Retinal Vessel Characteristics and Eye Disease Pathogenesis: The Canadian Longitudinal Study on Aging

Alexis O'Neil

Thesis submitted to the University of Ottawa in partial fulfillment of the requirements for the Master of Science in Epidemiology

School of Epidemiology and Public Health

Faculty of Medicine

University of Ottawa

Preface to the Thesis

The research outlined in this thesis was approved by the University of Ottawa School of Epidemiology and Public Health. This thesis presents the original work and research undertaken by Alexis O'Neil in partial fulfillment of the requirements of the Master of Science in Epidemiology. Dr. Ellen Freeman, Dr. Marie-Hélène Roy-Gagnon, and Dr. David Maberley provided feedback and guidance on the completion of this thesis. Other co-authors including Roshan Welikala, Sarah Barman, Christopher G. Owen, Alicja Rudnicka, and Mohan Rakesh were involved in the development and use of QUARTZ and revision of the final manuscripts. Specific contributions will be outlined as a preface to each manuscript.

Manuscript 1

Title: Factors Associated with Retinal Vessel Traits in the Canadian Longitudinal Study on Aging

Authors: Alexis O'Neil, Roshan A. Welikala, Sarah Barman, Christopher G. Owen, Alicja R. Rudnicka, Mohan Rakesh, Marie-Hélène Roy-Gagnon, David Maberley, Ellen E. Freeman

Ethics: Approved by the University of Ottawa Research Ethics Board), January 2023.

Publication Status: Accepted by Investigative Ophthalmology and Visual Science (IOVS).

Manuscript 2

Ethics: Approved by the University of Ottawa Research Ethics Board, January 2023.

Title: Retinal Vessel Traits and Age-Related Eye Disease in the Canadian Longitudinal Study on Aging

Authors: Alexis O'Neil, Roshan A. Welikala, Sarah Barman, Christopher G. Owen, Alicja R. Rudnicka, Mohan Rakesh, Marie-Hélène Roy-Gagnon, David Maberley, Ellen E. Freeman

Publication Status: In Review by Clinical and Experimental Ophthalmology.

Abstract

Retinal vessel characteristics have been linked to various health and lifestyle factors and age-related eye diseases. However, most studies have been cross-sectional, limiting our understanding of how retinal vessels change over time and the temporality of vascular mechanisms underlying eye disease. Using data from the Canadian Longitudinal Study on Aging Comprehensive Cohort, this research cross-sectionally and longitudinally investigated the relationships between retinal vessel traits and 1) sociodemographic, health, and lifestyle factors, 2) the prevalence and 3-year incidence of glaucoma and changes in intraocular pressure and cup-to-disc ratio, and 3) the prevalence of macular degeneration at baseline and its 3-year incidence. We confirmed that retinal vessel traits are related to factors such as age, blood pressure, and inflammatory markers, and provided additional evidence for a pathogenic role of the retinal vasculature in eye disease development. This research offers a comprehensive analysis with implications for improved treatment and prevention strategies in ophthalmology.

(150 / 150 words)

Acknowledgements

I would like to express my sincere gratitude to Dr. Freeman. Your mentorship over the past two years has spanned from teaching introductory epidemiology to guiding me through the process of publishing a manuscript. You have shown me the possibility of integrating ophthalmology into epidemiological research, altering my career path completely. Thank you for the opportunity to learn, teach, and conduct research at your side.

My thanks also go to Dr. Marie-Hélène Roy-Gagnon and Dr. David Maberley for being invaluable members of my Thesis Advisory Committee. Without your expertise, this research would not have been possible.

Evan, your patient listening to my endless droning about retinal vessels, the woes of data analysis and thesis writing, and your help with various technical issues has truly been appreciated. Thank you for being my biggest cheerleader.

Mom and Dad, thank you for your unwavering support and encouragement. I am forever motivated and grateful for your genuine interest and enthusiasm for my current and future endeavors.

Lastly, I would like to acknowledge Bryan and Chris. Bryan, your work ethic and devotion to science never ceases to amaze and motivate me. It has been a privilege to learn, work, and grow together over the past seven years. Chris, you have brought me endless joy through this sometimes-lonely process with your art, laughter, and banter. Both of you have harshly and lovingly encouraged me to be where I am today, and for that, I am wholly grateful. I can not wait to see what we all accomplish moving forward.

Table of Contents

List of Tables

Chapter 6

Chapter 7

List of Figures

Chapter 4

Chapter 6

Appendix A

List of Abbreviations

ACG	Angle Closure Glaucoma
AMD	Age-Related Macular Degeneration
ARIC	Atherosclerosis Risk in Communities
AVR	Arterio-Venous Ratio
BCVA	Best Corrected Visual Acuity
BDES	Beaver Dam Eye Study
BMES	Blue Mountain Eye Study
BMI	Body Mass Index
CDR	Cup-to-Disc Ratio
CI	Confidence Interval
CIHR	Canadian Institutes of Health Research
CLSA	Canadian Longitudinal Study on Aging
CRAE	Central Retinal Artery Equivalent
CRP	C-Reactive Protein
CRVE	Central Retinal Vein Equivalent
DBP	Diastolic Blood Pressure
DCS	Data Collection Site
EPIC	European Prospective Investigation of Cancer
HDL	High Density Lipoprotein
HR	Hazard Ratio
IOP	Intraocular Pressure
LDL	Low Density Lipoprotein
MABP	Mean Arterial Blood Pressure
MESA	Multi-Ethnic Study of Atherosclerosis
NTG	Normal Tension Glaucoma
OAG	Open Angle Glaucoma
OCT	Optical Coherence Tomography
OR	Odds Ratio
QUARTZ	Quantitative Analysis of Retinal Vessel Topology and Size
RDD	Random Digit Dialing
RGC	Retinal Ganglion Cells
RNFL	Retinal Nerve Fiber Layer
RPE	Retinal Pigment Epithelium
SBP	Systolic Blood Pressure
SiMES	Singapore Malay Eye Study
UK	United Kingdom
WESDR	Wisconsin Epidemiological Study of Diabetic Retinopathy

Chapter 1: Introduction

The eye remains the only area where blood vessels can be directly and non-invasively visualized using optical methods such as fundus photography.[1, 2] Recent advancements in fundus image analysis, particularly with the integration of artificial intelligence and deep learning, have enabled automated, objective, and reproducible measurements of retinal vessel characteristics in large population-based studies.[3-5] These quantitative methods have been widely applied in epidemiological research, with growing evidence linking retinal vascular geometry—such as vessel diameter and tortuosity—to cardiovascular disease [6-13] and its risk factors. As such, changes in retinal vessel diameter have been linked to several factors, including age,[14-22] smoking,[15, 17, 23-27] body mass index (BMI), [15, 17, 20, 26-32] diabetes,[17, 27, 33, 34] and stroke.[8, 9, 35-40] Although less frequently studied, tortuosity of the retinal microvasculature has been linked to a similar set of determinants.[27, 29, 41-43]

Beyond systemic health, some studies suggest a vascular role in age-related eye diseases such as glaucoma and age-related macular degeneration (AMD). While AMD affects the central area of the retina, glaucoma manifests as damage to the optic nerve; both diseases have been related to cardiovascular signs and outcomes,[44-51] prompting a series of cross-sectional and, less frequently, longitudinal [52-58] studies to uncover the pathogenic role of the retinal vasculature. However, the causal relationship is still unclear due to conflicting results in the literature and overall lack of prospective studies. Understanding the pathogenesis of age-related eye diseases like AMD and glaucoma is becoming increasingly important to determine early identification and prevention measures in the context of an exponentially aging population.[59, 60] Thus, more longitudinal investigations are needed to clarify the relationship between ocular health outcomes and microvascular changes.

Objectives

The key knowledge gap that this thesis aims to address is that most population-based studies assessing associations with retinal vessel characteristics have been cross-sectional. With limited information on how vessels change over time and how this may affect the risk of ocular disease, we aimed to:

- **Objective one:** Cross-sectionally and longitudinally examine the relationship between sociodemographic, health, and lifestyle factors and retinal vessel traits (width, area, tortuosity).
- **Objective two**: Cross-sectionally and longitudinally investigate the relationship between retinal vessel characteristics and glaucomatous outcomes (intraocular pressure (IOP), cup-to-disc ratio (CDR), and glaucoma prevalence and incidence).
- **Objective three:** Examine the relationship between retinal vessel characteristics (width and tortuosity) and prevalence of AMD at baseline and its 3-year incidence.

Organization of the Thesis

This thesis is categorized as a secondary analysis of an existing dataset and organized into chapters. Chapters 2 and 3 present an in-depth review of the relevant existing literature as it relates to retinal vessels, how their characteristics are quantified, their determinants, and how they relate to age-related eye disease. Chapter 4 presents the guiding conceptual framework for the thesis. Chapter 5 provides background on the data source, the Canadian Longitudinal Study on Aging (CLSA). Chapters 6 and 7 each present one manuscript that directly addresses the objectives. The results as they relate to the objectives are discussed in chapter 8, in addition to comparisons to existing literature, strengths and limitations, significance, and future directions of the research.

Chapter 2: Retinal Vessel Overview

Anatomy of the Retinal Microvasculature

The retina is a thin, light-sensitive, tissue located at the posterior pole of the eye and plays an essential role in sight - it is responsible for transforming light into neural signals that can be interpreted by the brain. [61] As one of the most metabolically active tissues in the human body, the retina relies on a constant supply of oxygen and glucose, making its blood flow crucial for optimal visual function.[61]

The retinal blood supply is provided by the ophthalmic artery, a major branch of the internal carotid artery. [62, 63] The ophthalmic artery then divides into four smaller branches: the long and short posterior ciliary arteries, the anterior ciliary arteries, and the central retinal artery. The central retinal artery enters the eye at the optic disc, and divides into the superior and inferior retinal artery, each dividing further into nasal and temporal branches. These arteries progressively narrow into smaller vessels called arterioles, which are less than 200 μm in diameter, have thinner walls than arteries and lack an internal elastic layer. [64] The arterioles then merge into a dense network of capillaries, the smallest blood vessels (10-15 μm in diameter), where oxygen and nutrient exchange with the inner layers of retinal tissue occurs. This blood supply is characterized by a low flow rate and high degree of oxygen exchange. [62, 63, 65]

Deoxygenated blood then exits the capillaries and enters the venules, where it is returned to the heart, mirroring the pattern of the arterial supply. [64] The venules are slightly larger than the arterioles with a diameter of 30-300 μm, lack a smooth muscle layer, and converge into the central retinal vein. This vein then exits through the optic disc to drain into the superior ophthalmic vein or directly into the cavernous sinus. [61] The retinal vasculature is easily visualized at the posterior pole of the eye due to the transparent layers of the retina.

As an extension of the ganglion cell layer of the retina, the optic nerve provides a direct link from the eye to the rest of the visual pathway in the brain. Blood supply to the optic nerve is more complex, involving several branches of the internal carotid artery and the choroid. [63] The optic nerve head is supplied by capillaries of the retinal arterioles, the choroid, and short posterior ciliary arteries. [63] The region just beyond the optic nerve head is supplied by the pial vascular plexus (derived from the internal carotid artery) and branches from the central retinal artery. Venous drainage is achieved predominantly by the central retinal vein.

Regulation of Ocular Blood Flow

The retinal circulation lacks autonomic innervation so optimal blood flow is maintained through local regulatory mechanisms.[66] Dependant on perfusion pressure and resistance in the blood vessels,[67] these mechanisms include myogenic and metabolic control, flow-mediated vasodilation, and intercellular conduction.

Myogenic control ensures stable blood flow despite arterial pressure fluctuations by causing smooth muscle in blood vessels to contract in response to stretch, and relax when the vessel is no longer stretched.[66] Similarly, flow-mediated vasodilation occurs when increased blood flow induces shear stress on endothelial cells, triggering a vasodilatory response. This response is thought to be mediated by various factors including oxygen, carbon dioxide, angiotensin-II, adenosine, nitric oxide, and endothelin-1.[68] Local metabolic control adjusts blood flow to meet the oxygen demands of retinal tissue; when metabolic activity increases, parenchymal cells produce vasodilatory signals that enhance blood supply to the area.[66]

The intercellular conduction theory is thought to link these forms of local control, allowing them to work together to regulate blood flow.[66] Communication between endothelial cells may

facilitate the propagation of vasodilatory or vasoconstrictive signals along the vascular network.[66] This rapid transmission of signals allows for coordinated adjustments in blood flow across different regions of the retina. Thus, the regulation of retinal microvascular blood flow is highly complex and relies on the interaction of multiple mechanisms to ensure consistent oxygen and nutrient delivery under varying physiological conditions.

Methods of Viewing the Retinal Vasculature

Disruptions of the retinal vasculature can be sight threatening. For example, retinal vessel occlusions (strokes), diabetic retinopathy, and hypertensive retinopathy can all cause vision loss and have severe consequences on systemic health if the underlying diseases are not appropriately treated. [69] Each disease presents with distinct vascular changes that are visible upon fundus examination. Thus, visualizing the posterior pole of the eye has become a crucial part of routine eye exams, allowing for the diagnosis and monitoring of both ocular and systemic disease.

Historically, a device called an ophthalmoscope has been used to manually view the interior of the eye. The first report of using an ophthalmoscope for fundus examination was in 1851 by Hermann von Helmholtz.[70] The tool revolutionized 19th century ophthalmology, allowing physicians to view and describe retinal vessel occlusions, glaucoma, and diabetic retinopathy for the first time. While ophthalmoscopy is still used today, it is one of the top three hardest clinical skills to learn [71] and has limitations in screening for eye diseases like diabetic retinopathy, glaucoma and hypertensive retinopathy. [72] Physicians instead rely more on advanced imaging techniques such as fundus photography and optical coherence tomography (OCT) to view the posterior pole of the eye.

In 1926, Carl Zeiss developed the first commercially available fundus camera. [73] With advancements from film to digital technology, high-resolution fundus images can be captured non-invasively and stored to diagnose and monitor eye disease. Coloured filters for techniques like autofluorescence, fundus fluorescein angiography, and indocyanine green angiography can be applied to the camera system for visualization of specific aspects of the retinal vasculature and its pathologies. As a cost effective and simple technique, fundus photography continues to be used globally as a diagnostic tool in ophthalmology. [73]

In 1996, a newer method of retinal imaging, OCT, was first developed. [74] Using infrared light and the principle of optical scattering, OCT generates cross sectional images of the layers in the retina. OCT-angiography has been developed to visualize the retinal and choroidal vasculature by detecting movement of blood cells, and integration of Doppler imaging has further allowed for blood velocity and vessel pulsatility to be measured throughout the retina. [75] Now one of the most utilized forms of imaging technology, OCT has become indispensable in diagnosing and monitoring several diseases, including diabetic retinopathy and macular degeneration. [75]

Ophthalmic diagnostics have certainly been revolutionized by advanced imaging techniques. Fundus photography, in particular, has been integrated into data collection protocols in several population-based studies, providing researchers with access to detailed views of the retinal vasculature. [3, 5, 76] This, in addition to computerized methods of quantifying retinal vessel traits, has shifted research towards utilizing precise measurements of retinal vessel characteristics to gain insight into both ocular and systemic health.

Retinal Vessel Characteristics

Vessel Diameter

Assessments of retinal vessel diameter began with subjective methods. This is exemplified by Wagener *et al*'s technique (1947) of grading the degree of arteriolar narrowing by comparing arteriolar and venular diameter during ophthalmoscopic examination. [77] Since then, researchers have derived objective and repeatable measurements to analyze retinal vessel width.

A formula to do so was initially proposed by Parr and Spears. [78] This formula used measurements from the arteriole branches closest to the optic disc to derive an approximation of the width of the central retinal artery, the central retinal artery equivalent (CRAE). These measurements were initially obtained by manually analyzing fundus images with a slide projector system. Hubbard *et al* later modified the formula to include venules, thus being the advent of the central retinal vein equivalent (CRVE). [5] The arterio-venous ratio (AVR), a ratio derived from the CRAE and CRVE, was also introduced at this time. Recognizing that the original formulas were dependent on the number of blood vessels included in the analysis, Knudtson *et al* refined the method so measurements would only be based on the six largest arterioles and venules. [79] This improvement ensured consistency across eyes and standardized measurements across different cameras and software. Many retinal vessel analysis software available today continue to generate summary metrics of retinal vessel diameter using the revised Knudtson-Parr-Hubbard formula.

As mentioned, CRAE, CRVE, and AVR can be derived using these formulas. CRAE and CRVE directly reflect the central arterial inflow and veinous outflow of the retina. [80] Meanwhile, AVR is a non-specific parameter reflecting the general regulative state of the retinal circulation. Smaller AVR typically denotes narrowing of retinal arterioles; this assumes constant venular diameter [81] despite evidence showing arterioles and venules can be influenced by distinct factors

and exhibit different associations with disease. [82] Consequently, both narrower arterioles and wider venules can contribute to a smaller AVR, limiting its interpretation. Additionally, as a summary parameter, AVR has been shown to convey less information than the individual parts of the ratio.[81] Since differing pathophysiological responses may be obscured when using AVR alone, [81] arteriolar and venular diameter have increasingly been investigated separately.

Through such studies, it has been described that normal variation and pathogenic changes in vessel width are thought to reflect cumulative structural damage from multiple processes, including aging,[16, 19] genetics,[83-88] hypertension,[26, 89, 90] atherosclerosis,[91] inflammation,[17, 24, 26] and endothelial dysfunction.[17, 26] These processes may underly associations between retinal vessel diameter and several health and lifestyle factors, which will be discussed further later in his chapter.

Despite being widely used in population-based studies, measurements of vessel width still have some limitations to their use. First, there remain issues with magnification effects from cameras and ocular refractive media. The refraction status and axial length of the eye influence image scaling, as the size of an object in a photograph is proportional to its true size and inversely proportional to the eye's optical dimensions.[92] Thus, in longer eyes, the retinal vessels would naturally appear more narrow and be measured as such. This is exemplified by associations between narrower CRAE and CRVE and myopic refractions, though these often become nonsignificant when magnification or refraction is accounted for in regression models. [92, 93] To address this issue, approaches such as adjusting for axial length or refractive error in regression models [92] or applying correction formulas for magnification [93] have been developed. AVR has also been used in lieu of CRAE or CRVE, despite its previously mentioned limitations, since it is a dimensionless measure free from the effects of magnification. [94] However, Wong *et al* found

minimal differences in estimates when correcting for refraction or not and argued that magnification-related bias is unlikely to severely impact epidemiological studies where refractive status was not collected. [92]

Second, fundus images and vessel analysis software only offer a simplified view of the retinal vasculature. Static fundus images do not capture dynamic vessel changes related to the cardiac cycle. [82, 95, 96] Furthermore, analysis software typically measure the reflective red blood cell column in the vessels, ignoring the surrounding clear plasma zone, and underestimate lumen diameter. [81, 97] However, given that pulsation only causes small changes in the vessels and blood column is proportional to the lumen diameter, these measures remain good surrogates for overall vessel diameter.

These limitations of vessel diameter have prompted the development of other parameters to further characterize retinal vessels and provide a more comprehensive assessment of their variation.

Vessel Tortuosity

The principle of optimal blood flow suggests that the circulatory system is structured to deliver adequate blood supply with minimal energy expenditure. [33, 98] Deviations from the optimal architecture can lead to reduced and less efficient peripheral circulation. It is thought that vessel tortuosity may capture information about the optimality of the vascular pattern. Defined as a dimensionless measure of vessel curvature, higher tortuosity indicates a higher degree of twisting or curvature. Tortuosity has been shown to reflect a different aspect of pathology than vessel diameter, thus providing additional insights into microvascular variation. [41]

Complex mechanisms underlie changes in vascular tortuosity. Hypoxia may stimulate the production of angiogenic factors by the endothelial cells, leading to vessel elongation and increased curvature. [99, 100] More tortuous (curved) vessels are hypothesized to provide greater surface area and lower blood velocity, enhancing oxygen and nutrient extraction. [101-104] Degradation of elastin and weakening of the vessel wall from the effects of aging, hypertension, or increased blood flow can also promote reduced axial tension, lengthening of the vessel, and a subsequent increase in tortuosity. [105] To note, severe tortuosity can increase resistance to blood flow, which elevates shear stress in the vessels and contributes to the risk of thrombosis. [105]

Meanwhile, decreased tortuosity (straightening) of the blood vessels may be explained by endothelial dysfunction and atherosclerosis, and can lead to impaired blood flow and perfusion. [106] Moreover, tortuosity can be limited by the number of branches of the vessels, which serve as anchor points, and vessels with wider diameter are unable to curve at the same frequency as narrower vessels. [100] Specific health and lifestyle factors impacting vessel tortuosity will be described later in this chapter.

While tortuosity has been proposed as a valuable measure of vessel geometry, it also has several limitations. As explained in a systematic review by Kalitzeos *et al*, there is a lack of standardization in terms of image acquisition, calculation methods, and indices used across studies.[107] For example, tortuosity can be calculated simply as the ratio of the arc length to chord length, although this does not consider the number of inflection points along the vessel.[108] Thus, other common formulas using subdivided chord lengths or curvature-based methods have been developed.[108] Most software also rely on small areas to calculate tortuosity; therefore global measurements are generally absent in the literature.[107] Abdalla *et al* had similar reservations in their review, noting inconsistencies in how tortuosity is defined among various studies.[109] They

also emphasized that validation datasets are often small and that vessel dilation and elongation, factors that may influence tortuosity, are rarely accounted for, raising concerns about measurement accuracy. Therefore, further work to improve and standardize the measurement of tortuosity may advance its utility in ophthalmic research.

Vessel Area

While measures of vessel diameter and tortuosity have frequently been used in population-based studies, vessel area is infrequently considered. Two cross sectional studies assessing the validity of vessel analysis software have investigated retinal vessel area in association with various factors. In multivariate analysis of 250 subjects adjusting for age and gender, Maderuelo-Fernandez *et al* found that venular area was inversely associated with systolic blood pressure (SBP) (β= −0.114, 95% Confidence Interval (CI): −0.168, −0.061). [110] In the same study, a cardiovascular risk score based on the Framingham risk equation was inversely correlated with both arteriolar and venular area (Pearson correlation coefficient = -0.338 and -0.238, respectively). In a similar validation study, Fukutsu *et al* observed that age, SBP and diastolic blood pressure (DBP) were negatively correlated with both arteriolar and venular area ($P<0.05$ for Pearson correlation coefficients). [111] Therefore, both teams concluded that retinal vessel area could be a useful indicator of cardiovascular risk, similar to diameter and tortuosity.

However, the significance of vascular area, its impact on perfusion, circulation, and clinical relevance, remains to be elucidated. Three-dimensional renderings of the ocular vessels using OCT technology have been used as indicators of retinal vessel perfusion, [112] although, vessel area is a two-dimensional summary measure of vessels derived from en-face images of the retina. Since vessels are inherently three-dimensional dynamic structures, a two-dimensional measurement may

not fully represent the vascular structure and perfusion of retinal tissues. Thus, further research on how variation in vessel area may impact retinal function is needed.

Vessel Analysis Software and QUARTZ

Grading images by human observers is time consuming and would not be feasible in epidemiological studies with large sample sizes. Therefore, several computerized methods to quantify retinal vessel characteristics have been developed. The Vascular Assessment and Measurement Platform for Images of the Retina is a software application for semi-automatic quantification of retinal vessel traits. [113] The system detects retinal landmarks (optic disc, retinal zones, main vasculature), and quantifies parameters including vessel width, vessel branching coefficients and tortuosity measures. Similarly, Singapore 'I' Vessel Assessment Program automatically calculates arteriolar/venular calibre, tortuosity, branching angles, and fractal dimensions. [41] Interactive Vessel Analysis is another semi-automated software commonly used among population-based studies of retinal vessel characteristics, requires trained graders, and only provides measurements of CRAE, CRVE, and AVR. [17]

While useful, these methods are restricted to analysis within a focal area around the optic disc (0.5–1 disc diameter away from the disc margin), may have difficulty differentiating arterioles from venules, and offer few parameters beyond vessel diameter. [114] Beyond this, vessel diameter has mostly been limited to CRAE and CRVE measurements which do not capture variability across the image and often depend on the number of vessels analyzed. Long processing times, lack of automatic processing – necessitating subjective human input that affects both intra- and inter-grader agreement, and expensive costs, further limit the use and accessibility of these methods.

To overcome some of the limitations in traditional retinal vessel analysis, this research utilized QUARTZ (Quantitative Analysis of Retinal Vessel Topology and Size), a deep learning software, to analyze images from the CLSA. [4] Deep learning is a subset of machine learning where the system is trained to recognize patterns and analyze complex data using multiple layers of artificial neural networks. [115] QUARTZ was validated to analyze fundus photographs using images from the United Kingdom (UK) Biobank and has demonstrated its effectiveness through application in several large population-based studies. [4, 29, 42, 116]

QUARTZ is fully automatic and processes most images in about 2 minutes. The software reliably assesses image quality with a sensitivity of 91% and specificity of 100% in the CLSA, where 82.77% of images were of adequate image quality. [4] Subsequently, the software identifies and segments visible vessels across the image. Arterioles and venules are differentiated by applying a probability-based label to each centreline pixel within the segment. [4] A voting strategy is used to label the whole vessel segment as arteriole or venule, with an accuracy of 96% when using a cut-off probability of 0.8.

Vessel characteristics are then measured across the image. Vessel diameter is determined using a zero-crossings of the second derivative method, which estimates vessel edges by detecting changes in color and brightness. [117] Diameter is calculated as the distance between the edge points perpendicular to the centerline of the vessel. Meanwhile, tortuosity is measured using a subdivided chord length method, where a straight line (chord) is drawn from the start to the end of the vessel segment. [118] The actual path of the segment is compared to the chord length; if the vessel's actual length is longer than the chord, the vessel is more curved.

QUARTZ obtains thousands of measurements of diameter and tortuosity across the image. The mean diameter and tortuosity of each arteriole and venule are summarized and weighted by

the vessel segment length. Total vessel area is calculated from the average vessel width multiplied by length and summed across the vessel segments. The measurements obtained by the software represent global assessments of vessel characteristics, accounting for variability across the retina.

In using QUARTZ and similar analysis software, authors have uncovered significant associations between retinal vessel characteristics and several sociodemographic, health, and lifestyle factors.

Determinants of Retinal Vessel Characteristics

Age

Both arterioles and venules have been shown to narrow with increasing age in several population-based studies. [14-22] However, some variability in these associations has been reported. For example, only narrower arterioles were associated with age in the Inter99 Eye Study, [119] and in Type 1 diabetics, age was only associated with narrower venules and not arterioles. [28]

Although less frequently documented, vessel widening has also been observed with age. Using QUARTZ to provide a global measurement of vessel diameter, Tapp *et al* found that venules widened by 1.0 μm (95% CI: 0.9 μm to 1.2 μm) for each decade increase in age. [29] In a similar analysis, Tapp *et al* also noted that venules widened with age regardless of diabetes status, whereas arterioles narrowed with increasing age only in those without diabetes. [27] Owen *et al* observed that arterioles were narrower with increasing age, whereas venular width increased with age, [32] while Sun *et al* found the opposite pattern - wider arterioles and narrower venules were associated with increasing age. [31] Additional longitudinal analyses are needed to better understand the natural history of retinal vessel changes with age and the factors contributing to the variability in these associations.

Age has also been associated with changes in vascular geometric pattern. In the UK biobank, Tapp *et al* found that for each decade rise in age, venular tortuosity increased by 2.5% (95% CI: 2.1% to 2.8%). [29] Using the same dataset, more tortuous venules were consistently associated with increasing age, whereas more tortuous arterioles were only associated with age in those without diabetes. [27] Owen *et al* also observed that arterioles were more tortuous with increasing age. [32] These results were not consistent with results from Cheung *et al*, who reported the opposite; more tortuous venules were associated with younger age, and less tortuous arterioles were associated with older age. [41] Overall, the existing evidence mostly supports that vessels become more tortuous with age.

Sex

Several population-based studies have reported that women tend to have wider arteriolar diameter than men. [17, 26, 30, 31] Meanwhile, one study found that men had wider CRAE, [20] and one study reported no significant sex differences in arteriolar diameter. [14] Men consistently exhibit wider venules than women, [14, 15, 26, 30] though some studies have found no significant differences in venular diameter. [17, 20, 31] Regarding tortuosity, Cheung *et al* reported that female gender was associated with more tortuous venules. [41] Variation in vessel characteristics may emerge with age, as multiple studies in children found no significant differences between males and females in vessel diameter nor tortuosity. [120-122]

Ethnicity

Several studies have assessed ethnic differences in diameter and tortuosity in children. In a multi-ethnic population of Singaporean children, Chinese children had narrower arteriolar and

venular diameter in comparison to Malay and Indian Children. [120] Similarly, the Sydney Childhood Eye Study, Asian and Middle Eastern children had wider arteriolar and venular diameter than White children. [121] In the Child Heart and Health Study in England study, vessel tortuosity did not differ significantly among White, South Asian (Indian, Pakistani, Bangladeshi), and African Caribbean (black African, Caribbean, or British) children. [122] Whereas other Asian children (Afghani, Chinese, and Turkish) had less tortuous vessels.

These ethnic patterns have been shown to persist into adulthood. Data from the Multi-Ethnic Study of Atherosclerosis (MESA) showed that Black and Hispanic people had wider arteriolar diameter than White people. [17] In the same study, Black, Hispanic, and Chinese people all had wider venular diameter than white people. Additionally, a study of healthy Asian adults found that Chinese people had the narrowest CRAE among Indians and Malays, and also had the most tortuous arterioles and venules. [123] Lastly, in the Atherosclerosis Risk in Communities Study (ARIC), African Americans had wider CRAE and CRVE in comparison to White people. [26]

Racial differences in retinal vasculature have been proposed to be due to a variety of reasons. First, identification of vessels and measurement of diameter may differ between ethnic groups due to differences in retinal and iris pigmentation. [121] Ethnic groups also differ in susceptibility to cardiovascular disease, potentially impacting susceptibility to changes in the retinal vasculature. [124] Lastly, variation in retinal vasculature is largely influenced by genetics. In a twin study conducted by Taarnøj *et al*, the heritability of retinal artery and vein diameters was found to be 70% and 83%, respectively. [125] Additionally, several genome-wide association studies have identified links between various gene loci and the diameters and tortuosity of retinal arterioles and venules. [83-88]

Blood Pressure and Hypertension

Mean Arterial Blood Pressure (MABP) represents the average blood pressure throughout the circulatory system and is a commonly used summary measure of systolic and diastolic blood pressure. [126] In the Funagata Study, higher MABP corresponded to narrower CRAE and CRVE; this association was stronger with CRAE. [18] These results were confirmed by von Hanno *et al* [30] and persisted in those with Type 1 and Type 2 diabetes in the Wisconsin Epidemiological Study of Diabetic Retinopathy (WESDR) study. [21, 28] Narrower arterioles alone have consistently been associated with higher MABP across several studies, [16, 20, 26, 31, 43] while either wider [20, 26, 43] or narrower [15] venules have been reported. In a meta-analysis of five studies, higher MABP was inversely associated with CRAE only (β= -3.07, 95% CI, −3.73 to −2.40). [127] Thus, it is generally well-established that retinal vessels narrow with increasing MABP.

There is evidence to suggest age differences in the relationship between blood pressure and retinal vessel diameter. Four studies have reported that with increasing age, the association between retinal arteriolar diameter and higher MABP weakens. [16, 22, 25, 31] Similarly, a meta-analysis of participant-level data found that the association between CRAE and hypertension was stronger in participants under the age of 60 years old, and became nonsignificant in those above the age of 70. [128]

In some studies, systolic and diastolic blood pressure have been assessed separately. Interestingly, Dervenis *et al*, [14] Ikram *et al*, [25] and Leung *et al* [19] observed wider CRAE was cross-sectionally associated with higher SBP while narrower CRAE and CRVE were associated with DBP. In contrast, analysis of data from the UK Biobank showed that both narrower arterioles and venules were linked with higher systolic and diastolic blood pressure. [42] This relationship persisted regardless of diabetes status in a separate study using data from the UK biobank. [27] Owens *et al* similarly observed that arteriolar width was inversely associated with SBP and DBP, however

narrower venules were only slightly associated with DBP. [32] Elevated SBP was associated with narrower arterioles alone across other studies as well. [17, 119, 129]

The longitudinal relationship between blood pressure and retinal vessel changes has been investigated. Narrower CRAE and focal arteriolar narrowing have consistently been associated with incident hypertension. [129-135] As such, in a meta-analysis of six studies with participant level data across 10,229 subjects, narrower CRAE was associated with a higher odds of developing hypertension (overall adjusted Odds Ratio (OR)=1.65, 95% CI: 1.53,1.79). [128] In the same study, having narrower CRAE at baseline was associated with a 5-year increase in SBP. [128] To further support these findings, Xing *et al* suggested that retinal arteriolar narrowing may precede hypertension, since linkage regions of retinal vessel diameter and essential hypertension overlapped in their genome-wide linkage study. [136]

Few studies have looked at retinal vessel tortuosity in association with blood pressure and hypertension. In the UK biobank, increased arteriolar and venular tortuosity were associated with higher SBP and DBP. [42] This was expanded on in a later analysis of the UK biobank data, where Tapp *et al* found that associations with tortuosity were only significant in those without diabetes; still, increased SBP and DBP were both associated with more tortuous arterioles and venules. [27] In the European Prospective Investigation of Cancer (EPIC)-Norfolk eye study, however, arteriolar and venular tortuosity were positively associated with SBP, but not DBP. [32] In contrast, the Singapore Malay Eye Study found that less tortuous arterioles and more tortuous venules were associated with higher MABP. [41, 43] Based on the existing literature, those with higher blood pressure tend to have more tortuous vessels.

Diabetes

Prevalence of diabetes has been linked to wider arterioles [17, 27, 33, 34] or venules. [17, 33] Interestingly, those with diabetes in the Australian Diabetes, Obesity, and Lifestyle Study exhibited wider CRAE, but this association disappeared after adjusting for CRVE. [137] In addition, with a median follow-up of 10 years, incidence of diabetes was not associated with arteriolar width in a meta-analysis of 5 studies with participant level data for 18,771 subjects. [138] Instead, wider retinal venular diameter alone corresponded to an increased risk of diabetes (overall Hazard Ratio (HR)=1.09, 95%CI: 1.02, 1.15). [138] Wider vessels have also been associated with several diabetes-related factors such as higher hemoglobin A1c, [27, 30, 32] poorer glycemic control, [21] high serum glucose, [139] and diabetic retinopathy. [33, 140, 141]

While most evidence suggests a relationship between vessel widening and diabetes, some prospective studies have indicated the opposite. The 10-year incidence of diabetes was associated with narrower arteriolar diameters, but not venular diameters, in the Beaver Dam Eye Study (BDES). [142] Additionally, narrower arterioles, as indicated by smaller AVR, were associated with incidence of diabetes over a mean follow-up time of 3.5 years in the ARIC study. [143] Although, this should be interpreted with caution as smaller AVR may also reflect venular widening, as previously described. Associations between both CRAE and CRVE and incident diabetes were not statistically significant in the Rotterdam study. [144] Overall, the literature reveals inconsistent associations between retinal vessel diameter and diabetes, with most cross-sectional studies reporting wider vessels, contrasted by some longitudinal observations of narrowing.

In terms of tortuosity, Owen *et al* observed that venules were 6.5% more tortuous (95% CI: 2.8%, 10.4%) among those with type 2 diabetes compared to with those without in the EPIC-Norfolk study. [32] Tapp *et al* confirmed this relationship using data from the UK biobank. [27] In contrast, Cheung *et al* observed that venular tortuosity was not associated with diabetes, and less

tortuous arterioles were associated with diabetes. [33] The studies differ in the ethnicity of their cohorts and the methods of vessel analysis software, potentially leading to contrasting results.

Stroke

Stroke has generally been associated with wider venular diameter [8, 9, 35-37] and narrower arterioles. [8, 37-40] In a meta-analysis of 6 cohort studies comprised of 20,798 subjects, wider venular diameter was associated with incident stroke (pooled HR = 1.15, 95% CI: 1.05, 1.25), whereas arteriolar diameter was not. [145] Wider CRVE was consistently associated with 22-year stroke mortality in the WESDR.[146] Null associations between stroke and vessel diameter have also been reported.[13, 147]

Very few studies have assessed the relationship between stroke and vessel tortuosity. Tortuosity as a continuous variable was not associated with the risk of stroke in the Singapore Malay Eye Study. [35] Meanwhile, Sandoval-Garcia *et al* found that more tortuous arterioles were associated with stroke in a study of participants with type two diabetes. [147] This is in agreement with findings from Owen *et al*, where more tortuous arterioles were associated with prevalent stroke in the EPIC Norfolk study. [32]

Heart Disease

Corresponding well to the stroke findings outlined above, Wong *et al* reported that narrower arterioles and wider venules were also associated with the 5-year development of heart disease in the Cardiovascular Health Study. [9] This relationship may be impacted by sex, as some studies have found that it only persists in females. [6-8] This was the case in a meta-analysis by McGeechan *et al* composed of participant-level data from 22,159 subjects from 6 population-based

studies - wider retinal venules and narrower retinal arterioles were associated with increased risk of coronary heart disease in women, but not in men. [10] However, in a more recent analysis of the 16-year follow-up ARIC data, incidence of heart failure was associated with narrower arterioles and wider venules in both sexes, suggesting that observed sex differences may attenuate over time.[11] In the same study, adverse cardiac remodeling (left ventricular hypertrophy and systolic and diastolic dysfunction) showed similar associations. Mortality due to cardiovascular outcomes has also been associated with narrower arterioles and wider venules in the ARIC and Rotterdam studies. [12, 13]

Prevalent myocardial infarction and incident coronary events were not associated with vessel tortuosity in the EPIC-Norfolk Study [32] and the Edinburgh Type 2 Diabetes Study, [147] respectively. However, an Australian clinic-based study found that straighter venules were associated with prevalent coronary artery disease, but only in women. [148] Additionally, in a recent genome-wide association study, genetic variants linked to retinal venular tortuosity were also associated with coronary artery disease. [86] Further research is needed to clarify how vessel tortuosity relates to cardiovascular disease.

Cholesterol

Serum total cholesterol is comprised of two main lipoproteins: high-density lipoprotein (HDL) and low-density lipoprotein (LDL). These components have distinct associations with vessel diameter. HDL ("good") cholesterol is consistently associated with narrower venules [15, 17, 20, 25, 27, 30-32, 119, 149] and narrower arterioles. [25-27, 32, 119, 149] Null associations between HDL cholesterol and arterioles have also been reported. [20, 30, 31, 139] Meanwhile, increased LDL ("bad") cholesterol is generally linked to wider venules, as observed in the Singapore Malay Eye Study

(SiMES), [31] MESA, [17] UK Biobank, [27] and a Singaporean population-based study. [20] Despite defined relationships with its components, total cholesterol as a whole is generally not associated with vessel diameter; [15, 25, 119, 149] however, higher levels of total cholesterol have been linked to wider venules in a Singaporean population [20] and narrower arterioles in the EPIC-Norfolk study [32] and UK Biobank.[27] Given the differing effects of HDL and LDL on the risk of heart disease, [150] it naturally follows that the components of cholesterol may also affect the microvasculature through separate mechanisms.

Additionally, HDL cholesterol was inversely associated with venular tortuosity in the Singapore Malay study [41] and EPIC Norfolk study. [32] In data from the UK Biobank, Tapp *et al* found that HDL cholesterol and total cholesterol were both inversely associated with venular tortuosity, but only in participants without diabetes. [27] In contrast, in a population of children from the UK, more tortuous arterioles were associated with higher total cholesterol and levels of LDL cholesterol, not HDL cholesterol (venules were not assessed in this study). [118] The natural history of how retinal vessel geometry is impacted by cholesterol remains unclear.

Body Mass Index

Associations between body composition and retinal vessel traits have been studied in children and adults alike. Among three studies of either Australian or Singaporean subjects, children with higher BMI tended to have wider venules [151, 152] and narrower arterioles. [23, 151, 152] Similar trends have been seen in adults - higher BMI has consistently been associated with wider venular diameters. [15, 17, 20, 26-32] This relationship was stronger in men than women in data from the Tromsø study. [30] Adults with higher BMI also tend to have narrower arterioles, [20, 23, 25-27, 29] but null associations have also been observed. [17, 31] Longitudinally, Wang *et al* reported that wider

venules at baseline were associated with incident obesity (OR=1.8; 95% CI: 1.0, 3.1) and weight gain (OR=1.7; 95% CI: 0.9, 3.2) over five years using data from the BMES; this relationship persisted in those with and without diabetes and hypertension.[153] Meanwhile, arteriolar diameter was not related with 5-year incidence of obesity or weight gain. The relationship between retinal vessel diameter and body composition is generally consistently documented in the literature.

Conversely, findings on the link between retinal vessel tortuosity and BMI are mixed. In two separate analyses of data from the UK biobank, those with higher BMI had more tortuous venules; [27, 29] no association was observed with arteriolar tortuosity. Meanwhile, data from the Singapore Malay Eye Study showed that less tortuous arterioles were associated with higher BMI.[41] Thus, the effect of body composition on retinal vessel geometry remains ambiguous due to limited reports in the literature.

Inflammation

Inflammation has frequently been studied as a determinant of retinal vessel traits. Wider venules have consistently been associated with inflammatory markers. [15, 17, 23-27] In a recent systematic review, 23 studies showed that increased levels of C-Reactive Protein (CRP) were associated with wider venules but had no association with retinal arterioles. [154] As such, in meta-analysis of 9 studies with 22,422 participant-level data, CRP was not associated with retinal arteriolar diameter but was modestly associated with venular diameter (correlation coefficient = 0.09, 95% CI: 0.05, 0.12). In the same study, IL-6 was only associated with venular diameter, and white blood cell count was linked with both venular and arteriolar diameter, with stronger associations seen for venular diameter. Prospectively, higher CRP has been associated with a greater decrease in CRVE over 15 years in the BDES. [15]

Only one study has investigated the relationship between inflammatory markers and retinal vessel tortuosity. Using data from the UK Biobank and stratifying by diabetes status, Tapp *et al* found that higher CRP was associated with more tortuous venules in those with and without diabetes.[27] White blood cell count and granulocyte count were associated with more tortuous venules and less tortuous arterioles. Associations were generally stronger in those with diabetes than without.

Smoking

Smoking status has consistently been associated with wider retinal blood vessels. Dervenis *et al* found that current smokers in the Thessaloniki cohort have wider arterioles and venules than non-smokers, with a stronger association among venules. [14] These findings have been confirmed within other population-based study cohorts including the EPIC-Norfolk study, [32] Inter99 study, [119] and several others.[15, 17, 20, 21, 25, 26, 28, 30, 31, 155]

This relationship seems to follow a dose-response pattern. Former smokers tended to have wider vessel diameter in the Tromsø, Rotterdam, ARIC, and BMES studies, but the association was stronger in current smokers as opposed to former.[25, 26, 30, 155] Similarly, widening of CRVE corresponded to a higher number of pack-years in the BDES, BMES, and in those with type two diabetes in the WESDR.[15, 21, 155] Furthermore, Myers *et al* observed that CRVE decreased with increasing time since smoking cessation, while the number of years participants smoked was positively related to wider CRVE. [15]

One study has previously assessed smoking's effect on vessel tortuosity. Owen *et al* found that venular and arteriolar tortuosity were positively associated with smoking, but only in women rather than men. Gender differences were partially explained by height. [32]

Alcohol

Compared to subjects who have never consumed alcohol, those who do consume alcohol tend to have either narrower arterioles alone, [14, 26, 155] or both narrower arterioles and venules. [17, 20] Null findings have also been noted. [25] To the best of our knowledge, no previous studies have investigated the effect of alcohol consumption on retinal vessel tortuosity.

Overall, retinal blood vessel characteristics, particularly diameter, have been extensively researched and linked to various demographic, health, and lifestyle factors. However, numerous discrepancies in published results make it challenging to ascertain the true impact of overall health on the retinal microvascular. Furthermore, limited reports on vessel tortuosity in relation to these factors provide little clarity on the role of vascular geometry.

Chapter 3: Age-Related Eye Disease and Blood Vessels

What is Glaucoma?

Glaucoma is the most common cause of irreversible vision loss worldwide. [60] In 2020, an estimated 3.61 million people were blind, and 4.14 million people were visually impaired due to glaucoma, accounting for 8% of all blindness in the world. The prevalence of glaucoma in Canada has been estimated to be between 2.7% and 3.9%. [156-158] This corresponds to a significant financial burden associated with various direct and indirect healthcare costs, including ocular hypotensive drugs, physician visits, glaucoma procedures, and loss of productivity. [159] In Canada, the treatment of glaucoma is estimated to be around C$300 million annually. [160] Not only does glaucoma pose a significant economic burden, but it is also associated with reduced quality of life and is a significant predictor of depression and other comorbidities. [161, 162]

Broadly, glaucoma can be classified into two categories, open angle glaucoma (OAG) or angle closure glaucoma (ACG), based on the anatomy of the anterior segment (Appendix 1).[163-165] Normally, aqueous humour is produced by the ciliary body, located behind the iris. The fluid then flows through the pupil into the anterior chamber and exits the eye through the trabecular meshwork at the angle formed between the iris and the cornea. In OAG, there is increased resistance to aqueous outflow within the trabecular meshwork, whereas in ACG, the angle becomes blocked due to the apposition of the iris or the lens. Typically, these disruptions in aqueous flow cause an increase in intraocular pressure (IOP), which is indeed the only modifiable factor for glaucoma targeted by treatment. [166] That being said, normal tension glaucoma (NTG), where the angle is open and IOP is normal, can occur. [167]

NTG, OAG, and chronic ACG are characterized by progressive degeneration of the optic nerve with apoptosis of retinal ganglion cells (RGC) leading to thinning of the retinal nerve fiber

layer (RNFL). [167] This is typically seen as rapid progressive cupping of the optic disc which is often measured as the cup-to-disc ratio (CDR); the average CDR of a normal eye is around 0.3 to 0.5, and higher CDRs can be indicative of glaucomatous damage. [164] Early glaucoma can be asymptomatic and remain undiagnosed until bilateral vision loss is noticed by the patient. [168, 169] As the disease progresses, irreversible visual field loss begins in the mid-periphery, progressing until only a small central or peripheral area of vision remains if left untreated. [164] Patients may also experience reduced contrast sensitivity, impaired colour perception, and difficulty reading.

Many risk factors have been associated with the development of the disease. Glaucoma is consistently associated with older age, Black race, elevated IOP, family history of glaucoma, and high myopia. [170-174] Mixed evidence exists for other risk factors including diabetes and migraine. Leske *et al* also showed that thinner central corneal thickness may be a predictive factor for glaucoma and its progression in those with ocular hypertension.[172]

While the causes of glaucoma are not fully understood, various hypotheses have been proposed to explain its occurrence and progression. For example, the mechanical theory of glaucoma suggests that elevated IOP compresses the optic nerve at the lamina cribrosa, causing mechanical strain on RGC axons and subsequently signalling cell apoptosis. [166, 175] While evidence for this theory exists, it does not explain all cases of glaucoma as some patients with elevated IOP do not develop the disease, some glaucoma patients do not have elevated IOP, and glaucoma can progress even after controlling IOP. Thus, pressure independent hypotheses have emerged including vascular, genetic, glymphatic, biochemical, autophagy dysregulation, and translaminar pressure. It is likely that a combination of these mechanisms contribute to the pathogenesis of glaucoma since no one theory fully explains its development. [176] For the purpose of this thesis, the vascular hypothesis of glaucoma will be elaborated.

Vascular Hypothesis of Glaucoma

The vascular theory of glaucoma postulates that RGC loss may occur due to impaired ocular blood flow from increased IOP or impaired autoregulation from vascular deregulatory factors. [177, 178] Vascular dysregulation and fluctuating vessel perfusion induce oxidative stress, mitochondrial dysfunction, ischemia, and ultimately, cell apoptosis at the optic nerve head. [178, 179] However, some controversy remains since reduced ocular blood flow may be a secondary consequence of RGC loss – less blood flow is required to satisfy the metabolic needs of fewer cells. [176] Therefore, more longitudinal studies are needed to determine if vascular changes precede the development of glaucoma or are a consequence of the disease process.

Supporting the vascular theory, numerous studies have linked blood pressure dysregulation with glaucoma. Separate systematic reviews indicated that reduced ocular perfusion pressure and dips in nocturnal blood pressure were associated with glaucoma [179] and its progression, [180] respectively. Other meta-analyses have revealed a 1.71-fold increased risk of glaucoma in hypertensive individuals [44] and a positive association between higher IOP and blood pressure. [45] Furthermore, one mendelian randomization study demonstrated a causal effect between high blood pressure and both RNFL thinning (a clinical sign of glaucomatous damage) independent of IOP, in addition to elevated IOP itself. [181] Therefore, dysregulation of blood pressure may certainly be a contributing pathogenic factor for glaucoma.

Beyond blood pressure, glaucoma has been associated with cardiovascular diagnostic signs and conditions. For example, in a recent analysis of data from the Vitamin D Assessment cohort, increased arterial stiffness predicted the 10-year incidence of glaucoma (HR= 1.40, 95%CI: 1.14-1.71). [182] Additionally, in a retrospective chart review including 540 eyes, those with a history of

cardiovascular disease had higher odds of developing rapidly progressive glaucoma in comparison to those without cardiovascular disease.[183] Reciprocally, having glaucoma has been associated with an increased risk of developing ischemic heart disease, coronary artery disease, myocardial infarction, and stroke. [46-48] Lastly, Funk *et al* noted that patients from the Mayo Clinic with low-tension glaucoma had a higher odds of having various systemic vascular conditions including diabetes, migraine, anemia, and Raynaud's syndrome.[184] Glaucoma and cardiovascular disease share several risk factors, potentially contributing to findings across published literature. [46]

Various properties of retinal vessels may be implicated in the vascular hypothesis of glaucoma. With the development of modern imaging technologies and software to analyze retinal images, links between glaucoma and specific retinal vessel characteristics have been identified and will be discussed below.

Cross-Sectional Findings:

Cross-sectional associations between retinal vessel diameter and glaucoma have been indicated in the literature. Narrower arterioles [185-188] and venules [185, 186, 188] have been associated with OAG in several studies. The Blue Mountains Eye Study showed that the narrowest quintile of CRAE was associated with a higher odds of having glaucomatous damage as indicated by visual field defects and optic disc cupping. [189] However, this association was only significant in those older than 65 years of age, in those with diabetes, and stronger in those with a history of smoking.[189]

As a significant risk factor for the development and progression of glaucoma, the relationship between IOP and retinal vessels has also been investigated. Amerasinghe *et al* observed that narrower venules corresponded with ocular hypertension (IOP > 21 mmHg) in eyes

without diagnosed glaucoma. [186] Only arteriolar narrowing was associated with increased IOP in a study by Freiberg *et al*. [190] This is only partially supported by findings from the WESDR, where those with higher IOP had narrower CRVE and wider CRAE. [21] In contrast, vessel width was not associated with ocular hypertension in a study by Rudnicka *et al*, [188] nor with IOP in a study by Klein *et al*.[56] Thus, while some evidence suggests a relationship between retinal vessel narrowing and elevated IOP, these findings have not been consistently reported.

Indicators of optic nerve health have also been associated with retinal vessel diameter. Narrower arterioles and venules have been associated with thinner RNFL thickness [99, 191, 192] and reduced neuroretinal rim area [193, 194] in children and adults. Horizontal cup diameter, cup area and volume, and the average nerve width were also positively associated with retinal venular diameter in 12 year old Australian adolescents. [194]

In terms of tortuosity, straighter arterioles were associated with glaucoma and elevated IOP in the Singapore Malay Eye Study. [195] Adults with thinner neuro-retinal rims and thinner ganglion cell layer thickness also tend to have less tortuous vessels. [99, 108] These studies are in contrast to the null results reported by Rudnicka *et al* in their analysis of the EPIC-Norfolk eye study; vessel tortuosity was not associated with glaucoma nor ocular hypertension ($P>0.05$). [188]

Longitudinal Findings:

There are very few longitudinal studies assessing the association between retinal vessel characteristics and the incidence of glaucoma. Two studies reported conflicting results, [57, 58] and only one study has investigated longitudinal changes in vessel characteristics in relation to IOP. [190]

For example, Kawasaki *et al* investigated the association between retinal vessel diameter and the 10-year incidence of primary OAG using data from 2417 participants in the Blue Mountain

Eye Study.[58] After adjustment for age, sex, family history of glaucoma, smoking, diabetes, hypertension, hypercholesterolemia, body mass index, spherical equivalent refraction, and cup-to-disc ratio, people with narrower CRAE had a higher 10-year incidence of glaucoma (OR per SD decrease = 1.77, 95% CI:1.12, 2.79). This was consistent even in subgroup analysis of eyes with low OAG risk (IOP <20 mmHg or CDR<0.6). Hypertension status did not modify this relationship. CRVE was not associated with the 10-year incidence of glaucoma (OR per SD decrease = 1.33, 95% CI: 0.86, 2.06).

In contrast, in an analysis of 3469 participants aged 55 years or older with a mean follow-up time of 6.5 years, Ikram *et al* found that neither arteriolar diameter (OR per SD decrease = 0.82, 95% CI: 0.66, 1.03) nor venular diameter (OR per SD increase = 1.20, 95% CI: 0.95, 1.53) were statistically significantly associated with incident OAG. [57] This was following adjustment for age, gender, follow-up time, diabetes, smoking, total-to-HDL cholesterol ratio, mean perfusion pressure, and intima media thickness. Baseline retinal vessel diameters did not predict changes in any optic disc metrics, including cup area, rim area, or vertical CDR.

Freiberg *et al* investigated the relationship between IOP with retinal vessel diameter and tortuosity after 3 to 6 years of follow-up. [190] In the analysis of data from 2089 participants of the Danish eye and vision cohort, elevated IOP at follow-up was associated with widening of arterioles, but not with changes in venular diameter. In addition, increased IOP at follow-up was associated with an increase in arteriolar tortuosity above baseline levels, and having higher IOP at baseline was associated with more tortuous venules at follow-up.

While some cross-sectional and longitudinal studies have suggested a role of vessel narrowing in the development of glaucoma and its associated signs, the published literature has generally been inconsistent, warranting further investigation.

What is Age-Related Macular Degeneration?

AMD is a leading cause of blindness in developed countries, particularly among people older than 65, and accounts for 8.7% of all blindness worldwide. [196] Estimates suggest that the total prevalence of AMD globally is approximately 8.69% and varies by age and ethnicity. [59] Projections indicate this number will rise to 300 million by 2040, driven by exponential aging of the world's population. [59] In Canada, AMD prevalence ranges between 3.2% - 8.7%, [158, 197] with an estimated incidence of 1.68%. [198] Canadian projections align with global trends, predicting an increase in prevalence to 10.9% by 2050. [197]

AMD imposes a significant burden on individuals and society alike. Increased rates of depression and anxiety are common among those with AMD, likely due to its chronic and progressive nature. [199, 200] In 2005, the economic impact of AMD in Canada, including unemployment and treatment costs, was estimated at $2.6 billion annually.[201] With an aging demographic, prevention of AMD is becoming increasingly important to limit morbidity.

AMD is a progressive dysfunction and degeneration of the photoreceptor cells in the macula region of the retina and is broadly categorized into early, intermediate, or late-stage AMD.[202] Although the development of drusen can be part of the normal aging process, it is also the hallmark sign of AMD-related change. Drusen appear as small yellow lesions that are an accumulation of debris (lipids, glycoproteins, immune components) excreted by the retinal pigment epithelium (RPE) and remain trapped between the RPE and Bruch's membrane. [65, 203] Early AMD is characterized by small to medium drusen (63-125 μm) with no visible retinal pigmentary changes. [202] In intermediate AMD, large drusen (>125 μm) are accompanied by retinal

pigmentary changes, such as non-geographic atrophy, hyperpigmentation, or RPE granularity, that can be visualized due to migration of RPE cells. [202, 203]

Advanced, or late stage, AMD can develop in one of two different forms: proliferative/neovascular (wet) AMD or non-proliferative (dry) AMD. [203] In those with non-proliferative AMD, areas of cell death in the choroid, photoreceptor layer, and RPE develop, and as they widen, are diagnosed as geographic atrophy. [75, 203, 204] Symptoms of dry AMD are chronic and progressive, ranging from no visual impairment to reduced central vision, general blurriness, decreased vision at night, visual distortions, and faded colour vision. [200, 202] Meanwhile, in neovascular AMD, hypoxia and oxidative stress are thought to cause upregulation of vascular endothelial growth factor, encouraging growth of abnormal choroidal or retinal blood vessels into the subretinal space. Subsequently, areas of fluid leakage, hemorrhage or lipid exudation develop. [75, 203] This is associated with rapid vision loss, as macular edema and intraocular hemorrhage can have significant impacts on the central vision. [202] Visual distortions are also commonly reported among wet AMD patients. [202]

No effective treatment for dry AMD has been found to date. However, changes in lifestyle such as nutritional supplements and smoking cessation have been recommended to prevent progression of the disease. [205] Meanwhile in neovascular AMD, injections of anti-vascular endothelial growth factor are the current gold standard treatment that provide the highest chance of improving visual acuity. [203]

AMD has several known risk factors that may contribute to its development. AMD is consistently associated with older age, family history of the disease, and previous cataract surgery.[206] AMD is more prevalent among people with European ancestry than in those with African ancestry [59, 206] and several major genes have been identified as risk factors for AMD.[207]

Smoking is a strong predictor of AMD development with an established dose-response among various studies. [50, 208-211] Adverse effects of being overweight or obese in addition to having higher levels of serum cholesterol have been related to AMD. [209, 212-215] Alcohol consumption has been related to the presence of soft drusen and AMD. [206, 209, 216, 217] Diabetes may be associated with late AMD, but the evidence is inconsistent among different study designs. [206]

AMD is a complex disease and the biological mechanisms leading to its development are not well understood. Generally, AMD development and progression are attributed to a multifactorial pathogenesis involving the vasculature and choriocapillaris (described below), and genetics, oxidative stress, and inflammatory factors.

Vascular Hypothesis of AMD

The modern hemodynamic theory of AMD was first proposed by Friedman in 1997. [218] Inspired by the mechanisms underlying atherosclerosis, his model hypothesized that lipid deposition causes increased resistance in the short posterior ciliary arteries, which supply the choroid. This may result in either decreased choroidal perfusion or an increase in choriocapillary pressure. The choroid supplies the outer layers of the retina; thus, in reducing the choroidal perfusion, RPE metabolism is impaired leading to cell atrophy. Meanwhile, an increase in choriocapillary pressure may reduce the rate of cellular byproduct removal, leading to the formation of drusen. This theory has recently been revised by Gelfand and Ambati in 2016. [219] They hypothesized that drusen accumulate as a result of local hemodynamic parameters within the choriocapillaris, such as impaired heat dissipation, shear stress, and endothelial dysfunction, which then determine the progression towards geographic atrophy or neovascular AMD.

These theories have been extended to the retinal circulation, where inhibition of retinal vessel perfusion may also contribute to the pathogenesis of AMD. As such, prevalence and incidence of AMD or AMD-related changes in the retina have been associated with high blood pressure, [209, 212] vascular stiffness, [220] carotid plaques, [221, 222] and cardiovascular disease. [49-51, 213, 223] A potential hemodynamic pathogenesis has also been supported by results showing that the presence of the cilioretinal artery, which is present in about 20% of people and is thought to provide an alternative route for blood supply from the choroid, is associated with a lower risk of developing choroidal neovascularization. [224, 225]

However, it should be noted that the causal association between cardiovascular disease, changes in the retinal vasculature, and AMD have yet to be fully understood. While the presented mechanisms would suggest AMD develops due to initial disruptions of the vasculature, Friedman also noted that changes in the choroid occasionally occur secondary to changes in the RPE, suggesting that the tissues rely on each other to remain fully functional. [218] Furthermore, some studies have found that AMD may precede cardiovascular diseases. [226, 227] There is contrasting evidence associating changes in the retinal microvasculature with AMD among several cross-sectional and longitudinal studies.

Cross-Sectional Findings

Cross-sectional studies examining the relationship between retinal vessel traits and AMD have been inconsistent. Some studies reported null associations between AMD and vessel diameter. [228-230] Meanwhile, others have shown vessel narrowing to be associated with AMD, as exemplified by Toulouie *et al*, who found that those with narrower CRAE were more likely to have central geographic atrophy. [225] Similarly, Klein *et al* showed that exudative AMD was linked with

focal arteriolar narrowing. [56] Furthermore, narrower arteriolar diameter has been linked with changes in retinal pigmentation, an early feature of AMD, in several studies. [56, 222, 231] However, other studies have reported contrasting findings. Wider CRAE corresponded with early AMD and the presence of drusen in the Handan Eye Study, [232] and wider CRVE was associated with early AMD in the Singapore Malay Eye Study. [231] Additionally, a study conducted in participants with human immunodeficiency virus found that both CRAE and CRVE were wider in those with AMD in comparison to those without. [233] Thus, the literature presents mixed evidence, with both wider and narrower vessels being cross-sectionally implicated in AMD.

To the best of our knowledge, no published studies have examined the association between AMD and retinal vessel tortuosity. However, conference proceedings using data from the Singapore Malay Eye Study suggested that more tortuous venules were weakly associated with signs of early AMD, even after adjustment for cardiovascular risk factors. [234] While these findings have not undergone peer review, they highlight the potential of venular geometry in AMD pathogenesis and warrant further investigation.

Longitudinal Findings

Six longitudinal studies have investigated the association between retinal vascular diameter and AMD. Five of these studies found no statistically significant associations between vessel diameters and incident AMD itself. [52-56] However, most of these studies reported statistically significant associations between arteriolar narrowing and other AMD-related signs.

Using data from the Blue Mountain Eye Study, there was no statistically significant association between baseline CRAE and the 5-year incidence of late or early-stage AMD. [52] However, focal retinal arteriolar narrowing was associated with the incidence of early-stage AMD

(OR=2.0, 95%CI: 1.2-3.2). The risk of developing late-stage AMD lesions (geographic atrophy or neovascularization) was higher in those with focal arteriolar narrowing at baseline after adjustment for age, but this association did not persist after adjustment for sex, smoking, and mean arterial blood pressure. This study was limited in the number of incident late-stage AMD cases.

In a more recent analysis of the BMES, Liew *et al* assessed the relationship between baseline arteriolar or venular diameter and the 10-year incidence of early or late AMD. [53] They found no statistically significant associations with CRAE or CRVE but confirmed that incident AMD was associated with focal arteriolar narrowing. On stratified analysis, the incidence of late-stage AMD was statistically significantly associated with focal arteriolar narrowing in those with hypertension, as opposed to those without. Additionally, an increased risk of 10-year incident pigment abnormalities (early AMD) was associated with both narrower arteriole and venule diameters.

Null results were found using data from the Rotterdam Study, where vessel diameter was not associated with incident AMD, progression of early to late AMD, nor the incidence of AMD-specific lesions. [54] However, in age stratified analysis, arteriolar narrowing did predict AMD in those 75 years of age or older.

The Beijing Eye Study was a prospective population-based study carried out in Northern China. [55] Diameters of the temporal and nasal, superior and inferior, vein and artery were not statistically significantly associated with incident early AMD ($P>0.05$) in unadjusted analysis. There was also no association found with general nor focal arteriolar narrowing ($P>0.05$). Most people in this study were under the age of 70, potentially resulting in limited numbers of AMD cases and statistical power. The authors also mention the potential of selection bias leading to

underestimating AMD incidence; participants who were excluded at follow-up had lower visual acuity at baseline which may have been due to having AMD.

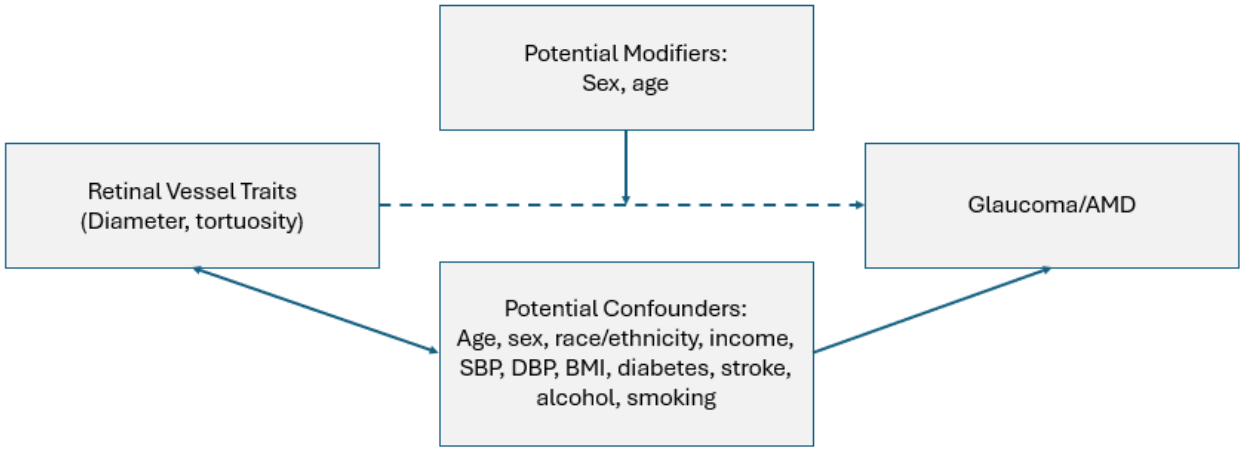
In a recent secondary analysis of the prospective Age-Related Eye Disease Study data, Toulouie *et al* assessed the association between several AMD outcomes and retinal vessel characteristics. [225] Their results indicated that those with narrower arterioles, in terms of both CRAE (β=-0.026, 95% CI: -0.045 to -0.007, P=0.007) and AVR (β=−5.794, 95% CI: -11.48 to -0.112, P=0.046), had higher incidence of central geographic atrophy. Lower AVR was also associated with greater AMD severity at baseline in multiple regression models. However, 5-year progression of AMD severity score, progression to choroidal neovascularization, and progression to central geographic atrophy were not associated with arteriolar nor venular diameter.

Similarly, in an analysis of data from the Beaver Dam Eye Study, CRAE was not cross-sectionally associated with any signs of, or diagnoses of, early or late AMD. [56] Focal arteriolar narrowing was associated with increased retinal pigment (OR=1.47, P=0.002), RPE depigmentation (OR=1.51, P=0.01), late AMD (OR=2.72, P<0.001), geographic atrophy (OR=1.93, P=0.10) and exudative AMD (OR=3.31, P<0.001) at baseline. Wider CRAE was also associated with 10-year incidence of RPE depigmentation, but not AMD itself. Associations remained similar after adjustment for smoking status, history of heavy drinking and vitamin use, and mean average blood pressure.

Overall, the published literature is inconsistent. Both narrowing and widening of vessels have been cross-sectionally linked with AMD. Longitudinal studies generally confirm the lack of an association between vessel diameter and incident AMD but highlight the potential role of arteriolar narrowing in predicting early AMD-related signs. Largely, evidence remains inconclusive regarding the role of retinal vessels in AMD.

Chapter 4: Conceptual Framework

Figure 4.1: Conceptual framework diagram for objectives 2 and 3



Objective 1 was descriptive in nature so we will only show a conceptual framework for Objectives 2 and 3 (Figure 1). The exposure of interest was the baseline retinal vessel trait (diameter or tortuosity). The outcomes at baseline were glaucoma, glaucoma-related outcomes (IOP or CDR), or macular degeneration. We also assessed 3-year development of the two eye diseases and 3-year change in IOP and CDR. Based on the literature review and methods used in previous studies, we selected potential confounders that are known risk factors for the diseases and have been associated with retinal vessel traits in prior research. Thus, the confounders we included were age, sex, ethnicity, income, SBP, DBP, BMI, diabetes, stroke, alcohol, and smoking. We do not believe that any of these variables act as mediators or colliders. We also assessed age and sex as potential effect modifiers by including interaction terms (e.g. sex*vessel diameter) in the statistical models and conducting stratified analyses.

Chapter 5: Data Source

The Canadian Longitudinal Study on Aging (CLSA) is a nationwide, prospective cohort study that investigates how biological, psychological, environmental, and social factors shape the trajectory of health and aging. [235] Research using data from the CLSA aims to enhance the health and quality of life of aging Canadians by identifying strategies for disease prevention and improving health services.

This thesis utilizes baseline and first follow-up data from the Comprehensive Cohort of the CLSA, which is comprised of 30,097 Canadians aged 45-85 years. [236] Data collection involved in-home interviews supplemented by physical assessments conducted at one of the Data Collection Sites (DCS) in 11 cities across Canada: Victoria, Surrey, Calgary, Winnipeg, Hamilton, Ottawa, Montreal, Sherbrooke, Halifax, and St. John's. The CLSA follow-up period will ultimately span over 20 years, with data collected every three years. Baseline assessments were completed between 2012 to 2015, while data was gathered between 2015 to 2018 for the first follow-up.

Sampling and Recruitment

The CLSA used two different sampling frames for the recruitment of participants into the Comprehensive Cohort: provincial health registration databases, and random digit dialling (RDD) of landline telephones. [237, 238] Due to unique differences across provincial databases, participants from Alberta and Quebec were recruited entirely through RDD. Eligible participants residing within 25-50km of a DCS were identified and randomly selected from provincial databases. For RDD, a random sample of landline phone numbers was chosen within the specified geographic areas and one individual from each eligible household was randomly invited to participate.

Sampling was stratified by province, age, and sex, resulting in 56 strata total. Selection probabilities were varied by strata to ensure the targeted number of participants were recruited in each group. [236, 238] Following preliminary analysis, data from the 2006 census was used to identify neighbourhoods of low socio-economic status which were then oversampled to ensure those groups were properly represented.

Sampling and Analytic Weights

Both analytic and sampling weights were calculated. [238] The sampling weights were calibrated by self-reported education, age, sex, and DCS location to account for the socio-demographic characteristics of the target population. The analytic weights were scaled to the provinces since the sampling rates were smaller in the larger provinces.

Response Rates

Of those initially contacted to participate in the CLSA, 45% provided consent for further contact. [238] However, only 10% completed the baseline assessment, which included both the in-home interview and a DCS visit. At the first follow-up, the overall retention rate was 94.5%, with 1.8% of Comprehensive Cohort participants having passed away since the baseline assessment. [236]

Ethics Approval

Written informed consent was obtained from participants during recruitment. Research Ethics Board approval was acquired for all CLSA sites in July 2010. The University of Ottawa Office of Research Ethics and Integrity gave approval for the present analysis in January 2023 (H-01-23-8331).

Chapter 6: Manuscript One

Preface to the Manuscript

Purpose: Answer thesis objective one.

Required Ethics Approval: After review by the University of Ottawa Office of Research Ethics and Integrity, approval was received January 2023 (H-01-23-8331).

Title: Factors Associated with Retinal Vessel Traits in the Canadian Longitudinal Study on Aging

Authors: Alexis O'Neil, Roshan A. Welikala, Sarah Barman, Christopher G. Owen, Alicja R. Rudnicka, Mohan Rakesh, Marie-Hélène Roy-Gagnon, David Maberley, Ellen E. Freeman

Authors and their Contributions:

- AO contributed to the analyses, interpretation of the data, drafting the manuscript and revision.
- RA, SB, CGO and ARR oversaw the development and use of QUARTZ on CLSA data and contributed to interpretation of the results and revision of the manuscript.
- MR and MHRG assisted with creating summary measures of the QUARTZ output, contributed to interpretation of the data and revision of the manuscript.
- DM contributed to interpretation of the data and revision of the manuscript.
- EEF contributed to the acquisition of funding, data analysis, and interpretation of the data and the revision of the manuscript.
- All authors gave final approval for the version published and agree to be accountable for all aspects of the work.

Publication Status: Published by Investigative Ophthalmology and Visual Science (DOI: 10.1167/iovs.66.3.13).

Factors Associated with Retinal Vessel Traits in the Canadian Longitudinal Study on Aging

Alexis O'Neil[1], Roshan A. Welikala[2], Sarah Barman[2], Christopher G. Owen[3], Alicja R. Rudnicka[3], Mohan Rakesh[1], Marie-Hélène Roy-Gagnon[1], David Maberley[4], Ellen E. Freeman[1,4,5]

[1] School of Epidemiology and Public Health, University of Ottawa, Ottawa; [2] School of Computer Science and Mathematics, Kingston University London, UK; [3] Population Health Research Institute, St George's School of Health and Medical Sciences, City St George's, University of London, London, UK; [4] Department of Ophthalmology, University of Ottawa, Ottawa; [5] Ottawa Hospital Research Institute, Ottawa

Running Title: Factors Associated with Retinal Vessel Traits

Abstract (250) & Main Text (3162) Word Count

Number of Tables (4) & Figures (3)

Funding details: This work was supported by the CIHR under Grant PJT-183690

Commercial Relationships Disclosure: A O'Neil, none; RA Welikala, none; S Barman, none; CG Owen, none; AR Rudnika, none; M Rakesh, none; MH Roy-Gagnon, none; D Maberley, none; EE Freeman, none

Corresponding Author:

Ellen Freeman, 600 Peter Morand Crescent, Office 301H, School of Epidemiology and Public Health, University of Ottawa, Ottawa, Canada, eefreeman@gmail.com

ABSTRACT

Purpose: To determine the factors cross-sectionally and longitudinally associated with retinal vessel diameter, total area, and tortuosity in the Canadian Longitudinal Study on Aging (CLSA).

Methods: Of the 30,097 adults between ages 45 and 85 years old in the CLSA Comprehensive Cohort, 26,076 had at least one retinal image gradable by QUARTZ, a deep learning algorithm that automatically assessed image quality, distinguished between arterioles and venules, and estimated retinal vessel traits over the entire retina. Questions were asked about demographic, lifestyle, and medical factors. Blood pressure, cholesterol, and C-reactive protein were measured. Participants returned for follow-up three years later. Multiple linear regression was used to provide adjusted estimates.

Results: Current smoking was strongly associated with wider arteriolar and venular diameters and their widening over 3 years ($P<0.05$). Current smoking was also associated with a larger arteriolar and venular area and a 3-year increase in venular area ($P<0.05$). Obesity was positively associated with venular diameter, total venular area, 3-year change in total venular area, and venular tortuosity ($P<0.05$). Diastolic blood pressure was negatively associated with both arteriolar and venular diameter, area, and tortuosity, both cross-sectionally and longitudinally ($P<0.05$). Diabetes was associated with wider arteriolar diameters cross-sectionally, and type 1 diabetes was associated with 3-year widening of arteriolar diameters ($P<0.05$).

Conclusions: This work provides comprehensive information on the factors associated with retinal vessel traits and their change. Factors like smoking, obesity, blood pressure, and diabetes were longitudinally related to retinal vessel traits, which play a role in the development of eye disease.

INTRODUCTION

Modern digital fundus photography and image analysis software provide a unique, non-invasive opportunity to visualize and obtain objective measurements of the retinal vasculature. [1-3] Retinal arterioles and venules are believed to reflect the health of the systemic microcirculation, which may play a key role in the development of many ocular and systemic diseases. [4,5] Therefore, the retinal vasculature may provide a valuable method for assessing the overall health of the microvascular system by enabling the detection of structural and pathological changes.

The retinal vasculature is thought to be affected by aging, hypertension, inflammation, arteriosclerosis, and endothelial dysfunction although the full extent of factors is still under investigation. [6] Many prior epidemiological studies examining factors associated with retinal vessel traits in adults have been cross-sectional. [6-9] These studies have indicated that retinal arteriolar and venular diameter have distinct factors associated with them indicating the importance of examining them separately. [6] For example, Tapp *et al* found that body mass index (BMI) and other measures of adiposity were associated with arteriolar and venular diameter in opposite directions. [10] Racial and ethnic differences in vessel diameter have been identified. [6] However, most studies only had a few racial and ethnic groups that could be examined at a time.

While many studies have focused on retinal vessel diameter, other parameters such as tortuosity and area have been less frequently researched. Prior research has reported an association between systolic blood pressure and venular area. [11] Retinal vessel tortuosity, or curvature, can indicate the health of the vasculature by providing insight into hemodynamics and perfusion. Its associated factors include age, sex, blood pressure, and body mass index. [9,10]

The Canadian Longitudinal Study on Aging (CLSA) is a population-based study that collected comprehensive health related data from a large cohort of Canadians and is comprised of

many racial and ethnic groups. [12] Despite the abundance of retinal images collected by the study, these data have been underutilized by researchers. To address this, we examined the factors associated with retinal vessel diameter, total area, and tortuosity, as well as their changes over a three-year period in a Canadian population.

METHODS

Study Population and Design

We conducted both cross-sectional and longitudinal analyses using data from the Canadian Longitudinal Study on Aging (CLSA) Comprehensive Cohort. [12] This study includes 30,097 Canadian adults aged 45 to 85 years, recruited through random sampling of provincial healthcare databases and random digit dialing of landline phones. Baseline data were gathered between 2012 and 2015 through in-home interviews and physical assessments at CLSA Data Collection Sites (DCS) in cities across Canada, including Victoria, Vancouver, Surrey, Calgary, Winnipeg, Hamilton, Ottawa, Montreal, Sherbrooke, Halifax, and St. John's. Follow-up data were collected in the same manner three years later, between 2015 and 2018.

Participants were eligible if they were aged 45-85 years, lived in the community, spoke English or French, and resided within 25-50 km of a data collection site. Exclusion criteria included cognitive impairment, full-time membership in the Canadian Armed Forces, living on a federal First Nations reserve or settlement, residing in a long-term care facility, or not being a permanent resident or citizen. Written informed consent was obtained for all participants. Research Ethics Board approval was acquired for all CLSA sites in July 2010. The University of Ottawa Office of Research Ethics and Integrity gave approval for the present analysis in January 2023 (H-01-23-8331).

Data Collection

Retinal images

Nonmydriatic retinal images were taken in each eye at both baseline and follow-up visits using a Topcon TRC-NW8 fundus camera with the Nikon D90 camera attached. Images were macular-centered and had a 45-degree field-of-view. Most images were saved in JPEG format with a resolution of 4288 x 2848 pixels. Images from one site were saved with a resolution of 4928 x 3264 pixels. These were resized to a resolution of 4288 x 2848 pixels.

Retinal vessel traits

Retinal images were processed using a deep learning algorithm called QUARTZ (Quantitative Analysis of Retinal Vessel Topology and Size). [1,13] Within the QUARTZ vessel analysis module, [1] vessel width was measured using zero-crossings of the second derivative and tortuosity was measured using a subdivided chord length method. The methods used within the vessel analysis module have been previously well documented and validated. [13] QUARTZ measures vessels across the inner retinal layer (supplied by the ophthalmic artery via the central retinal artery) contained in fundus images, which include arterioles, pre- and post-capillaries, and venules. On the CLSA data, QUARTZ can determine inadequate image quality with a sensitivity of 91% and a specificity of 100%. QUARTZ can also distinguish between an arteriole and a venule with 88% accuracy, increasing to 96% using a probability cutoff of 0.8. [1] QUARTZ obtained thousands of measures of diameter and tortuosity from across the entire retinal image. The mean diameter, area, and tortuosity of each arteriole and venule were then summarized weighted by the vessel segment length for each image. Area was calculated from the average vessel width

multiplied by length, summed across vessel segments. Diameter and area are given in pixels and pixels^2, respectively, while tortuosity is a unitless measure.

Demographic, health, and lifestyle data

Demographic data including age, sex, income, and race/ethnicity were collected at baseline during the in-home visit using the interviewer administered questionnaire. Income was assessed by asking participants "What is your best estimate of the total household income received by all household members, from all sources, before taxes and deductions, in the past 12 months?". Participants were grouped into 5 categories: >$150 K, $150-$100 K, $100-$50 K, $50 K-$20 K, Refused.

Smoking status was assessed by asking participants "Have you smoked at least 100 cigarettes in your life?" and "At the present time, do you smoke cigarettes daily, occasionally (at least once in last 30 days), or not at all (not in last 30 days)?". We defined current smokers as those who had smoked at least 100 cigarettes and currently smokes daily or occasionally. Former smokers were those who reported smoking at least 100 cigarettes in life but had not smoked in the last 30 days. Frequency of alcohol consumption was similarly assessed by asking participants "About how often during the past 12 months did you drink alcohol?". We categorized responses into never, occasionally (zero to three times a month), weekly (between one and five times a week), or daily (six or more times a week).

During the DCS interviewer-led questionnaire, participants were asked to report doctor diagnoses of several health conditions including heart disease (yes, no), stroke (yes, no) or diabetes (none, Type 1, Type 2, or neither). Height and weight were measured using standardized procedures during the DCS visit. Body mass index (BMI) was calculated as weight (kg)/height

squared (m^2) and classified according to the World Health Organization categories (underweight <18.5 kg/m^2, normal weight 18.5–24.9 kg/m^2, overweight 25.0–29.9 kg/m^2, and obese ≥30.0 kg/m^2). Blood pressure was measured using the VSM BpTRU monitor (Medaval, Dublin, Ireland). Six blood pressure measurements were acquired, and an average of measurements two through six was used.

Blood tests

Non-fasting venipuncture blood was collected from each participant during the DCS visit and analysed at each DCS laboratory. Several soluble and cellular biomarkers were measured in serum including total cholesterol and the concentration of high-sensitivity C-reactive protein (hs-CRP) using the Cobas 8000 modular analyzer (Roche Diagnostics).

Statistical Analysis

The normality of the retinal vessel outcome measures was assessed using standardized normal probability plots. Diameter and area were normally distributed while tortuosity measures were multiplied by 1000, and the natural log was taken to achieve normality. Multiple linear regression models were used to examine the associations with each retinal vessel trait. Both baseline and 3-year changes in retinal vessel traits were examined. Change models were adjusted for the baseline value of the retinal vessel trait. Regression models were run on right eyes since the sample sizes were slightly bigger for right eyes. The complex survey design (weights and strata) was accounted for in all analyses.

RESULTS

Of the 30,097 CLSA participants, 26,076 (87%) had at least one acceptable QUARTZ image quality score and were therefore included in subsequent analyses. At Round 2, 92% returned to follow-up. Those with an acceptable QUARTZ quality score were compared to those without acceptable scores in Table 1. Those with an acceptable score were substantially younger and less likely to have diabetes than those who were excluded. Also, shown in Table 1 are the descriptive statistics for the retinal vessel traits for right and left eyes to show their similarity. Values for right and left eyes were highly correlated ($r>0.60$). Only right eyes were used in subsequent analyses.

Using linear regression, variables in cross-sectional analyses which showed statistically significant positive associations with arteriolar diameter included (Table 2): older age, female sex, Black race, Southeast Asian, South Asian, Arab and West Asian, Latin American, Other, and Mixed ethnicities (compared to White race), lower income, smoking, and diabetes ($P<0.05$). For example, Type 1 diabetes was associated with greater arteriolar diameter compared to people without diabetes ($\beta=0.67$, 95% CI 0.31, 1.04) (Table 2 and Figure 1). Negative statistically significant associations were found with diastolic blood pressure ($P<0.05$). For example, each 10mmHg increase in diastolic blood pressure was associated with a narrower arteriolar diameter ($\beta=-0.34$, 95% CI -0.39, -0.29). Weekly and daily drinkers of alcohol had thinner arterioles than never drinkers ($P<0.05$). Similar but fewer positive statistically significant associations were found with venular diameter (Table 2) including: older age, female sex, Black race and Latin American ethnicity (compared to White race), lower income, obesity (Figure 2), and smoking while a negative statistically significant association was found with diastolic blood pressure (Figure 3) ($P<0.05$).

There were fewer associations when examining vessel diameter using the longitudinal data (Table 2). Older age, female sex, current smoking, and Type 1 diabetes were positively statistically

significantly associated with 3-year change in arteriolar diameter while diastolic blood pressure was negatively associated ($P<0.05$). For example, Type 1 diabetes was associated with a 3-year increase in arteriolar diameter compared to people without diabetes ($\beta=0.45$, 95% CI 0.09, 0.81). Similarly, older age and former and current smoking were positively statistically significantly associated with 3-year change in venular diameter while diastolic blood pressure was negatively associated ($P<0.05$).

There was a statistically significant positive association between arteriolar area and current smoking status, cholesterol, and hs-CRP ($P<0.05$) (Table 3). For example, higher hs-CRP levels were associated with a greater arteriolar area ($\beta=0.18$, 95% CI 0.01, 0.36). Older age, female sex, Black race, East Asian, Southeast Asian, and South Asian ethnicities (compared to White race), low and missing income (compared to high income), systolic blood pressure, diastolic blood pressure, and daily alcohol use (compared to never drinkers) were negatively associated with arteriolar area ($P<0.05$). Like arteriolar area, venular area was negatively associated with older age, female sex, South Asian ethnicity, and systolic blood pressure ($P<0.05$). In contrast, venular area was positively statistically significantly associated with the following variables: Arab and West Asian and Latin American ethnicities (compared to White race), overweight and obesity, former and current smoking, hs-CRP and cholesterol ($P<0.05$).

Using longitudinal data, arteriolar area had very few statistically significant associations (Table 3). Three-year change in arteriolar area was negatively associated with older age and higher diastolic blood pressure ($P<0.05$). Meanwhile, the 3-year change in venular area was positively statistically significantly associated with lower income, overweight and obesity, and former and current smoking ($P<0.05$). For example, obesity was associated with a 3-year increase in venular area compared to people with normal BMI ($\beta=3.52$, 95% CI 1.51, 5.51). The 3-year change in

venular area was also negatively associated with older age, female sex, East Asian ethnicity (compared to White race), underweight BMI (compared to normal), diastolic blood pressure, type 1 diabetes, and hs-CRP concentration ($P<0.05$).

In Table 4, we present associations for vascular tortuosity using both cross-sectional and longitudinal data. Arteriolar tortuosity was statistically significantly negatively associated with diastolic blood pressure, and East Asian, Southeast Asian, South Asian, Arab and West Asian, and mixed ethnicities (compared to White race), while systolic blood pressure, stroke and cholesterol were positively associated ($P<0.05$). Venular tortuosity was positively associated with older age, overweight and obesity, systolic blood pressure, current smoking status, and hs-CRP ($P<0.05$). For example, current smokers had more venular tortuosity than never smokers ($\beta=0.04$, 95% CI 0.02, 0.06). East Asian and Arab/West Asian ethnicities and diastolic blood pressure were negatively associated with venular tortuosity ($P<0.05$).

Longitudinally, arteriolar tortuosity was positively associated with female sex, Other ethnicity (compared to White race), and heart disease and negatively associated with Mixed ethnicity and missing income data (compared to high income) (Table 4) ($P<0.05$). 3-year change in venular tortuosity was positively associated with lower income, and systolic blood pressure, but negatively associated with older age, East Asian ethnicity, and diastolic blood pressure ($P<0.05$).

No statistically significant interactions by age (<65, ≥ 65) or sex were detected.

DISCUSSION

This work is one of the few very large studies to comprehensively examine risk factors for retinal vessel traits in adults using both cross-sectional and longitudinal data. We have both identified new associations and confirmed previously reported associations.

Several demographic variables were associated with the retinal vessel traits and their changes. We found that older age was associated with wider arteriolar and venular diameter and widening over the 3 years while other studies found a narrowing with age. [6] These differences may be due to the way the retinal vessels were measured. We used QUARTZ, which obtains thousands of measures of diameter from across the entire retinal image while other studies only measured vessels near the optic disk and then used models to project the diameter of the CRAE or CRVE. It's possible that the CRAE and CRVE do narrow with age but that there is a widening of the arterioles and venules more broadly across the retina. Our results are partially supported by findings from the UK Biobank using QUARTZ, which also showed that venular diameter widened with age. [9] We found that older age was associated with a reduction in retinal arteriolar and venular area, both cross-sectionally and over a three-year period, which is consistent with other research. [11,14,15]

We also saw several associations with race/ethnicity and arteriolar traits such that people from almost every other racial/ethnic group had wider, less tortuous arterioles than White people. No prior study has included as many racial and ethnic groups as the CLSA, but this finding is consistent with prior research. [6,7,16] These associations persisted after adjustment for cardiovascular risk factors like smoking, obesity, blood pressure, and diabetes. Some have proposed that racial and ethnic differences in vessel diameter could be due to measurement error due to ocular pigmentation; [17] however, differences in vessel geometry could also be due to genetic differences. [18-20] Lower incomes were also associated with wider vessels although not in longitudinal analyses. Lower income may be a marker for a less healthy lifestyle, more medical comorbidity, or living in a less healthy environment.

Lifestyle factors were also associated with retinal vessel traits. Current smoking was strongly associated with wider arteriolar and venular diameters and their widening over 3 years. Current smoking was also associated with a larger arteriolar and venular area and a 3-year widening of venular area. This is consistent with prior research. [6,21-23] Proposed biological mechanisms include impaired autoregulation of retinal vessel diameter, [24] response to nicotine-induced tissue hypoxia, [25] and inflammation. [26,27] For example, smoking is thought to cause vascular injury which elevates inflammatory markers. [28] A systematic review found that inflammatory markers like CRP are associated with wider retinal vessels. [29] By contrast, weekly and daily alcohol use were associated with narrower arteriolar diameters cross-sectionally but not longitudinally. The MESA study also showed a negative association between alcohol and arteriolar diameter. [7] Excessive alcohol consumption has been shown to constrict retinal vessels in healthy volunteers. [30] The mechanism of this constriction may be through activation of the sympathetic nervous system and its effect on vascular smooth muscle cells. [31] Obesity was positively associated with venular diameter, total venular area, 3-year change in total venular area, and venular tortuosity. Tapp et al also found a positive association between body mass index and venular diameter in the UK Biobank. [10] Several studies have found a positive association between obesity and CRVE in children, adolescents, and adults. [6,32] The effect of obesity on the eye has been described in a review. [33]

Medical factors were also associated with retinal vessel traits. Diastolic blood pressure was fairly consistently negatively associated with diameter, area, and tortuosity, both cross-sectionally and longitudinally. These results are consistent with prior literature. [6] Systolic blood pressure was associated with area and tortuosity but only cross-sectionally. The effect of hypertension on the eye has been described. [34,35] Diabetes was associated with wider arteriolar

diameters cross-sectionally while type 1 diabetes was specifically associated with 3-year widening of arteriolar diameters. This dilatation of retinal arterioles has been observed in previous studies [6] and in people with early-stage diabetic retinopathy. [36] The widening is thought to be due to changes in retinal metabolism and can lead to hyper-perfusion and tissue damage. [36] The concentration of CRP was consistently positively associated with retinal venular traits in our cross-sectional analyses but was negatively associated with venular area in longitudinal analyses. Previous studies have also found stronger associations between inflammatory biomarkers and venular traits compared to arterial traits. [29] Widening of venules is thought to be caused by endothelial dysfunction leading to vessel remodeling; increased nitric oxide production may also be involved. [29,37]

Strengths of this research include the use of a large, national, longitudinal dataset with extensive questionnaire, physical exam, and blood data. We used a deep learning approach called QUARTZ to provide retinal vessel data on the entire retina rather than only the area around the optic disk. We had data on 9 different racial and ethnic groups, more than any other study has presented. A limitation of this work is that some people did not have an image that could be evaluated by QUARTZ. They tended to be older and more likely to have diabetes than those who did have a gradable image. Some people did not return to follow-up or did not have a retinal image taken at follow-up, so our sample size is reduced for the longitudinal analyses. The majority of the sample was White so our sample size in the other racial/ethnic groups is small. Some exposure or confounder data were collected by self-report (smoking, alcohol, stroke, diabetes), which could lead to misclassification. However, any misclassification would be unlikely to differ by the value of the retinal vessel trait. This would lead to bias towards the null meaning our measures of

association are conservative. Finally, without data on refractive error or axial length, we were unable to convert from pixels to micrometers.

In conclusion, this work provides comprehensive information on the factors associated with retinal vessel traits. Factors like smoking, alcohol, blood pressure, obesity, and diabetes were related to retinal vessel traits, which may increase the risk of subsequent eye disease. [4]

ACKNOWLEDGMENTS

This research was made possible using the data/biospecimens collected by the Canadian Longitudinal Study on Aging (CLSA). Funding for the Canadian Longitudinal Study on Aging (CLSA) is provided by the Government of Canada through the Canadian Institutes of Health Research (CIHR) under grant reference: LSA 94473 and the Canada Foundation for Innovation, as well as the following provinces, Newfoundland, Nova Scotia, Quebec, Ontario, Manitoba, Alberta, and British Columbia. This research has been conducted using the CLSA Baseline Comprehensive Dataset version 7.0, Follow-up 1 Comprehensive Dataset version 4.0, Baseline Retinal Scans, and Follow-up 1 Retinal Scans under Application Number 2209017. The CLSA is led by Drs. Parminder Raina, Christina Wolfson, and Susan Kirkland. The funders had no role in the design, analysis, or the interpretation of results. The opinions expressed in this manuscript are the author's own and do not reflect the views of the Canadian Longitudinal Study on Aging. Data are available from the Canadian Longitudinal Study on Aging (www.clsa-elcv.ca) for researchers who meet the criteria for access to de-identified CLSA data. This research project was further funded by CIHR operating grant PJT-183690 led by Dr. Ellen Freeman.

Table 6.1: Selected characteristics of those with at least 1 image with an acceptable QUARTZ image quality score

	Acceptable Score (n=26,076)	Unacceptable Score (n=4,021)	P-value
Age, Years	58.8 (9.7)	67.4 (10.8)	<0.001
Female sex	52.6%	51.2%	0.261
Race/ethnicity			0.712
White	93.8	93.3	
Black	0.8	1.2	
East Asian	0.8	0.8	
Southeast Asian	0.4	0.3	
South Asian	0.9	0.9	
Arab and West Asian	0.5	0.4	
Latin American	0.4	0.3	
Other	0.8	0.9	
Mixed	1.8	1.9	
BMI, kg/m2	28.4 (5.6)	28.6 (5.5)	0.005
SBP, mmHg	120.0 (16.3)	124.3 (18.0)	<0.001
DBP, mmHg	74.7 (10.0)	72.9 (10.4)	<0.001
Smoking			<0.001
Never	44.3%	39.3%	
Former	44.3%	47.5%	
Current	11.4%	13.2%	
Alcohol			<0.001
Never	2.4%	4.2%	
Occasional	42.7%	46.4%	
Weekly	41.5%	33.0%	
Daily	13.4%	16.4%	
Diabetes			<0.001
None	83.2%	76.7%	
Type 1	0.6%	1.2%	
Type 2	8.8%	14.3%	
Neither Type	7.4%	7.8%	
Arteriolar diameter OD, pixels	15.6 (1.6)		
Arteriolar diameter OS, pixels	16.1 (1.8)		
Venular diameter OD, pixels	17.4 (1.8)		
Venular diameter OS, pixels	17.7 (1.9)		
Arteriolar area OD, kilopixel^2	236.69 (44.76)		
Arteriolar area OS, kilopixel^2	226.03 (48.35)		
Venular area OD, kilopixel^2	263.43 (47.90)		
Venular area OS, kilopixel^2	255.24 (49.30)		
Arteriolar tortuosity* OD	1.2 (0.4)		
Arteriolar tortuosity* OS	1.2 (0.4)		
Venular tortuosity* OD	1.2 (0.2)		
Venular tortuosity* OS	1.2 (0.2)		

*Tortuosity measures were multiplied by 1000 and the natural log taken to achieve normality

BMI=body mass index; SBP=systolic blood pressure; DBP=diastolic blood pressure; OD=right; OS=left

Table 6.2: Difference in retinal vessel diameter by sociodemographic factors and clinical variables in cross-sectional and longitudinal analyses*

	Arteriolar Diameter n=21,754		Venular Diameter n=21,754		Arteriolar Diameter Change n=16,796		Venular Diameter Change n=16,796	
	β	95% CI	β	95% CI	β	95% CI	β	95% CI
Age, Per 10 Years	0.21	0.17, 0.25	0.38	0.34, 0.43	0.12	0.08, 0.15	0.27	0.23, 0.31
Female sex	0.12	0.06, 0.18	0.24	0.18, 0.31	0.07	0.01, 0.12	0.05	-0.01, 0.11
Ethnicity								
White	Ref		Ref		Ref		Ref	
Black	1.27	0.83, 1.71	0.37	0.07, 0.68	0.19	-0.29, 0.67	0.06	-0.24, 0.37
East Asian	0.11	-0.19, 0.41	-0.02	-0.34, 0.29	0.26	-0.10, 0.63	0.33	-0.15, 0.80
Southeast Asian	0.95	0.13, 1.77	0.28	-0.25, 0.81	0.42	-0.02, 0.86	0.67	-0.21, 1.55
South Asian	1.05	0.65, 1.46	0.22	-0.38, 0.81	0.23	-0.16, 0.62	0.14	-0.23, 0.50
Arab and West Asian	0.58	0.23, 0.93	-0.09	-0.46, 0.29	0.26	-0.02, 0.55	0.06	-0.23, 0.34
Latin American	0.60	0.24, 0.95	0.41	0.08, 0.74	0.21	-0.05, 0.47	-0.03	-0.47, 0.40
Other	0.81	0.29, 1.34	0.36	-0.02, 0.75	-0.11	-0.44, 0.22	-0.10	-0.46, 0.26
Mixed	0.40	0.18, 0.63	-0.02	-0.32, 0.27	0.15	-0.04, 0.33	0.02	-0.21, 0.24
Income								
High 0	Ref		Ref		Ref		Ref	
1	0.04	-0.03, 0.11	0.05	-0.02, 0.12	0.04	-0.01, 0.10	0.03	-0.03, 0.09
2	0.14	0.06, 0.23	0.19	0.09, 0.29	-0.00	-0.08, 0.08	0.02	-0.08, 0.11
Low 3	0.24	0.05, 0.44	0.24	0.05, 0.43	-0.05	-0.23, 0.13	0.05	-0.13, 0.23
Missing	0.12	-0.02, 0.26	0.01	-0.12, 0.15	-0.06	-0.18, 0.06	-0.09	-0.21, 0.03
BMI								
Underweight	-0.04	-0.41, 0.32	0.04	-0.45, 0.53	0.29	-0.21, 0.80	0.30	-0.22, 0.82
Normal	Ref		Ref		Ref		Ref	
Overweight	-0.04	-0.11, 0.03	0.06	-0.02, 0.14	-0.01	-0.07, 0.05	-0.01	-0.08, 0.06
Obese	0.07	-0.02, 0.15	0.16	0.07, 0.25	-0.01	-0.08, 0.06	0.06	-0.02, 0.14
SBP, per 10mmHg	-0.02	-0.05, 0.01	0.02	-0.01, 0.05	0.02	-0.01, 0.05	0.02	-0.01, 0.05
DBP, per 10mmHg	-0.34	-0.39, -0.29	-0.15	-0.20, -0.10	-0.05	-0.09, -0.01	-0.04	-0.09, 0.00
Smoking								
Never	Ref		Ref		Ref		Ref	
Former	0.10	0.04, 0.16	0.10	0.04, 0.17	0.02	-0.03, 0.07	0.06	0.01, 0.12

Current	0.72	0.60, 0.83	0.61	0.49, 0.73	0.28	0.18, 0.38	0.24	0.13, 0.34
Alcohol								
Never	Ref		Ref		Ref		Ref	
Occasional	-0.18	-0.47, 0.12	-0.09	-0.35, 0.17	0.04	-0.23, 0.30	-0.04	-0.33, 0.26
Weekly	-0.33	-0.62, -0.03	-0.20	-0.46, 0.06	0.03	-0.24, 0.29	-0.08	-0.37, 0.22
Daily	-0.38	-0.69, -0.08	-0.23	-0.50, 0.04	-0.02	-0.30, 0.25	-0.08	-0.39, 0.23
Diabetes								
None	Ref		Ref		Ref		Ref	
Type 1	0.67	0.31, 1.04	-0.05	-0.53, 0.43	0.45	0.09, 0.81	0.29	-0.09, 0.68
Type 2	0.26	0.14, 0.37	0.01	-0.12, 0.13	0.10	-0.01, 0.21	-0.09	-0.21, 0.02
Neither Type	0.13	0.02, 0.24	0.03	-0.09, 0.15	0.07	-0.01, 0.16	0.08	-0.04, 0.20
Stroke	0.10	-0.22, 0.41	0.09	-0.20, 0.38	0.17	-0.02, 0.36	0.27	-0.07, 0.61
Heart Disease	0.07	-0.04, 0.17	-0.03	-0.16, 0.09	0.05	-0.05, 0.14	0.01	-0.10, 0.13
hs-CRP	0.00	-0.00, 0.01	0.01	0.01, 0.02	0.00	-0.00, 0.01	-0.00	-0.01, 0.00
Cholesterol	-0.02	-0.05, 0.01	0.00	-0.03, 0.03	0.02	-0.01, 0.04	0.01	-0.01, 0.04

*Estimates are adjusted for each variable in the table and province; change models also adjusted for baseline vessel trait

BMI=body mass index; SBP=systolic blood pressure; DBP=diastolic blood pressure; CRP=c-reactive protein; Ref=reference

Table 6.3: Difference in retinal vessel area (kilopixels) by sociodemographic factors and clinical variables in cross-sectional and longitudinal analyses*

	Arteriolar Area n=21,754		Venular Area n=21,754		Arteriolar Area Change n=16,796		Venular Area Change n=16,796	
	β	95% CI	β	95% CI	β	95% CI	β	95% CI
Age, Per 1 Year	-1.72	-1.84, -1.61	-1.49	-1.61, -1.37	-0.66	-0.76, -0.55	-0.96	-1.07, -0.86
Female sex	-3.01	-4.86, -1.30	-10.80	-12.73, -8.87	-1.07	-2.42, 0.33	-3.31	-4.90, -1.72
Ethnicity								
White	Ref		Ref		Ref		Ref	
Black	-17.20	-28.48, -5.82	7.58	-6.72, 21.89	1.13	-7.52, 9.79	-5.73	-17.89, 6.44
East Asian	-23.43	-30.88, -15.99	3.37	-4.72, 11.47	-4.15	-11.89, 3.58	-16.99	-28.82, -5.16
Southeast Asian	-12.01	-23.40, -0.62	-32.33	-74.29, 9.62	0.85	-12.36, 14.08	-3.15	-23.31, 17.01
South Asian	-28.48	-38.82, -18.14	-17.43	-32.37, -2.48	-7.03	-16.43, 2.37	-12.53	-25.76, 0.70
Arab and West Asian	-4.97	-18.89, 8.95	17.21	4.46, 29.95	4.46	-5.23, 14.15	9.23	-4.38, 22.86
Latin American	9.37	-1.50, 20.24	11.61	0.31, 22.91	4.79	-2.80, 12.39	6.85	-5.86, 19.55
Other	-4.43	-15.65, 6.78	-2.98	-16.19, 10.23	-0.25	-11.25, 10.75	0.55	-6.87, 7.98
Mixed	-0.67	-8.46, 7.11	6.56	-0.18, 13.30	-1.07	-6.05, 3.91	-4.25	-10.70, 2.01
Income								
High 0	Ref		Ref		Ref		Ref	
1	-0.64	-2.61, 1.33	-0.57	-2.61, 1.48	-1.35	-2.92, 0.21	0.23	-1.49, 1.96
2	0.62	-1.92, 3.16	1.06	-1.82, 3.93	-0.92	-2.94, 1.09	1.50	-0.82, 3.81
Low 3	-5.99	-10.58, -1.39	-2.40	-7.61, 2.82	2.07	-2.07, 6.22	4.91	1.11, 8.70
Missing	-4.04	-7.57, -0.53	0.82	-2.83, 4.47	-0.87	-3.91, 2.17	0.13	-3.01, 3.32
BMI								
Underweight	-1.66	-11.58, 8.27	-1.16	-9.83, 7.51	-2.20	-10.89, 6.48	-10.21	-18.70, -1.72
Normal	Ref		Ref		Ref		Ref	
Overweight	-0.42	-2.41, 1.57	3.16	1.11, 5.21	0.17	-1.43, 1.76	2.59	0.84, 4.34
Obese	-1.87	-4.23, 0.48	6.76	4.16, 9.35	-0.57	-2.32, 1.19	3.52	1.51, 5.51
SBP	-0.20	-0.28, -0.12	-0.23	-0.32, -0.15	0.05	-0.02, 0.13	0.01	-0.07, 0.09
DBP	-0.70	-0.83, -0.57	0.09	-0.05, 0.22	-0.30	-0.41, -0.20	-0.16	-0.28, -0.04
Smoking								
Never	Ref		Ref		Ref		Ref	

Former	1.37	-0.31, 3.05	2.21	0.46, 3.96	1.06	-0.32, 2.44	1.60	0.13, 3.07
Current	11.90	8.69, 15.11	20.03	16.60, 23.47	-0.54	-3.13, 2.06	3.48	0.28, 6.68
Alcohol								
Never	Ref		Ref		Ref		Ref	
Occasional	-1.42	-7.61, 4.76	3.58	-2.74, 9.89	-3.75	-8.79, 1.28	0.94	-5.99, 7.88
Weekly	-3.97	-10.21, 2.26	3.11	-3.22, 9.43	-4.21	-9.28, 0.86	1.96	-4.99, 8.91
Daily	-8.10	-14.64, -1.57	1.28	-5.27, 7.82	-5.20	-10.58, 0.18	1.45	-5.73, 8.64
Diabetes								
None	Ref		Ref		Ref		Ref	
Type 1	-1.54	-14.76, 11.69	-14.72	-31.67, 2.24	-2.88	-12.21, 6.45	-14.86	-24,19, -5.53
Type 2	-2.21	-5.39, 0.95	1.02	-2.24, 4.28	-2.40	-5.02, 0.22	-1.02	-4.27, 2.23
Neither Type	-1.68	-5.00, 1.65	0.65	-2.76, 4.06	1.61	-0.94, 4.14	1.19	-1.51, 3.90
Stroke	-7.93	-14.99, -0.87	-2.00	-11.73, 7.71	0.84	-7.48, 9.16	3.14	-4.40, 10.69
Heart Disease	0.62	-2.43, 3.68	-1.37	-4.95, 2.20	-1.19	-3.63, 1.24	-1.58	-4.40, 1.23
hs-CRP	0.18	0.01, 0.36	0.30	0.13, 0.47	-0.10	-0.21, 0.02	-0.26	-0.43, -0.09
Cholesterol	1.51	0.72, 2.30	1.92	1.08, 2.75	0.14	-0.51, 0.79	-0.44	-1.14, 0.26

* Estimates are adjusted for each variable in the table and province; change models also adjusted for baseline vessel trait

BMI=body mass index; SBP=systolic blood pressure; DBP=diastolic blood pressure; CRP=c-reactive protein; Ref=reference

Table 6.4: Difference in retinal vessel tortuosity by sociodemographic factors and clinical variables in cross-sectional and longitudinal analyses*

	Arteriolar Tortuosity n=21,698		**Venular Tortuosity n=21,672**		**Arteriolar Tortuosity Change n=16,704**		**Venular Tortuosity Change n=16,670**	
	β	**95% CI**	**β**	**95% CI**	**β**	**95% CI**	**β**	**95% CI**
Age, per 10 years	-0.00	-0.01, 0.01	0.01	0.00, 0.02	-0.00	-0.00, 0.00	-0.01	-0.01, -0.01
Female sex	0.02	-0.00, 0.03	0.00	-0.01, 0.01	0.01	0.00, 0.02	-0.00	-0.01, 0.00
Ethnicity								
White	Ref		Ref		Ref		Ref	
Black	0.00	-0.09, 0.09	0.00	-0.05, 0.05	0.01	-0.03, 0.06	0.01	-0.01, 0.04
East Asian	-0.39	-0.45, -0.33	-0.05	-0.10, -0.01	-0.01	-0.05, 0.03	-0.03	-0.06, -0.01
Southeast Asian	-0.24	-0.37, -0.12	0.06	-0.15, 0.27	-0.02	-0.07, 0.03	-0.02	-0.08, 0.05
South Asian	-0.18	-0.24, -0.11	-0.01	-0.08, 0.05	-0.03	-0.06, 0.01	0.00	-0.03, 0.04
Arab and West Asian	-0.21	-0.32, -0.11	-0.06	-0.10, -0.01	0.01	-0.03, 0.05	-0.00	-0.04, 0.03
Latin American	-0.06	-0.14, 0.02	-0.01	-0.06, 0.04	0.01	-0.02, 0.04	-0.04	-0.08, -0.01
Other	-0.10	-0.20, 0.00	-0.04	-0.08, -0.00	0.05	0.01, 0.09	0.01	-0.02, 0.04
Mixed	-0.06	-0.13, -0.00	0.02	-0.03, 0.07	-0.02	-0.04, -0.00	0.01	-0.01, 0.03
Income								
High 0	Ref		Ref		Ref		Ref	
1	-0.00	-0.02, 0.01	-0.00	-0.01, 0.01	-0.00	-0.01. 0.00	-0.00	-0.01, 0.00
2	-0.01	-0.03, 0.01	0.00	-0.01, 0.02	-0.00	-0.01, 0.01	0.01	-0.00, 0.02
Low 3	0.01	-0.03, 0.05	0.02	-0.01, 0.05	-0.01	-0.04, 0.01	0.02	0.00, 0.04
Missing	0.01	-0.02, 0.04	0.02	-0.01, 0.04	-0.02	-0.04, -0.01	-0.00	-0.02, 0.01
BMI								
Underweight	0.00	-0.07, 0.07	0.04	-0.02, 0.11	0.00	-0.05, 0.05	0.00	-0.03, 0.04
Normal	Ref		Ref		Ref		Ref	
Overweight	-0.02	-0.03, 0.00	0.02	0.01, 0.03	0.00	-0.00, 0.01	0.00	-0.00, 0.01
Obese	-0.02	-0.04, 0.00	0.04	0.03, 0.05	-0.00	-0.01, 0.01	-0.00	-0.01, 0.01
SBP, per 10 mmHg	0.01	0.01, 0.02	0.01	0.01, 0.02	0.00	-0.00, 0.00	0.01	0.00, 0.01
DBP, per 10 mmHg	-0.02	-0.04, -0.01	-0.03	-0.03, -0.02	-0.00	-0.01, 0.00	-0.01	-0.01, -0.00
Smoking								
Never	Ref		Ref		Ref		Ref	

Former	-0.00	-0.02, 0.01	0.00	-0.01, 0.01	-0.00	-0.01, 0.01	0.00	-0.00, 0.01
Current	0.01	-0.01, 0.04	0.04	0.02, 0.06	0.01	-0.01, 0.02	0.00	-0.01, 0.02
Alcohol								
Never	Ref		Ref		Ref		Ref	
Occasional	0.00	-0.05, 0.05	0.00	-0.03, 0.04	0.00	-0.02, 0.02	-0.01	-0.03, 0.01
Weekly	0.01	-0.04, 0.06	0.00	-0.03, 0.04	0.01	-0.02, 0.03	-0.00	-0.02, 0.02
Daily	-0.01	-0.07, 0.04	0.00	-0.04, 0.04	0.02	-0.01, 0.04	-0.00	-0.02, 0.02
Diabetes								
None	Ref		Ref		Ref		Ref	
Type 1	0.07	-0.04, 0.17	0.03	-0.03, 0.08	0.04	-0.01, 0.09	0.01	-0.03, 0.05
Type 2	-0.02	-0.05, 0.01	0.00	-0.01, 0.02	0.01	-0.00, 0.02	0.01	-0.01, 0.02
Neither Type	-0.01	-0.03, 0.02	-0.00	-0.02, 0.01	0.01	-0.00, 0.02	-0.00	-0.01, 0.01
Stroke	0.07	0.00, 0.14	0.01	-0.04, 0.06	-0.00	-0.05, 0.04	-0.01	-0.03, 0.02
Heart Disease	-0.01	-0.03, 0.02	-0.00	-0.02, 0.02	0.01	0.00, 0.03	-0.00	-0.01, 0.01
hs-CRP, per 10 units	0.01	-0.00, 0.03	0.02	0.01, 0.03	-0.00	-0.01, 0.00	-0.00	-0.01, 0.01
Cholesterol	0.01	0.00, 0.01	-0.00	-0.01, 0.00	0.00	-0.00, 0.01	-0.00	-0.00, 0.00

* Estimates are adjusted for each variable in the table and province; change models also adjusted for baseline vessel trait

BMI=body mass index; SBP=systolic blood pressure; DBP=diastolic blood pressure; CRP=c-reactive protein; Ref=reference

Figure 6.1: Mean arteriolar diameter by diabetes status using cross-sectional data

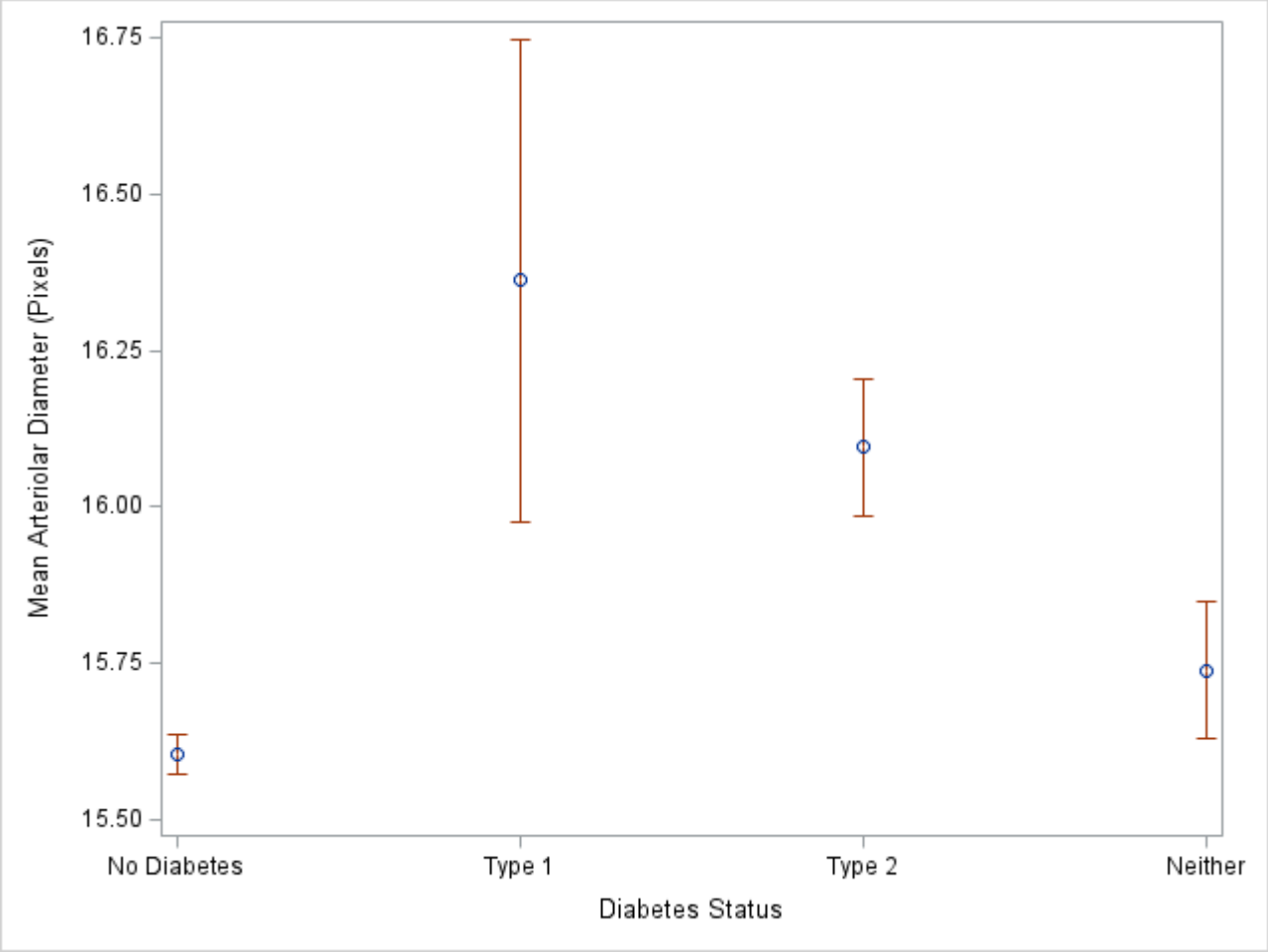


Figure 6.2: Mean venular diameter by BMI category using cross-sectional data

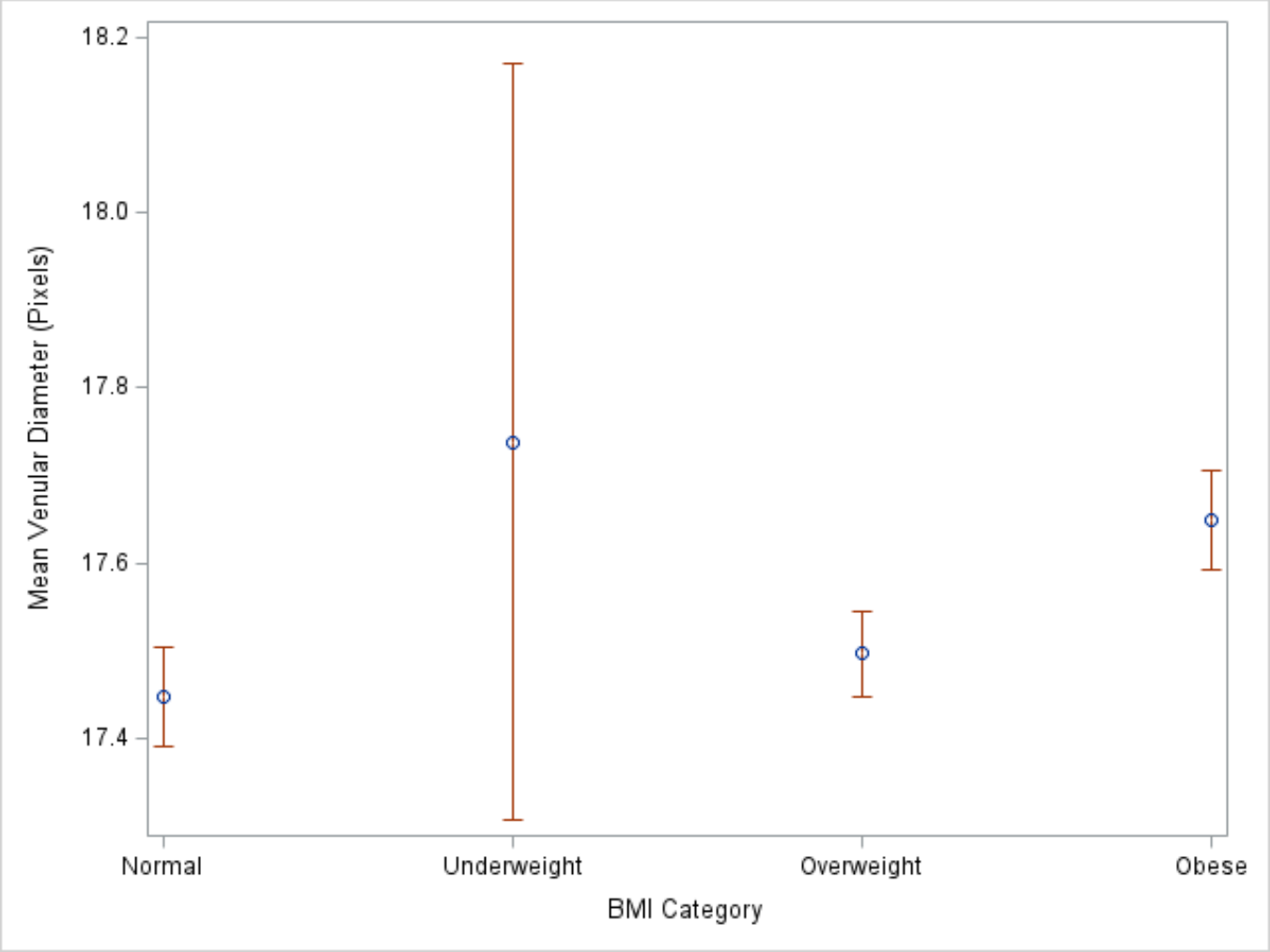
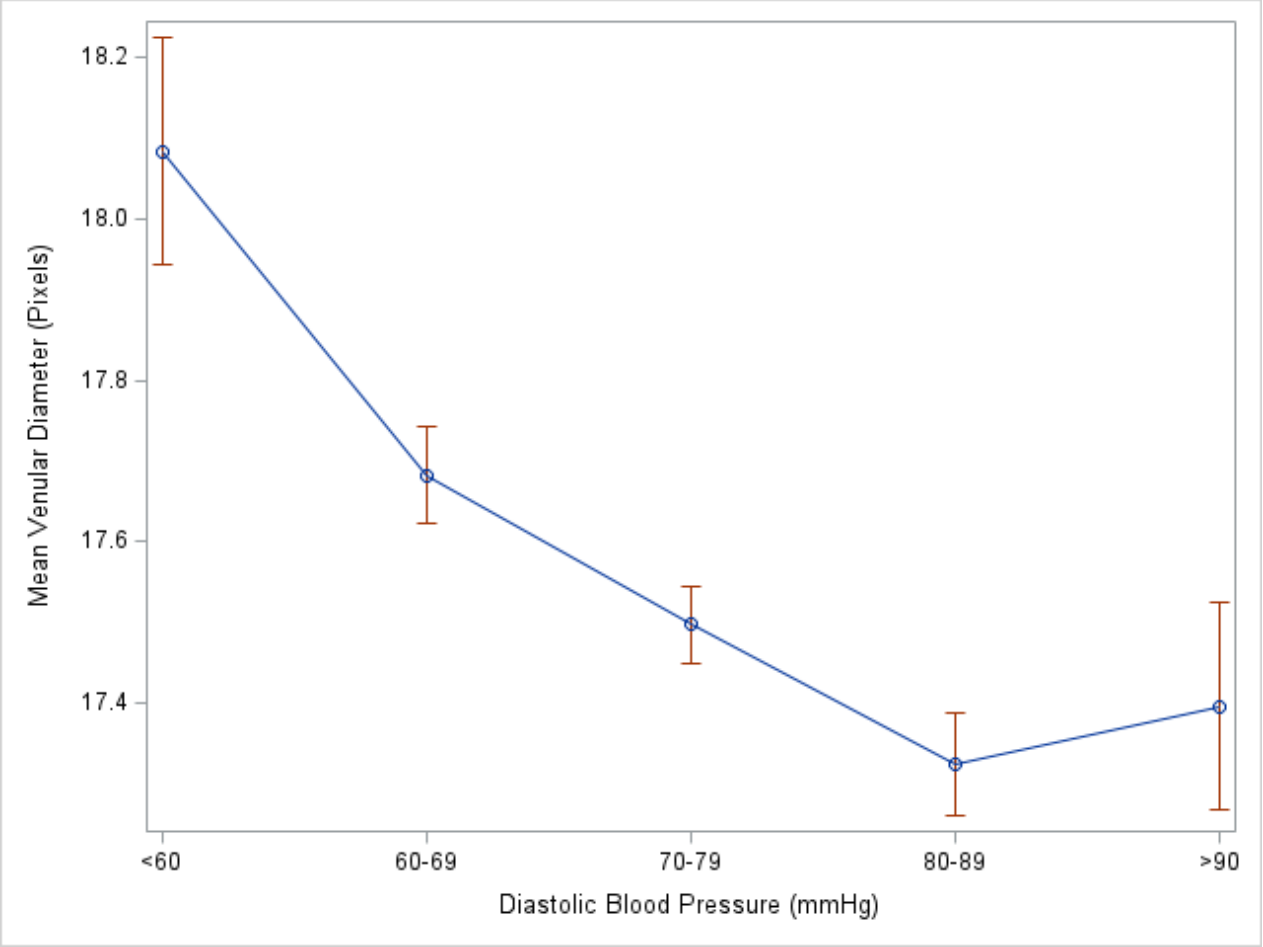


Figure 6.3: Mean venular diameter by diastolic blood pressure using cross-sectional data



REFERENCES

1. Welikala R FJ, Johnson G, Rahman F, Podoleanu R, Remagnino P, Freeman EE, Chambers R, Bolter L, Anderson J, Olvera-Barrios A, Warwick A, Foster PJ, Shakespeare R, Ganguly R, Egan C, Tufail A, Owen CG, Rudnicka AR, Barman SA. Artificial Intelligence-Enabled Retinal Vasculometry at Scale Utilizing the UK Biobank, CLSA, and NEL DESP Datasets. presented at: IEEE-EMBS International Conference on Biomedical and Health Informatics (BHI'24); Nov 10-13 2024; Houston, USA.
2. Perez-Rovira A, MacGillivray T, Trucco E, et al. VAMPIRE: Vessel assessment and measurement platform for images of the REtina. *Annu Int Conf IEEE Eng Med Biol Soc*. 2011;2011:3391-4. doi:10.1109/IEMBS.2011.6090918
3. Brazionis L, Quinn N, Dabbah S, et al. Review and comparison of retinal vessel calibre and geometry software and their application to diabetes, cardiovascular disease, and dementia. *Graefes Arch Clin Exp Ophthalmol*. Aug 2023;261(8):2117-2133. doi:10.1007/s00417-023-06002-7
4. Newman A, Andrew N, Casson R. Review of the association between retinal microvascular characteristics and eye disease. *Clin Exp Ophthalmol*. Jul 2018;46(5):531-552. doi:10.1111/ceo.13119
5. Wong TY, Kamineni A, Klein R, et al. Quantitative retinal venular caliber and risk of cardiovascular disease in older persons: the cardiovascular health study. *Arch Intern Med*. Nov 27 2006;166(21):2388-94. doi:10.1001/archinte.166.21.2388
6. Sun C, Wang JJ, Mackey DA, Wong TY. Retinal vascular caliber: systemic, environmental, and genetic associations. *Surv Ophthalmol*. Jan-Feb 2009;54(1):74-95. doi:10.1016/j.survophthal.2008.10.003
7. Wong TY, Islam FM, Klein R, et al. Retinal vascular caliber, cardiovascular risk factors, and inflammation: the multi-ethnic study of atherosclerosis (MESA). *Invest Ophthalmol Vis Sci*. Jun 2006;47(6):2341-50. doi:10.1167/iovs.05-1539
8. Kaushik S, Kifley A, Mitchell P, Wang JJ. Age, blood pressure, and retinal vessel diameter: separate effects and interaction of blood pressure and age. *Invest Ophthalmol Vis Sci*. Feb 2007;48(2):557-61. doi:10.1167/iovs.06-0893
9. Tapp RJ, Owen CG, Barman SA, et al. Associations of Retinal Microvascular Diameters and Tortuosity With Blood Pressure and Arterial Stiffness: United Kingdom Biobank. *Hypertension*. Dec 2019;74(6):1383-1390. doi:10.1161/HYPERTENSIONAHA.119.13752
10. Tapp RJ, Owen CG, Barman SA, et al. Retinal Vascular Tortuosity and Diameter Associations with Adiposity and Components of Body Composition. *Obesity (Silver Spring)*. Sep 2020;28(9):1750-1760. doi:10.1002/oby.22885
11. Maderuelo-Fernandez JA, Garcia-Garcia A, Chamoso P, et al. Automatic image analyser to assess retinal vessel calibre (ALTAIR). A new tool to evaluate the thickness, area and length of the vessels of the retina. *Int J Med Inform*. Apr 2020;136:104090. doi:10.1016/j.ijmedinf.2020.104090
12. Raina P, Wolfson C, Kirkland S, et al. Cohort Profile: The Canadian Longitudinal Study on Aging (CLSA). *Int J Epidemiol*. Dec 1 2019;48(6):1752-1753j. doi:10.1093/ije/dyz173
13. Welikala RA FM, Habib M, Daniel-Tong S, Yates M, Foster P, Whincup PH, Rudnicka A, Owen CG, Strachan DP, Barman SA. . Automated Quantification of Retinal Vessel Morphometry in the UK Biobank Cohort. presented at: Seventh International Conference on Image Processing Theory, Tools and Applications 2017;

14. Fukutsu K, Saito M, Noda K, et al. A Deep Learning Architecture for Vascular Area Measurement in Fundus Images. *Ophthalmol Sci*. Mar 2021;1(1):100004. doi:10.1016/j.xops.2021.100004
15. Maloca PM, Feu-Basilio S, Schottenhamml J, et al. Reference database of total retinal vessel surface area derived from volume-rendered optical coherence tomography angiography. *Sci Rep*. Mar 7 2022;12(1):3695. doi:10.1038/s41598-022-07439-2
16. Li X, Wong WL, Cheung CY, et al. Racial differences in retinal vessel geometric characteristics: a multiethnic study in healthy Asians. *Invest Ophthalmol Vis Sci*. May 1 2013;54(5):3650-6. doi:10.1167/iovs.12-11126
17. Rochtchina E, Wang JJ, Taylor B, Wong TY, Mitchell P. Ethnic variability in retinal vessel caliber: a potential source of measurement error from ocular pigmentation?--the Sydney Childhood Eye Study. *Invest Ophthalmol Vis Sci*. Apr 2008;49(4):1362-6. doi:10.1167/iovs.07-0150
18. Xing C, Klein BE, Klein R, Jun G, Lee KE, Iyengar SK. Genome-wide linkage study of retinal vessel diameters in the Beaver Dam Eye Study. *Hypertension*. Apr 2006;47(4):797-802. doi:10.1161/01.HYP.0000208330.68355.72
19. Tomasoni M, Beyeler MJ, Vela SO, et al. Genome-wide Association Studies of Retinal Vessel Tortuosity Identify Numerous Novel Loci Revealing Genes and Pathways Associated With Ocular and Cardiometabolic Diseases. *Ophthalmol Sci*. Sep 2023;3(3):100288. doi:10.1016/j.xops.2023.100288
20. Jensen RA, Sim X, Smith AV, et al. Novel Genetic Loci Associated With Retinal Microvascular Diameter. *Circ Cardiovasc Genet*. Feb 2016;9(1):45-54. doi:10.1161/CIRCGENETICS.115.001142
21. Yuen VL, Zhang XJ, Ling X, et al. Effects of firsthand tobacco smoking on retinal vessel caliber: a systematic review and meta-analysis. *Graefes Arch Clin Exp Ophthalmol*. May 2024;262(5):1397-1407. doi:10.1007/s00417-023-06223-w
22. Kifley A, Liew G, Wang JJ, et al. Long-term effects of smoking on retinal microvascular caliber. *Am J Epidemiol*. Dec 1 2007;166(11):1288-97. doi:10.1093/aje/kwm255
23. Liang C, Gu C, Wang N. Retinal Vascular Caliber in Coronary Heart Disease and Its Risk Factors. *Ophthalmic Res*. 2023;66(1):151-163. doi:10.1159/000526753
24. Wimpissinger B, Resch H, Berisha F, Weigert G, Schmetterer L, Polak K. Response of retinal blood flow to systemic hyperoxia in smokers and nonsmokers. *Graefes Arch Clin Exp Ophthalmol*. Jul 2005;243(7):646-52. doi:10.1007/s00417-004-1083-8
25. Mlinar T, Debevec T, Kapus J, et al. Retinal blood vessel diameters in children and adults exposed to a simulated altitude of 3,000 m. *Front Physiol*. 2023;14:1026987. doi:10.3389/fphys.2023.1026987
26. Klein R, Klein BE, Knudtson MD, Wong TY, Tsai MY. Are inflammatory factors related to retinal vessel caliber? The Beaver Dam Eye Study. *Arch Ophthalmol*. Jan 2006;124(1):87-94. doi:10.1001/archopht.124.1.87
27. Ambrose JA, Barua RS. The pathophysiology of cigarette smoking and cardiovascular disease: an update. *J Am Coll Cardiol*. May 19 2004;43(10):1731-7. doi:10.1016/j.jacc.2003.12.047
28. Wang F, Hadzic S, Roxlau ET, et al. Retinal tissue develops an inflammatory reaction to tobacco smoke and electronic cigarette vapor in mice. *J Mol Med (Berl)*. Oct 2021;99(10):1459-1469. doi:10.1007/s00109-021-02108-9

29. Liu M, Lovern C, Lycett K, et al. The association between markers of inflammation and retinal microvascular parameters: A systematic review and meta-analysis. *Atherosclerosis*. Nov 2021;336:12-22. doi:10.1016/j.atherosclerosis.2021.09.025
30. Zhuang X, He G, Zeng Y, et al. Quantitative evaluation of choroidal and retinal microvasculature post-alcohol consumption: A pilot study. *Microvasc Res*. Mar 2024;152:104629. doi:10.1016/j.mvr.2023.104629
31. Spaak J, Merlocco AC, Soleas GJ, et al. Dose-related effects of red wine and alcohol on hemodynamics, sympathetic nerve activity, and arterial diameter. *Am J Physiol Heart Circ Physiol*. Feb 2008;294(2):H605-12. doi:10.1152/ajpheart.01162.2007
32. Kochli S, Endes K, Infanger D, Zahner L, Hanssen H. Obesity, Blood Pressure, and Retinal Vessels: A Meta-analysis. *Pediatrics*. Jun 2018;141(6)doi:10.1542/peds.2017-4090
33. Cheung N, Wong TY. Obesity and eye diseases. *Surv Ophthalmol*. Mar-Apr 2007;52(2):180-95. doi:10.1016/j.survophthal.2006.12.003
34. Wong TY, Mitchell P. The eye in hypertension. *Lancet*. Feb 3 2007;369(9559):425-35. doi:10.1016/S0140-6736(07)60198-6
35. Lehmann MV, Schmieder RE. Remodeling of retinal small arteries in hypertension. *Am J Hypertens*. Dec 2011;24(12):1267-73. doi:10.1038/ajh.2011.166
36. Bek T. Diameter Changes of Retinal Vessels in Diabetic Retinopathy. *Curr Diab Rep*. Aug 8 2017;17(10):82. doi:10.1007/s11892-017-0909-9
37. Nguyen TT, Wong TY. Retinal vascular manifestations of metabolic disorders. *Trends Endocrinol Metab*. Sep 2006;17(7):262-8. doi:10.1016/j.tem.2006.07.006

Chapter 7: Manuscript Two

Preface to the Manuscript

Purpose: Answer thesis objective two and three.

Required Ethics Approval: After review by the University of Ottawa Office of Research Ethics and Integrity, approval was received January 2023 (H-01-23-8331).

Title: Retinal Vessel Traits and Age-Related Eye Disease in the Canadian Longitudinal Study on Aging

Authors: Alexis O'Neil, Roshan A. Welikala, Sarah Barman, Christopher G. Owen, Alicja R. Rudnicka, Mohan Rakesh, Marie-Hélène Roy-Gagnon, David Maberley, Ellen E. Freeman

Authors and their Contributions:

- AO contributed to the analyses, interpretation of the data, drafting the manuscript and revision.
- RA, SB, CGO and ARR oversaw the development and use of QUARTZ on CLSA data and contributed to interpretation of the results and revision of the manuscript.
- MR and MHRG assisted with creating summary measures of the QUARTZ output, contributed to interpretation of the data and revision of the manuscript.
- DM contributed to interpretation of the data and revision of the manuscript.
- EEF contributed to the acquisition of funding, data analysis, and interpretation of the data and the revision of the manuscript.
- All authors gave final approval for the version published and agree to be accountable for all aspects of the work.

Publication Status: The manuscript presented in this thesis formed the basis for the paper published by Clinical and Experimental Ophthalmology (DOI: 10.1111/ceo.14566)

Retinal Vessel Traits and Age-Related Eye Disease in the Canadian Longitudinal Study on Aging

Alexis O'Neil[1], Roshan A. Welikala[2], Sarah Barman[2], Christopher G. Owen[3], Alicja R. Rudnicka[3], Mohan Rakesh[1], Marie-Hélène Roy-Gagnon[1], David Maberley[4], Ellen E. Freeman[1,4,5]

[1] School of Epidemiology and Public Health, University of Ottawa, Ottawa; [2] School of Computer Science and Mathematics, Kingston University London, UK; [3] Population Health Research Institute, St George's School of Health and Medical Sciences, City St George's, University of London, London, UK; [4] Department of Ophthalmology, University of Ottawa, Ottawa; [5] Ottawa Hospital Research Institute, Ottawa

Running Title: Retinal Vessel Traits and Eye Disease

Abstract (248) & Main Text (2860) Word Count

Number of Tables (5)

Funding details: This work was supported by the CIHR under Grant PJT-183690

Commercial Relationships Disclosure: A O'Neil, none; RA Welikala, none; S Barman, none; CG Owen, none; AR Rudnika, none; M Rakesh, none; MH Roy-Gagnon, none; D Maberley, none; EE Freeman, none

Corresponding Author: Ellen Freeman, 600 Peter Morand Crescent, Office 301H, School of Epidemiology and Public Health, University of Ottawa, Ottawa, Canada, eefreeman@gmail.com

ABSTRACT

Purpose: To cross-sectionally and longitudinally examine whether retinal vessel traits are associated with glaucoma-related outcomes (glaucoma, cup-to-disc ratio (CDR), and intraocular pressure (IOP)) and age-related macular degeneration (AMD).

Methods: Baseline and 3-year follow-up data from the 30,097 participants of the Canadian Longitudinal Study on Aging were used. QUARTZ, a deep learning algorithm, was used to extract data from retinal images including arteriolar and venular diameter, tortuosity, and vertical CDR. Glaucoma and AMD were self-reported. Intraocular pressure was measured. Multiple linear and logistic regression were used to adjust for demographic, lifestyle, and clinical factors.

Results: Greater venular tortuosity was associated with a smaller CDR (β=-0.034, 95% CI -0.041, -0.027) and lower IOP (β=-0.39, 95% CI -0.67, -0.10) at baseline. Using longitudinal data, greater venular tortuosity was associated with a reduced 3-year development of glaucoma (OR=0.52, 95% CI 0.31, 0.87) and a 3-year reduction in the CDR (β=-0.006, 95% CI -0.010, -0.002). Having wider arterioles was associated with a lower odds of glaucoma (OR=0.90, 95% CI 0.85, 0.96) at baseline but there was no association using longitudinal data. Wider venular diameter was associated with a higher odds of AMD at baseline (OR=1.11, 95% CI 1.04, 1.18) and a higher odds of the 3-year development of AMD (OR=1.15, 95% CI 1.07, 1.24).

Conclusions: This work provides additional longitudinal analysis of the relationship between retinal vessel traits and age-related eye disease. Understanding how changes in the retinal microvasculature affect the development of eye disease may lead to better treatment and prevention strategies.

INTRODUCTION

Glaucoma and age-related macular degeneration (AMD) are two of the leading causes of visual impairment and blindness in the world. [1] Glaucoma is characterised by a progressive loss of retinal ganglion cells, which can be visualized on ophthalmic examination as optic disc cupping and measured by the cup-to-disc ratio. [2] High intraocular pressure (IOP) is a major risk factor for the development of glaucoma and remains the only modifiable factor targeted by treatment. Meanwhile, AMD is a progressive degeneration of multiple retinal tissues including the photoreceptors, retinal pigment epithelium, Bruch's membrane, and the choriocapillaris due to various age-related changes. [3] Choroidal neovascularization and geographic atrophy can occur in late-stage AMD, causing vision to deteriorate. Disruptions of the retinal vasculature have been found in both diseases. [4,5]

Because most prior research has been cross-sectional, the temporal relationship of the disease and the vascular disruptions are not entirely clear. For example, arteriolar narrowing has been found in glaucoma patients. [4] However, it is not clear if that narrowing leads to glaucoma or if the decreased metabolic needs of a glaucomatous retina lead to the narrowing. Since cross-sectional studies can suffer from reverse causality, longitudinal studies are critical to establish the temporality of retinal vessel diameter and age-related eye disease. Two longitudinal studies examined the link between retinal vessel diameter and the incidence of glaucoma with one of them finding a statistically significant association and one finding null results. [6,7] Five longitudinal studies examined retinal vessel diameter and the incidence of AMD or its signs with all but one finding null results. [8-12] However, longitudinal studies require much larger sample sizes than cross-sectional studies to be adequately powered. Using longitudinal data from the 30,097 participants of the Canadian Longitudinal Study on Aging, [13] we examined the relationship between retinal

vessel traits including diameter and tortuosity (i.e. curvature) and age-related eye diseases including glaucoma and AMD.

METHODS

Study Population and Design

Data originating from the Canadian Longitudinal Study on Aging (CLSA) Comprehensive Cohort were used for both cross-sectional and longitudinal analyses. [13] The CLSA recruited 30,097 Canadian adults aged 45 to 85 years through random sampling of provincial healthcare databases and random digit dialing of landline phones. Data collection began at baseline between 2012 and 2015 through in-home interviews and physical assessments at CLSA data collection sites in cities across Canada, including Victoria, Vancouver, Surrey, Calgary, Winnipeg, Hamilton, Ottawa, Montreal, Sherbrooke, Halifax, and St. John's. Follow-up data were collected in the same manner three years later, between 2015 and 2018.

Eligible participants were aged 45 to 85, community dwelling, and were fluent in either English or French. Participants were also required to live within 25 to 50 km of a data collection site. Exclusion criteria included cognitive impairment, full-time membership in the Canadian Armed Forces, living on a federal First Nations reserve or settlement, residing in a long-term care facility, or not being a permanent resident or citizen. Written informed consent was obtained for all participants. Research Ethics Board approval was acquired for all CLSA sites in July 2010. The University of Ottawa Office of Research Ethics and Integrity gave approval for the present analysis in January 2023 (H-01-23-8331).

Data Collection

Retinal images

A Topcon TRC-NW8 fundus camera with an attached Nikon D90 camera was used to take nonmydriatic retinal images in each eye. Images were centered on the macula and had a 45-degree field-of-view. The large majority of the images were saved in JPEG format with a resolution of 4288 x 2848 pixels. Images from one site were saved with a resolution of 4928 x 3264 pixels and were resized to 4288 x 2848 pixels to be consistent with the other images.

Retinal vessel traits

Retinal image analysis in this study was performed using QUARTZ (Quantitative Analysis of Retinal Vessel Topology and Size) was used. [14,15] QUARTZ takes measurements across the inner retinal layer, encompassing arterioles, pre- and post-capillaries, and venules. In the CLSA data, QUARTZ can evaluate inadequate image quality with a sensitivity of 91% and a specificity of 100%. [14] Furthermore, QUARTZ distinguishes between arterioles and venules with an accuracy of 88%, which improves to 96% when a probability cutoff of 0.8 is applied. [14] QUARTZ produced thousands of measures of diameter and tortuosity from across the entire retinal image. These measurements were then summarized by calculating the mean diameter and tortuosity for each arteriole and venule, weighted by their respective segment lengths. The units for diameter are pixels while tortuosity is a unitless measure with greater scores indicating curvier vessels. Measurements of the optic nerve vertical cup-to-disc ratios were also obtained from QUARTZ. Larger cup-to-disc ratios may indicate glaucoma.

Other Ocular Data

Participants were asked at baseline and follow-up if they had ever been diagnosed by a doctor with glaucoma or AMD. Corneal-compensated intraocular pressure was measured in each eye using the Reichart Ocular Response Analyzer (Reichart Technologies, Depew, NY, USA). Pinhole-corrected visual acuity was measured in each eye using the illuminated Early Treatment of Diabetic Retinopathy Study chart with scores converted to logMAR for analysis.

Demographic, health, and lifestyle data

At baseline, demographic information such as age, sex, income, and race/ethnicity were gathered during the in-home visit using the interviewer administered questionnaire. Income data were obtained by asking participants "What is your best estimate of the total household income received by all household members, from all sources, before taxes and deductions, in the past 12 months?". Participants were categorized into 5 groups: >$150 K, $150-$100 K, $100-$50 K, $50 K-$20 K, Refused.

Participants were asked two questions to ascertain smoking status, including "Have you smoked at least 100 cigarettes in your life?" and "At the present time, do you smoke cigarettes daily, occasionally (at least once in last 30 days), or not at all (not in last 30 days)?". Current smokers were defined as those who had smoked at least 100 cigarettes and currently smokes daily or occasionally. Former smokers were those who reported smoking at least 100 cigarettes in life but had not smoked in the last 30 days. Similarly, participants were asked "About how often during the past 12 months did you drink alcohol?" to assess frequency of alcohol consumption. We categorized responses into never, occasionally (zero to three times a month), weekly (between one and five times a week), or daily (six or more times a week).

During the interviewer-led questionnaire, participants were asked to report doctor diagnoses of health conditions. Responses with respect to diabetes status were categorized as none, Type 1, Type 2, or neither type. Height and weight were measured using standardized procedures. Body mass index (BMI) was calculated as weight (kg)/height squared (m^2) and classified according to the World Health Organization categories (underweight <18.5 kg/m^2, normal weight 18.5–24.9 kg/m^2, overweight 25.0–29.9 kg/m^2, and obese ≥30.0 kg/m^2). [16] Blood pressure was measured using the VSM BpTRU monitor (Medaval, Dublin, Ireland). Six readings were taken. The average of the second through sixth measurement was used for analysis.

Statistical Analysis

Those with and without glaucoma/AMD were compared. Multiple linear regression models were used to examine the associations between each baseline retinal vessel trait and the IOP, CDR and the 3-year change values. Change values were calculated by taking the follow-up value minus the baseline value. Multiple logistic regression models were used to assess the associations between each baseline retinal vessel trait and baseline eye disease (glaucoma or AMD) or the 3-year development of eye disease. All models contained both the arteriolar and venular trait, as researchers have noted that they may confound each other. [17,18] Models were adjusted for age, sex, race, income, BMI, systolic and diastolic blood pressure, stroke, smoking, alcohol, and province. Models for the 3-year change outcomes were also adjusted for the baseline level of the outcome to account for floor/ceiling effects (i.e. those who already have narrow vessels may be less likely to become narrower over time). Regression models for CDR and IOP were run for right eyes only since it is not possible to account for both the complex survey design (weight and strata)

and the correlation between eyes. The complex survey design (weights and strata) was accounted for in all analyses. Stata/SE Version 16.1 for Windows was used.

RESULTS

Of the 30,097 CLSA participants, 26,076 (87%) had at least one acceptable QUARTZ image quality score. Of those with acceptable QUARTZ image quality scores who answered the question on glaucoma (n=25,942), those with and without a report of glaucoma are compared in Table 1. Those who reported a diagnosis of glaucoma were older, more likely to be female, more likely to have diabetes, to have higher IOP, and worse cup-to-disc ratio. They also had wider arteriolar and venular diameter. Those who reported a diagnosis of AMD (Table 2) had worse visual acuity, were older, more likely to be female, more likely to have diabetes, and had wider venular diameter. A high percentage of CLSA participants returned to follow-up (92%). Of those with retinal images, there were 464 people (2.1%) with incident glaucoma and 415 with incident AMD (1.9%).

The cross-sectional adjusted associations between retinal vessel traits and glaucoma-related outcomes are shown in Table 3. After adjustment for demographic, lifestyle, and medical variables, having wider arterioles was associated with a lower odds of glaucoma (OR=0.90, 95% CI 0.85, 0.96) while having wider venules was associated with a higher odds of glaucoma (OR=1.09, 95% CI 1.04, 1.15). Neither arteriolar nor venular tortuosity were associated with glaucoma (P>0.05). Both wider arterioles and wider venules were associated with a smaller cup-to-disc ratio (β=-0.002, 95% CI -0.003, -0.001 and β=-0.001, 95% CI -0.002, -0.000 respectively). Arteriolar and venular tortuosity were associated with cup-to-disc ratio but in opposite directions (β=0.009, 95% CI 0.005, 0.013 and β=-0.034, 95% CI -0.041, -0.27, respectively). Wider venules

were inversely associated with IOP (β=-0.19, 95% CI -0.23, -0.15) while arterioles were not associated with IOP. Both arteriolar and venular tortuosity were associated with IOP (β=-0.26, 95% CI -0.44, -0.08 and β=-0.39, 95% CI -0.67, -0.10, respectively).

The longitudinal adjusted associations between retinal vessel traits and glaucoma-related outcomes are shown in Table 4. Unlike our cross-sectional associations between vessel diameter and glaucoma (Table 3), there were no statistically significant associations between retinal vessel diameter and the 3-year development of glaucoma. To determine whether the cross-sectional association between arteriolar diameter and glaucoma at baseline was due to reverse causality, we examined whether glaucoma at baseline was associated with 3-year change in arteriolar diameter. In fact, it was strongly associated (β=-0.21, 95% CI -0.37, -0.05) (data not shown) indicating that the cross-sectional association may have been due at least in part to reverse causality. Venular tortuosity was strongly inversely associated with the 3-year development of glaucoma (OR=0.52, 95% CI 0.31, 0.87). Also, venular tortuosity was inversely associated with 3-year change in cup-to-disc ratio (β=-0.006, 95% CI -0.010, -0.002). Venular diameter and arteriolar tortuosity were inversely associated with 3-year change in IOP (β=-0.07, 95% CI -0.11, -0.03 and β=-0.18, 95% CI -0.36, -0.01).

The cross sectional and longitudinal adjusted associations between AMD and retinal vascular diameter and tortuosity are shown in Table 5. Having wider arterioles was associated with a lower odds of AMD at baseline (OR=0.85, 95% CI 0.79, 0.91). By contrast, wider venular diameter was associated with a higher odds of both baseline AMD and the 3-year development of AMD (OR=1.11, 95% CI 1.04, 1.18 and OR=1.15, 95% CI 1.07, 1.24, respectively). Arteriolar tortuosity was not associated with AMD, while more tortuous retinal venules were strongly

associated with a lower odds of having AMD at baseline (OR=0.45, 95% CI 0.29, 0.69) but not with the 3-year development of AMD (OR=0.64, 95% CI 0.37, 1.10).

DISCUSSION

We have identified many cross-sectional and some longitudinal associations between retinal vessel traits and age-related eye disease. Sometimes the cross-sectional and longitudinal associations were consistent and other times they were not.

First, using cross-sectional data, arteriolar diameter was inversely associated with glaucoma and cup-to-disc ratio. This is consistent with most prior literature included in a systematic review and a more recent study by Rudnicka *et al* that reported that narrower arterioles were associated with glaucoma. [4,19] However, in our study, arteriolar diameter was not associated with the 3-year development of glaucoma or 3-year change in cup-to-disc ratio. To check if reverse causality was in part causing the cross-sectional association, we examined whether glaucoma at baseline was associated with the 3-year narrowing of retinal arterioles and indeed it was. Therefore, the cross-sectional association that we found between arteriolar diameter and glaucoma may have only been due to reverse causality. To date, to our knowledge, only one longitudinal study has established a statistically significant association between arteriolar diameter (CRAE) and the incidence of glaucoma. [6]

Evidence supporting a vascular pathogenesis of glaucoma and also the potential for vascular changes to develop secondary to glaucoma is discussed in a review. [20] In short, the vascular theory of glaucoma proposes that low perfusion pressure, impaired vascular autoregulation, and faulty neurovascular coupling may cause glaucoma. [20] However, there is also evidence in humans and animal models that the opposite is true: that glaucoma could cause

vascular abnormalities perhaps in part because glaucomatous retinal ganglion cells may need reduced blood supply. [20] More longitudinal studies are necessary to disentangle the temporal relationship between vessel abnormalities and glaucoma.

Venular diameter was positively associated with both baseline AMD and the 3-year development of AMD. Most prior studies in a recent systematic review have not found a statistically significant association between vessel width and signs of AMD although three studies are consistent with our findings. [21] For example, Jeganathan et al found in a cross-sectional study that widened venular diameter was associated with early AMD (OR=1.52, 1.11, 2.09). [22] Liew et al found in a longitudinal study that wider venules were associated with the 10-year development of RPE abnormalities (RR=1.1, 95% CI 1.0, 1.3). [10] Also, Wickremasinghe et al found that wider venular diameter was associated with worse response to bevacizumab in patients with neovascular AMD. [23] By contrast, we found that arteriolar diameter was negatively associated with baseline AMD. This is consistent with findings from a systematic review by Toulouie et al, where narrower CRAE was associated with central geographic atrophy in eyes with AMD. [24] Additionally, several studies using optical coherence tomography angiography have demonstrated an association between AMD and reduced vascular density. [5] Trinh et al proposed that reduced vascular density might be due in part to vessel thinning or constriction. [25]

In addition to vessel diameter, we also examined tortuosity. We found that venular tortuosity was negatively associated with the 3-year development of glaucoma and change in cup-to-disc ratio. This is consistent with Wu et al [26] and Koh et al [27] who found that less tortuosity (straighter vessels) was associated with glaucoma and a thinner neuroretinal rim, respectively. We found that increased venular tortuosity was also inversely associated with baseline AMD. We are not aware of any prior studies that examined tortuosity and AMD. Ischemic heart disease death

is also associated with straighter retinal vessels. [28] Witt et al proposed that reduced tortuosity may cause endothelial dysfunction and impairments in oxygenation in the microvasculature. [28]

Finally, we also examined the association between retinal vessel traits and IOP and the 3-year change in IOP. There is evidence to suggest that some topical hypotensive medications for glaucoma treatment, particularly beta-blockers and marginally prostaglandin analogues, are associated with narrowing of the retinal vessels. [29,30] However, regarding the reverse direction, only one previous study, to our knowledge, has assessed retinal vessel traits and their longitudinal relation to change in IOP. [31] That study found no longitudinal associations between retinal vessel diameter and IOP change. However, they did show that increased IOP at follow-up was associated with increased arteriolar tortuosity, while higher IOP at baseline was associated with more tortuous venules at follow-up. We were unable to replicate these findings in our study and instead found that arteriolar tortuosity was inversely associated with IOP and its 3-year change. This is consistent with findings from a cross-sectional study conducted by Wu et al, who found that arteriolar tortuosity was lower in eyes with higher IOP. [26]

Strengths of this research include the use of a large, national, longitudinal dataset with comprehensive physical exam and questionnaire data, the use of a deep learning approach called QUARTZ to provide retinal vessel data on the entire retina rather than only the area around the optic disc, and the assessment of multiple ocular outcomes. A limitation of this work is that glaucoma and AMD were assessed by self-report of a doctor's diagnosis, which is less valid than an eye exam or a medical record. However, for glaucoma, we had supplementary data from the retinal image and the physical exam such as CDR and IOP. Another limitation is that we do not know in which eye the person had glaucoma or AMD. Other analyses for CDR and IOP were limited to right eye data only to match the eye used for the retinal vessel traits. Furthermore, we

lacked data on axial length so were unable to convert pixels to microns. Finally, statistical power was reduced for our longitudinal analyses due to losses to follow-up and a smaller number of incident versus prevalent cases of glaucoma and AMD.

This work provides additional longitudinal analysis of the relationship between retinal vessel traits and age-related eye disease. Our strongest and most consistent evidence is that wider retinal venules are associated with AMD and that venular tortuosity is associated with glaucoma and glaucoma-related traits. Understanding how changes in the retinal microvasculature affect the development of eye disease is crucial to improving treatments and prevention strategies.

ACKNOWLEDGMENTS

This research was made possible using the data/biospecimens collected by the Canadian Longitudinal Study on Aging (CLSA). Funding for the Canadian Longitudinal Study on Aging (CLSA) is provided by the Government of Canada through the Canadian Institutes of Health Research (CIHR) under grant reference: LSA 94473 and the Canada Foundation for Innovation, as well as the following provinces, Newfoundland, Nova Scotia, Quebec, Ontario, Manitoba, Alberta, and British Columbia. This research has been conducted using the CLSA Baseline Comprehensive Dataset version 7.0, Follow-up 1 Comprehensive Dataset version 4.0, and Baseline Retinal Scans under Application Number 2209017. The CLSA is led by Drs. Parminder Raina, Christina Wolfson, and Susan Kirkland. The funders had no role in the design, analysis, or the interpretation of results. The opinions expressed in this manuscript are the author's own and do not reflect the views of the Canadian Longitudinal Study on Aging. Data are available from the Canadian Longitudinal Study on Aging (www.clsa-elcv.ca) for researchers who meet the criteria for access to de-identified CLSA data. This research project was further funded by CIHR operating grant PJT-183690 led by Dr. Ellen Freeman.

Table 7.1: Selected characteristics of those with and without a report of glaucoma at baseline

	Glaucoma (n=1,169) Mean (SD) or %	**No Glaucoma (n=24,773) Mean (SD) or %**
Age, Years	66.89 (10.50)	58.50 (9.58)
Female sex	58.17%	52.46%
Race/ethnicity		
White	93.53%	93.82%
Non-white	6.47%	6.18%
BMI, kg/m2	28.62 (6.11)	28.43 (5.69)
SBP, mmHg	123.09 (18.08)	119.84 (16.22)
DBP, mmHg	72.32 (10.75)	74.75 (9.90)
Smoking		
Never	41.24%	44.50%
Former	48.69%	44.06%
Current	10.06%	11.43%
Alcohol		
Never	2.40%	2.37%
Occasional	49.24%	42.37%
Weekly	32.42%	41.94%
Daily	15.95%	13.31%
Diabetes		
None	73.30%	83.69%
Type 1	1.51%	0.56%
Type 2	17.10%	8.37%
Neither	8.09%	7.38%
IOP, mmHg OD	17.38 (4.91)	15.61 (3.94)
IOP, mmHg OS	17.71 (5.43)	16.03 (3.94)
CDR OD	0.50 (0.12)	0.44 (0.09)
CDR OS	0.50 (0.12)	0.44 (0.09)
Arteriolar diameter OD, pixels	15.88 (1.99)	15.65 (1.64)
Arteriolar diameter OS, pixels	16.27 (2.16)	16.10 (1.78)
Venular diameter OD, pixels	18.10 (2.33)	17.51 (1.81)
Venular diameter OS, pixels	18.38 (2.64)	17.75 (1.91)
Arteriolar tortuosity* OD	1.24 (0.45)	1.23 (0.39)
Arteriolar tortuosity* OS	1.26 (0.46)	1.23 (0.39)
Venular tortuosity* OD	1.19 (0.32)	1.18 (0.24)
Venular tortuosity* OS	1.20 (0.29)	1.20 (0.25)

*tortuosity measures were multiplied by 1000 and the natural log taken to achieve normality

BMI=body mass index; SBP=systolic blood pressure; DBP=diastolic blood pressure; IOP=intraocular pressure; CDR=cup-to-disc ratio; OD=right eye; OS=left eye

Table 7.2: Selected characteristics of those with and without a report of AMD at baseline

	AMD (n=979) Mean (SD) or %	**No AMD (n=24,952) Mean (SD) or %**
OD pinhole visual acuity, logMAR	0.21 (0.25)	0.08 (0.15)
Age, Years	68.39 (11.95)	58.54 (9.54)
Female sex	59.22%	52.47%
Race/ethnicity		
White	94.55%	93.78%
Non-white	5.45%	6.22%
BMI, kg/m2	28.61 (6.45)	28.44 (5.68)
SBP, mmHg	124.92 (20.28)	119.84 (16.19)
DBP, mmHg	72.79 (10.95)	74.72 (9.92)
Smoking		
Never	41.78%	44.40%
Former	49.25%	44.11%
Current	8.98%	11.48%
Alcohol		
Never	2.93%	2.36%
Occasional	46.84%	42.52%
Weekly	33.12%	41.79%
Daily	17.11%	13.34%
Diabetes		
None	76.20%	83.56%
Type 1	0.29%	0.59%
Type 2	14.19%	8.53%
Neither	9.32%	7.32%
Arteriolar diameter OD, pixels	15.70 (1.96)	15.66 (1.64)
Arteriolar diameter OS, pixels	16.14 (2.27)	16.10 (1.79)
Venular diameter OD, pixels	18.15 (2.41)	17.52 (1.82)
Venular diameter OS, pixels	18.52 (2.67)	17.76 (1.92)
Arteriolar tortuosity* OD	1.22 (0.43)	1.23 (0.39)
Arteriolar tortuosity* OS	1.23 (0.44)	1.24 (0.39)
Venular tortuosity* OD	1.14 (0.30)	1.18 (0.24)
Venular tortuosity* OS	1.16 (0.31)	1.20 (0.25)

*tortuosity measures were multiplied by 1000 and the natural log taken to achieve normality

AMD=age-related macular degeneration; BMI=body mass index; SBP=systolic blood pressure; DBP=diastolic blood pressure; OD=right eye; OS=left eye

Table 7.3: Cross-sectional associations between retinal vessel traits and glaucoma outcomes

Model*	Vessel Trait†	Glaucoma n=23,993		CDR n=23,824		IOP n=23,279	
		OR	95% CI	β	95% CI	β	95% CI
1	Arteriolar diameter	0.90	0.85, 0.96	-0.002	-0.003, -0.001	0.04	-0.01, 0.09
	Venular diameter	1.09	1.04, 1.15	-0.001	-0.002, -0.000	-0.19	-0.23, -0.15
2	Arteriolar tortuosity	1.08	0.85, 1.36	0.009	0.005, 0.013	-0.26	-0.44, -0.08
	Venular tortuosity	0.92	0.56, 1.50	-0.034	-0.041, -0.027	-0.39	-0.67, -0.10

*Each model run separately containing both the arteriolar and venular trait; models also adjusted for age, sex, race, income, BMI, systolic and diastolic blood pressure, stroke, smoking, alcohol, and province

† Per 1 pixel increase in diameter or 1 unit increase in tortuosity

CDR=cup-to-disc ratio; IOP=intraocular pressure; CI=confidence interval; OR=odds ratio

Table 7.4: Longitudinal associations between baseline retinal vessel traits and glaucoma outcomes

Model*	Vessel Trait†	Development of Glaucoma n=20,994		CDR Change n=18,108		IOP Change n=19,386	
		OR	95% CI	β	95% CI	β	95% CI
1	Arteriolar diameter	1.06	0.94, 1.18	-0.000	-0.001, 0.000	-0.02	-0.06, 0.02
	Venular diameter	1.01	0.93, 1.10	-0.000	-0.001, 0.000	-0.07	-0.11, -0.03
2	Arteriolar tortuosity	1.12	0.79, 1.58	0.001	-0.001, 0.003	-0.18	-0.36, -0.01
	Venular tortuosity	0.52	0.31, 0.87	-0.006	-0.010, -0.002	-0.24	-0.50, 0.01

*Each model run separately containing both the arteriolar and venular trait; models also adjusted for age, sex, race, income, BMI, systolic and diastolic blood pressure, stroke, smoking, alcohol, and province; change models also adjusted for baseline level of outcome

† Per 1 pixel increase in diameter or 1 unit increase in tortuosity

CDR=cup-to-disc ratio; IOP=intraocular pressure; CI=confidence interval; OR=odds ratio

Table 7.5: Cross-sectional and 3-year longitudinal associations between baseline retinal vessel traits and baseline AMD and the 3-year development of AMD

Model*	Vessel Trait†	AMD at Baseline n=24,000		Development of AMD n=20,854	
		OR	95% CI	OR	95% CI
1	Arteriolar diameter	0.85	0.79, 0.91	0.98	0.89, 1.07
	Venular diameter	1.11	1.04, 1.18	1.15	1.07, 1.24
2	Arteriolar tortuosity	0.96	0.75, 1.22	1.23	0.85, 1.78
	Venular tortuosity	0.45	0.29, 0.69	0.64	0.37, 1.10

*Each model run separately containing both the arteriolar and venular trait; models also adjusted for age, sex, race, income, BMI, systolic and diastolic blood pressure, stroke, smoking, alcohol, and province

† Per 1 pixel increase in diameter or 1 unit increase in tortuosity

AMD=age-related macular degeneration; CI=confidence interval; OR=odds ratio

REFERENCES

1. Flaxman SR, Bourne RRA, Resnikoff S, et al. Global causes of blindness and distance vision impairment 1990-2020: a systematic review and meta-analysis. *Lancet Glob Health*. Dec 2017;5(12):e1221-e1234. doi:10.1016/S2214-109X(17)30393-5
2. Weinreb RN, Aung T, Medeiros FA. The pathophysiology and treatment of glaucoma: A review. *JAMA*. 2014;311:1901-1911. doi:10.1001/jama.2014.3192
3. Fleckenstein M, Schmitz-Valckenberg S, Chakravarthy U. Age-Related Macular Degeneration: A Review. *JAMA*. Jan 9 2024;331(2):147-157. doi:10.1001/jama.2023.26074
4. Chan KKW, Tang F, Tham CCY, Young AL, Cheung CY. Retinal vasculature in glaucoma: a review. *BMJ Open Ophthalmol*. 2017;1(1):e000032. doi:10.1136/bmjophth-2016-000032
5. Taylor TRP, Menten MJ, Rueckert D, Sivaprasad S, Lotery AJ. The role of the retinal vasculature in age-related macular degeneration: a spotlight on OCTA. *Eye (Lond)*. Feb 2024;38(3):442-449. doi:10.1038/s41433-023-02721-7
6. Kawasaki R, Wang JJ, Rochtchina E, Lee AJ, Wong TY, Mitchell P. Retinal vessel caliber is associated with the 10-year incidence of glaucoma: the Blue Mountains Eye Study. *Ophthalmology*. Jan 2013;120(1):84-90. doi:10.1016/j.ophtha.2012.07.007
7. Ikram MK, de Voogd S, Wolfs RC, et al. Retinal vessel diameters and incident open-angle glaucoma and optic disc changes: the Rotterdam study. *Invest Ophthalmol Vis Sci*. Apr 2005;46(4):1182-7. doi:10.1167/iovs.04-1459
8. Klein R, Klein BE, Tomany SC, Wong TY. The relation of retinal microvascular characteristics to age-related eye disease: the Beaver Dam eye study. *Am J Ophthalmol*. Mar 2004;137(3):435-44. doi:10.1016/j.ajo.2003.10.020
9. Wang JJ, Mitchell P, Rochtchina E, Tan AG, Wong TY, Klein R. Retinal vessel wall signs and the 5 year incidence of age related maculopathy: the Blue Mountains Eye Study. *Br J Ophthalmol*. Jan 2004;88(1):104-9. doi:10.1136/bjo.88.1.104
10. Liew G, Kaushik S, Rochtchina E, Tan AG, Mitchell P, Wang JJ. Retinal vessel signs and 10-year incident age-related maculopathy: the Blue Mountains Eye Study. *Ophthalmology*. Sep 2006;113(9):1481-7. doi:10.1016/j.ophtha.2006.03.051
11. Ikram MK, van Leeuwen R, Vingerling JR, Hofman A, de Jong PT. Retinal vessel diameters and the risk of incident age-related macular disease: the Rotterdam Study. *Ophthalmology*. Apr 2005;112(4):548-52. doi:10.1016/j.ophtha.2004.10.038
12. You QS, Xu L, Yang H, et al. Five-year incidence of age-related macular degeneration: the Beijing Eye Study. *Ophthalmology*. Dec 2012;119(12):2519-25. doi:10.1016/j.ophtha.2012.06.043
13. Raina P, Wolfson C, Kirkland S, et al. Cohort Profile: The Canadian Longitudinal Study on Aging (CLSA). *Int J Epidemiol*. Dec 1 2019;48(6):1752-1753j. doi:10.1093/ije/dyz173
14. Welikala R FJ, Johnson G, Rahman F, Podoleanu R, Remagnino P, Freeman EE, Chambers R, Bolter L, Anderson J, Olvera-Barrios A, Warwick A, Foster PJ, Shakespeare R, Ganguly R, Egan C, Tufail A, Owen CG, Rudnicka AR, Barman SA. Artificial Intelligence-Enabled Retinal Vasculometry at Scale Utilizing the UK Biobank, CLSA, and NEL DESP Datasets. presented at: IEEE-EMBS International Conference on Biomedical and Health Informatics (BHI'24); Nov 10-13 2024; Houston, USA.
15. Welikala RA FM, Habib M, Daniel-Tong S, Yates M, Foster P, Whincup PH, Rudnicka A, Owen CG, Strachan DP, Barman SA. . Automated Quantification of Retinal Vessel Morphometry

in the UK Biobank Cohort. presented at: Seventh International Conference on Image Processing Theory, Tools and Applications 2017;
16. WHO. A Healthy Lifestyle - WHO Recommendations. https://www.euro.who.int/en/health-topics/disease-prevention/nutrition/a-healthy-lifestyle/body-mass-index-bmi
17. Liew G, Wong TY, Mitchell P, Wang JJ. Are narrower or wider retinal venules associated with incident hypertension? *Hypertension*. Aug 2006;48(2):e10; author reply e11. doi:10.1161/01.HYP.0000231652.97173.4c
18. Sun C, Wang JJ, Mackey DA, Wong TY. Retinal vascular caliber: systemic, environmental, and genetic associations. *Surv Ophthalmol*. Jan-Feb 2009;54(1):74-95. doi:10.1016/j.survophthal.2008.10.003
19. Rudnicka AR, Owen CG, Welikala RA, et al. Retinal Vasculometry Associations With Glaucoma: Findings From the European Prospective Investigation of Cancer-Norfolk Eye Study. *Am J Ophthalmol*. Dec 2020;220:140-151. doi:10.1016/j.ajo.2020.07.027
20. Wang X, Wang M, Liu H, et al. The Association between Vascular Abnormalities and Glaucoma-What Comes First? *Int J Mol Sci*. Aug 25 2023;24(17)doi:10.3390/ijms241713211
21. Newman A, Andrew N, Casson R. Review of the association between retinal microvascular characteristics and eye disease. *Clin Exp Ophthalmol*. Jul 2018;46(5):531-552. doi:10.1111/ceo.13119
22. Jeganathan VS, Kawasaki R, Wang JJ, et al. Retinal vascular caliber and age-related macular degeneration: the Singapore Malay Eye Study. *Am J Ophthalmol*. Dec 2008;146(6):954-9 e1. doi:10.1016/j.ajo.2008.07.006
23. Wickremasinghe SS, Busija L, Guymer RH, Wong TY, Qureshi S. Retinal venular caliber predicts visual outcome after intravitreal ranibizumab injection treatments for neovascular AMD. *Invest Ophthalmol Vis Sci*. Jan 3 2012;53(1):37-41. doi:10.1167/iovs.11-7689
24. Toulouie S, Chang S, Pan J, Snyder K, Yiu G. Relationship of Retinal Vessel Caliber with Age-Related Macular Degeneration. *J Ophthalmol*. 2022;2022:8210599. doi:10.1155/2022/8210599
25. Trinh M, Kalloniatis M, Nivison-Smith L. Vascular Changes in Intermediate Age-Related Macular Degeneration Quantified Using Optical Coherence Tomography Angiography. *Transl Vis Sci Technol*. Jul 2019;8(4):20. doi:10.1167/tvst.8.4.20
26. Wu R, Cheung CY, Saw SM, Mitchell P, Aung T, Wong TY. Retinal vascular geometry and glaucoma: the Singapore Malay Eye Study. *Ophthalmology*. Jan 2013;120(1):77-83. doi:10.1016/j.ophtha.2012.07.063
27. Koh V, Cheung CY, Zheng Y, Wong TY, Wong W, Aung T. Relationship of retinal vascular tortuosity with the neuroretinal rim: the singapore malay eye study. *Invest Ophthalmol Vis Sci*. Jul 2010;51(7):3736-41. doi:10.1167/iovs.09-5008
28. Witt N, Wong TY, Hughes AD, et al. Abnormalities of retinal microvascular structure and risk of mortality from ischemic heart disease and stroke. *Hypertension*. May 2006;47(5):975-81. doi:10.1161/01.HYP.0000216717.72048.6c
29. Wong TY, Knudtson MD, Klein BE, Klein R, Hubbard LD. Medication use and retinal vessel diameters. *Am J Ophthalmol*. Feb 2005;139(2):373-5. doi:10.1016/j.ajo.2004.08.008
30. Howard KP, Klein BE, Dreyer JO, Danforth LG, Klein R. Cross-sectional associations of medication and supplement use with retinal vascular diameter in the Beaver Dam Eye Study. *JAMA Ophthalmol*. Jan 2014;132(1):23-31. doi:10.1001/jamaophthalmol.2013.6326

31. Freiberg J, Welikala R, Rovelt J, et al. Longitudinal associations of retinal vessel morphology with intraocular pressure and blood pressure at follow-up visit-Findings from a Danish eye and vision cohort, Project FOREVER. *Acta Ophthalmol*. Jul 2 2024;doi:10.1111/aos.16737

Chapter 8: Discussion

This thesis aimed to address three objectives in its investigation of retinal vessel traits in relation to sociodemographic, health, and lifestyle factors, and age-related eye disease. This discussion section includes our findings as they relate to each objective, comparison of results to previous literature, strengths and limitations, implications and significance of our work, and future research.

Objective One*:* *Cross-sectionally and longitudinally examine the relationship between sociodemographic, health, and lifestyle factors and retinal vessel traits (width, area, tortuosity).*

Vessel Diameter

Older age was significantly associated with having wider arteriolar (β=0.21, 95% CI= 0.17, 0.25) and venular diameter (β=0.38, 95% CI= 0.34, 0.43). Longitudinally, for every 10-year increase in age at baseline, arteriolar diameter widened by 0.12 pixels (95% CI: 0.08, 0.15) at follow-up, while venular diameter increased by 0.27 pixels (95% CI: 0.23, 0.31). This is in partial confirmation of other reports of arteriolar [31] and venular [27, 29] widening with increasing age. However, these findings are not consistent with those of several other epidemiological studies reporting that vessels narrow with age.[14-22, 190] As mentioned in manuscript one, we hypothesize that CRAE and CRVE may narrow with age but that there is a widening of the arterioles and venules more broadly across the retina.

We confirmed that White people generally have narrower arterioles than people of other races and ethnicities. For example, on average, Black people had wider arterioles by 1.27 pixels (95% CI: 0.83, 1.71) in comparison to White people. This is consistent with findings from a study of Australian children [121] and adults in the MESA and ARIC study.[17, 26]

SBP was not associated with arteriolar or venular diameter in cross-sectional or longitudinal analyses. Previous literature has been heterogenous in this sense, with studies suggesting higher SBP may be associated with wider venules [128] and either wider [14, 19, 25] or narrower arterioles. [17, 32, 42, 119, 128, 129] In contrast, we observed that DBP was consistently negatively associated with arteriolar and venular diameter, both cross- sectionally and longitudinally. For example, for every 10mmHg increase in DBP, venules narrowed by 0.15 pixels (95% CI: -0.20, -0.10). This is in agreement with several studies. [14, 19, 25, 27, 32, 42] Interestingly, previous studies have shown that MABP has been associated with narrower vessels, [18, 21, 28, 30] potentially being driven by the association between the vessels and DBP over SBP.

There were few associations between systemic health conditions and vessel diameter. Stroke and heart disease were not associated with vessel diameter. Diabetes was consistently associated with wider arteriolar diameters cross-sectionally, while type 1 diabetes was specifically associated with 3-year widening of arteriolar diameters (β=0.45, 95% CI: 0.09, 0.81). Venular diameter was not associated with diabetes in cross sectional or longitudinal analysis. These results are in agreement Tapp *et al* [27] and Islam *et al*.[34] However, other studies have indicated that both wider venules and arterioles are associated with diabetes, [17, 33] meanwhile a meta-analysis showed only venules were associated with incident diabetes. [138] We also noted that obesity was positively associated with venular diameter (β =0.16, 95% CI: 0.07, 0.25). Obesity and variation in body composition have repeatedly been associated with wider venules across several previous studies. [15, 17, 20, 26, 28-31]

The concentration of CRP was positively associated with retinal venular diameter in cross-sectional analyses; for every mg/L increase in CRP serum concentration, venular area was 0.30

kilopixel^2 (95% CI: 0.13, 0.47) larger. Markers of inflammation, including CRP, have consistently been associated with wider venules in the literature. [15, 17, 23-26] We were unable to corroborate findings by Myers *et al*, who saw that higher CRP was associated with a greater decrease in CRVE over 15 years. [15]

As lifestyle factors, smoking and alcohol consumption were associated with vessel diameter. Both current and former smoking were associated with wider arteriolar and venular diameters, and their widening over 3 years. The associations were generally stronger among current smokers compared to former smokers; in cross-sectional analysis, the effect on arteriolar diameter was β=0.10 (95% CI: 0.04, 0.16) for former smokers versus β=0.72 (95% CI: 0.60, 0.83) for current smokers. This pattern has been observed previously. [15, 17, 20, 21, 25, 26, 28, 30-32, 119, 155] Although, the literature also reports that venules are more strongly associated with smoking than arterioles, which we were not able to verify. In addition, we confirmed that arteriolar diameter was inversely associated with daily and weekly alcohol consumption (β=-0.33, 95% CI: -0.62, -0.03 and β=-0.38, 95% CI: -0.69, -0.08, respectively). [14, 17, 20, 26]

Vessel Area

We directly confirmed that smaller arteriolar and venular area may be a marker of age, systolic blood pressure, and diastolic blood pressure.[110, 111] Assuming that vessel area and diameter are proportional, our results are largely supported by literature that has previously assessed associations between vessel diameters and health and lifestyle factors. Larger venular area, like wider venular diameter, may be a marker of obesity, [15, 17, 20, 26, 28-31] smoking, [15, 17, 20, 21, 25, 26, 28, 30-

[32, 119, 155] and inflammation, [15, 17, 23-26] while smaller arteriolar area, like narrower arteriolar diameter, may be a marker of alcohol consumption, [14, 17, 20, 26] and stroke. [8, 37-40]

Interestingly, both larger arteriolar and venular area were associated with higher total cholesterol. This is partially supported by other studies in the literature. Wider venules [20] and narrower arterioles [27, 32] have been associated with total cholesterol. Meanwhile, high HDL ("good") cholesterol is consistently associated with narrower venules [15, 17, 20, 25, 27, 30-32, 119, 149] and narrower arterioles. [25-27, 32, 119, 149] Increased LDL ("bad") cholesterol is generally linked to wider venules. [17, 20, 27, 31] Future research should perhaps focus on the relationship between individual components of cholesterol and blood vessels.

Total vessel area remains an under-measured vascular parameter. However, as a potential indicator of retinal perfusion, it may provide additional information on how various factors influence retinal blood supply.

Vessel Tortuosity

Other race/ethnicities generally had less tortuous arterioles than White people. For example, arterioles were less tortuous (β=-0.39, 95% CI: -0.45, -0.33) in East Asians in comparison to White people. This is in contrast to a European study of children, where vessel tortuosity did not differ appreciably amongst most ethnic groups.[122] Perhaps this difference does not become apparent until later in life.

Being overweight (β=0.02, 95% CI: 0.01, 0.03) or obese (β=0.04, 95% CI: 0.03, 0.05) was positively associated with venular tortuosity cross-sectionally. This is consistent with findings from Tapp *et al* in two separate analyses of data from the UK biobank .[27, 29] We were unable to confirm results from Cheung *et al*, who observed that higher BMI was associated with less tortuous arterioles. [41] No consensus has yet been reached on how BMI impacts vessel tortuosity. However, our results, being in agreement with those from Tapp *et al*, may indicate that BMI has a more significant impact on venular tortuosity over that of arterioles.

Higher SBP was also associated with more tortuous arterioles and venules (β=0.01, 95% CI: 0.01, 0.02, for both), and increasing venular tortuosity over three years (β=0.01, 95% CI: 0.00, 0.01). Meanwhile, diastolic blood pressure showed the opposite association; it was negatively associated with tortuosity cross-sectionally and longitudinally associated with reduced venular tortuosity over three years (β=-0.01, 95% CI: -0.01, -0.00). Our results partially agree with findings from an analysis of data from the UK biobank, where Tapp *et al* observed that higher SBP was associated with more tortuous arterioles. [42] However, they also reported that higher DBP was associated with more tortuous venules, which we were not able to confirm. Additionally, in two studies using data from the Singapore Malay Eye Study, Cheung *et al* reported opposite results to ours, where less tortuous arterioles and more tortuous venules were associated with higher MABP.[41, 43] Therefore, more studies to better understand changes in vessel tortuosity in response to high blood pressure are warranted.

Those with stroke at baseline had slightly more tortuous arterioles (β=0.07, 95% CI: 0.00, 0.14) in comparison to those without stroke. Similar findings were reported by Owen *et al* [32] and

Sandoval-Garcia *et al*. [147] Meanwhile, tortuosity was not associated with stroke risk in an analysis of data from the Singapore Malaya Eye Study. [35]

In our study, current smoking was cross-sectionally associated with more tortuous venules (β=0.04, 95% CI: 0.02, 0.06). Owen *et al* previously observed that venular and arteriolar tortuosity was associated with smoking, but only in women compared to men. [32]

Lastly, higher CRP concentration was also associated with more tortuous venules in cross-sectional analysis (β=0.02, 95% CI: 0.01, 0.03). This is consistent with a large study (n=50,233) using data from the UK biobank, where higher CRP, as opposed to lower levels, was associated with a higher percent difference in venular tortuosity. [27]

Objective Two*:* *Cross-sectionally and longitudinally investigate the relationship between retinal vessel characteristics (width and tortuosity) and glaucomatous outcomes (IOP, CDR, and glaucoma prevalence).*

In manuscript #2, in confirmation with the existing literature, we found that those with narrower arterioles had higher odds of having glaucoma (OR=0.90, 95% CI: 0.85, 0.96) [185-189] and higher CDR (β=-0.002, 95% CI: -0.003, -0.001) [186, 189] at baseline. This is also supported by cross-sectional findings linking narrower arterioles to thinner RNFL thickness [99] and thinner neuroretinal rim. [108] Upon longitudinal analysis, we found that vessel diameter was not associated with the three-year development of glaucoma. This was in agreement with Ikram *et al*, who also reported null associations between arteriolar and venular diameter and incident glaucoma. [57] However, our

results were inconsistent with one other longitudinal study by Kawasaki *et al*, who found a positive relationship between narrower CRAE and incident glaucoma. [58]

When we looked at the reverse association with change in arteriolar diameter as the outcome, and baseline glaucoma as the exposure, the association was statistically significant; those with glaucoma at baseline had a higher degree of vessel narrowing over three years. This suggests that reverse causality may be implicated in the cross-sectional associations seen in our own study and those reported in the literature. Further longitudinal studies are needed to disentangle the temporal relationship between arteriolar narrowing and glaucoma.

We were unable to confirm results of three previous studies indicating that those with glaucoma may have narrower venules. [186, 188, 239] These studies did not adjust for arterial diameter in their models, which may be why we found the opposite; in adjusting for the fellow vessel, wider venules were cross-sectionally associated with glaucoma independent from the effects of arteriolar diameter (OR=1.09, 95% CI: 1.04, 1.15). Meanwhile, we saw that venular diameters were inversely associated with IOP (β=-0.19, 95% CI: -0.23, -0.15) and its three-year change (β=-0.07, 95% CI: -0.11, -0.03) which is more consistent with the literature; [21, 186] although, null results have also been reported. [188, 190] Further investigation into these relationships is warranted to clarify the role of venular changes in glaucoma pathogenesis.

Venular tortuosity was inversely associated with CDR (β=-0.034, 95% CI: -0.041, -0.027), its three-year change (β=-0.006, 95% CI: -0.010, -0.002), and the 3-year development of glaucoma (OR=0.52, 95% CI: 0.31, 0.87). This is consistent with cross sectional findings relating less tortuous venules to a thinner neuroretinal rim [108] and primary OAG [195] in the SiMES. Straighter venules have also been associated with thinner RGC thickness, an early structural change in glaucoma. [99] Meanwhile, Rudnicka *et al* observed that straighter arterioles and venules were not

associated with glaucoma. [188] Overall, there is some evidence to suggest that straighter vessels may be an indicator of early glaucomatous changes and glaucoma itself.

Lastly, we observed that IOP (β=-0.26, 95% CI: -0.44, -0.08) and its three-year change (β=-0.18, 95% CI: -0.36, -0.01) were inversely associated with arteriolar tortuosity. Conversely, no association was found between ocular hypertension and vessel tortuosity in the EPIC-Norfolk Eye study. [188] Conflicting evidence was also reported by Freiburg *et al*; they did not observe cross-sectional associations between tortuosity and IOP but did report that an increase in IOP from baseline to follow-up was associated with more tortuous arterioles. Higher IOP at baseline was also associated with more tortuous venules at follow- up. While our results are not consistent with previous studies, we present further evidence to suggest a vascular role in IOP variation.

Objective Three*:* *Examine the relationship between retinal vessel characteristics (width and tortuosity) and prevalence of AMD at baseline and 3-year follow-up.*

This objective was addressed in manuscript #2. We observed that those with narrower arteriolar diameter had higher odds of having AMD at baseline (OR=0.85, 95% CI: 0.79, 0.91). This is consistent with some cross-sectional studies noting that narrower arterioles were associated with either AMD itself, [225] or AMD-related pigmentary changes. [222, 231] Meanwhile, several others have reported null [56, 228-231] or the opposite effects [232, 233] in terms of AMD or AMD-related outcomes and arteriolar diameter. While we did not find any longitudinal associations between arteriolar diameter and AMD, other studies have seen increased risk of AMD or related outcomes with focal arteriolar narrowing [53, 56] or narrower CRAE. [53, 54]

We also saw that wider venular diameter was positively associated with AMD at baseline (OR=1.11, 95% CI: 1.04, 1.18) and its 3-year development (OR=1.15, 95% CI: 1.07, 1.24). The literature presents conflicting evidence for this relationship. Our findings are consistent with data from the Singapore Malay Eye Study, where wider venules were associated with prevalent early AMD. [231] However, null results were reported with respect to venular diameter and AMD prevalence in the Handan Eye Study [232] and the Singapore Indian Eye Study. [229] Longitudinally, incidence of early AMD-related pigment abnormalities has been linked to wider venules in the BMES, [53] whereas incidence of AMD itself is generally not associated with venular diameter.[53-55, 225]

Lastly, we observed that less tortuous venules were associated with AMD at baseline (OR=0.45, 95% CI: 0.29, 0.69). To the best of our knowledge, this is a novel finding, and no other published studies have assessed this relationship. It should, however, be noted that our results disagree with conference proceedings using data from the Singapore Malay Eye Study that suggested the opposite. [234] Further investigation is therefore warranted, as both studies suggest a role of vessel geometric pattern in AMD pathogenesis, despite opposing results.

Strengths

The CLSA's large sample size and national scope make it a uniquely valuable resource for Canadian researchers. The study provides comprehensive data on participants, including self-reported survey responses and objective physical assessments. This enabled us to examine a wide range of factors related to retinal vessel traits and adjust for several confounding factors when assessing their relationship with glaucoma and AMD. Furthermore, access to longitudinal data allowed us to assess how retinal vessel traits changed over a three-year period. We were also able

to determine development of AMD and glaucoma over three years by selecting a cohort free of either disease at baseline, offering insights into the temporal vascular mechanisms underlying age-related eye diseases. The CLSA also maintained a high retention rate at the three-year follow-up period (95%), [236] providing adequate power to detect statistically significant longitudinal associations.

In addition, the use of QUARTZ allowed us to extract retinal vessel data across the entire retina, rather than a restricted area around the optic nerve; this provided a more global assessment of retinal vessels in comparison to other methods. Lastly, in our statistical analysis, we included both the arteriolar and venular trait in regression models when assessing their relationship with the eye disease outcomes. Not only did this address the potential confounding relationship between the vessels, [81] it may also negate biasing effects from unmeasured parameters such as genetic factors. [34]

Limitations

The research presented in this thesis has several limitations. First, the CLSA had a participation rate of 45% with an overall response rate of only 10%, introducing the possibility of selection bias. [238] Additionally, while the CLSA is a nationwide study, it is not nationally representative, and the results may not apply to provinces/territories and populations excluded from the comprehensive cohort. This, in addition to having small sample sizes in some ethnic groups, limits the external validity of this research.

The CLSA also did not collect axial length, preventing us from converting arteriolar and venular diameter and area measurements from pixels into micrometers. Pixels are not a clinically relevant unit of measurement; therefore, the clinical applicability of this research is limited.

While the use of QUARTZ is a strength of the present analysis, our results may differ from those found in the existing literature due to differences in methodology. Measurements from different vessel analysis software have generally been shown to lack agreement. [240, 241] This is likely due to differing algorithms for vessel segmentation, vessel labeling, and estimating vascular diameter/tortuosity. [241] Since QUARTZ uniquely analyzes retinal vessel traits across the entire fundus image rather than a focal area around the optic disc, direct comparisons to the existing literature are even more problematic. [32] Additional factors contributing to heterogeneous results may include differences in study populations, disease definitions and diagnostics, and different methods in controlling for the many confounding factors that can affect retinal vessel characteristics. [232] The lack of standardization across several methods in this area of research contribute to lack of homogeneity.

Both manuscripts heavily relied on self-reported health and lifestyle factors. Specifically, in manuscript 2, glaucoma and AMD were not based on a standardized set of diagnostic criteria, nor were they confirmed by an ophthalmologist's assessment. The validity of self-reported eye disease, although infrequently studied, is low. For example, Linton *et al* reported that the sensitivity of in-person self-reported AMD in the BDES was 17.9%, suggesting that individuals who have the disease tend not to report it. [242] In contrast, specificity was quite high (98.7%), indicating that when participants did report having AMD, it was likely to be accurate. It is generally accepted that self-reported eye disease underestimates the true prevalence, leading to nonsignificant or erroneous results. [242] In the present study, it is unlikely that misclassification of eye disease, or other factors, differed based on retinal vessel parameters, therefore conservatively biasing results towards the null.

Furthermore, diagnosis of AMD and glaucoma were not assessed at the eye-level, and we used data from the right eye only. Since both glaucoma and AMD can affect the eyes unilaterally or asymmetrically, it is possible that the outcomes were non-differentially misclassified, again biasing our results towards the null. Additionally, the CLSA did not differentiate between the types and severity of glaucoma or AMD. We were therefore unable to run subgroup analyses on the types or severity of the eye diseases, potentially limiting the generalizability of our study since our results may not be the same across all types/severities of eye disease.

Significance and Implications

This research is the first to investigate retinal vessel characteristics in relation to a comprehensive set of factors and age-related eye disease in a Canadian population. By leveraging previously underutilized retinal images from the CLSA alongside its longitudinal data, this research provides additional insight into how health and lifestyle determinants are related to the retinal microvasculature over time. Furthermore, we have advanced the understanding of the vascular mechanisms underlying glaucoma and AMD. While more research is needed to translate our findings into clinical applications, some future implications of this research involve the screening, prevention, and treatment of both systemic and ocular disease.

Both glaucoma and macular degeneration can be asymptomatic in their early stages, often remaining undiagnosed while vision is lost. [168, 169] It is also understood that early detection and treatment of cardiovascular disease reduces the risk of adverse cardiovascular events and their associated chronic disabilities. [243] Thus, strategies for early diagnoses are crucial. If a causal association truly exists between changes in the retinal vasculature and subsequent disease development, fundus images and retinal vessel analysis could serve as a quick, non-invasive, and

cost-effective screening tool. By flagging patients with early microvascular signs for additional assessment, they could be identified, monitored, and treated sooner. Thus, integration of retinal vessel analysis into screening strategies for both ocular and systemic disease may be a valuable future clinical application.

Similarly, incorporating retinal vessel traits into prediction models may help identify individuals at risk of developing disease, allowing for targeted monitoring and earlier intervention for those with higher risk profiles. For example, it has been shown that including retinal vessel traits into stroke models improved prediction of its incidence by approximately 10% beyond the use of traditional risk factors.[35, 145] Applying this approach to eye disease and other chronic conditions could reduce their burden through proactive healthcare interventions.

The assessment of retinal vessel traits could also improve treatment strategies. Should a causal association exist between early microvascular changes and disease development, it may be possible to target these vascular issues directly, providing an alternative treatment pathway for disease management. Retinal vessels may also reflect a patient's response to treatment, and may therefore serve as a valuable tool for tracking treatment effectiveness. [244] For instance, retinal vessel narrowing may be reversed in response to blood pressure reduction.[245] Thus, measurement of retinal vessel characteristics has the potential to be incorporated into treatment strategies.

The research presented in this thesis lays the foundation for future clinical applications of retinal vessel analysis. While more longitudinal studies are needed to fully understand the relationships between health and the retinal microvasculature, this field of study could change efforts to screen for, prevent, and treat systemic and ocular disease. This may lead to earlier diagnoses, more targeted interventions, and ultimately, improved patient outcomes and reduced healthcare burdens.

Conclusions and Future Directions

Data from the CLSA has confirmed several previously known factors contributing to retinal vessel diameter and tortuosity. We also offered new insights into vessel area as a marker of cardiovascular risk factors. Lastly, in agreement with previous studies, we have highlighted the potential role of the retinal vasculature in the pathogenesis of AMD and glaucoma and changes in IOP and CDR in a Canadian population. As an immediate next step, formal mediation analyses could further clarify the role of the microvasculature in relation to known risk factors that impact both the retinal vessels and the development of age-related eye disease.

The CLSA is an ongoing prospective cohort study that will continue to follow its participants over 20 years. Continued analysis of new longitudinal data from the CLSA offers a valuable opportunity to further understand the natural history of retinal vessel changes over time in the face of sociodemographic, health, and lifestyle factors and how this may contribute to the development of ocular disease. Additionally, we have been notified that an updated version of QUARTZ is now able to convert pixel measurements into micrometers by standardizing images to the size of the optic disc. This advancement may allow for more clinically relevant interpretations of retinal vessel data; future studies using retinal images from the CLSA should prioritize using this to enhance the translational potential of the findings.

On a broader note, the scope of ophthalmological data collected by Canadian studies can be expanded moving forward. Future investigators of population-based studies should consider incorporating more comprehensive ocular testing, like visual fields, OCT, measurements of axial length or refraction, and physician diagnoses of eye disease based on established criteria into their protocols. This may enhance the clinical relevance of research findings. For example, data

collected from OCT images like RNFL and RGC thickness may further clarify structural impacts of blood vessel changes while visual field information may provide a functional understanding. This will also help overcome the limitations of self-reported eye-disease faced in the present research by providing more objective quantified endpoints for more certain conclusions.

Moreover, working towards standardized protocols in this area of research as a whole is imperative. This includes establishing consistent statistical methods and retinal vessel analysis techniques using verified software. This will improve comparability of results across research groups and accelerate the translation of research findings into clinical practice.

References

1. Wang J, Wang YX, Zeng D, Zhu Z, Li D, Liu Y, et al. Artificial intelligence-enhanced retinal imaging as a biomarker for systemic diseases. Theranostics. 2025;15(8):3223-33.
2. Rim TH, Lee G, Kim Y, Tham Y-C, Lee CJ, Baik SJ, et al. Prediction of systemic biomarkers from retinal photographs: development and validation of deep-learning algorithms. The Lancet Digital Health. 2020;2(10):e526-e36.
3. Foong AWP, Saw S-M, Loo J-L, Shen S, Loon S-C, Rosman M, et al. Rationale and Methodology for a Population-Based Study of Eye Diseases in Malay People: The Singapore Malay Eye Study (SiMES). Ophthalmic epidemiology. 2007;14(1):25-35.
4. Welikala RA, Fajtl J, Johnson G, Rahman F, Podoleanu R, Remagnino P, et al. Artificial Intelligence-Enabled Retinal Vasculometry at Scale Utilizing the UK Biobank, CLSA, and NEL DESP Datasets. IEEE-EMBS International Conference on Biomedical and Health Informatics. 2024.
5. Hubbard LD, Brothers RJ, King WN, Clegg LX, Klein R, Cooper LS, et al. Methods for evaluation of retinal microvascular abnormalities associated with hypertension/sclerosis in the atherosclerosis risk in communities study11The authors have no proprietary interest in the equipment and techniques described in this article. Ophthalmology. 1999;106(12):2269-80.
6. McGeechan KM, Liew GMM, Macaskill PP, Irwig LMP, Klein RMDMPH, Sharrett ARMDD, et al. Risk Prediction of Coronary Heart Disease Based on Retinal Vascular Caliber (from the Atherosclerosis Risk In Communities [ARIC] Study). The American journal of cardiology. 2008;102(1):58-63.
7. Wong TY, Klein R, Sharrett AR, Duncan BB, Couper DJ, Tielsch JM, et al. Retinal Arteriolar Narrowing and Risk of Coronary Heart Disease in Men and Women: The Atherosclerosis Risk in Communities Study. JAMA : the journal of the American Medical Association. 2002;287(9):1153-9.
8. Seidelmann SB, Claggett B, Bravo PE, Gupta A, Farhad H, Klein BE, et al. Retinal Vessel Calibers in Predicting Long-Term Cardiovascular Outcomes. Circulation. 2016;134(18):1328-38.
9. Wong TY, Kamineni A, Klein R, Sharrett AR, Klein BE, Siscovick DS, et al. Quantitative Retinal Venular Caliber and Risk of Cardiovascular Disease in Older Persons: The Cardiovascular Health Study. Archives of Internal Medicine. 2006;166(21):2388-94.
10. McGeechan K, Liew G, Witteman JCM, Breteler MMB, Shaw J, Zimmet P, et al. Meta-analysis: Retinal Vessel Caliber and Risk for Coronary Heart Disease. Annals of internal medicine. 2009;151(6):404-13.
11. Chandra A, Seidelmann SB, Claggett BL, Klein BE, Klein R, Shah AM, et al. The association of retinal vessel calibres with heart failure and long-term alterations in cardiac structure and function: the Atherosclerosis Risk in Communities (ARIC) Study. European journal of heart failure. 2019;21(10):1207-15.
12. Mutlu U, Ikram MK, Wolters FJ, Hofman A, Klaver CCW, Ikram MA. Retinal Microvasculature Is Associated With Long-Term Survival in the General Adult Dutch Population. Hypertension (Dallas, Tex 1979). 2016;67(2):281-7.
13. Wang JJ, Liew G, Klein R, Rochtchina E, Knudtson MD, Klein BEK, et al. Retinal vessel diameter and cardiovascular mortality: pooled data analysis from two older populations. European heart journal. 2007;28(16):1984-92.

14. Dervenis N, Coleman AL, Harris A, Wilson MR, Yu F, Anastasopoulos E, et al. Factors Associated With Retinal Vessel Diameters in an Elderly Population: The Thessaloniki Eye Study. Investigative ophthalmology & visual science. 2019;60(6):2208-17.
15. Myers CEM, Klein RMDMPH, Knudtson MDMS, Lee KEMS, Gangnon RP, Wong TYMDP, et al. Determinants of Retinal Venular Diameter: The Beaver Dam Eye Study. Ophthalmology (Rochester, Minn). 2012;119(12):2563-71.
16. Wong TY, Klein R, Klein BEK, Meuer SM, Hubbard LD. Retinal Vessel Diameters and Their Associations with Age and Blood Pressure. Investigative Ophthalmology & Visual Science. 2003;44(11):4644-50.
17. Wong TY, Islam FMA, Klein R, Klein BEK, Cotch MF, Castro C, et al. Retinal Vascular Caliber, Cardiovascular Risk Factors, and Inflammation: The Multi-Ethnic Study of Atherosclerosis (MESA). Investigative Ophthalmology & Visual Science. 2006;47(6):2341-50.
18. Kawasaki R, Wang JJ, Rochtchina E, Taylor B, Wong TY, Tominaga M, et al. Cardiovascular Risk Factors and Retinal Microvascular Signs in an Adult Japanese Population: The Funagata Study. Ophthalmology (Rochester, Minn). 2006;113(8):1378-84.
19. Leung H, Wang JJ, Rochtchina E, Tan AG, Wong TY, Klein R, et al. Relationships between Age, Blood Pressure, and Retinal Vessel Diameters in an Older Population. Investigative Ophthalmology & Visual Science. 2003;44(7):2900-4.
20. Jeganathan VSE, Sabanayagam C, Tai ES, Lee J, Sun C, Kawasaki R, et al. Effect of blood pressure on the retinal vasculature in a multi-ethnic Asian population. Hypertension Research. 2009;32(11):975-82.
21. Klein R, Klein BEK, Moss SE, Wong TY, Sharrett AR. Retinal vascular caliber in persons with type 2 diabetes : The wisconsin epidemiological study of diabetic retinopathy: XX. Ophthalmology (Rochester, Minn). 2006;113(9):1488-98.
22. Kaushik S, Kifley A, Mitchell P, Wang JJ. Age, Blood Pressure, and Retinal Vessel Diameter: Separate Effects and Interaction of Blood Pressure and Age. Investigative Opthalmology & Visual Science. 2007;48(2):557.
23. Liu M, Lycett K, Moreno-Betancur M, Wong TY, He M, Saffery R, et al. Inflammation mediates the relationship between obesity and retinal vascular calibre in 11-12 year-olds children and mid-life adults. Scientific reports. 2020;10(1):5006-.
24. Daien V, Carriere I, Kawasaki R, Cristol J-P, Villain M, Fesler P, et al. Retinal vascular caliber is associated with cardiovascular biomarkers of oxidative stress and inflammation: the POLA study. PloS one. 2013;8(7):e71089-e.
25. Ikram MK, De Jong FJ, Vingerling JR, Witteman JCM, Hofman A, Breteler MMB, et al. Are Retinal Arteriolar or Venular Diameters Associated with Markers for Cardiovascular Disorders? The Rotterdam Study. Investigative Opthalmology & Visual Science. 2004;45(7):2129.
26. Liew G, Sharrett AR, Wang JJ, Klein R, Klein BEK, Mitchell P, et al. Relative Importance of Systemic Determinants of Retinal Arteriolar and Venular Caliber: The Atherosclerosis Risk in Communities Study. Archives of Ophthalmology. 2008;126(10):1404-10.
27. Tapp RJ, Owen CG, Barman SA, Strachan DP, Welikala RA, Foster PJ, et al. Retinal microvascular associations with cardiometabolic risk factors differ by diabetes status: results from the UK Biobank. Diabetologia. 2022;65(10):1652-63.
28. Klein R, Klein BEK, Moss SE, Wong TY, Hubbard L, Cruickshanks KJ, et al. Retinal vascular abnormalities in persons with type 1 diabetes: The Wisconsin Epidemiologic Study of Diabetic Retinopathy: XVIII. Ophthalmology (Rochester, Minn). 2003;110(11):2118-25.

29. Tapp RJ, Owen CG, Barman SA, Welikala RA, Foster PJ, Whincup PH, et al. Retinal Vascular Tortuosity and Diameter Associations with Adiposity and Components of Body Composition. Obesity. 2020;28(9):1750-60.
30. von Hanno T, Bertelsen G, Sjølie AK, Mathiesen EB. Retinal vascular calibres are significantly associated with cardiovascular risk factors: the Tromsø Eye Study. Acta Ophthalmologica. 2014;92(1):40-6.
31. Sun C, Liew G, Wang JJ, Mitchell P, Saw SM, Aung T, et al. Retinal Vascular Caliber, Blood Pressure, and Cardiovascular Risk Factors in an Asian Population: The Singapore Malay Eye Study. Investigative Ophthalmology & Visual Science. 2008;49(5):1784-90.
32. Owen CG, Rudnicka AR, Welikala RA, Fraz MM, Barman SA, Luben R, et al. Retinal Vasculometry Associations with Cardiometabolic Risk Factors in the European Prospective Investigation of Cancer—Norfolk Study. Ophthalmology (Rochester, Minn). 2019;126(1):96-106.
33. Cheung CY-l, Lamoureux E, Ikram MK, Sasongko MB, Ding J, Zheng Y, et al. Retinal vascular geometry in Asian persons with diabetes and retinopathy. Journal of diabetes science and technology. 2012;6(3):595-605.
34. Islam FMA, Nguyen TT, Wang JJ, Tai ES, Shankar A, Saw SM, et al. Quantitative retinal vascular calibre changes in diabetes and retinopathy: the Singapore Malay eye study. Eye (London). 2009;23(8):1719-24.
35. Cheung CY-L, Wan Ting TAY, Ikram MK, Yi Ting ONG, De Silva DA, Khuan Yew C, et al. Retinal Microvascular Changes and Risk of Stroke: The Singapore Malay Eye Study. Stroke (1970). 2013;44(9):2402-8.
36. Ikram MK, De Jong FJ, Bos MJ, Vingerling JR, Hofman A, Koudstaal PJ, et al. Retinal vessel diameters and risk of stroke : The Rotterdam Study. Neurology. 2006;66(9):1339-43.
37. Yatsuya H, Folsom AR, Wong TY, Klein R, Klein BEK, Sharrett AR. Retinal Microvascular Abnormalities and Risk of Lacunar Stroke. Stroke. 2010;41(7):1349-55.
38. Kawasaki R, Jing XIE, Cheung N, Lamoureux E, Klein R, Klein BEK, et al. Retinal Microvascular Signs and Risk of Stroke: The Multi-Ethnic Study of Atherosclerosis (MESA). Stroke (1970). 2012;43(12):3245-51.
39. Wieberdink RG, Ikram MK, Koudstaal PJ, Hofman A, Vingerling JR, Breteler MMB. Retinal Vascular Calibers and the Risk of Intracerebral Hemorrhage and Cerebral Infarction: The Rotterdam Study. Stroke (1970). 2010;41(12):2757-61.
40. Mitchell P, Wang JJ, Wong TY, Smith W, Klein R, Leeder SR. Retinal microvascular signs and risk of stroke and stroke mortality. Neurology. 2005;65(7):1005-9.
41. Cheung CY-lP, Zheng YMD, Hsu WP, Lee MLP, Lau QPB, Mitchell PMDP, et al. Retinal Vascular Tortuosity, Blood Pressure, and Cardiovascular Risk Factors. Ophthalmology (Rochester, Minn). 2011;118(5):812-8.
42. Tapp RJ, Owen CG, Barman SA, Welikala RA, Foster PJ, Whincup PH, et al. Associations of Retinal Microvascular Diameters and Tortuosity With Blood Pressure and Arterial Stiffness. Hypertension. 2019;74(6):1383-90.
43. Cheung CY, Tay WT, Mitchell P, Wang JJ, Hsu W, Lee ML, et al. Quantitative and qualitative retinal microvascular characteristics and blood pressure. Journal of hypertension. 2011;29(7):1380-91.
44. Nislawati R, Taufik Fadillah Zainal A, Ismail A, Waspodo N, Kasim F, Gunawan AMAK. Role of hypertension as a risk factor for open-angle glaucoma: a systematic review and meta-analysis. BMJ open ophthalmology. 2021;6(1):e000798-e.

45. Zhao D, Cho J, Kim MH, Guallar E. The Association of Blood Pressure and Primary Open-Angle Glaucoma: A Meta-analysis. American journal of ophthalmology. 2014;158(3):615-27.e9.
46. Choi JA, Lee S-N, Jung S-H, Won H-H, Yun J-S. Association of glaucoma and lifestyle with incident cardiovascular disease: a longitudinal prospective study from UK Biobank. Scientific Reports. 2023;13(1):2712.
47. Chen Y-Y, Hu H-Y, Chu D, Chen H-H, Chang C-K, Chou P. Patients with Primary Open-Angle Glaucoma May Develop Ischemic Heart Disease More Often than Those without Glaucoma: An 11-Year Population-Based Cohort Study. PLOS ONE. 2016;11(9):e0163210.
48. Wang M, Chen N, Sun B-C, Guo C-B, Zhang S, Huang M-J, et al. Association between glaucoma and risk of stroke: A systematic review and meta-analysis. Frontiers in neurology. 2023;13:1034976-.
49. Feng J, Xie F, Wu Z, Wu Y. Age-related macular degeneration and cardiovascular disease in US population: an observational study. Acta Cardiologica. 2024;79(6):665-71.
50. Cackett P, Wong TY, Aung T, Saw S-M, Tay WT, Rochtchina E, et al. Smoking, Cardiovascular Risk Factors, and Age-related Macular Degeneration in Asians: The Singapore Malay Eye Study. American journal of ophthalmology. 2008;146(6):960-7.e1.
51. Wieberdink RG, Ho L, Kamran Ikram M, Koudstaal PJ, Hofman A, De Jong PTVM, et al. Age-Related Macular Degeneration and the Risk of Stroke The Rotterdam Study. Stroke (1970). 2011;42(8):2138-42.
52. Wang JJ. Retinal vessel wall signs and the 5 year incidence of age related maculopathy: the Blue Mountains Eye Study. British Journal of Ophthalmology. 2004;88(1):104-9.
53. Liew G, Kaushik S, Rochtchina E, Tan AG, Mitchell P, Jie Jin W. Retinal vessel signs and 10-year incident age-related maculopathy : The blue mountains eye study. Ophthalmology (Rochester, Minn). 2006;113(9):1481-7.
54. Ikram KM, Van Leeuwen R, Vingerling JR, Hofman A, De Jong PTVM. Retinal vessel diameters and the risk of incident age-related macular disease : The Rotterdam study. Ophthalmology (Rochester, Minn). 2005;112(4):548-52.
55. You QS, Xu L, Yang H, Li YB, Wang S, Wang JD, et al. Five-Year Incidence of Age-related Macular Degeneration: The Beijing Eye Study. Ophthalmology (Rochester, Minn). 2012;119(12):2519-25.
56. Klein R, Klein BEK, Tomany SC, Wong TY. The relation of retinal microvascular characteristics to age-related eye disease: the Beaver Dam eye study. American Journal of Ophthalmology. 2004;137(3):435-44.
57. Ikram MK, De Voogd S, Wolfs RCW, Hofman A, Breteler MMB, Hubbard LD, et al. Retinal Vessel Diameters and Incident Open-Angle Glaucoma and Optic Disc Changes: The Rotterdam Study. Investigative Opthalmology & Visual Science. 2005;46(4):1182.
58. Kawasaki R, Wang JJ, Rochtchina E, Lee AJ, Wong TY, Mitchell P. Retinal vessel caliber is associated with the 10-year incidence of glaucoma: the Blue Mountains Eye Study. Ophthalmology (Rochester, Minn). 2013;120(1):84-90.
59. Wong WL, Su X, Li X, Cheung CMG, Klein R, Cheng C-Y, et al. Global prevalence of age-related macular degeneration and disease burden projection for 2020 and 2040: a systematic review and meta-analysis. The Lancet Global Health. 2014;2(2):e106-e16.
60. Bourne RRA, Jonas JB, Friedman D, Nangia V, Bron A, Tapply I, et al. Global estimates on the number of people blind or visually impaired by glaucoma: A meta-analysis from 2000 to 2020. Eye. 2024.

61. Remington LA. Clinical anatomy and physiology of the visual system. 3rd ed. St. Louis: Elsevier/Butterworth-Heinemann; 2012.
62. Forrester JV, Dick AD, McMenamin PG, Roberts F, Pearlman E. Chapter 1 - Anatomy of the eye and orbit. In: Forrester JV, Dick AD, McMenamin PG, Roberts F, Pearlman E, editors. The Eye (Fourth Edition): W.B. Saunders; 2016. p. 1-102.e2.
63. Riva CE, Schmetterer L. Chapter 16 - Microcirculation of the Ocular Fundus. In: Tuma RF, Durán WN, Ley K, editors. Microcirculation (Second Edition). San Diego: Academic Press; 2008. p. 735-65.
64. Archer DB, Gardiner TA, Stitt AW. Functional Anatomy, Fine Structure and Basic Pathology of the Retinal Vasculature. Retinal Vascular Disease: Springer Berlin Heidelberg; 2007. p. 3-23.
65. Lens A, Nemeth SC, Ledford JK. Ocular anatomy and physiology. Second edition. ed. Boca Raton: CRC Press; 2024.
66. Kiel JW. The ocular circulation. San Rafael, Calif.?: Morgan & Claypool; 2011.
67. Forrester JV, Dick AD, McMenamin PG, Roberts F, Pearlman E. Chapter 4 - Biochemistry and cell biology. In: Forrester JV, Dick AD, McMenamin PG, Roberts F, Pearlman E, editors. The Eye (Fourth Edition): W.B. Saunders; 2016. p. 157-268.e4.
68. Luo X, Shen Y-m, Jiang M-n, Lou X-f, Shen Y. Ocular Blood Flow Autoregulation Mechanisms and Methods. Journal of Ophthalmology. 2015;2015(1):864871.
69. Biousse V, Bruce BB, Newman NJ. Ophthalmoscopy in the 21st century: The 2017 H. Houston Merritt Lecture. Neurology. 2018;90(4):167-75.
70. Ivanišević M. First look into the eye. European journal of ophthalmology. 2019;29(6):685-8.
71. The Stanford Medicine 25 [Available from: https://stanfordmedicine25.stanford.edu/the25.html.
72. Benbassat J, Polak BCP, Javitt JC. Objectives of teaching direct ophthalmoscopy to medical students. Acta ophthalmologica (Oxford, England). 2012;90(6):503-7.
73. Panwar N, Huang P, Lee J, Keane PA, Chuan TS, Richhariya A, et al. Fundus Photography in the 21st Century—A Review of Recent Technological Advances and Their Implications for Worldwide Healthcare. Telemedicine journal and e-health. 2016;22(3):198-208.
74. Girach A, Sergott RC. Optical coherence tomography. Cham: Springer; 2016.
75. Boopathiraj N, Wagner IV, Dorairaj SK, Miller DD, Stewart MW. Recent Updates on the Diagnosis and Management of Age-Related Macular Degeneration. Mayo Clinic Proceedings: Innovations, Quality & Outcomes. 2024;8(4):364-74.
76. CLSA. Data Collection Site (DCS) SOP (Vision-Retinal Camera). 2015.
77. Wagener HP, Clay GE, Gipner JF. Classification of Retinal Lesions in the Presence of Vascular Hypertension: Report submitted to the American Ophthalmological Society by the committee on Classification of Hypertensive Disease of the Retina. Trans Am Ophthalmol Soc. 1947;45:57-73.
78. Parr JC, Spears GFS. General Caliber of the Retinal Arteries Expressed as the Equivalent width of the Central Retinal Artery. American Journal of Ophthalmology. 1974;77(4):472-7.
79. Knudtson MD, Lee KE, Hubbard LD, Wong TY, Klein R, Klein BEK. Revised formulas for summarizing retinal vessel diameters. Current eye research. 2003;27(3):143-9.
80. Hanssen H, Streese L, Vilser W. Retinal vessel diameters and function in cardiovascular risk and disease. Progress in Retinal and Eye Research. 2022;91:101095.

81. Liew G, Sharrett AR, Kronmal R, Klein R, Wong TY, Mitchell P, et al. Measurement of Retinal Vascular Caliber: Issues and Alternatives to Using the Arteriole to Venule Ratio. Investigative Ophthalmology & Visual Science. 2007;48(1):52-7.
82. Sun CMDMPH, Wang JJMP, Mackey DAMD, Wong TYMDP. Retinal Vascular Caliber: Systemic, Environmental, and Genetic Associations. Survey of ophthalmology. 2009;54(1):74-95.
83. Ikram MK, Xueling S, Jensen RA, Cotch MF, Hewitt AW, Ikram MA, et al. Four Novel Loci (19q13, 6q24, 12q24, and 5q14) Influence the Microcirculation In Vivo. PLoS Genetics. 2010;6(10):e1001184.
84. Sim X, Jensen RA, Ikram MK, Cotch MF, Li X, Macgregor S, et al. Genetic Loci for Retinal Arteriolar Microcirculation. PLoS ONE. 2013;8(6):e65804.
85. Jensen RA, Sim X, Smith AV, Li X, Jakobsdóttir J, Cheng C-Y, et al. Novel Genetic Loci Associated With Retinal Microvascular Diameter. Circulation: Cardiovascular Genetics. 2016;9(1):45-54.
86. Veluchamy A, Ballerini L, Vitart V, Schraut KE, Kirin M, Campbell H, et al. Novel Genetic Locus Influencing Retinal Venular Tortuosity Is Also Associated With Risk of Coronary Artery Disease. Arteriosclerosis, Thrombosis, and Vascular Biology. 2019;39(12):2542-52.
87. Tomasoni M, Beyeler MJ, Vela SO, Mounier N, Porcu E, Corre T, et al. Genome-wide Association Studies of Retinal Vessel Tortuosity Identify Numerous Novel Loci Revealing Genes and Pathways Associated With Ocular and Cardiometabolic Diseases. Ophthalmology science (Online). 2023;3(3):100288-.
88. Jiang X, Hysi PG, Khawaja AP, Mahroo OA, Xu Z, Hammond CJ, et al. GWAS on retinal vasculometry phenotypes. PLOS Genetics. 2023;19(2):e1010583.
89. Leung H, Wang JJ, Rochtchina E, Wong TY, Klein R, Mitchell P. Impact of current and past blood pressure on retinal arteriolar diameter in an older population. Journal of hypertension. 2004;22(8):1543-9.
90. Wong TY, Hubbard LD, Klein R, Marino EK, Kronmal R, Sharrett AR, et al. Retinal microvascular abnormalities and blood pressure in older people: the Cardiovascular Health Study. British journal of ophthalmology. 2002;86(9):1007-13.
91. Klein R, Sharrett AR, Klein BEK, Chambless LE, Cooper LS, Hubbard LD, et al. Are Retinal Arteriolar Abnormalities Related to Atherosclerosis? Arteriosclerosis, Thrombosis, and Vascular Biology. 2000;20(6):1644-50.
92. Wong TY, Wang JJ, Rochtchina E, Klein R, Mitchell P. Does refractive error influence the association of blood pressure and retinal vessel diameters? the blue mountains eye study. American journal of ophthalmology. 2004;137(6):1050-5.
93. Yii F, Strang N, Moulson C, Dhillon B, Bernabeu MO, Macgillivray T. The Optical Nature of Myopic Changes in Retinal Vessel Caliber. Ophthalmology Science. 2025;5(1):100631.
94. Cheung N, Wong TY. The retinal arteriole to venule ratio: informative or deceptive? Graefe's Archive for Clinical and Experimental Ophthalmology. 2007;245(8):1245-6.
95. Chen HC, Patel V, Wiek J, Rassam SM, Kohner EM. Vessel diameter changes during the cardiac cycle. Eye. 1994;8(1):97-103.
96. Knudtson MD, Klein BEK, Klein R, Wong TY, Hubbard LD, Lee KE, et al. Variation associated with measurement of retinal vessel diameters at different points in the pulse cycle. British journal of ophthalmology. 2004;88(1):57-61.

97. Cheung CY-l, Ikram MK, Sabanayagam C, Wong TY. Retinal Microvasculature as a Model to Study the Manifestations of Hypertension. Hypertension. 2012;60(5):1094-103.
98. Rossitti S, LÖFgren J. Optimality principles and flow orderliness at the branching points of cerebral arteries. Stroke (1970). 1993;24(7):1029-32.
99. Tham Y-C, Cheng C-Y, Zheng Y, Aung T, Wong TY, Cheung CY. Relationship Between Retinal Vascular Geometry With Retinal Nerve Fiber Layer and Ganglion Cell-Inner Plexiform Layer in Nonglaucomatous Eyes. Investigative Ophthalmology & Visual Science. 2013;54(12):7309-16.
100. Ciurică S, Lopez-Sublet M, Loeys BL, Radhouani I, Natarajan N, Vikkula M, et al. Arterial Tortuosity: Novel Implications for an Old Phenotype. Hypertension. 2019;73(5):951-60.
101. Hartnett ME, Martiniuk D, Byfield G, Geisen P, Zeng G, Bautch VL. Neutralizing VEGF Decreases Tortuosity and Alters Endothelial Cell Division Orientation in Arterioles and Veins in a Rat Model of ROP: Relevance to Plus Disease. Investigative Ophthalmology & Visual Science. 2008;49(7):3107-14.
102. Goldman D, Popel AS. A Computational Study of the Effect of Capillary Network Anastomoses and Tortuosity on Oxygen Transport. Journal of theoretical biology. 2000;206(2):181-94.
103. Tomita Y, Kubis N, Calando Y, Dinh AT, Méric P, Seylaz J, et al. Long-Term in Vivo Investigation of Mouse Cerebral Microcirculation by Fluorescence Confocal Microscopy in the Area of Focal Ischemia. Journal of cerebral blood flow and metabolism. 2005;25(7):858-67.
104. Malek J, Azar AT, Tourki R. Impact of retinal vascular tortuosity on retinal circulation. Neural Comput Appl. 2015;26(1):25–40.
105. Han H-C. Twisted Blood Vessels: Symptoms, Etiology and Biomechanical Mechanisms. Journal of Vascular Research. 2012;49(3):185-97.
106. Yamakawa K, Ahmed Bhutto I, Lu Z, Watanabe Y, Amemiya T. Retinal vascular changes in rats with inherited hypercholesterolemia - Corrosion cast demonstration. Current eye research. 2001;22(4):258-65.
107. Kalitzeos AA, Lip GYH, Heitmar R. Retinal vessel tortuosity measures and their applications. Experimental eye research. 2013;106:40-6.
108. Koh V, Cheung CY-L, Zheng Y, Wong TY, Wong W, Aung T. Relationship of Retinal Vascular Tortuosity with the Neuroretinal Rim: The Singapore Malay Eye Study. Investigative Opthalmology & Visual Science. 2010;51(7):3736.
109. Abdalla M, Hunter A, Al-Diri B, editors. Quantifying retinal blood vessels' tortuosity - Review2015: IEEE.
110. Maderuelo-Fernandez JA, Garcia-Garcia A, Chamoso P, Recio-Rodríguez JI, Rodríguez-González S, Patino-Alonso MC, et al. Automatic image analyser to assess retinal vessel calibre (ALTAIR). A new tool to evaluate the thickness, area and length of the vessels of the retina. International journal of medical informatics (Shannon, Ireland). 2020;136:104090-.
111. Fukutsu K, Saito M, Noda K, Murata M, Kase S, Shiba R, et al. A Deep Learning Architecture for Vascular Area Measurement in Fundus Images. Ophthalmology science (Online). 2021;1(1):100004-.
112. Maloca PM, Feu-Basilio S, Schottenhamml J, Valmaggia P, Scholl HPN, Rosinés-Fonoll J, et al. Reference database of total retinal vessel surface area derived from volume-rendered optical coherence tomography angiography. Scientific Reports. 2022;12(1).
113. Perez-Rovira A, MacGillivray T, Trucco E, Chin KS, Zutis K, Lupascu C, et al., editors. VAMPIRE: Vessel assessment and measurement platform for images of the REtina2011: IEEE.

114. Fraz MM, Welikala RA, Rudnicka AR, Owen CG, Strachan DP, Barman SA. QUARTZ: Quantitative Analysis of Retinal Vessel Topology and size – An automated system for quantification of retinal vessels morphology. Expert Systems with Applications. 2015;42(20):7221-34.
115. LeCun Y, Bengio Y, Hinton G. Deep learning. Nature. 2015;521(7553):436-44.
116. Welikala RA, Fraz MM, Foster PJ, Whincup PH, Rudnicka AR, Owen CG, et al. Automated Retinal Image Quality Assessment on the UK Biobank Dataset for Epidemiological Studies. Computers in Biology and Medicine. 2015.
117. Welikala RA, Foster PJ, Whincup PH, Rudnicka AR, Owen CG, Strachan DP, et al. Automated arteriole and venule classification using deep learning for retinal images from the UK Biobank cohort. Computers in Biology and Medicine. 2017;90:23-32.
118. Owen CG, Rudnicka AR, Mullen R, Barman SA, Monekosso D, Whincup PH, et al. Measuring Retinal Vessel Tortuosity in 10-Year-Old Children: Validation of the Computer-Assisted Image Analysis of the Retina (CAIAR) Program. Investigative ophthalmology & visual science. 2009;50(5):2004-10.
119. Drobnjak D, Munch IC, Glümer C, Faerch K, Kessel L, Larsen M, et al. Retinal Vessel Diameters and Their Relationship with Cardiovascular Risk and All-Cause Mortality in the Inter99 Eye Study: A 15-Year Follow-Up. Journal of Ophthalmology. 2016;2016(2016):1-8-178.
120. Cheung N, Islam FMA, Saw SM, Shankar A, de Haseth K, Mitchell P, et al. Distribution and Associations of Retinal Vascular Caliber with Ethnicity, Gender, and Birth Parameters in Young Children. Investigative Ophthalmology & Visual Science. 2007;48(3):1018-24.
121. Rochtchina E, Wang JJ, Taylor B, Wong TY, Mitchell P. Ethnic Variability in Retinal Vessel Caliber: A Potential Source of Measurement Error from Ocular Pigmentation?—The Sydney Childhood Eye Study. Investigative Opthalmology & Visual Science. 2008;49(4):1362.
122. Owen CG, Rudnicka AR, Nightingale CM, Mullen R, Barman SA, Sattar N, et al. Retinal Arteriolar Tortuosity and Cardiovascular Risk Factors in a Multi-Ethnic Population Study of 10-Year-Old Children; the Child Heart and Health Study in England (CHASE). Arteriosclerosis, Thrombosis, and Vascular Biology. 2011;31(8):1933-8.
123. Li X, Wong WL, Cheung CY-l, Cheng C-Y, Ikram MK, Li J, et al. Racial Differences in Retinal Vessel Geometric Characteristics: A Multiethnic Study in Healthy Asians. Investigative Ophthalmology & Visual Science. 2013;54(5):3650-6.
124. Anand SS, Yusuf S, Vuksan V, Devanesen S, Teo KK, Montague PA, et al. Differences in risk factors, atherosclerosis, and cardiovascular disease between ethnic groups in Canada: the Study of Health Assessment and Risk in Ethnic groups (SHARE). The Lancet (British edition). 2000;356(9226):279-84.
125. Taarnhøj NCBB, Larsen M, Sander B, Kyvik KO, Kessel L, Hougaard JL, et al. Heritability of Retinal Vessel Diameters and Blood Pressure: A Twin Study. Investigative Opthalmology & Visual Science. 2006;47(8):3539.
126. DeMers D, Wachs D. Physiology, Mean Arterial Pressure. StatPearls. National Library of Medicine: StatPearls Publishing; 2023.
127. Chew SKH, Xie J, Wang JJ. Retinal Arteriolar Diameter and the Prevalence and Incidence of Hypertension: A Systematic Review and Meta-analysis of Their Association. Current Hypertension Reports. 2012;14(2):144-51.
128. Ding J, Wai KL, McGeechan K, Ikram MK, Kawasaki R, Xie J, et al. Retinal vascular caliber and the development of hypertension: a meta-analysis of individual participant data. Journal of hypertension. 2014;32(2):207-15.

129. Wong TY, Klein R, Sharrett AR, Duncan BB, Couper DJ, Klein BEK, et al. Retinal Arteriolar Diameter and Risk for Hypertension. Annals of Internal Medicine. 2004;140(4):248-55.
130. Wong TY, Shankar A, Klein R, Klein BEK, Hubbard LD. Prospective cohort study of retinal vessel diameters and risk of hypertension. BMJ. 2004;329(7457):79-82.
131. Smith W, Wang JJ, Wong TY, Rochtchina E, Klein R, Leeder SR, et al. Retinal Arteriolar Narrowing Is Associated With 5-Year Incident Severe Hypertension: The Blue Mountains Eye Study. Hypertension (Dallas, Tex 1979). 2004;44(4):442-7.
132. Wang JJ, Rochtchina E, Liew G, Tan AG, Wong TY, Leeder SR, et al. The Long-term Relation among Retinal Arteriolar Narrowing, Blood Pressure, and Incident Severe Hypertension. American journal of epidemiology. 2008;168(1):80-8.
133. Ikram MK, Witteman JCM, Vingerling JR, Breteler MMB, Hofman A, de Jong PTVM. Retinal Vessel Diameters and Risk of Hypertension: The Rotterdam Study. Hypertension (Dallas, Tex 1979). 2006;47(2):189-94.
134. Kawasaki R, Cheung N, Wang JJ, Klein R, Klein BEK, Cotch MF, et al. Retinal vessel diameters and risk of hypertension: the Multiethnic Study of Atherosclerosis. Journal of hypertension. 2009;27(12):2386-93.
135. TANABE Y, KAWASAKI R, WANG JJ, WONG TY, MITCHELL P, DAIMON M, et al. Retinal Arteriolar Narrowing Predicts 5-Year Risk of Hypertension in Japanese People: The Funagata Study. Microcirculation. 2010;17(2):94-102.
136. Xing C, Klein BEK, Klein R, Jun G, Lee KE, Iyengar SK. Genome-Wide Linkage Study of Retinal Vessel Diameters in the Beaver Dam Eye Study. Hypertension (Dallas, Tex 1979). 2006;47(4):797-802.
137. Tikellis G, Wang JJ, Tapp R, Simpson R, Mitchell P, Zimmet PZ, et al. relationship of retinal vascular calibre to diabetes and retinopathy: the Australian Diabetes, Obesity and Lifestyle (AusDiab) study. Diabetologia. 2007;50(11):2263-71.
138. Sabanayagam C, Lye WK, Klein R, Klein BEK, Cotch MF, Wang JJ, et al. Retinal microvascular calibre and risk of diabetes mellitus: a systematic review and participant-level meta-analysis. Diabetologia. 2015;58(11):2476-85.
139. Wong TY, Duncan BB, Golden SH, Klein R, Couper DJ, Klein BEK, et al. Associations between the Metabolic Syndrome and Retinal Microvascular Signs: The Atherosclerosis Risk in Communities Study. Investigative Ophthalmology & Visual Science. 2004;45(9):2949-54.
140. Klein R, Klein BEK, Moss SE, Wong TY, Hubbard L, Cruickshanks KJ, et al. The Relation of Retinal Vessel Caliber to the Incidence and Progressionof Diabetic Retinopathy: XIX: The Wisconsin Epidemiologic Study of Diabetic Retinopathy. Archives of Ophthalmology. 2004;122(1):76-83.
141. Kifley A, Wang JJ, Cugati S, Wong TY, Mitchell P. Retinal Vascular Caliber, Diabetes, and Retinopathy. American Journal of Ophthalmology. 2007;143(6):1024-6.
142. Wong TY, Shankar A, Klein R, Klein BEK, Hubbard LD. Retinal Arteriolar Narrowing, Hypertension, and Subsequent Risk of Diabetes Mellitus. Archives of Internal Medicine. 2005;165(9):1060-5.
143. Wong TY, Klein R, Sharrett AR, Schmidt MI, Pankow JS, Couper DJ, et al. Retinal Arteriolar Narrowing and Risk of Diabetes Mellitus in Middle-aged Persons. JAMA : the journal of the American Medical Association. 2002;287(19):2528-33.

144. Ikram MK, Janssen JAMJL, Roos AME, Rietveld I, Witteman JCM, Breteler MMB, et al. Retinal Vessel Diameters and Risk of Impaired Fasting Glucose or Diabetes: The Rotterdam Study. Diabetes. 2006;55(2):506-10.
145. McGeechan K, Liew G, Macaskill P, Irwig L, Klein R, Klein BEK, et al. Prediction of Incident Stroke Events Based on Retinal Vessel Caliber: A Systematic Review and Individual-Participant Meta-Analysis. American journal of epidemiology. 2009;170(11):1323-32.
146. Klein R, Klein BEK, Moss SE, Wong TY. Retinal vessel caliber and microvascular and macrovascular disease in type 2 diabetes XXI: The wisconsin epidemiologic study of diabetic retinopathy. Ophthalmology (Rochester, Minn). 2007;114(10):1884-92.
147. Sandoval-Garcia E, McLachlan S, Price AH, MacGillivray TJ, Strachan MWJ, Wilson JF, et al. Retinal arteriolar tortuosity and fractal dimension are associated with long-term cardiovascular outcomes in people with type 2 diabetes. Diabetologia. 2021;64(10):2215-27.
148. Wang SB, Mitchell P, Liew G, Wong TY, Phan K, Thiagalingam A, et al. A spectrum of retinal vasculature measures and coronary artery disease. Atherosclerosis. 2018;268:215-24.
149. Leung H, Wang JJ, Rochtchina E, Wong TY, Klein R, Mitchell P. Dyslipidaemia and microvascular disease in the retina. Eye. 2005;19(8):861-8.
150. Link JJMD, Rohatgi AMD, de Lemos JAMD. HDL Cholesterol: Physiology, Pathophysiology, and Management. Current problems in cardiology. 2007;32(5):268-314.
151. Cheung N, Saw SM, Liew G, Liu EY, Hodgson L, Mitchell P, et al. Childhood Vascular Risk Factors and Retinal Vessel Caliber. Asia-Pacific Journal of Ophthalmology. 2012;1(4):193-7.
152. Taylor B, Rochtchina E, Wang JJ, Wong TY, Heikal S, Saw SM, et al. Body mass index and its effects on retinal vessel diameter in 6-year-old children. International Journal of Obesity. 2007;31(10):1527-33.
153. Wang JJ, Taylor B, Wong TY, Chua B, Rochtchina E, Klein R, et al. Retinal Vessel Diameters and Obesity: A Population-Based Study in Older Persons. Obesity. 2006;14(2):206-14.
154. Liu M, Lovern C, Lycett K, He M, Wake M, Wong TY, et al. The association between markers of inflammation and retinal microvascular parameters: A systematic review and meta-analysis. Atherosclerosis. 2021;336:12-22.
155. Kifley A, Liew G, Wang JJ, Kaushik S, Smith W, Wong TY, et al. Long-term Effects of Smoking on Retinal Microvascular Caliber. American journal of epidemiology. 2007;166(11):1288-97.
156. Perruccio AVM, Badley EMD, Trope GEMBP. Self-reported glaucoma in Canada: findings from population-based surveys, 1994–2003. Canadian journal of ophthalmology. 2007;42(2):219-26.
157. Table 13-10-0466-01 Healthy aging indicators. Canada: Statistics Canada; 2010.
158. Aljied R, Aubin M-J, Buhrmann R, Sabeti S, Freeman EE. Prevalence and determinants of visual impairment in Canada: cross-sectional data from the Canadian Longitudinal Study on Aging. Canadian Journal of Ophthalmology. 2018;53(3):291-7.
159. Harasymowycz P, Birt C, Gooi P, Heckler L, Hutnik C, Jinapriya D, et al. Medical Management of Glaucoma in the 21st Century from a Canadian Perspective. Journal of Ophthalmology. 2016;2016:1-22.
160. Iskedjian M, Walker J, Vicente C, Trope GE, Buys Y, Einarson TR, et al. Cost of Glaucoma in Canada: Analyses Based on Visual Field and Physician's Assessment. Journal of glaucoma. 2003;12(6):456-62.

161. Quaranta L, Riva I, Gerardi C, Oddone F, Floriano I, Konstas AGP. Quality of Life in Glaucoma: A Review of the Literature. Advances in therapy. 2016;33(6):959-81.
162. Lin H-C, Chien C-W, Hu C-C, Ho J-D. Comparison of Comorbid Conditions between Open-Angle Glaucoma Patients and a Control Cohort: A Case-Control Study. Ophthalmology (Rochester, Minn). 2010;117(11):2088-95.
163. Stein JD, Khawaja AP, Weizer JS. Glaucoma in Adults—Screening, Diagnosis, and Management: A Review. JAMA. 2021;325(2):164-74.
164. Allingham RR, Shields MB. Shields' textbook of glaucoma. 7th ed. Philadelphia?: Lippincott Williams & Wilkins; 2019.
165. Weinreb RN, Aung T, Medeiros FA. The Pathophysiology and Treatment of Glaucoma: A Review. JAMA : the journal of the American Medical Association. 2014;311(18):1901-11.
166. Faiq M, Sofi R. A Glimpse into the mysteries of glaucoma: From theories to clinics. Oman journal of ophthalmology. 2019;12(1):1-3.
167. Schuster AK, Erb C, Hoffmann EM, Dietlein T, Pfeiffer N. The Diagnosis and Treatment of Glaucoma. Deutsches Ärzteblatt international. 2020;117(13):225-34.
168. Shaikh Y, Yu F, Coleman AL. Burden of Undetected and Untreated Glaucoma in the United States. American Journal of Ophthalmology. 2014;158(6):1121-9.e1.
169. Chua J, Baskaran M, Ong PG, Zheng Y, Wong TY, Aung T, et al. Prevalence, Risk Factors, and Visual Features of Undiagnosed Glaucoma: The Singapore Epidemiology of Eye Diseases Study. JAMA Ophthalmology. 2015;133(8):938-46.
170. Kwon YH, Fingert JH, Kuehn MH, Alward WLM. Primary Open-Angle Glaucoma. New England Journal of Medicine. 2009;360(11):1113-24.
171. Kapetanakis VV, Chan MPY, Foster PJ, Cook DG, Owen CG, Rudnicka AR. Global variations and time trends in the prevalence of primary open angle glaucoma (POAG): a systematic review and meta-analysis. British Journal of Ophthalmology. 2016;100(1):86-93.
172. Leske MCMDMPH, Heijl AMDP, Hyman LP, Bengtsson BP, Dong LP, Yang ZP. Predictors of Long-term Progression in the Early Manifest Glaucoma Trial. Ophthalmology (Rochester, Minn). 2007;114(11):1965-72.
173. Shan S, Wu J, Cao J, Feng Y, Zhou J, Luo Z, et al. Global incidence and risk factors for glaucoma: A systematic review and meta-analysis of prospective studies. Journal of global health. 2024;14:04252.
174. Tham Y-C, Li X, Wong TY, Quigley HA, Aung T, Cheng C-Y. Global prevalence of glaucoma and projections of glaucoma burden through 2040: a systematic review and meta-analysis. Ophthalmology (Rochester, Minn). 2014;121(11):2081-90.
175. Davis BM, Crawley L, Pahlitzsch M, Javaid F, Cordeiro MF. Glaucoma: the retina and beyond. Acta Neuropathologica. 2016;132(6):807-26.
176. Ahmad SS. Controversies in the vascular theory of glaucomatous optic nerve degeneration. Taiwan journal of ophthalmology. 2016;6(4):182-6.
177. Chan KKW, Tang F, Tham CCY, Young AL, Cheung CY. Retinal vasculature in glaucoma: a review. BMJ Open Ophthalmology. 2017;1(1):e000032.
178. Flammer J, Mozaffarieh M. The Mechanism of Glaucomatous Damage to the Optic Nerve. European Ophthalmic Review. 2009.
179. Kim KE, Oh S, Baek SU, Ahn SJ, Park KH, Jeoung JW. Ocular Perfusion Pressure and the Risk of Open-Angle Glaucoma: Systematic Review and Meta-analysis. Scientific reports. 2020;10(1):10056-.

180. Bowe A, Grünig M, Schubert J, Demir M, Hoffmann V, Kütting F, et al. Circadian Variation in Arterial Blood Pressure and Glaucomatous Optic Neuropathy—A Systematic Review and Meta-Analysis. American Journal of Hypertension. 2015;28(9):1077-82.
181. Rajasundaram S, Segrè AV, Gill D, Woolf B, Zekavat SM, Burgess S, et al. Independent Effects of Blood Pressure on Intraocular Pressure and Retinal Ganglion Cell Degeneration: A Mendelian Randomization Study. Investigative Ophthalmology & Visual Science. 2024;65(8):35.
182. Beros AL, Sluyter JD, Hughes AD, Hametner B, Wassertheurer S, Scragg RKR. Arterial Stiffness and Incident Glaucoma: A Large Population-Based Cohort Study. American Journal of Ophthalmology. 2024;266:68-76.
183. Chan TCW, Bala C, Siu A, Wan F, White A. Risk Factors for Rapid Glaucoma Disease Progression. American Journal of Ophthalmology. 2017;180:151-7.
184. Funk RO, Hodge DO, Kohli D, Roddy GW. Multiple Systemic Vascular Risk Factors Are Associated With Low-Tension Glaucoma. Journal of glaucoma. 2022;31(1):15-22.
185. Gao J, Liang Y, Wang F, Shen R, Wong T, Peng Y, et al. Retinal Vessels Change in Primary Angle-Closure Glaucoma: The Handan Eye Study. Scientific Reports. 2015;5(1):9585.
186. Amerasinghe N, Aung T, Cheung N, Fong CW, Wang JJ, Mitchell P, et al. Evidence of Retinal Vascular Narrowing in Glaucomatous Eyes in an Asian Population. Investigative Opthalmology & Visual Science. 2008;49(12):5397.
187. Wang S, Xu L, Wang Y, Wang Y, Jonas JB. Retinal vessel diameter in normal and glaucomatous eyes: the Beijing eye study. Clinical & Experimental Ophthalmology. 2007;35(9):800-7.
188. Rudnicka AR, Owen CG, Welikala RA, Barman SA, Whincup PH, Strachan DP, et al. Retinal Vasculometry Associations With Glaucoma: Findings From the European Prospective Investigation of Cancer–Norfolk Eye Study. American journal of ophthalmology. 2020;220:140-51.
189. Mitchell P, Leung H, Wang JJ, Rochtchina E, Lee AJ, Wong TY, et al. Retinal vessel diameter and open-angle glaucoma: The Blue Mountains Eye Study. Ophthalmology. 2005;112(2):245-50.
190. Freiberg J, Welikala R, Rovelt J, Barman SA, Owen CG, Rudnicka AR, et al. Longitudinal associations of retinal vessel morphology with intraocular pressure and blood pressure at follow-up visit—Findings from a Danish eye and vision cohort, Project <scp>FOREVER</scp>. Acta Ophthalmologica. 2025;103(1):33-42.
191. Cheung N, Huynh S, Wang JJ, Taylor B, Islam FMA, Saw SM, et al. Relationships of Retinal Vessel Diameters with Optic Disc, Macular and Retinal Nerve Fiber Layer Parameters in 6-Year-Old Children. Investigative Ophthalmology & Visual Science. 2008;49(6):2403-8.
192. Zheng Y, Cheung N, Aung T, Mitchell P, He M, Wong TY. Relationship of Retinal Vascular Caliber with Retinal Nerve Fiber Layer Thickness: The Singapore Malay Eye Study. Investigative Ophthalmology & Visual Science. 2009;50(9):4091-6.
193. Zhang Q, Jan C, Guo CY, Wang FH, Liang YB, Cao K, et al. Association of intraocular pressure-related factors and retinal vessel diameter with optic disc rim area in subjects with and without primary open angle glaucoma. Clinical & Experimental Ophthalmology. 2018;46(4):389-99.
194. Samarawickrama C, Huynh SC, Wang JJ, Pai A, Joachim N, Burlutsky G, et al. Relationship between Retinal Structures and Retinal Vessel Caliber in Normal Adolescents. Investigative Ophthalmology & Visual Science. 2009;50(12):5619-24.

195. Wu RMDP, Cheung CY-LP, Saw SMMDP, Mitchell PMDP, Aung TMDP, Wong TYMDP. Retinal Vascular Geometry and Glaucoma: The Singapore Malay Eye Study. Ophthalmology (Rochester, Minn). 2013;120(1):77-83.
196. Resnikoff S, Pascolini D, Etya'ale D, Kocur I, Pararajasegaram R, Pokharel GP, et al. Global data on visual impairment in the year 2002. Bulletin of the World Health Organization. 2004;82(11):844-51.
197. Deloitte. The cost of vision loss and blindness in Canada: Canadian Council of the Blind. 2021.
198. Kahiel Z, Aubin M-J, Buhrmann R, Kergoat M-J, Freeman EE. Incidence of visual impairment in Canada: the Canadian Longitudinal Study on Aging. Canadian Journal of Ophthalmology. 2022;57(1):2-7.
199. Gale RP, Finger RP, Eldem B, Aslam T, Barratt J, Daien V, et al. The management of neovascular age-related macular degeneration: A systematic literature review of patient-reported outcomes, patient mental health and caregiver burden. Acta Ophthalmologica. 2023;101(1):e26-e42.
200. Schultz NM, Bhardwaj S, Barclay C, Gaspar L, Schwartz J. Global Burden of Dry Age-Related Macular Degeneration: A Targeted Literature Review. Clinical Therapeutics. 2021;43(10):1792-818.
201. Brown MM, Brown GC, Stein JD, Roth Z, Campanella J, Beauchamp GR. Age-related macular degeneration: economic burden and value-based medicine analysis. Canadian journal of ophthalmology. 2005;40(3):277-87.
202. Li W. Age-related macular degeneration. Amsterdam: Elsevier; 2022.
203. Fineman MS, Ho AC, Rapuano CJ. Retina. 3rd ed. Philadelphia: Wolters Kluwer Health; 2018.
204. Ryan SJ. Retina. 5th ed. London?: Saunders; 2013.
205. Stahl A. The Diagnosis and Treatment of Age-Related Macular Degeneration. Deutsches Ärzteblatt international. 2020.
206. Chakravarthy U, Wong TY, Fletcher A, Piault E, Evans C, Zlateva G, et al. Clinical risk factors for age-related macular degeneration: a systematic review and meta-analysis. BMC Ophthalmology. 2010;10(1):31.
207. Schwartz SG, Hampton BM, Kovach JL, Brantley JMA. Genetics and age-related macular degeneration: a practical review for the clinician. Clinical ophthalmology (Auckland, NZ). 2016;10:1229-35.
208. Tan JSL, Mitchell P, Kifley A, Flood V, Smith W, Wang JJ. Smoking and the Long-term Incidence of Age-Related Macular Degeneration: The Blue Mountains Eye Study. Archives of Ophthalmology. 2007;125(8):1089-95.
209. Erke MG, Bertelsen G, Peto T, Sjølie AK, Lindekleiv H, Njølstad I. Cardiovascular risk factors associated with age-related macular degeneration: the Tromsø Study. Acta Ophthalmologica. 2014;92(7):662-9.
210. Myers CE, Klein BEK, Gangnon R, Sivakumaran TA, Iyengar SK, Klein R. Cigarette smoking and the natural history of age-related macular degeneration: the Beaver Dam Eye Study. Ophthalmology (Rochester, Minn). 2014;121(10):1949-55.
211. Chakravarthy U, Augood C, Bentham GC, de Jong PTVM, Rahu M, Seland J, et al. Cigarette Smoking and Age-Related Macular Degeneration in the EUREYE Study. Ophthalmology. 2007;114(6):1157-63.

212. Klein R, Klein BEK, Tomany SC, Cruickshanks KJ. The association of cardiovascular disease with the long-term incidence of age-related maculopathy: The Beaver Dam eye study. Ophthalmology (Rochester, Minn). 2003;110(4):636-43.
213. Fraser-Bell S, Wu J, Klein R, Azen SP, Hooper C, Foong AWP, et al. Cardiovascular Risk Factors and Age-related Macular Degeneration: The Los Angeles Latino Eye Study. American Journal of Ophthalmology. 2008;145(2):308-16.
214. Hyman L, Schachat AP, He Q, Leske MC, Group ftA-RMDRFS. Hypertension, Cardiovascular Disease, and Age-Related Macular Degeneration. Archives of Ophthalmology. 2000;118(3):351-8.
215. Adams MKM, Simpson JA, Khin Zaw A, Makeyeva GA, Giles GG, English DR, et al. Abdominal Obesity and Age-related Macular Degeneration. American journal of epidemiology. 2011;173(11):1246-55.
216. Chong EWT, Kreis AJ, Wong TY, Simpson JA, Guymer RH. Alcohol Consumption and the Risk of Age-Related Macular Degeneration: A Systematic Review and Meta-Analysis. American Journal of Ophthalmology. 2008;145(4):707-15.e2.
217. Klein R, Klein BEK, Tomany SC, Moss SE. Ten-year incidence of age-related maculopathy and smoking and drinking: The Beaver Dam Eye Study. American journal of epidemiology. 2002;156(7):589-98.
218. Friedman E. A Hemodynamic Model of the Pathogenesis of Age-related Macular Degeneration. American journal of ophthalmology. 1997;124(5):677-82.
219. Gelfand BD, Ambati J. A Revised Hemodynamic Theory of Age-Related Macular Degeneration. Trends in molecular medicine. 2016;22(8):656-70.
220. Cougnard-Grégoire A, Delyfer M-N, Korobelnik J-F, Rougier M-B, Malet F, Le Goff M, et al. Long-Term Blood Pressure and Age-Related Macular Degeneration: The ALIENOR Study. Investigative Opthalmology & Visual Science. 2013;54(3):1905.
221. Vingerling JR, Dielemans I, Bots ML, Hofman A, Grobbee DE, De Jong PTVM. Age-related macular degeneration is associated with atherosclerosis : the Rotterdam Study. American journal of epidemiology. 1995;142(4):404-9.
222. Klein R, Clegg L, Cooper LS, Hubbard LD, Klein BEK, King WN, et al. Prevalence of Age-related Maculopathy in the Atherosclerosis Risk in Communities Study. Archives of Ophthalmology. 1999;117(9):1203-10.
223. Duan YMDMS, Mo JMDP, Klein RMDMPH, Scott IUMDMPH, Lin H-MP, Caulfield JMS, et al. Age-Related Macular Degeneration Is Associated with Incident Myocardial Infarction among Elderly Americans. Ophthalmology (Rochester, Minn). 2007;114(4):732-7.
224. Snyder K, Yazdanyar A, Mahajan A, Yiu G. Association Between the Cilioretinal Artery and Choroidal Neovascularization in Age-Related Macular Degeneration. JAMA Ophthalmology. 2018;136(9):1008.
225. Toulouie S, Chang S, Pan J, Snyder K, Yiu G. Relationship of Retinal Vessel Caliber with Age-Related Macular Degeneration. Journal of Ophthalmology. 2022;2022:1-8.
226. Sun C, Klein R, Wong TY. Age-related Macular Degeneration and Risk of Coronary Heart Disease and Stroke: The Cardiovascular Health Study. Ophthalmology. 2009;116(10):1913-9.
227. Wu J, Uchino M, Sastry SM, Schaumberg DA. Age-related macular degeneration and the incidence of cardiovascular disease: a systematic review and meta-analysis. PloS one. 2014;9(3):e89600-e.

228. Xu L, Wang S, Li Y, Jonas JB. Retinal Vascular Abnormalities and Prevalence of Age-related Macular Degeneration in Adult Chinese: The Beijing Eye Study. American Journal of Ophthalmology. 2006;142(4):688-9.
229. Chin YC, Wong TY, Cheung CMG, Cheung CY-L, Zheng Y, Mitchell P, et al. Retinal Vascular Caliber and Age-related Macular Degeneration in an Indian Population from Singapore. Ophthalmic Epidemiology. 2014;21(4):224-9.
230. McGowan A, Silvestri G, Moore E, Silvestri V, Patterson CC, Maxwell AP, et al. Retinal Vascular Caliber, Iris Color, and Age-Related Macular Degeneration in the Irish Nun Eye Study. Investigative Ophthalmology & Visual Science. 2015;56(1):382-7.
231. Jeganathan VSE, Kawasaki R, Wang JJ, Aung T, Mitchell P, Saw S-M, et al. Retinal Vascular Caliber and Age-related Macular Degeneration: The Singapore Malay Eye Study. American Journal of Ophthalmology. 2008;146(6):954-9.e1.
232. Yang K, Zhan SY, Liang YB, Duan X, Wang F, Wong TY, et al. Association of dilated retinal arteriolar caliber with early age-related macular degeneration: the Handan Eye Study. Graefe's Archive for Clinical and Experimental Ophthalmology. 2012;250(5):741-9.
233. Jabs DA, Van Natta ML, Pak JW, Danis RP, Hunt PW. Association of Retinal Vascular Caliber and Age-Related Macular Degeneration in Patients With the Acquired Immunodeficiency Syndrome. Investigative Ophthalmology & Visual Science. 2018;59(2):904-8.
234. Cheung CM, Cheung CYL, Liu EY, Mitchell P, Wang JJ, Hamzah HB, et al., editors. Retinal Vascular Tortuosity and Age-Related Macular Degeneration: The Singapore Malay Study. Investigative Ophthalmology & Visual Science; 2010.
235. Raina PS, Wolfson C, Kirkland SA, Griffith LE, Oremus M, Patterson C, et al. The Canadian Longitudinal Study on Aging (CLSA). Canadian Journal on Aging / La Revue Canadienne du Vieillissement. 2009;28(3):221-9.
236. Raina P, Wolfson C, Kirkland S, Griffith LE, Balion C, Cossette B, et al. Cohort Profile: The Canadian Longitudinal Study on Aging (CLSA). International journal of epidemiology. 2019;48(6):1752-3j.
237. Raina P, Wolfson C, Kirkland S, Griffith LE. The Canadian Longitudinal Study on Aging (CLSA) Report on Health and Aging in Canada. Canadian Longitudinal Study on Aging.
238. CLSA. Sampling and Computation of Response Rates and Sample Weights for the Tracking (Telephone Interview) Participants and Comprehensive Participants. Canadian Longitudinal Study on Aging; 2024 February 13.
239. Tham Y-C, Siantar RG, Cheung CY-L, Tan S-P, Koh VT, Aung T, et al. Inter-Relationships Between Retinal Vascular Caliber, Retinal Nerve Fiber Layer Thickness, and Glaucoma: A Mediation Analysis Approach. Investigative ophthalmology & visual science. 2016;57(8):3803-9.
240. French C, Heitmar R. Comparison of Static Retinal Vessel Caliber Measurements by Different Commercially Available Platforms. Optometry and vision science. 2021;98(9):1104-12.
241. Mautuit T, Cunnac P, Cheung CY, Wong TY, Hogg S, Trucco E, et al. Concordance between SIVA, IVAN, and VAMPIRE Software Tools for Semi-Automated Analysis of Retinal Vessel Caliber. Diagnostics (Basel). 2022;12(6):1317.
242. Linton KLP, Klein BEK, Klein R. The Validity of Self-reported and Surrogate-reported Cataract and Age-related Macular Degeneration in the Beaver Dam Eye Study. American journal of epidemiology. 1991;134(12):1438-46.

243. Cohn JN, Hoke L, Whitwam W, Sommers PA, Taylor AL, Duprez D, et al. Screening for early detection of cardiovascular disease in asymptomatic individuals. American Heart Journal. 2003;146(4):679-85.
244. Cheung CY, Ikram MK, Klein R, Wong TY. The clinical implications of recent studies on the structure and function of the retinal microvasculature in diabetes. Diabetologia. 2015;58(5):871-85.
245. Hughes AD, Stanton AV, Jabbar AS, Chapman N, Martinez-Perez ME, McG Thom SA. Effect of antihypertensive treatment on retinal microvascular changes in hypertension. Journal of hypertension. 2008;26(8):1703-7.

Appendix 1: Anterior Chamber Anatomy and Pathology

Supplemental Figure 1: Anterior chamber angle anatomy and normal and pathological fluid dynamics (Image from Weinreb *et al* [165])