The Use Of Static Objective Retinal Vessel Analysis In Optometric Practice

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Thesis summary:

At present optometric examination of the retinal microcirculation consists of a subjective assessment of artery size when compared to a neighbouring vein; the arterio-venous ratio (AVR). Despite it's documented limitations, the AVR still features in UK optometric clinical guidance. An objective method of recording the central retinal artery and vein equivalent sizes (CRAE and CRVE) has been used for research purposes for two decades but has yet to be validated in a clinical setting. A review of the present literature identified correlations between CRAE and CRVE and three key cardiovascular pathologies; hypertension, diabetes mellitus and stroke. A methodological study was undertaken to establish whether retinal photographs acquired in clinical practice yielded results with reproducibility comparable with those in the literature. It was shown that calibre measurements do not fluctuate significantly with the use of mydriatics, or subject to minor temporal fluctuations. When the technique was applied in clinical practice, a cross-sectional cohort (n = 271) revealed vessel correlations with systemic biomarkers in agreement with those identified in the literature review. CRAE was seen to be reduced in those with raised blood pressure, and objective AVR was reduced in those with increased cardiovascular risk (QRISK). There did not appear to be significant fluctuations in vessel measurements when the cohort was observed longitudinally across a period of 12 – 24 months, suggesting changes either too slow or too small to be detected at present; supporting theories of gradual morphological changes. When considered as part of scope of primary care, improved cardiovascular assessment through retinal vessel analysis (especially when supplemented with blood pressure and cardiovascular risk calculations) relieves pressure on GPs and identifies a significant number of previously undiagnosed and unmanaged cases of cardiovascular disease. The results build a strong case for the incorporation of objective retinal vessel analysis into routine optometric clinical guidelines.

Keywords: Optometry, Retinal Vessels, Cardiovascular Disease, Multi-disciplinary Care

Dedication

I would like to extend my thanks and appreciation to a number of individuals who have contributed, supported or assisted throughout the development of this thesis. Firstly, to the staff at Aston University; particularly Richard Armstrong for his invaluable help with the intricate world of statistics and Rob Cubbidge, for his insight and support throughout. Next, I would like to thank my colleagues at the University of Hertfordshire; Joy, Lynne, Amy, Sheila and the rest of the team for their help and support with getting this manuscript finished whilst juggling my lecturing commitments. I would also like to thank Davis Optometrists, particularly my willing and enthusiastic patients, who kindly gave up their time to provide their data; this PhD would not have been possible without them. Nor would it have been possible without the financial support from the College of Optometrists; I am very thankful for the opportunity they have given me. I am immensely grateful to the team at Imedos; Cornelia, Benedikt, Walthard, Volker and the rest of the team who have helped provide valuable technical insight and support, making me feel extremely welcome in the process. To my wonderful family, particularly my parents who have always supported me and taught me to do what makes me happy, and my friends; both those going through the same PhD stresses (Katrin, Emma and Maryam), and those who could provide the much needed distractions from a seemingly endless workload (Aiden, Emily, Will and Matt). I would also thank Bill, Lee, John and the other members of CADSAC for introducing me to the joys of scuba diving. Finally, I would like to extend my heartfelt thanks to my supervisor, Rebekka, for her continued friendship, encouragement and support throughout; the PhD has been an incredible experience and I look forward to the research that lies ahead.

Contents

Nomenclature

AI Artificial Intelligence

ARIC Atherosclerosis Risk in Communities Study [United States]

AusDiab The Australian Diabetes, Obesity and Lifestyle Study [Australia]

AVR Arteriole-venule ratio

BDES Beaver Dam Eye Study [United States]

BMES Blue Mountains Eye Study [Australia]

CHS Cardiovascular Health Study [United States]

CRA Central retinal artery

CRAE Central retinal artery equivalent

CRV Central retinal vein

CRVE Central retinal vein equivalent

CSF Cerebro-spinal fluid

DES Diabetic Eye Screening programme

DM Diabetes Mellitus

DR Diabetic retinopathy

FAN Focal Arterial Narrowing

FLEMENGHO Flemish Study on Environmental, Genetics and Health Outcome Study [Belgium]

FPG Fasting Plasma Glucose

Funagata The Funagata Study [Japan]

GAN Generalised Arterial Narrowing

GP General Practitioner

HbA1c Glycosylated haemoglobin

HR Hazard Ratio

HT Hypertension

IOP Intra-ocular pressure

IVAN Interactive Vessel Analysis (Wisconsin, US)

KWB Keith-Wagener-Barker Classification

MABP Mean Arterial Blood Pressure

MESA Multi-Ethnic Study of Atherosclerosis [United States]

MP Mega-pixels

MSE Mean Spherical Equivalent

NHS National Health Service

NICE National Institute for Health and Care Excellence

NP-DR Non-proliferative diabetic retinopathy

O-AVR Objective arterio-venous ratio

OBF Ocular Blood Flow

OGTT Oral Glucose Tolerance Test

OR Odds Ratio

P-DR Proliferative diabetic retinopathy

PEDCS Pittsburgh Epidemiology of Diabetes Complications Study

RaRo Rate Ratio

Rotterdam The Rotterdam Study [Netherlands]

RR Risk Ratio

SiMES Singapore-Malay Eye Study [Singapore]

SIVA Singapore 'I' Vessel Assessment (Singapore)

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1 Introduction

1.1 Overview

The eye is often referred to as a *'window to the body'*. This may be an overstatement, but it is a well-documented fact that the eye, in particular the retina with it's rich vascular tree, serves as the only location where the microcirculation can be observed *in vivo*[1, 2]. Since the retinal vasculature is connected to the body's macrocirculation, systemic changes both physiological and pathological can manifest in the eye. Optometrists in primary care are well situated to screen patients on a routine, continual basis for such changes. Of particular consideration are those conditions which directly affect the circulation; i.e. cardiovascular disease.

1.2 Projected Impact of Cardiovascular Disease

Diabetes mellitus (DM), hypertension (HT) and stroke are three common, systemic cardiovascular conditions intrinsically linked to one another. Prevalence amongst adults (16+) of the over-arching term 'cardiovascular disease' (including stroke, angina, coronary heart disease, myocardial infarction) in England for the year 2017 was calculated to be 13.5%, with numbers increasing in-line with age. Age-standardised death rates from cardiovascular disease in the UK was 246 per 100,000 in the same year[3]. Aside from 1918, CVD has been the leading cause of death in America since 1900, with the incidence being higher than the next four leading causes combined (cancer, chronic lower respiratory disease, accidents, DM)[4]. Diabetes mellitus (Types I and II) currently affects 3.8 million people in the UK alone, and prevalence is expected to nearly double by 2035 (although it is thought that around half of the Type II DM diagnoses could be avoided if childhood obesity is sufficiently curtailed)[5]. In the UK each week, there are 169 limb amputations, 530 heart attacks and 680 strokes as a result of DM. In short, it is thought that more than 500 diabetics die prematurely per week[5]. Diabetes mellitus has been cited as an independent risk factor for the development of cardiovascular disease and hypertension[6, 7]. Conversely hypertension and anti-hypertensive medication are known to be a risk factor for DM[8, 9]. The increase in DM prevalence is set to require a further 7% of the National Health Service (NHS) budget by 2036[10]. This poses serious financial implications since diabetic retinopathy complications alone in 2010/11 cost the NHS £57,741,842[10]. Spending on Type II DM alone has increased rapidly in the UK over recent years. Between 2012 and 2016, there has been a 25% increase in spending on DM treatment; split between an increase in diagnoses and the prescribed treatment. Expenditure for diabetic drugs in 2015/16 was £873,000,000[11]. Per year,

total hospital costs are estimated to be £32bn; diabetes-related treatments made up 16.25% (£5.2bn) of that budget[12]. Hypertension is equally prevalent, affecting more than 50% of those aged 65 and over in the UK[13]. With an ageing population, incidence figures are set to rise for conditions such as DM, hypertension and cardiovascular disease. Their complex inter-relationship will undoubtedly exacerbate such figures. Clearly there will be a much greater need for prompt diagnosis and more efficient monitoring of individuals in order to best allocate NHS services and treatment. Examination of the retinal vasculature as a means of monitoring DM is relatively quick and cheap to perform. Current costs of the retinopathy screening service are only predicted to rise by just over £1million over the next twenty years, whereas diagnostic costs as a whole will rise by over £7.5million[10]. Optometrists have been identified as a key component in diabetic patient care, in both detection and subsequent diagnosis of DM, but also in monitoring for signs of progression in existing cases of DM[14]. More generally, multi-disciplinary care can improve the efficiency healthcare with better patient outcomes (such as slowed rate of renal function decline in chronic kidney disease[15]), although it has been identified that inter-professional communication is key to keep informed about that patient's well-being[16]. This would be especially pertinent in a community setting where primary healthcare providers may well be situated at different sites (e.g. GP, nurse, pharmacist, optometrist). There is an increasing shift towards the use of practice nurses and healthcare assistants (HCAs) in UK GP-based hypertension monitoring schemes[17]. In context to cardiovascular disease, early detection of vascular changes through optometric examination can help improve detection and diagnosis rates and initiate earlier interventions[18]. Changes to vascular morphology in cardiovascular disease is well-known[2, 1, 19], but in order for this to be effectively monitored, there needs to be an accurate and clinically appropriate method for recording them.

1.3 Overview: Hypertension

When the heart pumps, expelling oxygenated blood into the arterial system, the maximum pressure that this travels at is referred to as the *systolic* blood pressure. When the heart relaxes, and there is a surge of blood returning to the heart in the venous system, the blood is at its lowest pressure; the *diastolic* pressure. The Seventh Joint National Committee on the Prevention, Detection, Evaluation and Treatment of High Blood Pressure in the United States published a revised classification for blood pressure and hypertension based on blood pressure readings alone (*see Table 1*)[20]. Whilst there has since been an eighth JNC report, the classification criteria for hypertension remains unchanged[21]. The diagnosis of hypertension is made when the average of two or more readings over two successive visits

are repeatedly high[22]. The classification in the UK (*see Table 2*), outlined by the National Institute for Health and Care Excellence; NICE, is the same as that published in the JNC report, with the addition of a fifth category; '*Severe hypertension*' (sometimes referred to as '*malignant hypertension*', however for consistency the NICE terminology will be used). To reflect changes observed throughout the day, the European Society of Hypertension (ESH) and European Society of Cardiology (ESC) have also published guidance on defining hypertension based on the setting of BP measurement (*see Table 3*). Ambulatory blood pressure refers to the continuous monitoring of blood pressure over a given period, rather than an isolated 'window' during the day.

Classification	**Systolic (mmHg)**		**Diastolic (mmHg)**
Normotension	<120	*and*	<80
Pre-hypertension	120-139	*or*	80-89
Stage 1 hypertension	140-159	*or*	90-99
Stage 2 hypertension	≥160	*or*	≥100

Table 1: Classification of blood pressure in persons aged 18 and over - JNC-7 Report (USA). [20]

Classification	**Systolic (mmHg)**		**Diastolic (mmHg)**
Normotension	<120	*and*	<80
Pre-hypertension	120-139	*or*	80-89
Stage 1 hypertension	140-159	*or*	90-99
Stage 2 hypertension	160-179	*or*	100-109
Severe hypertension	≥180	*or*	≥110

Table 2: Classification of blood pressure in persons aged 18 and over - NICE Guidelines (UK). [23]

	Systolic (mmHg)		**Diastolic (mmHg)**
Office BP	≥140	*and/or*	≥90
Ambulatory BP:			
Daytime (awake)	≥135	*and/or*	≥85
Night-time (asleep)	≥120	*and/or*	≥70
24-hr	≥130	*and/or*	≥80
Home BP	≥135	*and/or*	≥85

Table 3: ESH/ESC hypertension definitions for office and out-of-office blood pressure readings. (*Note the definition for 'Office BP' is coincident with NICE and JNC definitions of 'Stage I hypertension'*) [24]

Pre-hypertension as a category was created since those persons with blood pressure readings of 130-139/80-89mmHg are twice as likely to develop hypertension as those with blood pressure below those values[20]. This category is of particular relevance to the optometrist since accurate retinal vessel analysis with this group of patients has the potential to monitor and detect pathological changes sooner or even before pathological damage has taken place; a previous study has shown that simply measuring blood pressure alone in optometric practice identifies one in 8 '*undiagnosed hypertensives*' (where BP was >140/80mmHg)[18]. This statistic could be suggested to underestimate those at risk, since the category of '*pre-hypertension*' was not recognised by the study. By identifying those patients at particular risk of going on to develop hypertension, a thorough assessment of the vascular status and calibre is of great importance. Patient education and notification of the GP or physician at this stage allows for the early intervention and possible curtailing of any further increase in (and microvascular damage arising from) blood pressure. Despite raised pressure being the driving force of hypertension, the majority of complications that arise are thrombotic (stroke, myocardial infarction) in origin, rather than haemorrhagic (as one would expect with raised pressure)[25, 26]. This has been termed the "Thrombotic (or Birmingham) Paradox" and is an important consideration when evaluating patient risk.

Despite it's widespread prevalence, the aetiology and mechanism of hypertension is still not well understood; even the action of the first-line recommended treatment (β-blockers) remains unknown[27]. There is a variety of factors known to influence hypertensive susceptibility; however the understanding of genetic influence is currently limited[22]. Whilst it is known that blood pressure traits can run in families, the specific genes dictating this have yet to be identified. In a theoretical population of normotensive persons, the blood pressure would be normally distributed, thus the genetic influence to predispose those subjects to fall either side of the peak of distribution needs to be explored[22]. There are a number of factors known to be hypertensinogenic; obesity, insulin resistance, high alcohol intake, high salt intake and low potassium and calcium intakes; and some of these factors can be additive (e.g. obesity and alcohol intake combined carry a greater risk)[20, 22]. Environmental aspects such as stress and a sedentary lifestyle are also associated with hypertension development[22].

Hypertension arises due to an imbalance between cardiac output (which generally remains unchanged) and peripheral vascular resistance[28]. This manifests as constriction of small arterioles. Exactly when these changes occur in the timeline of hypertension is still unknown, however it is believed that a structural change takes place prior to a clinical increase in blood pressure[29, 30]. Since small arterioles contain smooth muscle, one theory suggests that early hypertensive changes arise due to a local spike in intracel-

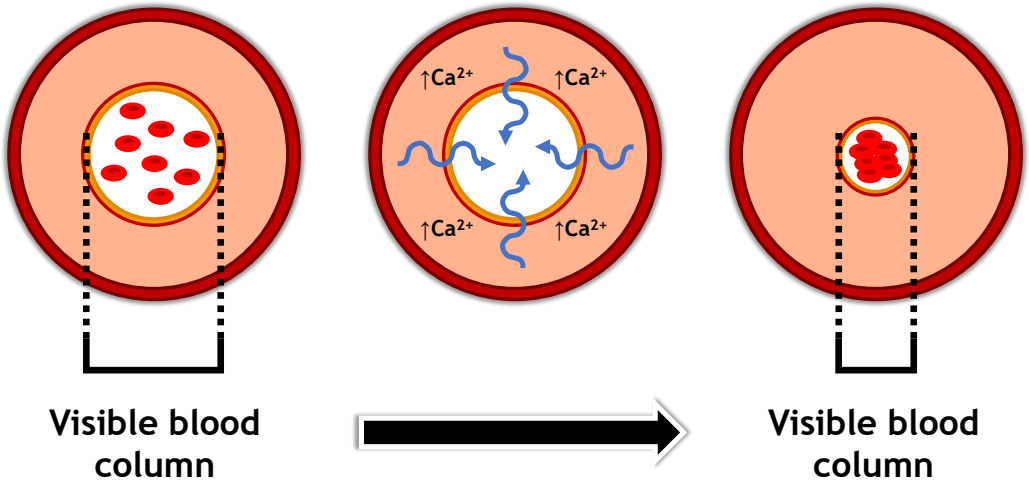

Figure 1: Development of hypertension. An increase in calcium within the muscular tunic of the arteries prompts inward muscular contraction and reduction of vessel lumen. Since the same volume of blood is travelling through the vessel, this raises the pressure at which it is travelling, and promotes the cascade of events associated with hypertensive damage (damage through increased sheer stress and vascular resistance).

lular calcium, contracting the muscle which leads to an irreversible thickening of the vessel wall with a reduction in vessel lumen and (in the case of retinal arteries) reduced visible blood column (*see Figure 1*). This is in addition to the subsequent sequelae associated with hypertensive pathophysiology[28]. Regardless of the anatomical uncertainty, there is a long-standing and well-documented association between increased blood pressure and narrow arteries[31, 32, 33, 34]. The arteries are known to stiffen with age as well as decrease their lumenal diameter. This results in a pressure-load increase and a decreased ability for vascular deformation to take place in an effort to absorb this increase in pressure (and thus raised vascular resistance)[34]. Atherosclerosis is a common process with strong associations with hypertension but remains a distinct pathophysiological entity. What begins as a 'fatty streak' within the intima and inner media of larger artery walls gradually develops into a fibrous plaque which, as it increases in size, protrudes into the lumen of the vessel; thus increasing blood turbulence and subsequent endothelial damage. A resultant increase in blood pressure locally due to the constriction is worsened as the atherosclerotic plaque increases in size (*see Figure 2*)[34]. As such, since the visible portion of the retinal vessels is the blood column itself, a reduction in visible calibre should be expected in hypertension[35].

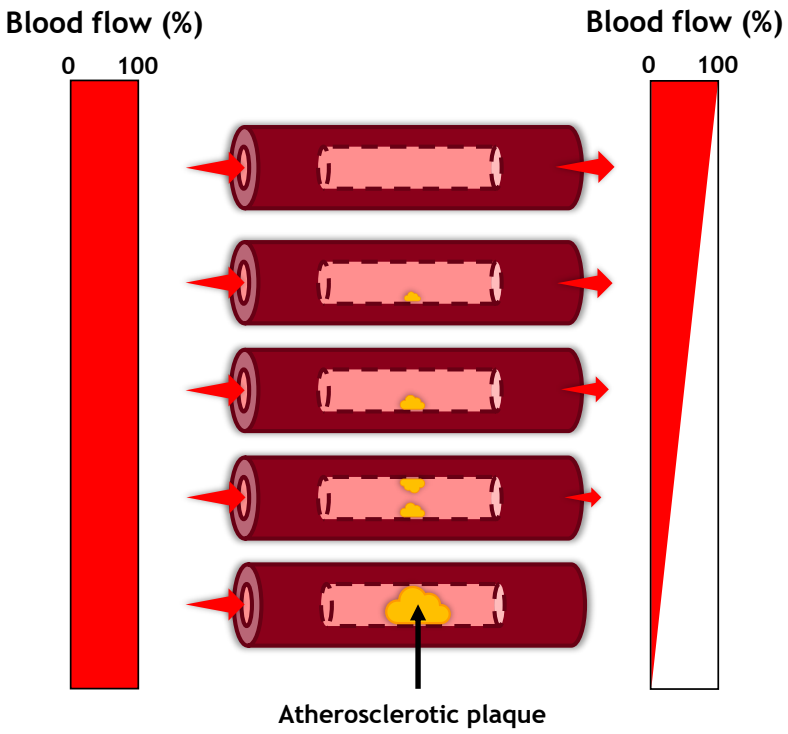

Figure 2: Development of atherosclerotic plaque. Gradual deposition of a 'fatty streak' restricts lumen diameter, particularly once it begins to fibrose. This eventually leads to a focal rise in blood pressure, itself producing further damage to the vessel wall.

1.4 Overview: Diabetes Mellitus

Diabetes mellitus is a collection of pathologies, characterised by abnormal insulin function. Since insulin is involved in the metabolism of sugars within the blood, the resultant hyperglycaemia in DM leads to long-term damage and failure of particular organs; the kidneys, nervous system and heart as well as the vascular network and the eyes (the latter being both highly innervated and vascularised)[36, 37]. Diabetes mellitus is commonly referred to as either being type I ('*insulin-dependent*') or type II ('*non-insulin-dependent*'), however a number of further sub-types such as gestational and drug-induced diabetes mean that the use of these two types in isolation is not strictly accurate. The terms '*type I*' and '*type II*' are still extensively prevalent, even in research. Recently, emphasis has been placed on the identification of pre-diabetes, particularly those patients with impaired glucose tolerance (IGT) and impaired fasting glucose (IFG), both of which carry an increased risk of diabetogenesis and the development of other cardiovascular conditions. The International Diabetes Federation held a Consensus Workshop and part of their findings suggested considering both IGT and IFG as actual diseases instead of risk factors[37]. Diabetes mellitus is also known to accelerate the vascular ageing process, particularly the development of atherosclerosis. Studies have shown that with both Type I and II DM free from other cardiovascular pathologies, vascular stiffness is increased compared to normoglycaemics[7].

1.4.1 Type I diabetes mellitus

Prevalence of Type I diabetes mellitus is estimated to be 5-10% of diabetics[36]. The full aetiology of Type I DM is still not fully understood, with aspects such as geographic prevalence variations (incidence in Scandinavian countries is markedly higher than oriental countries) currently remaining unexplained - although this does suggest a possible genetic element[38]. Whilst the exact trigger for diabetogenesis remains largely unclear, the subsequent pathological process is better understood; an autoimmune destruction of the β-cells in the pancreas which produce insulin [36, 38]. This destruction, which can take a variable time course, generally leads to an absolute deficiency of insulin, thus necessitating the lifelong dependency on insulin therapy. Since it is effectively an autoimmune condition, co-morbidities of pathologies such as thyroid dysfunction, myasethenia gravis and pernicious anaemia are common[36].

1.4.2 Type II diabetes mellitus

Type II DM is considerably more common, with prevalence being 90-95% of those with diabetes mellitus[39]. Just as with Type I DM, regional variations suggest a genetic element to DM development, however Type

II DM shows a much greater association with environmental and lifestyle factors such as weight. Approximately 80% of persons with Type II DM are obese, which carries a certain level of resistance to the action of insulin[36, 39]. The key difference between Type I and Type II is that in Type II there is no pathological destruction of the insulin-producing β-cells in the pancreas; patients therefore have a relative insulin deficiency, as opposed to an absolute deficiency[36]. Given the gradual decline in insulin secretion, symptoms may take years to develop despite microvascular damage taking place.

1.4.2.1 Young-onset Type II diabetes mellitus

Distinction should be made for the growing number of cases of Type II DM developing in younger persons and adolescents. Historically, the distinction between Types I and II has largely revolved around age of onset, with Type II being classically referred to as 'age-onset' DM. This is typified in study methodologies, where those wanting to make distinctions between DM types will do so based on age of onset, even in the cases of those which are specifically looking at a diabetic cohort (such as the Wisconsin Epidemiologic Study of Diabetic Retinopathy; WESDR)[40, 41, 42, 43]. It has also been suggested that children diagnosed as diabetic may have been classified erroneously as Type I simply because of their age[44]. As rates of child and adolescent obesity are rising, it is suggested that increased insulin resistance is developing in these individuals leading to young-onset Type II DM[45, 46]. Whilst these may not be insulin-dependent, and therefore modifiable lifestyle changes can be implemented in an attempt to improve patient health, it has been shown that adolescents in the United States with Type II DM are losing on average 15 years from remaining life expectancy when compared to similarly aged non-diabetic adolescents (and DM-related complications developing by the age of 40)[47]. It has been suggested that young-onset Type II DM should be an additional cohort in studies of cardiovascular disease since they appear to undergo a more aggressive disease process compared to older adults developing Type II DM[48].

1.4.3 Diagnosis

The American Diabetes Association (ADA) recommend that DM be diagnosed using one of three tests, and a positive diagnosis only be given if the results are consistently high at a second visit[36]. The least effective test is the casual plasma glucose (CPG) test. This is taken at any time of day, with no consideration given to when the last meal was consumed. The traditionally used test is the fasting plasma glucose (FPG) test, which requires the patient to not have eaten within the preceding 8 hours. The final method, which has specific guidelines provided by the World Health Organisation (WHO), is useful for diagnosis

of impaired glucose tolerance, but is time consuming and potentially problematic in a patient with DM, since it involves the loading of a patient with a concentrated sugar solution. The oral glucose tolerance test (OGTT) is performed 2 hours following the patient's consumption of a solution containing 75g of anhydrous glucose dissolved in water. The results and their meanings are summarised in Table 4. The ADA and WHO also recommend against the use of glycosylated haemoglobin (HbA1c) measurements in the diagnosis of DM[36, 49], although the WHO have relaxed this stance more recently, advising that HbA1c can be used provided there is no better alternative and a minimum of two readings are acquired[50]. The UK's NICE guidance for diabetes does not stipulate it's own classification of diagnosis; simply referring to those in need of diagnosis as '*presenting with hyperglycaemia*' (and making reference to the WHO criteria (*summarised in Table 4*))[51, 52].

Test	Result	Diagnosis
CPG	**≥200 mg/dl (11.1 mmol/l)**	**Diabetes**
FPG	≤100 mg/dl (5.6 mmol/l)	Healthy
	100-125 mg/dl (5.6-6.9 mmol/l)	IFG
	≥126 mg/dl (7.0 mmol/l)	**Diabetes**
OGTT	<140 mg/dl (7.8 mmol/l)	Healthy
	140-199 mg/dl (7.9-11.1 mmol/l)	IGT
	≥200 mg/dl (11.1 mmol/l)	**Diabetes**

Table 4: A summary of diagnostic tests for diabetes mellitus. 'CPG' = Casual Plasma Glucose (*reading taken at any time*), 'FPG' = Fasting Plasma Glucose (*reading taken 8 hours following last meal*), 'OGTT' = Oral Glucose Tolerance Test (*reading taken 2 hours following patient loading of 75g anhydrous glucose solution*). 'IFG' = Impaired Fasting Glucose, 'IGT' = Impaired Glucose Tolerance.

1.4.4 Monitoring

In the UK, the NHS oversees a comprehensive programme to monitor diabetics following diagnosis. An established retinopathy screening service involves patients having dilated fundus photographs taken and sent to an analysis centre for grading by trained observers, to evaluate both risk of sight-threatening diabetic retinopathy but also because the retina acts a surrogate for the rest of the circulatory system. This is recommended to take place within 3 months of diagnosis[53]. Similar automatic enrolment into a regular monitoring scheme is also in place for the diabetic foot[54], however NICE only recommend offering screening for chronic kidney disease[55]. Principally, patients are educated and encouraged to undertake home monitoring of blood glucose levels, especially when taking insulin. More recent treat-

ments include insulin pumps which operate continuously to supply the required dosage. Stability of blood glucose can be evaluated through the glycosylated haemoglobin (HbA1c) test, which provides an accurate indication to blood glucose levels over the preceding 8-12 weeks (based on the lifespan of erythrocytes (red blood cells))[56]. HbA1c is recorded in mmol/mol, however it was previously specified in percentage (%), thus patients may be more familiar with one or other format. The WHO suggest an HbA1c of 6.5% (48mmol/mol) as the cut-off for diabetic diagnosis. Diabetes UK suggest that when a person with Type II DM (and not taking any treatment, i.e. 'diet-controlled') has an HbA1c which falls below 6.5% (48mmol/mol) they are considered to be in remission[57]. Those at risk of Type II DM are encouraged to aim for an HbA1c of ≥6.0% (42mmol/mol).

1.4.5 Systemic complications of diabetes mellitus

Diabetes mellitus affects both the micro- and macro-circulation throughout the body, and are summarised in Figure 3. Kidney failure (nephropathy) is characterised by the presence of protein in urine (proteinuria). The risk of this complication rises exponentially with duration of DM, and can lead to premature death due to coronary heart disease (reiterating the complex interaction of cardiovascular disease). Diabetic neuropathy is also a complication, where peripheral regions such as the hands and feet are most at risk, and healing is also impaired[58]. This causes particular problems with the diabetic foot due to a combination of poor circulation and reduced nerve sensitivity.

1.4.6 Ocular complications of diabetes mellitus

The retinal vasculature is one of the main sites of diabetic complications, and several countries including the UK have a routine screening program to track the progress of the characteristic diabetic retinopathy (DR) as a surrogate for diabetic control. Duration of DM becomes an increasing risk factor for developing retinopathy; type I DM poses the greatest risk with 60% prevalence at 30 years duration of DM[59]. Briefly, the retinopathy consists of two stages; non-proliferative DR (NP-DR) and proliferative DR (P-DR).

1.4.6.1 Non-proliferative diabetic retinopathy The earliest stage of NP-DR consists of initial microaneurysms which are localised out-pouchings of the capillary wall. To the observer, these appear as small dots and are the first sign that vessel permeability is compromised. This can be more pronounced in cases of retinal haemorrhages, whereby the type of haemorrhage indicates it's location within the retinal architecture. In the nerve fibre layer they appear as feathery streaks (flame haemorrhages); intra-

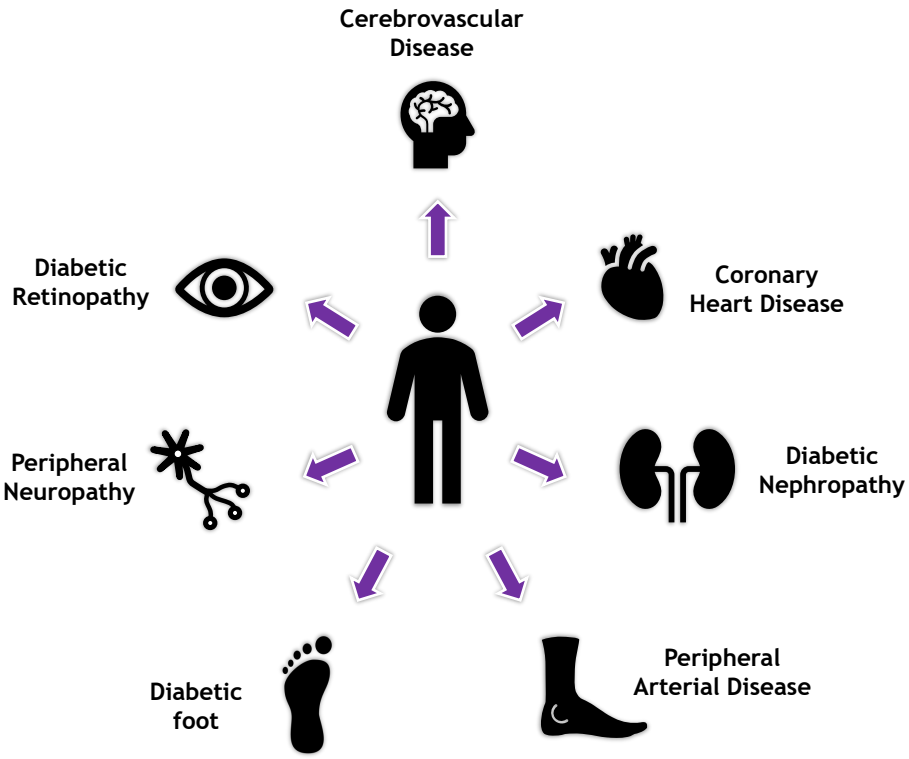

Figure 3: Systemic complications of diabetes mellitus. These occur at various vascular (and nervous) beds throughout the body with some being easier to image and monitor than others.

retinally they arise from the venous end of capillaries and are more concentrated, thus appear as more defined bleeds (dot and blot haemorrhages); and haemorrhages into the middle retinal layers signify haemorrhagic retinal infarcts and appear as darker, deeper bleeds. Waxy, yellow lesions (exudates) and neuronal debris / local infarcts (cotton-wool spots) can also be seen. All of these signs correspond to 'mild DR'. In 'moderate DR', there is an increased number of the retinopathy signs, as well as venous beading and 'generalised dilation'. This can be further classified as 'severe' and 'very severe', with the risk of the DR progressing to proliferative increasing proportionally to the amount of retinopathy present. A subtle dilation of the arteries is also suggested to be an early signifier of ischaemic dysfunction, progressing to 'silver-wiring' as a result of narrowing in cases of significant ischaemia[59].

1.4.6.2 Proliferative diabetic retinopathy The progression of retinopathy to P-DR signifies that the retinal circulation has been compromised to such an extent that perfusion is severely impaired in at least one quarter of the retina. The ocular response to this is to produce a network of new blood vessels following the release of a stimulating hormone, vascular endothelial growth factor (VEGF). These vessels can appear at the optic nerve, elsewhere on the retina, or extend as far forward as the iris. These vessels are prone to leaking and haemorrhage and so complications such as vitreous haemorrhage are a major concern.

Thus, depending on the stage of retinopathy, the alterations in vascular calibre are different, for both the arteries and veins.

1.4.6.3 Diabetic macular oedema Another complication of diabetes and retinopathy is macular oedema (DMO). DMO occurs as a result of capillary leakage and blood-retinal-barrier breakdown within the macular region, although the detailed pathophysiology remains unclear[60]. It can also occur at any point within the standard DR progression, and is assessed and graded separately to DR as a result[61]. Clinically, this accumulation of sub-retinal fluid disrupts vision, resulting in it being the major cause of vision loss among diabetics[60]. Whilst arterial compliance (i.e. rigidity) was found to be reduced in cases of DMO, there was no significant difference in measured vessel diameter[62]. DMO incidence is reportedly variable depending on type and onset of DM; highest in late-onset, insulin-dependent (25.4% over 10 years) and lowest amongst those who were late-onset, insulin-independent (13.9%)[63].

1.5 Overview: Stroke

Stroke occurs when there is a disruption to the blood supply to the cerebral tissue, arising through either blockage and reduction of cerebral blood flow (ischaemic stroke) or rupture of a cerebral blood vessel due to localised blockage and subsequent build-up of pressure (haemorrhagic stroke) (*see Figure 4*). This can occur at any time and it is estimated that every 40 seconds, someone in the United States suffers a stroke. Of the 795,000 stroke cases annually, 610,000 are first-time incidents[64]. Unfortunately, only half of those occur in hospital; highlighting that early detection of risk could prove vital in curtailing such statistics[4]. There can be a number of causes for a stroke incident, and there are various systems for classification; including the Lausanne Stroke Registry (LSR), Oxfordshire Community Stroke Project (OSCP) and Trial of Org 10172[1] in Acute Stroke Treatment (TOAST). Each classification system (with their own advantages and short-comings) makes use of a variety of diagnostic tests as well as clinical findings. LSR, whilst being the first classification system proposed, features a large number of stroke sub-types (7), increasing the chances of a patient falling into multiple categories[65]. Opting to focus more on the clinical assessment of a stroke victim, the OSCP classification system has the advantage of being applicable in the earliest stages of stroke, before such investigations as MRI and CT scanning have been performed. Whilst inter-observer reliability has only been found to be moderate, the system lends itself well to a clinical (rather than research) setting[66].

1.6 The Cardiovascular System: gross anatomy

The heart serves as a pump, sending blood throughout the body via a network of vessels. Arteries carry a quarter of the total blood volume away from the heart under high pressure, considered a 'pressure reservoir'. The blood then passes through a network of capillaries at target organs, whereby there is a transfer of nutrients and waste products. Blood is then returned to the heart, under less pressure (and three quarters of the total blood volume; a 'volume reservoir') in the veins[34]. To reflect these different roles, arteries and veins have different anatomy, which is summarised in Figure 6. Blood passes through the heart twice during circulation; once, where blood is pumped from the right ventricle to the lungs in order to acquire fresh oxygen and deposit waste carbon dioxide, before returning to the more muscular left ventricle whereby the blood is then pumped a second time into the systemic circulation[67]. The blood vessels, as they get further from the heart, decrease in diameter individually, however their combined diameter is actually increased compared to the originating aorta. This results in a net drop in pressure

[1] A low molecular weight heparin-derived medication given to stroke patients within 24 hours of stroke onset.

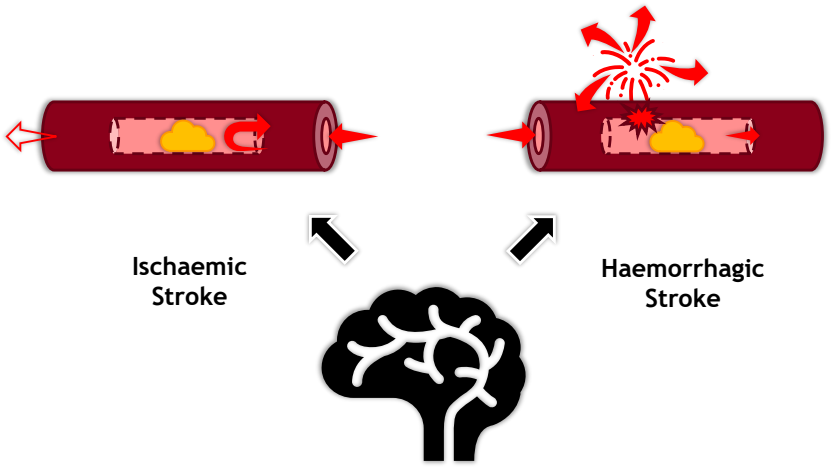

Figure 4: Types of stroke. Note that both types involve a focal reduction in vessel lumen which either prevents flow of blood (ischaemic stroke), or causes a rupture of the vessel wall (haemorrhagic stroke).

and velocity[34]. Arteries, by their nature and function, have a much more complex physiology than veins. Blood destined for the ocular circulation passes out of the heart into the aorta, which measures around 25mm in diameter[68]. In turn, it passes into the internal carotid arteries which run perpendicularly upwards through the neck to supply the head. The first branch of the internal carotid artery is the ophthalmic artery which provides blood to the eyeball, extraocular muscles and adnexa. Due to its near right-angle branching from the internal carotid artery, stenosis is highly common in the ophthalmic artery[69]. One significant branch of the ophthalmic artery (though only carrying 20% of the overall blood flow to the eye) is the central retinal artery (CRA). This pathway is summarised in Figure 5.

1.7 Vascular supply to the ocular circulation: The Central Retinal Artery

The CRA travels alongside the optic nerve before, 1.25cm posteriorly to the eyeball, penetrating the optic nerve's dura and arachnoid sheaths to travel alongside the nerve itself. The CRA, along with the exiting central retinal vein (CRV), penetrate the eyeball at the laminar cribrosa. The anatomy of the CRA and its branches is considered to be analogous to the cerebral vasculature, both through functionality and post-mortem studies[70, 71]. Functionally, both vascular beds feed high-oxygen-demand structures whilst also providing a tight barrier between the blood and its surrounding matter; the blood-retinal- and blood-brain-barriers. In cases of cerebral haemorrhage and infarction it has been shown that the

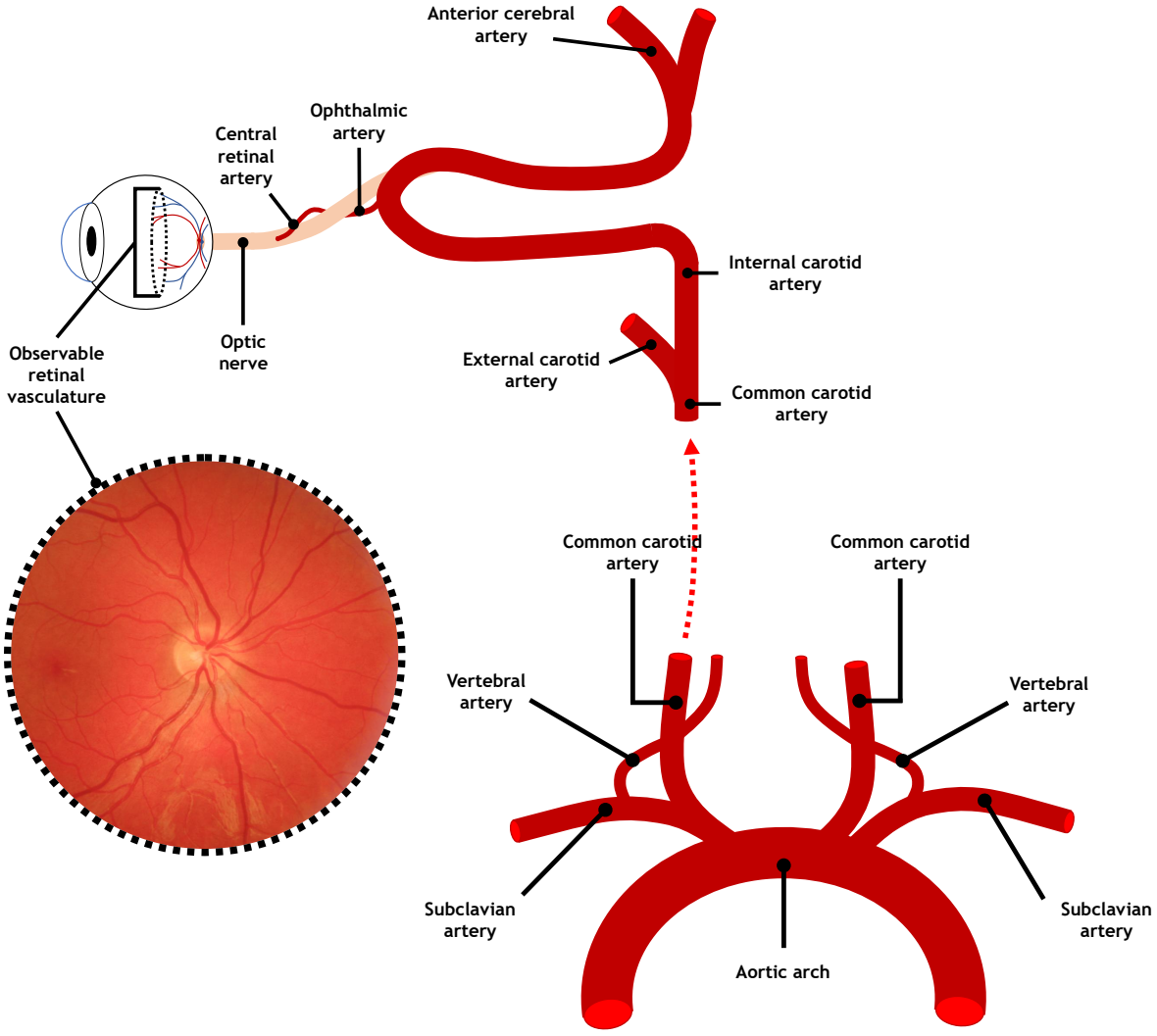

Figure 5: Origins of the ocular blood supply. Note the gradual reduction in vessel diameter from the aortic arch upwards to the ophthalmic artery and it's subsidiary, the central retinal artery. Retinal vessel architecture is analogous to cerebral vasculature, which is also supplied by the internal carotid artery.

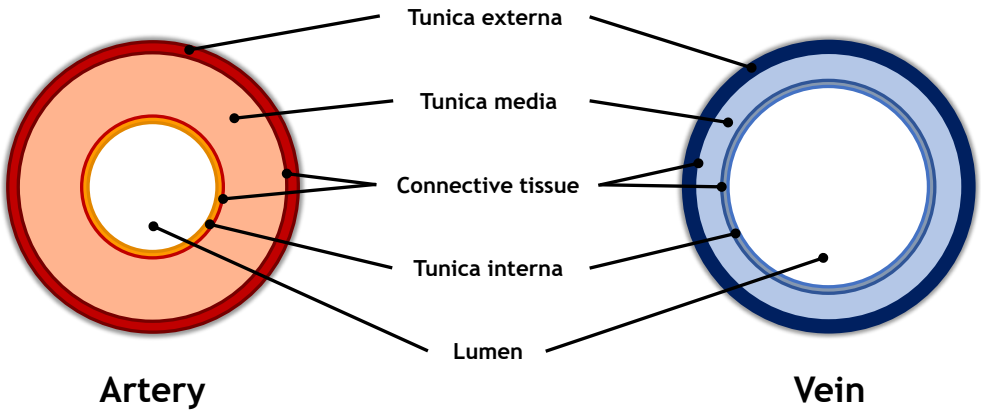

Figure 6: Blood vessel anatomy. Arteries feature a much more prominent muscular tunica media compared to veins of equivalent size. The relative lumen diameters partially explain why blood travels at higher pressure in the arteries, since the volume of blood is contained within a smaller diameter vessel. Stiffening of the muscular tunic will also cause further reduction of arterial lumen. The different architecture of the vessel types reflects it's function and is also the root of different vessel responses in pathology.

fibrous and fibro-hyalinoid thickening in the cerebral arteries occurred simultaneously in the retinal arteries[71]. The retinal circulation does differ however. Right-angle branchings, as observed on the retina, are not found in the cerebral circulation, and whilst the cerebral circulation has an abundance of collateral loops, the retinal arteries can be considered 'end-arteries' as there are no anastomoses[71, 72]. This puts the retina at greater risk of damage through occlusion. Irreparable damage is thought to occur one hour post-occlusion[73]. There is a distinction in the anatomy between the pre- and post-laminar portions of the CRA. Before the laminar cribrosa, the CRA consists of a single-layer endothelium, internal elastic laminar, media (containing layers of smooth muscle), external elastic laminar (albeit ill-defined) and a mostly collagen-based adventitia. Post-laminarly (i.e. intra-retinally) the CRA loses the elastic laminar, one argument for the subsequent retinal branches to be considered arterioles instead of arteries; and its media gets progressively thinner (due to a reduction in layers of smooth muscle). Overall, the post-laminar CRA shrinks in both diameter and wall thickness, with its wall-to-lumen ratio reducing from 1:4 to 1:10[74]. Although the wall naturally thickens with age, a post-laminar increase in wall-to-lumen ratio has been shown to be indicative of increased cerebrovascular risk; further evidence to support the proposed homogeneity with the cerebral vasculature[75]. Having pierced the posterior

aspect of the globe, the CRA bifurcates into a superior and inferior branch, before further dividing to serve the temporal and nasal aspects of each hemisphere. In the peripapillary region, the CRA has a lumen of approximately 100μm and a wall of 15μm (by contrast, pre-laminar adult CRAs have a lumen of approximately 200μm and a wall of 32μm, thickening with age by up to 5μm)[74]. The CRA branches progressively lose their artery-like structure as they reach the peripheral retina. Smooth muscle layers are reduced from 5-7 at the optic disc to 1-2 layers in the periphery, making them more arteriolar in nature. The designation of whether the retinal vessels are truly 'arterial' or 'arteriolar' is not definitive[74, 33]. It is agreed that the CRA itself and the initial bifurcations close to the disc can be considered arteries. This is supported by pathologic studies, since the retinal findings associated with cerebral changes were restricted to the peripapillary region only[71]. Vessels more proximal to the ora serrata correlated much less. It could therefore be argued that, rather than a clear divide, there is a progressive transition from artery to arteriole. This is of particular importance when considering the action of pathologies upon the retinal vasculature; changes detected in the peripapillary region may not be reflective of the outer reaches of the retina. The ora serrata itself is avascular, and the peripheral retina approaching it has a much lower metabolic demand; thus less blood is required which results in a reduced calibre[76]. Given that the CRA contains smooth muscle, it is at risk of systemic pathologies affecting muscular arteries, such as giant cell arteritis (GCA)[74]. The CRA is also accompanied by a sympathetic nerve plexus as it approaches the eye, but does not enter it. This is a contrast to the choroidal blood supply (which supplies the outer aspect of the retina). Sympathetically, the CRA is innervated by post-ganglionic nerves originating in the superior cervical ganglion[73]. Once the CRA passes the lamina cribrosa and loses it's innervation, it becomes reliant upon autoregulation. Autoregulation ensures that within an optimum perfusion pressure range flow remains constant[77]. As a result, retinal blood flow has been shown to remain constant despite changes to mean blood pressure of up to 41% above baseline[78]. Oxygen levels in the bloodstream are also affected by autoregulation, since both retinal arteries and veins have been shown to constrict under hyperbaric conditions[79]. The precise mechanisms behind autoregulation are unclear, however evidence suggests that there is input from various hormones (such as nitric oxide, prostaglandins, endothelins and lactate) as well as metabolic feedback from the retina[19]. The arteries themselves occupy the inner half of the retina, so supply of nutrients does not extend to the nuclei of the photoreceptors (a task fulfilled by the choroidal vasculature)[69]. Retinal arteries are known to have an increased reflex, the arteriolar light reflex, and this is a sign that has been used in the quantification of pathological changes such as hypertensive retinopathy. The actual underlying physiological explanation

for it's appearance has been disputed; historically being attributed to sclerotic changes within the vessel wall[33, 80]. Contemporary theories have suggested it occurs due to the convex and relatively slow-moving blood column, enhancing the illuminating light into a significantly visible reflex[81, 82].

1.8 Vascular drainage from the ocular circulation: The Central Retinal Vein

Blood is transferred from the arterial arm to the venous drainage aspect via a network of capillaries. These capillaries are much thinner vessels, with diameters between 3 and 6µm, and a much simpler architecture. The retinal capillaries consist of an endothelium, which maintains the integrity of the blood-retinal-barrier, intramural pericytes and a basement membrane[74]. Deoxygenated blood then passes into the venous system to be removed from the eye. Commencing posterior to the ora serrata, the retinal veins collect blood from capillary arcades, increasing towards the optic nerve head. The veins are distributed in a similar branching pattern to the arteries[73]. As venous blood volume increases towards the optic nerve, there is an increase in the wall and diameter of the vein branches, with a lumen of around 200µm prior to vein's exit through the laminar cribrosa[74]. Upon leaving the orbit, the central retinal vein (CRV) exits the optic nerve to drain into the superior ophthalmic vein. By exiting the optic nerve, the CRV travels in the subarachnoid space (as well as the CRA), meaning it is exposed to the pressure of cerebrospinal fluid (CSF)[73]. Whilst the CRA remains unaffected due to both it's substantial vessel wall and high arterial blood pressure within, the CRV is much more sensitive to changes in CSF. As intraocular pressure (IOP), CRV pressure and CSF pressure are much closer to one another, spontaneous pulsation of the CRV is often visible in healthy normal eyes[83]. Anatomically, the intra-retinal portion of the CRV consists of an endothelium, a thin basement membrane, a media (with widely spaced pericytes) and the adventitia (which accounts for two thirds of the vessel wall)[74]. Significantly, the wall lacks an internal elastic laminar and smooth muscle, rendering it much less able to respond to changes in environment. As such, the mechanisms behind changes in retinal venular calibre are much less understood[1, 19].

1.9 Subjective Retinal Vessel Calibre Assessment

Whilst it is, of course, critical to *observe* the retinal vasculature, it is of equal importance to be able to *record* and *quantify* it; particularly in the case of follow-up and long-term monitoring. Inconsistencies, inter- and intra-observer disagreement and learning effects are inherent problems of subjective methods which can largely be mitigated by an objective approach. Until the advent of digital photography, objective analysis of the retinal vasculature was a lengthy and clinically impractical process. For

instance, when Parr and Spears developed their objective formulae, photographic negatives needed to be mounted on a glass slide and viewed through an engineers' measuring projector[84]. Alternatives included a microscope and screw micrometer to measure each vessel individually. This meant there was a greater emphasis on subjective analysis, which falls into two categories; subjective *measuring* and subjective *grading*. Interestingly, subjective grading (often according to one of the earliest grading scales; the Keith-Wagener-Barker classification) still features alongside direct ophthalmoscopy in clinical management guidelines of systemic hypertension[24, 20, 23].

1.9.1 Subjective Measuring

1.9.1.1 Arterio-Venous Ratio Since a graticule cannot be physically placed within the eye to obtain a true measurement, any size measurements made subjectively within the eye need to use a landmark as reference (for instance disc diameter). Whilst the disc diameter is a good static landmark, it's size is considerably greater than the blood vessels, making it unsuitable for measuring vessel calibre. Gowers described a method for estimating the calibre of the retinal arteries as a ratio of the neighbouring veins, now commonly referred to as the arteriole-venule ratio (AVR), in 1876[31]. He stated that in an eye free from pathology the ratio was approximately two-thirds to three-quarters (or 2:3 to 3:4). The AVR is advantageous in that the objects being measured (i.e. arteries and veins) are both subject to the same distortive effects exhibited by the patient's refractive error and therefore do not require correction factors. This has even been demonstrated when examining photographs with modern objective formulae; where individual artery and vein diameters were found to require a correction factor, the ratio of the two did not[85]. It is also condenses both aspects of the circulation into a single value, allowing for quick assessment in the consulting room. Subjectivity is a major limitation of the AVR; with a lack of agreement between observers[86]. What is considered to be a 'normal' value differs between sources, and Gowers initial description of a normal AVR of "$\frac{2}{3}$ to $\frac{3}{4}$" is in itself a very broad range, although this is the most commonly-referenced value in cases of 'healthy normals'[87, 88, 31]. Both a wide range of quoted 'normative values' and reported considerable difficulties in selecting truly comparable vessels pose further issues to the technique[87]. There is an implicit assumption with the AVR that venous calibre remains unchanged with age or blood pressure, hence its utilisation as a static landmark for tracking arteriolar changes[89]. Various large-cohort studies have shown however, using more advanced methods, that changes to the retinal venular calibre do occur during hypertension and other pathologies[1, 2, 90, 91, 92]. Furthermore, patients suffering from co-morbid changes to both arms of the retinal circulatory system will confound

the AVR further. Bi-directional calibre changes (such as an artery decrease and venous increase) will result in an exaggerated change in AVR, whilst parallel changes (such as an increase in both artery and venous calibre) will result in no net change to the recorded AVR. The AVR has likely remained a staple for the optometrist due to its relatively simple premise and universal understanding. Optometrists in the United Kingdom are primary care practitioners and so can sometimes be the first medical professionals to detect a systemic pathology. There is an inference that AVR is a useful tool by its continued reference in pathologic studies. The AVR's value to the optometrist is made questionable by its marked absence from various (cardiovascular) pathology textbooks; common disease reference and anatomy handbooks, and more specialised retinal vascular publications, make no mention of the AVR[74, 93, 94, 95, 73, 96, 97]. This continues through to optometric referral criteria and clinical decision making, neither of which are influenced by AVR. Even optometric practice guidelines in the United Kingdom remain vague with regards to recording retinal vascular features such as AVR; principally, the only recommendation made by the College of Optometrists is to record the AVR for each eye and "*any unusual features*". Given it's absence from any referral or management guidelines, the clinical value of recording the AVR is debatable. Expressed as a ratio of one to another, calculating the widths of a retinal artery and it's equivalent vein is not as simple as the premise sounds. The key to an accurate AVR is the selection of vessels; particularly that they are arise from the same number of divisions (e.g. third bifurcation). The location for vessel selection varies between sources, with some optometric textbooks quoting a region between 1 and 2 disc diameters from the disc margin[93]. The problems from not having a standardised location are likely to be compounded by the fact that truly comparable vessels are not always neighbouring. They can be difficult to detect and would require intensive scrutiny of fundus photographs. Stokoe and Turner highlighted this problem and detailed no less than 11 separate problems with vessel selection (*see Figure 7*)[87].

1.9.1.2 Subjective-Objective Measuring Prior to the widespread use of fundus photography, vessel measurement by means of traditional ophthalmoscopic techniques were explored. Such devices were cumbersome and resulted in being a curiosity rather than a widely used instrument. Lobeck described an instrument which employed the optical separation technique[98]. It's mechanism is shown in Figure 8. The optic disc is imaged through a bi-prism which is adjusted until the outer aspect of one hemisphere is touching the inner aspect of the other, and the associated distance is read from a scale. The same method is then applied to the vessel in question, and it's diameter is calculated relative to the disc's diameter. As Lobeck states, this technique results in a measurement independent of refractive error. Mikuni adapted

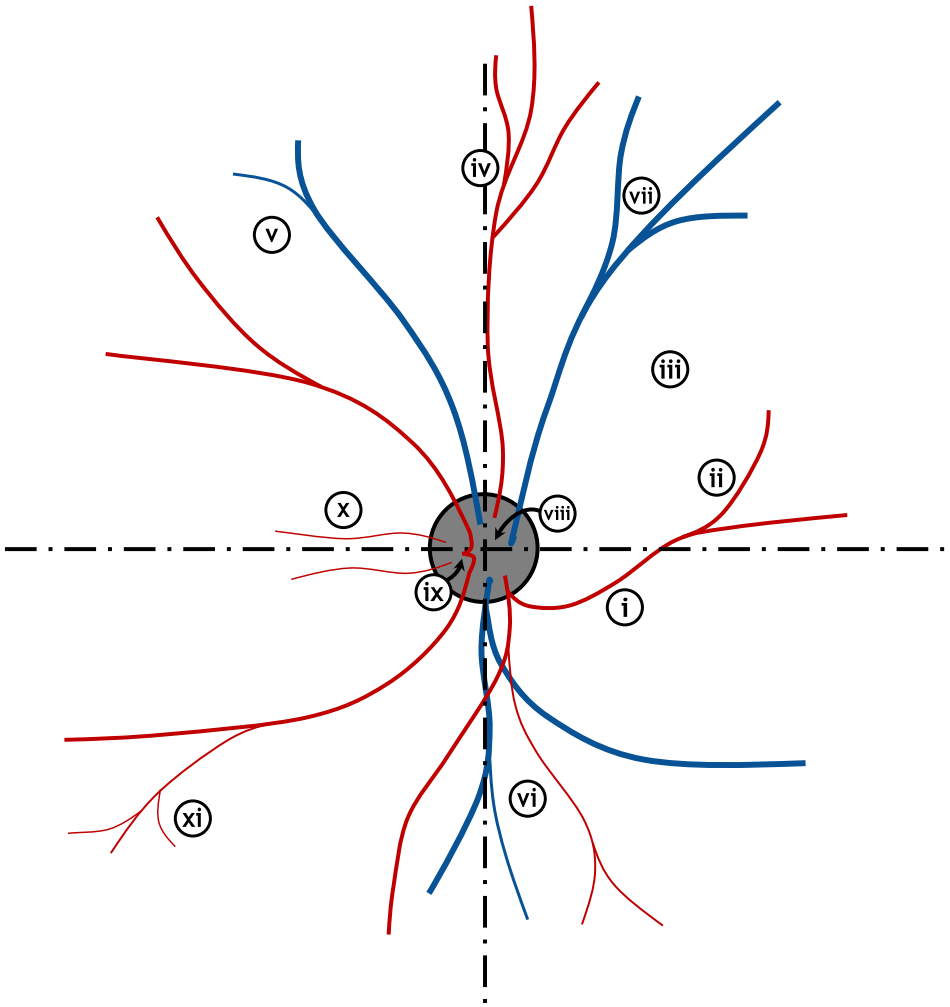

Figure 7: Issues associated with vessel selection in the subjective AVR. **i** – lower branch supplying upper hemisphere; **ii** – no vessel pair; **iii** – unequal ratio of arteries to veins; **iv** – multiple branching makes vessel selection difficult; **v** – one vessel doesn't branch evenly; **vi** – both vessels don't branch evenly; **vii** – vessel trifurcates; **viii** – central retinal artery / vein may branch posterior to optic nerve head; **ix** – superior and inferior vessel branch arise from common artery branch; **x** – unpaired arteries (*not cilio-retinal arteries*) run horizontally; **xi** – numerous small branches with questionable significance.

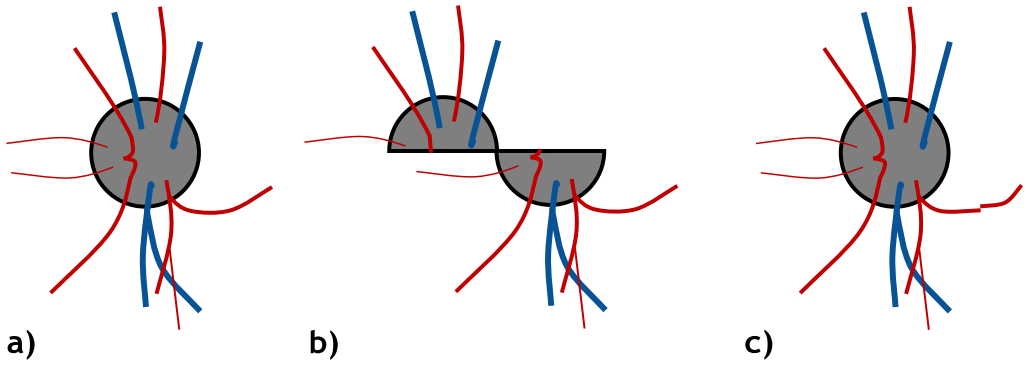

Figure 8: Lobeck's optical separation method for vessel measurement. a) shows the disc and vessel branches. b) shows the determination of the disc diameter by optical separation. c) shows the principle being applied to a vessel for a comparative calibre measurement to be made, based on the disc diameter.

this concept several years later (*see Figure 9*) to describe an ocular with built-in arrows which are adjusted to the diameter of the optic disc, and then the desired blood vessel[99]. Mikuni's instrument was found to have greater accuracy than Lobeck's, however neither instrument reached widespread usage.

1.9.2 Subjective Grading

1.9.2.1 Keith-Wagener-Barker Classification Since the blood vessels are known to change as part of the pathologic process, vessel diameter changes were integrated into broader grading scales. The work of Keith, Wagener and Barker (KWB) in their 1939 paper outlining a classification system for retinal hypertensive changes is still used today[32, 100, 101, 102]. They proposed four stages of hypertension, graded I to IV, which looks not just at a single factor (i.e. arteriolar narrowing), but the larger clinical picture for that patient. The retinal details are described in Table 5.

Given that the KWB grading system was devised at a time when hypertension was not well understood or easily treated, the spread of severity was much broader (3 year survival was 70% for Grade I but only 6% for Grade IV)[32]. This means that when applying the KWB classification today, there will be a much greater emphasis on the first two grades of the system. There are a number of other difficulties with the KWB classification. Whilst the authors acknowledge that grading numerically circumvents the use of confusing descriptive terms, the broad nature to their classification criteria has resulted in alterations

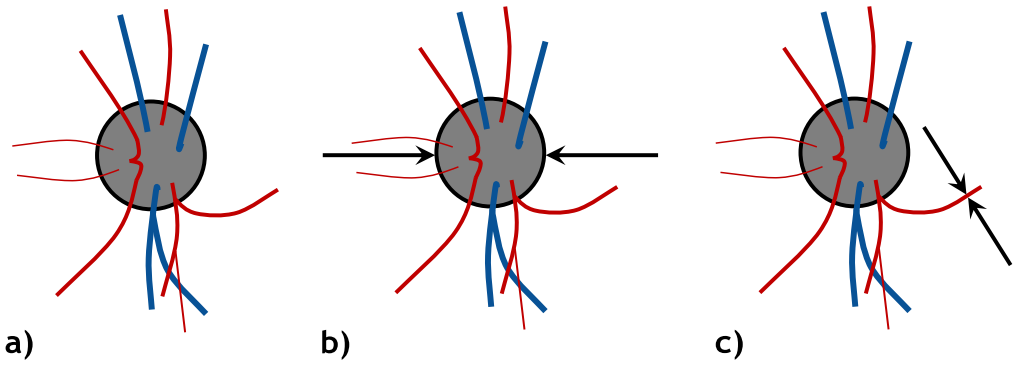

Figure 9: Mikuni's adaptation of the Lobeck ocular for vessel measurement. a) shows the disc and vessel branches. b) shows determination of the disc diameter by placing of the arrows at the disc margin. c) shows the principle being applied to a vessel for a comparative measurement to be made, based on the disc diameter.

Grade I	Mild generalised arteriolar narrowing
Grade II	Definite focal narrowing and AV nipping
Grade III	Grade II + retinal haemorrhages, cotton wool spots and exudates
Grade IV	Grade III + papilloedema

Table 5: The Keith-Wagener-Barker (KWB) classification of arterial hypertension.

by subsequent users[33, 101]. Distinguishing the subtleties between Grades I and II can be challenging for the observer[101]. Greater distinction between the mild presentations is of much more importance today given the improvements in diagnosis and treatment of hypertension. Another limitation is that the severity of retinopathy does not always correlate easily with the severity of the hypertension[101]. Indeed, the original KWB classification neglects to outline a definitive method for separating the retinal signs of Grade I and II (arguably the two most important grades of the classification today)[32, 103]. A later study with more emphasis on those first two grades used the absence (Grade I) or presence (Grade II) of arteriolar sclerosis[103]. What is important however, is that the classifications make reference to 'arteriolar narrowing' in very general terms. Whilst the AVR had been used for nearly 50 years by the time of Keith, Wagener and Barker, it does not feature in their classification. Interestingly, a potential solution

Grade I	Earliest increase in arteriolar reflex with minimal AV compression
Grade II	More marked reflex increase and AV compression
Grade III	Copper-wiring with advanced AV compression
Grade IV	Silver-wiring

Table 6: Scheie's modification to the original KWB classification.

to the problem of distinguishing between Grade I and II would undoubtedly involve measurement or quantification of the arterial calibre. This was proposed by Wagener and colleagues in 1947, where they suggested either using the AVR or comparing observed arterioles to 'normal' from memory[104]. Wagener et al. do acknowledge the principle limitation of the AVR for quantifying arteriolar narrowing; that there needs to be consideration for venular dilation, although give no remedial action besides using intuition as to whether the arteries are narrower than normal. Studies into the KWB classification and it's usefulness in modern patient management have demonstrated these drawbacks[101, 105].

1.9.2.2 Scheie Modification Scheie later refined the KWB classification in 1953[33]. The changes outlined in the original KWB classification give an indication of the severity of the hypertension, but not the duration. Scheie argued that severity of arteriolar sclerosis is of equal importance, since this serves as a reasonable index for the duration of the condition, and therefore proposed that in conjunction to the KWB grading of hypertension, the patient should also be graded on arteriolar sclerosis. Scheie stressed that since this can develop equally in mild hypertension over a number of years and acute malignant hypertension over a much shorter period, the two values together give a more accurate picture. Scheie's modification (or rather, supplementation) to the KWB classification is shown below in Table 6.

Whilst grading allows for a more consistent and comprehensive picture to be built up of the retinal status, condensing the entire retinal landscape (including its vasculature) into a single grade or number is too general, and still remains a major drawback of this technique. Retinal changes can differ between quadrants of the same retina, let alone between eyes. The classification systems are difficult to navigate, and modifications are still common, meaning that comparisons between studies is difficult.

1.9.2.3 Neubauer Classification The subjective grading landscape changed little in the following half-century, with the existing grading scales either having modifications applied or their applicability

to clinic questioned. Scales such as the one proposed by Neubauer (*see Table 7*) aimed to refine the classification initially outlined by Keith, Wagener and Barker[106]. Here, distinction is made between the vessel wall changes (*fundus hypertonicus*) and the accompanying ('classic') signs of advanced hypertensive retinopathy. Whilst this refinement would pose a cumbersome and time-consuming tool if applied routinely in clinic, it does highlight two key points; firstly that different vessel types respond differently to vascular pathology, and secondly that a more structured and systematic approach for examination of those vessels (particularly splitting the vessel tree into arteries and veins) is required.

1.9.2.4 Dodson et al. Prognosis-based Classification Dodson and colleagues proposed a simple, clinic-focussed grading system which was driven by overall prognosis[107]. Here the authors argue that a simple scale is all that is required for clinical requirements; the ultimate focus being on the individual's prognosis. Their justification for the division between grades being that BP is universally correlated with arterial narrowing, whilst the subsequent signs (including haemorrhages and cotton-wool spots) are more indicative of advanced BP-related damage.

1.9.2.5 Mitchell and Wong Simplified Classification Whilst the refinement proposed by Neubauer resulted in a more detailed and comprehensive classification (with resulting impracticalities for clinic), Mitchell and Wong's refinement of the KWB classification (*see Table 9*) aims to distil the grading down to more general grouping but with a greater emphasis on the cross-over with systemic cardiovascular health[108]. Whilst it lacks the specificity required to track subtle changes between visits, it offers an approach with greater inter- and intra-grader reproducibility than it's KWB forerunner[101]. Interestingly, this study also highlighted (albeit with single observers) that an optometrist had a greater level of agreement with the gold standard grader than the retina specialist ophthalmologist (kappa 0.784 vs. 0.683 respectively). As with all grading scales, the intended outcome dictates it's suitability; the requirements for individual one-off grading differ from long-term monitoring; the latter requiring a much greater level of resolution to detect minor changes which may have little effect on broad classifications.

Grade I	} ***fundus hypertonicus*** **(mild)**
Grade II	
Grade III	} ***hypertensive retinopathy*** **(severe)**
Grade IV	

Stage I	
Optic disc	*Normal; mild hyperaemia*
Major retinal vessels	*Normal; barely detectable light reflex changes*
Arterioles	*Barely detectable narrowing*
Precapillaries	*Normal*
Capillaries	*Visible on optic disc*
Venules	*Mild tortuosity (eventually)*
Veins	*Mild enlargement (eventually)*
Parenchyma	*Normal*
Stage II	
Optic disc	*Normal, mild hyperaemia*
Major retinal vessels	*Obvious increased light reflex changes; obvious narrowing of vessel wall (focal irregularities)*
Arterioles	*Obvious increased light reflex; obvious narrowing of vessel wall (focal / continuous irregularities)*
Precapillaries	*Narrowed; irregular light reflexes*
Capillaries	*Few peripapillary loops*
Venules	*Mild tortuosity*
Veins	*Mild enlargement (eventually)*
Parenchyma	*Mild haemorrhage; few cotton-wool spots (eventually)*
Stage III	
Optic disc	*Often unclear margins; slightly prominent (eventually)*
Major retinal vessels	*Mild / severe narrowing and focal constriction; irregular light reflexes*
Arterioles	*Moderate / severe narrowing; irregular light reflexes*
Precapillaries	*Narrowed; visible alterations of vessel walls*
Capillaries	*Ectatic (dilated / distended)*
Venules	*Mild tortuosity*
Veins	*Mild enlargement (eventually)*
Parenchyma	*Focal / grouped haemorrhage; cotton-wool spots; (pre-)thrombosis; capillary bleeding; hard (circinate) exudates*
Stage IV	
	Stage III + *papilloedema; peripapillary exudates; exudative retinal detachment (eventually)*

Table 7: Neubauer's modification to the Keith-Wagener-Barker classification. Note that each stage is sub-categorised by vessel type affected.

Grade	Retinal Signs	Hypertension	Prognosis
A 'non-malignant'	Generalise arteriolar narrowing Focal narrowing (*not nipping*)	Established	Dependent on level of BP; age and other cardiovascular risk factors important
B 'malignant'	Haemorrhages, hard exudates, cotton-wool spots ± papilloedema	Accelerated / malignant (*with bilateral retino-vascular damage*)	Untreated: 2-year mortality Treated: average survival >12 years

Table 8: Dodson et al.'s prognosis-based classification.

	Retinal Signs	Systemic Associations
None	No detectable signs	None
Mild	Generalised / focal arteriolar narrowing; AV nicking; copper wiring	Modest association with risk of (sub-)clinical stroke; coronary heart disease; death
Moderate	Haemorrhage (dot, blot, flame); microaneurysm; cotton-wool spot	Strong association with (sub)clinical stroke; cognitive decline; CVD-related death
Malignant	*Moderate* + papilloedema	Strong association with death

Table 9: Mitchell and Wong's simplified classification.

1.10 Objective Retinal Vessel Calibre Formulae

There have been many attempts to develop an objective method for recording vessel calibre since the the invention of the ophthalmoscope[35]. Technological constraints were the main limitation and with the advent of digital image analysis, there was a surge of literature highlighting a number of ways to measure vessel calibre accurately[1, 35, 109, 110, 111]. The wide variety in vascular patterns with no common landmark also hindered progress. As well as highlighting the difficulties in suitable vessel selection for the AVR, Stokoe and Turner also showed the huge variety in vessel distribution amongst humans[87]. The central retinal artery branches differently in people, and whilst it has been argued that the overall blood volume in the retina is the same for people[112], the way in which the vessels are distributed is not so consistent[74, 73]. This relationship is analogous to the appearance of trees. Whilst the arrangement of branches can differ significantly, the one biological constant true for all trees is that they originate from a single trunk; this is also true for the central retinal artery (and vein); *see Figure 10*). Since the

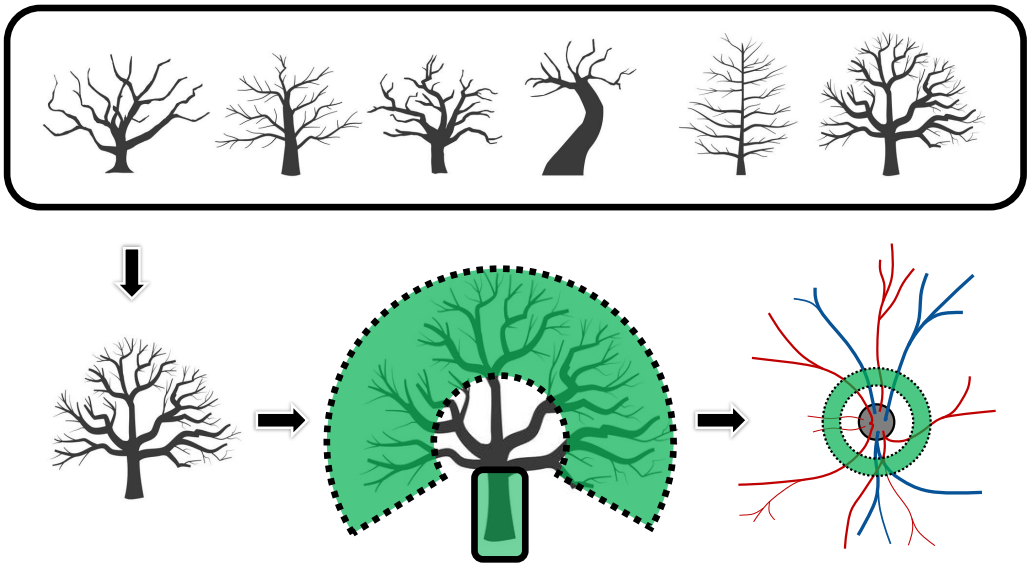

Figure 10: The variable branching pattern of trees is analogous to the retinal vasculature. Note the varying appearance of the trees (*top*), but the commonality of a single trunk. The key to objective retinal vessel analysis lies in taking measurements of the visible branches (*dashed lines*) and calculating the dimensions of the parent trunk (or central retinal artery/vein) (*solid line*).

single-most constant feature of human retinal vasculature are these single parent vessels (central retinal artery and vein), the logical solution for a population-wide measurement should focus on these vessels (rather than their highly variable daughter vessels). The difficulty in this approach is the obscuration of these vessels, since they invariably lie at, or posterior to, the lamina cribrosa and so aren't not easily viewed with standard diagnostic imaging equipment. The solution to deriving an objective formula lies in exploring the relationship between the visible branches of the central retinal artery and vein (as seen ophthalmoscopically) and the dimensions of their parent vessels. An effective objective method therefore has two requirements; detailed, cost-effective image capture (i.e. digital fundus photography) and a standardised routine for vessel calibre measurement (i.e. objective formulae).

1.10.1 Parr & Spears

A breakthrough in objective formulae resulted from the work of Parr and Spears in the early 1970's[84, 112]. They used the basic relationship between the width of a parent trunk and it's branches in what is termed the branching coefficient, where W is the width of the parent trunk, w^1 the wider branch width and w^2

the narrower branch width.

$$Branching\ coefficient = \frac{w_1^2 + w_2^2}{W^2}$$

The main issue with this calculation for the retinal vessels is that the central retinal artery (CRA) is often obscured from view on ophthalmoscopy or fundus photography since it bifurcates posteriorly to the laminar cribrosa. This means that the true width of the CRA (W) has to be estimated instead ($\hat{W}$). Parr and Spears discovered that there was an inverse relationship between the branch vessel width asymmetry and the branching coefficient. They established a new relationship between $\hat{W}$, w_1 and w_2 (with the use of several constants), in the form of a new equation[84]:

$$\hat{W} = \sqrt{0.87\,w_1^2 + 1.01\,w_2^2 - 0.22\,w_1 w_2 - 10.76}$$

The equation is used for each arterial branch of the CRA until the values are condensed down into a single value; the central retinal artery equivalent (CRAE). Parr and Spears suggested that the vessel width measurements should be taken at the point closest to the disc margin where the vessels were a) sharply in focus and b) level with the retinal plane. As such, the objectivity of their formula is undermined by the lack of standard location for measurement, as well as vessel inclusion and exclusion criteria; particularly a minimum vessel width and number of vessels to be included in the formula.

1.10.2 Hubbard et al.

Hubbard et al. used the Parr-Spears equation in the Atherosclerosis Risk in Communities (ARIC) study and made some improvements to the technique, as a way of addressing the shortcomings described above[113]. Firstly they defined a specific region from which the measurements would be taken; an annulus between ½ and 1 disc diameter away from the optic disc margin (*see Figure 11*). Because of the laborious nature in selecting the correct vessels for the original Parr-Spears equation, Hubbard proposed that the widest and narrowest arteries within the annulus be selected, regardless of whether they were associated branch pairs. In the paper, an equivalent formula is also suggested for calculating the central retinal vein equivalent (CRVE), thus giving two formulae:

$$(CRAE)\ A_{\hat{W}} = \sqrt{0.87\,w_1^2 + 1.01\,w_2^2 - 0.22\,w_1 w_2 - 10.76}$$

$$(CRVE)\ V_{\hat{W}} = \sqrt{0.72\,w_1^2 + 0.91\,w_2^2 + 450.05}$$

The generated CRAE and CRVE, as well as giving information specific to each arm of the retinal circulation, can then be combined to produce an objective AVR (O-AVR). Whilst this analysis cannot be performed as readily as an ophthalmoscopic examination, it clearly circumvents the major pitfalls of the original subjective technique. Knudtson and colleagues reported that a drawback of the CRAE and CRVE equations is that they allow for a variable number of vessels to be analysed[114]. The summary values generated are likely to be affected by a higher number of vessels used in the calculation. Given that the distribution of vessels is so variable between persons (as shown by Stokoe and Turner[87]), this is clearly a source for error. Hubbard's equations also rely heavily upon the inclusion of constants which means that the equivalents calculated will be dependent upon the inputted unit (e.g. μm, pixels etc.). The solution to this lay in rewriting the equations with a simplified approach.

1.10.3 Knudtson et al.

Knudtson et al. proposed to simply restrict the measurements to the six largest arteries and veins that fell within the measurement annulus. Knudtson also suggested taking the branching coefficients calculated from the same dataset that Hubbard used to develop his formula and inserting these into the original branching coefficient equation. These produced the refined equations:

$$A_{\hat{W}} = 0.88 \times \sqrt{w_1^2 + w_2^2}$$

$$V_{\hat{W}} = 0.95 \times \sqrt{w_1^2 + w_2^2}$$

These latest two equations mean that CRAE and CRVE can be determined with any given inputted units, plus the results are less susceptible to physiological differences in vascular tree distributions. When compared, Knudtson et al. acknowledge that these new formulae result in statistically different calibres from those derived through Parr-Hubbard formulae (CRAE was found to decrease on average by -19.7μm whilst CRVE increased by +12.6μm[114]), and AVR was reported as being more in line with what is 'expected' at 0.69 (instead of 0.85 as calculated with Parr-Hubbard). A further study has demonstrated

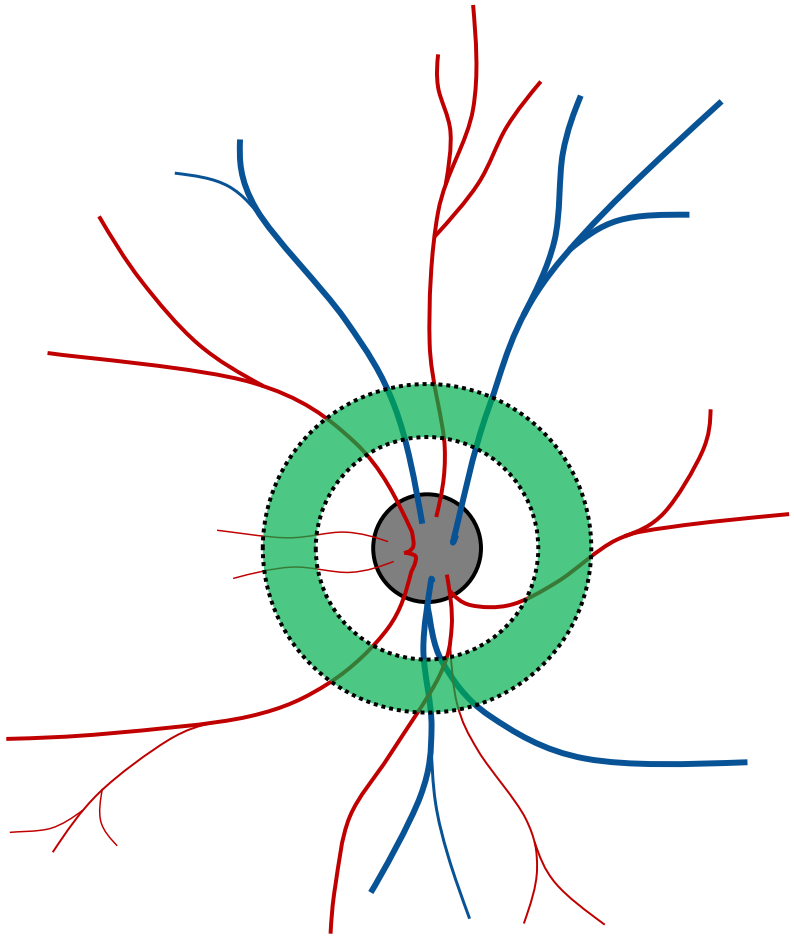

Figure 11: Measurement zone for objective vessel measurement. (½ - 1 disc diameter)

that whilst absolute summary values for arteries and veins may be different, calculated AVR remains relatively unchanged provided an equal number of vessels are selected (the authors noted a similar disparity in AVR depending on formulae used)[115]. AVR has been reported elsewhere as being in the region of 0.73-0.76 in healthy, non-hypertensive subjects using a different objective approach[116]. Since no single set of formulae has been adopted as a 'gold standard' good practice would suggest recording which formulae have been applied to derive objective measurements.

1.10.4 Future formulae

Since the objective formulae have been adapted already following Hubbard's first iteration of them in 1999, it shows that the theory behind the technique is still undergoing evolution. As such, new or refined formulae are to be expected in the coming years. Researchers from the Singapore Eye Study have assessed how an extended zone (0.5-2.0 disc diameters) gives similar results to the original ARIC annulus[117]. The authors state that since a larger section of the vessel is being sampled, the overall measurement has a greater representation of vascular calibre (although it's uptake in research has yet to occur). Adjustments to the formulae themselves has also taken place. The arterial formula has already been adapted further to account for asymmetrical vessel branching[118]. The authors suggest using a linear regression model to better account for asymmetrical branching in the arteries, whereas Knudtson's formulae was developed using an average branching coefficient. This suggests reworking the formula individually for each patient, which is a time-consuming process when applied practically. Another consideration for this approach is that vascular branching can change as a result of pathology (i.e. fractal dimensions), so would impact this method[119]. The authors state that there was no such association with the venous branching, which when their separate morphologies are considered, suggests that the formulae may have to be evolved independently.

1.11 Study Hypotheses and Aims

Optometrists are in a unique position to routinely screen and monitor their patients for signs of both local and systemic pathologies; examples of the latter often being able to manifest themselves with little or no symptoms. At present, the NHS runs a 'health check' scheme based with local General Practitioners (GPs). Running for those aged between 40 and 74, the scheme is designed to detect early signs of stroke, kidney disease, heart disease, type II DM and dementia, and is eligible to those without a pre-existing condition every 5 years. The test comprises of general health and family history questions,

a blood test, and measurements of height, weight and blood pressure. The scheme is designed to allow earlier intervention or management through lifestyle changes. All persons meeting the criteria are automatically invited to attend these checks, although it is also possible to request a check[120]. In addition to this 5-yearly scheme, regular examination by other healthcare practitioners (including optometrists) has the potential to detect earlier signs of pathology and manage accordingly; particularly given the increased frequency of appointments (generally every 1 - 2 years). In line with clinical guidelines laid out by the College of Optometrists, an appropriate history and symptoms will include details surrounding any existing cardiovascular disease and/or risk factors such as family history and lifestyle (including smoking status, alcohol intake etc.,). Also, an adequate examination of the ocular structures will include observation and interpretation of the retinal vasculature (albeit with subjective means). As more optometric practices become equipped with fundus cameras and Optical Coherence Tomographers (OCTs), and more emphasis is placed upon inter-professional co-operation of patient welfare, added to the overburdened healthcare system and spiralling incidence rates of (co-morbid) cardiovascular disease, it is hypothesised that an attempt should be made to move away from subjective grading of the retinal vasculature by optometrists. This would be in favour of an objective assessment, producing values which can be integrated with other CVD metrics such as blood pressure and risk calculation in an attempt to improve sensitivity and specificity of cardiovascular disease detection. Since a standardised objective approach of retinal vessel analysis exists in the research community, it will be evaluated whether this method can make the transition into clinical practice. This thesis will ask the following questions, whilst making continued reference to three leading forms of CVD; hypertension, diabetes mellitus and cardiovascular disease risk / stroke:

1. What are the current associations between objective retinal vessel calibre and CVD reported in the literature?

2. What is the protocol for using objective retinal vessel analysis in clinical practice? How repeatable is it?

3. Are the same cross-sectional associations found in a clinical practice as in the literature?

4. How effective/useful is objective retinal vessel analysis in long-term / inter-eye examination period follow-up of patients?

5. Can objective retinal vessel analysis be used in multi-disciplinary patient healthcare?

2 Associations of Retinal Vessel Calibre with Cardiovascular Disease: A Systematic Literature Review

2.1 Introduction

Since the ARIC study first published the use of objective vessel formulae, there has been a wealth of literature produced on the subject[113]. The use of the Parr-Hubbard, and latterly Knudtson, formulae has all but become the gold standard in measuring retinal vessel calibre, and it is this source which will be used in the subsequent practice-based study. Since objective analysis has not been used in UK optometric practice, and there is no research documenting its use, it would be difficult to determine a positive result from using the technology with no real benchmark in practice. Thus, to best demonstrate the efficacy of objective analysis in an optometric practice, the results obtained in practice will be compared to those established in the scientific literature. This requires a systematic literature review examining the findings of large, population-based studies investigating retinal vascular calibre and three key cardiovascular pathologies; diabetes mellitus, hypertension and stroke, asking the specific question: how do retinal vessel calibres correlate with these conditions? It can then be demonstrated whether results obtained in 'sub-optimal' conditions (i.e. routine optometric practice) reflect those found in a controlled research setting. The literature review, therefore, is key to establishing the benchmarks in static retinal vessel analysis.

2.2 Methods

A systematic search was performed using the academic journal search engines PubMed, ScienceDirect and Google Scholar for publications dating between December 1999 and August 2019. The searches aimed to locate papers which investigated associations between retinal vessel calibre and cardiovascular disease. The electronic search produced initial results which were then evaluated manually to discover those which met the inclusion criteria. Search terms included; "*retina**", "*vasc**", "*calib**", "*vessel*", "*diamet**", "*microvasc**", "*hypertens**", "*blood pressure*", "*diabetes*", "*diabetic*", "*type 1*", "*type 2*", "*stroke*", "*cerebrovasc**" and "*cardiovascular*".

2.2.1 Inclusion criteria

Only large cohort studies were included, and their methodology had to include retinal photography, either on (digitised) photographic negatives or digital images. Since there are no specific criteria that stipulate when a cohort study can be considered 'large', an arbitrary defining value of n = >100 was set for inclusion. Only papers citing the objective Parr-Hubbard formulae or the Knudtson refinements were included. Since Hubbard et al. published their formulae in December 1999, this provided the earliest time frame for the literature search. Papers which used either formulae but only presented data in the form of AVR were still included (though any inferences to artery calibre changes were noted). Only papers reporting associations with blood pressure (BP) / HT, DM and stroke were included as were papers looking at a variety of cardiovascular diseases or pathologies, but still had specific results for any of these three conditions. Papers located through references within sources used were also included.

2.2.2 Exclusion criteria

Papers referencing further modifications or refinements of the Parr-Hubbard/Knudtson formulae were discounted since there is not currently widespread uptake; any potential co-founding effects on results are, as yet, undetermined. Papers referencing retinal vessel calibre but pre-dating the work of Hubbard et al. were discounted for the same reason. Individual case studies were not included. Similarly, cohort studies with a sample size smaller than 100 persons were also discounted. Results from meta-analyses were not included.

2.2.3 Study Selection & Data Processing

Study selection and data processing was undertaken according to the Preferred Reporting Items for Systematic reviews and Meta-Analyses (PRISMA) Statement[121]. Initial search terms generated a high volume of results, which required refinement and filtering. This was initially performed based upon article title. In cases were the title alone did not indicate whether vascular calibre was included in the methodology, a review of the abstract took place. For those remaining papers, a review of the full-text was then performed to identify whether the methodology was correct, and published results were comparable within the present review. Each paper was summarised on a pro-forma for consistent data extraction.

2.3 Results

Following a systematic review of the journal search engines and applying the above criteria, a total of 48 papers were included in the subsequent review (the selection process is summarised in Figure 12). Manual exclusion of papers (n = 21) was principally due to non-standardised methods being applied to quantify retinal vessel calibre (such as length-to-diameter ratios, subjective grading of arteriolar narrowing or modified Parr-Hubbard / Knudtson formulae), but also included insufficient methodological information (analysis technique, imaging details) and small sample sizes (i.e. <100). Five studies using pooled data or meta-analyses were also excluded. The main details for each of the studies, including country of origin, date and at what stage the ophthalmological focus was incorporated, are summarised in Table 10. Associations between each of the three cardiovascular conditions are also tabulated below; for hypertension (Tables 11 and 12), diabetes mellitus (Tables 13, 14, 15 and 16) and stroke (Tables 17 and 18). The results for DM were categorised into sub-groups. Firstly, showing the association between retinal vessel calibre and incident diabetes / impaired fasting glucose (Table 13). Secondly, the association between retinal vessel calibre in cases of existing diabetes mellitus and progression of diabetic retinopathy (Table 14); and finally the association between retinal vessel calibre and systemic diabetic complications in cases of existing diabetes mellitus (Tables 15 and 16).

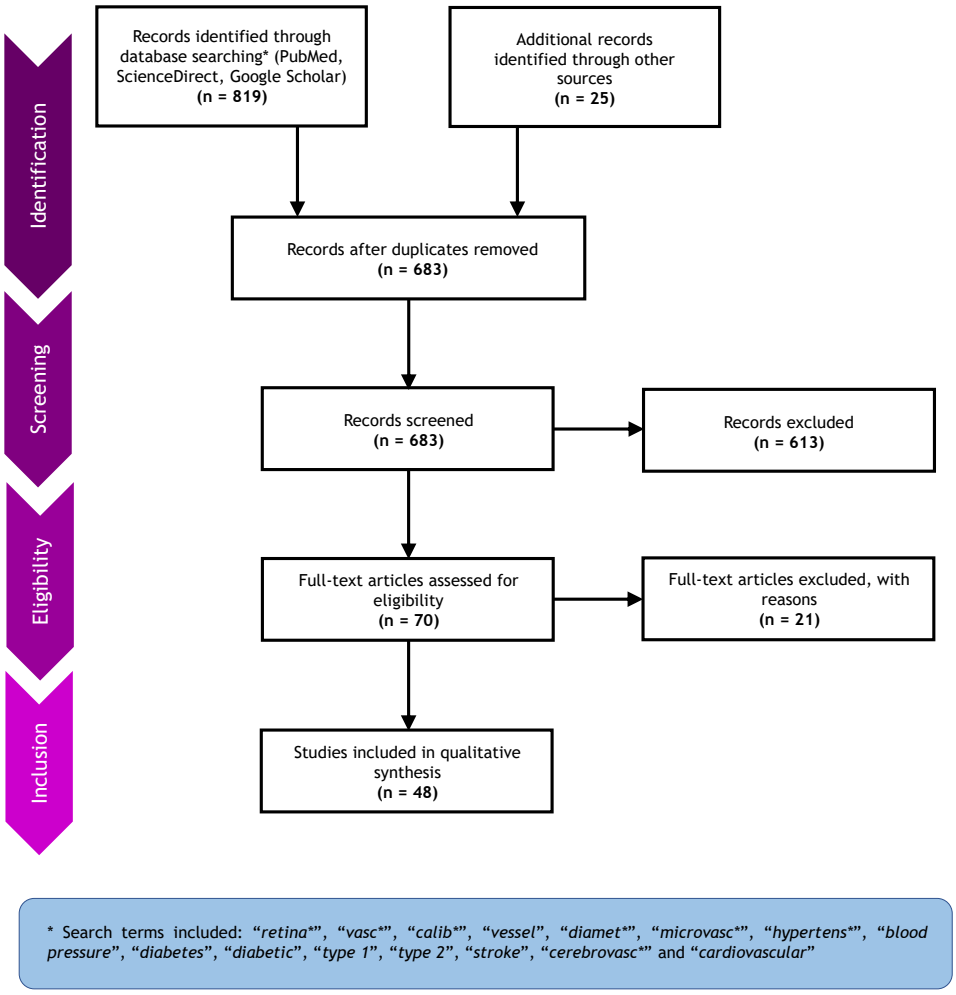

Figure 12: PRISMA summary of papers identified for literature review and inclusion process.

Study	Country	Baseline visit (Baseline Ophthalmological aspect)	Review	Ophthalmological focus
ARIC	United States	1987-89 (1993-95)	3 years (1990-92); 6 years (1993-95); 9 years (1996-98); 14 years (2011-13)	Integrated
AusDiab	Australia	1999-2000	5 years (2004-05); 12 years (2011-12)	From baseline
BDES	United States	1988-90	5 years (1993-1995); 10 years (1998-2000); 15 years (2003-2005); 20 years (2008-2010)	Principal focus
BMES	Australia	1992-94	5 years (1997-99); 10 years (2002-04)	Principal focus
CHS	United States	1988-89 (1997-98)	Annually; 'Major' components at 10 years (1999)	Integrated
DCPD1987	Denmark	1987-89 (1995)	1995; 2011	Integrated
DMP	Australia	2009-10	[*Cross-sectional*]	From baseline
FLEMENGHO	Belgium	1985-90 (2008-12)	Continual (invitation for specific tests)	Integrated
Funagata	Japan	1990-92 (2000-02)	5 years (2007-09)	Integrated
Fyn County	Denmark	1975 (Original study); 1981-82 (2007)	Invitation for specific tests	Integrated
MCRS	Australia / Singapore	2005-07	[*Cross-sectional*]	From baseline
MEAP	Singapore	2005-06	[*Cross-sectional*]	From baseline
MESA	United States	2000-02 (2002-04)	2 years (2002-04); 4 years (2004-05); 5 years (2005-07); 10 years (2010-11)	Integrated
New Jersey 725	United States	1993-97	6 years (1999-2003)	From baseline
PEDCS	United States	1986-88	Biennially [*no end date specified*]	From baseline
Rotterdam	Netherlands	1990-93	3-4 yearly reviews [*on-going*]	From baseline
SiMES	Singapore	2004-06	6 years (2011-13; SiMES-2)	Principal focus
Thessaloniki	Greece	2014	[*Cross-sectional*]	From baseline
WESDR	United States	1980-82	4-yearly (1984-86); (1990-92); (1995-96); (2000-01); (2006-07); (2012-14)	Principal focus
Westmead	Australia	1990-94	5 years (1995-98); 9 years (1999-2002)	From baseline

Table 10: Summary of population-based studies included in review

Study	Year	Type	Subjects	Formula	CRAE†	CRVE†	AVR†	†*Defined as*:
ARIC[113]	1999	Long.	9,040	PH	↓	Slight ↓[a]	↓	AVR -0.02 per +10mmHg MABP. CRAE and CRVE presented graphically
BDES	2003[122]	Cross.	4,247	PH	↓	–	↓	CRAE -4.9µm per +10mmHg MABP
	2004[123]	Long.	2,451	K	–	–	↓	Lowest quartile AVR (0.50-0.67) vs. highest (0.76-1.04) = OR 1.82 10-year incident HT
BMES	2003[124]	Cross	3,614	PH	↓	–	↓	Normotensive CRAE 189µm; Untreated HT CRAE 183µm
	2004[29]	Long.	1,269	PH	↓	–	↓	Normotensive CRAE 198.6µm; Incident severe HT CRAE 192.0µm
CHS	2002[125]	Long.	2,405	PH	↓	–	↓	OR 'GAN' per current 10mmHg BP (syst.) 1.11; per past 10mmHg BP (syst.-diast.) 1.15-1.30
	2003[126]	Cross.	2,050	PH	↓	–	↓	OR 'GAN' 1.07 with HT (compared to normotensive)
	2006[127]	Cross.	1,888	PH	↓	–	↓	Sig. difference between 1st and 4th quartile of CRAE (*unspecified*)
FLEMENGHO[128]	2014	Long.	514	K	↓	–	↓	CRAE -2.87µm per +15.1mmHg BP (syst)
...								

Table 11: Summary of findings linking blood pressure and retinal vessel calibre. **ARIC** = Atherosclerosis Risk in Communities study; **BDES** = Beaver Dam Eye Study; **BMES** = Blue Mountains Eye Study; **CHS** = Cardiovascular Health Study; **FLEMENGHO** = Flemish Study on Environmental, Genetics and Health Outcome study. '**Long.**' = longitudinal study. '**Cross.**' = cross-sectional study. '**PH**' = Parr-Hubbard; '**K**' = Knudtson. '**CRAE**' = central retinal artery equivalent; '**CRVE**' central retinal vein equivalent; '**AVR**' = arteriovenous ratio. '↓' denotes a statistically significant (p=≤0.05) decrease. '**MABP**' = Mean Arterial Blood Pressure. '**BP**' = blood pressure. '**HT**' = hypertension. '**OR**' = Odds ratio. '**GAN**' = Generalised Arterial Narrowing. '**syst**.' = systolic. '**diast.**' = diastolic. ([a]- approx. 4µm ↓ in CRVE over 45mmHg in ↑ MABP)

Study	Year	Type	Subjects	Formula	CRAE†	CRVE†	AVR†	†*Defined as*:
...								
Funagata	2006[129]	Cross.	921	PH	↓	–	↓	CRAE -2.8µm per +10mmHg BP AVR -0.01 per +10mmHg BP
	2010[130]	Long.	581	K	↓	–	↓	Per CRAE SD↓ (*unspecified*) = OR 1.53 of HT
MESA[131]	2006	Cross	5,979	K	↓	–	↓	CRAE -1.27µm per +10mmHg BP (syst)
Rotterdam	2004[132]	Cross.	5,674	K; L	↓	Slight ↓[b]	↓	CRAE/AVR -1.1µm/-0.0035 per +10mmHg BP (syst.); -2.1µm/-0.008 per +10mmHg BP (diast.)
	2006[133]	Long.	1,900	K; L	↓	–	↓	BP (syst./diast.) +1.1/+0.6mmHg per -1SD CRAE (*unspecified*)
SiMES[134]	2008	Cross.	3,019	K	↓	–	↓	CRAE -2.86µm per +10mmHg MABP
Thessaloniki[135]	2014	Cross	223	PH	↓	–	–	CRAE↓ -4.97 & AVR↓ -0.04 (Dx'd HT vs. undx'd)

Table 12: Summary of findings linking blood pressure and retinal vessel calibre cont[d]. **Funagata** = The Funagata Study; **MESA** = Multi-Ethnic Study of Atherosclerosis; **Rotterdam** = The Rotterdam Study; **SiMES** = The Sinagpore-Malay Eye Study. '**Long.**' = longitudinal study. '**Cross.**' = cross-sectional study. '**K**' = Knudtson, '**PH**' = Parr-Hubbard, '**L**' = Littmann. '**CRAE**' = central retinal artery equivalent; '**CRVE**' central retinal vein equivalent; '**AVR**' = arteriovenous ratio. '↓' denotes a statistically significant (p=≤0.05) decrease. '**BP**' = blood pressure. '**syst**.' = systolic. '**diast.**' = diastolic. '**SD**' = standard deviation. '**MABP**' = Mean Arterial Blood Pressure. '**Dx'd**' = diagnosed. 'Undx'd' = undiagnosed. ([b]- approx. 0.5µm ↓ in CRVE per +10mmHg MABP, became non-significant when stratified by age.)

Study	Year	Type	Subjects	Formula	CRAE†	CRVE†	AVR†	†*Defined as*:
ARIC[136]	2002	Long.	7,993	PH	**(↓)**	–	↓	Per SD↓ AVR (-0.08) = OR 26%↑ risk DM (Implying a reduction in CRAE)
AusDiab[137]	2008	Long.*	803	K	↓	–	–	Lowest tertile CRAE (≤167µm) vs. highest (≥188µm) = OR 2.21 incident DM
BDES[138]	2005	Long.*	3,251	K	↓	–	↓	Per SD↓ CRAE (unspecified) = OR 11%↑ risk DM; Per SD↓ AVR (0.07) = OR 16%↑ risk DM
BMES[90]	2008	Long.*	2,123	PH; B	–	↑	–	Per SD↑ CRVE (+20µm) = OR 1.59 risk IFG <70 years old; OR 1.47 risk IFG >70 years old
MESA[139]	2012	Long.	4,955	K	↓	–	–	Lowest tertile CRAE (≤139µm) vs. highest (≥150µm) = HR 1.60 incident DM [Caucasians only]
Rotterdam[140]	2006	Long.*	2,309	K; L	–	↑	↓	Per SD↑ CRVE (+19.9µm) = OR 13%↑ risk IFG; Per SD↓ AVR (-0.06) = OR 29%↑ risk IFG; 19%↑ DM

Table 13: Summary of findings linking incident diabetes/impaired fasting glucose and retinal vessel calibre. **ARIC** = Atherosclerosis Risk in Communities study; **AusDiab** = The Australian Diabetes, Obesity and Lifestyle Study; **BDES** = Beaver Dam Eye Study; **BMES** = Blue Mountains Eye Study; **MESA** = Multi-Ethnic Study of Atherosclerosis; **Rotterdam** = The Rotterdam Study. '**Long.**' = longitudinal study. '*' denotes follow-up ≥5 years. '**PH**' = Parr-Hubbard; '**K**' = Knudtson; '**B**' = Bengtsson; '**L**' = Littmann. '**CRAE**' = central retinal artery equivalent; '**CRVE**' central retinal vein equivalent; '**AVR**' = arteriovenous ratio. '↓' denotes a statistically significant (p=≤0.05) decrease. '↑' denotes a statistically significant (p=≤0.05) increase. '**SD**' = standard deviation. '**OR**' = Odds ratio. '**HR**' = Hazard ratio. '**DM**' = diabetes mellitus. '**IFG**' = impaired fasting glucose.

Study	Year	Type	Subjects	Formula	CRAE†	CRVE†	AVR†	†*Defined as*:
AusDiab[141]	2007	Cross.	1,998	K	(↑)	–	-	Per SD↑ CRAE = OR 1.64 of DR (*non-significant when correct for CRVE*)
BMES[41]	2008	Cross.	2,992	PH	↑	↑	–	↑CRAE +15.2µm non-DR *vs.* minimal NP-DR; ↑CRVE +40.8µm non-DR *vs.* severe NP-DR
MESA[142]	2008	Cross.	5,976	K	–	↑	–	↑CRVE +8.0µm non-DR *vs.* early severe DR
SiMES[143]	2017	Long.	427	K	↑	–	–	Per SD↑ CRAE (+11.4µm) RR 1.67 (referable DR); RR 2.22 (VTDR)
WESDR	2003[43]	Cross.	996	K	↓	↑	↓	Per DR level ↑ = ↓CRAE (-3.23µm); ↑CRVE (+1.07µm); ↓AVR (0.017)
	2004[42]	Long.	996	PH	↑	↑	↓	Highest quartile CRAE (≥235.6µm) *vs.* lowest (≤208.1µm) = RR 2.04 progression of DR; Highest quartile CRVE (≥260.7µm) *vs.* lowest (≤233.1µm) = RR 2.33 progression of DR
Westmead[40]	2008	Long.	645	K	↑	–	–	Per SD↑ CRAE (+18.90µm) = HR 1.46 of DR (2.33 when adjust for renal function)

Table 14: Summary of findings linking existing diabetes mellitus, and retinal vessel calibre and diabetic retinopathy status. **AusDiab** = The Australian Diabetes, Obesity and Lifestyle Study; **BMES** = Blue Mountains Eye Study; **MESA** = Multi-Ethnic Study of Atherosclerosis; **SiMES** = The Singapore-Malay Eye Study; **WESDR** = The Wisconsin Epidemiologic Study of Diabetic Retinopathy. **Westmead** = Westmead Children's Hospital, Australia. '**Cross**.' = cross-sectional study. '**Long.**' = longitudinal study. '**K**' = Knudtson; '**PH**' = Parr-Hubbard.'**CRAE**' = central retinal artery equivalent; '**CRVE**' central retinal vein equivalent; '**AVR**' = arteriovenous ratio. '↑' denotes a statistically significant (p=≤0.05) increase. '↓' denotes a statistically significant (p=≤0.05) decrease. '**SD**' = standard deviation. '**OR**' = Odds ratio. '**DR**' = Diabetic Retinopathy. '**NP-DR**' = Non-proliferative Diabetic Retinopathy. '**P-DR**' = Proliferative Diabetic Retinopathy. '**VTDR**' = vision-threatening diabetic retinopathy. '**RR**' = Risk ratio. '**HR**' = Hazard ratio.

Study	Year	Type	Subjects	Formula	CRAE†	CRVE†	AVR†	†*Defined as*:
DCPD1897[144]	2014	Long.	185	K	↓	↑	–	16-year incidence per 10µm↓ CRAE OR 2.96 (peripheral neuropathy); 2.63 (nephropathy) 16-year incidence per 10µm↑ CRVE OR 1.52 (peripheral neuropathy); 1.76 (nephropathy)
DMP[145]	2013	Cross.	289	K	↓	↑	–	Per SD↓ CRAE (*unspecified*) = OR 1.66 erectile dysfunction Per SD↑ CRVE (*unspecified*) = OR 1.58 erectile dysfunction
Fyn[146]	2009	Cross.	188	K	↓	–	↓	Per SD↓ CRAE (-17.4µm) = OR 2.17/3.17 nephropathy/macrovascular disease; Per SD↓ AVR (-0.07) = OR 1.48/1.79 nephropathy/macrovascular disease
MEAP[147]	2009	Cross.	3,748	K	↑	↑	–	Per SD↑ CRAE (*unspecified*) = OR 1.18 DM (1.30 for Indians); Per SD↑ CRVE (*unspecified*) = OR 1.15 DM (1.33 for Indians)
MESA[142]	2008	Cross.	5,976	K	↑	↑	–	CRAE in whites DM *vs.* non-DM = 144.3µm *vs.* 141.8µm; CRVE in Hispanics/Chinese DM *vs.* non-DM = 221.8µm *vs.* 218.9µm/221.0µm *vs.* 214.8µm

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Table 15: Summary of findings linking existing diabetes mellitus, retinal vessel calibre and systemic diabetic complications. **DCPD1987** = Danish Cohort of Pediatric Diabetes 1987; **DMP** = Diabetes Management Project; **Fyn** = Fyn County, Denmark; **MEAP** = Multi-Ethnic Asia Population (*combined data from Singapore Prospective Study Program and Singapore Cardiovascular Cohort Study 2*); **MESA** = Multi-Ethnic Study of Atherosclerosis; '**Cross**.' = cross-sectional study. '**Long.**' = longitudinal study. '**K**' = Knudtson; '**PH**' = Parr-Hubbard.'**CRAE**' = central retinal artery equivalent; '**CRVE**' central retinal vein equivalent; '**AVR**' = arteriovenous ratio. '↓' denotes a statistically significant (p=≤0.05) decrease. '↑' denotes a statistically significant (p=≤0.05) increase. '**SD**' = standard deviation. '**OR**' = Odds ratio. '**DM**' = Diabetes mellitus.

Study	Year	Type	Subjects	Formula	CRAE†	CRVE†	AVR†	†*Defined as*:
...								
New Jersey 725[148]	2012	Long.	468	K	↓	↑	–	6-year incidence per SD↓ CRAE = OR 1.60 (CVD†; -15.0µm), OR 1.94 (LEAD†; -14.4µm), OR 1.33 (all-cause mortality‡; -16.3µm) 6-year incidence per SD↑ CRVE = OR 1.41 (incident HT†; +30.3µm), OR 1.37 (all-cause mortality‡; +31.3µm)
PEDCS[149]	2009	Cross.	448	K	↓	–	–	Per SD↓ CRAE (*unspecified*) = HR 1.42 CAD (♀ = 1.92; ♂ = 1.13)
WESDR	2003[43]	Cross.	996	K	↓	–	↓	Presence of proteinuria = -8.0µm CRAE; -0.021 AVR
	2004[150]	Long.	557	PH	–	↑	–	Per SD↑ (*unspecified*) CRVE = RR 1.23/1.22 incident gross proteinuria/renal insufficiency
	2007[151]	Long.	533	K	(↓)	↑	–	$\frac{1}{4}$*vs.*$\frac{2-4}{4}$CRAE (*unspecified*) HR 2.20 14-year lower limb amputation^; 1.18 22-year mortality^; 1.47 22-year stroke mortality^ $\frac{4}{4}$*vs.*$\frac{1-3}{4}$CRVE (*unspecified*) HR 2.08 10-year proteinuria; 1.72 22-year stroke mortality

Table 16: Summary of findings linking existing diabetes mellitus, retinal vessel calibre and systemic diabetic complications cont^{d}. **New Jersey 725** = New Jersey 725 Study; **PEDCS** = Pittsburgh Epidemiology of Diabetes Complications Study; **WESDR** = The Wisconsin Epidemiologic Study of Diabetic Retinopathy. '**Cross**.' = cross-sectional study. '**Long.**' = longitudinal study. '**K**' = Knudtson; '**PH**' = Parr-Hubbard.'**CRAE**' = central retinal artery equivalent; '**CRVE**' central retinal vein equivalent; '**AVR**' = arteriovenous ratio. '↓' denotes a statistically significant (p=≤0.05) decrease. '↑' denotes a statistically significant (p=≤0.05) increase. '**SD**' = standard deviation. '**OR**' = Odds ratio. '**CVD**' = Cardiovascular Disease. '**LEAD**' = Lower Extremity Arterial Disease. '**DM**' = Diabetes mellitus. '**HR**' = Hazard ratio. '**CAD**' = coronary artery disease. '♀' = Female; '♂' = Male. '**RR**' = Risk ratio. '†' = corrected for diabetic retinopathy severity. '‡' = corrected for mean arterial blood pressure. '^' = significance lost when corrected for level of diabetic retinopathy.

Study	Year	Type	Subjects	Formula	CRAE†	CRVE†	AVR†	†*Defined as*:
ARIC	2001[92]	Long.	10,358	PH	–	–	↓	Lowest $\frac{1}{5}$ *vs.* highest $\frac{1}{5}$ (*unspecified*) AVR = RR 1.24 incident stroke
	2002[152]	Long.	1,684	PH	**(↓)**	–	–	GAN (lowest 20th percentile) = RR 7.0 incident stroke (with presence of WML and GAN)
	2005[153]	Cross.	1,684	PH	–	–	↓	Lowest $\frac{1}{5}$ *vs.* highest $\frac{1}{5}$ (*unspecified*) AVR = OR 1.74 incident stroke (3.74 with HT; 0.64 without HT)
BDES[154]	2003	Long.	1,611	PH	**(↓)**	–	↓	GAN = OR 1.5 cardiovascular-death, including stroke (OR 2.7 without HT/DM)
BMES[155]	2005	Long.	3,654	K	–	–	**n.s.↓**	*Non-linear increasing trend between GAN and RR of incident stroke*
CHS	2003[126]	Long.	2,050	PH	–	–	**n.s.↓**	*Non-significant association between GAN and incident stroke (p=0.698) - OR = 1.10*
	2006[156]	Cross.	1,717	PH	↓	–	↓	Per SD↓ CRAE (-19.3µm) = OR 1.17 5-year incident cerebral infarct; 1.17 5-year worsening WMG Per SD↓ AVR (-0.084) = OR 1.18 prevalent cerebral infarct; 1.26 5-year incident infarct
	2006[127]	Long.	1,992	PH	–	↑	**n.s.↓**	$\frac{4}{4}$ *vs.* $\frac{1}{4}$ CRVE (*unspecified*) = RaRo 2.2 5-year incident stroke $\frac{1}{4}$ *vs.* $\frac{4}{4}$ *AVR (unspecified) = RaRo 1.7 5-year incident stroke (p=0.06)*

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Table 17: Summary of findings linking stroke and retinal vessel calibre. **ARIC** = Atherosclerosis Risk in Communities study; **BDES** = Beaver Dam Eye Study; **BMES** = Blue Mountains Eye Study; **CHS** = Cardiovascular Health Study. '**Cross**.' = cross-sectional study. '**Long.**' = longitudinal study. '**PH**' = Parr-Hubbard; '**K**' = Knudtson. '**CRAE**' = central retinal artery equivalent; '**CRVE**' central retinal vein equivalent; '**AVR**' = arteriovenous ratio. '↓' denotes a statistically significant (p=≤0.05) decrease. '↑' denotes a statistically significant (p=≤0.05) increase. '**n.s.**' = Non-significant. '**RR**' = Risk ratio. '**OR**' = Odds ratio. '**HT**' = Hypertension. '**GAN**' = Generalised Arterial Narrowing (given as lowest $\frac{1}{5}$ AVR). '**WML**' = white matter lesion. '**DM**' = Diabetes mellitus. '**SD**' = standard deviation. '**WMG**' = White Matter Grade. '**RaRo**' = Rate ratio.

Study	Year	Type	Subjects	Formula	CRAE†	CRVE†	AVR†	†*Defined as*:
...								
MCRS[157]	2009	Cross.	1,321	K	–	↑	↓	CRVE in lacunar stroke *vs*. other stroke types = 214.8µm *vs*. 208.8µm; AVR in lacunar stroke *vs*. other stroke types = 0.62 *vs*. 0.68
Rotterdam	2006[158]	Long.	5,540	K; L	–	↑	↓	Per SD↑ CRVE (+20.9µm) = HR 1.13/1.15 incident stroke/cerebral infarct Per SD↓ AVR (-0.06) = HR 1.17 incident cerebral infarct
	2010[159]	Long.	5,518	K: L	↓	↑	–	Per SD↓ CRAE (-14.4µm) = HR 1.12/1.25/1.15 all strokes/intracerebral haemorrhage/anti-coagulant medication related haemorrhage Per SD↑ CRVE (+14.4µm) = HR 1.20/1.53/2.48 all strokes/intracerebral haemorrhage/anti-coagulant related haemorrhage
	2019[160]	Long.	3,219	K:L	↑	↑	–	Uppermost tertile CRAE (163-212µm): aHR 2.04 (lower global brain perfusion and TIA) Uppermost tertile CRVE (247-336µm): aHR 2.30 (lower global brain perfusion and TIA)
WESDR[161]	2004	Long.	871	PH	–	–	**n.s.**	*AVR only quoted in analysis. No association found.*

Table 18: Summary of findings linking stroke and retinal vessel calibre cont[d]. **MCRS** = Multi-Centre Retina & Stroke Study. **Rotterdam** = The Rotterdam Study. **WESDR** = The Wisconsin Epidemiologic Study of Diabetic Retinopathy. '**Long.**' = longitudinal study. '**K**' = Knudtson; '**L**' = Littmann; '**PH**' = Parr-Hubbard. '**CRAE**' = central retinal artery equivalent; '**CRVE**' central retinal vein equivalent; '**AVR**' = arteriovenous ratio. '↑' denotes a statistically significant (p=≤0.05) increase. '↓' denotes a statistically significant (p=≤0.05) decrease. '**n.s.**' = Non-significant. '**SD**' = standard deviation. '**HR**' = Hazard ratio. '**aHR**' = Adjusted hazard ratio. '**TIA**' = Transient ischaemic attack.

2.4 Discussion

2.4.1 Association of blood pressure and retinal vessel calibre

Retinal arterial narrowing with increasing BP has been well documented over the past 150 years, and is arguably the best-understood relationship between retinal blood vessels and a pathology. Prior to objective assessment, methods for evaluating the retinal blood vessels employed varying degrees of subjectivity, yet still managed to report significant reductions in arterial calibre in cases of HT. This inverse relationship is also clearly demonstrated with the objective formulae. A strong inverse relationship between BP and CRAE is shown, with no effects seen with CRVE, in Tables 11 and 12. In this particular instance, the inverse relationship between BP and CRAE could explain the similar finding with decreased AVR as Gowers first proposed; however the AVR does not make explicit whether there has been a narrowing of the retinal arteries or a dilation of the retinal veins[31]. To demonstrate that the inverse relationship exists across ethnicities, data from several studies around the world were combined into a meta-analysis[162] . Although each study confirmed the association individually, the meta-analysis showed that this finding is independent of ethnicity, reporting an overall CRAE decrease of 3.07µm per 10mmHg increase in mean arterial BP. As a general rule, direct comparisons between studies is difficult for three reasons. Firstly, different methodologies were employed across the studies. Some groups opted for dilated, stereoscopic photographs (Beaver Dam Eye Study (BDES), Blue Mountains Eye Study (BMES) and Rotterdam Study (Rotterdam)), dilated fundus photographs (Singapore–Malay Eye Study (SiMES)), whereas others used dark-adapted, non-mydriatic fundus photography (ARIC, Cardiovascular Health Study (CHS), Flemish Study on Environment, Genes and Health Outcomes (FLEMENGHO), Funagata Study (Funagata), the Multi-Ethnic Study of Atherosclerosis (MESA) and Thessaloniki studies). The effect of illumination on the definition of the vessel profiles through these different approaches, and how it may affect subsequent measurements, has not been investigated. Similarly, BP was measured differently across the studies. ARIC and MESA, both studies with a focus on CVD, took three readings and averaged the final two (and also the Thessaloniki study)[135, 131, 113]; BDES, CHS, FLEMENGHO, Rotterdam and SiMES all took two readings with a 5-minute interval [154, 125, 128, 132, 134], whereas BMES and Funagata both took a single BP reading [124, 129]. The fewer readings taken increases the chances that individual subjects may be misclassified, particularly in the cases of single BP readings. Subsequent vessel analysis also differed between reports. There is equal preference between the studies using both Parr–Hubbard (ARIC, BDES, BMES, CHS, Funagata and Thessaloniki) and Knudtson (BDES, FLEMENGHO, Funagata, MESA, Rotterdam and SiMES) formulae. The Rotterdam study added Littmann's correction factor into

their analysis – thus their findings are corrected for magnification errors – whereas the remaining studies did not. Correction formulae are aimed at mitigating the magnification errors that can arise from including a variety of refractive errors in the dataset. The two correction formulae featured in the literature reviewed here are the Bengtsson and Littmann formulae. Both formulae were developed for use with specific camera models, as camera optics differ between manufacturers and models. As explained in Chapter 3.1.2 (Correction factors), telecentric cameras have an extended depth of field, meaning that objects at various distances are equally focused on the photographic plane. As such, perspective of depth is lost. This can be achieved by a number of optical arrangements within a camera system, hence why the formulae are specific for a certain camera type. Incorrect application of the formulae (for instance, when a different imaging system is used) has the potential to attenuate otherwise significant findings. Littmann's formula was written specifically for a Zeiss Oberkochen camera. The stereo camera used by the Rotterdam study, although not explicitly named, was not the same camera used by Littmann, thus the constants within the formula will potentially be meaningless. Also, whilst the majority of refractive details required for the formula were collected in the methodology, recording of axial length, a key aspect of ocular magnification and requirement for the Littmann formula, is not mentioned[163, 164]. The second consideration to be made when comparing the studies directly is the different environments and times in which the studies were undertaken. As shown in Tables 11 and 12, the investigations took place across the globe across a 20-year period, so external factors such as treatment and diagnosis approaches, as well as genetic predispositions to CVD, must be considered. A final point is the variety of endpoints reported. There is a clear association between increasing BP and decreasing CRAE; however, the extent to which these two are linked varies across studies. Those which use a decreased AVR as a sign of generalised arteriolar narrowing are of particular concern since, as explained previously, an AVR decrease does not specify whether the change in vessel calibre has arisen from the arteries or veins. Given the increasing evidence documenting changes in venular calibre, this is particularly problematic[1]. Perhaps of most use to the optometrist are the findings reported by the authors of the reports for BMES, FLEMENGHO, Funagata, MESA, Rotterdam and SiMES. Here, reductions in CRAE in relation to a fixed increase in BP (10mmHg) are given (and shown in Table 19). This is of particular interest since the definition of a 'normal' or 'pathologic' vessel calibre is practically impossible given the wide range of variables to be considered (age, gender, ethnicity, co-morbidity, etc.). Despite no 'normative range' existing, the results from the Thessaloniki study should be approached with caution since they differ hugely from those quoted in other studies, possibly suggesting an error in either methodology or reporting (mean

Study	CRAE change [μm] (per +10mmHg BP)
BDES	-4.90
FLEMENGHO	-1.90
Funagata	-2.80
MESA	-1.27
Rotterdam	-1.10
SiMES	-2.86
*Meta-analysis**	-3.07

Table 19: Summary of CRAE changes relative to a +10mmHg increase in blood pressure. (**Meta-analysis combining data from BMES, Funagata, MESA and Rotterdam studies*)

non-hypertensive CRAE and CRVE are given as 91.44μm and 118.88μm respectively)[135]. Difficulty in defining a 'normal' vessel calibre is similar to there being no single 'normal' cup-to-disc ratio when examining the optic nerve head. Similarly to cup-to-disc ratio, vessel diameter changes for an individual between examinations are far more clinically relevant. In addition, such a value would carry greater clinical significance with the patient's BP readings from each examination.

2.4.2 Association of diabetes mellitus and retinal vessel calibre

Diabetes mellitus has several subcategories, each characterised by abnormal or total absence of insulin function, resulting in a range of signs and symptoms. The classification of DM based on blood serum glucose levels is widely accepted (*see Table 4*)[36], although the literature quotes DM status as either present or absent, rather than on a continuous scale of blood serum glucose, such as with BP. As such, correlations with DM and retinal vascular calibre are generally quoted in probability scores such as odds ratio (OR), hazard ratio (HR) and risk ratio (RR). Such ratios are important to clinicians assessing the retinal vasculature. Since retinal vessel calibre could be used to aid diagnosis and management in patients as well as ongoing monitoring of existing conditions, knowing the likelihood of a particular pathology being associated with a calibre change is very useful. The associations between retinal vascular calibre and DM can be broadly split into two categories. The first category encompasses the link between retinal vessel calibre and the future development of DM (or incident DM), whereas the secondly category includes retinal vessel calibre, existing DM and the complications thereof (including DR, nephropathy, neuropathy and other cardiovascular events). The exact physiological processes behind diabetic vascular changes are not fully understood and often contradictory, and this is reflected by an inconsistency

in the findings of the included literature[1].

2.4.2.1 Incident diabetes The less well-defined correlation between retinal vascular calibre and DM extends to its development (or diabetogenesis). Both arms of the circulation appear to be implicated, which further demonstrates the importance of considering artery and vein calibres separately. It has previously been demonstrated that, with increased levels of glycaemia, vasoregulatory responses are diminished, and as such an increase in vascular calibre would be expected in cases of incipient DM [165]. Despite this, the ARIC study reports a reduction in CRAE with incident DM [136]. However, since ARIC's findings are inferred through a significant reduction in AVR, and venous calibre changes have since been partially associated with DM development, the findings should be taken with caution. Perhaps of significance is the fact that venous calibre changes only became significant when measurements were corrected for refractive error (BMES and Rotterdam) [140, 90]. Whilst the BMES study used the telecentric Zeiss FF3 camera (for which the Bengtsson correction formulae were developed) in their methodology, the camera used in the Rotterdam study, as explained previously, was potentially not the Zeiss Oberkochen telecentric camera to which the Littmann formula exclusively applies.

The time course of diabetogenesis must also be considered when evaluating the literature. All studies including incident DM used only the baseline photograph for calibre measurements, despite some studies (BDES and BMES) taking photographs at each visit. The conclusions drawn from a single data point when remarking on a change over time (i.e. the development of DM) carry limited weight, since causality cannot be proven. The significant calibre associations with incident DM may reflect true, prediabetic changes in the vascular architecture, or they may simply signify a natural predisposition to the condition. This is especially true of those studies which had a follow-up greater than 5 years (Australian Diabetes, Obesity and Lifestyle Study (AusDiab), BDES, BMES and Rotterdam [137, 138, 90, 140]), where the time span between baseline retinal photography and diagnosis of DM is greater. By excluding potentially confounding results; ARIC for only inferring a reduction in CRAE, and Rotterdam for potentially misapplying a correction formula; three papers remain citing a reduction in CRAE as being indicative of incident DM (AusDiab, BDES, MESA), with only one showing an increase in CRVE (BMES). The findings of MESA were only significant in the Caucasian population, and since the cohorts for the other studies were also predominantly Caucasian, the possibility of an ethnic influence cannot be discounted[139]. Disagreement between studies demonstrates both the need for consistency in methodology (whether or not to apply a correction formula) and further study into the longitudinal monitoring of cohorts for individual changes. This would have the potential to answer the cause-or-effect question with regard to

where retinal vessel calibre changes fall within the process of diabetogenesis.

Detection of incident diabetes by the optometrist is difficult without blood samples, and the findings from studies are not as unequivocal as with BP. Here, an awareness of risk factors and family history becomes very important in conjunction with a thorough evaluation of the retina; not just the vasculature. Understanding that vessel changes for incident diabetes predominantly involve arterial narrowing aids differential diagnosis, particularly in cases where BP may remain unchanged from previous examinations. Importantly, though, venular changes should not be ignored, and patients should be monitored and referred accordingly (particularly if they are symptomatic).

2.4.2.2 Existing diabetes mellitus and diabetic retinopathy status The non-linearity between retinal vessel calibre and DM is less pronounced when connected with DR status. Of the six studies included, five reported an increase in CRVE with worsening DR status, with none reporting a contradictory decrease. CRAE is less clear cut, with four studies reporting an increase in calibre, and a decrease only in one. Interestingly, it is the Wisconsin Epidemiological Study of Diabetic Retinopathy (WESDR) study which reports both a decrease (cross-sectionally[43]) and increase (longitudinally[42]) in CRAE with increasing RR of DR progression; where the methodology and cohort remain the same. It is therefore reasonable to suggest that vessels are changing throughout the course of DM, although in this particular case the WESDR authors fail to quote individual long-term changes in CRVE and worsening DR and so a single time point for a patient will yield different results when compared to a full time course. For example it has been shown that, in acute instances of induced hyperglycaemia where retinal arterial calibre remains static, venular calibre decreases [166]. Whilst this only demonstrates short-term changes in vascular architecture, these may contribute to long-term effects. From the data included it can be suggested that CRVE increases are correlated with worsening DR, and CRAE increases are also a likely sign of this. The follow-up study for SiMES (SiMES-2) reported a similar increase in CRAE when subjects were stratified by DR category. For those considered to have vision-threatening DR (VTDR), CRAE increases per SD (+11.4µm) were associated with a Risk Ratio of 2.22%; referable DR a Risk Ratio of 1.67% and statistically insignificant correlations below that[143]. No such correlations were observed for CRVE amongst the same cohort. Changes seen in DM and DR are numerically larger and thus more pronounced than in HT. BMES state an increase in CRVE of 40.8µm between no DR and severe non-proliferative DR [41]. Such values are of greater significance since the ability to detect these clinically is much more feasible, whilst others, such as WESDR, which reported an increase in CRVE of 1.07µm per increasing level of DR, are so small that such an ability to detect these vessel changes clinically is arguably impossible[43].

Whilst such values may shed light upon the underlying physiology of diabetic progression, their use in informing clinical decision making is likely to be much less significant.

Interestingly, the Westmead Children's Hospital (Westmead) study highlighted a statistical difference in the correlation between increasing CRAE and DR progression between males and females[40]. Although there was no statistical interaction based on gender ($p \leq 0.10$), incident DR HRs were 4.39 in females and 2.44 in males. The cohort was evenly split between males and females (45.6% males); however there is evidence which suggests females are at higher risk of developing microvascular disease [167]. A direct comparison with the findings of the Westmead study should be performed with caution, since the average age is considerably younger than the other studies in Table 14 (13.5 years old, compared to 58.07 years (AusDiab), ≥49 years (BMES), 45–84 years (MESA), 29 years (WESDR)), and so age-related, physiological changes to the vascular tree need to be considered. There is an established DR screening programme in the UK available to those with DM, whereby signs of DR (microaneurysms, haemorrhages, exudates, etc.) are a method for evaluating stability of the condition as a whole. Retinal vascular calibre is not yet incorporated into this scheme. Both the presence and progression of DR act as a good surrogate for the state of DM as a whole. Studies into the prevalence of DR have reported that diabetogenesis can occur up to 12 years prior to formal diagnosis and/or presence of DR [168]. This reiterates the long-term effects of DM upon the vascular system. One issue with the literature in Table 14 is the indiscriminate grouping of both Type I and Type II DM into the same dataset. The authors of the AusDiab study state that unpublished data showed no significant difference when Type I and II diabetics were compared and thus the two sets were amalgamated [141]. Similarly, the MESA study made no distinction between the two types [142]. Whilst the WESDR and Westmead studies both specifically used Type I diabetics only, based on age of diagnosis (≤30 and 10–12 years old, respectively), the authors of BMES used only those with Type II DM. Again, BMES based the status of Type II on the age of participants (≥49 years old); whether those with long-standing Type I DM from childhood were excluded is not made clear. Whilst there does not seem to be any difference between the two subtypes (for instance, both WESDR (Type I only) and BMES (Type II only) found a significant increase in both CRAE and CRVE with worsening stages of DR), the underlying physiology of the two conditions may differ, particularly since Type I DM onset is predominantly in younger years. Duration of the condition is therefore an important consideration, particularly in older cohorts. Similarly, time of recruitment could also have an influence on the results. As Nguyen et al. report, to make a direct comparison between two studies such as MESA and WESDR is potentially confounded by the fact that recruitment and data collection took place 20

years apart. Treatment regimes and medications, diagnostic techniques and general awareness will have altered in the intervening years, and this could impact upon how well controlled the DM is. The studies included focus predominantly upon the earlier stages of DR. BMES, for instance, reports differences in vessel calibre between no DR and severe non-proliferative DR (i.e. prior to any damaging ischaemia taking place). Whilst these findings are still sizeable (CRVE increased by +40.8μm), the understanding of more advanced DR and how vessel calibre changes correlate with this remain less well documented.

Patients with established DM will often have regular DR screening, but this does not mean that a thorough inspection of the retina and its vasculature is not warranted at routine eye examinations. Unfortunately, since both CRAE and CRVE have been shown to increase with worsening DR, these changes could be lost when performing an AVR. Having accurate measurements for each arm of the circulation separately is of much greater clinical value, and can only be produced with objective analysis. Given that DM itself is a significant risk factor for developing HT and CVD, continual monitoring of the retinal vascular system and systemic BP will ensure that health changes can be detected as soon as possible. Patient education is also key with regard to the DR screening programme, particularly since other CVD could manifest in the eye involving parameters not currently measured by the DR screening service, and so regular eye examinations are still very important.

2.4.2.3 Existing diabetes mellitus and systemic complications Diabetic complications target a multitude of systemic organs making direct comparison of these problematic. However it can be considered that the systemic complications arise through a common causality – worsening or progressing DM. The Fyn County study demonstrated a decrease in CRAE and increased OR of nephropathy or macrovascular disease [146]. This finding was also reported by the Danish Cohort of Pediatric Diabetes 1987 (DCPD1987) study, Pittsburgh Epidemiology of Diabetes Complications (PEDCS) study and WESDR; all of which correlate a reduction in CRAE with peripheral neuropathy, coronary artery disease and proteinuria[144, 149, 151, 43]. Additionally, the Diabetes Management Project (DMP) reported a correlation between reduced CRAE (by unspecified SD increments) with a 66% increased risk of erectile dysfunction amongst Type II DM males[145]. These reductions in CRAE are most likely attributable to the underlying microvascular pathology (arteriosclerosis) or increased peripheral resistance to blood flow, of which having DM makes the individual more susceptible, rather than a direct measure of the DM itself. These findings help to support the theory of an interrelationship between CVD, and that having one (e.g. DM) puts the patient at greater risk of co-morbidity (e.g. coronary artery disease). DCPD1987, Fyn County and EDC present their findings in the form of ratios, which are of much greater use to the clinician

when evaluating the progressive changes of an individual. For instance (as shown by the Fyn County study), knowing that a 17.4µm reduction in CRAE carries an OR of 2.17 and 3.17 for nephropathy and macrovascular disease respectively helps to inform and direct patient management. In this case, the patient is three times more likely to have macrovascular disease compared to a normoglycaemic patient, potentially warranting further medical investigation. This approach was taken further by Broe et al., who stratified results by +/-10µm changes in calibre. They also attempted to define cut-off values of absolute risk, based on Area Under Curve (AUC) calculations, however significance was lost through small sample sizes[144]. A drive towards clinically relevant cut-off values and risk-based ratios lend themselves well into a clinical practice setting. The findings of the Multi-Ethnic Asian Population (MEAP) study found less significant calibre changes associated with increasing levels of fasting plasma glucose [147]. The CRAE increase with increasing fasting plasma glucose lost significance when corrected for CRVE. Venular increase was more statistically significant; however, after similar correction for CRAE, an increase of +0.51µm per unit increase in fasting plasma glucose is very small, arguably falling below levels of clinical significance. The stronger findings amongst the Indian population within the study could possibly be attributed to regional differences in diagnosis and management of DM. Ethnic variations must be considered, since increased levels of retinal pigmentation will make vessel edge detection more difficult. It has been shown, however, that iris colour has a greater association with levels of retinal pigmentation than ethnicity [169]. Ethnic variation is highlighted in the MESA study, where the increase in CRAE amongst diabetics was only reported in the Caucasian cohort, whilst the increase in CRVE was only amongst the Hispanic and Chinese populations [142]. Genetic variance and susceptibility are possibilities, as well as environmental factors such as diagnosis and treatment strategies. Although MESA took place entirely within the USA, different socio-economic groups could still impact upon results. The New Jersey 725 Study, which had an African American cohort found similar correlations to those reported elsewhere with Caucasian-based cohorts; namely a decrease in CRAE and an increase in CRVE. In this case, baseline CRAE was found to be decreased in those with a 6-year incidence of CVD or Lower Extremity Arterial Disease (LEAD), even when corrected for DR classification, thus suggesting a vessel change independent of other local changes. CRAE was also reduced for subjects with a 6-year all-cause mortality when corrected for Mean Arterial Blood Pressure (MABP). Conversely, significantly larger CRVE at baseline was associated with a 6-year incidence of hypertension (30.3µm) when corrected for DR classification, and all-cause mortality (31.3µm) when corrected for MABP. These findings suggest that some of the microvascular changes occur irrespective of ethnicity. Increased RRs of both protein-

uria and long-term risk of stroke mortality were both associated with increases in CRVE by the WESDR studies [151, 150]. Whilst increases in CRVE are well associated with numerous aspects of DM (and associated systemic complications), the underlying mechanisms are still unclear[144, 145, 147, 142, 151, 150]. Dilation of the retinal veins has been theorised to result from hypoxia and subsequent lactic acidosis [151]. Similarly, signs of inflammation through the presence of leukocytes and C-reactive proteins [117, 132, 170] and endothelial dysfunction [171, 172, 173] have been attributed to the abnormal vascular reactivity. All of these potential causes for changes in CRVE, whilst visualised and measured at the level of the retina, are almost certainly taking place in organs throughout the body, hence the correlation between CRVE changes and increased risk of developing micro- and macrovascular complications. It must also be noted, however, that it has been demonstrated that venular diameter decreases in episodes of acute hyperglycaemia[166].

Different outcomes between studies make it difficult to give a single sign to look for when relating retinal vessel calibre to systemic changes resulting from DM. This is confounded by the number of various endpoints covered by systemic changes, including nephropathy, neuropathy, proteinuria, macrovascular disease, limb amputation and death. As with the other aspects of DM, the awareness that both arterial and venular calibre can alter following a change in DM status is important. Effective monitoring for patients can be performed on an individual basis, recording BP at each examination as well as obtaining objective measurements of vessel diameters so that both arterial and venular aspects of the circulation can be considered and monitored over time in isolation. Focus on risk and odds ratios plus absolute AUC-derived cut-offs highlight the potential clinical applications of vessel calibre in monitoring patients.

2.4.3 Association of stroke and retinal vessel calibre

There is a growing number of papers citing correlations between stroke risk and/or incidence and retinal vascular calibre. This area is in continued need of further research given the strong anatomical links between the retinal and cerebral vasculatures (and the comparative ease of imaging the former). In brief, both vascular beds are responsible for supplying high oxygen demand tissue whilst maintaining a strong barrier between the blood and its surrounding matter (the blood–retinal and blood–brain barriers). Anatomically, the two systems share many parallels (*as explained in Chapter 1.7. Vascular supply to the ocular circulation: The Central Retinal Artery*)[70]. Post-mortem studies of cerebral haemorrhage and infarction have revealed that the fibrous and fibro-hyalinoid thickening that occurred in the cerebral arteries was also evident in the retinal arteries [71]. Given the considerable ease and lower cost of retinal

photography compared to magnetic resonance imaging, its use in clinical practice is of great significance in the detection and prediction of potential cerebrovascular incidents. The data included in Tables 17 and 18 do not reveal a significant trend at first, however consideration of the development of the analysis technique aids the interpretation of findings. Interestingly, there was the greatest number of manually excluded papers for this category, particularly those reporting pooled data and meta-analyses.

Earlier studies, particularly the ARIC, BDES, BMES, CHS and WESDR studies, all make reference to the AVR only, or used changes in AVR as a surrogate for arterial narrowing, reflecting the infancy of objective retinal vessel analysis at this time [156, 153, 155, 161, 152, 92, 154]. Such measures and assumptions of venular calibre stasis imply a greater emphasis on, or interest in, the arterial arm of the circulation at a time when understanding of venular changes was limited. Therefore, any venular changes are likely to have been missed in these analyses or could result in spurious AVR-related findings. Changes in CRVE, particularly increases, were generally found when correction was made for refraction. In this particular instance, however, the ambiguity over the measurements taken and type of camera used means the use of the correction factor has the potential to attenuate findings. Despite these potential limitations, the Rotterdam study demonstrates a strong association between the retinal veins and stroke [160, 159, 158]. Whilst there is a significant reduction in CRAE associated with a 12% increased risk of stroke and intracerebral haemorrhage[158], there is a much stronger correlation between increasing CRVE and stroke. In fact, Wieberdink et al. reported a 2.5-times increased risk of anticoagulant-related (suggesting an already compromised cardiovascular system) cerebral haemorrhage, with just a +14.4µm increase in CRVE amongst the Rotterdam cohort[159]. More recently, Fani et al. associated increases in both CRAE and CRVE with global reduced brain perfusion and TIA, although interestingly there was no such correlation with ischaemic stroke[160]. This could suggest that dilated vessels pose a greater risk of transient, rather than permanent, ischaemia. Similarly, the findings of Lindley et al. found the retinal microvascular changes to be different depending on stroke type; in this case a significantly wider CRVE and reduced AVR was observed in patients suffering from lacunar stroke when compared to other stroke types[157]. Lacunar stroke occurs when there is an occlusion to a single perforating artery supplying the deep tissue within the brain, and is of particular interest when comparing to the retinal circulation as it affects the smaller arteries of the brain (40–200µm), making them closely related to those of the superficial retina; in structure, function and even their diameters. This study also noted that findings were more pronounced when diabetic patients were excluded from the analysis (odds ratio of CRVE being in the uppermost quintile in patients with lacunar stroke compared to other stroke types was 1.35 for the whole

cohort, compared to 2.22 for the non-diabetic sample (66.84% of the whole cohort)), suggesting a distinct vascular change. Again, the exact mechanisms of such changes are not well understood, but since multivariate analysis accounted for other CVD risk factors, a true relationship between venular dilation and stroke is highly likely; larger vessel calibres could reflect maximal dilation leaving no autoregulatory reserve which would normally be employed to increase arterial flow during periods of low perfusion. These findings demonstrate that further research needs to be directed towards the underlying physiology of the various subtypes of stroke, but also shows that the retinal vasculature has the potential to play a key role in this avenue of investigation.

A subsequent meta-analysis has combined the data of both the BDES and BMES studies included here in an attempt to explore whether a larger dataset would reveal any links[174]. Separately, the studies revealed few significant data relating to vessel calibre and stroke. When the data were pooled however, there was a slight increase in HRs for stroke deaths; 1.09 per standard deviation increase in CRVE. Unfortunately, this does not account for non-fatal strokes, the incidence of which could be higher and of equal clinical value. The authors report that, when the data were stratified by age, significance was attenuated in the >70-years-old cohort, suggesting an age-related interaction with vessel calibres.

Given the similar anatomical structure shared by the retinal and cerebral vascular beds, optometry has the potential to enhance screening and monitoring in cerebrovascular pathologies like stroke. It appears that there is growing evidence to demonstrate that venular changes are of equal (if not greater) significance when screening for signs of stroke. Consideration must be given to the complex interaction of cardiovascular diseases, and further work is needed to explore whether retinal arterial narrowing is truly part of stroke pathophysiology, or an interaction of hypertensive changes which pre-dispose the patient to future incidence of stroke. This is similarly demonstrated by the findings of Lindley et al., when diabetic patients were excluded from analysis; demonstrating that there appears to be some overlap between the conditions and their effects on the retinal circulation.

Optometrists should appreciate that signs indicative of stroke may possibly manifest visibly in the retinal vasculature. Routine BP measuring, as well as an up-to-date general health and medication list, all help to highlight cardiovascular risk factors, particularly since a +14.4µm increase in CRVE has been associated with a 2.5-times increased risk of stroke amongst those taking anticoagulants. Regular retinal imaging of patients is very important, especially if objective retinal vessel measurements are being recorded, since these allow for much smaller changes in calibre to be detected in individual vessels. The associations of retinal vessels and stroke show, just as with DM, that the retinal veins should be con-

sidered with equal importance as the retinal arteries. Since stroke is a single event but multi-factorial in it's origins, the use of cardiovascular risk calculations may well be of additional use to identify those at risk patients.

2.4.4 Conclusions

There are several advantages to having a range of large cohort studies performed in this area. Firstly, and most obviously, is that the large samples have the ability to reflect trends and associations across a population, with a higher level of statistical certainty. The collective findings are able to greatly improve our understanding of the physiology of the retinal vasculature and how it responds to pathology. Secondly, the methodologies employed provide a detailed insight into the general health of the studied cohorts. Having a wide range of blood tests and other investigations performed greatly supports the independent associations between the pathologies and changes in retinal vessel calibre. By following up subjects also helps inform our longer-term understanding of pathological processes, and identifies the risks of developing particular conditions or complications. Thirdly, the global spread of studies shows that these findings are not confined to a particular ethnicity or environment. This is particularly true of the association with hypertension.

There are, however, a number of disadvantages that also need to be considered. Importantly, whilst values of vessel calibre have been defined which are *statistically* significant, what values can we deem to be *clinically* significant? These are in fact two separate values; whilst changes in the region of 2-5µm are deemed a significant change in response to a +10mmHg increase in blood pressure, is the method of objective analysis currently sensitive enough to reliably and repeatedly detect this level of change? If the margin of error for repeated measures is even similar to these values, then any statistically significant change will be lost in the margin for error of the measurement. As such, further research is required into the actual measurements that are taken to demonstrate that current methods are sensitive enough to repeatedly detect such changes. Secondly, the extent of co-morbidity also needs to be fully evaluated. Given the inter-relationship of conditions such as HT, DM and stroke, the pathological processes involved in one are undoubtedly likely to affect another, in which case this interaction needs to be fully explored. Finally, the question of cause or effect needs to be answered. Whilst it is unquestionable that retinal vessel calibres change in association with pathology; whether this occurs as a result of the pathology, or is actually part of the initial cause of the pathology is not fully understood. The number of excluded meta-analyses due to differing methodologies demonstrates a significant need for

greater standardisation with the technique to allow for truly comparable findings. The results of the above studies tell us a great deal about these associations, but actual individual changes over time need much more attention in future studies. We know that arterial calibre is reduced in hypertensives, but for an individual does this occur prior to the pressure raising, or as a result of it? Do the vessels of that individual stabilise with medical treatment, do they continue to constrict or do they return to their original calibre? Whilst we know the answers in a statistical sense for the general population, we still do not have much evidence on the subject for an individual over time.

The present literature review has also highlighted the need to evaluate the entire cardiovascular profile of a patient. With the interaction between the three pathologies evaluated here, this is undoubtedly increased when considering a wider variety of cardiovascular diseases. Evaluating a patient's retinal vessel calibre will need to be supported by an assessment and (quantification) of their overall cardiovascular health; especially since the technology needs to be used in a predictive rather than retrospective manner. The variety of findings (decreased CRAE, increased CRVE, decreased AVR etc.,) also suggests that in the first instance, retinal vessel calibres should be used to simply identify the risk of general cardiovascular disease, rather than pinpoint the specific condition, allowing for earlier intervention and management.

3 Materials & Methods

3.1 Image Acquisition

3.1.1 Fundus photography

Whilst images have been acquired of the living human fundus from as early as 1886, it has taken advances in both optics and image capture methods to make the fundus camera a near-routine instrument in the optometric practice[175]. The underlying optics of fundus photography is highly complex, and specific lens systems are not covered in detail by any fundus camera manufacturers for obvious reasons. Principally however, all fundus cameras consist of a dual-lens system; an initial ophthalmoscope lens which produces an aerial image of the retina, and a secondary conventional, camera-based lens array which captures this aerial image (either on photographic film or CCD chip and digitized)[176]. Imaging the human eye is fraught with problems; the retina is a curved surface and so is not easily imaged on a flat plane without exposing the image to a large amount of aberrations, both through the imaging system and the eye itself. The optics of the eye, which contains a number of refractive surfaces and interfaces, introduces further reflections, aberrations and other image degradations which must be corrected for in order to produce a high resolution image. As shown by Figure 13 on the following page, a pupil aperture within the camera prevents excess light being focussed and allows for coincident focus. The curvature of the fundus can be corrected by using a field lens; a construction of ancillary lenses which aim to focus all light bundles (which will originate from different points on the curved fundus) onto a single, flat plane. The resulting aerial image of the fundus can then be focussed onto the camera diaphragm by a secondary series of lenses; the photographic apparatus. This 'flattening' of the image whilst still retaining focus is a form of telecentricity; an approach which, whilst ensuring consistent levels of image focus, introduces it's own series of problems which are considered below in Chapter 3.1.2 Correction factors. The advent of digital photography in the 1990s meant that fundus cameras could be used more routinely and efficiently in optometric practice. The time required to get images developed (which meant problems with imaging were not immediately apparent to the photographer), the issues with storage and recall of photographic prints and the associated costs were greatly mitigated by digitizing the process[177].

Fundus photography is now widespread in UK optometric practice. The availability of cameras and particularly the improvement in optics to allow for clear image acquisition in the undilated eye have meant that the procedure is fast becoming a routine element of the eye exam. It's ease of use to obtain high quality images, even with minimal training, have resulted in a number of optometrists and

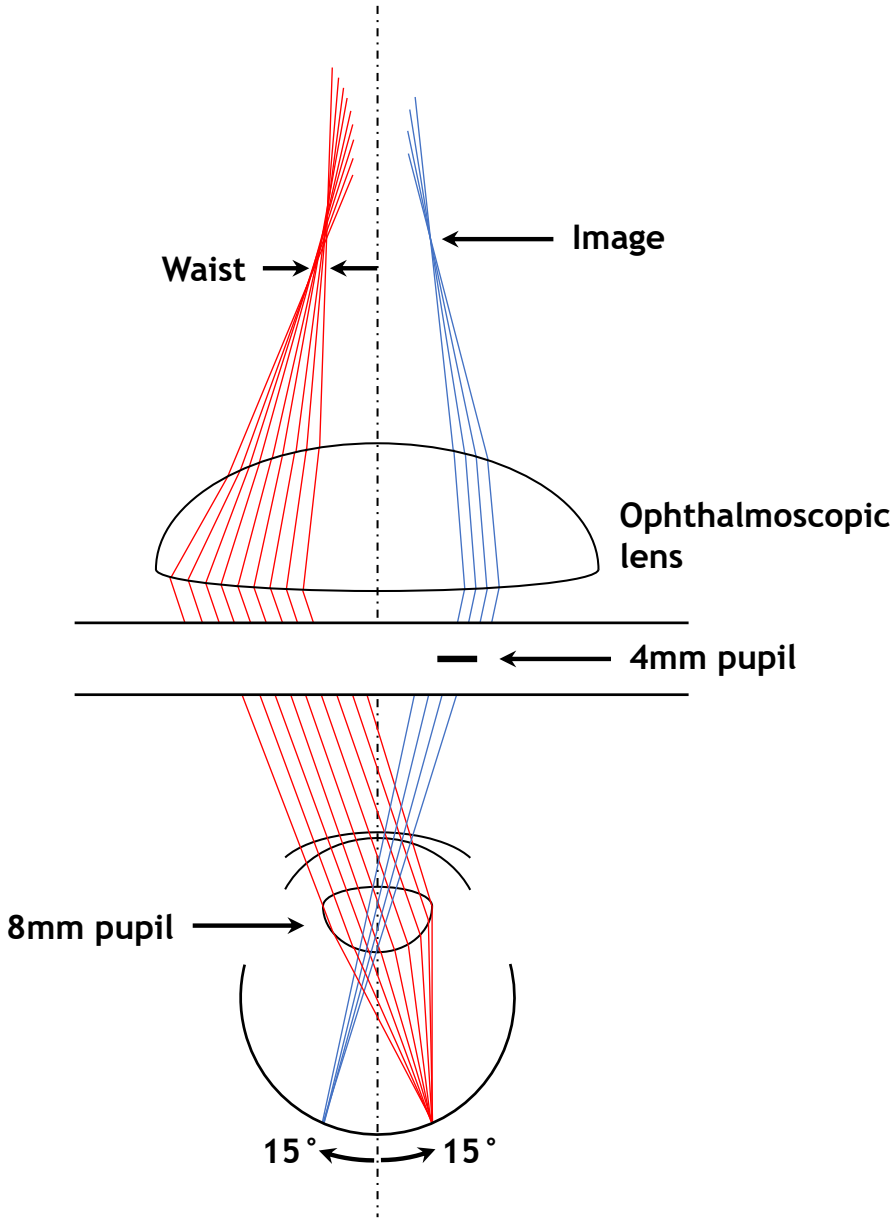


Figure 13: Ray diagram illustrating the ophthalmoscope portion of a fundus camera. If all light leaving the eye through an 8mm pupil is captured, there is no single point of focus; instead producing waists of light rays. A pupil within the camera reduces the light-ray bundle, resulting in a sharp aerial image which can then be focussed onto the camera diaphragm by a secondary lens array.

hospital eye departments delegating the duty to non-qualified ancillary staff[178]. Whilst large cohort studies predominantly use single photographs in their analysis (*see Chapter 2 (Associations of Retinal Vessel Calibre with Cardiovascular Disease: A Systematic Literature Review) for more detail*), optometrists have the major advantage that they have an individual catalogue of photographs for each patient, spaced over time. Whilst objective analysis is not currently performed by UK optometrists, successful implementation of the technique would mean that historic photographs for a patient could also be analysed. Modern non-mydriatic fundus cameras save digital files for photographs, and both full colour and red free photographs are required for successful objective analysis. In order to preserve as much image data as possible, photographs need to be saved in the largest (preferably uncompressed) file type, such as Tagged Image File Format (TIFF), since the compression associated with smaller file sizes causes a loss of resolution and data.

3.1.2 Correction factors

Whilst the fundus allows for an *in vivo* examination of the vascular system (particularly for taking measurements), there needs to be consideration for the eye's optics. Primarily, the measurements made of the vessels are being made indirectly. This is because a graticule cannot be placed within the eye and so measurements from a fundus photograph are made from an image on a single plane. This presents a number of optical influences upon the produced image. Firstly, the object being imaged (the retinal surface) is a concave surface, presented on a flat plane (either photographic film, or more recently digital imaging), so is susceptible to optical aberrations. The image is also larger than the object (and depending upon digital image file format, can be enlarged to a considerable degree). This means any optical imperfections in the system will be magnified and exaggerated. The dimensions of the eye being imaged are also important. Axial length and refractive error can both manipulate the final image. Refractive error (and so indirectly axial length) has been of particular focus for the development of correction factors in an attempt to standardise analysis of images. Two formulae receive most prominence in the literature; the work of Bengtsson[179] and Littmann[180]. Both formulae, however, are only applicable if the photograph is taken on a telecentric fundus camera. In short, telecentric camera systems use an array of lenses to enhance the depth of focus, resulting in a range of depths being in focus simultaneously[181]. The principle of telecentricity is shown in Figure 14.

This is particularly important for a retinal photograph as it is a concave surface being imaged. An example of how a telecentric system allows for multiple foci is the imaging of a deep optic cup. Both the rim

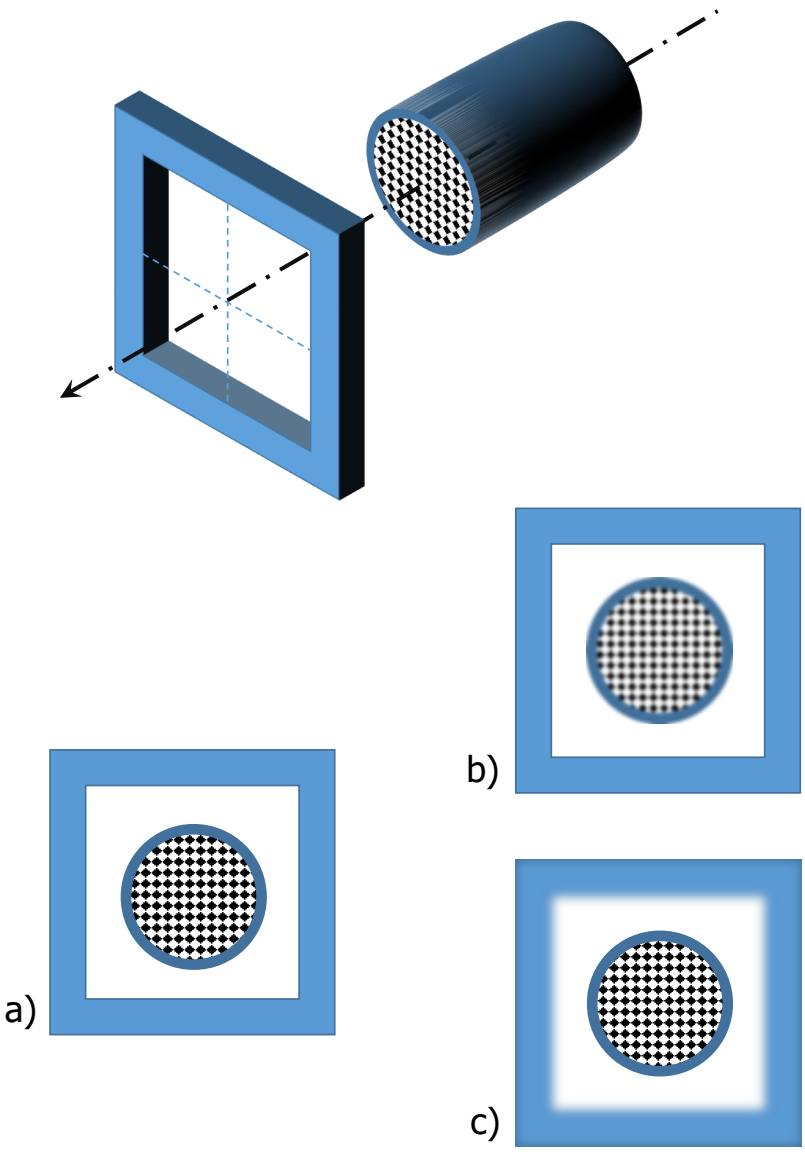


Figure 14: Telecentric image focus. **a)** shows a telecentric image, with both objects equally in focus. **b)** and **c)** show how conventional cameras are only able to focus one object at a time. Note that depth information is lost with a telecentric image.

and centre of the cup are equally in focus despite being at different distances from the camera. This basic optical problem can be combatted by various lens setups within the imaging system, with telecentric lenses being just one example. As such, for the Bengtsson or Littmann factors to be used correctly, it first must be confirmed that the camera used is indeed telecentric, and that the use of any constants apply specifically to the individual camera model. If not, any 'corrections' will potentially attenuate findings. The Rotterdam study is one such large-scale study investigating vessel calibre to acknowledge the potential need to use correction factors to their data[140, 133, 182, 158]. Unfortunately their camera (an unspecified Topcon stereo-fundus camera) was potentially not telecentric in design. The authors of the Rotterdam papers reference Littmann's original paper outlining his formula, however it is not directly stated in the original Littmann paper that there is the inherent need for the system being used to be telecentric. The Rotterdam study did not use a Zeiss Oberkochen camera like the one used by Littmann and as such, the constant value given by Littmann (which is specific for the Oberkochen camera alone) will be meaningless when applying it to the stereo-images acquired for the Rotterdam study. This has the potential to attenuate any significant findings. The Blue Mountains Eye Study however, did use a telecentric Zeiss FF3 fundus camera for their imaging[85], and so the use of a telecentric-specific formula (*Bengtsson*) is justified (despite their showing refraction had no effect on the measured outcome).

The large population-based studies make reference to the use of correction factors in their analyses, and this is indeed necessary when comparing data across a population to find a common outcome. How relevant this is for use in everyday optometric practice is debatable. This would depend upon what the vessel measurements would be used for. If an individual's CRAE and CRVE were measured and were to be compared against a pre-defined 'normal' then correction for ametropia would be required. However, CRAE and CRVE is more likely to be used to monitor an individual patient case in optometric practice. Just as optic nerve head analyses can be used to monitor for signs of incipient glaucomatous optic neuropathy, a comparison of individual data against a 'normal' population is of limited use. Greater insight for the clinical management of a patient will be in the long-term individual changes in vessel calibre. Since the range for clinical 'normal' is so widespread, individual changes in vessel calibre (which may be clinically significant) may still fall within what is considered 'normal'. Taking the studies linking vessel calibre and hypertension as an example, those providing 'normal' data as a comparison (i.e. normotensive subjects), the combined normative data covers a range of 53.9µm[133, 122, 124]. 'Normal' therefore covers a wide range, and since the same studies quote clinically significant changes in vessel calibre as values less than 10µm, this can be problematic.

When interpreting the results for an individual over time, there is much less need for correction factors since the magnification effects will be less changeable. There are of course instances when this will not be the case, particularly with laser refractive surgery and intraocular lens implants. In both cases there will be considerable changes to the optical parameters of the eye, and as such a conversion for 'before' and 'after' may indeed be necessary. Since the formulae require such measurements as axial length (not routinely recorded by optometrists), the practical execution of these corrections in practice may still only be a theoretical consideration.

3.1.3 Fundus photography protocol for current study

For the following studies, a Topcon OCT Maestro was used for data collection:

- Chapter 4. Repeatability and Variability in Objective Static Retinal Vessel Calibre Measurement (page 100)
- Chapter 5. Correlations Between Retinal Blood Vessel Calibre and Cardiovascular Metrics Observed in Optometric Practice (page 116)
- Chapter 6. Longitudinal Changes in Retinal Blood Vessel Calibre Observed Between Routine Optometric Eye Examinations (page 140)

The Maestro is able to take 45º colour photographs under non-mydriatic conditions, also taking simultaneous OCT scans. Full-size colour and red-free optic nerve head-centred photographs were exported as TIFF files; the largest, uncompressed format available. Since photographs were exported and analysed at a later date, patients could not be recalled for dilation, thus giving a reliable indication of what image quality could be analysed. Topcon recommend a pupil size of 2.50mm or greater[183]. Patients had their pupils measured prior to imaging to ensure that their pupils were of adequate size. Those with smaller pupils were offered the opportunity to rebook and receive pharmacological dilation.

Patients were seated in a room and dark adapted for a minimum of five minutes prior to the images being taken. Images were taken according to the instructed procedure in the equipment's documentation and any settings changed as suggested (i.e. small pupil function enabled when prompted). Patients were encouraged to keep their eyes closed for several minutes in between image capture of each eye. Photographs were reviewed for general quality at the time of acquisition, and if they were deemed too poor, repeat images were taken (or the patient was rescheduled for pharmacological dilation).

3.2 Image Analysis

3.2.1 Vessel selection

Selection of blood vessels from a 2D image can be achieved through a number of computational algorithms, and the process is not restricted to determination of vessel calibres only; branching patterns and angles, fractal dimensions and vessel tortuosity all utilise vessel segmentation algorithms. The method of vessel selection can vary between programs, but retinal vessel-based algorithms have been broadly categorised into one of six categories; pattern recognition, matched filtering, vessel tracking, mathematical morphology, multiscale approaches and model-based approaches[184]. Vessel segmentation and selection from images is not confined to ophthalmology, and so algorithms and approaches have a number of applications with both 2- and 3-dimensional images[185]. Selection of retinal blood vessel edges poses additional issues over simply skeletonising the vessel tree. An 'optimally' illuminated retinal blood vessel will have a Gaussian function cross-sectional intensity profile. For retinal veins, there is a more obvious hue and contrast change with the surrounding retinal tissue. However this is less apparent with the arteries, and is further confounded by an often prominent central reflex, resulting in a double-Gaussian profile. There is no one cut-off point for identifying the edge as a point on the Gaussian function, rather it is often defined as occurring at the local gradient maximum or minimum; i.e. the point of maximal contrast on the function[186]. Cross-sectional intensity profiles are illustrated in Figure 15.

As an experimental variable it is a theoretical consideration when interpreting findings, however it is a factor controlled by the software designers.

3.2.2 Software available

As uptake of objective analysis has increased in research, a number of software platforms have emerged which allow users to analyse retinal photographs and extract such details as vessel calibre. The software packages vary in terms of analysis features and output as well as accessibility. On a basic level, it is possible to use freeware image editors such as ImageJ. Here, images can be imported and custom algorithms applied to extract vessel widths, and the approach has very good agreement with existing semi-automatic analysis software (Spearman correlation 0.90)[187]. Whilst this approach is cost effective, both it's limited capacity and laborious nature make it an impractical option for mass screening. There are also more specialist, freeware packages which are run through the MatLab platform. Examples of this approach

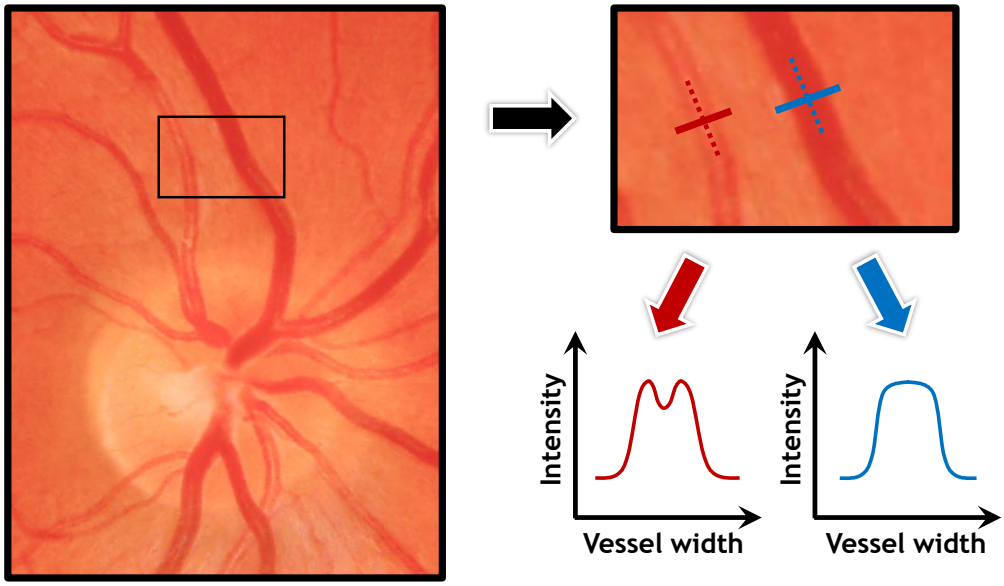


Figure 15: Cross-sectional vessel intensity profiles. Artery profile is shown in red (note the double-Gaussian peak corresponding to the arteriolar light reflex), and vein profile is shown in blue. Intensity profiles (solid lines) traverse the vessel perpendicular to it's centreline (dotted line).

are VAMPIRE (Vessel Assessment and Measurement Platform for Images of the REtina), ARIA (Automated Retinal Image Analyser), QUARTZ (Quantitative Analysis of Retinal Vessel Topology and Size), VesselFinder and RISA (Retinal Image multiScale Analysis)[188, 186, 189, 190, 191]. RISA and VesselFinder have both been trialled in studies into retinopathy of prematurity. The authors of VesselFinder report that the algorithm responsible for determining vessel diameter produces only an average width measurement, rather than a collection of measurements along the vessel length. Whilst the authors of ARIA cite this as a potential issue, measurements taken for determining CRAE and CRVE only require an average calibre for each branch which is then fed into the objective formulae. The advantage of all of these packages is that they are freely available to download, and so the costs incurred to the optometrist are relatively low. A downside to these packages are their (current) lack of widespread use, so their reliability and reproducibility have not yet fully been explored.

The final group of software packages are the commercially available platforms. IVAN (Interactive Vessel ANalysis) and RA (Retinal Analysis), both based in Wisconsin, US and SIVA (Singapore 'I' Vessel Assessment), based in Singapore, are the three software platforms most commonly referred to with the large cohort studies. Whilst images can be sent to the centre in Wisconsin for analysis, it is possible to

purchase a license for SIVA. Such software, given it's extensive coverage in the academic literature, has the advantage of being internationally recognised and it's reliability much more robust. Other organisations offer software for purchase (to be used in clinical practice or research), and these include Imedos' VesselMap (Imedos Systems, Jena, Germany) and vito's iFlexis (Belgium).

Whilst vessel selection algorithms may differ, the general principle for establishing vessel calibre remains constant and is summarised in Figure 16. A section of vessel is selected (within the measurement annulus; *see Figure 11*) and the software then takes multiple edge-to-edge pixel measurements along the section which can then be converted into μm if required. A limiting factor is therefore, especially with digital image acquisition, the pixel resolution of the imaging system (see Figure 17). From this, an average width is generated for the vessel section and this value is then used in subsequent analyses. A worked example in Figure 18 shows how a number of vessel width measurements produce an average with a very low standard deviation (1.08μm) and coefficient of variation (1%). Software has internal criteria regarding the minimum length of vessel that can be used, although it is not vital that the entire width of the annulus is selected. It is worth noting that whilst this approach helps to quantify vessel calibre (and therefore phenomena such as GAN) it does not account for local changes, such as Focal Arterial Narrowing (FAN).

There is currently limited evidence comparing the outcome from different measurement programmes. A detailed study by Yip et al. concluded that the measurements from three leading platforms (SIVA, IVAN and RA (Retinal Analysis)), whilst they differed in their absolute measurements of vessel calibre, all retained the same systemic associations (including age, blood pressure, BMI, cholesterol and glucose levels)[192]. This finding was echoed by McGrory et al. who evaluated a wider range of vascular parameters (vessel calibre, tortuosity and fractal dimension), but suggest there is less agreement between software measurements and systemic associations[193]. It should be noted that the more general measures with clinical applications such as blood pressure and glucose levels, produced the same associations regardless of software. The implication being that more detailed measures (such as c-reactive protein, interleukin-6 and carotid intima-media thickness) require further investigation into software-dependent bias, with more general measures producing repeatable results.

3.2.3 Image analysis protocol for current study

As a commercially available platform but which has also been referenced in the literature, VesselMap was selected for image analysis. Whilst commercially available, iFlexis had not been referenced in the

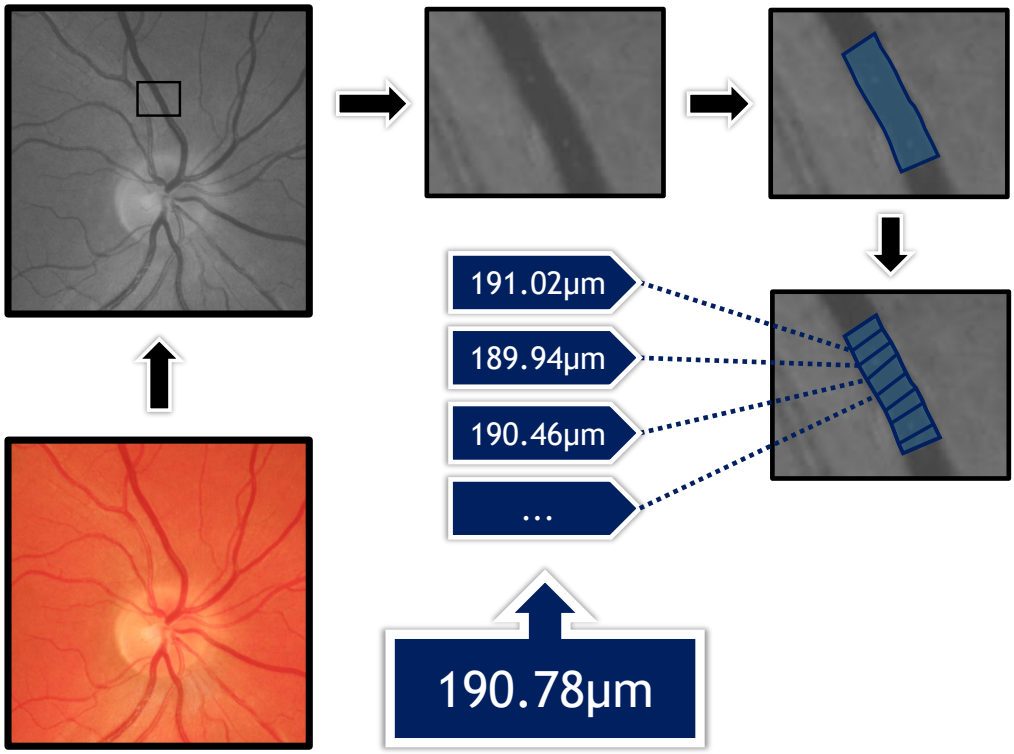


Figure 16: Vessel selection and measurement. Once a section of vessel has been marked, multiple edge-to-edge pixel measurements are taken, converted into micrometres (µm) and averaged to give a single width measurement for the vessel.

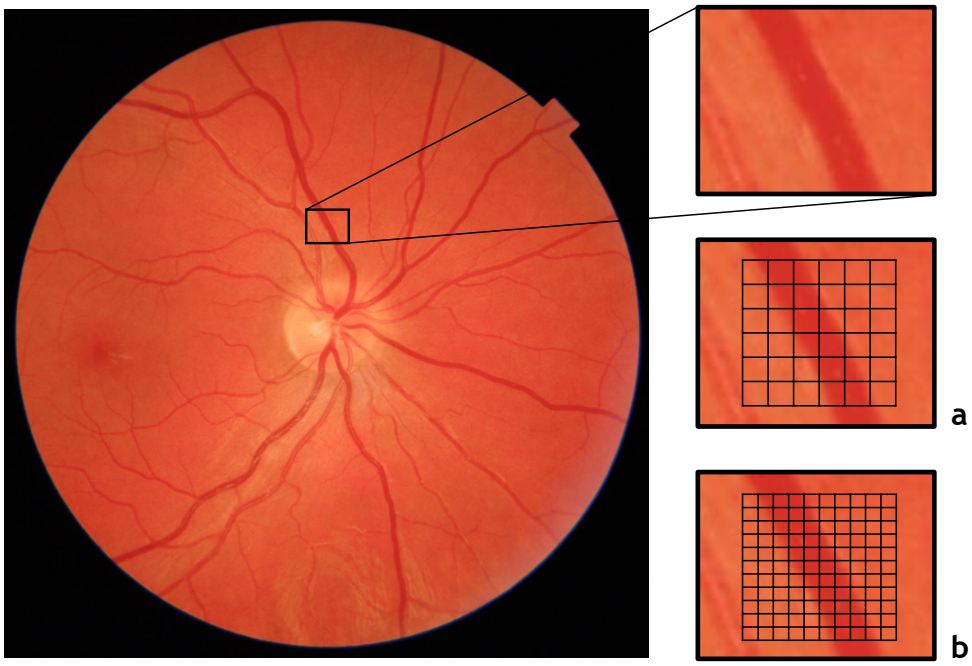


Figure 17: Image resolution as a limiting factor for determination of vessel edge. Splitting an image down into a grid pattern (for illustrative purposes larger than actual pixel size), the sampling of the vessel edge is less precise with (a) a lower (3MP) resolution compared to (b) higher (5MP) resolution.

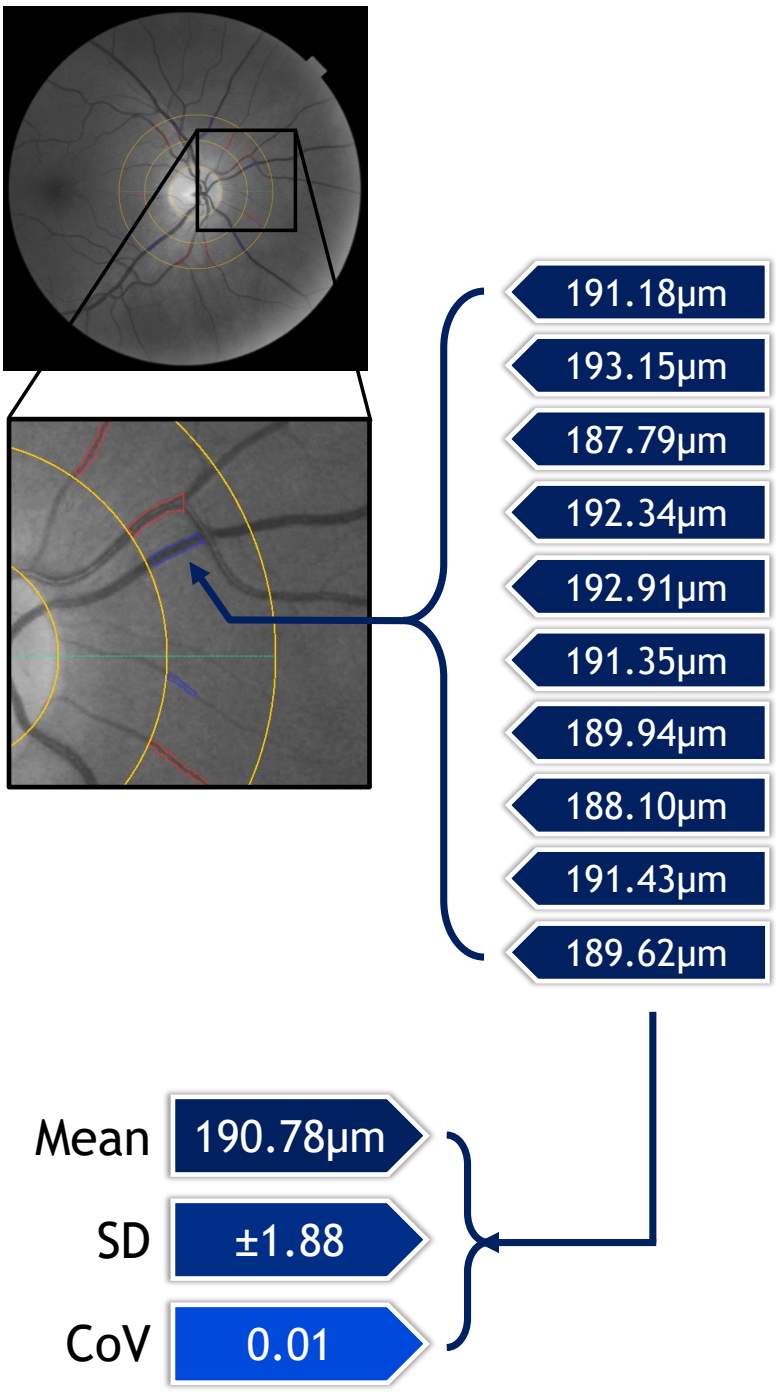


Figure 18: Worked example of vessel width measurements. For a section of vessel, a number of widths have been taken (in micrometres; µm) and the mean, standard deviation (SD) and coefficient of variation (CoV) calculated and shown.

academic literature at the time of study design and would therefore have required an extensive repeatability study prior to it's application. Exported images (in TIFF format) were imported into VesselMap. Only red-free images were analysed. Colour photographs were used for reference to ensure arteries and veins were selected correctly. Having set the designated grading area (*see Figure 11*), arteries and veins were selected. In order to facilitate future comparisons of individual vessels, a clockwise protocol was adopted, selecting the first artery and vein above the 9 o'clock position on the photograph, and working around from that vessel. Prior to vessel selection, a visual inspection to ensure the correct number of paired vessels could be selected was performed (i.e. to not end up with an unequal number of arteries and veins selected). The individual vessel calibres and a reference screenshot of the selected vessels were then exported for statistical analysis. Contrast enhancement was only used when a vessel was visible on the photograph but could not be selected by the software. In such cases, a note was made in the results that contrast enhancement had been employed.

3.2.4 VesselMap protocol

Retinal vessel analysis as performed in VesselMap is a straightforward process. First, the patient details (name, date of birth and eye(s) imaged) are entered into the system and, if an external imaging system is used, the photographs imported into patient record. In order for analysis to be performed, the image must be imported in it's red-free format to allow maximal contrast of the vessel edge. As part of this, properties of the image itself are also input; including the camera used and the size of image in degrees. Next, the image is opened in the analysis tab. At this point, the software will internally flag up images which are deemed not suitable for grading (if the contrast is too high or too low). The grader is prompted here whether they wish to proceed anyway as it is possible to adjust brightness and contrast of the image within VesselMap, as well as select vessels manually.

The first step of analysis is the placement of the optic disc zone over the patient's optic nerve head (although the software automatically positions the zone over the disc, meaning the grader has to simply check that it is correctly positioned). Since VesselMap employs the original Parr-Hubbard formulae, an optic disc zone of a fixed diameter equivalent to 1850μm as specified in the original paper[113]. In subjects with a larger visible disc, the zone is placed as close to the geometric centre of the disc as possible (similarly if the disc is actually smaller than the 1850μm ring). This differs from other analysis packages which require plotting of the disc margin manually (either on a point by point basis or an adjustable circle). Whilst this latter approach reflects an individualised, relative measurement (i.e. the

zone equates to the individual's own disc diameter), the original Parr-Hubbard approach ensures a more standardised location and lacks the need to subjectively define where the disc margin lies (a particular issue in those with indistinct margins). This is of particular benefit in subsequent analyses of the same person, as the measurement zone needs to be consistent across images.

Once the disc zone is placed, the measurement annulus automatically positions itself and the grader is then prompted to select all arteries within that zone. This is done by simply clicking on any part of the vessel which travels through the measurement zone and it is not possible to select vessels which fall outside of this. Edge detection algorithms then select the whole vessel as it runs across the measurement zone. The Parr-Hubbard (and Knudtson) formulae are derived from the trunk vessel rather than branches, therefore if a vessel branches within the annulus, only the trunk should be selected. The exception to this is given as if the trunk extends only briefly into the measurement zone; in which case the two daughter branches should be selected instead. The selected vessel then appears in the data table next to the image, with a unique vessel number and it's measured calibre (in μm). Assessing the selected vessel, the grader has several options:

a) The vessel has been correctly selected and measures more than 40μm: ***no action required.***

b) The vessel has been correctly selected but measures less than 40μm: ***vessel deselected and not included in analysis.***

c) The vessel has been correctly selected but extends beyond a branching within the measurement zone: ***region of vessel selected needs to be reduced to a point before vessel branching.***

d) The vessel selection extends onto the retina (often an underlying choroidal vessel): ***vessel needs reselecting, or vessel edge needs to be defined manually.***

e) The vessel has not been selected (generally due to poor contrast with neighbouring retina): ***vessel edge needs to be defined manually.***

Manual selection of the vessel involves a calliper being placed at one edge of the vessel (the software automatically zooms in to give a much better assessment of the vessel boundary) and dragged across the width of the vessel. Following this, the software will either use this measurement singly, or it will try to employ edge detection either side of the calliper to select a section of vessel. All of the arteries within the annulus are selected and once this has been completed, all of the veins can then be selected in the same fashion. The software colour codes selected vessels to aid identification (red for arteries, blue for veins,

and grey for vessels where type is unclear). Once all of the arteries and veins are selected, the grader can select the option to calculate AVR and the formulae are run on the vessels selected. An AVR is overlaid onto the image, and the tabulated vessel measurements (numbered and identified as either arteries or veins) can then be exported into spreadsheet software for analysis, including the calculated CRAE and CRVE measurements. Reports can also be generated at this point to plot changes and correlate with normative data (*as shown in Appendix 6. VesselMap Report*).When subsequent visits are to be analysed, the software prompts the grader to use the previous selection, and will both automatically position the measurement zone and reselect the same vessels. This then allows for comparative measurements to be acquired and greatly reduces the analysis time.

In total, from the moment of image import to the generation of summary measurements, the analysis of an image takes in the region of 2-3 minutes, depending on the complexity of the vessel arrangement (particular care has to be taken on tortuous and crossing vessels) and the contrast and reflex of the vessels. This contrasts to the original ARIC approach which cites analysis of an image can take approximately 10-15 minutes per eye[113]. Similarly, the iFlexis platform (which employs the Knudtson formulae instead) allows for quick analysis and is perhaps better suited to batch analysis. Here, the grader imports a spreadsheet populated with patient names and the file extensions of the images they wish to use. The images are imported in full colour and the software again automatically places the optic nerve head ring. Here, however, the grader will check the positioning in each image, but can also adjust the size of the ring to reflect different sizes of optic nerve heads. The measurement zone then corresponds to the individual's optic nerve head diameter, rather than the fixed value as specified by ARIC. iFlexis has a greater level of automation as it then pre-selects the vessels and defines them as arteries or veins. The grader simply has to check each image a second time to verify that all vessels have been selected and correctly identified by type. Whilst all vessels are selected, by definition the Knudtson formulae only makes use of the 6 largest vessel measurements, but this can be based numerically rather than subjectively choosing the six largest. Using a full colour image allows for an easier identification of arteries and veins, but also potentially retains maximum image resolution too (since red-free images tend to simply have extracted the red channel, thus removing 1/3 of the image). Whilst iFlexis requires a double-pass check on the part of the grader (first for disc position and size, second for vessel selection), both are confirmatory checks on automated selection and so allow for large batches of images to be measured in a shorter period of time (per image is in the region of 1-2 minutes).

3.3 Sample size and patient recruitment

Determination of an adequate number of subjects required to make statistically robust conclusions is an important consideration. This is particularly relevant if conclusions are to be made against previously reported findings and comparisons made. As much as the research question may be to establish whether a particular reported finding was similarly observed in optometric practice, it is of equal importance to establish whether the cohort study possessed the robustness to significantly demonstrate this.

To determine a sample size, several key factors have to be considered[194]:

- **Statistical power:** the ability of a test to detect a true / positive difference against a control or null hypothesis; *a true positive*. This is generally given as 80%; which is to say that the test is accurate in four of 5 cases.

- **Statistical significance:** this is essentially the opposite of power in that it is the level at which a true / positive difference is reported when no such difference exists; *a false negative*. Reported as the 'p value', these are generally set at 5% (p = 0.05) as '*significant*' or 1% (p = 0.01) as '*highly significant*'.

- **Prevalence rate:** this is sometimes referred to as the 'underlying population event rate', and is not determined by convention as with the previous two factors. Generally, prevalence is determined through the findings of previous studies but care must be taken as differences in samples or methodologies may mean that the two are not directly comparable. Some studies will re-run calculations throughout a study to ensure that changes in event rate are considered.

- **Expected effect size:** this is the amount that the study sample is expected to differ from the control or null hypothesis. This factor has a great impact on the calculated sample size as there is an inverse relationship between them; the smaller the effect size, the larger the sample required to detect it.

With values for these variables, it is then possible to input them into a standard sample size calculation formula[195]:

$$n = \frac{2(Z_\alpha + Z_{1-\beta})^2(\sigma)^2}{\Delta^2}$$

Where:

- $Z_{\alpha}$is related to statistical significance and whether the effect is expected to be one- or two-tailed [this value is obtained from pre-calculated tables].
- $Z_{1-\beta}$is calculated based on the statistical power [this value is obtained from pre-calculated tables].
- σ is prevalence rate as determined through previous studies and work [this is calculated from the estimated standard deviation of measurement(s) taken]
- Δ is the expected effect size in decimal form [this too is derived from previous work, however consideration must be given for differences in samples used].

3.3.1 Sample size for the present studies

As the present studies are observational, it becomes problematic to determine an adequate sample size. This is further compounded by the wealth of evidence to demonstrate that vessel calibre changes are multifactorial, and even with three discrete pathologies it is difficult to determine clear-cut correlations. A further issue is the disparity between clinical and statistical significance. For instance, the literature review has demonstrated that vessel calibre changes specifically in relation to +10mmHg increases in blood pressure are within the region of 3-5µm; yet the sensitivity of measurements may not be of sufficient detail or accuracy to record this.

Whereas correlations for HT are perhaps more straightforward, with arterial calibre and blood pressure as the two measurements, other conditions such as DM and stroke have a number of metrics contributing to their development, and as such would require a multitude of sample size calculations to factor these in. With respect to an observational study, it is not possible to foresee all possible comorbidities and influences upon vessel calibre which renders determination of sample size difficult.

The potential disadvantages of a lack of sample size calculation should not be discounted however, and need to be borne in mind when interpreting findings from such these studies. Since no previous studies have been undertaken in UK optometric practice, any findings of statistical significance can first be compared with previously established correlations (*as reported in the Chapter 2. Associations of Retinal Vessel Calibre with Cardiovascular Disease: A Systematic Literature Review*) to look for signs of general agreement, but they will also highlight areas of further study to be undertaken within similar conditions. Narrowing down the scope of study will then make calculation of sample size more feasible and carry greater insight.

3.3.2 Patient recruitment

The sample enrolled in the present studies were recruited from a single, independent practice based in Leicestershire (Davis Optometrists, Market Harborough). Patients were initially notified of the prospective study upon point of appointment booking, as the practice adopts a pre-arranged appointment system. The study was mentioned and additional information was given if required. Upon arrival for the appointment, patients were given a further opportunity to raise questions with the principle investigator (CF). Informed consent was then given prior to the examination taking place, but patients were advised that their routine examination would take place regardless of consent being given. Consent was reaffirmed at any subsequent examination, and patients were allowed to withdraw themselves from the study at any time.

The restricted locality and ethnicity (Market Harborough is a predominantly Caucasian neighbourhood) is advantageous in that it reflects the geographic variance in health as calculated by the QRISK algorithms. It does, however, potentially limit the nationwide applicability of findings since it is restricted to a single locality with a predominant ethnicity.

3.4 Additional ocular parameters

3.4.1 Tonometry

Fluid dynamics have demonstrated that there is a correlation between the intraocular pressure (IOP) and rate of ocular blood flow (OBF) within the eye[196]. There is also longitudinal evidence which suggests that decreases in both CRAE and CRVE is associated with increased risk of developing glaucoma over a 10-year period (77% per -15.1µm for CRAE and 33% per -22.4µm for CRVE)[197]. This suggests initial vascular changes prior to clinical detection of glaucoma, since a cross-sectional correlation has been established previously[198, 199]. Considered the 'gold standard' in IOP measurement, contact tonometry involves physical applanation of the cornea with a gradually increasing weight until it is coincident with the outward pressure from within the eye. This requires the additional use of a single-use applanator head, topical anaesthetic and staining agent (sodium fluorescein); all additional costs for the practitioner. Additionally, the technique is not always possible on patients unable to be seated at a slit lamp; however a hand-held equivalent (Perkins tonometer) is available which correlates strongly with Goldmann [200]. Non-contact tonometry (NCT) is a widely used surrogate for contact (Goldmann) tonometry in optometric practice, with 78% of optometrists using it as their primary source of intraocular pressure meas-

urement (whilst only 16% use contact tonometry as their routine method), and 68% of pressure-related glaucoma referrals contain NCT-derived measurements[201, 202, 203]. In short, the technique involves a measured impulse of air being directed towards the cornea whilst an illumination system produces an image upon the cornea. As the pressure increases to an extent which distorts the cornea, this reflected image is changed on the photodetector array within the instrument. At this point of distortion, the instrument samples the pressure of air being released by the instrument and determines the intraocular pressure[204]. Despite the most recent update of the NICE Guidelines for glaucoma referral stipulating that IOP must be measured with applanation tonometry, the correlations between non-contact tonometry and applanation tonometry are very high[205]. Corneal thickness and curvature is known to affect both methods, however NCT has been shown to yield higher results compared to applanation tonometry in patients with increased corneal thickness[206, 207].

3.4.1.1 Tonometry protocol for current study Intraocular pressure was recorded as a routine aspect of the eye examination. Non-contact tonometry was performed with a calibrated Keeler Pulsair 3000 in reduced room illumination and the patient seated. Three readings were taken of each eye and averaged for subsequent analysis. In cases of IOP reading above 24mmHg repeatedly, patients were re-assessed with Goldmann applanation tonometry (and referred according to NICE guidelines if pressure was found to be continually high[205]). Corneal thickness was not measured during the study.

3.4.2 Refraction

Associations with particular groups based on refractive status (e.g. myopia) have been explored previously, although have not always shown a significant association[208, 209]. Correction for magnification aberrations induced by refractive error is theoretically possible (see above; Section 3.1.2 (Correction factors)), however is not always possible due to the imaging system being used and the availability of additional ocular measurements.

3.4.2.1 Refraction protocol for current study A complete subjective refraction was completed on all participants. This was recorded for each visit in terms of a sphero-cylindrical prescription which was later condensed to a mean spherical equivalent (MSE) ($sphere + (\frac{1}{2}\, cylinder)$) for purposes of statistical analysis.

3.5 Cardiovascular metrics

3.5.1 Sphygmomanometry

The measurement of blood pressure is key to monitoring cardiovascular health, and is a measurement easily performed in the consulting room. Whilst the mercury sphygmomanometer is still considered the gold standard (despite being outnumbered in UK GP practices by their digital equivalent 4:1[210]) it's use is being phased out under an EU directive surrounding their containing mercury[211]. Also the practicality of performing this technique quickly and repeatedly on all or most patients seen in practice is problematic and time consuming. As such, simpler devices such as oscillometric (digital) sphygmomanometers provide a method of quick and (generally) reliable assessment of blood pressure, including over other devices such as aneroid sphygmomanometers[212, 210]. Briefly, an aneroid sphygmomanometer amplifies the pressure changes in the constricted blood vessel by means of a series of gears connected to an external dial showing the pressure reading, whereas an oscillometric device contains a transducer which detects the pressure wave within the compressed vessel and uses an internal algorithm to determine the points relating to cardiac systole and diastole, thus negating the input of an observer[213]. Guidelines relating to accurate, diagnostic determination of blood pressure recommend measuring pressure in the brachial artery of the upper arm, although it is possible to determine blood pressure from other locations[23]. Inter-arm differences in systemic blood pressure are widely acknowledged but not fully appreciated in clinical practice[214, 215, 216, 217]. A recent UK-based study reports a 'significant improvement' in that 52% of practices interviewed measure inter-arm difference as part of hypertension diagnosis[17]. Of particular note is that the origins of the inter-arm differences are relatively unknown; it is not merely a case of the left arm being closer to the heart. Neither the NICE or JNC guidelines for hypertension specify an arm to record blood pressure from, however NICE and AHA both advise that when formally diagnosing hypertension, blood pressure readings should be obtained from both arms[23, 218, 219, 212]. A recent, UK-based study of blood pressure measurements taken in primary care identified that significant inter-arm differences occur in a minority of patients, although incidence increases in the presence of cardiovascular pathology[220].

3.5.1.1 Sphygmomanometry protocol for current study In accordance to guidelines, blood pressure was recorded following more than five minutes of being seated. To ensure a maximum period of rest had been attained, the blood pressure readings were taken at the end of the eye examination. This also ensured that a good patient rapport had been established and all other tests had been completed which

may cause the participant undue stress or apprehension (particularly non-contact tonometry). Patients were instructed not to talk whilst the measurements were being taken. Three readings were obtained; each interspersed with a minute interval, on a digital oscillometric sphygmomanometer (A&D Medical UA-767). Arm selection was governed by the configuration of the consulting room, with the sphygmomanometer positioned to the right of the patient, however in line with NICE and JNC guidelines, this was not deemed a critical factor. The first reading was discounted and an average of the second and third readings was used in subsequent analysis (in line with other cardiovascular studies).

3.5.2 Pulse Oximetry

Pulse oximetry (PO) is a quick, non-invasive test that can easily be performed in practice with relatively little training. An awareness of the instrument, its methods and its drawbacks is a vital part of using the instrument effectively in practice. The pulse oximeter is a simpler version of the laboratory-based multi-wavelength co-oximeter, often considered the 'gold standard' for oximetry measurements[221]. A blood gas analyser requires actual blood samples to determine oxygenation levels, so the pulse oximeter is advantageous in that it provides a non-invasive instantaneous reading[222]. A pulse oximeter uses light of known wavelengths to penetrate through a cutaneous vascular bed such as the earlobe or finger in order to give the level of oxygen saturation in the bloodstream. In-depth discussions on the theory behind pulse oximetry have been published elsewhere[221, 222]. Essentially, light (typically in the red/near-infra-red portion of the spectrum) is transmitted at two wavelengths, designed to be at the peak of the absorption spectrum for either oxyhaemoglobin (O_2Hb) or reduced (deoxy-) haemoglobin (Hb). The wavelengths – generally 660nm (where Hb absorbs light approximately ten times more than O_2Hb) and 940nm (where O_2Hb absorbs more than Hb) – have the added benefit that they are able to penetrate tissues deeper than other parts of the visual spectrum. A comparison of the transmission data for the two wavelengths can then be used, by route of a ratio between the signals, to determine a level of oxygen saturation, or the functional saturation. The pulse oximeter is limited in it's ability to give precise levels of oxygen saturation because of it's method of sampling. The co-oximeter as well as measuring levels of O_2Hb and Hb also measures two other substances; carboxyhaemoglobin (COHb) and methaemoglobin (MetHb), giving fractional saturation. In a young, healthy, non-smoking individual, the levels of both COHb and Hb are minimal (less than 1%), hence the pulse oximeter works as a good screening instrument; operating on the assumption that of the four components, only O_2Hb and Hb are present in significant levels. However, both COHb and MetHb interfere with the readings generated by a pulse

oximeter as they also interact with the transmitted light wavelengths. This means that in case where there is excessive COHb (particularly in heavy smokers) or MetHb (through pharmaceuticals such as anaesthetics or nitrates), the reading generated by a pulse oximeter may not be a true reflection of the levels of oxygen in the bloodstream. COHb absorbs light from the 660nm source similarly to O_2Hb and so if present in supra-normal concentrations in the bloodstream, can lead to an over-estimation of oxygen saturation on the pulse oximeter. MetHb has a similar absorbance of 660nm to Hb, but crucially it also causes a greater impedance of the 940nm source. This has an additive effect at driving the overall ratio of signals between the two sources towards one, thus giving an under-estimation of the oxygen saturation level.

Other artefacts present in the bloodstream can also influence pulse oximetry readings. Patients with hyperbilirubinaemia will have higher levels of COHb due to an increase in carbon monoxide produced by the breakdown of haemoglobin. Intravenous dyes can also reduce oximetry measurements. Methylene blue can reduce levels to as low as 1%, though dyes encountered in optometry are much less (indocyanine green) or even negligible (fluorescein)[221].

In an optometric setting however, where the oximeter would simply be an auxiliary measurement, these drawbacks and limitations are of limited significance, but an awareness of the potential pitfalls is undoubtedly necessary, particularly in key patient groups, e.g. heavy smokers. The pulse oximeter is standardised to a calibration curve developed using a cohort of young, healthy adults. Because studies cannot ethically induce extreme levels of hypoxia onto participants, this calibration curve is extrapolated, meaning that it's efficacy with very low levels of oxygen saturation will be less (and indeed reports have shown that with low levels of oxygen saturation, the pulse oximetry levels show significant bias). Of note for clinical practice is that patients encountered routinely by optometrists are from all age ranges, with many suffering a variety of pathologies. This can be considered to be a drawback of the pulse oximeter, since measurements of an elderly patient will not equate to those of the young calibration cohort. However, the measurements (when taken on a routine basis) will be an individual measure and whilst comparisons across the patient base will not be possible, comparisons for an individual patient may highlight changes to the cardiovascular system between examinations.

Many modern pulse oximeters also feature a plethysmograph, which monitors the change in volume of the area being measured to provide a surrogate measure of beats per minute. This measure gives a good indication of a patient's pulse, however if systemic blood pressure is being measured in the consulting room, then this measure, which is known to have a number of confounding variables, is not of

vital importance[221].

3.5.2.1 Pulse Oximetry protocol for current study Since there are no guidelines for the recording of fingertip pulse oximetry, a routine was standardised for the present study. A digital finger-tip pulse oximeter (RisingMed RPO-8B) was applied to the right index finger (or nearest available digit) for a minimum of 30 seconds, or until both the oximetry and pulse rate readings stabilised. Patients were encouraged to rest their hand on the desk and refrain from talking as the measurement was taken. The measurement was taken in ambient room lighting.

3.5.3 Cardiovascular Risk Calculators

The Mayo Clinic has released an online risk calculator which requires simple health and lifestyle metrics to be entered in order to produce a quantifiable risk 'score' of developing cardiovascular disease. The calculator is based on an amalgamation of algorithms from a range of Framingham-based calculators; Framingham Heart Study Cardiovascular Disease 10-Year BMI-Based Risk Score Calculator, Framingham Heart Study General Cardiovascular Disease 30-Year Lipid-Based and BMI-Based Calculators, and ACC/AHA Pooled Cohort Equations CV Risk Calculator. Since the baseline Framingham Study was run in the United States between 1968 and 1975, it is perhaps not surprising that it's calculations have been shown to over-estimate risk in European populations[223, 224]. To address this, a UK-based calculator, QRISK, was devised based on contemporary data obtained nationwide through GP surgeries to reflect the varying regional and ethnic pathology incidences[225]. This was expanded to include a number of additional risk factors including Type II diabetes mellitus, treated hypertension, rheumatoid arthritis, renal disease and atrial fibrillation[226]. Risk stratification in a sample cohort (also of UK-based data) resulted in 41.1% of Framingham-derived 'high-risk' subjects being down-classified to 'low-risk', with a subsequent 10-year observed risk of 16.6%, reflecting increased classification accuracy . This has resulted in its incorporation into NICE Guidelines as a clinical tool[227, 23]. A newer iteration of the calculator (QRISK3) was released in 2018, however NICE guidelines have yet to be updated to incorporate this. The updated algorithm incorporates more condition-specific parameters which correlate with an increased CVD risk including; migraine, systemic lupus erythematosus (SLE), use of atypical antipsychotic and corticosteroids, severe mental illness and erectile dysfunction[228]. The new calculator also requires the standard deviation of two or more blood pressure readings to reflect blood pressure variability.

A more specific risk calculator for risk of progression of diabetic retinopathy (the RetinaRisk Calcu-

lator) can also be applied to those participants with existing diabetes mellitus. The RetinaRisk app is a smartphone-based system which allows for practitioners to individually stratify patients‘ risk of developing sight-threatening diabetic retinopathy[229]. ‘Sight-threatening’ DR was defined as proliferative retinopathy and/or clinically significant macular oedema. The algorithm has been validated against cohorts across Europe showing a similar level of accuracy and reduction in recommended visits for controlled cases when compared to fixed-interval annual screening[230, 231, 232]. When re-calibrated to quantify risk based on the ‘at risk’ categories in the UK’s grading system for DR, the calculator offered a 40% reduction in screening frequency compared to fixed-interval annual screening, posing a significant cost-saving potential[233]. Integration of risk calculators into optometric practice simply requires adding the necessary questions into routine history and symptom taking at the beginning of an eye examination.

3.5.3.1 Cardiovascular Risk Calculator protocol for current study In order to fulfil the criteria of the risk calculator, the specific data required was built into an extended history and symptoms with participating patients. The questions were not always optimum for an ophthalmological focus. For instance, questions pertaining to recent cigarette smoking ask if more than 100 cigarettes have been smoked in the last year, whereas it will be within a shorter time-period that cigarette smoke and nicotine will affect retinal vessel calibre (less than 24 hours). However, since the calculator is designed to quantify risk of cardiovascular disease, the questions were asked as stated in the calculator itself. These are shown in Appendix 5. Patient Questionnaire.

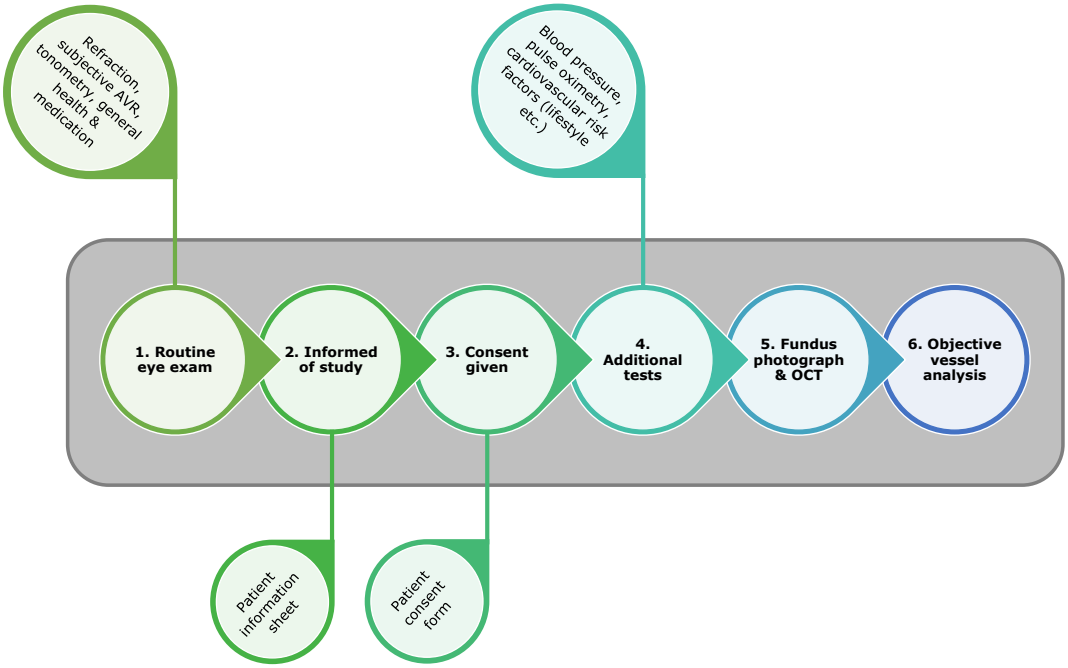


Figure 19: A flow-chart of methods for patient enrolment.

4 Repeatability and Variability in Objective Static Retinal Vessel Calibre Measurement

4.1 Introduction

A photograph captures the retinal vascular tree at a single point in time, with measurements taken only applicable to that particular moment. Before cross-sectional or longitudinal correlations between vessel calibre and ocular and systemic parameters can be explored, the validity of individual measurements needs to be determined. The cardiovascular system is dynamic, with pulse waves travelling throughout the body following cardiac systole. Indeed, spontaneous pulsation of the central retinal vein is often directly observable on ophthalmoscopy; a physiologically normal sign in approximately 90% of patients[83, 234]. The muscular anatomy of the arterial system particularly, as outlined in Chapter 1.6 (The Cardiovascular System: gross anatomy) on page 28, goes some way in maintaining the pressure wave that propagates in the ventricles of the heart. The comparative lack of muscular tunic in the venular system means it is less adept at responding to significant pressure changes and predominantly relies on autoregulatory vasodilation (through mediators such as nitric oxide and prostaglandins). These distinct anatomies mean that vessel diameters are not absolute and raise the question whether a single photograph can be affected by this. The cardiac pulse cycle and associated changes therefore become a consideration when measuring retinal blood vessel calibre statically (i.e. through photographic analysis). Previous studies, through various setups with fundus cameras attached to electrocardiograms (ECGs), demonstrate that it is possible to photographically measure significant changes in vessel diameters through the cardiac cycle[235, 236]. Chen et al. suggest three factors which can cause transient changes in retinal vessel diameter[235];

- **pulsatility** (due to direct changes in blood pressure through the cardiac cycle);
- **autoregulation** (a chemically-mediated change in vessel diameter in response to more pronounced / prolonged periods of pressure changes to ensure constant rates of perfusion);
- **vasomotion** (oscillative changes in vessel diameter that occurs independently from changes in blood pressure or the cardiac cycle).

Whilst their studies took place prior to the advent of the objective technique being employed here (and the authors do not elucidate how measurements were accurately obtained in μm) the percentage changes

in vessel diameter across the cardiac cycle offer confirmation that the vessel diameter *does* alter in line with stages of the cardiac cycle. When a similar study was run by Knudtson et al. in 2004, they observed that only large venules (most likely due to their size and significant contrast to surrounding retina) had a significant change in diameter when linked with pulse cycle[236]. However, it should be noted that in this particular study, images were captured on a Canon D-30 digital camera, with a resolution of only 3.0 mega-pixels (MP). Given that vessels of approximately 75µm were being evaluated, camera resolution has the potential to significantly limit quality and accuracy of such measurements. Pauli et al. demonstrated that compared to a 7MP reference image to a 3MP and 5MP version, there was an apparent 'narrowing' of vessels with decreasing image resolution[237]. For CRAE this was seen as a change from -1.57µm to -2.77µm. A larger variation was seen in CRVE, with a 5MP image underestimating vessel calibre by -1.93µm, raising to -4.89µm in a 3MP image. Compensation for cardiac cycle has also been demonstrated in cases of externally induced diameter change (e.g. isometric exercise, O_2 inhalation), although it was shown that if the effect on vessel diameter was significant enough, such as in the case of 60% O_2 inhalation, it was significant even when cardiac cycle was *not* compensated for[238]. Few studies have addressed the question of room illumination on vessel diameter measurements, and those which have tend to focus on the alteration in calibre measurements as subjects are exposed to sudden changes in ambient lighting (e.g. from dark to bright illumination). Of those studies, there appears to be a mild reduction in venular calibre when subjects are dark adapted (in the order of 1.5-2.8%)[239, 240]. Volumetrically this will have an increased effect on blood flow (the primary driving force for the change, responding to increased O_2demand), however if conditions for imaging subjects are kept constant, this effect should be distributed evenly across a cohort. Ambient illumination, as well as directly affecting diameters, also impacts on pupil size, itself another variable in determining image quality. There is limited evidence around the impact of pupil size on retinal vessel measurements, however studies involving the grading of diabetic retinopathy (DR) have been more extensive. Gradability is the ability to detect signs of DR, which includes small signs such as microaneurysms. Gradability can, therefore, be taken as a reasonable surrogate for ability to objectively analyse an image; i.e. vessel calibre measurements. It has been shown that there is a clear correlation between mydriasis and a greatly reduced number of ungradable images. Up to one quarter of images taken in non-mydriatic conditions were classified as ungradable, which reduced to less than 5% with the use of pharmacological dilation[241, 242]. Both of these studies reported that media opacity (i.e. cataract) was the leading cause of ungradable images when acquired under mydriasis (78% had early-moderate cataracts[241]), and subjects over the age of 40

years have been reported to be increasingly less likely to produce gradable images when not dilated[243].

A number of variables were identified which could impact on retinal vessel measurements and are summarised in Figure 20. Firstly, to reflect standard UK practice whereby patients are not routinely dilated, the use of non-mydriatic photography needs to be compared with dilated images from the same subjects. If non-mydriatic imaging is to be used and compared with the findings of large cohort studies which employed pharmacological dilation, there needs to be no significant difference in vessel measurements for both conditions. Secondly, to determine if a single image is sufficient, or whether multiple images need to be acquired for an individual. This relates to the dynamic nature of the microcirculation and ultimately depends upon the sensitivity of the measurements; is the current approach of static retinal vessel analysis accurate enough to detect minor fluctuations in vessel diameter arising from the cardiac pulse cycle? For a single image to be sufficient, there needs to be minimal variation in vessel calibre across a sequence of images taken in quick succession. An eye examination considers the ocular state at that particular visit, and is making assumptions that there has been little or no change in the time preceding and following the appointment. The third variable to consider is how stable vessel calibre measurements are over short periods of time; do they change day-to-day? For static retinal vessel analysis to be utilised in clinical practice, the measurements need to be consistent over short time scales (in order to then identify clinically significant changes between examinations). This means that there should be no statistically significant difference in measured vessel calibre for an individual over an interval of several days.

As well as image acquisition, the image analysis process also produces a number of potential variables. The selection of vessels themselves, influenced by the internal edge detection algorithm within the software, needs to be independently verified to demonstrate that it is capable of repeatedly producing the same measurement from the same selected vessels. Additionally, the formulae used to transform the individual vessel measurements into CRAE and CRVE values have been previously shown to influence the outcome value[115, 114]. Whether this is also the case in a clinic setting needs to be determined, as different software platforms employ the different algorithms. Finally, the grader themselves could potentially introduce variability to the measurements produced. Experienced graders are often employed in studies, whereas this may not be the case in practice (particularly in the early days of application, where the primary clinician will also be inexperienced). How sensitive, therefore, is objective vessel analysis to grader experience? These three image analysis variables can be investigated by using a single image but analysing it multiple times; any significant differences highlighting variables which will need

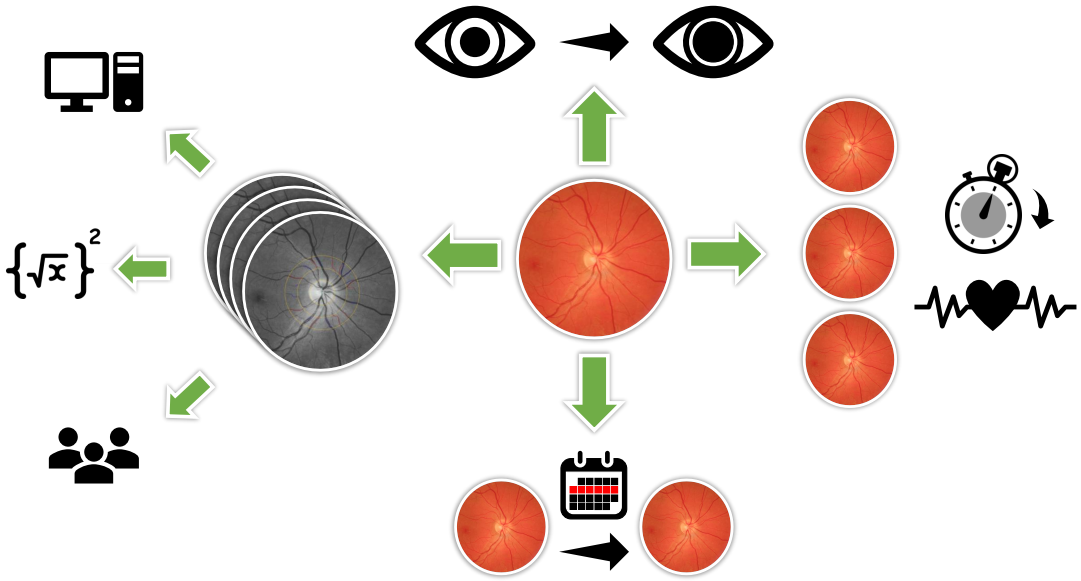


Figure 20: A summary of the potential variables encountered during image acquisition and analysis (Clockwise from top: i) effect of pupil dilation: undilated vs. pharmacologically dilated; 2) cardiac pulse cylce and small-scale temporal variations; 3) moderate temporal variations; 4) image anaylsis: software-related intra-image variability, formulae used and inter-observer agreement).

accounting for in future work.

4.2 Methods

All participants were enrolled from the prospective cohort study (*see Chapter 5. Correlations Between Retinal Blood Vessel Calibre and Cardiovascular Metrics Observed in Optometric Practice*) and had provided signed consent. The methodology study was covered by the over-arching ethics application which was approved by Aston University (*see Appendix Appendix 1. Ethical Approval*) and conformed to the tenets of the Declaration of Helsinki.

Participants had blood pressure and non-contact tonometry performed prior to assessment. All subjects had a thorough eye examination before participation to exclude underlying ocular pathology.

4.2.1 Effect of dilation

Each subject was seated in the dark for 5 minutes in order to achieve maximal natural pupil dilation. Pre-mydriatic pupil diameters were all larger than the minimum specified by the camera (2.50mm[183]);

subjects' pupils having been measured prior to imaging. Subjects with a pre-mydriatic pupil size less than 2.50mm in either eye were excluded from the study.

4.2.2 Sequential imaging

Ten, 45º disc-centred full-colour fundus images of the right eye were captured with a Topcon 3D Maestro (Visit 1). The images were acquired in rapid succession (although each individual photograph had to be manually captured). In cases where the pupil diameter was small, a pause of up to 30-seconds was employed to allow some recovery dilation. After the images were acquired, 0.5% Tropicamide (Bausch & Lomb) was instilled into the right eye. Following full pharmacological mydriasis, subjects were then re-imaged. A further 10 disc-centred photographs were taken. For each subject, a total of 20 images were captured; 10 undilated, and 10 dilated.

4.2.3 Repeated imaging

The procedure of non-mydriatic imaging followed by dilated imaging was repeated one week later (Visit 2), at the same time of day as Visit 1. Following completion of imaging, a total of 40 images were acquired for each subject; 20 from Visit 1 and 20 from Visit 2.

4.2.4 Image analysis

All images were exported both in full-colour (for vessel identification) and red-free (for analysis) in the maximum uncompressed file format size (Tagged Image File Format; TIFF). These were then imported into VesselMap v3.0 (Imedos Systems, Jena) and objective analysis was performed on all of the images acquired. Initial grading was performed by an experienced grader (CF), having trained on a sample bank of >150 unrelated images. To explore variability in image analysis, the sharpest dilated and undilated image of the sequences from Visit 1 were selected; reflecting a practice-based scenario. In order to evaluate inter-grader experience and variability, a trainee with no prior exposure to the software was recruited to also analyse the images (KH). The grader was shown the software's instruction and grading protocol, and a single image was graded as a demonstration. The trainee then graded the same bank of undilated images (to reflect image type most commonly found in clinical practice). The experienced grader was not present for this analysis. Summary CRAE, CRVE and AVR values were also calculated using the Knudtson formula for comparison. These were calculated externally to VesselMap, using the absolute vessel measurements from each image and selecting the six largest of each type (as per the formula).

Knudtson values were calculated from a single image (first undilated and dilated images on Visit 1) and average vessel measurements across the sequence of 10 images from Visit 1 (undilated and dilated).

4.2.5 Statistical analysis

Statistical analysis was performed using SPSS v25.0 (IBM). Since the same population was used for different variables, paired sample t-tests were used to compare measurements for undilated and dilated images, measurements from Visit 1 and Visit 2 and measurements produced from both Parr-Hubbard and Knudtson formulae. These were also plotted graphically using Bland-Altman difference-versus-mean plots. Coefficients of variation were used to quantify variance across the sequence of 10 images (to demonstrate cardiac pulse cycle) and across a sequence of 10 analyses of the same image (to demonstrate difference in vessel selection). Paired t-tests were then employed to compare the coefficients of variation for both undilated and dilated conditions. Finally, Pearson's correlations were used to compare the measurements taken by the experienced and trainee graders. Significance was set at $p = < 0.050$.

4.3 Results

Twenty Caucasian subjects participated in the study; 9 male and 11 female. All participants were free from cardiovascular pathology. Age ranged from 23 to 61 years (average 40.1 years ±13.7). Refractive error ranged between +1.00 and -7.75D (MSE) (average -1.20D MSE ±2.16). Blood pressure (systolic and diastolic) averaged 116mmHg (±11.0) and 73mmHg (±9.3) respectively, with an average beats per minute (BPM) of 68.6 (±9.3). Of the 800 images acquired, 7 were deemed ungradable due to image quality. Vessel calibre measurements were normally distributed; confirming the assumptions used in paired samples t-test models.

4.3.1 Effect of dilation

Average undilated baseline CRAE was 178.8µm (±15.8); CRVE was 211.0µm (±15.3) and AVR was 0.85 (±0.06). Average mydriatic baseline CRAE was 176.7µm (±15.8); CRVE was 210.0µm (±17.7) and AVR was 0.84 (±0.07). 7 images were ungradable due to image quality, six (85.71%) of which were acquired undilated. For Visit 1, paired samples t-tests revealed a significant difference between CRAE measurements taken in undilated and dilated conditions ($t(19) = 2.170$, 95% CI 0.07 to 4.07, $p = 0.043$), however both CRVE and AVR measurements were statistically insignificant ($p = >0.050$). For Visit 2, there was no significant difference between measurements taken from undilated or dilated images for CRAE, CRVE or

AVR (p = >0.050). Bland-Altman difference-versus-mean plots show a mean bias of +2.07µm (Limits of Agreement: ±8.38) and +1.13µm (Limits of Agreement: ±5.00) for CRAE on Visit 1 and Visit 2 respectively. For CRVE, a mean bias of +1.02µm (Limits of Agreement: ±10.70) and +0.86µm (Limits of Agreement: ±8.27) were found on Visits 1 and 2. These are illustrated in Figure 21.

4.3.2 Cardiac pulse & small-scale temporal variations

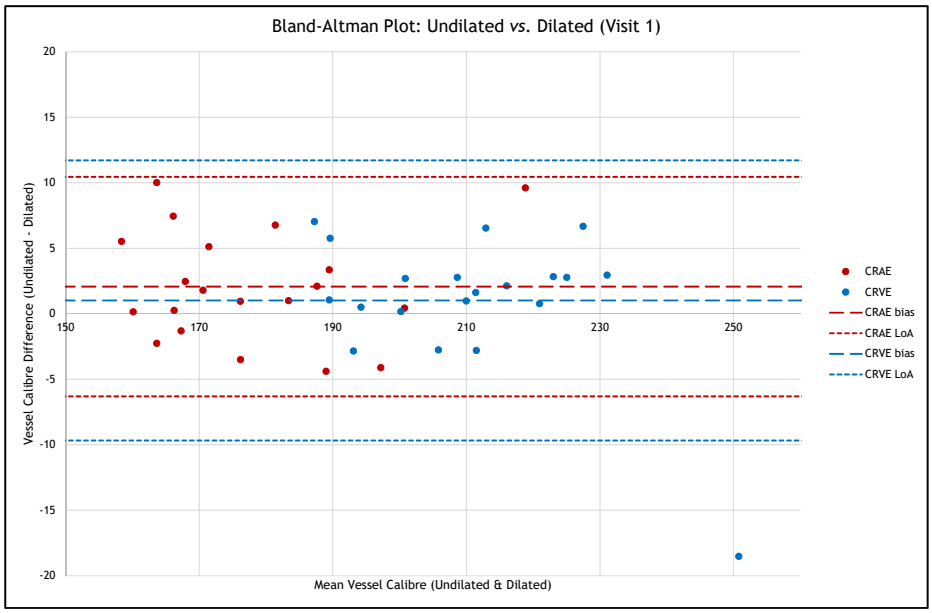
Across a sequence of 10 images for each subject, undilated CRAE measurement range varied, on average, by 16.19µm per individual, with an average range of 13.53µm when dilated. For CRVE, the average range of undilated measurements was 13.38µm, and dilated range was 12.71µm. Mean coefficient of variation for CRAE was 2.62% (±1.31; 95% CI 2.05 to 3.20) undilated, and 2.24% (±0.89; 95% CI 1.85 to 2.63) for dilated. For CRVE, mean coefficient of variation was 1.93% (±0.88; 95% CI 1.55 to 2.32) and 1.84% (±1.00; 95% CI 1.40 to 2.28) for undilated and dilated conditions respectively. A paired samples t-test comparing undilated and dilated coefficients of variation across an image sequence showed no significant difference on Visit 1 for CRAE or CRVE, and only borderline significance for CRAE on Visit 2 ($t(19) = 2.21$, $p = 0.043$); CRVE measurements for Visit 2 showed no significant difference between dilated or undilated.

4.3.3 Moderate temporal variations

When comparing results from Visit 1 and Visit 2, paired samples t-tests (for both undilated and dilated measurements) were non-significant for all three variables; CRAE, CRVE and AVR (p = >0.050). Bland-Altman difference-versus-mean plots (see Fig 22) for both undilated and dilated measurements demonstrate a mean bias of -0.46µm (Limits of Agreement: ±10.21) and -1.41µm (Limits of Agreement: ±7.94) for CRAE, and -1.62µm (Limits of Agreement: ±9.07) and -1.77µm (Limits of Agreement: ±11.49) for CRVE.

4.3.4 Image analysis and intra-image variability

4.3.4.1 Vessel selection Repeat analysis of a single image yielded an average intra-image measurement range of 9.56µm and 5.55µm for CRAE (undilated and dilated), and 5.10µm and 4.19µm for CRVE (undilated and dilated). Mean coefficient of variation for CRAE was 1.48% (±1.01; 95% CI 1.04 to 1.92) (undilated) and 0.92% (±0.56; 95% CI 0.68 to 1.17) (dilated), and for CRVE was 0.70% (±0.45; 95% CI 0.50 to 0.89) (undilated) and 0.57% (±0.38; 95% CI 0.40 to 0.74) (dilated). A paired samples t-test between undilated and dilated coefficients of variation was significant for CRAE ($t(19) = 2.58$, $p = 0.018$) but insignificant for CRVE (p = >0.050).



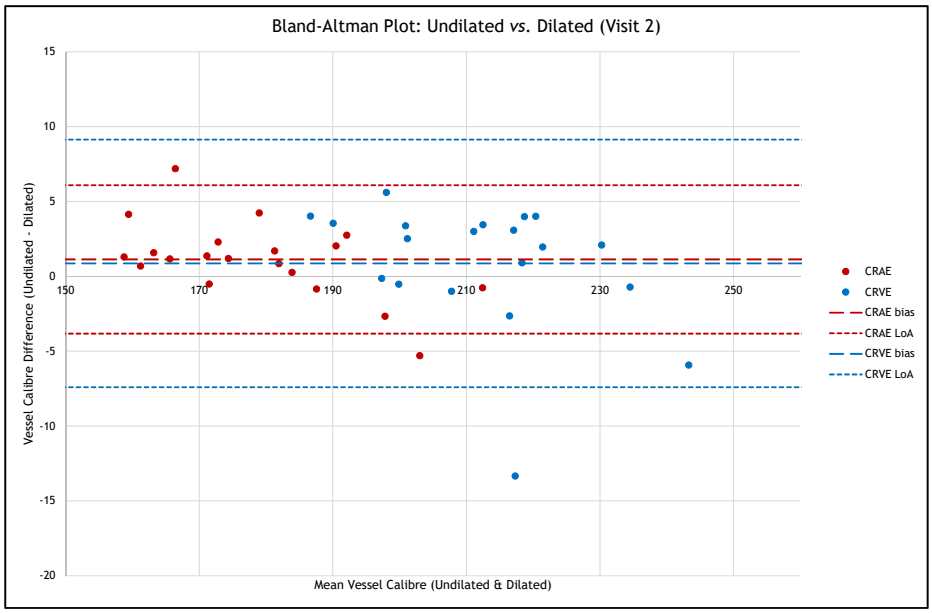
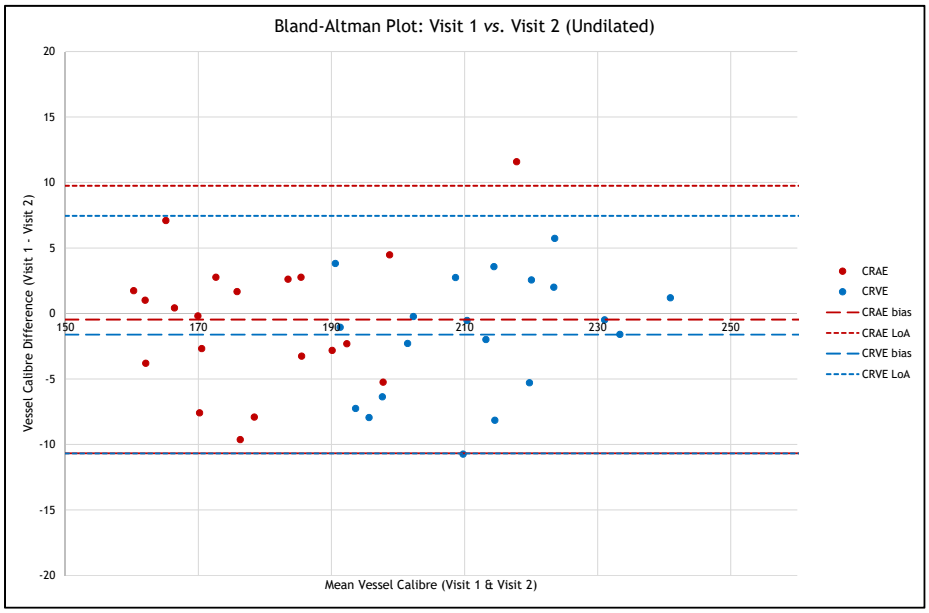


Figure 21: Bland-Altman difference-versus-mean plots showing the bias between CRAE and CRVE measurements taken from images when subjects are either undilated or dilated for Visit 1 (*upper panel*) and Visit 2 (*lower panel*). CRAE results are recorded in red, and CRVE in blue. In both graphs, note the mean bias as shown by the longer dashes, and the upper and lower limits of agreement (LoA) denoted by the smaller dashes.



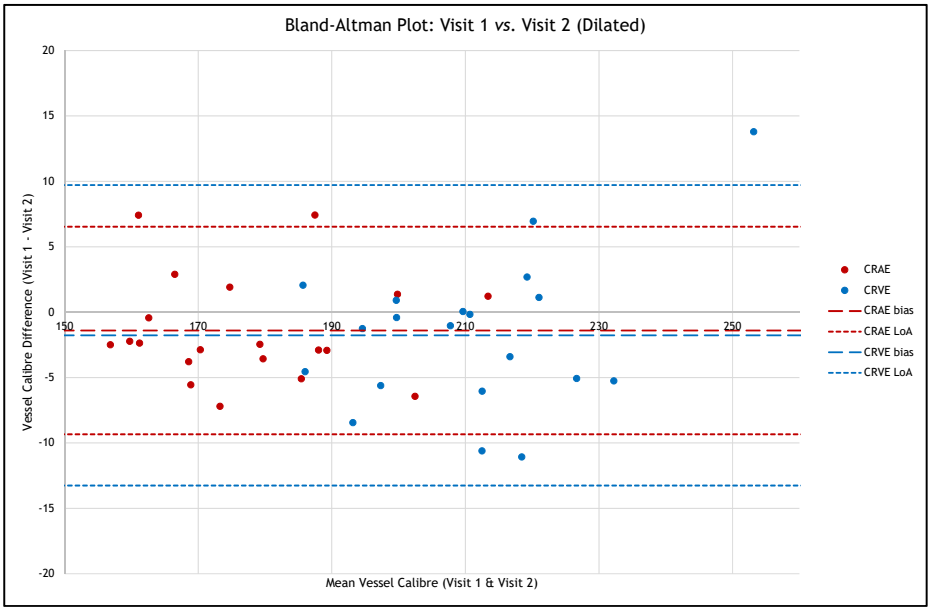


Figure 22: Bland-Altman difference-versus-mean plots showing the mean bias between CRAE and CRVE measurements taken of subjects on Visit 1 and Visit 2. Results are shown for both undilated (*upper panel*) and dilated (*lower panel*) conditions. CRAE results are shown in red, and CRVE in blue. Mean bias is denoted by longer dashes, and upper and lower limits of agreement (LoA) shown by smaller dashes. Note the near-coincident lower limits of agreement for CRAE (-10.67μm) and CRVE (-10.69μm) on the upper panel.

4.3.4.2 Formulae Using Knudtson formulae and comparing with the original Parr-Hubbard measures produced by VesselMap, paired t-tests revealed highly significant differences for all measures (p = <0.001); CRAE, CRVE and AVR across condition types (undilated, dilated, single image, average across image sequence). When the difference in calculations (Parr-Hubbard versus Knudtson) for each subject was compared to the number of vessels included in their Parr-Hubbard calculation, there was no significant correlation between number of arteries and CRAE or AVR (p = >0.050), however there was a significant correlation between the number of veins selected and difference in AVR between the two formulae (p = <0.003 in all conditions), which is summarised in Table 20.

	Single Image					
	Undilated			**Dilated**		
	CRAE	**CRVE**	**AVR**	**CRAE**	**CRVE**	**AVR**
Arteries	*0.096 (n.s.)*	*-0.076 (n.s.)*	*0.191 (n.s.)*	*0.041 (n.s.)*	*-0.88 (n.s.)*	*0.076 (n.s.)*
Veins	0.531 (0.016)	*-0.145 (n.s.)*	0.692 (0.001)	0.445 (0.050)	*-0.132 (n.s.)*	0.678 (0.001)
	10-Image Average					
	Undilated			**Dilated**		
	CRAE	**CRVE**	**AVR**	**CRAE**	**CRVE**	**AVR**
Arteries	*0.197 (n.s.)*	*-0.029 (n.s.)*	*0.228 (n.s.)*	*0.005 (n.s.)*	*-0.075 (n.s.)*	*0.008 (n.s.)*
Veins	0.526 (0.017)	*-0.144 (n.s.)*	0.632 (0.003)	0.493 (0.027)	*-0.092 (n.s.)*	0.778 (<0.001)

Table 20: Pearson correlations between difference in vessel calibre (depending on formulae applied) and number of vessels included. Significance shown in parentheses. Values in italics are non-significant (p = >0.050).

Comparison of the two formulae (Parr-Hubbard and Knudtson), based on measurements taken from undilated photographs (Visit 1) is demonstrated graphically in the Bland-Altman plot in Figure 23.

4.3.4.3 Observers There was a strong correlation for measurements taken by an experienced grader and trainee. Pearson correlations (r) were similar for all three measurements; 0.87 (CRAE), 0.87 (CRVE) and 0.84 (AVR). Bland-Altman difference-versus-mean plots for experienced grader versus trainee measurements demonstrate a mean bias of +5.03µm (Limits of Agreement: ±19.10), +6.13µm (Limits of Agreement: ±14.71), and -0.002 (Limits of Agreement: ±0.01) for CRAE, CRVE and AVR respectively. These are shown below in Figure 24.

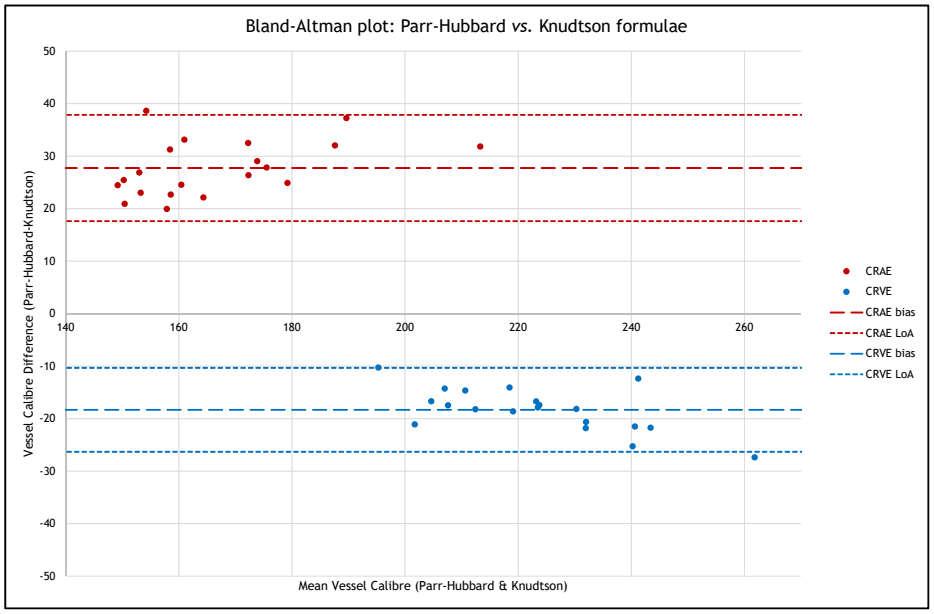


Figure 23: Bland-Altman difference-versus-mean plots showing the mean bias between undilated CRAE and CRVE measurements generated by the original Parr-Hubbard and revised Knudtson formulae. CRAE results are shown in red, and CRVE in blue. Mean bias is denoted by longer dashes, and upper and lower limits of agreement (LoA) shown by smaller dashes. Note that vessel measurements (artery and vein) appear to change in opposite directions.

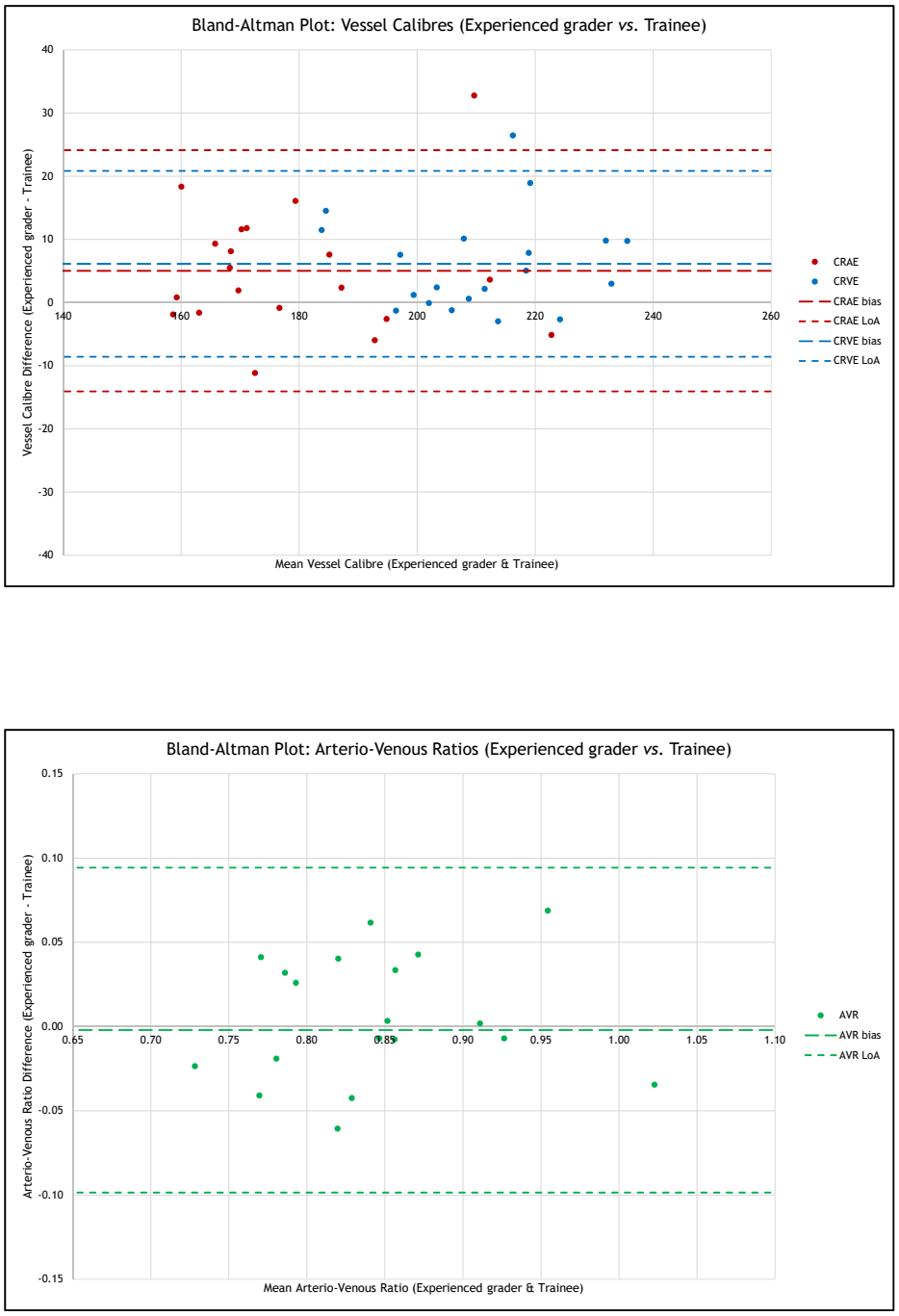


Figure 24: Bland-Altman difference-versus-mean plots showing the mean bias between CRAE and CRVE (upper panel) and AVR (lower panel) measurements taken by an experienced grader and a trainee. CRAE results are shown in red, CRVE in blue and AVR in green. Mean bias is denoted by longer dashes, and upper and lower limits of agreement (LoA) shown by smaller dashes.

4.4 Discussion

Retinal imaging is becoming a routine element of an eye examination in the UK, and since pharmacological dilation is not typically performed without prior indication, it is important to establish whether image analysis techniques are as robust without mydriasis. Much of the research around retinal vessel calibres was performed under mydriasis, and indeed the manufacturer's recommended protocol for image analysis software suggests patients be dilated. To this end, image quality, and by extension accuracy of vessel calibre measurements, needs to be maximised to give as little variation as possible. Paired samples t-tests highlight that both CRVE and AVR are not significantly influenced by the presence or absence of pharmacological dilation. For CRAE, there appeared to be a significant difference in measurements on Visit 1 but not on Visit 2. Difference-versus-mean plots for undilated vs. dilated (Figure 21) show on both days however that CRAE has narrower spacing of limits of agreement than CRVE (±8.35µm vs. ±10.70µm respectively for Visit 1). Whilst there was a slight increase in the mean bias for CRAE (+2.07µm) compared to CRVE (+1.02µm), consideration then needs to be made for what is considered to be *clinically* significant over *statistically* significant. A similar (slightly larger) level of agreement has been demonstrated by photographing the same retinae on different cameras, where CRAE had a mean bias of +0.17µm (Limits of Agreement: ±10.15) and CRVE a mean bias of -2.32µm (Limits of Agreement: ±11.76)[244]. The difference shown to be statistically significant easily fall within the variation demonstrated by multiple imaging under the same conditions, where variation for an individual averaged a range of 16.19µm. Interestingly, the spacing of the Limits of Agreement is considerably less than those reported in a large-scale study comparing the agreement between two other measurement platforms (SIVA and VAMPIRE)[193]. Whilst the cohort used here is much smaller, a cross-platform comparison including VesselMap is required to further explore this difference.

Variation across a sequence of images taken in quick succession should be considered to determine whether a single image (and subsequent vessel measurement) is sufficient in clinical practice (for instance, multiple readings are required for non-contact tonometry). Few studies have looked at the variation of vessel measurements across a sequence of isolated images, with most studies instead looking at video recordings, such as those taken with the Dynamic Vessel Analyzer (DVA, Imedos Systems, Jena)[239, 245]. A study using photographs from a similar number of subjects (n = 32) by Von Hanno et al. found a very similar level of change in vessel diameter change for CRVE (1.7% over 6 images with prior exposure to light); however they found there was an insignificant change in CRAE[240]. The very low coefficient of variation across a sequence, in the region of 3%, is additional evidence to support the

continued use of a single photograph for analysis. Even when taking the single-most largest recorded vessel in the present study (CRVE: 272μm; Subject PM), a 3% variation (±8.16μm) in individual vessel measurement still falls within the limits of agreement demonstrated in the Bland-Altman plots above. These findings support the work of Fuchs et al. who demonstrated that blood pressure at the time of image acquisition does not have a transient impact on vessel calibre[246].

A difference in vessel selection for arteries and veins could possibly explain the difference in significance levels for their measurements. A study into reproducibility of vessel calibre measurements from images taken on different (non-mydriatic) cameras also reported a more significant variation in CRVE measurements compared to CRAE[244]. This would be contrary to the theory that veins are easier for a software algorithm to define because of the greater colour contrast between the deoxygenated blood column and surrounding pigmented retina[169].

4.4.1 Study limitations

It should be noted that the cohort used was exclusively Caucasian, thus a multi-ethnic sample needs to be considered in order to establish whether these relationships exist consistently before these findings can be considered definitive and used in practice. Anatomical differences in vessel architecture are unlikely, however the role of retinal pigmentation and subsequent contrast with retinal vessels seen with varying ethnicities may influence measurement accuracy and consistency. A subsequent study should also explore the role played by iris pigmentation, to identify whether greater variability exists on an ethnicity basis or more locally in terms of levels of ocular pigmentation.

Additionally, the present study only examined those free from pathology (both systemic and local to the ocular circulation). Pathologies affecting the vasculature may impact on the variability of measurements, particularly in cases of endothelial dysfunction. Targeted methodological studies which run a similar experiment with pathological samples will establish whether the limits of clinical significance described above are the same even in diseased vessels.

Imedos (the developers of VesselMap) do not offer a structured training program in order to use their software (by contrast, SIVA host a several weeks long training course in Singapore for those wishing to use their software) which makes definition of an 'experienced' grader difficult. This could have been mitigated with more rigorous assessment of the trial bank of images analysed by the grader (it should be noted that the study by Neubauer and colleagues adopted a similar approach to train graders; selecting an arbitrary bank of >100 unrelated images to analyse[187]). Comparison (by means of a paired samples t-

test) of vessel calibre measurements from the first images within the sample bank to measurements from the same images following completion of the entire training bank to statistically demonstrate whether any measurable learning effect exists would have been a simple solution to this problem. It should be noted, however, that the results from intra-image analysis go some way in demonstrating that there is very little (0.57-1.48% coefficient of variation) difference in the calibres measured in an image when the same vessels are selected multiple times. Another advantage of adopting the Parr-Hubbard approach is that all vessels measuring >40µm are selected within the measurement annulus, eliminating the need for a subjective selection of the six largest vessels, leaving just the variation within the software analysis package to impact on the final measured calibre.

4.4.2 Conclusion

Despite the lack of ethnic diversity amongst the present sample, the population used is otherwise reflective of that encountered in routine optometric practice. A range of ages, refractive errors and pupil sizes (although not measured beyond checking they were above the minimum required diameter) demonstrates the versatility of this approach when applied to the consulting room. Further work will need to be undertaken to explore whether the presence of cardiovascular disease has a significant impact upon these findings. Such studies are potentially more difficult to undertake in optometric practice, as they require recruited subjects to present specifically as part of the study; in the present case, on multiple occasions. However, it forms a positive method of patient retention since they are having a greater level of interaction with the practice and their clinician, Overall patient interest and satisfaction after taking part in the present study was, anecdotally, high as they felt a positive return from partaking in research about their own eye examinations.

VesselMap applies the Parr-Hubbard formulae vessel measurements, however more recent publications have applied revised formulae proposed by Knudtson et al.[114]. Manual calculation with the revised formulae revealed altered vessel calibres in the same region as those originally outlined in Knudtson et al.'s paper. The authors reported changes in calculated CRAE (-19.7µm) and CRVE (+12.6µm); these were of a similar magnitude in the present study for both CRAE (-27.7µm) and CRVE (+18.3µm). This bidirectional change would result in an overall lower AVR, more in line with the classically accepted 0.67 or $\frac{2}{3}$[87, 88, 31].

It has been shown that, for a Caucasian sample, free from cardiovascular disease, static retinal vessel analysis can be easily performed in clinical practice, without the need for pharmacological dilation

(unless otherwise indicated) or the need for multiple images. Furthermore, aside from improved image quality, there does not appear to be a significant impact on measured retinal vessel calibre with the use of dilating agents. The use of a single image to obtain measurements also improves it's clinical application; negating the need for averaging of multiple measurements which does not lend itself to a busy clinic environment. The robustness of measurements acquired several days apart also adds strength to the measurements taken and suggests that they are representative of the subject's retinal microcirculation at the time of examination. The repeatability of analysis software has also been independently confirmed, demonstrating it's clinical utility regardless of grader experience. In summary, the study's findings suggest that objective retinal vessel analysis can be performed in clinic to produce results comparable with those acquired in more rigorous laboratory-based conditions.

5 Correlations Between Retinal Blood Vessel Calibre and Cardiovascular Metrics Observed in Optometric Practice

5.1 Introduction

Cardiovascular disease is a multifaceted problem; both through aetiology and pathophysiology. It cannot always be determined through a single measurement (such as blood pressure) and the whole patient case needs to be considered. This includes both physical characteristics; such as height, weight, blood pressure, cholesterol levels etc., but also lifestyle characteristics such as diet, level of exercise and smoking status. This has been reflected in the development of cardiovascular risk calculators, since they employ a combination of measured data (such as blood pressure, blood cholesterol, heigh and weight) and anecdotal data (such as intake of fruit and vegetables and level of exercise)[247, 248]. This is leading patient care away from isolated measures, since even measurements such as Body Mass Index (BMI) have been shown to lack specificity and sensitivity when quantifying levels of obesity alone[249]. The correlations between cardiovascular pathologies and retinal blood vessels have been documented previously (*see Chapter 2 (Associations of Retinal Vessel Calibre with Cardiovascular Disease: A Systematic Literature Review)*), and whilst retinal vessel health should be recorded as part of a routine eye examination, there is currently no clinical management guidance on how this can be performed to the same standard (i.e. objective retinal vessel analysis) as shown in the literature[250]. No research currently exists to demonstrate the utilisation of objective retinal vessel analysis in routine clinical practice despite it's acknowledgement as a future direction of research[89]. In addition to the identification of cardiovascular correlations, it has been shown that the techniques involved in objective retinal vessel analysis can be performed in practice with similar levels of repeatability (*see Chapter 4 (Repeatability and Variability in Objective Static Retinal Vessel Calibre Measurement)*). It then needs to be determined whether the technique yields findings similar to those reported in the literature when performed in clinical practice. Since no management guidelines currently exist, benchmarks established for three cardiovascular pathologies (hypertension, diabetes mellitus and stroke) will be used in line with the previous literature review. Additionally, multi-variable modelling (analogous to the QRISK calculation) will be performed to explore any inter-correlations of cardiovascular metrics and retinal vessel calibre, and an attempt will be made to apply intervention criteria (or 'cut off' values) to measurements taken in order to highlight potentially 'at risk' individuals based on their retinal vessel measurements and cardiovascular risk profile.

5.2 Methods

For detailed methodological information see Chapter 3 (Materials & Methods). Cross-sectional participants were recruited throughout the study course, with data from their baseline visit being used. All participants were recruited in optometric practice. Exclusion criteria was limited to those not covered by the ethical approval (i.e. minors and those unable to give informed consent) and those with significant media opacities resulting in poor quality and ungradeable photographs, even with pharmacological dilation. Briefly, the baseline visit involved collecting data regarding health, family history and lifestyle; refractive status; ocular health; and cardiovascular measures.

5.2.1 Patient selection

Patients were enrolled prospectively between October 2015 and September 2018 as they attended for routine eye examinations. Inclusion criteria was at least one photographable eye (with or without the aid of dilation), the ability to record systemic blood pressure and completion of an eye examination (plus additional cardiovascular-related questions). Exclusion criteria was limited to those subjects not covered by ethical approval (i.e. minors and vulnerable adults) (*see Appendix Appendix 1. Ethical Approval*).

5.2.2 Patient Health History

In line with both College of Optometrists guidelines on history and symptom collection, and also utilising questions featured in both the Mayo Clinic and QRISK cardiovascular health risk calculators, patients reported anecdotally their general health, including present medication, diagnosed conditions (and whether they were being treated) and any conditions currently undiagnosed but borderline or under investigation[251, 247, 248]. Family history was also explored with additional emphasis on cardiovascular health and occurrence of any cardiovascular events, especially in younger family members. Participants were asked lifestyle questions, including past or present smoking status, average weekly alcohol intake (approximate number of units), daily diet (approximate amount of both saturated fats and portions of fruit and vegetables per day, on a scale of '*Few*' (≤1), '*Some*' (2-4) or '*A lot*' (≥5), daily caffeine intake (*1-2 cups, 3-5 cups, >5 cups*) and weekly exercise (*Nil*; *<75 minutes vigorous/<150 minutes moderate*; *>75 minutes vigorous/>150 minutes moderate*). Anecdotal height and weight were also recorded. Examples of all categories were given to participants to improve consistency of self-reporting (*see Appendix 5. Patient Questionnaire for detail*).

5.2.3 Refractive status

All participants underwent a routine objective refraction (static retinoscopy) followed by subjective refraction by an experienced optometrist (CF). In cases of severe amblyopia or reduced vision or other instances where a balance lens was prescribed, refraction was recorded as the objective refraction obtained. Previous refractive status for participants having undergone refractive surgery (laser refractive surgery, cataract/clear lens extraction etc.,) was also noted.

5.2.4 Ocular health

Routine undilated (except in cases of poor fundal view) ophthalmoscopic examination was performed on all participants using a slit lamp biomicroscope and 78D condensing lens. Fundus photographs were then obtained after pupil diameter had recovered sufficiently following ophthalmoscopy. 45°, full colour disc- and macular centred fundus photographs were obtained using a Topcon 3D Maestro. Non-contact IOP was measured with a calibrated Pulsair 3000 obtaining three readings per eye.

5.2.5 Cardiovascular measures

Digital sphygmomanometry was performed with the subject seated at the end of the examination, with time having elapsed following non-contact IOP recording particularly (approximately 5 minutes) to allow blood pressure of subjects disliking this procedure to return to normal. Subjects were instructed to remove thick sleeves and to remain still and quiet during measurement. The cuff was applied level to the heart and three readings were taken, with a minute interval between each. The average of the final two readings was then used in analysis. If there was a difference of ≥10mmHg between readings, further readings were taken until three within this range were obtained. Heart rate (in BPM) was also recorded. Finger-tip pulse oximetry was obtained from the index finger of the right hand; applied for 30 seconds and a reading taken when measurement had stabilised.

5.2.6 Cardiovascular risk and ARIC-derived cut-offs

Cardiovascular risk calculations were determined using both the Mayo Clinic's 30-Year Heart Disease Risk Calculator and the QRISK2 calculator. The latter was employed rather than the newest for two reasons; firstly NICE guidelines have not been updated to incorporate this newer algorithm so clinical management will still be based upon the results obtained with QRISK2, and secondly because the present study was already running when QRISK3 was released, and some of the required data was not

AVR Quintiles	
Uppermost	0.90642
	0.86090
	0.82157
Lowest	0.77746

Table 21: ARIC AVR quintiles as used for quantifying risk of incident stroke.

included in the analysis. NICE state that primary threshold for prevention of cardiovascular disease is for QRISK2 calculations of 10% or greater[227]. Thus, 10% can be considered the cut-off for significant risk of cardiovascular disease.

No intervention criteria exists for retinal vessel calibres, either for arteries and vein measurements, or combined AVR. The ARIC study stratified their cohort (n = 10,358) by AVR quintiles, reporting risk ratios between upper and lower quintiles (quintile cut-offs unpublished, obtained direct from original statistician, shown in Table 21 (D.Couper, personal communication, July 2nd, 2019))[92]. Exclusion criteria for deriving quintiles was a positive history of stroke prior to the third ARIC visit (when the retinal photographs were obtained, *see Table 10 (Summary of population-based studies included in review)*). The ARIC study reported an 18% increased risk of stroke with subjects in the lowest quintile AVR when compared to the uppermost. In the absence of official classification criteria for vessel measurements, the use of a significant cardiovascular event (i.e. stroke) as calculated by the original study serves as an initial benchmark. This is a similar approach to the reports generated with VesselMap, however those incorporate unpublished data from a German cohort to account for age (*see Appendix 6. VesselMap Report; enlargement in Figure 25*).

5.2.7 Image acquisition and analysis

All images were exported both in full colour (for vessel reference) and red-free (for measurement) in uncompressed TIFF files. Images were then imported into VesselMap v3.0 for analysis. Analysis was performed by an experienced grader (CF), having practiced on a bank of >150 images prior to analysis. Images were batch analysed at time points several months after image acquisition with no access to patient notes.

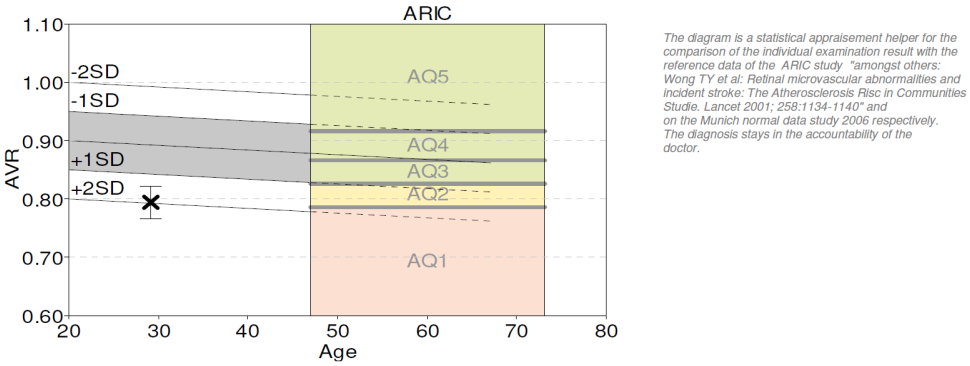


Figure 25: Enlargement of VesselMap report describing ARIC-based quintiles.

5.2.8 Statistical analysis

Statistical analysis was performed using SPSS v25.0 (IBM). Given that some degree of vessel measurement variability has been demonstrated previously (*see Chapter 4.3, Repeatability and Variability in Objective Static Retinal Vessel Calibre Measurement: Results*), inter-eye (OU) averages were used when drawing comparisons with systemic parameters. In order to use this approach, cases of isolated pathology which may bias one eye's measurements (both local pathology such as central retinal vein occlusion (CRVO) and systemic pathology such as carotid artery stenosis) were excluded from the analysis, and a paired samples t-test performed on the remaining data to demonstrate agreement between both eyes. To identify variables for inclusion in analysis, correlation matrices were employed, with a minimum significance set at $<p = 0.050$. For statistical models including refractive error (i.e. MSE) as a co-variable, one randomly selected eye was chosen for each subject (given the strong correlation between refractive error/ocular magnification and absolute vessel calibre measurements shown in the literature[252, 209, 208, 85, 179]). Normality of data distribution was established through Levene's test for Homogenity of Variance. A one-way ANOVA was used to determine whether vessel calibre (CRAE, CRVE and AVR) was significantly different between each NICE hypertension classification. A *post-hoc* Tukey's Honestly Significant Difference (HSD) test was used to explore the extent of calibre differences with increasing blood pressure category. Forward stepwise linear regression was used to construct models identifying variables which correlated most strongly with vessel calibre. Independent t-tests were used to explore differences between upper and lower ARIC-derived quintiles (since the cut-off values are derived from a separate population, data from the present subjects can be considered parametric provided

Variable	n	Mean (SD)	Minimum	Maximum
Gender: Female [male]	166 [104]	–	–	–
Age (*years*)	270	58.33 (± 15.60)	16	93
Blood pressure: Systolic (*mmHg*)	270	130.81 (± 19.45)	87.00	189.00
Blood pressure: Diastolic (*mmHg*)	270	81.78 (± 12.13)	52.00	117.00
Height (*m*)	269	1.72 (± 0.06)	1.55	1.93
Weight (*kg*)	269	75.60 (± 10.55)	44.00	114.30
BMI (kg/m^2)	269	25.46 (± 3.38)	17.63	37.32
OU CRAE (μm)	251	172.31 (± 18.16)	118.44	234.31
OU CRVE (μm)	251	201.39 (± 18.58)	152.11	268.93
OU AVR	251	0.86 (± 0.07)	0.70	1.05
QRISK2 10-year risk (%)	246	12.71 (± 12.05)	<1.00	62.70
Mayo Clinic 30-year risk (%)	242	24.58 (± 17.77)	<1.00	85.00
Right MSE (*D*)	269	-0.33 (± 2.35)	-10.13	+6.38
Left MSE (*D*)	269	-0.25 (± 2.26)	-9.50	+6.75

Table 22: Summary of cross-sectional participant data, showing variables (with units in parentheses), number of included participants (n), mean, standard deviation (SD) and minimum and maximum values.

they are normally distributed).

5.3 Results

A total of 270 subjects were included in the cross-sectional analysis. Summary data is shown in Table 22. Mean participant age was 58.3 ±15.6 years (range 16 - 93), and there were 166 females included in the study (104 males). Mean systolic blood pressure was 130.81mmHg ±19.45 (range: 87.00 - 189.00mmHg), and diastolic was 81.78mmHg ±12.13 (range: 52.00 - 117.00mmHg). Mean BMI was 25.46 ±3.38 (range: 17.63 - 37.32). Mean inter-eye average (OU) retinal vessel measurements were 172.31 ±18.16 (CRAE), 201.39 ±18.58 (CRVE) and 0.86 ±0.07 (AVR). Calculated QRISK 10-year cardiovascular risk was, on average, 12.71% ±12.05 whilst Mayo Clinic 30-year cardiovascular risk was 24.58% ±17.77. Mean right and left MSE were -0.33 ±2.35 (range: -10.13 - +6.38) and -0.25 ±2.26 (range: -9.50 - +6.75) respectively.

Paired sample t-tests were performed between measurements from right and left eyes (on subjects with calibre measurements for both eyes only). There was no significant difference between eyes for CRAE, CRVE or AVR (p = >0.050). Bland-Altman difference-versus-mean plots for right and left eye measurements demonstrate a mean bias of -0.33µm (Limits of Agreement: ±23.17), -0.51µm (Limits of

Agreement: ±22.45), and +0.0009 (Limits of Agreement: ±0.12) for CRAE, CRVE and AVR respectively. These are shown below in Figure 26. As such, all subsequent analyses employed inter-eye average (OU) measurements of CRAE, CRVE and AVR.

5.3.1 Hypertension and NICE-based blood pressure classification

All 270 subjects were included in blood pressure-based analysis, including those with existing cardiovascular disease (hypertension, diabetes mellitus, etc.). Within the 270 included for analysis, 66 subjects were already diagnosed with hypertension. Breakdown of characteristics when grouped by NICE hypertension classification is shown in Table 23. Distribution of blood pressure classification, stratified by present or absent hypertensive diagnosis is shown in Table 24. Of the 66 diagnosed hypertensives, 32 (48.48%) presented with blood pressure classified as Stage I hypertension or greater. Only 12 subjects (18.18%) had blood pressure comparable to normotension. When stratified by NICE Guideline classification for blood pressure, one-way ANOVA was highly significant ($p = <0.001$) for CRAE and AVR. There was no significant ($p = > 0.050$) correlation with CRVE. Levene's Homogeneity of Variance was non-significant ($p = >0.050$) for all variables prior to stratification (CRAE, CRVE, AVR). Table 25 summaries these results. A second one-way ANOVA was run using only the first 4 classification categories due to the very small sample size for 'Severe hypertension' ($n = 6$); there was no change in overall significance for any vessel measurement ($p = <0.001$), however the value of F increased by approximately an order of 2.00 for each parameter. *Post-hoc* Tukey's showed a greater difference in CRAE and AVR when there was a larger difference between NICE hypertension classification groups. On average, CRAE was +16.41µm greater for Grade I (normotensive) participants compared to Grade IV (Stage II hypertensives), with AVR being 0.07 lower in Grade I compared to Grade IV. The progressive decrease in CRAE with increasing NICE hypertension classification is demonstrated graphically in Figure 27.

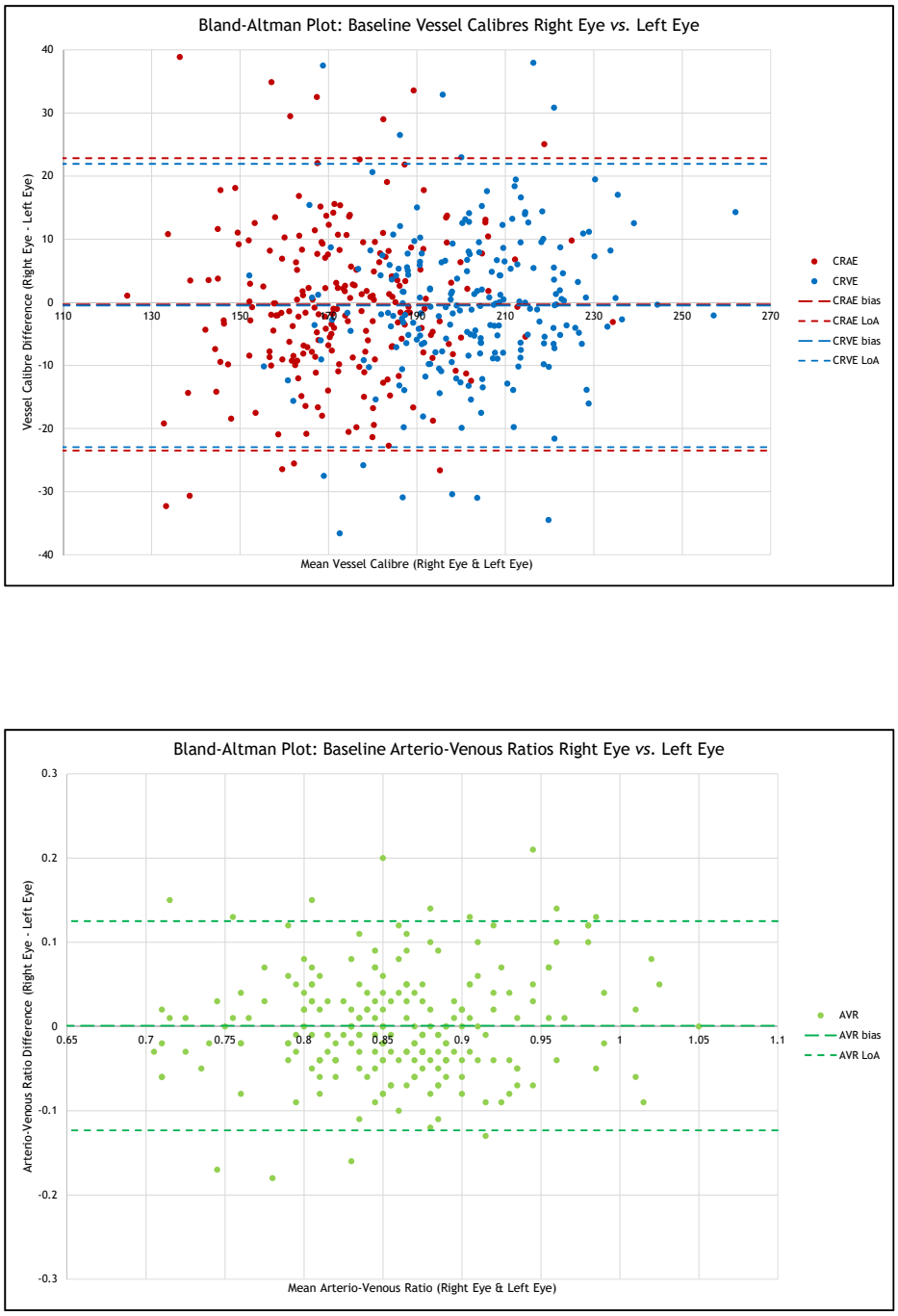


Figure 26: Bland-Altman difference-versus-mean plots showing the mean bias between baseline CRAE and CRVE (upper panel) and AVR (lower panel) measurements from right and left eyes. CRAE results are shown in red, CRVE in blue and AVR in green. Mean bias is denoted by longer dashes, and upper and lower limits of agreement (LoA) shown by smaller dashes. Note the near coincident mean bias for CRAE and CRVE.

Variable	Classification category				
	1	2	3	4	5
Gender: Female [male]	43 [25]	53 [40]	47 [27]	18 [11]	5 [1]
Age (*years*)	52.71 (± 18.23)	57.28 (± 15.48)	63.03 (± 12.59)	61.72 (± 11.39)	64.00 (± 10.88)
Blood pressure: Systolic (*mmHg*)	108.87 (± 8.41)	125.96 (± 7.02)	142.36 (± 9.24)	160.24 (± 14.27)	169.56 (± 13.08)
Blood pressure: Diastolic (*mmHg*)	69.27 (± 7.17)	80.44 (± 6.73)	87.13 (± 8.45)	96.09 (± 10.27)	108.61 (± 7.37)
Height (*m*)	1.72 (± 0.07)	1.73 (± 0.06)	1.72 (± 0.05)	1.72 (± 0.05)	1.71 (± 0.04)
Weight (*kg*)	72.03 (± 10.74)	75.85 (± 9.26)	77.20 (± 9.62)	78.65 (± 13.41)	77.79 (± 10.65)
BMI (kg/m^2)	24.40 (± 3.28)	25.10 (± 3.91)	25.81 (± 4.31)	26.48 (± 4.23)	26.69 (± 3.48)
OU CRAE (μm)	180.40 (± 16.16)	175.19 (± 16.80)	165.45 (± 18.49)	163.98 (± 16.32)	168.38 (± 9.42)
OU CRVE (μm)	203.60 (± 18.15)	201.43 (± 19.43)	199.87 (± 17.88)	201.48 (± 18.66)	194.84 (± 11.01)
OU AVR	0.89 (± 0.05)	0.87 (± 0.06)	0.83 (± 0.06)	0.82 (± 0.06)	0.87 (± 0.05)
QRISK2 10-year risk (%)	7.82 (± 9.46)	12.34 (± 12.62)	15.36 (± 12.76)	16.49 (± 9.03)	18.97 (± 11.77)
Heart Age (*years*)	52.64 (± 16.01)	61.17 (± 14.64)	66.66 (± 11.18)	69.11 (± 9.97)	71.33 (± 10.95)
Mayo Clinic 30-year risk (%)	13.47 (± 11.84)	24.23 (± 16.26)	29.27 (± 17.62)	36.38 (± 19.91)	41.17 (± 10.04)
Controlled Mayo Clinic 30-year risk (%)	11.26 (± 9.08)	15.89 (± 10.52)	15.28 (± 10.63)	15.03 (± 9.50)	11.67 (± 4.96)

Table 23: Mean parameters when split for each NICE hypertension category.

BP	n	Hypertension Dx'd	Normotension		Pre- hypertension		≥Stage I hypertension	
			Dx'd	Undx'd	Dx'd	Undx'd	Dx'd	Undx'd
BP_{syst}	270	66	12	78	22	73	32	53
BP_{diast}			27	90	22	66	17	48

Table 24: Baseline blood pressure categorised by hypertensive status. '**Dx'd**' = diagnosed. '**Undx'd**' = undiagnosed.

Variable	F	Significance
OU CRAE	8.56	<0.001
	(11.20)	(<0.001)
OU CRVE	*0.49*	*0.747*
	(0.43)	*(0.730)*
OU AVR	12.39	<0.001
	(16.42)	(<0.001)

Table 25: One-way ANOVA for retinal vessel calibre and NICE Guideline hypertensive classification, showing CRAE, CRVE and AVR for inter-eye average measurements (OU). Values in parentheses for one-way ANOVA using only first four categories for comparison, due to small sample size (6) for fifth category: *Severe hypertension*. Values in italics are non-significant (p = >0.050).

	Difference in classification			
	1-2	**1-3**	**1-4**	**1-5**
OU CRAE	*5.21*	14.95	16.41	*12.01*
OU CRVE	*2.17*	*3.72*	*2.12*	*8.76*
OU AVR	*0.02*	0.06	0.07	*0.02*

Grade	NICE Classification
1	Normotensive
2	Pre-hypertensive
3	Stage I hypertension
4	Stage II hypertension
5	Severe hypertension

Table 26: Post-hoc Tukey's HSD demonstrating the increasing degree of calibre difference (CRAE, CRVE and AVR) with increasing NICE Guideline hypertension classification. Values in italics are non-significant (p = >0.050).

Forward stepwise linear regression using data from one randomly selected eye for each subject produced three models which correlated CRAE with age, refractive error and systolic blood pressure, summarised in Table 27. Age alone accounted for 15.5% of the total variance (p = <0.001), and this almost doubles with the inclusion of refractive error (R^2 27.1%, p = <0.001). Systolic blood pressure accounts for a further 4% of variance, however inclusion of this variable reduces the overall prediction confidence (F = 36.5, from F = 44.9 in Model 2). Variables excluded from the analysis were diastolic blood pressure

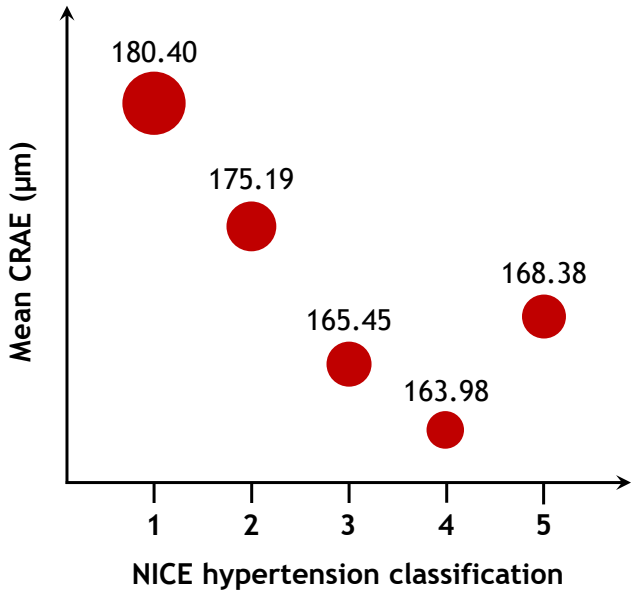


Figure 27: Mean CRAE (in µm) when stratified by NICE hypertension classification. Red circles graphically demonstrate a reduction in artery calibre with increasing hypertension classification [not to scale]. Note the slight increase in artery calibre noticed in the uppermost category.

and level of exercise. Similarly, forward stepwise linear regression produced three models correlating CRVE with age, refractive error and smoking status (also summarised in Table 27). Age alone accounted for 10.5% of overall variability (p = <0.001). With the inclusion of MSE, this model accounted for 22.8% of total variance (p = <0.001). The addition of smoking status into Model 3 accounts for 24.9% of total variance, however reduces the prediction confidence from F = 35.6 to F = 26.6. Excluded variables were systolic blood pressure, weight and alcohol intake.

5.3.2 Diabetes mellitus and retinopathy risk

Twenty-five (9.26%) subjects from the cohort reported having DM (Type I: 16; Type II: 9; *summarised in Table 28*). Persons with DM ranged from 16 to 87 years (mean: 59.8 years ±18.15), with mean duration of Type I DM being 14.25 years ±9.88 and Type II DM was 5.29 years ±3.61. Due to a small sample size, statistical interpretation of these findings will be limited. Mean age of subjects with Type I DM was over a decade less than those with Type II DM (55.44 years vs. 67.56), with mean age of the remaining cohort sitting in between the two (58.18 years). CRAE was slightly reduced in Type II DM compared to both Type I DM and Non-DM subjects. When compared to the non-diabetic cohort, CRVE was slightly raised in the Type I DM sub-group, whereas it was reduced amongst those with Type II DM. AVR appeared to

CRAE	R^2	F	Significance
Model 1 (Age)	15.5%	44.6	<0.001
Model 2 (Age + MSE)	27.1%	44.9	<0.001
Model 3 (Age + MSE + BP_{syst})	31.2%	36.5	<0.001

CRVE	R^2	F	Significance
Model 1 (Age)	10.5%	28.5	<0.001
Model 2 (Age + MSE)	22.8%	35.6	<0.001
Model 3 (Age + MSE + smoking)	24.9%	26.6	<0.001

Table 27: Forward stepwise linear regression correlating cross-sectional cardiovascular variables with vessel calibre.

remain similar across all three categories, although was slightly reduced in Type II DM (-0.03). There was a substantial increase in calculated QRISK risk amongst those with diabetes. Type I DM QRISK was, on average, double that of the non-DM sub-group (22.19% vs. 11.67%). There was an even larger shift seen amongst those with Type II DM (29.13%). This increase in QRISK was reflected by an equal increase in calculated Heart Age for both groups when compared to those where DM was not present. Mean blood pressure, both systolic and diastolic, was consistent across all three groups. Reported HbA1c was higher amongst those with Type I DM (73.09mmol/mol) than Type II DM (48.93mmol/mol). Calculated RetinaRisk, i.e. the risk of developing sight threatening (proliferative) retinopathy in 1 and 5 years was greater amongst those with Type I DM than those with Type II (5 year risk 7.65% vs. 4.25%). Incidence of co-morbidity amongst those with DM (*as shown in Table 29*) was greater (8 of 9 subjects) amongst those with Type II DM, compared to those with Type I DM (12 of 16). For both types, this was predominantly hypertension and hyperlipidaemia.

Status	n (*male* / *female*)	Age	DM duration	CRAE	CRVE	AVR
DM-I	16 (*7* / *9*)	55.44 (± 19.81)	14.25 (± 9.88)	175.89 (± 20.03)	207.99 (± 23.58)	0.85 (± 0.04)
DM-II	9 (*6* / *3*)	67.56 (± 11.14)	5.29 (± 3.61)	161.78 (± 10.70)	194.87 (± 15.85)	0.83 (± 0.04)
Non-DM	245 (*91* / *154*)	58.18 (± 15.28)	–	172.43 (± 18.05)	201.16 (± 18.10)	0.86 (± 0.07)

Status	QRISK2	Heart Age	BP_{syst}	BP_{diast}	HbA1c	Retina Risk (1 year)	Retina Risk (5 years)
DM-I	22.19 (± 17.00)	72.00 (± 11.16)	131.24 (± 16.89)	81.94 (± 11.44)	73.09 (± 27.05)	1.53 (±1.68)	7.65 (± 8.38)
DM-II	29.13 (± 12.28)	77.38 (± 6.26)	132.38 (± 5.06)	82.96 (± 11.37)	48.93 (± 9.56)	0.85 (± 0.48)	4.25 (± 2.41)
Non-DM	11.67 (± 11.06)	60.73 (± 14.69)	130.72 (± 19.84)	81.72 (± 12.73)	–	–	–

Table 28: Descriptive statistics of patients with diabetes mellitus. '**DM-I**' = Type I diabetes mellitus; '**DM-II**' = Type II diabetes mellitus; '**Non-DM**' = non-diabetic.

	Hypertension	Hyperlipidaemia	Other	Nil
DM-I (/16)	7	6	6	4
DM-II (/9)	4	5	2	1

Table 29: Co-morbidity within subjects with diabetes mellitus.

5.3.3 Cardiovascular risk and ARIC-based stratification

Of the cross-sectional cohort, 125 subjects fell within the age range included in the original ARIC study (51-70 years). Five subjects were excluded from analysis as they did not have vessel data due to image quality, leaving 120 for the subsequent analysis. These subjects were stratified according to ARIC-derived AVR quintiles. This is shown graphically in Figures 28 and 29; for the whole study cohort and that falling within the same age range as the original ARIC study. Normal distribution was confirmed through normal quantile-quantile plots and Kolmogorov-Smirnov testing for each variable (blood pressure (systolic and diastolic), CRAE, CRVE, AVR, QRISK, Mayo Clinic 30-year Risk). Pulse oximetry correlations were insignificant (p = >0.050). Independent t-tests were run on the each variable for the two groups (upper and lower quintiles) (*see Table 31*). Levene's Test for Equality of Variance showed that for all significant t-

test results, equal variance could be assumed. For those variables with non-normal distribution (QRISK Risk Ratio, Mayo Clinic Controlled 30-year Risk and NICE hypertension classification), Mann-Whitney U-tests were performed instead (*see Table 32*). Significant differences between upper and lower quintiles were found in a number of cardiovascular metrics. Blood pressure, both systolic and diastolic, was found to be increased in subjects falling in the lower quintile compared to the upper (+18.44mmHg (± 5.08) systolic; +9.17mmHg (± 3.50) diastolic). On average, this makes participants in the lower quintile of AVR data one NICE hypertension classification grade higher than the upper quintile. QRISK scores were 4.40% (± 2.16) greater in the lowest quintile. Mean QRISK for the upper quintile was 9.88% (± 4.89), whilst the lower quintile was 14.28% (± 7.34). This finding was also observed, albeit to a greater extent between the quintiles, with Mayo Clinic 30-year risk calculations (17.14% (± 6.32)). Upper AVR quintile for Mayo Clinic 30-year risk averaged 19.21% (± 16.67), whilst lower quintile had a mean of 36.35% (± 21.17). Whilst AVR is derived from both CRAE and CRVE, independent t-tests showed opposite changes in the different vessel types; CRAE was found to be decreased in the lower quintile (-26.05µm (± 5.31)), whilst CRVE was found to be increased (+19.59µm (± 5.73)); an overall reduction in AVR was found in the lowest quintile compared the highest (-0.21 (± 0.01)). Mann-Whitney U-tests showed a significant difference in both QRISK Risk Ratio (U = 44.5, p = 0.001) and NICE hypertension classification (U = 60.0, p = 0.001), but no significant difference in Mayo Clinic Controlled 30-year Risk.

5.4 Discussion

5.4.1 Hypertension and NICE-based blood pressure classification

Blood pressure has been shown to have a robust inverse correlation with retinal artery calibre. Historically this was reported through a reduction in AVR (employing the erroneous assumption that venular calibre remains unchanged), although latterly this has been more specifically driven towards the arteries in isolation (CRAE). One-way ANOVA confirms both of these findings; that there is a reduction in CRAE and AVR with increased blood pressure. It is perhaps interesting to observe that there seemed to be an apparent increase in CRAE for those subjects in the highest NICE classification category (*Figure 27*). Of the six subjects in this category, only 2 had received a formal diagnosis of hypertension (although one of these was not taking their medication). The magnitude of blood pressure is perhaps indicative of significant cardiovascular compromise. Whilst not directly comparable, it has been shown that, for advanced hypertensives, a loss in autoregulation results in increased arteriolar lumenal diameter in the kidney[253]. Since the retinal circulation also utilises autoregulation, a similar mechanism may be the

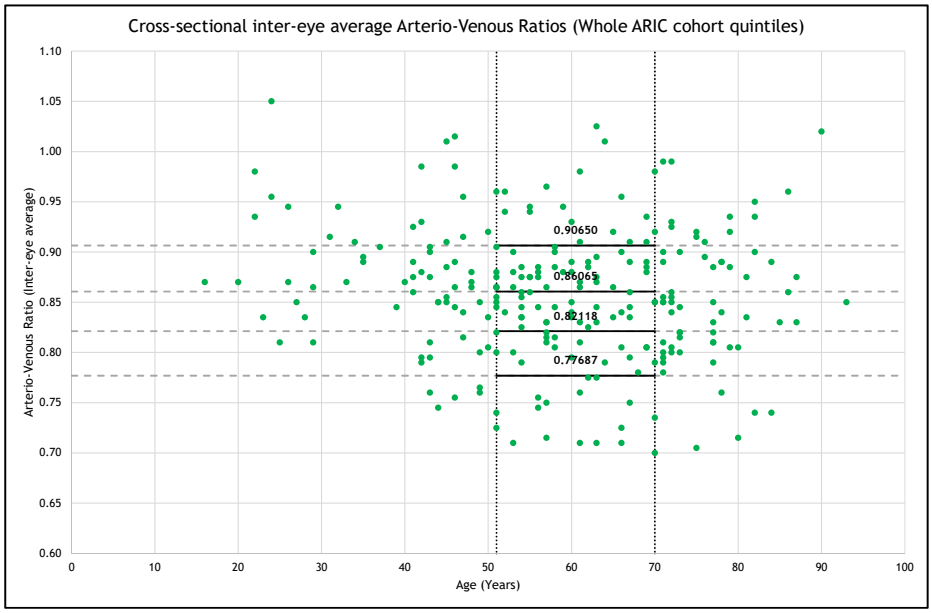


Figure 28: Scatterplot of AVRs for cross-sectional cohort with reference ARIC-based quintiles overlaid. Quintile cut-offs displayed above each line. Black regions represent the age range used by the ARIC study (51-70 years).

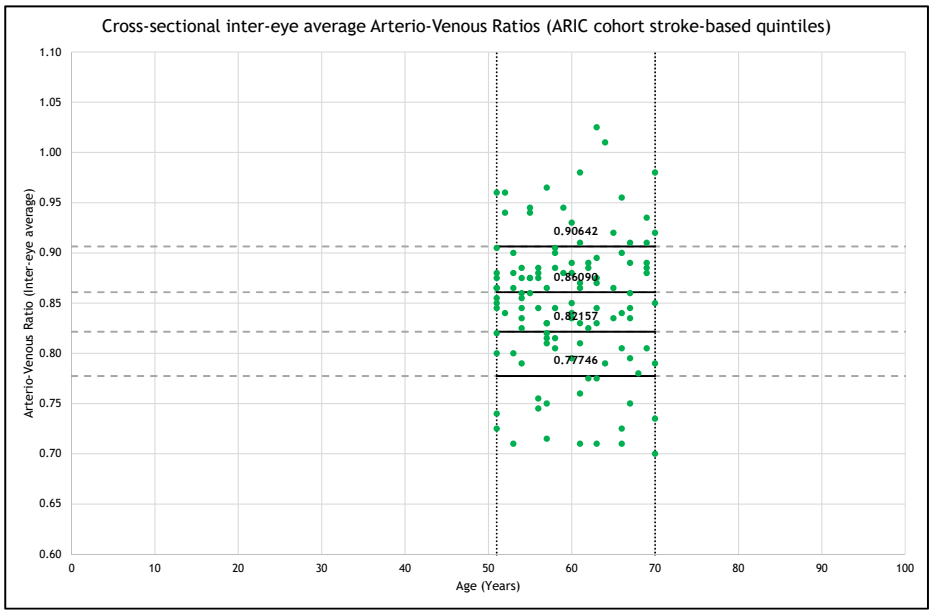


Figure 29: Scatterplot of AVRs for ARIC-equivalent age range only (51-70 years) and ARIC-based quintiles of CVD event (stroke) overlaid. Quintile cut-offs displayed above each line.

Variable	Whole cohort (SD)	Lower AVR quintile (<0.77746) (SD)	Upper AVR quintile (>0.90642) (SD)
n	125	17	19
Gender: Female [Male]	72 [48]	10 [7]	12 [7]
Age (*years*)	59.59 (± 6.04)	60.59 (± 6.12)	61.37 (± 6.38)
BP_{syst} (*mmHg*)	134.44 (± 18.72)	150.65 (± 13.85)	132.21 (± 16.33)
BP_{diast} (*mmHg*)	85.23 (± 11.30)	91.65 (± 2.25)	82.47 (± 11.45)
BPM	68.34 (± 9.56)	67.31 (± 9.44)	69.68 (± 9.23)
Height (*m*)	1.772 (± 0.05)	1.73 (± 0.06)	1.72 (± 0.04)
Weight (*kg*)	77.10 (± 10.35)	79.38 (± 12.19)	72.85 (± 10.97)
BMI (kg/m^2)	25.93 (± 3.31)	26.50 (± 3.58)	24.67 (± 3.23)
CRAE (μm)	170.60 (± 15.24)	152.62 (± 13.24)	178.66 (± 17.95)
CRVE (μm)	201.30 (± 16.41)	207.82 (± 17.54)	188.23 (± 16.78)
AVR	0.85 (± 0.07)	0.73 (± 0.02)	0.95 (0.03)
QRISK calculation (%)	10.77 (± 7.18)	14.28 (± 7.34)	9.87 (± 4.89)
QRISK Risk Ratio	1.48 (± 0.81)	1.99 (± 0.91)	1.24 (± 0.39)
QRISK Heart Age (*years*)	63.77 (± 8.10)	68.50 (± 6.76)	64.18 (± 7.44)
Mayo Clinic 30-year Risk (%)	28.36 (± 19.04)	36.35 (± 21.17)	19.21 (± 16.67)
Mayo Clinic 30-year Controlled Risk (%)	16.90 (± 11.43)	16.88 (± 13.84)	11.47 (± 11.20)
NICE hypertension classification	2.48 (± 1.02)	3.35 (± 0.61)	2.26 (± 1.10)

Table 30: Descriptive data for included cohort based on ARIC age range (51-70 years); split into whole cohort, upper and lower quintiles. Original ARIC quintile cut-offs shown in parentheses.

Variable	Mean Difference (SE)	Significance (p)
BP_{syst}	18.44 (± 5.08)	0.001
BP_{diast}	9.17 (± 3.50)	0.013
CRAE	-26.05 (± 5.31)	<0.001
CRVE	19.59 (± 5.73)	0.002
AVR	-0.21 (± 0.01)	<0.001
QRISK calculation	4.40 (± 2.16)	0.050
Mayo Clinic 30-year Risk	17.14 (± 6.32)	0.010

Table 31: Independent t-tests comparing subjects falling into the upper and lower ARIC-derived quintiles.

Variable	Test Statistic (U)	Significance
QRISK Risk Ratio	44.5	0.001
Mayo Clinic Controlled 30-year Risk	*131.0*	*0.346*
NICE hypertension classification	60.0	0.001

Table 32: Mann-Whitney U-test comparing subjects falling into upper and lower ARIC-derived quintiles (*for those parameters not deemed to be normally distributed in either or both categories*). Values in italics are non-significant (p = >0.050).

Variable	Lower AVR quintile		Upper AVR quintile	
	Diagnosed	Undiagnosed	Diagnosed	Undiagnosed
n	10	7	4	15
Mean BP_{syst}	148.00 (± 10.92)	155.00 (± 15.53)	137.00 (± 18.45)	131.00 (± 14.69)
Mean BP_{diast}	93.00 (± 10.29)	90.00 (± 6.56)	83.00 (± 12.09)	82.00 (± 6.61)
≤ Pre-hypertensive (Grade III)	1	1	3	11
≥ Stage I hypertensive (Grade IV)	9	**6**	1	**4**
QRISK	13 (± 7.16)	16 (± 6.68)	14 (± 4.15)	9 (± 4.57)

Table 33: Blood pressure characteristics stratified by upper and lower ARIC-derived AVR quintiles. Quintiles split on basis of formal hypertension diagnosis. Note the number of undiagnosed cases with blood pressure falling in Stage I hypertension category or above (highlighted in **bold**) relative to the total number of subjects in that cohort.

root of this finding. Alternatively, at least two of the subjects in this category reported disliking the procedure of sphygmomanometry. White-coat hypertension is a recognised and well-documented phenomenon (>20/10mmHg between clinic and average daytime ambulatory blood pressure) and may also account for the apparent increased arterial calibre, since the blood pressure measurement will not truly reflect actual pressure[254, 13]. Additionally, the altered light reflex within an advanced atherosclerosed arteriole may yield spurious results during the measurement process, with incorrect selection of the visible blood column and poorer contrast with surrounding retinal tissue. Ultimately, greater numbers will be required to investigate this finding further. Since there was a comparatively small group in the top blood pressure category (≥180/110mmHg; n=6), exploration on whether this sample size had an effect on the overall significance of the test revealed a persistent significance even when this uppermost category was omitted from the test. To further explore the inverse correlation between blood pressure and CRAE and AVR, a post-hoc Tukey's test showed, perhaps unsurprisingly, that the effect was larger (for both measures) when the blood pressure categories were further apart. This reinforces the linear nature that there is a greater reduction in CRAE (and AVR) with an increased level of blood pressure. Clinically this suggests that an optometrist should expect to see a greater reduction in artery calibre with higher blood pressure. This is a well-known phenomenon already, however static analysis allows for a definitive quantification of this rather than subjective 'intuition'.

Whilst there was no significant correlation between CRVE and blood pressure, the veins are known to be affected by other aspects of cardiovascular disease which can arise through raised blood pressure (see below). The lack of direct correlation between CRVE and blood pressure is also a finding extensively reported elsewhere (*see Chapter 2.4.1. Association of blood pressure and retinal vessel calibre*).

5.4.2 Diabetes mellitus and retinopathy risk

The relative number of subjects with DM in the study cohort (9.25%) was reasonably reflective of the incidence of DM within the UK (6%), however for the present study it meant there were comparatively fewer subjects in order to perform statistical analyses. General observations of the results do give some insight however and are suggestive that the data is reflective of the different pathophysiological processes associated with DM and DR. The mean age for Type II DM for instance was greater than both Type I and the non-DM sub-groups, which tallies with this type of DM having a tendency to develop in later life (when considering young-onset Type II DM as a separate pathological entity). Because of this, the likelihood of co-morbidity increases, which is again reflected in both the raw data of co-morbid

incidence (Table 29) and the significantly increased QRISK calculations. Given the shorter average duration of Type II DM, the slight decrease in CRAE and CRVE compared to Type I and non-DM subjects is similar to that previously reported as being associated with 'incident DM'; theorised as the early vascular changes in DM (*see Chapter 2.4.2* (*Association of diabetes mellitus and retinal vessel calibre*)). Also, the trend continues with an increased CRVE relative to both Type II and non-DM subjects, in what could be considered more 'established' cases of DM. Duration of DM is known to be a significant risk factor for developing DR, and this is reflected in the higher estimated risk as determined by the RetinaRisk algorithm for both 1 and 5 years. This is perhaps also influenced by the higher mean HbA1c amongst the Type I DM subjects compared to those with Type II. HbA1c has a strong, independent positive correlation with development or progression of DR, especially once levels rise above 48-51mmol/mol (6.5-6.8%); and is seen in both types of DM[255, 256, 257, 258, 259]. Although a much larger diabetic sample is required, RetinaRisk has the potential to help formulate a management plan for patients at the time of their eye examination. Although the application is designed to be patient-facing, usage of the internal algorithm has demonstrated a significant improvement in frequency of retinopathy assessments in individuals; lower risk patients are seen at greater intervals than those at higher risk. In optometric practice, the RetinaRisk application could be used in conjunction with retinal vessel calibres to formulate a management plan and decide on the review interval for the patient. More work is required to correlate the two in order to establish structured management and intervention pathways, but in principle the approach would have a very useful clinical application. Whilst the cohort size is limited, the initial conclusions appear to follow the established trends reported elsewhere. A focussed study using a much larger number of subjects with DM is required to produce more definitive statistics, but should also focus on the sub-types of DM, since it is likely that the underlying pathological processes may have different effects on the retinal microcirculation.

5.4.3 Cardiovascular risk and ARIC-based stratification

Whilst methods of categorising and stratifying cardiovascular risk and blood pressure exist with relevant intervention criteria, no such framework currently exists for retinal vessel calibre. This reflects their current standing in the management of patients in that the retinal vessel appearance is simply a confirmation or incidental observation, additional to the diagnostic criteria. The conclusions from the original ARIC study were based upon the formulae derived by Hubbard et al., and comparison to these findings are a good initial step in defining cut-off values and intervention thresholds. As cardiovascular

disease is a multi-faceted condition, a binary end-point was adopted to allow simpler classification of cardiovascular risk based upon vessel calibre (i.e. incident stroke). The original ARIC study reported that those subjects in the lowest quintile of AVR were 18% more at risk of incident stroke than those in the uppermost quintile. This was much greater for those without pre-existing cardiovascular disease (RR 2.85). The present study cohort indicated a similar trend when comparing other metrics from upper and lower AVR quintiles. Blood pressure, one of the key contributors to cardiovascular risk, was found to be elevated between the two quintiles. Both average systolic and diastolic blood pressure for the lowest quintile (150.65mmHg / 91.65mmHg respectively) fall within NICE guidelines 'Stage I hypertension' classification; thus requiring intervention. Conversely, for the uppermost quintile, mean systolic and diastolic blood pressures would both be considered 'pre-hypertension'. A greater proportion of subjects in the lower quintile were currently undiagnosed for hypertension, yet their blood pressure was recorded above the Stage I hypertension threshold (140/90 mmHg); 6 of the seven subjects met this criteria (85.71%), compared to only 26.67% of the upper AVR quintile cohort. This is supported by an increased QRISK score between the quintiles. Whilst the mean difference is only 4.40%, the absolute mean values for each quintile straddle the NICE intervention threshold of 10%. Taking only the undiagnosed cases of hypertension, the mean QRISK score is almost double in the lower AVR quintile when compared to the upper (16% vs. 9%).

Whilst AVR has been criticised previously for it's lack of specificity regarding the vessel type affected, independent t-testing showed that as well as general changes in AVR, there were bi-directional changes in CRAE and CRVE with lower ARIC-derived AVR quintiles. A number of factors could explain this. Hypertension is considered the leading modifiable risk factor associated with stroke[260, 261]. As such, those most at risk of incident stroke will be more likely to have raised blood pressure. As mentioned previously, a strong inverse correlation exists between raised blood pressure and reduced CRAE (and AVR). This could explain the increased incidence of Stage I and II hypertension in the lowest quintile, as well as increased QRISK scores. Whilst the underlying pathophysiology is not understood, a positive correlation between CRVE and stroke has previously been reported (*see Section 2.4.3 (Association of stroke and retinal vessel calibre)*). Differences in the classification of stroke exist, with ARIC classifying as either ischaemic or haemorrhagic (with the Rotterdam study adopting a similar stratification[158, 159]), whilst others opt for a more general grouping of 'stroke'[155, 262]. Arguably since an optometrist is not able to perform the requisite investigations to determine stroke sub-type (and the clinical intervention for risk of stroke is not dependent on type[263]) even a broad grouping of stroke is sufficient (although Wieberdink

et al. suggest that increased CRVE is more indicative of haemorrhaging stroke, particularly in those taking anti-coagulants[159]). More recent studies investigating retinal vessel calibre and stroke have pointed towards an, as yet, unexplained increase in CRVE with increased risk of stroke[158]. Both hypoxia and similarities to early stages of DR progression have been suggested as possible mechanisms[158, 127]. Both arterial narrowing and venular dilation, as reported previously in the literature as being associated with stroke and cardiovascular risk, have been demonstrated in the present cohort in the lowest quintile of AVR, itself associated with incident stroke.

The ARIC-derived quintiles are not perfect. Just as the Mayo Clinic Cardiovascular Risk calculations over-estimate when compared to a locally derived formulae (QRISK), the same population-bias could be attributed to these quintile cut-offs. Whilst AVR is less susceptible to magnification errors or overall image size, methodological differences (such as measurement software) exist between the present study and ARIC which could impact on the accuracy of the cut-offs. The cut-offs are also linear across the ARIC age-range, yet natural ageing is known to affect vascular calibre, independent of ethnicity or any pathology process[187, 264, 265, 131]. For use as a clinical decision making tool, cut-off values would need to consider this natural ageing process (and be extended beyond the current range).

5.4.4 Study limitations

Undertaking such a study in clinical practice means that, by it's very nature, methodological issues may impact upon the validity of the results. As discussed in Chapter 3.3 (Sample size and patient recruitment), to carry greater statistical weight a sample size calculation should be performed in order to demonstrate whether the cohort used is large enough to yield meaningful results. Since the present study is observational, and for reasons discussed in the aforementioned section, determination of a sample size in this instance is difficult. Whilst the results found have been found to be statistically significant (and it is therefore possible to suppose that these findings are clinically significant), it cannot be said with absolute confidence that they are applicable to the population as a whole; only to the cohort in the present study. However, what these results demonstrate (particularly those in smaller sub-samples such as the diabetic cohort) is areas for future research to focus on; with a more targeted research question, it becomes more feasible to perform a sample size calculation.

Of equal consideration is the ethnic representation of the present sample. A restricted geographic sample is advantageous when correlating with other variables which are equally stratified by location (i.e. QRISK), and also since if any ethnic variations do exist, when considered as one sample they have

the potential to attenuate any ethnic-specific correlations. The lack of diversity within the sample does, however, prevent any generalisation of findings. Correlations within the present sample, whether in agreement or not with the established literature, cannot be expanded to relate to the entire UK population as it does not truly reflect the genetic and ethnic diversity within it. Subsequent studies, most likely adopting a multi-centre approach, will need to test for any geographic or ethnic variations in the correlations reported here. Whilst a geographic element may exist, it is more likely that levels of retinal pigmentation may indirectly influence results by enhancing (or reducing) vessel edge contrast. It is only possible at present, therefore, to apply observed correlations to the localised Caucasian population.

Finally, with respect to calculated inter-eye average vessel calibre measurements, the impact of localised vascular pathology on these measurements needs to be fully determined. In the present study, these effects were mitigated by simply removing the affected eye from analysis. Whilst it was demonstrated with the present sample that there is good agreement between eyes (including those with local pathology), the potential influence of local pathology needs to be explored to discover if there is a level of disease severity where summary measurements are impacted by significantly different results.

5.4.5 Conclusion

Having inter-eye (OU) averages enables clinicians to explore systemic associations with the retinal vasculature whilst also attempting to reduce the intra-eye variations (*as described in detail in Chapter 4 (Repeatability and Variability in Objective Static Retinal Vessel Calibre Measurement)*). The cross-sectional cohort demonstrates a good level of agreement with the existing research literature. Whilst the methodology may not be as stringent as lab- and hospital-based studies, the correlations with cardiovascular metrics (blood pressure, stroke and diabetes) reported elsewhere have been reproduced with similar effects in the present study. Finger-tip pulse oximetry was not found to be correlated significantly with any ocular vascular parameters, and this is perhaps reflective of the limitations of the device for accurate classification. Instead, the pulse oximeter can be used as a gross assessment of pulmonary exchange, thus enabling elimination of this as a potential co-founder. The findings suggest that routine objective measurement of retinal vessel calibre could be used in conjunction with blood pressure measurement and cardiovascular risk calculations. A patient falling in the upper quintile of AVR is, on average, 4% less at risk of a cardiovascular event compared to a patient in the uppermost quintile. There is also a much greater incidence of potentially undiagnosed hypertension in the lowest quintile. Using both retinal vascular calibres and dedicated algorithms to evaluated subjects with DM is also useful. Whilst

more extensive work is required to produce statistically robust results, the present findings appear to support those theories already established elsewhere. Despite a current lack in professional guidance and intervention strategies based upon retinal vascular measurements, their use in clinical practice can serve two purposes. Firstly, they enable a clinician to accurately quantify the vessels and evaluate both arms of the circulation in relation to ocular and systemic factors. Secondly, they can serve as a reinforcer for patients when management is being given, such as in cases of undiagnosed hypertension. Here the blood pressure measurement itself is backed up with a sign of structural change which is strongly associated with the increase in pressure. Whilst the ARIC quintile system has not been validated against a UK-based population, it serves as a starting point from which clinicians can start to stratify their patients and make more reasoned clinical judgements based on retinal vessel calibre measurements. The current findings suggest that it is feasible to apply cut-off values to retinal vascular measurements to help refine individual patient management and clinical decision making.

6 Longitudinal Changes in Retinal Blood Vessel Calibre Observed Between Routine Optometric Eye Examinations

6.1 Introduction

Following cross-sectional associations between retinal vascular calibre and both local and systemic pathologies, the clinical usefulness of objective vessel analysis lies in it's application in the long-term monitoring of patients. Of the studies undertaking longitudinal observation of participants (*see Chapter 2 (Associations of Retinal Vessel Calibre with Cardiovascular Disease: A Systematic Literature Review)*) the average interval between visits was between 3 and 5 years, whereas patients will typically visit their optometrist every 1 to 2 years. This interval could, therefore, still be considered 'short-term' in relation to these studies. Since small intervals in time between image acquisition (several seconds and several days) have been examined to demonstrate levels of repeatability for a single visit (*see Chapter 4* (*Repeatability and Variability in Objective Static Retinal Vessel Calibre Measurement*)), the inter-examination vessel measurements also need to be explored. Applying the 'single visit' variability as a benchmark, measurements and correlations falling outside of this range could point towards areas of improved detection and diagnostic capabilities.

Previous repeatability studies have highlighted that vessel selection is a key detractor to intra- and inter-grader reproducibility[113, 187]. Since patients in practice will be presenting usually 12 months later for a follow-up examination, consistent vessel re-selection needs to be ensured and a method of recalling the previous examination's vessel selection is required.

When considering correlations between retinal vessel calibres and systemic cardiovascular health, there are three principle metrics which can change; vessel calibre, blood pressure, and cardiovascular risk score. Each of these variables can be measured both continuously and categorically, however it is the latter which forms the backbone of clinical management guidelines (where applicable). Retinal vessel calibre can be measured with absolute values (μm) or with an ARIC-based score. Blood pressure's measurement in millimetres of mercury (mmHg) can then be grouped into tension categories (hypo-, normo- and (stage I / II / severe) hypertension) which carry different management criteria. This also applies to cardiovascular risk score, with UK NICE guidelines referencing values from the QRISK2 calculator; specifically recommending intervention when risk exceeds 10% (a lowering of risk from previous guidance with a 20% threshold)[227, 23].

Metric	Measurement unit	Intervention
Vessel calibre	µm	–
Blood pressure	mmHg	• Stage I (**>140/90mmHg**) • Between 40 and 80yrs: Cardiovascular risk > **20%**[23]
Cardiovascular risk	%	**≥10%**[227]

Table 34: Measurement and points of intervention for cardiovascular metrics. Note the current lack of guidance regarding vessel calibre changes.

6.2 Methods

For detailed methodological information see Chapter 3 (Materials & Methods). Longitudinal participants were recruited throughout the study course and advised to return for a routine follow-up examination in line with College of Optometrists guidance for frequency of eye examinations[266], with data from their baseline visit being used. All participants were recruited in optometric practice. Exclusion criteria was limited to those not covered by the ethical approval (i.e. minors and those unable to give informed consent) and those with significant media opacities resulting in poor quality and ungradeable photographs, even with pharmacological dilation. Briefly, the baseline visit involved collecting data regarding health, family history and lifestyle; refractive status; ocular health; and cardiovascular measures.

6.2.1 Patient Health History

In line with both College of Optometrists guidelines on history and symptom collection, and also utilising questions featured in both the Mayo Clinic and QRISK cardiovascular health risk calculators, patients reported anecdotally their general health, including present medication, diagnosed conditions (and whether they were being treated) and any conditions currently undiagnosed but borderline or under investigation. Family history was also explored with additional emphasis on cardiovascular health and occurrence of any cardiovascular events, especially in younger family members. Participants were asked lifestyle questions, including past or present smoking status, average weekly alcohol intake (approximate number of units), daily diet (approximate amount of both saturated fats and portions of fruit and vegetables per day, on a scale of '*Few*' (≤1), '*Some*' (2-4) or '*A lot*' (≥5), daily caffeine intake (*1-2 cups, 3-5 cups, >5 cups*) and weekly exercise (*Nil*; *<75 minutes vigorous/<150 minutes moderate*; *>75 minutes*

vigorous/>150 minutes moderate). Anecdotal height and weight were also recorded. Examples of all categories were given to participants to improve consistency of self-reporting (*see Appendix 5. Patient Questionnaire for detail*).

6.2.2 Refractive status

All participants underwent a routine objective refraction (static retinoscopy) followed by subjective refraction by an experienced optometrist (CF). In cases of severe amblyopia or reduced vision or other instances where a balance lens was prescribed, refraction was recorded as the objective refraction obtained. Previous refractive status for participants having undergone refractive surgery (laser refractive surgery, cataract/clear lens extraction etc.,) was also noted.

6.2.3 Ocular health

Routine undilated (except in cases of poor fundal view) ophthalmoscopic examination was performed on all participants using a slit lamp biomicroscope and 78D condensing lens. Fundus photographs were then obtained after pupil diameter had recovered sufficiently following ophthalmoscopy. 45°, full colour disc- and macular centred fundus photographs were obtained using a Topcon 3D Maestro. Non-contact IOP was measured with a calibrated Pulsair 3000 obtaining three readings per eye.

6.2.4 Cardiovascular measures

Digital sphygmomanometry was performed with the subject seated at the end of the examination, with time having elapsed following non-contact IOP recording (approximately 5 minutes) to allow blood pressure of subjects disliking this procedure to return to normal. Subjects were instructed to remove thick sleeves and to remain still and quiet during measurement. The cuff was applied level to the heart and three readings were taken, with a minute interval between each. The average of the final two readings was then used in analysis. If there was a difference of ≥10mmHg between readings, further readings were taken until three within this range were obtained. Heart rate (in BPM) was also recorded. Fingertip pulse oximetry was obtained from the index finger of the right hand; applied for 30 seconds and a reading taken when measurement had stabilised.

6.2.5 Cardiovascular Risk and ARIC-derived cut-offs

As explained previously in Section 5.2.6 (*Cardiovascular risk and ARIC-derived cut-offs*), in the absence of official intervention criteria for vessel calibres, the original study from which the formulae were derived and validated offers the benchmark of predicting incident stroke; itself a surrogate measure of 'cardiovascular risk'. The absolute cut-off values for the quintiles were obtained from the study's named statistician, and these values were applied as the cut-offs (shown in Table 21).

6.2.6 Image analysis

All images were exported both in full colour (for vessel reference) and red-free (for measurement) in uncompressed TIFF files. Images were then imported into VesselMap v3.0 for analysis. Analysis was performed by an experienced grader (CF), having practiced on a bank of >150 images prior to analysis. Images were batch analysed at time points several months after image acquisition with no access to patient notes.

6.2.7 Statistical analysis

Statistical analysis was performed using SPSS v25.0 (IBM), however this was limited by the numbers of participants involved. Natural drop-out of participants over the course of the study and the uneven follow-up period, with some participants presenting much sooner than others, had a significant impact on the final numbers included for analysis (and thus prevented any complex statistical interpretation). There was no significant variation between right and left eye measurements, therefore inter-eye (OU) averages were used for systemic correlations (with eyes affected by localised pathology excluded). To explore variance of vessel calibres over time, Pearson correlations were used initially to determine whether time interval needed to be considered a co-variable (and thus necessitating the need for an ANCOVA instead). Examination time interval and absolute change in vessel calibres were insignificant ($p = >0.050$) for all three measures (CRAE, CRVE, AVR) in either eye. Correlations between examination interval and vessel calibres remained insignificant even when the largest interval for each participant was used (i.e. between initial and final visits). Bland-Altman difference-versus-mean plots were used to explore measurement differences across examinations.

6.3 Results

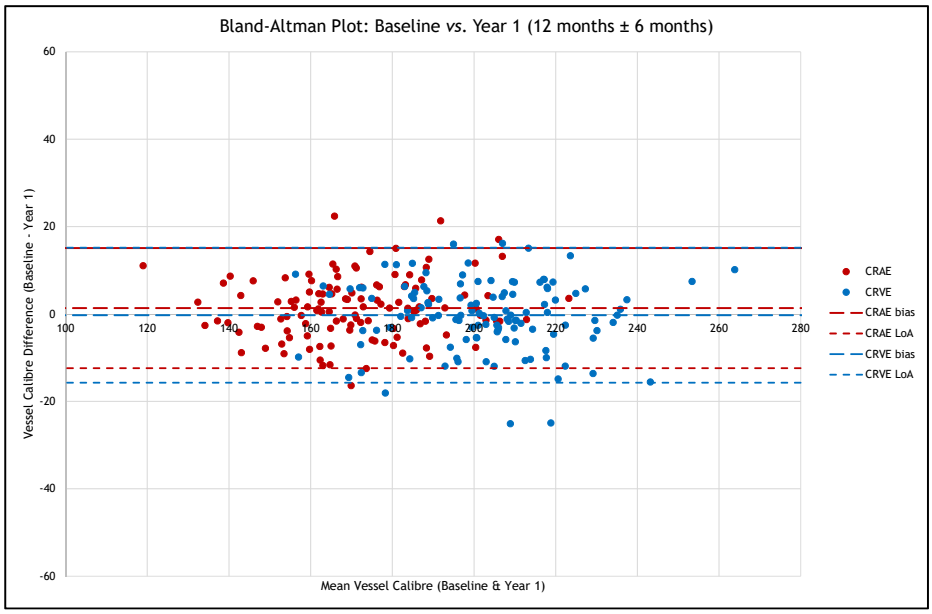
Analyses were broken down into two categories; 12 months (± 6 months) from baseline, and 24 months (± 6 months) from baseline. 122 subjects were included in the 12 month follow-up, and 55 in the 24-month follow-up; 11 of whom it was their first follow-up (i.e. they were not included in the 12-month follow-up).

Of those subjects who did not return for follow-up examination, 9 were enrolled too close to the end of data collection (i.e. <6 months). Two subjects died before they could attend follow-up appointments. There were no statistically significant differences in characteristics between those who returned and those who did not aside from blood pressure; both systolic (t = -2.288, p = 0.023, mean difference = -5.53mmHg) and diastolic (t = -3.037, p = 0.003, mean difference = -4.55mmHg).

Bland-Altman plots (*see Figure 30*) comparing baseline and maximum interval show wider spaced limits of agreement compared to those taken in the Methodology study (*see Chapter 4*), with a similar distribution of data points. When stratified by time interval between visits (12 months ± 6 months and 24 months ± 6 months), Bland-Altman plots then show closer spaced limits of agreement across the initial twelve-month period (CRAE LoA: ±13.74; CRVE LoA: ±15.45) compared to across the twenty four-month period (CRAE LoA: ±20.14; CRVE LoA: ±20.36).

6.3.1 NICE-based blood pressure classification

Hypertensive status of subjects ranked by their absolute change in blood pressure are shown in Table 36. These are further sub-classified into diagnosed hypertensives and those without. Absolute changes (in mmHg) are shown for both systolic and diastolic blood pressure. Clinically, changes in NICE Guideline classification are shown in Table 37 (for 12 months) and Table 38 (for 24 months). ANOVA revealed no significant correlation between change in blood pressure (systolic or diastolic) or beats per minute (p = >0.050) over the study course. Small sample sizes for larger changes in blood pressure prevent robust statistical analysis being performed. For both 12- and 24-month follow-up, a greater number of subjects had a reduction in blood pressure (systolic and diastolic) compared to an increase (65 vs. 41 for 12 months and 28 vs. 16 for 24 months respectively). There was also a large number of undiagnosed Pre-hypertensives and Stage I hypertensives identified in both the 12-month and 24-month follow-up (these were mixtures of both categories at baseline and/or follow-up); and this was a greater proportion than those with diagnosed hypertension. Of the 31 subjects with hypertension diagnosed throughout the study, 10 saw a raise in their blood pressure over a 12-month period and 5 of the 16 diagnosed at



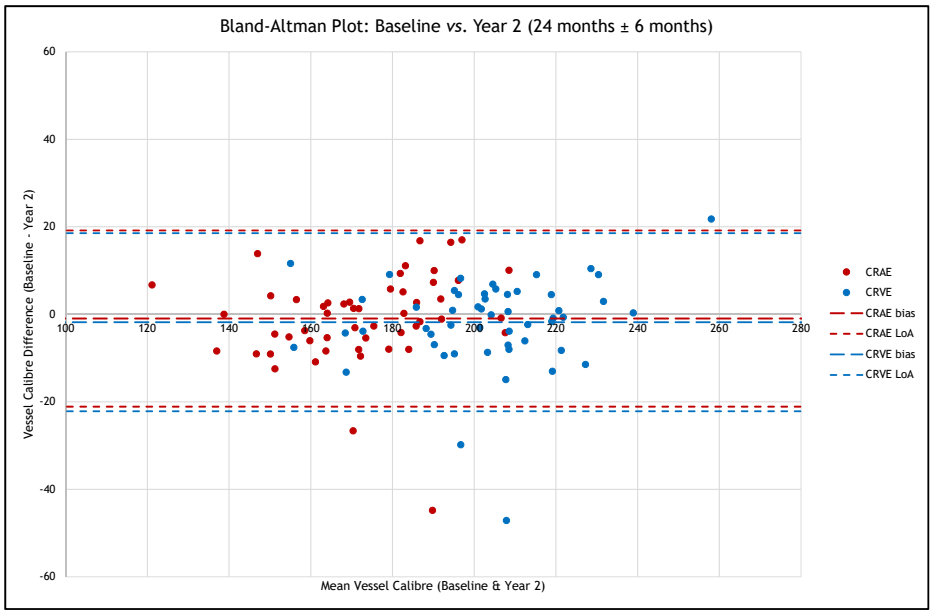


Figure 30: Bland-Altman difference-versus-mean plots showing the mean bias between baseline CRAE and CRVE and 12-month (upper panel) and 24-month (lower panel) follow-up. CRAE results are shown in red and CRVE in blue. Mean bias is denoted by longer dashes, and upper and lower limits of agreement (LoA) shown by smaller dashes. Note the near coincident 12-month upper LoA for CRAE and CRVE.

24-months saw a similar increase. Greater numbers of diagnosed hypertensives at both 12- and 24-month follow-up saw either a plateau or decrease in their blood pressure compared to baseline (16 and 8 respectively). With reference to NICE classification of blood pressure, only 2 subjects saw an increase of two categories over a 12-month period (and also a 24-month period). In both cases, this saw a mean increase of QRISK calculation of +10.60 and +9.00% for 12- and 24-months respectively. Mean QRISK for these individuals at 12-months was 48.30% and 30.00% at 24-months (both higher than the 20% intervention threshold). These changes were associated with a severe change in systolic blood pressure (+45.67 and +20.50mmHg respectively) and only a mild to moderate increase in diastolic blood pressure (+3.17 and +11.92mmHg respectively). Subjects who rose by +1 NICE category over the first 12 months had a mild decrease in CRAE (-4.07μm ±5.99) and a slight increase in CRVE (+2.49μm ±10.25). Conversely, over a 24-month follow-up, CRVE was seen to decrease (-5.05μm ±5.63), whilst CRAE only saw a very small overall reduction (-0.52μm ±4.65). For those individuals who dropped by -1 NICE category similar changes were observed. Over 12-months, CRAE decreased on average by -1.22μm ±8.18, whereas across 24-months follow-up, this same drop in category saw an average CRAE increase of +4.34μm ±4.92. Over these same periods, CRVE saw only a mild increase in the first 12-months from baseline (+0.09μm ±8.08) and a slightly larger increase over 24-months (+2.91μm ±5.90). For individuals whose blood pressure fell in NICE hypertension category, there was a progressive decrease in the calculated QRISK and Heart Age. For those who fell by a single blood pressure category, there was a -0.65% and -1.16% over 12- and 24-months respectively. The effects were doubled for individuals falling by two NICE hypertension categories over the same time periods (-3.27% and -3.63% respectively). Only those individuals whose blood pressure category did not change over the 12-month review period had a mean QRISK that was less than the 10% intervention threshold (9.45% ±10.12). QRISK was higher for those individuals with extreme changes in blood pressure (i.e. +/- two NICE categories); with a mean baseline QRISK of 37.70% (± 8.00) for those who rose by 2 categories, and a mean baseline QRISK of 24.57% (± 7.24) for those whose blood pressure fell by two categories. This effect was seen at a similar magnitude for the 24-month follow-up also (21.00% (± 6.40) and 22.57% (± 9.11) for +2 and -2 categories respectively). Real age-heart age disparity was greatest amongst those who rose by two categories (+7.50 years) over 12-months, but was greatest for those who fell by two categories over 24-months (+8.67 years); although the same category for 12-month follow-up also saw a 6 year real age-heart age disparity. CRAE and CRVE were approximately 6μm greater in those individuals who saw no change to their blood pressure classification over the initial 12-months of the study. For those individuals who were reviewed at 24 months, mean baseline CRAE

Interval	n	Normotension		Pre-hypertension		≥Stage I hypertension	
		Dx'd HT	Undx'd	Dx'd HT	Undx'd	Dx'd HT	Undx'd
12 months	122	5	36	10	37	16	16
24 months	55	2	13	7	16	7	10

Table 35: Cohort sizes based on NICE hypertension classification, split between those with pre-diagnosed hypertension (Dx'd HT) and undiagnosed (Undx'd). Data shown for both 12- and 24-month follow-up.

and CRVE were slightly greater (by a similar amount) in the +1 category, rather than no change.

6.3.2 Diabetes mellitus and retinopathy risk

Mean baseline data is summarised in Table 39 and average changes are shown in Table 40. Nineteen participants enrolled reported having either Type I (n = 10) or Type II (n = 9) diabetes mellitus. More males had Type II DM compared to females (6 vs. 3). Those with Type I DM were of a younger mean baseline age (57.40 years ±19.91 vs. 67.56 years ±11.14) and had a longer duration of the condition (13.80 years ±10.58 vs. 5.29 ±3.61) compared to Type II DM. Mean baseline HbA1c was higher amongst those with Type I DM (73.43mmol/mol ±29.52) and also increased by a greater amount over the follow-up period (+6.70mmol/mol ±33.37). Baseline BMI was greater amongst those with Type I DM compared with Type II (28.15 ±4.10 vs. 25.96 ±3.41); all but one subject reported 'no change' to their weight at subsequent follow-up visits. There was no significant correlation between BMI and HbA1c for all diabetic patients, or when split into Type I and Type II (p = >0.050). Systolic and diastolic blood pressure at baseline were very similar between both groups (129.77mmHg ±17.34 vs. 131.78mmHg ±5.06 and 79.50mmHg ±12.19 vs. 83.30mmHg ±10.76 respectively). Systolic blood pressure was seen to decrease during follow-up amongst the Type II DM cohort (-4.04mmHg ±11.27); with there being little mean change in the Type I cohort (+0.12mmHg ±16.85). Bi-directional changes were seen in diastolic blood pressure, with Type I DM seeing a slight increase (+2.30mmHg ±9.37) and Type II a slight decrease (-1.96mmHg ±8.25). Baseline QRISK score was higher than the specified 10% intervention threshold, more so with Type II DM (27.36% ±12.62) than Type I DM (21.12% ±12.02); QRISK was seen to increase in both groups, but this was also more so amongst Type II DM (+1.46% ±3.17 vs. +0.56% ±0.66). Baseline Heart Age was lower amongst Type I DM (73.17 years ±10.38), however there was also a greater mean baseline real age-heart age disparity compared to Type II DM (18.00 years ±3.06 vs. 9.11 years ±6.64). Mean baseline CRAE was less (and less variable) amongst those with Type II DM (161.31 ±10.17) than those with Type I DM (175.06 ±21.37), and

12 Months								
BP change	**n**	**Dx'd HT**	**Normotension**		**Pre-hypertension**		**≥Stage I hypertension**	
			Dx'd HT	Undx'd	Dx'd HT	Undx'd	Dx'd HT	Undx'd
BP_{syst}↑	41	10	1	11	2	14	7	6
BP_{diast} ↑	46	10	2	16	4	14	4	6
BP_{syst} =	4	1	0	2	0	1	1	0
BP_{diast} =	3	0	0	1	0	2	0	0
BP_{syst} ↓	65	16	2	28	9	15	5	6
BP_{diast} ↓	61	15	9	31	1	11	5	4

24 Months								
BP change	**n**	**Dx'd HT**	**Normotension**		**Pre-hypertension**		**≥Stage I hypertension**	
			Dx'd HT	Undx'd	Dx'd HT	Undx'd	Dx'd HT	Undx'd
BP_{syst}↑	16	2	0	4	0	6	2	4
BP_{diast} ↑	15	5	0	5	3	4	1	2
BP_{syst} =	0	0	0	0	0	0	0	0
BP_{diast} =	3	0	0	2	0	1	0	0
BP_{syst} ↓	28	8	2	8	3	12	3	0
BP_{diast} ↓	26	5	1	15	2	2	1	4

Table 36: Hypertensive status classified by overall blood pressure change between visits (*upper: 12 months from baseline; lower: 24 months from baseline*). Data is split between those with pre-diagnosed hypertension (Dx'd HT) and those without any diagnosis (Undx'd), and shows data for increases (↑), decreases (↓) and no change (=) to both systolic (BP_{syst}) and diastolic (BP_{diast}) blood pressures.

12 Months (Baseline data)											
Change in NICE classification	**n**	**QRISK**	**Age**	**BMI**	**Heart Age**	**Risk Ratio**	**CRAE**	**CRVE**	**AVR**	**BP_{syst}**	**BP_{diast}**
+2	2	37.70 (± 8.00)	76.00 (± 4.00)	24.75 (± 0.52)	83.50 (± 1.50)	2.05 (± 0.95)	180.69 (± 44.31)	198.49 (± 29.73)	0.90 (± 0.09)	127.33 (± 9.67)	89.33 (± 0.33)
+1	15	13.48 (± 9.46)	61.73 (± 11.82)	26.55 (± 3.21)	65.47 (± 13.08)	1.43 (± 0.81)	169.08 (± 22.67)	199.56 (± 24.64)	0.85 (± 0.07)	130.66 (± 13.76)	79.96 (± 9.56)
0	72	9.45 (± 10.12)	54.27 (± 17.65)	24.89 (± 3.03)	57.00 (± 15.87)	1.33 (± 1.00)	175.80 (± 18.56)	205.25 (± 20.09)	0.86 (± 0.07)	123.43 (± 17.43)	76.92 (± 10.22)
-1	28	17.89 (± 13.77)	62.92 (± 13.02)	26.19 (± 2.79)	67.42 (± 14.02)	1.54 (± 1.11)	167.58 (± 18.99)	199.48 (± 16.67)	0.84 (± 0.06)	136.13 (± 14.62)	86.62 (± 11.71)
-2	3	24.57 (± 7.24)	69.33 (± 3.86)	25.93 (± 1.56)	75.33 (± 4.64)	1.50 (± 0.14)	159.37 (± 1.96)	194.25 (± 7.08)	0.82 (± 0.03)	157.89 (± 14.93)	94.89 (± 8.17)
-3	1	30.00	72.00	28.64	73.00	1.00	176.13	219.02	0.81	110.00	64.00
-4	1	35.00	79.00	22.50	79.00	1.00	161.21	183.36	0.88	119.67	74.33

Measured change over 12 Months									
Change in NICE classification	**n**	**QRISK**	**Heart Age**	**Risk Ratio**	**CRAE**	**CRVE**	**AVR**	**BP_{syst}**	**BP_{diast}**
+2	2	+10.60 (± 2.20)	+1.50 (± 1.50)	+0.45 (± 0.15)	-0.98 (± 2.57)	+5.40 (± 1.61)	-0.03 (± 0.01)	+45.67 (± 14.67)	+3.17 (± 1.17)
+1	15	+1.71 (± 1.61)	+1.53 (± 1.02)	+0.01 (± 0.14)	-4.07 (± 5.99)	+2.49 (± 10.25)	-0.03 (± 0.03)	+6.58 (± 8.24)	+5.86 (± 5.95)
0	72	+0.63 (± 1.43)	+0.72 (± 1.67)	-0.02 (± 0.21)	-0.80 (± 6.62)	-0.23 (± 7.39)	<-0.01 (± 0.04)	-1.38 (± 8.49)	-0.77 (± 6.40)
-1	28	-0.65 (± 1.64)	-0.62 (± 1.94)	-0.22 (± 0.36)	-1.22 (± 8.18)	+0.09 (± 8.08)	-0.01 (± 0.04)	-11.15 (± 8.49)	-5.88 (± 7.18)
-2	3	-3.27 (± 0.82)	-1.67 (± 0.47)	-0.30 (± 0.08)	+0.94 (± 4.55)	-1.37 (± 4.02)	+0.01 (± 0.01)	-29.67 (± 10.17)	-10.89 (± 3.19)
-3	1	-8.30	-5.00	-0.50	-9.04	+10.39	-0.09	-56.33	-18.33
-4	1	-9.50	-5.00	-0.50	-2.70	-0.76	-0.01	-65.33	-22.33

Table 37: Descriptive baseline data and average changes based on change in NICE hypertension classification over 12-month period.

24 Months (Baseline data)										
Change in NICE classification	**n**	**QRISK**	**Age**	**Heart Age**	**Risk Ratio**	**CRAE**	**CRVE**	**AVR**	**BP_{syst}**	**BP_{diast}**
+2	2	21.00 (± 6.40)	70.00 (± 3.00)	76.00 (± 4.00)	1.80 (± 0.90)	167.40 (± 3.37)	203.34 (± 1.54)	0.83 (± 0.01)	118.33 (± 1.00)	67.67 (± 4.33)
+1	9	17.20 (± 13.04)	62.80 (± 11.50)	66.40 (± 14.84)	1.48 (± 1.04)	177.83 (± 16.55)	211.96 (± 11.06)	0.84 (± 0.11)	116.87 (± 15.24)	74.00 (± 7.73)
0	27	11.86 (± 11.75)	56.31 (± 18.47)	59.22 (± 16.93)	1.37 (± 0.84)	170.03 (± 21.34)	199.72 (± 24.02)	0.85 (± 0.06)	127.88 (± 15.69)	74.63 (± 11.24)
-1	14	14.23 (± 11.72)	61.07 (± 12.33)	64.67 (± 14.72)	1.31 (± 0.51)	169.04 (± 17.27)	202.70 (± 18.13)	0.84 (± 0.06)	140.35 (± 16.35)	89.21 (± 9.05)
-2	3	22.57 (± 9.11)	65.33 (± 4.19)	74.00 (± 5.10)	2.00 (± 1.07)	164.40 (± 20.97)	200.47 (± 18.85)	0.82 (± 0.03)	151.00 (± 7.62)	93.89 (± 11.12)

Measured change over 24 Months									
Change in NICE classification	**n**	**QRISK**	**Heart Age**	**Risk Ratio**	**CRAE**	**CRVE**	**AVR**	**BP_{syst}**	**BP_{diast}**
+2	2	+4.05 (± 2.75)	+1.00 (± 2.00)	-0.10 (± 0.10)	+3.64 (± 0.26)	-1.41 (± 9.60)	+0.02 (± 0.04)	+20.50 (± 13.50)	+11.92 (± 0.08)
+1	9	+3.34 (± 2.79)	+1.78 (± 2.78)	+0.08 (± 0.20)	-0.52 (± 4.65)	-5.05 (± 5.63)	+0.02 (± 0.03)	+20.00 (± 8.46)	+10.80 (± 7.39)
0	27	+0.52 (± 0.91)	+0.07 (± 5.49)	-0.11 (± 0.44)	+9.33 (± 34.10)	+12.30 (± 41.23)	+0.03 (± 0.16)	-1.45 (± 7.76)	-0.79 (± 6.06)
-1	14	-1.16 (± 1.64)	0.00 (± 1.56)	-0.21 (± 0.29)	+4.34 (± 4.92)	+2.91 (± 5.90)	+0.01 (± 0.03)	-14.74 (± 7.90)	-7.86 (± 3.44)
-2	3	-3.63 (± 1.99)	-2.33 (± 1.25)	-0.50 (± 0.36)	+5.94 (± 4.22)	+0.71 (± 8.56)	0.03 (± 0.06)	-30.33 (± 7.46)	-12.22 (± 10.96)

Table 38: Descriptive baseline data and average changes based on change in NICE hypertension classification over 24-month period.

both Type I and II DM saw a similar change in CRAE during follow-up (+2.64µm ±4.18 and +1.65µm ±7.16 respectively). Mean baseline CRVE was also reduced amongst those with Type II DM (195.45µm ±15.04) by almost 10µm compared to those with Type I DM (204.70µm ±21.76). Baseline AVR was very similar between both cohorts; 0.85 (± 0.04) and 0.83 (± 0.04) for Types I and II DM respectively. Calculated 1-year RetinaRisk at baseline was greater amongst those with Type I DM (1.49% ± 1.95). RetinaRisk was also seen to rise by a greater amount amongst those with Type I DM compared to Type II (+1.37 ±1.50 vs. +0.50 ±1.23). The distributions for these calculations was the same for 5-year RetinaRisk as the algorithm simply increases them by a factor of five.

6.3.3 ARIC-based stratification and cardiovascular risk

Changes in recorded AVR over time for those subjects falling within the ARIC age-range are shown in Figure 31. Given that follow-up time varied between subjects, with some presenting more frequently than others, statistical analyses becomes complex and lacks the sample size to strengthen these more elaborate tests. Those subjects in the upper and lower quintiles could be further classified by the time point in which they featured in these categories. Subjects could either remain in the quintile for the duration of follow-up (i.e. consistent); there could be an increase in AVR, which would lead to a rise in quintile (NB; for those in the lowest quintile, this effectively means that they are no longer in this quintile, whereas for the upper quintile this means a subject's AVR has now entered this range); there could be a decrease in AVR, leading to a fall in quintile, or there could be a non-linear change ("other"). The distribution of individuals into each of these categories is shown in Table 41. Cardiovascular and retinal vessel measurements for each upper and lower quintiles, stratified by longitudinal change category are shown in Tables 42 and 43.

Considering each progression type (consistent, rise, fall, other) is limited due to sample size, it is still possible to make general observations. Those AVRs in the uppermost quintile were generally more stable (i.e. all visits measured as the uppermost quintile; 6 of 11 subjects) whilst the AVRs in the lowest quintile had a greater number whereby the AVR increased to above the quintile cut-off during follow-up (5 of 15 subjects). Both mean systolic and diastolic blood pressure were lower in the entire upper quintile cohort (127.95 / 80.94mmHg), falling into the 'pre-hypertensive' NICE classification. This is contrasted to the lower quintile, where mean systolic and diastolic blood pressure fell within the 'Stage I hypertension' category (145.27 / 88.19mmHg). Blood pressure was highest amongst those who were in the uppermost quintile at baseline but fell below the cut-off during follow-up (148.21 / 90.17mmHg),

DM	n	Gender: Female [Male]	Age	Duration	HbA1c	BMI	BP_{syst}	BP_{diast}	QRISK	Heart Age
Type I	10	6 [4]	57.40 (±19.91)	13.80 (± 10.58)	73.43 (± 29.52)	28.15 (± 4.10)	129.77 (± 17.34)	79.50 (± 12.19)	21.12 (± 12.02)	73.17 (± 10.38)
Type II	9	3 [6]	67.56 (± 11.14)	5.29 (± 3.61)	48.93 (± 9.56)	25.96 (± 3.41)	131.78 (± 5.06)	83.30 (± 10.76)	27.36 (± 12.62)	76.67 (± 6.24)

DM	CRAE	CRVE	AVR	RetinaRisk (1 year)	RetinaRisk (5 years)
Type I	175.06 (± 21.37)	204.70 (± 21.76)	0.85 (± 0.04)	1.49 (± 1.95)	7.44 (± 9.76)
Type II	161.31 (± 10.17)	195.45 (± 15.04)	0.83 (± 0.04)	0.85 (± 0.48)	4.25 (± 2.41)

Table 39: Descriptive mean baseline data for individuals with Type I and Type II diabetes mellitus (DM).

DM	n	Interval	HbA1c	BP_{syst}	BP_{diast}	QRISK	Heart Age
Type I	10	1.53 (± 0.48)	+6.70 (± 33.37)	+0.12 (± 16.85)	+2.30 (± 9.37)	+0.56 (± 0.66)	+0.60 (± 2.24)
Type II	9	1.47 (± 0.61)	+4.81 (± 25.16)	-4.04 (± 11.27)	-1.96 (± 8.25)	+1.46 (± 3.17)	+0.11 (± 1.85)

DM	CRAE	CRVE	AVR	RetinaRisk (1 year)	RetinaRisk (5 years)
Type I	+2.64 (± 4.18)	+5.86 (± 8.87)	-0.01 (± 0.03)	+1.37 (± 1.50)	+6.85 (± 7.48)
Type II	+1.65 (± 7.16)	-0.85 (± 7.69)	+0.01 (± 0.04)	+0.50 (± 1.23)	+2.50 (± 6.14)

Table 40: Mean changes observed in individuals with Type I and Type II diabetes mellitus.

however both systolic and diastolic blood pressure dropped amongst these individuals on average by -13.21 and -7.67mmHg respectively. A similar drop in blood pressure was observed amongst individuals who were in the lowest ARIC quintile at baseline, but later rose above the cut-off during follow-up (-12.52 and -4.67mmHg respectively); this was accompanied by a slight drop in calculated QRISK (-0.32%) and heart age (-0.20 years). There were no significant changes in pulse oximetry measurements over the 12- or 24-month intervals in either quintile ($p = >0.050$).

All QRISK scores for those in the uppermost quintile were below the 10% intervention threshold suggested by NICE Guidelines. The highest scores were amongst those who were consistently in the upper quintile and those who fell below the quintile cut-off. The largest change in QRISK score for the upper quintile was seen in those who were consistently in this category (+1.97%). Real age-heart age disparity was greatest amongst the 'Rise' category in the upper quintile (+4.34 years), however this category had the largest mean decrease throughout the duration of the study (-4.00 years). QRISK was consistently greater than the 10% intervention threshold for the lower quintile, except for those individuals who rose above the quintile cut-off throughout the follow-up period. Those who were consistently in the lowest quintile had a mean QRISK score of 15.73% (compared to mean QRISK of 8.63% for those who were consistently in the uppermost quintile), but it was those who's AVR changes fell into a non-linear 'Other' change pattern who had the highest QRISK score (22.37%) and largest increase in QRISK across the duration of the study (+2.67%). Subjects in this category also had the highest recorded real age-heart age disparity (+10 years), which also rose during the follow-up period (+1.33 years), albeit on the same level as those who were consistently recorded in the lowest quintile (+1.25 years).

CRAE was greater for the upper quintile than lower (176.06 vs. 159.43μm), and was highest amongst those individuals who were consistently recorded as having an AVR in the uppermost quintile (183.71μm). Conversely, the lowest mean CRAE was recorded amongst those who were consistently in the lowest quintile (150.52μm). CRAE was seen to rise amongst those individuals whose AVR rose into the uppermost quintile throughout the study (mean change of +2.10μm), whilst the biggest decrease in CRAE was seen amongst individuals whose AVR fell into the lowest quintile after baseline (-5.90μm). CRVE was seen to be lower amongst the uppermost quintile compared to the lowest (189.95 vs. 203.63μm). In conjunction with the raised CRAE, this explains the higher overall AVR amongst this cohort. CRVE was greatest amongst those whose AVR rose into the uppermost quintile, although this category saw the largest overall decrease in CRVE across the follow-up (-8.27μm). CRVE was seen to increase by +8.14μm in those subjects were AVR fell below the upper quintile cut-off. For the lowest quintile, CRVE was

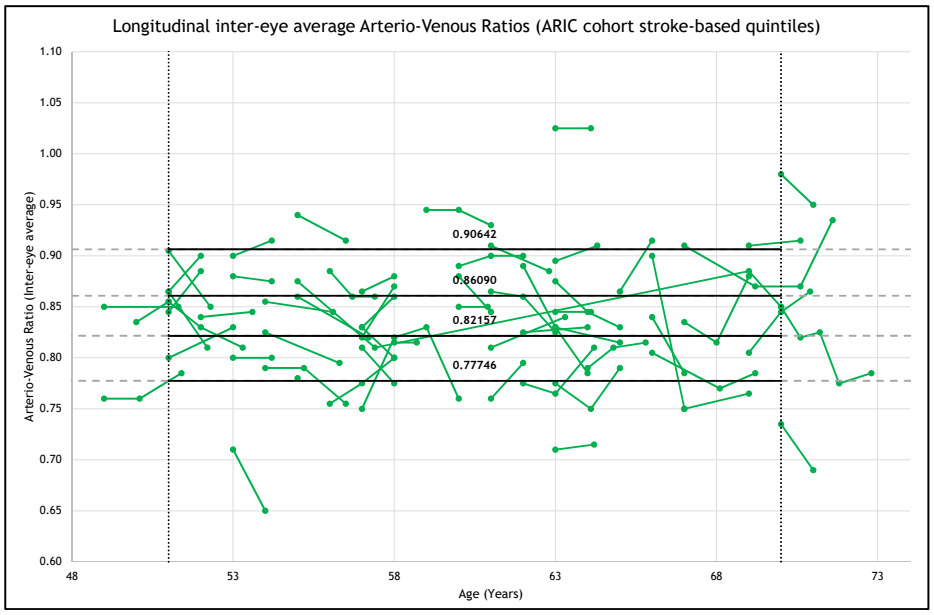


Figure 31: Scatterplot of longitudinal changes in AVR for ARIC-equivalent age range only (51-70 years) with ARIC-based quintiles of CVD event (stroke) overlaid. Quintile cut-offs displayed above each line.

largest for the consistent and non-linear 'Other' categories, being 207.19µm and 208.50µm respectively. The largest longitudinal change over time was seen amongst the sub-group whose AVR fell below the quintile cut-off throughout the study (+11.24µm). AVR was seen to increase amongst those who rose into the upper quintile throughout the study (+0.03) and fell by an equal amount for those whose AVR fell below the upper quintile cut-off as the study progressed. Consistent upper quintile individuals saw a mean decrease of -0.01 in AVR, which was similarly seen amongst those consistently in the lowest quintile (-0.02). The biggest change in AVR throughout the study was seen amongst those whose AVR fell below the lower quintile cut-off during follow-up (-0.07), which was in contrast to those whose AVR rose above the lowest quintile (+0.03).

6.4 Discussion

6.4.1 Hypertension and NICE-based blood pressure classification

The present study highlights the variability in blood pressure measurements over moderate time intervals, with a significant number of individuals (40.98% at 12-months and 50.91% at 24-months) meas-

	Upper Quintile (≥ 0.90642)	Lower Quintile (≤ 0.77746)
n	11	15
Consistent	6	4
Rise	3	5
Fall	2	3
Other	0	3

Table 41: Distribution of subjects in upper and lower ARIC quintiles, based on longitudinal changes in AVR.

uring in a different blood pressure category to that of their baseline one. Whilst a number of factors would also need to be considered, including the time of readings and a detailed analysis of the patient's cardiovascular health to identify any asymptomatic changes, this supports the argument that optometrists can assist in the management of patients through regular recording of blood pressure. This is also highlighted by the high proportion of subjects meeting the NICE hypertension classification, but are currently undiagnosed. Of the 89 subjects seen at the 12-month review (of a total of 122), only 16 (18%) registered a blood pressure within the 'normotensive' classification range. Of those whose blood pressure was higher than this, half fell into the 'Stage I hypertension' classification or higher. Sub-analysis of these groups could potentially highlight morphological changes observed in the retinal vasculature, although greater numbers are required to account for the variable duration of the diagnosed hypertensives to whom they would be compared (since overall duration of hypertension and intervention will both influence this group).

Interestingly, for those individuals who rose by one NICE category over 24 months saw a mean change in systolic blood pressure of 20.00mmHg (± 8.46). Whilst this change itself is similar to the interval between some NICE categories, it should be noted that *which* categories individuals rose between has not been factored for. Whilst the mean change is similar to those individuals who rose by 2 categories (+20.00 vs. +20.50mmHg), and indeed baseline mean measurement are similar (116.87 (±15.24) vs. 118.33 (±1.00) mmHg respectively), there is increased variability in those who only changed by one category, as demonstrated by the increased standard deviation. Despite this consideration, it can be supposed that a number of those subjects who rose by one category are actually close to the threshold for a further category increase, and thus the number of those rising by two categories may not be fully reflective of the cardiovascular changes observed.

Retinal vessel measurements do not appear to show a consistent correlation with changes in blood

	Age	BP_{syst}	BP_{syst} change	BP_{diast}	BP_{diast} change	QRISK	QRISK change	Heart Age	Heart Age change
Upper Quintile	61.55 (± 5.93)	127.95 (± 16.57)	-6.98 (± 10.87)	80.94 (± 10.23)	-3.18 (± 6.88)	8.67 (± 3.07)	+1.00 (± 2.92)	63.36 (± 6.11)	+0.50 (± 3.32)
Consistent	61.33 (± 6.70)	121.94 (± 14.04)	-3.22 (± 10.89)	78.94 (± 7.03)	-2.08 (± 7.39)	8.63 (± 3.47)	+1.97 (± 3.27)	61.83 (± 7.29)	+2.00 (± 2.71)
Rise	60.33 (± 5.25)	126.44 (± 16.02)	-12.00 (± 11.33)	78.78 (± 9.36)	-2.00 (± 6.33)	6.80 (± 1.50)	-1.55 (± 0.55)	64.67 (± 3.68)	-4.00 (± 2.00)
Fall	64.00 (± 3.00)	148.21 (± 3.54)	-13.21 (± 0.54)	90.17 (± 13.83)	-7.67 (± 2.67)	9.40 (± 2.50)	+0.65 (± 0.95)	66.00 (± 3.00)	+0.50 (± 1.50)
Lower Quintile	60.93 (± 6.17)	145.27 (± 14.50)	-5.22 (± 11.76)	88.19 (± 9.71)	-1.27 (± 9.28)	14.15 (± 8.62)	+0.95 (± 2.22)	67.13 (± 7.99)	+0.73 (± 1.29)
Consistent	63.25 (± 6.42)	152.50 (± 3.33)	-1.48 (± 5.10)	93.00 (± 4.52)	-7.23 (± 1.60)	15.73 (± 6.85)	+1.05 (± 0.39)	68.00 (± 6.75)	+1.25 (± 1.09)
Rise	58.20 (± 5.19)	145.45 (± 3.33)	-12.52 (± 6.41)	90.80 (± 11.42)	-4.67 (± 6.56)	11.46 (± 9.03)	-0.32 (± 0.56)	65.80 (± 9.22)	-0.20 (± 0.40)
Fall	59.00 (± 5.10)	130.25 (± 13.42)	+3.97 (± 7.99)	78.94 (± 4.07)	+5.50 (± 5.53)	8.27 (± 1.52)	+1.23 (± 0.69)	61.00 (± 3.74)	+1.00 (± 0.82)
(Other)	64.33 (± 5.44)	150.33 (± 18.46)	-7.22 (± 18.44)	86.67 (± 8.97)	+5.56 (± 12.89)	22.37 (± 7.34)	+2.67 (± 4.21)	74.33 (± 3.09)	+1.33 (± 1.89)

Table 42: Cardiovascular parameters and longitudinal changes for upper and lower ARIC quintiles.

	CRAE	CRAE change	CRVE	CRVE change	AVR	AVR change
Upper Quintile	176.06 (± 18.75)	-1.33 (± 6.15)	189.95 (± 18.24)	-0.70 (± 8.86)	0.93 (± 0.04)	-<0.01 (± 0.03)
Consistent	183.71 (± 18.33)	-2.08 (± 7.83)	191.40 (± 15.98)	+0.31 (± 9.43)	0.96 (± 0.04)	-0.01 (± 0.01)
Rise	174.42 (± 6.56)	-2.35 (± 3.34)	197.17 (± 11.10)	-8.27 (± 1.41)	0.89 (± 0.02)	+0.03 (± 0.02)
Fall	159.39 (± 20.63)	+2.10 (± 2.09)	175.50 (± 23.40)	+8.14 (± 0.58)	0.91 (± 0.01)	-0.03 (± 0.01)
Lower Quintile	159.43 (± 11.90)	-0.72 (± 6.03)	203.63 (± 16.27)	+3.26 (± 10.68)	0.79 (± 0.06)	-0.02 (± 0.05)
Consistent	150.52 (± 5.26)	-1.54 (± 4.68)	207.19 (± 8.84)	+5.09 (± 12.05)	0.73 (± 0.02)	-0.02 (± 0.03)
Rise	151.97 (± 19.04)	+3.51 (± 6.56)	198.64 (± 24.14)	-3.36 (± 7.38)	0.77 (± 0.01)	+0.03 (± 0.01)
Fall	169.60 (± 4.90)	-5.90 (± 3.36)	203.85 (± 5.93)	+11.24 (± 9.91)	0.83 (± 0.05)	-0.07 (± 0.05)
(Other)	166.37 (± 7.79)	-1.47 (± 3.38)	208.50 (± 6.90)	+3.87 (± 6.80)	0.80 (± 0.04)	-0.03 (± 0.03)

Table 43: Retinal vessel parameters and longitudinal changes for upper and lower ARIC quintiles.

pressure beyond those who saw a reduction in blood pressure category (i.e. their blood pressure was higher at baseline than follow-up) were observed to have a slightly reduced baseline CRAE compared to those whose blood pressure remained stable throughout. A limitation of the current study is that stable blood pressure as defined by NICE classification categories does not account for those with persistent hypertension, whether or not it is being treated. Due to the cohort size, splitting the categories down to number of changes around each classification criteria would result in extremely small numbers, further weakening any statistical arguments.

6.4.2 Diabetes mellitus and retinopathy risk

Both mean baseline age and duration of DM were reflective of the different pathogeneses of Type I and II DM. Those with Type I were generally younger (with a mean difference from Type II DM of 10.16 years) and had a longer duration of the condition, thus increasing the age gap at diagnosis. The larger number of males with Type II DM is in line with reported incidence rates and a suggested higher susceptibility (particularly at lower BMI) which is also observed in the present cohort[267]. The increased duration of Type I DM, along with higher BMI, could also be reflected in the higher QRISK calculation for this cohort. Prolonged disease duration, with potential decreased responsiveness to treatment, could account for an impaired cardiovascular system and also be related to the increased BMI of these subjects. This is further demonstrated by the increased real age-heart age disparity amongst these subjects; almost double that of the Type II cohort. Previous epidemiological studies have shown duration of DM to be a significant risk factor for further development of cardiovascular disease, with DM onset >10 years prior comparable to a past history of heart attack and increased mortality[268, 269, 270]. Increased DM duration, HbA1c and BMI are all considered to be independent risk factors for developing diabetic retinopathy[161, 271, 272, 273]. These are all reflected in the RetinaRisk calculations (which includes the duration and HbA1c); however the algorithm does not account for BMI. It could therefore be considered supportive evidence of the independent link between BMI and risk of DR development/progression that the RetinaRisk calculation is indeed higher in the subjects with a higher mean BMI, despite no individual correlation between BMI and HbA1c. Baseline retinal vessel measurements were not dissimilar to the rest of the cohort (*see Table 44*).

Consideration must be given, however, to the large non-diabetic cohort versus the relatively small diabetic ones, plus the wide age-range and co-morbid nature of these groups; all of which have not been accounted for. The relative narrowing of CRAE and CRVE amongst those with Type II DM compared to

	n	CRAE	CRVE	AVR
Type I DM	10	175.06 (± 21.37)	204.70 (± 21.76)	0.85 (± 0.04)
Type II DM	9	161.31 (± 10.17)	195.45 (± 15.04)	0.83 (± 0.04)
Non-DM	245	172.43 (± 18.06)	201.16 (±18.10)	0.86 (±0.07)

Table 44: Baseline retinal vessel data for both diabetic and non-diabetic cohorts.

Type I could reflect the earlier microvascular alterations described in Chapter 2, Associations of Retinal Vessel Calibre with Cardiovascular Disease: A Systematic Literature Review (*see Section 2.4.2. Association of diabetes mellitus and retinal vessel calibre*) particularly given the much shorter duration amongst this group. However, it could equally be explained by physiological differences observed in older subjects, since the mean age of those with Type II DM at baseline was a decade greater than that of Type I. In order to determine true changes observed in relation to pathologies such as DM, an accurate method of accounting for what could be considered 'natural ageing' also needs to be employed. At present, the changes observed, however significant, still fall below the threshold of measurement error and would thus be considered clinically insignificant at this stage.

6.4.3 Cardiovascular risk and ARIC-based stratification

In the absence of official values relating to retinal vessel calibre (either individual CRAE / CRVE or an aggregate such as AVR), the use of values based on the original study whereby the formulae used were derived and tested offers a starting point for stratification. Whilst this is based on an AVR and doesn't give as much detail for the arteries and veins independently, it has the advantage of supporting evidence in a larger cohort. The associations seen with this approach in the present cohort cross-sectionally has good agreement with those reported by the ARIC study and elsewhere (*see Section 5.4.3 Cardiovascular risk and ARIC-based stratification*). A number of these trends have been observed longitudinally also. Blood pressure was lower in the upper quintile category, with the majority of individuals registering as 'pre-hypertensive', in contrast to the lowest quintile were individuals were 'Stage I hypertension'. This is in agreement with the established correlation between raised blood pressure and risk of stroke. Interestingly, those individuals who were in the uppermost quintile at baseline, but proceeded to drop below the cut-off (i.e. 'fall') had a mean blood pressure in the 'Stage I category' which saw a subsequent drop to the 'Pre-hypertensive' category over the same time course. Whilst cohort numbers are small for this category (n = 2), the conjunction of change in ARIC quintile and significant drop in blood pressure may warrant further investigation into cardiovascular status. All subjects who rose to a higher ARIC quin-

tile throughout the study saw a decrease in blood pressure, systolic more than diastolic. These changes were, on average, greater than 10mmHg (although the standard deviations are also relatively high, as a result of small cohort numbers), i.e. the changes would see a down-grading of the individuals' NICE blood pressure classification. This is echoed by decreases in calculated QRISK for these subjects, and in the case of the lower quintile, bringing those individuals closer to the 10% intervention cut-off. There is an opposite increase in QRISK for both those individuals who either fell a quintile throughout the study, and those who remained consistently in the same quintile throughout. This poses the question whether the changes observed in QRISK calculation are of a magnitude to reflect a clinical change in the individual (i.e. the 'Fall' categories) or whether this is associated with natural ageing (i.e. the 'consistent' categories). Again, both greater numbers and a more specific array of cardiovascular measures (including blood samples and less reliance on anecdotal reporting from patients) would be required to further investigate this point. Calculated Heart Age, also derived through the QRISK algorithm, changed to different extents depending on quintile category. Those consistently in the uppermost AVR quintile saw a 2-year increase in their Heart Age over the course of the study; however this was very similar to their actual baseline age (61.83 vs. 61.33 years respectively). Those whose AVR rose into the top ARIC quintile over the course of the study saw, on average, a 4-year reduction to their calculated Heart Age, which was 4 years above their actual age at baseline (therefore, when factoring in the duration of the follow-up, their final Heart Age would, on average, be less than their real age, implying a greater level of cardiovascular health). There was a much greater real age-heart age disparity amongst individuals in the lowest ARIC quintile; suggesting poorer cardiovascular health. Those who were consistently in the lowest quintile had, on average, a Heart Age 4.75 years greater than their actual age. The greatest disparity was, however, amongst those individuals who's AVR had a non-linear change across the course of the study (i.e. their AVR was intermittently graded as being in the lowest quintile). This was again limited to a small sample size (n = 3), however it suggests an interesting sub-group warranting further attention. This group had the highest QRISK scores (22.37%) and also the most variable blood pressure at baseline (systolic blood pressure: 150.33 (± 18.46) mmHg). This was not reflected in their retinal blood vessel measurements, which did not alter hugely from the other categories in this quintile. This is perhaps unsurprising, since changes to the microcirculation are likely to not be as turbulent as other cardiovascular metrics in the given timescale.

CRAE was markedly reduced in the lowest quintile (150.52μm ± 5.26) compared to the uppermost quintile (183.71μm ± 18.33). The reverse was seen, albeit to a lesser extent (thus producing the different

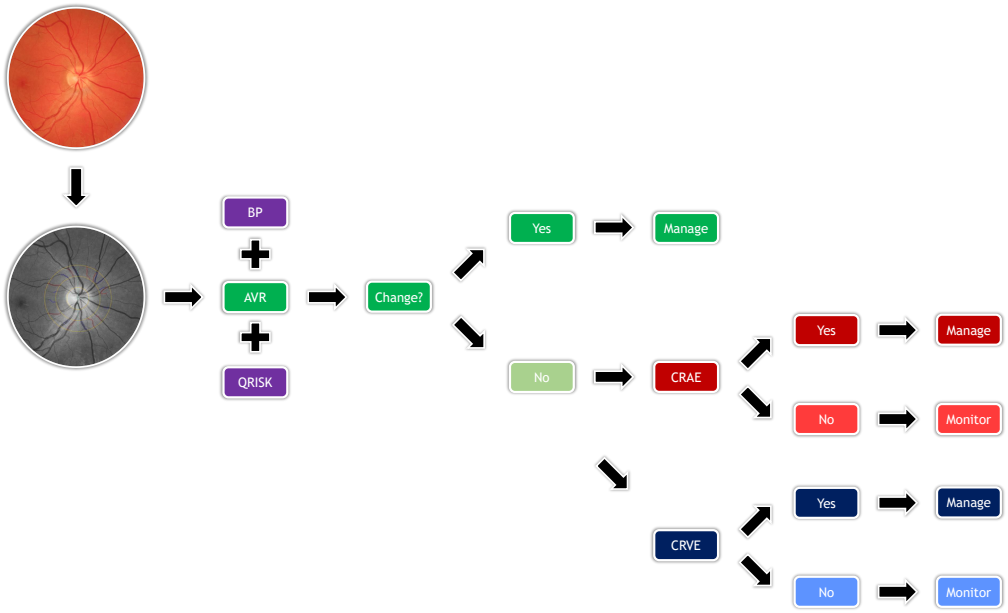


Figure 32: Summary flowchart for interpretation of vessel changes. A colour fundus photograph is analysed and the AVR produced and supplemented with blood pressure (BP) data and a QRISK cardiovascular risk calculation. If changes in AVR are not observed, individual vessel changes (CRAE and CRVE) are prompted to fully exclude any vessel-specific calibre alterations. The decision to monitor or intervene / manage is then instigated.

AVRs by which the groups were stratified) with CRVE. In the lowest quintile CRVE was slightly wider (207.19µm ± 8.84) compared to the uppermost quintile (191.40µm ± 15.98). The greater standard deviation should also be noted for CRVE which, despite the greater contrast with the background retina, appears to be a common finding in retinal vessel measurement repeatability and accuracy. The difference in individual vessel width suggests that arterial narrowing plays a greater role in the stratification by AVR compared to CRVE. This is supported by the significant difference in mean blood pressure readings across the two quintile extremes. Whilst the present study is limited by sample size, the good agreement of blood pressure, QRISK, Heart Age with changes in retinal vessel parameters (particularly CRAE and AVR), suggests that there is merit in stratifying patients by a pre-defined cut-off value for AVR. The use of AVR can precede any vessel-specific observations, as summarised in Figure 32.

6.4.4 Study limitations

The most significant limitation of the present study is the lack of patient retention within the sample. Availability to see the same optometrist, responsiveness of patients to reminder letters, and general patient movement meant that there was a severe drop in the number of repeat visits included in the study. This was further compounded by the less than regular nature at which patients present for a 'routine' eye examination, which is more sporadic than the recommended review interval. Some patients presented earlier or later than their allocated review appointment (for instance with a subjective change in vision, or simply wishing to replace existing spectacles) which meant that follow-up time was not confined to fixed intervals of 12 or 24 months. Combined with the lack of consistent review, this resulted in a greatly diminished sample for subsequent analysis.

Just as with the limitations outlined in Chapter 5.4.4, the issue of patient drop-out is doubly affected by the lack of adequate sample size (as determined through a sample size calculation; *see Chapter 3.3 (Sample size and patient recruitment)*). Similar to the cross-sectional study, significant findings and trends reported within this chapter at least serve to highlight areas which will require future study of a more focussed design. For instance, observing a sample of patients with significant cardiovascular risk profiles and whether changes can be detected amongst that sample depending on whether their risk profile improves, worsens or plateaus. Particularly if vessel calibres and AVRs are to have intervention guidelines in-line with established metrics like QRISK, longitudinal data is very important for subsequent visit management. Such studies would need to be undertaken alongside observation of a comparative 'healthy' sample to determine whether normal physiological ageing of vessels results in clinically significant changes to measured calibres. As with the previous cross-sectional study, generalisation of findings with the present cohort is restricted to the localised Caucasian population and further work is needed to discover any regional variance in findings.

6.4.5 Conclusion

Whilst cross-sectional correlations are readily demonstrated and replicated in a clinical practice setting by objective retinal vessel analysis (*see Chapter 5 Correlations Between Retinal Blood Vessel Calibre and Cardiovascular Metrics Observed in Optometric Practice*), it appears that these correlations do not fluctuate over moderate time intervals (i.e. 12 - 24 months); an interval which could be considered the average inter-examination period in routine practice. Stability and consistency of findings at this level is key both in the cases of known pathology and physiological 'good health'. Microvascular changes have

generally been observed over extended periods (such as 5 or even 10 year intervals), which is suggestive of more gradual remodelling. This would support the lack of clear trend(s) observed in the present cohort. Consistency in vessel calibre measurements across this time scale then allows for clinically significant changes to be highlighted and managed accordingly. It should also be noted that preference is placed upon the term 'consistency' in measurements, since 'stability' has an indirect implication against the activity of any pathological process. The present study's findings have been hampered most significantly by small numbers. Robustness of statistical analysis requires greater numbers and so future longitudinal studies should focus on increased participant numbers. Sub-division of cohorts into distinct pathology groups (hypertension, Type I diabetes mellitus, etc.) will then allow for identification of condition-specific changes. It should also be noted that this approach is not able to account for changes which occur for part of the inter-examination period, such as bouts of altered exercise levels or periods of dieting. Above all, work to identify (and ultimately account for) physiological ageing of the retinal microcirculation in the absence of disease is paramount. Just as limits of error have been identified from a number of sources at a single visit (*see Section 4.4 Discussion*), extrapolation of these limits needs to be made for longitudinal observation.

Clinically, this longitudinal study observed a lack of variation in vessel calibre despite blood pressure changes, which suggests that the structural change either precedes the rise in blood pressure, or is happening at too slow (or too little) extent to be detected by this current approach. With reference to the extensive pool of literature correlating reduced CRAE with increased blood pressure (*see Section 2.4.1. Association of blood pressure and retinal vessel calibre*), when quoting change in blood pressure in the order of +10mmHg, an average CRAE change was reported as -3-4μm; suggesting that an increase in blood pressure with a magnitude of +40mmHg would need to be required in order to cause a calibre change with enough clinical significance to fall outside the current limits of error. The present study also highlights the continued agreement of blood pressure, QRISK and stratification of patients by AVR. A clinician can therefore use AVR as an initial marker for change, before focussing on more vessel specific changes.

7 Incorporation of Retinal Blood Vessel Calibre Measurements into Multi-Disciplinary Patient Care

7.1 Introduction

Multi-disciplinary care is being heralded as a possible future solution to the currently over-burdened general practitioner (GP) based model of healthcare. It has been identified that with the current number of new GPs there will be a shortfall in provision of services for the population[274]. Annually, there are over 300 million GP consultations (compared to 23 million A&E presentations), despite funding for the latter increasing twice as fast over the same period[275, 276]. In an effort to tackle this, a number of approaches are being taken to promote multi-disciplinary care; utilising other competent healthcare providers to work collaboratively for a patient. This is in addition to the NHS aim to provide an additional 10,000 staff into GP practices by 2021, with half of those being doctors and the remaining consisting of other healthcare providers such as pharmacists and nurse practitioners[277]. An independent report by the King's Fund identified the need (and ability to integrate) multi-disciplinary patient care and that it was a viable and worthwhile solution to meet the ever-increasing financial burdens upon the NHS[278]. Over the last few years, the NHS, in collaboration with the National Association of Primary Care (NAPC) proposed the primary care home concept which focuses heavily on a localised healthcare network[279]. The combination of primary, secondary and tertiary care aims to bring a wealth of expertise and clinical skill to improve the management of patients, but also ease the burden on current go-to healthcare environments such as GP surgeries and accident and emergency. Whilst the Optical Confederation, an organisation representing a number of professional bodies within UK optometry, are listed as an associate of the NAPC, there is currently limited interaction of optometrists in the 'general' care of patients[276]. Minor Eye Conditions Schemes (MECS) exist on a regional basis and act as a buffer to prevent less serious eye complaints from arriving in ophthalmology accident and emergency. Although there is currently limited data, the use of MECS-based schemes has been shown to generally reduce the number of first-time and follow-up attendees at hospital ophthalmology departments[280]. MECS-based care could be considered 'later' within the pathological process, since the patient is symptomatic upon presentation which implies that a process has already been underway prior to this point. Relating this to cardiovascular health, the events which would render the patient symptomatic (such as stroke or heart attack) would firstly not involve the optometrist, but more significantly, pose a life-threatening risk to the patient. The cost implications of such an event are also much greater than if prophylactic inter-

vention were offered at an earlier point. A local scheme in the West Midlands has developed over the past few years to partially address this point. The Healthy Living Optician scheme in Dudley involves optometric practices providing health checks such as blood glucose and cholesterol levels, but also lifestyle management including weight loss and smoking cessation[281]. The scheme has yet to be rolled out nationally, but it demonstrates the ability to develop and integrate such a scheme. The Mayo Clinic Heart Disease Risk Calculator calculates 30-year risk of a cardiovascular event also provides a Controlled-Risk Factor grade; the 30-year risk if the modifiable (i.e. lifestyle) parameters were improved. From the cross-sectional population described in Chapter 5, *Correlations Between Retinal Blood Vessel Calibre and Cardiovascular Metrics Observed in Optometric Practice*, the modifiable risk was, on average, 11.30% less than their 'current' risk. Whilst not directly comparable, the QRISK calculator also provides a score for a 'healthy individual' of the same age, gender and ethnicity. Since this includes the presence (or absence) of pathology, it is not strictly the same as modifiable risk, but it gives some indication as to the disparity between what the individual's present cardiovascular risk is compared to an age-matched healthy individual.

7.2 Hypertension

Management of newly-diagnosed cases of hypertension is increasingly being delegated to other healthcare practitioners within a GP's practice. 73% of practices in a UK-based study reported changes to their hypertension management structure between 2011-16, with more responsibility placed upon nurses and healthcare assistants[17]. In this same study there was increased interest in a pharmacist-led hypertension clinic (19 of 117 practices), suggesting an appetite for shared-care beyond the GP's practice. The present studies have shown that optometrists are capable of recording structural microvascular differences observed in those with hypertension, in line with that reported elsewhere. Short-term structural changes have been reported with commencement of anti-hypertensive treatment[282]. Further studies need to demonstrate whether these changes are observable in practice with the current methodology (Hughes et al's study employed length-to-diameter ratio (LDR) to quantify arterial narrowing), but the finding suggests a possible role for optometrists in the co-management of recently diagnosed hypertensives. It has also been shown that dietary advice (adopting the Dietary Approaches to Stop Hypertension (DASH) approach) to normotensive UK adults was both well-tolerated but also resulted in a reduction in blood pressure[283]. Interestingly, mean pre-intervention blood pressure was 122.0/82.8mmHg which is actually considered 'pre-hypertensive' under NICE Guidelines, however this reduced to 117.4/78.9mmHg

following 30 days on the DASH diet.

7.2.1 Retinal vessel calibres and current hypertension intervention guidelines

Cross-sectionally, it has been shown that retinal vessel calibres (specifically arterial calibres) are strongly correlated with increasing blood pressure. This correlation appears to be unaffected by the cardiac cycle as measurements do not change significantly across a rapid sequence of images, and is supported by work elsewhere which suggests transient fluctuations in BP throughout the day also do not impact on retinal vessel calibre correlations[246]. Despite retinal arterial narrowing and increasing blood pressure being correlated for over 150 years, there is no clinical management guideline at present which includes this link, and it is simply a supplementary finding, or there is simply a suggestion that the patient will get their blood pressure checked by the GP. With over-burdened GP practices, an appetite for multi-disciplinary care with regards to hypertension has been demonstrated with other primary care professionals (pharmacists) suggested as a possible option for easing the workload of GPs[17]. As an alternative, optometrist-led hypertension clinics would have the added benefit of supplementing blood pressure measurements with structural measurements of the microcirculation (i.e. CRAE). This presents significant advantages over the conventional method of simply measuring blood pressure. In addition, the present cross-sectional study has highlighted a significant proportion of potentially undiagnosed hypertensives (62.35% of all those with a blood pressure considered as 'Stage I hypertension' or higher), as well as an even larger proportion of pre-hypertensives (76.84%)) (*see Section 5.3.1. Results: Hypertension and NICE-based blood pressure classification*). This is a finding reported previously when optometrists have measured blood pressure in practice, with a similar percentage (67%) also being undiagnosed[18]. With hypertension being predominantly asymptomatic, the benefit of incorporating routine hypertensive monitoring into optometric care (over other primary care providers such as pharmacists) is the routine nature with which patients present. The NHS run their Health Check service, which screens patients aged between 40 and 74 years for early signs of cardiovascular disease. This scheme, whilst adopting a proactive approach, is not all-encompassing. Patients are invited to attend screening; between 2012 and 2017 this amounted to over 11.5 million invitations[284]. On average, only 44.7% of those invited attended. The rate of non-attendance was much worse in the younger age bracket, with a yearly average of 207,797 persons aged 40-49 attending for their health check, with 650,001 not attending. This is in contrast to those aged 60-69 years, where average for attendance and non-attendance over the same period were 266,376 and 225,878 respectively. The NHS have not yet published data to

demonstrate how successful the Health Check programme has been at identifying those patients who would otherwise have gone undiagnosed, but whatever the success rate the attendance suggests that over half of England's population within the eligible age-range have gone unchecked. As such, screening as part of an annual (or biennial) eye examination has the additional benefit of potentially detecting hypertensive changes, both through physical examination of the retinal microcirculation (via objective measurement independent of clinician experience) and measurement of blood pressure. It is difficult to determine the overlap of non-attenders for a Health Check appointment and those presenting for a routine eye examination, but since Health Check is available to those of a presbyopic age, there is a significant chance that they may present in practice for a routine eye examination, even if for a one-off visit. Just as changes in IOP, whilst within the NICE Guidelines 'optimum' range, can still indicate (pre-)glaucomatous changes for that individual, the same can be said for a pre-hypertensive patient. It is only through having a catalogue of readings of successive visits that any gradual changes may be detected. For instance, a patient who is considered hypotensive could have a large increase in blood pressure and still remain within the 'normotensive' classification despite their change being significant and potentially warranting a cardiovascular examination. This particular instance would greatly benefit from regular measurement of blood pressure and review of those values by an optometrist in conjunction with an examination of their retinal microcirculation. Given the number of co-variables which may (or may not) influence retinal vascular calibre, it becomes difficult to attempt to provide an optimum value under which all measurements of CRAE should be considered at risk. Rather, an intuitive approach would need to be taken, considering both previous measurements of vessel calibre, blood pressure and the patients' general health and well-being. However, further studies observing patients over extended periods in an attempt to emulate the large cohort studies that have reviewed subjects after 5 or 10 years, would potentially allow practitioners to begin to discriminate between pathological and physiological changes in vessel calibre. At present it is possible to correlate blood pressure and vessel calibre cross-sectionally, and whilst it is unlikely to set an absolute change in vessel calibre (i.e. reduction in CRAE) suggestive of incipient hypertension without accompanying blood pressure measurements, it will be perfectly feasible to develop a structured intervention and management profile which incorporates retinal vessel calibre changes. The NHS has proposed that the introduction of more clinician pharmacists into GP practices who can help monitor and intervene earlier with common-yet-severe conditions such as hypertension is one way that multi-disciplinary care can ease the burden on GPs[275]. An additional proposition to this should be the use of optometrists for the routine screening of those individuals not currently attending

GP practices, and are therefore not going to access this service.

7.3 Diabetes mellitus

Individualised care regimes based on risk calculations have been shown to decrease the number of review appointments in low-risk cases and conversely increase appointment frequency for high-risk cases amongst diabetic patients[233, 230, 231, 232]. Of all the pathologies associated with cardiovascular disease, DM has the strongest multi-disciplinary links incorporating optometric care; from diagnosis and monitoring through to outcome-based patient management. It has recently been shown that incorporating retinal vessel parameters (including CRAE and CRVE) greatly improved the classification of CVD risk when included alongside conventional biomarkers such as estimated Glomerular Filtration Rate (eGFR) and high-sensitivity C-reactive protein (hsCRP) by between 17.0-19.1% (Net Reclassification Improvement)[285]. At present in the UK, those with DM are screened routinely by the Diabetic Eye Screening (DES) Programme on an annual basis (or more frequently as appropriate)[286]. The defining criteria for evaluating images omits any reference to vessel calibre (subjective or objective), despite the documented evidence of calibre changes in diabetes[287]. Individualised review periods based on algorithms have been shown to reduce screening frequency by up to 61%, a benefit for both the patient and the health service. These algorithms have since been incorporated into the RetinaRisk app, and have the ability to highlight patients considered 'at risk' for DR progression. As a patient group, those with DM are benefiting from a further evaluation of DR status by presenting for routine eye examinations in between DES visits. Since visits to DES are primarily concerned with evaluating DR, the additional CVD risk factors can be evaluated by the optometrist and actioned accordingly.

7.3.1 Retinal vessel calibres and current diabetes mellitus intervention guidelines

Algorithm-based risk calculators such as RetinaRisk can be refined as additional factors are identified. Since it has been shown that retinal vascular signs (in conjunction with established biomarkers of DM) improve the calculation of CVD risk, further work into retinal vessel calibre changes in DM is required to explore this relationship further. Whilst it is possible to quantify other vessel properties (including tortuosity, branching angle and fractal dimension), none have gained such widespread application as calibre measurements suggesting this characteristic be the initial site of attention for further refinement of risk calculations[288]. The use of specialist risk calculators like RetinaRisk (although designed for the patient) can be used in practice by an optometrist to help determine follow-up and recall periods

for diabetic patients to minimise risk of retinopathy going undetected. Greater distinction also needs to be made in order to reflect the underlying subtype of DM, particularly to reflect the recognition of young-onset Type II DM as a pathological process distinct from the classic 'age-onset' Type II DM. Further improvements in the classification of DM subtypes may also help to explain the sometimes conflicting findings observed in diabetic cohorts. A number of deep learning programmes using artificial intelligence (AI) have been used on DM and DR datasets with promising results[289, 290, 291]. Similar to the NHS's defining criteria of DR, there is no accounting specifically for vessel calibre with AI, although since the system works on a pixel-by-pixel approach and detects local changes, it may be that calibre is inadvertently being incorporated into these models. The present defining criteria for DR still features subjective elements and the inclusion of definitive measurements has the potential to further identify at risk patients, and also document changes to (and stabilisation of) the microvasculature longitudinally. As with hypertension, the definition of optimum calibres is unlikely, especially given the multiple co-morbidities associated with DM, but clinically significant changes to vessel calibre can be identified and correlated with existing measures of DM, with subsequent inclusion into DR defining (and intervention) criteria.

7.4 Cardiovascular risk

The risks associated with cardiovascular disease can be broadly split into two categories; those which are pathological and those which are modifiable. Pathological risk factors include genetic predisposition, co-morbidity, height (if considering BMI-based calculations of risk) etc., and whilst they cannot be altered they need to be considered when determining an individual's risk. Modifiable risk factors are those which equally important in risk calculations, but their impact can be, to some extent, controlled or mitigated by the individual's lifestyle. This includes diet, alcohol and caffeine intake, smoking status (or exposure to passive smoke) and amount of exercise. These are all integrated into general risk calculations, since pathologies of the cardiovascular system are so diverse it would not be practical to establish individual risk for each element of CVD. At a primary care level, identification of those at most risk is key since the modifiable risk factors for each form of CVD are generally the same, and early intervention is key.

7.4.1 Retinal vessel calibres and current cardiovascular risk intervention guidelines

In order to reflect the geographic consideration built into the present QRISK algorithms, studies of retinal vascular calibre could benefit from a multi-centre approach to investigate whether retinal vessel trends are equally variable depending on location also. The use of multiple clinics across the UK would provide a greater number of subjects, but also reflect this geographic (and socio-economic) diversity which has been accounted for in QRISK. Just as QRISK has been shown to have a greater accuracy than Framingham-based calculations, the same could be the case when using the ARIC-derived AVR cut-off values for stratifying risk. Whilst these values have been shown to support findings based on cardiovascular risk alone, there is undoubtedly room for refinement in those values; particularly to reflect the multiple variables which can impact upon cardiovascular risk. As such, it may well be that a tailored approach of intervention or cut-off values is required, with a QRISK-style algorithm requiring input of certain metrics to derive the values (such as age, gender, smoking status, medical history etc.). Since these are shared variables between both retinal vessel assessment and QRISK calculation, a simple solution would be the development of a combined platform which generates both values for an individual simultaneously.

Many of the large cohort studies covered in the literature review have integrated a battery of blood tests (particularly cholesterol, HbA1c and c-reactive protein) into their methodology to evaluate cell-specific correlations with cardiovascular pathology and retinal vessel diameter. The most recent figures available from the NHS (for the year 2015-16) show that the unit cost for phlebotomy is £3 per patient, with additional haematology costs of a further £3[292]. Whilst a low unit cost, the combined total spend on those two services cost the NHS £145,471,320.00 in that year alone. Taking the number of potentially undiagnosed hypertensives detected in the present study as an example, there are potential merits in optometrists also performing such routine blood work on patients whom may be identified as 'at risk' through combined QRISK and retinal vessel assessment (blanket screening would be not be financially viable). However, the additional costs of such an expansion to the clinical remit of an optometrist would be significant (education and training, indemnity insurance, and also clinic preparation to provide sterile environments for sample collection and handling; plus clinical waste disposal). As such, further work to explore whether such detail would provide clinically useful information needs to be undertaken, if only to demonstrate that correct signposting to the appropriate medical practitioner (i.e. GP) to perform these additional tests is sufficient. Alternatively, regional blood specialists in optometry may provide an intermediate solution if there was significant demand. The use of finger-tip pulse oximetry has shown

that tests can be used to give a gross overview of patient health, but their lack of correlation to any retinal vascular measurements suggests limited application.

7.5 Implementation into clinical practice

Implementation requires the purchase of the necessary equipment. In this instance, vessel analysis is more of an adjunct to fundus photography, rather than the incorporation of an entirely new examination. In order to build vessel analysis into practice, the principle investment will be in the analysis software. VesselMap is a one-off cost of £10,000 (iFlexis is significantly cheaper at £3,000, although this only covers a three-year period). Using the present enrolled cohort (n = 270), it would take 3 years at a charge of £12.35 per patient to fully recoup this expenditure. By means of a worked example, the practice involved in the present study saw, on average, 12 patients per day. Extending this over a 5-day week for 48 working weeks in a year results in an average patient load of 2,880. Assuming a similar age distribution to the enrolled cohort, 74.1% (n = 200) fall into the NHS Health Check age bracket (40 - 74 years). Spreading the (significant) cost of implementation (i.e. software purchase, since accurate blood pressure monitors are relatively inexpensive) across these patients (n = 2,134), a single charge of £4.69 per patient would cover the cost of implementation within a single year. The practice used for the present study employs a 45-minute eye examination time, which renders integration of such a technique relatively straightforward as there is sufficient time with each patient. Faster-paced clinics, operating on a 25- or even 20-minute appointment schedule may find such implementation difficult; however, even by extending each appointment by 5 minutes to obtain accurate blood pressure readings would still result in a greater number of patients seen and thus a faster return on recouping costs of implementation. Since the cost of implementation is a single payment (the software is not fixed-term licence), additional charges would simply be remunerating the clinician for the (additional) service provided. The increased risk of litigation due to additional tests and management options is negligible provided clinicians are adequately trained. The Association for Optometrists (who provide a medico-legal protection for a significant proportion of the UK optometric workforce) have already stated that provided optometrists are suitably trained and registered, they will extend cover to include the measurement of systemic blood pressure in the consulting room[293].

As has been shown in Chapter 4 (Repeatability and Variability in Objective Static Retinal Vessel Calibre Measurement), there is very good agreement between an experienced and novice grader. Although further study is required, this suggests that vessel selection and analysis could be delegated to an ad-

equately trained optical assistant or ophthalmic photographer. This would then mean that the image and accompanying vessel data could be recalled by the optometrist without having to use clinic time. Interpretation of the data would need to incorporate the additional information obtained throughout the eye examination. By obtaining blood pressure, this information can be used in it's own right and also when combined with data from history and symptoms to calculate a QRISK value. As demonstrated in Chapter 5. Correlations Between Retinal Blood Vessel Calibre and Cardiovascular Metrics Observed in Optometric Practice, use of AVR quintiles can highlight those patients at greater cardiovascular risk. Once these patients have been identified, attention can be drawn to the measurements to the vessel types specifically (arteries and veins). Here, the optometrist can check individual vessel measurements to identify any of clinical significance (i.e. which fall outside the values of 'internal noise' as determined in Chapter 4. Repeatability and Variability in Objective Static Retinal Vessel Calibre Measurement). This, combined with knowledge obtained from both Chapters2 (*Associations of Retinal Vessel Calibre with Cardiovascular Disease: A Systematic Literature Review*) and 5 (*Correlations Between Retinal Blood Vessel Calibre and Cardiovascular Metrics Observed in Optometric Practice*), it can then be determined which arm of the retinal microcirculation has experienced a calibre change, and a number of potential influencing factors considered.

Two worked examples of possible pathways are displayed in Figure 33. In the first example, AVR is highlighted as being in the lowest ARIC quintile, and the change is then identified as a reduction in CRAE (when compared to the previous examination). Blood pressure is measured (and found to be slightly elevated, although still considered as 'pre-hypertensive'), and QRISK is calculated to be less than the 10% intervention threshold. As a result the GP need only be notified, and the patient could be monitored in practice by the optometrist (with appropriate lifestyle advice as is suitable for borderline hypertensives). In the second example, the same increase in BP is noted, but here vessel changes are instead due to a CRVE increase, and an increased QRISK profile suggests intervention is required (and that anatomical changes are perhaps not solely due to raised blood pressure). The result is that the GP is requested to do a cardiovascular work-up in order to attempt to identify the possible cause. These two examples demonstrate the diagnostic usefulness of vessel calibre measurement (and the retention of AVR as an overall indicator), but also how they can help to stratify other clinical measures such as blood pressure. This has the potential to streamline the referral process as well as reduce GP chair-time to manage pre-hypertensives, which the Healthy Living Optician Scheme has demonstrated can be effectively 'out-sourced' to community optometrists.

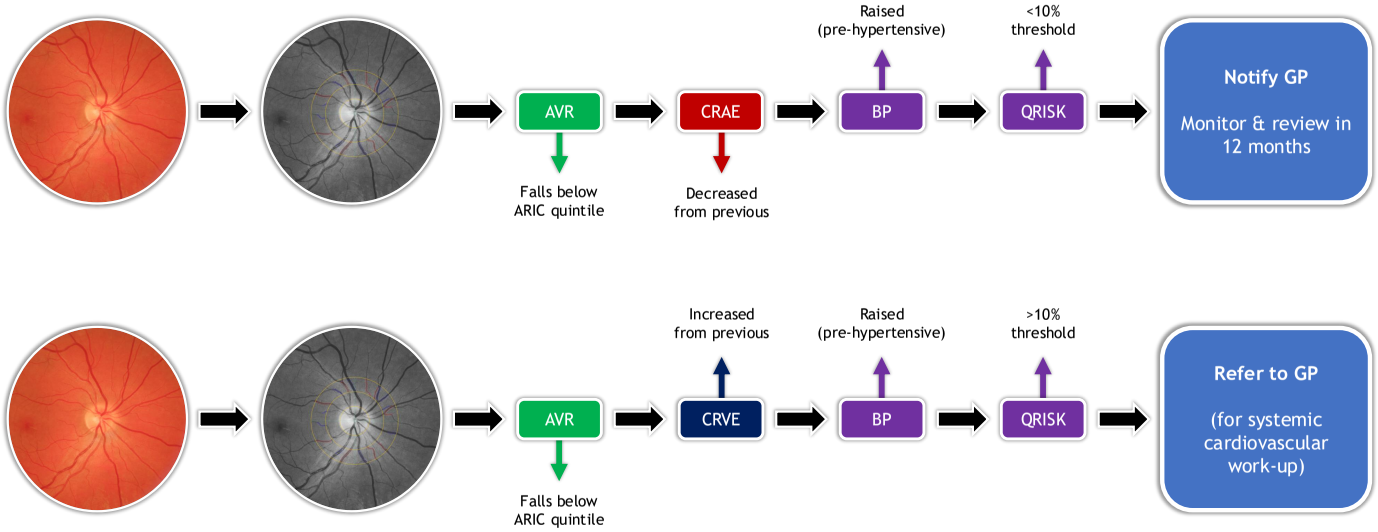


Figure 33: Example management flowchart based on retinal vessel analysis and integration with existing cardiovascular risk metrics. Note how blood pressure is measured as raised in both cases, but the change in vessel calibre helps to formulate a management plan, the former involving monitoring in practice, the latter requiring further investigative tests.

Fundamental to the uptake of objective retinal vessel analysis into clinical practice is the clinical guidance provided by the College of Optometrists. Currently, guidelines advocate the use of subjective analysis (AVR) and descriptions. Implementation of objective retinal vessel analysis into clinical guidance would require a revision of the current standards. In doing so there are two avenues which need to be considered. Firstly, the evidence base demonstrating the applicability and clinical utility of the technology would need to be increased from clinic-based data. The data presented within this thesis forms the first step in this, however further validation across imaging modalities and diverse populations will add weight to the argument to incorporate this approach into clinical guidance. Secondly there is a need to both educate and address the concerns of optometrists and clinicians. Despite it's well-documented drawbacks, the AVR is deeply ingrained in the optometrists' toolkit. To move away from this requires (re-)education of clinicians to improve their understanding of cardiovascular physiology in order to interpret the findings of objective analysis. A general awareness of disease processes and how different vessel types are affected is a key element to this interpretation. There would also need to be a move towards educating clinicians to take blood pressure routinely and starting to consider systemic health and cardiovascular risk, but also to be comfortable with understanding the results and developing a management plan. This would extend to raising awareness across healthcare disciplines (particularly GPs) about the scope of practice of optometrists and how their continued monitoring of patients' retinal microcirculation (in conjunction with systemic biomarkers) can feed into multi-disciplinary care. Aligning any changes to optometric clinical management guidelines with pre-existing pathways such as NICE Guidelines and the use of the QRISK algorithms will not only aid cross-disciplinary interaction and understanding, but also reinforce the level of competence in increased optometric involvement.

7.6 Conclusion

Whilst there is inconclusive evidence regarding the use of different vessel analysis platforms, further work needs to be undertaken to determine the levels of agreement between platforms. If clinical guidance is to be based upon retinal vessel measurements, the reproducibility of those measurements needs to be consistent across platforms, and if this is not the case, a standardised procedure (including approved platforms and protocols) needs to be devised. A pilot study focussing on image fixation highlights this need, since spurious results can result simply from using a macular-centred image rather than a disc-centred one[294]. Consideration for changing camera systems also needs to be made. As instruments improve, or even as CCD chips within existing setups are replaced, a method of cross-system

calibration needs to be developed in order to allow sequential images acquired on different systems to be compared. A reference image or grating of known dimensions that can be imaged through both fundus camera systems could yield individualised system-to-system correction factors which would allow historical images to be analysed and the measurements remain comparable. This would require high accuracy production methods since measurements are on a micrometre level, but it is an essential consideration for application in clinical practice. Instruments are not replaced frequently, so such a calibration process could be provided by the company supplying the analysis software.

Further work is required to document physiological changes in retinal vasculature. This allows for age-related corrections (or considerations) to measurements taken over time in practice. Discernment between *pathological* and *physiological* changes to vessel calibre could then potentially be made. Large cohort studies such as those outlined in Chapter 2. Associations of Retinal Vessel Calibre with Cardiovascular Disease: A Systematic Literature Review will generally quote baseline retinal vessel measurements. Whilst this can provide detail for both cross-sectional and longitudinal observations, it does not provide any clarity over the rate at which individual vessel measurements change over time. Since practice-based settings have been shown to produce comparable results in this thesis, studies which would follow patients for a number of years and track those changes individually are much more suited to being undertaken in optometric practice.

This thesis has highlighted issues with current practice guidelines surrounding retinal blood vessel assessment and identified a potential replacement strategy; objective retinal vessel analysis. This alternative has a wealth of literature supporting it's use and application in research. The method has been shown to be as repeatable and reproducible in a clinical practice setting as the original research settings, particularly with undilated patients (as is routine in UK optometric practice). Correlations already established in the literature have also been reproduced cross-sectionally (particularly between hypertension and cardiovascular risk), and shown to be relatively static over the standard 12-24 month eye examination interval. This has been accompanied by growing evidence that optometrists should be taking greater consideration of patients' overall cardiovascular health and risk status, and the assessment of the retinal microcirculation is greatly improved with the addition of simple measures such as systemic blood pressure and QRISK calculations. The routine nature of eye examinations offers a fantastic opportunity to not only identify a significant number of previously undiagnosed hypertensives, but also to monitor patients non-invasively over time and thus ease the burden upon GPs practices. With this initial groundwork completed it has been shown that objective retinal vessel analysis is capable of

making the transition from a predominantly research-based setting and move across into routine screening and monitoring of patients. There is also the opportunity for a wealth of research to be conducted within clinical practice focussing on particular sub-groups within the population to better our understanding of the retinal microcirculation. Objective retinal vessel analysis has an enormous potential to help tackle rising incidences of cardiovascular disease and strengthen multi-disciplinary patient-centred healthcare.

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8 Appendix 1. Ethical Approval

Aston University Ethics Committee
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Chairperson: [redacted]

Secretary: [redacted]

6th May 2015

Dr Rebekka Heitmar

School of Life and Health Sciences

Dear Rebekka

Study Title: 'Evaluation of objective versus subjective retinal vascular assessment in practice to update current practice'

REC Reference: Ethics Application 778

Protocol Number:

Confirmation of ethical opinion

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

The project is approved until the completion date specified on the form (March 30 2021) provided it is commenced within two years of the date of this letter and you are required to notify the Committee when the project is completed.

Approved documents

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

Document	Version	Date
University Ethics Application Form	One	19/03/2015
Protocol	Version 1.0	19/03/2015
Medical History Questionnaire	One	19/03/2015
Participant Information Sheet	Version 1.0	19/03/2015
Patient Consent Form	Version 1.0	19/03/2015
Insurance Certificate for Christian French	One	19/03/2015
Participant information sheet	Two	09/04/2015
Patient Consent Form	Two	09/04/2015
Copy of email confirming Indemnity Cover	One	09/04/2015
Reminder Letter Template	One	09/04/2015

Figure 34: Ethical approval document

Fig. 8 cont^d

Point by point response to the committee's comments for application No 778	One	09/04/2015
GP letter	Version 1.0	09/04/2015
Reminder Letter Template	Two	21/04/2015
Participant Information Sheet	Three	21/04/2015
Patient Consent Form	Three	21/04/2015
Reminder Letter Template	Three	06/05/2015
Participant Information Sheet	Four	06/05/2015

Statement of compliance

The Committee operates in accordance with the Aston University Ethics policy and procedures:

http://www1.aston.ac.uk/registry/for-staff/regsandpolicies/ethics-policy-and-procedures/

Reporting Requirements

The details of the investigation will be placed on file. You should notify the Secretary of the University Ethics Committee of any adverse events which occur in connection with this study and/or which may alter its ethical consideration, and/or any difficulties experienced by the volunteer subjects.

If you intend to make any future protocol amendments these must be approved by the Ethics Committee prior to implementation. You should also seek approval for any extension of the approved completion date.

Membership

The members of the University Ethics Committee present at the meeting are listed below:

- [redacted]Professor of Occupational Health & Safety, Aston University
- [redacted]Programme Director Postgraduate Logistics & Transport Management, Aston University
- [redacted]AHRIC Director, Aston University
- [redacted]Reader in Biosciences, Aston University
- [redacted]Director of Governance, Aston University

REC reference: **Ethics Application 778**
Please quote this number on all correspondence

With the Committee's best wishes for the success of the project

Yours sincerely

[redacted]

Secretary of the Ethics Committee

Email: [redacted]

9 Appendix 2. Patient Information Sheet

Evaluation of objective versus subjective techniques for retinal blood vessel analysis

Patient volunteer information sheet

Lead researchers: Christian French MOptom (Hons), MCOptom

Dr Rebekka Heitmar PhD, Dipl.Ing.(FH) AO, PGCertPP, MCOptom, FHEA

Invitation

We would like to invite you to take part in a research study. Before you decide whether to take part, we would like to explain to you why the research is being done and also what it will involve for you. One of the team will go through the sheets with you and answer any questions that you should have. This should take a few minutes. Please feel free to speak to anyone about the study.

Please feel free to ask any questions if something is not clear.

What is the study about?

A lot of health information can be gained from studying the eyes, particularly the retina. It allows for non-invasive viewing of the blood vessels, which is not possible anywhere else in the human body. Traditionally, this was done with a special torch, the ophthalmoscope. Technology, however, has advanced beyond this, to allow us to take photographs and even take 3D scans of the retinal layers. With these images we are able to perform digital analysis to give much more accurate measurements of the blood vessels. This study then hopes to investigate:

1. How does computer-performed vessel analysis compare to an optometrist looking at the blood vessels themselves? Are the measurements that the computer makes **repeatable** and **reliable**?

2. How do the blood vessel measurements compare to the patient's **general health**? Are the measurements able to pick up **subtle changes** before they would normally be seen? This would mean medical treatment could be provided earlier and so reduce potential damage caused to the eye and the body.

Figure 35: Patient Information Sheet

3. Do the results from (**1**) and (**2**) mean we should **change** the existing **national practice guidelines** to ensure this technology is used across the country?

So with the study, we hope to show that by using a computer to analyse your retinal photographs, we will get much more accurate measurements, but also be able to link this to your general health. This means if there are any changes, we can provide healthcare earlier and much more effectively. If our study is successful, we will be able to update the guidelines of practice, which would mean that optometrists across the country would be using this technology to help their patients.

Do I have to take part?

Whilst we would really appreciate your time and participation in the study, there is **no obligation** to take part in this study. Even if you initially consent to take part but change your mind at a later date, you are perfectly **entitled to withdraw yourself** and your data from the study. This will in no way affect the level of treatment and care that you will receive on subsequent visits to the practice.

Why do you need me in the study?

Every person is different; from gender and age to their lifestyle and genetic background. The more people that are included in the study means that we will have a better range of data and be able to make our findings much more accurate and certain. We hope to recruit 500 people into the study.

What will I need to do?

Because we hope for the results of this study to improve future eye examinations performed across the country, the 'extras' being performed today are to be kept to a minimum. It will still be a full comprehensive eye examination that you receive, but we shall take photographs of the back of your eyes, check your blood pressure you for and **may** also have to put some dilating eye drops in. The dilating drops ensure that we can get good quality, clear photographs of the back of your eyes. However, this will **only** be necessary if we cannot get clear photographs.

Fig. 9 cont^d

If I need to have the drops, what do I need to know about them?

The drops are the standard drops that are used by optometrists, called **Tropicamide 0.5%**. These drops only affect your eyes and do so by making your pupil get larger. This means that your vision can be blurry for around six hours afterwards and you can be sensitive to lights. They take about twenty minutes to work after they have been put in your eyes and their effect wears off after several hours. Although they can be a little uncomfortable when they are first put into your eyes, this wears off very quickly (within a minute). We strongly advise against driving in this time. If you have driven today that is not a problem, we can simply book you in for another time to do the photographs and do the other tests today. There are no long-term after effects of the drops.

Will you be using my personal records?

Your eye examination record, which will include information about your general, eye and family health history, will still only be accessible by your optometrist. The information that we will need to use for the study will be made **anonymous** so that we are able to use it without the worry that you can be identified by it. We may need to refer to specific details (such as your age or an existing health condition) but there will be **no personal identifiers included** (such as name or address). Only the lead researchers will have access to the list which links the personal records to the study data and it will **never be published**.

Are there any risks by taking part in the study?

No. The study is what we call an 'observational study', which means that we do not interfere with our subjects, we simply use the data that we would be taking normally (and possibly take some extra readings, such as blood pressure).

What are the benefits of taking part in the study?

On a personal level, you will be having a thorough eye examination that will be supplemented by an in-depth analysis of your retinal photographs and scans which has the potential to highlight any illnesses or changes earlier than we could previously. You will also be having your blood pressure checked.

Fig. 9 cont^d

By taking part you will also be helping to advance the profession of optometry across the UK (and possibly worldwide) and allow for eyecare providers across the country to expand and improve the level of care that they offer the public. This will allow for earlier and more accurate diagnoses and treatment.

Who has reviewed the study?

Aston University's ethical approval committee has approved the study. Their primary concerns are not the research, but the safety, rights and welfare of subjects taking part in the study. They have also approved all written information about this study (including this information sheet and accompanying consent form).

Who is funding the study?

The study has partly been funded by the **College of Optometrists** in London. They are in charge of the education of all optometrists in the UK and any optometrist working in the UK must have passed their exams (shown by having the letters *MCOptom* or *FCOptom*) and they also set out the guidelines for the way in which optometrists are expected to work.

What will happen with the information collected?

The (anonymous) data will be analysed and presented in a final report which shall be published. Findings will also be published in research articles in scientific journals that are used across the globe. We will be more than happy to discuss the outcome of the study with you at a convenient time and send a copy of any articles that we may publish if you so wish.

Will I get any documents or paperwork to keep?

You will be given a copy of this information sheet, along with a signed copy of your consent form. Please carefully retain these documents.

Fig. 9 cont^{d}

So in a nutshell, what does the study need from me?

The study is looking at blood vessel analysis and trying to improve the way that it is performed. Subjects taking part will have:

- a comprehensive eye examination – including vision, eye health and eye pressure check
- photographs taken of the back of the eyes
- blood pressure measured

The data collected will be made anonymous and used in a collection of data to allow us to look for trends and assess the validity of this new technique.

What if I have further questions, today or at a later date?

Contact either **Christian French** or **Rebekka Heitmar**, both of whom will gladly assist with any questions.

Christian French (*Davis Optometrists*) Telephone: 01858 462061 [Market Harborough practice]

[redacted]

Dr Rebekka Heitmar (*Aston University*) [redacted]]

[redacted]

Should, for any reason, you wish to make a complaint please contact the practice manager for Davis Optometrists who is not involved in the study and so will be able to resolve matters in an unbiased fashion. The practice manager, [redacted], can be contacted on the details below:

[redacted] (*Davis Optometrists*) Telephone: 01536 512792 [Kettering practice]

[redacted]

We hope that you will be able to take part in the study, and thank you in advance for your time.

Fig. 9 cont^d

10 Appendix 3. Patient Consent Form

Evaluation of objective versus subjective retinal blood vessel analysis

Patient volunteer consent form

Patient Name: _____

Investigator Name: _____

Please read each statement carefully and initial in the box provided.

1. I confirm that I have read the *Patient information leaflet* (Version 1.1 – dated 15.09.2015) for the above study. I have had the opportunity to consider the information, ask questions and had them answered satisfactorily. ☐

2. I understand that my participation is voluntary and that I am free to withdraw at any time without giving a reason and without my optometric care or legal rights being affected. ☐

3. I understand the relevant sections of my medical notes and data collected during the study may be looked at by members of the research team, regulatory authorities or the NHS Trust where it is relevant to my taking part in this research. I give my permission for these individuals to have access to my records. ☐

4. I agree to my GP being informed of my participation in the study. ☐

5. I agree to take part in the above study. ☐

_____ _____ ___:___
Signature of the **patient** **Date:** day/month/year **Time:** hr:min

_____ _____ ___:___
Signature of the **consent taker** **Date:** day/month/year **Time:** hr:min

[When completed: 1 for participant, 1 for researcher site file, 1 (original) to be kept in clinical record]

Patient volunteer consent form - Version 1.1 - 15.09.2015 [As per NRES template Version 3.6.1, March 2011]

Figure 36: Patient Consent Form

11 Appendix 4. Patient Record Card

DAVIS OPTOMETRISTS

EVALUATION OF OBJECTIVE VERSUS SUBJECTIVE TECHNIQUES FOR RETINAL BLOOD VESSEL ANALYSIS

Aston University

REF N^{O}: **D.O.B.:** **VISIT N^{O}:**

☐ EYE EXAM ☐ SUPPLEMENTARY TESTS ☐ OTHER:

ENROLMENT ☐ ROUTINE ☐ SPECIAL INTEREST (*SPECIFY*):

PAPERWORK ☐ INFORMATION SHEET GIVEN ☐ CONSENT FORM SIGNED ☐ BOS

IMAGING ☐ FUNDUS PHOTOGRAPHY ☐ OCT (PRACTICE:) ☐ DILATED?

BLOOD PRESSURE
1. _____
2. _____ (TIME:)
3. _____
4.

PULSE OXIMETRY (TIME:)

INTRAOCULAR PRESSURE _____ (TIME:) ***MEAN SPHERE EQUIVALENT*** _____

G.H. ☐ DM (T1 / T2) ☐ HT ☐ Chol.
☐ Cardiac ☐ Renal ☐ Hepatic ☐ Resp. ☐ Blood
☐ MI ☐ TIA ☐ Cor. bypass ☐ Stent ☐ Ab. aortic aneur. ☐ Periph. art dis.
Meds:

F.Hx ☐ DM (T1 / T2) ☐ HT ☐ Chol.
☐ Cardiac ☐ Renal ☐ Hepatic ☐ Resp. ☐ Blood
☐ MI ☐ TIA ☐ Cor. bypass ☐ Stent ☐ Ab. aortic aneur. ☐ Periph. art dis.

Lifestyle: ☐ Smoke? ☐ Alcohol? ☐ Caffeine?
☐ Veg. ☐ Sat. fat ☐ Exercise
Height: _____ Weight: _____

SIGNED: DATE:

Figure 37: Patient Record Card

12 Appendix 5. Patient Questionnaire

Evaluation of objective versus subjective retinal blood vessel analysis

Medical History Questionnaire

Please answer all of the questions below. If you are unsure of what to put for any section, please leave it blank and speak with a member of the research team. If you are unsure of any dates, please write an approximate.

Name: _____ D.O.B.: ___ / ___ / ___ Age: ____

Today's date: ___ / ___ / ___ Record No. (For Admin use only): _____

Refractive Status:

☐ Emmetrope ☐ Low Myope (< -3.00 MSE) ☐ Low Hypermetrope (< +3.00 MSE)

☐ High Myope (> -3.00 MSE) ☐ High Hypermetrope (> +3.00 MSE)

Ocular Pathology: (*Write '**R**' (right); '**L**' (left); '**U**' (both) in the box*)

☐ Glaucoma ☐ Cataract ☐ Amblyopia

☐ Macular Degeneration (Wet) ☐ Macular Degeneration (Dry) ☐ Diabetic Retinopathy

☐ Hypertensive Retinopathy ☐ Other: _____

Ocular Surgery: (*Write '**R**' (right); '**L**' (left); '**U**' (both) in the box*)

☐ Cataract extraction (Date: _____) ☐ Laser refractive surgery (Date: _____)

☐ Diabetic laser treatment (Date: _____) ☐ Other: _____

Figure 38: Patient Questionnaire

General (cardiovascular) Health: (*Include even if taking medication for*)

- ☐ Diabetes - Type I / Type II (*please circle*) Diagnosed: _____
- ☐ Hypertension (High blood pressure) Diagnosed: _____
- ☐ Hyperlipidaemia (High cholesterol) Diagnosed: _____
- ☐ Cardiac / heart problems Diagnosed: _____
- ☐ Renal / kidney problems Diagnosed: _____
- ☐ Hepatic / liver problems Diagnosed: _____
- ☐ Blood disorder (Specify: _____) Diagnosed: _____
- ☐ Lung disease / COPD / Emphysema Diagnosed: _____
- ☐ Sleep apnoea Diagnosed: _____
- ☐ History of myocardial infarction (heart attack) Date: _____
- ☐ History of cerebrovascular accident (stroke) Date: _____
- ☐ History of vascular surgery (heart surgery, stent etc.) Date: _____
- ☐ Other (including vascular surgery – heart operation, stent etc.) _____

_____

_____

Prescribed Medication:

_____

_____

_____

Over-the-counter (OTC) Medication:

_____

_____

Allergies:

_____

Fig. 12 cont[d]

Habits:

☐ Smoker Started: _____ Quit: _____

☐ Alcohol Approx. units per week: _____ (1pt beer / 1 glass wine = 2.3 units; 1 spirit = 1 unit)

☐ Caffeine Cups of coffee per day: _____ Cups of tea per day: _____

Social:

Occupation: _____ (Current / Retired)

Exercise (types and duration per week): _____

Family History:

☐ Diabetes - Type I / Type II (*please circle*) Relative(s): _____

☐ Hypertension (High blood pressure) Relative(s): _____

☐ Hyperlipidaemia (High cholesterol) Relative(s): _____

☐ Cardiac / heart problems Relative(s): _____

Father Age: _____ Alive / Deceased Cause of death: _____

Mother Age: _____ Alive / Deceased Cause of death: _____

Brother Age: _____ Alive / Deceased Cause of death: _____

Brother Age: _____ Alive / Deceased Cause of death: _____

Brother Age: _____ Alive / Deceased Cause of death: _____

Sister Age: _____ Alive / Deceased Cause of death: _____

Sister Age: _____ Alive / Deceased Cause of death: _____

Sister Age: _____ Alive / Deceased Cause of death: _____

Child Age: _____ Alive / Deceased Cause of death: _____

Child Age: _____ Alive / Deceased Cause of death: _____

Child Age: _____ Alive / Deceased Cause of death: _____

Fig. 12 cont^d

Symptoms: (*Please tick any which you experience*)

☐ Chest discomfort	☐ Shortness of breath	☐ Irregular / rapid heart beat
☐ Fainting / blackouts	☐ Breathing problems at night	☐ Palpitations
☐ Light headedness	☐ Leg / arm pain with exertion	☐ Hand pain with cold exposure
☐ Easy bruising / bleeding		

Many thanks for filling in this questionnaire. Please remember that this information is confidential and will be fully anonymised before any analysis takes place.

Date	Amendment / Addition	Initial

Fig. 12 cont^{d}

13 Appendix 6. VesselMap Report

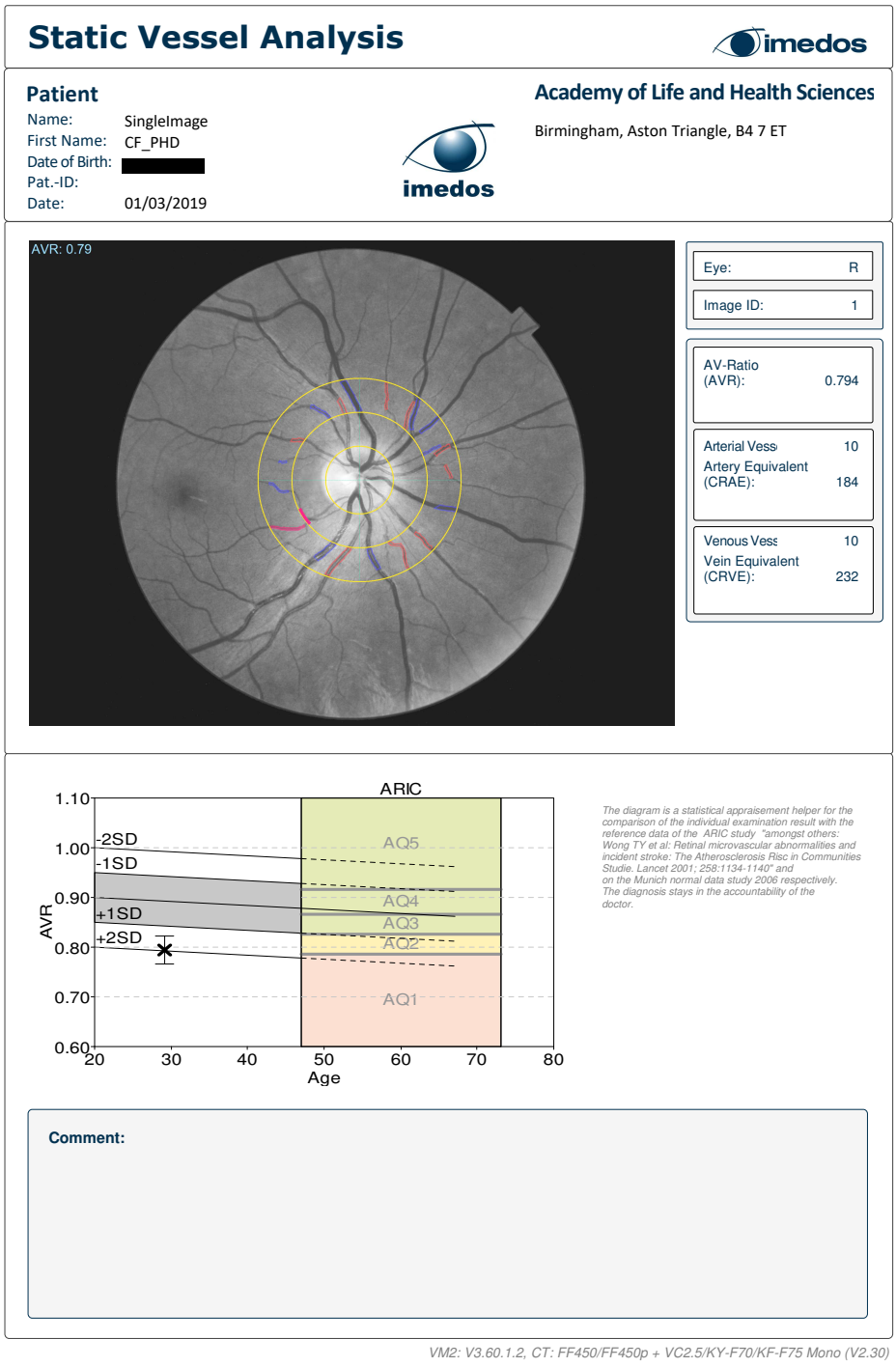

Figure 39: Sample report generated by VesselMap v3.0 (Imedos Systems, Jena)

14 Appendix 7. Poster Presentation: BCOVS (2015)

CONFERENCE: British Congress of Optometry and Visual Science (BCOVS)

DATE: 7th / 8th September, 2015

VENUE: City University, London, UK

TITLE: Evidence-based practice: How do we assess retinal vessels in practice?

Abstract

This prospective study will evaluate the current methods used in practice to assess retinal blood vessels (primarily the subjective-based arteriole-venule ratio (AVR) which has been shown to have a number of limitations) alongside newer, objective techniques. Objective analysis has been used in clinical research for approximately 20 years, though it has yet to be used routinely in optometric practice. There is a wealth of academic literature that advocates the use of objective vessel analysis, particularly Central Retinal Artery/Vein Equivalents (CRAE and CRVE), including links with various cardiovascular diseases.

This study will examine 300 patients seen in independent practice for routine eye examinations over a course of several visits. Alongside 'routine' procedures such as ophthalmoscopy (including subjective vessel assessment) and fundus photography/OCT, supplementary data will be obtained from patients; including blood pressure. Data analysis of photographs obtained will produce objective vessel analysis results. This will concentrate on generating CRAE and CRVE values. These can also be used in generating an objective AVR, allowing a direct comparison between subjective and objective AVRs for the same patient.

Despite still using a subjective measure for vessel analysis, optometric practice has the capacity to integrate advanced retinal vessel analysis, and this will hopefully aid clinical decision making; for example monitoring/detecting changes due to systemic pathologies. By accompanying vessel analysis with supplementary data such as blood pressure, medication and general health, it will hopefully be shown that these additional measures provide an extra level of efficiency and accuracy for optometrists with their patient management and referrals.

15 Appendix 8. Poster Presentation & Rapid Fire Oral Presentation: EVER (2016)

Conference: European Association for Vision and Eye Research (EVER)

Date: 5th-8th October, 2016

Venue: Acropolis Convention Centre, Nice, France

Title: Static Retinal Vessel Analysis in Routine Optometric Practice

Abstract

Title: Static Retinal Vessel Analysis in Routine Optometric Practice

Purpose: To evaluate the use of objective retinal vessel calibre measurements in optometric practice and its utility in clinical decision making.

Methods: A sub-sample [n=56] was extracted from a prospective study including patients booked for routine eye examinations in optometric practice. All participants underwent a standard examination including subjective refraction and slit lamp biomicroscopy. Undilated fundus photography and/or optical coherence tomography (OCT) was also performed. Optic nerve-centred (camera angle: 50 degrees), red-free photographs were analysed using VesselMap software (*Imedos, Germany*) to give objective vessel calibre measurements (central retinal artery and vein equivalents (CRAE / CRVE)).

Results: Mean age of the cohort was 56 years (range: 21-82yrs; consisting of 32 women and 24 men). Univariate analysis showed a significant association between systolic blood pressure and CRAE which was lost in multivariate analysis (p=0.02). Stepwise forward multiple regression analysis found age to be significantly, negatively associated with CRAE (CRAE: 157au (SD ±20); β=-0.54; p<0.001) and CRVE (β=-0.56; p<0.001), whereas BMI was positively associated with CRVE (198au (SD ±20) au; β=1.84; p=0.005) only. Two patients were measured twice: on initial presentation, one with a significant retinal haemorrhage and one with unilateral papilloedema; both showed normalisation of vessel diameters on follow up.

Conclusions: Participants with the largest CRVE had the highest BMI and/or were diabetic. Cross-sectional results from this sample are in agreement with results published from large cohort studies,

including the negative association with age and CRAE. Retinal vessel calibres can help provide information on a patient's vascular system and systemic health, and therefore be a useful tool to refine optometric referrals and aid patient monitoring.

16 Appendix 9. Poster Presentation & Rapid Fire Oral Presentation: EVER (2017)

Conference: European Association for Vision and Eye Research (EVER)

Date: 27th-30th September, 2017

Venue: Acropolis Convention Centre, Nice, France

Title: Cross-sectional Static Retinal Vessel Analysis in Routine Optometric Practice

Abstract

Title: Cross-sectional Static Retinal Vessel Analysis in Routine Optometric Practice

Purpose: To demonstrate the clinical significance of objective static retinal vessel analysis when utilised in routine optometric practice.

Methods: A cross-sectional sample of patients seen in routine UK optometric practice [n=225] underwent a standard eye examination including subjective refraction and slit-lamp biomicroscopy. Undilated, optic nerve-centred fundus photographs were obtained (camera angle: 50 degrees). Red-free photographs were analysed using iFlexis software (*Vito, Belgium*) to give objective retinal vessel calibre measurements (central retinal artery/vein equivalents; CRAE/CRVE), as described by Knudtson et al.

Results: The mean age of the cohort was 59 years (range: 16-90 years ±15) and comprised of 137 women and 88 men. BMI ranged between 17.6 – 37.3 kg/m2. Average systolic blood pressure was 131mmHg ±20 and diastolic blood pressure was 82mmHg ±12. Refractive error (MSE) ranged from -10.00 to +7.00D. Mean CRAE was found to be 143AU ±17, CRVE was 210AU ±22, with the ratio of the two (AVR) averaging 0.68 ± 0.07. When compared, there was significant agreement between right and left eyes (P<0.001), however this only accounted for around half of the agreement (r2 = 0.42 (CRAE); 0.50 (CRVE). Bland-Altman plots demonstrate good agreement between eyes but with widely spaced limits of agreement. Stepwise forward multiple regression analysis found a significant (p<0.0001) correlation between CRAE and age and MSE; and between CRVE and age, MSE and BMI.

Conclusions: Correlation between right and left eyes demonstrates that one eye does not fully account for the observations in the other when performing static retinal vessel analysis. Established

associations between age and both aspects of the retinal circulation are confirmed. The effect of refractive error upon the retinal circulation is also an important consideration for static retinal vessel analysis.

17 Appendix 10. Poster Presentation: ARVO (2018)

Conference: Association for Research in Vision and Ophthalmology (ARVO)

Date: 29th April-3rd May, 2018

Venue: Hawaii Convention Centre, Honolulu, Hawaii

Title: Do Static Retinal Vessel Diameters Compliment Cardiovascular Risk Calculations?

Abstract

Title: Do Static Retinal Vessel Diameters Compliment Cardiovascular Risk Calculations?

Purpose: Online cardiovascular risk calculators are freely available to input known health metrics and determine the likelihood of developing a cardiovascular incident in the future. Static retinal vessel measurements are known to change as a result of cardiovascular disease. A cross-sectional study was performed to establish whether vessel calibre compliments these risk calculations.

Methods: 263 subjects were enrolled from routine optometric practice in the UK. As well as a comprehensive eye examination (including refraction and fundus examination), OCT and fundus photographs were acquired, as well as blood pressure. A full history of general health, family history and lifestyle questions (including smoking status, diet and exercise) were also collected. Red-free, 50-degree, optic nerve head centred photographs were analysed using semi-automated retinal analysis software. Cardiovascular risk was calculated with the Mayo Clinic's online 30-year risk calculator.

Results: Overall average age was 58.5 years (range: 16-93), males (n=104) 58.8 (SD) and for females (n=159) 58.3 (SD). BMI ranged from 17.6 to 37.3, with male and female averages of 26.2 and 24.6. Mean 30-year cardiovascular risk was 27.5%, and higher in men (Males: 36.3%; Females: 22.1%; p=<0.001). The was no gender difference in retinal vessel calibres (CRAE and CRVE (p=<0.001)). In univariate analysis however, reduced CRAE correlated with increased 30-year risk (R=-0.26; p=0.004), in females only. BMI was linked with increased 30-year risk and CRVE (R=0.52 (p=<0.001); R=0.19 (p=0.017) respectively). In a stepwise forward multiple regression model significance was retained for BMI and age in men only.

Conclusions: Whilst there are multiple relationships between retinal vessel calibres and individual cardiovascular risk factors (BMI, age, gender, etc.) this relationship is lost in some multivariate models

accounting for these risk factors. Nonetheless, the present study highlights the value of retinal vessel calibres for their potential to refine existing cardiovascular risk stratification and how individual risk factors are strongly linked to vessel calibre changes which can potentially impact on the development of future ocular and systemic vascular pathology.

18 Appendix 11. Poster Presentation: ARVO (2019)

Conference: Association for Research in Vision and Ophthalmology (ARVO)

Date: 28th April-2nd May, 2019

Venue: Vancouver Convention Centre, Vancouver, Canada

Title: Repeatability of Static Retinal Vessel Analysis in a clinical practice setting

Abstract

Title: Repeatability of Static Retinal Vessel Analysis in a clinical practice setting

Purpose: Static objective retinal blood vessel analysis is widely used in research yet hasn't transitioned into clinical practice. Reduced retinal artery and vein calibres have been associated with hypertension, diabetes and coronary heart disease-related deaths. Findings like this suggest retinal vessel calibre measurements have great potential as a clinical screening and monitoring tool. Despite strong evidence of repeatability in a research setting, there is little evidence to demonstrate that this approach is equally robust in a clinic (where conditions could be considered sub-optimal; e.g. undilated patients).

Methods: 20 subjects had 10 retinal images captured in quick succession (both undilated and dilated with Tropicamide 0.5%) on Day One with a Topcon 3D Maestro and were then re-imaged seven days later. Central Retinal Artery and Vein Equivalents (CRAE and CRVE) and Arterio-Venous Ratios (AVR) were calculated for each image using VesselMap v3.0 (*Imedos Systems, Jena*). Intra-image measurement variance was also calculated through multiple vessel analysis for a single image of each subject.

Results: Mean age was 40 years ±14, with 11 females and 9 males. Overall average MSE was -1.20D (±2.22D). No subjects had existing cardiovascular disease. Average undilated baseline CRAE was 178.8µm (±15.8); CRVE was 211.0µm (±15.3) and AVR was 0.85 (±0.06). Average mydriatic baseline CRAE was 176.7µm (±15.8); CRVE was 210.0µm (±17.7) and AVR was 0.84 (±0.07). Both dilated and undilated measurements for CRAE and CRVE were compared in a Bland-Altman plot, with a bias of +2.07µm and +1.01µm respectively. Repeated measures ANOVA did not reveal a statistical significance between measurements taken on Day One or Day Seven, either dilated or undilated (p=>0.05).

Conclusions: It has been demonstrated that there is no significant difference in measurements taken from images acquired with or without the use of mydriatics. Furthermore successive image capture showed a negligible effect induced by the cardiac pulse cycle. Finally, short-term time intervals (one week) did not impact significantly on retinal vessel measurements. In summary, it has been shown that vessel analyses conducted on images (both dilated and undilated) acquired in clinic with an OCT-fundus camera show similar levels of variance and repeatability to those previously reported.

19 Appendix 12. Publication: *Associations of retinal vessel calibre with cardiovascular disease: A systematic literature review* (2017)

Journal: Optometry in Practice

Date (Volume:Issue, pages): 2017 (18:3, 155-170)

Authors: Christian JD French; Rebekka Heitmar

Title: Associations of retinal vessel calibre with cardiovascular disease: A systematic literature review

Abstract

Optometrists have to examine the retina and its vasculature as part of a standard eye examination. Added to this, a thorough history and symptoms at the beginning of an examination will include both general health and family health history. All of these factors can highlight potential risks or issues with the cardiovascular system, but are optometrists making full use of the technology available to them in the investigation of cardiovascular disease (CVD)? Since the Atherosclerosis Risk in Communities (ARIC) study first published the use of objective formulae to determine static retinal vessel calibre, there has been a wealth of literature produced on the subject (Hubbard et al. 1999). The use of the Parr–Hubbard, and latterly Knudtson, formulae has all but become the gold standard when measuring retinal vessel calibre. Objective retinal vessel analysis is not currently performed in UK optometric practice, principally since there is no research demonstrating its suitability. Since optometrists are primary healthcare clinicians, detecting and monitoring systemic health changes, as well as ocular conditions, are important aspects of their practice.