion pressure and hypoxia may occur (Vingerhoets, 2001). In addition to cerebrovascular insufficiency, as a generalized systemic process, either symptomatic or significant but yet clinically unrecognized atherosclerotic disease may co-exist within the same individual, and one manifestation of macrovascular disease should promote investigation of the similar disease in other arterial beds (Phillips, 2001). In fact, evidence of coronary artery disease has frequently been reported in patients who have had transient ischaemic attacks (TIA) and cerebral stroke (Russell, 1983), and is also found in up to 90% of individuals with symptomatic peripheral arterial disease

(Vogt et al., 1992). Furthermore, in coronary heart disease, such as myocardial infarction, atherosclerosis-induced cardiac pathology may predispose to cardiogenic cerebral emboli and possibly reduced ventricular function which may lead to either acute or chronic state of cerebral hypoxia and hypo-perfusion.

From these perspectives, different major clinical types of macrovascular disease may be associated with impaired neuropsychological functioning. To date, however, this potential source of variance in cognitive ability has generated somewhat limited interest in population-based studies. Specifically, longitudinal data on the pattern of cognitive decline in older individuals with macrovascular disease are lacking. Based on current investigations, findings are contradictory. For example, in survivors of stroke, some cross-sectional data showed reduced performance on both global (Zhu et al., 1998; Kase et al., 1998) and domain-specific tasks including verbal memory and executive function (Rafnsson et al., 2007) relative to stroke-free or healthy controls, whilst others failed to provide evidence in support of deficits in specific cognitive domains (Srikanth et al., 2003). Stroke has also been found to be associated with an increased decline in global cognitive function (Kalmijn et al., 1996; Kase et al., 1998; Haan et al., 1999). In the Zutphen Elderly Study, elderly male stroke patients were at 4.3 times increased odds of a decline in MMSE scores over three years after adjusting for age, education and baseline MMSE test scores (Kalmijn et al., 1996). Moreover, in the Cardiovascular Health Study (Haan et al., 1999), elderly subjects with prevalent stroke declined faster over a period of seven years in mean digit symbol test scores, compared with stroke-free participants (-1.96 points vs. -0.28). These effects were independent of age, sex, race, education and incident stroke.

A more recent investigation was based on 452 elderly men and women in the Edinburgh Artery Study, who were cognitively tested on two separate occasions, at baseline and four years later (Rafnsson et al., 2007). When compared with subjects free of any cardiovascular disease (CVD), and after adjusting for demographic characteristics, depression and major vascular risk factors, subjects with prevalent or incident stroke showed a significantly steeper 4-year decline in verbal memory

performance, but not on tasks assessing other domains, including nonverbal reasoning, executive function and processing speed. Contrary to these findings, an analysis based on 1224 older adults in the Longitudinal Aging Study Amsterdam did not find an association between stroke and 3-year cognitive decline in any domain-specific tests (Dik et al., 2000).

Given the inconsistencies in the relationship between stroke and cognitive function, a number of methodological limitations need to be noted. For example, apart from incomplete neuropsychological assessment, the timing of cognitive testing relative to the onset of stroke varies across studies. Although compared with hospitalized patients, population-recruited stroke survivors may be considered as having milder stroke pathology, particularly high rates of impairment may be observed where cognitive testing is performed very early following the event, which is largely influenced by acute confusional or sensorimotor state. In addition, brain imaging data have been collected in only a very few studies. As a result, specific information on aetiological type, lesion size and location of the stroke is generally not available. Despite assumed ischaemic stroke in the majority of cases, it is possible that subtype and lesion site may affect cognitive outcomes to a different extent. In some analyses, TIA patients have been included whereas elsewhere these have been deliberately excluded. The result of their inclusion may be dilution of any cognitive effects seen among cases.

Relatively few studies have examined cognitive function in the context of macro-vascular pathology other than stroke. In population samples, different indices of coronary heart disease (CHD), such as the presence of angina, myocardial infarction (MI) or CHD, have cross-sectionally been associated with worse performance on mental status tests in some studies (Breteler et al., 1994; Elwood et al., 2002) but not in others (Petrovitch et al., 1998; Ahto et al., 1999; Tilvis et al., 2004). Negative effects of CHD on measures of specific cognitive function, including verbal, mathematical reasoning and executive functioning have been found in other cross-sectional studies (Elwood et al., 2002; Verhaeghen et al., 2003). Furthermore, in most population-based follow-up studies, cognitive assessment has

been limited to global cognitive measures alone, such as the MMSE (Zhu et al., 1998; Haan et al., 1999; Eslinger et al., 2003; Piguet et al., 2003; Tilvis et al., 2004). No association between CHD and cognitive decline has been observed in the majority of these studies (Zhu et al., 1998; Eslinger et al., 2003; Piguet et al., 2003).

Only three studies have collected longitudinal data using a battery of cognitive tests. Haan et al. (1999) found a steeper 7-year decline in digit symbol test, in the presence of either major ECG abnormalities or cognitive heart failure at baseline. In contrast, neither clinically verified angina nor MI at baseline or follow-up was found to be associated with a greater 4-year decline in domain-specific measures of fluid intelligence (Rafnsson et al., 2007). A similar null finding was observed in another study (Verhaeghen et al., 2003). It should be noted that few studies have adequately taken into account the potential overlap between different CHD syndrome groups or between subjects with other manifestations of atherosclerotic disease and thus the possibility of confounding by previous stroke has not always been sufficiently excluded. Moreover, on several occasions, control subjects have not been entirely free of clinical vascular disease.

Studies of cognitive function in people with PAD are also scarce and most early studies were not designed to study cognitive function in relation to PAD per se. In population-based samples, the presence of PAD has been determined either from clinical symptoms of arterial narrowing in the lower limbs (intermittent claudication (IC)), or from measurements of the ankle brachial index (ABI). Intermittent claudication has been found to be cross sectionally associated with worse performance on global (Tilvis et al., 2004) and domain-specific tasks (Singh-Manoux et al., 2003). However, another cross-sectional report failed to show the effects of IC on cognitive test (Elwood et al., 2002). Similarly in longitudinal data, the findings of association between IC and decline in global mental status were conflicting (Piguet et al., 2003; Tilvis et al., 2004). More recently, in Edinburgh Artery Study, among stroke-free subjects, elderly individuals with symptomatic PAD (IC) had a significantly greater 4-year decline in verbal memory functioning (Rafnsson et al., 2007). An inverse association between ABI and cognitive performance on non-verbal

intelligence, executive function and information processing speed has also been observed in a cross-sectional study (Price et al., 2006). ABI has also been associated with a progressive decline in global mental status (Breteler et al., 1994; Haan et al., 1999). These studies may suffer from confounding by concomitant vascular risk factors. For example, disease categories are non-mutually exclusive of other manifestations of macrovascular disease, including stroke. As with studies on CHD, only on rare occasions have control subjects been free of any clinical cardiovascular disease.

Very few previous studies have presented detailed data on the relation between macrovascular disease and cognitive function in people with Type 2 diabetes. In a recent study, the presence of any macrovascular atherosclerotic disease appeared to be the most consistent determinant of impaired cognition on a composite score in Type 2 diabetetic patients (Manschot et al., 2007). Another cross-sectional analysis (Umegaki et al., 2008), involving 907 elderly Japanese people with diabetes, also showed the presence of stroke was associated with cognitive impairment (defined as an MMSE score of 24 or less) (OR 3.01, 95%CI 1.58, 5.75). Given the established relationship of Type 2 diabetes and macrovascular disease, it is important to determine the role of concomitant atherosclerotic disease, cerebrovascular or otherwise, on the cognitive function of people with Type 2 diabetes.

Microvascular disease

It has been shown that up to one-third of symptomatic stroke can be attributed to disease of the small arteries/arterioles of the cerebral circulation (Greenberg, 2006). People with Type 2 diabetes are especially prone to ischaemic strokes due to small vessel disease, which result in a different stroke pattern from non-diabetic subjects (Bell, 1994; Megherbi et al., 2003; Karapanayiotides et al., 2004). Cerebral microvascular disease is commonly characterised by stenosis or occlusion of small penetrating arteries with sudden or chronic ischaemia leading to complete infarction (lacunar infarcts) and/or incomplete infarction of cerebral white matter. These end arteries tend to arise directly from the large arteries and have few, if any, branches or distal collateral supply. However, morphological changes of these microvessels are difficult to visualise in life and clinical features develop insidiously

(Farrall and Wardlaw, 2009). Postmortem findings generally reflect late-stage changes which mask the earlier events.

Two major pathological changes have been described which contribute to occlusion or hypoperfusion of small vessels, resulting in cerebral ischaemia. Similar to atherosclerosis in large vessels, the first and more frequent is arteriosclerosis in arterioles which further leads to a number of pathophysiological alterations, such as thickening of vessel walls, disturbance of the blood brain barrier and drop of perfusion rate. As a result, 'incomplete infarctions' may develop, producing ischaemic demyelination, astrogliosis, oligodendrocyte and axonal loss. The second is lipohyalinosis which manifests as a progressive disorganization of small artery walls, with subintimal deposits of a hyaline fibrinoid substance (Fisher, 1968; Lammie, 2000; Román et al., 2002). This is considered to be the major pathological feature leading to lacunar infarction (Lammie, 2002).

Although the initial steps in the development of microvascular disease remain unclear, increased permeability of the blood-brain barrier has been proposed to play an important role, in which vascular leakage caused by microvascular endothelial dysfunction and damage may allow plasma components of the blood to enter into the arteriolar wall (with damage, thickening and eventual disintegration), followed by perivascular oedma-related lesions in brain parenchyma (Farrall and Wardlaw, 2009).

On magnetic resonance imaging (MRI) of the brain, cerebral microvascular disease can be frequently observed, particularly in adults over the age of 60. White matter lesions or abnormalities are seen as focal and diffuse hyperintensities on T2-weighted MRI scans, typically located in the extensive periventricular areas and the deep regions of anterior limb of the internal capsule. This is possibly related to local cerebral blood flow being lowest in periventricular and deep white-matter regions, as these are perfused by long, narrow, non-collateral end-arterioles (de Pantoni et al., 1996). The severity of white-matter lesions (WML) is directly proportional to the degree of stenosis of the multiple medullary arterioles due to arteriosclerosis. Lacunar infarcts or lacunes are small cavitated ischaemic infarcts,

resulting from occlusion of the lenticulostriate, thalamo-perforating and long medullary arterioles (Fisher, 1982). They are typically located in the thalamus, caudate nucleus, globus pallidus, internal capsule and frontal white matter. They are found in 10% to 31% of all symptomatic strokes, but most lacunes are clinically silent (Fisher, 1965; Sacco et al., 1991; Longstreth et al., 1998). Lacunar infarcts may be silent, but they may contribute to gradual cognitive decline (Patton, 2006). Further, lacunar infarcts in the white matter and brainstem can be distinguished from white matter lesions of hypodensity on T1-weighted MRI.

Both lacunar infarcts and WML associated with small vessel disease are predominantly found in the sub-cortical associative areas of the brain, involving the basal ganglia, cerebral white matter and the brain stem. Thus these ischaemic lesions may particularly interrupt the prefrontal subcortical loops and disconnect the prefrontal cortex or anterior cingulated cortices from their basal ganglia or thalamocortical connections, which lead to impaired prefrontal lobe/executive functioning, including impaired information processing (Mori, 2002). Apart from having a direct effect on cognitive function, lacunes and WML are frequently found in the presence of Alzheimer's disease and these lesions may also affect cognition by interacting with neurodegenerative pathology such as amyloid plaques and neurofibrillary tangles.

There is growing evidence from population-based studies that cerebral small vessel disease, as visualised on MRI as WML load or progression and/or lacunar infarcts may contribute to cognitive impairment (Longstreth et al., 1996; de Groot et al., 2000; Au et al., 2006) and cognitive decline associated with ageing (Garde et al., 2000; Garde et al., 2005; Prins et al., 2005; Schmidt et al., 2005; van den Heuvel et al., 2006). Conventional MRI is unlikely to identify the full spectrum of small vessel disease related cerebral damage. More sophisticated techniques, such as diffusion tensor and magnetization transfer-imaging (MTI) have proved superior regarding the detection and quantification of subtle loss of white matter tract integrity, especially in the so-called normal-appearing white matter, which has been related to cognitive decline in elderly individuals (Schmidt et al., 2005; Shenkin et al., 2005). Another

pathological correlate of cognitive impairment in subjects with small vessel disease which eludes detection by MRI is microinfarcts in the gray matter (Kövari et al., 2007).

These neuro-imaging techniques have provided invaluable tools in advancing our understanding of cerebral vascular pathology. However, they are often expensive and available only in highly specialized settings, and therefore are not suitable candidates for more widespread screening of large numbers of ostensibly healthy people from the general population. Moreover, cerebral small vessels themselves are too small to visualise in detail using current neuro-imaging methods. A simpler, more accessible and sensitive technique is required. Small vessel disease is not restricted to the brain, but as an expression of a systemic condition, also affects other organs such as the retina and the kidney (Thompson and Hakim, 2009). In particular, the retinal vasculature can be directly visualised non-invasively in vivo and also photographed with ease because of its essential two-dimensional nature. More importantly, retinal and cerebral small vessels share similar embryological origin, size (50-250μm), structure (endarteries lacking anastomoses), and the blood-brain and blood-retinal barrier (Patton et al., 2005; Lloyd et al., 1995; Wong and Mitchell, 2007). As a consequence, there is increasing interest on the role of retinal microvascular abnormalities as a phenotypic marker of the condition of the cerebral microvasculature and their relationship with cognitive dysfunction. The association of these retinal vascular signs with cognitive dysfunction is systematically reviewed in the next chapter.

1.5. Overview of retinal microvascular abnormalities

The retina and the brain are highly metabolically active tissues with demanding requirements on metabolites via specialised vascular networks (Patton et al., 2005). From an embryological perspective, the retina is an extension of the diencephalon, and both organs share a similar pattern of vasculisation during development. There is a close anatomical correlation between both macrovascular and microvascular blood supply to the brain and to the retina. Both vascular

networks have similar vascular regulatory processes (Patton et al., 2005). Retinal blood vessels are the only structures that are visible by clinical examination because they convey erythrocytes containing the visible pigment haemoglobin. Abnormalities of the retinal circulation frequently highlight perturbation of the systemic circulation due to conditions such as diabetes and hypertension. In the last decades, advances in digital retinal photography and imaging techniques have allowed precise characterisation of subtle retinal vascular changes in large populations. This section provides a brief overview of the anatomical and physiological homology between the retinal and cerebral microvasculatures. It also describes the classification, pathophysiology and epidemiology of various retinal microvascular abnormalities, in particular, those which are related to diabetes.

1.5.1. The normal retinal and microvascular arrangement

The human eye functions similarly to a camera. Light comes in through the cornea, pupil and lens at the front of the eye just as the lens of the camera lets in light to the film (Osareh, 2004). The light is then focused on the retina like the film of a camera. This information will then be processed and sent to the brain via the optic nerve, connecting the eye to the brain, and finally the image is perceived in the brain (Osareh, 2004). Figure 6 illustrates a cross section of the human eye and highlights the main components.

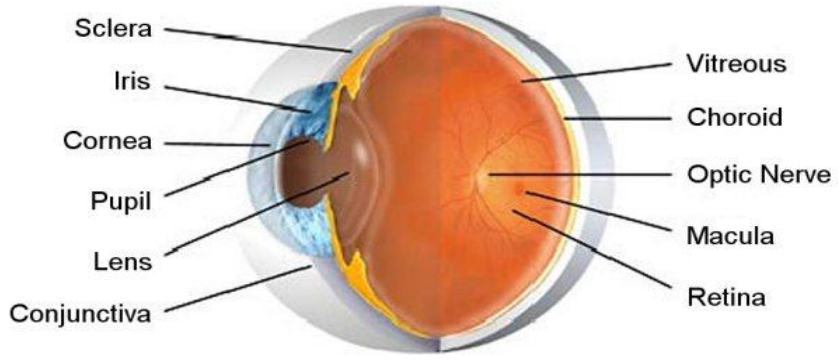


Figure 6: A cross section of the human eye with various ocular components (Sources: http://www.freedomscientific.com/resources/vision-anatomy-eye.asp)

The retina is a thin multi-layered neural sensory tissue between the retinal

pigmented epithelium and the vitreous body, covering the inside wall at the back of the eye. The retina has four major types of cells: neurons (photoreceptors, bipolar cells, amacrine and ganglion cells), vascular (pericytes and endothelial), macroglial cells (Muller cells and astrocytes), and microglia (which act as phagocytes). The photoreceptor cells (mainly the rods and cones) are responsible for translating the light beams they receive into electrical impulses and then chemical signals, eventually transmitting this neural information to the brain where they are interpreted into images. The outlying parts of the retina are responsible for peripheral vision while the central and round area, called the macula, is in charge of detailed central vision which allows us to see detail and perform tasks that require central vision such as reading (Osareh, 2004). Within the macula is a small depression called the fovea, which corresponds to the region of the retina with highest sensitivity. The optic disc or optic nerve head is the entry and exit site of blood vessels and optic nerve fibres from the retina to the optic nerve (Osareh, 2004). It is a brighter region than the rest of the ocular fundus with a usually round shape. In the centre of the optic disc, there is a depression called the optic cup. Figure 7 shows highlighted regions of macular, fovea and optic disc components in a normal fundus image.

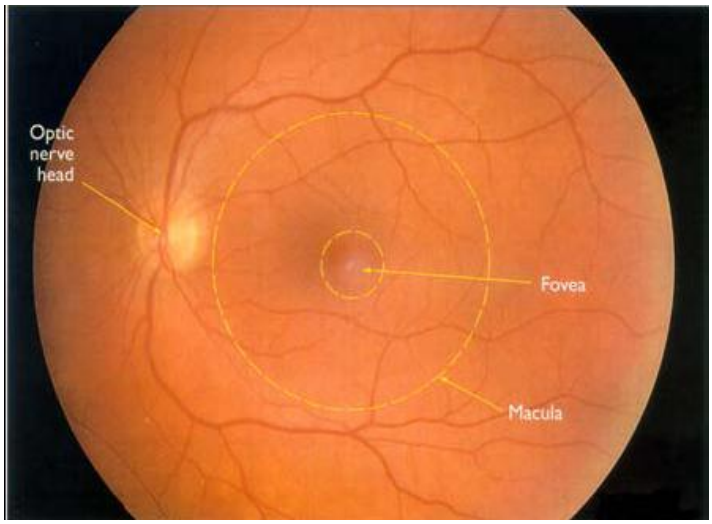


Figure 7: Posterior pole of the eye showing macular, fovea and optic disc (Reproduced from Osareh, 2004)

In humans, the retinal vessels occupy and nourish the inner half of the neural retina. They are highly organised and have two distinct layers of microvessels:

the superficial layer in the nerve fibre/ganglion cell layer, and the deeper layer extending into the inner nuclear and outer plexiform layers (Figure 8). The retinal vasculature is separated from the surrounding neural components by the cytoplasm of Muller cells and glial cells. In general, the retinal blood vessels are derived from the central retinal artery and vein and extend outward from the optic disc in all directions spreading their network across the retina. The retinal circulation is a relatively low-flow and high-oxygen-extraction system. Retinal vessel density is greater in the central retina and decreases towards the retinal periphery with the extreme part avascular and also sparing the fovea. Specific regions of the retina identified as dominating the oxygen requirements of the retina include the inner segments of the photoreceptors and the inner and outer plexiform layers.

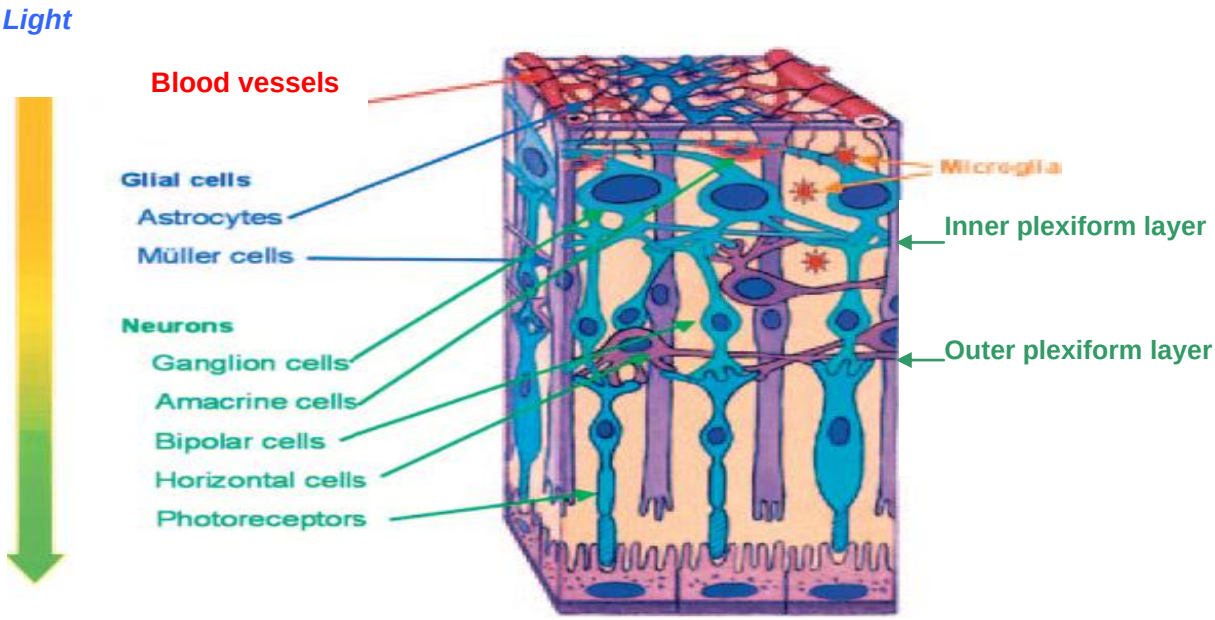
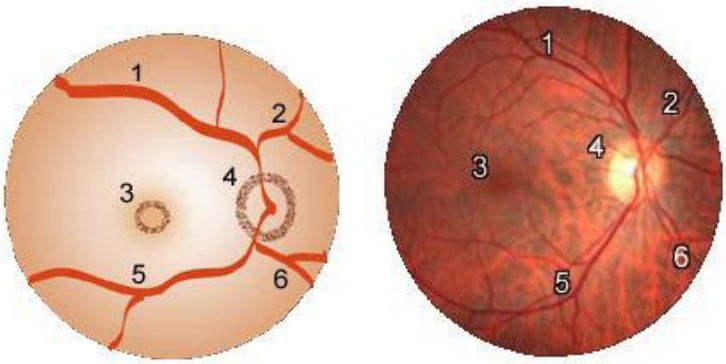


Figure 8: Organisation and structure of retina showing different cell types. The retinal vessels comprise two layers of microvessels: a superficial layer in the ganglion cell layer and a deep layer in the inner and outer plexiform layers (Adapted from Gardner and Aiello, 2000)

The central retinal artery is a direct branch of the ophthalmic artery arising from bifurcations adjacent to the optic disc to form an intraretinal, end artery microvascular system. The retinal branch 'arteries' lack an internal elastic lamina and therefore they are arterioles anatomically (Archer et al., 2007). These arterioles are around 200 µm in diameter and bifurcate to third and fourth orders in the peripheral

retina and finally to pre-capillary arterioles. Contrary to this arrangement, in the central retina small pre-capillary arterioles appear abruptly from the larger radial arteries. It is also notable that retinal arterioles often present 90^{o} vascular branching patterns, in which the smaller arterioles tend to come off at right angles from the parent stem (Cogan and Kuwabara, 1984). Although the arrangement of retinal vessels is unique for each person, the large blood vessels, in general, occupy the innermost portion of the retina while smaller blood vessels, the capillaries are found between the nerve fibre and inner nuclear layers. The diameter of the capillary network varies in different parts of the retina: the deep outer mesh ranges from 15-130µm while the superficial network averages about 65µm (Wise et al., 1971). The retinal capillary wall is lined by a monolayer of endothelial cells encompassed by a basement membrane and surrounded by pericytes.

The capillaries drain blood flow into the venular system, which is localized in the deeper retina (inner plexiform layer), and eventually to the retinal veins (Archer et al., 2007). Due to the lack of a well-developed smooth muscle covering, they have a larger diameter than arteries ranging from 30 to 300µm (Archer et al., 2007). The central retinal vein lies within the optic disc and is drained by the ophthalmic vein and cavernous sinus. As retinal blood vessels are end arteries in nature without anastomotic connections, occlusion of these vessels leads to cessation of retinal blood flow and subsequent destruction of the inner layers of the retina (Patton et al., 2005). Figure 9 shows a normal human retinal image from the right eye with different structures including blood vessels and the optic disc.



(a) (b)

Figure 9: A normal retinal image from the right eye. Diagram of the retinal (a) and

retinal image (b) (Reproduced from Osareh, 2004)
The main retinal components numbered in Figure 9 are as follows:
1- Superior temporal blood vessels; 2- Superior nasal blood vessels; 3- Fovea; 4- Optic disc; 5- Inferior temporal blood vessels; 6- Inferior nasal blood vessels

1.5.2. Anatomical and physiological similarities of the cerebral and retinal microcirculation

'Barrier' circulations

The intraretinal circulation is structurally and functionally analogous to the cerebral microcirculation in that they both have similar and important properties of 'barrier' endothelia (Patton et al., 2005). This barrier function serves to prevent non-specific permeation of the neuronal milieu by macromolecules and restrict the transfer of small hydrophilic and blood cells, yet facilitates exchange of respiratory gases, amino acids, salts, sugars and some peptides, and helps buffer variations in blood composition in the brain and retina (Archer et al., 2007). The endothelial cells of the cerebral and retinal capillaries form a continuous and single layer around the capillary lumen. They are non-fenestrated with each cell being fused to juxtaposed neighbours by tight junctional complexes, which are composed of several proteins, such as occludin, junctional adhesion molecules and zonulae occludens. These tight junctions form the mechanical component of the blood-brain and inner blood-retinal barriers (Patton et al., 2005). In addition, instead of static lipid membrane barriers, barrier endothelia are dynamic interfaces with specific and elective membrane transport acting as a metabolic component of the barrier. They have specific carrier-mediated transport proteins, providing transport of nutrients such as glucose and amino acids across the tight junctions, as well as enzymatic degradation of molecules crossing the blood-brain and blood-retinal barriers.

This barrier is thought to be maintained in part by other constituents of the cerebral and retinal microvasculaures, including pericytes, basement membranes and the surrounding neuroglia, that promote tight junction integrity and the non-fenestrated phenotype of the endothelium. For example, pericytes, which are

embedded within a common basement membrane with the endothelial cell, provide structural support to the microvasculature as well as having contractile properties by acting as the capillary counterparts of vascular smooth muscle cells. Retinal pericytes occur in a 1:1 ratio with endothelial cells, which is greater than in their counterparts of cerebral capillaries and is also a unique feature of this microvasculature in humans. The basement membrane, a connective tissue sheath surrounds the capillaries and anchors vessels to adjacent tissues (Patton et al., 2005). Both cerebral and retinal astrocytes (known as perivascular end feet) may not only play a role in the development of endothelial zonule occludens and specific barrier proteins, but also in angiogenesis, inducing endothelial cell and pericyte differentiation (Patton et al., 2005). Figure 10 illustrates the mechanical and metabolic components of the blood-brain and blood-retinal barriers and the influence of glial cells on these barriers.

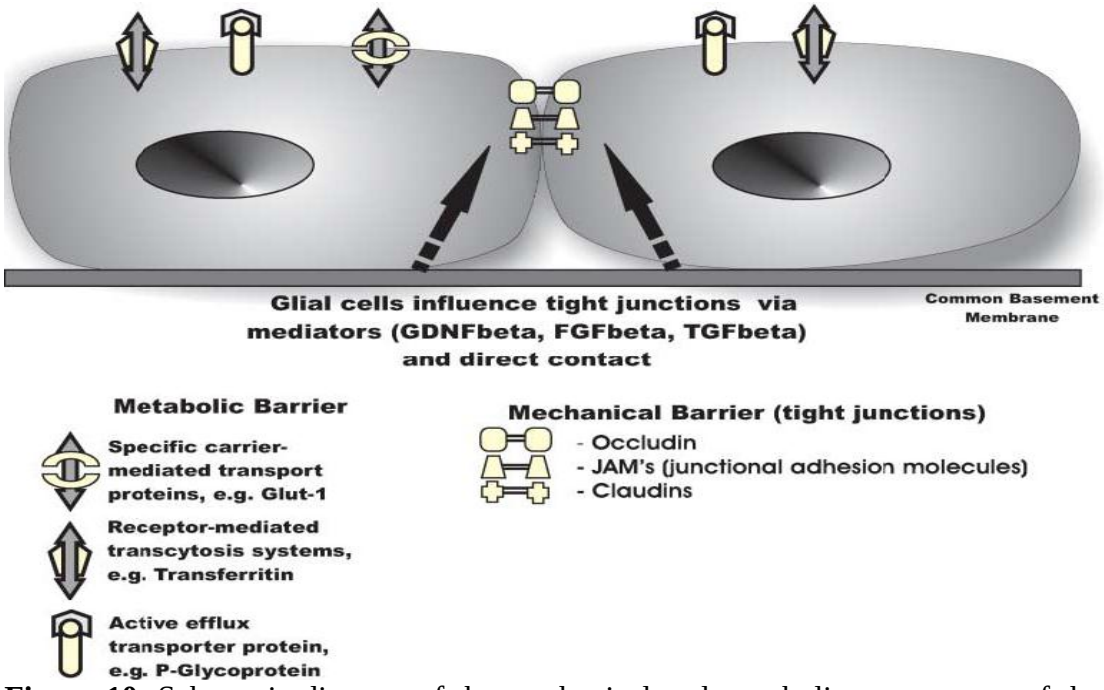


Figure 10: Schematic diagram of the mechanical and metabolic components of the blood-brain and blood-retinal barriers and the influence of glial cells on these barriers (Reproduced from Patton et al., 2005)

Regulation of cerebral and retinal circulations

The retinal vasculature lacks autononomic innervation (except for the

choroidal circulation extending into the outer layers of the retina) and therefore blood flow into the capillary beds is not under neurogenic control but autoregulated via local myogenic and metabolic mechanisms. Likewise, there is no autonomic innervation to the cerebral microvasculature and the local process of control or autoregulation is a property of both circulations. More specifically, the perfusion pressure of the cerebral or retinal circulation is related to systemic blood pressure and tissue pressure by the following relationship (Figure 11):

Cerebral/ocular blood flow = (mean arterial blood pressure-intracranial/intraocular pressure)/ vascular resistance

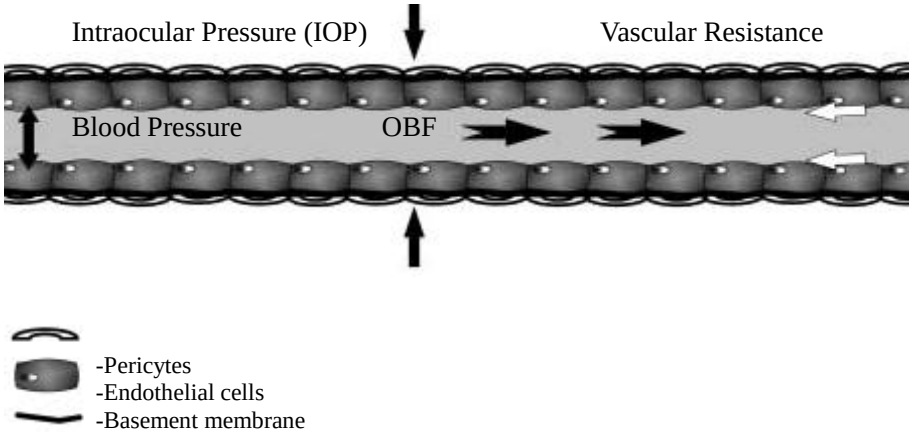


Figure 11: Schematic diagram of the retinal microvessel and ocular blood flow (OBF) as an example (Reproduced from Patton et al., 2005)

In order for the retinal and cerebral circulations to maintain a constant flow over a range of systemic blood pressures, their vascular resistance has to be altered accordingly. This is largely mediated by the vascular smooth muscle of the arterioles and pericytes, resulting in changes in luminal diameters. In particular, in response to a high intraocular or intracranial pressure, the myogenic capacity of both vascular smooth muscles and pericytes to contract produces increased vascular tone and facilitates a sensitive decrease of luminal diameter in retinal and cerebral blood vessels. In addition, cerebral and retinal blood flow is related to local metabolic needs, which depends on regional neuronal activity (Patton et al., 2005). For example, in the retina, blood flow has been found to be greater in the temporal retinal (containing the metabolically active macula) than in the nasal retina (Patton et al.,

2005). This metabolic-driven activity in parenchyma can also be achieved by the sensitivity of the component smooth muscle cells in the arterioles to endothelial generated vasodilators and constrictors, such as nitric oxide (NO), endothelins and bradykinin. The degree of autoregulation within the retinal vascular circulation varies with region supplied by the superficial capillary bed being better regulated than the deeper capillary bed (Yu et al., 1994).

1.5.3. Classification of retinal microvascular abnormalities

The retinal vasculature offers an opportunity to examine the effects of various systemic and metabolic diseases on the microcirculation. With the development of retinal photographic techniques, including high-resolution digital imaging, subtle retinal vascular changes can be assessed with accuracy and high reproducibility. Broadly these retinal changes in older people can be divided into three groups:

1) classic retinal vascular changes in diabetes and hypertension (i.e., diabetic and hypertensive retinopathy)
2) isolated retinopathy signs in individuals without clinical diabetes or hypertension (e.g., microaneurysms, retinal haemorrhags, or cotton-wool spot)
3) changes in retinal vascular network geometry, which include arteriolar and venular calibre changes, and changes in retinal vascular architecture (e.g., retinal tortuosity, arteriolar bifurcation angles)

Of the various retinal microvascular abnormalities, only the spectrum of retinal vascular changes related to Type 2 diabetes is reviewed in this thesis as this is most relevant to the original research work undertaken.

1.5.4. Pathophysiology of retinal vascular changes in Type 2 diabetes

In Type 2 diabetes, chronic hyperglycaemia triggers a cascade of molecular events that cause microvascular damage. In particular, persistent hyperglycaemia in poorly controlled diabetes may result in biochemical alterations and altered

hemodynamics of the retinal vasculature, which lead to chronic hypoxia. When compensatory pathways fail to resolve this retinal hypoxia, a spectrum of pathological processes may develop. Diabetic retinopathy is primarily a disorder of the retinal circulation that compromises the delivery of oxygen and nutrients to meet the high metabolic demands of the retina. It is one of the most common microvascular complications of diabetes that can lead to irreversible visual loss. Even in diabetic people without clinical retinopathy, retinal microvasculature may have been affected, such as the 'dynamic' or 'static' changes of the retinal vascular calibre size. In turn, these distinct and subtle changes may provide prognostic information regarding the risk of diabetic retinopathy. The pathophysiological changes and possible mechanisms underlying diabetic retinopathy and variation of retinal vascular calibre are described.

Diabetic retinopathy

The pathophysiology of diabetic retinopathy (DR) is complex. The vascular disruptions of diabetic retinopathy are primarily characterised by abnormal vascular flow, increased vessel permeability, and/or occlusion or closure of capillaries. Histologically, a hallmark of early DR is the change in the structure and cellular composition of the microvasculature, including endothelia cell damage, loss of intramural pericytes and thickening of capillary basement membrane. As a consequence, breakdown of the inner blood-retinal barrier may occur, resulting in accumulation of extracellular fluid and plasma proteins. Loss of contractile function in pericytes may weaken the vessel wall as well as disturb blood flow pattern. Another common feature in early DR is the presence of acellular capillaries, which are degenerate and non-perfused capillaries with only a basement membrane tube remaining (Kern and Mohr, 2007). When patches of these capillaries increase and become confluent, the terminal arterioles that supply these capillaries often become occluded. In addition, local accumulation of leukocytes, platelets and erythrocytes may also contribute to capillary occlusion. As a result of occluded capillaries, retinal ischaemia stimulates a pathologic neovascularisation mediated by angiogenic factors, such as vascular endothelial growth factor (VEGF). Endothelial proliferation occurs following ischaemia with the subsequent preretinal new vessel proliferation in an

attempt to supply oxygenated blood to the hypoxic retina. This neovascularisation is the predominant feature of later, advanced stage of DR. Hemorrhaging of new vessels into the vitreous may also lead to tractional retinal detachment.

Cytotoxic effects of glucose

The exact mechanisms by which elevated glucose initiates the vascular disruption in DR remain poorly defined and several pathways have been implicated (Figure 12). For instance, hyperglycaemia may trigger apoptosis in endothelial cells. Retinal capillary endothelial cells and pericytes have been found to die by an apoptotic-like process in diabetic humans, and in diabetic or experimentally galactosemic rats (Mizutani et al., 1996). High ambient glucose levels increase glucose flux though the polyol pathway, which operates in both endothelium and pericytes that express aldose reductase (Knott and Forrester, 2003). Thus glucose may also be toxic by generating free redicals whose activity is increased because of depletion of glutathione (a powerful free-redical scavenger) by polyol pathway overactivity (Knott and Forrester, 2003). Excessive production of free radicals may lead to oxidative stress, changes in blood rheology and haemodynamics. In addition, increased intracellular glucose levels may activate protein kinase C, which can have various deleterious effects, such as basement membrane thickening and prolonged retinal circulation time. For example, activation of PKC could alter the expression of endothelium-derived vasoactive factors, leading to an increase of vascocontrictor actions of endothelin-1 and a decrease in the vasodilatory function of NO. Such alterations in vascoreactivity may further promote retinal vasoconstriction and increased resistance to blood flow, thus prolonging retinal circulation time (Way et al., 2001). More importantly, activation of PKC can induce endothelial cells to release entothelin-1, which is a potent vasoconstrictor that may decrease retinal perfusion, and vascular endothelial growth factors (VEGFs) that may break down the blood-brain barrier and also lead to neovascularisation (Knott and Forrester, 2003). Further, VEGF is thought to act via its receptor to phosphorylate specific tight-junction proteins, leading to separation of the tight junctions and eventually the break-down of the blood-retina barrier. Increased blood glucose concentration can also lead to formation of advanced glycation end-products (AGEs) by non enzymatic binding of glucose to protein side chains. Glycation of basement membrane

components may alter the membrane's electrochemical charge characteristics leading to increased vascular permeability, and also affect endothelial function.

Inflammatory mechanisms

There is increasing evidence that inflammation may also play an important role in the pathogenesis of DR (Ciulla et al., 2003). The stimulus for these inflammatory-like changes may be high glucose concentration. Leukostasis is an important component of the inflammatory process, and adhesion of leukocytes to the vascular wall has been found to be significantly increased in retinas of diabetic animals (Miyamoto and Ogura, 1999). Diabetes has been reported to increase expression of ICAM-1 (intracellular adhesion molecule-1) in the retina and interaction of this protein with the CD18 adhesion molecule on monocytes and neutrophils contributed to the diabetes-induced increase in leukostasis within retinal vessels (Joussen et al., 2002). Leukostasis has been postulated to be an important factor in death of retinal endothelial cells in diabetes (Miyamoto and Ogura, 1999; Joussen et al., 2001; Joussen et al., 2004). Large leukocytes cannot pass through unobstructured capillary beds and become logged in the narrow lumen, resulting in blockage of the affected capillary and reducing the retinal perfusion. In particular, leukocytes in diabetes are less deformable (Miyamoto and Ogura, 1999) and this, together with their inappropriate adherence and inherent capacity to produce toxic superoxide radicals has important implications for capillary non-perfusion, endothelial cell damage, and vascular leakage in the retinal microcirculation.

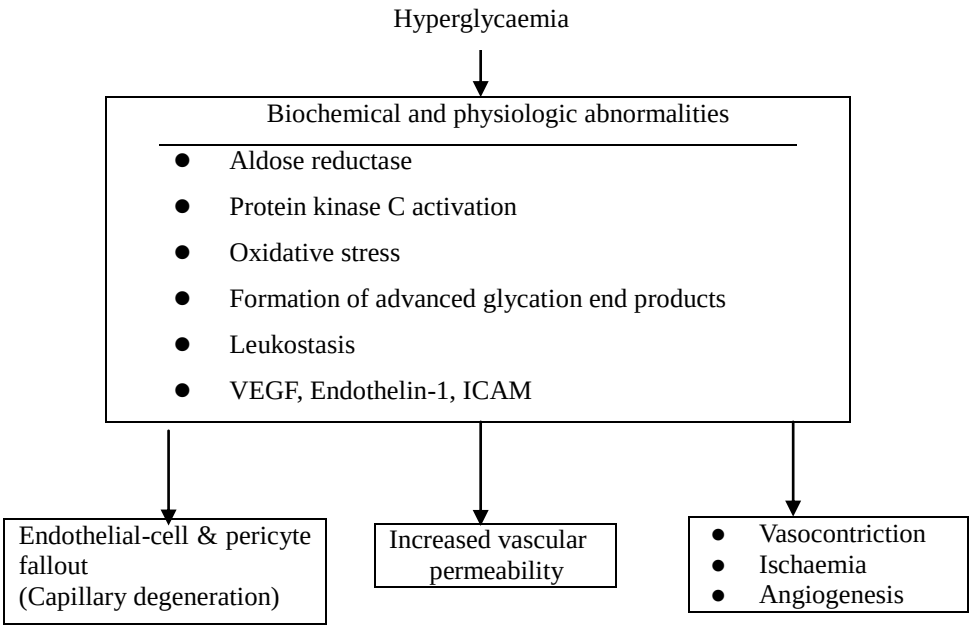


Figure 12. Possible pathways of glucose-induced damages in retinal vascular cells in diabetic retinopathy. VEGF, vascular endothelium-derived growth factor; ICAM, intracellular adhesion molecule.

Retinal vascular calibre changes

As described earlier, the end-artery system of the retina lacks any obvious autonomic nerve supply, and blood flow into the capillary beds is tightly regulated in response to the metabolic needs of the retinal parenchyma. This is achieved, in large part, by the regulatory capacity of the component smooth muscle cells (VSMc) in the retinal arteries and arterioles, which are highly sensitive to endothelial-generated vasodilators and vasocontrictors (Gardiner et al., 2007). Downstream from the precapillary arteriole, pericytes of the retinal capillary possess contractile properties. Together with VSMc, pericytes exert a major impact on retinal hemodynamics and fine control of blood flow through the capillaries (Gardiner et al., 2007).

Persistent dilatation of retinal arterioles has been well observed in diabetes (Kristinsson et al. 19997). Although the precise mechanisms through which diabetes triggers retinal arteriolar dilation remains unclear, Gardiner and his colleagues (Gardiner et al., 2007; Curtis et al., 2003) have proposed that the retinal arteriolar

smooth muscle cells become resistant to the vasoconstricting actions of endothelin-1. Retinal arteriolar dilation in diabetes may be further promoted by an inhibition of Ca^{+2} influx channels on smooth muscle cells and this occurs via hyperglycaemia-mediated overactivation of protein kinase C (Curtis and Scholfield, 2004). Arteriolar dilation and the associated increase in retinal blood flow may be exacerbated in the later stages of diabetic retinopathy through a drop in retinal oxygen tension secondary to capillary non-perfusion (Gardiner et al., 2007). According to the laws of Poiseuille's, arteriolar dilation decreases resistance to flow and increases hydrostatic pressure in the capillaries and venules, leading to increase in the rate of fluid movement from the vessel into the tissue (Gardiner et al., 2007). In this scenario, retinal arteriolar dilation may also account, at least in part, for early increases in retinal capillary permeability in diabetes. Furthermore hemodynamic abnormalities associated with diabetes can be accentuated by the increases in the retinal capillary and venular diameters that are seen in people with diabetes. These possibly represent a direct consequence of arteriolar dysfunction. Alternatively, retinal venular dilation may also reflect inflammatory processes implicated in the pathogenesis of impaired glucose metabolism or be related to endothelial dysfunction, reflecting an increased production of nitric oxide secondary to higher levels of cytokines (Caballero, 2005; Kolodjaschna et al., 2004; Wilkinson-Berka, 2004).

Most recently, it has been argued that before the onset of diabetic retinopathy, there is an early reduction in retinal perfusion and arteriolar calibre, followed by a gradual increase in both as the complication progresses (Curtis et al., 2009). Perhaps the discrepancy is due to different definitions of 'early', according to TA Gardiner (personal communication, 20 November 2009). The early dilatation which Gardiner referred to in the original studies was based on observation in human studies (perhaps several years of diabetes) and that was always in conflict with the very early observations of constriction observed in diabetic rats (only weeks after induction of diabetes). Alternatively, at the early stage there may be tissue hypoxia induced by arteriolar constriction that in the later stages progresses to hypoxia due to vessel loss. The normal vascular response to hypoxia is dilatation but in very early diabetic retinopathy the hypoxia is actually the result of arteriolar constriction and

vessels that do not respond to the hypoxia that they are causing. That constriction and failure to respond to the resultant hypoxia is due to hyperglycaemia-induced arteriolar dysfunction (Curtis et al., 2009).

1.5.5. Clinical stages and features of diabetic retinopathy

The natural history of diabetic retinopathy has been classically divided into an early, nonproliferative stage, and a later, proliferative stage, including a spectrum of retinal vascular signs. Currently it can be diagnosed either through ophthalmoscopy or retinal photography. Although there is a lack of pathognomonic signs, the individual features are associated in distinct diagnostic patterns. Except for microaneurysms, it is unusual for one clinical sign to occur independently (Towler and Lightman, 2003).

Nonproliferative diabetic retinopathy

In this stage, microaneurysms and other individual lesions caused by microvascular leakage, including hard exudates and haemorrhages, are the features of so-called 'background diabetic retinopathy'. They may appear and regress without obvious warning signs. Unless maculopathy coexists, affecting the vision, patients are often unaware that they suffer from the disease until it progresses to more severe levels. When microvascular occlusion becomes more extensive, lesions including venous beading or looping, intraretinal microvascular abnormalities (IRMAs) and abundant cotton-wool spots are common. These changes, previously termed 'preproliferative retinopathy' indicate worsening retinal ischaemia and carry a high risk of progression to neovascularization and the proliferative phrase of diabetic retinopathy (Towler and Lightman, 2003).

Microaneurysms

Microaneurysms are outpouchings of capillaries and are the earliest clinically detectable signs of diabetic retinopathy. They arise mostly due to asymmetrical dilatations of weakened capillary walls or endothelial buds attempting to revascularise an ischemic retina. They appear as tiny red spots, varying in size from 10 to 150 μm and commonly temporal to the macula (Figure 13 (b)). Although microaneurysms are not static features and may even disappear, sudden appearance

of numerous microaneurysms is an indication of worsening retinal ischaemia.

Haemorrhages

Haemorrhages occur due to the rupture of weakened capillaries. They appear either as small red dots or blots indistinguishable from microaneurysms or as larger flame-shaped haemorrhages (Figure 13 (b)). The former are often present within the densely packed deeper layers of the retina while the latter occur in the more superficial nerve fiber layer. As the retinal vessels become more damaged and leaky, their numbers increase.

Hard exudates

Hard exudates (HE) are the result of precipitation of lipoproteins and other circulating proteins through abnormally leaky retinal vessels including microaneurysms. They are typically manifested as random yellowish/white patches of varying sizes, shapes and locations (Figure 13 (c)). These are either seen as individual spots, clusters, or are found in large rings around leaking capillaries. Hard exudates encroaching upon the macula may severely compromise vision. Although exudates may absorb spontaneously, they usually tend to increase in volume in an untreated retina

Cotton-wool spots

Cotton-wool spots (CWS) come about due to occlusion of retinal pre-capillary arterioles supplying the nerve fibre layer, with concurrent swelling of local nerve fibre axons. These abnormalities usually appear as little fluffy round or oval areas in the retina with a whitish colour, usually adjacent to an area of haemorrhage (Figure 13 (c)).

Intraretinal microvascular abnormalities

Intraretinal microvascular abnormalities (IRMAs) are clusters of irregular vascular segments within the retina (Figure 13 (d)). They may result from dilated preexisting capillaries or represent limited new vessel formation. They often arise within large areas of capillary non-perfusion and when several are present they indicate severe non-proliferative diabetic retinopathy that may rapidly progress to the proliferative stage.

Venous changes: venous beading, looping or reduplication

Venous beading (VB) occurs where there is widespread capillary ischaemia adjacent to a vein and may represent foci of endothelial cell proliferation that have failed to develop into new vessels. It consists of localised dilatation in venous calibre with an appearance resembling a string of sausages (Figure 13(e)), which is often found adjacent to IRMA. Venous beading is a sign of severe non-proliferative diabetic retinopathy and a strong predictor of neovascularisation. Venous loops, also known as omega loops, appear as an abrupt, curving deviation of a vein from its normal path. They are less frequent than beading. Venous reduplication may arise from revascularisation around areas of venous occlusion. They appear as dilatation of a pre-existing channel or the proliferation of a new channel of similar calibre adjacent to the original vein.

Proliferative retinopathy

This stage is characterised by new vessel formation which appears as arcade of abnormal structures normally occuring on the optic disc (new vessel disc or NVD) or elsewhere on the retina (new vessel elsewhere or NVE). Abnormal new vasculature may threaten vision due to bleeding which causes complications such as preretinal haemorrhage and glucoma. Fibrous tissue and haemorrhages may also accompany and can lead to tractional retinal detachment. NVD carries a worse prognosis than NVE and if left untreated often leads to vitreous haemorrhage and an increased chance of blindness.

New vessels elsewhere or on the disc

New vessel formation is stimulated by retinal ischaemia of its inner layers secondary to regional closure of the capillary bed. Retinal hypoxia triggers a variety of vasoproliferative factors, such as vascular endothelia growth factors (VEGFs), resulting in a neovascular response locally and by diffusing through the vitreous to other areas of retina, to the optic disc and into the anterior chamber. New vessels elsewhere (NVE) are on the surface of the retina or further forward in the vitreous cavity. They appear as very fine, branching or loops of vessels that develop from the venous sides of the capillary network adjacent to an area of retinal ischaemia (Figure 13 (f)). In the absence of stereo biomicroscopic examination, it may be difficult to

distinguish subtle new vessels from IRMA. New vessels on the disc (NVD) lie on the disc or within 1 disc diameter of its margin.

Fibrous proliferation elsewhere or on the disc

This fibrous tissue occurs as a supportive cellular matrix accompanying retinal neovascularization, which persists even if the new vessels involute. They appear as grayish-white sheets or fine strands opaque enough to be definitely seen (Figure 13 (g)).

Preretinal haemorrgage

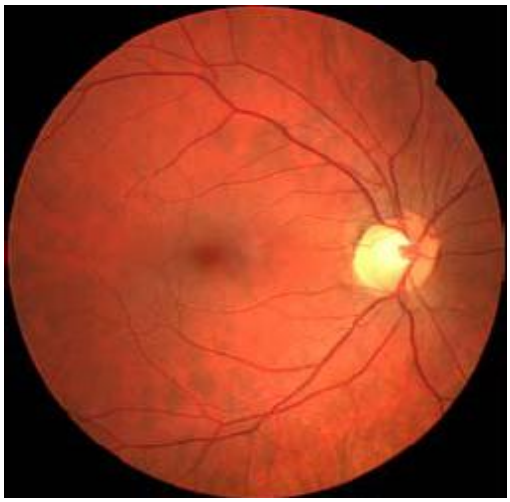
Preretinal haemorrhages (PRH) are located just anterior to the retina or under the internal limiting membrane. They may remain encapsulated and appear as round, oval or linear patches, or boat-shaped haemorrhages with a fluid level which obscures the underlying retina (Figure 13 (h)). They may reabsorb slowly or may need laser or surgical treatment.

Vitreous haemorrhage

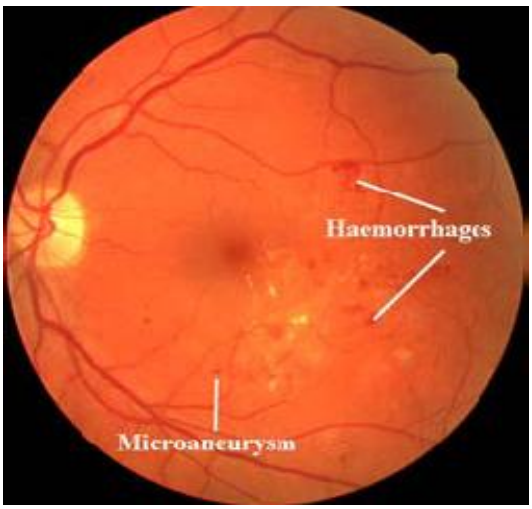
Vitreous haemorrhages (VH) extend further forward into the vitreous cavity than PRH (Figure 13 (i)). They arise from bleeding from new vessels anterior to the retina as well as fibrovascular proliferations.

Diabetic maculopathy

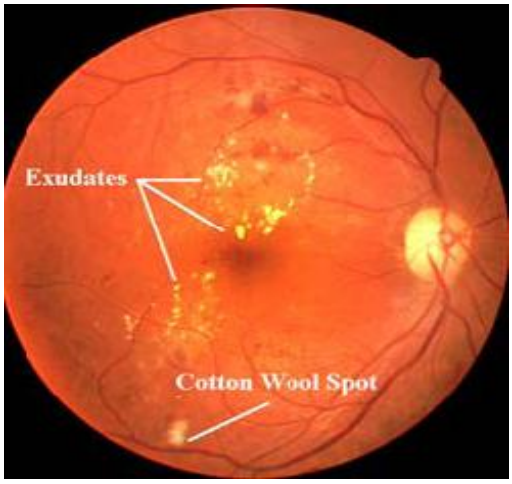
Diabetic maculopathy is characterised by macular oedema and/or ischaemia. It can accompany any stage of DR and may affect central vision (Hudson, 2008). Clinically significant macular oedema comprises any one of the following: 1) Retinal thickening within 500 μm of the fovea; 2) Hard exudates within 500 μm of the fovea; 3) Retinal thickening that is larger than one disc diameter, any part of which is within one disc diameter of the fovea.



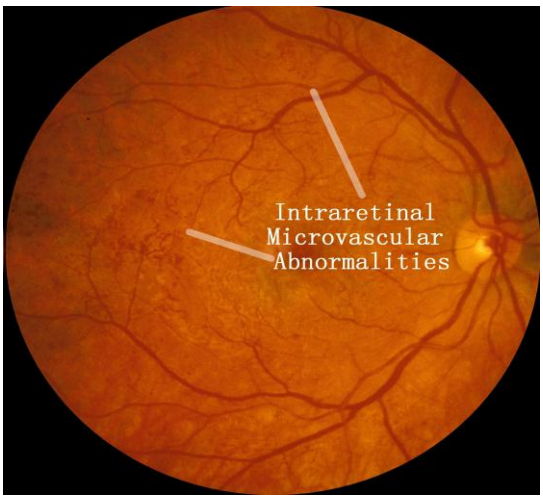
(a)


Haemorrhages
Microaneurysm

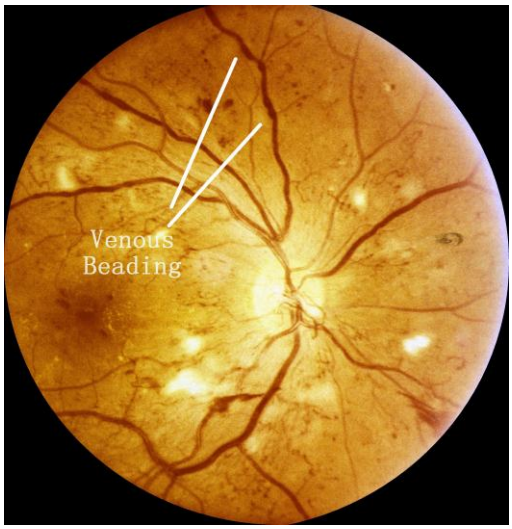
(b)


Exudates
Cotton Wool Spot

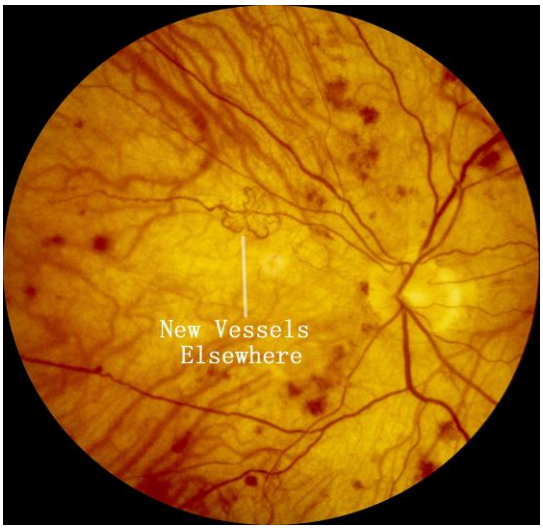
(c)


Intraretinal
Microvascular
Abnormalities

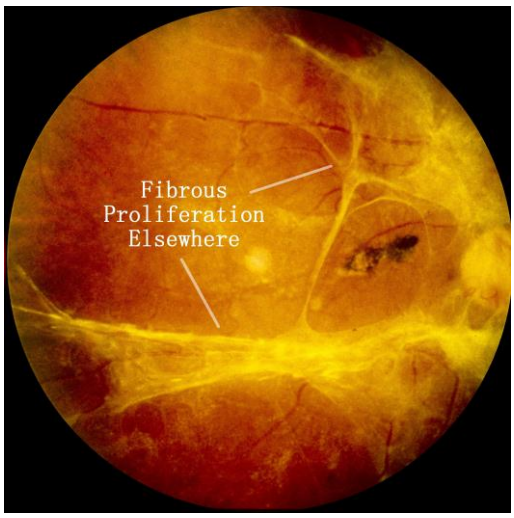
(d)


Venous
Beading

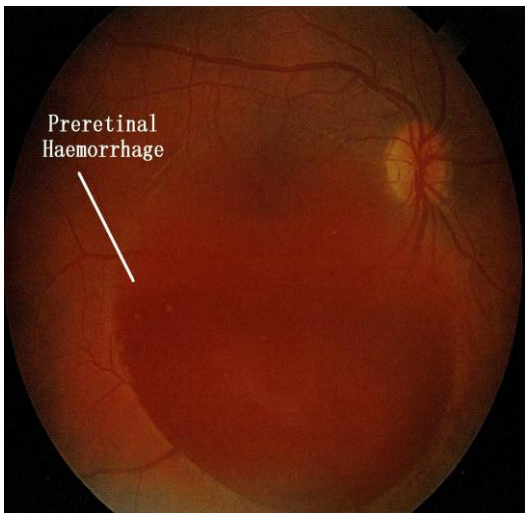
(e)


New Vessels
Elsewhere

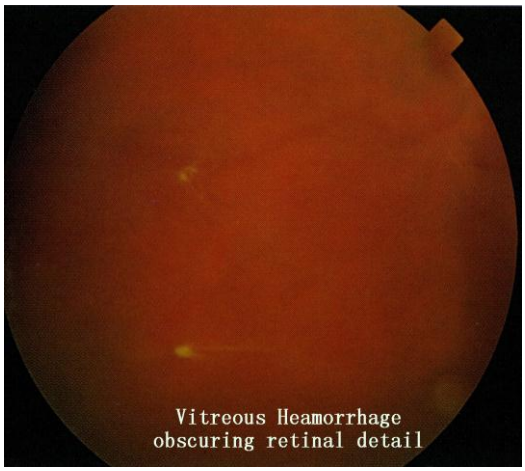
(f)



(g)



(h)



(i)

Figure 13: Normal and abnormal diabetic retinopathy (DR) images. A typical normal retinal image (a), DR showing microaneurysms and haemorrhages (b), DR with cotton wool spots and exudates (c), intraretinal microvascular abnormalities (IRMAs) (d), venous beading (e), new vessels elsewhere (f), fibrous tissue elsewhere (g), preretinal haemorrhage (the uncoagulated blood) (h), and vitreous haemorrhage obscuring retinal detail (i) (Adapted from Towle & Lightman, 2003; Osareh, 2004; http://www.glostruphospital.dk/menu/Afdelinger/Oejenafdelingen/Oculus/EURODIAB.htm)

1.5.6. Prevalence and incidence of diabetic retinopathy

The prevalence and incidence of DR in diabetic populations have been measured in several epidemiological studies using a variety of ascertainment methods. Some have used direct opthalmoloscopic examination whereas others have

employed a number of different retinal photographic techniques (e.g. stereoscopic vs. nonstereoscopic, the number of fields taken). More reports have combined the results of Type 1 and Type 2 diabetes, although most have concentrated on Type 2. Few studies have focused on older people with diabetes. In general, the prevalence of DR varies with the age of the diabetic cohort studied, diabetes duration, the risk factor profile of the cohort, the grading systems used to measure DR and definitions for diabetes and DR severity.

Many epidemiological studies have assessed the prevalence of DR by using the modified Airlie House classification and its extension of the Early Treatment Diabetic Retinopathy Study (ETDRS) classification systems (ETDRS Report No.10, 1991). Overall, the estimated prevalence of DR (of any level of severity) assessed by using this classification system ranges from 18.2 to 54.1% (Varma et al., 2004). For example, in the Beaver Dam Study of 445 diabetic individuals aged 43-86 years the prevalence of any DR was 36.8%, with 1.8% of subjects having proliferative retinopathy (PDR) (Klein et al., 1992a). Similarly, the Los Angeles Latino Eye Study on over 1187 diabetic individuals (age $\geq$ 40 years) estimated the prevalence of any DR at 45.8% and PDR at 5.6% (Varma et al., 2004). The Wisconsin Epidemiologic Study of Diabetic Retinopathy (WESDR) reported a higher prevalence of 50% for people who had a diagnosis of diabetes aged between 50 and 69 years (Klein et al., 1984). This higher prevalence may reflect poorer diabetes management and the late diagnosis of diabetes in the earlier years. Table 2 lists the population-based studies that have estimated the prevalence of DR using the ETDRS classification system or other method in people with Type 2 diabetes. This shows that the prevalence of any DR varied across studies with an overall increase according to mean duration of diabetes in study participants. For example, the prevalence of any DR in people with newly diagnosed diabetes was lower than in those with known diabetes and varied from 1.5 to 15.8%. The differences in reported prevalences of retinopathy in people with newly discovered Type 2 diabetes may be in part due to variations in the time between onset and detection of DM, reflecting variations in the access to and availability of medical care, as well as to different definitions of DM (Klein et al., 1992a).

Age- and gender- related differences have not been reported consistently and in most reports, it was difficult to seperate out the influence of Type 1 diabetes, as the results were not stratified by type of diabetes. Based on data from the Los Angeles Latino Eye study (Varma et al., 2004) in which Type 1 diabetes (n=30) made up only 2.5% of all diabetics (n=1217), the prevalence of any DR and PDR rose steadily with every 10-year increase in age from the age of 40 years (from 40.7 to 55.0% for DR, 4.4 to 8.2% for PDR) whereas a decrease in prevalence of any DR or PDR was seen among those aged 80 years and older (40.0% and 3.3% respectively). The age-adjusted prevalence of any DR was similar in men (49.6%) compared with women (45.0%). In support of these findings, Leske (Leske et al. 1999) reported 28.9% prevalence of any DR in men and 28.2% in women and there was no age-specific difference when a very small percentage of Type 1 diabetics (1.9%) were included. A lower prevalence of DR in age groups over 80 relative to younger age groups in the Latino Eye study may stem from either increased mortality in diabetic patients with the most severe disease and complications, or could reflect a reduced prevalence of DR in older diabetics with newly diagnosed diabetes. With respect to race, in the National Health and Nutrition Examination Survey (NHANES) III, Whites had a lower prevalence rate of any DR relative to either Hispanics or African Americans. Specifically Hispanics experienced the highest prevalence rates irrespective of duration of diabetes (Harris et al., 1998; Varma et al., 2004).

Compared with prevalence studies, there are far fewer reports on the incidence and progression of diabetic retinopathy in population-based studies. In the WESDR, incidence of any DR was based on 533 diabetic patients diagnosed at age 30 years or older and DR was assessed by 7-field retinal photographs using ETDRS protocols. The 10-year cumulative incidence of DR was 73% and progression to PDR was 17% for patients who were free of DR at the baseline examination (Klein et al., 1994a). In the Liverpool Diabetic Eye study of 3742 patients with Type 2 diabetes aged 56-72 years old the 5-year cumulative incidence of any DR was 30.5% based on 3-field photographic gradings (Younis et al., 2003). In another study in Taiwan, of 471 Chinese diabetic patients aged over 40 years old, the 4-year

cumulative incidence of any DR was 4.8% and progression to PDR 1.5% when DR was determined by opthalmoscopy (Chen et al., 1995). These results show that the incidence in populations may vary greatly depending on geographical regions, socioeconomic factors and characteristics of the diabetic population as well as the epidemiological methods used, such as definition of diabetes and detection and classification of DR (The Eye Disease Prevalence Research Group, 2004). Whether there is a variation with age or gender remains unclear based on the limited data.

Table 2: Prevalence of diabetic retinopathy in middle-aged and older people with Type 2 diabetes from different population-based studies

Study (Author, year)	Setting	No. of participants with DM & Age (years)[a]	Method of Quantifying Characteristics	DM duration (years)[b]	Prevalence of DR (%)	
					Any DR	PDR
WESDR (Klein et al., 1984)	Wisconsin USA	1,370 MW 54.8±12.4 (Dx)	Photographic grading, 7 fields	10.6±8.2	46.6 66.9 (Dx 30-49 yrs) 50 (Dx 50-69 yrs) 34.2 (Dx ≥70 yrs)	6
(Segato et al., 1991)	Italy	N/A MW ≥70			24.6	
Beaver Dam Study (Klein et al., 1992a)	Beaver Dam Wisconsin USA	445 MW ≥43	Photographic grading, 3 fields	New Dx Pervious Dx	10.2 38.7	0 1.7
(Chen et al., 1992)	Taiwan China	527 MW ≥40	Ophthalmoscopic grading	New Dx <4 5-9 >10	23 35.3 49.3 55.1 50/26.8 (M/W, 40-49 yrs) 37.1/32.7(M/W, 50-59 yrs) 38.3/38.6 (M/W, 60-69 yrs) 33.3/42.1 (M/W, 70+yrs)	 0.5 3.2 4.0 0/0 1.4/1.0 0.9/3.4 2.4/8.8
Exeter Diabetic Retinopathy Screening (EDRS) Programme (Ling et al., 2002)	Exeter UK	671 MW N/A	Photographic grading, 1 field		27.4	2
Blue Mountains Study (Mitchell et al., 1998)	Sydney Australia	249 MW ≥49	Photographic grading, 5 fields	New Dx Pervious Dx	15.8 35.7	

NHANES III (Harris et al., 1998)	USA	1180 MW ≥40 Blacks Latinos Non-Hispanic whites	Photographic grading, 1 field	 New Dx Pervious Dx New Dx Pervious Dx New Dx Pervious Dx	 1.5 26.5 9.9 33.4 7.7 18.2	
Barbados Eye Study (Leske et al., 1999)	USA	624 MW ≥40 Blacks/mixed	Photographic grading, 2 fields	 New Dx Pervious Dx	27.5 4.2 30.5	
(de Fine Olivarius et al., 2001)	Denmark	105 MW ≥80	Ophthalmoscopic grading		8.6	0
Los Angeles Latino Eye Study (Varma et al., 2004)	Los Angeles USA	1187 MW ≥40	Photographic grading, 7 fields		45.8	5.6
(Kato et al., 2002)	Tokyo Japan	3028 MW ≥40	Ophthalmoscopic grading	 ≤5 >5	43(65+ yrs) 23(65+ yrs) 44(65+ yrs) 40 (40-64 yrs)	16(65+ yrs) 23(40-64 yrs)
Australian Diabetes, Obesity and Lifestyle study (AusDiab) (Tapp et al., 2003)	Australia	333 MW 65±11[c]	Photographic grading, 2 fields	7(0-15)[c] 0-4 5-9 10-19 ≥20	21.9 9.2 23.1 33.3 57.1	2.1
Arhus County study (Hove et al., 2004)	Arhus County Denmark	378 MW 65±12	Photographic grading, 2 fields	9±8	31.5	2.9

[a, b]min-max or ±SD; [c]Diagnosed with DR only; DR: diabetic retinopathy; Dx: diagnosed; M: men; W: women; NHANES III:Third National Health and Nutrition Examination Survey; WESDR: The Wisconsin Epidemiologic Study of Diabetic Retinopathy.

1.6. Diabetic retinopathy, retinal vascular calibre and systemic factors

Two decades ago, the Framingham Heart and Eye Study proposed that diabetic retinopathy signs may reflect generalised microangiopathic processes that affect not only the eyes but also organs elsewhere in the body (Hiller et al., 1988). In recent years, using standardised and advanced retinal photographic methods such as high-resolution digital imaging to evaluate retinal vascular changes in diabetes, population-based studies have more precisely quantified the associations of these retinal vascular changes with a diverse range of environmental and genetic factors as well as systemic vascular complications. It has now become evident that diabetic retinopathy and other subtle vascular signs including arteriolar and venular calibre changes, might be biomarkers of underlying widespread deleterious effects of abnormal glucose metabolism on the systemic microcirculation and disease (Cheung and Wong, 2008). This section provides a brief summary of associations between diabetic retinopathy, changes in retinal vessel calibre and systemic factors, including vascular risk factors and vascular disease. Other retinal microvascular abnormalities such as venular tortuosity and changes in arteriolar bifurcation geometry are described in the next section.

1.6.1. Associations with age, gender and ethnicity

As described earlier, the relationship between diabetic retinopathy and age or gender in Type 2 diabetes is not clear based on available data. However, an inverse association between age and retinal vascular calibre has been consistently demonstrated across different study populations including in people with Type 2 diabetes (Wong et al., 2003a; Leung et al., 2003a; Wong et al., 2006a; Kawasaki et al., 2006; Klein et al., 2006). In the Wisconsin Epidemiological Study of Diabetic Retinopathy (WESDR), retinal arteriolar and venular calibre decreased from 2.0-3.0 μm for each decade increase in age, independent of sex, refractive error, arterial blood pressure and other risk factors (Klein et al., 2006). Moreover, after accounting for the correlation between retinal arteriolar and venular calibre, the inverse association between retinal vascular (both arteriolar and venular) calibre and age was

largely unchanged (Kaushik et al., 2007).

Whereas many studies have examined the relationship between age and retinal vascular calibre, few population-based studies have evaluated the influence of sex or ethnicity on retinal vascular calibre, and none of them focused on diabetic people. Results of studies from the general population are inconsistent. In the Blue Mountains Eye study (Leung et al., 2003a), both mean retinal arteriolar calibre and AVR (a ratio between the average diameters of arterioles with respect to venules) were consistently higher in women than men across all age groups, in line with findings from the Cardiovascular Health Study and MESA (Wong et al., 2003b; Wong et al., 2006a) but these sex differences could not be confirmed in reports from other studies (Wong et al., 2005a; Leung et al., 2004). There is no adequate explanation for these inconsistent findings for men and women.

With respect to ethnicity, both retinal arteriolar and venular calibre were significantly greater in black and Hispanic participants than in white and Chinese Americans in the general population (Wong et al., 2006a). Also as mentioned earlier, a higher prevalence of diabetic retinopathy was found in Hispanic and African Americans than Whites in people with Type 2 diabetes (Harris et al., 1998; Varma et al., 2004). A greater exposure to both genetic and/or environmental risk factors leading to obesity, diabetes, hypertension and hyperlipidaemia, may in part account for the observed ethnic differences in retinal vascular changes. It is also possible that these differences may partly reflect variations in inheritance of vascular risk factors, susceptibility to their effects, or other processes not examined in these studies, such as potential measurement error in quantifying retinal vascular calibre caused by ocular pigmentation (Rochtchina et al., 2008).

1.6.2. Associations with duration of diabetes and glycaemic control

There is clear evidence that longer duration of diabetes and poorer glycaemic control are the major risk factors not only for the incidence and prevalence of diabetic retinopathy but also progression of existing retinopathy. For example, the

Australian Diabetes, Obesity and Lifestyle study (Ausdiab) showed a linear trend between DR prevalence and diabetes duration in people with Type 2 diabetes. The prevalence of any DR was less than 10% in those with diabetes duration of less than 5 years, but more than 50% in those with diabetes duration of 20 years or longer (Tapp et al., 2003). The importance of glycaemic control as indexed by glycosylated haemoglobin has been demonstrated in large-scale observational studies (Klein et al., 1988) and in two pivotal clinical trials, including the United Kingdom Prospective Diabetes Study (UKPDS) in patients with Type 2 diabetes (DCCT research group, 1993; UKPDS 33, 1998). In the UKPDS, there was a reduction in the 12-year rate of progression of diabetic retinopathy of 21% and reduction in need for laser photocoagulation of 29% in the intensive vs. the conventional treatment group (UKPDS 33, 1998). However, in a more recent report from the Action in Diabetes and Vascular Disease (ADVANCE) clinical trial with 11,140 patients with Type 2 diabetes, further intensive glucose control (near normalisation of blood glucose defined as keeping HbA1c levels close to 6%) had no effect in incidence and progression of DR (Patel et al., 2008). Moreover, a pooled analysis of 11,000 participants from three population cohorts showed that retinopathy lesions characteristic of diabetes were found in 7.4% to 13.4% of non-diabetic participants and were present even in individuals with normal blood glucose independent of blood pressure (Wong et al., 2008). These results, together with the observed limit to the risk reduction for DR through tighter glucose lowering, suggest that processes other than hyperglycaemia may also contribute to the development of DR (Nguyen and Wong, 2009).

In general, patients with shorter duration of diabetes (<5 years) show a constriction of the major arteries and arterioles (Wong et al., 2002a). With more prolonged diabetes duration and concomitant presence of clinical retinopathy, arterial vessels begin to dilate accompanied or followed by venular dilatation (Curtis et al., 2009). In the WESDR, each 10-year increase in diabetes duration was associated with a 0.26μm increase in arteriolar calibre in people with Type 2 diabetes (Klein et al., 2006). In Type 1 diabetes, wider venular calibre was associated with longer duration of diabetes (Klein et al., 2003). Wider retinal arterioles also have been found to be associated with incidence of retinopathy (Klein et al., 2007; Rogers et al., 2008;

Cheung et al., 2008), whereas wider venules have been associated with progression of retinopathy (Klein et al., 2004a) in people with diabetes. Similarly, higher glycosylated haemoglobin was associated with both wider retinal arteriolar and venular calibre in Type 1 diabetes (Klein et al., 2003) and wider venular calibre in Type 2 diabetes or individuals without diabetes (Klein et al., 2006; Nguyen et al., 2008a).

1.6.3. Associations with hypertension

Raised blood pressure is another major risk factor for both the initial development of diabetic retinopathy and its subsequent progression. Impaired retinal vascular autoregulation, endothelial dysfunction and increased expression of vascular endothelial growth factors secondary to hypertension may play at least a partial role in this association in diabetes (Klein and Klein, 2002). The UKPDS trial also showed blood pressure control reduced the risk of DR independent of glycaemia levels in patients with Type 2 diabetes (UKPDS 38, 1998). However, similar to tighter glucose lowering, there seems to be a threshold limit to the risk reduction for DR by further reduction of blood pressure (Patel et al., 2008; Mitchell and Wong, 2008), which suggests other processes apart from elevated blood pressure and hyperglycaemia may also be involved.

In distinct contrast to the association of hyperglycaemia with retinal arteriolar calibre, an inverse relationship between elevated current blood pressure and retinal arteriolar narrowing has been well established in general populations and in diabetic adults (Sharrett et al, 1999; Wong et al., 2002b; Klein et al. 1994b; Wong et al., 2003a; Leung et al., 2003a; Wong et al., 2006a; Klein et al., 2006). It has long been known that generalised retinal arteriolar narrowing is an early characteristic sign of hypertensive retinopathy (Wong and Mitchell, 2004). Impaired endothelium-dependent vasodilatation in essential hypertension caused by decreased nitric oxide levels may play a role in the narrowing of retinal arteriolar calibre (Sun et al., 2009). Moreover, lower AVR and narrowed arteriolar calibre have been found to be independently associated with raised past blood pressure levels, measured up to 10 years prior to the retinal assessment, suggesting that retinal arteriolar calibre

changes reflect persistent damage from long-term hypertension (Sharrett et al., 1999; Leung et al., 2004; Sun et al., 2009). Of greater significance are prospective findings that retinal arteriolar narrowing is also a pre-clinical marker of hypertension risk in initially normotensive individuals (Ikram et al., 2006a; Smith et al., 2004; Wong et al., 2004a, b) which further supports the hypothesis that peripheral vascular resistence, reflected by retinal arteriolar narrowing, is an important contributing factor for hypertension (Nguyen et al., 2007). Contrary to the findings in arterioles, retinal venules tend to widen rather than narrow with increasing blood pressure and wider retinal venular calibre predicted incident hypertension (Ikram et al., 2006b; Liew et al., 2006).

1.6.4. Associations with dyslipidaemia

The role of higher blood lipids levels, including total cholesterol, low-density lipoprotein (LDL), and triglycerides, in the development of retinal hard exudates has been well recognised in people with diabetes (Klein et al., 1991; Chew et al., 1996; van Leiden et al., 2002). Recent results from the Fenofibrate Intervention and Event Lowering in Diabetes (FIELD) study suggest that lipid-lowering therapy may reduce retinopathy requiring laser treatment in people with Type 2 diabetes (Keech et al., 2005).

Dyslipidaemia may also have an effect on retinal vascular calibre size in the general population. For example, larger retinal venular calibre has been reported to be independently related to higher triglyceride and lower HDL cholesterol levels (Sun et al., 2009). It has been proposed that inflammation and endothelial dysfunction might play a role in these relationships. As yet, no such data are available in people with diabetes.

1.6.5. Associations with obesity

The influence of obesity on diabetic retinopathy has been investigated in several studies. Although a positive relationship between higher body mass index and increased risk of diabetic retinopathy has been demonstrated in most studies (UKPDS 34, 1998; Zhang et al., 2001; Chaturvedi et al., 2001; Keen et al., 2001;

Henricsson et al., 2003), conflicting data were reported in the WESDR (Klein et al., 1984; Klein et al. 1997). In older-onset diabetic patients (diagnosed as having diabetes at age 30 years or older), there was a 3-fold increase in risk of developing retinopathy for those who were underweight (BMI <20 kg/m^2). On the other hand, obesity (BMI >31.0 kg/m^2 for men and 32.1 kg/m^2 for women) was not found to associate with either progression or severity of retinopathy. It has been suggested that underweight may be a marker of the more 'severe' phase of diabetes, or an indicator of late-onset type 1 diabetes (Klein et al., 1997). More recently, larger waist-to-hip ratio but not body mass index has been reported to be independently associated with a number of incident individual retinopathy signs in a non-diabetic population (van Leiden et al., 2002). Obesity correlates with many common vascular risk factors of diabetic retinopathy, including hyperglycaemia, hyerlipidemia and hypertension. It may also have a more direct effect on the development of DR, such as inducing oxidative stress and increasing expression of the vascular endothelial growth factors (VEGFs) (Cheung and Wong, 2007).

Both cross-sectional and prospective studies have shown that obesity is associated with larger retinal venular calibre and lower AVR in the general population (Wang et al., 2006) and in people with diabetes (Klein et al., 2003; Klein et al., 2006). Elevated markers of endothelial activation and chronic vascular inflammation have been observed to correlate with retinal venular dilatation. Furthermore, obesity is also linked with increased blood volume and leptin levels, which might modulate vascular calibre through local mechanisms involving nitric oxide release (Oren et al. 1996; Vecchione et al., 2002; Caballero et al., 2005; Surmacz, 2007). These factors may interact to contribute to the larger venular diameter in obese individuals.

1.6.6. Associations with smoking

The effect of cigarette smoking has been examined, producing contradictory findings. Some cross-sectional studies reported that cigarette-smoking was associated with an increased risk of proliferative diabetic retinopathy (Patekau et al., 1977; Muhlhauser et al., 1986), whereas others found no significant association with DR

(Moss et al., 1991). An inverse relationship of smoking status was also detected in relation to the incidence and progression of retinopathy in Type 2 diabetic subjects (Stratton et al., 2001). Possibly, a positive association of smoking with proliferative DR is partly due to elevated carboxyhaemoglobin levels induced by smoking, which may further hamper oxygen delivery to a hypoxic sensory retina and eventually lead to retinal neovascularization (Muhlhauser et al., 1986).

In the WESDR of Type 2 diabetic population, current cigarette smoking was associated with both larger retinal arteriolar and venular calibre, and the number of pack-years smoking was linearly related to larger venular calibre (Klein et al., 2006). These results are in good agreement with cross-sectional findings from Type 1 diabetic people and non-diabetic population (Klein et al. 2003; Klein et al., 2000; Kifley et al., 2007). While the mechanisms involved in retinal vascular dilatation in smokers are not yet known, venular widening has been well observed clinically in cigarette smokers (Stefansson et al., 1983; Rosenberg et al., 2005; Jeganathan et al., 2005). It has been postulated that the association of smoking with venular dilatation may involve higher carbon monoxide levels and endothelium-dependent relaxation, which may result in a decrease in oxygen supply to retinal thus leading to retinal venular dilatation (Stefansson et al., 1983; Rahman et al., 2007).

1.6.7. Associations with alcohol intake

The role of alcohol intake in diabetic retinopathy also remains uncertain. For example, an English clinic-based prospective study of 296 diabetic men free of retinopathy at baseline examination found that heavy drinkers (>10 pints beer per day) were three times as likely to develop exudates or proliferative DR (Young et al., 1984). Conversely, WESDR investigators found no association between alcohol intake (classified as none, light, moderate, and heavy) and the incidence or progression of DR (Moss et al. 1994). The discrepancy in results could have arisen from differences in classification or cut-off levels of the exposure variable, with less statistical power to detect a significant association in the WESDR due to relatively low exposure levels compared with the British cohort (McCarty et al., 2001).

So far only a few studies have examined the association of alcohol drinking with retinal vascular calibre in the general population and results are inconsistent. The AVR was low in those who drink over 20 grams per day in the Rotterdam Study, and lower AVR was ssociated with alcohol consumption in the ARIC study (Ikram et al., 2004; Klein et al., 2000). Although the former study did not find an effect of alcohol consumption on retinal arteriolar or venular calibre, cross-sectional data from the MESA indicated that current alcohol intake was related to smaller retinal arteriolar calibre (Wong et al., 2006a). The exact mechanism of the influence of alcohol on the variation of retinal vascular calibre, however, remains to be elucidated.

1.6.8. Associations with coronary heart disease

It is now clear that in people with Type 2 diabetes, diabetic retinopathy signs are associated with an increased risk of myocardial infarction (MI), coronary heart disease (CHD) and heart failure (Cheung and Wong, 2008). For instance, in the ARIC study, the presence of any DR was associated with a twofold higher risk of incident coronary heart disease (defined as MI, fatal CHD, and myocardial revascularization) and incident MI, threefold higher risk of fatal CHD, and fourfold higher risk of congestive heart failure (Cheung et al., 2007a; Wong et al., 2005b). These results were independent of diabetes duration, glycaemic control and other cardiovascular risk factors. There was also a graded association between increasing diabetic retinopathy severity and increasing risk of coronary heart disease (Cheung et al., 2007a). The findings are consistent with data from the World Health Organization Multinational Study of Vascular Disease in Diabetes (WHO-MSVDD) (Fuller et al., 2001), and other studies demonstrating associations of not only NDPR but also PDR with ischaemic heart disease (Miettinen et al., 1996; Faglia et al., 2002; Juutilainen et al., 2007).

These associations, together with other observations such as an association of DR with impaired myocardial perfusion and severity of coronary artery stenosis on angiography, support the concept that microvascular and macrovascular complications of diabetes share common pathogenic mechanisms beyond the

traditional cardiovascular risk factors adjusted for in most studies. It has been suggested that retinopathy is a manifestation of generalised vascular dysfunction caused by endothelial dysfunction or genetically determined alterations in the basement membrane metabolism associated with hyperglycaemia (Cheung and Wong, 2008). These vascular effects may increase arterial or arteriolar wall permeability and leakage, which further facilitate the pathogenic cascade of atherosclerosis formation in large arteries (Norgaz et al., 2005; Cheung et al., 2007a). Alternatively, rather than being a simple indicator of a more adverse cardiovascular profile, microvascular disease (indicated by the presence of retinopathy) may play a causal role by providing the inflammatory drive in the development of atherosclerotic disease in people with Type 2 diabetes.

Other circulatory mechanisms may also underlie a causal link between diabetic retinopathy and coronary heart disease. For example, diabetic retinopathy may represent widespread systemic microcirculatory (resistance vessel) disease, and places an increased impedance burden on the diabetic heart, predisposing to the development of diabetic cardiomyopathy and heart failure (Cheung and Wong, 2008).

There is also good evidence from prospective studies for an association between retinal vascular calibre and coronary heart disease. For example, prospective data from the Cardiovascular Health Study demonstrated that both smaller retinal arteriolar calibre and larger venular calibre, which contributed to a lower AVR, were independently associated with 5-year risk of coronary heart disease in elderly people (Wong et al., 2006b). In individuals with Type 1 diabetes, lower AVR was associated with incident myocardial infarction (Klein et al., 2004b). However, no such data are available to date in Type 2 diabetes, except for the findings that both smaller retinal arterioles and larger venules predicted 22-year cardiovascular mortality caused by stroke in a Type 2 diabetic cohort (Klein et al., 2006).

1.6.9. Associations with stroke and sub-clinical cerebrovascular disease

In 1975, an autopsy study of patients with stroke showed a close correlation of retinal and cerebral arteriolar pathology (Goto et al., 1975). Since then, the presence of retinopathy has been shown to be associated with stroke, particularly in people with hypertension (Svärdsudd et al., 1978; Petitti and Bhatt, 1995; Nakayama et al., 1997). New population-based studies, using standardised photographic assessment of retinal images to ascertain retinopathy lesions, have confirmed these early observations in diabetes (Cheung and Wong, 2008).

In the WESDR, PDR was associated with mortality from stroke in people with Type 2 diabetes, independent of diabetes duration, glycaemic control and other risk factors (Klein et al., 1992b; Klein et al., 1999). Data from the WHO-MSVDD demonstrated that the presence of retinopathy was also associated with incidence of stroke in both men and women with Type 2 diabetes (Fuller et al., 2001). More recently, in the ARIC study with 1, 617 middle-aged white and black Americans with Type 2 diabetes, retinopathy signs (the presence of individual lesions or any diabetic retinopathy) were associated with two to four-fold higher risk of incident ischaemic stroke (Wong et al., 2001a; Cheung et al., 2007b).

In a sub-study of the ARIC cohort including non-diabetic people, participants had cranial magnetic resonance imaging (MRI) scans (Wong et al., 2002c; Wong et al., 2003c). The presence of retinopathy was associated with presence and severity of cerebral white matter lesions and cerebral atrophy on MRI. Furthermore, there was a synergistic interaction between the presence of retinopathy and white matter lesions on the subsequent risk of clinical stroke (Wong et al., 2002c). Participants with retinopathy or white matter lesions alone had about twofold increase in stroke risk, but participant with both retinopathy and cerebral lesions had higher risk than those with white lesions only and even more than 18 times higher than those without either phenotype. This suggests that subclinical cerebrovascular disease may be more severe or extensive in people with both cerebral and retinal markers of microvascular pathology. Findings from the ARIC study are further reinforced by data from other studies (Petitti and Bhatt, 1995; Wong et al., 2003b; Wong et al., 2003d).

Associations of retinopathy signs with stroke, white matter lesions and cerebral atrophy could reflect a contribution of small vessel disease (evident in the retina) to the pathophysiology of a wide spectrum of cerebrovascular conditions in people with Type 2 diabetes (Cheung and Wong, 2008). It is also possible that these cerebral conditions may be related to a break-down of the blood-brain barrier given that diabetic retinopathy (e.g. retinal micoraneurysms, haemorrhages and cotton wool spots) is usually due to a disruption in the blood retina barrier (Wardlaw et al., 2003).

As mentioned, in Type 2 diabetes, both smaller retinal arterioles and larger venules predicted the future risk of stroke death. There is also considerable evidence supporting an association of altered retinal vascular calibre with both clinical and subclinical stroke in the general population. Prospective data from both the Rotterdam and Cardiovascular Health studies have consistently shown that larger retinal venular calibre is associated with an increased risk of clinical stroke (Ikram et al., 2006c; Wong et al., 2006b). A more recent meta-analysis from 6 longitudinal studies has further confirmed these findings (McGeechan et al., 2009). Furthermore, the Rotterdam study has demonstrated that wider retinal venules are also associated with cerebral infarction, MRI-defined whiter matter lesions and lacunar infarction (Ikram et al., 2006c; Ikram et al., 2006d). Conversely, no association between arteriolar caliber and incident stroke or progression of cerebral small vessel disease was observed in these studies (McGeechan et al., 2009).

1.6.10. Associations with depression

In people with Type 2 diabetes, an association between retinopathy and depression was reported in a meta-analysis of cross-sectional data from population and clinic-based studies (de Groot et al., 2001). However, given the cross-sectional nature of these studies the temporal relationship between diabetic retinopathy and depression remains unknown. This has exacerbated the argument on potentially underlying mechanisms linking the two conditions. For example, it has been speculated that depression may precede and accelerate the development of diabetic retinopathy either by its mediating effect on negative health behaviors such as

physical inactivity and lack of adherence to medications, or by a more direct influence of psychologically induced biochemical and physiological changes affecting the systemic vasculature (de Groot et al., 2001). An alternative hypothesis is that diabetic retinopathy may cause distress to patients resulting in symptoms of depression (Miyaoka et al., 1997). On the other hand, and supported by recent findings from brain imaging studies, it is also possible that the microvascular disease evident as retinopathy is also present in the brain where it plays a role in the development of depression in people with Type 2 diabetes. Thus, cerebral microvascular disease has been suggested to contribute to the development of late-life depression, a subtype sometimes referred to as 'vascular depression' (Tiemeier et al., 2004). In support of such a vascular hypothesis, studies have shown that both silent and symptomatic brain infarcts are associated with subsequent depression (Chemerinski and Robinson, 2000), and that magnetic resonance imaging (MRI)-defined white matter lesions and deep grey-matter abnormalities are more frequent in depressed than in non-depressed individuals (Lyness et al., 1998).

Although a vascular role in depression remains both conceptually attractive and biologically plausible in Type 2 diabetes, it has yet to be shown conclusively in clinical studies and in particular needs to be confirmed in large population-based studies (Baldwin, 2005; Thomas et al., 2004; Nguyen et al. 2008b). Recently, a clinic-based cross-sectional study in patients with Type 2 diabetes showed that wider retinal arteriolar calibre was associated with increasing severity of depression after adjusting for age, gender and duration of diabetes, but not after further adjusting for other vascular risk factors (Nguyen et al., 2008b). Whether the lack of association was mainly due to controlling for the confounding effect of poorer diabetes control and a high frequency of vascular risk factors or to insufficient statistical power given a small sample size (n=99) remains to be determined in future investigations.

1.7. Measurement of retinal vascular changes in diabetes

In general, clinical examination and grading of diabetic retinopathy is a

complex task due to variable retinal vascular characteristics of the disease. Various techniques have been applied to examine the retina, including direct or indirect ophthalmoscopy and fundus photography. Direct ophthalmoscopy is limited in this respect because it has a small field of view and lacks stereoscopic viewing making the direct observation of pre-retinal proliferation and macular oedema difficult or impossible. Stereoscopic slit-lamp biomicroscopy using indirect or corneal contact lenses is the most accurate clinical method. Retinal photography (now largely digital) has been preferred in large scale community programs and epidemiological studies because of the need for lasting documentation such as for quality assurance and demonstrating disease progression over time. In this context, mydriasis reduces the technical failure rate of ungradable photographs compared with undilated fundus examination.

Systematic evaluation of the diabetic fundus begins with the identification of the number and location of individual lesions by class (e.g. haemorrhage, hard exudate), then summarises this information to determine the severity of retinopathy. Retinopathy grading is based on the concept that a hierarchy of stages can be defined, where the higher the grade, the higher the risk of suffering visual loss (Larsen and Soliman, 2007). Although the grading scale reflects the natural course of the disease in its unrelenting and most devastating form, in reality, diabetic retinopathy can regress both spontaneously and in particular, after timely therapeutic intervention (Larsen and Soliman, 2007). In epidemiological studies, the purpose of using a validated grading scale is to assess retinopathy levels and retinopathy progression with high sensitivity and reproducibility.

With advances in digital retinal photography and new imaging technology, more precise assessment of the subtle changes seen in the retinal microvasculature has been made available, notably quantifying retinal vascular calibre in objective methods. Relying on an accurate measurement of retinal vessel calibre, other parameters of retinal vascular architecture, such as arteriolar bifurcation angles can also be derived and are gaining interest as potential markers of systemic disease. This section describes the several grading systems developed for diabetic retinopathy and

quantitative evaluation of retinal vascular network geometry which have been used in large epidemiological studies.

1.7.1. Grading of diabetic retinopathy

Early Treatment Diabetic Retinopathy Study Grading

The extension of the Airlie House classification that was developed for the Early Treatment Diabetic Retinopathy Study (ETDRS) currently stands as the recognised Gold Standard system for grading DR, in terms of its outcome related evidence-base from randomised clinical trials (ETDRS Report No. 10, 1991). On the basis of a prospective evaluation of the relative risk of retinopathy progression and visual loss, a retinopathy severity scale was developed that divides diabetic retinopathy into 13 levels ranging from absence of retinopathy to severe vitreous haemorrhage (ETDRS Report No. 12, 1991). This scale can be used to describe overall retinopathy severity and change in severity over time (ETDRS Report No. 12, 1991; ETDRS Report No. 18, 1998).

ETDRS fundus photography consists of seven standard dilated stereoscopic photographic fields, each covering 30 degrees (Figure 14). The photographs are recorded on photographic film as diapositives and each stereoscopic pair of diapositives is inspected against light boards using stereo viewers (5×magnification). When needed, a comparison is performed with standard reference photographs. The grade for each lesion is recorded separately for each standard field based on a multi-step scale including absent, questionable, definitely present but less than a standard photograph A or a written definition, equal to or worse than the standard photograph A but less severe than the standard photograph B, equal to or worse than the standard photograph B. For some lesions a standard photograph provides the dividing lines between grades whereas for others a written definition is used. The lesion grades are summarised for each eye into an ETDRS level that defines the diabetic retinopathy severity.

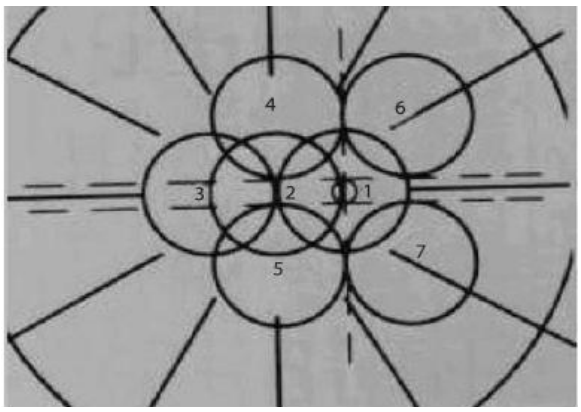


Figure 14: Fundus photography in the Edinburgh Type 2 Diabetes Study (ET2DS): location of the seven 30-degree fields. Field 1 is centred on the optic disc. Field 2 is centred on the centre of the macula. Field 3 is centred just temporal to the macula. Fields 4–7 are tangential to horizontal lines passing through the upper and lower poles of the disk and to a vertical line passing through its centre (Reproduced from DRSRG Report No.7, 1981)

The inter-grader reliability of this scale has been assessed by comparing independent duplicate gradings of photographs. Complete agreement on level was observed in 53% eyes and agreement within 1 step was observed in 88% (ETDRS Report No. 12, 1991). Overall, agreement between graders was substantial with a weighted kappa of 0.65. When the scale was used for assessing overall retinopathy severity from both eyes for each patient, classifying patients by retinopathy level in the worse eye retained substantial reliability between independent gradings with a weighted kappa of 0.71 (ETDRS Report No. 12, 1991).

The ETDRS scale does not cover macular oedema, which is graded separately. Using a grid overlaid on one photograph in Field 2 of the stereo pair, the grader evaluates the size and location of thickened retina, the maximum thickness of the thickened retina, the degree of cystoid oedema, and the location of the hard exudate that is often found with it (Larsen and Soliman, 2007). Diabetic macular oedema in the ETDRS is defined as thickening and/or hard exudate within 1 disc diameter of the center of the macula, given that the total amount of hard exudate within the Field 1 photograph exceeds that in a certain standard photograph.

For individual lesions, including macular oedema, retinal haemorrhages and/or microaneurysms, hard exudates, new vessels and fibrous proliferations, the inter-grader reliability of the ETDRS system has been found to be substantial, with a weighted kappa varying from 0.61 to 0.80 (ETDRS Report No. 10, 1991). For other lesions, such as cotton wool spots, intraretinal microvascular abnormalities, and venous beading, the inter-grader reliability was moderate, with a weighted kappa

varying from 0.41 to 0.60.

Despite the reference standard in clinical trials, the ETDRS fundus photography places considerable demand on the patient and the resources and the grading is also complex and time consuming (Larsen and Soliman, 2007). As a result, abbreviated protocols have been developed, two of which will be described below.

EURODIAB Grading

The EURODIAB grading system is a simplified system, with reference to seven-field 30^{o} ETDRS classification, but adapted to facilitate large epidemiological studies, in which investigators may have limited experience in retinal photography (Aldington et al., 1995). The photographic protocol involves two 45^{o} colour diapositive photographs, consisting of one macular field photograph with the centre of the optic disc at the nasal end of the horizontal meridian of the viewing field and one disc/nasal field with the optic disc one disc-diameter in from the temporal edge of the field on its horizontal meridian (Figure 15). The diabetic retinopathy severity scale comprises no retinopathy (level 0), non-proliferative (level 1 to 3), and proliferative (level 4 to 5) retinopathy (Table 3). Non-proliferative retinopathy is defined as the presence of one or more microaneurysms, haemorrhages, and/or hard exudates. Proliferative retinopathy is defined as any new vessels, fibrous proliferations, preretinal haemorrhage, vitreous haemorrhage, or photocoagulation scars. As a consequence of not taking stereo retinal photographs, macular oedema (thickening) is not gradable. An overall retinopathy level is allocated from top down after grading of individual lesion types (Aldington et al., 1995). This means that the first step is to determine whether proliferative diabetic retinopathy is present. In the event that it is, the retinopathy level of the eye is 5, otherwise one proceeds to evaluate if the eye is level 4, and so on.

The EURODIAB grading system has been well validated by comparing it with the reference standard of 7-field 30^{o} stereo photography. Within-observer reliability yields an unweighted kappa of 0.85 and between observer reliability a value of 0.83 (Aldington et al., 1995). It should be noted that the unweighted kappa

statistics provides an estimate of the proportion of exact agreement achieved beyond that expected by chance, thus compensating for the problem of agreement that looks better than it really is because the characteristic is rare. Weighted kappa is more suitable for ordinal scales as it takes the degree of disagreement into account and provides partial disagreements when agreement is not complete.

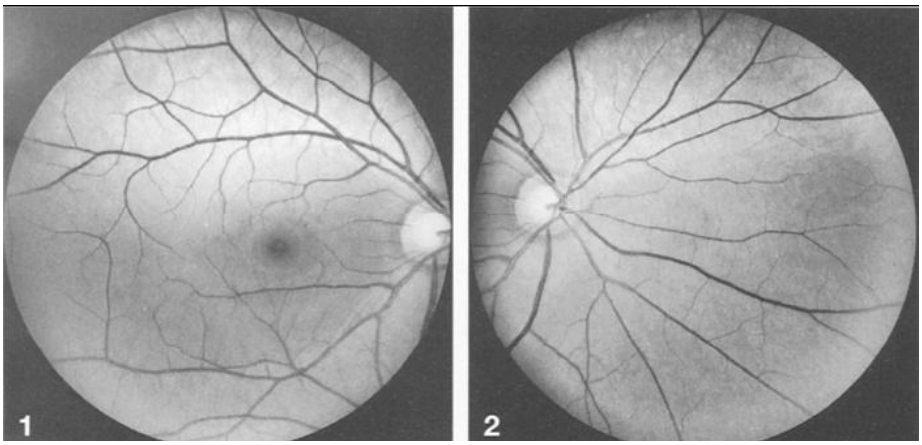


Figure 15: Example of 45^{o} retinal photograph of macular field (1) and disc/nasal field (2) of right eye (from colour transparency) (Reproduced from Aldington et al., 1995)

Table 3: General comparison of gradings between ETDRS and EURODIAB system

ETDRS Level	ETDRS severity	EURODIAB level
10-15	No retinopathy	0
20-35	Mild non-proliferative	1
43-47	Moderate non-proliferative	2
53	Severe non-proliferative	3
61-85	Proliferative (or photocoagulated)	4 (photocoagulated), 5

International Clinic Diabetic Retinopathy Severity Scale

The International Clinical Diabetic Retinopathy and Diabetic Macular Edema Disease Severity Scale has been developed by a consensus panel of experts to provide a severity scale for use in clinical practice, such as retinopathy screening and communication among health care professionals (Wilkinson et al., 2003). With reference to the ETDRS grading system, the classification for diabetic retinopathy comprises five stages of disease severity which are further divided into three stages

of low risk, a fourth stage of severe non-proliferative retinopathy, and a fifth stage of proliferative retinopathy (Table 4). Diabetic macular oedema is classified as apparently present or apparently absent. If training and equipment allow the grader to make a valid decision, macular oedema is further categorised based on its distance from the central macula (fovea) (Larsen and Soliman, 2007). This simplified staging system may help standardise clinical retinopathy grading given that many nominally different systems are in use but vary little in their fundamental concept (Larsen and Soliman, 2007).

Table 4: Diabetic retinopathy disease severity scale (Reproduced from Wilkinson et al., 2003)

Proposed Disease Severity Level	Findings Observable on Dilated Ophthalmoscopy
No apparent retinopathy	No abnormalities
Mild nonproliferative diabetic retinopathy	Microaneurysms only
Moderate nonproliferative diabetic retinopathy	More than just microaneurysms but less than severe nonproliferative diabetic retinopathy
Severe nonproliferative diabetic retinopathy	Any of the following: more than 20 intraretinal haemorrhages in each of 4 quadrants; definite venous beading in 2+ quadrants; Prominent intraretinal microvascular abnormalities in 1+ quadrant. And no signs of proliferative diabetic retinopathy
Proliferative diabetic retinopathy	One or more of the following: neovascularisation, vitreous/ preretinal haemorrhage

1.7.2. Measuring retinal vessel calibre from retinal photography

The apparent calibre of a normal retinal vessel is believed to be the width of the streaming column of reflective erythrocytes viewed perpendicularly to the flow direction (Figure 16). By ophthalmoscopy or fundus photography, the surrounding plasma zone and the vessel wall are transparent, and the former accounts for less than 10% of the internal diameter. However, erythrocyte column width is considered to be a good surrogate measure of internal vessel calibre as it appears to be proportional to internal retinal vessel calibre in animal studies and the ratio of the width of the plasma layer to internal vessel calbre for both arterioles and venules stays constant

(Bulpitt et al., 1970). Existing techniques for measuring blood vessel width consist of two steps which may both affect the apparent blood vessel width. Step one is the acquisition of the vessel image, such as using fundus photography or fluorescein angiograms, and step two is the measurement of the vessel width. Photography is the most common technique used to acquire retinal vscular images.

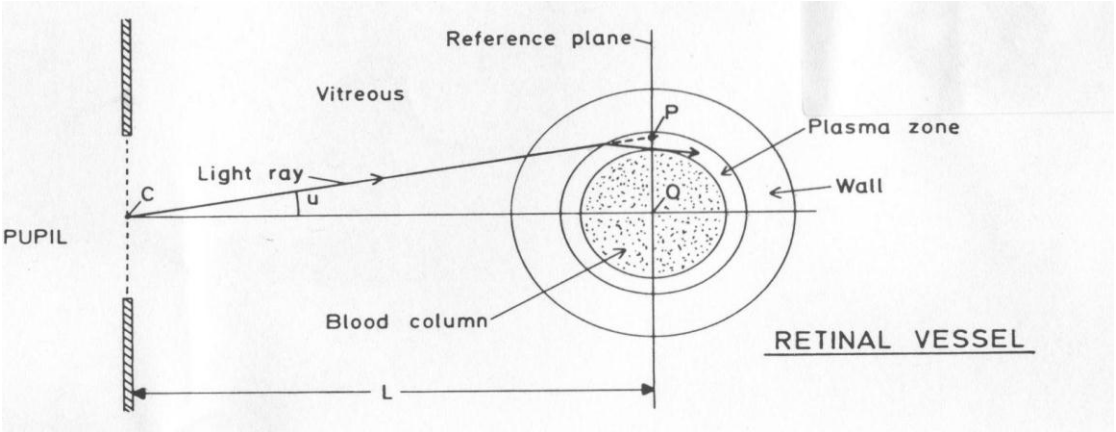


Figure 16: A simplified diagram showing the apparent and true width of the blood column based on. When an image of a blood vessel is viewed from point C (the centre of the pupil), the apparent edge of the blood column is given by the point P, which is the interaction point between the incoming part of the ray and the chosen reference plane. (Reprinted from Brinchmann-Hansen and Heier, 1986)

In general, retinal arterioles are often small and require high-resolution images to measure accurately. Also, to enhance the contrast between the retinal arterioles and the background, red-free images are often preferable or the green channel from the RGB colour image can be used. Although conventional fundus photography can be converted to digitalised image using a high resolution digital photographic scanner, direct digital capture has advantages, including immediate photographic evaluation and repeat capture for optimum image quality (Patton, 2006). Attempts at quantifying retinal arteriolar calibre were first proposed by Wagener et al in 1947 (Wagener et al., 1947). Later with the introduction of retinal photography, Parr and colleagues (Parr and Spears, 1974 a, b) developed the Central Retinal Artery Equivalent (CRAE) measurement by using micrometric techniques, in which measurements were performed in enlarged projected images with callipers.

Following the widespread use of digital imaging systems, more objective and reliable methods including those based on intensity profiles of retinal blood vessels (microdensitometry) have superseded previous micrometric techniques. Using computer-assisted programme, microdensitometry techniques are semi-automated, requiring some observer input to select which cross-section of blood vessel to measure in a grayscale image, which consists of many elements or pixels. The location of each pixel can be identified with spatial coordinates and each has a defined intensity also known as its grey value (Patton et al., 2005). Due to its variable grey level at each pixel point, a cross-section of a retinal blood vessel has a typical intensity profile, very similar to a distinct double Gaussian curve against the background intensity of the surrounding retina. Mathematically, this profile can be described by subtracting a smaller central Gaussian function (representing the central light reflex from retinal arterioles) from a single Gaussian model, to correct for the absence of the central reflex in older eyes (Patton et al., 2005). The single Gaussian model is expressed by the following equation:

$$f(x) = a_1 e^{-\left(\frac{x-a_2}{a_3}\right)^2} + a_4$$

Where a_1 is the the amplitude of the peak of the profile, a_2 is the the location of the peak of the curve, a_3 is a specific variable indicating the width or spread of the Gaussian curve and a_4 is the background retinal intensity (Patton et al., 2005).

The double-Gaussian profile can be subjected to image analysis to estimate the width of the blood vessel, most commonly done by measuring the width of the vessel at half the height of the peak of the intensity profile (half-height method). This strategy minimises any effect of de-focusing at image acquisition due to medial opacities, such as cataracts (Patton et al., 2005). In addition to the evaluation for CRAE, a similar formula for venules (central retinal venule equivalent, CRVE) was developed by Hubbard et al. in 1999. By combining the two measures, the AVR was the ratio of the CRAE/CRVE. The Parr and Spear and Hubbard formulas for CRAE and CRVE were derived by examining numerous vascular junctions in retinal images and calculating the relationship between individual trunk vessels and their respective branch vessels using a least root mean square deviation model that best fit the observed data. The retinal photographs used in both groups were from a young,

healthy, normotensive population.

Recently, the Parr-Hubbard formulae have been further improved by Knudtson et al. to overcome some of their limitations (Knudtson et al., 2003). For example, the Parr-Hubbard formulae contain constants that require using measurements scaled only in micrometers. Thus to be able to use the formulas, a conversion from pixel to micrometers is required, which could be an inexact summarised conversation. An estimate of the pix-to-micrometre ratio is usually calculated based on an assumed average optic disc diameter of 1850μm (Hubbard et al., 1999). In addition, the formulae were dependent on the number of retinal vessels measured. Knudtson et al. therefore developed a set of revised formulae for summarising retinal vascular calibre, and showed clear superiority over the Parr-Hubbard formulae. In particular, Knudtson (Knudtson et al., 2003) incorporated the concept of 'branching coefficient' which was firstly proposed by Young in 1809. In a young, normotensive healthy population, Knudtson arbitrarily chose to measure the six largest arterioles and venules in each retinal image and calculated the observed 'branching coefficient' (e.g. 1.28 for arterioles which was against the theoretical value of 1.26) based on the previous formula. By placing the calculated empirical values into the branching coefficient formula, they calculated that (Patton et al., 2005):

For arterioles: $D_0=0.88* \sqrt{(D_1^2+D_2^2)}$ [$0.88=\sqrt{1/1.28}$]

For venules: $D_0=0.95* \sqrt{(D_1^2+D_2^2)}$ [$0.95=\sqrt{1/1.11}$], where D_0 is the width of trunk vessel and $D_1$and D_2 are the two branch vessels.

More recently, Patton (Patton et al., 2006a) suggested another revised formula for more accurate estimation of arteriolar branching coefficient. Instead of adopting a constant value of 1.28, this formula used a linear regression model to incorporate the asymmetry index of the vessel branches being measured. However, whether the newer formulae are more accurate and have a better predicative value for outcomes compared with older ones remains to be determined by future work. Moreover, these estimates of retinal vessel calibre measurements have not been validated against entire diameters of these vessels in vivo, such as measurements from fluorescein angiographs which include the plasma zone.

Computerised quantification of retinal vessel calibre

As stated earlier, digital images are formed in such a way that makes them accessible to simple and complex mathematical manipulation (Patton et al., 2006b). After a series of image processing, enhancement, restoration and segmentation, image quality can be improved and subjected to further analysis for quantitative measurements. Retinal vasculature is measured from the gray-scale image in which the optic disc is centred. This field provides the most detail from the retinal vasculature as it enters and leaves the eye, and serves as the starting point for all further analysis of retinal vascular network geometry. Within a circular zone defined as between 0.5 to 1 disc diameter from the optic disc margin, the calibre of all individual arterioles and venules are measured through vessel tracking technique in a semi-automatic fashion. For example, after manually selecting a starting and ending position of a vessel segment, the software commences the generation of a series of cross-sectional intensity profiles perpendicular to the vessel. For each intensity profile created, a 'best-fit' Gaussian curve function is incorporated onto the data. The vessel calibre is calculated as the mean width of the intensity profiles at half the height of the intensity profile peak. These individual retinal vessel measurements are then summarised using formulae as described earlier reflecting the average arteriolar and venular calibre of that eye, taking into account branching patterns.

Reliability of retinal vessel calibre measurements

As mentioned previously, compared with observer-driven micrometric techniques, computer driven microdensitometry has been shown to be more accurate in the presence of focusing errors (Brinchmann-Hansen, 1986). The coefficient of variation for the computer driven microdensitometry has been calculated as 1.5-7.5% compared with 6-34% for the observer-driven technique (Patton et al., 2006b). Also there was a tendency of the latter method to underestimate the size of small vessels.

Several large, population-based epidemiological studies have examined the reliability of computer-assisted retinal vessel measurements (Hubbard et al., 1999; Couper et al., 2002; Sherry et al., 2002; Wong et al., 2002c; Wong et al., 2003b;

Ikram et al., 2004; Wong et al., 2006a; van Hecke et al., 2006). Overall, these retinal vascular indices, including CRAE, CRVE and AVR, have demonstrated substantial reproducibility, with both intra- and inter-grader correlation coefficients ranging form 0.67 to 0.99. For instance, in the ARIC study (Hubbard et al., 1999) correlation analysis (R) was used to evaluate inter-observer agreement (R=0.74, 0.77 and 0.79, for CRAE, CRVE and AVR, respectively, n=151 eyes). For intra-observer agreement, R=0.69, 0.89 and 0.84 for CRAE, CRVE and AVR respectively.

In the Blue Mountains Eye Study which used a similar system and the same Parr-Hubbard formula, intraobserver reliability weighted kappa values ranged from 0.8 (for trunk AVR ratios) to 0.93 (for CRVE measures) (Sherry et al., 2002). In addition, R^2 correlation analysis showed agreement ranging from 0.79 to 0.92. For interobserver reliability, kappa ranged from 0.71 (for trunk AVR ratios) to 0.9 (for CRVE measures), and correlation statistics showed R^2 ranging from 0.78 to 0.9. Although it is unclear as to why the kappa statistic normally applied for the analysis of agreement of categorical data was chosen, these values suggested a good agreement using computer-assisted measurements (Patton et al., 2005).

Similarly, using the Knudtson's revised formula, the Rotterdam Study reported correlation coefficients for intra-observer agreement ranging from 0.67 to 0.80 for CRAE, 0.91 to 0.94 for CRVE and 0.75 to 0.84 for AVR. For intra-observer agreement these were 0.69 to 0.88, 0.90 to 0.95 and 0.72 to 0.90, respectively (Ikram et al., 2004). Thus the findings suggest that these measurements provide a highly reliable research tool for objective assessment of vascular changes.

Other methodological issues in measuring retinal vessel calibre

Although measurements of retinal vascular calibre have now been used widely in epidemiological studies of cardiovascular and ocular disease, there are several methodological issues in quantification of these parameters from retinal photographs. First, to date, no age, sex, or blood pressure-level specific reference levels of retinal vascular calibre have been established (Sun et al., 2009). Existing retinal vascular imaging research has generally focused on the relative differences in

retinal vascular calibre changes in the study population (e.g. people with wider quartile of retinal venular calibre are more likely to have or develop cardiovascular disease than those with narrower quartile calibre). Normal reference ranges may facilitate comparison between studies and are essential for implementation of measurements in clinical practice. However, it may be difficult to obtain standard values across different populations given the various systemic and environmental influences on the variation of retinal arteriolar and venular calibre measurements (Sun et al., 2009). In particular, in adult populations, it is difficult to fully account for the confounding effect of vascular and ocular disease processes.

Second, all images captured from the retina are subject to image magnification, depending on the distance from the camera to the eye as well as the refractive error of the eye. Magnification error could therefore be an obstacle when comparing retinal vascular calibre size directly between individuals, especially when the study population has large variation in refraction status. Therefore, several attempts have been made to adjust for magnification effects, such as using ocular biometric parameters (e.g., axial length), plain films or alternative geometric attributes that are dimensionless in nature, including length-diameter ratio, AVR and bifurcation angle. Among these, the AVR is the most commonly used measure.

A smaller AVR was initially thought to reflect generalised arteriolar narrowing, as venular calibre was assumed to be relatively constant. However, recent studies suggest that the interpretation of the AVR may be overly simplistic and that it has significant limitations, including the inability to capture the separate information of individual arteriolar and venular calibre components (Liew et al., 2007). For example, both narrower arterioles and wider venules may contribute to a smaller AVR. Thus a smaller AVR cannot differentiate specific changes in arteriolar and venular calibre. Furthermore, narrowed retinal arterioles and widened venules may carry different information and use of a summary measure of AVR may lead to incorrect inferences on associations with different systemic diseases. Whereas smaller arterioles are associated with hypertension or even predict its development, larger venules have been associated with inflammation, smoking, obesity and

hyperglycaemia (Sun et al., 2009). On the other hand, the major reason for the initial use of AVR (to correct for magnification differences) may not be as important as previously thought (Liew et al., 2007). Several population-based studies found no relationship between refraction/axial length and retinal vascular calibre after ocular magnification had been controlled for, suggesting no biological link between ocular dimension and structural changes in the retinal vasculature (Wong et al., 2004c; Cheung et al., 2007 c, d). It has also been proposed that in the absence of data on refractive error, bias from magnification difference is not profound in most eyes within the refractive power range of ±3 D (Wong et al., 2004c).

Third, a potential confounding effect between retinal arteriolar and venular calibre has been addressed. Liew et al. observed that retinal arteriolar and venular calibre size are highly correlated and individuals with narrower arterioles also tend to have narrower venules, presumably due to their shared genetic and environmental determinants (e.g., diet, health and growth) as well as magnification artifacts (Liew et al., 2007). One approach to account for this confounding effect has been to adjust for both fellow vessels simultaneously in the statistical analysis (e.g., controlling for retinal arteriolar calibre in analysis of retinal venular, and vice versa). For example, an association between retinal venular narrowing and incident hypertension was initially reported in the Rotterdam Study (Ikram et al., 2006a). However, when using this new analytical method by adjusting for retinal arteriolar and venular calibre all together, retinal venular narrowing was no longer associated with incident hypertension (Ikram et al., 2006b).

Finally, there are other potential problems with image analysis including the fact that retinal vessel calibres vary according to the cardiac cycle and degree of fundus pigmentation. The ARIC study showed that vessel pulsation was detectable, mainly in the largest venules of the retina (Knudtson et al., 2004). In addition, small changes in retinal vessel calibre can be accurately detected by multiple frame electrocardiograph synchronized fundus photographs. In this context, measurement of vessel calibre from a single, static retinal image at a random point in the pulse cycle may introduce measurement error (Dumskyj et al., 1996). However, given that

the variation caused by cardiac pulse appears to be small and random, it may only contribute to the non-differential misclassification and tend to bias the associations found to the null (Sun et al., 2009).

The effect of variation in retinal background colour on retinal vessel calibre was observed in the Sydney Myopia Study (Rochtchina et al., 2008). They found that retinal vascular calibre was significantly wider in children of East Asian ethnicity than those of white children, which was in accordance with the findings from another two population-based studies suggesting racial/ethnic differences in retinal vascular calibre (Cheung et al., 2007e; Wong et al., 2006a). However, when the analysis was limited to children with a dark brown iris as a surrogate marker of retinal pigment colour, there was no such difference between white and East Asian children. It was therefore hypothesised that the reported racial/ethnic differences may be due to differences in retinal pigmentation (Sun et al., 2009). Retinal pigmentation may be a potential source of measurement error in the variation of retinal vascular calibre across different ethnicities. Alternatively, given iris color itself is a complex human trait with very high heritability, genetic differences may also explain partly the observed association (Sun et al., 2009).

1.7.3. Measuring retinal arteriolar bifurcation geometry from retinal photography

The ultimate purpose of the arterial system is to supply sufficient blood flow to capillaries in all parts of the body to enable adequate exchange of substances through the capillary walls. It has long been known that when in a healthy state, an arterial structure tends to conform to an idealised optimal structure in order to achieve the greatest efficiency, i.e. by minimising the costs involved across the vascular network. These costs include the shear stress experienced by the blood vessels due to vascular blood flow, volume of the vascular tree, pumping power across the vascular network, and surface area of the lumen (Patton et al. 2005). Specifically, this physiological principle has a great impact on connecting large and small vessels at bifurcations. An optimal branching pattern of the arterial network would facilitate both translational intravascular blood flow and transmural diffusion,

with the minimisation of energy costs. Geometric features of such branching networks that may determine the degree to which it is optimised include the angle between daughter blood vessels as they bifurcate from the parent vessel, and the relationship between the calibre of the trunk and branch vessel (Patton et al. 2005).

Arteriolar branching coefficient and bifurcation angles

Young first proposed the concept of branching coefficient as a means of expressing the ratio of the area of the blood circulation before and after a vascular dichotomous junction (Young, 1809). He defined branching coefficient as $(D_1^2+D_2^2)/D_0^2$, where D_1 and D_2 are calibre of two branch vessels respectively, and D_0 is the calibre of the trunk vessel. Young also derived a theoretical optimum value of 1.26 for the branching coefficient, which was later further explained by Murray in 1926. In his published work, Murray (Murray, 1926 a, b) described the design of an optimal branching vascular system, which minimises the power required to overcome viscous drag in a fluid and the metabolic energy required to maintain the volume of blood and tissues of the vessel segments. He calculated that the most efficient circulation can be achieved if blood flow is proportional to the cubed power of the vessel's radius, which is known as 'Murray's law'. For example, at a vascular bifurcation, costs are increased when branch calibre, relative to the calibre of the trunk vessel, are large (viscous drag is minimised at the expense of increased metabolic costs) or very small (reduced metabolic costs but increased viscous drag). In this scenario, Murray's law relates the calibre of the trunk and branch vessels at a vascular junction in an optimal circulation, such that the cube of the calibre of the trunk vessel equals the sum of the cubes of the branch calibre. This can be expressed as $D_0^3=D_1^3+D_2^3$ (where D_1 and D_2 are calibre of two branch vessels respectively, and D_0 is the calibre of the trunk vessel). The exponent of 3 is also known as optimal junctional exponent, predicting constant shear stress throughout the network (Sherman, 1981). Both human and animal studies have shown values very close to this ideal value for the retinal circulation as well as the coronary circulation (Patton et al., 2005).

For a symmetrical, dichotomous junction where the two branch vessels are

equal in calibre ($D_1=D_{2)}$, the equation can be reduced to $D_0^3=2D_1^3$. Thus it can be shown that the area ratio of $(D_1^2+D_2^2)/D_0^2=2^{1/3}(\approx 1.26)$, which is in agreement with the theoretical value of optimal branching coefficient (BC) as given by Young.

Rather than using the absolute value of the BC, it has been suggested that it may be important to analyse the degree of deviation of the BC from the idealised optimum (i.e. 1.26). Figure 17 demonstrates that a deviation in either direction would be associated with suboptimality, either of power losses or volume (Patton, 2006). The deviation of retinal arterial branching coefficients from optimal values has been shown to be associated with cognitive ability scores (Patton et al., 2007). Other similar but more complex measures of BC have also been developed. These optimality parameters, measuring the extent to which the measures (e.g. junctional exponents) deviate from the theoretical predicted optimum, are found to be associated with advancing age (Stanton et al., 1995a), higher body mass index (Hughes et al., 2009), and in association with peripheral vascular disease (Chapman et al., 2002) and mortality from ischemic heart mortality (Witt et al., 2006).

The angle subtended between two daughter vessels at a vascular junction has also been found to have an optimal value (approximately 75^o) depending on which costs (surface, volume, drag or power) are considered and the degree of asymmetry between the two daughter vessels (Patton et al., 2006). As shown in Figure 17, the costs are minimised as shown by the confluence of the curves, when branching coefficient (area ratio) approximates to 1.26 and the angle approximates 75^o for power losses and volume, as predicted by Murray. Animal studies have further corroborated that mean bifurcation angles approximate to 75^o (Patton et al., 2006). Additional work by Frame and Sarelius has shown that angles are not anatomically invariant or static, but change significantly with blood flow and that the resultant increase in vessel diameter results in changes of angles of up to 50^o (Frame and Sarelius, 1993). This change is in both directions with some angles decrease while others increase.

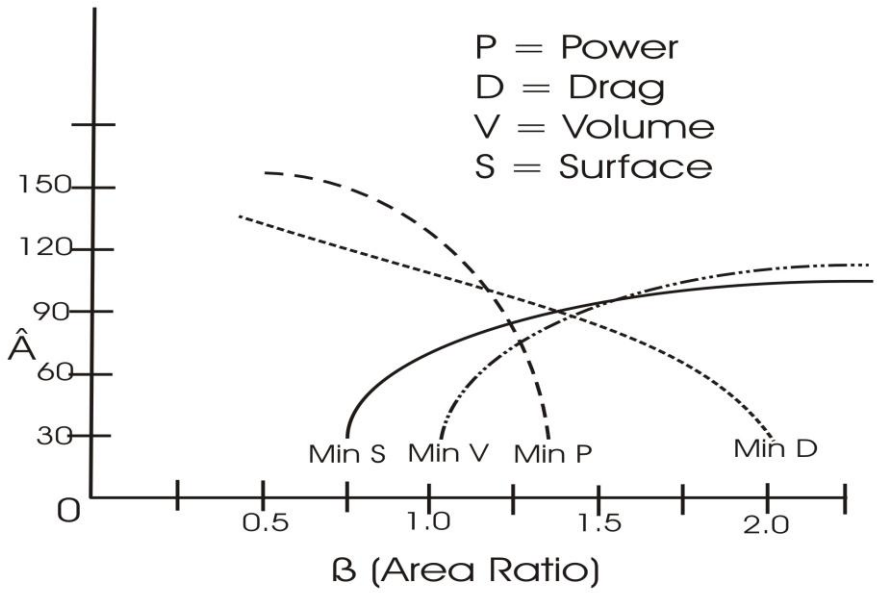


Figure 17: Graph showing the relationship between β (an area ratio also known as branching coefficient, $(D_1^2+D_2^2)/D_0^2$) and A (angle between D1 and D2), for the costs of power losses, drag, volume and surface area (Reproduced from Patton et al., 2006)

Several studies have demonstrated that retinal arteriolar bifurcation angles are reduced in hypertension (Stanton et al., 1995a), increasing age (Stanton et al., 1995b), and in men with low birth weight (Chapman et al., 1997). Deviation of angles from optimality has been found to be associated with logical memory scores in elderly people (Patton et al., 2007). By contrast, other studies found no associations between the angle at vascular bifurcation and vascular risk factors, peripheral arterial disease or other cardiovascular disease (Chapman et al. 2002; Witt et al., 2006; Hughes et al., 2009).

Given that the endothelium may play a significant role in optimisation of vascular geometry by nitric oxide and endothelin-1 release (Griffith and Edwards, 1990; Griffith et al., 1991), it is possible that changes in retinal arterial bifurcation optimality may reflect endothelial dysfunction. Also altered shear stress across the network, which is associated with altered optimal geometry, may further compound the effects on the vascular endothelium and cause endothelial inflammation through increased expression of proinflammtory genes, increased production of free radicals but decreased levels of important intracellular antioxidants (Patton et al., 2007). The

area of greatest wall shear stress has been determined both in vivo and by computational fluid dynamics as occurring within one vessel diameter of a vessel branch (Noren et al., 2000; Patton et al., 2007). Despite these hypothesises, it remains unclear how angles at vascular junctions may serve as independent predictors of systemic disease and further studies are needed to explore these relationships.

Computerised quantification of retinal arteriolar bifurcation geometry

Image analysis has been used to calculate the branching coefficient by measuring the widths of the trunk and branch vessels and the angles between branch retinal arterioles at bifurcation. Depending on formulae, different methods have been applied to estimate arteriolar angles. For example, Chapman et al. measured the bifurcation angle by subtracting the sum of the angles measured between trunk and each branch arterioles from 360^{o}. Patton et al. directly calculated the total bifurcation angle between the two branch vessels using the cosine rule (Patton, 2006).

Reliability of retinal arteriolar bifurcation geometry measurements

Given the lack of accepted formulae for quantifying branching angles or coefficients, the variance could be, at least in part, attributed to the variable measurement methods. Also, very little data is available on the reliability of these measurements. Chapman et al. reported an intra-observer reliability for bifurcation angles as a within-subject standard deviation of 2.66^{o} (mean angle 68.9^{o}; coefficient of variation 3.86%) (Chapman et al., 2002). Patton et al. estimated both intra- and inter-observer reliability based on intra-class coefficient (ICC) and coefficient of repeatability (2SD of the differences) between the repeated measures. For intra-observer reliability of angle measurements, the ICC was 0.96 with a coefficient of repeatability of 10.4^{o}. Equivalent figures for inter-observer reliability were 0.95 and 9.2^{o}. For intra-observer reliability of the arterial branching coefficient, the ICC was 0.55 and coefficient of repeatability 0.67. Given the small range of branching coefficient measurements in this cohort (1.1-2.2), the investigators pointed out that the intra-reliability was moderately poor and predicted that the likelihood of the

inter-observer reliability would be even worse. When using the alternative 'micrometric technique' to measure junctional vessel width, ICC of intra-observer reliability was 0.65, with a coefficient of repeatability of 0.54. For inter-observer reliability, ICC was 0.62 and the coefficient of repeatability was 0.61.

1.7.4. Measuring other retinal quantitative parameters

Retinal venular tortuosity

Venous beading, as a feature of diabetic retinopathy, has recently been measured quantitatively, especially within computer-assisted screening programmes (Patton et al., 2006). Earlier measures assumed blood vessels as one-dimensional curves and applied the 'distance metric' (defined as the ratio of the vessel length to the chord length between the end points). Recently, the perceived tortuosity of blood vessels has been proposed to be affected by vessel thickness as well (Azegrouz et al., 2006). Using a thickness dependent estimation method, an excellent overall agreement (92.4%) for vascular tortuosity was achieved in comparison with clinical judgement (Azegrouz et al., 2006). Despite a promising diagnostic tool in clinics, no study has explored the association between venular tortuosity and systemic risk factors and diseasease.

Fractal geometrical analysis

In addition to individual quantitative parameters of the retinal vascular network, a single 'global' measure combining these parameters with the branching pattern of the retinal vasculature has been developed. The retinal vascular tree is a complex branching structure that could not be adequently described by using the classic Euclidean geometrical terms (e.g. length, area, or volume) (Cheung et al., 2009). Fractals represent one type of non-Euclidean geometric entity and can be defined as geometric patterns whose parts resemble the whole (i.e. the property of self-similarity). Fractal dimension is a measure of geometric complexity of a spatial object (e.g. retinal vascular tree), describing how thoughly the pattern fills two-dimensional spaces. Fractal analysis has been used to characterise the complexity or density of the retinal vessel branching network from digital retinal images. For instance, a less complex, less dense, and lower fractal dimension may

indicate rarefaction or loss of vessels, whereas a more complex, denser, and higher fractal dimension may imply a microvascular proliferation (Liew et al., 2008a) (Figure 18).

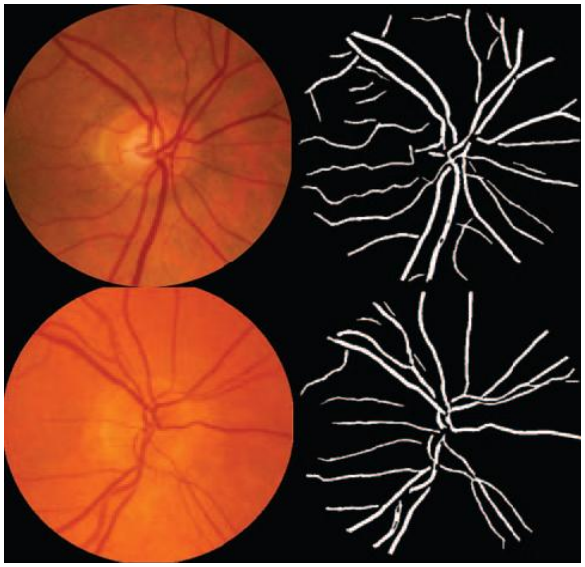


Figure 18: Fractal pattern of retinal vessels. The upper series shows an eye with higher fractal dimension and more complex branching pattern, whereas the lower series shows one with lower fractal dimension and less complex branching pattern (vascular rarefaction) (Reprinted from Liew et al., 2008a)

Recently it has been shown that fractal analysis of the retinal vasculature from fundus photographs can be performed reliably and efficiently using a novel computer-based program (Liew et al., 2008b). The variations in retinal vascular fractal dimension have been found to be correlated with several biological parameters such as age and blood pressure. They may potentially be a sensitive indicator of deviation from normal or optimised architecture and, thus, early microvascular disease possibly due to retinal hypoxia (Liew et al., 2008a). However, very few studies, mostly clinic-based, have examined the role of retinal vessel fractal analysis in diabetes and cardiovascular disease. In a cross-sectional study of 729 patients with Type 1 diabetes aged between 12 and 20 years, higher retinal vascular fractal dimension was associated with an increased odds of non-proliferative diabetic retinopathy (OR of 4^{th} vs. 1^{st} quartile of fractal dimension 3.9; 95%CI, 2.0, 7.6) (Cheung et al., 2009). However, a further follow up of these patients for three years failed to confirm a longitudinal association between retinal vascular fractal dimension and risk of developing early diabetic retinopathy (Lim et al., 2009). No study to date has been performed in people with Type 2 diabetes.

'Dynamic' measures of retinal vascular calibre

As well as measuring static structural features from retinal images, other newer techniques, such as scanning laser Doppler flowetry and Dynamic Vessel Analyser, have been developed to measure dynamic changes in the retinal microcirculation. The laser Doppler flowetry can assess retinal vascular hemodynamic by calculating retinal blood flow, volume and velocity (Patton et al., 2006). However, the measurements obtained from this approach shows variable reproducibility (Patton et al., 2006). The Dynamic Vessel Analyser (Imedos, Jena, Germany) allows real-time assessment of retinal vascular diameters and has been previously used to measure retinal vessel dilatation in response to diffuse luminance flicker (Nguyen and Wong, 2009). This dynamic response may reflect instrinic endothelial function possibly due to the role of nitric oxide in flicker-light-induced retinal vasodilatation. Small clinical studies using the analyser have shown that impaired dilatation to this flicker is associated with systemic diseases such as diabetes and diabetic retinopathy (Mandecka et al., 2007). Furthermore, impaired flicker-induced vasodilatation has been found to be associated with wider arteriolar and venular diameters from retinal images in people with diabetes (Nguyen et al., 2009).

1.8. Chapter summary

Type 2 diabetes is a chronic, complex metabolic disorder characterised by abnormally high blood glucose levels. Type 2 diabetes is a common condition, currently affecting approximately 10% of people aged over 65 years in the UK and this prevalence is predicted to double over the next 20 years, with a particular increase in elderly people. There is good evidence that Type 2 diabetes is associated with an increased risk of age-related cognitive impairment and an enhanced risk of age-related cognitive decline, in addition to higher incidences of stroke and dementia. Given the ageing and increasing diabetic population, the deletious effects of Type 2 diabetes on the brain is an emerging public health concern.

Current knowledge on the relationship between risk factors and the

development and progression of diabetes-related intellectual dysfunction has until now been based on relatively limited and often contradictory evidence. However, there is increasing evidence that several potentially modifiable diabetes-related risk factors may be important and have implications for strategies aimed at delaying or preventing cognitive deterioration in people with Type 2 diabetes.

One outcome of the research effort to date has been an increase in studies suggesting that cerebral small vessel disease with white matter lesions and lacunar infarcts may be a vascular cause of cognitive impairment in the elderly population. However, cerebral small vessels themselves are too small to visualise in detail using current neuroimaging methods. Given the close anatomical and physiological homology between the retinal and cerebral vasculatures both in health and in disease states, the retinal microvasculature is considered to be a potential surrogate biomarker of altered cerebral vasculature and therefore may demonstrate an association with cognitive function. Moreover, the retinal microcirculation is the only vasculature that can be visualised and photographed in vivo in humans.

This thesis focuses on qualitative and quantitative parameters of retinal vascular signs in relation to cognitive function in people with Type 2 diabetes. Qualitative parameters include the presence and severity of diabetic retinopathy. Quantitative parameters reflect more objective and subtle retinal vascular changes, and comprise retinal vessel calibre, arteriolar bifurcation angles and branching coefficient. A number of epidemiological studies have used standard photography and computerised image analysis to detect associations between these retinal vascular changes and systemic vascular risk factors and cardiovascular disease in both the general population and in diabetic patients. However, the epidemiological research on retinal vascular signs in relation to cognitive function, in particular in people with Type 2 diabetes is sparse. These studies are reviewed systematically in the next chapter.

Chapter 2

Retinal Microvascular Abnormalities and Cognitive Dysfunction: Systematic Review

2.1. Introduction

The following review provides a systematic overview of published epidemiological research relevant to the analysis performed in this dissertation. This systematic review aims to describe pertinent studies to date which have contributed to understanding the association between retinal microvascular abnormalities as proxy markers of cerebral small vessel disease and risk of cognitive impairment and decline in patients and in the general population. Data will be extracted including details on study design, sample size, methods of assessing retinal microvascular abnormalities, cognitive function and/or impairment, and results. The main methodological issues that have arisen in these studies will be discussed. By presenting the existing body of research, the relevance of the analysis performed in this dissertation can be assessed.

2.2. Methods

2.2.1. Criteria for considering studies for the review

I aimed to include all published studies that investigated the association between retinal microvascular abnormalities and cognitive dysfunction or dementia in adult subjects (middle-aged or older), available for review by July 2007. Given the relative scarcity of studies in this field, criteria for the inclusion of studies were kept broad. Studies had to meet the following pre-determined inclusion criteria: a) observational epidemiological design (i.e. cross-sectional, case-control, cohort), b) inclusion of a cognitive function assessment tool that was either a structured neuropsychological test or a clinical evaluation of dementia, c) assessment of the retinal microvasculature (either characteristics associated with generalised retinopathy or changes specific to arterioles or venules, such as focal arteriolar

narrowing and venular tortuosity) but not other retinal conditions, which fail to offer a direct measure of retinal vascular signs (such as age-related maculopathy), d) provision of data relating retinal microvascular lesions to cognitive function. Studies in which participants were selected on the basis of existing infectious disease due to a virus or bacteria (e.g. cytomegalovirus or diphtheria) were excluded because cognitive dysfunction in these subjects is likely to have specific pathology peculiar to the infectious disease.

2.2.2. Search strategy for identification of studies

Studies were identified by computerised searches of PubMed (Medline) (1950-2007), EMBASE (1980-2007), PsycINFO (1950-2007), BIOSIS Previews (1969- 2007) and Web of Science (1945- 2007), by checking the reference lists of relevant original and review articles, by reference to conference proceedings, and by tracking articles that cited retrieved studies using Science Citation Index. A detailed 'topic only' search strategy was adopted as it proves the most sensitive but not specific in order to produce the maximum number of papers on this topic. The search string for each database was similar with the terms "retinopath* OR (retina* AND (microvascu* OR arteriol* OR venul* OR vascu*)) AND (cognit* OR Alzheim* OR dementi* OR memor* OR neuropsycholog*)". Searches were limited to papers published in English and human studies.

2.2.3. Selection of studies

The titles and abstracts of all articles identified were screened and potentially relevant full-text articles were retrieved and assessed according to the inclusion criteria.

2.2.4. Quality of studies

The quality of studies was assessed independently by myself and a second reviewer, using a scoring system based on previously published guidelines (Critical Appraisal Skills Programme, 2004; Tooth et al., 2005). Studies were assessed for both internal validity (assessment of exposure, outcome status, covariates, analytical approach) and external validity (recruitment, sample selection, participation at

follow-up) (Figure 19). Studies scoring less than 6 out of a possible maximum 9 for case control or cross sectional studies, and less than 7 out of 11 for cohort studies were excluded from further analysis. Disagreements in scoring were resolved by discussion or arbitrated by a third reviewer.

2.2.5. Data extraction

The following information was extracted from included studies: method of assessment of retinal microvascular abnormalities, measurement of cognitive function, and all relevant results, including adjustment for potential confounding factors (see Appendix A). Data was extracted by myself and checked by the third reviewer.

2.2.6. Analysis

Owing to a high degree of heterogeneity between studies, including study designs, type of population, measures of retinal vascular characteristics, types of neuropsychological tests and covariates included in analyses, meta-analysis of study results was not possible. A descriptive or non-quantitative synthesis approach was therefore used.

Participants selection and recruitment (maximum 2 points)

- Well-defined population sample (the final study sample was likely to be representative of the target population) (1)
- (Baseline) response rate 70% or more (1)

Participation at follow-up (maximum 2 points)

- Follow-up period more than one year (1)
- Loss to follow-up less than 30-40% (death was excluded from the estimate) (1)

Retinal microvascular abnormalities assessment (maximum 3 points)

- Retinopathy status based on self-report or medical records (0)

or

- Ophthalmoscopic examination (1)

or

- Retinal photography and grading based on a standardised protocol (2)
- Assessors blinded to cognitive status (1)

Cognitive function and/or dementia assessment (maximum 3 points)

- Only a global functioning test (1)

or

- Neuropsychological battery (2)

or

- Dementia based on medical records (0)

or

- Dementia diagnosed by active screening with specific criteria (1)

or

- Dementia based on world-wide recognized criteria by a central consensus committee (2)
- Assessors blinded to ocular findings and other major clinical disease status (1)

Adjustment for confounding (maximum 1 point)

- Effect of main confounders not investigated (at baseline) (0)

or

- Main confounders reported but not adjusted in the final analysis (0)

or

- Main confounders reported and adequately adjusted in the analysis (1)

Eligible studies: scoring ≥7/11 for cohort studies and ≥ 6/9 for case-control/cross-sectional studies

Figure 19: Quality assessment of studies

2.3. Results

2.3.1. Description of studies

My PubMed search yielded 180 hits, EMBASE 262 hits, PsycINFO 46 hits, BIOSIS Previews 172 hits and Web of Science 198 hits. Overall I identified 474 different citations and, following assessment of titles and abstracts, reviewed 16 potentially relevant full text articles. The numbers of studies identified at each stage of the systematic review are shown in Figure 20.

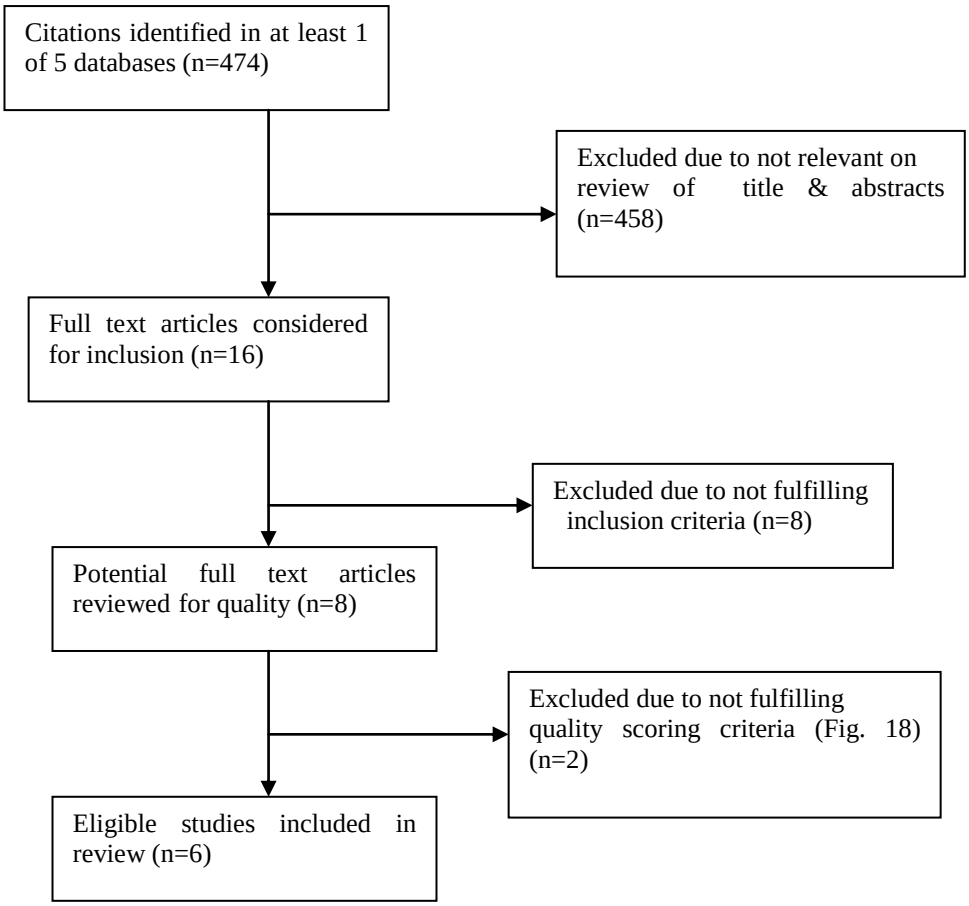


Figure 20: Flow chart of eligibility of studies

Excluded studies

Of the full text articles reviewed, three large population-based studies were excluded because only age-related maculopathy was examined for an association with cognitive function or Alzheimer's disease (Klaver et al., 1999; Wong et al.,

2002d; Clemons et al., 2006). Another three longitudinal studies which assessed the relationship between retinopathy of prematurity and cognitive outcome were also excluded because subjects were aged less than 6 years (Msall et al., 2000; Böhm et al., 2002; Mikkola et al., 2005). One cross sectional study was excluded because subjects were selected according to the presence of HIV infection in addition to retinal vascular abnormalities (Freeman et al., 2004). Two articles referred to the same study in cross sectional and longitudinal design respectively, and only the results of the longitudinal study were included (Ryan et al., 1993; Ryan et al., 2003). Of the eight studies identified as meeting our inclusion criteria (Wong et al., 2002e; Ryan et al., 2003; Ferguson et al., 2003; Tekin et al., 2004; Kadoi et al., 2005; Rotkiewicz-Piorun et al., 2006; Baker et al., 2007; Patton et al., 2007), two were subsequently excluded as they did not fulfill the quality criteria. In one, retinopathy status relied on self-reporting without any objective assessment (Rotkiewicz-Piorun et al., 2006). In the other, potential confounding variables were reported but not controlled in further analysis (Tekin et al., 2004).

Included studies

Data from 6 studies were included in the review and the key details of these studies are summarized in Table 5 & 6.

Study design and populations

The 6 included studies comprised 2 prospective cohort studies in which cognitive function was measured both at baseline (when retinal images were taken) and at follow-up (Ryan et al., 2003; Kadoi et al., 2005), and 4 cross sectional studies (Wong et al., 2002e; Ferguson et al., 2003; Baker et al., 2007; Patton et al., 2007). Three studies were population-based (Wong et al., 2002e; Baker et al., 2007; Patton et al., 2007) and 3 hospital or clinic-based (Ferguson et al., 2003; Ryan et al., 2003; Kadoi et al., 2005).

The populations sampled came from the US, UK and Japan. The clinic and hospital based studies involved patients with type 1 or type 2 diabetes (Ferguson et al., 2003; Ryan et al., 2003) and patients with type 2 diabetes after coronary artery bypass graft surgery (Kadoi et al., 2005). The age range of participants (at baseline or

at the time of survey) was from 18-35 years (Ryan et al., 2003) to 83-84 years (Patton et al., 2007) and included both men and women. In the prospective studies, duration of follow-up was 6 months and 7 years respectively (Kadoi et al., 2005; Ryan et al., 2003) with losses to follow-up estimated to be 5% and 39%.

In 3 of the 4 cross sectional studies, retinal examination was undertaken at the same time as the cognitive testing. In the other study, the cognitive function tests were performed either 3 years before or after retinal photography and the average test scores were used in the analysis (Wong et al., 2002e). One prospective study in which cognitive abilities were assessed both at baseline and 7 years follow-up, examined the presence of retinopathy biennially as well as at baseline (Ryan et al., 2003).

Two studies explicitly reported that retinopathy signs were graded without the examiner being aware of cognitive status and other clinical characteristics (Wong et al., 2002e; Kadoi et al., 2005), and two studies reported that the examiners who administered the cognitive tests were unaware of diabetic status and the presence of retinopathy (Ferguson et al., 2003; Kadoi et al., 2005).

Table 5: Included clinic/hospital-based studies investigating cognitive performance in relation to retinal microvascular abnormalities

References (year, location)	Study design	Study sample	Age (at baseline)	Assessment of retinal microvascular abnormalities	Cognitive measure & outcome	Key findings	Comments & quality rating[a]
Ferguson et al. (2003, UK)	cross-sectional Cognitive & Retinal assessments under-taken simultaneously	clinic-based 74 men & women,with type 1 diabetes	20-45 years	Ophthalmoscopic & photographic assessment for retinal microaneurysms (MA, two or more in one eye) Classified as present vs. absent	WAIS-R (PC, BD, OA &DSS) IT&CRT(ms) VFT PASAT(2s errors)	Z score for MA vs. no MA in PC: -0.13 vs. 0.07, P=0.39 BD: -0.55 vs.0.32, P<0.01 OA: -0.13 vs.0.07, P=0.44 DSS: -0.28 vs. 0.19, P=0.04 IT: 0.29 vs. -0.15, P=0.03 CRT : 0.44 vs.-0.23, P=0.003 VFT: 0.07 vs. -0.04, P=0.72 PASAT: 0.30 vs. -0.16, P=0.03	MA related to poorer cognitive measures on 2 of 4 tests of performance IQ (BD, DSS), information processing speed & attention/concentration. Hypertension, neuropathy, microalbuminuria, advanced retinopathy, CNS disease, alcohol or drug abuse excluded. Adjustment for severe hypo-glycaemia exposure, sex, premorbid IQ & duration of diabetes. **8/9**
Ryan et al. (2003,US)	prospective cohort Cognitive function measured at baseline & after 7 years; Retinopathy assessed at baseline & biennially	clinic-based 103 men & women,with type 1 diabetes	40 years	Photographic grading of proliferative retinopathy (PDR) Classified as present vs. absent	DVT, DSS, GPT, TMTB A composite Z score derived from the four tests[b]	Z change score over 7 years from baseline for 1)PDR at baseline vs. no PDR -0.50 vs. -0.22, P<0.02 2) incident PDR (follow-up) vs. no PDR -0.56 vs. -0.22, P<0.02	PDR related to greater decline in cognitive measures of psycho-motor efficiency. Alcohol or drug abuse, head trauma, PD excluded. Adjustment for incident (AN, DSP, ON, CAD, PVD), SBP, and duration of diabetes. **7/11**
Kadoi et al. (2005, Japan)	prospective cohort Cognitive function	hospital-based 180 men &	64±11 years	Ophthalmoscopic grading of retinopathy	MMSE, RAVLT, GPT, TMTA, TMTB, DSF	OR for retinopathy vs. no retinopathy	Retinopathy related to an increased risk of short and

	measured preoperatively and at 7 days & 6 months after surgery; Retinopathy assessed preoperatively	women with type 2 diabetes scheduled for CABG surgery	Classified as present vs. absent	Cognitive impairment defined as a decline from preoperative testing of more than 1SD on at least 2 of the 6 tests	at 7 days: 2.0(95%CI 1.3, 3.0) P=0.01;
					at 6 month: 2.1(95%CI 1.2,2.7) P<0.01;
					at both: 2.4 (95%CI 1.4, 2.9) P<0.01
						long term cognitive impairment after CABG surgery.
						PD, renal or active liver disease, type 1 diabetes excluded.
						Adjustment for AAA, hypertension, HbA1c, insulin therapy, Svo2 less than 50% time.
						8/11

AAA, ascending aorta atherosclerosis; AN, autonomic neuropathy; CABG, coronary artery bypass grafting; CAD, coronary artery disease; CI, confidence interval; DSP, distal symmetric polyneuropathy; HbA_1, glycosylated haemoglobin; ON, overt nephropathy; OR: odds ratio; PD, psychiatric disorder; PVD, peripheral vascular disease; SBP, systolic blood pressure; SD, standard deviation; Svo_2, jugular venous oxygen saturation

[a] Boldface number indicates quality rating; [b] Tests of VPAL, WSTM, RCFM, SDPA ,TPT, WAIS-R (OA, BD), WCR were also administered but not used in analysis of a relationship with retinopathy, which was judged by authors due to no group effect shown between the diabetics and health controls on the measures

Table 6: Included population-based studies investigating cognitive performance in relation to retinal microvascular abnormalities

Referencs (year)	Study (country)	Study design	Study sample	Age	Assessment of retinal microvascular abnormalities	Cognitive measure & outcome	Key findings	Comments & quality rating[a]
Wong et al. (2002)	ARIC study (US)	cross-sectional Cognitive function assessed at 3 years before or after retinal images taken (averaged test scores used in analysis)	8,734 men & women, sampled from 4 US communities	51-70 years	Photographic grading of 1) any retinopathy 2) microaneurysm 3) retinal haemorrhage 4) soft exudates 5) focal arteriolar narrowing (FAN) 6) AV nicking Computer-assisted grading of 7) generalised arteriolar narrowing, summarised by AVR and defined as lowest 20th percentile of AVR distribution Classified as present vs. absent	DWR, DSS, VFT Cognitive impairment defined as scores 2SD or lower than the mean scores for each test	in DWR OR for presence vs. absence of 1) any retinopathy 2.6 (95%CI 1.3 to 2.9) 2) microaneurysms 3.0 (95%CI 1.8 to 5.0) 3) retinal haemorrhage 3.4 (95%CI 2.0 to 5.8) 4) soft exudates 3.1 (95%CI 1.5 to 6.2) 5) AV nicking 1.2 (95%CI 0.8 to 1.6) 6) FAN 0.6 (95%CI 0.4 to 0.9) 7) generalized arteriolar narrowing 1.0 (95%CI 0.8 to 1.5) Similar results for DSS and VFT	Retinal vascular signs except AV nicking & arteriolar narrowing, related to lower cognitive test scores. Prevalent stroke, taking CNS relevant medication excluded. Adjustment for age, sex, race, field centre, education, occupation, diabetes, fasting glucose, hypertension, carotid IMT, cigarette smoking, alcohol intake, fasting total and HDL cholesterol and triglyceride levels. **7/9**
Baker et al. (2007)	Cardiovascular Health Study (US)	cross-sectional Cognitive& Retinal assessments under-taken simultaneously	2,211 (1,767 in analysis for dementia) men & women, sampled from a defined sample of Medicare	69-97 years	Photographic grading of 1) any retinopathy 2) focal arteriolar narrowing (FAN) 3) AV nicking Classified as present vs. absent Computer-assisted grading of 4) CRAE 5) CRVE Measured in micrometers and	3MSE, DSS Clinical diagnosis of dementia[b]	in DSS Adjusted mean scores for presence vs. absence of 1) any retinopathy 39 vs. 42 P<0.02 2) FAN 42 vs. 42 P=0.62 3) AV nicking 41 vs. 42 P=0.20 1st to 5th quintile of	Any retinopathy related to lower DSS scores; focal arteriolar narrowing related to an increased risk of dementia. Both retinal vascular signs related to an increased risk of dementia in hypertensive people. Prevalent of stroke, taking anti-psychotics or anti-depressants excluded.

			eligible persons from 4 US communities		categorised into quintiles		4)CRAE or 5)CRVE between-group difference P=0.48 or P=0.64 separately in dementia OR for presence vs. absence of 1) FAN 1.99 (95%CI 1.11 to 3.56); 2)FAN in hypertensives 3.02 (95%CI 1.51-6.02); 3) any retinopathy in hypertensives 2.10 (95%CI 1.04-4.24) No statistically significant results for 3MSE	Adjustment for age, sex, race, field center, education level, internal carotid IMT, BMI, hypertension, diabetes, and cigarette smoking. **6/9**
Patton et al. (2007)	Lothian Birth Cohort 1921 Study (UK)	cross-sectional Cognitive &Retinal assessments under-taken simultaneously	321 men & women born in 1921, participated in a mental survey of 1932 and residing in the Lothian region of Scotland	83-84 years	Computer-assisted grading of 1) CRAE 2) CRVE 3) AVR 4) Suboptimal branching coefficient (SBC) 5) Suboptimal bifurcation angles (SBA) CRAE & CRVE measured in pixels, SBA in degree; all measured on continuous scale	MMSE LMT VFT RPM General cognitive ability score (g) extracted from the 4 tests	Effect size (η^2) of the variance in g accounted for by SBC: 0.034 P=0.02; in LMT by SBA: 0.026 P=0.03; in VFT by SBC: 0.037 P=0.01 No statistically significant results for RPM or with CRVE, CRAE, and AVR.	Suboptimal BC related to lower scores in g & VFT; suboptimal BA related to lower scores in LMT Adjustment for sex, diabetes, cerebrovascular disease, SBP, DBP, alcohol use, smoking, APOE e4 status, near visual acuity, social class, education, IQ at age 11 **7/9**

ARIC, the Atherosclerosis Risk in Communities Study; APOE, apolipoprotein E;BMI, body mass index; DBP, diastolic blood pressure; HDL, high-density lipoprotein; IMT, intima-media wall thickness; IQ, intelligence; SBP, systolic blood pressure; SD, standard deviation

[a] Boldface number indicates quality rating; [b] Based on detailed neuropsychological testing and established by a consensus committee

Assessment of retinal microvascular abnormalities

Both the techniques and grading methods used to evaluate retinal microvascular signs varied between the studies. In one study, the combination of ophthalmoscopic examination with digital retinal photography was adopted (Ferguson et al., 2003), whereas in the remaining studies, stereoscopic color fundus photography alone was used. Prior to retinal photography, pharmacologic dilatation of the pupil(s) was performed in two studies (Ryan et al., 2003; Patton et al., 2007). The angle used for the field of view in photography differed, ranging from 30^{o} (Ryan et al., 2003, Kadoi et al., 2005; Ferguson et al., 2003) to 45^{o} (Wong et al., 2002e; Baker et al., 2007) or 50^{o} (Patton et al., 2007). Three studies reported that only one eye was assessed (Wong et al., 2002e; Baker et al., 2007; Patton et al., 2007) (either randomly or arbitrarily based on laterality) but the other studies didn't provide such information.

In the studies using a fully manual grading system, subjects were categorized either according to the presence of diabetic retinopathy varying with severity (Ryan et al., 2003, Kadoi et al., 2005; Ferguson et al., 2003), or into one of several defined groups, including the presence of: 1) any specific retinopathy signs (microaneurysms, retinal haemorrhages, cotton wool spots, soft exudates, hard exudates, macular edema, venous beading etc.); 2)arteriovenous nicking; 3) focal arterial narrowing (Wong et al., 2002e; Baker et al., 2007) (Table 7). At least three different classification systems for retinal vascular signs based on previous diabetic or hypertensive retinopathy studies were used to develop standardised grading protocols in five of the studies. Computer-assisted imaging techniques were applied in three studies to quantify retinal vessel caliber and other parameters of retinal vascular network geometry (Wong et al., 2002e; Baker et al., 2007; Patton et al., 2007). One study focusing on computer-based quantitative measures alone assessed 5 continuous variables: the central retinal arterial equivalent (CRAE), central retinal venular equivalent (CRVE), the arteriovenous ratio (AVR), suboptimal geometry of arteriolar branching coefficients and angles (Patton et al., 2007) (Table 7).

Table 7: Terminology and definitions of retinal microvascular abnormalities used in included studies

Retinal microvascular signs	Definitions	References
Background diabetic retinopathy	The presence of two or more microaneurysms in one eye	Ferguson et al.
Proliferative diabetic retinopathy (PDR)	A grading level at 60 or higher in one eye, or less than 60 but with panretinal photocoagulation scars consistent with laser therapy[a]	Ryan et al.
Diabetic retinopathy	A grading level greater than 10 (10=no retinopathy)[b]	Kadoi et al.
Any retinopathy	The presence of any of the following lesions (not explicitly arteriolar in nature): microaneurysms, retinal haemorrhages, soft exudates, hard exudates, macular edema, intraretinal microvascular abnormalities (tortuous intraretinal vessels), venous beading, new vessels at the disc or elsewhere, vitreous haemorrhage, disc swelling , or laser photocoagulation scars[b]	Wong et al. Baker et al.
Focal arteriolar narrowing (FAN)	An arteriole estimated to be 50-μm diameter or greater has a constricted area of 2/3 or less the width of proximal and distal vessel segments[c]	Wong et al., Baker et al.
Arteriovenous nicking (AV nicking)	A venule is constricted on both sides of its crossing under an arteriole in a specified region[c]	Wong et al., Baker et al.
Generalised arteriolar narrowing	The lowest 20th percentile of the sample arteriole-to-venule ratio (AVR) distribution measured on a continuous scale via a computer-assisted technique	Wong et al.
Arteriovenous ratio (AVR)	A ratio between the summarized caliber measurements of the arterioles (CRAE) with respect to the venules (CRVE)[d]	Wong et al. Patton et al.
Central retinal arterial equivalent (CRAE)	Combined calibre measurements of individual arterioles coursing through a circular zone defined as between 0.5 and 1 disc diameter from the optic disc margin[d]	Wong et al. Baker et al. Patton et al.
Central retinal venular equivalent (CRVE)	Combined calibre measurements of individual venules coursing through a circular zone defined as between 0.5 and 1 disc diameter from the optic disc margin[d]	Wong et al. Baker et al. Patton et al.
Arteriolar branching coefficient (BC)	A ratio expressing the relationship between trunk vessel (W) and two branch vessels (w_1 and w_2) on vessel widths at arteriolar branching points: $BC=\left(w_1^2 + w_2^2\right)/W^2$	Patton et al.
Arteriolar bifurcation angles (BA)	An angle (degree) measured at arteriolar branching points between the two branch arterioles	Patton et al.
Suboptimal branching coefficient	The degree of deviation of the BC from optimality (i.e., a theoretical value of 1.26)	Patton et al.
Suboptimal angles	The difference of the degree of BA from optimality (i.e., a theoretical and empirical value of 75^o)	Patton et al.

Note. [a] the Wisconsin Epidemiologic Study of Diabetic Retinopathy Classification and Grading System (ETDRS, 1980); [b] the Diabetic Retinopathy Study and the Early Treatment Diabetic

Retinopathy study grading scale (ETDRS Report No.12, 1991); [c] the Modified Airlie House Classification of Diabetic Retinopathy (ETDRS Report No. 10, 1991); [d] Methods for evaluation of retinal microvascular abnormalities associated with Hypertension/Sclerosis in the Atherosclerosis Risk in Communities Study (Hubbard et al., 1999)

Assessment of cognitive function and impairment

Cognitive function was assessed using four primary methods: (1) specific cognitive tests (Table 8); (2) a mean/composite score on two or more tests in the battery (Ryan et al., 2003); (3) general cognitive ability score (g) generated by extracting a component reflecting the variance shared by all tests (Patton et al., 2007); (4) clinical assessment alone (e.g. diagnosis of dementia) (Baker et al., 2007). Results were reported as either continuous or categorical outcomes. More than 17 different psychometric tests were used among the 6 studies reviewed. To help organize discussion, many of these neuropsychological tests can be classified into the cognitive domain that they examined, including attention/concentration, information processing speed, manual dexterity, frontal lobe/executive function, verbal memory, performance IQ, and mini-mental state examination (Table 8) (Strachan et al., 1997; Lezak, 1995). In one study (Baker et al., 2007) with dementia as outcome, patients were identified by an initial screening across the whole study population, then by a more detailed diagnostic examination by neurologists and psychiatrists in patients suspected of cognitive impairment, and dementia was defined in terms of the criteria from the *Diagnostic and Statistical Manual of Mental Disorders, 4th edition* (American Psychiatric Association, 1994).

Three of the studies including one of the prospective studies divided the participants into two groups based on whether or not they experienced cognitive impairment or decline (during follow up). Cognitive impairment was defined in a variety of ways that included: (1) a decline by a particular amount relative to the baseline test scores (Kadoi et al., 2005); (2) test scores below a particular threshold score (Wong et al, 2002e); or (3) the presence of clinically diagnosed dementia (Baker et al., 2007). Three studies used variations of the Mini-Mental Examination (MMSE) test: two used it as a continuous variable to assess general cognitive functioning (Kadoi et al., 2005; Baker et al., 2007) whereas another study only included it to screen subjects for dementia (Patton et al., 2007).

Two studies assessed premorbid intellectual ability in addition to performance IQ, by using psychometric testing strategy. One administered the vocabulary-based National Adult Reading test (NART) at the time of study and adjusted the scores in the analysis (Ferguson et al., 2003). The other study used the Moray House Test scores at age 11 years as a direct estimate of premorbid IQ (Patton et al., 2007).

Table 8: Cognitive function assessment tools used in studies evaluating the relation to retinal vascular signs

Cognitive Domain[a]	Test Name	References
Attention/Concentration	Paced Auditory Serial Additional Task (PASAT)	Ferguson et al.
	Digit Span Forward (DSF)	Kadoi et al.
	DigitVigilance Test[b] (DVT)	Ryan et al.
	Part A of the Trail Making test (TMTA)	Kadoi et al.
	Part B of the Trail Making test[b] (TMTB)	Ryan et al., Kadoi, et al.
Information processing speed	Digit Symbol Subtest[b,c] (DSS)	Wong et al., Ferguson et al.,
	Inspection time & Median four-choice reaction time (IT & MRT)	Ryan et al, Baker et al Ferguson et al.
Manual dexterity	Grooved Pegboard[b] (GPT)	Ryan et al., Kadoi et al.
Frontal lobe/executive function	Verbal Fluency Test (VFT)	Wong et al., Patton et al. Ferguson et al.
Performance IQ	Object Assembly (OA) and Block Design subtests (BD): ([WAIS-R])	Ryan et al., Ferguson et al. Ferguson et al.
	Picture completion (PC)	Patton et al.
	Raven's Progressive Matrices (RPM)	
Verbal Memory	Delayed Word Recall Test (DWR)	Wong et al.
	Rey auditory verbal learning test (RAVLT)	Kadoi et al.
	Logical Memory (LMT)	Patton et al.
Mini-Mental State Examination (MMSE)	MMSE	Kadoi et al., Patton et al., Baker et al.
	Modified Mini-Mental State Examination (3MSE)	

Note. WAIS-R= Wechsler Adult Intelligence Scale-Revised; [a] Classification of cognitive domain based on descriptions by Lezak (1995) and widely used definitions; [b] Tests used as part of Psychomotor Efficiency domain in Ryan et al.; [c] Test used as part of performance IQ domain in Ferguson et al.

2.3.2. Findings from studies on diabetic patients

The results of the studies on subjects with Type 1 or Type 2 diabetes are shown in Table 5.

In 74 patients with Type 1 diabetes (Ferguson et al., 2003), the presence of retinal microaneurysms was associated with poorer performance on some tests of performance IQ (Block Design, Digital Symbol Test), information processing speed and attention /concentration but not on other tests of performance IQ (Picture Completion, Object Assembly) or frontal/executive functions. In a second study of 103 patients with Type 1 diabetes (Ryan et al., 2003), the presence of proliferative diabetic retinopathy (PDR) at baseline or development of the condition during follow-up was associated with poorer performance on measures of 'psychomotor efficiency' (a composite score based on the results of 4 tests: Digit Vigilance, Digital Symbol Test, Grooved Pegboard and Part B of the Trail Making). Compared with diabetic people without retinopathy at either assessment, there was a decline in subjects with PDR (change in test score -0.50 or -0.56 vs. -0.22, both $P<0.02$) over 7 years. In both studies, the association remained significant after adjusting for a variety of demographic and several important diabetes-related variables. The magnitude of the cognitive deficits observed in these young adult subjects was moderate to large, with effect sizes for the group difference ranging from about 0.3 to 0.7 SD (according to Cohen's [1988] terminology).

In 180 patients with Type 2 diabetes (Kadoi et al., 2005), after adjustment for a number of biomedical and demographic variables, the presence of diabetic retinopathy assessed prior to coronary artery bypass grafting surgery was associated with an increased risk of cognitive decline (compared with pre-operative cognitive performance) after 7 days (OR 2.0, 95%CI 1.3, 3.0) and 6 months (OR 2.1, 95%CI 1.2, 2.7) following surgery.

2.3.3. Findings from studies on population-based samples

The results from population-based studies are shown in Table 6.

In the Atherosclerosis Risk in Communities Study of 8,734 middle-aged men and women free of stroke (Wong et al., 2002e), the presence of any retinopathy, microaneurysms, retinal haemorrhages or soft exudates was associated with an increased risk of impaired performance on tests of verbal memory (Delayed Word

Recall), information processing speed (Digital Symbol Test) and frontal lobe/executive function (Verbal Fluency Test). The presence of arteriolar narrowing or AV nicking was not associated with these tests. Subjects with any of these retinal vascular lesions were 2.6 (OR, 95%CI 1.3, 2.9) to 3.4 (OR, 95%CI 2.0, 5.8) times more likely to have cognitive impairment than people without these lesions independent of other biomedical, psychosocial and demographic covariates.

In the Cardiovascular Health Study (Baker et al., 2007) of 2,211 men and women, the presence of any retinopathy was associated with poorer performance on information processing speed (Digital Symbol Test, adjusted mean test scores 39 vs. 42, P=0.02), but not on the MMSE. No association was found with the presence of focal arteriolar narrowing, AV nicking or quintiles of retinal caliber. In a hypertensive subgroup, the presence of any retinopathy (multivariable-adjusted OR, 2.10 95%CI 1.04, 4.24) or focal arteriolar narrowing (OR, 3.02 95%CI 1.51, 6.02) was associated with an increased risk of dementia, but no association was found with the presence of other retinal vascular signs.

In the Lothian Birth Cohort 1921 (LBC1921) study of 321 elderly men and women (Patton et al, 2007), deviation from optimality of the retinal vascular network geometry was associated with lower cognitive ability scores, but no association was found for retinal arteriolar or venular calibers. After controlling for mental ability at age 11, APOE e4 status and a variety of other demographic, behavioral and biomedical covariates, sub-optimality of angles at arteriolar bifurcations (from theoretically derived-optimum values) was associated with poorer verbal memory (Logical Memory) but not other cognitive modalities including general cognitive ability (g), frontal lobe/executive function (Verbal Fluency Test) or performance IQ (Raven's Matrices), and suboptimal branching coefficient (from theoretically derived-optimum values) was associated with poorer general cognitive ability and verbal fluency but not verbal memory or performance IQ. The effect size of the variance in cognitive test scores explained by the retinal vascular abnormalities was small, ranging from 2.6% to 3.7%.

2.4. Discussion

The preceding systematic review aimed to describe and examine relevant, published research that has investigated the association between retinal vascular signs and cognitive dysfunction. To date, research in this area is very limited. Findings are consistent with the hypothesis that retinal microvascular abnormalities are associated with cognitive impairment and dementia in diabetic patients and in the general population, although findings are not conclusive due to varied and sometimes poor methodology in the included studies. The presence of retinal vascular signs was mostly associated with poorer verbal memory, information processing speed (Digit Symbol Test) and frontal lobe function (Verbal Fluency Test) in the general population, and with poorer information processing speed in patients with Type 1 diabetes. Cognitive dysfunction among diabetic patients with retinal vascular signs was also suggested on measures of attention and concentration. However, these findings were inconsistent, possibly due in part to a lack of precision in linking cognitive tests to function (e.g. using only a composite test score rather than individual ones).

A number of important methodological issues need to be considered when interpreting the findings of this review and understanding inconsistencies in the findings from different studies. These issues are also important for planning future research in this area.

2.4.1. Study design

Half of the studies were clinic-based on diabetic patients, using variable inclusion criteria that limited generalization of the results. Diabetes-related cognitive impairment may have different underlying mechanisms from cognitive dysfunction in the general population and may differ in patients with Type 1 diabetes, Type 2 diabetes and postoperatively. However, population-based studies are scarce so far restricted to a cross-sectional design, which makes it impossible to determine the temporal relationship between retinal vascular disease and cognitive dysfunction (though it is perhaps unlikely that cognitive impairment leads directly to retinal vascular abnormalities).

Although longitudinal studies are superior to cross sectional studies when investigating ageing or cohort effects (Allen et al., 2004), the longitudinal studies in this review may have underestimated the impact of retinal vascular abnormalities on cognitive function in patients with diabetes because of survival bias or learning effects. Diabetic patients with retinopathy at baseline were more likely not to attend for follow-up (including deaths), when follow-up success itself could well be linked to good cognitive function. However, the two studies did not track retinopathy information among patients lost to follow-up, as the cognitive effects of retinopathy were not their primary focus (Ryan et al., 2003; Kadoi et al. 2005). This could introduce bias given one study had a proportion of losses to follow-up as high as 39% (Ryan et al., 2003). Learning effects (Morris et al., 1999) may also obscure any true underlying cognitive decline particularly when intervals between test sessions are short and tests are administered multiple times, even if parallel versions are applied (Rasmussen et al., 2001).

2.4.2. Measuring retinal microvascular abnormalities

Compared with ophthalmoscopic examination, the assessment of retinal photographs has been suggested as a more reliable method for measuring a number of retinal vascular signs, including generalized and focal arteriolar narrowing, arteriovenous (AV) nicking, isolated retinal haemorrhages, microaneurysms and cotton wool spots (Couper et al., 2002; Wong et al., 2001b; Wong, 2004; Porta et al., 2005). Computer-assisted measures of retinal vascular widths (to define generalized arteriolar narrowing, venular dilatation and other measures of retinal vascular network geometry), have been found to detect subtle microvascular changes with low intra and inter-observer variability (Couper et al., 2002; Sherry et al., 2002; Wong et al., 2003b,c). Grading of other abnormalities (such as focal arteriolar attenuation and retinal haemorrhages) is still largely done manually and therefore highly operator dependent, though automated techniques are being developed. The reliability of grading of focal retinal abnormalities has also been shown to be dependent on the classification system used (Boehm et al., 2002). Despite the use of standardised protocols, differing methods and grading systems for measuring retinal

microvascular abnormalities could explain at least in part some of the inconsistent findings between studies in this review.

2.4.3. Measuring cognitive function

Many observed changes in cognitive function of interest in studies are generally small in comparison with the entire range of function (Morris et al., 1999). However, mild cognitive impairment is an established risk factor for the subsequent development of dementia (Petersen et al., 2001) and may therefore be considered to be clinically relevant (Allen et al., 2004). This requires measurement of cognitive function that use validated (sensitive to small increments of change) and reliable psychometric tests, covering the range of different domains of interest including memory, reasoning and language (Areosa et al., 2003). In two of the studies, less than 3 neuropsychological tests were used and only a narrow range of cognitive domains could be assessed (Wong et al., 2002e; Baker et al., 2007). In one of the studies, MMSE scores were used to examine the relationship with retinal vascular signs (Baker et al., 2007). However, the MMSE is not designed to measure subtle changes in cognitive decline, but to screen for dementia (Folstein et al., 1975; Tombaugh and McIntyre, 1992). This simple test is likely to produce a ceiling effect in measurement and has reduced sensitivity to detect a mild change in cognitive performance in people with high educational achievement (Allen et al., 2004)

The majority of cross-sectional studies of cognitive function relied on cognitive performance at a single time point, which does not capture the pathological process under study--cognitive decline or change in cognitive function over time. Pre-morbid cognitive ability represents the highest level of ability attained prior to any cognitive decrements taking place and is also known to account for a substantial proportion of the variance in cognitive function scores in old age (Strachan et al., 1997; Deary 2000; Deary et al., 2003). However, such prior cognitive data are rarely available for most people, especially from a previous period of good health (Deary et al., 2004a). Some studies estimated prior ability by a patient's education and occupation history, but this is limited in that these features may be influenced by lack of opportunity rather than lack of ability (Nelson and Willison, 1991). The National

Adult Reading Test (NART) could be a more useful measure as word reading ability is preserved even in generalized cognitive decline (Nelson and Willison, 1991). Adjusting scores on a fluid mental test for NART has also been shown to be a valid, instantaneous measure of lifetime cognitive change or cognitive decline (Deary et al., 2004a; McGurn et al., 2004). Only one study adjusted for pre-morbid IQ using cognitive data from youth of early life for aging cohort in an attempt to get a direct measure of lifetime cognitive decline (Patton et al., 2007).

2.4.4. Confounding and effect modification

Most studies assessed important potential confounders such as age, gender, lifestyle factors and medical conditions, including diabetes, diabetes-specific variables (such as duration of diabetes and HbA1c levels), hypertension, dyslipidemia and other cerebrovascular or cardiovascular disease. Patients with neurological conditions (including a history of stroke) that might interfere with cognitive abilities were also excluded from the majority of studies. However, subjects with a history of chronic alcohol or drug abuse were only variably excluded. In addition, inadequate adjustment was generally made for visual acuity and depression. Some of the cognitive tasks may require at least moderate visual function to complete and if diabetic people with severe retinopathy have greatly impaired vision, their scores on the relevant cognitive testing could be affected. Two of the studies collected data on visual acuity (Ryan et al., 2003; Patton et al., 2007) and one study controlled for this in assessing the relationship between retinal vascular abnormalities and cognitive function (Patton et al., 2007). Depression may confound the association between retinal microvascular disease and cognitive dysfunction because depression can be mistaken for dementia (and vice versa), and is associated with deficits of memory and learning, and occurs more frequently in those with diabetes-related complications including retinopathy (Reding et al. 1985; Austin and Mitchell, 1997; Visser et al., 2000; Anderson et al., 2001; Roy et al., 2007). Two studies assessed the potential effects of low mood (Ryan et al., 2003; Ferguson et al., 2003).

Studies which attempted to evaluate effect modification looked at

interaction with diabetes and hypertension and found that retinal microvascular disease may be linked to an increased risk of cognitive impairment in those with high blood pressure or diabetes (Wong et al., 2002b; Baker et al, 2007). Such analyses may provide insights into etiologic mechanisms. However, the relationship between retinal vascular disease and cognitive dysfunction in other different sub-groups of the population (e.g. younger and older, males and females) has not been well examined, as most studies lacked sufficient statistical power for analysis.

2.4.5. Limitations of review

It should be noted that I reviewed only published studies and it is possible that studies which did not show an association between retinal vascular disease and cognitive dysfunction may not have been published as often as studies that did. It was not possible to test for this formally (e.g. by use of a funnel plot) because studies were too few and too methodologically heterogeneous.

2.5. Chapter summary

There is some epidemiologic evidence from six published studies suggesting that the presence of retinal microvascular abnormalities may be associated with an increased risk of cognitive impairment or dementia in both diabetic patients and in the general population. However, findings are still inconclusive regarding the true nature and importance of this association, as previous findings are limited by substantial differences in methodology between studies, small size of some of the studies, limited controlling for potential confounding factors and variability in the range of cognitive domains and retinal microvascular abnormalities measured. In particular, no study to date has explored such an association in a large group of patients with Type 2 diabetes.

Therefore, the epidemiological study and the analysis which are presented in Chapters 3 to 5, appear to be well suited to contribute to the body of knowledge in this field as the ongoing study is based on a uniquely large sample size of 1,066 older people with Type 2 diabetes designed to investigate the association between

microvascular disease and cognitive decreaments, using a battery of neuropsychological tests, standardised retinal vascular assessment, and measurement of a wide range of potential confounding factors.

Chapter 3

Aims and Objectives

3.1. Introduction

Chapter 1 highlighted the fact that Type 2 diabetes is associated with poor cognitive ability especially in late adulthood. However, few data are available on the risk factors associated with the development of cognitive impairment in Type 2 diabetes. Although cerebral microvascular disease has been hypothesised to contribute to the deleterious effect of diabetes on cognition, very little epidemiological data has been reported to support such an association, particularly in large numbers of people with Type 2 diabetes. The systematic review in Chapter 2 demonstrated that retinal microvascular abnormalities, which are prevalent in Type 2 diabetes, have been proposed as markers of cerebral microvascular disease. Such abnormalities have therefore been hypothesised as potential risk factors for cognitive dysfunction in a general population and in people with diabetes. However, their relationship with cognitive impairment in people with Type 2 diabetes has not been well-established due to scarce data from relatively small clinical samples.

3.2. Aim

The principal aim of this thesis was to determine whether several parameters of retinal microvascular abnormality were related to cognitive ability, to estimated lifetime cognitive decline, and to cognitive impairment (based solely upon cognitive test performance) in people with Type 2 diabetes in order to explore possible mechanisms involved in the aetiology of diabetes-related cognitive impairment. Data from the cross-sectional phase of the Edinburgh Type 2 Diabetes Study, an ongoing population cohort with plans to undertake repeat cognitive testing at four years follow-up, were used (the ET2DS is described in detail in the following chapter). The following parameters of retinal microvascular abnormality were assessed: diabetic retinopathy, retinal vessel calibres summarised as the central retinal arterial and venular equivalents (CRAE and CRVE, respectively), arterio-venous ratio (AVR),

and retinal arteriolar bifurcation geometry expressed as absolute and deviation of the median branching coefficient (BC) and median angle (BA). The main outcomes examined were cognitive ability (scores on neuropsychological tests), lifetime cognitive decline estimated by adjusting scores on a fluid mental test for pre-morbid ability (the Mill-Hill Vocabulary Scale) and the presence of cognitive impairment defined as scores 1.5 SD or lower than the mean score of the group.

3.3. Objectives

1. To estimate the total prevalence of diabetic retinopathy and the prevalences of diabetic retinopathy according to severity level in older people with Type 2 diabetes.

2. To examine the distribution of the following quantitative parameters of retinal vascular network geometry in elderly people with type 2 diabetes:

(a) CRAE, CRVE, and AVR
(b) absolute value of arteriolar branching coefficient (BC) and angle (BA)
(c) deviation of the BC from optimality (sub-optimal BC)
(d) deviation of the BA from optimality (sub-optimal BA)

3. To determine the association of the presence and severity of diabetic retinopathy and other retinal vascular abnormalities as described above with

(i) sociodemographic characteristics (age, sex, educational level, occupational social class)
(ii) conventional cardiovascular risk factors (alcohol consumption, smoking habit, obesity, brachial arterial blood pressure and blood lipids)
(iii) cardiovascular disease (myocardial infarction or angina pectoris, stroke or transient ischaemic attack, and peripheral arterial disease)
(iv) diabetes history and control (duration of diabetes, HbA1c level and treatment modality)
(v) mood and prior cognitive ability level (depression and peak prior cognitive ability)

4. To determine the association between current cognitive ability and

(i) sociodemographic characteristics (age, sex, educational level, occupational social class)

(ii) conventional cardiovascular risk factors (alcohol consumption, smoking habits, obesity, brachial arterial blood pressure and blood lipids)

(iii) cardiovascular disease (myocardial infarction or angina pectoris, stroke or transient ischaemic attack, and peripheral arterial disease)

(iv) diabetes history and control (duration of diabetes, HbA1c level and treatment modality)

(v) mood and prior cognitive ability level (depression and peak prior cognitive ability)

5. To determine the association of the presence of diabetic retinopathy and severity of diabetic retinopathy with cognitive ability, estimated lifetime cognitive decline and cognitive impairment

6. To determine the association of quantitative parameters of retinal vascular network geometry (CRAE, CRVE, AVR, absolute and suboptimal BA and BC) with cognitive ability, estimated lifetime cognitive decline and cognitive impairment

7. To determine the independence of associations between the retinal microvascular abnormalities as above and cognitive function after adjusting for psychosocial factors, cardiovascular risk factors, cardiovascular disease and diabetes control.

Chapter 4

Methods

4.1. Introduction

This chapter describes the baseline methodology of the Edinburgh Type 2 Diabetes Study, which is the first population-based epidemiological study in the United Kingdom to focus principally on cognitive dysfunction in people with Type 2 diabetes. Specifically, the chapter provides information on recruitment of the study population as well as the baseline clinical examinations. In addition, the grading protocols used for diabetic retinopathy and the assessment of other retinal vascular abnormalities are described. Finally the chapter presents the methods used to define cardiovascular variables which were used in subsequent analyses, and the statistical analysis plan. The Edinburgh Type 2 Diabetes study was originally designed by other investigators and Section 4.2.1 and 4.2.2 were mainly taken from the ET2DS Methods paper (Price et al., 2008).

4.2. The Edinburgh Type 2 Diabetes Study

4.2.1. Study design and subject recruitment

The principal aim of the Edinburgh Type 2 Diabetes Study (ET2DS) was to investigate potentially modifiable risk factors for cognitive dysfunction in people with Type 2 diabetes. The ET2DS commenced as a cross-sectional survey in 2006 and continues as a 4-year longitudinal study. The study population of the ET2DS comprised 60 to 74 year-old inhabitants of Lothian, Scotland with established Type 2 diabetes. A random sample of participants stratified by sex and 5-year age bands was selected from the Lothian Diabetes Register (LDR), which is a computerised database containing clinical details on over 20,000 patients with known Type 2 diabetes. Comparison of age-sex specific prevalences of diabetes recorded on the LDR with those from other data sources in Scotland suggests that the LDR captures almost everyone with diagnosed diabetes in Lothian (Price et al., 2008). The sample

size of 1,000 was estimated on the basis of the number required to conduct a baseline and a subsequent follow-up study with sufficient statistical power to detect any risk factor that contributed 1% or more to the variance in continuous outcome measures (i.e. cognitive test scores).

All eligible subjects were approached about attending a research clinic for medical examination (n=5,454). Following publicity in the local media, letters of invitation to participate in the study were sent by the custodians of the Lothian Diabetes Register. Subjects not replying to the invitation letter, with letters returned by the Post Office, or refusing to participate were replaced by another randomly selected subject from the same sex and 5-year age band. To preserve patient confidentiality, only the names and addresses of subjects who expressed interest in participating were forwarded to the ET2DS team. On receipt of an affirmative reply, an appointment was booked for the research clinic, and a map, along with a questionnaire to be completed and returned was sent. Transport to and from the clinic was provided for those having difficulty in attending the clinic. Travelling expenses were paid if required. Respondents who did not attend the clinic were offered a second appointment both by telephone and mail, and reminder calls on the day preceding clinic appointments were made (Price et al., 2008). The overall subject recruitment is summarised in Figure 21.

Of the 1,077 subjects attending the baseline clinic, four were subsequently excluded because they were unable for physical or emotional reasons to complete the cognitive or clinical examination. In addition, seven subjects were excluded as they did not meet the criteria for Type 2 diabetes after detailed review of medical notes, searching of electronic databases and, where necessary, discussion with the general practitioner (Price et al., 2008). Of these seven subjects, two diet-treated patients with HbA1c <6.5% at the research clinic could not be confirmed as having diabetes, four were felt to have Type 1 diabetes due to starting on insulin within 1 year of diagnosis or being treated with insulin and diagnosed under the age of 35 years, and one subject had a previous pancreatic neuroendocrine tumour. This left 1,066 subjects who were both willing and eligible to take part in the study and who formed the total study population of the ET2DS. The study was approved by Lothian

Medical Research Ethics Committee and informed consent was obtained from each participant.

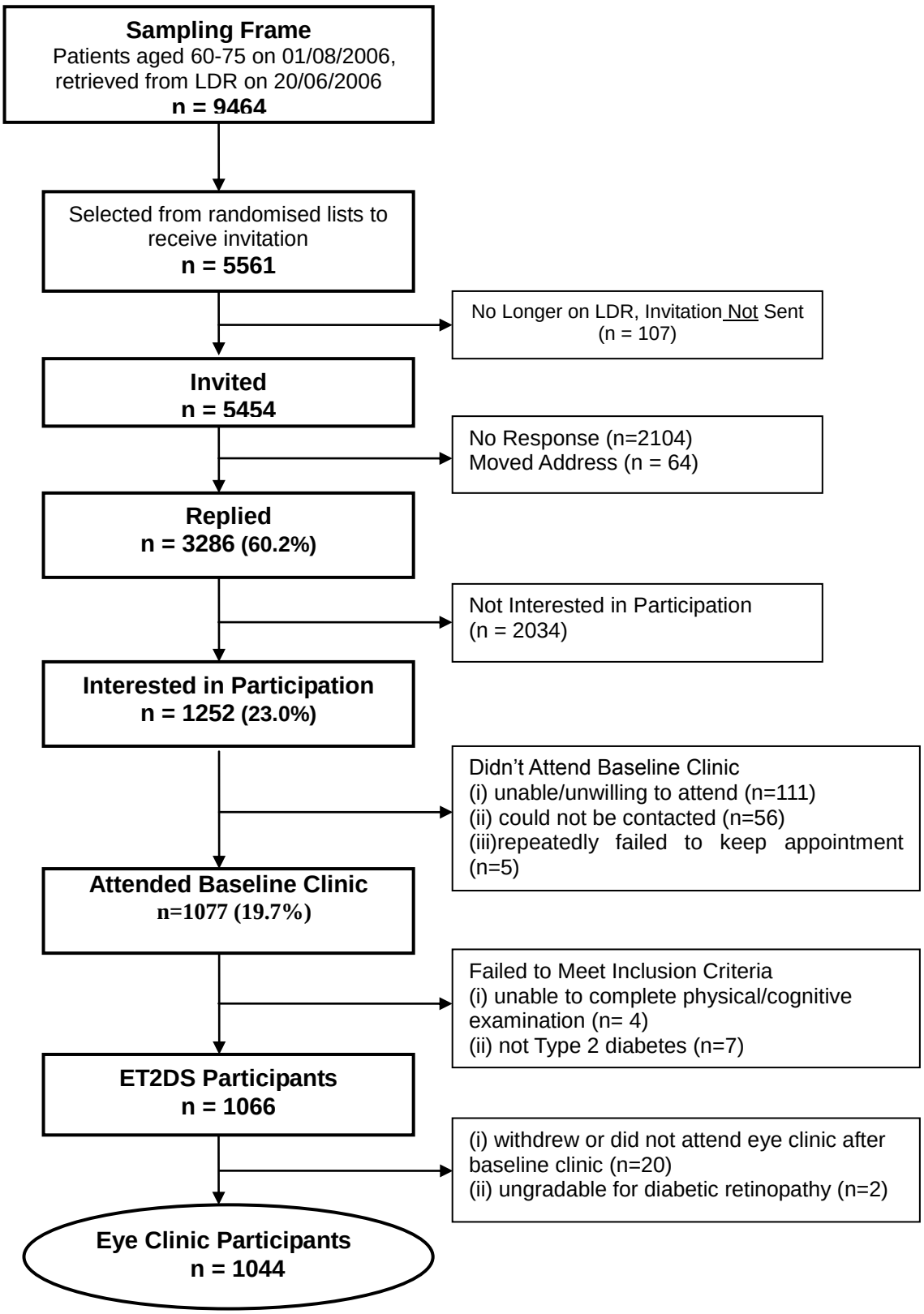


Figure 21: Recruitment summary of the Edinburgh Type 2 Diabetes Study

4.2.2. Clinical examination

Physical examinations were conducted each weekday morning from August 2006 to September 2007 at a research clinic. All subjects were asked to fast from 11pm the previous evening and a venous blood sample was taken for subsequent measurements of biochemical, inflammatory and genetic factors. Before the clinical procedures were performed, a self-administered questionnaire was filled in by each participant and read over for completeness by one of research nurses.

Questionnaire

The self-administered questionnaire contained validated questions enquiring about personal characteristics (age, sex, and marital status), highest education level completed, occupational social class, smoking history, alcohol consumption, diabetes history and control (age at onset of diabetes, current treatment by insulin injections or tablets), other medication use and recall of a doctor's diagnosis of cardiovascular disease (myocardial infarction, angina pectoris, stroke, transient ischaemic attack, and peripheral arterial disease) (Appendix D). The WHO Chest Pain (Rose, 1962) and Edinburgh Claudication Questionnaires (Leng and Fowkes, 1992) were also completed.

Physiological testing

Standing height (without shoes) was measured to the nearest 5mm using a wall-mounted vertical metal ruler. Weight without outdoor clothing and shoes was measured to the nearest 0.1kg on mechanical scales (SECA 761). Waist circumference was measured during exhalation at the level midway between the lower rib margin and the iliac crest (pre-marked at the mid-axillary line on each side of the subject), with the subject standing with their feet 30cm apart. Hip circumference was taken with the subject in the same standing position, by wrapping the tape measure around the buttocks and lowering or raising the tape until the maximum circumference was located and the tape fitted comfortably. Both measurements were taken using a non-expandable tape measure and the average of two readings taken to the nearest 0.5cm was used as the final measurement. Systolic and diastolic brachial blood pressure (phase V) were measured in the right arm only to the nearest 2mmHg with subjects in the supine position, using a standard

stethoscope and an aneroid, 6 inch dial desk standing sphygmomanometer (Acceson ™, AC Crossor &Son (Surgical) Ltd, Harlow, UK).

A resting 12-lead electrocardiogram (ECG) was recorded according to standard procedures (Marquette MAC1200 machine) and independently coded by three clinical members of the research team using the Minnesota coding system (http://www.epi.umn.edu/ecg/; Prineas et al, 1982).

Blood sampling and laboratory assays

A 40ml sample of venous blood was taken. All samples were obtained between 09:00am and 10.00am to minimise diurnal variation. HbA1c was measured by ion exchange high performance liquid chromatography (HPLC) on a fully automated analyser (HA 8160) manufactured by Arkray, Japan and distributed by Menarini Diagnostics. Cholesterol and HDL cholesterol were measured enzymatically using dry slide technology on the Vitros 5.1 FS analyser manufactured by Johnson and Johnson.

4.2.3. Cognitive testing

Research nurses were trained in cognitive testing and then observed for validation by one ET2DS investigator who is an expert in psychological testing. A battery of psychometric tests was administered at the research clinic in a standardised order during one clinical session in a mildly warm, quiet and well-lit room. The test running time was about one hour. Prior to testing, distance visual acuity was examined by using the Snellen Chart as well as near vision by applying Sussex test-types for both eyes. Corrected visual acuity worse than 6/36 for distance vision or inability to read large print text were used as exclusion criteria as at least moderate visual function was required to complete some of the cognitive tasks. Capillary blood glucose was also measured to exclude prevailing hypoglycaemia. The neuropsychological session was rescheduled if antecedent hypoglycaemia (blood glucose < 4.0 mmol/l) occurred. The cognitive function test battery used in the ET2DS was chosen to be sensitive to small-to-moderate difference in ability, comprising eight neuropsychological tests, a test assessing subjects' pre-morbid

ability and a scale aimed at measuring mood states. The individual parts of the test battery are described below. Copies of each of these are supplied in secions 1 to 10 of Appendix E.

The Hospital Anxiety and Depression Scale

The Hospital Anxiety and Depression Scale (HADS) is a short, well accepted questionnaire consisting of two sub-scales, used for evaluating current anxiety and depression levels in non-psychiatric patients (Zigmond & Snaith, 1983). Each sub-scale is comprised of 7 items on the basis of the psychic symptoms of anxiety or depression, and each item is rated on a four-point scale (0-3). The range of total scores for either anxiety or depression is between 0 and 21 with non-cases and possible cases falling into the range 0-7 and 8-10, and 'probable cases' having a total score of 11 or more. The HADS has been found to be a valid measure in identifying a probable case of anxiety or depression with good sensitivity and specificity although it does not allow a definite diagnose, and only gives a dimensional rather than a categorical representation of mood (Herrmann, 1997; Snaith, 2003).

The Mini-Mental State Examination

The Mini-Mental State Examination (MMSE) is a brief and general cognitive measure with components for orientation, concentration, language, praxis and immediate and delayed memory with a maximum (best) score of 30. It is widely used for assessing cognitive status among older people and particularly as a screening test for dementia. An MMSE score of <24 is often used to indicate significant cognitive impairment.

The Mill Hill Vocabulary Scale

The Mill Hill Vocabulary Scale (MHVS) is a brief self-administered reading test used as a measure of acquired verbal knowledge. The MHVS, in common with other vocabulary tests, is primarily an indicator of previous ("best ever" or premorbid) cognitive ability, which changes little with age (Salthouse, 2004; Schaie, 2005). A combined version of the Junior and Senior Form A synonyms of the Mill Hill vocabulary scale was used (Raven et al., 1998). Subjects are presented with a word and asked to identify the closest synonym by choosing one of six given words. The

number of correctly sorted words comprises the score (the maximum score was 44). It has been used to compare the characteristics of the population and to enable assessment of the impact of any difference in previous cognitive ability on changes in cognitive performance over time. The test has been shown previously to correlate highly with other tests of previous cognitive ability that are used in a similar manner (Crawford et al., 2001)

The Wechsler Memory Scale-III: Logical Memory Test (Immediate & Delayed Recall)

The Wechsler Memory Scale-Revised 3rd Edition is a revised version of the Wechsler Memory Scale which is a widely used clinical instrument for evaluation of major dimensions of memory functions in adolescents and adults (Wechsler, 1987). The Logical Memory (LMT) test, a sub-test from the WMS-III, is a test of verbal declarative memory. It measures immediate (short-term) free recall after auditory presentation and delayed (long-term) recall. With high interrater reliability, this test has been demonstrated to be a valid and sensitive measure (Spreen & Strauss, 1998).

One brief story (story A) with 25 elements is read aloud to the subject, and after reading, the subject is asked to recall as much as possible (Logical Memory Part I). Delayed Recall (Logical Memory Part II) is tested and recorded in the same way after approximately 40 minutes, when the participant is asked to recall the story from memory without hearing the story again. Participants are not informed in advance that they will be re-tested on the recall of the story. Prose recall is rarely word for word in adults, which normally is divided into ‘gist’ recall and ‘verbatim’ exact word recall (Haaland et al., 1983). In the ET2DS population for scoring, both of gist and verbatim recall words were counted. Participants are scored based on the number of story elements recalled correctly (one point for each correct element) and the points are summed for the immediate and delayed recall, respectively.

The Trail Making Test-Part B

The Trail Making-Part B Test from the Halstead Reitan battery (Reita, 1958) assesses mental flexibility, including complex visual scanning and a motor component that measures visual conceptual and visual motor tracking. Subjects are

required to draw a line between numbers and letters in an alternating order and in as short a time as possible. Scores are calculated by recording the time (in seconds) it takes to complete Part B (errors are not counted). In the ET2DS, before undertaking the test, subjects were given a simple sample to enable a short practice trial and to ensure they had fully understood the test directions.

The Wechsler Memory Scale-III: Faces & Family Pictures Test (Immediate & Delayed Recall)

The Faces and Family Pictures task is a sub-test of the WMS-III designed for assessing non-verbal memory using facial recognition format. Subjects are shown a series of 24 pictures of human faces and then, in subsequent immediate and delayed (following an approximately 30 minutes delay) tests, they are asked to select these faces from a larger series of 48 pictures, including both target and distractor faces. The total number of correct responses comprises the immediate and delayed scores respectively (the overall maximum score on each test is 48).

The Wechsler Adult Intelligence Scale-III: Matrix Reasoning Test

The Matrix Reasoning is a sub-test from the Wechsler Adult Intelligence Scale 3rd Edition (WAIS-III) (Wechsler, 1981) and constitutes a measure of non-verbal reasoning designed to measure a person's ability to reason by induction (Raven et al., 1977). It requires the subject to make sense of complex situations and identify spatial, design and numerical relationships between apparently unrelated structures, ranging from the very obvious and concrete to the very complex and abstract (Lezak, 1995). In each item of the test, participants examine a pattern arrayed in matrix with a portion missing. The participant has to work out the rules on the array of the elements in matrix, apply them to find out what the missing portion should look like, and select the correct piece from the five answer options. The number of correctly completed items comprises the total score (the maximum score is 26).

The Wechsler Adult Intelligence Scale-III: Digit Symbol-Coding Test

The Digit Symbol-Coding Test (DST), a subtest from the WAIS-III is a test

of coding performed at speed (information processing speed). This is a paper-and-pencil task requiring timed translation of numbers (1-9) to symbols using an information key that pairs each number with a unique symbol printed at the top of the page. Participants are asked to fill in empty boxes with the respective code, with empty boxes below a given selection of random numbers. After an initial 7 boxes which are used for practice only, and are not counted towards the overall score, the participants are then asked to complete the remaining boxes. In total, there are a maximum of 133 boxes to fill in. The test is scored as the number of symbols the participant correctly matches to their corresponding numbers in 120 seconds.

The Borkowski Verbal Fluency Test

The Borkowski Verbal Fluency Test (BVFT) is a phonemic test used as a brief cognitive assessment of executive function (the speed and flexibility of verbal thought processes or language ability related to executive function such as formation of appropriate strategies for words retrieval; lexical fluency tasks involving specific regions of right and left frontal lobes). This phonemic fluency is opposed to semantic fluency where the test taker generates words according to categories. The test has established reliability and validity (Spreen & Strauss, 1998), and age, level of education and gender play a role in verbal fluency task performance (Loonstra et al., 2001). Participants are asked to vocally generate as many words as possible in one minute that begin with a particular letter of the alphabet. The test is repeated for three different letters (C, F and L), which are chosen on the basis of the frequency of English words beginning with these letters (Rafnsson, 2007). The score is the number of appropriate words named, excluding proper nouns (names of persons and places), numbers, repeats, and the same words with different suffixes (e.g. play, player and playing). The total test score is the sum of all words produced in each of the three letters.

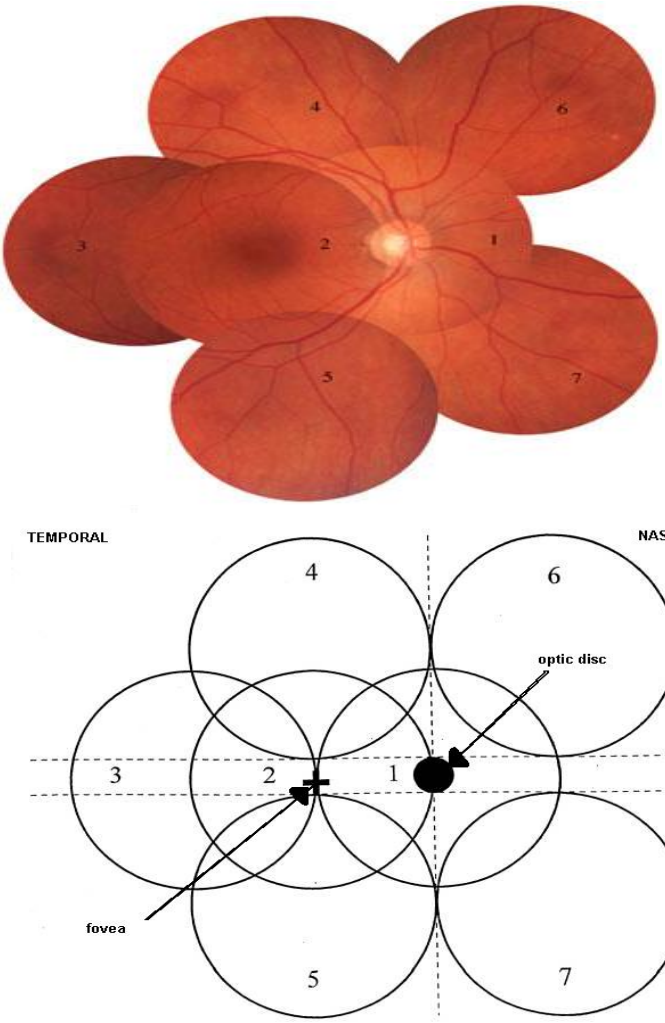
The Wechsler Adult Intelligence Scale-III: Letter-Number Sequencing Test

The Letter-Number Sequencing test is another subtest of WAIS-III used for assessing working memory, temporary storage, active maintenance and information organization. The participant is read mixed strings of numbers and letters (in

alternating order) with increasing lengths (from 2 to 8 digits). The participant is asked to repeat the numbers first, in numerical order, and then the letters in alphabetical order. For example, T-9-A-3 will be reported as 3-9-A-T. The test is divided into 7 groups and each group composes of three different strings. Group 1 consists of one letter / one number string (x 3), which is used only as a trial to ensure the participants understand the instructions of the task. Following this, group 2 consists of either 1 letter / two numbers, or two letters / one number. The process will continue and become progressively more difficult, until the seventh group, which consists of 8 constituent for each of the three strings (Patton, 2006). For each group, if the participant fails on all three strings, the test will be discontinued. The total score is calculated by adding up the correct response of each string and the maximum is 21.

4.2.4. Retinal photography

Approximately two or three weeks after their initial visit to the research clinic, subjects returned to a separate research clinic based within the major ophthalmology department for NHS Lothian for digital retinal photography. If subjects cancelled or did not attend they were contacted to arrange another appointment. Photography was conducted between 9.30 am and 3.00 pm each weekday by a single specially trained medical photographer. Subjects had 1% tropocamide drops instilled into both eyes if possible to allow pupillary dilatation before image capture unless ocular opacities (eg. cataract) or false eye existed. Pupils were inspected after approximately 20 minutes and if dilatation was insufficient, a further 1% tropocamide dose was given. Subsequent to this, standard 7-field non-stereoscopic retinal colour photographs of both eyes were taken at 35^{o} using a high resolution digital retinal camera (TOPCON TRC-50FX, Topcon Optical Company, Tokyo, Japan). The seven standard photographic fields are shown in Figure 22. All images were stored as JPEG or TIFF format files (both are high resolution digital images) on the study computer attached to the retinal camera, backed up onto an external drive and subsequently transferred to a dedicated server at the University of Edinburgh for long term back up and storage.



(a)

(b)

Figure 22: Diagrams (a and b) showing 7-field definition in right eye. Field 1 is centred on the optic disc, field 2 on the macular. Field 3 is temporal to the macula. Fields 4 to 7 are tangential to horizontal lines passing through the upper and lower poles of the disc and to a vertical line passing through its centre (Sources: www.bt-asia.com/visdr_medicalprof.htm; Diabetic retinopathy grading protocol for ET2DS, 2007)

4.3. Grading for diabetic retinopathy

Prior to grading, two sets of grading equipment (including computers with large colour monitors for viewing photographs and laptops for entering data) were set up. Electronic data entry forms were created for each batch of 50 subjects at the study office, and supplied to the graders with subject ID number and initials shown

(Diabetic retinopathy grading protocol for ET2DS, 2007). All photographs were graded by two trained optometrists, working independently and according to the scale described by the Early Treatment Diabetic Retinopathy Study (ETDRS) (ETDRS Report No. 10, 1991) research group, which was an adaption of the modified Airlie House classification of diabetic retinopathy. All fields were graded regardless of their deviation from the field definition. Throughout the grading process, graders checked photos for quality, and a paper/acetate record of the position and nature of artefacts (e.g. due to dust on the camera) was maintained to minimise the chances of artefacts being mistakenly graded as features. Characteristic lesions of diabetic retinopathy were recorded for seven fields in each eye. The final level retinopathy score for each eye was generated automatically through a series of calculations embedded in the data entry form. Discrepancies in the final level score at subject level between the graders were resolved in the first instance by discussion between the graders. Unresolved discrepancies were further reviewed and arbitrated by a consultant ophthalmologist.

Diabetic retinopathy was considered to be present if microaneurysms alone, or with any of the following characteristic lesions (as defined by ETDRS scale) were present: 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. Standard retinal colour photographs (No.1-14) of the Modified Airlie House Classification of Diabetic Retinopathy (The Diabetic Retinopathy Study Research Group, 1981) were used as references to define grades of lesions. Lesions were graded using the following descriptive terms:

a. Grade 0= None /Absent (A), when the lesion was absent or the grader was less than 50% certain that it was present;

b. Grade 1=Questionable, (i) when the grader was 50% to 90% certain that the lesion was present, or (ii) an abnormality was definitely present but its nature was uncertain (in this case the grader assigned the grade ‘questionable’ for the characteristic considered to be most likely and ‘absent’ for the ones least likely);

c. Grade 2 to 5= Definite, when the grader was greater than 90% certain that the

lesion was present. In addition, the following grading codes were used to express the severities of the lesion, which were defined in terms of the number or retinal area present, or the photographic standards related: grade 2= definitely present; grade 3= moderate; grade 4=severe; grade 5= very severe;

d. Cannot grade, when no lesion was seen and 100% of the field was missing (e.g. due to obscuration from vitreous haemorrhage).

- e.g. Hard exudates grading – Standard Photographs (SP) 3 4 5 (2-7 fields were graded)

CODE	CODE DEFINITION
0	No hard exudates
1	Questionable hard exudates
2	Definite hard exudates < SP 3
3	Definite hard exudates ≥ SP 3 but <5
4	Definite hard exudates ≥SP 5 but < 4
5	Definite hard exudates ≥SP 4
x	Cannot grade

Each abnormality was graded separately for each of the seven fields in each eye. Features graded in the seven fields included:

- fields 2-7: haemorrhages and/or microaneurysms, hard exudates, cotton wool spots, venous calibre abnormalities, intra retinal microvascular abnormalities, new vessels elsewhere, fibrous proliferations elsewhere, preretinal haemorrhages, and vitreous haemorrhage
- field 1 only: new vessels at the disc, fibrous proliferations at the disc

For lesions graded only in field 1, the grading code as described above was used directly to indicate the severity of that lesion for the eye. For lesions graded in multiple fields, a summary scale of the grades was assigned which combined the maximum severity in any field with the number of non overlapping fields (i.e. fields 3-7) in which that maximum was present (Table 9). Any field in which a lesion was graded could establish the maximum grade, but for two or more fields to be recorded as having the maximum, both or all had to be in non overlapping fields 3 to 7. On the basis of summary grade of each lesion in 7 fields, the final diabetic retinopathy level

was defined for each eye, varying from level 10 (no diabetic retinopathy) to level 81 (advanced proliferative diabetic retinopathy). For the purpose of this study a score of 81 was added for panretinal photocoagulation scars only if the laser treatment was for diabetic retinopathy (e.g. photocoagulation scars for other conditions such as retinal vein occlusion were not graded as 81). Table 10 presents the final diabetic retinopathy severity scale, which was originally from ETDRS report No. 12 (ETDRS Report No. 12, 1991) and modified for use in ET2DS.

Table 9: Scale for characteristics graded in multiple fields (Adapted from the ETDRS report No.10, 1991)

Grade	Maximum Severity	No. of Fields with Maximum Severity
A	Absent	1 to 5 fields
Q/1	Questionable	1 field
Q/2-3		2 or 3 fields from fields 3 to 7
Q/4-5		4 or 5 fields from fields 3 to 7
D/1	Definite	1 field
D/2-3		2 or 3 fields from fields 3 to 7
D/4-5		4 or 5 fields from fields 3 to 7
M/1	Moderate	1 field
M/2-3		2 or 3 fields from fields 3 to 7
M/4-5		4 or 5 fields from fields 3 to 7
S/1	Severe	1 field
S/2-3		2 or 3 fields from fields 3 to 7
S/4-5		4 or 5 fields from fields 3 to 7
VS/1	Very severe	1 field
VS/2-3		2 or 3 fields from fields 3 to 7
VS/4-5		4 or 5 fields from fields 3 to 7

Table 10: Modified final diabetic retinopathy severity scale for each eye in ET2DS (Replicated from Diabetic retinopathy grading protocol for ET2DS, 2007)

LEVEL	SEVERITY	DEFINITION
10	DR absent	Microaneurysms and other characteristics absent/questionable[#]
14	DR Questionable	HE, CWS or IRMA definite: MA absent/questionable[#]
15	DR Questionable	Haemorrhage(s) definite: MA absent/questionable[#]
20	MA only	MA definite, other characteristics absent
35*	Mild NPDR	One of more of the following: a. Venous loops ≥ D/1 b. CWS, VB or IRMA = Q c. Retinal haemorrhage present d. HE ≥ D/1 f. CWS ≥ D/1
43	Moderate NPDR	One (only) of the following: a. H/MA = M/4-5 – S/1 or b. IRMA = D/1-3
47	Moderately severe NPDR	Both Level 43 characteristics and/or one (only) of the following: a. IRMA = D/4-5 b. HMA = S/2-3 c. VB = D/1
53	Severe NPDR	One or more of the following: a. At least 2 of the last 3 Level 47 definitions b. HMA ≥ S/4-5 c. IRMA ≥ M/1 d. VB ≥ D/2-3
61	Mild PDR	a. FPD or FPE present (with NVD and NVE absent) b. NVE = D
65	Moderate PDR	Either of the following: a. NVE ≥ M/1 or NVD = D; and VH and PRH = A or Q b. VH or PRH = D and NVE < M/1 and NVD absent
71	High Risk PDR(1)	Any of the following: a. VH or PRH ≥ M/1 b. NVE ≥ M/1 and VH or PRH ≥ D/1 c. NVD = 2 and VH or PRH ≥ D/1 d. NVD ≥ M
75	High Risk PDR(2)	NVD ≥ M and VH or PRH ≥ D/1
81	Advanced PDR	a. Retina obscured due to VH b. Photocoagulation scars in any field in the absence of any evidence of vein occlusion or other non-diabetic retinopathy reason for such treatment. Subject considered to have received laser treatment for diabetic retinopathy, hence classification as advanced PDR
x	Cannot grade	No characteristics graded for eye concerned

* NPDR Levels 35 and above require presence of micro-aneurysms; [#] for Levels 10,14 and 15, wherever absence of a characteristic forms part of a set of criteria; questionable is interpreted as absent; DR, diabetic retinopathy; HE, hard exudates; CWS, cotton wool spots; IRMA, intraretinal microvascular abnormalities; MA, microaneurysms ; NPDR, nonproliferative DR; VB, venous beading; HMA, haemorrhages/microaneurysms; PDR, proliferative DR; NVE, new vessels elsewhere; NVD, new vessels at the disc; FPE, fibrous proliferations elsewhere; FPD, fibrous proliferations at the disc; VH,vitreous haemorrhage; PRH, preretinal haemorrhage.

4.4. Assessing parameters of retinal vascular network geometry

Retinal photographs containing the optic disc in the centre field were chosen and used to assess parameters of retinal vascular network geometry. Measurement was performed on one randomly selected eye or the eye with the best photographic quality. All parameters of retinal vascular network geometry were measured by myself using operator-directed image analysis and a custom-written (by TM) software package within the MATLAB environment on a personal computer with a high-resolution 17 inch monitor (MathWorks Inc., Natick, Massachusetts). The red, green, and blue (RGB) colour image was converted to a grayscale image prior to analysis, and image contrast was enhanced by contrast-limited adaptive histogram equalization. The original colour image was used as a reference to identify an arteriole or a venule on the grayscale image.

Retinal vessel width was recorded for each individual. In addition, the retinal arteriolar bifurcation angles and branching coefficients were measured.

4.4.1. Measurements of retinal vessel width

In accordance with the Atherosclerosis Risk in Communities (ARIC) study (Hubbard et al., 1999), after defining the boundary of the optic disc manually, a concentric circular grid centered on the optic disc was overlaid each retinal photograph to define the measurement area (Figure 23). The six largest arterioles and venules were then selected in the area between 0.5 and 1 disc diameter from the optic disc margin. For each vessel, a portion of vessel segment which was uniform in thickness and not obscured by any crossing or fairly close vessels was selected by means of a mouse-driven cursor to mark edges. A series of lines perpendicular to the vessel were created automatically at regular intervals using an imaging analysis programme (Figure 24). The intensity profile of each created line was fitted to a double-Gaussian curve with microdensitomety and the width of the vessel was taken at half the height of the fitted curve (Figure 25). The overall width of the vessel was derived from the mean width value of these profile cross-sections. The measurement

was rejected and repeated if less than 3 of the fitted curves were, on inspection, deemed to be a good fit (the majority of poor fits related to background 'noise' producing artifact of vessel profiles) or accurate, or if the standard deviation of the calculated widths was greater than 10% (an arbitrarily chosen cut-off based on previous studies) of the mean widths measured.

Computer-assisted measurements of individual arterioles and venules were each summarized according to formulas developed by Hubbard et al (1999), Knudtson et al (2003) and Patton et al (2007) to provide the average calibre of retinal arterioles (central retinal arteriolar equivalent [CRAE]) and venules (central retinal venular equivalent [CRVE]) in that eye. These summary indices were also expressed as the retinal arteriole-to-venule ratio, which is a measure of relative caliber of retinal arterioles to venules, and compensate for possible magnification differences between eyes.

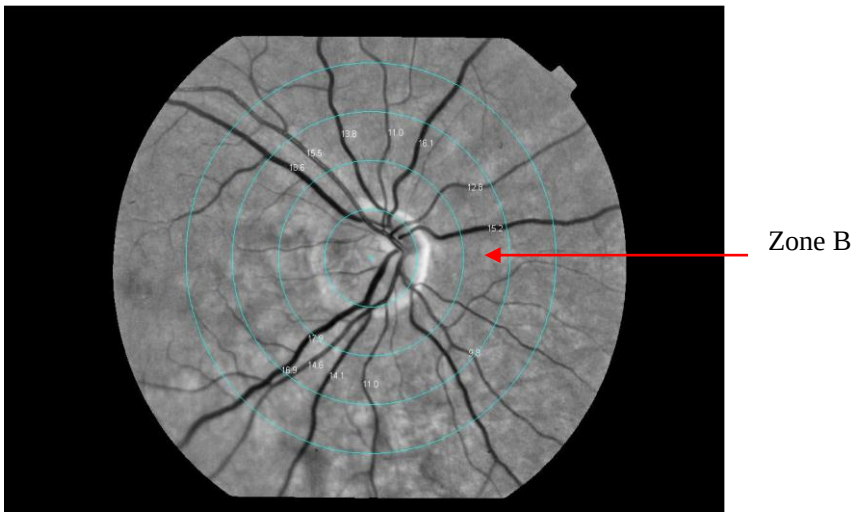


Figure 23: Retinal vessel diameter measurements were performed in Zone B, which is 0.5 to 1.0 disc diameters from the optic disc margin.

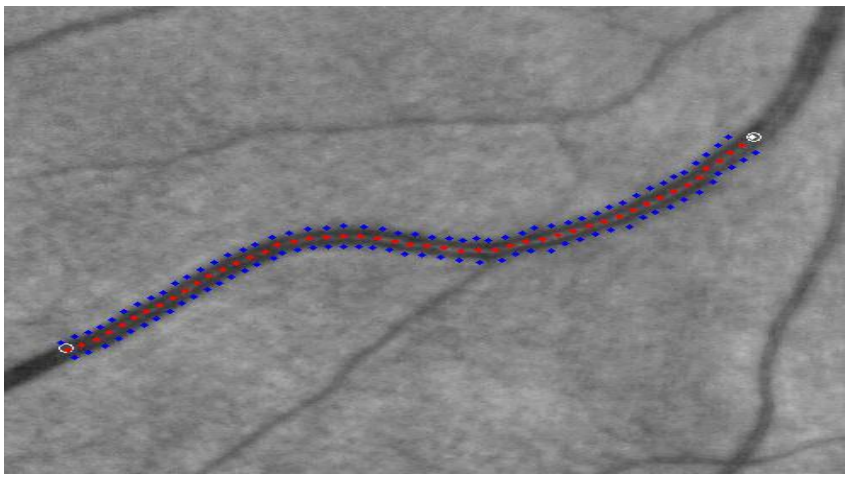


Figure 24: Automatic tracking of a segment of blood vessel. Red dots correspond to vessel centre points and blue dots to vessel edges determined at regular intervals along the segment, having been given starting and ending points.

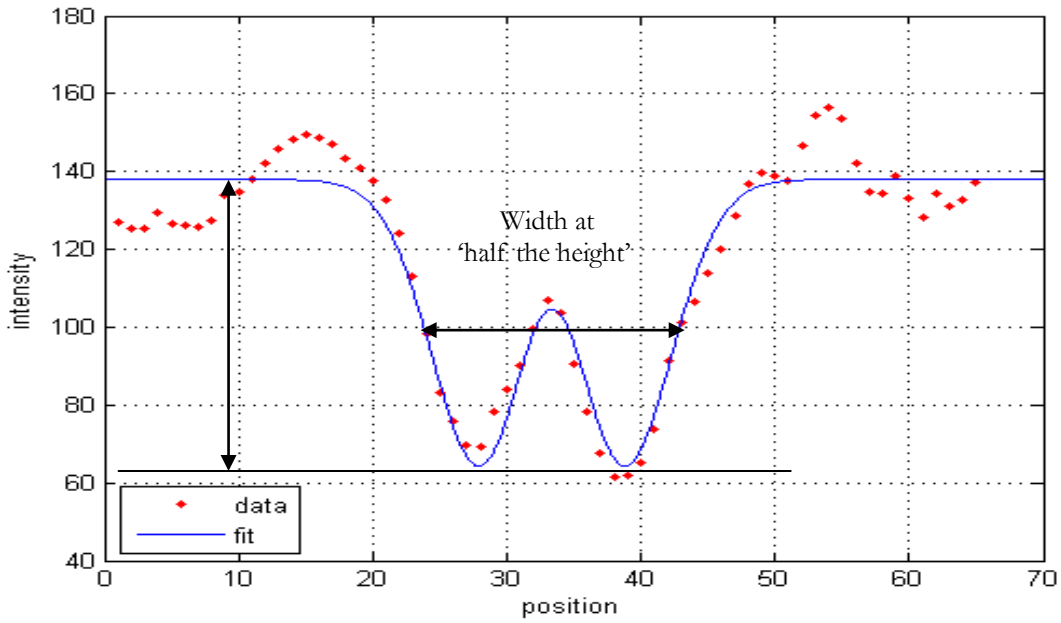


Figure 25: A typical intensity profile of a cross-section of a retinal vessel. A curve of best fit has been placed over the actual intensity data, showing a double-Gaussian configuration. The height of the intensity profile was calculated by subtracting the nadir intensity from the background intensity measured across the vessel. Vessel diameter is the width of the curve at half the height.

4.4.2. Measurements of retinal arteriolar bifurcation geometry

Other parameters of retinal vascular network geometry measured and quantified were the angle subtended between two retinal arterioles and the branching coefficient based on vessel widths between the parent vessel and the two daughter vessels across the arteriolar bifurcation (Figure 26a).

As stated in Chapter 1, vascular topographical geometry tends to conform to optimal principals to minimise physical properties such as shear stress and volume across vascular network (Murray, 1926a, b; Patton et al., 2005). According to Murray's law, the most efficient circulation across a vascular network can be achieved if blood flow is proportional to the cubed power of the vessel's radius (i.e., the junction exponent =3 for the formula $D_0^x = D_1^x + D_2^x$ where x is the junction exponent, D_0 is the diameter of parent vessel, and D_1 & D_2 are the diameters of the two daughter vessels). An empirically derived method to express Murray's law is to use the Branching Coefficient (BC), where $BC = (D_1^2 + D_2^2) / D_0^2$ (Figure 26b).

At least 3 arteriolar junctions that were of good photographic image quality were assessed. To calculate the BC, the area that contained the arteriolar junction to be measured was selected, which then opened in a new window. By means of a mouse-driven cursor, the two edges of the parent vessel were marked by locating one point at the midpoint of the junction and the other at some distance beyond the junction. Another two points were defined in each of two daughter vessels, with the point at the junction fixed. The diameters of the parent and daughter vessels were then calculated by deriving the mean value of several (mostly 5) parallel lines perpendicular to the vessel, each line being 5-10 pixels away from its neighbor (Figure 27). The intensity profile of each created line was fitted to a double-Gaussian curve and the width of the vessel was taken at half the height of the fitted curve. Having measured the vessel widths, the middle points of the daughter vessels (red points in Figure 27) were identified and marked simultaneously by a vessel tracking technique in the above process. A line fixed in the selected bifurcation was drawn through the accepted middle points of each daughter vessel and the angle between the two lines was measured by using the cosine rule and presented in degrees (Figure

28). The median BC and the median bifurcation angle of the 3 arteriolar junctions were taken as the final measurements respectively.

To standardise procedures and to overcome the difficulty of drawing a line in the daughter vessel when either of the daughter vessel segments curved immediately after the bifurcation point, width measurements were arbitrarily defined after taking into account the influence of the bifurcation on the shear stress in the daughter vessels. Widths were measured in daughter vessels along a distance of 2 times of the parent width, and the vessel tracking started at a distance of 0.75 to 1.5 times of the parent width from the bifurcation point.

The measurements were rejected and the bifurcation was remeasured or declared unevaluable if

(i) less than 3 of the fitted curves were on inspection deemed to be good fit or accurate

or

(ii) the standard deviation of the calculated widths was greater than 10% of the mean widths measured in either of branch vessels (parent and daughter vessels)

Although images with the best quality (from either eye) were chosen, the number of measurable arteriolar bifurcations did not reach the required minimum of 3 in 60% of the images. Using multiple branching point measurement from individual image has been shown to provide a more reliable estimate (compared with one measurement) and to minimise a great deal of variation between different images (Patton et al., 2006b). In one previous study, the average of measurements (median value) from 5 bifurcations was used (Patton et al., 2007). However, scrutiny of the ET2DS retinal photographs in the first instance showed that it was difficult to identify or measure the five most proximal bifurcations from the optic disc margin, largely caused by the more strict semi-automatic method (instead of manual estimation) applied in our study (comparison of the two methods is discussed in Chapter 6). Thus the measurement was restricted to at least the three arteriolar angles.

More specifically, the failure to get a measure on arteriolar bifurcation could be due to:

(1) Arteriolar bifurcations were obscured by other vessels which were crossing or fairly close

(2) Branch vessel segments (parent and daughter vessels) were too thin (<10 pixels) to be accurately measured (i.e. their vessel edges were often not sufficiently resolved to be able to measure with good accuracy)

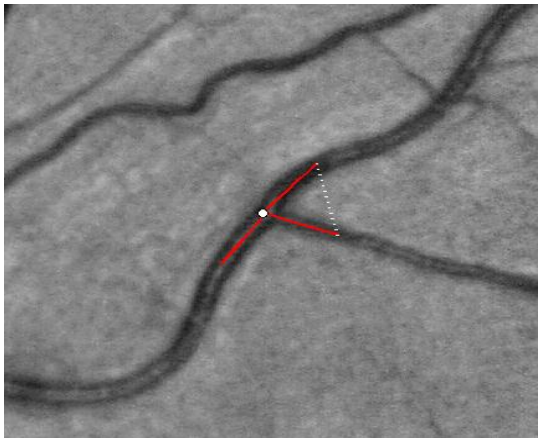
(3) Branch vessel segments were not uniform in thickness (focal narrowing or distension)

(4) Background 'noise' weakened the contrast at some points in vessel segments such that vessel edges could not be identified correctly

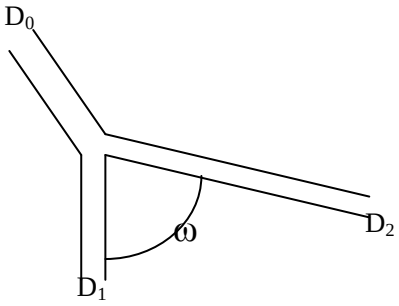
(5) Too little contrast between vessel segment and the background intensity of the surrounding retina (retinal pigment epithelium) disabled the measures

(6) Either the daughter or parent vessel segments were too short to measure

(7) Bifurcation was beyond the 35 degree image field



(a)



(b)

Figure 26: A schematic representation (b) of an arterial bifurcation in digitized retinal image (a). D_0=parent diameter, D_1=1st daughter diameter, D_2=2nd daughter diameter, ω=bifurcation angle and branching coefficient = $(D_1^2 + D_2^2) / D_0^2$.

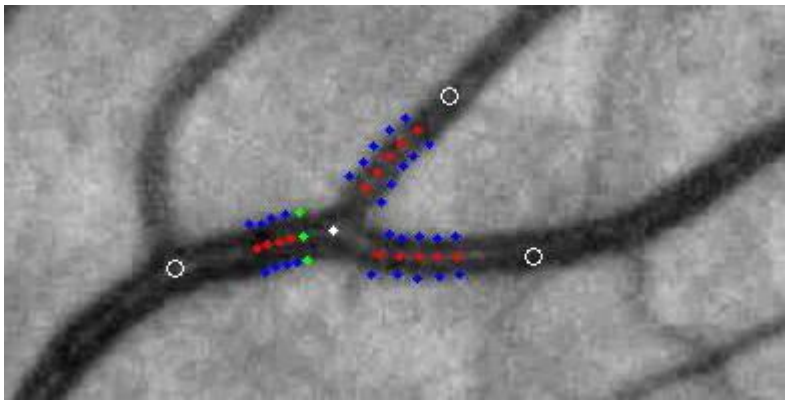
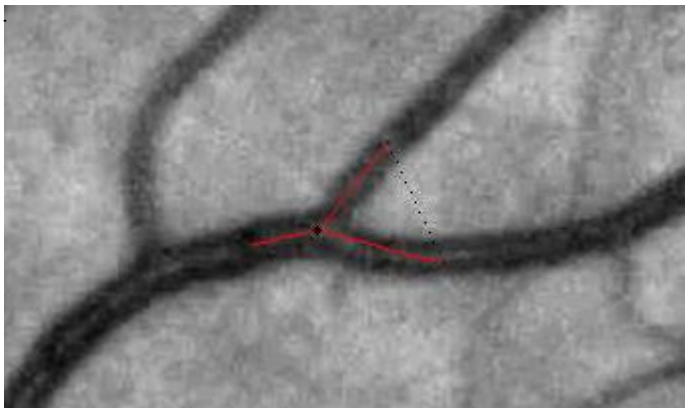
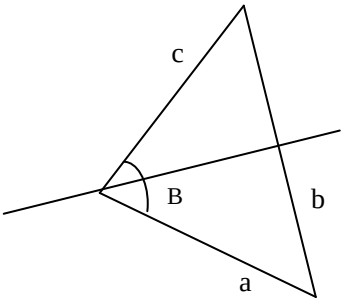


Figure 27: An example of an arteriolar bifurcation: having selected 4 points in the parent and daughter vessels, 5 parallel lines perpendicular to each vessel were drawn.





The Cosine Rule
$b^2=a^2+c^2-(2ac*CosB)$
$CosB= (a^2+c^2- b^2)/ 2ac$

Figure 28: An example of an arteriolar bifurcation demonstrating the Cosine Rule used to calculate the angle subtended by the two arteriolar daughter vessels

4.4.3. Reliability of retinal vascular parameter measurements

A quality control session with a random subsample of 20 digital retinal images was designed with repeat measurment performed after a 10-week interval to assess intraobserver reliability of retinal vessel width parameters (i.e. CRVE, CRAE, AVR, BC and BA). Inter-observer variability of measurements on these parameters was assessed at a later date by another trained grader measuring a separate set of 30 images. The intraclass correlation coefficient (ICC) (ratio of between-groups variance to the total variance) and 'Limits of agreement' were used to evaluate the

intra- and inter-observer reliability.

4.5. Derivation of variables used in analysis

Data obtained from the baseline questionnaire and data collection forms were coded and entered onto a master Microsoft Access database by members of the ET2DS research team. The results of plasma assays were entered onto the same master database either from paper records (biochemistry and haematology) or from electronic files provided by the laboratories. For retinopathy grading, data on individual retinal characteristics for each relevant photographic field were entered directly onto a specially designed spreadsheet (Microsoft Excel). The overall retinopathy grade for the right and left eyes for each subject was calculated automatically and these results were transferred to a separate master database. Data on retinal quantitative parameters were entered onto a second Microsoft Excel file at the time of assessment.

The majority of data from paper records were double entered and discrepancies resolved by reference to the original paper documentation. For the retinopathy grading data, a random sample of 50 records were double-checked by myself. Data from assessment of retinal vascular parameters were checked by reviewing the saved 'measurement log' or retinal images. All data from Access Database or Excel spreadsheets were exported into SPSS version 14.0 which was used for statistical description and analysis (SPSS, 2005). Study ID numbers (a unique five digit number assigned to each patient) were used to match the retinal data set with cognitive and other clinical data collected from the baseline examination. The complete data set was inspected for errors (e.g. values outside the possible range for a given variable) and missing values.

4.5.1. Diabetic retinopathy and retinal vascular parameters

The final level of diabetic retinopathy of the more severely affected eye (higher score) was used for assigning a grade to a participant. This 'worse eye' method provided the overall measurement representing the presence and extent of

diabetic retinopathy. When the severity of diabetic retinopathy could not be graded in an eye, it was considered to have a score equivalent to that in the opposite eye. Any diabetic retinopathy was defined as level 20 and above. This was further divided into mild nonproliferative diabetic retinopathy (NPDR) (levels 20 to 35), moderate-severe NPDR (levels 43 to 53) and proliferative retinopathy (levels 61 to 81). The latter two were combined into moderate-severe DR due to small numbers in the proliferative retinopathy category (n=15, levels 61 to 81).

In order to achieve the most efficient circulation across a vascular network, the theoretical and optimal BC (assuming a symmetrical dichotomous vessel branching where branch D_1 = branch D_2) should approximate 1.26. Similarly, both theoretical and empiric studies have shown an optimized arteriolar bifurcation (BA) geometry of approximately 75^o to minimise shear stress and work across the vascular network (Patton et al., 2007). To express the degree of deviation of the actual measurements (i.e. absolute BC or BA) from optimality, two 'sub-optimal' variables, measuring the magnitude of varying quantity, were derived. The sub-optimal branching coefficient was calculated by using the root mean square of the difference of the median BC from 1.26 (i.e. $\sqrt{(1.26\text{-branching coefficient})^2}$) and sub-optimal branching angle was calculated by using the root mean square of the difference of the median BA from 75^o (i.e. $\sqrt{(75\text{-branching angle})^2}$. The root mean square was used to remove the negative sign of the deviation as 'quantity' was the interest of the study.

In order to enable a comparison with other published studies, the dimensions measured on the image (pixels) for retinal vascular width were converted into actual measurements (micrometers) by using a reference value of the average optic disc (OD) diameters (1850μm) derived from the ARIC study. Thus, the mean optic disc diameter using microdensitometry in the series of images (mean OD=327 pixels) in the ET2DS was transformed by multiplying the value in pixel by 5.7 (1850/327), to obtain a measure in micrometers.

4.5.2. Cognitive variables

Given the moderate to high correlation between the immediate and delayed sub-components of the Faces task (r=0.55), these were combined to form a total score, pertaining to 'immediate and delayed non-verbal memory'. A similar approach was taken for the immediate and delayed recall sub-components of the Logical Memory Test (r=0.87), giving an overall score for Logical Memory. All of the other cognitive test scores were retained as individual measures. Each of the cognitive test scores was normally distributed except for the Trail-Making Test which was normalised through a natural logarithm transformation.

Scores on the individual tests were used to calculate a general intelligence factor score (g) representing the amount of shared variance between all of the cognitive tests. This was generated by Principal Components Analysis (PCA) from the scores of the 7 correlated cognitive tests (Logical memory, Verbal Fluency, Faces, Digit Symbol, Matrix Reasoning, Letter Number Sequencing and Trail-Making) (Table 11). Scores were saved on the first unrotated principal component. Each cognitive test loaded strongly on this component which accounted for 44.03% of the total variance among the 7 test scores, further validating the use of the general factor. It should be noted that memory test scores as indexed by Faces and Logical Memory contributed relatively lower to 'g' than other cognitive test scores, which may reflect possible distinction between memory and fluid intelligence (Deary et al., 1998). A scree plot was examined to confirm the results from the PCA factor extraction. Table 12 shows how much each cognitive test contributed to calculation of 'g'. Prior to PCA, the data were checked and adequately met the criteria for PCA with respect to the large sample size, factorability of the correlation matrix measure of sampling adequacy, Bartlett's test of sphericity on the adequacy of the intercorrelations among the cognitive test scores (Rafnsson, 2006). Scatter plots of all cognitive outcome combinations revealed no non-linear relationships and there were no outliers.

Table 11: Pearson's correlations of cognitive test scores

	Mill Hill Vocabulary	Verbal Fluency	Faces	Trail Making (ln)	Logical Memory	Matrix Reasoning	Digit Symbol	Letter-Number Sequencing
Mill-Hill Vocabulary	1	0.44	0.28	-0.37	0.38	0.45	0.37	0.40
Verbal Fluency			0.22	-0.39	0.25	0.36	0.40	0.46
Faces				-0.26	0.24	0.24	0.29	0.20
Trail Making (ln)					-0.28	-0.46	-0.63	-0.50
Logical Memory						0.28	0.27	0.31
Matrix Reasoning							0.38	0.40
Digit Symbol								0.40

P values all significant at the 0.001 level

Table 12: Contributions of each cognitive test to 'g'

	Adjusted R^2	P-value
Verbal Fluency	0.448	<0.001
Faces	0.206	<0.001
Trail Making Test($\log_e$)	0.630	<0.001
Logical Memory	0.279	<0.001
Matrix Reasoning	0.443	<0.001
Digit Symbol Test	0.569	<0.001
Letter-Number Sequencing	0.503	<0.001

Cognitive impairment was defined as scores 1.5SD or lower than the mean scores of the total sample on individual cognitive tests. The cut-off of 1.5 standard deviations was selected as it was consistent with one of the recommendations for the diagnostic criteria for mild cognitive impairment (MCI) (Petersen et al., 1999). The full diagnostic criteria for MCI could not be used as other standardised information on functional ability or activities of daily living were not collected in the ET2DS.

4.5.3. Demographic, diabetic and vascular variables

Education, social class and deprivation

Level of education (highest level completed) was categorised as a three-level variable with secondary and primary school combined (university, other professional or technical qualification, secondary & primary school). Social class

was coded according to the Registrar General's classification with female married subjects classified by their husband's occupation (OPCS, 1980). Retired and currently unemployed participants were coded according to their last held job. Thus level of occupational social class was grouped as a five-level variable with social class IIIN and IIIM combined (I, II, IIIN &IIIM, IV and V). In addition, a Scottish Index of Multiple Deprivation (SIMD, 2006) quintile score was calculated by a research statistician and allocated to each subject from 1 (most deprived) to 5 (least deprived), based on the link of postcode with 37 indicators of deprivation across seven domains.

Smoking

Smoking habits were self-reported and included information on both current and lifetime smoking status. Smoking status was coded as a three-level variable (current, ex and never smoker). Subjects were classified as smokers if they smoked any of cigarettes, cigars or pipes and as current smokers if they answered that they were currently smoking or had given up in the past 6 months. For purpose of analysis, a dichotomous variable was also created (smoker and never smoker). Pack-years of smoking was derived as an index of life time smoking, by calculating the number of 20-cigarette packs per day smoked, multiplied by the number of years being a smoker at current or former levels. A zero value was entered for life-long non-smokers. The above measure on pack-years smoking has been used in previous epidemiological studies in which it was judged to be sufficiently valid because the self-reported smoking history correlated with plasma thiocyanate levels (Fowkes et al., 1992).

Alcohol consumption

Alcohol intake was also self-reported and included information on both beverage-specific and global questions. The number of drinks consumed over the past week was recorded in three categories: beer, spirits and wine. One unit of alcohol was defined as a half a pint of beer, one glass of wine or a single measure of spirits. Subjects were also asked whether this represented a typical week's consumption, and if not, whether they usually drank more or less. A typical week's alcohol consumption was calculated by adding up the units of alcohol consumed per

week.

In addition, the alcohol intake over the past year was recorded using an 'expanded quantity frequency' (QF) (Dawson, 2003) approach with respect to:
(a) the frequency of drinking alcohol (never, monthly or less, 2 to 4 times a month, 2 to 3 times a week, 4 to 5 times a week, and 6 or more times a week)
(b) the quantity of standard drinks, as defined by a can or bottle of beer, a glass of wine, one cocktail or a measure of spirits (like scotch, gin or vodka), on a typical drinking day (0, 1-2 drinks, 3-4 drinks, 5-6 drinks, 7-9 drinks and 10 or more drinks)
(c) the frequency of atypical or high-risk drinking (≥ 6 drinks) (never, less than monthly, monthly, weekly, daily or almost daily).
Frequency of drinking at each quantity level was converted into the number of drinking days per year by using midpoints for each frequency category (e.g. 2 -3 times a week =2.5×52=130 days per year) (Goddard 2007).

Two components of annual alcohol intake were derived:
(1) the typical quantity using the midpoint of the quantity category times the frequency of drinking that typical quantity (i.e. the overall frequency drinking minus the frequency of atypical or high-risk drinking)
(2) the high-risk drinking quantity (6 drinks) times the frequency of high-risk drinking. The annual volume of alcohol an individual consumed (standards drinks per year) was the sum of these two components ((Dawson, 2003). To enable a comparison with published studies, the average weekly alcohol intake was calculated by dividing the annual volume by 52.

Diabetes treatment

Based on self-reported mode of diabetes treatment and listed medications, diabetes treatment was classified into three groups, (i) diet control only, (ii) hypoglycaemic oral agents without insulin and (iii) insulin injection with or without oral agents. A dichotomous variable was also created to compare subjects who were using insulin with those who were not.

Obesity, hypercholesterolaemia and hypertension

Two indicators were derived to assess obesity. Body Mass Index (BMI) was defined as the weight in kilograms divided by square of the height in meters. Waist to hip ratio (WHR) was calculated as the waist circumference divided by the hip circumference. Hypercholesterolaemia was defined as plasma total cholesterol ≥ 5 mmol/L or when subject self-reported medication prescribed by a doctor to lower blood lipids level. Hypertension was defined as systolic blood pressure ≥140mmHg or diastolic blood pressure ≥85mmHg or when subject self-reported medication prescribed by a doctor to lower blood pressure.

Cardiovascular disease categories

Information about the following cardiovascular events was obtained: myocardial infarction (MI), angina, stroke, transient ischaemic attacks (TIA) and intermittent claudication (IC). The data used to define a cardiovascular event were collected from three sources:

(a) Baseline questionnaire and 12-lead ECG
(b) Hospital discharge data from Information and Statistics Division (ISD) of NHS Scotland
(c) Clinical notes

(a) Baseline questionnaire and ECG

Medical history items in the self-administered questionnaire addressed subject's recall of diagnoses by a doctor and/or treatment for angina, coronary heart disease/MI, stroke, TIA, and peripheral arterial disease. In addition, the WHO's Chest Pain and Edinburgh Claudication Questionnaires were completed by subjects to assess the symptoms of pain in the chest and legs (Rose, 1962) (Appendix B). The ECG was coded as coronary artery ischaemia if Minnesota codes were 1.1 to 1.3; 4.1 to 4.2; 5.1 to 5.3; 7.1, and coded as diagnostic Q-waves (indicating a definite MI) if Minnesota codes were 1.1.1 to 1.2.5; 1.2.7; or 9.2 plus 5.1 or 5.2 (Prineas et al., 1982).

(b) Hospital discharge data

ISD provided information on medical and surgical discharges from Scottish

hospitals since 1981 for all study participants. All discharges with ICD-10 (International Classification of Diseases, 10th revisions) codes for cardiovascular disease (I20-I25, I61, I63-I66, I252, I679, I694, G45 and G659) were extracted manually by research staff.

(c) Clinical notes

When necessary to confirm the diagnosis, participants who had a cardiovascular event identified from either the baseline questionnaire or the ISD computer print-out were further investigated by reviewing case notes from hospital records or clinical details from the Lothian Diabetes Register.

Myocardial infarction was recorded if two out of the first 3 of the following criteria were met, or if both the first and last criteria were met:

(i) Self-report (subject's recall of a doctor's diagnosis) of heart attack
(ii) myocardial infarction indicated by WHO chest pain questionnaire
(iii) ECG evidence of ischaemia
(iv) prior hospital discharge code for MI (ICD10 codes I21-I23, I252)

Angina was recorded if two out of the first three of the following criteria were met, or if both the first and last criteria were met:

(i) self report of doctor-diagnosed angina or taking regular anti-anginal medication
(ii) angina indicated on WHO Chest Pain Questionnaire
(iii) Ischaemic ECG codes
(iv) prior hospital discharge code for ischemic heart disease (ICD10 codes I20-I25).

Stroke was recorded if two out of three following criteria were present:

(i) subject recall of a doctor's diagnosis of stroke
(ii) prior hospital discharge code consistent with stroke (ICD10 codes I61, I63-I66, I679, I694)
(iii) confirmation by clinical notes review that event not due to transient ischemic attack (TIA).

Transient ischaemic attack was recorded if two out of three of the following

criteria were present:

(i) subject recall of a doctor's diagnosis of stroke

(ii) prior hospital discharge code consistent with TIA (eg. ICD10 codes G45, G659)

(iii) confirmation by clinical notes review that event due to TIA.

TIA was also recorded if subjects volunteered a self-reported history of 'TIA', 'mini-stroke' or 'slight stroke' on the questionnaire.

Intermittent claudication was recorded if a positive response (i.e. definite and atypical) to the Edinburgh Claudication Questionnaire was present.

Five major cardiovascular disease (CVD) categories were further identified: (i) coronary artery disease (myocardial infarction and/or angina); or (ii) cerebrovascular disease (stroke and/or transient ischaemic attacks); or (iii) peripheral arterial disease (intermittent claudication); or (iv) any CVD (MI, angina, stroke, TIA, or IC); and (v) 'healthy' group without CVD (no coronary artery disease, or cerebrovascular disease, or intermittent claudication). Assignment to any of the CVD groups was based on the presence of the disease noted for each case, yet acknowledging there was a considerable overlap between some CVD exposure groups given that multiple events co-existed in a substantial number of subjects ever reporting symptomatic CVD (Rafnsson, 2006).

4.6. Data analysis

Variables used in the statistical analyses were examined and described. In addition to descriptive statistics, multivariate analysis was performed to assess the relationship between retinal parameters and cognitive outcomes after controlling for potential confounding variables.

4.6.1. Data description

Prior to undertaking any statistical analysis, the dataset was examined for extreme values/outliers and missing data. Categorical variables were described with respect to the frequency (percentage/number). The distribution of continuous

variables was explored by inspecting histograms first. Any outliers were corrected if necessary or omitted from subsequent analysis. Continuous variables showing a positively skewed distribution on histogram plots were normalised using square root or logarithm transformation. The transformed variables were used in all subsequent analyses. For descriptive purposes, the median and the interquartile range (IQR) were quoted for the skewed variables and the mean and standard deviation (SD) were used for other continuous variables.

4.6.2. Bivariate statistical analysis

Pearson's r correlation coefficients were used to examine the direction and strength of the relationship between continuous variables (e.g. between quantitative retinal vascular parameters and continuous cardiovascular risk factors). The Chi-square test (or Chi-square test for trend in the case of ordinal variables) was used to test the statistical significance of differences in categorical risk factors. The Mann-Whitney U Test or Kruskal-Walli test was applied to assess the differences in median levels of risk factors by diabetic retinopathy status (presence or severity). The independent t-test or ANOVA was used to compare the mean levels of each cognitive function test variable and other normally distributed risk factors.

4.6.3. Multivariate statistical analysis

Analysis of covariance (ANCOVA) was used to examine the statistical significance of mean differences in both general and specific cognitive functions between subjects (i) with and without diabetic retinopathy and (ii) with different severity levels of diabetic retinopathy while adjusting for potential confounding factors. Multiple linear regression analysis was used to assess the independent contribution of (i) diabetic retinopathy and (ii) quantitative retinal vascular parameters to estimated change in both general and specific cognitive abilities while adjusting for possible confounders. In a further set of examinations, the multiple linear regression-based analyses above were repeated using the general cognitive factor-adjusted standardised residuals of each cognitive test as dependent variable.

In the modeling process (in both the ANCOVA and multiple linear

regression analyses), potential confounding variables were introduced into the models generally in three cumulative steps. In the first model, age and sex were adjusted for. Further, adjustment for the Mill-Hill Vocabulary Scale was used to control for the effects of pre-morbid cognitive ability. By controlling for a well-validated estimate of peak prior ability, it was possible to assess the impact of retinal vascular disease on the imputed lifetime decline from best-ever level of cognitive function to that actually measured here in old age (Deary et al., 2004a). Finally, adjustment was made for potential confounding by education level, vascular risk factors (alcohol intake, smoking, waist to hip ratio, systolic blood pressure and HDL cholesterol), the presence of cardiovascular disease (MI and/or angina, stroke and/or TIA), mood level (depression), and diabetes-related variables (duration of diabetes and HbA1c).

Variables were selected as possible confounders if univariate association with retinal variables (between group differences or correlation) was significant or if from previous literature a variable was known to be associated with both retinal variables and cognitive test performance. They were incorporated into the age, sex, and Mill-Hill adjusted model cumulatively in a predetermined sequence, with the most distal factors on the hypothesized causal pathway added first and then all other factors except for glycaemic control and duration of diabetes (Victoria et al., 1997).

In people with diabetes, cognitive ability is associated with hyperglycaemia and longer duration of diabetes. Longer duration of diabetes reflects many biological changes and pathologic processes, including hyperglycaemia and development of diabetic retinopathy. Hyperglycaemia may cause diabetic retinopathy. Thus analyses adjusted for HbA1c level and duration of diabetes may lead to an over-adjustment of the association between diabetic retinopathy and cognition to some extent. As a result, these two diabetes-related variables were added to the model in the final step to examine their impact on the association of diabetic retinopathy with cognitive ability over and above the effect of other variables. B values (unstandardised and standardised regression coefficients) were reported, as was the proportion of variance explained by each model.

Logistic regression was performed in order to further examine the clinical relevance of the relationship between cognitive impairment and diabetic retinopathy adjusted for age, sex and the Mill-Hill Vocabulary Scale. This analysis was not anticipated a priori, but was done as a descriptive exercise, mainly driven by significant results of the previous multiple linear regression analysis which seemed to indicate the presence of diabetic retinopathy was associated with poorer cognitive ability scores. Although this was a less efficient use of the available data than using all test scores as continuous scores, this step allowed the increased risk of cognitive impairment for diabetic retinopathy to be quantified (Price et al., 2006). Variables were then entered into the multivariate model using the 'ENTER' procedure and odds ratios (95%CI) for cognitive impairment were calculated.

With all covariates in the model, the interaction between all main effects was tested. The interaction term was not significant in all analyses except for sex and diabetic retinopathy in relation to performance on the general cognitive ability factor (g) and Verbal Fluency test. These data were analysed separately for men and women. A two sided p value <0.05 was taken to denote statistical significance throughout all analyses. However, given that many statistical tests were performed (e.g. there were 7 retinal vascular parameters and each correlated with 8 cognitive variables), the prudent approach here was to acknowledge that very low p values were more likely to indicate replicable associations (Deary et al., 2006).

4.6.4. Model assumptions

Prior to the above analyses, model assumptions were assessed and found to be met.

For independent t-test and the analysis of variance, normality of distributions was examined by plotting histogram or box-whisker plot. The homogeneity of variance was tested by visualising the standard deviations of mean scores or using the Levene's test.

For multiple linear regressions, standardised residual scatter plots were visually inspected for violations of normality, linearity, and homoscedasticity. Collinearity was checked by scanning the correlation matrix between independent variables ($r > 0.9$) or by looking at the variance inflation factor ($VF < 10$) and none of the variables constituted a combination of other independent variables. The data were visually inspected for outliers as a part of the univariate description of the data.

Prior to logistic modeling, variables were checked for any 'zero cells' which would result in spurious coefficients (Hosmer & Lemeshow, 1989). The assumption that predicator variables are linearly related to the log odds (B) of the outcome was tested by looking at separate log odds plots of outcome by covariates for all ordered categorical and continuous variables. The model's goodness-of-fit (Hosmer & Lemeshow, 2000) was assessed by looking at the Hosmer & Lemeshow statistic.

Chapter 5

Study Results

5.1. Introduction

This chapter presents results on the relationship between retinal microvascular abnormalities, vascular risk factors and CVD, and cognitive outcomes in elderly participants with Type 2 diabetes in the ET2DS. Descriptive data on the ET2DS population are presented first. Correlations between all parameters of retinal microvascular abnormality and the association of these parameters with socio-demographic, vascular risk factors and CVD are presented next. This is followed by the association of cognitive parameters with the sociodemographic and vascular variables. Finally, univariate and multivariate analyses of the association between retinal microvascular abnormalities and cognitive function are presented.

5.2. Study population

5.2.1. Representativeness

Crude levels of sociodemographic and diabetes-related factors in those who had been invited to take part in the study but had not participated (n=4,385) compared with those subjects who participated in the ET2DS (n=1,066) are shown in Table 13. Age, median duration of diabetes, total cholesterol, HbA1c and treatment status (% on insulin) did not differ greatly between participants and non-participants. The mean age of the study population was 67.4 years and approximately 51% of subjects were men. Table 14 shows that there was a slight under representation of females aged 70-74 years. The proportion of men in the study participant group was greater than in the non-participant group (51.3% vs. 41.9%) due to the stratified sampling process (response rates were higher for men than for women). Subjects from the most deprived SIMD categories (SIMD 2006 quintiles, 1&2) made up 31.4% of participants, which was lower than the proportion of non-participants in these quintiles (42.7%). The difference in deprivation profile between participants

and non-participants could be explained by the replacement of subjects who refused to participate in the study being age and sex stratified, but not socio-economic position such as the same postcode area. Mean systolic blood pressure of participants (133.3mmHg) was also slightly lower than that of non-participants (137.2 mmHg).

Table 13: Socio-demographic and diabetes-related factors in participants and non-participants

	Participants	Non-participants
	Mean/Percent (n)[a]	Mean/Percent (n)[b]
Mean age, years	67.4	67.9
Male sex, % (n)	51.3 (547)	41.9 (1839)
Deprivation rank, % (n)		
1st quintile (most deprived)	11.9 (127)	16.8 (736)
2nd quintile	19.5(208)	25.9 (1134)
3rd quintile	17.6 (188)	18.7 (820)
4th quintile	18.2 (194)	17.8 (782)
5th quintile (least deprived)	32.7 (349)	20.8 (897)
Less than 5 years duration of DM, % (n)	48.4 (516)	48.7 (2135)
Insulin treatment, % (n)	17.4 (185)	16.1 (704)
Mean cholesterol, mmol/L	4.3	4.2
Mean Systolic blood pressure, mmHg	133.3	137.2
Mean HbA1c, %	7.4	7.4

[a] Maximum n=1066, minimum n=1028 for participants
[b] Maximum n=4385, minimum n=4020 for non-participants

Table 14: Age by sex distribution of the study participants

Age (years)	Males (%)	Females (%)
60-64	175 (32.0)	187 (36.0)
65-69	183 (33.5)	177 (34.1)
70-74	189 (34.6)	155 (29.9)
Total	547 (100)	519(100)

Assessment of missing data

Complete data were available for the following variables: age, sex, education level, treatment of diabetes, smoking status (current, former, never), hypertension and hypercholesterolaemia. Very small amounts of data (less than 3%) were missing for social class, duration of diabetes, BMI, WHR, annual volume of alcohol consumption, brachial blood pressure, total and HDL cholesterol. HbA1c

was not assessed in 38 (3.6%) subjects, and pack-years smoking was missing for 65 (6.1%) subjects. This was mostly due to an insufficient volume of plasma sent for HbA1c measurement or questions left blank in the smoking questionnaire. Typical week's alcohol consumption had a relatively high level of missing data (n=189, 17.7%) due to subjects in whom the previous week was not reported as 'typical' in terms of drinking (subjects were excluded from variable calculation). Given that typical week's alcohol intake correlated highly with average weekly alcohol intake in the past year ($r=0.92$, $p<0.001$), but the former had high degree of missing data, the variable of average weekly alcohol intake alone was used in subsequent statistical analysis.

The proportion of subjects with missing cognitive function test data ranged from 0.3% to 1.7%. The Letter-Number Sequencing test was most frequently missing, due to participant refusal or inability to attempt or complete the test. The LNS was used as a measure of auditory working memory which requires the concurrent retention and manipulation of information (Baddeley, 1986). In the LNS testing, the subject must recall the inter-mixed numbers and letters (verbally presented), stating the numbers in ascending numerical order followed by the letters in alphabetical order. The test was discontinued when the subject failed 3 consecutive trials of the same length. Thus, a number of factors could particularly contribute to the highest refusal/discontinuation rate in the LNS, including task difficulty, illiteracy, hearing impairment, cognitive impairment, fatigue, and anxiety or mood disturbance. Further analysis showed no relationship between missing values for the cognitive tests and demographic or vascular risk profile or retinal microvascular abnormalities (see Appendix G, Table g1).

Twenty-two subjects (2%) had missing diabetic retinopathy status, due to ungradable or unavailable retinal photographs. Characteristics of participants with and without gradable retinal photographs are described in Table 15. Subjects with gradable photographs (n=1044) performed better on Faces, Logical Memory and general cognitive ability tests, but did not differ from subjects (n=22) without gradable photographs with respect to any of the cardiovascular risk factors or other

cognitive test scores.

Missing data for the quantitative retinal vascular parameters was highest for arteriolar bifurcation angle and branching coefficient (58%), due to poor image quality or technical and practical difficulties in measurement (See Section 4.4.2). A comparison was made between subjects with and without missing individual retinal quantitative parameter with respect to characteristics of the cohort (Table 16 and 17). Subjects with retinal width or arteriolar bifurcation measurements had a lower prevalence of cardiovascular disease (e.g. stroke and/or TIA) and significantly better scores on most cognitive tests, but no major difference in other cardiovascular risk factors was detected. However, the magnitude of the differences on these cognitive measures was small.

Table 15: Characteristics for subjects with and without retinal photographs which were gradable for diabetic retinopathy

	Subjects with retinal grading (n=1044)[a]	Subjects with missing retinal grading (n=22)[b]
Age (years),mean (SD)	67.9(4.2)	68.0(4.3)
Sex, (% (n) men)	51.2 (535)	54.5(12)
BMI (kg/m^2), mean (SD)	31.4(5.7)	32.0(7.3)
WHR, mean (SD)	0.97(0.08)	0.94(0.08)
HbA1c, median (IQR)	7.2(6.7-7.9)	7.2(6.9-7.9)
Total cholesterol (mmol/L), mean (SD)	4.3(0.9)	4.6(1.1)
HDL cholesterol (mmol/L), mean (SD)	1.3(0.4)	1.4(0.3)
Smoker, %(n)[1]	60.9 (636)	59.1 (13)
Hypertension, %(n)	84.9 (886)	81.8 (18)
Cardiovascular disease, %(n)		
MI and/or Angina	31.2 (326)	18.2(4)
Stroke and/or TIA	8.6 (90)	13.6 (3)
Cognitive tests, mean (SD)		
Mill-Hill Vocabulary Scale	31.0(5.2)	29.2(5.8)
Verbal Fluency	36.9(12.8)	37.1(14.5)
Faces	66(7.8)	58.3(7.0)*
Trail Making Test (ln)	4.7(0.4)	4.8 (0.3)
Logical Memory	25.4(8.2)	20.0(5.9) *
Matrix Reasoning	12.9(5.3)	11.0(5.0)
Digit Symbol	49.3(14.8)	44.1(13.7)
Letter-Number Sequencing	9.7(2.8)	9.0(2.1)
General factor score (g)	0.0(1.0)	-0.53 (0.77)*

[1]Smoker=current or former smoker; * P <0.05; [a] Maximum n=1044, minimum n=1000 for subjects with retinal grading; [b] Maximum n=22, minimum n=21 for subjects without retinal grading

Table 16: Characteristics of subjects with and without retinal width measurements

	Subjects with retinal width measurement (n=980)[a]	Subjects with missing retinal width measurement (n=86)[b]
Age (years),mean (SD)	67.3(4.2)	68.1(4.5)
Sex, (% (n) men)	51.3 (503)	51.2 (44)
BMI (kg/m^2), mean (SD)	31.4(5.7)	31.5(5.8)
WHR, mean (SD)	0.97(0.08)	0.97(0.08)
Duration of DM (yrs), median (IQR)	6.6(3.9-11.2)	7.8(4.1-12.7)
HbA1c, mean (SD)	7.4(1.2)	7.6(1.3)
Total cholesterol (mmol/L), mean (SD)	4.3(0.9)	4.4(0.9)
HDL cholesterol (mmol/L), mean (SD)	1.3(0.4)	1.3(0.4)
Smoker, %(n)	61.4 (602)	54.7 (47)
Hypertension, %(n)	84.6 (829)	87.2 (75)
Cardiovascular disease, %(n)		
MI and/or Angina	31.2 (306)	27.9 (24)
Stroke and/or TIA	8.1 (79)	16.3 (14) *
Cognitive tests, mean (SD)		
Mill-Hill Vocabulary Scale	31.0(5.2)	30.2(5.2)
Verbal Fluency	36.9(12.7)	37.3(14.1)
Faces	66(7.8)	61.5(7.5) *
Trail Making Test (ln)	4.67(0.40)	4.85(0.5) *
Logical Memory	25.4(8.2)	23.6(7.9)
Matrix Reasoning	12.9(5.3)	11.7(5.0) *
Digit Symbol	49.6(14.7)	44.5(14.4) *
Letter-Number Sequencing	9.7(2.8)	9.3(2.6)
General factor (g)	0.03(1.0))	-0.34(1.0) *

[1]Smoker=current or former smoker; * P <0.05; [a] Maximum n=980, minimum n=938 for subjects with retinal width measurement; [b] Maximum n=86, minimum n=83 for subjects without retinal width measurement

Table 17: Characteristics of subjects with and without retinal bifurcation measurement

	Subjects with retinal BA measurement (n=444)[a]	Subjects with missing retinal BA measurement (n=622)[b]
Age (years),mean (SD)	66.9(4.0)	67.7(4.3)
Sex, (% (n) men)	50.9 (226)	51.6(321)
BMI (kg/m^2), mean (SD)	31.3(5.5)	31.6(5.8)
WHR, mean (SD)	0.96(0.07)	0.97(0.08)
HbA1c, mean (SD)	7.3(1.0)	7.4(1.2)
Total cholesterol (mmol/L), mean (SD)	4.3(0.9)	4.3(0.9)
HDL cholesterol (mmol/L), mean (SD)	1.3(0.4)	1.3(0.4)
Smoker, %(n)[1]	61.3 (272)	60.6 (377)
Hypertension, %(n)	83.6(371)	85.7(533)
Cardiovascular disease, %(n)		
MI and/or Angina	27.5 (122)	33.4 (208) *
Stroke and/or TIA	6.5(29)	10.3 (64) *
Cognitive tests, mean (SD)		
Mill-Hill Vocabulary Scale	31.3(5.1)	30.7(5.3)
Verbal Fluency	37.5(12.6)	36.5(13.0)
Faces	66.4(7.8)	65.4(7.9) *
Trail Making Test (ln)	4.6(0.4)	4.7(0.4) *
Logical Memory	25.7(8.4)	24.9(8.0)
Matrix Reasoning	13.1(5.4)	12.6(5.2)
Digit Symbol	49.8(14.5)	48.9(15.0)
Letter-Number Sequencing	9.95(2.6)	9.48(2.8) *
General factor score (g)	0.08(1.0)	-0.06(1.0) *

[1]Smoker=current or former smoker; * P <0.05; [a] Maximum n=444, minimum n=431 for subjects with retinal arteriolar bifurcation measurement; [b] Maximum n=622, minimum n=590 for subjects without retinal arteriolar bifurcation measurement

5.2.2. Characteristics of study population

Full characteristics of the study population are shown in Table 18. The median duration of diabetes was 6.7 years (IQR 3.9-11.3). Glycaemic control was assessed by HbA1c levels and diabetes treatment modality. The mean HbA1c level was 7.4% and 63.7% of the participants were treated with oral hypoglycaemic agents without insulin. A further 17.4% were receiving insulin injection with or without oral hypoglycaemic agents. Approximately 18.9% were diet controlled.

Regarding the highest level of education completed, 16 % of subjects had completed university and 28.8% had finished post school training of some profession. About 55.2% had completed secondary (54.5%) and primary school (0.7%) (these were combined due to the small number in primary school). Most social classes were well represented in the sample and 23.3% of the population was classified in social class I, 22.5% in class II, 28.8% in class III, 11.0% in class IV and 14.0% in class V.

Cardiovascular risk factors measured included smoking habit, alcohol consumption, obesity (assessed with body mass index and waist-to-hip ratio), cholesterol levels, and blood pressure. About 14.3% of the population were self-reported current smokers and 46.6% were former smokers, whereas 39.1% had never smoked. Alcohol intake referred to average weekly alcohol intake in the past year and 28.5% of subjects reported not having consumed any alcohol in the year prior to examination. A further 45.6% had consumed less than 7 drinks per week, 13.4% had had 7-15 drinks, and 9.9% had consumed more than 15 drinks of alcohol. Also, around 90% of the population had hypercholesterolaemia and 84.8% were suffering from hypertension. 93 subjects (8.7 %) had suffered a stroke or TIA, 150 (14.1 %) had met study criteria for diagnosis of myocardial infarction and 298 (28.0 %) had angina prior to the examination. In addition, 63 (5.9 %) subjects were suffering from intermittent claudication as identified by the Edinburgh Intermittent Claudication questionnaire.

Approximately 11.8% of the population had depression scores ≥8 indicating possible or probable clinical depression. The MMSE was used to screen for dementia and most individuals had close to the upper-limits of the scale (median 29, IQR 28 to 30), with only a very small proportion having an MMSE score below 24 (n=30), indicating severe cognitive impairment.

Table 18: ET2DS population characteristics

		Mean (SD)/Percent (n)
Age (years)		67.4 (4.2)
Males (%)		51.3 (547)
Duration of diabetes		6.7 (3.9-11.3) [a,b]
HbA1c (% of haemoglobin)		7.4(1.1)
Treatment of diabetes		
	Diet only	18.9 (201)
	Hypoglycaemic oral agents	63.7 (679)
	Insulin ± hypoglycaemic oral agent	17.4 (186)
Education Level		
	University	16.0 (171)
	Other professional/technical qualification	28.8 (307)
	Secondary & Primary school	55.2 (588)
Social Class		
	I	23.3 (248)
	II	22.5 (240)
	III	28.8 (307)
	IV	11.0 (117)
	V	14.0 (149)
Obesity Indices	Body Mass Index (kg/m^2)	31.4 (5.7)
	Waist to hip ratio	0.97(0.08)
Plasma Lipids	Total cholesterol (mmol/L)	4.3(0.9)
	HDL cholesterol (mmol/L)	1.3(0.4)
	Hypercholesterolaemia (%)	86.6 (923)
Smoking	Smoker (current or former)	60.9 (649)
	Pack-yrs smoking (among smokers)	29.1(13.8-45.0) [a]
Alcohol intake		
	Average weekly alcohol intake (standard drinks/week)	1(0-6.8) [a]
	Average weekly alcohol intake category (%)	
	Never	28.5 (304)
	Light drinker (≤7 drinks)	45.6 (486)
	Moderate drinker (7-15 drinks)	13.4 (143)
	Heavy drinker (>15 drinks)	9.9 (106)
Blood pressure	Systolic blood pressure (mmHg)	133.3(16.4)
	Diastolic blood pressure (mmHg)	69.1(9.0)
	Hypertension (%)	84.8 (904)

Cardiovascular disease	Stroke and/or TIA	8.7 (93)[c]
	Myocardial infarction and/or Angina	31.0 (330)[d]
	Intermittent caludication (IC)	5.9 (63)[e]
	Any CVD (MI, angina, stroke, TIA, and IC)	36.8 (392)
Mood and cognitive status	Depression score	3.0(1.0-6.0) [a]
	Depressive symptoms (≥8)	11.8 (126)
	Mini-Mental State Examination	29(28-30) [a]

[a]The median (IQR) was quoted for skewed variables

[b] Duration of diabetes= (age at examination-age at diagnosis); zero indicated duration was less than one year

[c] Cerebrovascular disease (n=93) included subjects with cerebrovascular disease (stroke and/or TIA) only (n=40), coronary artery disease and cerebrovascular disease (MI and/or angina, stroke and/or TIA) (n=38), cerebrovascular disease and peripheral arterial disease (stroke and/or TIA, and IC) (n=4), MI and/or angina, stroke and/or TIA and IC (n=11);

[d] Coronary artery disease (n=330) included subjects with coronary artery disease (MI and/or angina) only (n=251), coronary artery disease and cerebrovascular disease (MI and/or angina, stroke and/or TIA) (n=38), coronary artery disease and peripheral arterial disease (MI and/or angina, and IC) (n=30), MI or angina, stroke or TIA and IC (n=11);

[e] Peripheral arterial disease (n=63) included subjects with IC only (n=18), IC and coronary artery disease (IC, MI and/or angina) (n=30), IC and cerebrovascular disease (IC, stroke and/or TIA) (n=4), MI and/or angina, stroke and/ or TIA, and IC (n=11).

5.3. Retinal microvascular abnormalities

This section focuses on the prevalence of diabetic retinopathy, the distribution of parameters of retinal vascular network geometry, and the interrelationships between the various retinal microvascular abnormalities. It also describes the associations between retinal microvascular abnormalities and sociodemographic characteristics, vascular risk factors and cardiovascular disease.

5.3.1. The prevalence and severity of diabetic retinopathy

Of the 1,066 participants in the ET2DS, 20 did not undergo digital retinal photography, and 2 had no gradable photographs for diabetic retinopathy in either eye, leaving 1,044 participants who provided data for the analysis.

The crude prevalence of diabetic retinopathy (DR) at baseline was 32.5% (95%CI: 29.7, 35.3). Men were more likely to have diabetic retinopathy than women (35.1% vs. 29.7%) but the difference was of borderline statistical significance (P=0.058). There was no age-related trend in the prevalence of diabetic retinopathy

between 5-year age groups (P=0.86) (Figure 29). Mild NPDR was found in 28% of the population, moderate-severe NPDR in 3.1%, and proliferative diabetic retinopathy in 1.4% (Table 19).

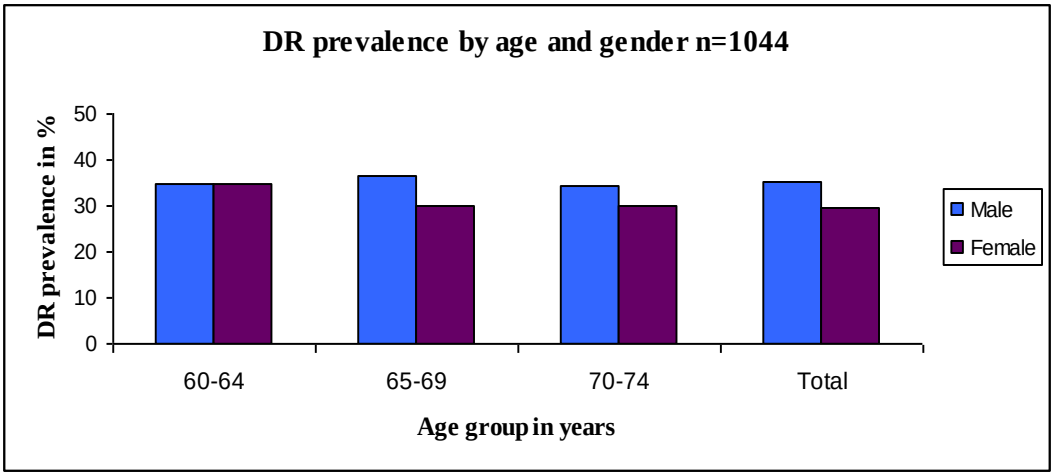


Figure 29: Diabetic retinopathy (DR) in ET2DS by age and gender

Table 19: Age- and gender-specific prevalence (95% Confidence Interval) of diabetic retinopathy in ET2DS

Severity of diabetic retinopathy (DR)	Age group (years)			Gender		Total (n=1044)
	60-64 (n=356)	65-69 (n=351)	70-74 (n=337)	Female (n=509)	Male (n=535)	
Any DR	31.7 (26.9, 36.5)	33.3 (28.4, 38.2)	32.3 (27.3, 37.3)	29.7 (25.7,33.7)	35.1 (31.1, 39.1)	32.5(29.7,35.3)
Mild NPDR	26.4(21.8, 31.0)	30.2 (25.4, 35.0)	27.3(22.5, 32.1)	25.7(21.9, 29.5)	30.1(26.2, 34.0)	28(25.3, 30.7)
Moderate-severe NPDR	3.4 (1.5, 5.3)	2.6(0.7, 4.5)	3.3(1.4, 5.2)	2.6(1.2, 4.0)	3.6(2, 5.2)	3.1(2.1, 4.2)
Proliferative DR	2.0(0.6, 3.4)	0.6(0.0, 1.4)	1.8(0.4, 3.2)	1.4(0.4, 2.4)	1.5(0.5, 2.5)	1.4(0.7, 2.1)

DR: diabetic retinopathy; NPDR: Non-proliferative diabetic retinopathy

5.3.2. Reliability of retinal quantitative parameters measurement

The Bland-Altman plot of the difference between the two repeats or raters measurements against their means was drawn and the range of the mean difference ±2 standard deviations (SDs) was examined for measuring agreement (Appendix H). The repeatability coefficient was also used to indicate that the difference between two measurements by the same rater or two raters was within 2SD for 95% of subjects.

Table 20 presents information on the reliability of retinal quantitative parameters as determined by replicate grading exercises. Results are divided into intra-observer (i.e., the same grader in our study at different time) and inter-observer (i.e., two graders) comparisons. In summary, there were both high intra- and inter-rater reliability in retinal width measurement compared with previous studies. The intra-rater reliability in branching angle and coefficient was lower than the values in a published study (Patton et al., 2007) which however used a different measuring method from the study here (0.53 vs. 0.65 in BC, 0.72 vs. 0.96 in BA). The inter-rater reliability for BC that ranged between 1.6 and 2.3 was also low given the two ratings by different raters fell within ±0.7 for 95% of subjects, and even poorer for branching angle, in which the two raters' measurements were not correlated at all.

Table 20: Intra- and Inter-observer reliability of quantitative measurements, described by intra-class correlation coefficient and coefficient of repeatability between two measures

Measurements	Intra-class Correlation Coefficient [a]		Repeatability Coefficient (±2SDs)	
	Intra-observer	Inter-observer	Intra-observer	Inter-observer
CRAE (pixels)	0.72	0.74	2.7 pixels	3.6 pixels
CRVE (pixels)	0.95	0.67	2.6 pixels	5.6 pixels
Arteriole-to-venule ratio	0.92	0.79	0.07	0.14
Arteriolar branching angle (degree)	0.72	-0.14[b]	13.3[O]	N/A[b]
Arteriolar branching coefficient	0.53	0.69	0.36	0.7

CRAE: central retinal arteriolar equivalent; CRVE: central retinal venular equivalent; [a]All ICCs were significant at 0.05 level except for inter-observer reliability of arteriolar branching angle; [b]The ICC was non-significant (P=0.76) thus the Bland-Altman plot was not drawn.

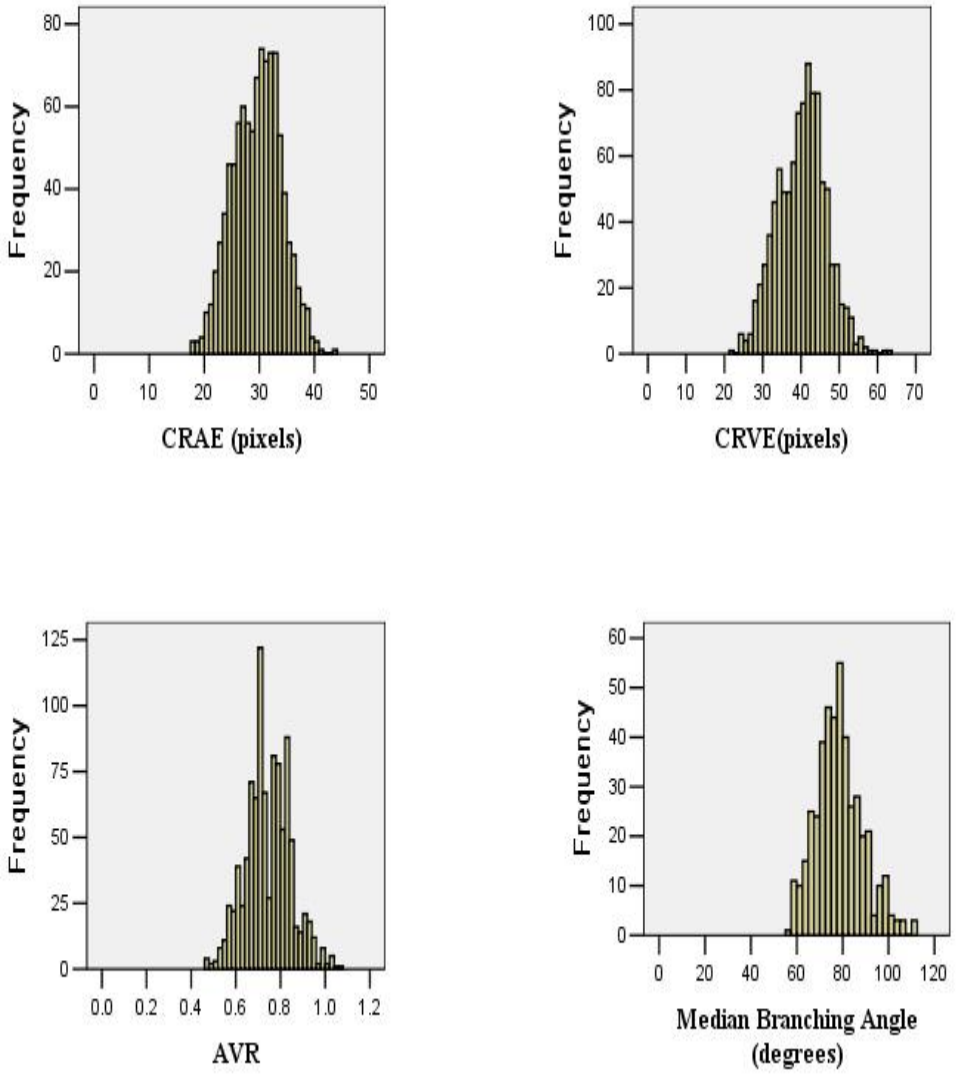
5.3.3. Distribution of retinal quantitative parameters

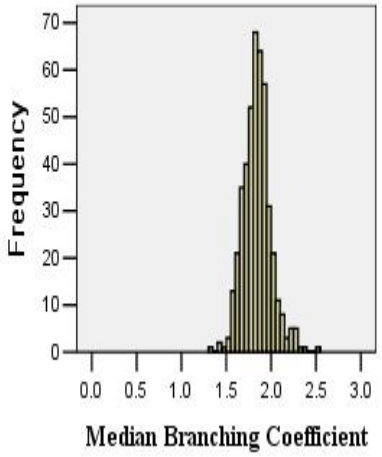
Of the 1,044 participants who had gradable photographs for diabetic retinopathy severity, 64 did not have gradable retinal vessels for analysis of any of the retinal vascular parameters in either eye. In all of the remaining 980 individuals, the retinal width parameters of CRVE, CRAE, and AVR were measured. However, of 980 images that were gradable for CRVE, CRAE and AVR, 536 were either of too poor quality to analyse 3 arteriolar junctions, or less than 3 arteriolar junctions were evaluable within the 35^{o} field. Thus for arterial branching angle and coefficient (BA & BC), only 444 subjects provided data for the analysis. The frequency of measurements was approximately equal for the right and left eyes (51% vs. 49% for left and right eye on retinal width measures, and 49.5 % vs. 50.5% on retinal angles).

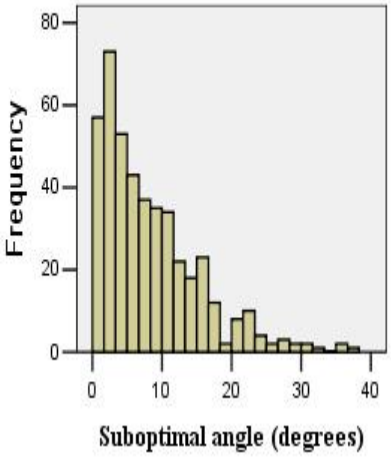
Frequency histograms for retinal parameters are shown in Figure 30. All parameters followed a normal distribution except for suboptimal branching angles (BA). The absolute values of deviations of the median angles from optimality were positively skewed and underwent natural log (n+1) transformation in subsequent analyses that assume approximate normality.

The mean (SD) or median (IQR) of each retinal vascular network parameter for all subjects and for males and females separately are shown in Table 21. For the vessel width measurements, the values in pixels translated into the following values in micrometers, (undertaken to aid comparison with other studies, based on an assumption of the average optic disc diameter (approximately 327 pixels) being equal to 1850μm): CRAE= 167.5μm and CRVE=227.8μm. There were no significant differences observed between males and females on any parameters.

Figure 30: Histograms of parameters of retinal vascular network geometry







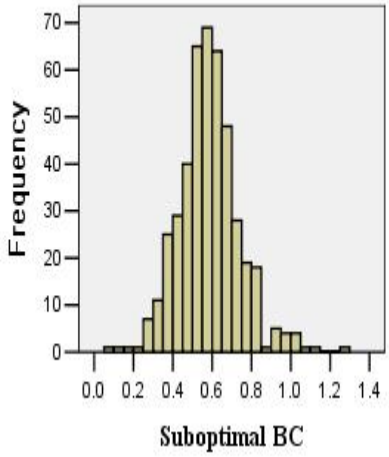


Table 21: Parameters of retinal vascular network geometry in males and females

	N	All subjects	N	Males	N	Females	P-value*
CRAE, pixels, Mean (SD)	980	29.6 (4.3)	503	29.5 (4.4)	477	29.8 (4.3)	0.25
CRVE, pixels, Mean (SD)	980	40.3 (6.3)	503	40.4 (6.4)	477	40.2 (6.2)	0.65
AVR, Mean (SD)	980	0.74 (0.10)	503	0.74 (0.10)	477	0.75 (0.10)	0.06
Median angle BA, degrees, Mean (SD)	444	78.5 (10.3)	226	78.4(10.5)	218	78.6(10.1)	0.78
Median BC, Mean (SD)	444	1.84 (0.16)	226	1.85 (0.15)	218	1.84 (0.16)	0.50
Suboptimal BA, degrees, Median(IQR)	444	6.5 (2.9-11.9)	226	6.8 (2.8-12.0)	218	6.1 (3.1-11.8)	0.85
Suboptimal BC, Mean (SD)	444	0.58 (0.16)	226	0.59 (0.15)	218	0.58 (0.16)	0.50

*P-value for difference between males and females was calculated with independent t-test; transformed suboptimal BA was used

5.3.4. Correlations between parameters of retinal microvascular abnormalities

Overall, most quantitative retinal vascular parameters showed significant inter-correlation (Table 22). There was a perfect correlation for suboptimal BC and median BC (r=1.00, P<0.01). Suboptimal BC measured the degree of deviation of the BC from optimality (i.e.1.26). All the values of median BC were greater than 1.26, suggesting they were all abnormal relative to 1.26. Thus suboptimal BC and the absolute BC were not two separate sources of information and median BC alone was used in subsequent analysis. For other parameters, the strongest associations were between CRAE and CRVE (r=0.61, P<0.01), AVR and CRVE (r=-0.51, P<0.01) and suboptimal BA and median BA (r = 0.50, P<0.01). Suboptimal BA and median BA did not show strong associations with other parameters.

There were no significant differences in mean CRAE, CRVE, AVR, median BA or median BC between subjects with and without diabetic retinopathy. Suboptimal BA was significantly higher in those with diabetic retinopathy based on grading level either from the matched eye (P=0.007) or from the worse eye (P=0.019) (Table 23).

When the presence of diabetic retinopathy was further categorized into mild and moderate-severe diabetic retinopathy, none of the parameters except for suboptimal BA showed a clear trend across the worsening categories of diabetic retinopathy. Based on gradings from the worse eye, the mean suboptimal BA increased from 0.82 (95%CI 0.78, 0.86) to 0.94 (95%CI 0.79, 1.08) between the absent and moderate-severe groups, with an intermediate value for mild diabetic retinopathy (mean 0.89, 95%CI 0.83, 0.95), indicating that increased abnormality in arteriolar branching angles was associated with more severe diabetic retinopathy (Table 24). Although the trend was also significant based on the gradings from the single matched eye, the group means were not apparently in a correct order but with the mild diabetic retinopathy group having the highest and non-diabetic retinopathy group the lowest. This was possibly due to the small number of subjects having moderate-severe diabetic retinopathy (n=14). Further tests for reversals (Page et al.,

2003) by testing each adjacent pair of means (e.g. no vs. mild, mild vs. moderate-severe) were all non-significant supporting a monotonic trend. However, increasing severity of diabetic retinopathy was not associated with changes in mean CRAE or CRVE (Figure 31). For both CRAE and CRVE, the standard deviation was nearly constant across levels of severity except for level 53, which had a very small number of subjects (n=3 based on grading from the single matched eye and n=4 based on grading from the worse eye).

Table 22: Pearson correlation coefficients between retinal vascular parameters

	CRAE (pixels)	CRVE (pixels)	AVR	Median BA (degrees)	Median BC	Suboptimal BA[a] (degrees)	Suboptimal BC
Central Retinal Venular Equivalent	**0.61**[†]	---					
Arteriovenous ratio	**0.36**[†]	**-0.51**[†]	---				
Median arteriolar branching angle	-0.02	**-0.10**[*]	**0.11**[*]	---			
Median arteriolar branching coefficient	**-0.14**[†]	**-0.18**[†]	0.08	0.08	---		
Suboptimal branching angle	-0.04	**-0.10**[*]	0.08	**0.50**[†]	-0.01	---	
Suboptimal branching coefficient	**-0.14**[†]	**-0.18**[†]	0.08	0.08	**1.00**[†]	-0.01	---

CRAE: Central Retinal Arteriolar Equivalent; CRVE: Central Retinal Venular Equivalent; AVR: Arteriovenous ratio; BA: arteriolar branching angle; BC: arteriolar branching coefficient; [a] transformed suboptimal BA was used; [†] significant at 0.01 level; [*]significant at 0.05 level

Table 23: Retinal width and arteriolar bifurcation parameters according to presence of diabetic retinopathy

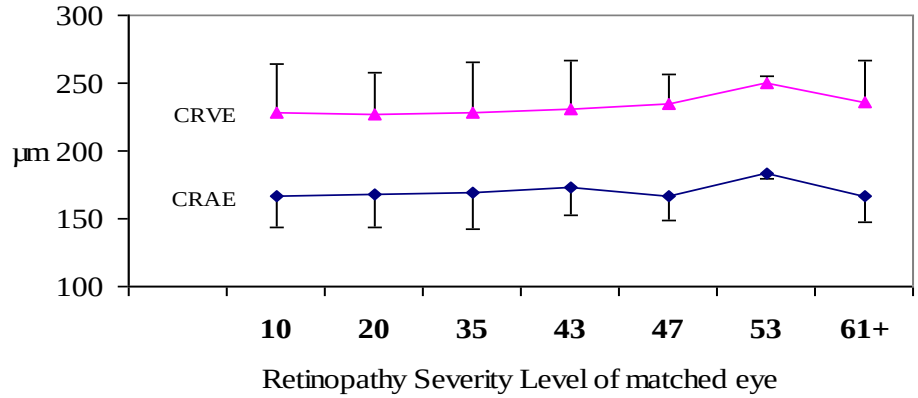
	Retinopathy of the matched single eye			Retinopathy of the worse eye		
	Absent	Present	P value	Absent	Present	P value
Retinal width parameters[a]						
CRAE, µm, Mean (SD)	167 (25)	169 (24)	0.35	167 (24)	168 (25)	0.57
CRVE, µm, Mean (SD)	228 (36)	227 (34)	0.76	228 (37)	227 (34)	0.64
AVR, Mean (SD)	0.74 (0.10)	0.75 (0.10)	0.65	0.74 (0.10)	0.75 (0.10)	0.36
Retinal bifurcation parameters[b]						
Median BA, degrees, Mean (SD)	78.0 (10.0)	80.0 (11.1)	0.09	78.0 (10.0)	79.0 (11.0)	0.16
Median BC, Mean (SD)	1.8 (0.2)	1.8 (0.2)	0.52	1.8 (0.2)	1.8 (0.2)	0.58
Suboptimal BA, Mean (SD)[c]	0.82 (0.3)	0.92(0.3)	**0.006**	0.82 (0.3)	0.89 (0.3)	**0.026**

[a]N=980 for subjects with retinal width measurements, of which 233 subjects had diabetic retinopathy based on the grading from the matched single eye whereas 323 subjects had diabetic retinopathy from the worse eye; [b]N=444 for subjects with retinal arteriolar bifurcation measurements, of which 109 subjects had diabetic retinopathy based on the grading from the matched single eye whereas 155 subjects had diabetic retinopathy from the worse eye; [c] Transformed variable was used

Table 24: Retinal width and arteriolar bifurcation parameters according to diabetic retinopathy severity

	Retinopathy severity of the matched single eye				Retinopathy severity of the worse eye			
	None	Mild DR	Moderate-severe DR	P for trend	None	Mild DR	Moderate-severe DR	P for trend
Retinal width parameters[a]								
CRAE, µm, Mean (SD)	167 (25)	168 (26)	171 (19)	0.28	167 (24)	168 (26)	170 (21)	0.50
CRVE, µm, Mean (SD)	228 (36)	227 (34)	235 (30)	0.53	228 (37)	226 (34)	232 (31)	0.92
AVR, Mean (SD)	0.74 (0.10)	0.75 (0.10)	0.74 (0.07)	0.83	0.74 (0.10)	0.75 (0.10)	0.74 (0.07)	0.56
Retinal bifurcation parameters[b]								
Median BA, degrees, Mean (SD)	78.0 (10.0)	79.6 (11.1)	82.3 (10.0)	0.06	78.0 (10.0)	79.1 (10.7)	81.7 (10.9)	0.09
Median BC, Mean (SD)	1.85 (0.2)	1.83 (0.2)	1.84 (0.2)	0.58	1.85 (0.2)	1.83 (0.2)	1.84 (0.1)	0.62
Suboptimal BA, Mean (SD)	0.82 (0.3)	0.93 (0.3)	0.87 (0.3)	**0.02**	0.82 (0.3)	0.89 (0.3)	0.94 (0.3)	**0.02**

[a]N=980 for subjects with retinal width measurements, of which 233 subjects had diabetic retinopathy based on the grading from the matched single eye whereas 323 subjects had diabetic retinopathy from the worse eye; [b]N=444 for subjects with retinal arteriolar bifurcation measurements, of which 109 subjects had diabetic retinopathy based on the grading from the matched single eye whereas 155 subjects had diabetic retinopathy from the worse eye; [c] Transformed variable was used



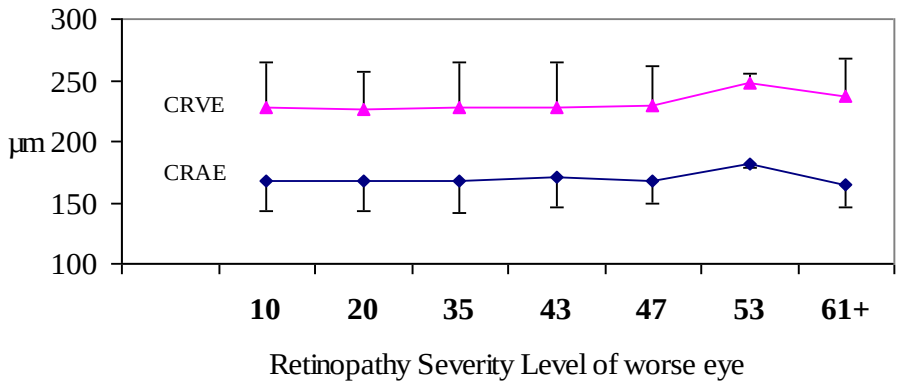


Figure 31: Mean central arteriolar equivalent (CRVE) and central retinal venular equivalent (CRVE) and standard deviations (SDs) by retinopathy severity level. Error bars indicate the SDs at each point, shown only one side for simplicity

5.3.5. Associations of diabetic retinopathy with sociodemographic variables, vascular risk factors and cardiovascular disease

Table 25 presents levels of sociodemographic variables, vascular risk factors and cardiovascular disease in subjects with and without diabetic retinopathy. Subjects with diabetic retinopathy were slightly more likely to be male (55.5%) compared to those without diabetic retinopathy (49.2%, P=0.06). A higher proportion of subjects with diabetic retinopathy were on insulin treatment (31.9% vs. 10.5%, P<0.001) and they had a longer duration of diabetes (median 10.3 vs. 5.5, P<0.001) and higher mean HbA1c levels (mean 7.6 vs. 7.3, P<0.001). Mean waist-to-hip ratio was higher in subjects with diabetic retinopathy (mean 0.98 vs. 0.96, P=0.001), whereas mean diastolic blood pressure was significantly lower (mean 67.8 vs. 69.7, P=0.002). Of those subjects with diabetic retinopathy, 13.6% had suffered a stroke or TIA compared with 6.2% in the group without diabetic retinopathy (P<0.001). No statistically significant differences were found for other variables, including education, social class, smoking habits, alcohol intake, BMI, systolic blood pressure, hypertension, plasma cholesterol, coronary artery disease or peripheral arterial disease.

Subjects with diabetic retinopathy were further divided into those who had mild non-proliferative diabetic retinopathy (NPDR), and those who had moderate to severe NPDR or PDR. Levels of variables were calculated across retinopathy severity categories (Table 26). A significant trend across worsening retinopathy categories was found for diabetes-related variables, including the median duration of diabetes and mean HbA1c, with longer duration of diabetes and worse glycaemic control in the mild diabetic retinopathy group and even longer duration and worse glycaemic control in the moderate-severe NPDR or PDR group. The percentage of subjects on insulin treatment and mean waist to hip ratio were significantly higher in the mild retinopathy group and even higher in the group with moderate to severe retinopathy. Conversely, mean diastolic blood pressure was significantly lower in the mild retinopathy group and even lower in the moderate to severe group, possibly because of the higher percentage of subjects in the diabetic retinopathy groups who

were on treatment for hypertension. There was no significant relationship across diabetic retinopathy severity categories for BMI, alcohol intake, pack-years smoking, systolic blood pressure, and plasma cholesterol. The prevalence of any cardiovascular disease, in particular cerebrovascular disease, was significantly higher in the diabetic retinopathy group, with the moderate to severe diabetic retinopathy group having the highest prevalence.

Table 25: Levels of socio-demographic variables, vascular risk factors and cardiovascular disease by presence of diabetic retinopathy

	No Diabetic Retinopathy (N=705)	Diabetic Retinopathy (N=339)	P-value for difference†
Sociodemographic Variables			
Age, yrs, mean (SD)	67.3(4.2)	67.4(4.2)	0.89
Sex, male, n (%)	347(49.2)	188(55.5)	0.06
Education level, n (%)			
University	113(16)	56(16.5)	0.26
Other profession/technical qualification	192(27.2)	108(31.9)	(Trend)
Secondary& Primary school	400(56.7)	175(51.6)	
Social Class, n (%)			
I	157(22.3)	85(25.3)	0.55
II	166(23.6)	70(20.8)	(Trend)
III	202(28.7)	100(29.8)	
IV	80(11.4)	36(10.7)	
V	99(14.1)	45(13.4)	
Diabetes-related Variables			
Diabetes duration, yrs, median (IQR)	5.5(3.4-9.4)	10.3 (5.9-15.8)	**<0.001**
Treatment, n (%)			
Diets	168(23.8)	28(8.3)	**<0.001**
Oral hypoglycaemics only	463(65.7)	203(59.9)	
Insulin ± oral hypoglycaemics	74(10.5)	108(31.9)	
HbA1c (% of haemoglobin), mean (SD)	7.3(1.1)	7.6(1.2)	**<0.001**
Vascular Risk Factors			
Body Mass Index, kg/m^2, mean (SD)	31.4(5.5)	31.4(6.0)	0.94
Waist-hip ratio, mean (SD)	0.96(0.07)	0.98(0.08)	**0.001**
Smoking status, n (%)			
Never	268(38.0)	140(41.3)	0.25
Former	334(47.4)	156(46.0)	(Trend)
Current	103(14.6)	43(12.7)	
Pack-years smoking, median (IQR)	9(0-32)	4.5(0-30)	0.17
Average alcohol intake, drinks/week, median (IQR)	1.0(0-7)	0.6(0-5)	0.23
Alcohol (drinks consumed per week), n (%)			
Never	198(28.9)	100(30.1)	0.22
≤7	314(45.8)	160(48.2)	(Trend)
7-15	97(14.2)	44(13.3)	
>15	76(11.1)	28(8.4)	
Systolic blood pressure, mmHg, mean (SD)	133.3(15.7)	133.3(17. 7)	0.97
Diastolic blood pressure, mmHg, mean (SD)	69.7(9.1)	67.8(8.8)	**0.002**
Hypertension, n (%)	594(84.3)	292(86.1)	0.43
Total cholesterol, mmol/L, mean (SD)	4.3(0.9)	4.2(0.9)	0.15
HDL cholesterol, mmol/L, mean (SD)	1.30(0.4)	1.27(0.4)	0.25
Total/HDL cholesterol ratio, mean (SD)	3.5(1.1)	3.5(1.1)	0.75
Hypercholesterolaemia, n (%)	634(89.9)	305(90.0)	0.98
Cardiovascular Disease			
Myocardial infarction and/or Angina, n (%)	213(30.2)	113(33.3)	0.31
Stroke and/or TIA, n (%)	44(6.2)	46(13.6)	**<0.001**
Intermittent caludication, n (%)	39(5.5)	23(6.8)	0.42
Any CVD (MI, angina, stroke, TIA, or IC), n (%)	249 (35.3)	136 (40.1)	0.13

median (IQR= interquartile range); †For categorical variables P values were calculated with the chi-square test, for quantitative and normally distributed variables, with t-test and for skewed variables with Mann-Whitney U test

Table 26: Levels of socio-demographic & diabetes-related variables, vascular risk factors and cardiovascular disease by severity of diabetic retinopathy

	No diabetic retinopathy (N=705)	Mild diabetic retinopathy (N=292)	Moderate-severe NPDR-PDR (N=47)	P value for trend†
Sociodemographic Variables				
Age, yrs, mean (SD)	67.3(4.2)	67.4(4.2)	67.1(4.2)	
Gender, male, n (%)	347(49.2)	161(55.1)	27(57.4)	0.96
Education level, n (%)				0.06
University	113(16)	47(16.1)	9(19.1)	
Other profession/technical qualification	192(27.2)	96(32.9)	12(25.5)	0.31
Secondary& Primary school	400(56.7)	149(51.0)	26(55.3)	
Social Class, n (%)				
I	157(22.3)	74(25.6)	11(23.4)	
II	166(23.6)	57(19.7)	13(27.7)	0.44
III	202(28.7)	87(30.1)	13(27.7)	
IV	80(11.4)	30(10.4)	6(12.8)	
V	99(14.1)	41(14.1)	4(8.5)	
Diabetes Variables				
Diabetes duration, yrs, median (IQR)	5.5(3.4-9.4)	9.3 (5.1-14.4)	17.1(12.1-22.9)	**<0.001**
Treatment, n (%)				
Diets	168(23.8)	27(9.2)	1(2.1)	**<0.001**
Oral hypoglycaemics only	463(65.7)	185(63.4)	18(38.3)	
Insulin ± oral hypoglycaemics	74(10.5)	80(27.4)	28(59.6)	
HbA1c (% of haemoglobin), mean (SD)	7.3(1.1)	7.5(1.1)	8.4(1.4)	**<0.001**
Vascular Risk Factors				
Body Mass Index, kg/m^2, mean (SD)	31.4(5.5)	31.3(6.1)	32.3(5.3)	0.52
Waist-hip ratio, mean (SD)	0.96(0.07)	0.97(0.08)	0.99(0.09)	**0.001**
Smoking status, n (%)				
Never	268(38.0)	115(39.4)	25(53.2)	0.11
Former	334(47.4)	139(47.6)	17(36.2)	

Current	103(14.6)	38(13.0)	5(10.6)	
Pack-years smoking, median (IQR)	9(0-32)	5.1(0-30)	0(0-12)	0.10
Average alcohol intake, drinks/week, median (IQR)	1(0-7)	0.6(0-6.8)	1(0-3.8)	0.29
Alcohol (drinks consumed per week), n (%)				
Never	198(28.9)	88(30.9)	12(25.5)	0.23
≤7	314(45.8)	133(46.7)	27(67.4)	
7-15	97(14.2)	39(13.7)	5(10.6)	
>15	76(11.1)	25(8.8)	3(6.4)	
Systolic blood pressure, mmHg, mean (SD)	133.3(15.8)	133.4(17.4)	132.8(19.9)	0.97
Diastolic blood pressure, mmHg, mean (SD)	69.7(9.1)	67.9(8.4)	67.2(10.9)	**0.002**
Hypertension, n (%)	594(84.3)	247(84.6)	45(95.7)	0.15
Total cholesterol, mmol/L, mean (SD)	4.3(0.9)	4.2(0.9)	4.3(1.0)	0.20
HDL cholesterol, mmol/L, mean (SD)	1.30(0.4)	1.29(0.4)	1.17(0.4)	0.08
Total/HDL cholesterol ratio, mean (SD)	3.5(1.1)	3.5(1.1)	3.9(1.2)	0.11
Hypercholesterolaemia, n (%)	634(89.9)	258(88.4)	47(100.0)	0.38
Cardiovascular Disease Variables				
Myocardial infarction and/or Angina, n (%)	213(30.2)	92(31.5)	21(44.7)	0.11
Stroke and/or TIA, n (%)	44(6.2)	39(13.4)	7(14.9)	**<0.001**
Intermittent caludication, n (%)	39(5.5)	19(6.5)	4(8.5)	0.35
Any CVD (MI, angina, stroke, TIA, or IC), n (%)	249(35.3)	112(38.4)	24(51.1)	**0.046**

† For categorical variables P values were calculated with the chi-square test, for quantitative and normally distributed variables, with ANOVA test and for quantitative and skewed variables with Jonckheere-Terpstra test.

5.3.6. Associations of retinal quantitative parameters with sociodemographic variables, vascular risk factors and cardiovascular disease

Tables 27 to 30 present the association of parameters of retinal vascular network geometry with sociodemographic and vascular variables, both before and after adjustment for age and sex.

Higher mean arteriolar diameter (CRAE) was significantly associated with lower social class (P for trend=0.003), insulin treatment use (P for trend=0.008), former or current smoking (highest in current smokers, P for trend=0.01) and prevalent coronary heart disease (MI and/or angina, P=0.002). Conversely, lower mean CRAE level was associated with the presence of hypertension (P=0.002).

Increased mean venular diameter (CRVE) was associated with higher HbA1c levels, waist-hip ratio, body mass index, and lifetime smoking (r ranged from 0.08 to 0.11, P<0.05), as well as with prevalent coronary artery disease (P=0.001). Mean CRVE was highest in current smokers (P for trend<0.001). Conversely, decreased CRVE was associated with increasing age (r=-0.09, P<0.01).

Trends towards higher mean CRAE and CRVE in subjects with cerebrovascular disease and peripheral arterial disease did not reach statistical significance, possibly because of a small number of these events.

Lower mean AVR (as a relative measure that combined information from both CRAE and CRVE) was associated with increased waist-hip ratio, lifetime smoking and average alcohol intake (r ranged from -0.07 to -0.10, p<0.05) as well as with the presence of hypertension (P=0.001). Mean AVR was lowest in current smokers (P for trend=0.006). Higher AVR was associated with increasing age (r=0.07, P<0.05) and higher education level (P for trend=0.03).

Decreased mean arteriolar branching coefficient was associated with higher plasma total cholesterol (r=-0.1, P<0.05). Raised mean of deviation level from

optimality of the arteriolar angle was associated with lower education level (P for trend=0.002), lower social class (P for trend=0.01), and prevalent peripheral arterial disease (P=0.01).

All the above associations remained largely unaffected by adjustment for age and sex, except for the associations between AVR, alcohol intake and education level, which both became attenuated and no longer statistically significant. There were no other significant associations between retinal width, arteriolar bifurcation parameters and risk factors.

Table 27: Pearson correlation coefficients between retinal vascular parameters and continuous vascular risk factors

	CRAE (μm)	CRVE (μm)	AVR	median BA (degrees)	median BC	Suboptimal BA[a]
Age (years)	-0.04	**-0.09**†	**0.07***	-0.03	0.03	-0.01
Duration of DM (years)√	0.01	-0.01	0.03	-0.06	-0.06	-0.03
HbA1c	0.05	**0.08***	-0.04	0.03	-0.05	0.01
WHR	0.01	**0.09**†	**-0.10**†	-0.03	0.05	-0.05
BMI (kg/m^2)	0.05	**0.08**†	-0.04	-0.02	0.02	0.001
Pack-years smoking √	0.04	**0.11**‡	**-0.09**†	-0.03	0.02	0.03
Average alcohol intake (drinks/week)√	-0.05	0.01	**-0.07***	-0.01	0.00	0.003
Total cholesterol (mmol/L)	-0.04	-0.02	-0.02	0.08	**-0.10***	-0.01
HDL cholesterol (mmol/L)	-0.02	-0.01	-0.01	0.003	-0.07	0.02
Total/HDL cholesterol ratio	-0.02	-0.01	-0.01	0.04	0.03	-0.02
Systolic blood pressure (mmHg)	-0.04	-0.05	0.02	0.02	-0.02	0.04
Diastolic blood pressure(mmHg)	-0.01	0.02	-0.03	-0.01	0.01	-0.04

[a] Transformed variable was used; CRAE: Central Retinal Arterial Equivalent; CRVE: Central Retinal Venular Equivalent; AVR: Arteriovenous ratio; BA: arteriolar branching angle; BC: arteriolar branching coefficient; Correlation coefficients in bold are statistically significant at $^{\ddagger}P\leq0.001$, $^{\dagger}P\leq0.01$, *$P<0.05$.

Table 28: Age-sex adjusted partial correlation coefficients between retinal vascular parameters and continuous vascular risk factors

	CRAE (µm)	CRVE (µm)	AVR	median BA (degrees)	median BC	Suboptimal BA[a]
Age (years)[b]	-0.03	**-0.09**†	**0.07***	-0.03	0.03	-0.01
Duration of DM (years)√	0.01	-0.01	0.03	-0.06	-0.06	-0.04
HbA1c	0.04	**0.07***	-0.04	0.03	-0.05	0.02
WHR	0.03	**0.09**†	**-0.07***	-0.03	0.05	-0.07
BMI (kg/m^2)	0.05	**0.08***	-0.04	-0.03	0.03	0.005
Pack-years smoking √	0.05	**0.11**‡	**-0.07***	-0.03	0.01	0.04
Average alcohol intake (drinks/week)√	-0.05	0.003	-0.05	-0.001	-0.01	0.005
Total cholesterol (mmol/L)	-0.05	-0.03	-0.02	0.08	**-0.10***	0.001
HDL cholesterol (mmol/L)	-0.03	-0.004	-0.03	0.00	-0.06	0.02
Total/HDL cholesterol ratio	-0.02	-0.02	0.001	0.04	0.02	-0.01
Systolic blood pressure (mmHg)	-0.04	-0.05	0.01	0.02	-0.02	0.04
Diastolic blood pressure(mmHg)	-0.01	0.00	-0.01	-0.01	0.02	-0.05

[a] Transformed variable was used; [b] For the age category, adjustments were made for gender only; CRAE: Central Retinal Arterial Equivalent; CRVE: Central Retinal Venular Equivalent; AVR: Arteriovenous ratio; BA: arteriolar branching angle; BC: arteriolar branching coefficient; Correlation coefficients in bold are statistically significant at ‡P≤0.001, †P≤0.01 *P<0.05.

Table 29: Crude mean retinal width and arteriolar bifurcation parameters according to categorical variables

	n	Central Retinal Arteriolar Equivalent (µm)		Central Retinal Venular Equivalent (µm)		Arteriole-to-Venule Ratio		n	Arteriolar Bifurcation Angles (degrees)		Arteriolar Branching Coefficient		Suboptimal Arteriolar Angles[a]	
		Mean (SD)	P value	Mean (SD)	P value	Mean (SD)	P value		Mean (SD)	P value	Mean(SD)	P value	Mean (SD)	P value
Education level														
University	149	168 (24)	0.56	230 (31)	0.29	0.73 (0.1)	**0.03**	73	78(9)	0.26	1.86(0.13)	0.36	0.74(0.4)	**0.002**
Other profession qualification	276	166 (25)		227 (35)		0.74 (0.1)		129	78(10)		1.85 (0.17)		0.83(0.3)	
Secondary & Primary school	508	168 (24)		227 (37)		0.75 (0.1)		242	79(11)		1.84 (0.15)		0.88(0.3)	
Social class														
I	225	166 (24)	**0.003**	228 (35)	0.07	0.74 (0.1)	0.32	110	79(9)	0.47	1.84(0.15)	0.28	0.77(0.3)	**0.01**
II	220	165 (25)		225 (37)		0.75 (0.1)		106	77(10)		1.84 (0.13)		0.79(0.4)	
III	285	166 (24)		227 (35)		0.74 (0.1)		123	81(11)		1.85 (0.17)		0.94(0.3)	
IV	108	172 (26)		230 (35)		0.75 (0.1)		45	76(10)		1.84 (0.18)		0.89(0.3)	
V	138	173 (24)		233 (36)		0.74 (0.1)		59	80(9)		1.87 (0.16)		0.85(0.3)	
Diabetes Treatment														
Diet	183	166 (25)	**0.008**	229 (34)	0.17	0.73 (0.10)	0.20	79	79(10)	0.50	1.85(0.14)	0.51	0.84(0.3)	0.56
Oral hypoglycaemics only	629	167 (25)		226 (37)		0.75 (0.10)		292	78(10)		1.85 (0.16)		0.84(0.3)	
Insulin ± oral hypoglycaemics	168	173 (23)		234 (32)		0.74 (0.09)		73	80(10)		1.84 (0.14)		0.87(0.4)	
Smoking status														
Nonsmoker	378	166 (24)	**0.01**	223 (34)	**<0.001**	0.75 (0.1)	**0.006**	172	78(10)	0.54	1.84(0.15)	0.70	0.83(0.3)	0.26
Former smoker	465	167 (25)		227 (35)		0.74 (0.1)		205	79(10)		1.84 (0.16)		0.85(0.4)	
Current smoker	137	174 (24)		242 (39)		0.73 (0.1)		67	79(10)		1.85 (0.16)		0.88(0.3)	
Alcohol intake (drinks/week)														
Never	282	169(24)	0.31	227(36)	0.10	0.75(0.10)	**0.023**	129	78(9.4)	0.91	1.85(0.14)	0.99	0.82(0.3)	0.95
≤7	441	168(24)		229(34)		0.74(0.10)		199	79(11)		1.83(0.16)		0.87(0.3)	
7-15	131	164(25)		223(38)		0.74(0.11)		61	77(10)		1.85(0.18)		0.82(0.3)	
>15	100	167(25)		234(34)		0.72(0.09)		46	78(11)		1.85(0.11)		0.82(0.4)	
Hypercholesterolemia status														
Yes	885	168 (25)	0.35	228(36)	0.55	0.74 (0.1)	0.87	398	78(10)	0.63	1.84 (0.16)	0.18	0.84(0.4)	0.91
No	95	165 (23)		226(36)		0.74 (0.1)		46	79(10)		1.87 (0.14)		0.84(0.3)	
Hypertension status														
Yes	829	167(24)	**0.006**	228(38)	0.95	0.74 (0.1)	**0.001**	371	79(9)	0.96	1.81(0.15)	0.07	0.84(0.4)	0.57

No	151	173 (23)		228(35)		0.77 (0.1)		73	78(11)		1.85 (0.16)		0.86(0.3)	
Coronary artery disease														
Yes	306	171 (24)	**0.002**	233(35)	**0.001**	0.74 (0.1)	0.51	122	78(10)	0.38	1.83(0.16)	0.40	0.84(0.3)	0.80
No	674	166 (24)		225(36)		0.74 (0.1)		322	79(10)		1.85(0.15)		0.85(0.3)	
Cerebrovascular disease														
Yes	79	168 (25)	0.97	229(38)	0.78	0.74 (0.1)	0.94	29	78(9)	0.78	1.85(0.18)	0.86	0.82(0.3)	0.61
No	901	167(25)		228(35)		0.74 (0.1)		415	79(10)		1.84 (0.15)		0.85(0.3)	
Peripheral arterial disease														
Yes	59	170 (23)	0.26	228(33)	0.65	0.75 (0.1)	0.56	26	78(14)	0.9	1.85(0.14)	0.82	1.0(0.4)	**0.02**
No	921	167(25)		228(36)		0.74 (0.1)		418	79(10)		1.84 (0.16)		0.83(0.3)	
Any CVD														
Yes	358	170 (24)	**0.008**	232(35)	**0.003**	0.74 (0.1)	0.49	142	79(10)	0.67	1.84(0.15)	0.47	0.85(0.3)	0.44
No	622	166 (25)		225(35)		0.75 (0.1)		302	78(10)		1.85(0.16)		0.84(0.3)	

[a] Transformed variable was used; CVD: cardiovascular disease

Table 30: Age-and sex- adjusted mean retinal width and arteriolar bifurcation parameters according to categorical variables

	n	Central Retinal Arteriolar Equivalent (µm)		Central Retinal Venular Equivalent (µm)		Arteriole-to-Venule Ratio		n	Arteriolar Bifurcation Angles (degrees)		Arteriolar Branching Coefficient		Suboptimal Arteriolar Angles	
		Mean	P value	Mean	P value	Mean	P value		Mean	P value	Mean	P value	Mean	P value
Education level														
University	149	168	0.55	231	0.40	0.73	0.051	73	78	0.23	1.86	0.33	0.74	**0.002**
Other profession qualification	276	166		227		0.74		129	78		1.85		0.83	
Secondary & Primary school	508	168		227		0.75		242	79		1.84		0.88	
Social class														
I	225	166	**0.005**	228	0.07	0.74	0.41	110	79	0.48	1.84	0.25	0.77	**0.01**
II	220	165		224		0.75		106	77		1.83		0.79	
III	285	166		227		0.74		123	80		1.85		0.94	
IV	108	172		231		0.75		45	76		1.85		0.89	
V	138	173		233		0.75		59	80		1.87		0.85	
Diabetes Treatment														
Diet	183	166	**0.009**	229	0.20	0.73	0.17	79	79	0.51	1.85	0.5	0.84	0.6
Oral hypoglycaemics only	629	167		226		0.75		292	78		1.85		0.84	
Insulin ± oral hypoglycaemics	168	173		234		0.74		73	80		1.84		0.87	
Smoking status														
Nonsmoker	378	166	**0.005**	223	**<0.001**	0.75	**0.024**	172	78	0.75	1.84	0.85	0.83	0.26
Former smoker	465	167		228		0.74		205	79		1.84		0.85	
Current smoker	137	174		241		0.73		67	79		1.85		0.88	
Alcohol intake (drinks/week)														
Never	282	169	0.17	227	0.93	0.75	0.13	129	78	0.99	1.85	0.81	0.81	0.92
≤7	441	168		229		0.74		199	79		1.83		0.87	
7-15	131	164		223		0.75		61	78		1.85		0.82	
>15	100	168		233		073		46	78		1.85		0.82	
Hypercholesterolemia status														
Yes	885	168	0.35	228	0.55	0.74	0.87	398	78	0.63	1.84	0.18	0.84	0.91
No	95	165		226		0.74		46	79		1.87		0.84	
Hypertension status														
Yes	829	167	**0.006**	228	0.95	0.74	**0.001**	371	79	0.96	1.81	0.07	0.84	0.57

No	151	173		228		0.77		73	78		1.85		0.86	
Coronary artery disease														
Yes	306	172	**0.001**	234	**<0.001**	0.74	0.51	122	78	0.38	1.83	0.40	0.84	0.80
No	674	166		225		0.74		322	79		1.85		0.85	
Cerebrovascular disease														
Yes	79	168	0.97	229	0.78	0.74	0.94	29	78	0.78	1.85	0.86	0.82	0.61
No	901	167		228		0.74		415	79		1.84		0.85	
Peripheral arterial disease														
Yes	59	170	0.26	228	0.65	0.75	0.56	26	78	0.9	1.85	0.82	1.0	**0.019**
No	921	167		228		0.74		418	79		1.84		0.83	
Any CVD														
Yes	358	171	**0.003**	233	**0.001**	0.74	0.49	142	79	0.67	1.84	0.47	0.85	0.44
No	622	166		225		0.75		302	78		1.85		0.84	

CVD: cardiovascular disease

5.4. Cognitive outcomes

This section describes the distribution and the interrelationships of the cognitive test results. Also, it presents their associations with sociodemographic characteristics, vascular risk factors and cardiovascular disease.

5.4.1. Distribution of cognitive test scores and correlations between different tests and depression

As an early step in the statistical analysis of the ET2DS data, the central tendency and distribution of each of the cognitive test scores was determined in the 1,044 subjects who had retinal data. Results from each of the cognitive tests and g were normally distributed, except for TMT which was normalised through a natural log (ln) transformation. As defined, the mean general cognitive factor score for the sample was 0.00 (SD±1.00) and valid data were available for 1,000 subjects. Mean scores for each of the individual tests are given in Table 31.

All of the cognitive tests demonstrated statistically significant and at least moderate, positive correlations between each other apart from TMT, for which the correlations were negative. TMT was measured as time spent to complete the task and therefore a lower score or shorter time indicated better performance. The depression score showed generally weaker correlations with the cognitive test variables. Of these, the strongest correlation was between depression and ‘g’ ($r=-0.19$, $P<0.001$).

Table 31: Mean (SD), cognitive test scores and Pearson correlation coefficients between cognitive test and depression (N=1,044)

			Correlation Coefficient								
Test	Mean (SD)	N	Mill-Hill	Verbal Fluency	Faces	Trail-Making Test B (ln)	Logical Memory	Matrix Reasoning	Digit Symbol	Letter-Number Sequencing	Depression Score[a]
Mill-Hill Vocabulary	31.0 (5.2)	1028		0.43‡	0.28‡	-0.37‡	0.39‡	0.45‡	0.38‡	0.40‡	-0.13‡
Verbal Fluency	36.9 (12.8)	1038			0.22‡	-0.40‡	0.25‡	0.36‡	0.41‡	0.46‡	-0.11‡
Faces	66 (7.8)	1037				-0.26‡	0.23‡	0.23‡	0.29‡	0.19‡	-0.06
Trail-Making Test B (ln)	4.7 (0.4)	1030					-0.28‡	-0.47‡	-0.63‡	-0.50‡	0.17‡
Logical Memory	25.4 (8.2)	1029						0.28‡	0.27‡	0.31‡	-0.09†
Matrix Reasoning	12.9 (5.3)	1030							0.39‡	0.40‡	-0.15‡
Digit Symbol	49.3 (14.8)	1035								0.40‡	-0.18‡
Letter-Number Sequencing	9.7 (2.8)	1026									-0.14‡
General factor ‘g’	0.0 (1.0)	1000	0.57‡	0.68‡	0.45‡	-0.80‡	0.53‡	0.67‡	0.76‡	0.71‡	-0.19‡

‡P≤0.001, †P≤0.01, *P<0.05; [a] The Hospital Depression Scale was logarithmically transformed on the n+1 scores (to avoid a logarithmic transformation of a score of zero) and the transformed variable was used here.

5.4.2. Associations of cognitive function with sociodemographic variables, vascular risk factors and cardiovascular disease

Tables 32-35 show the associations of cognitive test scores and depression with sociodemographic characteristics, vascular risk factors and cardiovascular disease, both before and after adjustment for age and sex (for categorical variables, only age- and sex-adjusted associations are shown). All the cognitive tests and the general intelligence factor (g) were significantly associated with age, with increasing age associated with poorer performance. Neither the Mill-Hill Vocabulary Scale (MHVS) nor the depression scale was correlated with age. Mean scores for the MHVS and MR were significantly higher in men than in women whereas for Faces, LMT, DST and depression, mean scores were higher in women than in men.

After adjustment for age and sex, lower mean 'g' scores were significantly associated with longer duration of diabetes, higher waist to hip ratio, body mass index, and lifetime smoking (r ranged from -0.17 to -0.09, $P<0.05$), as well as with insulin treatment use ($P=0.002$), prevalent coronary heart disease ($P<0.001$) and cerebrovascular disease ($P<0.001$) (Tables 36-38). Higher mean 'g' scores were associated with higher alcohol intake ($r=0.15$, $P<0.001$), HDL cholesterol level ($r=0.10$, $P<0.01$), higher education level ($P<0.001$) and higher social class ($P<0.001$). The individual tests contributing to the association between 'g' and these risk factors differed for each factor. For example, the inverse association of 'g' with duration of diabetes was mainly due to poorer individual test scores on VFT, Faces, DST, LNS (r ranged from -0.15 to -0.08, $P<0.001$) and TMTB ($r=0.12$, $P<0.001$); with waist to hip ratio, tests for VFT, Faces, MR, DST, LNS (r ranged from -0.15 to -0.09, $P<0.01$) and TMTB ($r=0.16$, $P<0.001$); and with cerebrovascular disease, tests for VFT, TMTB, MR, DST and LNS (all significant at $P<0.05$) (Table 33-35).

In addition to individual tests contributing to the associations between 'g' and risk factors, significant negative associations were found between VFT and HbA1c ($r=-0.07$, $P<0.05$), and MR and total/HDL cholesterol level ($r=-0.06$, $P<0.05$). Poorer individual test scores on TMTB ($P=0.01$), MR ($P=0.003$) and DST ($P<0.001$)

were associated with current smoking. Conversely, higher mean MR scores were associated with the presence of hypertension (P=0.005), possibly because of a high percentage of subjects on treatment for increased blood pressure.

Lower mean MHVS was associated with higher waist to hip ratio, body mass index and lifetime smoking (r ranged from -0.15 to -0.08, P<0.01), as well as with prevalent coronary heart disease (P<0.001). Higher scores on MHVS were associated with higher alcohol intake (r=0.14, P<0.001) and HDL cholesterol (r=0.08, P<0.001), and with higher education and social class levels (P<0.001 for both). Higher depression levels were associated with longer duration of diabetes, higher HbA1c, waist to hip ratio, body mass index, lifetime smoking and total/HDL cholesterol ratio (r ranged from 0.09 to 0.20, P<0.01), as well as with current smoking (P<0.001) and all prevalent cardiovascular diseases (i.e. coronary artery disease, cerebrovascular disease and peripheral arterial disease) (P<0.001). Lower depression levels were associated with higher HDL cholesterol (r=-0.13, P<0.001) and higher education and social class levels (P<0.001).

Neither systolic nor diastolic blood pressure was correlated with any of the cognitive measures or depression. There were no other significant associations between cognitive tests and risk factors.

Table 32: Pearson correlation coefficients between vascular risk factors, cognitive tests and depression

	Verbal Fluency	Faces	Trail Making (ln)	Logical Memory	Matrix Reasoning	Digit Symbol	Letter-Number Sequencing	g	Mill-Hill	Depression[a]
Age (years)	**-0.08**†	**-0.16**‡	**0.21**‡	**-0.09**†	**-0.17**‡	**-0.21**‡	**-0.16**‡	**-0.24**‡	0.002	-0.01
Duration of DM (years)√	**-0.10**‡	**-0.13**‡	**0.12**‡	-0.02	-0.02	**-0.15**‡	**-0.08**†	**-0.14**‡	-0.03	**0.08**†
HbA1c	**-0.07***	-0.02	0.02	0.01	-0.01	-0.02	-0.04	0.03	-0.06	**0.13**‡
WHR	**-0.08***	**-0.16**‡	**0.14**‡	**-0.08**†	-0.02	**-0.19**‡	-0.05	**-0.15**‡	**-0.09**†	**0.11**‡
BMI (kg/m^2)	-0.04	0.03	0.01	0.04	-0.06	-0.03	-0.001	0.03	**-0.09**†	**0.22**‡
Pack-years smoking √	-0.01	**-0.14**‡	**0.13**‡	-0.04	**-0.08**†	**-0.18**‡	-0.01	**-0.12**‡	**-0.07***	**0.12**‡
Average alcohol intake√ (drinks/week)	**0.13**‡	0.04	-0.04	**0.06***	**0.16**‡	0.04	**0.15**‡	**0.14**‡	**0.17**‡	**-0.09**†
Total cholesterol (mmol/L)	0.04	0.05	-0.04	0.02	0.03	**0.07***	0.03	**0.07***	0.03	0.002
HDL cholesterol (mmol/L)	**0.09**†	0.05	-0.03	0.05	0.04	**0.10**†	0.02	**0.09**†	**0.07***	**-0.10**
Total/HDL cholesterol ratio	-0.05	-0.01	0.00	-0.04	-0.03	-0.05	0.01	-0.04	-0.03	**0.10**†
Systolic blood pressure (mmHg)	-0.06	0.01	0.03	-0.00	-0.02	-0.02	-0.04	-0.04	-0.02	-0.03
Diastolic blood pressure(mmHg)	-0.02	0.01	0.00	-0.04	0.05	0.02	0.03	0.02	0.03	-0.05

Correlation coefficients in bold are statistically significant at $^{\ddagger}P\leq0.001$, $^{\dagger}P\leq0.01$, *$P<0.05$; [a] Transformed variable was used.

Table 33: Age-sex adjusted partial correlation coefficients between vascular risk factors, cognitive tests and depression

	Verbal Fluency	Faces	Trail Making (ln)	Logical Memory	Matrix Reasoning	Digit Symbol	Letter-Number Sequencing	g	Mill-Hill	Depression[a]
Age (years)[b]	**-0.08**†	**-0.15**‡	**0.21**‡	**-0.09**†	**-0.18**‡	**-0.21**‡	**-0.16**‡	**-0.24**‡	-0.001	-0.005
Duration of DM (years)√	**-0.11**‡	**-0.11**‡	**0.11**‡	-0.01	-0.01	**-0.13**‡	**-0.07***	**-0.12**‡	-0.03	**0.09**†
HbA1c	**-0.07***	-0.04	0.04	0.003	-0.01	-0.05	-0.05	-0.05	-0.06	**0.12**‡
WHR	**-0.10**‡	**-0.10**†	**0.16**‡	-0.05	**-0.11**‡	**-0.15**‡	**-0.09**†	**-0.17**‡	**-0.15** ‡	**0.18**‡
BMI (kg/m^2)	-0.06	0.04	**0.06***	0.001	**-0.07***	**-0.11**‡	-0.03	**-0.09**†	**-0.08**†	**0.20**‡
Pack-years smoking √	-0.02	**-0.11**‡	**0.13**‡	-0.02	**-0.14**‡	**-0.16**‡	-0.04	**-0.13**‡	**-0.10** †	**0.15**‡
Average alcohol intake√ (drinks/week)	**0.13**‡	**0.11**‡	-0.04	**0.11**‡	**0.11**‡	**0.09**†	**0.14**‡	**0.15**‡	**0.14** ‡	-0.06
Total cholesterol (mmol/L)	0.03	0.01	-0.02	-0.01	0.04	0.03	0.03	0.05	0.05	-0.02
HDL cholesterol (mmol/L)	**0.11**‡	0.01	-0.03	0.04	**0.09**†	**0.08***	0.04	**0.10**†	**0.08**†	**-0.13**‡
Total/HDL cholesterol ratio	-0.05	-0.01	0.02	-0.04	**-0.06***	-0.06	-0.02	-0.06	-0.04	**0.10**†
Systolic blood pressure (mmHg)	-0.05	0.02	0.02	0.01	-0.01	0.001	-0.03	-0.02	-0.02	-0.03
Diastolic blood pressure(mmHg)	-0.04	0.02	0.02	-0.04	0.004	0.02	-0.001	-0.01	0.01	-0.03

Correlation coefficients in bold are statistically significant at ‡P≤0.001, †P≤0.01, *P<0.05; [a] Transformed variable was used; [b] For the age category, adjustments were made for gender only.

Table 34: Age- and sex- adjusted mean (Standard Error) differences in cognitive test scores by sociodemographic characteristics and vascular risk factors

		Mill-Hill Vocabulary Scale		**Verbal Fluency**		**Faces**		**Trail Making** (ln)		**Logical Memory**		**Matrix Reasoning**		**Digit Symbol**		**Letter-Number Sequencing**		**General Factor (g)**		**Depression** (log)	
	n	Mean(SE)	P	Mean(SE)	P	Mean(SE)	P	Mean(SE)	P	Mean(SE)	P	Mean(SE)	P	Mean(SE)	P	Mean(SE)	P	Mean(SE)	P	Mean(SE)	P
Gender			**0.01**		0.56		**<0.001**		0.28		**0.002**		**<0.001**		**<0.001**		0.21		0.18		**0.001**
Male	535	31.4(0.2)		37.2(0.6)		64.6(0.3)		4.70(0.02)		24.6(0.3)		13.6(0.2)		47.1(0.6)		9.8(0.1)		-0.04(0.04)		0.58(0.01)	
Female	509	30.5(0.2)		36.7(0.6)		67.4(0.4)		4.67(0.02)		26.2(0.4)		12.1(0.2)		51.6(0.7)		9.6(0.1)		0.04(0.04)		0.63(0.01)	
Education level			**<0.001**		**<0.001**		**<0.001**		**<0.001**		**<0.001**		**<0.001**		**<0.001**		**<0.001**		**<0.001**		**<0.001**
University	169	35.1(0.4)		43.9(1.0)		67.7(0.6)		4.55(0.03)		28.2(0.6)		15.6(0.4)		55.8(1.1)		11.0(0.2)		0.62(0.08)		0.56(0.02)	
Other profession qualification	300	32.3(0.2)		37.6(0.7)		67.3(0.5)		4.64(0.02)		26.6(0.5)		13.9(0.3)		50.7(0.8)		10.1(0.1)		0.17(0.05)		0.57(0.02)	
Secondary & Primary school	575	29.1(0.2)		34.6(0.5)		64.8(0.3)		4.74(0.02)		23.9(0.3)		11.5(0.2)		46.8(0.6)		9.1(0.1)		-0.27(0.04)		0.64(0.01)	
Social class			**<0.001**		**<0.001**		**<0.001**		**<0.001**		**<0.001**		**<0.001**		**<0.001**		**<0.001**		**<0.001**		**<0.001**
I	242	34.1(0.3)		40.6(0.8)		67.9(0.5)		4.57(0.02)		27.6(0.5)		14.9(0.3)		53.8(0.9)		10.6(0.2)		0.44(0.06)		0.56(0.02)	
II	236	31.6(0.3)		38.8(0.8)		66.6(0.5)		4.62(0.03)		25.9(0.5)		13.9(0.3)		53.3(1.0)		10.1(0.2)		0.28(0.07)		0.57(0.02)	
III	302	30.5(0.3)		35.7(0.7)		65.6(0.4)		4.69(0.02)		25.1(0.8)		12.4(0.3)		47.1(0.8)		9.4(0.1)		-0.13(0.05)		0.64(0.02)	
IV	116	29.5(0.4)		33.4(1.1)		64.9(0.7)		4.79(0.04)		23.4(0.8)		11.4(0.5)		46.3(1.4)		9.2(0.2)		-0.37(0.09)		0.63(0.03)	
V	144	27.0(0.5)		33.3(1.1)		64.1(0.7)		4.85(0.03)		23.0(0.7)		9.9(0.4)		43.0(1.2)		8.5(0.2)		-0.57(0.08)		0.66(0.02)	
Diabetes Treatment			0.64		**0.004**		0.27		**0.01**		0.54		0.88		**0.002**		**0.047**		**0.002**		**<0.001**
Diet	196	31.2(0.4)		38.9(0.9)		66.4(0.6)		4.63(0.03)		25.3(0.5)		12.7(0.4)		51.6(1.0)		10.0(0.2)		0.13(0.07)		0.57(0.02)	
Oral hypoglycaemics only	666	30.9(0.2)		36.8(0.5)		65.9(0.3)		4.68(0.02)		25.5(0.3)		13.0(0.2)		49.3(0.6)		9.7(0.1)		0.01(0.04)		0.60(0.01)	
Insulin ± oral hypoglycaemics	182	30.9(0.4)		35.1(0.9)		65.8(0.6)		4.74(0.03)		24.9(0.6)		12.6(0.4)		47.2(1.1)		9.4(0.2)		-0.18(0.08)		0.68(0.02)	
Smoking status			0.19		0.54		0.76		**0.01**		0.35		**0.003**		**<0.001**		0.49		0.12		**<0.001**
Nonsmoker	408	31.0(0.3)		36.8(0.6)		66.4(0.4)		4.65(0.02)		25.4(0.4)		13.4(0.3)		51.4(0.7)		9.6(0.1)		0.07(0.05)		0.57(0.01)	
Former smoker	490	31.1(0.2)		36.6(0.6)		65.7(0.4)		4.69(0.02)		25.1(0.4)		12.6(0.2)		48.3(0.7)		9.6(0.1)		-0.04(0.05)		0.62(0.01)	
Current smoker	146	30.5(0.4)		38.4(1.1)		65.8(0.6)		4.75(0.04)		26.0(0.7)		12.0(0.4)		47.1(1.2)		10.1(0.2)		-0.05(0.08)		0.66(0.02)	
Hypercholesterolemia status			0.45		0.89		0.78		0.97		0.35		0.20		0.91		0.54		0.98		0.50
Yes	939	30.9(0.2)		36.9(0.4)		66.0(0.3)		4.68(0.1)		25.3(0.3)		12.9(0.2)		49.3(0.5)		9.7(0.1)		0.001(0.03)		0.61(0.01)	
No	105	31.3(0.5)		36.7(1.2)		66.2(0.7)		4.68(0.4)		26.1(0.8)		12.2(0.5)		49.5(1.4)		9.8(0.3)		0.003(0.10)		0.59(0.03)	
Hypertension status			0.69		0.64		0.31		0.10		0.47		**0.005**		0.86		0.76		0.37		0.80
Yes	886	31.0(0.2)		37.0(0.4)		66.1(0.2)		4.67(0.01)		25.4(0.3)		13.0(0.2)		49.4(0.5)		9.7(0.1)		0.01(0.03)		0.61(0.01)	
No	158	30.8(0.4)		36.4(1.0)		65.4(0.6)		4.73(0.03)		24.9(0.6)		11.8(0.4)		49.1(1.3)		9.7(0.2)		-0.07(0.08)		0.60(0.02)	

Table 35: Age- and sex- adjusted mean (Standard Error) differences in cognitive test scores according to cardiovascular disease status

	n	Mill-Hill Vocabulary Scale Mean(SE)	Verbal Fluency Mean(SE)	Faces Mean(SE)	Trail Making (ln) Mean(SE)	Logical Memory Mean(SE)	Matrix Reasoning Mean(SE)	Digit Symbol Mean(SE)	Letter-Number Sequencing Mean(SE)	General Factor (g) Mean(SE)	Depression (log) Mean(SE)
Coronary artery disease (MI and/or Angina)											
Yes	326	30.0(0.3)	36.6(0.7)	65.2(0.5)	4.73(0.02)	24.7(0.4)	12.0(0.3)	46.6(0.7)	9.4(0.2)	-0.19(0.06)	0.70(0.01)
No	718	31.4(0.2) ‡	37.0(0.5)	66.4(0.3) *	4.66(0.02) *	25.7(0.3)	13.2(0.2) ‡	50.6(0.6) ‡	9.8(0.1)	0.09(0.04) ‡	0.57(0.01) ‡
No CVD[a]	659	31.5(0.2) ‡	37.5(0.5)	66.5(0.3) *	4.64(0.02) †	25.7(0.3)	13.3(0.2) ‡	51.0(0.6) ‡	9.9(0.1) *	0.11(0.04) ‡	0.56(0.01) ‡
Cerebrovascular disease (Stroke and/or TIA)											
Yes	90	30.0(0.6)	34.1(1.4)	65.1(0.8)	4.84(0.05)	24.1(0.8)	11.8(0.5)	43.5(1.5)	8.8(0.3)	-0.45(0.12)	0.70(0.03)
No	954	31.0(0.2)	37.2(0.4) *	66.1(0.3)	4.67(0.01) ‡	25.5(0.3)	12.9(0.2) *	49.9(0.5) ‡	9.8(0.1) †	0.04(0.03) ‡	0.60(0.01) ‡
No CVD[a]	659	31.5(0.2) *	37.5(0.5) *	66.6(0.3)	4.65(0.02) ‡	25.9(0.3)	13.4(0.2) †	51.4(0.6) ‡	9.9(0.1) ‡	0.14(0.04) ‡	0.56(0.01) ‡
Peripheral arterial disease (IC)											
Yes	62	30.9(0.6)	36.7(2.0)	65.4(1.0)	4.72(0.05)	25.6(1.0)	12.3(0.6)	48.4(1.6)	9.5(0.4)	-0.12(0.1)	0.74(0.03)
No	982	31.0(0.2)	37.0(0.4)	66.1(0.3)	4.68(0.01)	25.4(0.3)	12.9(0.2)	49.4(0.5)	9.7(0.1)	0.01(0.03)	0.60(0.01) ‡
No CVD[a]	659	31.5(0.2)	37.5(0.5)	66.6(0.3)	4.64(0.02)	25.8(0.3)	13.4(0.2)	51.2(0.6)	9.9(0.1)	0.14(0.04)	0.56(0.01) ‡
Any CVD											
Yes	385	30.1(0.3)	36.0(0.7)	65.1(0.4)	4.74(0.02)	24.7(0.4)	12.0(0.3)	46.6(0.7)	9.4(0.1)	-0.21(0.05)	0.68(0.01)
No CVD[a]	659	31.5(0.2) ‡	37.5(0.5)	66.5(0.3) †	4.65(0.02) ‡	25.7(0.3)	13.3(0.2) ‡	51.0(0.6) ‡	9.9(0.1) †	0.13(0.04) ‡	0.56(0.01) ‡

MI: myocardial infarction; IC: intermittent claudication; CVD: cardiovascular disease; TIA: Transient Ischaemic Attack [a] No CVD: No MI, Angina, stroke, TIA or IC; P values for difference between individual CVD group with the group free of any CVD (no CVD) or with the group free of specific CVD, which were significant at ‡≤ 0.001 level, †≤ 0.01 level, and * < 0.05 level

5.5. Diabetic retinopathy and cognitive function

This section describes the associations between diabetic retinopathy and cognitive function, measured as current cognitive ability and estimated lifetime cognitive decline. Univariate analysis for the cognitive ability and diabetic retinopathy associations is presented first, followed by the multivariate analysis for associations between estimated cognitive decline and diabetic retinopathy. Further, associations between any non-general factor cognitive elements (using the general factor-adjusted standardised residuals as outcome) and diabetic retinopathy are shown. Finally, sensitivity analyses by excluding subjects with cerebrovascular disease or severe cognitive impairment are performed to examine further the associations between cognitive decline and diabetic retinopathy.

5.5.1. Associations of diabetic retinopathy with current cognitive ability

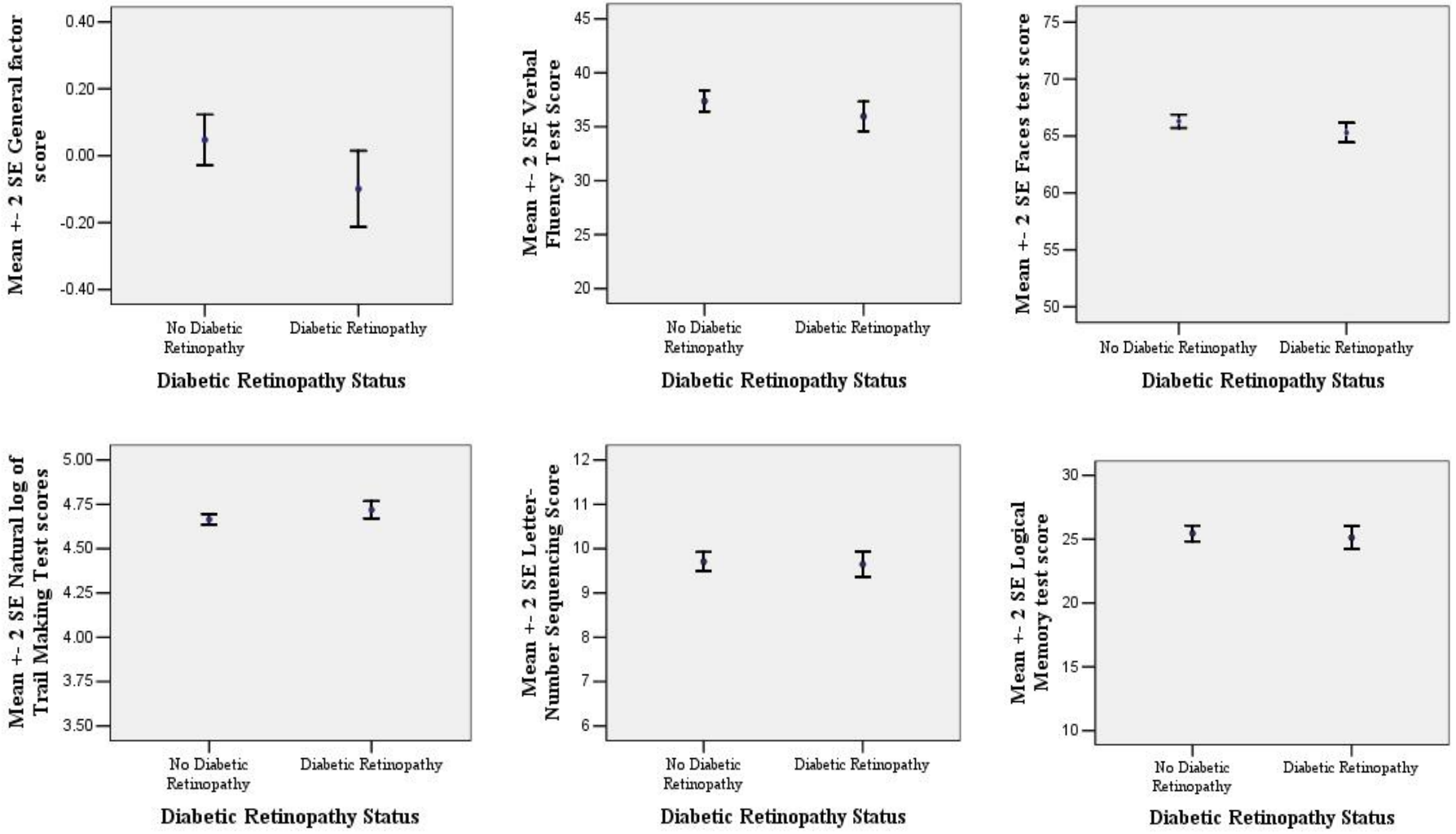
Figure 32 presents crude mean cognitive test scores and depression scores in subjects with and without diabetic retinopathy. Subjects with diabetic retinopathy had significantly lower mean scores on ‘g’ (P=0.03) and the Digit Symbol Test (P=0.001). Subjects with diabetic retinopathy also had poorer test scores for VFT, Faces and TMTB, but differences were not statistically significant. There was no significant difference in mean MHVS, LM, MR LNS or depression scores. When evaluated separately by sex (Table 36), the differences in g, DST and VFT scores were each found to be significant only in males (P=0.036, 0.005, and 0.02 respectively). In each case, men with diabetic retinopathy had decreased ability.

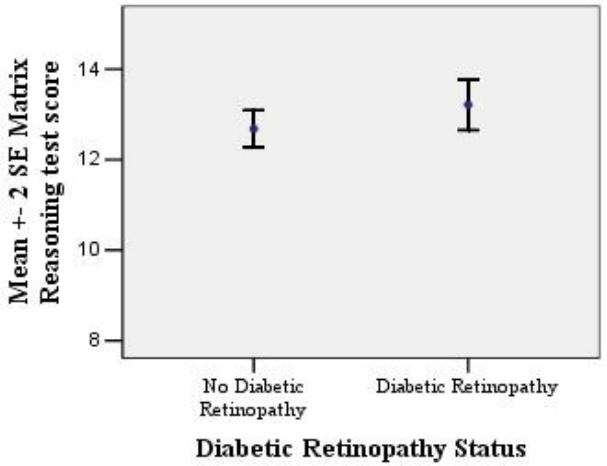
When diabetic retinopathy was categorised into mild retinopathy and moderate to severe diabetic retinopathy, there was a significant trend across worsening severity of diabetic retinopathy for ‘g’ (P=0.005), Faces (P=0.02), TMTB (P=0.005) and DST (P<0.001), with the moderate to severe diabetic retinopathy group having poorest cognitive test performance (Figure 33). The trend also approached statistical significance for the VFT (P=0.05). When evaluated separately by sex (Table 36), a significant trend was found only in men for ‘g’ (P=0.008), VFT (P=0.008) and

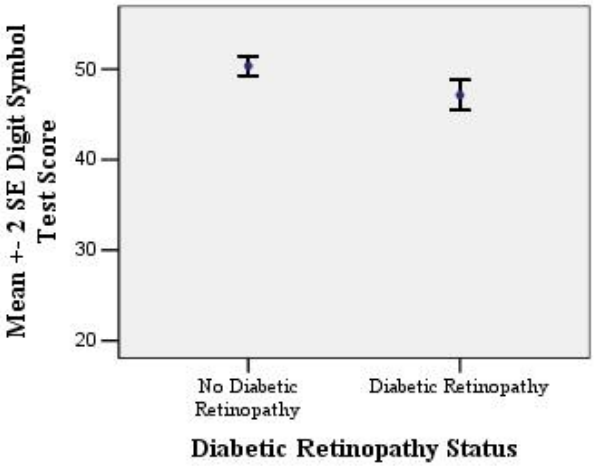
TMTB (P=0.02) scores, whereas the trend for DST remained significant in both men and women. A trend in mean Faces in males also approached statistical significance (P=0.08).

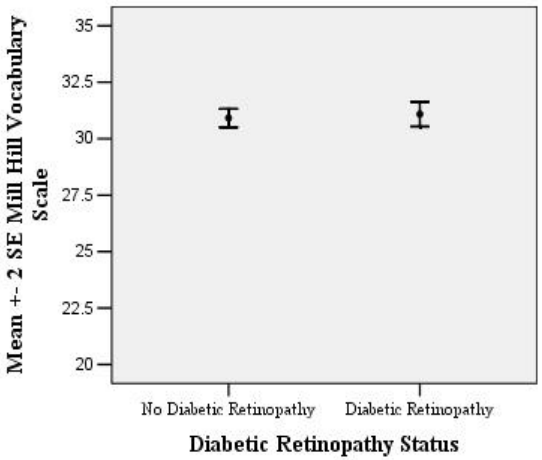
After adjustment for age and sex, the associations of diabetic retinopathy with current cognitive ability were essentially unchanged except for the VFT, in which a trend with increasing severity of diabetic retinopathy became significant (P=0.04) (Table 37).

Figure 32: Crude mean (±2SE) of cognitive test performances according to presence of diabetic retinopathy




Mean +- 2 SE Matrix Reasoning test score
14
12
10
8
No Diabetic Retinopathy
Diabetic Retinopathy
Diabetic Retinopathy Status


Mean +- 2 SE Digit Symbol Test Score
50
40
30
20
No Diabetic Retinopathy
Diabetic Retinopathy
Diabetic Retinopathy Status


Mean +- 2 SE Mill Hill Vocabulary Scale
35
32.5
30
27.5
25
22.5
20
No Diabetic Retinopathy
Diabetic Retinopathy
Diabetic Retinopathy Status

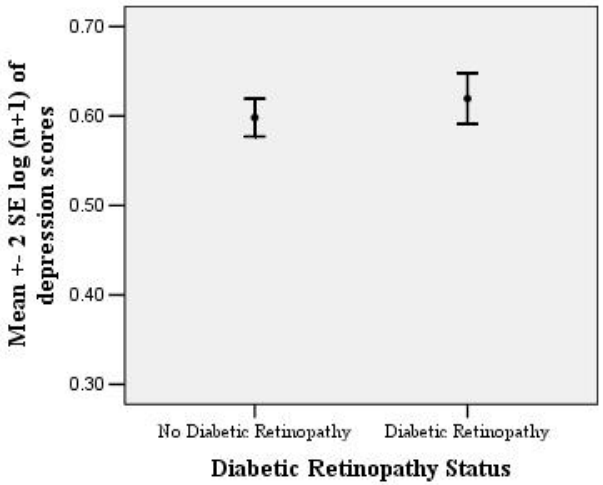
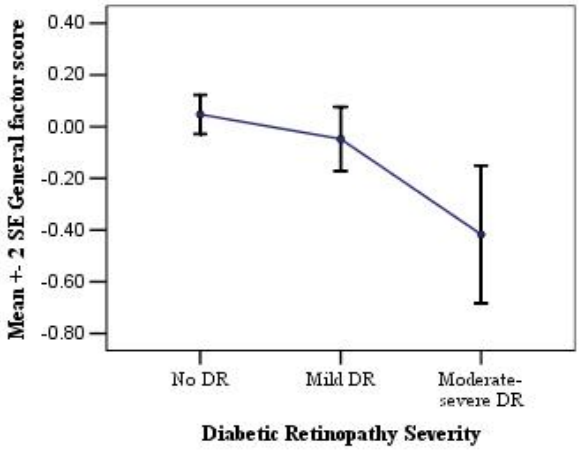
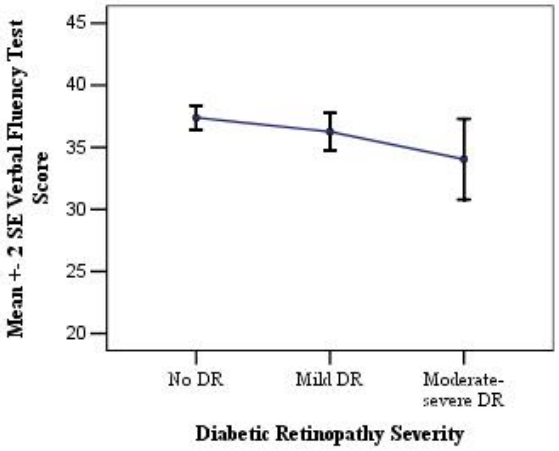

Mean +- 2 SE log (n+1) of depression scores
0.70
0.60
0.50
0.40
0.30
No Diabetic Retinopathy
Diabetic Retinopathy
Diabetic Retinopathy Status

Figure 33: Crude Mean (±2SE) Cognitive Test performances by severity of diabetic retinopathy.



P for trend=0.005



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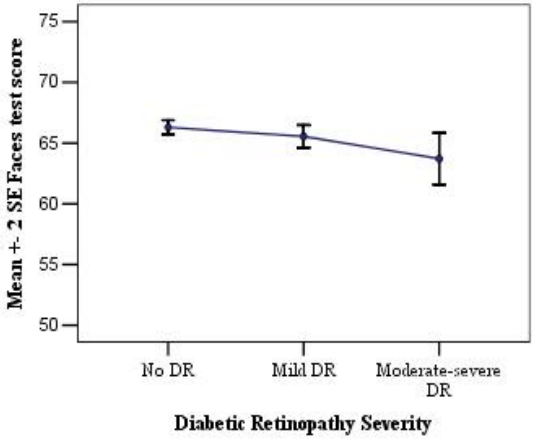

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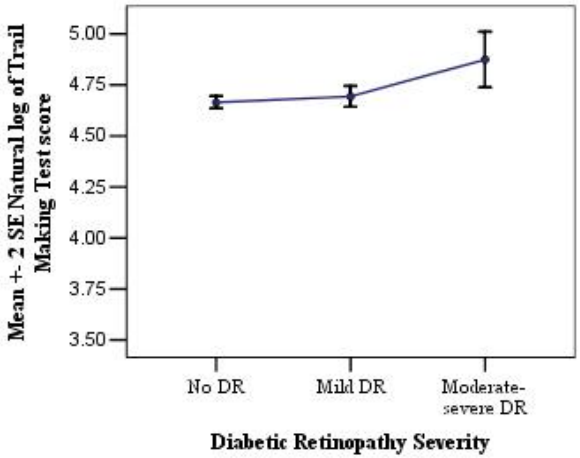

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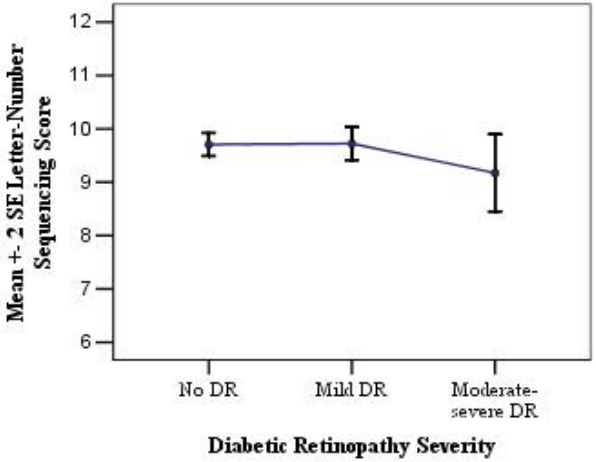
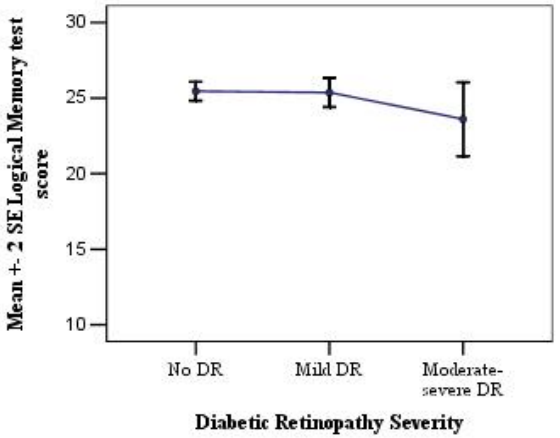


P for trend=0.46



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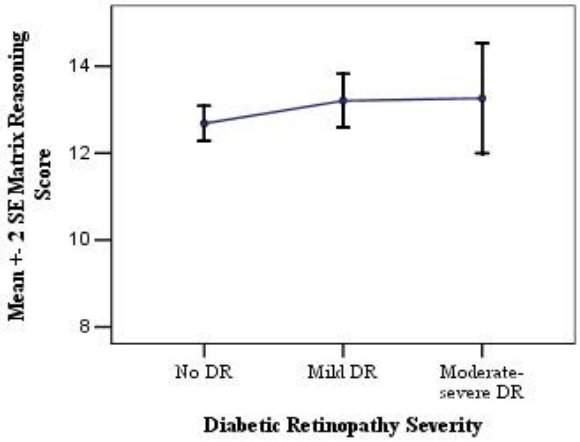
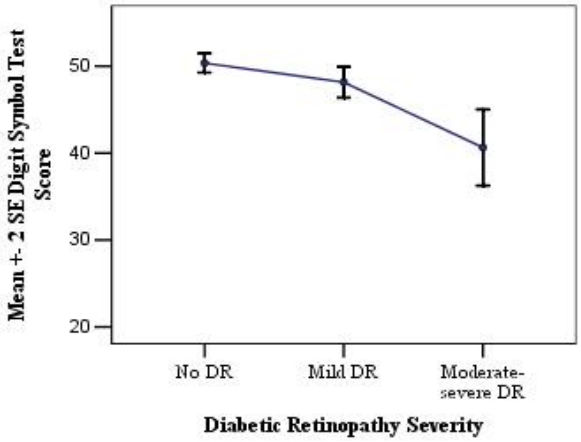
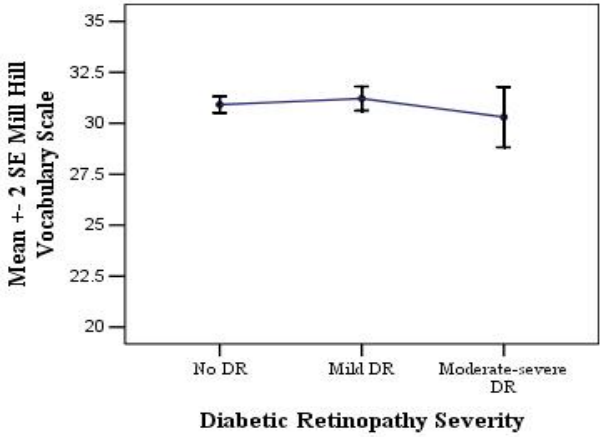


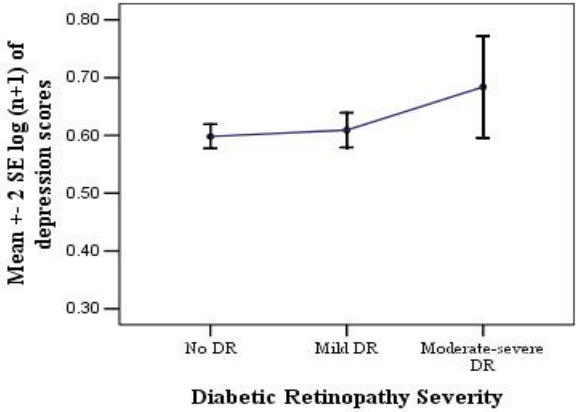
P for trend=0.15



P for trend<0.001



P for trend=0.93



P for trend=0.09

Table 36: Crude mean (95%CI) of cognitive test scores by diabetic retinopathy for men and women separately

		Presence of Diabetic Retinopathy (DR)			Severity of Diabetic Retinopathy (DR)			
		DR Absent	**DR Present**	**P value**	**No DR**	**Mild DR**	**Moderate-Severe DR**	**P value for trend**
Mill-Hill Test	Male	31.1(30.5, 31.7)	31.9(31.1, 32.6)	0.52	31.1(30.5, 31.7)	32.0(31.2, 32.7)	31.3(29.1, 33.5)	0.23
	Female	30.7(30.2, 31.3)	30.1(29.3, 30.9)	0.23	30.7(30.2, 31.3)	30.3(29.4, 31.2)	29.1(27.1, 31.0)	0.14
Verbal Fluency	Male	38.0(36.7, 39.4)	35.4(33.5, 37.2)	**0.02**	38.0(36.7, 39.4)	35.9(33.9, 38.0)	31.9(27.8, 36.1)	**0.008**
	Female	36.8(35.4, 38.1)	36.7(34.7, 38.7)	0.94	36.8(35.4, 38.1)	36.7(34.5, 38.9)	36.8(31.4, 42.2)	0.96
Faces	Male	65.0(64.2, 65.8)	63.8(62.8, 64.9)	0.10	65.0(64.2, 65.8)	64.0(62.9, 65.1)	63.0(60.0, 66.0)	0.08
	Female	67.6(66.8, 68.4)	67.1(65.8, 68.5)	0.53	67.6(66.8, 68.4)	67.5(66.0, 69.0)	64.7(61.5, 67.9)	0.28
Trail Making Test (ln)	Male	4.68(4.64, 4.72)	4.74(4.68, 4.80)	0.11	4.68(4.64, 4.72)	4.71(4.64, 4.78)	4.91(4.74, 5.08)	**0.02**
	Female	4.65(4.61, 4.69)	4.69(4.62, 4.77)	0.30	4.65(4.61, 4.69)	4.67(4.59, 4.75)	4.83(4.59, 5.07)	0.13
Logical Memory	Male	24.8(23.9, 25.6)	24.2(23.0, 25.3)	0.42	24.8(23.9, 25.6)	24.2(23.0, 25.5)	24.0(21.1, 26.9)	0.42
	Female	26.1(25.3, 27.0)	26.3(24.9, 27.7)	0.79	26.1(25.3, 27.0)	26.9(25.4, 28.3)	23.1(18.6, 27.6)	0.71
Matrix Reasoning	Male	13.4(12.9, 14.0)	13.7(13.0, 14.4)	0.54	13.4(12.9-14.0)	13.9(13.1-14.7)	12.6(11.1-14.1)	0.88
	Female	12.0(11.4, 12.5)	12.6(11.7, 13.4)	0.25	12.0(11.4, 12.5)	12.3(11.4, 13.3)	14.2(11.9, 16.4)	0.12
Digit Symbol	Male	48.2(46.7, 49.7)	44.6(42.6, 46.6)	**0.005**	48.2(46.7, 49.7)	45.6(43.5, 47.8)	38.1(32.7, 43.4)	**<0.001**
	Female	52.4(50.9, 53.9)	50.3(47.6, 52.9)	0.14	52.4(50.9, 53.9)	51.3(48.4, 54.1)	43.9(36.2, 51.5)	**0.038**
Letter-Number Sequencing	Male	9.8(9.5. 10.1)	9.7(9.3, 10.1)	0.81	9.8(9.5, 10.1)	9.8(9.4, 10.3)	9.2(8.1, 10.3)	0.56
	Female	9.6(9.3, 9.9)	9.5(9.2, 9.9)	0.75	9.6(9.3, 9.9)	9.6(9.2, 10.0)	9.2(8.2, 10.1)	0.59

General factor score	Male	0.01(-0.09, 0.12)	-0.17(-0.32,-0.0)	**0.036**	0.01(-0.09, 0.12)	-0.12(-0.28, 0.04)	-0.53(-0.87, -0.18)	**0.008**
	Female	0.08(-0.03, 0.18)	-0.005(-0.18,0.1)	0.42	0.08(-0.03, 0.18)	0.04(-0.15, 0.23)	-0.28(-0.73, 0.18)	0.23

Table 37: Age-sex adjusted mean (95%CI) of cognitive test scores and depression by presence and severity of diabetic retinopathy

	Presence of Diabetic Retinopathy (DR)			**Severity of Diabetic Retinopathy (DR)**			
	DR Absent (N=705)	**DR Present (N=339)**	**P value**	**No DR (N=705)**	**Mild DR (N=292)**	**Moderate-Severe DR (N=47)**	**P value for trend**
Mill-Hill Test	30.9(30.5, 31.3)	31.0(30.5, 31.6)	0.73	30.9(30.5, 31.3)	31.2(30.6, 31.8)	30.2 (28.7, 31.8)	0.95
Verbal Fluency	37.4(36.4, 38.3)	35.9(34.6, 37.3)	0.085	37.4(36.4, 38.3)	36.3(34.8, 37.7)	33.9(30.2, 37.6)	**0.044**
Faces	66.3(65.7, 66.8)	65.5(64.6, 66.3)	0.10	66.3(65.7, 66.8)	65.7(64.8, 66.6)	63.8(61.6, 66.0)	**0.036**
Trail Making Test (ln)	4.67(4.64, 4.70)	4.72(4.67, 4.76)	0.05	4.67(4.64, 4.70)	4.69(4.64, 4.74)	4.88(4.76, 5.00) ‡	**0.004**
Logical Memory	25.4(24.8, 26.0)	25.2(24.3, 26.1)	0.69	25.4(24.8, 26.0)	25.5(24.5, 26.4)	23.6(21.3, 26.0)	0.39
Matrix Reasoning	12.7(12.3, 13.1)	13.1(12.6, 13.7)	0.21	12.7(12.3, 13.1)	13.1(12.5-13.7)	13.1(11.6-14.6)	0.25
Digit Symbol	50.3(49.3, 51.4)	47.4(45.8, 48.9)	**0.002**	50.4(49.3, 51.4)	48.4(46.8-50.0)	40.5(36.4-44.6) ‡	**<0.001**
Letter-Number Sequencing	9.7(9.5, 9.9)	9.6(9.3, 9.9)	0.68	9.7(9.5, 9.9)	9.7(9.4, 10.3)	9.1(8.3-9.9)	0.39
General factor score	0.05(-0.03, 0.12)	-0.10(-0.20, 0.01)	**0.03**	0.05(-0.03, 0.12)	-0.04(-0.17, 0.07)	-0.44(-0.73, -0.16)	**0.003**
HAD-Depression (log (n+1))	0.60(0.58-0.62)	0.62(0.59-0.65)	0.17	0.60(0.58, 0.62)	0.61(0.58, 0.64)	0.69(0.61, 0.77)	0.06

‡P≤0.001, †P≤0.01, *P<0.05 ANCOVA test of pair-wise difference in a given mean from the mean of the group without diabetic retinopathy

5.5.2. Associations of diabetic retinopathy with estimated lifetime cognitive decline

In a regression model containing age and sex, further adjustment for peak prior cognitive ability as estimated by performance on the Mill-Hill Vocabulary Scale (MHVS) allowed for the investigation of the relationship between diabetic retinopathy and the imputed decline from best-ever level of cognitive function, not just cognitive function at one time point (i.e. estimated lifetime cognitive decline). Table 38 shows the association between severity of diabetic retinopathy and estimated lifetime cognitive decline after adjustment for age, sex and the MHVS.

For five of the cognitive measures, including 'g', there was a statistically significant trend across diabetic retinopathy severity categories, with poorer performance in subjects with diabetic retinopathy, suggesting that severity of diabetic retinopathy was associated with estimated lifetime cognitive decline. The general intelligence score was significantly lower in the group with moderate-severe diabetic retinopathy (mean -0.36, 95%CI -0.60, -0.13) compared with the group without diabetic retinopathy (mean 0.05, 95%CI -0.01, 0.10, $P \leq 0.01$), with an intermediate score for the mild diabetic retinopathy group (mean -0.05, 95%CI -0.14, 0.04) (one-way ANOVA, P for linear trend=0.001).This trend in g across worsening diabetic retinopathy severity was mainly due to similar trends in the individual Verbal Fluency (P=0.022), Faces (P=0.024), Trail Making Test B (P=0.002) and Digit Symbol (P<0.001) tests. In particular, subjects with moderate-severe diabetic retinopathy had an adjusted mean score on the Digit Symbol test which was 9.2 points (0.7 SD)(P<0.01) (Cohen, 1988) lower than in subjects without retinopathy (Table 38) (mean scores 41.3, 95%CI 37.5, 45.0 and 50.5, 95%CI 49.5, 51.4 respectively).

There was no statistically significant trend with increasing severity of diabetic retinopathy on adjusted mean scores for the Logical Memory, Matrix Reasoning and Letter-Number Sequencing tests. Nor was there any evidence of gender by diabetic retinopathy interaction for these three individual cognitive tests. Therefore, no further analyses were carried out on these data.

Multivariate associations in the total sample

In addition to age, sex and peak prior cognitive ability, a series of multiple linear regression analyses were carried out to determine the importance of severity of diabetic retinopathy as an independent explanatory factor of cognitive test performance (Table 38-39). Education level was entered first into the model since it is considered as an additional marker of prior cognitive ability (together with vocabulary tasks, such as the MHVS). Cardiovascular risk factors and the presence of cardiovascular disease were then added in the second and third steps, since these vascular variables were either associated with retinal parameters in univariate associations or with both retinal variables and cognitive test performance in previous literature. Depression scores were entered in the fourth step since this mood variable was associated with both cognitive dysfunction and cerebral microvascular disease. In the final step, diabetes-related factors including HbA1c and duration of diabetes were added to determine whether the retinopathy-cognition association was independent of hyperglycaemia, which is considered as a causal factor in the development of diabetic retinopathy.

For all five cognitive measures, adjustment for education level in addition to age, sex and the MHVS, did not greatly alter the associations between diabetic retinopathy and estimated cognitive change. The addition of the five cardiovascular risk factors (smoking status, alcohol intake, waist to hip ratio, systolic blood pressure and total cholesterol level) in the second step led to a general attenuation in the associations, with the regression coefficients for the Verbal Fluency and Faces tests becoming non-significant. Additional adjustment for the presence of cardiovascular disease (coronary heart disease and cerebrovascular disease) resulted in further attenuation of the associations, but the associations of diabetic retinopathy with ‘g’, Trail Making and Digit Symbol Test remained statistically significant. Including the depression scores made very little difference to the associations. Finally, following the addition of HbA1c and duration of diabetes to the models, only the Digit Symbol Test remained statistically significantly associated with severity of diabetic retinopathy.

When the presence of diabetic retinopathy (absent vs. present) was fitted in the models instead of severity of diabetic retinopathy, the results were almost the same (Table 40). The Digit Symbol Test remained statistically associated with diabetic retinopathy but the association was less significant (P changed from <0.001 to <0.05).

Table 41 presents a summary of the final regression models. In the aforementioned final models, the significance of interaction terms between all main effects (explanatory variables) was tested. There was no evidence of an interaction between these factors in relation to cognitive performance on the Digit Symbol, Faces, and Trail-Making tests, and therefore none of them were included in the final models. However, both 'g' and the Verbal Fluency test had a significant interaction between sex and severity of diabetic retinopathy (P=0.006 for g, P=0.004 for VFT), which accounted for 0.8% to 0.9% of variation in the mean scores. This indicated that the association (magnitude or direction) between diabetic retinopathy and estimated lifetime cognitive decline in g and the VFT may differ for males and females in the population sample. Therefore the results for g and the VFT were shown separately for men and women. The final linear regression models are presented in a complete form in the attached Appendix I (Tables i1 to i5).

For the Digit Symbol test, the following contributed independently to the variance in the outcome in the final model: severity of diabetic retinopathy (B=-2.44/standardised beta=-0.09, P<0.01), age (B=-0.72/standardised beta=-0.21, P<0.001), male sex (B=-4.22/standardised beta=-0.14, P<0.001), MHVS (B=0.94/standardised beta=0.32, P<0.001), smoking status (B=-2.62/standardised beta=-0.09, P<0.01), waist to hip ratio (B=-1.31/standardised beta=-0.07, P<0.05), presence of stroke and/or TIA (B=-3.19/standardised beta=-0.06, P<0.05), depression (B=-6.19/standardised beta=-0.11, P<0.001) and duration of diabetes (B=-0.92/standardised beta=-0.06, P<0.05) (Table 41). Severity of diabetic retinopathy explained close to 1% of the variance in DST scores over and above the effect of all other factors and this final model accounted for over 26% of the total variance. When lifetime smoking was fitted in the model instead of smoking status,

the results were almost the same and the association between diabetic retinopathy and the DST remained significant (B decreased from -2.44 to -2.58, $P<0.05$). The adjusted mean (95%CI) DST scores in the final model according to severity of diabetic retinopathy are shown graphically on Figure 34.

Table 38: Mean differences (95%CI) in cognitive test scores by severity of diabetic retinopathy, adjusted for age, gender and peak prior cognitive ability (MHVS)

Cognitive Measure	No Diabetic Retinopathy (n=705)	Mild Diabetic Retinopathy (n=339)	Moderate-severe Diabetic Retinopathy (n=47)	P for trend
Verbal Fluency test	37.5(36.7, 38.4)	36.0(34.7, 37.4)	34.7(31.3, 38.0)	**0.022**
Faces test	66.3(65.8, 66.9)	65.6(64.8, 66.5)	64.1(62.0, 66.2)	**0.024**
Trail Making Test (ln)	4.66(4.63, 4.69)	4.69(4.65, 4.74)	4.86(4.75, 4.97) ‡	**0.002**
Logical Memory test	25.5(24.9, 26.0)	25.3(24.4, 26.1)	24.1(21.9, 26.3)	0.32
Matrix Reasoning test	12.7(12.4, 13.1)	13.1(12.5, 13.6)	13.4(12.1, 14.7)	0.16
Digit Symbol Test	50.5(49.5, 51.4)	48.3(46.8, 49.8) *	41.3(37.5, 45.0) †	**<0.001**
Letter-Number Sequencing test	9.7(9.5, 9.9)	9.7(9.4, 10.0)	9.3(8.5, 10.0)	0.38
General factor score	0.05(-0.01, 0.10)	-0.05(-0.14, 0.04)	-0.36(-0.59, -0.13) †	**0.001**

‡P≤0.001, †P≤0.01, *P<0.05 ANCOVA test of pair-wise difference in a given mean from the mean of the group without diabetic retinopathy

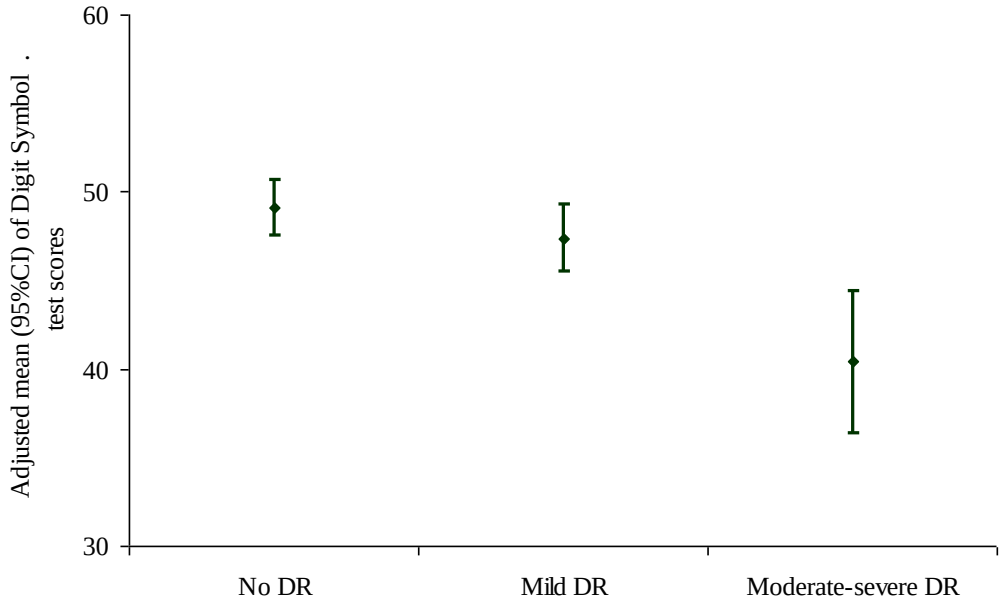


Figure 34: Multivariate adjusted mean (95%CI) Digit Symbol test scores according to severity of diabetic retinopathy

Table 39: Multivariate associations between severity of diabetic retinopathy and late-life cognition, and estimated lifetime cognitive decline

Severity of Diabetic retinopathy	General Factor (g)	Verbal Fluency	Faces	Trail Making Test B	Digit Symbol
		Unstandardised Beta Coefficient (Standard Error) (95%CI)			
Model I					
Age and sex adjusted	-0.16 (0.05) [†] (-0.26, -0.05)	-1.46 (0.70) * (-2.84, -0.09)	-0.92 (0.42) * (-1.74, -0.10)	0.07 (0.02) [†] (0.02, 0.11)	-3.35 (0.78) [‡] (-4.88, -1.82)
Model II					
Age, sex and MHVS adjusted	-0.14 (0.04) [‡] (-0.23, -0.06)	-1.45 (0.63) * (-2.69, -0.22)	-0.91 (0.40) * (-1.69, -0.12)	0.06 (0.02) [†] (0.02, 0.10)	-3.27 (0.71) [‡] (-4.67, -1.88)
Model III					
Age, sex, MHVS and all other variables adjusted					
Step 1					
+ Education level	-0.14 (0.04) [‡] (-0.23, -0.05)	-1.42 (0.64) * (-2.69, -0.16)	-0.82 (0.41) * (-1.62, -0.01)	0.06 (0.02) [†] (0.02, 0.10)	-3.39 (0.73) [‡] (-4.82, -1.95)
Step 2					
+ Vascular risk factors	-0.13 (0.04) [†] (-0.21, -0.04)	-1.24 (0.65) (-2.51, 0.04)	-0.70 (0.42) (-1.52, 0.11)	0.06 (0.02) [†] (0.02, 0.10)	-3.33 (0.73) [‡] (-4.77, -1.90)
Step 3					
+ Cardiovascular disease	-0.11 (0.04) * (-0.20, -0.02)	-1.14 (0.65) (-2.42, 0.14)	-0.69 (0.42) (-1.51, 0.13)	0.05 (0.02) * (0.01, 0.09)	-3.11 (0.73) [‡] (-4.55, -1.68)
Step 4					
+ Depression	-0.11 (0.04) * (-0.19, -0.02)	-1.11 (0.65) (-2.39, 0.17)	-0.68 (0.42) (-1.49, 0.14)	0.05 (0.02) * (0.01, 0.09)	-3.06 (0.73) [‡] (-4.48, -1.63)
Step 5					
+ HbA1c and duration of diabetes	-0.06 (0.05) (-0.15, 0.04)	-0.23 (0.72) (-1.64, 1.18)	-0.20 (0.46) (-1.11, 0.71)	0.03 (0.02) (-0.02, 0.08)	-2.44 (0.80) [†] (-4.01, -0.86)

[‡]P≤0.001, [†]P≤0.01, *P<0.05

Table 40: Multivariate associations between presence of diabetic retinopathy and late-life cognition, and estimated lifetime cognitive decline

Severity of Diabetic retinopathy	General Factor (g)	Verbal Fluency	Faces	Trail Making Test B	Digit Symbol
	Unstandardised Beta Coefficient (Standard Error)				
Model I					
Age and sex adjusted	-0.14 (0.07) * (-0.27, -0.01)	-1.52 (0.85) (-3.20, 0.15)	-0.89 (0.51) (-1.89, 0.11)	0.06 (0.03)* (0.003, 0.11)	-3.10 (0.95) ‡ (-4.96, -1.24)
Model II					
Age, sex and MHVS adjusted	-0.14 (0.05) * (-0.24, -0.03)	-1.67 (0.77) * (-3.17, -0.16)	-0.93 (0.49) (-1.89, 0.02)	0.06 (0.03)* (0.01, 0.10)	-3.15 (0.86) ‡ (-4.84, -1.45)
Model III					
Age, sex, MHVS and all other variables adjusted					
Step 1					
+ Education level	-0.14 (0.05) † (-0.25, -0.04)	-1.70 (0.78) * (-3.23, -0.17)	-0.86 (0.50) (-1.84, 0.12)	0.05 (0.03) * (0.002, 0.10)	-3.44 (0.88) ‡ (-5.71, -1.71)
Step 2					
+ Vascular risk factors	-0.12 (0.05) * (-0.23. -0.02)	-1.47 (0.78) (-3.01, 0.07)	-0.73 (0.50) (-1.71, 0.26)	0.05 (0.03) (-0.001, 0.10)	-3.35 (0.88) ‡ (-5.08, -1.62)
Step 3					
+ Cardiovascular disease	-0.10 (0.05) (-0.21, 0.001)	-1.33 (0.79) (-2.88, 0.22)	-0.72 (0.50) (-1.71, 0.27)	0.04 (0.03) (-0.01, 0.09)	-3.08 (0.88) ‡ (-4.82, -1.35)
Step 4					
+ Depression	-0.10 (0.05) (-0.20, 0.01)	-1.31 (0.79) (-2.85, 0.24)	-0.71 (0.50) (-1.70, 0.28)	0.04 (0.03) (-0.01, 0.09)	-3.05 (0.88) ‡ (-4.77, -1.33)
Step 5					
+ HbA1c and duration of diabetes	-0.03 (0.05) (-0.15, 0.08)	-0.33 (0.72) (-2.00, 1.35)	-0.17 (0.55) (-1.25, 0.90)	0.01 (0.03) (-0.04, 0.07)	-2.20 (0.95) * (-4.07, -0.33)

‡P≤0.001, †P≤0.01, *P<0.05

Table 41: Summary of multiple linear regression analysis for severity of diabetic retinopathy with the general intelligence factor and individual cognitive function tests as the dependent variables (Standardised Beta regression coefficients and standard errors)

Variables in final model	'g'		Verbal Fluency		Faces		Trail Making Test B		Digit Symbol Test	
	B (SE)	η^2	B (SE)	η^2	B(SE)	η^2	B(SE)	η^2	B (SE)	η^2
Diabetic retinopathy (increasing severity level)	-0.03(0.05)	0.001	-0.01 (0.72)	0.000	-0.01 (0.46)	0.000	0.04 (0.02)	0.002	-0.09 (0.80) †	0.011
Age	-0.23 (0.01) ‡	0.06	-0.07 (0.09) *	0.005	-0.13 (0.06) ‡	0.020	0.23 (0.003) ‡	0.051	-0.21 (0.10) ‡	0.044
Sex (male vs. female)	-0.09 (0.06) †	0.006	-0.04 (0.95)		-0.21 (0.61) ‡	0.030	0.01 (0.03)		-0.14 (1.05) ‡	0.014
MHVS	0.50 (0.01) ‡	0.228	0.37 (0.08) ‡	0.117	0.23 (0.05) ‡	0.047	-0.35 (0.003)‡	0.10	0.32 (0.09) ‡	0.09
Education (increasing level)	0.09 (0.04) †	0.007	0.09 (0.55) †	0.007	0.05 (0.36)		0.003 (0.02)		0.05 (0.62)	
√Alcohol intake	0.05 (0.02)		0.07 (0.26)*	0.004	0.07 (0.17)		0.05 (0.01)		0.02 (0.29)	
Smoking status (smoker vs. non-smoker)	-0.03 (0.05)		0.02 (0.81)		0.002 (0.52)		0.03 (0.03)		-0.09 (0.90) †	0.007
Waist to hip ratio	-0.06 (0.40)		-0.04 (0.59)		-0.04 (0.04)		0.08 (0.02) *	0.004	-0.07 (0.66) *	0.003
Systolic blood pressure	-0.02(0.002)		-0.06 (0.02)*	0.004	0.02 (0.02)		0.02 (0.001)		-0.01 (0.03)	
Total cholesterol	-0.01 (0.03)		0.01 (0.43)		-0.02 (0.28)		0.01 (0.01)		-0.02 (0.48)	
MI and/or Angina	0.003 (0.06)		0.07 (0.85)		-0.01 (0.55)		-0.01 (0.03)		-0.03 (0.95)	
Stroke and/or TIA	-0.08 (0.09) †	0.007	-0.05 (1.34)		0.001 (0.86)		0.07 (0.04) *	0.005	-0.06 (1.50) *	0.004
log Depression	-0.12 (0.10) ‡	0.015	-0.04 (1.46)		-0.05 (0.93)		0.12 (0.05) ‡	0.014	-0.11 (1.62) ‡	0.013
HbA1c	0.03 (0.02)		-0.01 (0.36)		0.02 (0.23)		-0.04 (0.01)		0.04 (0.40)	
√ Duration of diabetes	-0.07 (0.03) *	0.005	-0.07 (0.41) *	0.004	-0.10 (0.26) †	0.010	0.08 (0.03) *	0.005	-0.06 (0.46) *	0.004
Total adjusted R^2		0.426		0.206		0.140		0.223		0.263

‡P≤0.001, †P≤0.01, *P<0.05; Partial eta-squared (η^2) for diabetic retinopathy and variables significantly associated with cognitive outcome.

Multivariate associations for General Factor Scores and Verbal Fluency in men and women

Tables 42 to 45 show the association between severity of diabetic retinopathy and scores for ‘g’ and the VFT in men and women separately. A comparison of age adjusted mean ‘g’ scores in men revealed a significant trend across worsening severity of diabetic retinopathy (one-way ANOVA, P=0.003). Further adjustment for peak prior ability (MHVS) led to a slight increase in the observed difference. ‘g’ was significantly lower in the group with moderate-severe diabetic retinopathy (mean -0.61, 95%CI -0.91, -0.32; P<0.001) compared with the group without diabetic retinopathy (mean 0.04, 95%CI -0.05, 0.12), with an intermediate score for mild diabetic retinopathy group (mean -0.15, 95%CI 0.26, -0.03). Also in men, subjects with moderate-severe diabetic retinopathy had an age-adjusted mean verbal fluency score which, on average, was 6.5 points (P<0.05) lower than in subjects without diabetic retinopathy (Table 42). Following adjustment for peak prior ability, difference in test scores across worsening diabetic retinopathy severity categories became more obvious and P changed from 0.006 to <0.001. Mean VFT scores fell from 38.4 (95%CI 37.1, 39.6) to 31.7 (95%CI 27.2, 36.1) between the absent and moderate-severe diabetic retinopathy groups, with an intermediate score for the mild diabetic retinopathy group (mean 35.5, 95%CI 33.7, 37.3).

No statistical significant difference was found in age adjusted mean scores for ‘g’ and VFT between retinopathy absent and present subjects for women. Neither was there a linear trend with increasing severity of diabetic retinopathy. The difference was more attenuated and even reversed (subjects with mild or moderate-severe diabetic retinopathy had increasingly higher mean VFT scores) after further adjustment for the MHVS, and remained non-significant. Further multivariate analyses did not show any significant associations between retinopathy and ‘g’ and VFT in women and data were not shown in tables.

For the general intelligence factor and the Verbal Fluency test in men, adjustment for education level in addition to age and MHVS did not greatly alter the diabetic retinopathy-estimated cognitive change associations (Table 43).

Cumulatively, the inclusion of the five cardiovascular risk factors, the presence of coronary heart disease and cerebrovascular disease and depression in the subsequent steps led to a gradual and slight attenuation of the associations which, however, remained statistically significant. Finally, the addition of HbA1c and duration of diabetes to the models led to a further decrease in the associations with a considerable decline in the strength of the association for the Verbal Fluency Test (B increased from -3.10 to -1.99). However, both associations remained significant.

When the presence of diabetic retinopathy (absent vs. present) was fitted in the models instead of severity of diabetic retinopathy, the results became non-significant after further adjustment for HbA1c and duration of diabetes (Table 44).

Table 45 presents a summary of the final regression models. Further models were fitted including interactions between all main effects (explanatory variables), but none of them reached significance levels for inclusion in the final models. Severity of diabetic retinopathy remained independently associated with the outcome variable (B=-0.17/standardised beta=-0.10, P<0.05), accounting for about 1% of the variance in general cognitive factor over and above the effect of other factors, along with age (B=-0.06/standardised beta=-0.24, P<0.001), the MHVS (B=0.09/standardised B=0.46, P<0.001), education (B=0.14/standardised beta=0.11, P<0.01), alcohol intake (B=0.05/standardised beta=0.08, P<0.05), the presence of stroke and/or TIA (B=-0.31/standardised beta=-0.10, P<0.01) and depression (B=-0.36/standardised beta=-0.10, P<0.01). Taken together, approximately 43% (based on adjusted R^2) of the variance in general cognitive factor score was explained by the final regression model. Similarly in the final model, the following factors independently contributed to the variance in VFT scores: diabetic retinopathy (B=-1.99/standardised beta=-0.09, P<0.05), MHVS (B=0.88/standardised beta=0.36, P<0.001), alcohol intake (B=0.71/standardised beta=0.10, P<0.05) and systolic blood pressure (B=-0.09/standardised beta=-0.11, P<0.01). Diabetic retinopathy explained approximately 1% of variance in VFT over and above the effect of other factors whereas just over 20% of the variance was explained by the model as a whole.

The adjusted mean (95%CI) general factor and VFT scores of men and women (calculated for women) in the final models by severity of diabetic retinopathy are shown graphically on Figures 35 and 36. The final linear regression models in men are presented in a complete form in the attached Appendix I (Tables i6 to i7).

Table 42: Mean differences (95%CI) in 'g' and the Verbal Fluency test (VFT) scores by severity of diabetic retinopathy for men and women separately, adjusted for age and peak prior cognitive ability

	No Diabetic Retinopathy	Mild Diabetic Retinopathy	Moderate-severe Diabetic Retinopathy	P for trend
Men				
General factor score				
Age Adjusted	0.01(-0.09, 0.12)	-0.10(-0.25, 0.05)	-0.63(-1.0, -0.26) †	**0.003**
Age & MHVS Adjusted	0.04(-0.05, 0.12)	-0.15(0.26, -0.03) *	-0.61(-0.91, -0.32) ‡	<**0.001**
Verbal Fluency Test				
Age Adjusted	38.0(36.7, 39.4)	36.0(34.0, 38.0)	31.5(26.6, 36.4) *	**0.006**
Age & MHVS Adjusted	38.4(37.1, 39.6)	35.5(33.7, 37.3) *	31.7(27.2, 36.1) *	<**0.001**
Women				
General factor score				
Age Adjusted	0.08(-0.03, 0.19)	0.03(-0.15, 0.21)	-0.24(-0.69, 0.20)	0.22
Age & MHVS Adjusted	0.05(-0.03, 0.14)	0.07(-0.07, 0.21)	-0.05(-0.40, 0.30)	0.83
Verbal Fluency Test				
Age Adjusted	36.8(35.4, 38.1)	36.6(34.4, 38.8)	36.9(31.3, 42.5)	0.97
Age & MHVS Adjusted	36.6(35.5, 37.8)	36.9(34.9, 38.8)	38.7(33.7, 43.7)	0.53

‡P≤0.001, †P≤0.01, *P<0.05 ANCOVA test of pair-wise difference in a given mean from the mean of the group without diabetic retinopathy

Table 43: Multivariate associations between severity of diabetic retinopathy and late-life cognition, and estimated lifetime cognitive decline for 'g' and Verbal Fluency test in men

Severity of Diabetic retinopathy	General Factor (g)	Verbal Fluency
	Unstandardised Beta Coefficient (Standard Error) (95%CI)	
Model I		
Age adjusted	-0.21 (0.07) [†] (-0.35, -0.07)	-2.61 (0.95) [†] (-4.48, -0.75)
Model II		
Age and MHVS adjusted	-0.25 (0.06) [‡] (-0.36, -0.14)	-3.10 (0.87) [‡] (-4.80 -1.40)
Model III		
Age, MHVS and all other variables adjusted		
Step 1		
+ Education level	-0.24 (0.06) [‡] (-0.35, -0.12)	-3.05 (0.89) [‡] (-4.79, -1.30)
Step 2		
+ Vascular risk factors	-0.22 (0.06) [‡] (-0.33, -0.10)	-3.08 (0.89) [‡] (-4.83, -1.33)
Step 3		
+ Cardiovascular disease	-0.20(0.06) [‡] (-0.32, -0.09)	-2.95 (0.89) [‡] (-4.70, -1.20)
Step 4		
+ Depression	-0.20(0.06) [‡] (-0.32, -0.09)	-2.94 (0.89) [‡] (-4.69, -1.19)
Step 5		
+ HbA1c and duration of diabetes	-0.17(0.07) * (-0.29, -0.04)	-1.99(1.0) * (-3.97, -0.01)

[‡]P≤0.001, [†]P≤0.01, *P<0.05

Table 44: Multivariate associations between presence of diabetic retinopathy and late-life cognition, and estimated lifetime cognitive decline for 'g' and Verbal Fluency test (VFT) in men

Presence of Diabetic retinopathy	General Factor (g)	Verbal Fluency
	Unstandardised	Beta Coefficient (Standard Error)
Model I		
Age adjusted	-0.19 (0.09) * (-0.36, -0.02)	-2.65 (1.16) * (-4.94, -0.37)
Model II		
Age and MHVS adjusted	-0.25 (0.07) ‡ (-0.38, -0.11)	-3.42 (1.06) ‡ (-5.50, -1.34)
Model III		
Age, MHVS and all other variables adjusted		
Step 1		
+ Education level	-0.24 (0.07) ‡ (-0.37, -0.10)	-3.39 (1.08) † (-5.52, -1.26)
Step 2		
+ Vascular risk factors	-0.22 (0.07) † (-0.36, -0.08)	-3.42 (1.08) † (-5.54, -1.30)
Step 3		
+ Cardiovascular disease	-0.20(0.07) † (-0.34, -0.07)	-3.20 (1.08) † (-5.33, -1.08)
Step 4		
+ Depression	-0.20(0.07) † (-0.34, -0.07)	-3.20(1.08) † (-5.32, -1.07)
Step 5		
+ HbA1c and duration of diabetes	-0.15(0.08) (-0.30, 0.003)	-2.02 (1.2) (-4.38, 0.34)

‡P≤0.001, †P≤0.01, *P<0.05

Table 45: Summary of multiple linear regression analysis for severity of diabetic retinopathy with the general intelligence factor and Verbal Fluency test (VFT) as the dependent variables in men (Standardised Beta regression coefficients and standard errors)

	'g'		**Verbal Fluency**	
	B (SE)	η^2	B (SE)	η^2
Diabetic retinopathy (increasing severity level)	-0.10 (0.07) *	0.012	-0.09 (1.0) *	0.010
Age	-0.24 (0.01) ‡	0.07	-0.08 (0.13)	
MHVS	0.46 (0.01) ‡	0.20	0.36 (0.12) ‡	0.11
Education (increasing level)	0.11 (0.05) †	0.017	0.07 (0.77)	
√Alcohol intake	0.08 (0.02) *	0.011	0.10 (0.32) *	0.009
Smoking status (smoker vs. non-smoker)	0.02 (0.08)		0.05 (1.2)	
Waist to hip ratio	-0.04 (0.06)		-0.01 (0.09)	
Systolic blood pressure	-0.03 (0.002)		-0.11 (0.03) †	0.013
Total cholesterol	-0.01 (0.04)		-0.004 (0.64)	
MI and/or Angina	-0.01 (0.08)		0.08 (0.16)	
Stroke and/or TIA	-0.10 (0.11) †	0.012	-0.08 (1.68)	
log Depression	-0.10 (0.14) †	0.01	-0.04 (2.12)	
HbA1c	0.002 (0.03)		-0.02 (0.51)	
√ Duration of diabetes	-0.06 (0.04)		-0.07 (0.57)	
Total adjusted R^2		0.434		0.203

‡P≤0.001, †P≤0.01, *P<0.05; Partial eta-squared (η^2) for diabetic retinopathy and variables significantly associated with cognitive outcomes

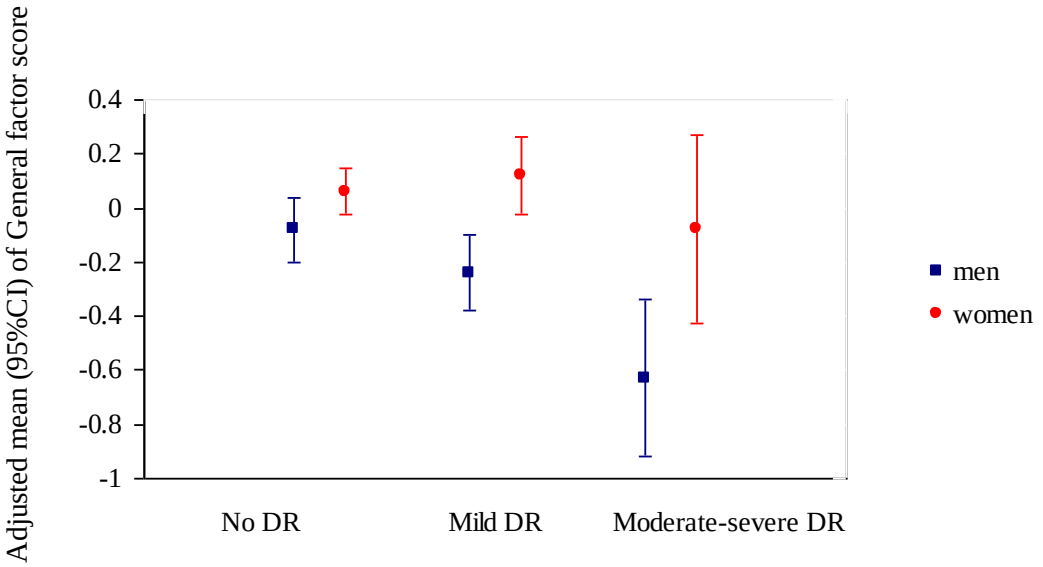


Figure 35: Multivariate adjusted mean (95%CI) general factor scores of men and women according to severity of diabetic retinopathy

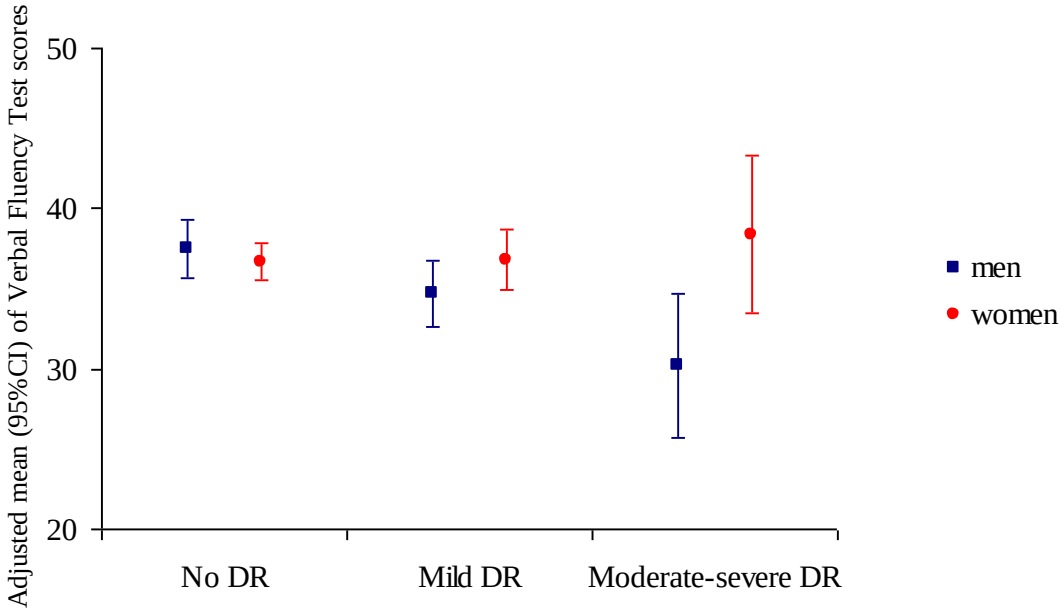


Figure 36: Multivariate adjusted mean (95%CI) Verbal Fluency test scores of men and women according to severity of diabetic retinopathy

5.5.3. General-factor adjusted residuals of individual cognitive tests by diabetic retinopathy status

Given the high loading of each of the tests on the general factor, further outcome variables were computed in order to examine any non-general factor cognitive elements associated with diabetic retinopathy. This was carried out by using multiple linear regressions whereby each of the cognitive tests (VFT, Faces, TMTB and DST) were regressed on the general cognitive ability factor each at a time. The new outcome variables in the current analysis comprised the general factor-adjusted standardised residuals for each cognitive test.

As shown in Table 46, in age and sex adjusted models, severity of diabetic retinopathy was significantly associated with the Digit Symbol test residual scores only but not with others. Further adjustment for peak prior ability did not have any impact on the relationships between diabetic retinopathy and the outcome variables. Addtional adjustment for other potential confounding factors changed the associations only very slightly. In none of the models did diabetic retinopathy exert independent effects on the outcomes except the DST residuals. In the final model (Table 47), diabetic retinopathy (standardised beta=-0.11, $P<0.01$) remained a significant independent predictor of the DST residual scores, explaining about 1% of variance in the dependent variable over and above the effect of other factors. The model as a whole accounted for approximately 6% of the total variance in the DST residuals. The final linear regression models are presented in a complete form in the attached Appendix I (Tables i8 to i11).

Table 46: Multivariate associations between severity of diabetic retinopathy and residuals of individual cognitive test scores regressed on 'g'

Severity of Diabetic retinopathy	Verbal Fluency (men)	Faces	Trail Making Test B	Digit Symbol
		Standardised Beta Coefficient (Standard Error)		
Model I				
Age and sex adjusted	-0.05 (0.08)	-0.03 (0.06)	0.03 (0.06)	-0.10 (0.06) †
Model II				
Age, sex and MHVS adjusted	-0.05 (0.08)	-0.03 (0.06)	0.04 (0.06)	-0.10 (0.06) †
Model III				
Age, sex, MHVS and all other variables adjusted				
Step 1				
+ Education level	-0.06 (0.08)	-0.03 (0.06)	0.03 (0.06)	-0.11(0.06) ‡
Step 2				
+ Vascular risk factors	-0.07 (0.08)	-0.02 (0.06)	0.04 (0.06)	-0.11(0.06) ‡
Step 3				
+ Cardiovascular disease	-0.07 (0.08)	-0.02 (0.06)	0.03 (0.06)	-0.11(0.06) ‡
Step 4				
+ Depression	-0.07 (0.08)	-0.02 (0.06)	0.03 (0.06)	-0.11(0.06) ‡
Step 5				
+ HbA1c and duration of diabetes	-0.07 (0.08)	-0.01 (0.06)	0.03 (0.06)	-0.11(0.06) †

‡P≤0.001, †P≤0.01, *P<0.05;

Table 47: Summary of multiple linear regression analysis for severity of diabetic retinopathy with residuals of individual cognitive test scores regressed on 'g' as the dependent variables (Standardised Beta regression coefficients and standard errors)

	Verbal Fluency (men)		**Faces**		**Trail Making Test B**		**Digit Symbol Test**	
Variables in final model	B (SE)	η^2	B(SE)	η^2	B(SE)	η^2	B (SE)	η^2
Diabetic retinopathy (increasing severity level)	-0.03 (0.09)	0.001	-0.01 (0.06)	0.000	0.03 (0.06)	0.001	-0.11 (0.06) †	0.009
Age	0.09 (0.01)		-0.04 (0.01)		0.07 (0.01) *	0.005	-0.06 (0.01)	
Sex (male vs. female)	-----		-0.19 (0.08) ‡	0.022	-0.10 (0.08) *	0.007	-0.11 (0.08) *	0.007
MHVS	0.08 (0.01)		0.02 (0.01)		0.07 (0.01) *	0.004	-0.09 (0.01) *	0.006
Education (increasing level)	-0.001 (0.07)		0.01 (0.05)		0.13 (0.05) ‡	0.013	-0.03 (0.05)	
√Alcohol intake	0.05 (0.03)		0.06 (0.02)		0.14 (0.02) ‡	0.016	-0.03 (0.02)	
Smoking status (smoker vs. non-smoker)	0.06 (0.11)		0.01 (0.07)		0.03 (0.07)		-0.10 (0.07) †	0.009
Waist to hip ratio	0.03 (0.08)		-0.00 (0.05)		0.05 (0.05)		-0.03 (0.52)	
Systolic blood pressure	-0.12 (0.003)*	0.013	0.03 (0.002)		-0.001 (0.002)		0.004 (0.002)	
Total cholesterol	0.003 (0.06)		-0.02 (0.04)		0.01 (0.04)		-0.01(0.04)	
MI and/or Angina	0.11 (0.10) *	0.012	-0.02 (0.08)		-0.01 (0.07)		-0.06 (0.08)	
Stroke and/or TIA	-0.01 (0.15)		0.04 (0.12)		0.03 (0.12)		-0.02 (0.12)	
log Depression	0.03 (0.19)		0.01 (0.13)		0.04 (0.13)		-0.03 (0.13)	
HbA1c	-0.04 (0.05)		0.01 (0.13)		-0.02 (0.03)		0.03 (0.03)	

√ Duration of diabetes	-0.04 (0.05)	-0.07 (0.04)	0.01 (0.04)	-0.01 (0.04)	
Total adjusted R^2	0.025	0.026	0.041		0.056

‡P≤0.001, †P≤0.01, *P<0.05; Partial eta-squared (η^2) for diabetic retinopathy and variables significantly associated with cognitive outcome.

5.5.4. Diabetic retinopathy and cognitive impairment

A binary variable indicating an impaired score on each of the four cognitive tests as well as the general intelligence factor was created. The proportions of impaired test scores are presented in Table 48.

Table 48: Proportions of impaired test scores on four cognitive tests and the general intelligence factor (N=1,044)

Cognitive Test	Cutoff value	n	Proportion of Individuals with impaired score
Verbal Fluency	18	77	7.4%[a]
Faces	54	73	7.0%
Trail Making (ln)	5.31	75	7.2%
Digit Symbol	27	64	6.1%
General factor score	-1.5	72	6.9%[b]

[a] In men, n=40 (7.5%); [b] In men, n=34 (6.7%)

In univariate analyses to determine the association between the presence of diabetic retinopathy and an impaired score on the four cognitive tests and the general cognitive ability factor (Table 49), odds ratios above the value of 1 were found for all cognitive measures. However, only for the Trail Making and the Digit Symbol tests, confidence intervals did not include the null value of one and were thus significant. Further adjustment for age and sex did not greatly alter the calculated odds ratios. The addition of the Mill-Hill Vocabulary Scale into the logistic regression models led to a slight increase in the odds ratios, with the associations for Trail Making and Digit Symbol tests becoming more significant (P values from 0.002 to 0.001 or from 0.001 to <0.001). The adjusted odds ratios for other cognitive tests remained non-significant. Results in the final models indicated that the odds of an 'impaired' score on the Trail Making test for individuals with diabetic retinopathy was 2.32 (95%CI 1.39, 3.90) times that of individuals without diabetic retinopathy. Similarly, diabetic retinopathy was also associated with an increased risk of impairment on the Digit Symbol test (odds ratio 2.71, 95%CI 1.57, 4.70) (Table 49). The odds ratios obtained from univariate and multivariate analyses are illustrated in Figure 37.

Table 49: Unadjusted and adjusted odds ratios (95%CI) for cognitive impairment in participants with diabetic retinopathy

	Unadjusted	Age & Sex Adjusted	Age, sex & Mill-Hill Adjusted
Verbal Fluency[a]	1.00 (0.51-1.96)	1.00 (0.51-1.97)	1.17 (0.58-2.37)
Faces	1.17 (0.71-1.93)	1.14 (0.69-1.88)	1.22 (0.73-2.06)
Trail Making	2.05 (1.27-3.28) †	2.10 (1.30-3.39) †	2.32 (1.39-3.90) ‡
Digit Symbol	2.37(1.43-3.94) ‡	2.34 (1.40-3.90) ‡	2.71 (1.57-4.70) ‡
General Factor-[a]	1.71 (0.85-3.44)	1.83(0.88-3.81)	2.32 (0.96-5.58)

[a]Test scores were calculated for men; ‡P≤0.001, †P≤0.01, *P<0.05.

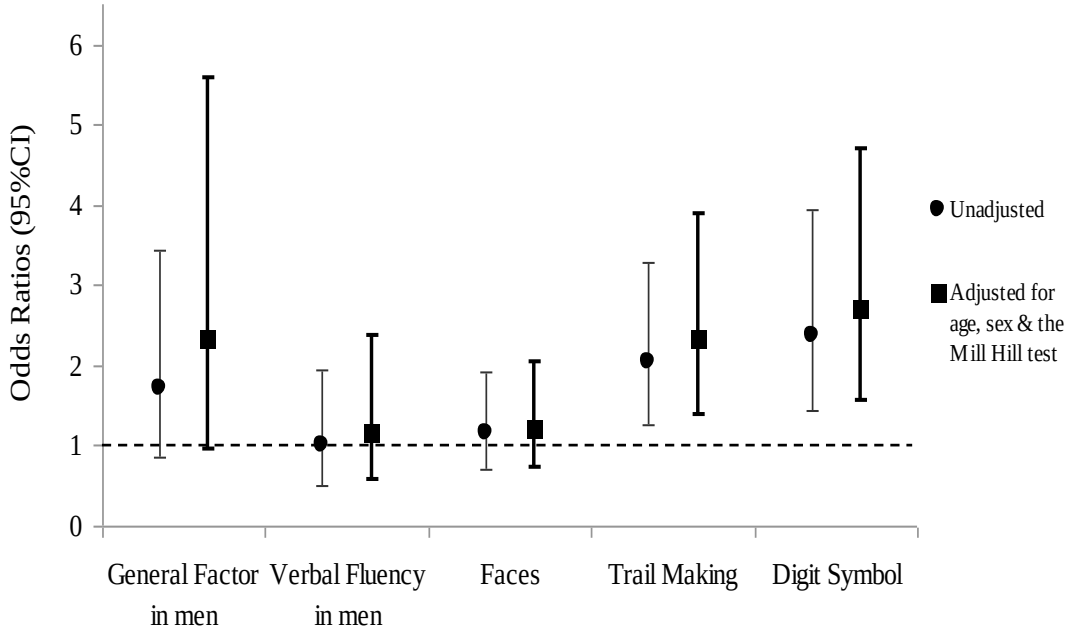


Figure 37: Odds ratios and 95%CIs for cognitive impairment in participants with diabetic retinopathy

5.5.5. Sensitivity analyses

Given that retinal capillary microaneurysm is usually the first visible sign of diabetic retinopathy, the presence of other characteristics (e.g. hard exudates, cotton wool spots) in the absence of microaneurysms was graded as 'possible diabetic retinopathy' (grading level 14 or 15). These isolated lesions could be principally caused by other pathphysiological process rather than diabetes itself, such as hypertension. When the analyses were repeated on a subgroup of 983 subjects (excluding subjects having possible diabetic retinopathy), the associations between severity of diabetic retinopathy and estimated cognitive decline were essentially unchanged after adjustment for age, sex and the MHVS (Table 50). After further adjustment for education level, vascular risk factors, cardiovascular disease and depression, diabetic retinopathy remained significantly associated with poorer performance on the Digit Symbol test ($P<0.001$) and in males, poorer scores for g ($P<0.01$) and Verbal Fluency test ($P<0.001$) (Table 50). However, the observed association between diabetic retinopathy and Trail Making Test became borderline non-significant (P for trend=0.054), indicating that this association was affected by subjects with 'possible diabetic retinopathy' to some extent. The associations for g, VFT and DST were also independent of glycaemic control (HbA1c) and duration of diabetes.

In diabetic individuals, a history of ischemic stroke is commonly associated cerebral microvascular disease (Cheung et al., 2007c). When the ET2DS population was limited to 954 subjects without stroke or TIA, the associations between diabetic retinopathy and cognitive decline were little changed (results adjusted for age, sex, the MHVS and other risk factors shown in Table 51). Diabetic retinopathy severity was significantly associated with worse performance on the Digit Symbol ($P<0.001$) and Trail Making ($P<0.01$) tests and in males, poorer scores for the general cognitive factor ($P<0.001$) and Verbal Fluency test ($p<0.001$). These associations for g, VFT and DST were independent of glycaemic control (HbA1c) and duration of diabetes.

When the analysis was limited to the 1,015 subjects with an MMSE scores≥24 (to reduce the probability that subjects with dementia were included),

similar results were found and the effect of diabetic retinopathy on estimated lifetime cognitive decline was little changed in multivariate adjusted analyses (Table 52).

Table 50: Multivariate associations between severity of diabetic retinopathy and late-life cognition, and estimated lifetime cognitive decline (subjects with possible diabetic retinopathy were excluded) (N=983)

Severity of Diabetic retinopathy	General Factor (g) (males)	Verbal Fluency (males)	Faces	Trail Making Test B	Digit Symbol
		Unstandardised Beta Coefficient (Standard Error)			
Model I					
Age and sex adjusted	-0.20 (0.07) †	-2.85(0.97) †	-0.95 (0.42) *	0.06 (0.02) †	-3.36 (0.79) ‡
Model II					
Age, sex and MHVS adjusted	-0.24 (0.06) ‡	-3.35 (0.88) ‡	-0.93 (0.40) *	0.06 (0.02) †	-3.27 (0.73) ‡
Model III					
Age, sex, MHVS and all other variables adjusted					
Step 1					
+ Education level	-0.22 (0.06) ‡	-3.31 (0.91) ‡	-0.83 (0.42) *	0.05 (0.02) *	-3.38 (0.75) ‡
Step 2					
+ Vascular risk factors	-0.21 (0.06) ‡	-3.37 (0.91) ‡	-0.72 (0.42)	0.05 (0.02) *	-3.34 (0.75) ‡
Step 3					
+ Cardiovascular disease	-0.19 (0.06) ‡	-3.22 (0.91) ‡	-0.71 (0.42)	0.04 (0.02) *	-3.14 (0.75) ‡
Step 4					
+ Depression	-0.19 (0.06) †	-3.20(0.91) ‡	-0.69 (0.42)	0.04 (0.02)	-3.07 (0.74) ‡
Step 5					
+ HbA1c and duration of diabetes	-0.15 (0.07) *	-2.15 (1.03) *	-0.18 (0.47)	0.02 (0.02)	-2.28 (0.82) †

‡P≤0.001, †P≤0.01, *P<0.05.

Table 51: Multivariate associations between severity of diabetic retinopathy and late-life cognition, and estimated lifetime cognitive decline in subjects free of stroke or TIA (N=954)

Severity of Diabetic retinopathy	General Factor (g) (males)	Verbal Fluency (males)	Faces	Trail Making Test B	Digit Symbol
		Unstandardised Beta Coefficient (Standard Error)			
Model I					
Age and sex adjusted	-0.26 (0.08) †	-3.42(1.02) †	-1.00 (0.45) *	0.07 (0.02) †	-3.31 (0.83) ‡
Model II					
Age, sex and MHVS adjusted	-0.27 (0.06) ‡	-3.67 (0.93) ‡	-0.92 (0.42) *	0.07 (0.02) †	-3.07 (0.76) ‡
Model III					
Age, sex, MHVS and all other variables adjusted					
Step 1					
+ Education level	-0.26 (0.06) ‡	-3.72 (0.95) ‡	-0.80 (0.44)	0.06 (0.02) †	-3.19 (0.78) ‡
Step 2					
+ Vascular risk factors	-0.24 (0.06) ‡	-3.73 (0.95) ‡	-0.66 (0.44)	0.06 (0.02) †	-3.21 (0.78) ‡
Step 3					
+ Cardiovascular disease	-0.24 (0.06) ‡	-3.71 (0.95) ‡	-0.66 (0.44)	0.06 (0.02) †	-3.20 (0.78) ‡
Step 4					
+ Depression	-0.24 (0.06) ‡	-3.69(0.95) ‡	-0.65 (0.44)	0.06 (0.02) †	-3.15 (0.78) ‡
Step 5					
+ HbA1c and duration of diabetes	-0.21 (0.07) †	-2.83 (1.05) †	-0.23 (0.48)	0.04 (0.02)	-2.56 (0.85) †

‡P≤0.001, †P≤0.01, *P<0.05.

Table 52: Multivariate associations between severity of diabetic retinopathy and late-life cognition, and estimated lifetime cognitive decline (N=1,015, subjects with MMSE <24 were excluded)

Severity of Diabetic retinopathy	General Factor (g) (males)	Verbal Fluency (males)	Faces	Trail Making Test B	Digit Symbol
		Unstandardised Beta Coefficient (Standard Error)			
Model I					
Age and sex adjusted	-0.24 (0.07) †	-3.03(0.95) †	-0.97 (0.42) *	0.07 (0.02) †	-3.44 (0.77) ‡
Model II					
Age, sex and MHVS adjusted	-0.26 (0.06) ‡	-3.24 (0.88) ‡	-0.89 (0.40) *	0.06 (0.02) †	-3.23 (0.72) ‡
Model III					
Age, sex, MHVS and all other variables adjusted					
Step 1					
+ Education level	-0.24 (0.06) ‡	-3.17 (0.90) ‡	-0.79 (0.42)	0.06 (0.02) †	-3.33 (0.74) ‡
Step 2					
+ Vascular risk factors	-0.22 (0.06) ‡	-3.19 (0.90) ‡	-0.66 (0.42)	0.06 (0.02) †	-3.25 (0.74) ‡
Step 3					
+ Cardiovascular disease	-0.21 (0.06) ‡	-3.10 (0.90) ‡	-0.64 (0.42)	0.05 (0.02) *	-3.02 (0.74) ‡
Step 4					
+ Depression	-0.21 (0.06) ‡	-3.09(0.90) ‡	-0.63 (0.42)	0.05 (0.02) *	-2.96 (0.73) ‡
Step 5					
+ HbA1c and duration of diabetes	-0.17 (0.07) *	-2.09 (1.02) *	-0.15 (0.46)	0.03 (0.02)	-2.41 (0.81) †

‡P≤0.001, †P≤0.01, *P<0.05.

5.6. Retinal vascular network geometry and cognitive function

This section describes the associations between the parameters of retinal vascular network geometry and cognitive function, measured as current cognitive ability and estimated lifetime cognitive decline. Univariate relationships between the quantitative retinal vascular parameters and cognitive ability are presented first, followed by the multivariate analyses for associations between these parameters and estimated cognitive decline. Finally, sensitivity analyses excluding subjects with severe proliferative diabetic retinopathy or severe cognitive impairment are performed to explore further associations between estimated cognitive decline and retinal parameters.

5.6.1. Associations of quantitative retinal vascular parameters with current cognitive ability

Pearson correlation coefficients were calculated for retinal vascular parameters and cognitive function variables (Table 53 and 54).

Higher arteriolar diameters (CRAE) were significantly associated with lower MHVS ($r=-0.09$, $P<0.01$) and Logical Memory ($r=-0.11$, $P<0.001$) scores and with higher depression scores ($r=0.07$, $P<0.05$). Increased venular diameters (CRVE) were only associated with lower scores for Logical Memory ($r=-0.07$, $P<0.05$). This implied that the wider retinal vessel width (an increase either in CRVE or CRAE) was significantly related to a worse performance on logical memory test. Raised deviation levels on suboptimal BA were associated with lower scores for MHVS ($r=-0.11$, $P<0.05$) and increased arteriolar angles were associated with lower scores for the Verbal Fluency Test ($r=-0.09$, $P<0.05$).

When the correlations were adjusted for both age and sex, the partial correlation coefficients (rs) remained significant between the CRAE, CRVE and logical memory test, and arteriolar angle and VFT. There were no other significant associations between retinal width, arteriolar bifurcation parameters and cognitive

tests.

5.6.2 Associations of quantitative retinal vascular parameters with estimated lifetime cognitive decline

After further adjustment for MHVS, the partial correlation coefficients declined and only the higher CRAE remained significantly associated with lower scores on logical memory (r=-0.09, P<0.01) (Table 53 and 54). When evaluated separately by sex (Table 55 and 56), significant negative correlations were found between Logical Memory and CRAE (r=-0.17, P<0.001) and CRVE (r=-0.13, P<0.01) in men after adjusting for age and MHVS score. Conversely, significant positive correlations emerged between the Faces test and CRAE (r=0.09, P<0.05) and CRVE (r=0.13, P<0.01) in women after adjustment for both age and MHVS. No other significant associations in either men or women were demonstrated. As a consequence, further multivariate analyses (a series of multiple linear regression analyses) were only carried out on the associations of CRAE and CRVE with Logical Memory test in men and Faces test in women but not on other data.

When further adjusted for education, vascular risk factors, cardiovascular disease and depression, both increased retinal arteriolar and venular diameters (CRAE and CRVE) remained significantly associated with poorer performance on the Logical Memory in men (P<0.01) (Table 57 and 58). In women, increased CRVE continued to be associated with better performance on Faces (P<0.01) whereas the association between CRAE and Faces became non-significant (Table 59 and 60). The addition of duration of diabetes and HbA1c did not change the associations greatly.

Lastly, whether CRAE and CRVE contributed separately to variance in performance on Logical Memory in men or Faces in women, was examined by adding the fellow vessel calibre in the multivariate models as a covariable. This was done because the retinal arteriolar and venular width were highly correlated (r=0.61, P<0.01) and had a similar profile of associations with cognitive test scores. Also findings from previous studies have demonstrated the main determinant of retinal arteriolar width is in fact venular width, presumably due to their shared genetic and

environmental factors as well as magnification artifacts (Hubbard et al., 1999; Liew et al. 2007). As shown in Tables 57 to 60, Model IV adjusted for variables in model III as well as the fellow vessel calibre (i.e. addition of CRAE in models for CRVE, and CRVE in models for CRAE). In this model, increased CRAE was independently associated with poorer Logical Memory scores ($P<0.05$) in men but increased CRVE was no long associated with LM. Similarly, only increased CRVE contributed significantly to higher Faces scores in women whereas the positive association with CRAE became reversed in direction and non-significant. The change in association between CRVE and LM was unlikely to be due to collinearity between these two correlated variables as the variance inflation factors for both terms were low (approximately 1.7).

As shown in Table 61, for the Logical Memory test, in this final model where both vessel calibres were simultaneously entered, CRAE (B=-0.04/standardised beta=-0.12, $P<0.05$) explained about 1% of the variance in LM scores in men over and above the effect of all other factors, along with the MHVS (B=0.44/standardised beta=0.29, $P<0.001$) which also exerted independent effects on LM scores. The model as a whole accounted for 14.1% of the variance in the outcome. For the Faces test (Table 62), CRVE (B=0.03/standardised beta=0.12, $P<0.05$) explained about 1% of the variance in Faces scores in women over and above the effect of all other factors and as a whole, the model accounted for 15.3% of the total variance.

Table 61 and 62 present a summary of the final regression models. Using the final models, the significance of interaction terms between all explanatory variables were tested. None of these reached significance levels for inclusion in the models. The final linear regression models in men and women are presented in a complete form in the attached Appendix I (Tables i12 to i15).

Table 53: Pearson correlation coefficients of retinal width parameters with cognitive test variables and depression (N=980)

	Depression	MHVS	Verbal Fluency	Faces	Trail Making (ln)	Logical Memory	Matrix Reasoning	Digit Symbol	Letter-Number Sequencing	g
CRAE										
Unadjusted	0.073*	-0.09†	-0.05	0.03	0.02	-0.11‡	-0.02	-0.05	-0.04	-0.05
Adjusted for age and sex			-0.04	0.02	0.04	-0.11‡	-0.02	-0.08	-0.04	-0.07*
Adjusted for age, sex and MHVS			-0.002	0.05	0.007	-0.09†	0.009	-0.05	-0.006	-0.02
CRVE										
Unadjusted	0.06	-0.05	-0.001	0.05	-0.01	-0.07*	0.01	0.001	-0.01	-0.001
Adjusted for age and sex			0.002	0.04	0.03	-0.07*	-0.01	-0.03	-0.01	-0.03
Adjusted for age, sex and MHVS			0.02	0.06	0.02	-0.06	0.009	-0.021	0.003	-0.006
AVR										
Unadjusted	0.01	-0.04	-0.06	-0.03	0.04	-0.03	-0.04	-0.06	-0.04	-0.06
Adjusted for age and sex			-0.05	-0.03	0.01	-0.04	-0.02	-0.05	-0.03	-0.05
Adjusted for age, sex and MHVS			-0.03	-0.02	-0.01	-0.03	-0.003	-0.03	-0.02	-0.03

‡P≤0.001, †P≤0.01, *P<0.05.

Table 54: Pearson correlation coefficients of retinal bifurcation parameters with cognitive test variables and depression (N=444)

	Depression	MHVS	Verbal Fluency	Faces	Trail Making (ln)	Logical Memory	Matrix Reasoning	Digit Symbol	Letter-Number Sequencing	g
Arteriolar bifurcation angle										
Unadjusted	-0.047	-0.09	-0.09*	0.01	0.004	0.03	-0.009	-0.08	-0.02	-0.05
Adjusted for age and sex			-0.10*	0.006	0.005	0.023	-0.02	-0.09	-0.035	-0.05
Adjusted for age, sex and MHVS			-0.07	0.03	-0.04	0.08	0.035	-0.066	0.006	0.010
Arteriolar branching coefficient										
Unadjusted	0.003	0.01	-0.04	0.004	0.04	0.01	0.06	-0.05	0.05	0.003
Adjusted for age and sex			-0.02	0.02	0.04	0.003	0.06	-0.035	0.06	0.008
Adjusted for age, sex and MHVS			-0.02	0.02	0.02	-0.02	0.08	-0.025	0.072	0.022
Suboptimal bifurcation angle (Ln)										
Unadjusted	-0.024	-0.114*	-0.002	0.014	-0.02	0.02	0.008	-0.030	-0.005	0.002
Adjusted for age and sex			-0.01	0.02	-0.03	0.02	0.007	-0.038	-0.007	0.002
Adjusted for age, sex and MHVS			0.05	0.06	-0.08	0.08	0.06	0.004	0.042	0.094

‡P≤0.001, †P≤0.01, *P<0.05.

Table 55: Pearson correlation coefficients of retinal width parameters with cognitive test variables and depression in men and women separately

	Depression	MHVS	Verbal Fluency	Faces	Trail Making (ln)	Logical Memory	Matrix Reasoning	Digit Symbol	Letter-Number Sequencing	g
Men										
CRAE										
Unadjusted	0.11*	-0.04	-0.07	-0.001	-0.002	-0.18 ‡	-0.03	-0.05	-0.03	-0.07
Adjusted for age			-0.07	0.01	-0.01	-0.18‡	0.003	-0.05	-0.03	-0.07
Adjusted for age and MHVS			-0.07	0.02	-0.02	-0.17‡	0.02	-0.04	-0.02	-0.06
CRVE										
Unadjusted	0.07	0.02	-0.01	0.02	0.07	-0.12†	0.003	0.05	0.001	0.02
Adjusted for age			-0.02	0.01	-0.06	-0.12†	0.02	0.04	-0.01	0.003
Adjusted for age and MHVS			-0.04	0.01	-0.05	-0.13†	0.01	0.02	-0.02	-0.03
AVR										
Unadjusted	0.04	-0.07	-0.09	-0.03	0.08	-0.07	-0.04	-0.13†	-0.05	-0.11*
Adjusted for age			-0.07	-0.01	0.06	-0.04	-0.02	-0.10*	-0.03	-0.09
Adjusted for age and MHVS			-0.05	0.004	0.03	-0.04	0.01	-0.07	-0.01	-0.04
Women										
CRAE										
Unadjusted	0.03	-0.14 †	-0.01	0.04	0.04	-0.04	-0.04	-0.06	-0.05	-0.04
Adjusted for age			-0.02	0.04	0.06	-0.06	-0.05	-0.09	-0.06	-0.06
Adjusted for age and MHVS			0.05	0.09*	0.02	0.002	0.001	-0.04	-0.01	0.02
CRVE										
Unadjusted	0.06	-0.13 †	0.01	0.09	0.06	-0.03	-0.02	-0.04	-0.03	-0.04
Adjusted for age			0.001	0.08	0.08	-0.04	-0.04	-0.07	-0.05	-0.05
Adjusted for age and MHVS			0.07	0.13†	0.05	0.02	0.02	-0.02	0.001	0.03
AVR										
Unadjusted	-0.04	0.02 †	-0.02	-0.05	-0.01	-0.01	-0.03	-0.02	-0.02	-0.02
Adjusted for age			-0.02	-0.05	-0.02	-0.01	-0.02	-0.01	-0.01	-0.01
Adjusted for age and MHVS			-0.03	-0.05	-0.03	-0.02	-0.04	-0.01	-0.02	-0.02

‡P≤0.001, †P≤0.01, *P<0.05.

Table 56: Pearson correlation coefficients of retinal bifurcation parameters with cognitive test variables and depression in men and women separately

	Depression	MHVS	Verbal Fluency	Faces	Trail Making (ln)	Logical Memory	Matrix Reasoning	Digit Symbol	Letter-Number Sequencing	g
Men										
Arteriolar bifurcation angle										
Unadjusted	0.005	-0.18†	-0.16*	0.04	0.05	0.04	-0.05	-0.12	-0.02	-0.08
Adjusted for age and sex			-0.17*	0.03	0.05	0.04	-0.05	-0.12	-0.02	-0.09
Adjusted for age, sex and MHVS			-0.11	0.08	-0.02	0.13	0.04	-0.07	0.07	0.04
Arteriolar branching coefficient										
Unadjusted	0.01	-0.06	-0.06	-0.08	0.09	-0.07	-0.01	-0.04	-0.06	-0.09
Adjusted for age and sex			-0.05	-0.07	0.09	-0.07	0.00	-0.03	-0.06	-0.09
Adjusted for age, sex and MHVS			-0.03	-0.05	0.02	-0.08	0.04	-0.02	-0.02	-0.03
Suboptimal bifurcation angle (Ln)										
Unadjusted	0.003	-0.16*	-0.10	0.01	-0.03	-0.02	-0.07	-0.08	-0.004	-0.05
Adjusted for age and sex			-0.01	0.02	-0.03	-0.02	-0.06	-0.07	0.002	-0.03
Adjusted for age, sex and MHVS			-0.09	0.06	-0.04	0.04	0.004	-0.02	0.07	0.08
Women										
Arteriolar bifurcation angle										
Unadjusted	-0.11	-0.01	-0.03	-0.02	-0.04	0.02	0.14	-0.05	-0.02	-0.02
Adjusted for age and sex			-0.03	-0.02	-0.03	0.02	0.03	-0.06	-0.02	-0.02
Adjusted for age, sex and MHVS			-0.03	-0.02	-0.04	0.02	0.03	-0.06	-0.02	-0.03
Arteriolar branching coefficient										
Unadjusted	0.01	0.07	-0.02	0.10	-0.02	0.08	0.12	-0.04	0.16*	0.09
Adjusted for age and sex			-0.02	0.10	-0.02	0.08	0.12	-0.04	0.16	0.09
Adjusted for age, sex and MHVS			-0.06	0.08	0.02	0.05	0.10	-0.07	0.15	0.06
Suboptimal bifurcation angle (Ln)										
Unadjusted	-0.05	-0.07	0.09	0.02	-0.01	0.06	0.09	0.01	-0.01	0.04
Adjusted for age and sex			0.09	0.02	-0.002	0.06	0.08	0.01	-0.01	0.03
Adjusted for age, sex and MHVS			0.13	0.04	-0.03	0.11	0.12	0.02	0.02	0.10

‡P≤0.001, †P≤0.01, *P<0.05

Table 57: Multivariate associations between retinal arteriolar width (CRAE) and late-life cognition, and estimated lifetime cognitive decline for Logical Memory in men

CRAE	Logical Memory	
	Unstandardised	Beta Coefficient (Standard Error)
Model I		
Age adjusted	-0.05 (0.01) ‡	
Model II		
Age and MHVS adjusted	-0.05 (0.01) ‡	
Model III		
Age, MHVS and all other variables adjusted		
Step 1		
+ Education level	-0.05 (0.01) ‡	
Step 2		
+ Vascular risk factors	-0.05 (0.01) ‡	
Step 3		
+ Cardiovascular disease	-0.05 (0.01) ‡	
Step 4		
+ Depression	-0.05 (0.01) ‡	
Step 5		
+ HbA1c and duration of diabetes	-0.05 (0.01) †	
Model IV		
All variables in Model III		
+ Retinal venular width (CRVE)	-0.04(0.02) *	

‡P≤0.001, †P≤0.01, *P<0.05

Table 58. Multivariate associations between retinal venular width (CRVE) and late-life cognition, and estimated lifetime cognitive decline for Logical Memory in men

CRAE	Logical Memory	
	Unstandardised	Beta Coefficient (Standard Error)
Model I		
Age adjusted	-0.03 (0.01) *	
Model II		
Age and MHVS adjusted	-0.03 (0.01) †	
Model III		
Age, MHVS and all other variables adjusted		
Step 1		
+ Education level	-0.03 (0.01) †	
Step 2		
+ Vascular risk factors	-0.03 (0.01) †	
Step 3		
+ Cardiovascular disease	-0.03(0.01) †	
Step 4		
+ Depression	-0.03(0.01) †	
Step 5		
+ HbA1c and duration of diabetes	-0.03(0.01) †	
Model IV		
All variables in Model III		
+ Retinal arteriolar width (CRAE)	-0.01 (0.01)	

‡P≤0.001, †P≤0.01, *P<0.05

Table 59: Multivariate associations between retinal arteriolar width (CRAE) and late-life cognition, and estimated lifetime cognitive decline for Faces test in women

CRAE	Faces	
	Unstandardised	Beta Coefficient (Standard Error)
Model I		
Age adjusted		0.01 (0.02)
Model II		
Age and MHVS adjusted		0.03 (0.01) *
Model III		
Age, MHVS and all other variables adjusted		
Step 1		
+ Education level		0.03 (0.02)
Step 2		
+ Vascular risk factors		0.03 (0.02)
Step 3		
+ Cardiovascular disease		0.02 (0.02)
Step 4		
+ Depression		0.02 (0.02)
Step 5		
+ HbA1c and duration of diabetes		-0.02 (0.02)
Model IV		
All variables in Model III		
+ Retinal venular width (CRVE)		-0.004(0.02)

‡P≤0.001, †P≤0.01, *P<0.05

Table 60: Multivariate associations between retinal venular width (CRVE) and late-life cognition, and estimated lifetime cognitive decline for Faces test in women

CRAE	Faces	
	Unstandardised	Beta Coefficient (Standard Error)
Model I		
Age adjusted		0.02 (0.01)
Model II		
Age and MHVS adjusted		0.03 (0.01) †
Model III		
Age, MHVS and all other variables adjusted		
Step 1		
+ Education level		0.03 (0.01) †
Step 2		
+ Vascular risk factors		0.03 (0.01) †
Step 3		
+ Cardiovascular disease		0.03 (0.01) †
Step 4		
+ Depression		0.03 (0.01) †
Step 5		
+ HbA1c and duration of diabetes		0.03 (0.01) *
Model IV		
All variables in Model III		
+ Retinal arteriolar width (CRAE)		0.03 (0.01) *

‡P≤0.001, †P≤0.01, *P<0.05

Table 61: Summary of multiple linear regression analysis for retinal width (CRAE and CRVE) with the Logical Memory Test as the dependent variables in men (Standardised Beta regression coefficients and standard errors)

	Logical Memory[1]		**Logical Memory[2]**		**Logical Memory[3]**	
	B (SE)	η^2	B (SE)	η^2	B (SE)	η^2
CRAE	-0.15 (0.01) †	0.027			-0.12 (0.01) *	0.011
CRVE			-0.13 (0.01) †	0.018	-0.05 (0.01)	0.002
Age	-0.04 (0.09)		-0.04 (0.09)		-0.04 (0.09)	
MHVS	0.29 (0.08) ‡	0.072	0.30 (0.08) ‡	0.074	0.29 (0.08) ‡	0.073
Education (increasing level)	0.06 (0.51)		0.07 (0.52)		0.06 (0.51)	
√Alcohol intake	0.06 (0.21)		0.07 (0.21)		0.06 (0.21)	
Smoking status (smoker vs. non-smoker)	0.06 (0.81)		0.06 (0.82)		0.06 (0.81)	
Waist to hip ratio	0.004 (0.06)		0.02 (0.06)		0.009 (0.06)	
Systolic blood pressure	-0.01 (0.002)		-0.006 (0.02)		-0.01 (0.02)	
Total cholesterol	-0.003 (0.43)		0.01 (0.43)		0.001 (0.43)	
MI and/or Angina	0.06 (0.77)		0.06 (0.77)		0.06 (0.77)	
Stroke and/or TIA	-0.06 (1.14)		-0.06 (1.14)		-0.06 (1.14)	
log Depression	-0.07 (1.38)		-0.08 (1.39)		-0.07 (1.39)	
HbA1c	-0.08 (0.34)		-0.07 (0.34)		-0.07 (0.34)	
√ Duration of diabetes	0.03 (0.36)		0.03 (0.36)		0.03 (0.36)	
Total adjusted R^2		0.142		0.134		0.141

[‡]P≤0.001, [†]P≤0.01, *P<0.05; Partial eta-squared (η^2) for CRAE, CRVE and variables significantly associated with cognitive outcomes. [1]Model adjusted for all the variables in the table except for CRVE; [2]Model adjusted for all the variables in the table except for CRAE; [3]Model adjusted for all the variables in the table including CRAE and CRVE

Table 62: Summary of multiple linear regression analysis for retinal width (CRAE and CRVE) with the Faces Test as the dependent variables in women (Standardised Beta regression coefficients and standard errors)

	Faces[1]		**Faces[2]**		**Faces[3]**	
	B (SE)	η^2	B (SE)	η^2	B (SE)	η^2
CRAE	0.05 (0.02)	0.003			-0.01 (0.02)	0.000
CRVE			0.11 (0.01) *	0.01	0.12 (0.01) *	0.009
Age	-0.06 (0.09)		-0.05 (0.09)		-0.05 (0.09)	
MHVS	0.28 (0.08) ‡	0.068	0.29 (0.08) ‡	0.073	0.29 (0.08) ‡	0.072
Education (increasing level)	0.09 (0.55)		0.09 (0.54)		0.09 (0.54)	
√Alcohol intake	0.10 (0.31) *	0.011	0.10 (0.31) *	0.011	0.10 (0.31) *	0.011
Smoking status (smoker vs. non-smoker)	0.00 (0.75)		-0.01 (0.75)		-0.01 (0.75)	
Waist to hip ratio	-0.04 (0.5)		-0.04 (0.52)		-0.04 (0.52)	
Systolic blood pressure	0.03 (0.02)		0.04 (0.02)		0.04 (0.02)	
Total cholesterol	-0.04 (0.4)		-0.03 (0.39)		-0.03 (0.40)	
MI and/or Angina	0.04(0.9)		0.03 (0.86)		0.03(0.87)	
Stroke and/or TIA	-0.03 (1.7)		-0.02 (1.66)		-0.02 (1.7)	
log Depression	-0.09 (1.4)		-1.0 (1.38)*	0.010	-1.0 (1.4) *	0.010
HbA1c	0.06 (0.4)		0.06 (0.4)		0.06 (0.4)	
√ Duration of diabetes	-0.15 (0.39) †	0.022	-0.14 (0.39)†	0.021	-0.14 (0.39) †	0.021
Total adjusted R^2		0.147		0.155		0.153

[‡]P≤0.001, [†]P≤0.01, *P<0.05; Partial eta-squared (η^2) for CRAE, CRVE and variables significantly associated with cognitive outcomes. [1]Model adjusted for all the variables in the table except for CRVE; [2]Model adjusted for all the variables in the table except for CRAE; [3]Model adjusted for all the variables in the table including CRAE and CRVE.

5.6.3. General-factor adjusted residuals of individual cognitive tests

The new outcome variables in the current analysis comprised the general factor-adjusted standardised residuals for the two individual cognitive tests: Logical Memory and Faces.

As shown in Tables 63 and 64, in age and sex adjusted models, increased CRAE was significantly associated with lower Logical Memory test residual scores in men, whereas increased CRVE was associated with higher Faces test residual scores in women. Further adjustment for peak prior ability did not greatly alter the associations although the association between CRVE and Faces residuals was a little strengthened. The cumulative adjustment for other potential confounding factors after control for age, sex and MHVS changed the associations only slightly. Finally, the addition of the fellow vessel width led to an attenuation of the associations which, however, remained statistically significant. In the final model (Table 65), CRAE (standardised beta=-0.13, $p<0.05$) continued to exert independent effects on the DST residual scores, explaining about 1% of variance in the dependent variable over and above the effect of other factors. The model as a whole accounted for approximately 2.6% of the total variance in the DST residuals. Similarly, CRVE (standardised beta=0.13, $p<0.05$) remained independently associated with Faces residuals, accounting for 1.1% of variance and the whole model explained about 3.1% of the total variance in the Faces test residuals.

Table 63: Multivariate associations between retinal arteriolar width (CRAE) and residuals of Logical Memory test scores regressed on 'g' in men

CRAE	Logical Memory
	Standardised Beta Coefficient (Standard Error)
Model I	
Age adjusted	-0.15 (0.002) †
Model II	
Age and MHVS adjusted	-0.15 (0.002) †
Model III	
Age, MHVS and all other variables adjusted	
Step 1	
+ Education level	-0.15 (0.002) †
Step 2	
+ Vascular risk factors	-0.15 (0.002) †
Step 3	
+ Cardiovascular disease	-0.16 (0.002) †
Step 4	
+ Depression	-0.15 (0.002) †
Step 5	
+ HbA1c and duration of diabetes	-0.15 (0.002) †
Model IV	
All variables in Model III	
+ Retinal venular width (CRVE)	-0.13(0.002) *

‡P≤0.001, †P≤0.01, *P<0.05

Table 64: Multivariate associations between retinal venular width (CRVE) and residuals of Faces test scores regressed on 'g' in women

CRVE	Faces
	Standardised Beta Coefficient (Standard Error)
Model I	
Age adjusted	0.12 (0.001) *
Model II	
Age and MHVS adjusted	0.13 (0.001) †
Model III	
Age, MHVS and all other variables adjusted	
Step 1	
+ Education level	0.14 (0.001) †
Step 2	
+ Vascular risk factors	0.14 (0.001) †
Step 3	
+ Cardiovascular disease	0.14 (0.001) †
Step 4	
+ Depression	0.14 (0.001) †
Step 5	
+ HbA1c and duration of diabetes	0.13 (0.001) †
Model IV	
All variables in Model III	
+ Retinal arteriolar width (CRAE)	0.13(0.002) *

‡P≤0.001, †P≤0.01, *P<0.05

Table 65: Summary of multiple linear regression analyses for retinal arteriolar and venular width with residuals of individual cognitive test scores regressed on 'g' as the dependent variables (Standardised Beta regression coefficients and standard errors)

Variables in Final Model	Logical Memory (men)		Faces (women)	
	B (SE)	η^2	B (SE)	η^2
CRAE	-0.13 (0.002) *	0.010	0.004 (0.003)	0.000
CRVE	-0.04 (0.002)	0.001	0.13 (0.002) *	0.01
Age	0.11 (0.01) *	0.012	0.06 (0.012)	
MHVS	0.04 (0.01)		0.06 (0.01)	
Education (increasing level)	-0.01 (0.07)		0.06 (0.07)	
√Alcohol intake	0.03 (0.03)		0.11 (0.04) *	0.012
Smoking status (smoker vs. non-smoker)	0.06 (0.11)		0.02 (0.10)	
Waist to hip ratio	0.03 (0.09)		-0.01 (0.71)	
Systolic blood pressure	0.01 (0.003)		0.06 (0.003)	
Total cholesterol	0.01 (0.06)		-0.02 (0.05)	
MI and/or Angina	0.09 (0.11)		0.02 (0.12)	
Stroke and/or TIA	-0.01 (0.06)		-0.003 (0.22)	
logDepression	-0.03 (0.19)		-0.04 (0.19)	
HbA1c	-0.07 (0.05)		0.03 (0.05)	
√ Duration of diabetes	0.09 (0.05)		-0.15 (0.05) †	0.019
Total adjusted R^2		0.026		0.031

‡P≤0.001, †P≤0.01, *P<0.05; Partial eta-squared (η^2) for CRAE, CRVE for diabetic retinopathy and variables significantly associated with cognitive outcome

5.6.4. Sensitivity analyses

When the analysis was limited to 954 subjects with an MMSE scores ≥24, CRAE was independently associated with Logical Memory in men (P<0.05), whereas CRVE was no longer associated with Faces in women (Table 66), suggesting that the latter association could at least in part be attributed to subjects with severe cognitive impairment.

In addition, for a few participants (n=14) who had advanced proliferative diabetic retinopathy with a grade of 81, arteriolar diameters can be narrowed mostly due to laser therapy. When the analysis was limted to 966 subjects not receiving laser treatment, the results were essentitally unchanged. The wider CRAE was independently associated with poorer Logicl Memory in men (P<0.05), whereas the wider CRVE was associated with better Faces test scores in women (P<0.05). Data were not shown in here.

Table 66: Multivariate associations between retinal vessel width and late-life cognition, and estimated lifetime cognitive decline (N=954, subjects with MMSE <24 were excluded)

	Logical Memory-CRAE (Male)	Faces-CRVE (Female)
	Standardised Beta Coefficient (Standard Error)	
Model I		
Age adjusted	-0.18 (0.01) ‡	0.08 (0.01)
Model II		
Age and MHVS adjusted	-0.16 (0.01) ‡	0.12 (0.01) †
Model III		
Age, MHVS and all other variables adjusted		
Step 1		
+ Education level	-0.17 (0.01) ‡	0.12 (0.01) †
Step 2		
+ Vascular risk factors	-0.17 (0.01) ‡	0.13 (0.01) †
Step 3		
+ Cardiovascular disease	-0.17 (0.01) ‡	0.13 (0.01) †
Step 4		
+ Depression	-0.16 (0.01) ‡	0.13 (0.01) †
Step 5		
+ HbA1c and duration of diabetes	-0.16 (0.01) †	0.11 (0.01) *
Model IV		
All variables in Model III		
+ Retinal fellow vessel width (CRVE/CRAE)	-0.12(0.02) *	0.11 (0.01)

‡P≤0.001, †P≤0.01, *P<0.05

5.7. Chapter summary

The main aim of this chapter was to present data from the sample of 1,044 Edinburgh Type 2 Diabetes Study subjects who underwent both cognitive function assessment and diabetic retinopathy grading. A comparison of socio-demographic and biomedical characteristics showed that subjects with diabetic retinopathy were slightly more likely to be male, had a longer duration of diabetes and a corresponding worse vascular risk factor profile compared with those without diabetic retinopathy. After age and sex adjustment, subjects with diabetic retinopathy had poorer mean scores on 'g' and on most of the individual cognitive tests. When analysed by severity of diabetic retinopathy (none, mild, and moderate-severe), these cognitive measures showed a significant relationship with increasing severity of diabetic retinopathy. Those with moderate-severe diabetic retinopathy had poorest performances on general cognitive ability factor 'g', which was mainly due to poorer scores on the individual Verbal Fluency, Faces, Trail Making and Digit Symbol tests.

In the total sample, after adjustment for MHVS to estimate lifetime cognitive decline, a trend toward worse performance across diabetic retinopathy severity categories remained significant for 'g' and the four individual cognitive tests. In multivariate analyses adjusting for other possible confounding factors (including vascular risk factors and prevalence of macrovascular disease), there was a significant interaction between diabetic retinopathy and sex for both the general factor score and the Verbal Fluency Test but not for other tests. Severity of diabetic retinopathy remained significantly associated with MHVS-adjusted scores for the Trail Making, Faces and Digit Symbol tests and, in males, for 'g' and the Verbal Fluency test. The multivariate associations of diabetic retinopathy with DST persisted after additional adjustment for HbA1c and duration of diabetes. In males, the associations of diabetic retinopathy with g and VFT remained. When a sensitivity analysis was performed excluding subjects with a history of cerebrovascular disease, the associations of diabetic retinopathy with 'estimated' lifetime cognitive decline were essentially the same.

Further analyses showed that, the associations with estimated cognitive

decline in all specific cognitive measures except for Digit Symbol test, principally attributed to the relationship between diabetic retinopathy and general cognitive ability. It was likely that the diabetic retinopathy-DST association resulted from this individual function per se.

For quantitative parameters of retinal vascular network geometry, both increased arteriolar and venular width were significantly associated with lower scores on Logical Memory in men, whereas they were associated with higher scores on Faces in women after adjustment for age and prior cognitive ability. When further adjusted for education, vascular risk factors, cardiovascular disease, depression and hyperglycaemia, the association of CRAE with Faces became non-significant whereas others remained statistically significant. After additional adjustment for the fellow vessel, increased CRAE was independently associated with poorer performance on Logical Memory in men and increased CRVE continued to exert independent and positive effects on Faces scores in women. When a sensitivity analysis was performed excluding subjects with an MMSE<24, the association of CRAE with Logical Memory was not altered, whereas the association of CRVE with Faces was attenuated beyond statistical significance, suggesting the latter association could at least in part be attributed to subjects with severe cognitive impairment. These associations could also attribute to the associations of retinal vascular width with non-general cognitive ability component. Other parameters did not show any significant associations with cognitive tests.

Chapter 6

Discussion

6.1. Introduction

In this chapter the main findings from the study are discussed. Findings are considered in relation to results from other published studies. General issues on the methodology and the analysis are addressed, and potential neuropathological mechanisms underlying the association between cognitive dysfunction and cerebral microvascular disease in diabetes are proposed.

6.2. Summary of main findings

6.2.1. Diabetic retinopathy and cognition

Diabetic retinopathy is the most common and specific microvascular complication of diabetes. Over and above its adverse impact on vision, the present study demonstrated that retinopathy signs were associated with greater estimated age-related decline in neuropsychological functioning in people with Type 2 diabetes. Subjects with diabetic retinopathy performed worse on the majority of cognitive tests undertaken compared with those without. These cognitive measures also showed a statistically significant relationship with increasing severity of diabetic retinopathy (none, mild, and moderate-severe). In multiple linear regression analyses, there was a statistically significant trend in cognitive function across severity of diabetic retinopathy for information processing speed and, in men, for general cognitive function and word fluency, independent of prior cognitive ability and socio-demographic factors, cardiovascular risk factors, macrovascular disease and hyperglycaemia. Diabetic retinopathy explained 1 to 2 % of the variation in these cognitive measures. As there were a limited number of subjects with proliferative diabetic retinopathy in the ET2DS, it is likely that the findings were heavily influenced by the cognitive experience of subjects with mild to moderate diabetic retinopathy. Further analyses examining the association between retinal

microvascular abnormalities and estimated cognitive change after controlling for performance on the general cognitive factor revealed that the association of diabetic retinopathy with cognition was principally explained by an association with general mental ability.

6.2.2. Retinal vascular network geometry and cognition

Larger retinal arteriolar and venular calibres were statistically significantly associated with lower scores on a test of verbal memory but not with the results of other tests in men. Multiple linear regression analyses demonstrated that larger retinal arteriolar calibre was statistically significantly associated with verbal memory (accounting for 1% of the total variance) even after adjustment for pre-morbid cognitive ability and once possible confounding by retinal venular calibre, vascular risk factors and vascular disease was taken into account. In contrast, findings did not support an independent association between retinal venular calibre and estimated cognitive change. The association of retinal arteriolar calibre with decline in verbal memory was not related to an association with general mental ability but was attributable to an association with this specific cognitive function itself. Parameters of retinal arteriolar bifurcation geometry were not associated with cognitive outcome.

6.2.3. Gender-specific findings

A striking finding was the significant interaction of diabetic retinopathy with gender. The negative associations of diabetic retinopathy with verbal fluency and general cognitive ability were statistically significant only in men. A non-significant trend towards a positive association between severity of diabetic retinopathy and verbal fluency was observed in women. This sex difference was not anticipated a priori and previous research has not examined whether gender modifies the relationship of diabetic retinopathy with cognition. There are also limited previous data comparing diabetes as a risk factor for cognitive dysfunction in men versus women. Despite a prior hypothesis that Type 2 diabetes may be a more potent risk factor for cognitive dysfunction in women than in men, given their higher risk of diabetes-related cardiovascular disease complications (Okereke et al., 2008), the existing literature generally supports the findings of similar relationships between

diabetes and cognitive function in men and women.

The sex-specific effect of diabetic retinopathy on cognitive functioning may be related to the fact that in the ET2DS, women were less likely to have diabetic retinopathy (29.7% vs. 35.1%, P=0.058), prevalent coronary heart disease (24.4% vs. 37.8%, P<0.001) and stroke and/or TIA (5.5 vs. 11.6, P<0.001) relative to men. However, adjusting for these macrovascular disease variables in the statistical models did not alter the sex-specific effects. It is also possible that other unknown risk factors or disease which were not examined in this analysis (e.g. carotid intima-medial thickness, estrogen use, physical activity), might have differed in male and female participants and could play a mediating role in such an association. Given the possible protective effects of estrogen on cognitive functioning in older women with Type 2 diabetes, it is also possible that use of hormone replacement therapy (HRT) by women in the ET2DS may be important. Estrogen may stimulate multiple neurotransmitters, enhance cerebral blood flow, act as an antioxidant and induce a highly significant increase in the morphological complexity of neurons associated with learning and memory (Coker and Shumaker, 2003). The degree to which these and other variables may help to explain sex-specific associations of diabetic retinopathy with cognitive function needs to be further explored. However, since there was no specific a priori reason for assuming that the association of diabetic retinopathy with cognitive function would be different between males and females, and given that there is no evidence from other studies suggesting such a gender difference in the general populations or in people with diabetes, caution is needed in interpreting the gender-diabetic retinopathy interaction until this finding is replicated in other large studies.

Another unexpected finding was the significant interaction of retinal vessel calibre with gender. The associations of both the increased retinal arteriolar and venular calibres with lower verbal memory scores were significant in men but not in women. On the other hand, larger retinal venular calibre was significantly associated with higher Faces test scores in women but not in men. This aspect has not been examined in previous studies and the reasons for these sex-specific effects are not

clear.

6.3. Comparison with previous studies, methodological issues and possible mechanisms

6.3.1. Diabetic retinopathy and cognition

Comparison with findings from previous studies

This is the first study to date that specifically addresses the association between retinal microvascular abnormalities and cognitive functioning in a large group of people with Type 2 diabetes. The consistency of the results with previous studies, including those on people with Type 1 diabetes and in the general populations is considered in detail below. However, comparison of results across studies is challenging given the methodological heterogeneity between studies including differences in the selection of population, the comparison group 'free' of disease used, the assessment and definition of diabetic retinopathy, the choice of cognitive measures and the approach to controlling for potential confounding factors. In addition, very few previous studies have used multi-field retinal photography and a detailed, validated grading system to evaluate diabetic retinopathy, or have comprehensively assessed cognitive change in a full range of major domains in relation to diabetic retinopathy in people with Type 2 diabetes.

Association with general cognitive ability

The present study used an approach for assessing general cognitive ability which is in line with current theoretical concepts of the hierarchical construct of human cognitive abilities (Rafnsson, 2006; Deary and Batty, 2007). General cognitive ability, as indexed by a general factor, was computed by subjecting individual mental tests to a principal component analysis and represented the variance common to the specific tests the ET2DS used. By contrast, of the limited number of previous studies on this topic, several have administered only the MMSE for assessing general mental ability. Such a global measure may not be well-suited

for this purpose (Morris et al., 1999). In particular, as a brief and summary measure of general cognitive function, the MMSE is likely to exhibit a ceiling effect (e.g. where the majority of values lie close to the maximum score of 30) when used in non-demented study populations. The MMSE is also relatively insensitive for the detection of subtle changes in cognitive function, such as those which might be expected in studies of cognitively-intact older people. In this context, the use of the MMSE may underestimate the true extent of cognitive impairment and decline.

Present findings of an inverse association between retinopathy severity and general ability scores are consistent with results from some but not all previous studies. In a sub-group of subjects with hypertension aged 49 years and older participating in the Blue Mountains Eye Study, Liew et al. (2009) reported that people with signs of retinopathy were significantly more likely to score below 23 on the MMSE. Retinopathy was defined as an ETDRS level of 15 or greater (presence of any, microaneurysms, haemorrhages, hard exudates, cotton wool spots or evidence of laser treatment for retinopathy). The analysis was cross sectional and change in MMSE performance over time was not assessed in relation to retinopathy status. The findings were based on a hypertensive sub-group and the associations were not found in people with diabetes or in the total study population. In cross-sectional analysis of the Cardiovascular Health Study, retinopathy was associated with lower mean score on the modified MMSE scale in people without stroke aged 69 to 97 years at recruitment. However, this crude association did not hold after adjustment for factors such as age, hypertension, diabetes and internal carotid intima-media thickness (Baker et al., 2007).

Studies whose results deviate from the present findings include a study by Umegaki et al. (2008), which reported no association between prevalent diabetic retinopathy and global cognitive dysfunction (MMSE<24) in elderly Japanese adults with diabetes. Also, in the Utrecht Diabetic Encephalopathy study, Manschot et al. (2007) found no significant difference in average performance on a composite cognitive score from five domains in a cross-sectional comparison of 122 older people with Type 2 diabetes with and without retinopathy. In addition to the

discrepancy between these studies and the ET2DS in measures of general cognitive ability, other differences between the studies may have contributed to the different findings. For example, the diagnosis of diabetic retinopathy was either based solely on a direct clinical ophthalmoscopic examination (Umegaki et al., 2008) or a single-field retinal photograph (Manschot et al., 2007), methods which are imprecise and less reliable compared with 7-field retinal photography (only imaging a small portion of the retina when compared with multiple field retinal photographs). Both previous studies may have underestimated the prevalence of retinopathy signs in older people with diabetes. The imprecision of retinopathy gradings may also have resulted in non-differential misclassification, which is likely to attenuate associations between retinal vascular abnormalities and cognition.

Association with specific cognitive ability

With respect to tests of specific cognitive domains, age and sex adjusted comparisons showed a significant trend with increasing severity of diabetic retinopathy for tests of non-verbal memory, mental flexibility, verbal fluency and information processing speed, with moderate to severe diabetic retinopathy groups having worst cognitive performance. Furthermore, severity of diabetic retinopathy was independently associated with greater estimated decline in information processing speed, and in men, for verbal fluency.

Findings were consistent with reports from five other studies, including those in people with Type 1 diabetes. Ferguson et al. (2003) reported an overall downward shift in the distribution of several test scores in relation to background diabetic retinopathy (presence of microaneurysms) in a cross-sectional analysis of 71 young people with Type 1 diabetes. The magnitude of the influence of diabetic retinopathy on cognitive performance was moderate to large (e.g. diabetic retinopathy explained about 7% of the total variance in information processing speed as measured by Digit Symbol test). By contrast, the effect sizes from the ET2DS did not exceed 2%. The smaller effect sizes in the ET2DS may reflect higher variability in cognitive performance among older patients with Type 2 diabetes. It is also possible that larger effect sizes observed by Ferguson et al. (2003) were caused by stringent selection of the ‘no retinopathy’ comparison group in which subjects had

less co-morbidity and, specifically, were free of other microvascular disease (e.g. neuropathy and nephropathy). The 'no retinopathy' group in the ET2DS was not necessarily 'microvascular disease-free'. For example, the comparison group included those who had 'possible diabetic retinopathy' (n=61) (i.e. the presence of other retinal vascular lesions including haemorrhages, hard exudates and cotton wool spots but in the absence of microaneurysms). Indeed, when the analyses were repeated after excluding those subjects, the associations of diabetic retinopathy with verbal fluency and information processing speed were slightly stronger. In addition, even those who did not have characteristics of diabetic retinopathy may have had other manifestations of systemic microvascular disease and this 'dilution' would have made differences between the groups less extreme, biasing the results toward the null and leading to possible underestimation of effect sizes.

In another set of analyses based on 103 young and middle-aged patients (mean age 40 yrs) with Type 1 diabetes, Ryan et al. (2003) demonstrated that the presence of proliferative diabetic retinopathy at baseline was associated with significantly greater decline in psychomotor efficiency over a follow-up of seven years. The cognitive domain of psychomotor efficiency was measured through the administration of multiple tests including the Digit Symbol test assessing information processing speed.

Although in the population-based Cardiovascular Health Study, Baker et al. (2007) did not find statistically significant multivariate-adjusted differences in mean global cognitive test scores between people with and without any retinopathy signs, the retinopathy groups did perform significantly worse on Digit Symbol test.

In the Atherosclerosis Risk in Communities (ARIC) Study based on a considerably larger sample than the ET2DS, Wong et al. (2002e) reported that retinopathy was independently associated with worse cognitive function on several tests in middle-aged people without stroke. Subjects with retinopathy had significantly lower scores on delayed verbal memory, executive function and information processing speed compared with retinopathy-free controls. These

associations were similar in people with and without diabetes and hypertension. Although differences in the measurement of verbal memory between the ARIC and the ET2DS need to be considered, these may not fully account for the discrepancy in findings (i.e. no such association was found in the ET2DS). In the ET2DS, verbal memory was assessed using immediate and delayed recall of one story (WMS-R Logical Memory Test) whereas Wong et al. (2002) administered the Delayed Word Recall Test, which is based on recalling a list of 10 words. It could be argued that recalling a list of random words (free recall) might pose different challenges for memory functioning than memorising a story which may have some cues in facilitating a recall (Rafnsson, 2006). Also, in the relatively healthier and younger cohort of the ARIC study, performance on cognitive tests is heavily influenced by prior mental ability level. Adjustment for differences regarding level of education in this study (Wong et al., 2002e) might not have provided adequate control for prior ability differences between subjects with and without retinopathy, given the potential wide variation of cognitive ability levels within the same education category. As a result, some residual confounding by prior ability level cannot be ruled out in the data by Wong et al. (2002e). Finally, of the associations found in a subgroup of subjects with diabetes in the ARIC study, lack of specific data regarding characteristics of diabetes (e.g. Type 1 or 2, duration of diabetes and glycaemic control) hinders further interpretation of the results in diabetic patients.

Subsequent findings from the 14-year follow-up of the ARIC study found that people with retinopathy showed a greater 10-year decline in verbal fluency and information processing speed compared with those without retinal microvascular abnormalities (Lesage et al., 2009). In contrast to the earlier cross-sectional associations (Wong et al., 2002e), there was no association of retinopathy with decline in delayed verbal memory. This may reflect different measures of cognitive change over time (actual measured change versus estimated change using a single time point). Longitudinal analysis of cognitive change tends to reduce the influence of extrinsic potential confounding factors (e.g. education) that cannot be completely accounted for with multivariate modeling in cross sectional studies. It is also suspected that the Delayed Word Recall applied in the ARIC study is a test of

episodic memory (a form of memory in which information is stored with 'mental tags') which is more typically affected in Alzheimer's disease rather than in cognitive impairment of vascular origin (Fleisher et al., 2007). Indeed, no significant association with verbal memory was found in the ET2DS and the pattern of cognitive decline associated with diabetic retinopathy was, in general, more indicative of vascular dementia.

In the ET2DS the associations of diabetic retinopathy with decline in specific cognitive abilities were largely attributed to the relationship of diabetic retinopathy with general cognitive ability rather than with the individual component tests per se. An exception was the DST (measuring information processing speed), with which diabetic retinopathy remained associated after controlling for the influence of the general factor. This implies that the DST might have variance in common with the general ability factor, but it could still have a different developmental trajectory such as a unique retinopathy-related influence, which is similar to that sometimes found with ageing (Salthouse, 1994). Given that DST is not only a measure of psychomotor speed, but also working memory (Joy et al., 2004), it is also possible that the assumption of non-overlap between component/factors in using principal component analysis without orthogonal rotations could be violated. In this case, non-orthogonal rotations may provide a better or informative model.

DST is a paper-pencil based test in which subjects are asked to translate numbers into symbols using a key in 120 seconds. Preliminary analysis in the ET2DS demonstrated that diabetic people with retinopathy were more likely to have peripheral neuropathy as measured by an increased vibration perception threshold at the great toe compared with those without retinopathy (55.2% vs. 45.8%, $P<0.05$). It is possible that people with diabetic retinopathy also had some sensorimotor difficulties related to neuropathy, which could have affected their performance on the DST. Specific information on functional impairment or disabilities, such as limited joint mobility or the presence of arthritis in the hands was not collected in the ET2DS. However, adjustment for neuropathy in a post hoc analysis did not alter the observed associations between DST and retinopathy. Whether unrecognised sensorimotor

dysfunctions mediated the association to some extent remains unclear.

Severe diabetic retinopathy, including maculopathy and proliferative retinopathy, can produce visual impairment, in particular affecting near visual acuity and resulting in reading difficulties. This could have influenced performance on the DST. However, this was unlikely to account for the associations reported here given that participants who had corrected visual acuity worse than 6/36 for distance vision or inability to read large print text were excluded. Subjects with poor visual acuity performed the DST with glasses and there was no significant difference in corrected near visual acuity between people with and without diabetic retinopathy. The DST-specific effects remain difficult to interpret until the DST can be grouped with other processing speed tests (e.g. inspection time and reaction time) to probe this cognitive domain.

Methodological issues

Confounding

A number of factors may confound the association between diabetic retinopathy and cognitive dysfunction in people with Type 2 diabetes. If not adequately controlled for, these confounding factors may give rise to spurious results. In particular, possible confounding by prior cognitive ability has been sufficiently taken into account in very few previous studies. Where attempted, adjustment has often been made for education or social class status, variables which may be influenced by factors such as lack of opportunity rather than lack of ability and which exhibit wide variation of ability levels within the same category. Statistical adjustment for differences in these characteristics may not adequately control for subject's prior level of mental ability. In contrast, findings in the ET2DS were unlikely to be explained solely by differences in prior cognitive ability between subjects with and without diabetic retinopathy because vocabulary ability assessed by using MHVS is preserved even in generalised cognitive decline.

The measurement of a wide range of diabetes-specific characteristics, conventional vascular risk factors and macrovascular diseases further allowed the

examination of the independence of association between severity of diabetic retinopathy and cognitive function in relation to these parameters. In particular, subjects with diabetic retinopathy had significantly longer duration of diabetes, higher mean HbA1c level, and greater waist-to-hip ratio and were more likely to suffer from cardiovascular disease, especially cerebrovascular disease, compared with those without diabetic retinopathy. The findings that retinopathy remained significantly associated with estimated cognitive decline after adjustment for these factors suggests that the association may depend on mechanisms resulting in cerebral small vessel disease eventually leading to insidious ischaemia and injuries in brain parenchyma rather than on any other concomitant influences such as hyperglycaemia and macrovascular disease.

The transient deleterious effects of acute hypoglycaemia are also unlikely to account for the associations between diabetic retinopathy and cognitive function, because blood glucose was measured before cognitive testing to ensure that subjects were not suffering from unrecognised hypoglycaemia.

Bias

There were twenty-two subjects who had cognitive testing but did not provide diabetic retinopathy status data mostly due to unavailable retinal photographs (non-participation). When comparing the characteristics of participants who had gradable retinal photographs to those not graded, those not graded were more likely to have prevalent stroke and/or TIA, and performed significantly worse on non-verbal memory, verbal memory and general cognitive ability. Given that the differences between the graded and ungraded groups in these cognitive abilities were moderate to large, this raises the possibility of selection bias. If those who did not have gradable retinal photographs had severe diabetic retinopathy, then the results presented here will have been biased towards the null or underestimating effect sizes. Because subjects with missing retinal data had a relatively higher proportion of cerebrovascular disease (13.6% vs. 8.6%) and neuropathy (72.7% vs. 51.2%), these subjects may well have had more severe diabetic retinopathy. Nonetheless, it is unlikely that this selection bias had a large impact on the results given that only 2% of subjects had missing data on diabetic retinopathy status.

Grading of diabetic retinopathy was performed independently by two optometrists according to a validated standardised classification system. It should be noted that graders assessed retinal vascular lesions with overlapping fields, not following the stringent field definitions (although the deviation from the field definition was noted for each field and was measured in terms of disc diameters). This may have led to the over-grading of images given that lesions could be counted in more than one field. This is more of a problem in the higher grades, only accounting for a very small proportion of subjects (Preston, 2008). However, to the extent that the diabetic retinopathy severity category included 'false severe' cases, any misclassification of cases would have reduced the observed associations towards the null value and have underestimated the effect sizes.

Possible mechanisms

In the early stages of diabetic retinopathy, a spectrum of retinal vascular signs such as microaneurysms, haemorrhages, cotton wool spots and hard exudates, result from microvascular closure and loss of vascular integrity. With increasing severity, retinal venous beading and intra-retinal microvascular abnormalities may develop, which usually precede frank new vessel formation and the onset of proliferative diabetic retinopathy. In addition to a break-down of the blood-retinal barrier, these ischaemic processes are likely in turn to damage the neural retinal tissues supplied.

As the retinal and cerebral microvasculature share common anatomy, embryology and physiology, these retinal vacular changes may provide an indirect marker of similar or concomitant changes in the brain microvasculature. As discussed in detail later, it is possible that instead of circumscribed vascular lesions, a disruption of the blood brain-barrier, and/or ischaemia from more generalised and widespread cerebral microvascular disease may lead to disruption of complex, intricate neural networks underlying general cognition by affecting the functional connectivity of different cerebral regions. More specifically, ischaemic changes disrupt the structural integrity of white matter tracts and subcortical gray matter,

which are essential for intact inter-regional communication of cortical and subcortical grey matter areas. As a result, they may affect not only the functional capacity of the white matter but also the processing ability of neuro-cognitive networks such as frontal-subcortical circuits, most notably leading to deficits in executive function and processing speed (Jokinen et al., 2006; Rafnsson, 2006; Verdelho et al. 2007).

As the findings were not attenuated after excluding subjects with recognised clinical stroke and/or TIA, perivascular brain damage and/or ischaemic injuries from unrecognised or asymptomatic cerebral small vessel disease (SVD) would appear to be a plausible explanation for the cognitive associations presented here.

The association between diabetic retinopathy and estimated decline in specific cognitive tests was largely mediated by the effects of diabetic retinopathy on the general cognitive factor. This suggests that those neurocognitive processes represented by general ability may be more susceptible to cerebral mcirovascular pathology. The additional effect on processing speed may be at least partially attributable to demyelination of neurons in relation to cerebral microvascular disease associated with Type 2 diabetes (Deary, 2000).

6.3.2. Retinal vessel calibre and cognition

Changes in retinal vessel calibre have been relatively understudied in cognitive ageing epidemiological investigations, in particular in diabetic patients. Three quantifiable parameters of retinal vessel calibre were examined in the ET2DS including the central retinal arteriolar equivalent (CRAE), central retinal venular equivalent (CRVE), and the arteriovenous ratio (AVR).

Comparison with findings from previous studies

The finding of an association between retinal arteriolar dilatation and lower scores for verbal memory has not been previously reported, although one larger population-based study found that retinal venular dilatation was associated with

lower MMSE scores (Liew et al., 2009). Findings in the ET2DS contrast with the majority of published evidence which did not find a significant association between any parameters of retinal vessel calibre and cognitive test performance (Wong et al., 2002e; Baker et al., 2007; Patton et al., 2007; Lesage et al., 2009). For example, in the ARIC study with a considerably larger sample than the ET2DS, no association was found between the AVR and risk of cognitive impairment in delayed components of verbal memory, information processing and verbal fluency (Wong et al., 2002e). However, as a ratio, the AVR is a crude summary measure. If both the CRAE and CRVE associate with cognition in the same direction as shown in the ET2DS, no relationship would be detected. Another possible reason for the lack of association seen in population-based studies is that retinal arteriolar dilatation might be a specific indicator of diabetes-related microvascular dysfunction, but not a physiological marker in non-diabetic people.

Methodological issues

Confounding

The association between retinal arteriolar dilatation and worse verbal memory is unlikely to be explained by confounding by differences in levels of mood, vascular risk factors or cardiovascular disease. Higher retinal arteriolar calibre was significantly associated with higher depression scores and the presence of coronary heart disease. Mean CRAE was raised in current smoker and lower mean CRAE was associated with the presence of hypertension. However, the finding of an independent relationship between increased CRAE and lower verbal memory scores suggests that, at least to a degree, the association may be due to underlying cerebral microvascular pathology rather than the concomitant influences of vascular risk factors and disease.

When excluding subjects with an MMSE scores<24, retinal venular calibre was no longer associated with Faces test scores in women. It is possible that the association could be mediated by subjects with severe cognitive impairment. After the exclusion of participants with prevalent stroke and/or TIA, the association between the CRAE and verbal memory became attenuated and lost statistical

significance. However, whether this was due to lack of statistical power or indicates that stroke plays an intermediate role remains uncertain.

In contrast to the associations of diabetic retinopathy with several of the cognitive tests, an association was found between retinal vessel calibre and memory function alone. However, no association between diabetic retinopathy and verbal memory was found. It is possible that before the onset of retinopathy, retinal arteriolar dilatation in diabetic subjects may contribute to variation in cognitive abilities. Although the data were not presented in this thesis, additional adjustment for diabetic retinopathy in analyses did not alter the significant association between CRAE and verbal memory. On the other hand, the ET2DS population comprised older diabetic patients without dementia. Memory is often the first domain of cognition that is affected in subjects with mild cognitive impairment, with otherwise relatively preserved cognitive and functional abilities (Nordahl et al., 2005; Patton, 2006). Furthermore, there may have been a relatively greater degree of variation of logical memory scores, compared with other cognitive domains tested in the present study, which may have increased the sensitivity to detect an association (Patton, 2006). After examining the coefficients of variation for each of the tests, this is possible but could not account fully for the observed association.

An important concern in controlling for anatomy and measurement error using the fellow vessel calibre is the potential for overadjustment when both are associated with cognition in the same direction. As there is a large proportion of shared variance between the CRAE and CRVE, and both are related to verbal memory, they may each cancel out the effect of the other, and both would have dropped out of the equation. Furthermore, there are other factors that were not controlled for that may influence retinal vessel calibre such as genetic, ocular (e.g. axial length) and other cardiovascular risk factors (e.g. inflammation markers). By adjusting for the fellow vessel, effects of other ‘non-measurable’ factors are negated.

Bias

Of the 1,044 subjects who had gradable retinal photographs for diabetic retinopathy status, 64 subjects had retinal images of insufficient quality in either eye

to make retinal vessel calibre measurements. This was mostly due to the high prevalence of medial opacities in this older group as well as technical reasons for failure to obtain the 6 measurable largest retinal arterioles or venules. When comparing the characteristics of participants included in this analysis with those excluded (no retinal photographs or ungradable retinal vessels), those excluded were more likely to have prevalent stroke and/or TIA, and performed significantly worse on non-verbal memory, information processing speed and general cognitive ability. The magnitude of the differences in these cognitive abilities between the analysed and un-analysed individuals (for retinal vessel calibre) was small to moderate. However, if people with greater variation in retinal vessel calibre (very high or low values) were excluded, and also had lower cognitive test scores on these domains, then the results presented here will have been biased towards the null or underestimating effect sizes, or possibly even shown associations in the opposite direction.

Before retinal photography, pharmacologic pupillary dilatation of both eyes was performed and a very low percentage of photographs (6.1%) were ungradable in the study. A semi-automated computer assisted method was used to measure retinal vessel diameters from digital retinal images with high resolution. Reliability of these retinal vessel measurements in the study was substantial, with intra- and inter-grader intra-class correlation coefficient ranged from 0.67 to 0.95.

In all ET2DS participants, retinal images from one randomly selected eye or the eye with the best image quality were measured. Findings from population-based epidemiological studies have demonstrated substantial correlation between right and left eyes for retinal arteriolar (Pearson correlation coefficients ranged from 0.70-0.72) and venular calibre (0.74-0.77), and moderate correlation for the AVR (0.49-0.64) (Leung et al., 2003b; Couper et al., 2002; Wong et al., 2004a). Slightly lower correlations for arteriolar calibre than for venular calibre are likely to reflect a greater precision when measuring venular calibre given the better and more distinct contrast between the retinal background and the venular blood column than the arteriolar blood column. As expected, lower correlation was found for the AVR as a ratio

exaggerates the errors of its components (Wong et al., 2004a). Although a high correlation between eyes would indicate that measurement of retinal vessel calibre in one eye might be adequate for studies evaluating the systemic associations of retinal vascular calibre, there is a potential for loss of statistical power to detect such associations due to lower precision in vessel calibre data compared with averaged measurements in two eyes. When measurements from both eyes are taken into account, there would be less of the total variation caused by measurement error (e.g. R^2 increased in the regression models that used the mean calibre of two eyes) (Wong et al., 2004a). Thus measuring one eye may have diminished the chances of finding significant associations, particularly if they are small.

Wong et al. (2004a) found that women had poorer correlations in retinal vessel calibre measurements between eyes than men. These differences may reflect greater variability of measurement in women, as a result of variations in biological and physiological factors, such as blood pressure, the cardiac cycle and other ocular disease. It is possible that measurements from one eye in women are more influenced by measurement error and such non-differential error would have tended to dilute associations toward the null. This may, at least in part, explain why an interaction was observed between retinal vessel calibre and gender and a significant association with cognition was found only in men in the ET2DS.

Retinal vascular calibre may change in an individual, even over a short period-for example, with the pulse cycle. Thus, taking photographs at untimed points in the dynamic pulse cycle may result in further random variation in the measurement of retinal vessel calibre. Such non-differential misclassification may also have masked or attenuated the associations towards null.

Possible mechanisms

Arteriolar dilatation is considered to be a sign of impaired arteriolar autoregulation in diabetes, which has been suggested to play an important role in the initiation and progression of diabetic retinopathy (Gardiner et al., 2007). Experimental studies indicate that arteriolar dilatation and the associated increase in

retinal blood flow are frequently found in the retinas of people with diabetes, which may reflect underlying arteriolar autoregulation dysfunction (Gardiner et al., 2007). This could be due to hyperglycaemia and hypoxia mediated endothelin-1 resistance and calcium-influx channel inhibition in smooth muscle cells. These processes could impair retinal arteriolar constriction. In turn, hyperperfusion could exacerbate retinal arteriolar dilatory response by a drop in retinal oxygen tension from capillary non-perfusion (Gardiner et al., 2007). On the other hand, retinal venular dilatation may represent a later sign of such haemodynamic abnormalities which possibly reflect a direct consequence of arteriolar dysfunction (Gardiner et al., 2007). Another possibility is that hypercapnia or elevated partial pressure of carbon dioxide in systemic arterial blood, may increase retinal blood flow as a result of retinal vasodilatation (Schmetterer and Polak, 2001). Because there is no reason to assume that participants were retaining carbon dioxide, this is unlikely to have a major effect on retinal arteriolar dilatory response. Based on these observations, retinal arteriolar dilatation could be a general marker of retinal ischaemia and by proxy cerebral ischaemia. It is also possible that retinal arteriolar dilatation in some way reflects cerebral arteriolar abnormalities in diabetes.

In this context, cerebral microvascular disease associated with diabetes may play a role in the episodic memory impairment characteristics of mild cognitive impairment (Nordahl et al., 2005). Findings from a number of epidemiological studies have demonstrated that memory is not solely dependent on the hippocampus and medial temporal lobe region in the brain, but that multiple cortical-subcortical circuits may be involved (Ungerleider, 1995; Nyberg et al., 1996; Tulving and Markowitsch, 1997; Nordahl et al., 2005; Buffon et al., 2006). It is possible that ischaemic injuries from cerebral small vessel disease may compromise frontal-lobe executive control processes that are critical for working memory (the ability to hold and manipulate information mentally as a component of executive function), which may in turn result in episodic memory (information encoding) deficits (Nordahl et al., 2005).

Recently, the cognitive profile in patients with CADASIL (the archetypal model of pure cerebral microangiopathy) showed that memory was affected in

approximately 70% of subjects, illustrating the role that cerebral small vessel disease may have on changes in memory (Buffon et al., 2006; Patton, 2006). CADASIL is a rare autosomal dominant microangiopathy, resulting from defects in the Notch3 gene. Notch3 encodes a cell surface receptor that is exclusively expressed in vascular smooth muscle cells and pericytes (Patton et al., 2005). Histopathological examinations have shown loss of vascular smooth muscle cells with perivascular fibrosis but with no evidence of vascular occlusion in both cerebral and retinal arterioles (Patton et al., 2005). Interestingly, the pathological changes of retinal arterioles in CADASIL are similar to the observations in diabetes, which lends further support to the relatively unappreciated role of arteriolar dysfunction in cerebral microvascular disease associated with diabetes.

6.3.3. Retinal arteriolar bifurcation geometry and cognition

Retinal arteriolar bifurcation geometry including median bifurcation angle (BA), median branching coefficient (BC) and suboptimal angles has not been explored in people with diabetes. In the ET2DS, none of the three parameters of arteriolar bifurcation were related to any measures of cognitive function.

Comparison with findings from previous studies

Null results in the present study are in conflict with cross-sectional findings from the Lothian Birth Cohort (LBC) 1921 study (Patton et al., 2007), the only other available investigation in non-demented elderly population. Patton et al. demonstrated that deviation of the median BC from optimality was significantly negatively associated with general cognitive ability and verbal fluency, whereas deviation of the angle at arteriolar bifurcation from optimality was significantly associated with verbal memory (Logical memory), with effect sizes ranging from 2% to 3%. Apart from differences in sample structure and disease profile, the discrepancy between the ET2DS and the LBC may also be partly attributed to the different methods in quantifying these retinal bifurcation parameters.

The ET2DS used a 35^{o} retinal image with optic disc in the centre to evaluate the parameters on at least 3 measurable arteriolar bifurcation angles. Using multiple

numbers of branching point measurements from one individual image has been shown to provide a more reliable estimate (compared with one single angle measurement) and to minimise a great deal of variation between different images (Patton et al., 2006). However, Patton et al. (2007) measured the median value from the five most proximal arteriolar bifurcations, which represented the first bifurcation of the arterioles from the optic disc margin. In the ET2DS, measurements were not restricted to the most proximal bifurcations but often extended to those which could most easily be evaluated given that on many occasions it was difficult to detect at least three arteriolar angles in the whole field of a retinal image. Although it has been shown in a healthy normotensive population that the calculated branching coefficient from peripheral arteriolar junctions was not different from more central arteriolar junctions (Patton et al., 2006), the relationship between bifurcation angles and degree of eccentricity remains uncertain. Using an animal model (isolated arterioles examined in vivo from cremasteric muscle) to evaluate actual measured angles, Frame and Sarelius (1993) found that arteriolar bifurcation angles vary systematically with position for sequential branches and that angles change with flow. It is possible that the three angle measures in the present study are subject to greater variability due to the random position of arteriolar bifurcations and their limited number.

Despite choosing the images with the best quality from either eye, in about 55% of the images, the number of measurable arteriolar bifurcations did not reach the required minimum of 3. The narrower field of view prohibited the observation of more arteriolar angles than were seen in the larger field (e.g. 45^{o}) of Patton's study. In addition, a semi-automatic method of measurement was applied, which was technically more demanding compared with the manual estimation used by Patton et al. (2007). However, this could be more accurate than the manual estimation given that identification of the three midlines in trunk and branch vessels is heavily dependent on arbitrary selection of the six edge points by the grader (Patton et al., 2007).

Methodological issues

Intra-grader reliability for arteriolar bifurcation angles and branching coefficient was moderate to high (intra-class correlation coefficient of 0.72 and 0.53). For inter-grader reliability, the intraclass correlation coefficient for branching coefficient was 0.69 and the Bland-Altman plot demonstrated a large degree of variability with a coefficient of repeatability of 0.7. This was considered moderately poor, in view of the small range of scale of the branching coefficient in this cohort. There was no correlation for angle measurements between the two graders. Although it should be noted that each median angle measurement reflects the three arteriolar junctions, and therefore will not solely reflect individual angle measurement difference but also the differing choice between individuals as to which are the three measurable junctions, this finding seriously questions the usefulness of this semi-automatic measure in epidemiological studies.

Within one junction measurement, the manual selection of the central bifurcation position as starting point is subjective and also variable between individual graders. It has been noted that the precise location of the centre of the bifurcation is important for angle calculation. One pixel of difference in angle vertex position can result in two to three degrees of difference in the calculated angle (Gao et al., 2000). More recently, Hughes et al. (2009) has developed a new method, in which the initial estimate provided by the grader acts only as a first step in the determination of the centre of the bifurcation and will be further refined by a computer programme in the subsequent steps. It could be more objective and efficient using such an automatic process compared with the semi-automatic method used in the ET2DS.

In all of the remaining 980 subjects who had gradable images for retinal vessel calibre measurements, 536 subjects had retinal images either of too poor quality to analyse all three arteriolar junctions or less than three arteriolar junctions were evaluable within the 35^{o} field. The high proportion of missing data for arteriolar bifurcation angle and branching coefficient raises concerns about selection bias. When comparing the characteristics of participants included in this analysis with those excluded (no retinal photographs or ungradable retinal arteriolar bifurcations),

those excluded were more likely to have prevalent coronary heart disease, stroke or TIA, and performed worse on all cognitive tests. However, differences between the graded and ungraded groups in these cognitive abilities were small.

6.4. General limitations

Several general study limitations deserve mentioning in relation to the findings presented above. Analyses were cross-sectional, limiting the ability to determine the temporal sequence of the associations reported. Lifetime imputed cognitive decline was estimated by including the MHVS as a covariable in analyses. However, to date only the difference between the NART and scores on the Raven's Standard Progressive Matrices (non-verbal general fluid reasoning) has been validated as a measure of lifetime change in cognitive function (Deary et al., 2004a). It is likely that the MHVS might not have adequately reflected the test components represented by other neuropsychological measures that were used in the study. The ET2DS is an ongoing four-year follow-up study and the actual cognitve change or decline from the baseline performance may provide a more objective outcome measure.

Associations may have been attenuated by potential survival bias. Diabetic retinopathy and severe cognitive impairment have been shown to be related to mortality in the diabetic population (McGuire et al., 2006). If people with more severe retinal microvascular abnormalities died, and also had worse cognitive test scores, those still living would have represented neither the most severe retinal vascular disease nor the most extreme cognitive ability scores. The original study cohort was recruited from the pool of surviving diabetic patients on the Lothian Diabetes Register. Participants in the ET2DS were less likely to be socially deprived and had slightly lower mean systolic blood pressure compared with non-participants. Other characteristics, including age, median duration of diabetes, total cholesterol, HbA1c and treatment status (percentage on insulin) were similar in the two groups. Thus, the ET2DS original sample was reasonably representative of the target population.

The cognitive test battery used in the present study included widely-used, validated measures of major components of human cognitive function which are known to be sensitive to the effects of biological ageing or neurological insults by diabetes. However, only one single test was used for assessing any particular cognitive domain. As a result, it is possible that only some or limited aspects of what may be considered complex mental functions were actually determined (Lezak, 1995). For example, executive functioning was assessed only by a single brief task of word fluency. It is possible that this measure itself did not sufficiently reflect the complexity and importance of this particular cognitive domain (Rafnsson, 2006). In addition, as a measure of high level of cognitive ability, digit symbol test is an obscure 'package of information processing speed' with a number of different fundamental components, such as nerve conduction velocity, inspection and reaction time. It may be more helpful for understanding age or diabetes-related variances in cognitive function if these specific processing parameters of the brain are assessed individually. For example, simpler reaction time indices have been shown to play an intermediate role in the association between white matter abnormalities (i.e. water diffusion tensor parameters) and higher level cognitive test scores associated with ageing (Deary et al., 2006).

Some potential confounders of the retinopathy-cognition associations presented were not taken into account in the multivariate analyses, including medication history and other ocular factors (e.g. intraocular pressure). As a consequence, the possible confounding of the associations reported herein by medication use, where diabetic patients with retinal microvascular abnormalities were more likely to be taking medication with an effect on interfering with cognitive performance, cannot be ruled out.

As many statistical tests were performed, it is possible that a type I error (incorrect rejection of a true null hypothesis) may have occurred i.e. some of the findings may be due to chance despite being statistical significant. Use of the commonly applied Bonferroni corrections to reduce the p-value taken as statistically

significant would have been inappropriate because the cognitive test variables were not independent (Deary et al., 2006). In addition, use of Bonferroni corrections may lead to additional problems, including increased likelihood of a type II error (incorrect acceptance of a null hypothesis when the alternative is true) (Rafnsson, 2006). The aim of the study was to investigate both overall cognitive ability and specific cognitive domains individually and so consider the role of individual associations in their own right (e.g. biological plausibility), rather than addressing a universal null hypothesis, i.e. where the null hypothesis states that there is no difference between groups for any of the variables under study. The relationships examined were specified as a priori thus minimising the number of possible analyses. The possibility of a type I error was further decreased by data reduction in generating a general cognitive factor.

In some instances, associations became stronger after controlling for potential confounding variables. While this may have occurred by chance, in some instances it may reflect the complexity of the associations between small vessel disease and cognitive outcomes in Type 2 diabetes. Findings from the stratified analyses should be interpreted with caution, as they were not specified a priori and there may be statistical power concerns following stratification.

Despite the exclusion of patients with overt dementia or AD, it is possible that some clinically cognitively impaired patients might have been included in the study. Only about 2.8% of the 1066 subjects scored less than 24 on the MMSE, a widely used cut-point for severe cognitive impairment. Impaired performance on the MMSE was not related to diabetic retinopathy status or to quantitative retinal vascular parameters. In sensitivity analyses, after excluding these patients, the significant associations highlighted and discussed were essentially unaltered.

6.5. Possible neuropathological mechanisms of cerebral microvascular disease in cognitive dysfunction in diabetes

To date, information on the microvascular contributions to cognitive dysfunction has arisen primarily from pathologic and brain imaging studies. The lack of structural neuroimaging in the ET2DS hindered the further interpretation of the potential neuropathological substrates underlying the relationships which were found. On the other hand, there are still no accepted standards for the lesion load on imaging that can explicitly characterise a person's cognitive dysfunction as vascular origin (Jellinger, 2007). However, it is likely that cerebral microvascular disease associated with diabetes, can induce diverse changes affecting fibres and tracts connecting eloquent cortical and subcortical grey matter areas, either visible or invisible on standard MRI (e.g. microinfarcts detectable only by direct microscopic examination of the brain), eventually leading to disruption of normal neurocognitive processes. Furthermore, a disintegration of the cerebral microcirculation's blood-brain barrier may be an important pathophysiological mechanism in the occurrence of cognitive dysfunction in Type 2 diabetes.

6.5.1. Neuropathological substrates

Cerebral microvascular disease accounts for about a third of acute cerebral ischaemic events (i.e. transient ischaemic attack or stroke) and contributes to the development of asymptomatic ischaemic injuries of the brain (Grau-Olivares and Arboix, 2009). These clinically overt or 'silent' lesions and their manifestations predominantly involve subcortical gray matter and white matter structures. The spectrum of the lesion loads seen on brain imaging includes lacunar infarcts, white matter lesions and microbleeds.

Lacunar infarcts

In general, people with Type 2 diabetes are 1.3 times more likely to have small subcortical infarcts (lacunar infarcts) than those without diabetes (van Harten et al., 2006). Lacunar infarcts may cause acute symptoms when they occur at strategic sites where descending and ascending long tracts are concentrated (Norrving, 2008). However, substantial evidence has clearly shown that direct ischaemic injuries to deep central gray matter mostly involving the basal ganglia and thalamus, occur most commonly without any apparent neurological symptoms

(clinically ‘silent’) (Vermeer et al., 2007; Norrving, 2008). On MRI scanning, subcortical infarcts are characterised by areas of focal hypertensities on T2 weighted images with corresponding hypointense lesions on T1 or FLAIR imaging, that are usually less than 15 mm in diameter. These anatomic regions are served by deep, small and non-branching end arteries, arising directly from large cerebral arteries.

Although emboli (from the heart or extracranial arteries) can cause lacunar inarcts, the vast majority of infarcts are attributable to cerebral microvascular disease (Wardlaw et al., 2009). It has been suggested that both arteriolosclerosis and lipohyalinosis may contribute directly to subcortical gray matter infarctions. For example, microatheromatous vessel pathology (atheromatous plaques) may produce lumen stenosis or occlusion of small penetrating arterioles which are lack of collateral supply. Lipohyalinosis pathology may lead either to thrombotic occlusion of the lumen or to mural destruction with formation of microaneurysms and haemorrhages. A popular hypothesis is that these cerebral microvascular abnormalities may cause an inability of the blood vessels to maintain and autoregulate cerebral blood flow and therefore predispose small regions of the brain parenchyma served by each vessel to ischaemia and necrosis (Thompson and Hakim, 2009). More recently, it has been proposed that vascular leakage is associated with small vessel disease in the brain, and damage to the blood-brain barrier could be an important mechanism in the initiation or worsening of cerebral microvascular disease (Farrall and Wardlaw, 2008). This is discussed below.

Lacunar infarcts and retinal microvascular abnormalities

There are limited data on the presence of symptomatic or silent small brain infarcts in relation to retinal microvascular abnormalities. In a recent small-scale analysis of older patients with lacunar and cortical ischaemic stroke, Doubal et al. (2009a) found no significant differences in proportions with retinopathy features (defined as the presence of haemorrhages, microaneurysms, and hard or soft exudates) between stroke subtypes. However, relative to large artery cortical ischaemic stroke, wider retinal venular diameter was significantly associated with lacunar stroke subtype (Doubal et al., 2009b). In another cross-sectional study with relatively larger sample size, the patients with lacunar stroke were more likely than those with other

stroke subtypes to have smaller AVRs, retinal arteriovenous crossing abnormalities and enhanced light reflex of the arteriolar wall (indicates arteriolar wall thickening) (Lindley et al., 2009).

With respect to asymptomatic infarcts, Kwa et al. (2002) reported that the prevalence of retinal exudates was significantly higher in patients with lacunar infarctions than those without any MRI-defined cerebral small vessel disease. In a large population-based cross-sectional study of middle-aged people free of stroke, the presence of retinal microvascular abnormalities (presence of arteriovenous nicking, focal arteriolar narrowing, blot haemorrhages and microaneurysms) was independently associated with higher prevalence of cerebral infarcts (most of which were lacunar)(Cooper et al., 2006). In the Cardiovascular Health Study, the smaller AVRs and the presence of arteriovenous nicking were independently associated with the presence of lacunar infarcts in elderly people (Longstreth et al., 2007). In longitudinal analyses from the Rotterdam Study, Ikram et al., (2006d) found wider retinal venular diameters were associated with a higher risk of 3.3-year progression of lacunar infarcts between two MRI scans. However, the association was attenuated beyond statistical significance after controlling for other cardiovascular risk factors.

Lacunar infarcts and cognitive dysfunction

There is epidemiological evidence that deep subcortical infarcts could induce measurable changes in higher cortical functioning as indexed by cognitive tests. In particular, deep gray matter (basal ganglia and thalamus) infarcts may be an important predicator (Gold et al., 2005). In a modest sample size (n=164) of healthy elderly people, the presence of subcortical brain infarctions (defined by pathological examination) has been associated (but non-significantly) with worse domain-specific cognitive test performance including working and semantic memory, perceptual speed and visual-spatial abilities. Conversely, no association was found with reduced global cognitive function based on a summary score of all tests (Schneider et al., 2003).

In other cross-sectional analyses, silent infarcts in the basal ganglia determined by MRI were associated with slower verbal memory retrieval speed and

worse executive functioning (O'Brien et al., 2002). Thalamic infarcts were related to worse focused attention in addition to impaired performance in verbal memory retrieval speed and executive functioning. In the Rotterdam Scan study, silent thalamic infarcts at baseline predicated a greater four-year decline in verbal memory performance, whereas the presence of silent infarcts that were not located in the thalamus (mostly located in the basal ganglia) were associated with a decline in psychomotor speed tasks (Vermeer et al., 2003). In addition to location, multiple silent infarcts compared with a single lesion were more strongly associated with cognitive decline. The effects were limited to those with incident silent infarcts, irrespective of whether they had silent lesion at baseline, suggesting a stepwise worsening in higher cortical functioning following silent subcortical infarction (Rafnsson, 2006). Manschot et al. (2006) demonstrated that in modestly-sized samples of subjects with Type 2 diabetes, the presence of infarcts was negatively and significantly associated with information processing. Negative associations were also shown in attention/executive function, abstract reasoning and memory albeit non-significant.

Given the complex cortico-subcortical circuitry networks connecting the superficial and deep structures, these subcortical infarcts may lead to wider-ranging effects on cortical functioning (Mori, 2002; Tekin and Cummings, 2002; Rafnsson, 2006). Specifically, Baron et al. (1986) observed that up to 20% reduction in metabolism in cortical areas both ipsi- and contralateral to unilateral thalamic infarcts. This pattern may reflect the fact that the thalamocortical connections comprise specific systems connecting relay nuclei with specific cortical areas as well as non-specific systems projecting diffusely into the cerebral cortex (Rafnsson, 2006). However, it remains unclear how exactly wide-ranging cortical inactivity might come about secondary to a focal thalamic infarction. As a result, several pathways have been proposed by Baron et al. (1986) which include: a) anterograde degeneration of thalamocortical terminals, b) retrograde degeneration of the corticothalamic neurons following damage to their thalamic terminals, c) transsynaptic degeneration of the cortical neurons, following damage to the thalamocortical system, or d) decreased activity of cortical neurons without structural

damage secondary to the loss of activating afferences from the thalamus (Rafnsson, 2006).

White matter lesions

Changes in the subcortical white matter, visualised as bright, hypertensive deep or periventricular white matter regions on T2-weighted MRI scans, are frequently observed in neurologically intact older people. White matter changes are not specific to infarcts, and frank infarction might be rare changes seen with neuroimaging than white matter lesions (WMLs) (Moorhouse and Rockwood, 2008).

Although diverse pathogenic processes may cause changes in the cerebral white matter, most lesions in the elderly are thought to be of vascular origin (Malloy et al., 2007) and the primary vascular mechanism is small vessel disease. For example, arteriolosclerosis with thickening (hyalinisation) of deep perforating (e.g. medullary) arterioles may induce complete or incomplete ischaemic infarctions and/or hypoperfusion-hypoxic injury such as diffuse rarefaction. These may then induce loss of axons, demyelination, inflammation (gliosis) and necrotic changes which are characteristic neuropathological correlates of vascular-related white matter abnormalities (Englund, 2002). Recently, it has been proposed that different from deep WMLs specifically due to small vessel disease, periventricular WMLs could be more likely determined by chronic hemodynamic insufficiency (e.g. focal or systematic hypoperfusion due to carotid atherosclerosis) or inability of blood brain barrier to absorb excessive interstitial fluid resulting from disruption of the ependymal lining (the blood-cerebrospinal fluid barrier) (Kim et al., 2008).

White matter lesions and retinal microvascular abnormalities

There have been a few studies looking at the association of diabetic retinopathy with WML, although some earlier studies may have been affected by confounding factors, such as the presence of hypertension and other co-morbidity. For example, in a small clinic-based study based on people with Type 1 diabetes who had had laser treatment for proliferative diabetic retinopathy, no association was found between retinopathy and cerebral MRI whiter matter abnormalities (Yousem et al., 1991). More recently, Ferguson et al. (2003) reported on a cross-sectional study

of young people with type 1 diabetes and found an association between the presence of microaneurysms and small focal white-matter hyperintensities in the basal ganglia.

In population-based studies, Kwa et al. (2002) found that the presence of retinal arteriolar abnormalities, including narrowing and arteriolosclerosis was significantly more frequent in patients with MRI signs of cerebral white matter lesions than those without any cerebral small vessel disease. In the ARIC study, people with retinopathy and other arteriolar abnormalities were 2.1 to 4.0 times more likely to have MRI-defined WMLs than people without these retinal signs, independent of age and vascular risk factors. In particular, the more severe signs of microvascular damage (microaneurysms, haemorrhages and exudates) were most strongly associated with WMLs (Wong et al., 2002c). In another cross-sectional analyses based on elderly people in the Cardiovascular Health Study, the smaller AVRs were independently associated with the more severe white matter grade (Longstreth et al., 2007). In longitudinal analyses from the Rotterdam Study, Ikram et al. (2006d) found wider retinal venular diameters were significantly associated with a higher risk of 3.3-year progression of both periventricular and subcortical WML between two MRI scans.

White matter lesions and cognitive dysfunction

There is well-established evidence on the significance of the cerebral white matter integrity for higher cortical functioning (Malloy et al., 2007). In a recent quantitative review of 23 empirical studies, the burden of white matter hyperintensities was also found to be associated with poorer cognitive performance on tasks of global cognitive function, processing speed, delayed memory and executive functioning (Dixon and Raz, 2000). Other studies have associated the presence or progression of white matter lesions with a greater decline in mental processing speed (van den Heuvel et al., 2006; van Dijk et al., 2008), memory (Silbert et al., 2008), conceptualisation and visuopractical skills (Schmidt et al., 2005) and executive functioning (Kramer et al., 2007). White matter abnormalities also contributed independently of childhood mental ability to lifetime decline in general cognitive ability but not individual mental functions per se (Deary et al., 2003).

Additional evidence comes from recent studies using diffusion tensor MRI (DTI) where markers sensitive to the detection of white matter microstructural integrity have been associated with cognitive outcomes (Rafnsson, 2006). As a result, Vernooij et al. (2009) reported white matter water diffusion parameters were significantly associated worse performance on tasks assessing global cognition, information processing speed and memory. While in normal-appearing white matter, diffusivity was related to executive function. Interestingly, Shenkin et al. (2005) showed a strong, consistent and negative relationship between diffusion indices and performance on executive functioning, which was not restricted to any of the anatomical regions examined, including the frontal lobe area.

In the light of these reports, white matter hyperintensities have an impact on higher cortical functions, particularly these may rely on either processing speed or the intact diffuse neural networks (e.g. executive functions) (Rafnsson, 2006). These findings further lend support to the neurobiological hypothesis that the loss of white matter integrity may lead to impaired information transfer between different cortical areas, from a loss of transfer speed in the case of demyelination to complete disconnection when axonal disruptions occur (Deary et al., 2009). Interactions between distant cortical areas are considered as crucial for the appearance of higher cognitive functions (Sullivan and Prefferbaum, 2006).

Importantly, there is very limited evidence regarding the importance of white matter lesion as a substrate of cognitive deficits in diabetic patients. In a small sample (n=92) older people with Type 2 diabetes, periventricular hypertintensities correlated significantly and negatively with motor speed tasks (van Harten et al., 2007). In another sample (n=113) of people with Type 2 diabetes, both the periventricular and deep white matter hypertintensities were significantly and inversely related to measures of information processing speed (Manschot et al., 2006). In addition, periventricular hypertintensities were associated with worse attention and executive function. Despite the paucity of data, both studies may emphasize the importance of white matter integrity for processing speed or executive function, implying that deficits in other cortical functions may be secondary to

suboptimal functioning of either of them in Type 2 diabetes.

Cerebral microbleeds

Another group of lesions that has received increasing attention is cerebral microbleeds (CMBs), which are recognised by small round homogenous low signal appearance on gradient-echo T2* MRI scans but are difficult to detect on standard T2-weighted MRI sequences. Demonstrated MRI-based microbleeds have been found in more than half of patients with lacunar strokes and are associated with increasing age, hypertension, diabetes, the presence of lacunar infarcts and white matter hyperintensities (Cordonnier et al., 2007). CMBs apparent on gradent echo MRI are characterised histopathologically by haemosiderin deposits due to frank microvascular haemorrhages or blood leakage from arterioles, with necrosis or infarction of the surrounding tissue (Cordonnier et al., 2007). Lipohyalinosis, ruptured arteriosclerotic mcirovessels and occasional amyloid deposits have been observed in the deep perforating arterioles feeding the areas of the brain surrounding CMBs.

Cerebral microbleeds and retinal microvascular abnormalities

To date, there is only one epidemiological study examining the relation of retinal microvascular signs to CMBs. In this large population-based study, Qiu et al. (2008) described multiple CMBs in one third of people (mean age 78.1 years) with retinal microaneurysms/haemorrhage and half of those with arteriovenous nicking. More specifically, those with diabetes, in combination with the presence of either retinal arteriovenous nicking (odds ratio, 2.47; 95%CI: 1.42, 4.31) or retinal microaneurysms/haemorrhage (odds ratio, 2.28; 95%CI: 1.24, 4.18) were significantly more likely to have multiple cerebral microbleeds.

Cerebral microbleeds and cognitive dysfunction

The existence and quantitative burden of microbleeds on cortical functioning have remained relatively unexplored. In a small case-control study (Werring et al., 2004), the presence of microbleeds was significantly associated with an increased risk of executive impairment, independent of the prevalent ischaemic stroke and white matter lesions. In addition, patients with executive dysfunction had

more microbleeds in the frontal region and in the basal ganglia. There was also a modest correlation between the number of microbleeds and the number of cognitive domains impaired. Although the confounding by lacunar infarcts cannot be excluded given that no T1-weighted imaging was performed to make a definitive count of lacunar infarcts, the authors proposed that microbleeds may influence cortical functioning by the effects of small focal destructive lesions accumulating in strategic subcortical and cortical structures, or important connection fibres (Werring et al., 2004). Whether such radiological abnormality is also associated with cognitive dysfunction in diabetic patients remains to be investigated.

6.5.2. Increased blood-brain barrier permeability

Fisher originally observed in his pathological work that a substantial proportion of lacunar infarcts were attributable to an intrinsic cerebral small arteriolar abnormality, characterised by vessel wall thicknening, focal arteriolar dilatation, striking loss of normal vessel wall architecture, and extravasation of blood components into and through the wall (Fisher, 1968). This is also described as 'segmental arteriolar disorganization', 'lipohyalinosis', or 'fibrinoid necrosis' (Fisher, 1968; Lammie, 2000). Recently, the intrinsic small vessel disease is recognised as a generalised rather than focal small vessel condition given that these changes are also associated with white-matter lesions (WMLs) and microbleeds (Wardlaw et al., 2008).

The main theories on what causes the cerebral micvascular abnormality include microatheroma, endothelial dysfunction or inflammation (Davis and Donnan, 2004). On the other hand, while ischaemia may be the primary mechanism whereby the microvascular disease damages the brain parenchyma, evidence for reduced blood flow is conflicting, and low cerebral blood flow could be consequence upon reduced tissue supply (Farrall and Wardlaw, 2008). Accumulating observations from pathological and imaging studies as well as animal models have suggested the primary event could be alteration of cerebral microvascular endothelial (i.e., blood-brain barrier [BBB]) dysfunction. The small-vessel endothelium becomes more permeable, allowing substance that should remain intravascular to enter and

damage the vessel wall (causing thickening, inflammation and eventual disintegration) and then into the perivascular parenchyma to cause the tissue lesions (Wardlaw et al., 2009). The BBB is crucial for the regulation of the normal neuronal and glial cell environment and is a key component of the 'neurovascular unit' (Farrall and Wardlaw, 2008). Elevated BBB permeability with normal ageing, vascular cognitive impairment, white matter lesions and AD has been increasingly recognised (Wardlaw et al., 2009). In particular, using magnetic resonance imaging on patients with Type 2 diabetes, Starr et al. (2003) demonstrated increased BBB permeability to gadolinium-diethylenetriamine penataacetic acid (DPTA) and concluded that although the openings in the BBB were to a small molecule (gadolinium-DTPA), clinical significance was substantial given their potential effects in the increased progressive cognitive impairment often seen in diabetic patients.

Several endogenous agents have been implicated in altered BBB permeability, possibly leading to cerebral microvascular disease. These include abnormally elevated inflammatory and endothelial activation markers, which have been found to be associated with established and progressive white-matter lesions (Abbott, 2000; Hassan et al., 2003; van Dijk et al., 2005).

Hyperglycaemia has been shown to damage endothelial cells in the BBB through activation of protein kinase C (PKC) and increased oxidative stress. Activation of PKC may enhance nuclear translocation of NF-κB which is a central transcription factor involved in the inflammatory response processes (Taguchi, 2009). Hyperglycaemis also induces excess production of reactive oxygen species (ROS), chemically reacting with and damaging DNA, proteins and lipids (Taguchi, 2009). Overproduction of ROS causes pathological changes in the endothelium of the BBB, promotes nuclear translocation of NF-κB, and activates microglia (Taguchi, 2009). Advanced glycation endproducts (AGEs) may also induce chronic inflammation involving NF-κB and further cause chronic pathological changes in both endothelium and neuronal cells.

6.6. Chapter summary

Analysis in the ET2DS has shown that both diabetic retinopathy and retinal vessel calibres are associated with a modest but significant estimated decline in a range of diverse cognitive functions in older people with Type 2 diabetes. In particular, these cognitive measures showed a significant relationship with increasing severity of diabetic retinopathy. Severity of diabetic retinopathy was related to a greater decline in information processing speed and, in men for general cognitive function and word fluency. Larger retinal arteriolar calibre was also associated with an increased decline in verbal memory in men. Retinal microvascular abnormalities explained about 1 to 2 % of the total variance in these cognitive measures.

Previous studies in people with Type 1 diabetes and in the general population that examined retinal microvascular abnormalities and cognitive function show results that are comparable with the ET2DS. The robust control for a large number of potential confounding factors suggest these associations may in fact rely on the underlying pathophysiological processes. Although structural neuroimaging was not conducted in the present study and prevented further interpretation of the results, the effects of cerebral microvascular disease as indicated by retinal vascular signs were mainly mediated through the relationship of these with general cognitive function. Given that general intelligence is thought to reflect neurocognitive processes depending on intact deep white and matter integrity, it is possible that cerebral microvascular disease associated with diabetes may affect subcortical structures and the disruption of the blood-brain barrier may be an important pathophysiological feature in the occurrence of cognitive dysfunction in people with Type 2 diabetes.

Chapter 7

Conclusions and Recommendations

In this chapter, the principal conclusions are summarised and the recommendations for future research are presented.

7.1. Conclusions

In the ageing population, Type 2 diabetes is an established risk factor for age-related cognitive impairment and decline, in addition to stroke and dementia. In people with diabetes, retinal microvascular disease is associated with reduced cognitive ability and with estimated cognitive decline, especially in men, and independent of a range of potential confounding factors. Given the homology between the retinal and cerebral microcirculations, this supports the hypothesis that cerebral microvascular disease is involved in the aetiology of diabetes-related cognitive decline during aging. Cerebral microvascular disease may induce neuropathological changes in white matter and deep subcortical gray matter structures which lead to deficits in higher cortical functioning. However, further investigation is required to confirm the direction and potential causal nature of the association between retinal microvascular disease and cognitive decline.

In people with Type 2 diabetes, larger retinal arteriolar calibre, but not other quantitative retinal abnormalities, may also be associated with a greater decline in cognitive ability. This is especially the case for verbal memory in men. However, the mechanisms underlying this association are unclear.

7.2. Recommendations for further research

1. The direction of the association between diabetic retinopathy and cognitive impairment, and whether the associations of diabetic retinopathy and retinal vessel calibre with estimated cognitive decline may be causal in nature, remain uncertain.

Cerebral microvascular disease, as indexed by retinopathy, may lead to cognitive decline, or cognitive decline may make diabetes management more difficult, in turn promoting the development of cerebral microvascular disease. It is also possible that a third unidentified factor is causing both diabetic retinopathy and the cognitive decline. Prospective studies would help to clarify the temporal sequence of these associations and the clinical significance of these small, early cognitive function changes. Such a follow-up project involving the present study population for actual cognitive change is underway.

From a clinical perspective, if the above findings are substantiated, diabetes-associated cognitive dysfunction may be amenable to treatment and preventive strategies targeted at small vessel disease and its risk factors in an ageing diabetic population. Such management strategies would need to be tested in randomised controlled trials of diabetic retinopathy risk reduction.

2. Computer-assisted retinal vessel calibre measurements have proven to be accurate and reproducible and may provide additional clues to understanding the pathology and consequences of cerebral microvascular disease. However, quantification of arteriolar bifurcation angles is imprecise and time-consuming. More objective and efficient methods need to be developed.

3. In the Edinburgh Type 2 diabetes study, retinal microvascular abnormalities were measured only at the baseline examination. Further studies are needed to investigate the progression and incidence of diabetic retinopathy and their associations with cognitive function.

4. The sex-specific effect of diabetic retinopathy and retinal vessel calibre on cognitive function merits further examination in order to understand the complex association between microvascular disease and cognitive function in people with diabetes. Large studies in both men and women with Type 2 diabetes should be continued.

5. The current findings are based on a primarily white sample representative of a

North European diabetic population. Replication of these findings in other racial/ethnic diabetic populations is required.

6. In further studies, the administration of a number of tests for each major cognitive domain, particularly executive function and information processing, should be considered as opposed to the use in the present study of only a single marker of each principal domain. Moreover, the impact of subtle cognitive deficits on activities of daily living needs to be assessed given the importance of self-management behaviors in diabetes and the high complexity of diabetes treatment regimens (e.g., blood glucose testing, meal planning and medication compliance) (Munshi et al., 2006). This may also provide insights into the extent to which decline in specific cognitive abilities may be predictive of further cognitive decrements in the diabetic population.

7. Few available previous studies have highlighted the importance of MRI-detected subcortical white matter and grey matter changes in patients with Type 2 diabetes (Manschot et al., 2006; van Harten et al., 2007). MRI studies need to be performed in older people with Type 2 diabetes recruited from different populations. They will provide complementary information to retinal vascular signs by using a different modality to fill gaps in knowledge on the relation between structural neuroimaging and neuropsychological outcomes in people with diabetes. When possible, future studies should adopt more sensitive neuroimaging techniques, such as diffusion tensor MRI, for the measurement of the integrate connectivity of cerebral white matter to determine the potential impact of early or very small changes in the white matter on cognitive function. Studies are also needed to relate established neuro-imaging markers of cerebral microvasuclar disease (such as cerebral white matter lesions and lacunar infarcts) with retinal microvascular abnormalities in diabetes.

8. Further animal/experimental studies are required to elucidate the pathophysiology of diabetic retinopathy as well as changes in retinal vessel calibre. Histo-pathological studies are also needed on the association between changes in cerebral and retinal microvasculature. A better understanding of both may shed light onto the complex pathogenesis of the consequences of cerebral microvascular disease.

9. Further genetic association studies may be informative. For example, genetic variants can serve as unconfounded markers of exposures (instrumental variables) for determining the causal effect of non-genetic risk factors on disease traits and clinical outcome. However, current genome-wide association studies on retinal vascular calibre genotypes are inconclusive (Sun et al., 2009). Whether retinal vascular calibres are intermediate phenotypes of diabetic retinopathy and cerebral microvascular disease needs to be further investigated.

10. Retinal microvascular abnormalities could serve as a useful biomarker of cerebral microvascular disease to improve risk stratification in people with diabetes. However, the utility of retinal vascular imaging for risk stratification should be judged by a demonstration of independent predicative value that would substantially add to conventional methods (Nguyen and Wong, 2009). Application of common analytical methods in different studies would facilitate data pooling to generate more valid risk estimates (Nguyen and Wong, 2009). Until the additional value of these potential retinal vascular parameters is examined, the cost-effectiveness of retinal photography in screening for cognitive impairment in diabetes and identifying people with unexplained cognitive impairment who might benefit from risk reduction modification cannot be determined.

11. Other systemic microvascular disease may be important but is not as specific as retinal microvascular abnormalities which serve a proxy of cerebral microvascular disease. Peripheral neuropathy (Perlmuter et al., 1984; Zaslavsky et al., 1995) and microalbuminuria (albumin-to-creatinine ratio, ACR) (Bruce et al., 2008) have also been found to predict the degree of cognitive slowing in people with Type 2 diabetes. A relationship between the number of microvascular complications and the magnitude of cognitive decline has been shown in patients with Type 1 diabetes. Evaluation of the additive or synergistic effects of these microvascular complications on cognitive decline is needed in order to promote risk prediction and stratification in people with diabetes.

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APPENDIX A: Data extraction sheet for studies on retinal microvasuclar disease and cognitive dysfunction

Ref ID: _____
Author: _____
Year: _____
Reviewer: _____

A) STUDY DESIGN

Cross-sectional Case-Control Prospective cohort

B) PARTICIPANTS SELECTION, RECRUITMENT & FOLLOW-UP

(1) Type of sample: General population/ Hospital/ Outpatient Clinic

(2) Principle clinical status of patients: Diabetes (Type 1/ Type 2/ Unspecified) / Hypertension / others_____

(3) No. of participants_____ (4) Age (mean/range) _____
(5) Sex: F/M/both
(6) (Baseline) response rate (%): _____/ Not reported

(7) Follow-up period: ____ / NA (8) Loss to follow-up (%):_____/ Not reported / NA

C) MEASURES OF RETINAL MICROVASCULAR DISEASE

(1) No. of eyes assessed: 1/2/Not reported
(2) Assessment tools: Photography / Ophthalmoscopy / Medical records/ Questionnaire
(3) Grading Methods: Adopted from Prior Classification System (name) _____ _____ _____/Computer-assisted / Not Reported
(4) Abnormalities measured:

-----***Any Retinopathy:*** generalized/focal arteriolar narrowing / arteriovenous nicking microaneurysm / retinal hemorrhage / soft or hard exudates/ cotton wool spots/ background
retinopathy / proliferative retinopathy / retinopathy/_____

------***Retinal vascular network geometry:*** AVR (the arteriole-to-venule ratio) / CRAE (central retinal arterial equivalent) / CRVE (central retinal venular equivalent) / Branching Coefficient / Bifurcation Angles /_____

(5) Assessors blinded: Yes/No/Not reported

D) MEASURES OF COGNITIVE FUNCTION

(1) Name of Neuropsychological Test:

(2) Assessment of Premobid intelligence: occupation / education / word reading test /
_____/ Not Reported

(3) Cognitive impairment defined as:

_____

(4) Assessors blinded: Yes/No/Not reported

E) RESULTS

Neuropsychological Domain	*RR/OR*	*Correlation Coefficient*	*Reference Category*	*CI*	*P value*	*Others*

Neuropsychological Domain	*Z scores*	*CI*	*Effect Size*	*P value*	*Others*

F) POTENTIAL CONFOUNDERS ADJUSTED FOR

Age / Sex / Premobid intelligence / Hypertension / Diabetes / Medical conditions (eg. dementia, depression, hyperlipemia, coronary heart disease, peripheral vascular disease and cerebrovascular disease) / Drugs with effects in the central nervous system (eg. beta-adrenoreceptor blocking agents and antidepressants) / Alcohol consumption / Smoking / Impaired sensory (eg. poor eyesight) / APOE ε4 status/ Others_____

_____

_____

_____

Scores: /

APPENDIX B: Documentation relating to the 2006-2007 survey of Edinburgh Type 2 Diabetes Study

B-1: Subject's information sheet, letter of invitation and reply form

Will my details be kept confidential?
All data collected from you will be kept confidential. Samples will be coded to remove identifying information before being sent to the laboratories. Only members of the research team will have access to named data. Data on computer will be accessible only by research team members and will be password protected. Written information will be stored securely in locked filing cabinets.

If you agree to take part in the study then please:

1. Complete the Reply Slip at the bottom of the invitation letter and tick the box to say you **do wish** to be contacted. Return in reply paid envelope.
2. Keep this information sheet for your own information and wait for the research team to contact you.

If you do not wish to take part then please:

1. Complete the Reply Slip and tick the box to say you **do not wish** to be contacted. Return in reply paid envelope (this will avoid you being accidentally contacted again).

If you change your mind

If you do decide to take part you are still free to withdraw from the study at any time without giving a reason. However, if you do decide to withdraw, it would be very helpful if you could contact the study office and let us know. A decision to withdraw at any time, or a decision not to take part, will not affect any health care you receive.

Thank you for reading this information sheet

MRC Medical Research Council

THE UNIVERSITY OF EDINBURGH

INFORMATION SHEET

Edinburgh Type 2 Diabetes Study

Diabetes is a major health problem in Scotland. It is important that we try to improve our understanding of the complications of diabetes so that new ways of preventing and treating these can be developed.

If you have any queries please contact :

Edinburgh Type 2 Diabetes Study Research Office
The University of Edinburgh
Teviot Place
Edinburgh EH8 9AG
Telephone: 0131-xxx-xxxx Email: xxxx@xx.xx.xx

Background information to the study
The Medical Research Council is funding a major new research project into the cause of complications associated with diabetes in Lothian. This study is looking at people with type 2 diabetes who are aged 60 to 75 and investigating them for disease of the blood vessels and changes in mental sharpness. We are collecting samples of blood and taking clinical measurements to look at which risk factors may contribute to these conditions in diabetic people.

Why have I been asked to take part?
You have been asked to take part because you are one of several thousand people living in Lothian who has diabetes and are aged 60 to 75. If you agree to take part you will be one of 1000 people we aim to recruit into the study.

How can I help with this study?
If you agree to take part, we will invite you to a research clinic at the Western General Hospital in Edinburgh where you will be met by one of our study nurses. They will ask you to:

1. Give your personal details and sign a consent form.
2. Give a blood sample of approximately 30mls (6 teaspoons).
3. Complete a questionnaire about your social and medical history. The nurse will offer help with this should you wish.
4. Undertake some interviews and questionnaires to test your memory, thinking skills and other aspects of mental function.
5. Allow us to take some simple clinical measurements on you, such as height, weight, waist and hip circumferences, blood pressure in your arm and ankle, an electrocardiogram (ECG, recording of the heart), non-invasive tests of feeling in your feet and body fat composition.
6. Provide us with specimens of urine and saliva.
7. Allow us to take pictures of the back of your eyes after dilating your pupils with eye drops (we will give you a separate 40 minute appointment at the Princess Alexandra Eye Pavilion in Edinburgh for this).

We will ask you to attend the research clinic in the morning and without breakfast (a simple breakfast will be provided in the clinic). The whole appointment should last around 3 hours and we will be happy to reimburse your travel expenses.

Blood, urine and saliva samples will be used in the laboratories to measure possible risk factors. Constituents of your blood sample (plasma and DNA) will be stored for measurement of additional risk factors at a later date, including genetic factors. Whole blood and/or blood cells will also be stored. The blood cells can be modified to produce cells which are capable of continuously renewing themselves and provide an on-going source of DNA for future analysis of genetic risk factors.

In 4 to 5 years time, we will contact you again to ask you to repeat some of the measurements, in order to see whether these have changed. In the intervening years, we will keep in touch with you, either by telephone or post. We may also wish to contact you to ask if you would be willing to participate in any additional related studies. You will not be asked to commit to this just now, but we will ask your permission to contact you again for this purpose (you will be free to refuse permission).

We will also ask for permission to look at your medical records (both your medical notes and any electronic medical records). This will enable us to follow your medical history and find out details about your diabetes without asking you each time.

Will the research have any implications to my health?
There is no health risk or risk of significant physical or psychological harm associated with participation in this study. Nevertheless, you should know that the University of Edinburgh has indemnity insurance cover for research work undertaken by its employees. Occasionally people experience reactions to the eye drops used for pupil dilatation.

However, this is very rare and experienced staff and facilities will be on hand to deal quickly and effectively with any such problem. Complaints from participants about their treatment by individual members of research staff will be dealt with sensitively by the research team.

There is no intended clinical benefit to you from taking part in this study. Your General Practitioner will be notified of your participation in the study and any information collected during the course of the study which could affect your clinical care will be forwarded to them.

^Name
^Address

^Date

Dear ^Title ^Name

Edinburgh Type 2 Diabetes Study

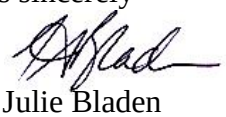
A major new research project into the effects of diabetes on blood vessels and mental function is taking place in Lothian and is called the Edinburgh Type 2 Diabetes Study. This study, which will involve 1000 people with Type 2 diabetes in Lothian, is being run by a team of researchers from Edinburgh University in collaboration with diabetes specialists in Lothian. It is funded by the Medical Research Council and has the support of NHS Lothian.

The purpose of this letter is to ask whether you would be interested in taking part in this research. Your name has been randomly picked from the Lothian diabetes register and will not be passed on to the research team without your permission If you do give permission you will then be invited to attend a clinic run by trained research nurses. More information about the project is given in the enclosed patient information sheet. Please note however that whether or not you wish to participate is entirely up to you and that a decision either way will not affect the care you receive from your GP or your hospital diabetes team if you attend hospital as an outpatient.

I would be very grateful if you could complete the slip below and return it in the reply paid envelope. Even if you do not wish to take part, please return the slip so that you will not be contacted again for this study. **If you have any queries, you can contact the research team directly on 0131-XXX-XXXX.**

I do hope that you will be able to help the research team and look forward to hearing from you.

Yours sincerely



Miss Julie Bladen
Lothian Diabetes Register Facilitator (with the support of Mary Scott, Diabetes MCN Manager)

---

^Title ^Name ^DOB
^Address ^Postcode

I **am interested** in taking part in the Edinburgh Type 2 Diabetes Study ☐

I **do not wish** to take part in the Edinburgh Type 2 Diabetes Study ☐

Signed…… Date…… Contact Telephone No……..

Please return to: Miss Julie Bladen, Lothian Diabetes Register Facilitator, Blackford Pavillion, Astley Ainslie Hospital, 133 Grange Loan, Edinburgh EH9 2HL Tel: 0131 – XXX- XXXX Fax. 0131-XXX- XXXX

For office use: Study no……

B-2: Letter of appointment and instructions for clinical examination

^Title ^Initial ^Surname
^Address
^Postcode

^Date

Dear ^Title ^Surname,

Thank you for agreeing to participate in the Edinburgh Type 2 Diabetes Study.

To confirm, we have arranged an appointment for you on:

^**Day** ^**Date** ^ **Month** at **9am**, at the **Wellcome Trust Clinical Research Facility**, **Western General Hospital** (Directions and a map are enclosed). Your appointment is likely to last until 12:30pm.

So that we can take a fasting blood sample from you, we will need you to fast for twelve hours prior to your appointment. If you take insulin or any other medication for diabetes in the morning, please bring this with you to the clinic and take it after you have provided a blood sample. (Please note: any other medication which is not for diabetes should be taken as normal). If you have any concerns or questions about this please call the study team. We will take blood samples as early as possible, and we will provide a light breakfast afterwards.

We have enclosed a questionnaire - please complete this and bring it to the clinic. If you are unable to complete the questionnaire or if you have any questions about it, a nurse will help you at the clinic.

We also need you to bring a small sample of urine to the clinic. We have enclosed a container, with instructions, for this purpose. Please follow these instructions carefully.

If you wear glasses for reading, please bring these with you to the clinic.

If you have any queries about the study, or if you are unable to attend your appointment, please telephone us at the number above.

With many thanks for your continued cooperation,

Evelyn Crooks
Edinburgh Type 2 Diabetes Study Team

Please Note: This is a fasted appointment. It is important that you have nothing to eat or drink (except for water) for the twelve hours preceding your appointment, i.e. after 9pm the previous evening.

B-3: Directions to the Wellcome Trust Clinical Research Facility

The Wellcome Trust Clinical Research Facility is at the Western General Hospital, situated between Crewe Road South and Telford Road in North Edinburgh. **The main entrance to the WTCRF is on the ground floor, off the north corridor between the Acute Receiving Unit (ARU) and the Alexander Donald Building (ADB) →** **Please see map.**

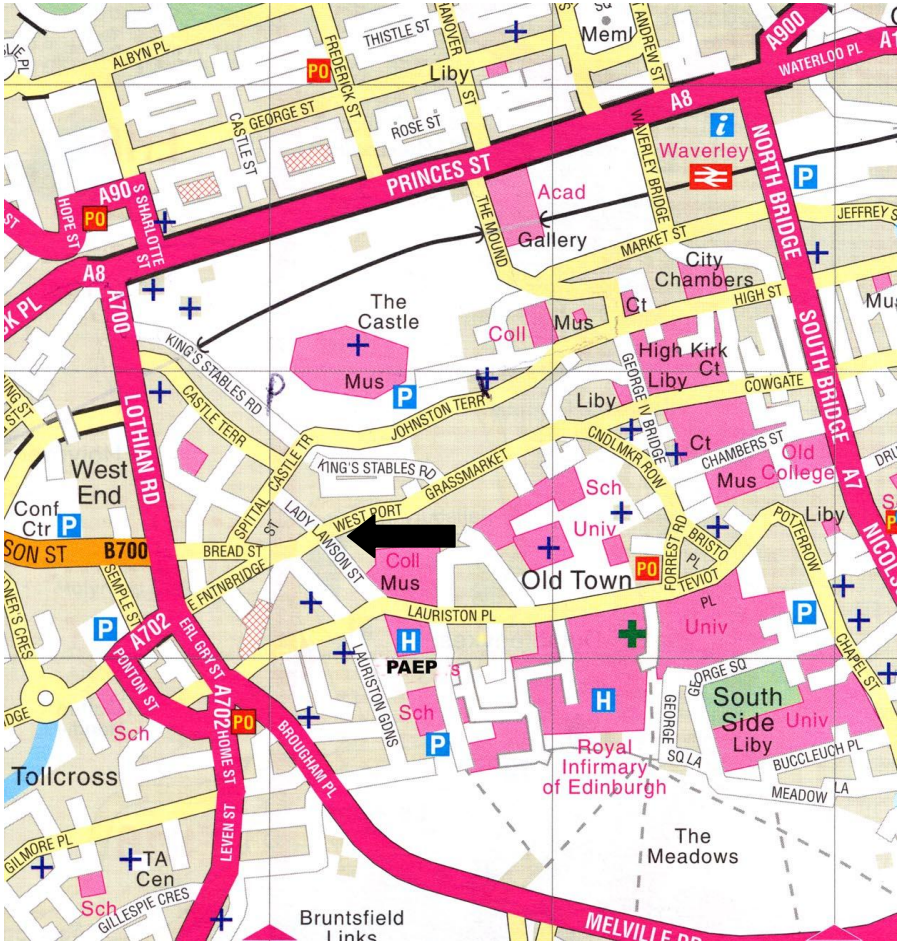
Directions:
Enter via Porterfield Road; follow the road to the right of the Outpatient building towards the Alexander Donald Building (ADB), under the walkway and past the Acute Receiving Unit (ARU) on the left. The WTCRF entrance is immediately on your left opposite the large blue building (Anne Ferguson Building). Enter through double glass doors and through the next set of turquoise doors.

Public transport:
The following buses stop at the hospital:

Lothian Regional Transport – 19,19A, 27, 28, 28B,29,29A, 37, 37A, 38, X29, X7, 42 For information about all bus services, contact Traveline on freephone 0800 232323.

Arriving by car or taxi:
Enter by Porterfield Road, as above. You can be dropped off directly outside the WTCRF. The hospital operates a pay and display system in public parking areas, as marked on the map.
Unfortunately, there is restricted parking in the hospital grounds so you may not be able to find a parking space easily especially at peak times (8.45am-3pm Monday to Friday).
If you need extra assistance with travel arrangements please contact the study team on 0131-xxx-xxxx.

B-4: Eye pavilion appointment card with a map of the area



Edinburgh Type 2 Diabetes Study
Eye Pavilion Appointment

Name……..

Date…….

Time…….……

The Eye Pavilion is situated on Chalmers Street, off Lauriston Place.

There is metered parking outside the hospital, or a car park 100 yards away at the bottom of the road. LRT Buses nos 23 and 27 go from the bottom of the Mound to Lauriston Place.

Once at the Eye Pavilion:
→Go past the reception area and take the lift or stairs to the ***first*** floor
→Go through the double doors, turn right and wait in the red chair area
→If you need to ask directions, say that you are from the Edinburgh Type 2 Diabetes Study and have an appointment with Dr. Halina Kaim

Please Note: Your appointment will take 40 mins to 1 hour. We will use eye drops to dilate your pupils, and we do not recommend that you drive for 2-3 hours afterwards.

Please call the Edinburgh Type 2 Diabetes Study team on 0131-XXX-XXXX if you have any queries or need to change your appointment

B-5: Consent form

The Edinburgh Type 2 Diabetes Study

Co-ordinating Centre Public Health Sciences University of Edinburgh
Teviot Place Edinburgh EH8 9AG Tel/Fax 0131-XXX-XXXX

Patient Identification Number:

(Circle YES or NO below)

1. I confirm that I have read and understand the information sheet dated 30th March 2006 (version 2) for the above study and have had the opportunity to ask questions. YES/NO
2. I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason, without my medical care or legal rights being affected. YES/NO
3. I give permission for responsible individuals from the research team to gather relevant medical information from any of my medical records, including NHS electronic medical records, and any medical information that may be held by the General Register Office for Scotland. YES/NO
4. I agree that the samples I give and the information gathered about me can be stored by the research team and used in future, related research, as described in the information sheet (this excludes the genetic information, for which there is a separate consent form). YES/NO
5. I understand that my GP will be contacted about my participation in the study and will be told about results of the examination which may be important for my clinical care. YES/NO
6. I agree to take part in the above study. YES/NO
7. I agree, if selected, to be invited to participate in related studies involving collection of additional information and/or samples on a separate occasion. YES/NO

_____	_____	_____
Name of Patient	Date	Signature
_____	_____	_____
Name of Person taking consent (Research nurse)	Date	Signature

Top copy to be retained by researcher, second copy to be retained by subject

APPENDIX C: Data collection form & check list used at clinical examination

EDINBURGH TYPE 2 DIABETES STUDY (Baseline)

Data Collection Form & Checklist

Subject Study No. …….

Date……

PART 1 – Consent and venepuncture

Recorder: DJ GB SL KB CM RM Other……. *(circle initials)*

☐ General consent form signed?

☐ Genetic consent form signed?

☐ Urine sample received from subject and **labelled**?

☐ Questionnaire part 1 received from subject, **checked** and **labelled**?

☐ Patient fasted and still to take diabetic medication (if appropriate)?

If any of the above NOT as per protocol, specify (giving reason and action taken)

…….

…….

Venepuncture

		Yes	No
1.	Was venepuncture completed and all tubes filled?	☐	☐
2.	Was venepuncture difficult/slow?	☐	☐

If any problems and/or tubes not filled, specify …….

…….

☐ **All** samples labelled?

☐ Correct brown, yellow and red tubes placed in specimen bag with

COMPLETED request form and urine specimen?

☐ Remaining samples taken to sample processing room and placed on ice?

Subject breakfast

☐ Subject reminded to take medication if necessary?

☐ Questionnaire part 3 (and pen) given to subject?

PART 2 – Cognitive function testing

Tester: DJ GB SL KB CM RM Other…….

Preliminary tests:

Distance vision

	YES	NO
Does subject normally wear glasses/contact lenses for distance vision?	☐	☐
If YES, subject tested wearing glasses/contact lenses?	■	☐

Right eye ……..

Left eye ……..

Near vision

	YES	NO
Does subject normally wear glasses for near vision?	☐	☐
If YES, subject tested wearing glasses?	■	☐

Right eye N ….

Left eye N ….

BM ☐☐.☐ *(If < 4.0, re-arrange appointment)*

Cognitive Test Battery:

Time tests started……am

Tick box if complete — If test incomplete/difficulty encountered, specify

☐ Hospital Anxiety & Depression Scale
……..

☐ Mini-Mental State Examination
……

☐ Logical memory 1 (WMS-III)
……

☐ Trail-Making Test B (TMB)
……

☐ Faces 1 (WMS-III)
……

☐ Matrix Reasoning (WAIS-III)
……

☐ Digit symbol test (WAIS-III)
……

☐ Borkowski Verbal Fluency Task
……

☐ Mill Hill Vocabulary Scale
……

☐ Letter-number sequencing (WAIS-III)
……

☐ Logical memory 2 (WMS-III)
……

☐ Faces 2 (WMS-III)
……

PART 3 –physiological testing

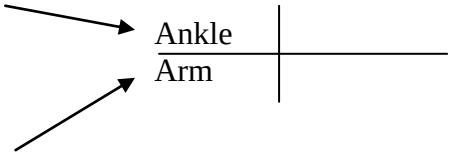
Time physiological testing started …….

Tester: DJ GB SL KB CM Other…….

Group A tests (subject lying down)

ABPI
BP measurements **using doppler**

	Left	Right	
Posterior tibial			LOWEST
Dorsalis pedis			
Brachial			HIGHER



Circle lowest ankle and higher arm reading

ABPI = ☐.☐☐

RESTING BLOOD PRESSURE

Right arm, **using stethoscope**

Observed

Systolic mmHg ☐☐☐ mm/Hg

Diastolic mmHg ☐☐☐ mm Hg

ECG

	Yes	No
ECG recorded and **labelled**	☐	☐

NEUROTHESIOMETRY

	Right big toe	Left big toe
Reading 1	☐☐.☐ volts	☐☐.☐ volts
Reading 2	☐☐.☐ volts	☐☐.☐ volts
Reading 3	☐☐.☐ volts	☐☐.☐ volts
Average	☐☐.☐ volts	☐☐.☐ volts

Is there any obvious FOOT ulceration?	Yes	No
Right foot	☐	☐
Left foot	☐	☐

Has subject **ever** had a FOOT ulcer?	Yes	No
Right foot	☐	☐
Left foot	☐	☐

Group B tests (subject standing)

WAIST CIRCUMFERENCE

Circumference mid-way between lower rib and iliac crest *(feet 30cm apart)*

Reading 1	☐☐☐.☐ cm	*(to nearest 0.5cm)*
Reading 2	☐☐☐.☐ cm	*(to nearest 0.5cm)*
Average	☐☐☐.☐ cm	

HIP CIRCUMFERENCE

Max circumference at hips *(feet together)*

☐☐☐ ☐

Reading 1 . cm *(to nearest 0.5cm)*

Reading 2 ☐☐☐ . ☐ cm *(to nearest 0.5cm)*

Average ☐☐☐ . ☐ cm

HEIGHT

Height (without shoes) ☐☐☐ . ☐ cm

WEIGHT

Weight (without coat and shoes) ☐☐☐ . ☐ kg

BIO-IMPEDENCE

(Shoes off, feet 30 cm apart) % fat

Reading 1 ☐☐ . ☐ %

Reading 2 ☐☐ . ☐ %

Reading 3 ☐☐ . ☐ %

Average ☐☐ . ☐ %

PART 4 – questionnaire checks and departure

Recorder: DJ GB SL KB CM Other…….

☐ Questionnaire part 2 completed with subject?

☐ Questionnaire part 3 completed and **checked**

OR

☐ sent home with subject?

☐ Appointment for eye pavilion made?

☐ Travel expenses offered?

APPENDIX D: Baseline questionnaire part 1 & 2

EDINBURGH TYPE 2 DIABETES STUDY

BASELINE

QUESTIONNAIRE

PLEASE NOTE: ONE OF OUR RESEARCH NURSES WILL GO OVER THE QUESTIONNAIRE WITH YOU AT THE CLINIC AND MAY ASK A FEW ADDITIIONAL QUESTIONS

THE INFORMATION IN THIS QUESTIONNAIRE IS HIGHLY CONFIDENTIAL AND IS PART OF A MEDICAL RESEARCH STUDY

The information you give in this questionnaire will be treated as strictly confidential and will be available only to your own doctor and the study team. The results of the research will appear only in the form of general statistics from which it will be impossible to identify you as an individual.

Please complete the following:

SURNAME: ……..

FORENAMES: ……..

DATE: ……..

If you have any difficulties in answering some of the questions, you will have a chance to discuss these with a member of the study team.

THANK YOU FOR YOUR CO-OPERATION IN THIS STUDY

For Office Use: Study No…....

BASELINE QUESTIONNAIRE

IT IS IMPORTANT TO ANSWER ALL THE QUESTIONS CAREFULLY. PLEASE TAKE YOUR TIME.

PERSONAL HISTORY

1. Please tick one box: Male ☐ Female ☐

2. Enter your date of birth: Day ☐☐ Month ☐☐ Year ☐☐

3. Please tick the box showing your present marital status:

- Married and/or living with long-term partner ☐
- Single ☐
- Widowed ☐
- Divorced or separated ☐

4. Please enter your address (including postcode) and telephone no.

Address: ……

……

Postcode: ……..

Telephone no: ……

5. Please enter the details of your GP

GP name: ……

Address: ……

……

EDUCATION

6. What is the HIGHEST level of education you and your spouse/ex-spouse or long-term partner have completed?

Please tick appropriate boxes:

	You	Spouse/ex-spouse /partner
University/college degree course	☐	☐
Other professional/technical qualification after leaving school	☐	☐

Secondary school	☐	☐
Primary school	☐	☐

ETHNICITY

7. What is your ethnic group?

Please choose ONE section from 1 to 5, then tick the appropriate box to indicate your ethnic Group

(i) White

☐ British
☐ Any Other White background, *please write in*
_____

(ii) Mixed

☐ White and Black Caribbean
☐ White and Black African
☐ White and Asian
☐ Any Other Mixed background, *please write in*
_____

(iii) Asian or Asian British

☐ Indian
☐ Pakistani
☐ Bangladeshi
☐ Any Other Asian background, *please write in*
_____

(iv) Black or Black British

☐ Caribbean
☐ African
☐ Any Other Black background, *please write in*
_____

(v) Chinese or other ethnic group

☐ Chinese
☐ Any Other, *please write in* _____

CURRENT EMPOYMENT STATUS

8. At the moment, what is the employment status of you and your spouse/ex-spouse or long-term partner?

	You	**Spouse/ex-spouse/partner**

Employed, full-time	☐	Employed, full-time	☐
Employed, part-time	☐	Employed, part-time	☐
Unemployed	☐	Unemployed	☐
Retired	☐	Retired	☐
A Housewife (full-time)	☐	A Housewife (full-time)	☐
Other	☐	Other	☐
please specify ……..		please specify ...…….…	

MEDICAL HISTORY

Diabetes history

9. When was your diabetes diagnosed (if known)? Year ……..……

10. What treatment do you receive currently for your diabetes?

(i) Tablets Yes ☐ No ☐

If 'yes', please give name(s) …….....

(ii) Insulin injections Yes ☐ No ☐

If 'yes',

(a) give total number of units per day ……..…units/day

(b) give date (year) when you started insulin year ……

11. Have you **ever** had an episode of low blood glucose (hypoglycaemia) when you have needed **someone else** to treat you eg. give sugary drink or glucagon? Yes ☐ No ☐ Don't Know ☐

If 'yes', how many times has this ever happened?

1-2 ☐
3-4 ☐
5 or over ☐

How many times has this happened **over the past year**?

1-2 ☐
3-4 ☐
5 or over ☐

12. Are you on any regular medical treatment from a doctor as follows:

	Yes	No	Don't Know
Aspirin?	☐	☐	☐
Drugs for angina (including spray)?	☐	☐	☐
Drugs to lower blood pressure?	☐	☐	☐
Drugs to lower cholesterol?	☐	☐	☐

(If you have answered YES to any of these, please include details below)

13. Give names of <u>all</u> current medication if possible (including regular skin creams, eye drops, inhalers, tablets and injections which may or may not be repeat prescriptions):

……

……...

……

……...

……

……...

……

……...

	Yes	No	Don't Know
14. Have you taken any oral steroids, used steroid inhalers or used steroid containing creams or eye drops in the last 3 months?	☐	☐	☐

Vascular Disease

15. Have you ever been <u>told by a doctor</u> that you have or have had any of the following?

	Yes	No	Don't Know
(i) Heart attack (coronary thrombosis, myocardial infarction)?	☐	☐	☐
(ii) Angina?	☐	☐	☐
(iii) Stroke?	☐	☐	☐
(iv) Hardening of the arteries in the legs?	☐	☐	☐
(v) High blood pressure?	☐	☐	☐

If you have answered 'yes' to any of the above, please give the year in which the event occurred and/or condition was diagnosed (as near as you can remember) and the name of the hospital/GP surgery where you were/are treated for the condition

Event/condition	Year of event/diagnosis	Hospital/GP surgery where treated
……	……	……

…… …….. ……

16. Have you ever undergone any of the following procedures/operations?

	Yes	No	Don't Know
(i) An operation or balloon treatment to relieve a blockage in the arteries of your heart (coronary by-pass or angioplasty)?	☐	☐	☐
(ii) An operation or balloon treatment to relieve a blockage in the arteries of your leg(s) , other than for varicose veins?	☐	☐	☐
(iii) Surgery to remove toes or leg (above or below the knee)?	☐	☐	☐
(iv) An operation or balloon treatment to relieve a blockage in the arteries of your neck (carotid surgery/angioplasty/stenting)?	☐	☐	☐

If you have answered 'yes' to any of the above, please give the year in which the procedure was performed and the name of the hospital you attended

Procedure/operation	Year performed	Hospital attended
……	……	……
……	……..	……

Liver Condition/Disease

17. Have you ever been told by a doctor that you have or have had any of the following?

	Yes	No	Don't Know
(i) Hepatitis?	☐	☐	☐
(ii) Cirrhosis of the liver?	☐	☐	☐
(iii) Any other disease/medical condition affecting the liver?	☐	☐	☐

If you have answered 'yes' to any of the above, please give the name of the condition, the year in which it was diagnosed (as near as you can remember) and the name of the hospital where you were/are treated for the condition

Name of condition	Year of diagnosis	Hospital where treated
……	……	……
……	……..	……

18. Have you ever had any of the following investigations of your liver

	Yes	No	Don't Know
(i) Abnormal blood tests of liver function?	☐	☐	☐
(ii) Liver biopsy?	☐	☐	☐

(iii) Scan (ultrasound or CT etc.) of the liver? ☐ ☐ ☐

(iv) Other investigation of the liver? ☐ ☐ ☐

If you have answered 'yes' to any of the above, please give the name of the investigation, the year in which it was done (as near as you can remember) and the name of the hospital where the test/investigation was performed

Name of investigation	Year done	Hospital where performed
……	…….	……
……	……..	……

Other Medical Conditions

		Yes	No	Don't Know
19.	Do you suffer from disease of the thyroid gland?	☐	☐	☐
20.	Do you have any other medical conditions not mentioned above?	☐	☐	

If yes, please specify: ……..

……

……..

ALCOHOL

21. **Current alcohol intake**

(i) Think back carefully over the last seven days. Please write in each column the exact number of alcoholic drinks you consumed on each day during the past week. If none consumed write '0' in the boxes.

Try to remember where and who you were with on each day. This may help you remember what you had to drink.

	Pints of beer, lager, cider etc	Single glasses of whisky, vodka, gin etc	Single glasses of martini, wine, sherry, etc
Monday	☐	☐	☐
Tuesday	☐	☐	☐
Wednesday	☐	☐	☐
Thursday	☐	☐	☐
Friday	☐	☐	☐
Saturday	☐	☐	☐
Sunday	☐	☐	☐

		Yes	No
(ii)	Would you say that last week was fairly typical of what you usually	☐	☐

have to drink in a week?

(iii) If last week was not typical, would you normally drink more or less in a week? More ☐ Less ☐

22. **Alcohol intake over past year**

(i) How often did you have a drink containing alcohol in the past year?
Consider a "drink" to be a can or bottle of beer, a glass of wine, or one cocktail or a measure of spirits (like scotch, gin, or vodka).

- never ☐
- monthly or less ☐
- 2 to 4 times a month ☐
- 2 to 3 times a week ☐
- 4 to 5 times a week ☐
- 6 or more times a week ☐

(ii) How many drinks did you have on a typical day when you were drinking in the past year?

- 0 drinks ☐
- 1 to 2 drinks ☐
- 3 to 4 drinks ☐
- 5 to 6 drinks ☐
- 7 to 9 drinks ☐
- 10 or more drinks ☐

(iii) How often did you have 6 or more drinks on one occasion in the past year?

- never ☐
- less than monthly ☐
- monthly ☐
- weekly ☐
- daily or almost daily ☐

23. Have you or your doctor ever considered that you suffer/have suffered in the past from an alcohol problem/excessive drinking? Yes ☐ No ☐

SMOKING

Smoking has been linked with many health problems. It is important that you answer the following section as accurately as possible.

24. Do you smoke at present? Yes ☐ No ☐

If no, proceed to Question 29

25. What do you usually smoke now?

	Yes	No
Cigarettes	☐	☐
Pipe	☐	☐
Cigars	☐	☐

26. How many do you usually smoke now?

Cigarettes per day …… cigarettes

Ozs. tobacco per week …… ozs.

Cigars per week …… cigars

27. For how many years during your life have you smoked cigarettes? …… years

28. How many cigarettes have you smoked on average per day during the period you have smoked? ……cigarettes

Now proceed to Question 34

	Yes	No
29. Have you ever smoked regularly?	☐	☐

If no, proceed to Question 34

30. What did you usually smoke?

	Yes	No
Cigarettes	☐	☐
Pipe	☐	☐
Cigars	☐	☐

31. How much did you smoke on average while you were a smoker?

Cigarettes per day …… cigarettes

Ozs. tobacco per week …… oz.

Cigars per week …… cigars

32. For how many years did you smoke cigarettes? …… years

33. If you smoked cigarettes, how long is it since you finally gave up?

……. years …… months

CHEST PAIN

		Yes	No
34.	Do you ever get pain or discomfort in your chest?	☐	☐

IF NO, PROCEED TO QUESTION 40

		Yes	No
35.	Do you get this pain or discomfort when you walk uphill or hurry?	☐	☐

IF NO, PROCEED TO QUESTION 40

		Yes	No
36.	Do you get it when you walk at an ordinary pace on the level?	☐	☐

37. When you get any pain or discomfort in your chest what do you do?

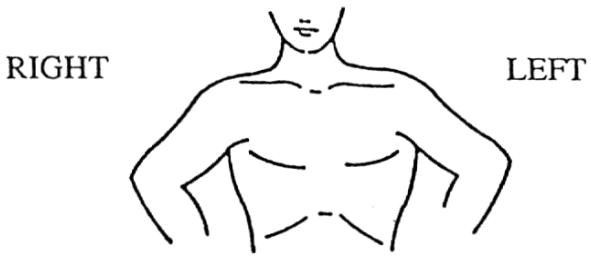
	Tick one
Stop	☐
Slow down	☐
Continue at the same pace	☐

		Yes	No
38.	Does it go away when you stand still or sit down?	☐	☐

How soon?

	Tick one
10 minutes or less	☐
More than 10 minutes	☐

39. Where do you get this pain or discomfort? Mark the place(s) with an 'X' on the diagram



			Yes	No
40.	(i)	Have you ever had a severe pain across the front of your chest lasting for half an hour?	☐	☐
	(ii)	What was the cause? ……		

LEG PAIN

		Yes	No	I am unable to walk
41.	Do you get a pain or discomfort in your leg(s) when you walk?	☐	☐	☐

If you answered 'yes' to question 41, please answer the following questions.

	Yes	No
(i) Does this pain ever begin when you are standing still or sitting?	☐	☐
(ii) Do you get it if you walk uphill or hurry?	☐	☐
(iii) Do you get it when you walk at an ordinary pace on the level?	☐	☐
(iv) Does the pain ever disappear while you are still walking?	☐	☐

(v) What do you do if you get it when you are walking?

	Tick one
Stop	☐
Slow down	☐
Continue at same pace	☐

(vi) What happens to it if you stand still?

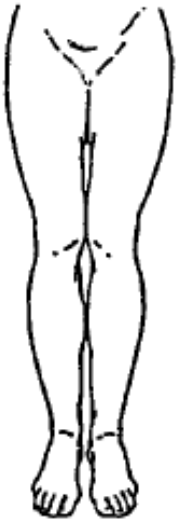
	Tick one
Usually continues for more than 10 minutes	☐
Usually disappears in 10 minutes or less	☐

(vii) Where do you get this pain or discomfort?

	Yes	No
(i) Do you get this pain in your calf (or calves)?	☐	☐

(ii) Please mark the place(s) where you get the pain with 'X' on the diagram below

Front



Back

THANK YOU FOR COMPLETING THIS QUESTIONNAIRE – PLEASE BRING IT WITH YOU TO YOUR APPOINTMENT AT THE WELLCOME TRUST CLINICAL RESEARCH FACILITY

EDINBURGH TYPE 2 DIABETES STUDY

BASELINE QUESTIONNAIRE
Part 2

THE INFORMATION IN THIS QUESTIONNAIRE IS HIGHLY CONFIDENTIAL AND IS PART OF A MEDICAL RESEARCH STUDY

The information you give in this questionnaire will be treated as strictly confidential and will be available only to your own doctor and the study team. The results of the research will appear only in the form of general statistics from which it will be impossible to identify you as an individual.

Please complete the following:

SURNAME: ……..

FORENAMES: ……..

DATE: ……..

THANK YOU FOR YOUR CO-OPERATION IN THIS STUDY

For Office Use: Study No…....

EMPLOYMENT STATUS / OCCUPATION

The following questions refer to your current main job, or (if you are not working now) to your last main job. Please complete for both yourself (I) and for your spouse/ex-spouse or long-term partner (II)

(I) Yourself (Please tick one box only per question)

1. What is (was) your employment status?

Employee ☐

Self-employed with employees ☐

Self-employed / freelance without employees (go to **question 4**) ☐

Housewife (go to **question 4**) ☐

No previous paid employment (excluding housewife) (go to **question 4**) ☐

2. Number of employees

For employees: indicate below how many people work (worked) for your employer at the place where you work (worked). Then go to question 3.
For self-employed: indicate below how many people you employ (employed). Then go to question 4.

1 to 24 ☐ 25 or more ☐

3. Do (did) you supervise any other employees?

A supervisor or foreman is responsible for overseeing the work of other employees on a day-to-day basis

Yes ☐ No ☐

4. Please tick one box to show which **best** describes the sort of work you do.

(If you are not working now, please tick a box to show what you did in your last job).

PLEASE TICK **ONE BOX ONLY**

Modern professional occupations
such as: teacher - nurse - physiotherapist - social worker - welfare officer - artist - musician - police officer (sergeant or above) - software designer ☐

Clerical and intermediate occupations
such as: secretary - personal assistant - clerical worker - office clerk - call centre agent - nursing auxiliary - nursery nurse ☐

Senior managers or administrators
(usually responsible for planning, organising and co-ordinating work and for finance)
such as: finance manager - chief executive ☐

Technical and craft occupations
such as: motor mechanic - fitter - inspector - plumber - printer - tool maker - electrician - gardener - train driver ☐

Semi-routine manual and service occupations
such as: postal worker - machine operative - security guard - caretaker - farm worker - catering assistant - receptionist - sales assistant ☐

Routine manual and service occupations ☐
such as: HGV driver - van driver - cleaner - porter - packer - sewing machinist - messenger - labourer - waiter / waitress - bar staff

Middle or junior managers ☐
such as: office manager - retail manager - bank manager - restaurant manager - warehouse manager - publican

Traditional professional occupations ☐
such as: accountant - solicitor - medical practitioner - scientist - civil / mechanical engineer

EMPLOYMENT STATUS / OCCUPATION (cont.)

(II) Your spouse/ex-spouse/long term partner (Please tick one box only per question)

5. What is (was) his (her) employment status?

Employee ☐

Self-employed with employees ☐

Self-employed / freelance without employees (go to **question 8**) ☐

Housewife (go to **question 8**) ☐

No previous paid employment (excluding housewife) (go to **question 8**) ☐

6. Number of employees

For employees: indicate below how many people work (worked) for his/her employer at the place where he/she work (worked). Then go to question 7.
For self-employed: indicate below how many people he/she employs (employed). Then go to question 8.

1 to 24 ☐ 25 or more ☐

7. Do (did) he/she supervise any other employees?

A supervisor or foreman is responsible for overseeing the work of other employees on a day-to-day basis

No ☐ Yes ☐

8. Please tick one box to show which **best** describes the sort of work he/she does.

(If not working now, please tick a box to show what he/she did in his/her last job).

PLEASE TICK **ONE BOX ONLY**

Modern professional occupations ☐
such as: teacher - nurse - physiotherapist - social worker - welfare officer - artist - musician - police officer (sergeant or above) - software designer

Clerical and intermediate occupations ☐
such as: secretary - personal assistant - clerical worker - office clerk - call centre agent - nursing auxiliary - nursery nurse

Senior managers or administrators ☐
(usually responsible for planning, organising and co-ordinating work and for finance)
such as: finance manager - chief executive

Technical and craft occupations ☐
such as: motor mechanic - fitter - inspector - plumber - printer - tool maker - electrician - gardener - train driver

Semi-routine manual and service occupations ☐
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such as: HGV driver - van driver - cleaner - porter - packer - sewing machinist - messenger - labourer - waiter / waitress - bar staff

Middle or junior managers ☐
such as: office manager - retail manager - bank manager - restaurant manager - warehouse manager - publican

Traditional professional occupations ☐
such as: accountant - solicitor - medical practitioner - scientist - civil / mechanical engineer

APPENDIX E: Cognitive test battery

E-1: Hospital Anxiety and Depression Scale

Tick the response which comes closest to how you have felt in the last few days.

I feel tense or 'wound up'.
Most of the time.....
A lot of the time.....
From time to time, occasionally....
Not at all.....

I still enjoy the things I used to enjoy.
Definitely as much.....
Not quite so much.....
Only a little.....
Hardly at all.....

I get a sort of frightened feeling as if something awful is about to happen.
Very definitely and quite badly....
Yes, but not too badly.....
A little, but it doesn't worry me....
Not at all.....

I can laugh and see the funny side of things.
As much as I always could.....
Not quite so much now.....
Definitely not so much now.....
Not at all.....

Worrying thoughts go through my mind.
A great deal of the time.....
A lot of the time.....
From time to time but not too often....
Only occasionally.....

I feel cheerful.
Not at all.....
Not often.....
Sometimes.....
Most of the time.....

I can sit at ease and feel relaxed.
Definitely.....
Usually.....
Not often.....
Not at all.....

I feel as if I am slowed down.
Nearly all the time.....
Very often.....
Sometimes.....
Not at all.....

I get a sort of frightened feeling like 'butterflies' in the stomach.
Not at all.....
Occasionally.....
Quite often.....
Very often.....

I have lost interest in my appearance.
Definitely.....
I don't take so much care as I should...
I may not take quite as much care.....
I take just as much care as ever.....

I feel restless as if I have to be on the move.
Very much indeed.....
Quite a lot.....
Not very much.....
Not at all.....

I look forward with enjoyment to things.
As much as I ever did.....
Rather less than I used to.....
Definitely less than I used to.....
Hardly at all.....

I get sudden feelings of panic.
Very often indeed.....
Quite often.....
Not very often.....
Not at all.....

I can enjoy a good book or radio or TV programme.
Often.....
Sometimes.....
Not often.....
Very seldom.....

Study No .....
Date.....

Score A.....
Score D.....

E-2: Mini-Mental State Examination

Mini-Mental State Examination **Trial No.....**
Date.....

Orientation-Time
Score one point for each correct answer

		Correct?	
1.	What day of the week is it?	Yes	No
	What date is it today?		
2.	Day	Yes	No
3.	Month	Yes	No
4.	Year	Yes	No
5.	What is the season? *(Allow flexibility when season changes)*	Yes	No

Total correct.....

Orientation-Place
Score one point for each correct answer

		Correct?	
6.	Can you tell me where we are now? For instance, what county or region we are in?	Yes	No
7.	What is the name of this town (city)?	Yes	No
8.	Which country are we in?	Yes	No
9.	What floor of the building are we on?	Yes	No
10.	What is the name of this place? (or: What is this address? If the subject is tested at home)	Yes	No

Total correct.....

Memory-registration

11. I am going to name three objects. After I have finished saying all three, I want you to repeat them. Remember what they are because I am going to ask you to name them again in a few minutes.

Name the following three objects taking one second to say each:
LEMON, KEY, BALL. Go!
Note items which are correct on the FIRST attempt and enter number correct under total.

	Correct?	
Lemon	Yes	No
Key	Yes	No
Ball	Yes	No

Total correct.....

1.

Attention and Calculation

12. Spell 'world' backwards

Correct?
Yes No

Total correct.....

Memory-Recall

13. Could you please tell me the three objects I named earlier

Correct?

Lemon	Yes	No
Key	Yes	No
Ball	Yes	No

Total correct.....

14. Could you please tell me what this is

Correct?

show WATCH	Yes	No
show PENCIL	Yes	No

Total correct.....

15. I would like you to repeat the following
'No ifs, ands or buts'

Correct?

Yes No

Total correct.....

16. Please read and obey the following:
Give the patient the paper with the printed sentence 'Close your eyes'
Ask the patient to read it and do what it says (only score when patient actually closes eyes)

Correct?

Yes No

Total correct.....

2.

Praxis-ideational

Read the following statement and then hand to the subject a sheet of paper. Make a point of handing to the subject's midline.

17. Please listen carefully to the instructions as I will explain it only once: I am going to give you a piece of paper. When I do, take the paper in your RIGHT hand. Fold the paper in half with both hands, and put the paper down in your lap.

Do not repeat instructions or coach. Score a move as correct only if it takes place n the correct sequence. Note each correct move and enter total number correct (Maximum score=3 points).

	Correct?	
Right hand	Yes	No
Folds	Yes	No
On lap	Yes	No

Total correct.....

Praxis-copying and drawing

	Correct?	
19. Copy this design (pattern)	Yes	No

Total correct.....

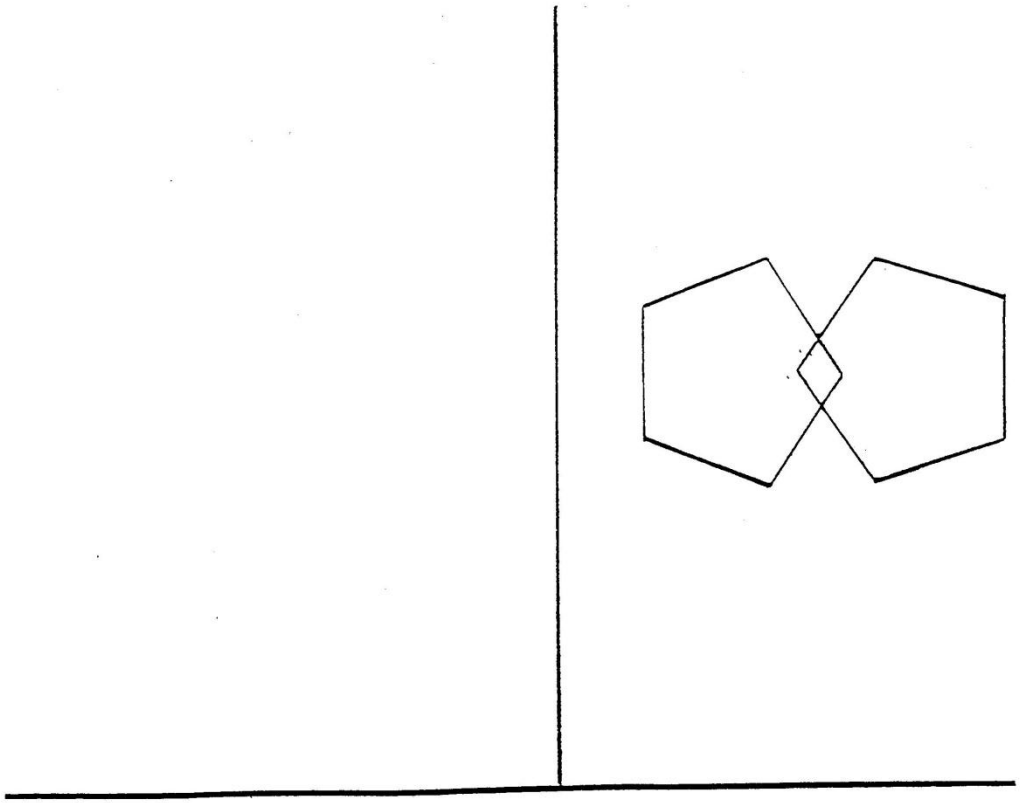
Praxis-Writing: Spontaneous

	Correct?	
20. Write a complete sentence in the space on the page indicated. It can be anything you like as long as it is a complete sentence.	Yes	No

Total correct.....

Overall total correct.....

3.



E-3: Mill Hill Vocabulary Scale

1	**TOMATO**	
fly		crack
wood		dunce
fruit		step
2	**REST**	
lie down		sing
go away		taste
run up		cry
3	**PATCH**	
switch		watch
hand		bang
mend		cook
4	**AFRAID**	
pleased		warm
goodness		horse
tired		frightened
5	**CRUEL**	
clean		green
pretty		found
water		unkind
6	**BLAZE**	
kitchen		flare
grass		roof
coat		side
7	**ACHE**	
screen		tree
prize		pain
boom		land
8	**SQUABBLE**	
quarrel		lift
bubble		photo
mould		saw
9	**RAGE**	
crease		love
invite		anger
rain		hoist
10	**SHRIVEL**	
linger		heed
volunteer		haunt
wither		shiver
11	**CONNECT**	
accident		join
lace		bean
flint		field
12	**PROVIDE**	
harmonize		commit
hurt		supply
annoy		divide
13	**STUBBORN**	
obstinate		steady
hopeful		hollow
orderly		slack
14	**SCHOONER**	
building		man
ship		singer
plant		scholar
15	**LIBERTY**	
worry		freedom
rich		serviette
forest		cheerful
16	**COURTEOUS**	
dreadful		proud
truthful		short
curtsey		polite
17	**RESEMBLANCE**	
attendance		fondness
assemble		repose
likeness		memory
18	**THRIVE**	
flourish		try
thrash		reap
think		blame
19	**PRECISE**	
natural		stupid
faulty		grand
small		exact
20	**ELEVATE**	
revolve		move
raise		work
waver		disperse
21	**DWINDLE**	
swindle		pander
diminish		wheeze
linger		compare
22	**LAVISH**	
unaccountable		selfish
romantic		lawful
extravagant		praise
23	**WHIM**	
complain		noise
tonic		fancy
wind		rush
24	**SURMOUNT**	
mountain		descend
overcome		concede
appease		snub
25	**BOMBASTIC**	
democratic		pompous
bickering		cautious
destructive		anxious
26	**RECUMBENT**	
fugitive		cumbersome
unwieldy		repelling
reclining		penitent
27	**ENVISAGE**	
contemplate		activate
surround		estrange
enfeeble		regress

1.

28 **TRUMPERY**

worthless	heraldry
etiquette	highest
amusement	final

29 **GLOWER**

extinguish	shine
disguise	gloat
aerate	scowl

30 **PERPETRATE**

appropriate	commit
propitiate	deface
control	pierce

31 **LEVITY**

parsimony	velleity
salutary	frivolity
alacrity	tariff

32 **LIBERTINE**

missionary	rescuer
profligate	canard
regicide	farrago

33 **AMULET**

savoury	jacket
flirtation	crest
cameo	charm

34 **QUERULOUS**

astringent	fearful
petulant	curious
inquiring	spurious

35 **TEMERITY**

impermanence	rashness
nervousness	stability
punctuality	submissiveness

36 **FECUND**

esculent	optative
profound	prolific
sublime	salic

37 **ABNEGATE**

contradict	decry
renounce	execute
believe	assemble

38 **TRADUCE**

challenge	attenuate
suspend	establish
misrepresent	conclude

39 **VAGARY**

vagabond	caprice
obscurity	vulgarity
evasion	fallacy

40 **SPECIOUS**

fallacious	coeval
palatial	typical
nutritious	flexible

41 **SEDULOUS**

rebellious	dilatory
complaisant	diligent
seductive	credulous

42 **NUGATORY**

inimitable	adamant
sublime	contrary
numismatic	trifling

43 **ADUMBRATE**

foreshadow	protect
detect	eradicate
elaborate	approach

44 **MINATORY**

implacable	diminutive
belittling	quiescent
depository	threatening

THE END

HAVE YOU ANSWERED <u>EVERY</u> QUESTION (EVEN IF GUESSING) AND ONLY UNDERLINED <u>ONE</u> ANSWER FOR EACH GROUP OF WORDS?

For Office Use: Study Number..... *.....

2.

E-4: Logical Memory Test

Logical Memory I

RECORDING:
Place a tick (✔) next to each story unit recalled verbatim. Write non-verbatim responses next to the story unit.

SCORING RULE:
0–1 pt. for each story or thematic unit
See Administration and Scoring Manual (Chapter 4 and Appendix A) for scoring criteria.

Story A

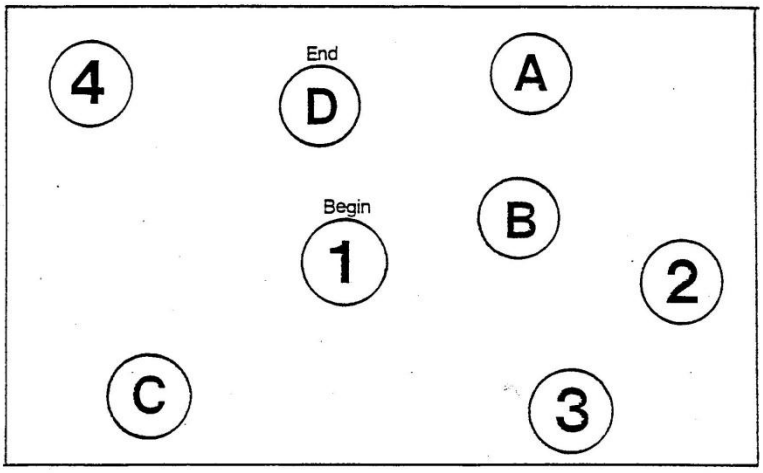
Anna Thompson of South London, employed as a cook in a school canteen, reported at the police station that she had been held up on the High Street the night before and robbed of fifty-six pounds. She had four small children, the rent was due, and they had not eaten for two days. The police, touched by the woman's story, made up a collection for her.

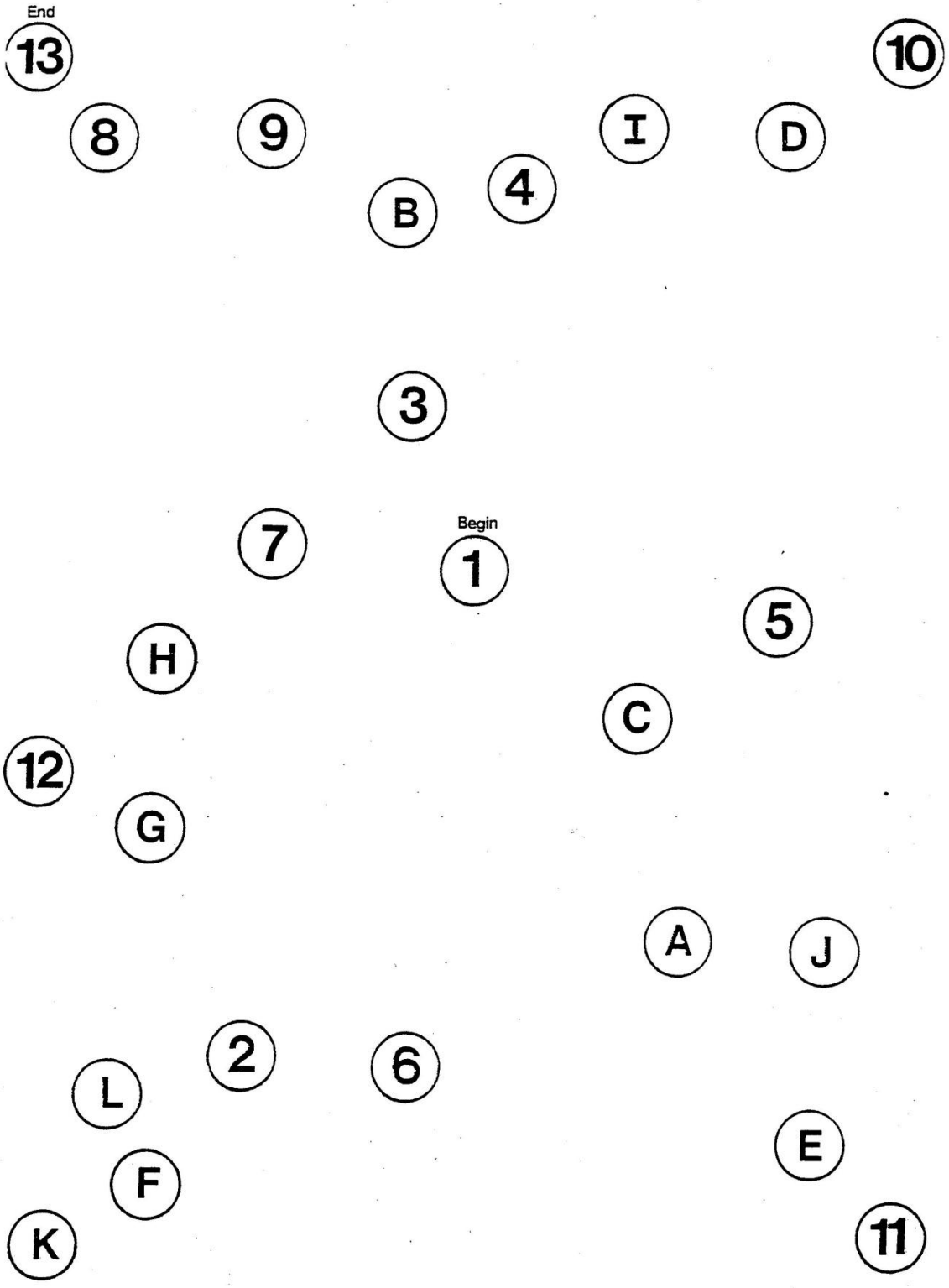
(Turn page to record Story A Responses.)

E-5: Trail-Making Test B

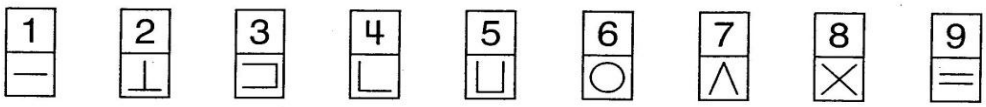
TRAIL MAKING

Part B




End
13
10
8
9
I
D
4
B
3
7
Begin
1
5
H
C
12
G
A
J
2
6
L
E
F
K
11

E-6: Digit Symbol-Coding Test



Sample Items

2	1	3	7	2	4	8	2	1	3	2	1	4	2	3	5	2	3	1	4

5	6	3	1	4	1	5	4	2	7	6	3	5	7	2	8	5	4	6	3

7	2	8	1	9	5	8	4	7	3	6	2	5	1	9	2	8	3	7	4

6	5	9	4	8	3	7	2	6	1	5	4	6	3	7	9	2	8	1	7

9	4	6	8	5	9	7	1	8	5	2	9	4	8	6	3	7	9	8	6

2	7	3	6	5	1	9	8	4	5	7	3	1	4	8	7	9	1	4	5

7	1	8	2	9	3	6	7	2	8	5	2	3	1	4	8	4	2	7	6

E-7: Borkowski Verbal Fluency Task

C	F	L
Total =	Total =	Total =

E -8: Letter-Number Sequencing Test

DISCONTINUE RULE
After failure on all 3 trials of an item.

SCORING RULE
0 or 1 pt. for each response.
Item score = Trial 1 + Trial 2 + Trial 3

	Trial	Item/Response	Trial Score (0 or 1)	Item Score (0, 1, 2, or 3)
1.	1	**L-2** (2-L)		
START	2	**6-P** (6-P)		
	3	**B-5** (5-B)		
2.	1	**F-7-L** (7-F-L)		
	2	**R-4-D** (4-D-R)		
	3	**H-1-8** (1-8-H)		
3.	1	**T-9-A-3** (3-9-A-T)		
	2	**V-1-J-5** (1-5-J-V)		
	3	**7-N-4-L** (4-7-L-N)		
4.	1	**8-D-6-G-1** (1-6-8-D-G)		
	2	**K-2-C-7-S** (2-7-C-K-S)		
	3	**5-P-3-Y-9** (3-5-9-P-Y)		
5.	1	**M-4-E-7-Q-2** (2-4-7-E-M-Q)		
	2	**W-8-H-5-F-3** (3-5-8-F-H-W)		
	3	**6-G-9-A-2-S** (2-6-9-A-G-S)		
6.	1	**R-3-B-4-Z-1-C** (1-3-4-B-C-R-Z)		
	2	**5-T-9-J-2-X-7** (2-5-7-9-J-T-X)		
	3	**E-1-H-8-R-4-D** (1-4-8-D-E-H-R)		
7.	1	**5-H-9-S-2-N-6-A** (2-5-6-9-A-H-N-S)		
	2	**D-1-R-9-B-4-K-3** (1-3-4-9-B-D-K-R)		
	3	**7-M-2-T-6-F-1-Z** (1-2-6-7-F-M-T-Z)		

Total Raw Score (Maximum = 21)

APPENDIX F: Diabetic retinopathy characteristics graded in the Edinburgh Type 2 Diabetes Study

1. NUMBER OF MICROANEURYSMS (MA Grade)

Features of a microaneurysm

- red spot
- sharp margins
- even density
- usually round (but may be fusiform – sausage shaped)
- usually red (but may be pink or dull white if opacified)

NB.:

- A red spot which is < 150 μ (125 μ ETDRS) (the width of a major vein at the disc margin) with sharp margins and even density is considered to be a microaneurysm. Otherwise it is assessed as a retinal haemorrhage.

- A red spot which is > 150 μ is assessed as a retinal haemorrhage unless features e.g. round shape, smooth margins etc suggest that it is likely to be a microaneurysm.

GRADE ALL FIELDS

	CODE DEFINITION
0	**No MA**
1	**Questionable MA**
2	**1 MA**
3	**2 MA**
4	**3 MA**
5	**4 MA**
6	**5 or more MA**
X	**Cannot grade**

2 HAEMORRHAGES AND MICROANEURYSMS H/MA

STANDARD PHOTOS 1 2A 2B

Notes:

- Preretinal haemorrhages are excluded
- Subretinal haemorrhages are excluded
- Microaneurysms that appear as white dots with no blood visible in a central lumen are graded as hard exudates
- All other haemorrhages and microaneurysms are included

GRADE FIELDS 2-7

	CODE DEFINITION
0	**No H/MA**
1	**Questionable H/MA**
2	**Definite H/MA < 1**
3	**Definite H/MA ≥ 1 < 2A**
4	**Definite H/MA ≥ 2A < 2B**
5	**Definite H/MA ≥ 2 B**
X	**Cannot grade**

3 HARD EXUDATES (HE)

STANDARD PHOTOS 3 4 5

Notes on Appearance:

- yellowish –white (white if regressing)
- waxy appearance
- sharp margins
- usually irregular shape
- don't have a border of pigment
- can have a varied appearance –
    - dots
    - patches: may be confluent
    - partial or complete rings surrounding microaneurysms

NB: include perivenous exudates

GRADE FIELDS 2-7

	CODE DEFINITION
0	**No hard exudates**
1	**Questionable hard exudate**
2	**Definite hard exudate < 3**
3	**Definite hard exudate ≥ 3 but < 5**
4	**Definite hard exudate ≥ 5 but < 4**
5	**Definite hard exudate ≥ 4**
X	**Cannot grade**

4 COTTON WOOL SPOTS (Soft Exudates, CWS)

STANDARD PHOTOS 8A 5

Notes:

- localised swellings in the nerve fibre layer
- round or oval
- white, pale yellow – white or greyish – white
- "feathery edges"
- frequently have striations parallel to nerve fibres
- a large CWS may displace a vessel
- may be –
    - haemorrhage on the distal side
    - IRMA on the proximal side
    - beading may also be present

GRADE ALL FIELDS

	CODE DEFINITION
0	**No cotton wool spot**
1	**Questionable cotton wool spot**
2	**Definite CWS < 8A**
3	**Definite CWS ≥ 8A < 5**
4	**Definite CWS ≥ 5**
X	**Cannot grade**

5 INTRARETINAL MICROVASCULAR ABNORMALITIES (IRMA)

STANDARD PHOTOS 8A 8B

Notes:

- tortuous intraretinal vascular segments
- vary in calibre from barely visible to 35 μm (31 μm ETDRS) (¼ width of a major vein at the disc margin) or occasionally larger.
- Punctate microaneurysms in the retina and new vessels on the surface of the retina are excluded.
- Without stereo it may be difficult to distinguish IRMA from new vessels. In general IRMA are more:
    - Delicate
    - Angular or jagged in their tortuosity
    - less likely to cross themselves or other retinal vessels
    - More likely to occur in relatively open areas between major vessels

GRADE FIELDS 2-7

	CODE DEFINITION
0	**No IRMA**
1	**Questionable IRMA**
2	**Definite IRMA < 8A**
3	**IRMA ≥ 8A but < 8B**
4	**IRMA ≥ 8B**
X	**Cannot grade**

6 VENOUS BEADING (VB)

STANDARD PHOTOS 6A 6B

Notes:

- localised increases in venous calibre which sometimes resemble a string of beads
- Grading is based on the total length of vein involved and the severity using standard photos.

GRADE FIELDS 3-7

	CODE DEFINITION
0	**No beading**
1	**Questionable beading**
2	**Definite beading < 6A**
3	**Beading ≥ 6A but < 6B**
4	**Beading ≥ 6B**
X	**Cannot grade**

7 VENOUS LOOPS AND/OR REDUPLICATION

Notes & Definitions:

Venous loop: An abrupt, curving deviation of a vein from its normal path

Venous reduplication: Dilation of a pre-existing channel or the proliferation of a new channel of similar calibre adjacent to the original vein.

NB: Grading is based on the calibre of veins involved - 35µm (31µm ETDRS) = ¼ width of a major vein at disc margin.

GRADE FIELDS 3-7

	CODE DEFINITION
0	**No loops or reduplications**
1	**Questionable loop(s) or definite loop(s) in a branch < 31 µm wide if the loop < 50% wider than the vein from which it arises.**
2	**Loops in a branch ≥ 31 µm wide or loop(s) in a branch < 31µm wide if the loop is ≥ 50% wider than the vein From which it arises**
3	**Reduplication of a small vein < 31 µm wide**
4	**Reduplication of a larger vein ≥ 31 µm wide**
X	**Cannot grade**

8 NEW VESSELS ELSEWHERE (NVE)

STANDARD PHOTO 7

Notes:

- New vessels that are clearly on the surface of the retina (i.e. not within the retina) or further forward in the vitreous cavity – except for those on the disc or within 1 disc diameter of its margin (or in the vitreous anterior to this area)

- However if new vessels located mostly elsewhere extend into the area between ½ - 1 DD from the disc margin and no other vessels are present closer to or on the disc, all the new vessels are included in the NVE category

- Grading is based on the area of retina covered by the new vessels using as cut points on the grading scale ½ disc area (DA) and NVE in standard photo 7

- I the absence of stereo it may be difficult to distinguish subtle new vessels from IRMA. In general new vessels are:

    - Bolder
    - More curvilinear
    - More likely to cross and recross both themselves and the retinal vessels
    - More likely to be sited over retinal vessels

GRADE FIELDS 1, 3-7

	CODE DEFINITIONS
0	**No NVE**
1	**Questionable NVE**
2	**Definite NVE < ½ DA**
3	**NVE ≥ ½ DA < photo 7**
4	**NVE ≥ photo 7**
X	**Cannot grade**

9 FIBROUS PROLIFERATIONS ELSEWHERE (FPE)

STANDARD PHOTO 11

Notes:

- Fibrous tissue opaque enough to be definitely seen, with or without accompanying new vessels including fibrous strands or sheets that comprise a thickened posterior hyaloid surface as well as completely atrophied new vessels (no visible red blood column present)

- Fibrous proliferations on the surface of the retina or further forward in the vitreous cavity – except for those on the disc or within 1 disc diameter of its margin (or in the vitreous anterior to this area)

- However if fibrous proliferations located mostly elsewhere extend into the are between ½ - 1 DD from the disc margin and no other fibrous proliferations are present closer to or on the disc, all fibrous proliferations are included in the FPE category

GRADE FIELDS 1, 3-7

	CODE DEFINITION
0	**No FPE**
1	**Questionable FPE**
2	**Definite FPE < ½ DA**
3	**FPE ≥ ½ DA < photo 11**
4	**FPE ≥ photo 11**
X	**Cannot grade**

10 PRERETINAL HAEMORRHAGE (PRH)

STANDARD PHOTOS 9 13

Notes:

- Round, oval or linear patches of haemorrhage just anterior to the retina or under its internal limiting membrane (Photo 9)
- Boat-shaped haemorrhage with a fluid level (Photo 13)
- Haemorrhage on the surface of detached retina is also considered to be PRH
- Grading is based on the area of retina covered by haemorrhage using standard photos 9 and 13 and the area of one half of the field for comparison

GRADE ALL FIELDS

	CODE DEFINITION
0	**No PRH**
1	**Questionable PRH**
2	**Definite PRH < photos 9 or 13**
3	**PRH ≥ photos 9 or 13 < ½ field**
4	**PRH ≥ ½ field**
X	**Cannot grade**

11 VITREOUS HAEMORRHAGE VH

Notes:

- Haemorrhage further forward in the vitreous cavity than PRH, including haemorrhage on or within fibrovascular proliferations.

- Grade 1 (Questionable) – Assigned when the haziness present could be due to haemorrhage, lens opacity, or poor focus.

  NB:If definite haemorrhage is present but whether it is PRH or VH is uncertain – the grade questionable may not be used for both. The grader must decide which definite grade is the best choice.

- Grade 2 (Definite) – When VH is definitely present but interferes with grading of < 1 DA of the field.
  - Diffuse VH (so thin that it does not prevent the grading of the fundus abnormalities) covering, but not obscuring, IDA or more of field (occasionally all of field)

- Grade 3 (Definite) – When VH is dense enough to obscure a major retinal vessel (to the extent that beading, narrowing etc cannot be graded) and extensive enough to obscure > 1 DA but < ½ field.

- Grade 4 (Definite) – When VH obscures ½ field but some detail can still be graded (eg fibrovascular proliferations)

- Grade 5 (Definite) – Only when all fundus details are obscured by VH.

- Grade X (Cannot grade) – Cannot grade for reasons other than VH with 2 exceptions (both in field 2 only):
  1. obscuration of the macula by VH is graded 2, definitely present.
  2. it might be possible to grade posterior vitreous detachment by inference from adjacent field (see below)

GRADE ALL FIELDS

	CODE DEFINITION
0	**No VH**
1	**Questionable VH**
2	**Definite VH obscuring < 1 DA**
3	**VH ≥ 1 DA < ½ field**
4	**VH ≥ ½ field, but some characteristics can still be graded**
5	**VH obscures the entire field**
X	**Cannot grade (for reasons other than VH)**

12 NEW VESSELS ON OR WITHIN 1DD OF DISC (NVD)

STANDARD PHOTOS 10A 10C

Notes:

- New vessels on the disc or within 1 disc diameter of its margin (or in the vitreous anterior to this area**)**
- NB: NVE can extend into an area between ½ - 1DD from the disc margin and as long as no other vessels are present closer to or on the disc – this would be classified as NVE.

GRADE FIELD 1 ONLY

	CODE DEFINITION
0	**No NVD**
1	**Questionable NVD**
2	**Definite NVD < 10 A**
3	**NVD ≥ 10 A < 10 C**
4	**NVD ≥ 10 C**
X	**Cannot grade**

13 FIBROUS PROLIFERATIONS AT THE DISC (FPD)

STANDARD PHOTOS 10B 7

Notes:

- White sheets or fine strands of fibrous tissue opaque enough to be definitely seen, with or without accompanying new vessels, including:
  - Fibrous strands or sheets that comprise a thickened posterior hyaloid surface
  - Completely atrophic new vessels (no visible red blood column present)

- Fibrous proliferations on or within IDD of the disc (or in the vitreous anterior to this area)

- NB : fibrous proliferations elsewhere can extend into the area between ½ - 1DD from the disc margin and as long as no other fibrous proliferations are present closer to or on the disc – this would be classified as fibrous proliferations elsewhere – FPE)

- Grading is based on the area involved by FPD, not their thickness or whiteness, using cut points on the scale 2 DA and standard photo 10B.

- If the FPD occur in fine strands, the grader mentally compresses them into a network of density similar to that of the NVE in standard photo 7 and estimates the area that would be covered.

GRADE FIELD 1 ONLY

	CODE DEFINITION
0	**No FPD**
1	**Questionable FPD**
2	**Definite FPD < photo 10B**
3	**FPD ≥ 10 B < 2DA**
4	**FPD ≥ 2 DA**
X	**Cannot grade**

14 MACULAR LASER SCARS

Notes:

Macular grid laser:
A grid of laser around the fovea used to treat central serous macular oedema (with or without the presence of exudates).

Focal laser:

- Usually within the temporal arcades but not centered on the fovea.
- Used to treat areas of leakage (usually exudates are present) away from the fovea but still within the temporal arcades
- May also be used to directly treat NVE in conjunction with pan retinal photocoagulation.

NB: Focal laser not included for an eye with no microaneurysms

GRADE FIELD 2 ONLY

	CODE DEFINITION
0	**No photocoagulation scars**
1	**Questionable photocoagulation scars**
2	**Definite photocoagulation scars**
X	**Cannot grade**

15 DIABETIC MACULOPATHY [EXUDATES]

Notes:

- Diabetic maculopathy is defined as hard exudates within 1 disc diameter of the centre of the macula.
- This characteristic does not form part of the ETDRS grading system, but provides a separate variable for use in the ET2DS.
- Hard exudates will be recorded in the same field <u>in addition</u> to this diabetic maculopathy characteristic, and will contribute to the final level grading system as usual.

GRADE FIELD 2 ONLY

	CODE DEFINITION
0	**No Diabetic Maculopathy**
1	**Questionable Diabetic Maculopathy**
2	**Definite Diabetic Maculopathy**
X	**Cannot grade**

16 PANRETINAL PHOTOCOAGULATION SCARS (PRP)

Notes:

- Usually for severe non proliferative or proliferative retinopathy
- An uneven pattern of burns in all 4 quadrants sparing the macula and the papillomacular bundle.
- Graded at the level of each individual field

GRADE FIELDS 3-7

	CODE DEFINITION
0	**No photocoagulation**
1	**Questionable photocoagulation**
2	**Definite photocoagulation**
X	**Cannot grade**

16 PANRETINAL PHOTOCOAGULATION SCARS (PRP)

Notes:

- Graded at the level of each eye, therefore proving a simple indication of whether or not PRPs were present for use in calculating final level score. (see notes on review of subjects with photocoagulation scars)

GRADE EACH EYE

	CODE DEFINITION
0	**No photocoagulation**
1	**Questionable photocoagulation**
2	**Definite photocoagulation**
X	**Cannot grade**

18 RETINA OBSCURED DUE TO VITREOUS HAEMORRHAGE

Notes:

- Provides an indication at the level of each eye of whether or not the retina is obscured by VH for use in calculating final level score.

GRADE EACH EYE

CODE	**DEFINITION**
Yes	
No	
x	**= not gradable**

19 RETINAL HAEMORRHAGE

Notes:

- Graded at the level of each eye, therefore proving a simple indication of whether or not retinal haemorrhage was present, for use in calculating final level scores (relevant to level 15 and 35 in particular).)

GRADE EACH EYE

	CODE DEFINITION
0	**No Retinal Haemorrhage**
1	**Questionable Retinal Haemorrhage**
2	**Definite Retinal Haemorrhage**
X	**Cannot grade**

APPENDIX G: Comparisons of characteristics between subjects with and without missing cognitive data

Table g1: Characteristics in subjects with and without Letter-Number Sequencing (LNS) test data

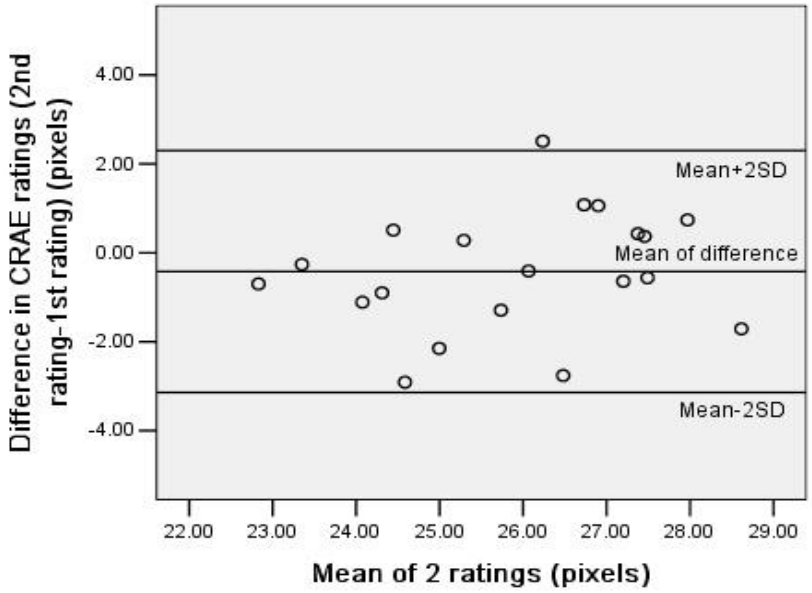
	Subjects with LNS values (n=1048)[a]	Subjects with missing LNS values (n=18)[b]
Age (years),mean (SD)	67.4(4.2)	67.1(4.7)
Sex, (% (n) men)	51.2 (537)	55.6(10)
BMI (kg/m^2), mean (SD)	31.5(5.7)	29.1(4.9)
WHR, mean (SD)	0.97(0.08)	1.00(0.08)
HbA1c, median (IQR)	7.4(6.7-7.9)	7.3(6.5-8.5)
Total cholesterol (mmol/L), mean (SD)	4.3(0.9)	4.7(0.9)
HDL cholesterol (mmol/L), mean (SD)	1.3(0.4)	1.4(0.3)
Smoker, %(n)[1]	61.2 (641)	44.4(8)
Hypertension, %(n)	84.9 (890)	77.8 (14)
Cardiovascular disease, %(n)		
MI and/or Angina	31.0 (325)	27.8 (5)
Stroke and/or TIA	8.8 (92)	5.6 (1)
Diabetic retinopathy, % (n)	38.1(391)	50.0(9)
Retinal width parameters		
Central Retinal Arteriolar Equivalent, μm, mean (SD)	29.6(4.3)	27.3(4.5)*
Central Retinal Venular Equivalent, μm, mean (SD)	40.3(6.3)	36.3(6.2)*
Aretrio-venous ratio	0.74(0.10)	0.76(0.14)
Retinal bifurcation parameters		
Median bifurcation angles, degrees, mean (SD)	78.5(10.2)	78.4(13.3)
Median branching coefficient, mean (SD)	1.8(0.2)	1.9(0.2)

[1]Smoker=current or former smoker; * P <0.05; [a] Maximum n=1048, minimum n=438 for subjects with LNS data; [b]Maximum n=18, minimum n=6 for subjects without LNS data.

APPENDIX H: Reliability of retinal vessel widths and arteriolar bifurcation measurements: Bland-Altman plots

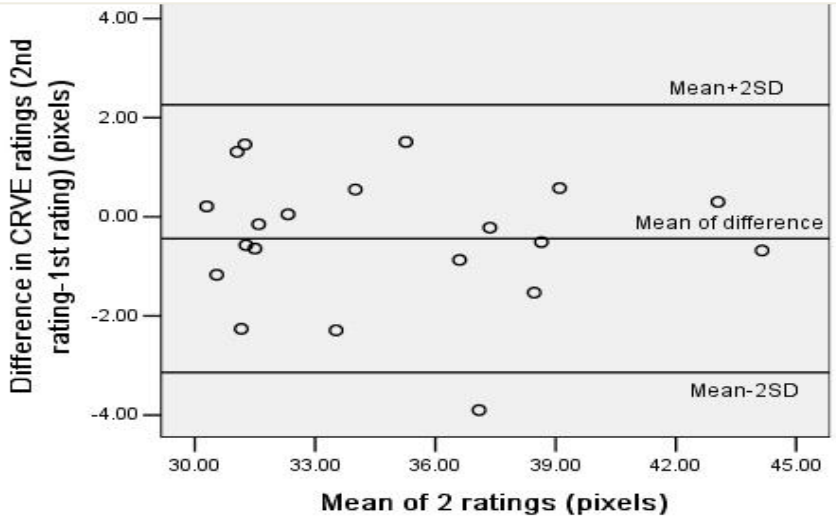
H-1: Intra-rater Reliability

Figure h1: Difference against mean for CRAE data (n=20)



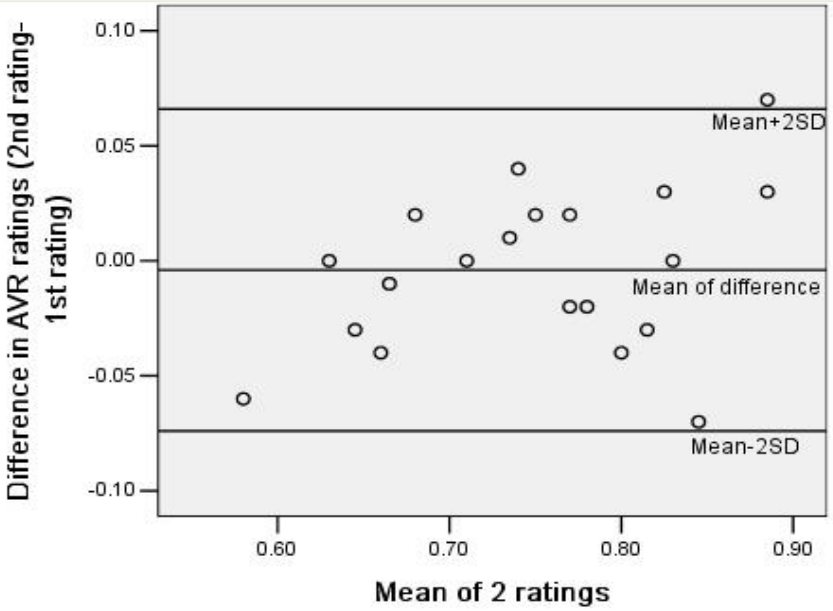
The mean difference was -0.42; the upper and lower limits of agreement (95% limits of agreement) were 2.3 and -3.1 respectively. For arteriolar vessels that range between 22.0 -29.0 pixels, 95% of the repeat measurements fell within ±2.7 pixels of the first measurements. There was no systematic bias and the size of differences did not change with increasing CRAE.

Figure h2: Difference against mean for CRVE data (n=20)



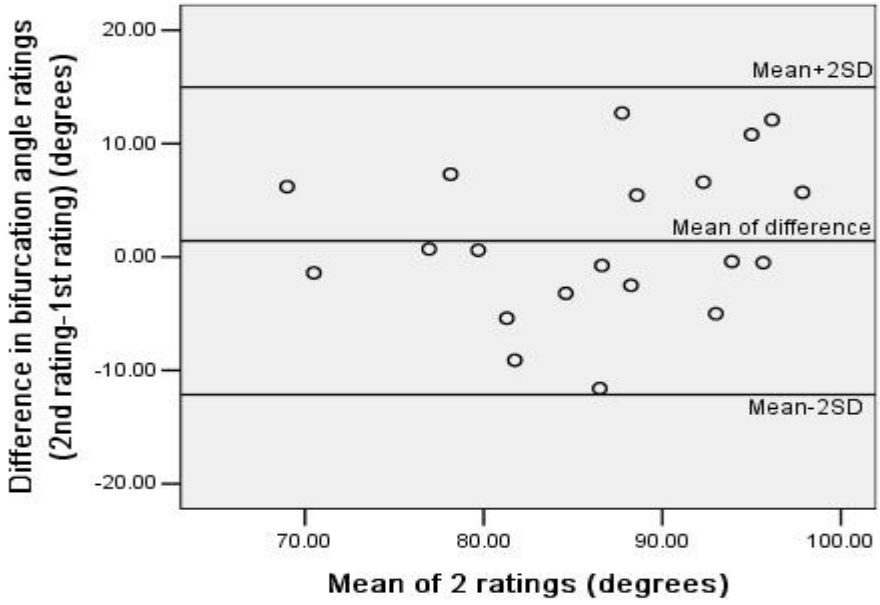
The mean difference was -0.44; the upper and lower limits of agreement (95% limits of agreement) were 2.26 and -3.14 respectively. For venular vessels that range between 30.0 -45.0 pixels, 95% of the repeat measurements fell within ±2.7 pixels of the first measurements. There was no systematic bias and the size of differences did not change with increasing CRVE.

Figure h3: Difference against mean for AVR data (n=20)



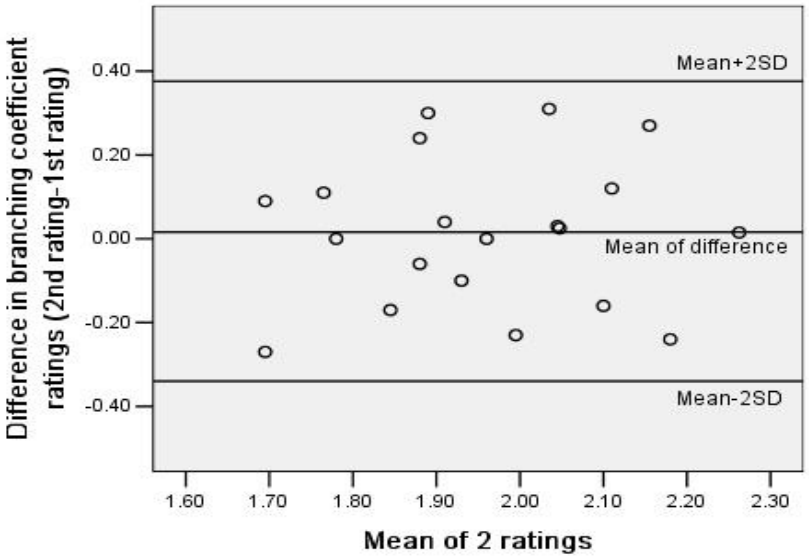
The mean difference was -0.004; the upper and lower limits of agreement (95% limits of agreement) were 0.066 and -0.074 respectively. For AVR that range between 0.58 -0.90, 95% of the repeat measurements fell within ±0.07 of the first measurements. There was no systematic bias and the size of differences did not change with increasing AVR.

Figure h4: Difference against mean for arteriolar bifurcation angle data (n=20)



The mean difference was 1.42; the upper and lower limits of agreement (95% limits of agreement) were 14.98 and -12.14 respectively. For bifurcation angle that range between 69-100^0, 95% of the repeat measurements fell within ± 13.6^0 of the first measurements. There was no systematic bias and the size of differences did not change with increasing bifurcation angle.

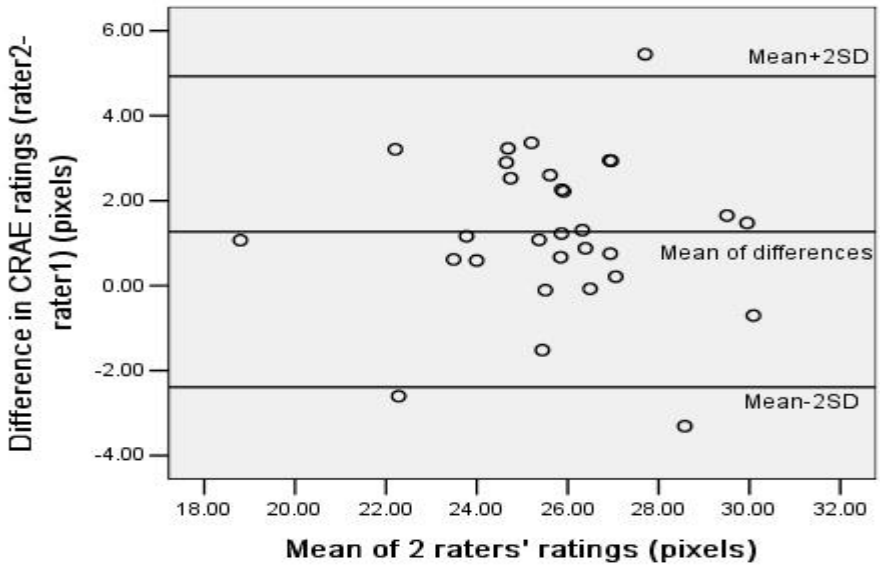
Figure h5: Difference against mean for arteriolar branching coefficient data (n=20)



The mean difference was 0.016; the upper and lower limits of agreement (95% limits of agreement) were 0.38 and -0.34 respectively. For branching coefficient that range between 1.6-2.3, 95% of the repeat measurements fell within ± 0.36 of the first measurements. There was no systematic bias and the size of differences did not change with increasing branching coefficient.

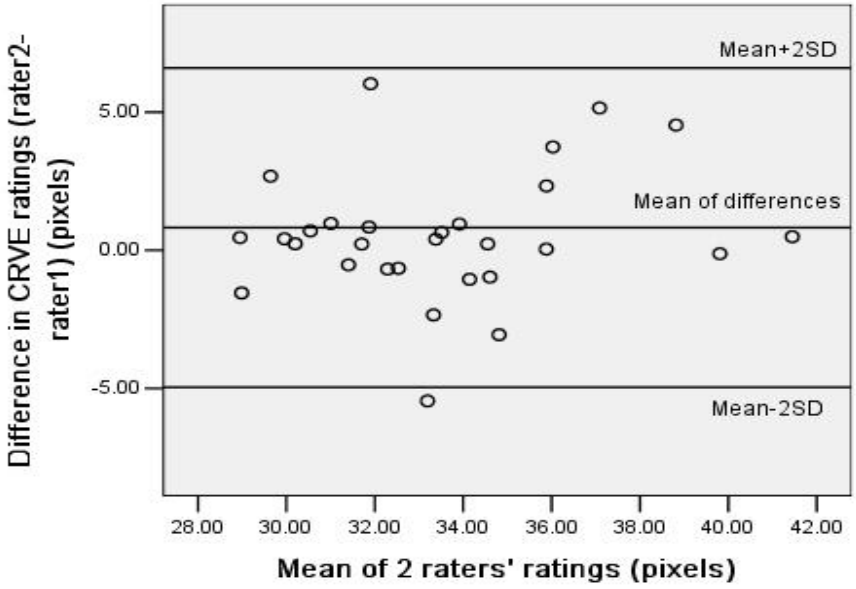
H-2: Inter-rater Reliability

Figure h6: Difference against mean for CRAE data (n=30)



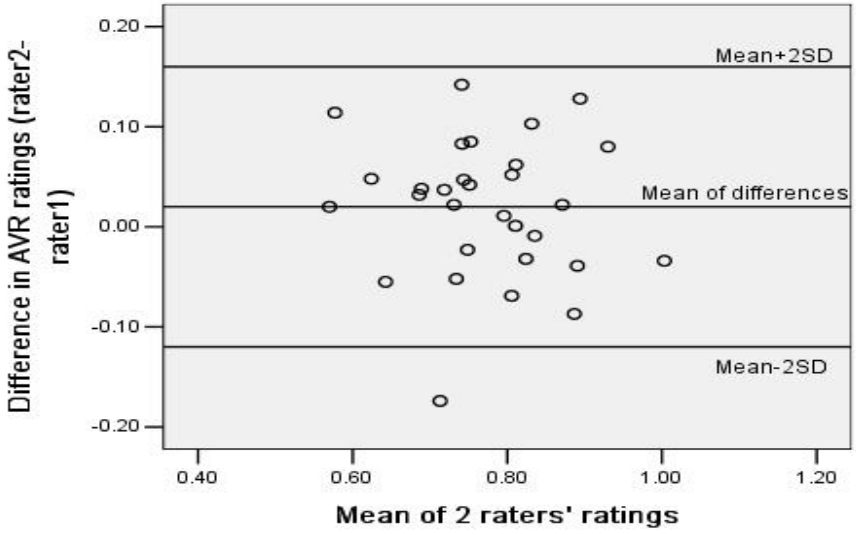
The mean difference was 1.27; the upper and lower limits of agreement (95% limits of agreement) were 4.9 and -2.4 respectively. For arteriolar vessels that range between 18-32 pixels, 95% of the repeat measurements fell within ±3.6 pixels of the first rater (rater of our study) measurements. There was no systematic bias and the size of differences did not change with increasing CRAE.

Figure h7: Difference against mean for CRVE data (n=30)



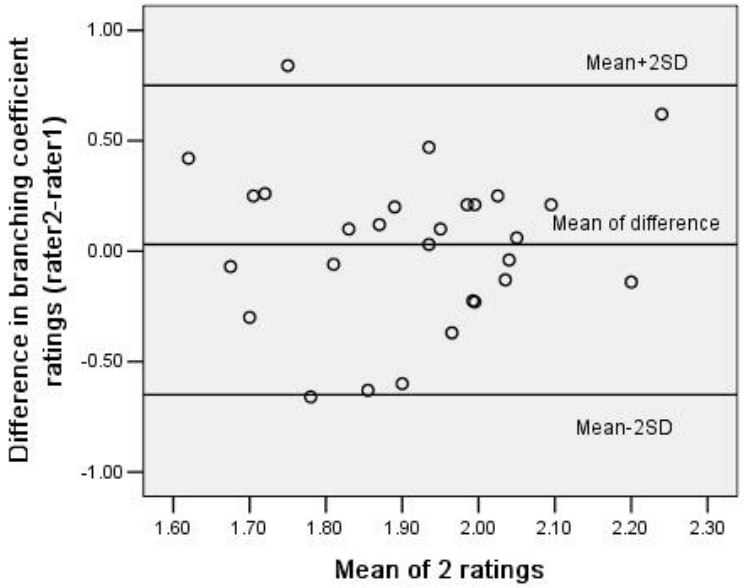
The mean difference was -0.82; the upper and lower limits of agreement (95% limits of agreement) were 6.5 and -4.8 respectively. For venular vessels that range between 28-42 pixels, 95% of the repeat measurements fell within ±5.7 pixels of the first rater's (rater of our study) measurements. There was no systematic bias and the size of differences did not change with increasing CRVE.

Figure h8: Difference against mean for AVR data (n=30)



The mean difference was 0.02; the upper and lower limits of agreement (95% limits of agreement) were 0.16 and -0.12 respectively. For AVR that range between 0.58 -1.00, 95% of the repeat measurements fell within ±0.14 of the first rater's (rater of our study) measurements. There was no systematic bias and the size of differences did not change with increasing AVR.

Figure h9: Difference against mean for branching coefficient data (n=30)



The mean difference was 0.03; the upper and lower limits of agreement (95% limits of agreement) were 0.65 and -0.75 respectively. For branching coefficient that range between 1.6-2.3, 95% of the repeat measurements fell within ±0.7 of the first rater's (rater of our study) measurements. There was no systematic bias and the size of differences did not change with increasing AVR.

APPENDIX I: Multivariate models

Table i1: Multivariate –adjusted regression analysis of general cognitive factor (g) in relation to severity of diabetic retinopathy

Model Term	**Model 1**		**Model 2**		**Model 3**		**Model 4**		**Model 5**		**Model 6**		**Model 7**	
	B	SE	B	SE	B	SE	B	SE	B	SE	B	SE	B	SE
Diabetic retinopathy	-0.16†	0.05	-0.14‡	0.04	-0.15‡	0.04	-0.13 †	0.04	-0.11*	0.04	-0.11*	0.04	-0.06	0.05
Age	-0.06‡	0.01	-0.06‡	0.01	-0.06‡	0.01	-0.06‡	0.01	-0.06‡	0.01	-0.06‡	0.01	-0.05‡	0.01
Sex (male vs. female)	-0.07	0.06	-0.17‡	0.05	-0.18‡	0.05	-0.13*	0.06	-0.11	0.06	-0.17 †	0.06	-0.18†	0.06
MHVS			0.11‡	0.01	0.10‡	0.01	0.10‡	0.01	0.10‡	0.01	0.10‡	0.01	0.10‡	0.01
Education					0.14‡	0.04	0.13‡	0.04	0.13‡	0.04	0.12 ‡	0.04	0.11 †	0.04
Alcohol intake							0.04*	0.02	0.03	0.02	0.03	0.02	0.03	0.02
Smoking (yes vs. no)							-0.10	0.05	-0.08	0.05	-0.06	0.05	-0.05	0.05
Waist to hip ratio (per 0.1 unit increase)							-0.10*	0.04	-0.09*	0.04	-0.06	0.04	-0.08	0.04
Systolic blood pressure							0.00	0.002	0.00	0.002	-0.001	0.001	-0.001	0.002
Total cholesterol level							0.004	0.03	0.003	0.03	0.006	0.03	-0.01	0.03
MI and/or angina									-0.06	0.06	-0.01	0.06	0.01	0.06
Stroke and/or TIA									-0.29‡	0.09	-0.26†	0.09	-0.27 †	0.09
Depression											-0.44 ‡	0.10	-0.44 ‡	0.10
HbA1c													0.03	0.02
Duration of diabetes													-0.07*	0.03
Adj.R^2	0.066		0.396		0.402		0.408		0.415		0.427		0.426	
Df	(3,988)		(4,987)		(5,946)		(10,941)		(12,939)		(13,938)		(15,900)	
F	24.46‡		163.70‡		128.85‡		66.62‡		57.13‡		55.49‡		46.30‡	
ΔR^2 (R square change)			0.33		0.008		0.009		0.007		0.013		0.004	
Df			(1,987)		(1,946)		(5,941)		(2,939)		(1,938)		(2,900)	
ΔF (F change)			541.29‡		13.49‡		3.02*		6.07 †		21.10‡		3.42*	

‡P≤0.001, †P≤0.01, *P<0.05; Figures are unstandardised Beta regression coefficients (B) and standard errors (SE)

Table i2: Multivariate –adjusted regression analysis of Verbal Fluency test in relation to severity of diabetic retinopathy

Model Term	**Model 1**	**Model 2**	**Model 3**	**Model 4**	**Model 5**	**Model 6**	**Model 7**
	B SE	B SE	B SE	B SE	B SE	B SE	B SE
Diabetic retinopathy	-1.46* 0.70	-1.45* 0.63	-1.52* 0.63	-1.24 0.65	-1.14 0.65	-1.11 0.65	-0.23 0.72
Age	-0.25† 0.10	-0.25† 0.09	-0.23† 0.09	-0.20* 0.09	-0.22* 0.09	-0.22* 0.09	-0.21* 0.09
Sex (male vs. female)	0.55 0.80	-0.32 0.72	-0.39 0.72	-0.49 0.91	-0.54 0.91	-0.85 0.91	-0.85 0.91
MHVS		1.07‡ 0.07	0.97‡ 0.08	0.95‡ 0.08	0.95‡ 0.08	0.94‡ 0.08	0.93‡ 0.08
Education			1.56** 0.53	1.51† 0.54	1.61† 0.54	1.55 † 0.54	1.53† 0.54
Alcohol intake				0.53* 0.25	0.54* 0.25	0.52* 0.25	0.51* 0.25
Smoking (yes vs. no)				0.02 0.79	0.02 0.79	0.15 0.79	0.45 0.79
Waist to hip ratio (per 0.1 unit increase)				-0.65 0.57	-0.69 0.57	-0.54 0.57	-0.03 0.57
Systolic blood pressure				-0.04 0.02	-0.04 0.02	-0.04 0.02	-0.05* 0.02
Total cholesterol level				0.18 0.42	0.19 0.42	0.21 0.42	0.08 0.42
MI and/or angina					1.49 0.82	1.72 0.84	1.89 0.84
Stroke and/or TIA					-2.31 1.32	-2.13 1.32	-2.20 1.32
Depression						-2.62 1.41	-2.0 1.41
HbA1c							-0.15 0.36
Duration of diabetes							-0.88* 0.41
Adj.R^2	0.008	0.196	0.202	0.205	0.208	0.210	0.206
Df	(3,1022)	(4,1021)	(5,978)	(10,973)	(12,971)	(13,970)	(15,929)
F	3.77*	63.50‡	50.71‡	26.31‡	22.48‡	21.06‡	17.31‡
ΔR^2 (R square change)		0.19	0.007	0.007	0.005	0.003	0.005
Df		(1,1021)	(1,978)	(5,973)	(2,971)	(1,970)	(2,929)
ΔF (F change)		240.04‡	8.00†	1.72	2.84	3.44	2.76

‡P≤0.001, †P≤0.01, *P<0.05; Figures are unstandardised Beta regression coefficients (B) and standard errors (SE)

Table i3: Multivariate –adjusted regression analysis of Faces test in relation to severity of diabetic retinopathy

Model Term	Model 1		Model 2		Model 3		Model 4		Model 5		Model 6		Model 7	
	B	SE	B	SE	B	SE	B	SE	B	SE	B	SE	B	SE
Diabetic retinopathy	-0.92*	0.42	-0.91*	0.40	-0.82*	0.41	-0.70	0.42	-0.69	0.42	-0.68	0.42	-0.20	0.46
Age	-0.28‡	0.06	-0.28 ‡	0.05	-0.28‡	0.05	-0.28‡	0.05	-0.27‡	0.06	-0.27‡	0.06	-0.25‡	0.06
Sex (male vs. female)	-2.7‡	0.48	-3.04 ‡	0.46	-3.10‡	0.46	-3.06‡	0.46	-3.02‡	0.59	-3.20‡	0.60	-3.26‡	0.61
MHVS			0.44‡	0.04	0.40‡	0.05	0.41‡	0.04	0.38 ‡	0.05	0.37 ‡	0.05	0.36 ‡	0.05
Education					0.49	0.35	0.41	0.34	0.45	0.34	0.42	0.39	0.52	0.36
Alcohol intake							0.32	0.16	0.30	0.16	0.29	0.16	0.32	0.17
Smoking (yes vs. no)							-0.11	0.50	-0.08	0.50	-0.01	0.51	0.03	0.52
Waist to hip ratio (per 0.1 unit increase)							-0.56	0.36	-0.53	0.36	-0.45	0.37	-0.38	0.38
Systolic blood pressure							0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.02
Total cholesterol level							-0.12	0.27	-0.13	0.27	-0.12	0.27	-0.21	0.28
MI and/or angina									-0.46	0.53	-0.34	0.53	-0.16	0.55
Stroke and/or TIA									-0.14	0.85	-0.04	0.85	0.03	0.86
Depression											-1.34	0.90	-1.51	0.93
HbA1c													0.14	0.23
Duration of diabetes													-0.78†	0.41
Adj.R^2	0.057		0.142		0.136		0.138		0.137		0.138		0.140	
Df	(3,1023)		(4,1022)		(5,979)		(10,974)		(12,972)		(13,971)		(15,930)	
F	21.8‡		43.4‡		32.01‡		16.77‡		14.03‡		13.14‡		11.22‡	
ΔR^2 (R square change)			0.085		0.002		0.006		0.001		0.002		0.008	
Df			(1,1022)		(1,979)		(5,974)		(2,972)		(1,971)		(2,930)	
ΔF (F change)			101.49‡		2.03		1.45		0.42		0.22		4.50*	

‡P≤0.001, †P≤0.01, *P<0.05; Figures are unstandardised Beta regression coefficients (B) and standard errors (SE)

Table i4: Multivariate –adjusted regression analysis of Trail Making Test B in relation to severity of diabetic retinopathy

Model Term	**Model 1**		**Model 2**		**Model 3**		**Model 4**		**Model 5**		**Model 6**		**Model 7**	
	B	SE	B	SE	B	SE	B	SE	B	SE	B	SE	B	SE
Diabetic retinopathy	0.07†	0.02	0.06 †	0.02	0.06†	0.02	0.06 †	0.02	0.05*	0.02	0.05*	0.02	0.03	0.02
Age	0.02‡	0.003	0.02‡	0.003	0.02‡	0.003	0.02‡	0.003	0.02‡	0.003	0.02‡	0.003	0.02‡	0.003
Sex (male vs. female)	0.02	0.02	0.05*	0.02	0.05*	0.02	-0.01	0.03	-0.02	0.03	0.01	0.03	0.01	0.03
MHVS			-0.03‡	0.002	-0.03‡	0.003	-0.03‡	0.003	-0.03‡	0.003	-0.03‡	0.003	-0.03‡	0.003
Education					-0.01	0.02	-0.01	0.02	-0.01	0.02	-0.002	0.02	0.001	0.02
Alcohol intake							0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Smoking (yes vs. no)							0.05	0.03	0.04	0.03	0.03	0.03	0.03	0.03
Waist to hip ratio (per 0.1 unit increase)							0.05†	0.02	0.05†	0.02	0.04*	0.02	0.04*	0.02
Systolic blood pressure							0.00	0.001	0.00	0.001	0.00	0.001	0.00	0.001
Total cholesterol level							-0.001	0.01	-0.001	0.01	-0.002	0.01	0.004	0.01
MI and/or angina									0.01	0.03	-0.01	0.03	-0.01	0.03
Stroke and/or TIA									0.11†	0.04	0.10*	0.04	0.11*	0.04
Depression											0.18‡	0.05	0.19‡	0.05
HbA1c													-0.01	0.01
Duration of diabetes													0.03*	0.01
Adj.R^2	0.053		0.194		0.191		0.199		0.203		0.215		0.223	
Df	(3,1014)		(4,1013)		(5,971)		(10,966)		(12,964)		(13,963)		(15,923)	
F	20.1‡		62.23‡		47.02‡		25.21‡		21.73‡		21.51‡		18.99‡	
ΔR^2 (R square change)			0.141		0.000		0.012		0.006		0.012		0.005	
Df			(1,1013)		(1,971)		(5,966)		(2,964)		(1,963)		(2,923)	
ΔF (F change)			178.15‡		0.142		2.93*		3.66*		15.06‡		2.88	

‡P≤0.001, †P≤0.01, *P<0.05; Figures are unstandardised Beta regression coefficients (B) and standard errors (SE)

Table i5: Multivariate –adjusted regression analysis of Digit Symbol Test in relation to severity of diabetic retinopathy

Model Term	Model 1		Model 2		Model 3		Model 4		Model 5		Model 6		Model 7	
	B	SE	B	SE	B	SE	B	SE	B	SE	B	SE	B	SE
Diabetic retinopathy	-3.35‡	0.78	-3.27‡	0.71	-3.39‡	0.73	-3.33‡	0.73	-3.11‡	0.73	-3.06‡	0.73	-2.44†	0.80
Age	-0.74‡	0.11	-0.75‡	0.10	-0.75 ‡	0.10	-0.79‡	0.10	-0.75‡	0.10	-0.76‡	0.10	-0.72‡	0.10
Sex (male vs. female)	-4.31‡	0.88	-5.34‡	0.81	-5.48‡	0.83	-3.77‡	1.02	-3.42‡	1.02	-4.18‡	1.03	-4.22‡	1.05
MHVS			1.14‡	0.08	1.02‡	0.09	1.00 ‡	0.09	0.96‡	0.09	0.94‡	0.09	0.94‡	0.09
Education					1.47*	0.61	1.27*	0.61	1.23*	0.61	1.09	0.60	0.94	0.62
Alcohol intake							0.25	0.28	0.16	0.28	0.12	0.28	0.19	0.29
Smoking (yes vs. no)							-3.27‡	0.89	-3.00‡	0.89	-2.69†	0.88	-2.62†	0.90
Waist to hip ratio (per 0.1 unit increase)							-1.60*	0.64	-1.40*	0.64	-1.03	0.64	-1.31*	0.66
Systolic blood pressure							0.01	0.03	0.01	0.03	0.01	0.03	-0.01	0.03
Total cholesterol level							-0.15	0.47	-0.17	0.47	-0.14	0.47	-0.32	0.48
MI and/or angina									-1.73	0.92	-1.14	0.92	-0.90	0.95
Stroke and/or TIA									-3.79*	1.48	-3.36*	1.47	-3.19*	1.50
Depression											-6.21‡	1.56	-6.19‡	1.62
HbA1c													0.47	0.40
Duration of diabetes													-0.92*	0.46
Adj.R^2	0.084		0.241		0.236		0.250		0.257		0.268		0.263	
Df	(3,1019)		(4,1018)		(5,975)		(10,970)		(12,968)		(13,967)		(15,926)	
F	32.35‡		82.00‡		61.70‡		33.67‡		29.27‡		28.64 ‡		23.37 ‡	
ΔR^2 (R square change)			0.141		0.005		0.017		0.009		0.012		0.004	
Df			(1,1018)		(1,975)		(5,970)		(2,968)		(1,967)		(2,926)	
ΔF (F change)			210.98‡		5.83*		4.52‡		5.68†		15.76 ‡		2.33	

‡P≤0.001, †P≤0.01, *P<0.05; Figures are unstandardised Beta regression coefficients (B) and standard errors (SE)

Table i6: Multivariate –adjusted regression analysis of general cognitive factor (g) in relation to severity of diabetic retinopathy in men

Model Term	Model 1		Model 2		Model 3		Model 4		Model 5		Model 6		Model 7	
	B	SE	B	SE	B	SE	B	SE	B	SE	B	SE	B	SE
Diabetic retinopathy	-0.21 †	0.07	-0.25‡	0.06	-0.24‡	0.06	-0.22‡	0.06	-0.20‡	0.06	-0.20‡	0.06	-0.17*	0.07
Age	-0.07‡	0.01	-0.06‡	0.01	-0.06‡	0.01	-0.06‡	0.01	-0.06 ‡	0.01	-0.06 ‡	0.01	-0.06‡	0.01
MHVS			0.11‡	0.01	0.11‡	0.01	0.09‡	0.01	0.09‡	0.01	0.09‡	0.01	0.09‡	0.01
Education					0.17‡	0.05	0.16 ‡	0.05	0.15†	0.05	0.15 †	0.05	0.14†	0.05
Alcohol intake							0.06†	0.02	0.05*	0.02	0.05*	0.02	0.05*	0.02
Smoking (yes vs. no)							0.01	0.08	0.03	0.08	0.03	0.08	0.04	0.08
Waist to hip ratio (per 0.1 unit increase)							-0.10	0.06	-0.09	0.06	-0.06	0.06	-0.06	0.06
Systolic blood pressure							-0.001	0.002	-0.001	0.002	-0.001	0.002	-0.002	0.002
Total cholesterol level							0.01	0.04	0.00	0.04	-0.001	0.04	-0.01	0.04
MI and/or angina									-0.06	0.07	-0.03	0.07	-0.02	0.08
Stroke and/or TIA									-0.32†	0.11	-0.30†	0.11	-0.31†	0.11
Depression											-0.34*	0.13	-0.36†	0.14
HbA1c													0.002	0.03
Duration of diabetes													-0.06	0.04
Adj.R^2	0.091		0.410		0.418		0.425		0.436		0.442		0.434	
Df	(2,501)		(3,500)		(4,480)		(9,475)		(11,473)		(12,472)		(14,450)	
F	26.22‡		117.74‡		87.87‡		40.78‡		34.99‡		33.01‡		26.38‡	
ΔR^2 (R square change)			0.319		0.014		0.013		0.013		0.008		0.003	
Df			(1,500)		(1,480)		(5,475)		(2,473)		(1,472)		(2,450)	
ΔF (F change)			272.37‡		11.77‡		2.21		5.48†		6.67*		1.12	

‡P≤0.001, †P≤0.01, *P<0.05; Figures are unstandardised Beta regression coefficients (B) and standard errors (SE)

Table i7: Multivariate –adjusted regression analysis of Verbal Fluency test in relation to severity of diabetic retinopathy in men

Model Term	**Model 1**		**Model 2**		**Model 3**		**Model 4**		**Model 5**		**Model 6**		**Model 7**	
	B	SE	B	SE	B	SE	B	SE	B	SE	B	SE	B	SE
Diabetic retinopathy	-2.61†	0.95	-3.10‡	0.87	-3.05‡	0.89	-3.08‡	0.89	-2.95‡	0.89	-2.94‡	0.89	-1.99*	1.0
Age	-0.35*	0.14	-0.28*	0.13	-0.28*	0.13	-0.25	0.13	-0.25*	0.13	-0.25	0.13	-0.25	0.13
MHVS			0.99 ‡	0.10	0.94 ‡	0.11	0.88 ‡	0.11	0.88 ‡	0.11	0.88 ‡	0.11	0.88‡	0.12
Education					1.04	0.74	1.17	0.75	1.28	0.75	1.24	0.75	1.16	0.77
Alcohol intake							0.75*	0.31	0.76*	0.31	0.75*	0.31	0.71*	0.32
Smoking (yes vs. no)							0.97	1.19	0.98	1.18	1.00	1.18	1.57	1.22
Waist to hip ratio (per 0.1 unit increase)							-0.22	0.86	-0.26	0.88	-0.06	0.09	-0.15	0.93
Systolic blood pressure							-0.09†	0.03	-0.08*	0.03	-0.09†	0.03	-0.09†	0.03
Total cholesterol level							0.07	0.62	-0.02	0.62	-0.03	0.62	-0.06	0.64
MI and/or angina									1.76	1.10	1.77	1.10	2.04	1.16
Stroke and/or TIA									-3.06	1.64	-2.89	1.65	-2.99	1.68
Depression											-2.09	2.04	-1.90	2.12
HbA1c													-0.25	0.51
Duration of diabetes													-0.83	0.57
Adj.R^2	0.022		0.191		0.189		0.202		0.207		0.207		0.206	
Df	(2,520)		(3,519)		(4,497)		(9,492)		(11,490)		(12,489)		(14,466)	
F	6.86‡		42.05‡		30.27‡		15.10‡		12.92‡		11.93‡		9.73‡	
ΔR^2 (R square change)			0.170		0.003		0.020		0.008		0.002		0.005	
Df			(1,519)		(1,492)		(5,490)		(2,489)		(1,970)		(2,466)	
ΔF (F change)			109.56‡		1.97		2.57*		2.66		1.05		1.37	

‡P≤0.001, †P≤0.01, *P<0.05; Figures are unstandardised Beta regression coefficients (B) and standard errors (SE)

Table i8: Multivariate –adjusted regression analysis of Verbal Fluency Test score residuals in relation to severity of diabetic retinopathy in men

Model Term	**Model 1**		**Model 2**		**Model 3**		**Model 4**		**Model 5**		**Model 6**		**Model 7**	
	B	SE	B	SE	B	SE	B	SE	B	SE	B	SE	B	SE
Diabetic retinopathy	-0.05	0.08	-0.05	0.08	-0.06	0.08	-0.07	0.08	-0.07	0.08	-0.07	0.08	-0.03	0.09
Age	0.10*	0.01	0.10*	0.01	0.10*	0.01	0.10*	0.01	0.10*	0.01	0.10*	0.01	0.09	0.01
MHVS			0.07	0.01	0.07	0.01	0.07	0.01	0.07	0.01	0.07	0.01	0.08	0.01
Education					-0.03	0.07	-0.02	0.07	-0.02	0.07	-0.02	0.07	-0.001	0.07
Alcohol intake							0.04	0.03	0.04	0.03	0.04	0.03	0.05	0.03
Smoking (yes vs. no)							0.04	0.11	0.04	0.11	0.04	0.11	0.06	0.11
Waist to hip ratio (per 0.1 unit increase)							0.05	0.08	0.05	0.08	0.05	0.08	0.03	0.08
Systolic blood pressure							-0.12†	0.003	-0.12†	0.003	-0.12†	0.003	-0.12*	0.003
Total cholesterol level							0.001	0.06	0.001	0.06	0.001	0.06	0.001	0.06
MI and/or angina									0.11*	0.10	0.11*	0.10	0.11*	0.10
Stroke and/or TIA									-0.01	0.15	-0.01	0.15	-0.01	0.15
Depression											0.01	0.18	0.03	0.18
HbA1c													-0.04	0.05
Duration of diabetes													-0.04	0.05
Adj.R^2	0.01		0.01		0.01		0.02		0.028		0.026		0.025	
Df	(2,501)		(3,500)		(4,480)		(9,475)		(11,473)		(12,472)		(14,450)	
F	3.27*		2.90*		2.16		2.10*		2.26*		2.07*		1.84*	
ΔR^2 (R square change)			0.003		0.003		0.020		0.008		0.002		0.003	
Df			(1,500)		(1,480)		(5,475)		(2,473)		(1,472)		(2,450)	
ΔF (F change)			1.41		0.35		2.03		2.91		0.09		0.82	

‡P≤0.001, †P≤0.01, *P<0.05; Figures were Standardised Beta regression coefficients and standard errors

Table i9: Multivariate –adjusted regression analysis of Faces Test score residuals in relation to severity of diabetic retinopathy

Model Term	**Model 1**		**Model 2**		**Model 3**		**Model 4**		**Model 5**		**Model 6**		**Model 7**	
	B	SE	B	SE	B	SE	B	SE	B	SE	B	SE	B	SE
Diabetic retinopathy	-0.03	0.06	-0.03	0.06	-0.03	0.06	-0.02	0.06	-0.02	0.06	-0.02	0.06	-0.01	0.06
Age	-0.05	0.01	-0.05	0.01	-0.05	0.01	-0.05	0.01	-0.05	0.01	-0.05	0.01	-0.04	0.01
Sex (Male vs. Female)	-0.17‡	0.06	-0.17‡	0.06	-0.17‡	0.06	-0.19‡	0.08	-0.19‡	0.08	-0.19‡	0.08	-0.19‡	0.08
MHVS			0.03	0.01	0.03	0.01	0.02	0.01	0.02	0.01	0.02	0.01	0.02	0.01
Education					0.00	0.05	-0.00	0.05	-0.00	0.05	-0.00	0.05	0.01	0.05
Alcohol intake							0.05	0.02	0.05	0.02	0.05	0.02	0.06	0.02
Smoking (yes vs. no)							0.01	0.07	0.01	0.07	0.01	0.07	0.01	0.07
Waist to hip ratio (per 0.1 unit increase)							-0.02	0.05	-0.02	0.05	-0.02	0.05	-0.00	0.05
Systolic blood pressure							0.04	0.002	0.04	0.002	0.04	0.002	0.03	0.002
Total cholesterol level							-0.01	0.04	-0.01	0.04	-0.01	0.04	-0.02	0.04
MI and/or angina									-0.03	0.07	-0.03	0.07	-0.02	0.08
Stroke and/or TIA									0.03	0.12	0.03	0.12	0.04	0.12
Depression											0.01	0.13	0.01	0.13
HbA1c													0.01	0.13
Duration of diabetes													-0.07	0.04
Adj.R^2	0.031		0.031		0.029		0.028		0.028		0.027		0.026	
Df	(3,988)		(4,987)		(5,946)		(10,941)		(12,939)		(13,938)		(15,900)	
F	11.54‡		8.91‡		6.77‡		3.76‡		3.28‡		3.03‡		2.60‡	
ΔR^2 (R square change)			0.001		0.003		0.020		0.008		0.002		0.004	
Df			(1,987)		(1,946)		(5,941)		(2,939)		(1,938)		(2,900)	
ΔF (F change)			1.02		0.00		0.76		0.86		0.14		1.70	

‡P≤0.001, †P≤0.01, *P<0.05; Figures were Standardised Beta regression coefficients and standard errors

Table i10: Multivariate –adjusted regression analysis of Trail Making Test B score residuals in relation to severity of diabetic retinopathy

Model Term	Model 1		Model 2		Model 3		Model 4		Model 5		Model 6		Model 7	
	B	SE	B	SE	B	SE	B	SE	B	SE	B	SE	B	SE
Diabetic retinopathy	0.03	0.06	0.04	0.06	0.03	0.06	0.04	0.06	0.03	0.06	0.03	0.06	0.03	0.06
Age	0.04	0.06	0.04	0.06	0.04	0.01	0.05	0.01	0.05	0.01	0.05	0.01	0.07*	0.01
Sex (Male vs. Female)	-0.02	0.06	-0.03	0.06	-0.03	0.07	-0.12†	0.08	-0.12†	0.08	-0.11†	0.08	-0.10*	0.08
MHVS			0.14‡	0.01	0.07*	0.01	0.07	0.01	0.07	0.01	0.07	0.01	0.07*	0.01
Education					0.12‡	0.05	0.12‡	0.05	0.12‡	0.05	0.12‡	0.05	0.12‡	0.05
Alcohol intake							0.13‡	0.02	0.13‡	0.02	0.13‡	0.02	0.13‡	0.05
Smoking (yes vs. no)							0.03	0.07	0.03	0.07	0.03	0.07	0.03	0.07
Waist to hip ratio (per 0.1 unit increase)							0.06	0.05	0.06	0.05	0.06	0.05	0.05	0.05
Systolic blood pressure							-0.01	0.002	-0.01	0.002	-0.01	0.002	-0.01	0.002
Total cholesterol level							0.01	0.04	0.01	0.04	0.01	0.04	0.01	0.04
MI and/or angina									-0.02	0.07	-0.02	0.07	-0.01	0.07
Stroke and/or TIA									0.04	0.12	0.03	0.12	0.03	0.12
Depression											0.03	0.12	0.04	0.13
HbA1c													-0.02	0.03
Duration of diabetes													0.01	0.01
Adj.R^2	-0.001		0.017		0.025		0.039		0.038		0.038		0.041	
Df	(3,988)		(4, 987)		(5,946)		(10,941)		(12,939)		(13,938)		(15,900)	
F	0.83		5.28‡		5.91‡		4.85‡		4.14‡		3.88‡		3.64‡	
ΔR^2 (R square change)			0.018		0.012		0.019		0.001		0.001		0.000	
Df			(1,987)		(1,946)		(5,941)		(2,939)		(1,938)		(2,900)	
ΔF (F change)			18.57		12.11‡		3.70†		0.63		0.72		0.21	

‡P≤0.001, †P≤0.01, *P<0.05; Figures were Standardised Beta regression coefficients and standard errors

Table i11: Multivariate –adjusted regression analysis of Digit Symbol Test score residuals in relation to severity of diabetic retinopathy

Model Term	Model 1		Model 2		Model 3		Model 4		Model 5		Model 6		Model 7	
	B	SE	B	SE	B	SE	B	SE	B	SE	B	SE	B	SE
Diabetic retinopathy	-0.10†	0.06	-0.10 †	0.06	-0.11‡	0.06	-0.11‡	0.06	-0.11‡	0.06	-0.1 ‡	0.06	-0.11†	0.06
Age	-0.05	0.01	-0.05	0.01	-0.06	0.01	-0.06	0.01	-0.07*	0.01	-0.07*	0.01	-0.06	0.01
Sex (Male vs. Female)	-0.18‡	0.06	-0.17‡	0.06	-0.17‡	0.06	-0.17‡	0.06	-0.10*	0.08	-0.10*	0.08	-0.11*	0.08
MHVS			-0.08*	0.01	-0.08*	0.01	-0.08*	0.01	-0.09*	0.01	-0.09*	0.01	-0.09*	0.01
Education					-0.01	0.05	-0.03	0.02	-0.02	0.05	-0.02	0.05	-0.03	0.05
Alcohol intake							0.04	0.11	0.04	0.11	0.04	0.11	-0.03	0.02
Smoking (yes vs. no)							-0.11†	0.07	-0.11†	0.07	-0.11†	0.07	-0.10†	0.07
Waist to hip ratio (per 0.1 unit increase)							-0.03	0.05	-0.03	0.05	-0.03	0.05	-0.03	0.05
Systolic blood pressure							0.02	0.002	0.02	0.002	0.02	0.002	0.004	0.002
Total cholesterol level							-0.01	0.04	-0.01	0.04	-0.01	0.04	-0.01	0.04
MI and/or angina									-0.07*	0.07	-0.06	0.07	-0.06	0.08
Stroke and/or TIA									-0.03	0.12	-0.03	0.12	-0.02	0.12
Depression											-0.03	0.12	-0.03	0.12
HbA1c													0.03	0.03
Duration of diabetes													-0.01	0.04
Adj.R^2	0.042		0.47		0.050		0.059		0.063		0.063		0.056	
Df	(3,988)		(4,987)		(5,946)		(10,941)		(12,939)		(13,938)		(15,900)	
F	15.52‡		13.14‡		11.1‡		6.98‡		6.33‡		5.92‡		4.65‡	
ΔR^2 (R square change)			0.006		0.000		0.014		0.006		0.001		0.001	
Df			(1,987)		(1,946)		(5,941)		(2,939)		(1,938)		(2,900)	
ΔF (F change)			5.78*		0.06		2.76*		2.92		0.98		0.35	

‡P≤0.001, †P≤0.01, *P<0.05; Figures were Standardised Beta regression coefficients and standard errors

Table i12: Multivariate –adjusted regression analysis of Logical Memory test in relation to retinal arteriolar width (CRAE) in men

Model Term	Model 1		Model 2		Model 3		Model 4		Model 5		Model 6		Model 7		Model 8	
	B	SE	B	SE	B	SE	B	SE	B	SE	B	SE	B	SE	B	SE
CRAE	-0.05‡	0.01	-0.05‡	0.01	-0.05‡	0.01	-0.05‡	0.01	-0.05‡	0.01	-0.05‡	0.01	-0.05†	0.01	-0.04*	0.02
Age	-0.15	0.09	-0.11	0.08	-0.10	0.08	-0.10	0.08	-0.08	0.08	-0.08	0.08	-0.08	0.09	-0.08	0.09
MHVS			0.51‡	0.06	0.47‡	0.07	0.45‡	0.07	0.45‡	0.07	0.45‡	0.07	0.44 ‡	0.08	0.44‡	0.08
Education					0.63	0.49	0.67	0.49	0.67	0.49	0.67	0.49	0.60	0.51	0.65	0.51
Alcohol intake							0.31	0.20	0.31	0.20	0.31	0.20	0.28	0.21	0.28	0.21
Smoking (yes vs. no)							0.89	0.78	0.92	0.78	0.97	0.79	1.0	0.80	1.04	0.81
Waist to hip ratio (per 0.1 unit increase)							-0.11	0.06	-0.13	0.06	0.07	0.06	0.05	0.06	0.01	0.06
Systolic blood pressure							-0.004	0.02	-0.004	0.02	-0.01	0.02	-0.01	0.02	-0.01	0.02
Total cholesterol level							-0.08	0.41	-0.14	0.41	-0.14	0.41	-0.02	0.43	0.01	0.43
MI and/or angina									0.60	0.72	0.81	0.74	0.94	0.77	0.98	0.77
Stroke and/or TIA									-1.57	1.10	-1.42	1.10	-1.49	1.14	-1.48	1.14
Depression											-2.04	1.32	-2.03	1.38	-2.07	1.39
HbA1c													-0.55	0.34	-0.52	0.34
Duration of diabetes													0.24	0.36	0.23	0.36
CRVE															-0.01	0.01
Adj.R^2	0.032		0.148		0.148		0.146		0.146		0.149		0.142		0.141	
Df	(2,487)		(3,486)		(4,466)		(9,461)		(11,459)		(12,458)		(14,435)		(15,434)	
F	9.08‡		29.30‡		21.33‡		9.91‡		8.33‡		7.86‡		6.31‡		5.93‡	
ΔR^2 (R square change)			0.117		0.003		0.007		0.004		0.004		0.005		0.001	
Df			(1,486)		(1,466)		(5,461)		(2,459)		(1,458)		(2,435)		(1,434)	
ΔF (F change)			67.27‡		1.68		0.80		1.21		2.38		1.40		0.73	

‡P≤0.001, †P≤0.01, *P<0.05; Figures are unstandardised Beta regression coefficients (B) and standard errors (SE)

Table i13: Multivariate –adjusted regression analysis of Logical Memory test in relation to retinal venular width (CRVE) in men

Model Term	Model 1		Model 2		Model 3		Model 4		Model 5		Model 6		Model 7		Model 8	
	B	SE	B	SE	B	SE	B	SE	B	SE	B	SE	B	SE	B	SE
CRVE	-0.03*	0.01	-0.03†	0.01	-0.03†	0.01	-0.03†	0.01	-0.03†	0.01	-0.03†	0.01	-0.03†	0.01	-0.01	0.01
Age	-0.17	0.09	-0.13	0.08	-0.12	0.08	-0.10	0.08	-0.10	0.09	-0.09	0.09	-0.09	0.09	-0.08	0.09
MHVS			0.52‡	0.06	0.47‡	0.07	0.46‡	0.07	0.46‡	0.07	0.46‡	0.07	0.45‡	0.07	0.44‡	0.08
Education					0.67	0.49	0.74	0.50	0.79	0.50	0.74	0.50	0.67	0.52	0.65	0.51
Alcohol intake							0.34	0.20	0.34	0.20	0.34	0.20	0.31	0.21	0.28	0.21
Smoking (yes vs. no)							1.05	0.79	1.08	0.79	1.13	0.79	1.12	0.82	1.04	0.82
Waist to hip ratio (per 0.1 unit increase)							0.06	0.06	0.04	0.06	0.03	0.06	0.02	0.06	0.01	0.06
Systolic blood pressure							-0.002	0.02	-0.002	0.02	-0.006	0.02	-0.003	0.02	-0.005	0.02
Total cholesterol level							0.07	0.41	0.02	0.42	0.02	0.42	0.11	0.43	0.01	0.43
MI and/or angina									0.65	0.73	0.91	0.75	0.99	0.77	0.98	0.77
Stroke and/or TIA									-1.60	1.11	-1.42	1.11	-1.49	1.14	-1.48	1.14
Depression											-2.39	1.32	-2.32	1.39	-2.07	1.39
HbA1c													-0.50	0.34	-0.52	0.34
Duration of diabetes													0.21	0.36	0.23	0.36
CRAE															-0.04*	0.02
Adj.R^2	0.016		0.137		0.136		0.137		0.138		0.142		0.134		0.141	
Df	(2,487)		(3,486)		(4,466)		(9,461)		(11,459)		(12,458)		(14,435)		(15,434)	
F	4.95†		26.96‡		19.54‡		9.28‡		7.84‡		7.49‡		5.98‡		5.93‡	
ΔR^2 (R square change)			0.123		0.003		0.010		0.005		0.006		0.004		0.009	
Df			(1,486)		(1,466)		(5,461)		(2,459)		(1,458)		(2,435)		(1,434)	
ΔF (F change)			69.58‡		1.89		1.06		1.29		3.26		1.10		4.59*	

‡P≤0.001, †P≤0.01, *P<0.05; Figures are unstandardised Beta regression coefficients (B) and standard errors (SE)

Table i14: Multivariate –adjusted regression analysis of Faces test in relation to retinal arteriolar width (CRAE) in women

Model Term	Model 1		Model 2		Model 3		Model 4		Model 5		Model 6		Model 7		Model 8	
	B	SE	B	SE	B	SE	B	SE	B	SE	B	SE	B	SE	B	SE
CRAE	0.01	0.02	0.03*	0.01	0.03	0.02	0.03	0.02	0.02	0.02	0.02	0.02	0.02	0.02	-0.004	0.02
Age	-0.13	0.09	-0.15	0.08	-0.14	0.08	-0.12	0.08	-0.13	0.09	-0.14	0.09	-0.10	0.09	-0.09	0.09
MHVS			0.57‡	0.07	0.52‡	0.08	0.49‡	0.08	0.49‡	0.08	0.47‡	0.08	0.44‡	0.08	0.45‡	0.08
Education					0.71	0.53	0.75	0.53	0.80	0.54	0.75	0.54	0.98	0.55	0.93	0.54
Alcohol intake							0.64*	0.31	0.61*	0.31	0.59	0.31	0.67*	0.31	0.68*	0.31
Smoking (yes vs. no)							-0.15	0.72	-0.10	0.72	0.10	0.73	-0.001	0.75	-0.15	0.75
Waist to hip ratio (per 0.1 unit increase)							-0.6	0.5	-0.6	0.5	-0.5	0.5	-0.41	0.52	-0.45	0.52
Systolic blood pressure							0.01	0.02	0.01	0.02	0.01	0.02	0.02	0.02	0.02	0.02
Total cholesterol level							-0.16	0.38	-0.16	0.38	-0.10	0.38	-0.32	0.38	-0.28	0.39
MI and/or angina									0.15	0.85	0.37	0.86	0.68	0.87	0.62	0.87
Stroke and/or TIA									-1.73	1.66	-1.58	1.66	-0.99	1.67	-0.86	1.67
Depression											-2.33	1.35	-2.71	1.39	-2.78*	1.39
HbA1c													0.41	0.35	0.40	0.35
Duration of diabetes													-1.20†	0.39	-1.18 †	0.39
CRVE															0.03*	0.01
Adj.R^2	0.002		0.132		0.130		0.131		0.130		0.134		0.147		0.153	
Df	(2,467)		(3,466)		(4,445)		(9,440)		(11,438)		(12,437)		(14,417)		(15,416)	
F	1.57		24.77‡		17.70‡		8.55‡		7.08‡		6.77‡		6.29‡		6.21‡	
ΔR^2 (R square change)			0.131		0.003		0.012		0.002		0.006		0.019		0.009	
Df			(1,466)		(1,445)		(5,440)		(2,438)		(1,437)		(2,417)		(1,416)	
ΔF (F change)			70.71‡		1.80		1.20		0.55		3.01		4.69*		4.33*	

‡P≤0.001, †P≤0.01, *P<0.05; Figures are unstandardised Beta regression coefficients (B) and standard errors (SE)

Table i15: Multivariate –adjusted regression analysis of Faces test in relation to retinal venular width (CRVE) in women

Model Term	Model 1		Model 2		Model 3		Model 4		Model 5		Model 6		Model 7		Model 8	
	B	SE	B	SE	B	SE	B	SE	B	SE	B	SE	B	SE	B	SE
CRAE	0.03	0.01	0.03†	0.01	0.03†	0.01	0.03†	0.01	0.03†	0.01	0.03†	0.01	0.03*	0.01	0.03*	0.01
Age	-0.12	0.09	-0.14	0.08	-0.12	0.08	-0.12	0.08	-0.12	0.08	-0.13	0.09	-0.09	0.09	-0.09	0.09
MHVS			0.57‡	0.07	0.53‡	0.08	0.50 ‡	0.08	0.50 ‡	0.08	0.48‡	0.08	0.45‡	0.08	0.45‡	0.08
Education					0.67	0.53	0.71	0.53	0.76	0.53	0.71	0.53	0.93	0.54	0.93	0.54
Alcohol intake							0.66*	0.31	0.63*	0.31	0.61	0.31	0.68*	0.31	0.68*	0.31
Smoking (yes vs. no)							-0.31	0.72	-0.26	0.72	-0.06	0.72	-0.15	0.75	-0.15	0.75
Waist to hip ratio (per 0.1 unit increase)							-0.63	0.50	-0.61	0.50	-0.52	0.51	-0.45	0.52	-0.45	0.52
Systolic blood pressure							0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
Total cholesterol level							-0.11	0.38	-0.07	0.38	-0.05	0.38	-0.29	0.39	-0.28	0.39
MI and/or angina									0.11	0.84	0.33	0.85	0.61	0.86	0.62	0.87
Stroke and/or TIA									-1.61	1.65	-1.44	1.65	-0.84	1.66	-0.86	1.67
Depression											-2.39	1.34	-2.76*	1.38	-2.78*	1.39
HbA1c													0.40	0.35	0.40	0.35
Duration of diabetes													-1.18	0.39	-1.18†	0.39
CRVE															-0.004	0.02
Adj.R^2	0.007		0.140		0.138		0.141		0.139		0.143		0.155		0.153	
Df	(2,467)		(3,466)		(4,445)		(9,440)		(11,438)		(12,437)		(14,417)		(15,416)	
F	2.76		26.43‡		18.91‡		9.17‡		7.57‡		7.24‡		6.66‡		6.21‡	
ΔR^2 (R square change)			0.134		0.003		0.013		0.002		0.006		0.018		0.000	
Df			(1,466)		(1,445)		(5,440)		(2,438)		(1,437)		(2,417)		(1,416)	
ΔF (F change)			73.05‡		1.62		1.33		0.48		3.21		4.56*		0.05	

‡P≤0.001, †P≤0.01, *P<0.05; Figures are unstandardised Beta regression coefficients (B) and standard errors (SE)

APPENDIX J: Published paper

Retinal microvascular abnormalities and cognitive dysfunction: a systematic review

J Ding, N Patton, I J Deary, et al.

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Retinal microvascular abnormalities and cognitive dysfunction: a systematic review

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ABSTRACT

Objective: To examine the evidence for an association between cognitive impairment or dementia and the presence of retinal microvascular abnormalities.

Methods: A systematic review of observational studies identified through searching five electronic databases and reference lists. Studies were required to have both a recognised cognitive function assessment (either a structured neuropsychological test or a clinical evaluation of dementia), and assessment of the retinal microvasculature (either characteristics associated with generalised retinopathy or changes specific to arterioles or venules).

Results: 6 studies were included. Studies were clinically and methodologically heterogeneous and of variable quality. Some degree of cognitive impairment was found to be associated with the presence of retinal microvascular abnormalities in all studies, although the extent of the association varied. The presence of retinal vascular signs was mostly associated with poorer verbal memory, mental speed and executive function in the general population, but not consistently associated with other cognitive modalities.

Conclusions: There is some evidence suggesting a positive association between retinal microvascular abnormalities and cognitive impairment or dementia in elderly people and in patients with diabetes. Findings are inconclusive, and further better designed studies are required, with standardised and objective retinal vascular assessment and a range of sensitive cognitive tests.

Cognitive impairment and dementia are important causes of morbidity and mortality in elderly people in Western countries. Apart from the effects of normal ageing, marked inter-individual differences in the rate of cognitive decline indicate that other age-associated pathologies may be involved, such as macro- or microvascular disease. Current primary prevention programmes, aimed at reducing the impact of macrovascular disease, have proved unsuccessful in reducing the risk of cognitive decline, other than by preventing major strokes.[1–5] Improved prevention of cognitive dysfunction therefore requires a better understanding of underlying pathogenic mechanisms, identification of novel risk factors and development of new approaches to recognition of people at high risk.[6]

Several pathobiological markers have been suggested as potential predictors of cognitive dysfunction. These include non-invasive measures of cerebral microvascular disease, such as white-matter hyperintensities and lacunar infarcts detected by magnetic resonance imaging (MRI), and retinal microvascular signs.[7–9] Of these, retinal microvascular signs may offer the most promise as a potential tool in clinical practice, as they can be simply and accessibly measured. Based on retinal photographs, retinal microvascular abnormalities, such as the presence of microaneurysms, soft exudates, haemorrhages and generalised arteriolar narrowing, predict incident stroke as well as MRI-identified subclinical stroke independent of other measured risk factors.[10–13] Recently, influenced by the homology between retinal vascular disease and cerebral small vessels disease, several studies have investigated retinal microvascular abnormalities and risk of cognitive impairment and dementia in patients and in the general population. These studies are reviewed systematically in this article.

METHODS

We aimed to include all published studies that investigated the association between retinal microvascular abnormalities and cognitive dysfunction in adult subjects, available for review by July 2007. Studies had to meet the following predetermined inclusion criteria: (a) observational epidemiological design (ie, cross-sectional, case-control, cohort), (b) inclusion of a cognitive function assessment tool that was either a structured neuropsychological test or a clinical evaluation of dementia, (c) assessment of the retinal microvasculature (either characteristics associated with generalised retinopathy or changes specific to arterioles or venules, such as focal arteriolar narrowing or venular tortuosity) but not other retinal conditions, which fail to offer a direct measure of retinal vascular signs (such as age-related maculopathy), (d) provision of data relating retinal microvascular lesions to cognitive function. Studies in which participants were selected on the basis of existing infectious disease due to a virus or bacteria were excluded because cognitive dysfunction in these subjects is likely to have specific pathology peculiar to the infectious disease.

Studies were identified by computerised searches of PubMed (Medline), EMBASE, PsycINFO, BIOSIS Previews, and Web of Science, by checking the reference lists of relevant original and review articles, by reference to conference proceedings, and by tracking articles that cited retrieved studies using Science Citation Index. A detailed "topic only" search strategy was adopted, as it proves the most sensitive. The search string for each database was similar with the terms "retinopath* OR (retina* AND (microvascu* OR arteriol* OR venul* OR vascu*)) AND (cognit* OR Alzheim* OR dementi* OR memor* OR neuropsycholog*)." Searches were limited to the English language.

Further details on the search strategy are available from the authors on request.

The titles and abstracts of studies identified were screened by one reviewer (JD), and potentially relevant full-text articles were retrieved and assessed according to the inclusion criteria. Data were extracted by two reviewers (RJM and JD), who also independently assessed methodological quality of studies, using a scoring system based on previously published guidelines.[14 15] The following information was extracted from included studies: method of assessment of retinal microvascular abnormalities, measurement of cognitive function, and all relevant results, including adjustment for potential confounding factors. Studies were assessed for both internal and external validity (Box). Studies scoring less than six out of a possible maximum nine for case control or cross-sectional studies, and less than seven out of 11 for cohort studies were excluded for further analysis. Disagreements in rating were resolved by discussion or arbitrated by a third reviewer (JP). Owing to a high degree of heterogeneity between studies, including study designs, type of population, measures of retinal vascular characteristics, types of neuropsychological tests and covariates included in analyses, meta-analysis of study results was not possible. A descriptive synthesis approach was therefore used.

RESULTS

The numbers of studies identified at each stage of the systematic review are shown in fig 1. Of the eight studies meeting our inclusion criteria,[16–23] two were subsequently excluded, as they did not fulfil the quality criteria.[16 17] The key details of the six included studies are summarised in tables 1, 2.

Study design and populations

The six included studies comprised two prospective cohort studies in which cognitive function was measured both at baseline (when retinal images were taken) and at follow-up,[18 19] and four cross-sectional studies.[20–23] Three studies were population-based[21–23] and three hospital- or clinic-based.[18–20] The populations sampled came from the US, UK and Japan. The clinic and hospital-based studies involved patients with type 1 or type 2[18 20] and patients with type 2 diabetes after coronary artery bypass grafting (CABG).[19] The age range of participants (at baseline) was 18–35 years[18] to 83–84 years[23] and all studies included both men and women. In the prospective studies, the duration of follow-up was 6 months and 7 years, respectively, with losses to follow-up estimated to be 5%[19] and 39%.[18]

In three of the four cross-sectional studies, a retinal examination was undertaken at the same time as the cognitive testing. In the other study, the cognitive function tests were performed 3 years before or after retinal photography, and the average test scores were used in the analysis.[21] One prospective study in which cognitive abilities were assessed both at baseline and at 7 years' follow-up examined the presence of retinopathy biennially as well as at baseline.[18]

Assessment of retinal microvascular abnormalities

Both the techniques and grading methods used to evaluate retinal microvascular signs varied between the studies. In one study, the combination of ophthalmoscopic examination with digital retinal photography was adopted,[20] whereas in the remaining studies, colour fundus photography alone was used. Prior to retinal photography, pharmacological dilation of the pupil(s) was performed in two studies.[18 23] The angle used for the field of view in photography differed, ranging from 30°[18–20] to 45°[21 22] or 50°.[23] Three studies reported that only one eye was assessed[21–23] (arbitrarily chosen) but the other studies did not provide such information.

In the studies using a fully manual grading system, subjects were categorised either according to the presence of diabetic retinopathy varying with severity,[18–20] or into one of several defined groups, including the presence of: (1) any specific retinopathy signs (microaneurysms, retinal haemorrhages, cotton wool spots, soft exudates, macular oedema, venous beading, etc); (2) arteriovenous nicking; (3) focal arterial narrowing (table 3).[21 22] At least three different classification systems for retinal vascular signs were used in five studies. Computer-assisted imaging techniques were applied in three studies to quantify retinal vessel calibre and other parameters of retinal vascular network geometry.[21–23] One study focusing on quantitative measures alone assessed five continuous variables: the central retinal arterial equivalent (CRAE), central retinal venular equivalent (CRVE), arteriovenous ratio (AVR), sub-optimal geometry of arteriolar branching coefficients and angles (table 3).[23]

Assessment of cognitive function and impairment

Cognitive function was assessed using four primary methods: (1) specific cognitive tests; (2) a mean/composite score on two or more tests in the battery;[18] (3) general cognitive ability score (g) generated by extracting a component reflecting the variance shared by all tests;[23] (4) clinical assessment alone (eg, diagnosis of dementia).[22] Results were reported as either continuous or categorical outcomes. More than 17 different psychometric tests were used among these studies. To help organise discussion, many of these neuropsychological tests can be classified into the cognitive domains that they examined, including attention/concentration, information processing speed, manual dexterity, frontal lobe/executive function, verbal memory, performance IQ, and mini-mental state examination (table 4).[28 29] In one study, patients with dementia were identified through a detailed diagnostic examination, and dementia was defined according to recognised international criteria.[22 30]

Three of the studies including one of the prospective studies divided the participants into two groups based on whether or not they experienced cognitive impairment or decline (during follow-up). Cognitive impairment was defined in a variety of ways that included: (1) a decline by a particular amount relative to the baseline test score;[19] (2) test scores below a particular threshold score;[21] or (3) the presence of clinically diagnosed dementia.

Findings from studies on patients with diabetes

The results of the studies on subjects with diabetes are shown in table 1.

In 74 patients with type 1 diabetes,[20] the presence of retinal microaneurysms was associated with poorer performance on some tests of performance IQ (Block Design, Digit Symbol Test), information-processing speed and attention but not on other tests of performance IQ or executive function. In a second study of 103 patients with type 1 diabetes,[18] the presence of proliferative diabetic retinopathy (PDR) at baseline or development of the condition during follow-up was associated with poorer performance on measures of "psychomotor efficiency" (a composite score based on the results of four tests: Digit Vigilance, Digit Symbol Test, Grooved Pegboard and Part B of the Trail Making). Compared with diabetic people without retinopathy at either assessment, there was a cognitive decline

Box 1 Quality assessment of studies

Participant selection and recruitment (maximum 2 points)

- Well-defined population sample (the final study sample was likely to be representative of the target population) (1)
- (Baseline) response rate 70% or more (1)

Participation at follow-up (maximum 2 points)

- Follow-up period more than 1 year (1)
- Loss to follow-up less than 30–40% (death was excluded from the estimate) (1)

Retinal microvascular abnormalities assessment (maximum 3 points)

- Retinopathy status based on self-report or medical records (0)
  or
- Ophthalmoscopic examination (1)
  or
- Retinal photography and grading based on a standardised protocol (2)
- Assessors blinded to cognitive status (1)

Cognitive function and/or dementia assessment (maximum 3 points)

- Only a global functioning test (1)
  or
- Neuropsychological battery (2)
  or
- Dementia based on medical records (0)
  or
- Dementia diagnosed by active screening with specific criteria (1)
  or
- Dementia based on worldwide recognised criteria by a central consensus committee (2)
- Assessors blinded to ocular findings and other major clinical disease status (1)

Adjustment for confounding (maximum 1 point)

- Effect of main confounders not investigated (at baseline) (0)
  or
- Main confounders reported but not adjusted in the final analysis (0)
  or
- Main confounders reported and adequately adjusted in the analysis (1)

Eligible studies: scoring ⩾7/11 for cohort studies and ⩾6/9 for case-control/cross-sectional studies

either in subjects with PDR at baseline (change in test score −0.50 vs −0.22, p<0.02) or in incident PDR (change in test score −0.56 vs −0.22, p<0.02) over 7 years. In both studies, the association remained significant after adjusting for a variety of demographic and several important diabetes-related variables. The magnitude of the cognitive deficits observed in these young adult subjects was moderate to large, with effect sizes for the group difference ranging from about 0.3 to 0.7 SD (according to Cohen's terminology[31]).

In 180 patients with type 2 diabetes,[19] after adjustment for a number of biomedical and demographic variables, the presence of diabetic retinopathy assessed prior to CABG surgery was associated with an increased risk of cognitive decline (compared with preoperative cognitive performance) after 7 days (OR 2.0, 95% CI 1.3 to 3.0) and 6 months (OR 2.1, 95% CI 1.2 to 2.7) following surgery.

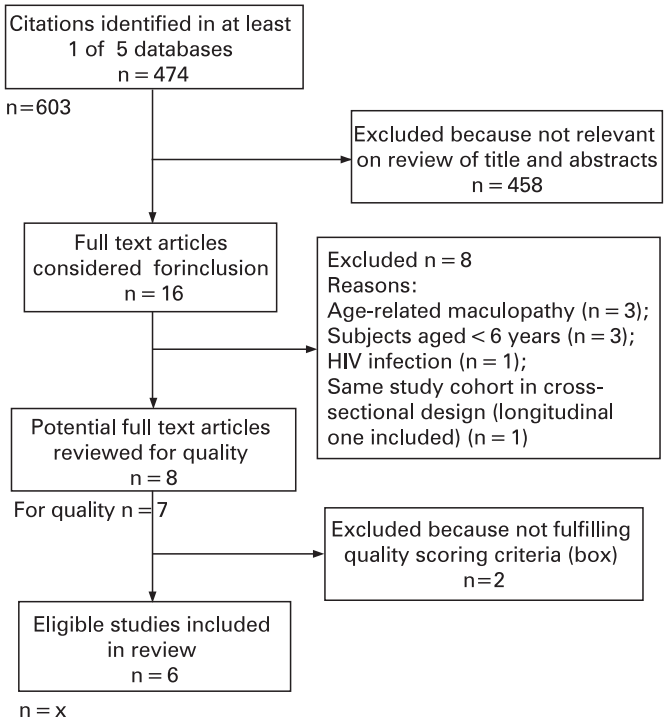

Figure 1 Flow chart of eligibility of studies.

Findings from studies on population-based samples

The results from population-based studies are shown in table 2.

In the Atherosclerosis Risk in Communities Study of 8,734 middle-aged men and women free of stroke,[21] the presence of any retinopathy, microaneurysms, retinal haemorrhages or soft exudates was associated with an increased risk of impaired performance on tests of verbal memory (Delayed Word Recall), information-processing speed (Digit Symbol Test) and executive function (Verbal Fluency Test). The presence of arteriolar narrowing or AV nicking was not associated with these tests. Subjects with any of these retinal vascular lesions were 2.6 (OR (95% CI 1.3 to 2.9)) to 3.4 (OR (95% CI 2.0 to 5.8)) times more likely to have cognitive impairment than people without these lesions independent of other covariates.

In the Cardiovascular Health Study[22] of 2,211 men and women, the presence of any retinopathy was associated with poorer performance on information processing speed (Digital Symbol Test, adjusted mean test scores 39 vs 42, p = 0.02), but not on the MMSE. No association was found with the presence of focal arteriolar narrowing, AV nicking or quintiles of retinal calibre. In a hypertensive subgroup, the presence of any retinopathy (multivariable-adjusted OR, 2.10 (95% CI 1.04 to 4.24)) or focal arteriolar narrowing (OR, 3.02 (95% CI 1.51 to 6.02)) was associated with an increased risk of dementia, but no association was found with the presence of other retinal vascular signs.

In the Lothian Birth Cohort 1921 study[23] of 321 elderly men and women, the deviation from optimality of the retinal vascular network geometry was associated with lower cognitive ability scores, but no association was found for retinal arteriolar or venular calibre. After controlling for a variety of other covariates, suboptimality of angles at arteriolar bifurcations (from theoretically derived-optimum values) was associated

Table 1 Included clinic/hospital-based studies investigating cognitive performance in relation to retinal microvascular abnormalities

References (location)	Study design	Study sample	Age (years at baseline)	Assessment of retinal microvascular abnormalities	Cognitive measure and outcome	Key findings	Comments and quality rating*
Ferguson *et al*[20] (UK)	Cross-sectional	Clinic-based 74 men and women, with type 1 diabetes	20–45	Ophthalmoscopic and photographic assessment for retinal microaneurysms (MA, two or more in one eye)	WAIS-R (PC, BD, OA and DSS)	Z score in subjects with MA versus without	MA related to poorer cognitive measures on two of four tests of performance IQ (BD, DSS), information-processing speed and attention/concentration
	Cognitive and retinal assessments undertaken simultaneously			Classified as present versus absent	IT and CRT (ms)	PC: −0.13 vs 0.07, p = 0.39	Hypertension, prevalent neuropathy, microalbuminuria, advanced retinopathy, CNS disease, alcohol or drug abuse excluded
					VFT	BD: −0.55 vs 0.32, p<0.01	Adjustment for severe hypoglycaemia exposure, gender, premorbid IQ and duration of diabetes; **8/9**
					PASAT (2 s errors)	OA: −0.13 vs 0.07, p = 0.44 DSS: −0.28 vs 0.19, p = 0.04 IT: 0.29 vs −0.15, p = 0.03 CRT: 0.44 vs −0.23, p = 0.003 VFT: 0.07 vs −0.04, p = 0.72 PASAT: 0.30 vs −0.16, p = 0.03	
Ryan *et al*[18] (US)	Prospective cohort	Clinic-based 103 men and women, with type 1 diabetes	40	Photographic grading of proliferative retinopathy (PDR)	Digit Vigilance Test, DSS, GPT, Part B of the Trail Making test	Z change score over 7 years from baseline in subjects with	PDR related to greater decline in cognitive measures of psycho-motor efficiency
	Cognitive function measured at baseline and after 7 years; retinopathy assessed at baseline and biennially			Classified as present versus absent	A composite Z score derived from the four tests	(1) PDR at baseline versus without (at both time points) −0.50 vs −0.22, p<0.02	Alcohol or drug abuse, head trauma, PD excluded
						(2) Incident PDR (follow-up) versus without (at both time points)−0.56 vs −0.22, p<0.02	Adjustment for incident (AN, DSP, ON, CAD, PVD), SBP, and duration of diabetes; **7/11**
Kadoi *et al*[19] (Japan)	Prospective cohort	Hospital-based 180 men and women with type 2 diabetes scheduled for CABG surgery	64 (SD 11)	Ophthalmoscopic grading of retinopathy	Mini-Mental State Examination, Rey auditory verbal learning test, GPT, Part A of the Trail Making test, Part B of the Trail Making test, Digit Span Forward	OR for cognitive impairment in subjects with retinopathy versus without at 7 days: 2.0 (95% CI 1.3 to 3.0) p = 0.01; at 6 month: 2.1 (95% CI 1.2 to 2.7) p<0.01; at both: 2.4 (95% CI 1.4 to 2.9) p<0.01	Retinopathy related to an increased risk of short- and long-term cognitive impairment after CABG surgery
	Cognitive function measured preoperatively and at 7 days and 6 months after surgery; retinopathy assessed preoperatively			Classified as present versus absent	Cognitive impairment defined as a decline from preoperative testing of more than 1 SD on at least 2 of the six tests		PD, renal or active liver disease, type 1 diabetes excluded
							Adjustment for AAA, hypertension, HbA1c, insulin therapy, Svo_2 less than 50% of time; **8/11**

*Bold numbers indicate the quality rating.
AAA, ascending aorta atherosclerosis; AN, autonomic neuropathy; BD, block design; CABG, coronary artery bypass grafting; CAD, coronary artery disease; CI, confidence interval; CRT, choice reaction time; DSP, distal symmetric polyneuropathy; DSS, Digit Symbol Subtest; GPT, Grooved Pegboard Test; HbA_1, glycosylated haemoglobin; IT, inspection time; OA, object assembly; ON, overt nephropathy; OR: odds ratio; PASAT, Paced Auditory Serial Additional Task; PC, picture completion; PD, psychiatric disorder; PVD, peripheral vascular disease; SBP, systolic blood pressure; Svo_2, jugular venous oxygen saturation; VFT, Verbal Fluency Test; WAIS-R, Wechsler Adult Intelligence Scale—Revised.

Table 2 Included population-based studies investigating cognitive performance in relation to retinal microvascular abnormalities

References	Study (country)	Study design	Study sample	Age (years)	Assessment of retinal microvascular abnormalities	Cognitive measure and outcome	Key findings	Comments and quality rating*
Wong *et al*[21]	ARIC study (US)	Cross-sectional	8,734 men and women, sampled from four US communities	51–70	Photographic grading of:	DWR	In DWR	Retinal vascular signs except AV nicking and arteriolar narrowing, related to lower cognitive test scores
		Cognitive function assessed at 3 years before or after retinal images taken (averaged test scores used in analysis)			(1) any retinopathy	DSS	OR for cognitive impairment in subjects with versus without:	Prevalent stroke, taking CNS-relevant medication excluded
					(2) microaneurysm	VFT	(1) any retinopathy 2.6 (95% CI 1.3 to 2.9)	Adjustment for age, sex, race, field centre, education, occupation, diabetes, fasting glucose, hypertension, carotid IMT, cigarette smoking, alcohol consumption, fasting total and HDL cholesterol and triglyceride levels; **7/9**
					(3) retinal haemorrhage	Cognitive impairment defined as scores 2SD or lower than the mean scores for each test	(2) microaneurysms 3.0 (95% CI 1.8 to 5.0)	
					(4) soft exudates		(3) retinal haemorrhage 3.4 (95% CI 2.0 to 5.8)	
					(5) FAN		(4) soft exudates 3.1 (95% CI 1.5 to 6.2)	
					(6) AV nicking		(5) AV nicking 1.2 (95% CI 0.8 to 1.6)	
					Computer-assisted grading of:		(6) FAN 0.6 (95% CI 0.4 to 0.9)	
					(7) generalised arteriolar narrowing, summarised by AVR and defined as lowest 20th percentile of AVR distribution		(7) generalised arteriolar narrowing 1.0 (95% CI 0.8 to 1.5) Similar results for DSS and VFT	
Baker *et al*[22]	Cardiovascular Health Study (US)	Cross-sectional	2,211 (1,767 in analysis for dementia) men and women, sampled from a defined sample of Medicare eligible	69–97	Classified as present versus absent Photographic grading of:	3MSE	In DSS	Any retinopathy related to lower DSS scores; focal arteriolar narrowing related to an increased risk of dementia; both retinal vascular signs related to an increased risk of dementia in hypertensive people
		Cognitive and retinal assessments undertaken simultaneously	Persons from four US communities		(1) any retinopathy	DSS	Adjusted mean scores in subjects with versus without	Prevalent of stroke, taking antipsychotics or antidepressants excluded
					(2) FAN	Clinical diagnosis of dementia†	(1) any retinopathy 39 vs 42 $p<0.02$	Adjustment for age, sex, race, field centre, education level, internal carotid IMT, BMI, hypertension, diabetes, and cigarette smoking; **6/9**
					(3) AV nicking		(2) FAN 42 vs 42 $p = 0.62$	
					Classified as present versus absent computer-assisted grading of		(3) AV nicking 41 vs 42 $p = 0.20$	

Continued

Table 2 Continued

References	Study (country)	Study design	Study sample	Age (years)	Assessment of retinal microvascular abnormalities	Cognitive measure and outcome	Key findings	Comments and quality rating*
					(4) CRAE		No statistically significant differences in the mean scores in the quintiles of CRAE or CRVE	
					(5) CRVE		OR for dementia in subjects with versus without	
					Measured in micrometres and categorised into quintiles		(1) FAN 1.99 (95% CI 1.11 to 3.56)	
							(2) FAN in hypertensives 3.02 (95% CI 1.51 to 6.02)	
							(3) any retinopathy in hypertensives 2.10 (95% CI 1.04 to 4.24)	
							No statistically significant results for 3MSE; no other statistically significant results for dementia	
Patton *et al*[23]	Lothian Birth Cohort 1921 Study (UK)	Cross-sectional	321 men and women born in 1921, participated in a mental survey of 1932 and residing in the Lothian region of Scotland	83–84	Computer-assisted grading of:	MMSE	Effect size (η^2) of the variance in g accounted for by SBC: 0.034 $p = 0.02$; in LMT by SBA: 0.026 $p = 0.03$; in VFT by SBC: 0.037 $p = 0.01$	Suboptimal BC related to lower scores in g and VFT; suboptimal BA related to lower scores in LMT
		Cognitive and retinal assessments undertaken simultaneously			(1) CRAE	LMT	No statistically significant results for RPM or with CRVE, CRAE and AVR	Adjustment for sex, diabetes, cerebrovascular disease, systolic blood pressure, DBP, alcohol use, smoking
					(2) CRVE	VFT		APOE e4 status, near visual acuity, social class, education, IQ at age 11; **7/9**
					(3) AVR	RPM		
					(4) SBC	General cognitive ability score (g) extracted from the four tests		
					(5) SBA			
					CRAE and CRVE measured in pixels, SBA in degree; all measured on continuous scale			

*Bold numbers indicate the quality rating.
†Based on detailed neuropsychological testing and established by a consensus committee.
APOE, apolipoprotein E; ARIC, the Atherosclerosis Risk in Communities Study; AVR, arteriovenous ratio; BA, bifurcation angles; BMI, body mass index; CRAE, central retinal arterial equivalent; CRVE, central retinal venular equivalent; DBP, diastolic blood pressure; DWR, Delayed Word Recall Test; FAN, focal arteriolar narrowing; HDL, high-density lipoprotein; IMT, intima-media wall thickness; IQ, intelligence; LMT, Logical Memory Test; 3MSE, Modified Mini-Mental State Examination; RPM, Raven's Progressive Matrices; SBA, suboptimal bifurcation angles; SBC, suboptimal branching coefficient; SBP, systolic blood pressure; VFT, Verbal Fluency Test.

Table 3 Terminology and definitions of retinal microvascular abnormalities used in included studies

Retinal microvascular signs	Definitions	References
Background diabetic retinopathy	Presence of two or more microaneurysms in one eye	Ferguson *et al*[20]
Proliferative diabetic retinopathy (PDR)	A grading level at 60 or higher in one eye, or less than 60 but with panretinal photocoagulation scars consistent with laser therapy*	Ryan *et al*[18]
Diabetic retinopathy	A grading level greater than 10 (10 = no retinopathy)†	Kadoi *et al*[19]
Any retinopathy	Presence of any of the following lesions (not explicitly arteriolar in nature): microaneurysms, retinal haemorrhages, soft exudates, hard exudates, macular oedema, intraretinal microvascular abnormalities (tortuous intraretinal vessels), venous beading, new vessels at the disc or elsewhere, vitreous haemorrhage, disc swelling, or laser photocoagulation scars†	Wong *et al*,[21] Baker *et al*[22]
Focal arteriolar narrowing (FAN)	An arteriole estimated to be 50 μm diameter or greater has a constricted area of 2/3 or less the width of proximal and distal vessel segments‡	Wong *et al*,[21] Baker *et al*[22]
Arteriovenous nicking (AV nicking)	A venule is constricted on both sides of its crossing under an arteriole in a specified region‡	Wong *et al*,[21] Baker *et al*[22]
Generalised arteriolar narrowing	Lowest 20th percentile of the sample arteriole-to-venule ratio (AVR) distribution measured on a continuous scale via a computer-assisted technique	Wong *et al*[21]
Arteriovenous ratio (AVR)	A ratio between the summarised calibre measurements of the arterioles (CRAE) with respect to the venules (CRVE)§	Wong *et al*,[21] Patton *et al*[23]
Central retinal arterial equivalent (CRAE)	Combined calibre measurements of individual arterioles coursing through a circular zone defined as between 0.5 and 1 disc diameter from the optic disc margin§	Wong *et al*,[21] Baker *et al*,[22] Patton *et al*[23]
Central retinal venular equivalent (CRVE)	Combined calibre measurements of individual venules coursing through a circular zone defined as between 0.5 and 1 disc diameter from the optic disc margin§	Wong *et al*,[21] Baker *et al*,[22] Patton *et al*[23]
Arteriolar branching coefficient (BC)	A ratio expressing the relationship between trunk vessel (W) and two branch vessels (w_1 and w_2) on vessel widths at arteriolar branching points: BC = $(w_1^2+w_2^2)/w^2$	Patton *et al*[23]
Arteriolar bifurcation angles (BA)	An angle (degree) measured at arteriolar branching points between the two branch arterioles	Patton *et al*[23]
Suboptimal branching coefficient	The degree of deviation of the BC from optimality (ie, a theoretical value of 1.26)	Patton *et al*[23]
Suboptimal angles	The difference of the degree of BA from optimality (ie, a theoretical and empirical value of 75°)	Patton *et al*[23]

*The Wisconsin Epidemiologic Study of Diabetic Retinopathy Classification and Grading System.[24]
†The Diabetic Retinopathy Study and the Early Treatment Diabetic Retinopathy study grading scale.[25]
‡The Modified Airlie House Classification of Diabetic Retinopathy.[26]
§Methods for evaluation of retinal microvascular abnormalities associated with Hypertension/Sclerosis in the Atherosclerosis Risk in Communities Study.[27]

with poorer verbal memory (Logical Memory) but not other cognitive modalities including general cognitive ability (g), executive function (Verbal Fluency Test) or performance IQ (Raven's Matrices); suboptimal branching coefficient (from theoretically derived-optimum values) was associated with poorer general cognitive ability and verbal fluency but not other tests. The effect size of the variance in cognitive test scores explained by the retinal vascular abnormalities was small, ranging from 2.6% to 3.7%.

DISCUSSION

This is the first systematic review of the association between retinal vascular signs and cognitive dysfunction. Findings are consistent with the hypothesis that retinal microvascular abnormalities are associated with cognitive impairment or dementia in patients with diabetes and in the general population, although findings are not conclusive due to varied and sometimes limited methodology in the included studies. The presence of retinal vascular signs was mostly associated with poorer verbal memory, information-processing speed (Digit Symbol Test) and executive function (Verbal Fluency Test) in the general population. Furthermore, the longitudinal studies in this review may have underestimated the association with retinal abnormalities and cognitive function in patients with diabetes because of survival bias. Patients with diabetes with retinopathy at baseline were more likely not to attend for follow-up (including deaths), when follow-up success itself could well be related to good cognitive function, but the two studies did not report the retinopathy status of individuals lost to follow-up.[18 19] Indeed, one study had a higher proportion of drop-outs.[18]

If there is indeed a direct relationship between retinal vascular abnormalities and cognitive function, this may reflect the homology between the retinal and cerebral microvasculatures.[9] Similar to changes in the retinal vasculature, these microvascular changes might lead to chronic ischaemia of the brain and the development of white-matter lesions. In epidemiological studies, the presence of retinal vascular lesions that reflect a breakdown of the blood–retinal barrier, including microaneurysms, retinal haemorrhages and soft exudates, was related most consistently to stroke, stroke mortality, white-matter lesions and cerebral atrophy.[10–13] Thus, the similar disruption of the blood–brain barrier of the cerebral microcirculation could be an important pathophysiological feature in the occurrence of cognitive impairment and dementia.[32]

Compared with ophthalmoscopic examination, the assessment of retinal photographs has been suggested as a more reliable method for measuring a number of retinal vascular signs, including generalised and focal arteriolar narrowing, arteriovenous (AV) nicking, isolated retinal haemorrhages, microaneurysms and cotton wool spots.[6 33–35] Computer-assisted measures of retinal vascular widths (to define generalised arteriolar narrowing, venular dilation and other measures of retinal vascular network geometry) have been found to detect subtle microvascular changes with low intra- and inter-observer variability.[10 13 35 36] Grading of other abnormalities (such as focal arteriolar attenuation and retinal haemorrhages) is still largely done manually and therefore highly operator-dependent, though automated techniques are being developed. The reliability of grading of focal retinal abnormalities has also been shown to be dependent on the classification system used.[37] Despite the use of standardised protocols, differing methods and grading systems for measuring retinal microvascular abnormalities could explain at least in part some of the inconsistent findings between studies in this review.

Many observed changes in cognitive function of interest in studies are generally small,[38] and mild cognitive impairment is an established risk factor for the subsequent development of dementia[39] This requires measurement of cognitive function that use validated (sensitive to small increments of change) and

Table 4 Cognitive function assessment tools used in studies evaluating the relation to retinal vascular signs

Cognitive domain*	Test name	References
Attention/concentration	Paced Auditory Serial Additional Task (PASAT)	Ferguson *et al*[20]
	Digit Span Forward (DSF)	Kadoi *et al*[19]
	DigitVigilance Test† (DVT)	Ryan *et al*[18]
	Part A of the Trail Making test (TMTA)	Kadoi *et al*[19]
	Part B of the Trail Making test† (TMTB)	Ryan *et al*,[18] Kadoi *et al*[19]
Information-processing speed	Digit Symbol Subtest†,‡ (DSS)	Wong *et al*,[21] Ferguson *et al*,[20] Ryan *et al*,[18] Baker *et al*[22]
	Inspection time and Median four-choice reaction time (IT and CRT)	Ferguson *et al*[20]
Manual dexterity	Grooved Pegboard Test† (GPT)	Ryan *et al*,[18] Kadoi *et al*[19]
Frontal lobe/executive function	Verbal Fluency Test (VFT)	Wong *et al*,[21] Patton *et al*[23]
		Ferguson *et al*[20]
Performance IQ	Object Assembly (OA) and Block Design subtests (BD): ([WAIS-R])	Ryan *et al*,[18] Ferguson *et al*[20]
	Picture completion (PC)	Ferguson *et al*[20]
	Raven's Progressive Matrices (RPM)	Patton *et al*[23]
Verbal Memory	Delayed Word Recall Test (DWR)	Wong *et al*[21]
	Rey auditory verbal learning test (RAVLT)	Kadoi *et al*[19]
	Logical Memory Test (LMT)	Patton *et al*[23]
Mini-Mental State Examination (MMSE)	MMSE	Kadoi *et al*,[19] Patton *et al*,[23] Baker *et al*[22]
	Modified Mini-Mental State Examination (3MSE)	

*Classification of cognitive domain based on descriptions by Lezak[29] and widely used definitions.
†Tests used as part of Psychomotor Efficiency domain in Ryan *et al*.
‡Test used as part of performance IQ domain in Ferguson *et al*.
WAIS-R, Wechsler Adult Intelligence Scale—Revised.

reliable psychometric tests. In one of the studies, MMSE scores were used as a measure of cognitive function.[22] However, the MMSE is a relatively insensitive test, which is designed to screen for dementia but not to measure subtle changes in cognitive decline.[40–42]

The majority of cross-sectional studies of cognitive function relied on cognitive performance at a single time point, which does not capture the pathological process under study—cognitive decline or change over time. Premorbid cognitive ability represents the highest level of ability attained prior to any cognitive decrements taking place and is also known to account for a substantial proportion of the variance in cognitive function scores in old age.[28 43 44] Some studies estimated prior ability by a patient's education and occupation history, but this is limited in that these features may be influenced by lack of opportunity rather than lack of ability.[28 45] The National Adult Reading Test (NART) could be a more useful measure, as word reading ability is preserved even in generalised cognitive decline.[45] Adjusting scores on a fluid mental test for NART has been shown to be a valid, instantaneous measure of lifetime cognitive change or decline.[46 47] Only one study adjusted for premorbid IQ in an attempt to get a direct measure of lifetime cognitive decline.[23]

Most studies assessed important potential confounders such as age, gender, lifestyle factors and medical conditions. Patients with neurological conditions (including a history of stroke) that might interfere with cognitive abilities were also excluded from all studies. However, subjects with a history of chronic alcohol or drug abuse were only variably excluded. In addition, inadequate adjustment was generally made for visual acuity and depression. Some of the cognitive tasks may require at least moderate visual function to complete and if diabetic people with severe retinopathy have greatly impaired vision, their scores on the relevant cognitive testing could be affected. Two of the studies collected data on visual acuity[18 23] and one study controlled for this.[23] Depression may confound the association between retinal microvascular disease and cognitive dysfunction because depression can be mistaken for dementia (and vice versa), and is associated with deficits of memory and learning, and occurs more frequently in those with diabetes-related complications including retinopathy.[48–53] Two studies assessed the potential effects of low mood.[18 20]

It should be noted that we reviewed only published studies, and it is possible that studies which did not show an association between retinal vascular disease and cognitive dysfunction may not have been published as often as studies that did. It was not possible to test for this formally (eg, by use of a funnel plot) because studies were too few and too methodologically heterogeneous. Clinic-based studies on patients with diabetes used variable inclusion criteria and this limited the generalisability of the results. Diabetes-related cognitive impairment may have different underlying mechanisms from cognitive dysfunction in the general population and may differ in patients with type 1 diabetes, type 2 diabetes and postoperatively. However, population-based studies are scarce so far restricted to a cross-sectional design, which makes it impossible to determine the temporal relationship between retinal vascular disease and cognitive dysfunction (though it is perhaps unlikely that cognitive impairment leads directly to retinal vascular abnormalities). Findings are not consistent regarding the importance of the association which are limited by substantial differences in methodology between the studies, small size of some of the studies, restricted controlling for potential confounding factors and variability in the range of cognitive domains and retinal microvascular abnormalities measured. Further better-designed studies are required, with a common, standardised and objective retinal vascular assessment, together with a range of sensitive cognitive tests.

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