Retinal microvascular calibre and blood pressure associated target organ damage: a bi-ethnic investigation within the SABPA study

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CONTRIBUTIONS OF THE AUTHORS

The following researchers contributed to the final product of this study:

- **Ms. Chrisna du Plessis:**

  Responsible for conducting the literature searches, statistical analyses, processing of data, design, planning and writing of the dissertation. My methodological contributions are indicated on p79.

- **Dr. Wayne Smith (Supervisor)**

  Dr. Smith supervised the writing of the literature review and manuscript. He also undertook interpretation of data and gave guidance regarding statistical analyses, initial planning and design of the manuscript apart from making recommendations and providing professional input.

- **Prof. Alta Schutte (Co-supervisor)**

  Prof. Schutte gave recommendations regarding the writing, construction and interpretation of the results and research.

This is a statement from the co-authors confirming their individual role in participation of this study and giving their permission that the manuscript may form part of this mini-dissertation.

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Background and aims

Cardiovascular disease (CVD) is a global leading cause of mortality. Regardless of the various factors that may contribute to CVD development, hypertension (HT) is known to be a leading risk factor, which was recently reported to be the highest in South Africa. The prevalence of HT is known to be the highest in black populations, when compared to whites. However, limited data exists regarding biomarkers that may contribute to the development of HT in black populations.

Since it is known that HT predisposes to distinctive structural and functional small vessel adaptations, the investigation of the microcirculation has gained increased interest. Furthermore, the use of retinal imaging, using new and improved equipment such as the dynamic retinal vessel analyzer (DVA), a funduscope with applicable software, has increased. The retina allows non-invasive observation of the microcirculation. Analysis of the retinal microvasculature provides valuable information about the structure as well as the function of the vessels and can easily be obtained repeatedly over time. Data from population-based studies have shown that retinal vascular changes are related to risk of both clinical and subclinical CVD.

The retina is distinctive in that it allows the direct sequelae of elevated BP to be visualised early, particularly changes in the retinal microvasculature. Changes in retinal vessel calibre have been linked to markers of target organ damage (TOD) such as left ventricular hypertrophy (LVH), vascular remodelling (increased carotid intima-madia thickness (cIMT), glomerular filtration rate (GFR), and nephropathy, as consequences of hypertension. However, findings regarding retinal vessel calibre and TOD remain limited and inconsistent.

In addition, the associations between retinal blood vessel calibre, and its functional capacity, assessed upon light flicker provocation, and blood pressure associated TOD, have not yet been established. According to a review by Ikram *et al.* it has been suggested that the DVA should be used in retinal calibre analysis for better functional and dynamic aspects of the retinal microvasculature. Taking the high prevalence of HT in blacks into consideration, as well as their tendency to have a higher risk for developing TOD such as LVH and kidney disease, the relationship between retinal vessel calibre and markers of HT associated TOD warrants further investigation.

Consequently, the aim of this study was to investigate the associations between blood pressure-related TOD with static and dynamic retinal calibre measurements in black and white participants. Our objectives were therefore to:

a) determine whether measurements of BP-associated TOD (Cornell product, carotid intima media thickness (CIMT) and estimated glomerular filtration rate (eGFR) are associated with retinal vessel calibre and light flicker-induced retinal calibre changes (arteriolar dilation, constriction and venular dilation); and

b) determine whether these relationships differ between a black and white population.

Methodology

The present study was performed using data obtained from the follow-up phase of the SABPA (Sympathetic Activity and Ambulatory Blood Pressure in Africans) study which was conducted in February to May of 2011 and 2012. A total of 156 black (80 men and 76 women) and 179 white (86 men and 93 women) school teachers (aged 23–68 years) took part in this sub-study. Ambulatory blood pressure, cIMT, electrocardiogram (ECG) derived Cornell product, and the eGFR were determined as measures of TOD. Retinal images (for the determination of retinal vessel calibres) and vessel functional responses to a light-flicker were captured using the DVA. Anthropometric, biochemical analysis and lifestyle factors were measured using standard procedures.

Results

A narrower central retinal artery equivalent (CRAE) and smaller arteriolar-to-venular ratio (AVR), and wider central retinal vein equivalent (CRVE) were independently associated with systolic blood pressure (SBP), whereas peak arteriolar dilation negatively associated with SBP only in black hypertensives (β=-0.12 ± 0.06; p=0.04). No independent associations were present between retinal vessel calibre and TOD measures. However, eGFR was positively associated with peak arteriolar dilation in the total group (β=0.29 ± 0.12; p=0.008), black men (β=0.29 ± 0.12; p=0.02), black women (β=0.29 ± 0.12; p=0.01), and black hypertensives (β=0.32 ± 0.12; p=0.007), and venular dilation in white hypertensives (β=0.24 ± 0.10; p=0.03). cIMTf associated negatively with peak arteriolar dilation in black hypertensives (β=-0.22 ± 0.10; p=0.03).

Conclusion

In general, static retinal vessel calibre was associated with BP but not with TOD. However, the functional response of retinal arteriolar calibre to a light flicker stimulus was positively and independently associated with renal function (eGFR), as well as negatively associated with cIMTf, predominantly in blacks.

OPSOMMING

Retinale mikrovaskulêre kaliber en bloeddruk-geassosieerde teikenorgaanskade:

'n bi-etniese ondersoek binne die SABPA studie

Doel en agtergrond

Kardiovaskulêre siektes is 'n wêreldwye oorsaak van hoë sterftesyfers, en daar is onlangs gerapporteer dat Suid Afrika die hoogste voorkoms het. Ten spyte van die verskeie faktore wat bydra tot die ontwikkeling van kardiovaskulêre siektes is hipertensie bekend as 'n belangrike risiko faktor. Die voorkoms van hipertensie is veral hoog in swart populasies, ook as dit vergelyk word met wit populasies. Data is egter beperk met betrekking tot biomerkers wat moontlik kan bydra tot die ontwikkeling van hipertensie in swart populasies.

Dit is bekend dat hipertensie aanleiding gee tot kenmerkende strukturele en funksionele veranderinge van die klein bloedvaatjies en daarom het die belanstelling in die ondersoek van die mikrosirkulasie drasties toegeneem. Die gebruik van retinale beelde het ook toegeneem, deur gebruik te maak van nuut-ontwikkelde en sensitiewe apparaat, soos die *dynamic retinal vessel analyzer (DVA)*– 'n fundoskoop met toepaslike sagteware. Die retina voorsien die ideale geleentheid om die mikrosirkulasie op 'n nie-ingrypende wyse te bestudeer. Analise van die retinale mikrovaskulêre sirkulasie verskaf waardevolle, herhaalbare inligting rakende strukturele en ook funksionele aspekte van die mikrosirkulasie. Data van populasie-gebasseerde studies het getoon dat retinale vaskulêre veranderinge verwant is aan die risiko van beide kliniese en subkliniese kardiovaskulêre siektes.

Die retina is kenmerkend aangesien dit die direkte gevolge van verhoogde blooeddruk vroeg vertoon deur veranderinge in die retinale mikrovaskulatuur. Veranderinge in die mikrovaskulatuur hou verband met merkers van teikenorgaanskade soos linker ventrikulêre hipertrofie, vaskulêre hermodellering (verhoogde karotid intima-media dikte), en nefropatie, as gevolge van hipertensie. Tog is bevindinge rakende retinale vat deursnee en

teikenorgaanskade beperk en teenstrydig. Verder is die assosiasies tussen retinale vat deursnee en die funksionele kapasiteit, wat ondersoek kan word deur middel van ‘n lig flikkertoets, en bloeddruk verwante teikenorgaanskade, nog nie vasgestel nie. In ‘n oorsig artikel deur Ikram *et al.* geskryf, word voorgestel dat die DVA gebruik word vir beter analise van beide die strukturele sowel as funksionele kapasiteit van die retinale mikrovaskulatuur.

Die hoë voorkoms van hipertensie by swartes, sowel as hulle geneigdheid tot ontwikkeling van teikenorgaanskade, soos linker ventrikulêre hipertrofie, motiveer dus dat die verwantskap tussen retinale vat kaliber en merkers van hipertensie geassosieerde teikenorgaanskade verder ondersoek word.

Gevolglik was die doelstelling van hierdie studie om die assosiasies tussen bloeddruk-verwante teikenorgaanskade met statiese en dinamiese retinale vat deursnee metings in swart en wit populasies te ondersoek. Ons doelwitte was;

a) om te bepaal of metings van bloeddruk geassosieerde teikenorgaanskade (Cornell produk, karotid intima-madia dikte, en beraamde glomerulêre filtrasie snelheid) assosieer met retinale vat deursnee, en ook met lig flikker geinduseerde retinale vat se deursnee veranderinge (arteriolêre- dilatasie, konstriksie en venulêre dilatasie), en

b) om te bepaal of hierdie verwantskappe verskil tussen die swart en wit populasie.

Metode

Die studie was uitgevoer met data verky vanaf die opvolg fase van die SABPA (*Sympathetic Activity and Ambulatory Blood Pressure in Africans*) studie wat uitgevoer is gedurende Februarie tot Mei 2011 en 2012. ‘n Totaal van 156 swart (80 mans en 76 vrouens) en 179 wit (86 mans en 93 vrouens) onderwysers (23-68 jaar) het deelgeneem aan die sub-studie. Ambulatoriese bloeddruk, karotid intima-media dikte, elektrokardiogram (EKG) afkomstige Cornell produk, en die beraamde glomerulêre filtrasietempo (eGFR) was bepaal as merkers van teikenorgaanskade. Retinale beelde is verkry deur middel van ‘n DVA (*Dynamic retinal*

vessel analyzer), vir die bepaling van retinale vat deursnee, en ook die funksionele respons teenoor ‘n ligflikker stimulus. Antropometriese, biochemiese analise en lewensstyl faktore was gemeet deur middel van standaardprosedures.

Resultate

‘n Vernoude sentrale retinale arteriolêre ekwivalent, en arteriolêre-venule verhouding, en ‘n wyer sentrale venulêre ekwivalent was onafhanklik geassosieer met sistoliese bloeddruk (SBP), terwyl piek arteriolêre dilatasie negatief geassosieer het met SBP net in swart hipertensiewes (β=-0.12 ± 0.06; p=0.04). Geen onafhanklike assosiasies tussen retinale vat deursnee en teikenorgaanskade merkers was gevind nie. Tog is ‘n positiewe assosiasie gevind tussen eGFR en piek arteriolêre dilatasie in swart mans (β=0.29 ± 0.12; p=0.008), swart vrouens (β=0.29 ± 0.12; p=0.01) en swart hipertensiewes (β=0.32 ± 0.12; p=0.007), en ook met venule dilatasie in wit hipertensiewes (β=0.24 ± 0.10; p=0.03). ‘n Negatiewe assosiasie tussen karotid intima-media dikte en piek arteriolêre dilatasie in swart hipertensiewes (β=-0.22 ± 0.10; p=0.03) is ook gevind.

Gevolgtrekking

Statiese retinale mikrovaskulêre vat deursnee het met bloeddruk geassosieer, maar nie met teikenorgaanskade nie. Tog het die funksionele respons van die retinale arteriolêre deursnee tydens ‘n ligflikker-stimulus, positief en onafhanklik geassosieer met nier funksie, en ook negatief geassosieer met karotid intima media dikte, veral in swart mense.

PREFACE

The article-format was chosen for this dissertation. This is format-approved and recommended by the North-West University (NWU), and embodies a motivation, literature review, a manuscript (ready for submission to a peer reviewed journal) and a concluding chapter. However, we added an additional chapter, to fully explain the methodology of the main apparatus used for this study (dynamic retinal vessel analyzer (DVA) (funduscope)).

This dissertation is written according to the requirements of the *Journal of Hypertension.*

- **Chapter 1:** Provides an introduction and short background which clarifies the purpose of this study, and the knowledge needed for interpretation of this study.

- **Chapter 2:** Contains a complete literature review of the topic, together with the aims, objectives and hypotheses.

- **Chapter 3:** Gives an outlay of the methodology of the SABPA study and the dynamic retinal vessel analyzer (DVA).

- **Chapter 4:** Provides the Author instructions of the *Journal of Hypertension.* This chapter also contains the manuscript, which includes an abstract, introduction, methods, results, discussion and acknowledgements of the research study.

- **Chapter 5:** Consists of the conclusions made regarding the main findings of this study, together with the recommendations and limitations.

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LIST OF ABBREVIATIONS

- **ABPM:** Ambulatory blood pressure measurements
- **AVR:** Arterio-venous ratio
- **BMI:** Body mass index
- **BP:** Blood pressure
- **CHD:** Coronary heart disease
- **CIMT:** Carotid intima-media thickness
- **CKD:** Chronic kidney disease
- **CRAE:** Central retinal arteriolar equivalent
- **CRP:** C-reactive protein
- **CRVE:** Central retinal venular equivalent
- **CSWA:** Cross-sectional wall area
- **CVD:** Cardiovascular disease
- **DBP:** Diastolic blood pressure
- **DVA:** Dynamic retinal vessel analyzer
- **eCcr:** Estimated creatinine clearance
- **eGFR:** Estimated glomerular filtration rate
- **FO:** Fundus oculi
- **GGT:** Gamma-glutamyltransferase
- **HbA1c:** Glycated hemoglobin
- **HDL:** High-density lipoprotein cholesterol
- **HIV:** Human immunodeficiency virus
- **HT:** Hypertension
- **LDL:** Low-density lipoprotein cholesterol
- **LVH:** Left ventricular hypertrophy
- **LV:** Left ventricle
- **NO:** Nitric oxide

- **SABPA:** Sympathetic activity and Ambulatory Blood Pressure in Africans
- **SBP:** Systolic blood pressure
- **TEE:** Total energy expenditure
- **TOD:** Target organ damage
- **WC:** Waist circumference

CHAPTER 1
Introduction and Motivation

Introduction and motivation

Globally cardiovascular disease (CVD) is a leading cause of mortality [1-3] and this is also applicable on sub-Saharan Africa. [4]. There are many risk factors that may contribute to the development of CVD, with hypertension (HT) itself being an important risk factor [5, 6]. In a study done in 2009 it was found that approximately 21% of the South African population was hypertensive [7]. More recent evidence suggests that the prevalence of HT is increasing and that currently South Africa has one of the highest prevalence of hypertension among low and middle –income countries [8]. Several recent isolated South African studies suggest that the prevalence of HT is high in black subjects [9-11], further indicating higher HT prevalence in blacks compared to whites [12-15].

Besides HT contributing to target organ damage (TOD) markers, such as left ventricular hypertrophy (LVH), proteinuria and renal failure, retinopathy and vascular wall remodelling, which are grouped under the term "target organ damage" (TOD) [16], it is known to have profound effects on both the structure and function of the microvasculature [17]. The presence of small vessel disease, specifically vasoconstriction, rarefaction, and narrowing of the peripheral small arteries and arterioles, is thought to be a key pathological characteristic of HT.

Arterioles are known to have a similar structure to small arteries and therefore provide access to study these small arterial and arteriolar changes [18]. In this regard, the use of retinal imaging and the measurements that can be obtained there from, such as with the dynamic retinal vessel analyzer (DVA), has gained increased interest. This method represents a unique and non-invasive way of studying the retinal microvasculature [19, 20]. Using this apparatus, information is gained regarding the 1) calibres of the central retinal artery and vein, and 2) the endothelial function of these arterioles and venules following a light flicker stimulus. Evaluating retinal vessel calibre and function is further thought to be a

useful prognostic marker of CVD as the retinal vessel calibre may provide information about the microvasculature of other organ systems (eg, in persons with hypertension, the retinal arteriole narrows and may also reflect general systemic pathologies [21). Retinal abnormalities are long known to be associated with both the presence and also severity of HT status and elevated blood pressure (BP) [22-26]. HT is generally characterised by a smaller CRAE (central retinal arteriolar equivalent) CRVE (central retinal venular equivalent) and a reduced AVR (arterio-venous ratio) [27].

Changes in retinal vessel calibre have also been linked to markers of TOD [28, 29], such as left ventricular hypertrophy [22, 30-34], vascular wall remodelling [16, 35], carotid artery disease [36, 37], renal disease [22, 32, 38, 39], and nepropathy, as a consequence of HT [19, 40-43]. However, findings regarding retinal vessel calibre and TOD remain limited and inconsistent [29-31, 43-47]. The information on light-flicker induced retinal vessel changes (dynamic calibre) and TOD is also scant but available information indicates that reduced vessel dilation is similarly observed in pathological states known to influence the functioning of the endothelium [48, 49].

For the purpose of this study we will specifically focus on the association between retinal vascular calibre (observed under resting conditions and in response to light-flicker induced changes in vessel calibre) and 1) the Cornell product as an indirect marker of left ventricular mass, 2) carotid intima-media thickness (cIMT) and 3) estimated glomerular filtration rate (eGFR)).

Motivation

Much attention has been given to the relationship between retinal vessel calibre changes and systemic pathology [50]. Although associations between retinal vessel calibre and BP related TOD have been observed these associations are not always consistent [29-31, 43-

47]. In addition, although retinal blood vessel functional responses to a light flicker stimulus are associated adversely with hypertension status, their relationship with markers of TOD has yet to be explored.

Sub-Saharan Africa and South Africa is plagued by a HT epidemic [5, 51, 52]. Considering South Africa's high prevalence of HT [10], especially in its black population, it is of concern that black ethnicity has been shown to be an independent risk factor for TOD such LVH [53]. Therefore, the relationship between retinal vessel calibre and markers of BP-associated TOD warrants further investigation in a population prone to CVD [54].

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CHAPTER 2
Literature review

2.1 Introduction

Cardiovascular disease (CVD), a global leading cause of mortality [1-3], also impacts significantly on South Africa, and the rest of sub-Saharan Africa. [4]. Sub-Saharan African countries are currently experiencing increasing epidemiological transitions which is characterized by increasing urbanization and changing lifestyle factors. This resulted in an increase in of non-communicable diseases, especially CVD [5]. Three times as many deaths from CVD now occur in developing countries as compared with developed countries [6]. Multiple risk factors contribute to this increasing burden, such as smoking, obesity, alcohol consumption, diet, low physical activity, psychosocial factors, diabetes and high lipid levels [5]. Hypertension (HT) itself constitutes as an important risk factor to this burden [7, 8]. A recent study revealed that among low and middle income countries, South Africa has the highest rate of hypertension [9] which is substantially higher than recently published estimates for South Africa and 11 other sub-Saharan African countries [10]. Several recent isolated South African studies further suggest that the prevalence of HT is high in black subjects [11-14]. When compared to whites, black subjects have a higher prevalence of HT than whites [14-16]. A recent 3 year follow up study by Hamer *et al*. (SABPA study) [17], demonstrated that, overall, black subjects had a greater progression/worsening of cardiovascular risk factors, compared to white subjects. In addition, hypertension contributes to early changes, such as left ventricular hypertrophy (LVH), proteinuria and renal failure, retinopathy and vascular wall remodelling, which are grouped under the term "target organ damage" (TOD) [19]. Since hypertension is known to be a significant threat towards cardiovascular health, the identification of possible biomarkers that may provide a quick and easy estimation of the degree of hypertension related TOD must be stressed.

Recently, the role of the microvasculature in CVD has gained increased interest. In particular, HT is associated with altered vascular structure and function of the microcirculation [20]. In this regard more emphasis has been placed on retinal imaging which represents a unique and non-invasive way of studying the retinal microvasculature.

One such apparatus is the Dynamic Retinal Vessel Analyzer (DVA), which is a funduscope with applicable software (Figure 1) [20, 21].

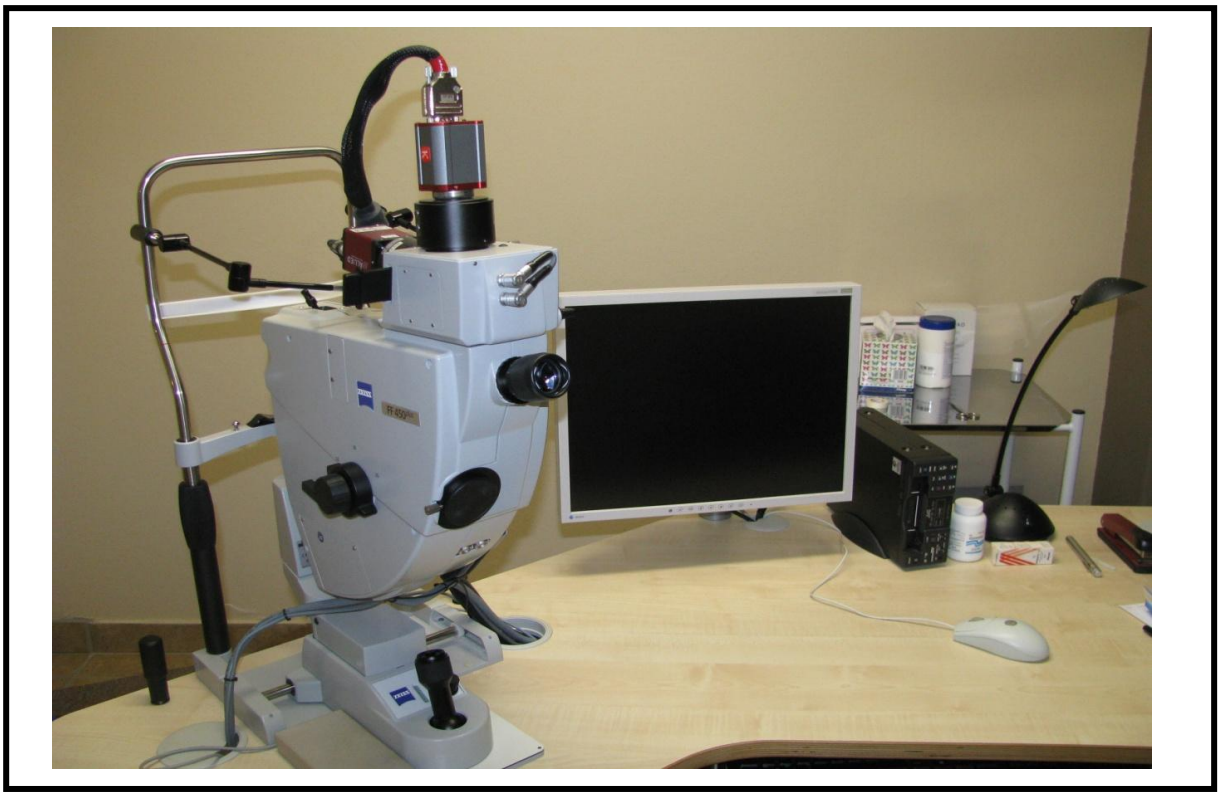

Figure 1: The dynamic retinal vessel analyzer (DVA)

It is known that the vasculature of the eye shares similar features with the vasculature of the heart and the cerebral circulation [22, 23]. However, some differences do exist [24], for example the retinal microvasculature is thought not to be innervated by the autonomic nervous system [25]. In the ocular vasculature a marked difference exists in the distribution of adrenergic innervations between the intraocular and the extraocular portions [25]. The arteriolar branches of the central retinal artery are devoid of adrenergic innervations while the choroidal vessels in the same provision are heavily innervated [25].

It is also speculated that the changes occurring in the retinal vascular network can be related to various systemic vascular conditions [22]. Arterioles are known to have a similar structure to small arteries (however less elastic) and muscular fibres, and therefore offer access to study these small arterial and arteriolar changes [26]. As such, the retinal vessels are a site that allows one to study these small arterial and arteriolar changes non-invasively [26].

Studies have shown that alterations in retinal vessel calibre are associated with CVD risk factors such as endothelial dysfunction [27, 28], inflammation [22], hyperglycaemia [29], obesity [30], HT [31], diabetes mellitus [32, 33] and certain markers of TOD [34, 35]. (A more specific breakdown of how artery and vein calibre changes are associated with CVD risk factors will be discussed in section 2.4). These vessel calibre changes have also been shown to be an early predictor of incident hypertension, coronary artery disease [27, 36-39], and stroke [40]. Furthermore, demographic, environmental, lifestyle (smoking and alcohol use) and genetic factors [41] may have an impact on retinal vessel calibres.

Despite findings linking changes in retinal vessel calibre with CVD [42-44], such as HT, coronary heart disease and stroke, findings linking retinal vessel calibre with TOD are discrepant [35, 45-51].

Besides the more general static characteristics of retinal blood vessels that have been measured (vessel calibres), functional measurement of the retinal microvasculature is also available now, which use a light-flicker to induce vessel dilation. However, to the best of our knowledge no information exists regarding the relationship between retinal blood vessel calibre, or their functional capacity with TOD and CVD markers in Sub-Saharan Africans. Most current research done on functional elements of retinal vascular calibre focuses on its relationship with endothelial dysfunction and systemic conditions [52, 53]. Taking the high prevalence of HT in blacks [12] into consideration, the relationship between retinal vessel calibre and markers of HT associated TOD warrants further investigation.

2.2 Vasculature of the eye

The vascular circulation of the eye comprises four parts: i) the anterior part of the eye, specifically the ciliary body that produces the aqueous humor, ii) a retinal circulation similar to the cerebral and coronary circulation (but lacks autonomic innervation, as mentioned before), iii) a choroidal vasculature with fenestrated capillaries and the greatest density of autonomic innervations in the body, and iv) the optic nerve head [54]. Blood supply to the

eye faces unique homeostatic challenges, i) the retina has the highest oxygen consumption per volume in the body, ii) the very exposed eye needs a constant temperature to function, and iii) the blood supply should not hinder the optical function. However, blood is supplied in the following ways: i) transparent parts such as the cornea and lens are supplied with necessary fluids by a transparent aqueous humor, ii) within the retina oxygen transport is facilitated by intracellular haemoglobin, and iii) the translucent retina has only a few blood vessels and the photoreceptors receive their oxygen and nutrition from the choroid, which in turn has the highest blood flow per volume in the body [24]. The retinal blood flow is auto-regulated, and within a certain range it is independent of perfusion pressure [55]. The major regulators of retinal blood flow are the vascular endothelial cells and neural and glial cells [56]. An illustration of the basic anatomy of the eye is depicted in Figure 2 and an Illustration of the internal carotid artery entering the optic nerve and linking with the central retinal artery is depicted in Figure 3 [57].

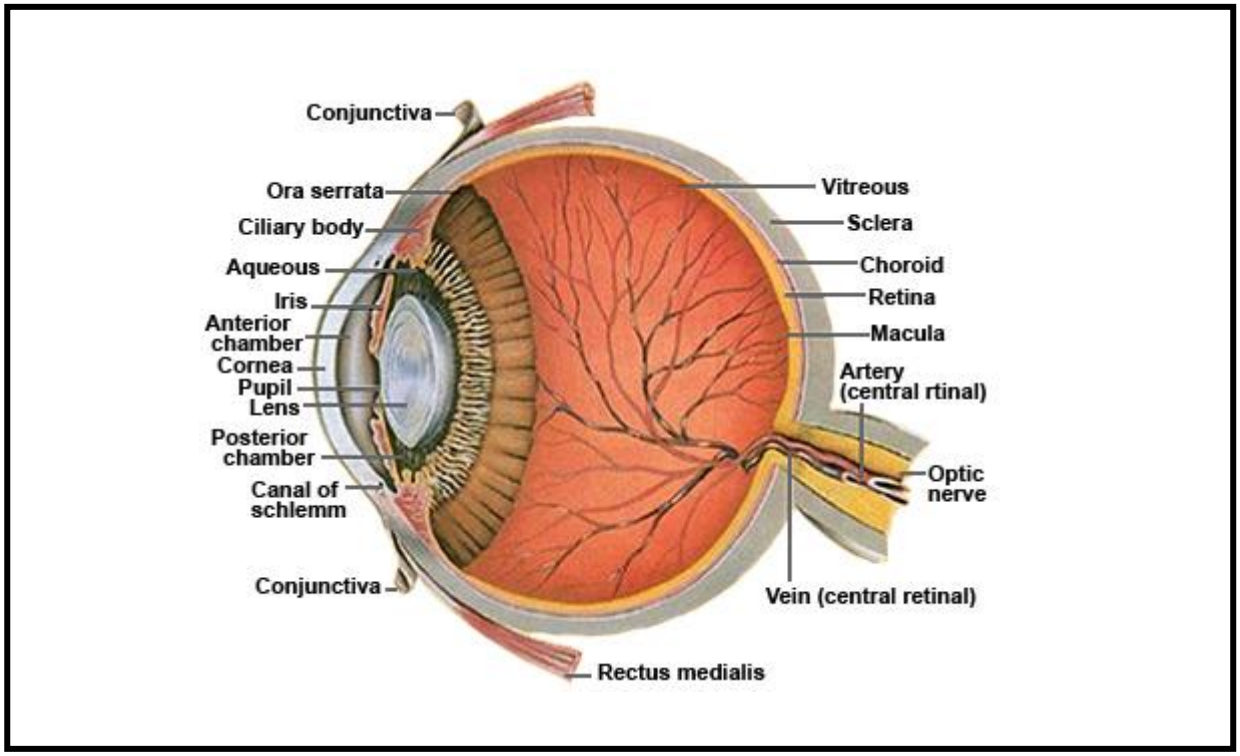

Figure 2: Anatomy of the eye [57].

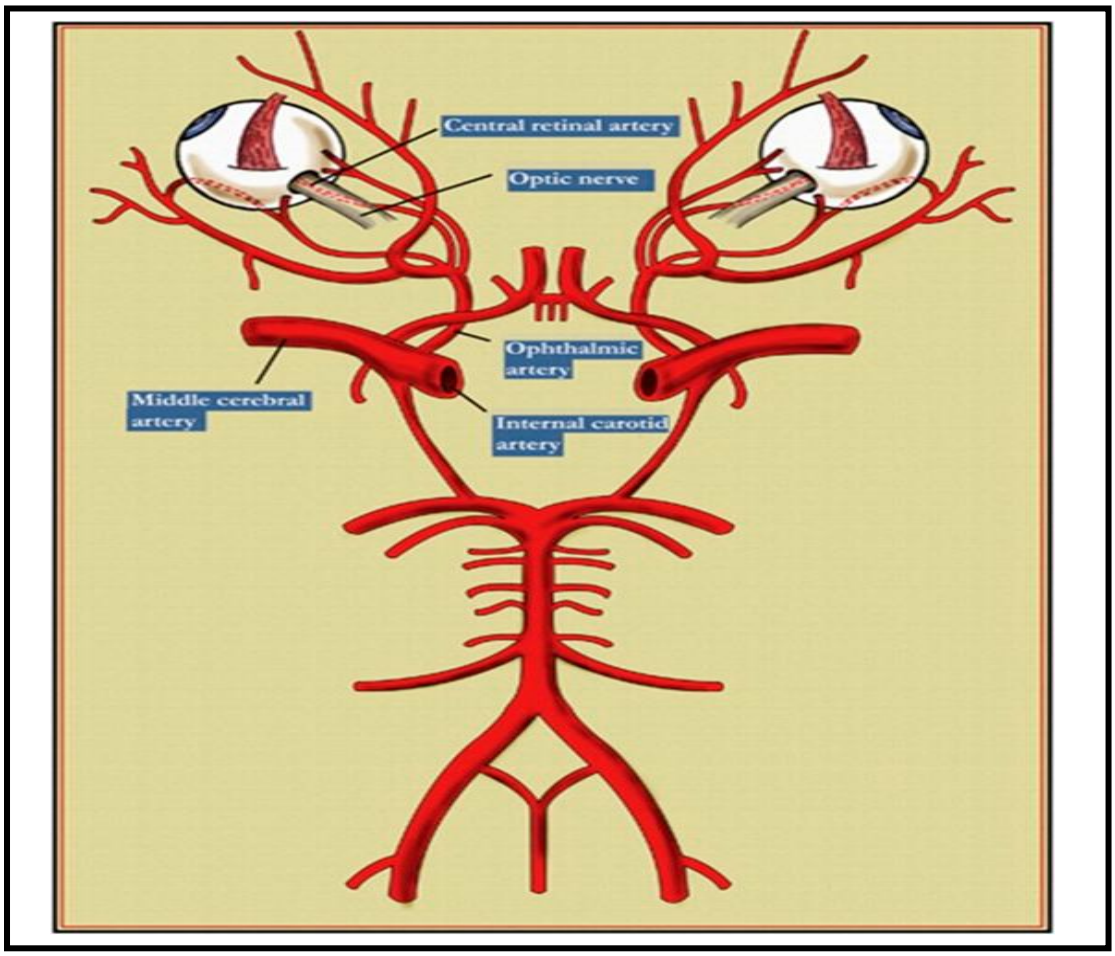

Figure 3: Internal carotid artery entering the optic nerve and linking with the central retinal artery [57].

2.3 Hypertension and the stages of retinopathy

As mentioned, HT has been reported to have various profound effects on the structure as well as the function of the eye [39]. Firstly the retinal, choroidal and optic nerve circulations undergo a series of pathophysiological changes in response to elevated blood pressure (BP), which will result in a range of clinical signs referred to as hypertensive retinopathy, hypertensive choroidaopathy, and hypertensive optic neuropathy [39]. Secondly HT is a well known important risk factor for the development of potentially blinding vascular eye diseases, such as retinal vein and artery occlusion, retinal-arteriolar emboli, and diabetic retinopathy [39]. Finally HT may also be a pathogenic factor for non-vascular ocular diseases, glaucoma and age-related macular degeneration [39]. Systemic cardiovascular

diseases such as arterial HT, coronary heart disease, diabetes mellitus as well as obesity are known to be well associated with structural vascular changes in the retina. This includes either narrowing of arterioles, dilation of venules or a decrease in the arteriolar-to-venular ratio (AVR) [24].

Hypertensive retinopathy in particular is a recognised cardiovascular risk stratification factor [59]. It is a condition in which the retinal circulation undergoes a series of changes in response to elevated BP. Hypertensive retinopathy encompasses a variety of retinal microvascular signs which include generalized retinal and focal arteriolar narrowing, arteriovenous nicking, retinal haemorrhages, microaneurisms and, in severe cases, optic disc and macular edema [60]. All of these signs develop due to acute and chronic elevations in BP [61]. Retinopathy can be divided into different stages. The initial response is diffuse and localized vasospasm of the retinal arterioles with consequent narrowing (generalized arteriolar narrowing and focal arteriolar narrowing (FAN), respectively). Generalized arteriolar narrowing is thought to reflect vasoconstriction as an autoregulatory response to attempt to control the volume of blood received by the retinal capillary bed. This usually occurs before the onset of sclerosis and can even be detected in children with HT [62]. Arteriovenous nicking occurs as a result of chronically elevated BP that will lead to compression of venules by structural changes in the arterioles. This will ultimately progress to an ‘exudative’ stage in which flame-shaped retinal haemorrhages and cotton wool spots are observed, and finally to a ‘malignant’ stage with optic disc and macular edema [39]. All aforementioned stages are usually not sequential and the signs reflecting the ‘exudative’ stage may also be seen in eyes without features of the ‘arteriosclerotic’ stage (AVN), and can therefore frequently be detected even in adults who are not hypertensive [63]. Racial differences may exist in the prevalence of retinopathy, where the highest rates are observed among Chinese (17.2%) and the lowest among white (11.9%) and black populations (13.9).

It has been reported that the classical Keith Wagener classification, applied to the analysis of retinal vascular lesions, has some limitations. Particularly in correctly evaluating the initial

phases of the vascular lesions. However, tools that allow estimation of the retinal arteriovenous index have been developed [38, 64-67]. This index and venous calibre have independently correlated to an increased risk of arterial HT, diabetes mellitus and cardio- and cerebrovascular disease [29, 33, 68, 69].

2.4 Introduction to the dynamic retinal vessel analyzer (DVA)

The retinal vessel imaging system (DVA) from Imedos systems allows for both static (vessel calibre assessed under normal resting conditions) and dynamic (a functional measure of calibre in response to a light flicker provocation) quantification of retinal vessel calibre [70, 71].

2.4.1 Changes in retinal vessel calibre (static measurements)

From a static retinal image, visual identification of retinopathy can be made. For the purpose of this dissertation focus will only be given to the direct quantification of retinal blood vessel calibres. Apparatuses such as the DVA enable the direct quantification of retinal vessel calibres. From a fundoscope image vessel calibre equivalent of the central retinal artery (CRAE) and central retinal vein (CRVE) are determined. The ratio of these 2 equivalents (CRAE/CRVE), namely the arteriolar-to-venular ratio (AVR) is then determined. (See Chapter 3 for a detailed description of determining these parameters). The Parr-Hubbard formulas for the summary measures CRAE and CRVE were derived from examination of a large number of retinal images using a root mean square deviation model that best fit observed data [66, 72, 73]. The methods that were used to quantify retinal vessel calibre were not independent of scale and were affected by the number of vessels. Recently, modified formulas for summarising retinal vascular calibre were developed by Knudtson *et al.* [74], which demonstrated a clear superiority over the previously used Parr-Hubbard formulas. These formulas correlate highly with the previously used Parr-Hubbard formulas but offer the advantage of being more robust against variability in the number of vessels observed, being independent of image scale, and being easier to implement [75]. Knudtson

et al. further makes use of the six largest arterioles and venules to compute retinal vascular measures. These retinal indices have been used in multiple large-scale epidemiological studies, which have demonstrated substantial reproducibility for these retinal vascular calibre measurements (intraclass correlation coefficient ranged from 0.80 to 0.99) [66], which provides further evidence that retinal photography offers a more sensitive and precise means of assessing architectural changes in the retinal vascular network [20, 76, 77].

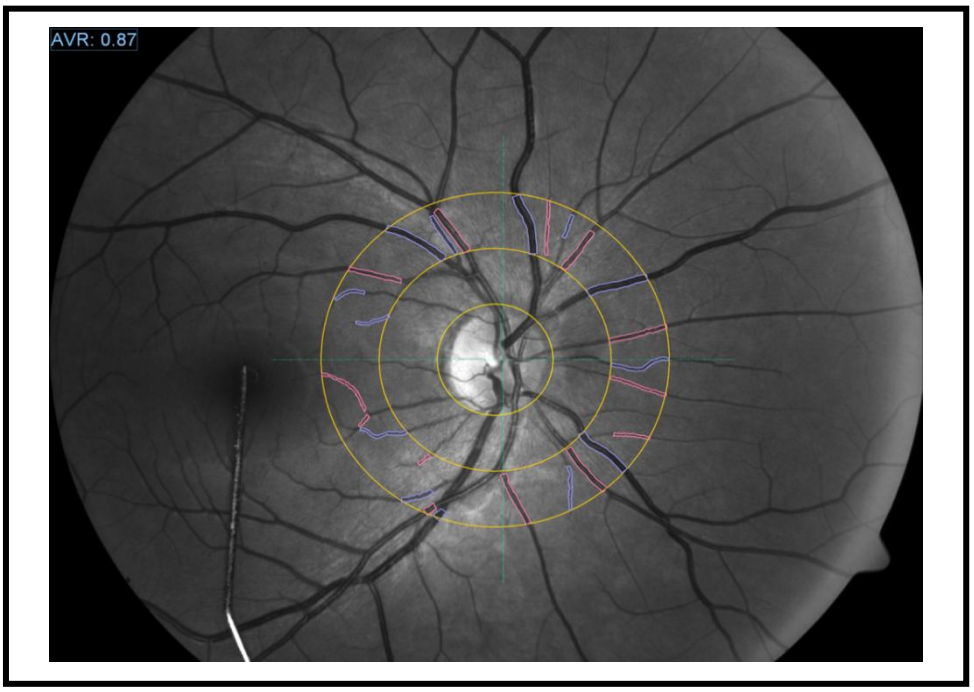

Figure 4: A monochrome fundus image indicating the measuring ring in which vessel segments are selected, where red and blue delineated vessels indicate the presence of arterioles and venules respectively.

The ocular microcirculation represents a preferential target for many systemic diseases, and changes in vessel structure can pre-date the development of hypertension by many years [78]. It has long been known that structural changes in the retinal vasculature have been recognized as an important predictor of systemic hypertensive damage [79, 80]. Various systemic cardiovascular diseases like arterial HT, coronary heart disease, or diabetes, as well as obesity are all associated with structural vascular changes in the retina [24]. It is important to note that arterioles and venules are differentially associated with cardiovascular

and metabolic conditions and, therefore, their individual relationship with various endpoints is of more value than investigating changes in AVR alone [38].

2.4.1.1 Retinal arteriolar narrowing

As systemic BP remains elevated, generalised retinal arteriolar narrowing are developed as a consequence of autoregulatory process that starts from vasospasm, and is followed by chronic arteriosclerotic changes, such as intimal thickening, media-wall hyperplasia and hyaline degeneration [41. It can also be explained by nitric oxide (NO)-dependent endothelial dysfunction, which is also a key feature of HT and may contribute to impaired endothelium-related vasodilatation [81].

2.4.1.2 Retinal venular widening

It has been reported that retinal venular widening is widely associated with carbohydrate intake, greater BMI, C-reactive protein (CRP) levels, lower birth weight [82], triglycerides, total cholesterol, and lower HDL levels [83]. Retinal venular widening is also reported to associate with increased risk of vascular dementia [84]. Moreover, newer data suggest that increased BP may also have a weak effect on retinal venules [38, 85, 86]. However, the mechanism for retinal venular widening remains complex and not easily understood [82]. It has been hypothesized that inflammatory induced endothelial dysfunction is the reason for retinal venular widening [38, 41, 87, 88], although not all studies found consistent relationships [89]. Most studies have only examined associations with nonspecific inflammatory markers (such as, white blood cell count, erythrocyte sedimentation rate) [38, 88].

2.4.2 Dynamic changes in retinal vessel calibre in response to light flicker provocation

The second measurement that can be performed by the DVA involves the dynamic assessment of retinal arteriole and venular calibre, using a light-induced-flicker stimulus. The light flicker has been shown to induce dilation of retinal arteries, a mechanism

dependent on endothelial nitric oxide synthase [90]. Thus inferences of the endothelial function of the retinal microcirculation can be made.

2.4.2.1 Flicker-light-induced retinal responses

The information on light-flicker induced retinal vessel changes (dynamic calibre changes) is scant. However, available information indicates that reduced vessel dilation is observed in pathological states associated with endothelial dysfunction [52, 53]. As such, reduced flicker light-induced vasodilation has previously been demonstrated in subjects with cardiovascular risk factors, such as diabetes (or diabetic retinopathy) [91, 92], HT, obesity and dyslipidaemia [94]. However, it can be improved with the relevant therapy [91, 93, 94]. For example, it is known that impaired response to light-flicker stimulation in hypertensive patients could be restored by angiotensin-II subtype 1 receptor blockade [95]. Visual stimulation of the retina primarily dilates capillaries as well as very small arterioles, thereby inducing a flow-mediated dilation of the larger retinal vessels [96] (figure 5). This response of retinal vessels to diffuse luminance flicker is measured non-invasively by the DVA apparatus, and may reflect endothelial function of the retinal microcirculation [71, 97], which plays a key role in the pathogenesis of vascular disease. Nitric oxide (NO) not only plays a role in the maintenance of retinal arterial and venous tone, but also in hyperaemic responses to flickering light, since the latter was abolished by systemic infusion of a NO-synthase inhibitor [98]. In this regard it has been demonstrated that NO is released in the retinal vasculature when stimulated by flickering light [90, 98-100]. Improvement was found in the retinal arteriolar architecture with successful treatment of HT [101]. Although the retinal microcirculation is proposed to self-adjust during diffuse luminance flicker, this response depends on endothelial function, as well as local variations in functional metabolic demand, and neurovascular coupling [53].

Associations between endothelial dysfunction and TOD markers have been reported, such as with LVH [102], eGFR [103, 104] and cIMT [105, 106]. In this regard, associations between dynamic retinal vessel calibre and TOD can be expected.

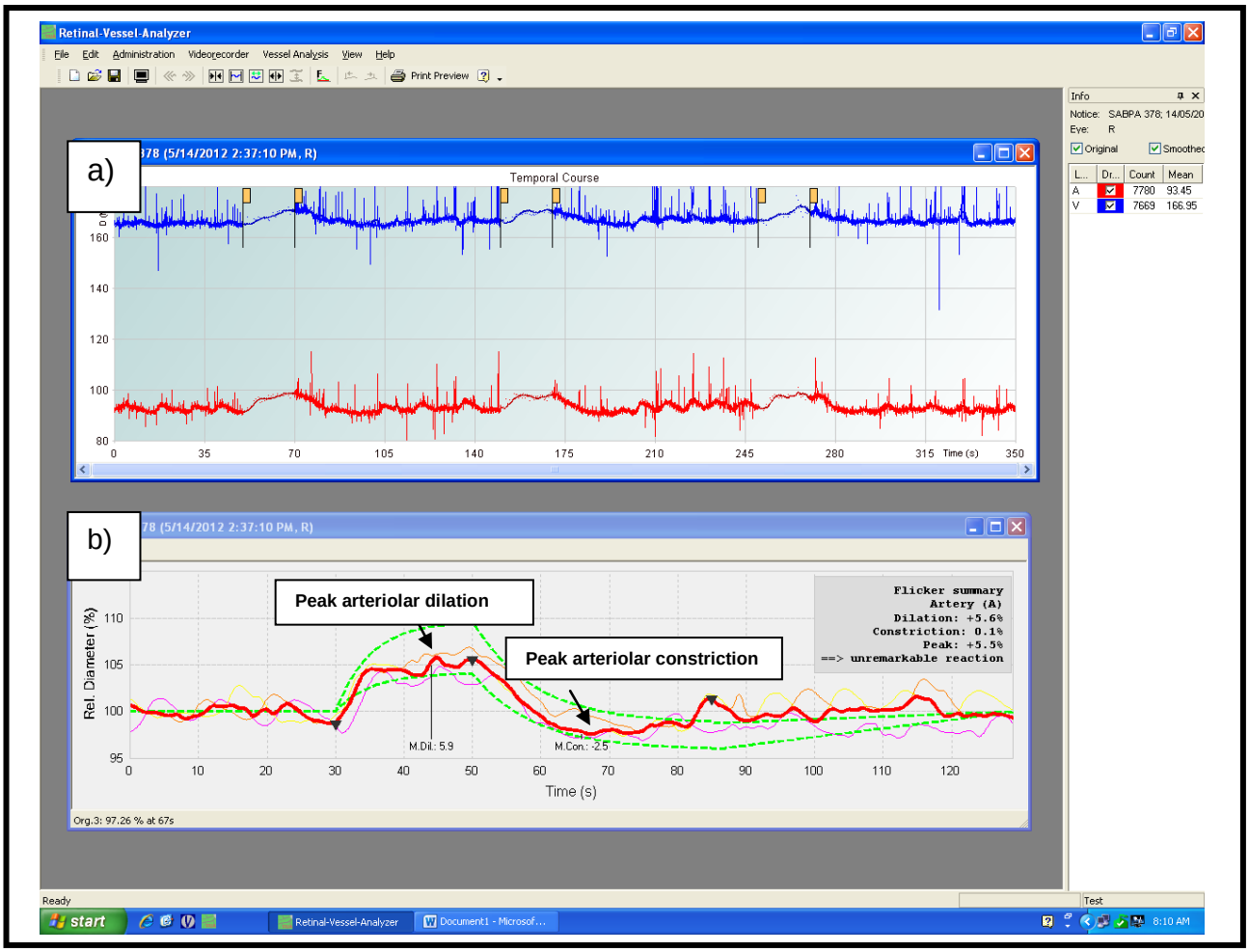

Figure 5: a) The temporal course of a completed examination, which reflects arterial (red line) and venule (blue line) diameter changes over time. (See chapter 3 for a better outlay of this figure). b) The vessel dilatory response to a light flicker protocol, which is reflected by three flicker cycles onto the same diagram, and summarises a profile as a thick red (artery) or blue (vein) line.

2.5 The retinal microvascular calibre and hypertension

The retinal microvasculature is distinctive in that it allows the direct sequelae of elevated BP to be visualised early [60]. Changes in retinal vessel calibre are long known to be strongly associated with both the presence and also severity of HT status and elevated BP [38, 66, 85, 87, 107].

2.5.1 Epidemiology

With the increased use of retinal photography in population based studies since the 1990's much data have been obtained regarding the manner in which systemic associations relate to hypertensive retinal vasculature changes [108]. For example, one of the primary features of essential HT is increased peripheral vascular resistance (PVR) in small vessels throughout the body. Since HT is associated with generalized retinal arteriolar narrowing, these systemic vascular changes may be visible non-invasively in the retinal microvasculature [109], and may therefore provide additional information in predicting cardiovascular diseases [110-114]. HT is generally characterised by a smaller CRAE [116], wider or unchanged CRVE and a reduced AVR [116]. It has been found that retinal arteriolar narrowing and arteriovenous (AV) nicking is related to past BP levels measured 3 and 6 years before retinal assessment, even after adjustments were made for current BP levels. This may suggest that arteriolar narrowing and AV nicking may be microvascular markers of cumulative hypertensive damage, since retinal arteriolar narrowing has been proposed to reflect structural damage from chronic HT [61, 85, 86, 108, 117, 118]. It is however important to note that studies have demonstrated that retinal artery narrowing is not only related to chronic exposure to HT, but might also precede the development of HT [119, 120]. Besides changes in CRAE and CRVE older studies have also investigated blood pressures association with the retinal vasculature in terms of the AVR. A summary of retinal AVR and its association with incident hypertension is indicated in figure 6 [41]. This figure is an illustration of longitudinal data from four population-based studies that have demonstrated that a smaller retinal arteriolar calibre (and smaller AVR) predicts the development of HT in initially normotensive subjects.

A reduced AVR has also been found to be associated with carotid stiffness [38], diabetes mellitus [32], heart failure [121], renal disease progression [122], metabolic syndrome [123], cerebral white matter lesions [124, 125] and cognitive disorder [126]. Adding to the prognostic potential of this measurement a reduced AVR has also been shown to associate

with cardiovascular morbidity and mortality [20, 127], future development of HT [128], or progression to more severe forms of HT [129], and stroke [130, 131].

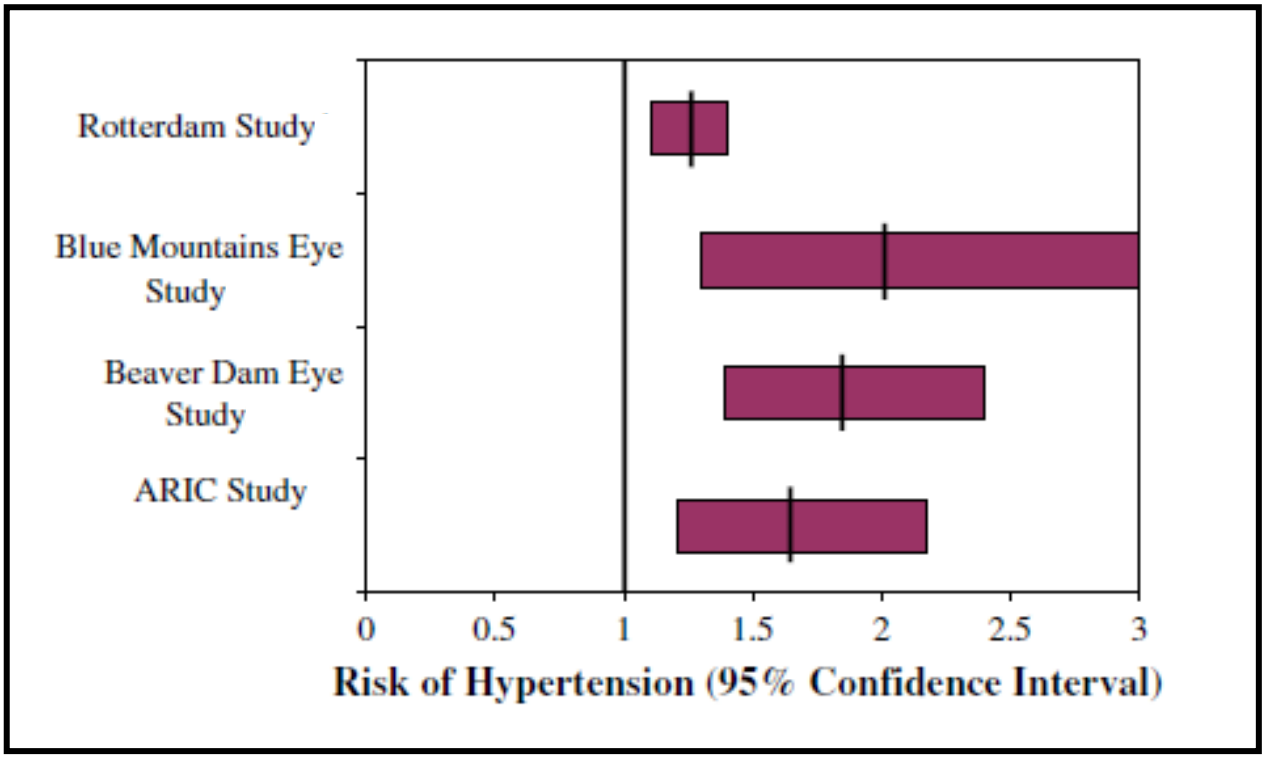

Figure 6: A summary of retinal AVR and incidence of hypertension [41].

2.5.2 Confounders of retinal vessel calibre measurements (age, sex, ethnicity, genetics and obesity)

The strength of associations between retinal microvascular abnormalities and HT is known to vary with age [132], ethnicity [133] and inconsistently with sex [107, 134 135]. Findings regarding retinal vascular calibres and their association with age are predominantly consistent, with retinal arteriolar diameters being narrower in older persons and in persons with higher BP [85, 86]. Data from large population based studies indicate that amongst middle-aged and elderly subjects an inverse association between retinal vascular calibres and age subsequently occurs, where older persons have both smaller retinal arteriolar and venular calibres [109]. Thus, age can be considered as a confounder when assessing the relationships between retinal vessel calibres and other variables. It is also suggested that the association between BP and retinal microvascular calibres weakens with ageing [132]

which may possibly be due to more pronounced arteriosclerosis (e.g. intimal thickening and medial hyperplasia, hyalinization, and sclerosis) of retinal arterioles in older persons [41]. This may prevent a degree of vasoconstriction [85], and might also be reflected by microvascular damage from elevated blood pressure.

Several studies suggest that ethnic differences may account for changes in retinal vascular characteristics [87, 133, 136, 137]. For example the MESA (Multi-Ethnic Study of Atherosclerosis) showed that compared with whites, blacks and Hispanics had larger retinal arteriolar calibres and blacks, Hispanics, and Chinese had larger retinal venular calibres than whites [133]. Data from ARIC and MESA studies also suggest that black ethnicity is associated with reduced AVR, compared to whites [87, 133]. Despite these potential ethnic differences in vessel calibre it has been suggested that retinal pigmentation (assessed by using iris pigmentation as a proxy), may influence the measure of retinal vessel calibre, possibly explaining in part the observed ethnic differences in retinal vascular calibres [138]. However, few studies have performed an in-depth investigation regarding the influence of ethnicity on retinal vascular measures [139].

Regarding genetics, the Beaver Dam Eye Study previously reported that retinal vascular calibres were more highly correlated between relatives than unrelated subjects, proposed due to shared genes [140]. This supports data from another previous twin study, indicating that 70% of the variance in retinal arteriolar calibre and 83% of the variance in retinal venular calibre was attributable to genetic factors [141]. More recent data from the Beaver Dam Eye Study, which was based on genome-wide linkage scans, further reinforced the genetic contribution to the variation in retinal vascular calibres independently of HT and other markers [142], and also showed that these linkage regions for retinal vascular calibres overlap with regions that has been shown to associate with essential HT, coronary heart disease (CHD), endothelial dysfunction and vasculogenesis [142].

Obesity may have profound effects on the eye but manifestations are poorly understood [143]. Larger venular calibre, but not arteriolar calibre has been reported to be related to

measures of obesity, such as greater body mass index and waist-to-hip ratio) and dyslipidemia (higher levels of plasma triglycerides and LDL cholesterol and lower levels of HDL cholesterol [38, 41]. A lack of regular moderate-to-vigorous intensity physical activity is closely related to obesity, and is known to be a modifiable risk factor for CVD [109]. Data from previous studies such as the Singapore Prospective Study program, ARIC- and MESA study have shown consistent relationships between lower levels of physical activity and wider retinal venular calibre [144-146].

2.6. Retinal vessel calibre and its association with stroke

The retinal vasculature is known to be morphologically and functionally related to the cerebral vessels because of the common origin from the internal carotid artery [147]. In this regard it has been suggested that the retinal vascular calibre may in particular present valuable insights into vascular pathology of the brain [148]. Multiple studies have reported a strong link between retinal microvascular changes and cerebrovascular disease [76, 125, 149]. According to the ARIC Study a smaller retinal AVR, which may reflect narrower arterioles or wider venules, was reported to be associated with an increased risk of stroke, especially cerebral infarction [76], which was also confirmed in the Cardiovascular Health study [150]. Despite the strong previous associations found, has not been consistent. In addition to previously mentioned findings, others have found that only wider retinal venules predict stroke [151, 152]. Another study suggests that both of these retinal vascular measures (narrower arterioles and wider venules) predict stroke mortality, but only for those younger than 70 years of age [153]. A meta-analysis, using published estimates of different studies, reported associations between only wider retinal venular caliber and stroke, with no associations for narrower retinal arteriolar caliber [154]. With regards to the relationship between individual vessel diameters and risk of stroke, various large population based studies have consistently demonstrated associations with larger venules [151, 152, 154]. The predictive value of smaller arterioles are less clear, for example, the Rotterdam Study found that a larger venular calibre was associated with a 12% higher risk of stroke and a

15% higher risk cerebral infarction with retinal arteriolar narrowing being neither related to the risk of stroke nor cerebral infarction [151]. McGeehan *et al*. [40] found similar results. Furthermore it has been found that retinal microvascular changes are associated with specific subtypes of stroke. Generalised arteriolar narrowing and venular widening are positively linked with lacunar stroke, and retinopathy signs are associated with non-lacunar thrombotic and cardio embolic strokes [155].

2.7 Retinal vascular calibres and target organ damage (TOD)

There are three key objectives in the assessment of a person with suspected HT: i) to confirm whether or not BP is elevated; ii) to document the presence or absence of BP-related TOD (e.g. left ventricular hypertrophy, hypertensive retinopathy, increased albumin:creatinine ratio); and iii) to evaluate the person's cardiovascular risk either due to established CVD or high CVD risk states (e.g. diabetes or chronic kidney disease (CKD) [156]. An illustration of various complications of hypertension associated target organ damage is shown in Figure 7 [181].

TOD is currently considered as an intermediate stage in the continuum of vascular disease and is a strong, independent predictor of total CV risk in individuals with high BP [157]. Elevated BP is associated with intermediate markers such as left ventricular hypertrophy (LVH) [158, 159] carotid intima-media thickness (cIMT) [160, 161] coronary atherosclerosis [162] or decreased glomerular filtration rate (GFR) [161] and also with CV morbidity and mortality [163-165].

Increasing evidence shows that values obtained from 24-hour ambulatory BP (ABPM) [166], shows a better correlation to TOD and cardiovascular morbidity-mortality [167-169] than home BP measurements. According to Mensah, Croft and Giles, the brain, heart, kidneys and vasculature are the primary target organs that are damaged by HT [170]. It is known that retinal microvascular abnormalities and/or calibre changes have been reported in subjects with HT and renal disease [87, 171-173], and also in subjects with carotid artery

disease [174, 175] and LVH [87, 172, 176-178]. These retinal microvascular changes are even present in subjects without HT [171, 179, 180].

For the purpose of this study we will specifically focus on the association between retinal vascular calibre (static and dynamic measurements) and markers of 1) left ventricular hypertrophy; 2) vascular remodelling; and 3) kidney function. To the best of our knowledge, the relationship between the retinal vessel light-flicker response and TOD has not yet been investigated

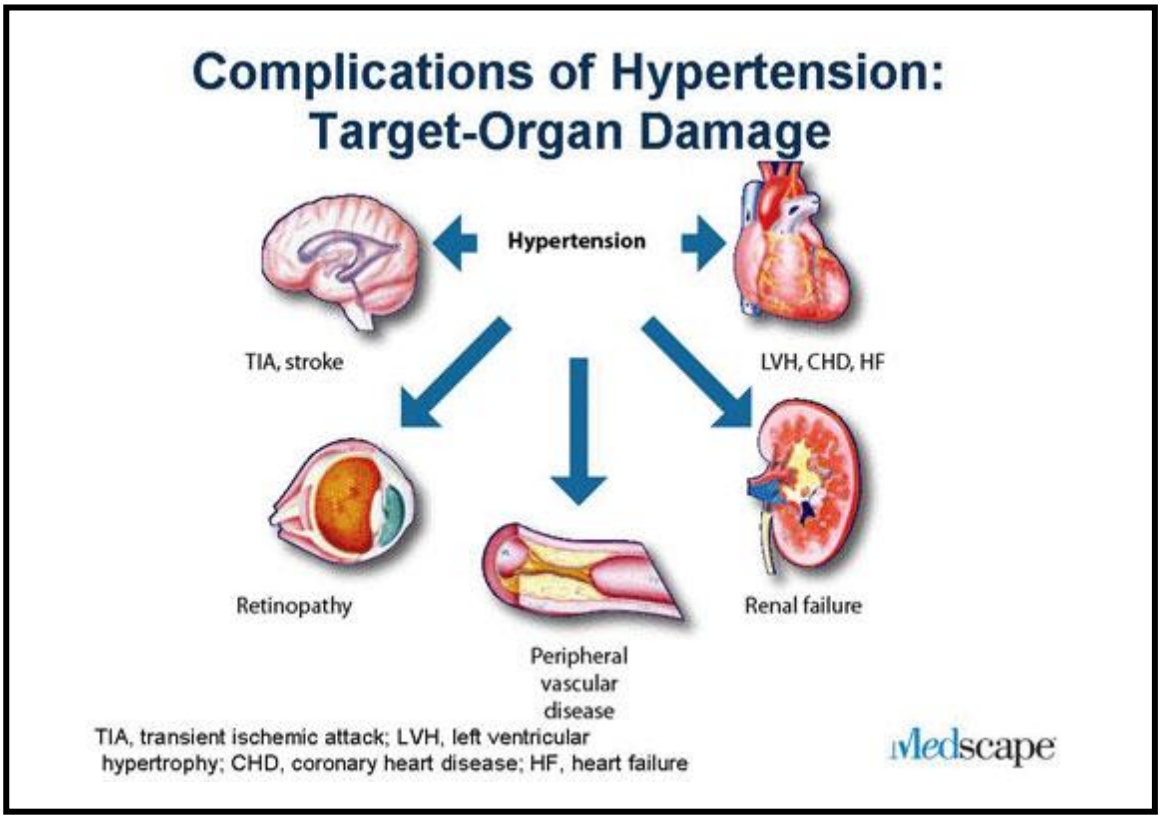

Figure 7: Complications of hypertension associated target organ damage [181].

2.7.1 Retinal vascular calibres and left ventricular hypertrophy

Left ventricular hypertrophy is an important marker of TOD and a powerful predictor of CVD [158]. LVH and retinal vascular disease, such as hypertensive retinopathy [182, 183], generalized and focal retinal arteriolar narrowing [50] appear early in the course of BP elevation and both changes develop in parallel [184].

LVH is generally detected by echocardiography or 12-lead electrocardiogram (ECG). In the absence of echocardiography data, an ECG can be performed to determine the Cornell product ((RaVL + SV3) × QRS ≥ 244.0 mV.ms^4 for men and (RaVL + SV3 + 0.8 mV) × QRS ≥ 244.0 mV.ms^4 for women) as an indirect marker of left ventricular (LV) mass [185-187]. The cut-off values for LVH defined by Cornell Product are >244mV.ms) [188, 227].

Microvascular disease is hypothesized to contribute to LV remodelling [189]. However, whether retinal microvascular calibre changes are related to early subclinical morphologic alterations and remodelling in the heart of individuals without symptomatic heart failure is unclear [189]. It has been reported that retinal microvascular abnormalities such as hypertensive retinopathy [184] and retinal arterial narrowing are related to LVH [50, 190]. In addition, an association between narrower retinal arterioles and LV concentric remodelling has been shown by Cheung *et al.* [189].

Findings of a recent study which investigated the association between AVR and LVH, in hypertensive patients, provides evidence that retinal AVR correlates with the presence of LVH [49]. They further suggest that AVR provides information of the LV geometric pattern in hypertensive patients. These cross-sectional studies are supported by findings from a one-year follow up study done by Coll-de-Tuero *et al.* [45] that subjects with a greater increase of AVR had significantly higher prevalence of left ventricular hypertrophy (LVH) and amount of TOD.

Changes in retinal vessel calibre, such as arteriolar narrowing, seen in the retina may represent widespread microcirculatory disease. In turn such changes places an impedance burden, in part through reflected waves, on the left ventricle [189]. This may result in an increased LV load and impinge in LV emptying, predisposing the LV to undergo physiological (adaptive) or pathological (maladaptive) concentric remodelling. Examining the association between retinal vessel calibre and LV remodelling may help understand the potential role of the microvasculature in the pathogenesis of early cardiac remodelling and subsequent development of heart failure [189].

However, in contrast to the previously mentioned studies, other studies failed to demonstrate a significant association between left ventricular concentric remodelling and retinal vessel calibre in subjects with essential HT [46, 47, 191]. These studies suggest that retinal vessel calibre measurements may be of limited value in identifying LVH in patients with hypertension. However, other researchers argue/believe that the microvasculature (AVR), may have an important role in global CV risk stratification and could possibly be used for optimising the hypertensive patient management [49]. Among South African hypertensives, black ethnicity was found to be an independent predictor of LVH, so hypertensive blacks may be more susceptible to TOD [192].

2.7.2 Retinal vascular calibres and carotid intima-media thickness

Measurement of the carotid intima-media thickness (cIMT) is a non-invasive method which is a valuable measurement of arterial wall thickening [193, 194]. Although a cIMT cutoff >0.9mm was taken as a conservative estimate of existing abnormalities in the 2007 Guidelines [157], the threshold value for high CV risk was higher in the elderly patients of the Cardiovascular Health Study and in the middle-aged patients of the European Lacidipine Study on Atherosclerosis (ELSA) study (1.06 and 1.16 mm, respectively) [187,189, 224]. Presence of a plaque can be identified by an IMT>1.5 mm or by a focal increase in thickness of 0.5mm or 50% of the surrounding carotid IMT value [188].

cIMT is a well known predictor of other forms of TOD, and some researchers argue that an increased cIMT may be indicative of subclinical atherosclerosis [195], which is the key underlining pathological process of CVD [38, 41, 87, 196]. Significantly, increased cIMT is reported to be a predictor of future cardiovascular events [197]. However, the association between abnormalities in medium sized vessels, such as carotid arteries and microvascular retinal changes, has been poorly investigated in hypertensive patients [47, 198]. Overall the evidence of a link between CIMT and retinal vascular calibres has not been consistent. Various large populations studies have found associations between retinal vascular calibre

and cIMT [38, 51, 199], whereas others found none [47, 89], and others only with the presence of retinopathy [200] and cIMT.

Studies reporting a relationship, demonstrate an inverse association of retinal arteriolar diameter, and a positive association of retinal venular calibre with cIMT in hypertensive subjects [51]. In line with these findings, the suggestion was made that vascular damage occurs parallel in large arteries and in the microcirculation [51]. Torres and co-workers suggest that hypertensive subjects may present similar pathological abnormalities in various vascular sites [201, 202]. They also concluded that their results strengthen the link between elevated BP and widespread vascular damage by demonstrating an independent association of markers of vascular damage in the retina and in the carotid arteries.

The Rotterdam Study has shown that lower retinal arteriolar diameters and AVR were related to increased cIMT [38]. It is suggested that increased cIMT may indicate a response of the artery wall to changes in shear and tensile stress due to HT [199].

However, in addition the Hoorn study found that retinal venular dilation was associated with an increased cIMT, however non-significantly after multivariable adjustments for cardiovascular risk factors, especially fasting insulin levels [203]. Lastly, the MESA (Multi-Ethnic study of Atherosclerosis) study has found an association between increased internal cIMT and the presence of retinopathy but not retinal vessel calibres [200]. Data from the Cardiovascular Health Study also revealed no consistent independent association between smaller AVR and measures of large artery atherosclerosis [89]. Since the AVR is a composite measure and does not reveal which component (CRAE or CRVE) is related, it is suggested that these retinal summary measures should be examined separately [109].

Cuspidi and colleagues also found no association with IMT and concluded that fundoscopic examination has a limited clinical value to detect target organ damage in patients with grade 1 and 2 HT [47].

2.7.3 Retinal vascular calibres and renal function

It has been estimated that by 2030, more than 70% of patients with end-stage renal disease are estimated to be living in low-income countries, such as Sub-Saharan Africa [225]. There are various potential causes of chronic kidney disease (CKD) in Sun-Saharan Africa, which makes kidney disease in this region especially burdensome [225]. Predictions show that by 2030, 18.65 million people in Sub-Saharan Africa will have diabetes, and similar objections have been made for hypertension, obesity and tobacco use [226]. An important difficulty of detecting kidney diseases in Su-Saharan Africa is the reliability and validated measures of kidney function [225]. Several methods exist to measure renal function, such as creatinine clearance, microalbuminuria, albumin-to-creatinine ratio, and glomerular filtration rate (GFR) [48, 204-208]. On a continent where chronic diseases and cardiovascular deaths are drawing increasing attention, CKD should not be overlooked [225]. Furthermore, the risk of cardiac death is increased by 46% in subjects with a glomerular filtration rate (GFR) between 30 and 60 ml/min per 1.73 m^2 [228].

Previous experimental studies have shown a link between retinal abnormalities, such as retinopathy [205] and AV nicking [209] with renal vasculature [210]. Edwards *et al.* [205] reported a significant association between retinopathy and renal function deterioration, possibly suggesting that common systemic microvascular processes may underlie the development of microvascular damage in the eye and kidneys [205, 211]. Wong *et al*. [209] found that subjects with AV nicking were more likely to develop renal dysfunction and Kaul *et al.* [210] concluded that the microvasculature is narrowed in patients with reduced eGFR. However, few studies have explored the independent association of retinal vascular abnormalities with kidney disease [109].

A smaller retinal arteriolar calibre was confirmed to be associated with a higher risk of developing chronic kidney disease (CKD) [212-214]. It has been hypothesised that vascular diseases in subjects with renal impairment, but not nephritis, involving the renal microcirculation are one of the possible causes of early decline in renal function [215-217].

This hypothesis is supported by experimental studies indicating microvascular alterations in the renal circulation in models of chronic renal failure [218], and the strong link between kidney disease and vascular risk factors, such as diabetes and HT [219-221]. However, it has been suggested that further studies are required to confirm these associations and further elucidate the exact link between changes in retinal vascular calibres and kidney disease [222].

It has been shown that even a moderate decrease in the GFR of the kidneys is associated with a cluster of haemodynamic and pro-atherogenic abnormalities such as elevations in BP, dyslipidaemia, hypercysteinaemia, insulin resistance, endothelial dysfunction, and systemic inflammation, all of which in turn may result in the development of cardiovascular complications [206]. Glomerular and retinal arterioles are small vessels, which may be mutually affected by systemic diseases including diabetes and HT [223]. It is known that the retinal vasculature is similar to the glomerular vascular bed in that vasculature tone increases in response to elevated BP due to auto-regulatory mechanisms [61]. Awua-Larbi *et al.* [223] found a higher prevalence of albuminuria among participants with narrower or wider CRAE. Retinal arteriolar and venular calibres were negatively correlated with kidney function, in a study performed in apparently healthy subjects with normal renal function (measured by eGFR). This may suggest common determinants of these preclinical target organ damages [48]. Sabanayagam *et al*. [213] provided evidence that the presence of a generalized retinal arteriolar narrowing, a vascular phenotype reflecting both functional and structural changes from a variety of cardiovascular risks, including high BP, increases the likelihood of CKD, defined by an estimated glomerular filtration rate less than 60 ml/min per $1.73m^2$.

In the ARIC study [209] lower arteriolar-to-venular ratio (AVR), arteriolar narrowing and venular widening, was associated with a longitudinal increase in serum creatinine. Serum creatinine levels are frequently used as a screening test for renal dysfunction. This study was contradicted by Cardiovascular Health Study In the one-year follow up study of Coll-de-

Tuero *et a.l* [45]; no association was found between AVR and development of renal function measured by glomerular filtration rate (GFR). However, their study was done using fundus oculi (FO) in hypertensive subjects by means of retinography and not retinal imaging.

2.8 Summary

Analysis of the retinal microvasculature provides valuable information about the structure as well as the function of the retinal vessels, and can easily be obtained repeatedly over time, although its clinical application has only recently gained attention [21]. The retina is therefore a unique site which may present as a window to the heart, brain and kidney, where the microcirculation can be imaged directly, and in this regard provide a window for detecting changes in microvasculature relating to the development of CVD such as arterial HT or coronary heart disease [24].

2.9 Motivation

Ultimately, retinal vessel imaging provides the potential to be used as a non-invasive, reliable method for assessing, as well as early detection of microvascular sequelae of HT, and possibly considered as a biomarker for TOD [60]. Early detection and adequate management of hypertensive TOD can slow or prevent damage, or even allow disease regression where organ damage is still at reversible stage. Therefore, the diagnosis of hypertensive target organ damage is of decisive importance, especially when taking the increasing susceptibility of HT [4] and TOD (LVH [192] and kidney disease [165]) of blacks, within South Africa, into consideration.

2.10 Aims and Objectives

The aim of this study was to investigate the associations between BP-related target organ damage with static and dynamic retinal calibre measurements in black and white participants.

2.10.1 The objectives were:

a) To determine whether measurements of BP-related TOD (Cornell product, cIMT, estimated GFR) are associated with
    - Retinal vessel calibre (CRAE, CRVE, AVR); and
    - Light flicker-induced retinal calibre changes (arteriolar dilation, constriction and venular dilation)
b) To determine whether these relationships differ between a black and white population.

2.11 Hypotheses

Taking our aims and objectives into consideration, we made the following hypotheses:

a) Both static and dynamic retinal calibre measurements will display adverse associations with markers of BP-associated TOD.
b) Associations between retinal vessel calibre measurements and TOD will be more outspoken in black participants.

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CHAPTER 3
Methodology Chapter

3.1 Methodology of the SABPA study

Only measurements relating to this particular sub-study of the SABPA (Sympathetic Activity and Ambulatory Blood Pressure in Africans) study will be discussed. (A complete outlay of the methodology for the SABPA study is further explained in a recently published article: [1]).

Study design and participants

Organisational procedure

The present study was performed using data obtained from the follow-up phase of a prospective cohort study, the SABPA study. This study started in 2008/2009 and the 3-year follow up phase was conducted in February to May of 2011 and 2012. All participants taking part in the baseline phase were then re-invited to take part in the study. The study had a successful follow-up with 87.8% of participants returning. A total of 173 black and 186 white school teachers (aged 23–68 years) from the Dr Kenneth Kaunda Education district of the North West province in South Africa, took part in this phase of the study. For our particular sub-study participants with epilepsy, missing DVA, cIMT, Cornell product and eGFR data were excluded. After the exclusion, a total of 334 participants (156 Black (80 men and 76 women) and 179 White (86 men and 93 women) were considered eligible for our sub-study. All participants signed an informed consent form and were fully informed about the objectives and procedures of the study prior to the measurements. The study complied with all applicable requirements of the US and international regulations, in particular the Helsinki declaration of 1975 (as revised in 2008) for the investigation of human participants. Ethical clearance for the SABPA study was obtained from the Health Research Ethics committee of the North-West University (NWU-00036-07-S6), and for this particular sub-study (NWU-00036-07-A6).

The two-day procedure commenced on a normal working day between 07h00-08h00 where an ambulatory blood pressure monitoring (ABPM) device was attached to the participant's non-dominant arm. They then proceeded with their daily routine or work schedule. At

approximately 14h00 participants were transported to the North-West University. Participants arrived at 15h00 for retinal vessel measurements. Between 17h00 and 18h00 participants were taken to the Metabolic Unit research facility of the North-West where they completed general health questionnaires. Before 20h30 they received a standardised dinner as well as a last beverage, coffee/tee and two biscuits. Thereafter they were asked to relax and to refrain from alcohol, smoking, caffeine or exercise. Participants were encouraged to go to sleep at 22h00. Participants were woken at 06h00 the following morning, where the ABPM device was removed and further measurements were performed. A registered nurse obtained a blood sample. Thereafter an accelerometer (Actical®, Mini Mitter, Montreal, Quebec) was fitted to the hip of the participant. This device was worn for 7 days.

Anthropometric measurements

Calibrated instruments were used to measure the participants' height (stature) (cm), weight (kg) and waist circumference (WC) (cm) whilst being in their underwear (Invicta Stadiometer, London; United Kingdom; IP 1465, Precision Health Scale, A&D Company, Tokyo, Japan; Holtain unstretchable flexible 7mm wide metal tape, Crosswell, Wales). WC was measured over the costal margin and the iliac crest. Body mass index (BMI) was calculated (kg/m^2). All measurements were performed by registered biokineticists and were done in triplicate according to standard performing procedure methods.

Biochemical measurements

Fasting blood samples were obtained by a registered nurse, with a sterile winged infusion set from the antebrachial vein branches. Fasting glucose, cholesterol, serum high density lipoprotein (HDL) cholesterol, high sensitivity C-reactive protein (CRP), gamma-glutamyl transferase (GGT), creatinine and triglyceride levels were determined, using multiple analysers (Cobas Integra 400 plus, Roche, Basel, Switserland; Beckman and Coulter time-end method (Unicel, DXC 800, Germany)). A turbidimetric inhibition immunoassay was used

to determine the percentage of glycated haemoglobin (HbA1c) on whole blood with the Roche Integra 400 (Roche, Basil, Switzerland). HIV status was determined with the First response HIV card Test 1-2.0 (PMC medical, India Pvt Ltd) and confirmed by means of the Pareekshak HIV triline test (UCB Pharma, India). Serum cotinine levels were determined with a homogeneous immunoassay (Automated Modular, Roche, Basel, Switzerland). Participants were classified as smokers based on the answers of the general health questionnaire. In an attempt to improve the accuracy of the questionnaire data, participants indicating that they were not smokers, but presented with a serum cotinine value above 6ng/ml (ethnic specific cut point), were classified as smokers [2]. Estimated glomerular filtration rate (eGFR) as calculated according to the MDRD formula (estimates glomerular filtration rate based on creatinine and patient characteristic): GFR = 175 x SerumCreatinine$^{-1.154}$ * age$^{-0.203}$ * 1.212 (if patient is black) * 0.742 (if female) [3]. All intra- and inter-coefficients of variation for assays were below 10%.

Cardiovascular measurements

Ambulatory blood pressure measurements (ABPM)

Ambulatory blood pressure measurements (Cardiotens CE120®, Meditech, Budapest, Hungary) were determined within 30 minute intervals during the day (08h00-22h00) and every hour during the night (22h00-06h00). The cuffs were attached to the participants' non-dominant arm and hip respectively. In the SABPA II study participants achieved the following average inflation rate in a 24h period: Blacks: 85% ± 9; and whites: 92% ± 8. It was expected of the participants to proceed with their daily routine and activities but to note any discomforts or abnormalities. The data were analysed using Cardio Visions 1.9.0 Personal Edition software. Twenty four hour ambulatory blood pressure values exceeding 130/80 mmHg were classified as hypertensive [4].

Carotid intima-media thickness (cIMT)

cIMT was obtained using a SonoSite Micromax ultrasound system (SonoSite Inc., Bothell, WA, USA) and a 6-13 MHz linear array transducer. Images from at least two optimal angles of the left and right common carotid artery were obtained. A single reader conducted measurements using a semi-automated program, namely the Artery Measurement Systems (AMS) II v1.139 (Chalmers University of Technology, Gothenburg, Sweden). A maximal of 10mm segment with good image quality was chosen for analysis. A program automatically identifies the borders of the intima-media of the near and far wall, and the inner diameter of the vessel and calculates the CIMT and the diameter from around 100 discrete measurements through the 10mm segment. This automated analysis was capable of being manually corrected if not found appropriate on visual inspection. Far wall measurements were used for the purpose of this study. Intra-observer variability for the far wall was 0.04mm between two measurements made 4 weeks apart on 10 subjects.

Cornell product

An ECG was performed to determine Cornell product as an indirect marker of left ventricular mass ((RaVL + SV3) × QRS ≥ 244.0 mV.ms^4 for men and (RaVL + SV3 + 0.8 mV) × QRS ≥ 244.0 mV.ms^4 for women) [5-7]. A 12-lead ECG of 6 cardiac cycles was performed (NORAV PC-ECG 1200, Israel).

3.2 Introduction of the DVA:

The retinal vessel imaging system (dynamic retinal vessel analyzer (DVA)) from Imedos Systems is a funduscope with various software packages, that allows non-invasive measurement of the retinal vessel calibres during resting conditions (static), and in response to light-flicker provocation (dynamic) [8, 9].

From the static retinal measurements information is obtained regarding the central retinal artery equivalent (CRAE), the central retinal vein equivalent (CRVE) and the ratio of the two variables, the arteriolar-to-venular ratio (AVR). The dynamic retinal measurement involves the

assessment of retinal arteriole and venular calibre, changes following a light-induced-flicker response, and information is obtained relating to the peak arteriolar dilation, peak venular dilation and peak arteriolar constriction during the prescribed protocol.

The DVA apparatus (figure 1) consists of a fundus camera, video recorder, real-time monitor, and personal computer with analysis software [10]. Illumination light of the fundus camera is reflected by the retina and blood vessels, which is delivered via the observation optical pathway to a charge-coupled device [10]. For dynamic measurements, an optoelectric shutter is inserted in the fundus camera, to interrupt the observation light. This shutter produces a bright-to-dark contrast of at least 25:1, generating a flicker frequency of 12.5 Hz, which has been shown to be within the range of maximal flicker stimulation for retinal vessels in human [9].

All measurements took place in a half-lit/dark room. In the following sections, a description of each DVA measurement will be provided.

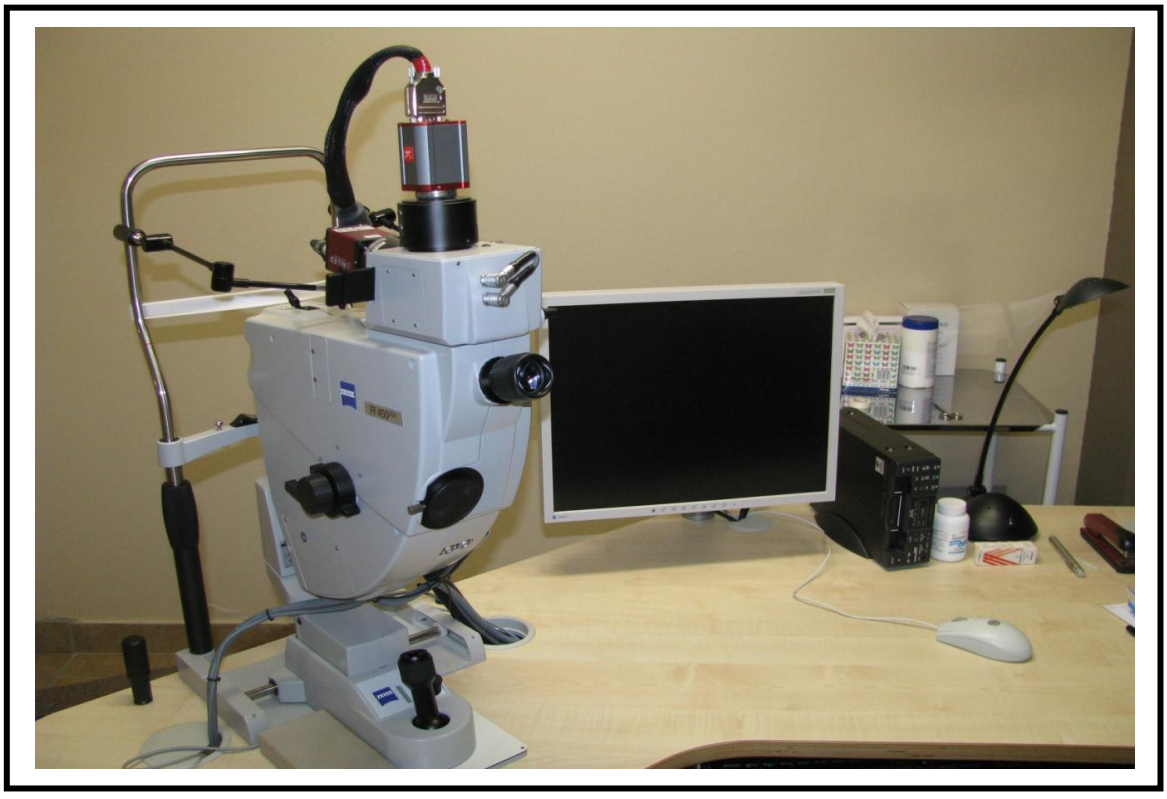

Figure 1: Illustration of the dynamic retinal vessel analyzer (DVA)

3.2.1 Protocol

3.2.1.1 Organizational procedures:

Prior to the measurements being performed, the following took place or were ensured:

- Subjects were asked whether they had a history of epilepsy. If they did, they are excluded from the measurement as the light flicker stimulus may induce an epileptic episode.
- One drop of Tropicamide (1%,Alcon) was administrated into the right eye to achieve mydriatic conditions. In the event of injury to the right eye, the left eye was used (performed by a registered nurse).
- The risk for acute anterior chamber angle glaucoma was tested by shining a light across the pupil. Risk was identified by the presence of a shadow cast on the opposite side of the pupil. This test was performed by a registered nurse
- Before measurements commenced, all procedures were explained to each participant.
- An eye-patch was placed over the untreated eye that was not going to be used (left eye).
- Participants were in a non-fasting state, but were not allowed to consume food or water, smoke or perform exercise 1hour prior to the measurement

3.2.1.2 Retinal photography and measurement of static and dynamic retinal vascular calibre

Fifteen minutes after a drop of Tropicamide (1% Alcon) was administrated, static and functional retinal measurements were performed according to a standardised Dynamic retinal vessel (DVA) protocol, using the DVA with a Zeiss Fundus Camera FF-450 Plus (Imedos, Jena, Germany).

Each measurement will be described briefly

Dynamic measurement:

For the **dynamic** measurement (using RVA 4.10 software, Imedos Systems, Jena, Germany), the fundus camera was set at an angle of 30°. The subject was requested to focuses on the tip of a fixation bar within the retinal camera to allow the researcher to perform measurements on the correct area of the retina. An example of the fixation bar as applied to the static measurement is shown in Figure 3. The fundus was then examined under green light (optimal for visualizing of vessels). A section from each of an arteriole and venule branch was selected from the superior temporal vessels. This selection must be between one half and two disk diameters from the optic disk margin. It was important to ensure the selected section of vasculature was free of side branches, and as straight as possible. Inferior temporal vessels segments were only used when measurements were not possible along the superior temporal vessels. The mean diameters of arteriolar and venular vessel segments are automatically recorded and calculated. After a 50 second baseline phase a light flicker stimulus was automatically induced, for 20 seconds followed by an 80 second recovery phase. Thereafter two more flicker cycles took place. The second and third baseline period was only 30 seconds long with a recovery period of 50 seconds

Following the measurement, the software automatically generates a summary curve of the 3 cycles. The raw data curves are also present on this graph. The reliability of the summary curve is assessed by its fit with the raw data curves (Figure 2). From this summary curve peak flicker induced vessel (peak- arteriolar and venular) dilation (expressed as a percentage of baseline values) and maximum arteriolar constriction (expressed as a percentage of baseline values), were manually determined. In the event that the summary curve was not reliable, the percentage peak vessel dilations and arteriolar constriction were calculated from the raw data. The programme summarises a layout of the measurement results. It gives an indication of the valid cycles, or amount of measurements, which must be greater than 30% to be reliable, and there has to be 3 cycles (3 flicker responses). For this

study, valid percentage measurements were obtained for all groups: blacks: 72% ± 13 (arterioles), 77% ± 10 (venules); Whites: 80% ± 9 (arterioles), 82% ± 8 (venules).

An option is available on the software, called *Temporary examination,* which indicates the amount of light shining on the fundus. The line should not exceed the middle mark (Figure 5). To ensure an optimal measurement, the subjects' eye needs to focus on the fixation device at all times (Figure 3). Adjustments with the camera's joystick are made as the participant moves.

Static measurement:

For the **static** measurement (using Visualis 2.81 software, Imedos Systems, Jena, Germany), the camera was set at an angle of 50°. A monochrome (Figure 4) and colour image (Figure 3) were captured for each participant. The flash intensity was adjusted accordingly. The monochrome image was used to calculate retinal vessel calibre equivalents and the colour image to determine the presence of retinopathy, and aid with the identification of vessel type.

The monochrome image was then further analyzed using VesselMap2 software Version 3.02 (Imedos Systems, Jena, Germany) to determine retinal vessel calibre equivalents. All vessels located within a standardized measuring zone of 0.5 – 1 optic disc diameters from the outer margin of the optic disk were selected and identified as either an arteriole or venule. Using a selection tool, each vessels' border was delineated automatically upon vessel selection. In the event that a vessels' border was not properly delineated, the procedure could be performed manually. The software then automatically selected the 6 largest arterioles and venules (Figure 4) and their calibres were processed via the Knudtson formula to provide information regarding central retinal artery equivalent (CRAE), central retinal vein equivalent (CRVE) and the arteriolar-to-venular ratio (AVR). CRAE and CRVE represent equivalents summarizing the caliber of the central retinal artery and central retinal vein respectively. The light which is reflected into the retina needs to be evenly distributed across the fundus, and there has to be enough light on the fundus to get a clear contrast

between tissue layer and vessel. It is also important to note that it is the column of red blood cells that is measured, and not really the outer walls of the artery/vein.

After the measurement a cotton wool eye patch was placed over the eye used in the measurement, to protect the retina from bright light.

All data analysis was performed by two trained researchers that were blinded to the health status of the participants. For each measurement obtained, calibers are calculated in measuring units (MU) as the dimensions of the eye are unknown. If the assumption is made that the dimensions of the eye are similar to the normal Gullstrand eye, then 1MU is equivalent to 1µM.

Currently, only three researchers at our institution are trained to perform measurements with the DVA, namely Dr. W. Smith, prof. L. Malan and prof. N.T Malan, who form part of the Hypertension in Africa Research Team (HART). They received training from Mr. D. Mueller*, Dr. Ing Vilser* and M. Reimann** (*From Imedos, Jena, Germany) (** From Dresden, Germany). To our knowledge, this is the only apparatus of its kind in Africa. I have been privileged to sit in during a few DVA measurements to improve my understanding of this apparatus. Although I was not primarily involved in obtaining the data of the SABPA study (since it was performed before I enrolled as a postgraduate student), I have gained training and knowledge of the necessary apparatuses and methods used in this sub-study. It includes the following: ECG- and blood pressure measures, as well as blood and urine sampling in the laboratory. I also gained experience by directly interacting with participants, as well as showing my commitment as a HART team member to the current ongoing African Predict study, currently performed by HART.

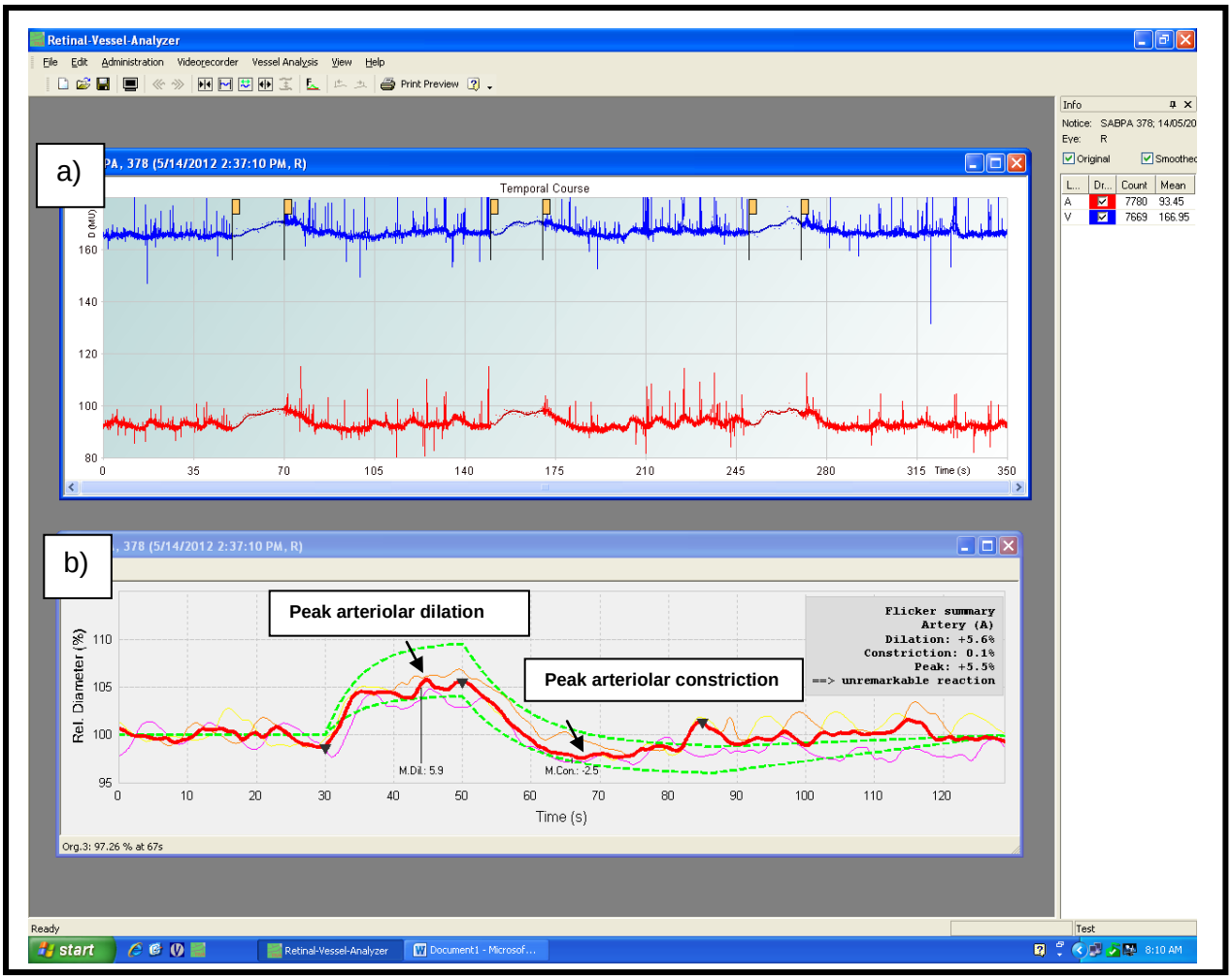

Figure 2: a) The temporal course of a completed examination, which reflects arterial (red line) and venule (blue line) diameter changes over time; b) The arteriole flicker summary obtained following 3 cycles of light flicker provocation. The thick red line represents the summary of the 3 individual flicker cycles.

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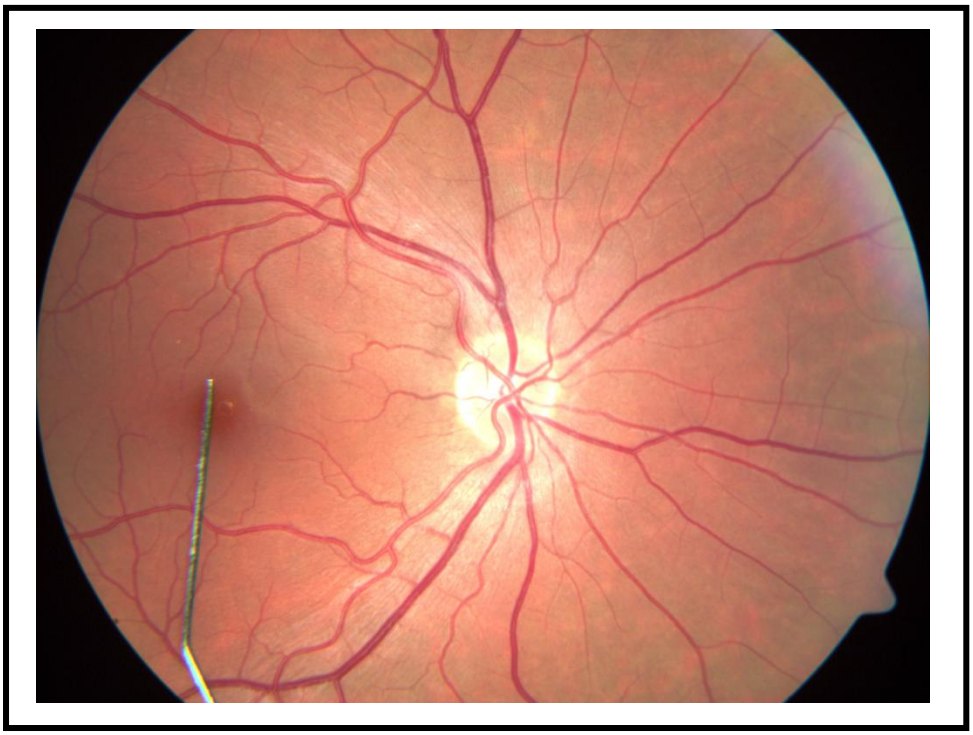

Figure 3: Colour image, to illustrate the retinal blood vessels, with the subject focusing on the tip of the fixation bar within the retinal camera.

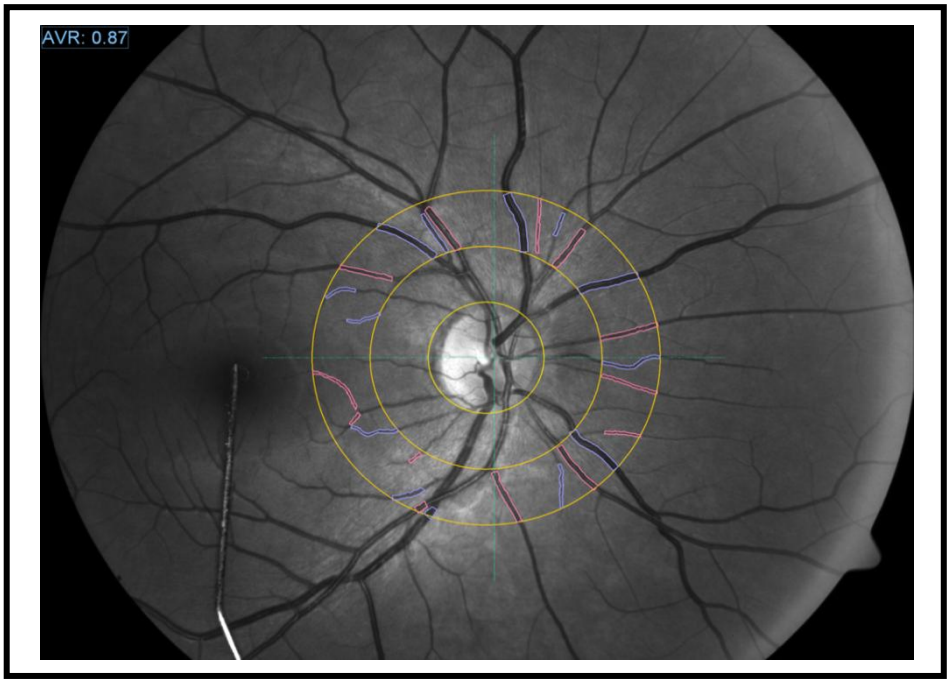

Figure 4: A monochrome image, where selection is made of an arteriole (red) and venule (blue) segment between one half and two disk diameters from the optic disk margin to begin the examination.

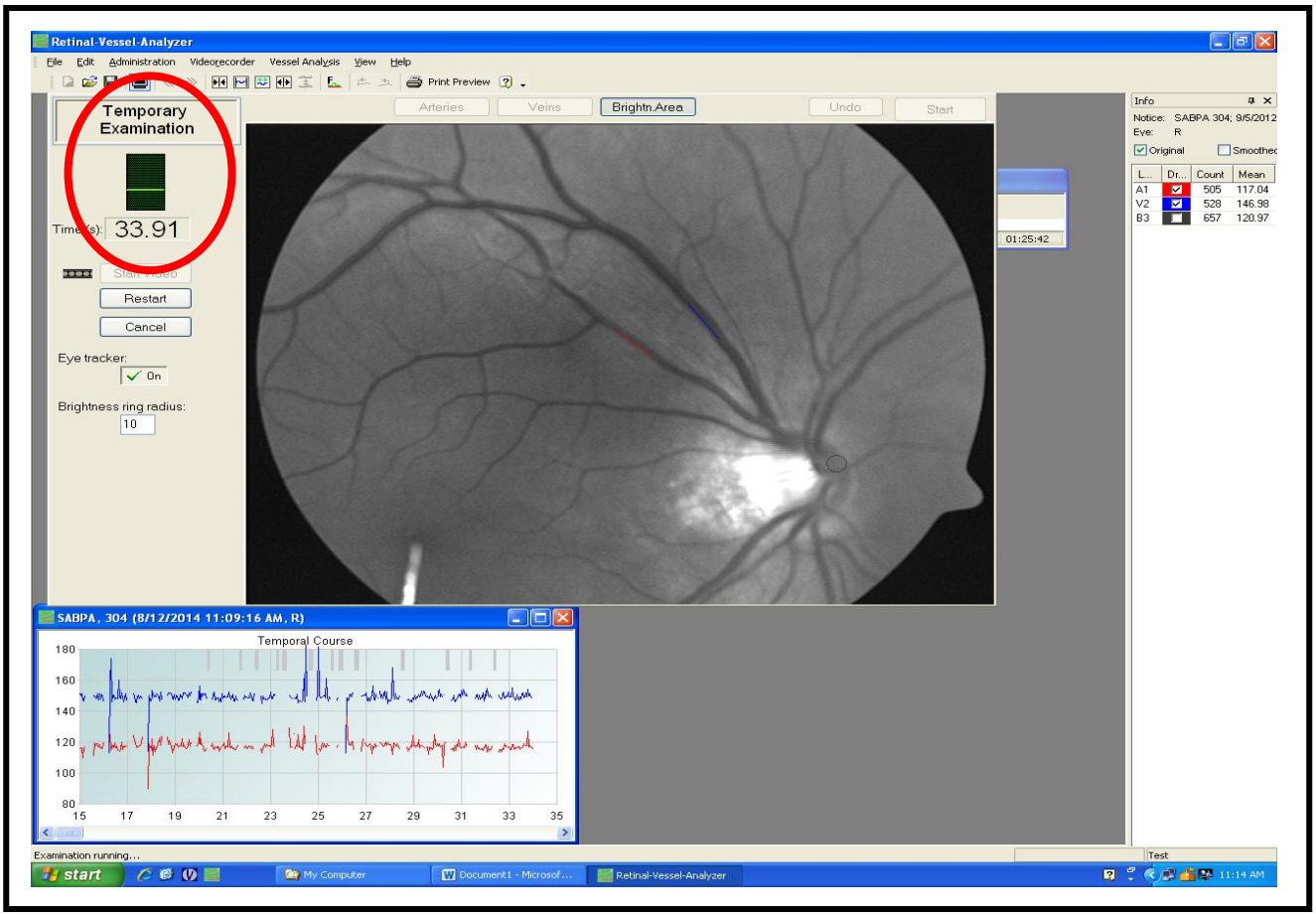

Figure 5: Illustration of the Temporary examination, to indicate the amount of light.

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CHAPTER 4
Manuscript

INSTRUCTIONS FOR AUTHORS

Journal of Hypertension

Title Page

The title page should display:

- Full title of the paper, no more than 20 words.
- A brief short title, which will be used as running head (consisting of not more than 40 characters, including spaces)
- All authors' names: the full first name, middle initial(s) and last (family name, in capital letters) name of each author should appear.
- Disclose funding received for this work from any of the following organizations.
- A statement on potential conflicts of interest.
- Word count: list full word count (including references, but not tables and legends)
- Number of tables
- Number of figures
- Number of supplementary file

Abstract

A structured abstract of no more than 250 words. The abstract should state the Objective(s), basic Methods, main Results and the principal Conclusions.

Condensed abstract

No more than 100 words and should briefly summarise main findings.

Key Words

The abstract should be followed by a list of 3–10 keywords.

Abbreviations and symbols

Use only standard abbreviations. Avoid abbreviations in the title and abstract.

Text

Full papers may be divided into sections headed Introduction, Methods (including ethical and statistical information), Results and Discussion (including a conclusion), although reviews may require a different format.

Acknowledgements

Acknowledgements should be made only to those who have made a substantial contribution to the study.

References

References should be numbered in the order in which they appear in the text. They should be assigned Arabic numerals, which should be given in brackets, e.g. [17].

Articles in journals

Zhou M-S, Schulman IH, Raij L. Vascular inflammation, insulin resistance, and endothelial dysfunction in salt-sensitive hypertension: role of nuclear factor kappa B activation. J Hypertension 2010; 28:527–535

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Grassi G, Vailati S, Bertinieri G, Seravalle G, Stella ML, Dell'Oro R, et al. Heart rate as a marker of sympathetic activity. J Hypertens 1998; 16:1635–1639.

Supplements:

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Hostetter TH, Kren S, Ibrahim HN. Mineralocorticoid receptor blockade in the remnant kidney model [Abstract]. J Am Soc Nephrol 1999; 10:85A.

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Chapter in a book:

Wakhloo AK. Carotid artery revascularization. In: Kandarpa K (editor). Peripheral Vascular Interventions. Philadelphia: Lippincott Williams & Wilkins; 2008. pp. 137–153.

Retinal microvascular calibre and blood pressure associated target organ damage: a bi-ethnic investigation within the SABPA study

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Hypertension in Africa Research Team (HART)
North-West University; Potchefstroom; South Africa

Short title: Retinal vessel calibre and organ damage

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Conflict of interest: All authors declare no conflict of interest

Abstract word count: 250

Number of tables: 4

Number of figures: 0

Supplementary tables: 5

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ABSTRACT

Objectives: Controversial findings concerning the link between the microvasculature and blood pressure associated target organ damage (TOD) exist. This is important for South Africa, where blacks have a high hypertension prevalence. We determined whether measurements of TOD are associated with retinal vessel calibre and light flicker-induced retinal calibre changes, and explored whether these relationships differ among black and white South Africans.

Methods: We included 156 black and 179 white school teachers (23–68 years). Ambulatory blood pressure, retinal vessel calibre and retinal vascular responses to light-flicker provocation were measured. We measured Carotid intima-media thickness (cIMT), ECG derived Cornell product, and estimated glomerular filtration rate (eGFR).

Results: A narrower central retinal artery equivalent (CRAE), smaller arteriolar-to-venular ratio (AVR), and wider central retinal vein equivalent (CRVE) were independently associated with SBP, whereas peak arteriolar dilation associated negatively with SBP only in black hypertensives (β=-0.12 ± 0.06; p=0.04). No independent associations were present between retinal vessel calibre and TOD measures. However eGFR associated positively with peak arteriolar dilation in black men (β=0.29 ± 0.12; p=0.02) and women (β=0.29 ± 0.12; p=0.01), and black hypertensives (β=0.32 ± 0.12; p=0.007); and also with venular dilation in white hypertensives (β=0.24 ± 0.10; p=0.03). cIMTf associated negatively with peak arteriolar dilation in black hypertensives (β=-0.22 ± 0.10; p=0.03).

Conclusion: Overall, static retinal vessel calibre was associated with BP but not with TOD. However, the functional response of retinal arteriolar calibre to a light flicker stimulus was independently associated with renal function and cIMTf, predominantly in blacks.

Keywords: retinal vessel calibre, flicker induced retinal vessel dilation, ambulatory blood pressure, target organ damage

Condensed Abstract:

The use of retinal imaging allows non-invasive observation of the microcirculation. In this regard, we investigated the association between blood pressure-related TOD (Cornell product, carotis intima media thickness (CIMT) and estimated glomerular filtration rate (eGFR)) with static and dynamic retinal calibre measurements in black and white participants. Overall, static retinal vessel calibre was associated with blood pressure but not with TOD. However, the functional response of retinal arteriolar calibre to a light flicker stimulus was positively and independently associated with renal function (eGFR), as well as negatively associated with CIMTf, predominantly in blacks.

INTRODUCTION

Examination of the retinal vascular calibre, by means of retinal imaging, allows for the non-invasive determination of detailed retinal vascular characteristics [1-3]. Moreover, it has become a popular and reliable method to investigate insights into early microvascular effects before the onset of hypertension, which may help in the development of new treatment strategies targeted at the microcirculation [4]. Increasing evidence exist, albeit indirect, that structural changes in retinal arteries may resemble those in small resistance arteries, and also that systemic BP influences vascular remodelling in retinal arterioles in a similar manner to that in peripheral arteries [5, 6].

Besides contributing to markers of TOD, such as left ventricular hypertrophy (LVH) [7, 8], carotid intima-media thickness (cIMT) [9, 10] and decreased glomerular filtration rate [10], hypertension is known to have profound effects on both the structure and function of the microvasculature [11], as a result, changes in retinal vessel calibre strongly associate with both the presence and severity of hypertension [12-16]. Moreover, studies have shown that alterations in retinal vessel calibre are associated with systemic inflammation [3], endothelial dysfunction [17, 18], hyperglycemia [19], diabetes mellitus [20, 21], obesity [22], hypertension [3], and target organ damage (TOD) [23-30].

Changes in retinal vessel calibre have been linked to markers of TOD, such as LVH [25, 31], vascular remodelling (increased cIMT) and nephropathy, seen in hypertension [1, 27, 32-34]. However, findings regarding retinal vessel calibre and TOD remain limited and inconsistent [24-31, 35]. In addition the association between dynamic retinal vessel calibre changes in response to light-flicker provocation, and blood pressure associated target organ damage have not yet been established. Apart from retinal microvascular remodelling in terms of calibre changes as a consequence of hypertension, the response of the microvessels when stimulated, would increase our understanding of the vasodilatory capacity [36], dependent

on nitric oxide (NO) [37], which may reflect microvascular endothelial function [36]. Furthermore no information exists regarding these relationships between retinal vessel calibres and TOD in different ethnic groups suffering from a high prevalence of hypertension. Such is the case for the black population of South Africa [38], where the prevalence of hypertension is increasing [39], as well as their tendency to have a higher risk for developing TOD, such as LVH [40].

Therefore, taking the high prevalence of hypertension in black South Africans into consideration [39, 41-44] a better understanding on the microvascular changes accompanying hypertension and its related target organ damage is needed.

We therefore investigated the associations between BP-associated TOD with static and dynamic retinal vessel calibre measurements in black and white participants.

METHODS

Study design and participants:

Organisational procedure

We used data obtained from the follow-up phase of the SABPA (Sympathetic Activity and Ambulatory Blood Pressure in Africans) study, which started in 2008/2009 and was conducted in February to May of 2011 and 2012. The study had a successful follow-up with 87.8% of participants. A total of 173 black and 186 white school teachers (aged 23-68 ears) from the Dr Kenneth Kaunda Education district of the North West province in South Africa, took part in this phase of the study. The study complied with with all applicable requirements of the US and international regulations, in particular the Helsinki declaration of 1975 (as revised in 2008) for the investigation of human participants. Ethical clearance for the SABPA study was obtained from the Health research Ethics committee of the North-West University (NWU-00036-07-S6), and for this particular sub-study (NWU-00036-07-A6).

Details regarding the SABPA study protocol have been published elsewhere [45]. For our particular sub-study participants with epilepsy, missing retinal vessel calibre-, carotid intima-media thickness (cIMT), Cornell product and estimated glomerular filtration rate (eGFR) data were excluded. After the exclusion, a total of 334 participants (156 Black (80 men and 76 women) and 179 White (86 men and 93 women) were considered eligible for our sub-study.

Procedures and research method:

Questionnaires

Each participant completed a general health questionnaire which was used to determine lifestyle habits (such as physical activity, smoking and alcohol use) as well as medication use. Participants were classified as smokers based on the answers of the general health questionnaire. Because a large proportion of the participants had a cotinine value of 0ng/ml, we made use of self reported smoking values. We further improved the accuracy of the self reported smoking data by reclassifying non-smokers as smokers when their serum cotinine values were above 6ng/ml (ethnic specific cut point) [46].

Anthropometric measurements

Height, weight and waist circumference were measured using calibrated instruments (Invicta Stadiometer, IP 1465, Precision Health Scale, A&D Company, Tokyo, Japan; Holtain unstretchable flexible 7mm wide metal tape, Crosswell, Wales).

Biochemical measurements:

Fasting blood samples, fasting glucose, serum high density lipoprotein (HDL) cholesterol, C-reactive protein (CRP), gamma-glutamyl transferase (GGT), creatinine and triglyceride levels were obtained, using multiple analysers (Cobas Integra 400 plus, Roche, Basel, Switserland; Beckman and Coulter time-end method (Unicel, DXC 800, Germany)). The percentage of glycated haemoglobin (HbA1c) was determined from whole blood. HIV status was

determined with the First response HIV card Test 1-2.0 (PMC medical, India Pvt Ltd) and confirmed by means of the Pareekshak HIV triline test (UCB Pharma, India). Serum cotinine levels were determined with a homogeneous immunoassay (Automated Modular, Roche, Basel, Switzerland. Estimated glomerular filtration rate (eGFR) were calculated according to the MDRD formula [47]. All intra- and inter-coefficients of variation for assays were below 10%.

Retinal photography and measurement of retinal vascular calibre

Retinal photography was performed according to a standardised Dynamic retinal vessel (DVA) protocol with applicable software (RVA 4.10 software, Imedos Systems, Jena, Germany). Static and functional retinal measurements were taken using the DVA with a Zeiss Fundus Camera (FF-450 Plus).

For the dynamic measurement, two segments from each of an arteriole and venule branch were selected. The fundus camera was set at an angle of 30°. A light flicker stimulus was induced after a 50 second baseline phase, for 20 seconds followed by an 80 second recovery phase. The protocol was automatically repeated for a total of 3 cycles. Following the measurement, the software automatically generates a summary curve of the 3 cycles. From this curve, maximum flicker induced vessel (arteriolar and venular) dilation and maximum arteriolar constriction is manually determined. In the event that the summary curve did not accurately reflect the raw data, the endpoints were manually determined from the raw data curves. The percentage valid measurements during the dynamic measurement protocol were: blacks: 72% ± 13 (arterioles), 77% ± 10 (venules); Whites: 80% ± 9 (arterioles), 82% ± 8 (venules).

For the static measurement, a colour as well as monochrome image was captured. The monochrome image was then further analyzed using VesselMap2 software. The camera was set at an angle of 50°. Each retinal image then yields information regarding central

retinal arteriolar equivalent (CRAE), central retinal venular equivalent (CRVE) and the arteriolar-to-venular ratio (AVR). CRAE and CRVE represent equivalents summarizing the calibre of the central retinal artery and central retinal vein respectively. CRAE and CRVE were calculated with the Knudtson formula [48], where the 6 largest arterioles and venules in the measuring area were used to compute the equivalents.

All data analysis was performed by two trained researchers that were blinded to the health status of the participants.

Cardiovascular measurements:

Ambulatory blood pressure measurements (ABPM)

Ambulatory blood pressure measurements were obtained (Cardiotens CE120®, Meditech, Budapest, Hungary). In the SABPA II study, participants achieved the following average inflation rate in a 24h period: Blacks: 85% ± 9; and whites: 92% ± 8. Ambulatory blood pressure values exceeding 130/80 mmHg were classified as hypertensive [49].

Carotid intima-media thickness (cIMT)

cIMT was obtained using a SonoSite Micromaxx ultrasound system and a 6-13 MHz linear array transducer. Images from at least two optimal angles of the left and right common carotid artery were obtained. Following previous prescribed protocols [50], these segments were imaged and measured. A 10mm segment with good image quality was chosen for analysis. A program (Artery Measurement Systems (AMS) II v1.139 (Chalmers University of Technology, Gothenburg, Sweden)) automatically identifies the borders of the intima-media of the near and far wall, and the inner diameter of the vessel and calculates the CIMT and the diameter from around 100 discrete measurements through the 10mm segment. This automated analysis was capable of being manually corrected if not found appropriate on visual inspection. Far wall measurements were used for the purpose of this study.

Cornell product

An ECG was performed to determine Cornell product as an indirect marker of left ventricular mass ((RaVL + SV3) × QRS ≥ 244.0 mV.ms^4 for men and (RaVL + SV3 + 0.8 mV) × QRS ≥ 244.0 mV.ms^4 for women) [51]. A 12-lead ECG of 6 cardiac cycles was performed (NORAV PC-ECG 1200, Israel).

Statistical analyses:

The data were analyzed using Statistica v12.0 (Statsoft Inc., Tulsa, USA, 2010). Variables with non-Gaussian distributions, such as estimated glomerular filtration rate, waist circumference, body mass index (BMI), total energy expenditure (TEE), gamma glutamyl transferase (cGGT), C-reactive protein (CRP), high density lipoprotein (HDL), glucose, triglycerides, and total cholesterol-to-HDL ratio, were logarithmically transformed. Comparisons between groups were made using independent T-tests, where logged data was presented as geometric mean with 5th and 95th percentile intervals, and normal distributed values were presented as arithmetic mean and standard deviation. Chi-square tests were used to calculate and compare proportions between groups. Single, partial and forward stepwise multiple regression analysis were performed to test for independent associations between SBP, DVA parameters and TOD. We considered all the following variables for the multiple regression models: age, BMI, WC, BP medication, total cholesterol, total cholesterol:HDL, cGGT, glucose, smoking, CRP. However, covariates that were consistently associated with DVA parameters and TOD were included in the model and they were: age, waist circumference (WC), total cholesterol, systolic blood pressure (SBP), smoking and glucose. When working in the total study group or hypertensive black or white groups, ethnicity and/or sex were additionally included. CRAE and CRVE were included together in the multiple regression models as suggested by previous authors [52].

RESULTS

Interaction terms were introduced to test for the main effects of ethnicity and sex on the association between DVA parameters, SBP and TOD (Supplementary table S1). Only ethnicity emerged as having a significant interaction on the aforementioned relationships. We further stratified our ethnic groups by sex bacause of the significant influence of sex hormones on endothelial function [89, 90]. Therefore we divided our groups into sub-groups namely, black men and women, and white men and women.

Table 1 lists the characteristics of the study population. Overall, Black men and women presented higher ambulatory BP, CRVE and lower AVR, than their white counterparts (all $p<0.001$), with no ethnic differences regarding peak arteriolar or venular dilation and peak arteriolar constriction. Blacks also revealed higher values of Cornell product (tendency in men) and hypertension status, with higher eGRF values compared to whites. White men had higher cIMTf values than the blacks.

Partial correlations were performed to test for associations between retinal vessel calibre (both static and dynamic) measurements and TOD with SBP and DBP (Supplementary table S2), and also retinal vessel calibre with TOD (Supplementary table S3). This was followed up by forward stepwise multiple regression analysis (Table 2), to test for independent associations. SBP was chosen as it was more significantly associated with retinal vessel and TOD measurements than DBP in partial correlation analysis (Supplementary table S2).

Regarding static retinal vessel calibre, CRAE and AVR were negatively, and CRVE was positively associated with SBP in all groups, with the exception of white men and hypertensives, where only AVR was significant (Table 2). No significant associations were found regarding dynamic retinal vessel calibre and SBP, with the exception of peak arteriolar dilation which was negatively associated with SBP in the total ($\beta=-0.12 \pm 0.06$; $p=0.04$) and hypertensive black group ($\beta=-0.19 \pm 0.09$; $p=0.03$), while peak venular dilation was negatively associated with SBP in hypertensive blacks alone ($\beta=-0.21 \pm 0.10$; $p=0.04$) (Table 2).

Regarding TOD, Cornell Product was positively associated with SBP in the total group (β=0.29 ± 0.001; p<0.001, white women (β=0.25 ± 0.10; p=0.012), black men (β=0.52 ± 0.10; p<0.001), and the black hypertensive group (β=0.32 ± 0.09; p<0.001). CIMTf displayed positive associations with SBP in the white women (β=0.20 ± 0.09; p=0.024) and the white hypertensive group (β=0.34 ± 0.00; p<0.001) (Table 2).

We then explored whether markers of TOD were associated with static or dynamic calibre measurements obtained with the DVA (Tables 3-4). We observed no associations between markers of TOD and CRAE and CRVE or AVR (Table 3), in both partial correlation analysis (with the exception of CRAE with eGFR (r=-0.26; p=0.033) (Table S3) and in forward stepwise multiple regression analysis (Table 3), irrespective of the group divisions. However, when exploring the associations between TOD and dynamic retinal vessel calibres in response to light-flicker provocation, eGFR was positively associated with peak arteriolar dilation in the total group (β=0.10 ± 0.04; p=0.008), black men (β=0.29 ± 0.12; p=0.02), black women (β=0.29 ± 0.12; p=0.01), black hypertensives (β=0.32 ± 0.12; p=0.007), as well as cIMTf that negatively associated with peak arteriolar dilation in the black hypertensives (β=-0.22 ± 0.10; p=0.03) (Table 4).

Venular dilation showed a positive association with eGFR only in the total white hypertensive group (Supplementary table S4). No association was found between arterial constriction and TOD (Supplementary table S5).

A number of sensitivity analyses were performed to test the validity of our results. When we additionally added cGGT to the model, the significant results obtained remained robust. We further excluded HIV positive subjects. In this analysis, only the association between cIMTf and arteriolar dilation in the black hypertensives became non-significant (results not shown).

Table 1: Characteristics of black and white men and women

	MEN			WOMEN		
	WHITE	**BLACK**	***p***	**WHITE**	**BLACK**	***p***
N	86	80		93	76	
Age (years)	49.0 ± 10.0	46.0 ± 7.29	0.013	45.0 ± 9.67	49.0 ± 7.78	0.47
ANTHROPOMETRICAL MEASUREMENTS						
BMI (kg/m²)	29.8 (24.2; 39.7)	27.7 (20.43; 38.35)	**0.0112**	26.6 (20.29; 40.28)	31.9 (21.7; 43.1)	**<0.001**
Waist circumference (cm)	105.3 (20.27; 129.5)	97.3 (78.33; 125.85)	**0.0002**	85.6 (67.9; 120.27)	95.0 (69.7; 120.2)	**<0.001**
BIOCHEMICAL MEASUREMENTS						
Glucose (mmol/l)	4.50 (3.10; 6.75)	5.63 (4.50; 10.44)	**<0.001**	4.07 (2.94; 5.13)	5.08 (4.25; 7.71)	**<0.001**
Triglycerides (mmol/l)	1.18 (0.62; 2.80)	1.47 (0.69; 3.61)	**0.004**	0.83 (0.38; 2.22)	0.93 (0.43; 2.10)	0.14
CRP (mg/l)	1.16 (0.17; 7.05)	2.81 (0.30; 27.76)	**<0.001**	1.15 (0.14; 10.19)	4.72 (0.78; 17.7)	**<0.001**
Cholesterol (mmol/l)	4.17 ± 1.01	4.62 ± 1.03	**0.005**	-0.03 ± 1.09	4.42 ± 0.97	0.97
HDL cholesterol (mmol/l)	0.82 (0.55; 1.28)	0.88 (0.53; 1.53)	0.14	1.22 (0.8; 2.07)	1.03 (0.71; 1.61)	**<0.001**
Total chol:HDL	4.95 (3.04; 6.80)	5.15 (2.72; 8.83)	0.42	3.53 (2.34; 6.15)	4.18 (2.60; 6.20)	**<0.001**
TARGET ORGAN DAMAGE MEASUREMENTS						
Cornell product (mV.ms)	75.9 ± 31.78	86.9 ± 46.18	**0.07**	43.6 ± 30.3	55.0 ± 34. 0	**0.02**
CIMTf (mm)	0.75 ± 0.16	0.69 ± 0.11	**0.01**	0.69 ± 0.11	0.66 ± 0.12	0.20
eGFR (ml/min/1.73m²)	62.55 ± 129.28	123.4 ± 327.8	**<0.001**	62.84 ± 134.59	135.9 ± 403.9	**<0.001**
CARDIOVASCULAR MEASUREMENTS						
Ambulatory 24h SBP (mmHg)	128 ± 10	137 ± 16	**<0.001**	119 ± 11	131 ± 17	**<0.001**
Ambulatory 24h DBP (mmHg)	79 ± 7	87 ± 10	**<0.001**	72 ± 6.99	79 ± 10.4	**<0.001**
RETINAL VESSEL MEASUREMENTS						
CRAE (MU)	150.6 ± 11.93	148.1 ± 13.2	0.24	151.7 ± 11.1	151.7 ± 12.6	0.97
CRVE (MU)	237.9 ± 18.72	251. 8 ± 19.4	**<0.001**	235.5 ± 17.8	246.5 ± 21.1	**<0.001**
AVR	0.63 ± 0.04	0.59 ± 0.06	**<0.001**	0.65 ± 0.04	0.62 ± 0.06	**<0.001**
Arterial dilation (%)	3.85 ± 1.99	4.43 ± 2.63	0.12	4.11 ± 2.12	4.33 ± 2.50	0.56
Arterial constriction (%)	-1.93 ± 1.36	-1.71 ± 1.43	0.36	02.0 ± 1.47	-1.85 ± 1.43	0.36
Venular dilation (%)	4.22 ± 2.05	4.95 ± 1.75	0.20	4.86 ± 2.10	5.43 ± 2.69	0.14
LIFESTYLE MEASUREMENTS						
Hypertensive status n (%)	49 (56.98%)	64 (80.0%)	**0.002**	31 (33.3%)	46 (60.5%)	**<0.001**
HIV infected, n (%)	0 (0.00%)	12 (15%)	**<0.001**	0 (0.00%)	7 (9.33%)	**0.003**
Smoking, n (%)	14 (16.28)	27 (33.75%)	**0.009**	10 (10.7%)	10 (13.1%)	0.60
Alcohol intake, n(%)	52 (60.47%)	55 (68.75%)	0.27	22 (28.9%)	28 (30.1%)	0.87
cGGT (U/l)	26.7 (9.90; 97.30)	56.5 (19.30; 208.7)	0.34	14.29 (6.0; 39.6)	26.7 (10.8; 130.7)	0.40
TEE (kCal)	3762.82 (2398.57; 7382.14)	3240 (1971.14; 6102.33)	**0.009**	2743.8 (1736.43; 4124.43)	2930.6 (1887.1; 5070.6)	0.14
Anti-hypertensive medication, n(%)	24 (30.00%)	22 (25.5%)	0.53	27 (35.5%)	14 (15.1%)	**0.002**

Values are arithmetic mean ± SD, geometric mean (5^{th} to 95^{th} percentile interval) or n (%); BMI, body mass index; CRP, C-reactive protein; HDL, high density lipoprotein; SBP, systolic blood pressure; DBP, diastolic blood pressure; CIMTf, far wall carotid intima-media thicknes; eGFR, estimated glomerulur filtration rate; CRAE, central retinal artery equivalent; CRVE, central retinal vein equivalent; AVR, arteriolar-to-venular ratio; HIV, human immunodeficiency virus; cGGT, gamma glutamyl transferase; TEE, total energy expenditure.

Table 2: Independent associations between retinal vessel calibre measures and markers of target organ damage with 24hr SBP

	STATIC RETINAL CALIBRE			DYNAMIC RETINAL CALIBRE			TARGET ORGAN DAMAGE MEASURES		
	CRAE	**CRVE**	**AVR**	**ARTERIAL DILATION**	**VENULAR DILATION**	**ARTERIAL CONSTRICTION**	**CORNELL PRODUCT**	**CIMTf**	**eGFR**
	β ± SE	**β ± SE**	**β ± SE**	**β ± SE**	**β ± SE**	**β ± SE**	**β ± SE**	**β ± SE**	**β ± SE**
Total * (N303-332)									
R^2	0.36	0.39	0.28	0.10	0.04	0.11	0.25	0.26	0.59
SBP (mmHg)	**-0.33 ± 0.05 p<0.001**	**0.26 ± 0.05 p<0.001**	**-0.35 ± 0.06 p<0.001**	**-0.12 ± 0.06 p=0.04**	-0.10 ± 0.07 p=0.14	----	**0.29 ± 0.001 p<0.001**	0.06 ± 0.05 p=0.23	0.08 ± 0.04 p=0.06
White women (N=88-93)									
R^2	0.43	0.47	0.19	0.06	----	0.19	0.10	0.35	0.13
SBP (mmHg)	**-0.33 ± 0.08 p<0.001**	**0.35 ± 0.08 p<0.001**	**-0.45 ± 0.10 p<0.001**	**----**	**----**	**----**	**0.25 ± 0.10 p=0.012**	**0.20 ± 0.09 p=0.024**	----
White men (N=82-86)									
R^2	0.54	0.51	0.11	0.10	0.04	0.12	0.04	0.29	0.28
SBP (mmHg)	-0.15 ± 0.08 p=0.052	----	**-0.15 ± 0.11 p= 0.004**	----	----	----	----	0.10 ± 0.09 p=0.27	0.11 ± 0,10 p=0.26
Black women (N=61-73)									
R^2	0.32	0.22	0.13	0.16	0.01	0.10	0.16	0.32	0.06
SBP (mmHg)	**-0.40 ± 0.12 p=0.002**	**0.26 ± 0.12 p=0.03**	**-0.35 ± 0.12 p=0.006**	----	-0.16 ± 0.13 p=0.21	----	----	----	----
Black men (N=72-80)									
R^2	0.21	0.14	0.19	0.18	0.06	0.02	0.26	0.06	0.04
SBP (mmHg)	**-0.40 ± 0.11 p<0.001**	**0.14 ± 0.32 p=0.008**	**-0.42 ± 0.11 p<0.001**	-0.19 ± 0.11 p=0.09	-0.18 ± 0.12 p=0.12	----	**0.52 ± 0.10 p<0.001**	----	0.21 ± 0.12 p=0.08
Hypertensives:									
Total (black) (N=93-108)**									
R^2	0.20	0.13	0.11	0.29	0.04	0.06	0.23	0.12	0.04
SBP (mmHg)	**-0.24 ± 0.10 p=0.02**	0.17 ± 0.10 p=0.10	**-0.24 ± 0.10 p=0.02**	**-0.19 ± 0.09 p=0.03**	**-0.21 ± 0.10 p=0.04**	----	**0.32 ± 0.09 p<0.001**	----	0.14 ± 0.10 p=0.15
Total (white) (N=76-80)**									
R^2	0.43	0.41	0.07	0.01	0.06	0.15	0.16	0.33	0.14
SBP (mmHg)	**-0.22 ± 0.09 p=0.01**	0.17 ± 0.09 p=0.07	**-0.23 ± 0.11 p=0.04**	----	----	----	----	**0.34 ± 0.09 p<0.001**	0.16 ± 0.11 p=0.14

Values include β, Beta, SEM (Standard error mean) and p-value ; Independent variables included in the model; age, waist circumference (WC), total cholesterol, systolic blood pressure (SBP), smoking and glucose. *Model additionally included ethnicity and sex.;** Additionally adjusted for sex. CRAE, central retinal artery equivalent; CRVE, central retinal vein equivalent; AVR, arteriolar-to-venular ratio; CIMTf, carotid intima-media thickness; eGFR, estimated glomerular filtration rate. ----, variable did not enter the model.

Table 3: Independent associations between markers of TOD and CRAE, CRVE or AVR

	CORNELL PRODUCT	CIMTf	eGFR
CRAE and CRVE	β ± SE	β ± SE	β ± SE
Total*	**(N=303)**	**(N=303)**	**(N=303)**
R^2	0.26	0.28	0.60
	----	----	----
White women:	**(N=88)**	**(N=88)**	**(N=88)**
R^2	0.12	0.36	0.13
Static variables:			
CRVE	**----**	0.11 ± 0.09 p=0.22	**----**
White men:	**(N=82)**	**(N=82)**	**(N=82)**
R^2	0.04	0.28	0.31
Static variables:			
CRAE	**----**	-0.14 ± 0.10 p=0.15	**----**
Black women:	**(N=61)**	**(N=61)**	**(N=61)**
R^2	0.11	0.46	0.08
Static variables:			
CRVE	**-----**	**----**	0.20 ± 0.12 p=0.11
Black men:	**(N=72)**	**(N=72)**	**(N=72)**
R^2	0.24	0.07	0.14
Static variables:			
CRVE	-0.13 ± 0.11 p=0.21	**----**	-0.21 ± 0.12 p=0.07
CRAE	**----**	**----**	**----**
Hypertensives:			
Total (black)**	**(N=93)**	**(N=93)**	**(N=93)**
R^2	0.14	0.32	0.20
Static variables:			
CRVE	-0.17 ± 0.11 p=0.13	**----**	**----**
Total (white)**	**(N=72)**	**(N=72)**	**(N=72)**
R^2	0.14	0.32	0.20
Static variables:			
CRVE	-0.17 ± 0.11 p=0.13	**----**	**----**
AVR	β ± SE	β ± SE	β ± SE
Total*	**(N=303)**	**(N=303)**	**(N=303)**
R^2	0.26	0.28	0.60
	----	----	----
White women:	**(N=88)**	**(N=88)**	**(N=88)**
R^2	0.12	0.36	0.13
	----	----	----
White men:	**(N=82)**	**(N=82)**	**(N=82)**
R^2	0.05	0.28	0.31
Static variables:			
AVR	0.15 ± 0.12 p=0.19	----	----
Black women:	**(N=61)**	**(N=61)**	**(N=61)**
R^2	0.11	0.46	0.05
	----	----	----
Black men:	**(N=72)**	**(N=72)**	**(N=72)**
R^2	0.24	0.07	0.05
Static variables:			
AVR	----	----	----
Hypertensives:			
Total (black)**	**(N=93)**	**(N=93)**	**(N=93)**
R^2	0.27	0.15	0.05
Static variables:			
AVR	----	-0.15 ± 0.10 p=0.13	----
Total (white)**	**(N=76)**	**(N=76)**	**(N=76)**
R^2	0.13	0.32	0.20
	----	----	----

Values include β, Beta, SEM (Standard error mean) and p-value ; *Model additionally included ethnicity and sex. ;** Additionally adjusted for sex. CRAE, central retinal artery equivalent; CRVE, central retinal veinquivalent; AVR, arteriolar-to-venular ratio; CIMTf, carotid intima-media thickness; eGFR, estimated glomerular filtration rate. ----, Variable did not enter the model. Covariates include age, waist circumference, total cholesterol, systolic blood pressure, smoking and glucose.

Table 4: Independent associations between markers of TOD and arterial dilation

	CORNELL PRODUCT	CIMTf	eGFR
Arterial dilation	β= ± SE=	β= ± SE=	β= ± SE=
Total*	**(N=305)**	**(N=304)**	**(N=305)**
R^2	0.27	0.24	0.60
Dynamic variables:			
Arterial dilation	-0.06 ± 0.05 p=0.20	-0.05 ± 0.05 p=0.30	**0.10 ± 0.04 p=0.008**
White women:	**(N=90)**	**(N=90)**	**(N=90)**
R^2	0.11	0.35	0.13
Dynamic variables:			
Arterial dilation	----	-0.13 ± 0.09 p=0.16	----
White men:	**(N=80)**	**(N=80)**	**(N=80)**
R^2	0.04	0.28	0.31
Dynamic variables:			
Arterial dilation	----	----	0.15 ± 0.09 p=0.12
Black women:	**(N=62)**	**(N=61)**	**(N=62)**
R^2	0.24	0.34	0.17
Dynamic variables:			
Arterial dilation	**----**	-0.16 ± 0.12 p=0.18	**0.29 ± 0.12 p=0.01**
Black men:	**(N=73)**	**(N=73)**	**(N=73)**
R^2	**0.29**	**0.05**	**0.07**
Dynamic variables:			
Arterial dilation	----	----	**0.29 ± 0.12 p=0.02**
Hypertensives:			
Total (black)**	**(N=96)**	**(N=96)**	**(N=96)**
R^2	0.24	0.12	0.10
Dynamic variables:			
Arterial dilation	----	**-0.22 ± 0.10 p=0.03**	**0.32 ± 0.12 p=0.007**
Total (white)**	**(N=78)**	**(N=78)**	**(N=78)**
R^2	0.15	0.32	0.14
Dynamic variables:			
Arterial dilation	----	----	----

Values include β, Beta, SEM (Standard error mean) and p-value; *Model additionally included ethnicity and sex. ** Additionally adjusted for sex. ; CIMTf, carotid intima-media thickness; eGFR, estimated glomerular filtration rate. Covariates include age, waist circumference, total cholesterol, systolic blood pressure, smoking and glucose. ----, Variable did not enter the model.

DISCUSSION

We found that retinal vascular calibre (static measurement) was independently related to SBP, but not with markers of TOD. Nevertheless, the retinal vessels' functional response to light flicker stimulation was independently and positively associated with kidney function, specifically in black subjects. In addition, peak arteriolar dilation was negatively associated with cIMTf in black hypertensives.

The correlation found between retinal vessel calibre and BP further confirms findings from other clinical studies that have established links between retinal vascular calibre and BP [12-16]. We found that CRAE and AVR were negatively, and CRVE positively associated with SBP in most groups. This finding is consistent with studies suggesting that retinal arteriolar narrowing predicts the incidence of hypertension [53-55].

It is thought that changes in the retinal vascular calibre may be indicative of the systemic burden of elevated BP on the microvasculature [12-16, 56]. Changes observed in the retinal vascular calibre may therefore reflect the damage of HT not only in the vascular system, but also in other target organs [57]. Various studies have reported an association between static retinal vessel calibres and TOD. For example, generalised retinal arteriolar narrowing and/or AVR were related to increased carotid intima-thickness and stiffness [15], renal disease progression [58], and LVH [29, 31, 59]. However, this relationship remains limited and inconsistent [24-31]. In this regard, we initially expected to find associations between static measures and TOD, however, our results showed no independent associations between markers of TOD and static measures (CRAE and CRVE or AVR). A possible explanation may be that in our study measurements were taken from a general population, without specific advanced established diseases, and consequently the TOD markers measured in this study were subclinical, in contrast to other studies where clinically relevant TOD was present [29, 31, 59] or where subjects were elderly, such as the Rotterdam study [15] and Cardiovascular Health study [58]. In this regard, analysis of the retinal vascular calibre may

provide insight into early microvascular changes prior to the onset of clinical hypertension, and may help in the development of new treatment strategies targeted at the microcirculation [4].

To the best of our knowledge our study is the first to investigate the relationship between dynamic retinal vessel calibre changes in response to a light-flicker provocation and TOD. As such, there are no other studies to compare our findings with. Of note, we found associations between retinal vessel calibre changes, in response to a light-flicker provocation, and certain measures of TOD.

In particular, we found that peak arteriolar dilation was positively associated with eGFR in the total group, black men and women, and black hypertensives. Also, a positive association between eGFR was found with venular dilation in white hypertensives. Although the link between eGFR and the retinal calibre changes following flickering light provocation, have not previously been investigated, a link between static retinal vessel calibre measures and renal function has been observed. Daien *et al.* [28] found that, overall, CRAE and CRVE were positively correlated with glomerular filtration rate. Many other links have also been reported between static measures and renal function, such as smaller CRAE and higher risk of developing chronic kidney disease (CKD) [60-62]. It is also known that in patients with renal failure CRAE and CRVE were found to progressively decrease with the progression of CKD [63]. However, we found no association between eGFR and static measures. The observation of a relationship between peak arteriolar dilation and eGFR in the black groups where the average eGFR of the group was in the normal range suggests that the dynamic DVA measures may potentially provide prognostic information on kidney function in a hypertension prone group.

Our finding that a higher eGFR relates positively to peak retinal arteriolar dilation particularly in the black participants should be viewed in light of the elevated glomerular filtration rate

observed in our black vs. white groups. Black populations are prone to be salt sensitive [64], and hypertensive blacks are 3-5 times more likely to develop kidney diseases than whites [65]. As a consequence, renal hyperfiltration is a common occurrence which may be a response to increase sodium excretion and reduce blood pressure [66, 67]. Our finding in the black group may reflect that the microvasculature of the nephron and retina both respond actively by dilating in response to stress – observed by the hyperfiltration in the microvasculature of the nephrons corresponding with the retinal arteriolar dilation due to light flicker provocation. Prolonged hyperfiltration, although initially beneficial, may contribute to the development of deterioration of the kidneys, since it will result in long term damage to the glomeruli of the remaining nephrons, and eventually result renal failure [68, 69]. In blacks, their increased intake of salt may partly explain their high prevalence of hypertension [70]. In addition, studies in essential hypertensive patients suggest that both high salt intake and salt sensitivity are associated with impaired endothelial function [71]. Both HT, as well as salt-sensitive patients appear unable to up-regulate the production of nitric oxide (NO) in response to salt intake [71]. Recent results support the hypothesis that salt-sensitive hypertensive patients have greater impairment of the endothelial vasodilatory capacity [71]. Therefore, the relationship we see may have clinical significance to identify black participants at risk for kidney disease, but more studies are needed to explore this relationship.

In a similar manner to the result with eGFR, we are the first to show an associated between cIMTf and peak arteriolar dilation, but only in black hypertensive subjects. While there are no studies to compare our results with, it is worth mentioning that the relationship between static retinal vessel calibre measures and cIMT are controversial at best. Furthermore the association between abnormalities in medium sized vessels such as carotid arteries and microvascular retinal changes are poorly investigated in hypertensive patients [27, 72]. Nevertheless our finding between peak arteriolar dilation and cIMT in black hypertensives may relate their common link with endothelial function. Although the results were

independent of BP, BP may still have been involved in this association, as the result were present in the hypertensive group, and therefore may be in an environment of chronically increased BP. It is however well-known that a strong correlation exists between cIMT and hypertension [73], as such chronically elevated BP will result in arterial narrowing as a consequence of autoregulatory processes that start from vasospasm, and is followed by chronic arteriosclerotic changes, such as intimal thickening, media-wall hyperplasia and hyaline degeneration [74]. Torres and co-workers suggest that hypertensive subjects may present similar pathological abnormalities in various vascular sites [75, 76]. Consequently this may result in endothelial dysfunction, which is known to be an early marker for the atherosclerotic process [77]. It can also be explained by nitric oxide (NO)-dependent endothelial dysfunction, which is a key feature of HT and may contribute to impaired endothelium-related vasodilation [78]. However more studies are needed to establish this relationship, especially since exclusion of HIV subjects resulted in the relationship falling away.

We speculate that static retinal vascular calibre may correlate well with SBP, but its relationship with TOD may only manifest when TOD is at a more advanced stage. Dynamic retinal vascular calibre may be more sensitive to subclinical TOD because markers such as eGFR [81, 82] and cIMT [83, 84] are related to endothelial dysfunction (which is what the dynamic response measures), which plays a key role in the early pathogenesis of vascular disease.

Limitations to this study may include the use of the Cornell product as an indirect marker of left ventricular mass [51], in the absence of echocardiography. Secondly, the population was generally healthy, and thus presented with subclinical TOD. Perhaps a population with more established CVD and resultant TOD could give more significant results. Lastly, since this is a cross-sectional study, cause and effect cannot be established. A possible follow-up of 5 years may help to better understand and evaluate these observations and changes.

Despite the abovementioned limitations, this study also includes certain strengths, such as the use of 24h ambulatory blood pressure [85-87], the bi-ethnic population, and finally the use of recently revised formulas (Knudtson formula) for the calculation of retinal vessel calibre [88], which is more robust against variability in the number of vessels observed, and independent of image scale, therefore being easier to implement.

The relationship regarding retinal vascular calibre and TOD remains controversial, and further studies are needed to establish this relationship. However, we support literature indicating that assessment of retinal calibre is a useful tool in identifying systemic abnormalities of the microcirculation.

In conclusion, measures of static retinal vessel calibre were associated with ambulatory BP, but not with TOD. eGFR (predominantly in the blacks) associated positively with peak retinal arteriolar dilation. Therefore, assessment of the retinal vessel calibres' functional response to light is independently associated with kidney function, predominantly in blacks**.** cIMTf was also associated with peak retinal arteriolar dilation, in response to a light flicker provocation, however only in black hypertensives, which may be indicative of endothelial function, possibly as a consequence of chronically elevated BP. However, longitudinal studies are needed to clarify these relationships.

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CONFLICT OF INTEREST

None declared.

Table S1: Interaction terms to test for the main effects of ethnicity and sex on the association between DVA parameters, SBP and TOD

	TARGET ORGAN DAMAGE MEASURES		
	Cornell product	**CIMTf**	**eGFR**
CRAE			
Ethnicity	----	----	----
Sex	----	----	----
CRVE			
Ethnicity	----	**β=-0.58; p=0.02**	----
Sex	----	----	----
AVR			
Ethnicity	----	----	----
Sex	----	----	----
Arteriolar dilation:			
Ethnicity	----	----	**β=0.11; p=0.01**
Sex	----	----	----
Venular dilation:			
Ethnicity	----	----	----
Sex	----	----	----
Arteriolar constriction			
Ethnicity	----	----	----
Sex	----	----	----

CIMTf, carotid intima-media thickness; eGFR, estimated glomerular filtration rate. Covariates include age, waist circumference, total cholesterol, systolic blood pressure, smoking and glucose. ----, Variable did not enter the model

Table S2: Partial correlation coefficients, for the relationship between retinal vessel calibre measures and target organ damage with 24hr SBP and DBP

	STATIC RETINAL CALIBRES			DYNAMIC RETINAL CALIBRES		TARGET ORGAN DAMAGE MEASURES		
Total group:*	**CRAE**	**CRVE**	**AVR**	**Arteriolar dilation**	**Venular dilation**	**Cornell product**	**CIMTf**	**eGFR**
24-h SBP	**r=-0.26; p<0.001**	r=0.07; p=0.20	**r=-0.32; p<0.001**	r=-0.09; p=0.11	r=-0.10; p=0;09	**r=0.25; p<0.001**	r=0.11; p=0.06	r=0.47; p=0.39
24-h DBP	**r=0-.29; p<0.001**	r=0.02; p=0.74	**r=-0.31; p<0.001**	r=-0.05; p=0.39	r=-0.05; p=0.42	**r=0.23; p<0.001**	r=0.03; p=0.58	r=0.07; p=0.18
White women:								
24-h SBP	r=-0.12; p=0.28	r=0.17; p=0.11	**r=-0.32; p=0.003**	r=-0.05; p=0.65	r=0.06; p=0.61	r=0.12; p=0.26	r=0.16; p=0.13	r=-0.003; p=0.98
24-h DBP	r=-0.13; p=0.22	r=0.12; p=0.26	**r=-0.30; p=0.01**	r=0.01; p=0.94	r=0.10; p=0.35	r=0.15; p=0.17	r=0.04; p=0.73	r=0.04; p=0.74
White men:								
24-h SBP	**r=0.22; p=0.048**	r=-0.14; p=0.21	r=-0.10; p=0.37	r=0.06; p=0.59	r=-0.04; p=0.74	r=0.02; p=0.87	r=0.15; p=0.16	r=0.15; p=0.19
24-h DBP	r=-0.11; p=0.34	r=-0.12; p=0.14	r=0.07; p=0.53	r=-0.02; p=0.86	r=-0.01; p=0.94	r=0.09 p=0.42	r=0.01; p=0.96	r=0.16; p=0.14
Black women:								
24-h SBP	**r=-0.34 p=0.01**	r=0.03; p=0.80	**r=-0.33; p=0.01**	r=-0.08; p=0.55	r=-0.11; p=0.38	r=0.12; p=0.31	r=0.21; p=0.07	r=0.04;p=74
24-h DBP	**r=-0.41; p<0.001**	r=-0.05; p=0.68	**r=-0.33; p=0.01**	r=-0.05; p=0.71	r=-0.04 p=0.76	r=0.19; p=0.10	r=0.14; p=0.23	r=-0.03; p=0.79
Black men:								
24-h SBP	**r=-0.30; p=0.01**	r=0.16; p=0.17	**r=-0.40; p<0.001**	r=-0.21; p=0.08	r=-0.19; p=0.11	**r=0.48; p<0.001**	r=0.01; p=0.95	r=0.19; p=0.09
24-h DBP	**r=-0.40; p<0.001**	r=0.12; p=0.31	**r=-0.46; p<0.001**	r=-0.11; p=0.35	r=-0.19; p=0.11	**r=0.38; p<0.001**	r=-0.03; p=0.80	r=0.10; p=0.10

Adjustments were made for age and waist circumference (WC). *Total group additionally adjusted for sex and ethnicity. CRAE, central retinal artery equivalent; CRVE, central retinal vein equivalent; AVR, arteriolar-to-venular ratio; CIMTf, carotid intima-media thickness; eGFR, estimated glomerular filtration rate. Covariates include age, waist circumference, total cholesterol, systolic blood pressure, smoking and glucose.

Table S3: Partial correlation coefficients for the relationship between retinal vessel calibre measures and target organ damage

	TARGET ORGAN DAMAGE VARIABLES		
Total group:*	**Cornell product**	**CIMTf**	**eGFR**
Static variables:			
CRAE	r=-0.06; p=0.32	r=-0.07; p=0.23	r=0.02; p=0.77
CRVE	r=-0.02; p=0.70	r=-0.01; p=0.83	r=0.03; p=0.61
AVR	r=-0.03; p=0.55	r=-0.06; p=0.33	r=-0.02; p=0.71
Dynamic variables:			
Arteriolar dilation	r=-0.08; p=0.17	r=-0.05; p=0.42	**r=0.13; p=0.02**
Venular dilation	r=0.02; p=0.73	r=0.02; p=0.76	r=-0.01; p=0.81
Arteriolar constriction	r=-0.02; p=0.69	r=0.05; p=0.37	r=-0.00; p= 0.86
White women:			
Static variables:			
CRAE	r=-0.03; p=0.77	r=0.05; p=0.62	r=0.04; p=0.72
CRVE	r=0.07; p=0.50	r=0.14; p=0.19	r=0.09; p=0.42
AVR	r=-0.12; p=0.27	r=-0.10; p=0.35	r=-0.07; p=0.52
Dynamic variables:			
Arteriolar dilation	r=-0.09; p=0.42	r=-0.17; p=0.12	r=-0.01; p=0.91
Venular dilation	r=0.08; p0.45	r=0.05; p=0.62	r=0.16; p=0.13
Arteriolar constriction	r=-0.11; p=0.30	r=0.12; p=0.27	r=0.18; p=0.09
White men:			
Static variables:			
CRAE	r=0.04; p=0.72	r=-0.16; p=0.15	r=0.13; p=0.25
CRVE	r=-0.07; p=0.56	r=-0.07; p=0.52	r=-0.01; p=0.96
AVR	r=0.13; p=0.25	r=-0.10; p=0.37	r=0.16; p=0.15
Dynamic variables:			
Arteriolar dilation	r=-0.01 p=0.93	r=0.02; p=0.86	r=0.05; p=0.70
Venular dilation	r=0.00; p=0.97	r=0.08; p=0.49	r=0.01; p=0.91
Arteriolar constriction	r=0.03; p=0.77	r=-0.16; p=0.15	r=0.17; p=0.13
Black women:			
Static variables:			
CRAE	r=-0.04; p=0.77	r=-0.03; p=0.80	r=0.13; p=0.32
CRVE	r=-0.06; p=0.67	r=-0.03; p=0.83	r=0.15; p=0.26
AVR	r=-0.02; p=0.90	r=-0.00; p=0.99	r=-0.003; p=0.98
Dynamic variables:			
Arteriolar dilation	r=0.14; p=0.26	r=-0.07; p=0.57	**r=0.26; p=0.04**
Venular dilation	r=0.12; p=0.33	r=-0.04; p=0.74	r=0.06; p=0.64
Arteriolar constriction	r=0.01; p=0.93	r=0.19; p=0.14	r=-0.20; p=0.11
Black men:			
Static variables:			
CRAE	r=-0.16; p=0.19	r=-0.07; p=0.58	**r=-0.26; p=0.033**
CRVE	r=-0.06; p=0.62	r=0.02; p=0.87	r=-0.22; p=0.07
AVR	r=-0.09; p=0.47	r=-0.07; p=0.57	r=-0.07; p=0.54
Dynamic variables			
Arteriolar dilation	r=-0.22; p=0.06	r=-0.12; p=0.34	r=0.22; p=0.06
Venular dilation	r=-0.08; p=0.53	r=-0.08; p=0.50	r=-0.14; p=0.24
Arteriolar constriction	r=-0.03; p=0.80	r=0.10; p=0.39	r=-0.01; p=0.92

Adjusted for age and waist circumference (WC). *Total group additionally adjusted for sex and ethnicity. CRAE, central retinal artery equivalent; CRVE, central retinal vein equivalent; AVR, arteriolar-to-venular ratio; CIMTf, carotid intima-media thickness; eGFR, estimated glomerular filtration rate. Covariates include age, waist circumference, total cholesterol, systolic blood pressure, smoking and glucose.

Table S4: Independent associations between measures of target organ damage and peak venular dilation

	CORNELL PRODUCT	CIMTf	eGFR
Venular dilation	β= ± SE=	β= ± SE=	β= ± SE=
Total*	**(N=305)**	**(N=304)**	**(N=305)**
R^2	0.27	0.24	0.59
Dynamic variables:			
Venular dilation	0.05 ± 0.05 p=0.30	----	----
White women	**(N=90)**	**(N=90)**	**(N=90)**
R^2	0.11	0.34	0.15
Dynamic variables:			
Venular dilation	----	----	β=0.16 ± SE=0.10 p=0.10
White men:	**(N=80)**	**(N=80)**	**(N=80)**
R^2	0.04	0.28	0.30
Dynamic variables:			
Venular dilation	----	β=0.12 ± SE=0.10 p=0.23	----
Black women:	**(N=62)**	**(N=61)**	**(N=62)**
R^2	0.25	0.33	0.10
Dynamic variables:			
Venular dilation	β=0.18 ± SE=0.11 p=0.11	----	----
Black men:	**(N=73)**	**(N=73)**	**(N=73)**
R^2	0.29	0.05	0.02
Dynamic variables:			
Venular dilation	----	----	β=-0.17 ± SE=0.12 p=0.17
Hypertensives:			
Total (black)**	**(N=96)**	**(N=96)**	**(N=96)**
R^2	0.25	0.10	0.04
Dynamic variables:			
Venular dilation	β=0.16 ± SE=0.09 p=0.08	β=-0.10 ± SE=0.10 p=0.32	----
Total (white)**	**(N=78)**	**(N=78)**	**(N=78)**
R^2	0.15	0.32	0.18
Dynamic variables:			
Venular dilation	----	----	**β=0.24 ± SE=0.10 p=0.03**

Values include β, Beta, SEM (Standard error mean) and p-value; *Model additionally included ethnicity and sex. ** Additionally adjusted for sex. CIMTf, carotid intima-media thickness; eGFR, estimated glomerular filtration rate. Covariates include age, waist circumference, total cholesterol, systolic blood pressure, smoking and glucose . ----, Variable did not enter the model.

Table S5: Independent associations between measures of target organ damage and peak arteriolar constriction

	CORNELL PRODUCT	CIMTf	eGFR
Arteriolar constriction			
Total*	**(N=305)**	**(N=304)**	**(N=305)**
R^2	0.27	0.24	0.59
Dynamic variables:			
Arteriolar constriction	**----**	β=0.06 ± SE=0.05 p=0.24	**----**
White women:	**(N=90)**	**(N=90)**	**(N=90)**
R^2	0.11	0.34	0.15
Dynamic variables:			
Arteriolar constriction	**----**	β=0.11 ± SE=0.10 p=0.27	β=0.20 ± SE=0.10 p=0.07
White men:	**(N=80)**	**(N=80)**	**(N=80)**
R^2	0.04	0.29	0.30
Dynamic variables:			
Arteriolar constriction	**----**	β=-0.14 ± SE=0.10 p=0.17	**----**
Black women:	**(N=62)**	**(N=61)**	**(N=62)**
R^2	0.24	0.34	0.12
Dynamic variables:			
Arteriolar constriction	**----**	β=0.18 ± SE=0.11 p=0.12	β=-0.20 ± SE=0.12 p=0.10
Black men:	**(N=73)**	**(N=73)**	**(N=73)**
R^2	0.29	0.06	0.00
Dynamic variables:			
Arteriolar constriction	**----**	β=0.14 ± SE=0.12 p=0.23	**----**
Hypertensive subjects:			
Total (black)**	**(N=96)**	**(N=96)**	**(N=96)**
R^2	0.24	0.11	0.06
Dynamic variables:			
Arteriolar constriction		β=0.12 ± SE=0.10 p=0.23	β=-0.17 ± SE=0.10 p=0.09
Total (white)**	**(N=78)**	**(N=78)**	**(N=78)**
R^2	0.15	0.32	0.15
Dynamic variables:			
Arteriolar constriction	**----**	**----**	β=0.15 ± SE=0.11 p=0.19

Values include β, Beta, SEM (Standard error mean) and p-value; *Model additionally included ethnicity and sex. ** Additionally adjusted for sex. CIMTf, carotid intima-media thickness; eGFR, estimated glomerular filtration rate. Covariates include age, waist circumference, total cholesterol, systolic blood pressure, smoking and glucose . ----, Variable did not enter the model.

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CHAPTER 5
Conclusion, recommendations and limitations

5.1 Introduction

This chapter is a summary of the main findings of this study, which will be discussed and compared to relevant literature. Strengths and limitations of this study will be mentioned, followed by recommendations for future studies.

5.2 Discussion of the main findings and comparison to the relevant literature

Hypothesis 1: Both static and dynamic retinal calibre measurements and TOD will display adverse associations with markers of BP-related TOD

We firstly aimed to determine whether TOD markers (Cornell product, cIMT, estimated GFR) were associated with retinal vessel calibre (CRAE, CRVE, AVR); and light flicker-induced retinal vessel calibre changes (arteriolar dilation, constriction and venular dilation). We found that there were no independent associations regarding retinal vessel calibre and TOD measures. We did however find that eGFR was positively associated with peak arteriolar dilation in in the total group (β=0.29 ± 0.12; p=0.008), black men (β=0.29 ± 0.12; p=0.02), black women (β=0.29 ± 0.12; p=0.01), and black hypertensives (β=0.32 ± 0.12; p=0.007), and venular dilation in white hypertensives (β=0.24 ± 0.10; p=0.03). cIMTf negatively associated with peak arteriolar dilation in black hypertensives (β=-0.22 ± 0.10; p=0.03). In this regard, we partially accept our hypotheses, in that we did not find associations between static retinal vessel calibre with TOD, but associations were present between dynamic retinal vessel calibre and TOD, specifically eGFR, predominantly in blacks, and cIMT in black hypertensives.

Hypothesis 2: The associations between retinal vessel calibre measurements and TOD would be more outspoken in black participants

Taking into consideration the increasing prevalence of HT [1] and risk for developing TOD (LVH [2] and kidney disease [3]) in blacks, it was expected that associations between retinal vessel calibre measurements would be more outspoken in black participants. We therefore

partially accept our hypothesis in that similarities, as well as differences were present between blacks and whites. In both ethnic groups it was found that static retinal vascular calibre did not associate with TOD. However, associations between retinal vasculature's dilatory capacity in response to light flicker stimulation were more prominent in blacks.

5.3 Summary

The relationship regarding retinal vessel calibre and TOD still remains unclear, since current available literature is controversial [4-11]. Further supporting this controversy, we only found associations with dynamic retinal calibre measures, and not with static retinal calibre measures, whereas other studies only found links with static measures and TOD [9, 10, 12-14]. Since this study is cross-sectional, cause and effect cannot be established. However, a possible reason that associations were only found with dynamic measures and not with static retinal vessel calibres may partly be that the dilatory response of retinal vessels to diffuse luminance flicker, is thought to reflect endothelial function [15-17], dependent on the release of nitric oxide synthase [18-20]. Dynamic retinal vascular calibre may be more sensitive to subclinical TOD because markers such as eGFR [21, 22] and cIMT [23, 24] are related to endothelial dysfunction (which is what the dynamic response measures), and play a key role in the early pathogenesis of vascular disease.

5.4 Strengths

Strengths of this study include the bi-ethnic and population-based design with participants being free of advanced forms of CVD. The study also made use of new and improved technology (Dynamic retinal vessel analyzer) to measure static retinal vessel calibres, and more importantly dynamic retinal vessel measures. Another strength may include 24h BP monitoring, which shows a better correlation to TOD and cardiovascular morbidity-mortality than office BP, thereby excluding the white coat effect [26-28]. Lastly, we made use of more recently modified formulas for summarising retinal vascular calibre that has been developed

by Knudtson *et al.* [29], which demonstrates a clear superiority of their formula over the previously used Parr-Hubbard formulas. The formula proposed by Knudtson *et al.* correlates highly with the previously used Parr-Hubbard formula but offers the advantage of being more robust against variability in the number of vessels observed, being independent of image scale, and being easier to implement [30].

5.5 Limitations of this study

Firstly, this study is not longitudinal. The cross-sectional nature prevents us to establish cause and effect. Perhaps a follow up of five years or more will help to better understand and evaluate the relationships explored in this study. The absence of echocardiography data can also be seen as a possible limitation. Lastly, the participants were not randomly selected, however, teachers were selected to ensure similar high socio economic status in black and white groups.

5.6 Limitations regarding the overall use of retinal imaging (DVA)

Although retinal image analysis provides exciting and valuable possibilities in terms of cardiovascular risk stratification, its applicability in the clinic setting is yet to be established, due to limitations in methodology. Existing retinal vessel calibre research mostly focused on differences in retinal vascular calibres between large population groups. Retinal image analysis should in future aim to produce results that will increase the ability to assess the absolute risk in *individuals*, to aid the use of retinal vascular calibre measurements as a potential clinical risk stratification tool. Current population-based studies have used retinal vessel calibre measurements obtained from one retinal image, but the retinal calibre may vary up to 15% depending on the moment in the cardiac cycle when the image was taken [31]. Further standardisation is needed to improve accuracy and reliability of these measurements. Finally, despite the multiple amounts of population-based studies there is a lack of information about normative data for this measurement [32]. Correct definitions of

what are normal and what is abnormal is crucial for a clinical tool development [32]. According to Ikram *et al* [32], in this regard, the challenge has however been that it is difficult to completely control for the confounding effect of systemic (e.g. HT, smoking, diabetes or medication) processes on retinal vascular measurements. Examining retinal vascular calibre in healthy children and adults provide a better understanding of the vascular function [33].

5.7 Recommendations

Possible recommendations are that future studies also include diagnosis of retinopathy as this condition may be a better indicator for TOD than just changes in retinal vascular calibre [34]. Secondly, as mentioned, longitudinal studies may provide a better understanding of the underlying mechanisms regarding retinal vascular calibre and its association with TOD within an individual. Detailed evaluation of the retinal microvasculature has the potential to identify novel retinal vascular parameters in relation to CVD - both local as well as global vascular topographic features. This includes branching angles of blood vessels, fractal dimension and retinal vessel tortuosity [35] which will indicate how optimally designed and developed the retinal microvascular system is, and also reflect the state of the systemic and brain microcirculation. Another recommendation may be to use wall: lumen ratio using scanning laser Doppler flowmetry [36], which is a better indicator of the type of remodelling of the vascular wall. Assessments of wall: lumen ratio of retinal arterioles emerged as an attractive tool to identify treated patients with hypertension with increased cerebrovascular risk [36]. Vascular remodelling of small and large vessels in arterial hypertension is known to be an early sign of atherosclerosis. It is particularly an increase in the wall thickness:lumen ratio diameter that develops and maintains hypertension by increasing vascular resistance [37].

5.8 Chance and confounding

It is important to highlight certain factors that may have had a possible influence on the results of this study. This may include the applied methodology, statistical analysis and meaning of results.

Regarding results, the possibility of chance should also be taken into account. There is a possibility that one out of twenty associations, from regression analysis, may be due to chance. Additionally, multiple adjustments were made for certain factors in the regression analyses.

Salt sensitivity is associated with HT as well as increased GFR, and since salt sensitivity is more common in blacks, it may have influenced our findings. We did not test for salt sensitivity and therefore could not include this as a covariate in our adjusted analyses.

Although our results were of statistical significance, it does not necessarily indicate physiological significance.

Since this study is only based on participants from the North West Province, it is not representative of the whole of South Africa.

5.9 Conclusion

In conclusion, an association exists between retinal vessel calibres and BP but not with TOD. To the best of our knowledge, our study is the first to present an association between dynamic retinal vessel calibre and TOD, in that we showed that the retinal vessels' functional response to light flicker provocation may be indicative of renal function in blacks. The association found between cIMTf with peak retinal arteriolar dilation, in response to a light flicker provocation that were found only in black hypertensive, may be indicative of endothelial dysfunction, possibly as a consequence of chronically elevated BP. Although previous studies have reported AVR as the better predictor of systemic hypertensive damage and life prognosis [14], we found that dynamic measurements may also be useful,

possibly due to its evaluation of endothelial function [38, 39]. We therefore support that the retina provides an ideal opportunity to assess the microvasculature non-invasively, which may develop to become a valuable clinical tool, also in the hypertension prone black population in South Africa. The use of retinal vascular analysis may be of great value to assess the retinal vasculature's static and functional capacity, with regards to microvascular abnormalities, such as hypertension development or manifestation, and renal function.

5.10 References

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6 October 2014

Dear Dr Smith

Ethics Application: NWU-00036-07-A6 "SABPA Study"

Your application to include the sub-study "Retinal microvascular calibre and blood pressure associated target organ damage: a bi-ethnic investigation within the SABPA study" under the umbrella project has been approved by the HREC until 31/12/2015.

Yours sincerely

Prof Minrie Greeff
Health Research Ethics Committee Chairperson

Original details: Prof Minrie Greeff(10187308) C:\Users\13210572\Documents\ETIEK\2007 ETHICS\NWU-00036-07-A6 (W Smith-C du Plessis) - Approval letter.docm
6 October 2014

File reference: 9.1.5.3

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10th June 2014

Dear **Me C du Plessis (22117644)**

Master degree (Curriculum G855P)

Promoter Dr W Smith

LETTER OF CONSENT

Permission is hereby granted for the following study: *Retinal microvascular calibre and blood pressure associated target organ damage: a bi-ethnic investigation within the SABPA-study* (falls within the scope of the **S**ympathetic activity and **A**mbulatory **B**lood **P**ressure in **A**fricans (SABPA) Prospective cohort study).

Success with your research

Yours sincerely

Prof L Malan (SABPA Study, Principal Investigator)

(RN, HED, PhD)

(ESH Working Group: Hypertension and the Brain)

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Dr L Malan

Dear Dr Malan

6 February 2008

ETHICS APPROVAL OF PROJECT

The North-West University Ethics Committee (NWU-EC) hereby approves your project as indicated below. This implies that the NWU-EC grants its permission that, provided the special conditions specified below are met and pending any other authorisation that may be necessary, the project may be initiated, using the ethics number below.

Project title:.SABPA (Sympathetic activity and Ambulatory Blood Pressure in Africans)

***Ethics* number**: N W U - 0 0 0 3 6 - 0 7 - S 6
(Institution - Project Number - Year - Status)
Status: S = Submission; R = Re-Submission; P = Provisional Authorisation; A = Authorisation

Approval date: 12 November 2007 **Expiry date**: 11 November 2012

Special conditions of the approval (if any): None

General conditions:

While this ethics approval is subject to all declarations, undertakings and agreements incorporated and signed in the application form, please note the following:

- *The project leader (principle investigator) must report in the prescribed format to the NWU-EC:*
  - *annually (or as otherwise requested) on the progress of the project,*
  - *without any delay in case of any adverse event (or any matter that interrupts sound ethical principles) during the course of the project.*
- *The approval applies strictly to the protocol as stipulated in the application form. Would any changes to the protocol be deemed necessary during the course of the project, the project leader must apply for approval of these changes at the NWU-EC. Would there be deviated from the project protocol without the necessary approval of such changes, the ethics approval is immediately and automatically forfeited.*
- *The date of approval indicates the first date that the project may be started. Would the project have to continue after the expiry date, a new application must be made to the NWU-EC and new approval received before or on the expiry date.*
- *In the interest of ethical responsibility the NWU-EC retains the right to:*
  - *request access to any information or data at any time during the course or after completion of the project;*
  - *withdraw or postpone approval if:*
    - *any unethical principles or practices of the project are revealed or suspected,*
    - *it becomes apparent that any relevant information was withheld from the NWU-EC or that information has been false or misrepresented,*
    - *the required annual report and reporting of adverse events was not done timely and accurately,*
    - *new institutional rules, national legislation or international conventions deem it necessary.*

The Ethics Committee would like to remain at your service as scientist and researcher, and wishes you well with your project. Please do not hesitate to contact the Ethics Committee for any further enquiries or requests for assistance.

Yours sincerely

Prof M M J Lowes
(chair NWU Ethics Committee)

Language Editor's Certificate

TO WHOM IT MAY CONCERN

This document serves to confirm that I, the undersigned, a professional language practitioner* of

30 Kwartel Crescent, Rooihuiskraal, Centurion,

was responsible for the language editing (English) of the MSc dissertation of Ms. Chrisna du Plessis, a student of the NorthWest University, titled

"Retinal microvascular calibre and blood pressure associated target organ damage: a bi-ethnic investigation within the SABPA study"

(under the guidance of Dr. W. Smith and Prof. A.E. Schutte as Study Supervisors).

A S du T Sonnekus
(Dries Sonnekus)

Professional Language Practitioner/Text Editor
Tel: 012 661 5907

*Accredited by NWU to translate/edit study guides in various disciplines.