Retinal Haemodynamics in Primary Open Angle Glaucoma Patients with Differing Nocturnal Blood Pressure Profiles

by

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Author's declaration

I hereby declare that I am the sole author of this thesis. This is a true copy of the thesis including any final revisions, as accepted by my examiners.

I understand that my thesis may be made electronically available to the public.

Abstract

The impairment of ocular haemodynamics has been reported in primary open angle glaucoma (POAG) patients using various blood flow measurement techniques. Studies have shown hypotension may accelerate progression in POAG and a marked nocturnal blood pressure reduction (NBPR) was reported to further expedite the neuro-degenerative disease. It has been suggested that extreme NBPR may be a form of systemic vascular dysregulation that is manifested as an ocular dysregulation, but this hypothesis has never been tested. In addition, the relationship, if any, of retinal haemodyanamics to magnitude of the NBPR is also unknown. A prototype Doppler spectral domain optical coherence tomographer (SD-OCT) together with the more established bi-directional Laser Doppler velocimetry with simultaneous vessel densitometry (BLDV-SVD) methodology (Canon Laser Blood Flowmeter, CLBF) were utilized to see if the NBPR is associated to impaired retinal haemodynamics in glaucoma patients.

The specific aims of this thesis were: (1) To evaluate the association between retinal hemodynamics of healthy eyes with age, as derived by Doppler SD-OCT, (2) To evaluate retinal blood flow (RBF) in patients with early POAG and differing NBPR profiles, (3) To investigate the relationship between Doppler SD-OCT derived retinal haemodynamics and the retinal nerve fiber layer (RNFL) thickness in patients with early POAG and healthy age-matched controls, (4) To evaluate the vascular reactivity in patients with early POAG with different NBPR profiles using a normoxic hypercapnia provocation stimulus. Perfusion to the retina was not altered with increasing age among healthy people in the range of 20 to 70 years. Patients with early POAG who exhibited an exaggerated nocturnal reduction in mean arterial pressure also demonstrated lower RBF values as shown by the measurement of Doppler SD-OCT. The change in retinal

circulation in early POAG was related to reduced RNFL thickness and a larger venous area may be associated with thicker RNFL among controls. Patients with early POAG who exhibited an exaggerated NBPR also demonstrated disturbance of retinal vascular reactivity.

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My love and appreciation to my mum, my siblings, in laws, nephews and nieces; thank you for the inspiration.

Dedication

Dedicated to my *mak* Sa'adiah Abdul Hamid, my *abah* Yusof Alias Duriat, and the rest of the Yusof family.

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List of Abbreviations

ABP	Ambulatory blood pressure
ABPM	Ambulatory blood pressure monitoring
ANOVA	Analysis of variance
ANS	Autonomic nervous system
BLDV-SVD	Bi-directional Laser Doppler Velocimetry with simultaneous vessel densitometry
BP	Blood pressure
C_{aq}	Aqueous drainage
CCD	Charged coupled device
CCT	Central corneal thickness
CDI	Color Doppler Imaging
CLBF	Canon laser blood flowmeter
CO_2	Carbon dioxide
COV	Coefficient of variation
CRA	Central retinal artery
CSF	Cerebrospinal fluid
DBP	Diastolic blood pressure
DOCTORC	Doppler OCT of Retinal Circulation
EDCF	Endothelium derived constricting factors
EDRF	Endothelium derived relaxing factors
EDV	End diastolic velocity
EEG	Electroencephalography
eNOS	Endothelial NOS

ET-1	Endothelin-1
F_ACO_2	Alveolar CO_2 concentration
F_{aq}	Aqueous formation
$F_{ET}CO_2$	Fractional alveolar CO_2 concentration
FPA	Fundus pulsation amplitude
HeNe	Helium-Neon
HFA	Humphrey field analyzer
HRF	Heidelberg retinal flowmeter
ICC	Intra-class correlation
IDACO	International database in ABPM in relation to cardiovascular outcome
IOP	Intraocular pressure
L	Vessel's length
LDF	Laser Doppler flowmetry
LDV	Laser Doppler velocimetry
LPCA	Long posterior ciliary artery
MAC	Major arterial circle
MAP	Mean arterial pressure
MD	Mean deviation
NAION	Nonarteritic ischaemic optic neuropathy
NBP	Nocturnal blood pressure
NBPR	Nocturnal blood pressure reduction
NFL	Nerve fiber layer
NO	Nitric oxide
NREM	Non-random eye movement

NTG	Normal tension glaucoma
O_2	Oxygen
OA	Ophthalmic artery
OCT	Optical coherence tomographer
OHT	Ocular hypertension
ONH	Optic nerve head
OPP	Ocular perfusion pressure
P_a	Partial pressure
PDR	Proliferative diabetic retinopathy
PEEP	Positive end-expiratory pressure
$P_{ET}CO_2$	End-tidal CO_2 partial pressure
$P_{ET}O_2$	End-tidal O_2 partial pressure
P_{ev}	Episcleral venous pressure
POAG	Primary open angle glaucoma
POBF	Pulsatile ocular blood flow
PR	Pulse rate
PSD	Pattern standard deviation
PSV	Peak systolic velocity
Q	Blood flow
R	Resistance
RBF	Retinal blood flow
ReANOVA	Repeated measures of ANOVA
REM	Rapid eye movement
RNFL	Retinal nerve fiber layer

ROS	Reactive oxygen species
RVA	Retinal vessel analyzer
SAP	Standard automated perimetry
SBP	Systolic blood pressure
SD-OCT	Spectral domain ocular coherence tomography
SGD	Sequential gas delivery
SITA	Swedish interactive tresholding algorithm
SLDF	Scanning laser Doppler flowimetry
SLO	Scanning Laser ophthalmoscope
SPCA	Short posterior ciliary artery
TRBF	Total retinal blood flow
V_A	Arteriolar ventilation
VA	Visual acuity
VCO_2	Metabolic carbon dioxide production
Ve	Total ventilation
VF	Visual field
VO_2	Metabolic oxygen consumption
Vpds	Physiological dead space
η	Blood viscosity

1 Introduction

Vascular perfusion is necessary to maintain ocular function, while keeping the temperature constant and retaining the best optical quality.

1.1 Ocular blood supply

Blood travels from the heart to the eye flowing through the aorta, common carotid artery, internal carotid artery, and ophthalmic artery (OA). The OA branches into short posterior ciliary arteries (SPCA), long posterior ciliary arteries (LPCA), and central retinal artery (CRA) to form separate circulatory systems in the eye (Figure 1.1).

The CRA generates a retinal circulatory system that nourishes three quarters of the retina (layers except the RPE and the photoreceptors). The retina has low flow but highest oxygen consumption per tissue volume in the body,[1] and it accounts for 4% of the total blood supply to the eye. This circulatory vascular bed is thought to lack autonomic innervation and therefore perfusion is mainly regulated through local factors released by endothelial cells and local tissue.[2] The CRA pierces 12mm behind the optic nerve head (ONH) and travels in the centre of the optic nerve to surface on the retina. It then bifurcates into 4 major arteriolar branches typically supplying the superonasal-, superotemporal-, inferonasal-, and inferotemporal-retinal quadrant. The arterioles and venules from the CRA will stay in nerve fiber layer (NFL) and the capillary does not go further than the inner nuclear layer of the retina. Two capillaries bed forms separate layers with the superficial capillary plexus located at the level of ganglion cell and nerve fiber

layers, and a deeper plexus at inner nuclear layer. The CRA also sends pial branches for the optic nerve before entering the retina.[3]

The SPCAs and LPCAs form the uveal circulatory system which supplies the iris, ciliary body and choroid. Around 15 to 20 SPCAs pierce the sclera around the optic nerve, and forms the posterior choroid. The vessels terminate at the choriocapillaris, which in effect is a single layer capillary that supplies critical nourishment to the outer retina (the photoreceptors and RPE). The SPCAs also supply the ONH, particularly at the lamina cribosa layer and also via the pial vessels. The posterior choriocapillaris from the SPCA merge with the anterior choriocapillaris which is supplied by the LPCA. The LPCA together with anterior ciliary arteries (originating from the rectus muscles) forms the major arterial circle (MAC) of the iris. The choroidal vasculature has a high flow, due to low resistance, but has a low oxygen extraction of about 4%.[4, 5] The choroidal vessels supply 85% of the total blood flow to the eye.[2] This system has the greatest density of autonomic innervations in the body, in stark contrast to the inner retina vessels which have no functioning autonomic innervation.[1]

The blood from the inner retina drains via the central retinal vein, and superior ophthalmic vein. The uvea drains out the blood via 4 vortex veins, and then to either the superior or inferior ophthalmic vein. The anterior part of the eye drains the blood via anterior ciliary veins which are located in the rectus muscles, and then to the respective superior or inferior ophthalmic vein (Figure 1.1).

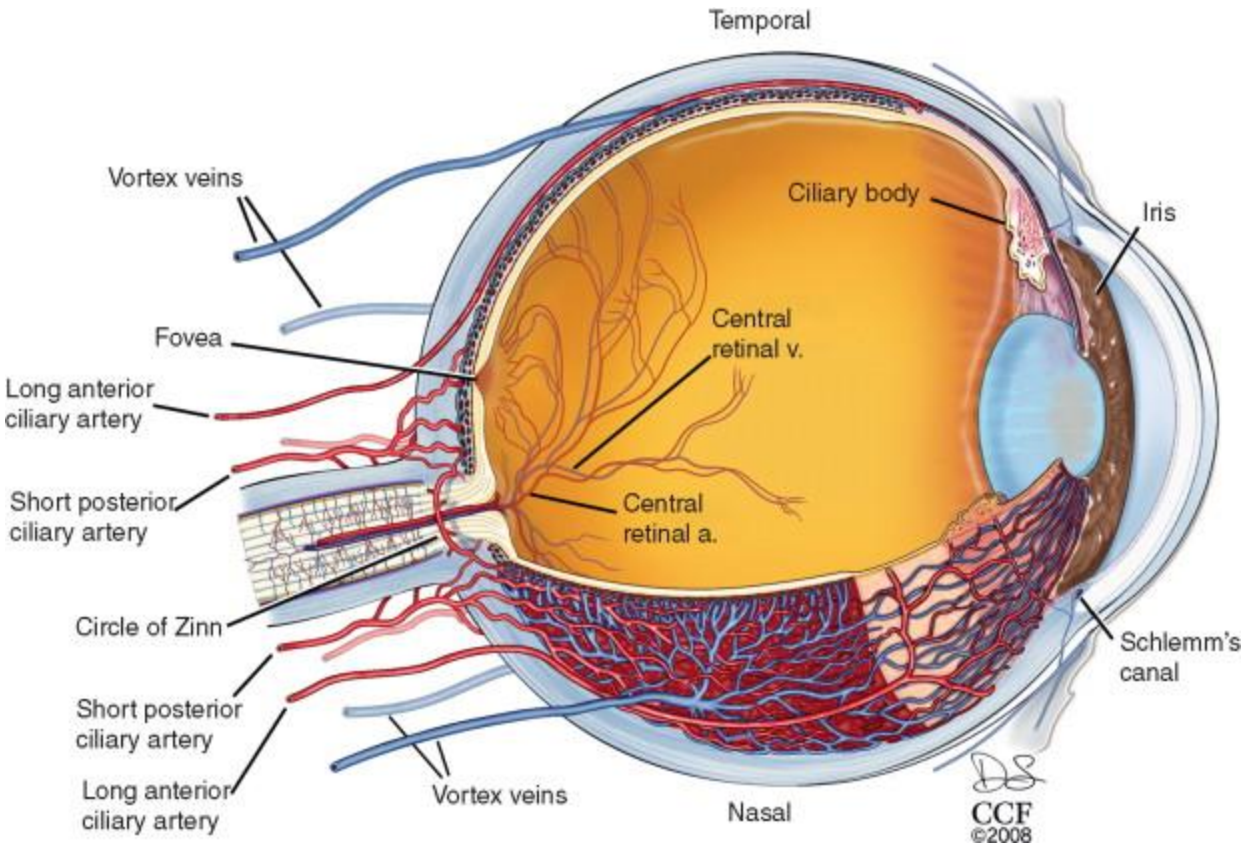

Figure 1.1: Anatomy of the ocular vascular supply. Drawings by Dave Schumick in Anand-Apte and Hollyfield[6]. [Image reproduced with permission from Elsevier Publications.]

1.2 Blood supply to the ONH

The ONH is divided into 4 regions, namely the (i) superficial NFL, (ii) prelamina layer, (iii) lamina cribosa area, and (iv) retrolamina area (Figure 1.2). The superficial NFL is supplied by the retinal arterioles and their branches, and in some individuals the temporal area of the NFL may also be supplied by the SPCAs or the cilioretinal artery.[3] The prelaminar region which consists of the nerve fibers receives its blood supply from the peri-papillary choroid (Figure 1.3). The CRA and peri-papillary choriocapillaris have no influence on this structure.[3, 7] The lamina

cribosa is an elastic collagenous structure continuous with the sclera. It has fenestrations for the axon bundles going out from the eye travelling to the cortex. This layer is supplied by centripetal branches from SPCA or sometimes from the intrascleral circle of Haller and Zinn (if it present, considering interindividual variations in form, position and branches[8]). The circle of Haller and Zinn also branches into the peri-papillary choroid.[9] Supply to the retrolamina region mainly comes from the centripetal system formed by the peri-papillary choroid with additional pial branches from the CRA or from the other orbital arteries (Figure 1.3).[3]

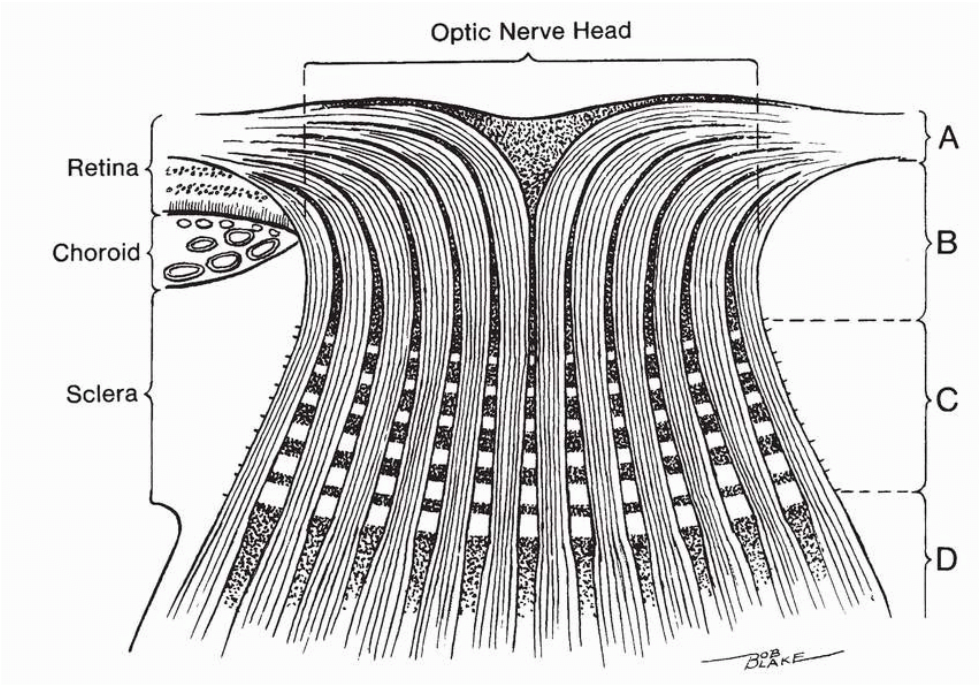

Figure 1.2: Divisions of the optic nerve head. (A) Surperficial nerve fiber layer. (B) Prelaminar region. (C) Lamina cribosa region. (D) Retrolaminar region. Adapted from Allingham et al.[10] [Image reproduced with permission from Lippincott Williams & Wilkins.]

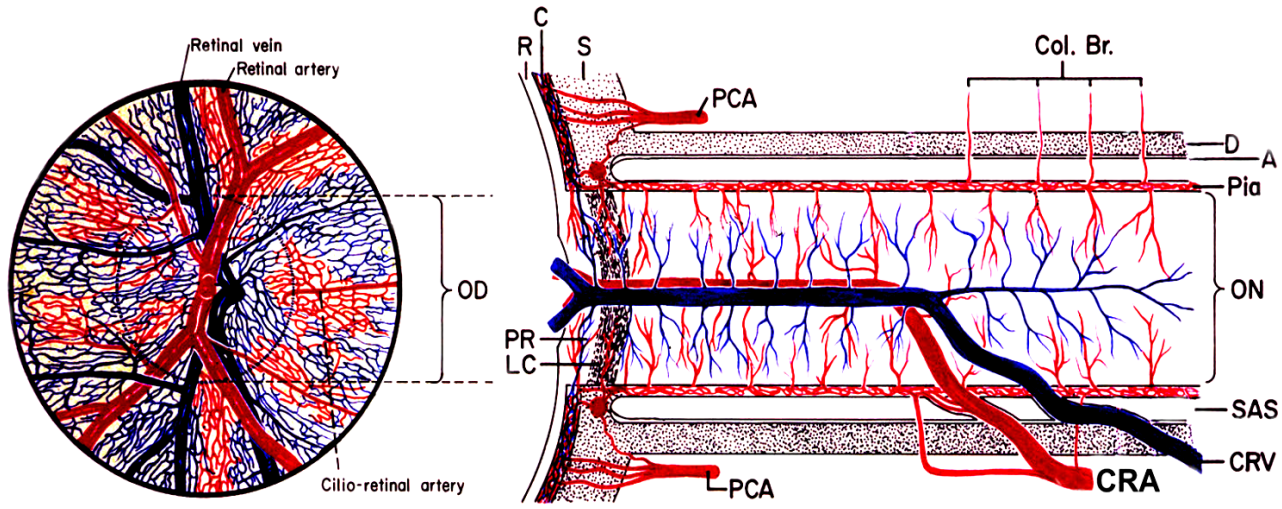

Figure 1.3: Abbreviations used in figure: A = arachnoid, C = choroid, CRA = central retinal artery, Col Br. = collateral branches, CRV = central retinal vein, D = dura, LC = lamina cribrosa, OD = optic disc, ON = optic nerve, ONH = optic nerve head, PCA = posterior ciliary artery, PR = prelaminar region, R = retina, S = sclera, SAS = subarachnoid space. Taken from Hayreh SS.[3] *[Image reproduced with permission from Elsevier Publications.]*

1.3 Retinal blood vessel structures

The blood vessel wall consists of: 1) innermost layer of endothelial cells, 2) middle layer of smooth muscle cells, and 3) outermost adventitia layer.[2] The endothelial layer consists of a single cell layer and is important in the regulation of blood flow by secretion of vasoreactive substances. The arteriolar middle smooth muscle cell layer around the optic disc is thicker than those located at the equator and peripheral retina (7 to 8 layers of smooth muscle cells immediately around the ONH, while only one to two layers of smooth muscle cells are present in the peripheral arterioles).[11] The smooth muscle cell layer controls lumen size as a result of

contraction or relaxation.[12] The adventitial layer consist of elastic collagen fiber that is important for structural function especially in the arterioles. Its contribution to vascular tone is unclear, but results from experimental tissue culture indicated that it can respond to vasoactive agents.[13] The diameter of the arterioles and venules diameter ranges from 20-150μm (with the venular system being actually a little larger in diameter than equivalent arteriole) and they become smaller in diameter as they course towards their final branch into the capillary beds. Arterioles in particular are important in regulating blood flow as it has the ability to change its diameter size, hence they are regarded as "resistance" vessels.

Capillaries are smaller in size, 6-10μm, and are composed of an endothelial cell layer, surrounded by basal lamina, and pericytes.[11] In the retina, capillaries are arranged in laminar fashion, with greater number of layers in the central retina than in the periphery.[14] Exchange of tissue metabolites and blood gasses is facilitated by the capillaries (hence regarded as "exchange" vessels), in conjunction with the pericytes allowing "fine tuning" of capillary perfusion.[14] The pericytes are paired with the endothelial cells at ratio 1:1, and this organization is unique to the retinal capillaries.[15] The capillaries are responsive to local blood gases concentrations and vasoreactive factor, thus playing a part in regulating retinal perfusion. Endothelial cells have tight junctions (zona-occludens) and form the blood-retina barrier, preventing leaking of macromolecules to the interstitial space.[11]

1.4 Retinal blood flow

Blood flow (Q) is the temporal quantity, in volume, of blood that passes through a vessel of a given diameter. Blood velocity is the speed of red blood cells that travel through a vessel. Two

condition of flow may happen in a vessel, namely the laminar flow, or the turbulent flow.[16] Laminar flow is a type of blood flow when concentric layers of blood move in parallel fashion through vessel and with the maximum velocity occurs in the centre of the vessel while the minimum velocity occurs at the vessels wall (reduced velocity due to friction; Figure 1.4). Turbulent flow has a random flow pattern and its occurrence is usually at vessel's bifurcation. Experimentally, plug flow may exist, characterized by constant and equal velocity throughout a vessel. But true type of flow does not occur in retinal vessels due to friction between vessel wall and blood column and the elevated viscosity of blood (relative to a liquid with Newtonian properties).

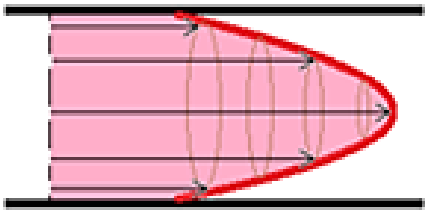

Figure 1.4: The laminar flow.

Following Ohm's law, Q is proportional to perfusion pressure (pressure difference along a blood vessel between two given points, ΔP), and is inversely proportional to vascular resistance, R (Equation 1.1). The law assumes a steady, laminar flow of a Newtonian fluid in a rigid, straight tube. Consequently, blood moving in a vessel will deviate from the relationship due to pulsitility and/or turbulent nature of the flow pattern. Regardless, this relationship is a fair estimation for studying blood flow in the microvasculature.

$$\boldsymbol{Q = \frac{\Delta P}{R}}$$ **Equation 1.1**

R depends on the vessel's length (L), blood viscosity (η), and fourth power of vessel's radius (r), as given by the formula:

$$\boldsymbol{R = \frac{8L\eta}{\pi r^4}}$$ **Equation 1.2**

Applying both formulas together, flow is proportionate to perfusion pressure and fourth power of capillary radius, and inversely proportional to vessel's length and blood viscosity.[17] This formula is known as Hagen-Poiseuille's law.

$$\boldsymbol{Q = \frac{\Delta P \pi r^4}{8\eta L}}$$ **Equation 1.3**

1.5 Ocular perfusion pressure (OPP)

OPP is the pressure that drives the blood through the intraocular vasculature. It is influenced by the resistance, which is effected by vessel lumen diameter.[4, 18] OPP is the difference between arterial and venular blood pressure (Pa and Pv, respectively).[4] Since there is no means of measuring the ocular blood pressure, a surrogate measurement from the brachial artery is taken to estimate the ophthalmic arterial pressure. However, the brachial arterial pressure is not equivalent to the ocular arterial pressure because of the gravitational effect also known as the hydrostatic column effect.[4] When a person is sitting or standing, the ocular arterial blood pressure is lower than the brachial arterial pressure due to the fact that the eye is situated higher

than the heart and therefore blood has to be pumped up to the eye against the force of gravity. The calculation of OPP using the brachial arterial pressure should be corrected for this reason.

Venous pressure in the eye is roughly equal to intraocular pressure (IOP),[19] although it is debatable. IOP has been shown to be a reasonable approximation to the venous pressure in animal studies, although there was a study that showed the venous pressure being higher than IOP in glaucomatous human eyes.[20] In the seating position, OPP can be calculated by: [18]

$$\boldsymbol{OPP = (\frac{95}{140} MAP) - IOP} \qquad \textbf{Equation 1.4}$$

where MAP stands for mean arterial pressure. In the supine position, OPP can be calculated by:

$$\boldsymbol{OPP = \left(\frac{115}{130} MAP\right) - IOP} \qquad \textbf{Equation 1.5}$$

The factor used for MAP in Equation 1.4 is usually simplified to 2/3 in most of the literature.

Increase of the IOP and/or the reduction of the BP will reduce the OPP. OPP reduction in turn will affect perfusion in tissue with vascular dysregulation. Flow reduction causes ischaemia leading to atrophy of ganglion cells.[21] Numerous epidemiological studies have indicated that reduction of OPP is a risk factor for incidence, prevalence and progression of glaucoma.[22-28] This suggests the involvement of impaired perfusion in pathogenesis of glaucoma.

1.6 Intraocular pressure (IOP)

IOP is the pressure that is generated by the fluid that fills the eye which is opposed by the rate of aqueous outflow and the rigidity of the outer coats of the globe. The amount of pressure depends on the formation (F_{aq}) and drainage (C_{aq}) rate of aqueous humor, and also on the episcleral venous pressure (P_{ev}).

$$\boldsymbol{IOP = \frac{F_{aq}}{C_{aq}} + P_{ev}} \qquad \textbf{Equation 1.6}$$

The aqueous humor is secreted by the ciliary body in the posterior chamber, specifically by non-pigmented epithelial cells.[29] Via a convection current, the aqueous moves to the anterior chamber primarily through the pupil. The fluid is drained mostly through the trabecular meshwork into Schlemm's canal, and a fraction into the suprachoroidal space known as uveoscleral drainage.[29]

Measurement of IOP is actually a measurement of the transcorneal pressure gradient between the atmosphere and IOP, using a tonometer.[30] The IOP is considered clinically normal between 10-21mmHg. However confounding factors such as the central corneal thickness, time of measurement, and body position may affect the measurement, hence, IOP values should be interpreted cautiously. Elevated IOP is the major modifiable risk factor for glaucoma.[4] IOP of more than 21mmHg usually calls for further investigation. An absence of glaucomatous optic neuropathy in the presence of elevated IOP is termed as ocular hypertension (OH). Reduction of IOP in people with OH has been shown to prevent or delay the onset of primary open angle glaucoma (POAG).[31] Normal tension glaucoma (NTG), according to some clinicians, is a subset

of POAG with IOP value of less than 21mmHg. Nevertheless, this classification is arbitrary as it is loosely based only on IOP values.

1.7 Blood pressure (BP)

BP refers to the pressure exerted by blood on the arterial walls as the heart beats. BP is maximal during ventricular contraction, i.e. systolic BP, and minimal during ventricular relaxation, i.e. diastolic BP. BP reduces in magnitude as it moves away from the heart. Clinically, systemic BP is measured at the brachial artery. BP is considered normal when systolic BP is <120 mmHg and diastolic BP is <80 mmHg. The diagnosis of pre-hypertension is when systolic BP is between 120 to 139 mmHg and diastolic BP is 80 to 89 mmHg. Stage I hypertension is when the systolic BP is 140-159 mmHg and diastolic BP is 90 to 99 mmHg, while stage II is >160 mmHg systolic BP and >100 mmHg diastolic BP.[32] Mean arterial pressure can be calculated by[29]:

$$\boldsymbol{MAP = Diastolic\ BP + \frac{1}{3}(Systolic\ BP - Diastolic\ BP)} \qquad \textbf{Equation 1.7}$$

Any association of BP and glaucoma is controversial (Table 1.1). Some authors found a significant association with hypertension, while others found a significant association with hypotension. A few epidemiological studies have even associated glaucoma to both hypertension and hypotension.[25, 33] Table 1.1 summarizes studies that have investigated any association between BP and glaucoma.

Studies	Findings
Thessaloniki Eye Study[34] Early Manifest Glaucoma Trial[23] The Barbados Eye Study[24] Leske et al.[35] Kaiser et al.[36]	Glaucoma is associated with arterial hypotension
Rotterdam Eye Study[37] Blue Mountains Eye Study[26] Beaver Dam Eye Study[38] Egna-Neumarkt Glaucoma Study[22]	Glaucoma is associated with arterial hypertension
Baltimore Eye Survey[33] Los Angeles Latino Eye Study[25]	Glaucoma is associated with both arterial hypotension and hypertension

Table 1.1: Summary of studies on blood pressure association with glaucoma.

1.7.1 Arterial hypotension and glaucoma

From the vascular perspective, low BP leads to reduction of OPP, which in turn leads to ischemia and neuropathy. It has been demonstrated that aggressive systemic hypertensive treatment exacerbates glaucoma progression. A population study on a non-glaucomatous cohort reported an increase in cupping and cup-to-disc ratio in treated hypertensive subjects, compared to untreated and normal subjects.[34] In another study on glaucoma patients, it was found that systemic diuretic usage was related to glaucoma development.[39] A similar result was reported by a 6 month longitudinal study which reported that degenerative changes and impaired blood flow were evident among glaucoma patients who take antihypertensive medication in the evenings.[40] This raises the concerns about any possible effect of hypertensive treatment on glaucoma.

1.7.2 Arterial hypertension and glaucoma

It is counter intuitive to associate hypertension to glaucoma, considering that high BP results in a high OPP.[18] The Baltimore Eye Study[33, 41] showed that relationship of hypertension to glaucoma is age dependent where it appears to be protective in younger patients but not in older patients. They speculated that blood vessels around the ONH experience atherosclerosis with age, becoming rigid and narrow, and thus compromising perfusion. Hypertension is therefore suggested to be a chronic risk factor that involves a long-term changes of the vascular beds which impairs perfusion.[42]

1.7.3 Effect of BP on tissue flow

It is expected that high arterial pressure will increase blood flow in tissues. The increase of perfusion pressure in a vessel will increase the blood velocity and at the same time will tend to distend the lumen size but this will be opposed by increase in tone, which in turn will increase flow. The relationship between BP and blood flow is exponential; increasing BP two fold will cause 4 to 6 folds of increase in flow.[16] However, this is not evident in a peripheral system like in the eye as autoregulation ensures stable perfusion despite BP changes. It was reported that retinal flow remains stable during dynamic exercise.[43] A study on healthy retinal arteriolar showed there was 2.5% arteriolar constriction with 21.8mmHg increase in MAP from baseline, suggesting a resistance to vasodilation in the retinal arteriolar vessels despite large change in MAP.[44] Choroidal flow[45, 46] and retrobulbar flow[47] was also reported to remain stable during dynamic exercise.

1.7.4 Effect of BP on IOP

Epidemiological studies have consistently reported an association between high BP and IOP.[22, 33, 37, 38] Animal studies have shown that the IOP is elevated upon stimulation of hypertension.[48, 49] It is, however, unclear how hypertension increases IOP. It has been proposed that BP elevation tends to elevate ciliary artery pressure which leads to elevation of ultrafiltration of aqueous production and hence IOP elevation. Alternatively, increased arterial pressure may also lead to a small elevation to the venous pressure, which may reduce the aqueous outflow and then increase IOP.[42] However, the change in IOP induced by BP is small (0.2-0.4mmHg IOP increase per 10mmHg BP increase),[50] thus it is unlikely that there is an association of glaucoma with high BP.

1.7.5 Ambulatory blood pressure monitoring (ABPM)

BP is influenced by factors which include physical and emotional status, environmental circumstances, the autonomic nervous system, the renin-angiotensin-aldosterone system, and neurohumoral factors.[51-53] BP measurement in a doctor's office may mask the real BP value where a "white coat reaction" has been reported to cause an increase of 20% in the BP value in suspected-hypertensive and hypertensive patients.[54] Masked hypertension on the other hand may also occur where a patient produces a normal office BP value despite having hypertension.[51, 53] As a result, a continuous BP measurement is pivotal which is permissible using ambulatory device. The International Database in Ambulatory BP monitoring in relation to Cardiovascular Outcome (IDACO) suggested normal daytime ambulatory BP should be <130/85 mmHg, nighttime BP <110/70 mmHg, and 24-hour BP <125/75 mmHg.[55]

1.8 Sleep

Sleep is a form of unconsciousness from which a person can be aroused by sensory or other stimuli (differently to coma which is unconsciousness without ability for arousal).[56] Sleep is important for homeostasis and restores natural balances among the neuronal centres.[56] Sleep can be categorized into rapid eye movement (REM) and non-REM type.[57, 58] These sleep cycles run cyclically throughout the night. Non-REM sleep is a restful, deep sleep. The peripheral vascular tone, blood pressure, respiratory rate and basal metabolic rate decreases during this sleep.[56] REM sleep carries 20-25% of the total sleep duration[57] and usually occurs during second half of the night. REM sleep is characterized by the rapid movement of the eyes, electroencephalography (EEG) arousal, and atonia.[58] This sleep is not so restful with heart and breathing rate becoming irregular, and active dreaming takes place.[56] REM sleep can further divided into tonic and phasic subtypes, where in the phasic stage the sympathetic activity increases and may exceed the level during wakefulness,[57, 58] potentially causes haemodynamic variability during REM sleep.

1.8.1 Circadian rhythm in pressure parameters

Circadian rhythm is defined as a biological cycle within a period of 24 hours and is pivotal to maintaining homeostasis in physiological systems. It has been established that IOP varies as a function of the circadian clock.[59] Recently, it has been indicated that blood pressure,[60] OPP[61]and ocular blood flow[62] also follows the circadian variation.

1.8.1.1 Circadian variation in BP

Classically, systolic BP is observed to rise abruptly after waking by 20-25 mmHg while diastolic BP rises by 10-15 mmHg.[63] BP climbs a little to reach a peak in the afternoon and then decline in the evening, continuing to reduce to its lowest during sleep. Typically, BP will fall during the first hour of sleep, then rise markedly in the morning upon waking. This nocturnal BP reduction (NBPR) normally value between 10 to 20% from the diurnal value,[51] and occurs in both normotensive and hypertensive individuals.[4] In some patients, high reduction (NBPR>20%) or low reduction (NBPR<10%) may also occur.[4, 60, 64, 65] Rarely, nocturnal blood pressure elevation may also occur.[66] Two thirds of the normal population is believed to have the normal NBPR value.[67] NBPR is calculated using the formula below:

$$\boldsymbol{NBPR}\ (\%) = \frac{\boldsymbol{Diurnal\ MAP - Nocturnal\ MAP}}{\boldsymbol{Diurnal\ MAP}} * \mathbf{100} \qquad \textbf{Equation 1.8}$$

Subjects with low NBPR have been shown to have poorer cardiovascular outcome.[51] It has been reported that low NBPR is associated with left ventricular hypertrophy,[68] silent cerebrovascular disease,[69] microalbuminuria,[70] and renal damage.[71] Reduced NBPR pattern may be due to autonomic dysfunction, steroid use, renal problems, bad sleep quality or postmenopausal state.[4] Advancing age[72] and female gender[73] are also associated with reduced NBPR. An exaggerated nocturnal hypotensive condition, in theory, may cause ischemia to organs such as the eye, especially in people with impaired autoregulation.[67, 74, 75] Subjects with high NBPR may also be accompanied with increased BP variability.[76] High NBPR may occur naturally, or may be induced by medication such as anti-hypertensive drugs. By calculation, the OPP is increased about 15mmHg when a person takes a supine position to sleep due to hydrostatic column

pressure.[4] Thus, the nocturnal blood pressure (NBP) should fall more than 15mmHg, or more than 20% to potentially cause an ischemic insult.

The decline in nocturnal BP is thought to be due to physiological attenuation in the sympathetic tone.[57, 58, 77] However, the sympathetic tone may also rise nocturnally during the phasic REM sleep.[58] This sympathetic activity fluctuation will result in a similar effect in the cardiovascular function, which may in turn result to a repetitive ischaemic-reperfusion cycle. Repetitive reperfusion has been shown to be damaging as it promotes the formation of free radicals and induce oxidative damage.[78] Patients with NTG have been shown to have different sympathetic activity during nocturnal hours, upon analysis of 48-hours heart rate variability.[79]

It has been implied that chronic hypertension may cause microvascular damage, while hypotension may cause local ischaemia. In situations where IOP is elevated, and/or vascular dysregulation exist, both hypertension and hypotension are possible causes of glaucomatous damage. The association between BP and glaucoma is inconclusive but most have associated hypotension to glaucoma. In addition, a high degree of BP variability has also been associated with glaucoma.[80] Table 1.2 summarizes the findings in the literature on NBP and its relationship to glaucoma. Also note the differences in ambulatory blood pressure monitoring (ABPM) methods between studies, which could affect the determination of NBP, or NBPR calculations. The location of ABPM measurement is also of importance since hospital setting measurement does not really reflect a patient's usual circadian rhythm. Sleep lab or hospital conditions may create environments that influence circadian rhythms.

Authors	Participants	BP measurement method	Findings
Kaiser et al.[36]	78 POAG 39 NTG 32 healthy	24-hours ABPM Night: 10pm-8am(60 min interval) HS	▪ Reduced diurnal and nocturnal systolic BP in NTG and progressive POAG
Hayreh et al.[81]	21 POAG 67 NTG 53 AION	24-hours ABPM Night: 11pm-6am (20 min interval) HS	▪ Greatest diastolic NBP reduction in NTG ▪ NBPR was associated with field progression in patients on antihypertensive medication
Bechetoille et al.[82]	16 NTG 16 POAG	24-hours ABPM Night: 10pm-6am (60 min interval) HS	▪ NBPR was not different between groups ▪ Reduced diurnal BP in NTG ▪ Greater systolic NBP variability in NTG
Orgul et al.[83]	9 POAG 11 NTG	24-hours ABPM Night: 10pm-8am (60 min interval) Measurement site unknown	▪ Reduced NBPR was correlated with vasospasm
Graham et al.[67]	38 NTG 46 POAG 11 healthy	24-hours ABPM Night: 10pm-6am (30 min interval) HS for glaucoma, HmS for healthy	▪ Greater reduction in NBP parameters relative to the diurnal's in progressive patients
Bressen-Dumont et al.[80]	83 POAG	24-hours ABPM Night: 10pm-6am (60 min interval) Measurement site unknown	▪ Greater BP variability in progressive patients ▪ Greater NBPR in progressive POAG
Meyer et al.[84]	20 NTG 20 healthy	24-hours ABPM Night: 10pm-6am (40 min interval) HS	▪ Greater NBPR in NTG
Detry et al.[85]	36 POAG	24-hours ABPM Night: 10pm-6am (interval not known) HS	▪ Patients with reduced NBPR associated with progressive glaucoma

Follman et al.[86]	100 glaucoma 60 healthy	24-hours ABPM Night: 10pm-6am (60 min interval) HS	▪ Reduced NBP in glaucoma ▪ Visual field loss was associated with reduced NBP
Harris et al.[87]	9 POAG 9 healthy	Manual BP at 9pm, 12am, 3am, and 6am HS	▪ No difference between diurnal and nocturnal BP
Collignon et al.[88]	51 POAG 19 NTG	24-hours ABPM Night: 10pm-6am (30 min interval) Low NBPR <5%, Normal 5-10%, High NBPR >10% HS	▪ High NBPR and low NBPR associated with progression
Hayreh et al.[75]	114 AION 131 NTG 30 POAG	24-hours ABPM Night: 11pm-6am (20 min interval) HS	▪ Reduced NBP in NTG ▪ NBPR was associated with field progression of patients on antihypertensive medication ▪ Reduced diastolic NBP in progressive patients
Kashiwagi et al.[89]	43 NTG 226 healthy	49-hours ABPM Night: according to subject sleeping time (30 min interval) Low NBPR <10%, Normal >10% HmS	▪ No differences in NBPR between groups. ▪ Greater NBPR in stable NTG than the progressive NTG ▪ BP fluctuated in progressive NTG
Harris et al.[90]	5 NTG 10 POAG 15 healthy	Night ABPM only from 10.30pm – 6am (20min) HS	▪ NBPR was not different between groups
Yazici et al.[91]	18 NTG 22 POAG 19 OHT	24-hours ABPM Night: 11pm-6am (30 min interval) HmS	▪ No differences in BP data between groups ▪ Individual BP data showed greater NBPR in NTG
Riccadonna et al.[92]	17 NTG 13 POAG 17 healthy	24-hours ABPM Did not mention nocturnal period. 30 min interval Measurement site unknown	▪ No differences in BP data between groups ▪ Reduction in diurnal heart rate variability in NTG ▪ Reduction in diastolic NBP variability in NTG

Liu et al.[61]	24 glaucoma 24 healthy	24-hours ABPM Night: 11pm-7am (120 min interval) HS	▪ Reduced NBP in glaucoma
Tokunaga et al.[93]	23 NTG 15 POAG	48-hours ABPM Night: according to subject sleeping time (30 min interval) Low NBPR <10%, Normal10-20%, High NBPR >20% HmS	▪ High NBPR and low NBPR was associated with progression
Plange et al.[94]	51 NTG 28 healthy	24-hours ABPM Night: 12am-6am (30 min interval) Low NBPR <10%, Normal >10% HS	▪ No difference in NBPR between group ▪ Higher diastolic NBP and nocturnal MAP in NTG ▪ Greater NBP variability in NTG
Joe et al.[95]	54 NTG	24-hours ABPM Night: 12am-6am (30 min interval) Low NBPR <5%, Normal 5-10%, High NBPR >10% HS but encourage for normal diurnal activities	▪ More than 50% patients have high NBPR ▪ BP variability was pronounced in patients with high NBPR
Kim et al.[96]	24 NTG 22 healthy	Manual BP until 11pm No measurement during sleep Low NBPR <5%, Normal 5-10%, High NBPR >10% HS	▪ No differences in BP parameters between groups ▪ NTG have larger morning BP reduction ▪ Patients with high NBPR showed variability in MAP and MOPP
Costa et al.[97]	29 POAG 24 healthy	Manual BP until 11pm Night: 12am-6am (120 mins) Low NBPR <10%, Normal 10-20%, High NBPR >20%	▪ 86% of POAG patients have low NBPR ▪ 96% of healthy controls have low NBPR

		HS	
Krasinska et al.[98]	69 POAG	24-hours ABPM Night: 12am-6am (120 mins) Low NBPR <10%, Normal 10-20%, High NBPR >20% HS	▪ Patient with >10% NBPR associated with nerve degeneration, and visual field deterioration

Table 1.2: Summary of findings relating NBPR and glaucoma, partially adapted from Werne et al.[65] and Graham et al. [99] [ABPM = ambulatory blood pressure monitoring, BP = blood pressure, NTG = normal tension glaucoma, POAG = primary open angle glaucoma, OHT = ocular hypertension, AION = anterior ischaemic optic neuropathy, NBPR = nocturnal blood pressure reduction, NBP = nocturnal blood pressure, HS = measurement at hospital, HmS = measurement at home]

1.8.1.2 Circadian variation in IOP

In healthy subjects, nocturnal IOP is higher than diurnal and it peaks towards the end of sleep before awakening.[100, 101] It has been shown that the nocturnal IOP, measured while a person is supine or sitting, is significantly higher than the diurnal value measured while a person is sitting.[102, 103] This elevation can partly be explained by the body posture (from sitting to supine when one sleeps), but the increment change can be detected even without postural change indicating IOP follows a true circadian rhythm.[104] The true reason behind the IOP increase at night is unknown, especially when the aqueous production is decreased nocturnally,[104] and episcleral venous pressure does not account for differences between different postures.

Circadian variation of the IOP has been demonstrated in patients with glaucoma as well, only the peaks occurring outside normal clinic hours.[105] Again, this observation suggests that IOP has real

circadian rhythm, and disturbance to this endogenous factor may be important in the pathogenesis of glaucoma. It is also observed that glaucomatous damage is associated with greater diurnal fluctuations, rather than a constant high pressure alone.[106-108] Considering the IOP having its peak value at night, glaucoma management should incorporate a 24-hour IOP measurement.

1.8.1.3 Circadian variation in OPP

By calculation, OPP decreases at night as a result of increasing IOP and decreasing BP, and this has been confirmed in healthy subjects[109] and also in glaucoma patients.[110] Glaucoma subjects with ocular vascular dysregulation may experience a severe reduction of OPP at night that may negatively impact the ONH morphology secondary to perfusion insufficiency. Sehi and co-workers[111] proposed that the diurnal OPP percentage changes as a risk factor for glaucoma. This is agreed by Choi and associates[112] as they identified that OPP fluctuations following circadian variation as the most consistent risk factor for the severity in glaucoma. They noticed that patients with greater variation in circadian OPP have the worst visual field and thinner RNFL. It was hypothesized that OPP variations lead to repetitive ischaemic-reperfusion injury. Sung and co-workers[113] in their 4 years longitudinal study also reported that OPP fluctuation as the most consistent prognostic factor for glaucoma progression. 1mmHg increase in OPP fluctuation was reported to associate with 27% higher hazard ratio of progression.

Choi and co-workers[114] measured 24-hour OPP on 132 NTG clustered patients based on NBPR status. They observed a significantly lower nocturnal OPP in patients with high NBPR compared to patients with normal NBPR and low NBPR. Sehi and co-workers[115] reported that the lowest OPP and the highest IOP was observed in the morning in their glaucoma patients. At the same

time, BP is relatively low in the early morning. This suggests that glaucoma patients may experience regulatory failure to maintain OPP in the face of changes in the IOP and BP that occur nocturnally.[65] Table 1.3 summarizes the findings in the literature regarding OPP circadian variation.

Study	Participants	Findings
Liu et al.[109]	16 young healthy 16 elderly healthy	▪ OPP increases at night in both groups ▪ Diurnal to nocturnal increase ratio is higher in the elderly
Okuno et al. [116]	12 NTG 12 healthy	▪ No diurnal variation in OPP
Sehi et al.[111, 115]	14 untreated POAG 14 healthy	▪ Diurnal increase in OPP for both groups ▪ OPP change is lower in glaucoma
Choi et al.[114]	132 NTG	▪ Circadian OPP fluctuation associated with patients with high NBPR and worse field defect ▪ High NBPR patients have reduced OPP at night
Choi et al.[112]	113 NTG	▪ Larger variability in OPP associated with field defect and morphological change
Kida et al.[101]	15 healthy young 15 healthy elderly	▪ No differences between diurnal and nocturnal OPP in elderly group, but reduced nocturnal OPP in younger group
Sung et al.[113]	101 NTG	▪ OPP fluctuation was associated with greater field progression ▪ 24-hour OPP fluctuation was highly predictive to filed progression
Renard et al.[117]	22 NTG	▪ Rhythm OPP was found in all subject, about equal distribution of acrophase diurnally and nocturnally ▪ OPP amplitude was not associated with progression
Ramli et al.[110]	72 NTG 55 healthy	▪ Night OPP is lower in the NTG group ▪ No difference in day OPP between the groups

Table 1.3: Summary of findings relating to circadian variation in OPP, partially adapted from Werne et al.[65] [NTG = normal tension glaucoma, POAG = primary open angle glaucoma, OPP = ocular perfusion pressure, NBPR = nocturnal blood pressure reduction]

1.8.1.4 Circadian variation in ocular haemodynamics

There should be relatively little variation of perfusion in a healthy eye, due to autoregulation that will ensure a steady RBF over a range of OPP. This has been confirmed in a few studies which found no variation in haemodynamics in healthy eyes,[62, 118] and by other centers with multiple glaucoma studies that included the recruitment of controls.[87, 90, 119] Kida and co-workers[101], however, reported that macula and ONH blood flow reduced at night only in their elderly healthy group despite a stable OPP. Their younger healthy groups on the contrary experienced a nocturnal reduction in OPP, but stable flow. They speculated that aging may compromise autoregulatory mechanisms.

In a compromised vascular bed, like that which is hypothesized to occur in some glaucoma patients, inefficiency or failure of the autoregulatory system may cause hypoxia or transient ischemia even in a steady perfusion state. It is difficult to interpret collective findings in the literature pertaining to perfusion circadian variability, considering the different techniques that have been used on different vascular beds. Regardless, there has been a general indication that perfusion is altered throughout the day in glaucomatous eyes.[111, 119] However, there were also studies that failed to find circadian variation in perfusion among glaucoma cohorts.[87, 90, 120] Table 1.4 summarizes the reports available in the literature pertaining perfusion variation following circadian rhythm.

Study	Participants	Method	Findings
Claridge et al.[120]	8 healthy 8 POAG 10 OHT	POBF	▪ No change in the haemodynamics from diurnal to nocturnal ▪ Large individual variation exist
Harris et al.[87]	9 healthy 9 POAG	CDI	▪ No change was observed to blood velocity in the ophthalmic artery ▪ Resistance decreases at night in both groups
Evans et al.[119]	20 healthy 20 POAG	CDI	▪ Blood velocity in ophthalmic and central retinal arteries reduces in glaucoma patients at night
Osusky et al.[62]	15 healthy	LCD	▪ Mean flow and volume decrease at midnight ▪ Velocity did not change at midnight
Gherghel et al.[121]	193 POAG	CDI	▪ Patients with high NBPR have lower blood velocity and higher resistance in the central retinal artery
Harris et al.[90]	15 healthy 15 glaucoma	CDI	▪ Blood velocity did not change in central retina, ophthalmic, and middle cerebral arteries during nocturnal BP hypotension
Sehi et al.[111]	14 healthy 14 untreated POAG	HRF	▪ Mean ONH rim flow did not change diurnally ▪ Diurnal change was present in region with greatest diurnal change in rim topography
Kida et al.[101]	15 healthy young 15 healthy elderly	LDF	▪ No difference between diurnal and nocturnal flow in younger group, but reduced nocturnal flow in elderly group
Karadag et al.[118]	59 healthy	CDI	▪ No significant difference in the haemodynamics between patients with normal NBPR (>10%) and low NBPR (<10%)
Krasinska et al.[98]	69 POAG with hypertension	CDI	▪ Hypertensive glaucoma patients with >10% NBPR have slower blood velocity, and higher resistance in ophthalmic and central retinal arteries
Krasinska et al.[40]	66 POAG with hypertension	CDI	▪ Taking antihypertensive medication for 6 months before sleep induces normal NBPR patients into having high NBPR ▪ Blood velocity decreases in the short posterior ciliary arteries in the high NBPR group relative to normal NBPR group, and relative before taking antihypertensive drugs

Table 1.4: Summary of findings relating to circadian variation in ocular haemodynamics, partially adapted from Werne et al.[65] *[NBPR = nocturnal blood pressure reduction, NTG = normal tension glaucoma, POAG = primary open angle glaucoma, BP = blood pressure, OPP = ocular perfusion pressure, CDI = color Doppler imaging, POBF = pulsatile ocular blood flow, HRF = Heidelberg retinal flowmeter, LDF = laser Doppler flowmetry]*

1.9 Retinal blood flow regulation

The retina lacks a functioning autonomic innervation, unlike the choroid. Perfusion control is regulated through local metabolic, myogenic, and humoral mechanisms.[11] These mechanisms may directly promote the release of endothelial vasoactive substances which cause dilation or constriction, by their action on the vascular smooth muscle cells.[11] The varying lumen diameter regulates the amount of blood that perfuses into the tissue.

1.9.1 Autoregulation

Autoregulation is "the ability of the vasculature to maintain blood flow at relatively constant levels despite moderate variations in perfusion pressure".[16] Autoregulation in a healthy retina and ONH is effective over a substantial range of systemic BP and IOP.[122, 123] It is reported that flow is constant until autoregulation breaks down when MAP rises 40% from baseline,[124, 125] and IOP rises above 30 mmHg.[126, 127] In the ONH, flow is constant until OPP increased 34% above baseline,[128] and IOP rises above 40-45 mmHg.[129, 130]

1.9.1.1 Metabolic autoregulation / Vascular reactivity

Metabolic autoregulation, plays an important role in the regulation of retinal and ONH perfusion. Flow is regulated in accordance to the metabolic demand. Perfusion is changed following accumulation or dissipation of metabolites that is produced by the retinal tissues especially the vascular endothelium. The concentration of oxygen (O_2), carbon dioxide (CO_2), nitric oxide, endothelin-1 or adenosine plays a role in metabolic autoregulation.[122] The study of metabolic

autoregulation, by means of varying the concentration of the metabolic components, is often termed as vascular reactivity.

Vascular reactivity is the magnitude of change in the retinal haemodynamics elicited by provocative stimuli. Provocations manipulating OPP, light flicker, cold-stress, or blood gas concentration may be used on healthy or diseased groups. Provocation studies in glaucoma will be elaborated at the end of this chapter.

1.9.1.2 Myogenic autoregulation

The myogenic response, also known as the Bayliss effect,[131] assesses the function of the vascular smooth muscles to contract or to relax in response, respectively to increase or decrease of the transmural pressure.[122] Via myogenic mechanisms, flow is kept constant despite an increase in arterial BP by smooth muscle contraction, thereby reducing flow. Conversely, smooth muscle relaxes at low arterial pressure to allow more flow. Pressure change activates the calcium ion channels in the vascular smooth muscle which mediate contraction or relaxation. Myogenic mechanism seems to be evident in all parts of the vascular system, and the magnitude of any myogenic-driven response may depend on vessels diameter.[132] Vasoactive substances from the endothelium are speculated to play a big role in the myogenic induced contraction.[133]

1.9.1.3 Humoral autoregulation

Circulating and locally produced hormones regulate the blood flow by signalling endothelial cells, vascular smooth muscle cells and pericytes.[134] Angiotensin-II receptor binding sites have been found to exist in ocular tissue, leading to evidence that the renin-angiotensin-system may

also play its part in ocular regulation.[135] Angiotensin-II is a potent vasoconstrictor, however, its administration in the human studies showed no alteration to choroidal and ONH flow.[136] Epinephrine causes vasoconstriction through the adrenergic receptors in the retina[137] but mediates dilation in other vascular beds.[138] In the same line, its effect on the retinal and ONH circulation is not well established.

1.9.1.4 Neurologic autoregulation

Unlike the retinal vessels, choroidal vessels are rich with neural innervations. Perfusion in the choroid, along with the posterior ciliary arteries and the extraocular CRA, are regulated by autonomic nerves.[134] Animal studies have shown that sympathetic stimulation reduces,[139] and denervation increases, choroidal flow.[140] It is also possible that parasympathetic excitement increases choroidal flow.[122]

1.9.2 Endothelial derived vasoactive factors

The endothelial cells lining the retinal vessels, stimulated by either chemical or physical stimuli, produce vasoactive substances in response to these stimuli to regulate the blood flow. These vasoactive substances induce primarily the vascular smooth muscle cells, and to a much lesser extent, the pericytes, to contract or relax. Blood flow is regulated by controlling the amount of flow by constricting or dilating the vessels. In the face of changes in systemic BP, IOP, or metabolic demands, different substances will be released accordingly to maintain stable perfusion. Two vasoactive groups being released to control blood flow are namely the endothelium derived constricting factors (EDCF) and the endothelium derived relaxing factors

(EDRF).[11] A balance between these factors regulate the vascular tone, thus reduction of one vaso-substance will result to vasoconstriction or vasodilation.[7]

EDCFs such as endothelin-1 (ET-1), angiotensin-II, cyclooxygenase products (i.e. thromboxane A2 and prostaglandin H2) promote vessels constriction.[2] ET-1 is the most potent constricting factor.[134] ET-1 binds to the vascular smooth muscle at ET_A receptor to mediate vasoconstriction, or on the endothelial cell itself at receptor ET_B to mediate vasodilatation.[134] Although it has been shown that ET-1 may mediate dilation or constriction, Rubanyi and Polokoff[141] debated that the overall response of ET-1 in vivo is sustained and powerful constriction. Studies have reported of increased levels of ET-1 in plasma,[142, 143] and aqueous[144] in glaucoma patients. Nicolela and co-workers[145] reported an increased plasma ET-1 after cold-provocation in POAG patients. A study by our group[146] reported a lower level of plasma ET-1 in untreated and newly treated POAG at baseline and normoxic hypercapnia condition, compared to controls and progressive POAG. On the other hand, other studies have reported of no significant difference in the ET-1 plasma level between POAG, NTG and healthy individuals.[147]

EDRFs such as nitric oxide (NO), prostacyclin, and endothelium-derived hyperpolarizing factors promote vessels dilation. NO is the most potent vasodilator released by the endothelium.[148] The NO activity contributes to autoregulation and provides protection against pathologic stressors implicated in diseases such as glaucoma, ischaemia and diabetes.[149] NO synthase (NOS) catalyses the conversion of L-arginine into NO and L-citrulline. NO formation increases the production of cyclic guanosine monophosphate (cGMP) leading to the reduction of calcium ions and thereby causing vasodilation.[150, 151] There was evidence which linked glaucoma patients with an altered ocular L-arginine/NO pathway.[152] Laude and associates[153] have reported a reduced

levels of cyclic GMP (NO activity indicator), together with a lower blood velocity in the ophthalmic artery of NTG patients. Additionally, there was also evidence of polymorphisms in the endothelium specific NOS (eNOS) in familial glaucoma.[154] Other studies also suggested polymorphisms in the eNOS in both high and normal tension glaucoma.[155]

1.10 Manipulation of blood gas

Vascular reactivity provocation can be achieved by changing the arterial partial pressures (Pa) of CO_2 and O_2 and this phenomenon has been extensively studied. Raising the $PaCO_2$ level while maintaining normal PaO_2 level is termed normoxic hypercapnia, and increasing PaO_2 level while maintaining $PaCO_2$ level is termed normocapnic hyperoxia. Hypercapnia induces vasodilation while hyperoxia induces vasoconstriction. The control of gases in most studies in the literature, however, has been generally poor although a few have published data to support or refute this statement. A few centers, including the Hudson Lab, have attempted to use a stable, standardized and repeatable breathing stimulus to assess reactivity that carefully controls inhaled gas mixtures.

1.10.1 Respiration, and ventilation gas exchange

Respiration is the process of supplying O_2, and removal of CO_2 in the body. O_2 and CO_2 are transferred between atmosphere and pulmonary capillaries followed by red blood cells and tissues. The total amount of air at a given time (total ventilation, Ve) during inspiration does not participate in alveolar gas exchange. The leftover gas forms the physiological dead space (Vpds) that remains in the airways and is exhaled first during expiration. The volume of air that

participates in alveolar gas exchange, termed as alveolar ventilation (V_A), is the difference between Ve and Vpds.[16]

The amount of CO_2 in expired gas represents the concentration of the gas in the alveoli (F_ACO_2) and can be presented in fractional value ($F_{ET}CO_2$). $PaCO_2$ concentration in the blood can be substituted with end-tidal partial pressure (P_{ET}) CO_2, which is the amount of exhaled CO_2, when using a partial rebreathing circuit. However, non-standardized techniques that use a non-rebreathing circuit or the manual addition of CO_2 into breathing air, show only an increase in $P_{ET}CO_2$, while tending to effectively fail to increase $PaCO_2$.[156, 157] Additionally, studies using hypercapnic stimuli fail to adequately consider concomitant O_2 control.[158-162] In order to accurately study vascular reactivity, it is important to use a standardized method to elicit repeatable stimuli to avoid any confounding effects.

The usage of a sequential gas delivery (SGD) breathing circuit with an automated gas sequencer has resulted in the desired control of the level of $P_{ET}O_2$ and $P_{ET}CO_2$ with precision. In the Hudson lab, a prospective gas targeting system (Respiract^{TM}, Thornhill, Toronto) has been used which allows independent control of PaO_2 and $PaCO_2$ to elicit repeatable provocation stimuli. It has been demonstrated that by using this gas blender, the $P_{ET}CO_2$ is correlated with $PaCO_2$.[156, 163] The SGD breathing circuit is a mask with a clamping ability to stably maintain normoxia/ normocapnia irrespective to minute ventilation changes (Figure 1.5). Respiract^{TM} uses metabolic O_2 and CO_2 consumption, resting and target P_{ET} data which has been pre-programmed in the software to determine the precise concentration of gas to be used to achieve a desired stimulus.

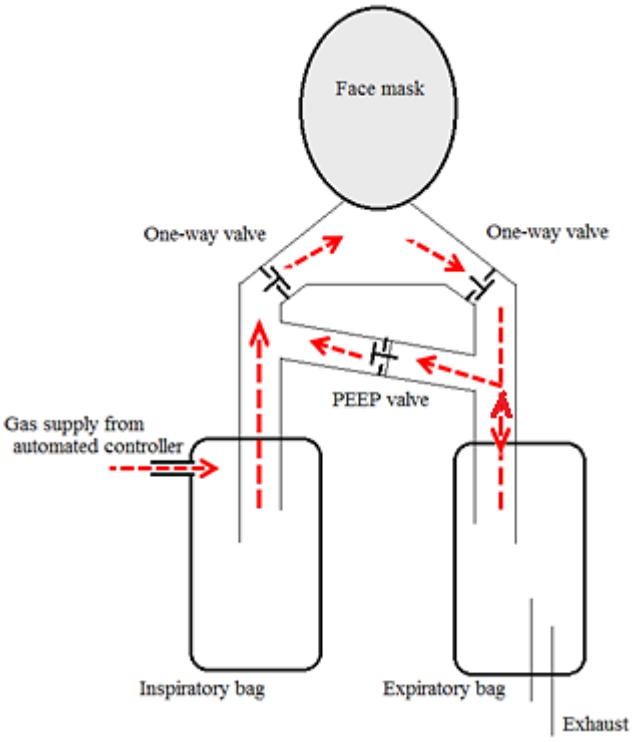

Figure 1.5: Schematic diagram of sequential gas delivery (SGD) circuit. An SGD breathing circuit comprised of two gas reservoirs and a face mask. The automatic gas sequencer supplies fresh gas into the inspiratory reservoir, while breathed gas fills the expiratory reservoirs. Each reservoir is connected to the mask with a separate one-way valve. The two reservoirs are interconnected by a positive end-expiratory pressure (PEEP) valve, which enables participants to rebreathe the exhaled gas in situations where fresh gas reservoir is emptied.

1.10.2 Normoxic hypercapnia

Normoxic hypercapnia induces vasodilation and increased blood flow in the human retina.[160-162, 164, 165] The involvement of NO has been implicated to this vasodilation,[166, 167] but the exact mechanism is still debated. However, Hessellund and co-workers[168] suggest that acidosis is responsible for the relaxation vascular tone in the porcine retina. This mechanism is associated with hyperpolarization in the smooth muscle cells and a constant decrease of Ca^{2+}. However most of the studies conducted to study retinal flow using hypercapnia did not control the

concomitant change in $P_{ET}O_2$, which may have confounded the magnitude of the vasoconstriction effect. It has been reported that hypercapnia may cause hyperventilation which affect $P_{ET}O_2$ level unpredictably.[169] The findings in the Hudson lab, using a stable normoxic hypercapnia stimulus, showed increased blood flow in the retinal arterioles.[170, 171]

1.10.3 Normocapnic hyperoxia

Normocapnic hyperoxia induces vasoconstriction and this has been shown in many studies.[159, 160, 171-175] The involvement of ET-1 is advocated to the constriction of the vessels.[176] However most studies did not control the $P_{ET}CO_2$ level. O_2 addition to breathing has been shown to induce hyperventilation thus resulting in a concomitant reduction of $PaCO_2$ pressure.[177] The findings in our lab, using a stable normocapnic hyperoxia stimulus, showed reduction in blood flow in the retinal arterioles.[171, 174]

1.11 Ocular blood flow quantification techniques

It is not surprising that there are only a relatively few techniques that are commercially available to non-invasively assess ocular blood flow given the intricate morphology of the ocular perfusion network. A relatively small number of techniques have been proven to be of value for the investigation of various aspects of ocular haemodynamics but all have their limitations in terms of interpretation and utility. The use of the current techniques is limited to research laboratories around the world because it has yet to be proven that retinal blood flow imaging has any value as a diagnostic procedure and because measurement variability can be very high for certain

techniques and the process of assigning a gold standard technique against which all other techniques can be assessed is on-going.

1.11.1 Color Doppler Imaging (CDI)

The CDI has been used extensively to study ocular haemodynamics and reactivity. It uses sound waves, inherent from the ultrasound technique, to measure retro bulbar blood velocity and vascular resistance.[178] Briefly, sound waves propagated from the probe into the eye are reflected from stationary structures without frequency change. However, sound waves reflected from moving particles such as blood, undergo frequency change. The frequency shift, the Doppler shift, is measured, and used to calculate blood velocity. The principles of Doppler will be elaborated later in this chapter.

Flow measurement is impossible as the technique is unable to measure vessel diameter. Velocity measurement is also restricted to big vessels such as the central retinal, SPC, LPC, and ophthalmic vessel(s) because of low resolution of the technique. The technique requires an experienced operator for a repeatable values.[179] The potential errors that may arise relate to how the measurement is carried out; (1) minimal pressure must be applied to the globe via the probe as not to induce an IOP rise and that impact OPP, (2) derived velocities values are dependent upon probe angle relative to moving blood cells, and (3) the ability to detect correctly and identify specific vessels from the sophisticated retro bulbar anatomical vascular structures can be very difficult.

Using CDI, glaucoma patients have been found to have a reduced velocity and higher resistance index value in comparison to healthy subjects.[180, 181] Progressive glaucoma was reported to have

reduced velocity in the CRA relative to healthy people.[182] In response to hypercapnia, a decreased magnitude of reactivity was evident in glaucomatous eyes compared to OHT and control subjects.[183]

1.11.2 Pulsatile ocular blood flowmeter (POBF)

The POBF estimates choroidal blood flow based on the ocular pulsatility, i.e. the changes in ocular volume and pressure during cardiac cycle.[184] The pneumatonometer is used to continuously measure IOP at a rate of 200Hz for 5 to 20 seconds, to produce a pulsatile waveform corresponding to systolic/diastolic time, ocular pulse amplitude and heart rate. Based on the assumption that there is a constant correlation between eye pressure and eye volume, it is taken that IOP is proportional to blood volume. The instrument calculates flow by analyzing the IOP waveforms during systolic and diastolic phase, assuming a general scleral rigidity and constant aqueous humor outflow.

This method does not measure flow directly, since it estimates flow by mathematical calculation from the ocular pulse volume variation. In addition, measurement is affected by scleral rigidity, axial length, refractive error, gender, BP, pulse rate, and age.[185, 185-187] Despite these limitations, measurement on patients with glaucoma have reported a reduced flow.[188] A study reported that POAG and OHT patients with IOP more than 25mmHg were found to have reduced flow, compared to OHT patients with IOP less than 25mmHg.[189, 190] The non significant difference observed upon comparison between OHT and POAG with IOP above 25mmHg, however, indicates that POBF reading is dependent on the IOP.

1.11.3 Blue field entoptic stimulation

The blue field entoptic phenomenon is based on the perception of leukocytes flowing in the retinal vessels in the macula. Blood velocity quantification using this phenomenon is subjective, where the subject notes the presence of leukocytes upon looking at diffuse blue light (430nm wavelength) in an area of 10 to 15° surrounding fixation.[191] Blood velocity is measured by asking the subject to make a match between his entoptic perception to stimulated moving particle field of different speeds.

This method requires patient's cooperation and perception, questioning its validity. Large variations between patients exist and data is limited in the perifoveal region.[192] The pathologic and physiologic state of the retina at any point in time will influence the measurement. Measurement on patients with cataract may be difficult. It is still debated whether leucocyte flux is proportional to blood flow under pathological conditions.[193]

1.11.4 Laser interferometry

Laser interferometry is a fundus pulsation technique that is used to determine choroidal flow.[194] Heartbeat-induced changes cause the distance between anterior corneal surface and retinal reflected light to pulsate i.e. this phenomenon is used to calculate pulsation amplitude. Using illumination from a single mode-diode laser (783nm wavelength), reflections from both the anterior cornea and retina form a non-localized concentric interference pattern. Distance change inflicted by rhythmic filling of blood during the cardiac cycle is analyzed as change in interference order. The systolic phase decreases the distance between cornea and retina, and vice

versa. A charged-coupled device (CCD) camera captures the interference image at high temporal resolution, which can be used to analyze the fundus pulsation amplitude (FPA).[195-197]

This method may be limited for patients with opaque media. Only the pulsatile component of flow is measured and the ratio between the pulsatile component and blood flow is unknown.[198] How the pulsatility is affected by diseases is also an interesting question. It has been reported that the FPA is decreased in POAG.[199]

1.11.5 Retinal vessel analyzer (RVA)

The RVA enables a continuous and real time diameter measurement of a vessel segment in relative units.[198] It comprises of a fundus camera, a video camera with a real time monitor and vessel diameter analysis software. Briefly, the brightness profile of an examiner-defined vessel is acquired and saved in a video recording device. The brightness profile, or densitometry image, is made by focusing wavelengths of 420 to 600nm perpendicular to the vessel. The wavelength values are chosen for optimal absorption by erythrocytes. Reflected light by different layers of retina and vessel is captured by a CCD sensor and is analyzed to determine vessel diameter. Twenty five diameter measurements are acquired per second[160, 161] and the system stops automatically if a subject blinks or moves. Measurement is resumed once a movement or blink has ceased.[200]

RVA usage may be limited in subjects with clear media and good fixation ability. Vessels that are close in proximity may also impossible to be measured as the RVA is unable to differentiate vessels apart.[201] Resolution is also limited (less than 1μm), thus measurement is only possible for

vessels greater than 90μm of diameter.[201] Measurement by the RVA represents the reaction of retinal vessel diameter, a haemodynamic parameter that is a surrogate of blood flow.

1.11.6 Laser Doppler techniques

1.11.6.1 Doppler shift effect

Light waves undergo a shift in frequency upon reflection from a moving particle (f'), relative to its pre-reflected state, or relative to reflected frequency from neighbouring stationary particles (f, Figure 1.6). This observation of frequency shift in waves is known as the Doppler shift (Δf).

$$\Delta f = f - f' \qquad \textbf{Equation 1.9}$$

The magnitude of shift is proportional to the velocity of moving particles. Doppler shift can be observed by illuminating volume of a retinal vessel. In a blood column experiencing Poiseuille's flow, the central maximum velocity (V_{max}) vector is considered. Assuming light illuminates an entire cross section of a vessel, light scattered from the moving blood is shifted in frequency by:

$$\Delta f = \frac{n . V_{max} (\cos\theta - \cos\vartheta)}{\lambda} \qquad \textbf{Equation 1.10}$$

where ϑ is the angle between velocity vector and incident light, and θ is the angle between velocity vector and reflected (scattered) light (Figure 1.5).[202] n is the refractive index of the medium and λ is the wavelength of the illumination light. Knowing the value of Doppler shift, maximum central line blood velocity, V_{max}, can be calculated.

$$V_{max} = \frac{\Delta f . \lambda}{n (\cos\theta - \cos\vartheta)} \qquad \textbf{Equation 1.11}$$

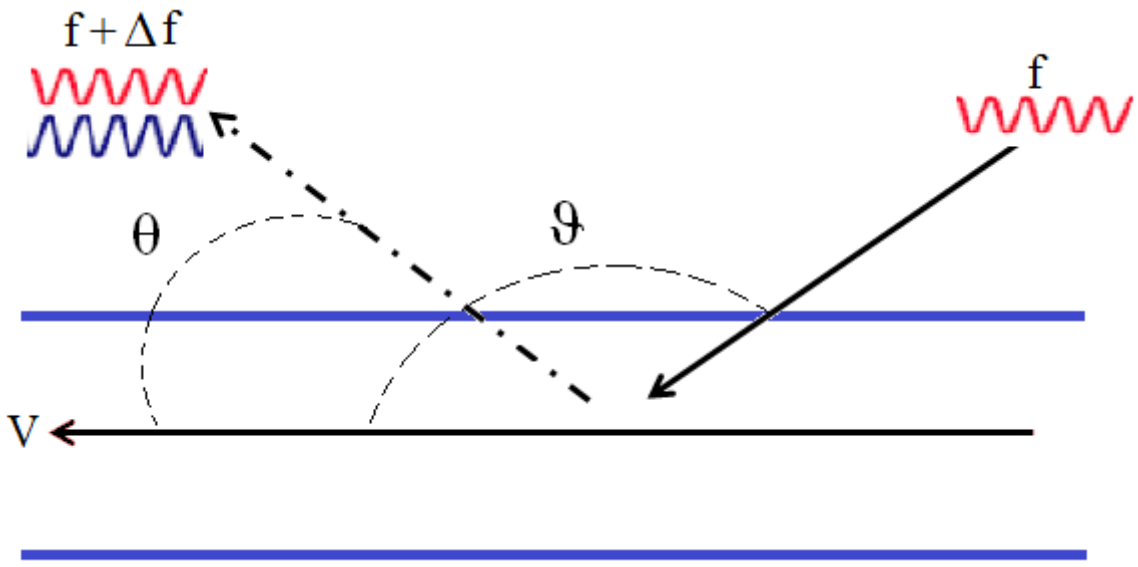

Figure 1.6: Illustration of the Doppler shift effect in a blood vessel.

1.11.6.2 Laser Doppler velocimetry (LDV)

The LDV uses the Doppler shift effect, enabling the measurement of absolute centre blood velocity of the retina. The maximum Doppler shift corresponds to the maximum centre velocity of a vessel. Most of the approaches for measuring centerline blood velocity in retinal arterioles and venules are based on the LDV technique.[184]

1.11.6.3 Laser Doppler flowmetry (LDF)

LDF is a non-invasive technique designed to measure relative blood velocity, volume and flow within a sampled tissue. The technique is based on light scattering properties in tissues, assuming that relative velocity and blood volume of the erythrocytes is related to complete randomization

of light directions after impinging the erythrocytes.[203] A 50μm diameter circular area on the retina is projected by laser, while a photodetector collects scattered light from 150μm circular area surrounding the laser spot. A larger scatter area indicates a Doppler shift from deeper sources in the retina. The photodetector analyses the Doppler shift to produce relative flow values.[204] Scanning laser Doppler flowmetry (SLDF) combines the LDF technology with scanning laser tomography. The Heidelberg Retina Flowmeter (HRF) is an example of a commercialized LDF, confocal version instrument (400μm focal plane thickness), which enables relative flow measurement of the retinal and ONH vasculature.[204]

Limitations of the LDF include; (1) only relatives flow values are measured, a by-product value from velocity and volume, (2) variation in scattering properties between subjects is high due to differing anatomy, (3) since the system is not depth plane specific, non-foveal measurement may be biased from both vasculature of retina and choroid.[184, 204] Findings from our lab using HRF showed that untreated POAG patients having the least reactivity to normoxic hypercapnia in the ONH compared to progressive POAG and controls.[146] In another study, LDF was used to measure 5 sites around the ONH in POAG and healthy group. Mean flow was reduced in the glaucoma group.[205] Upon comparison with glaucoma suspects, flow between a glaucoma group and a glaucoma-suspect group were not significantly different but the glaucoma-suspect group had lower flow than controls.[206]

1.11.6.4 Bi-directional laser Doppler velocimetry (BLDV)

The use of two photodetectors separated by a known angle enables a more accurate measurement of the centreline blood velocity. Unlike the LDV which is impacted by the Doppler angle because it uses a single photodetector, the BLDV (uses 2 photodetectors) is able to calculate the

Doppler angle from the difference in Doppler shift between 2 photodetectors.[207] This results in an accurate velocity calculation.

The maximum frequency shifts from the centreline vessel (δf_1 and δf_2) are detected simultaneously by photodetectors, K_1 and K_2, in two directions, separated by a fixed angle, $\Delta\alpha$ (Figure 1.7). Wave frequency can be calculated by dividing velocity by wavelength ($f = v/\lambda$), thus the maximum frequency shift from both photodetectors of a known separate angle can be calculated by:

$$\Delta f = \delta f_2 - \delta f_1 = \frac{(\alpha_2 - \alpha_1).v}{\lambda} \qquad \textbf{Equation 1.12}$$

where α_1 and α_2 are the angles between the velocity vector V_{max} and K_1 and K_2, respectively. Maximum velocity, V_{max}, can be calculated by:

$$V_{max} = \frac{(\lambda . \Delta f)}{n . \Delta\propto . \cos\beta} \qquad \textbf{Equation 1.13}$$

where Δf is taken from Equation 1.12.

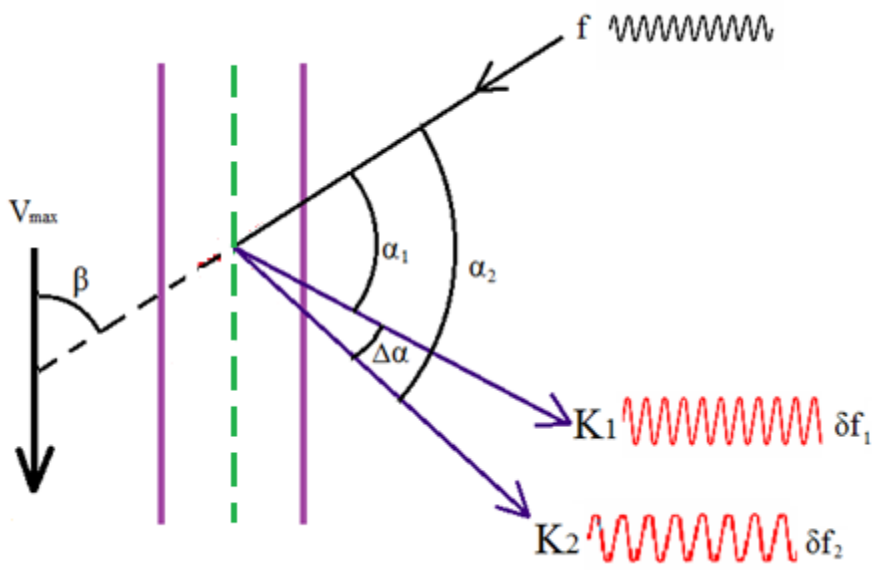

Figure 1.7: Illustration of the Doppler angle measurement in the bi-directional laser Doppler velocimetry.

An average flow in a Poiseuille condition is located in between of centreline and the vessel wall.[202] Thus, the mean velocity (V_{mean}) can be calculated by dividing half of the V_{max}.[17] If the cross sectional area (S) of where the fluid with the V_{mean} is known, then the flow rate (F) can be calculated by:

$$\boldsymbol{F = \frac{Vmax}{2} . S} \qquad \textbf{Equation 1.14}$$

Assuming that vessel has a circular cross section, F can be calculated by:

$$\boldsymbol{F = \frac{1}{2} . \pi . (D^2/4) . V_{mean} . 60} \qquad \textbf{Equation 1.15}$$

where D is vessel diameter.

1.11.6.5 Canon Laser Blood Flowmeter (CLBF)

The CLBF derives volumetric blood flow of the retina in absolute units (µL/min). It simultaneously measures blood velocity and diameter of a single vessel, to derive a flow value. The CLBF comprises a fundus camera incorporating a pair of photodectors as well as lasers. Velocity measurement is done every 0.02s across 2s measurement windows using red diode laser (675nm, 80µm x 50µm oval), producing a velocity-time tracing data. A separate green HeNe laser (543nm, 1500µm x 150µm rectangle), perpendicular to the measured vessel is used to measure diameter.[208] Diameter is measured every 4ms during the first and final 60ms of the 2s measurement window. The green laser also tracks eye movement and stabilizes laser position at the measurement site.[209] Two sequential measurements are acquired for the machine to give a valid averaged flow reading. The measurement is influenced by axial length and refractive error,[210] thus corrections are made by calibrating the optics accordingly. The software allows post-acquisition modification of velocity measurements, for example in cases where large tracking errors occur due to saccadic eye movement.[208]

The CLBF is limited to the measurement of one vessel at a time and it can only measure vessel diameters of 60µm or larger. Using systemic provocation, retinal vascular reactivity assessed using the CLBF is extrapolated on the assumption that a single site assessment is representative of global reactivity across the retina. Using normoxic hypercapnia as provocation, our group found that untreated and progressive POAG patients have decreased reactivity compared to controls and treated POAG.[146] Homeostatic baseline however revealed no significant difference between groups. In a different study, Berisha and co-workers[211] reported a significant lower

velocity and lower flow in the inferior temporal artery of early stage POAG patients compared to healthy controls.

1.11.6.6 Doppler spectral-domain optical coherence tomography (Doppler SD-OCT)

OCT is a high-resolution imaging methodology that can be used to quantify ocular morphology, particularly the ONH and retina. Consequently, it is routinely used in the clinical diagnosis and management of eye diseases. Of late, its fast acquisition and high resolution facility has also been applied to detect the Doppler shift in retinal vessels. OCT employs low coherence infrared light to image, which is divided into a sample arm and a reference arm/channel. Backscattered reflection from the sample arm (i.e. retina) interferes with that from the reference arm/channel, producing interference echoes. This interference echoes is detected by spectrometer, and Fourier transformed to create a measurement of light echoes versus depth, or A-scan.[212, 213] Combination of successive A-scans produce a B-scan image of the tissue. Doppler shift is measured by detecting the phase difference between sequential axial A-scans on each pixel.[214-216]

The RTVue SD-OCT (Optovue Inc., Freemont, CA) employs an 841nm light source with a bandwidth of 49nm. It has an axial resolution of 5.6μm in tissue and transverse resolution of 20μm. It uses a custom spectrometer with 1024-pixel line scan camera to detect backscattered light. The double circular scan protocol consist of two concentric rings of 3.4 and 3.75mm in diameter to measure total retinal blood flow.[217, 218] Centred on the ONH, the rings transect all vessels that traverse into and out from the eye at two locations. From the difference in the vessel positions in the concentric ring, the Doppler angle (θ) is measured for the Doppler shift calculation:

$$\Delta f = \frac{2V \sin \theta}{\lambda}$$ **Equation 1.16**

A smaller Doppler angle produces a weak Doppler shift, however an angle too large will cause extreme Doppler shift that may be out of the detection range of the SD-OCT. A reliable blood flow measurement needs a Doppler angle of between 5 to 15°. Four pairs (includes the inner and outer concentric circles) of scans are taken over two cardiac cycles in each scan (Figure 1.8).[216, 219] For measuring the total retinal blood flow, six scans are usually taken. During the blood flow scans, the Doppler angle is maximized by decentering scan angle minimally to the superonasal and inferonasal.

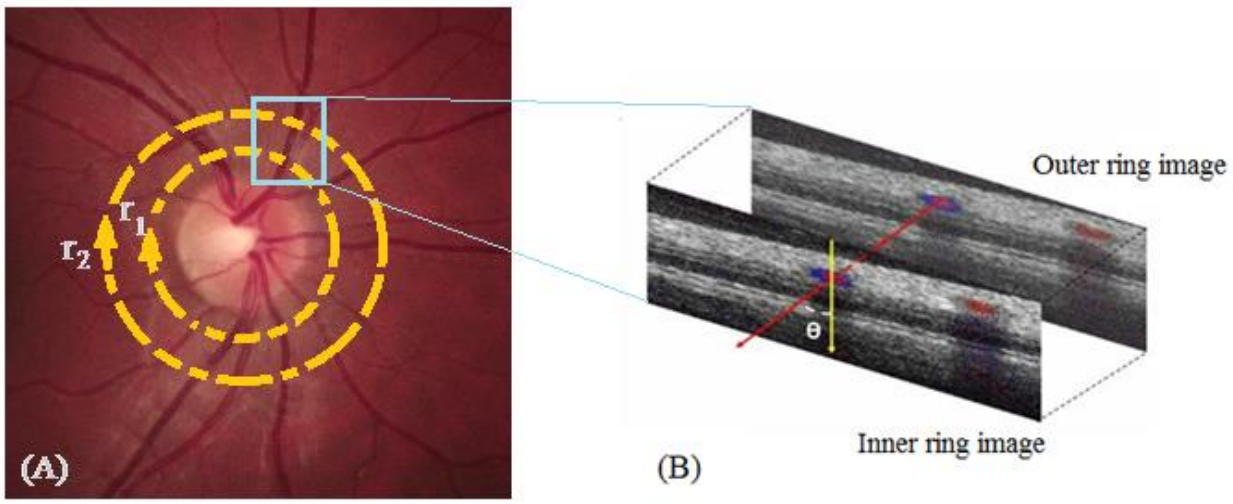

Figure 1.8: The blood flow double circular scan protocol. (A) Two concentric circles with different radius (r1 = 3.4mm and r2 = 3.75mm) centred on the optic nerve head transecting all vessels traversing into and out from the eye. (B) OCT images corresponding from each circular scan pattern showing vessels with Doppler shift (blue and red colour dots). Doppler angle is measured between OCT beam line (yellow) and vessel line (red). Adapted from Wang et al.[218]

A semi-automated software named the Doppler optical coherence tomography of retinal circulation, DOCTORC (Centre for Ophthalmic Optics and Lasers, CA, USA) is used to evaluate

the quality of the scans, and to calculate flow. A description of Doppler data grading and using the DOCTORC has appeared elsewhere.[220] Total retinal blood flow (TRBF) is calculated by summing flows from all detectable venules. Velocity is calculated by dividing flow in each valid venule by the cross sectional area of valid venule.[218, 220] This technique assumes that the venular flow is equal to the arteriolar flow, taking that inflow must be equal to outflow in any closed system that obeys law of conservation of mass.[218]

The TRBF using this technique was shown to have coefficient of variation (COV) value of 10.9% in healthy eyes, and 14.3% in diseased eye. This technique alsoshowed a good intra class correlation coefficient (ICC) of 0.85.[218] Our group found the COV median for young healthy subjects of 7.5% and for elderly healthy subjects of 9.2%.(Tayyari F., in press) These results indicate that the Doppler SD-OCT blood flow measurement is reliable and repeatable. Konduru and co-workers[220] studied the reproducibility of measurements derived from DOCTORC among two trained graders. They reported an ICC value of 0.933 with mean difference TRBF value of 3.84µL/min. Our group similarly compared haemodynamics results derived from DOCTORC from two, novice and experienced, graders. An overall analysis showed an ICC of 0.935 for the TRBF, with values from both graders being no different.[326] These results indicate that reproducible measurement is possible using semi-automated DOCTORC software. Retinal blood flow measurement using the Doppler SD-OCT has been shown to be lower in patients with POAG compared to healthy people. The reduction of flow was also correlated with the severity of field loss.[218]

1.12 Glaucoma

Glaucoma is an optic neuropathy that results in chronic, progressive loss of retinal ganglion cells and their neurons. The clinical characteristics include optic disc cupping, nerve fiber layer loss and visual field defects.[221] Glaucoma is one of the leading causes of irreversible blindness in the world.[222]

1.12.1 Classification

Classification of glaucoma is important to ensure the best clinical management available for a patient. There are different treatment options for different types of glaucoma. In general, glaucoma can be classified based on the anterior chamber angle, either an open angle or a closed angle. IOP elevation, a strong risk factor of this disease, is associated with the aqueous outflow anatomy structure. Both types of glaucoma may further be classified as primary or secondary. Secondary glaucoma may occur as a result of other eye disease or abnormality, or systemic diseases. Primary open angle glaucoma (POAG) may either be associated with an increased or a normal IOP, which results in the term high tension glaucoma or normal/low tension glaucoma (NTG), respectively. NTG is considered a spectrum of POAG. Eyes with high IOP but without glaucomatous damage are termed as ocular hypertensive (OHT). Other classifications are related to the onset of the disease, e.g. congenital or infantile glaucoma, and juvenile glaucoma.[223] In this thesis, the scope is confined to POAG, which includes both high and normal tension subdivision.

1.12.2 Risk factors of POAG

There is no real cure for glaucoma and current treatment modalities are aimed at preserving vision. Earlier detection of glaucoma is thus important for for a better prognosis. It is important to differentiate the risk factors that can lead to glaucoma, and the risk factors that lead to glaucoma progression.[224, 225] Risk factors that may lead to the disease include those that are associated with IOP elevation.

1.12.2.1 Age

Age is a major risk factor for the development and progression of glaucoma[42, 225] It has been noted that POAG seldomly occurs at ages younger than 40 years old.[222] It has been demonstrated that prevalence of glaucoma steadily increases with each decade after 55 years of age.[226, 227] A gradual rise of IOP and a reduced number of ganglion cells have been proposed as the causal factors.[225] Alternatively, it is hypothesized that the ganglion cells susceptibility to IOP insult increases with advancing age.[228] Ageing may also reduce the efficiency of blood flow autoregulation in the maintenance of stable perfusion in the face of OPP fluctuation.[42]

1.12.2.2 IOP

IOP has long been identified as a strong risk factor for the development and progression of glaucoma; the higher the IOP, the higher the likelihood of developing glaucomatous optic neuropathy (GON) and increased disease progression. Even in NTG, IOP may also play a role in the process of the disease.[229] Fluctuations of IOP may also contribute to the development and progression of glaucoma, and this includes circadian and day-to-day fluctuations.[224, 229] All of the

treatment modalities that are currently available involve reducing IOP. Reducing the IOP is of benefit even in OHT individuals. Longitudinal studies have indicated that the use of IOP lowering drugs delays or prevents POAG onset.[31]

1.12.2.3 Race

Although it is difficult to distinguish genetic risk factors from those by socioeconomic influences, POAG has been shown to be more prevalent among persons of African descent.[230, 231] Patients with African ancestry often have higher IOP and greater risk factor to experience GON.[225] The prevalence of POAG among black populations is at an earlier age compared to whites.[232] NTG is more prevalent among people of Japanese ancestry.[233]

1.12.2.4 Family history

The likelihood of an individual having POAG is increased with a family history of the disease.[234-236] Genetic studies have advocated protein coding defects, and gene mutations among POAG and NTG patients. Several genes loci such as MYOC and OPTN, and other chromosomal locations, have been subject of active investigations.[237] The interactions of genetic with environmental factors may play a part in glaucoma.[231]

1.12.2.5 Myopia

The Barbados Eye Study[238], Blue Mountains Eye Study[239], and Beijing Eye Study[240] all reported a higher prevalence of glaucoma among myopic eyes. Axial myopia is more susceptible to the development and progression of glaucoma. An elongated axial length has been associated with a thinner sclera and lamina cribosa,[241] that in turn exacerbates the IOP inflicted stress at the ONH.

The Kandy eye study from Sri Lanka, reported that longer axial length was a significant risk factor for glaucoma, together with age and IOP. [242] There were also reports of ONH connective tissues abnormalities in myopic eyes.[241, 243, 244]

1.12.2.6 Central corneal thickness (CCT)

Thinner CCT showed a strong relationship with the development of POAG.[24, 245] The Ocular Hypertension Treatment Study (OHTS) showed that CCT is a powerful predictor of POAG development, even after adjusting the effect of IOP, age, cup disc ratio, and pattern standard deviation.[246] The OHTS reported that patients with CCT of <555μm were at a 3 times greater risk of developing glaucoma than those with CCT of >588μm. The exact mechanism is still unclear. It is suggested that CCT may reflect scleral thickness, and thus a thin lamina cribosa may possesses a potential mechanical disadvantage.[247] Thinner structure can easily bend, hence may reduce the pores size hosting the axons traffic. This in turn causes kinking of the axons and blood vessels and triggers apoptosis and ischaemia.

1.12.2.7 Diabetes mellitus (DM)

The association between DM and glaucoma is controversial as some epidemiological studies have reported a negative relative risk,[248-253] while others found no association[254-257]. Experimental studies, however, suggest that diabetes may exaggerate the vulnerability of the eye towards insult such as increasing pressure.[258] Metabolic changes that occur in diabetes promote vascular damage and exacerbate glaucoma progression via ischaemic insult. The relationship between these two diseases has been reviewed elsewhere.[259]

1.12.2.8 Vasospasm

Vasospasm is considered as risk factor in glaucoma.[134, 260] Vasospasm or vascular dysregulation[260] can be described as an inappropriate constriction or insufficient dilation of a vessel to stimuli such as stress or coldness, which results to inadequate blood flow to tissue and consequent hypoxia/ischaemia. Simultaneous vessel dilatations in neighbouring tissues may occur as a by-product of dysregulation in vasospastic individuals.[261] Glaucoma patients who show evidence of vasospasm have a higher risk of field progression.[145] Vasospasm may affect the whole body but certain organs may be especially prone to vascular dysregulation.[262] It has been shown that women are more frequently affected by vasospasm, and this correlates with the observation that NTG is twice as common in women.[263] Vasospasm is also more common in Japanese than Caucasian patients, which again corresponding with the high prevalence of NTG in Japan.[262, 264] Pache and Flammer[260] in their review argued that vasospasm in glaucoma may occur due to imbalances of endothelial derived vasoactive factors, stemming from endothelial dysfunction. In addition, it is also suggested that vasospastic patients may experience more fluctuations in perfusion, which leads to reperfusion damage.

1.12.2.9 Migraine

Migraine has been proposed as a risk factor for glaucoma progression as it has been shown to have an association with field progression.[265] The Collaborative Normal-Tension Glaucoma Study Group in their analysis on field progression risk factors found that migraine was attributed for faster field deterioration.[266] Migraine has often been described as a spectrum of vasospastic disorders.[134] The occurrence has been shown to decrease with age.[260, 267] Its pathogenesis has been associated with functional vasospasm of the vessels in the brain.[260] It affects more women

than men and is more common in patients with NTG.[268] Literature on migraine and glaucoma shows varying results. Using headache questionnaires, a large sample size study by Phelps and Corbett,[269] showed higher prevalence of migraine-like symptom in NTG compared to OHT and controls. Smaller studies in Japan however did not find association.[270] The Beaver Dam Eye Study failed to associate migraine and glaucoma,[271] however, the Blue Mountains Eye Study suggested an association in patients of 70-79 years of age.[267]

1.12.2.10 Systemic BP

Systemic hypotension has been observed to be involved in glaucoma development and progression.[39, 40] Furthermore, nocturnal hypotension was shown to be more prevalent in progressive glaucoma.[67, 75, 84, 86, 98] Glaucoma patients with excessive nocturnal hypotension also showed reduced blood flow compared to glaucoma patients with normal and low nocturnal hypotension.[121]

1.12.2.11 Obstructive sleep apnea syndrome (OSAS)

In the recent decades, OSAS has been listed as a plausible risk factor for the development of glaucoma, especially NTG.[272-275] Significant associations were reported ranging from 5.7 to 27% in patients with OSAS.[57] OSAS can be described as repetitive collapse of the pharyngeal airway during sleep, with episodes usually terminating by arousal when the upper airway muscle tone increases.[276] The exact pathogenesis of OSAS in glaucoma is not clear.[277] It is assumed that OSAS may result in large swings in BP and severe hypoxia and hypercapnia. The repetitive nature of the episodes may cause reperfusion injury and contribute to ONH damage.[278] However, a review paper by Dhillon and co-workers[277] suggested that the proposed association between

OSAS and glaucoma is unlikely. They concluded that obstructive sleep apnea may not directly impact the development of glaucomatous optic neuropathy and anecdotally claimed association being related to the confounder of aging. This was supported by a very large retrospective study that was unable to identify an association between OSAS and glaucoma.[279]

1.12.3 Pathophysiology of POAG

There are many theories proposed to explain the pathogenesis of glaucoma, but the exact mechanisms behind the optic neuropathy remain unknown. The most discussed theories in the literature are the mechanical theory and the vascular theory. Most likely these mechanisms are not mutually exclusive although the relative contribution is unclear.

1.12.3.1 Mechanical and Vascular theory

The mechanical theory of glaucoma proposes that chronic IOP elevation promotes remodelling of the lamina cribosa, leading to mechanical compression of the ganglion axons. This lamina cribosa remodelling in turn affects the anterograde and retrograde axoplasmic transport, thus interrupting the transport of neurotrophic factors from the lateral geniculate nucleus to the ganglion cell body, and eventually causing ganglion cell death and apoptosis.[280] The central argument in this theory is that IOP elevation leads to GON, however it fails to explain glaucoma that occurs in the "normal range" of IOP, i.e the NTG. Glaucoma that occurs in normal IOP indicates involvement of other important factors in GON.

The vascular theory argues that the GON is a result of hypoxia/ischaemia that is secondary to perfusion insufficiency. Perfusion inadequacy may be a direct result of elevated IOP and/or other

risk factors such as vascular dysregulation or autoregulation deficit.[244] The lamina cribosa retrograde bowing induced by elevated IOP causes vessels to compress, affecting the perfusion and leading to hypoxia/ischaemia. Failure in autoregulation or vascular dysregulation causes tissue ischaemia and cellular injury, and eventually ganglion cell apoptosis. Indeed, ocular haemodynamic studies in the retinal,[217, 281] ONH,[199, 282, 283] choroidal,[188, 190] and retrobulbar[282, 284] showed low homeostatic values in glaucoma patients.

1.12.3.2 ONH insults in glaucoma

It is well-accepted that the primary insult of ganglion cell axons is at the lamina cribosa. Elevated IOP may be responsible for such insult modulated by the biomechanical properties of the lamina cribosa,[285] and the cerebrospinal fluid (CSF) pressure level.[286-289] CSF surrounds the ONH. CSF pressure has a positive relationship with the BP, where high BP causes CSF pressure to increase (to prevent dangerous levels of pressure in cerebral vasculature) and vice versa. The pressure gradient between the CSF pressure and IOP is called the trans-lamina cribosa pressure difference.[290] As such, low BP; by means of taking hypotensive medication, nocturnal hypotensive episode, or hypotension elicited by postural change, will reduce the pressure gradient. A low trans-lamina gradient may cause harm even when IOP in its "safe" value, as the relatively higher IOP pushes lamina cribosa outward and put pressure on the ganglion axons.[30, 291] It should be realized that glaucoma can happen at any level of IOP. Studies have shown that NTG and POAG patients have lower intra cranial pressure compared to OHT and controls.[286, 287]

Low OPP may cause primary insults by creating an ischaemic state in the ONH leading to cell death.[290] Again, low OPP may arise from systemic hypotension or elevated IOP. In theory, a, fully functional intrinsic autoregulation system should be able to keep perfusion stable.

Glaucomatous eye with abnormal autoregulation, or having vascular dysregulation, may be unable to keep perfusion constant. In addition, Chereacheanu and co-workers[290] suggested that reduced OPP may be associated with neurovascular coupling breakdown, where the hyperemic reaction to visual stimulation is defective (will be elaborated in 1.13.4).

In susceptible eyes with autoregulatory dysfunction, the tissues suffer from unstable perfusion in the face of systemic BP or IOP change. It is speculated that dysregulation may occur because of atherosclerosis of the vessels resulting from chronic hypertension. The hardening vessels thereby reduce available reserve to accommodate for the perfusion challenges. Autoregulatory dysfunction may also be caused by an abnormal vascular response to autonomic stimulation, local hormones and metabolites. As a result, the ONH may not only suffer from ischaemic insult, but also reperfusion injury due to inconsistent perfusion.[18]

Reperfusion injury refers to oxidative stress that is associated with the production of reactive oxygen species (ROS) such as free radicals, hydrogen peroxide, and singlet oxygen.[78] ROS are mainly produced in mitochondria.[292] The involvement of oxidative stress in GON due to hypoxia has long been debated,[293] and ischaemia and ischaemic-reperfusion are identified as ROS generators.[78] Considering the circadian variability in BP, OPP, IOP, and ocular haemodynamics that we have discussed earlier, reperfusion injury and oxidative stress, may contribute heavily to glaucoma pathogenesis. On the bright side, oxidative stress offers a potential area for neuroprotection in glaucoma. Strategies such as ROS inhibition, antioxidant supplement for ROS detoxification, or stimulation of gene expression for increasing mitochondrial antioxidant defence, may be a subject of interest in future glaucoma studies.[294] Interestingly, ginkgo biloba

has been used as an adjuvant therapy for glaucoma on the possibility of its neuroprotective effect,[295, 296] although it is controversial.

The cardiovascular system has a close relationship with the autonomic nervous system (ANS), regulating BP through sympathetic and parasympathetic system. Extreme nocturnal hypotension has been associated with glaucoma,[39, 67, 99] probably causing ischaemia to the ONH. Thus, ANS dysregulation has also been implicated in GON.[58] A few studies have reported systemic sympathetic and parasympathetic neuropathies in POAG patients.[92, 297-300] Although it is not directly measuring the ANS activity, the cold stress test has been shown to reduce ocular blood flow in POAG patients.[301] Studies pertaining to this issue are still lacking and a concrete involvement of ANS as a true risk factor is still to be proven. More research is needed to validate this matter.

1.12.4 Management

IOP is the only modifiable risk factor in glaucoma and is used to manage the disease. Thus, glaucoma management is confined to treat the IOP so that it goes lower than its current level, either by decreasing aqueous production or by increasing the outflow of the aqueous humor. It has been accepted that IOP lowering slows glaucoma progression and improves prognosis.[223] Only a few modalities to reduce IOP are available, including pharmacological agents, laser surgery, and incisional surgery. A detailed discussion of disease management is beyond the scope of this thesis.

1.13 Vascular reactivity in glaucoma

Vascular reactivity can be differentiated from autoregulation on the basis that it is driven by non-myogenic mechanisms. A myogenic response is studied by observing haemodynamics by manipulating OPP or cold-stress test, while metabolic response can be observed by measuring vascular reactivity to blood-gas change or flicker.

1.13.1 Haemodynamic regulation in response to perfusion pressure change

Myogenic response is the increase of the vascular resistance (by means of vasoconstriction) in response to perfusion pressure increase.[44] This normal retinal haemodynamic response is intrinsic to smooth muscle cells, which is independent of metabolic and hormonal influences.[15] The retinal myogenic response can be investigated by doing isometric/dynamic exercise or instigating posture changes including body inversion. BP increment, instigated by the exercise or postural change lead to OPP elevation.[122] Other methods to alter OPP include changing IOP via suction cup[302] and changing the arterial BP by thigh cuff technique[303].

Studies have indicated that glaucoma patients have an impaired autoregulatory response to OPP changes.[304] Evans and co-workers[305] using the CDI technique compared the change in the retrobulbar haemodynamics during different body postures. They saw a significant reduction in the resistance index of the CRA from sitting to supine in the healthy control group, but not in the glaucoma group. A similar observation was reported by Feke and co-workers[281] where they showed a broader range of responses in the retinal blood flow to OPP changes in glaucoma patients, compared to the healthy controls. An abnormal ocular haemodynamic response to an elevated IOP was also reported in a few studies recruiting glaucoma patients and healthy

controls.[306, 307] A study that induced BP elevation by isometric exercise found that NTG patients showed a trend for increased vascular resistance compared to healthy control subjects.[308]

1.13.2 Reactivity in response to hypercapnia

Hypercapnic provocation in a healthy eye causes vasodilation and increase in blood flow. The mechanism behind this response is not fully understood but it is thought to be primarily due to the NO system.[167] Studies using hypercapnia on glaucoma patients reported mixed results. The Hudson lab previously used the BLDV-SVD on untreated and progressive POAG patients and found the retinal arterial vascular reactivity was significantly reduced compared to healthy controls.[146] Additionally, using HRF on the ONH capillaries, the untreated POAG group showed the smallest magnitude of reactivity compared to treated POAG and controls. Hosking and co-workers[180] using the CDI technique found that POAG group showed increased velocity in the SPCA and CRA compared to the controls that showed only increased velocity in the CRA. A similar observation was reported by Harris and co-workers[309] in patients with NTG. An exaggerated response was noted in the NTG compared to the controls. They argued that the observation may be due to reduced baseline velocity in the patient group compared to controls. Sines and associates,[183] also using the CDI, reported a greater percent change of CRA resistive index in the OHT group compared to the POAG group. Of note, these studies did not use a partial rebreathing technique to reach the targeted $P_{ET}CO_2$ and $P_{ET}O_2$ level.

1.13.3 Reactivity in response to hyperoxia

Hyperoxia causes vasoconstriction in healthy eyes.[158, 160, 172-174, 310, 311] It is unfortunate that hyperoxia has been widely studied in glaucoma as the vasoconstricting effect of O_2 may cause

further harm to a compromised vascular bed like that proposed to occur in POAG. Hosking and associates[180] measured the retrobulbar reactivity using the CDI using 100% O_2 breathing. The control group showed reduction in blood velocity in the OA while glaucoma patients were not reactive to hyperoxia. They speculated that pre-existing vasospasms in the glaucomatous eye causes an inability to further constrict under hyperoxia. Harris and co-workers[312] studied the impact of hyperoxia on cerebral blood flow using a transcranial Doppler device on 16 POAG and 15 control subjects. While their control group showed reduction in velocity, hyperoxia did not cause significant change in POAG.

1.13.4 Reactivity in response to light flicker

Flicker stimulation increases retinal metabolism and leads to compensatory vasodilation and an increase in retinal blood flow.[313-318] The reactivity in veins is less pronounced than arteries with dilatation recorded between 1 to 7%.[15, 314] The underlying mechanism is thought to be due to a response called functional hyperemia; an increase of blood flow as the neuron gets activated.[319] This hyperemic response is also known as neurovascular coupling. In brief, glutamate that is released from the synapse activates N-methyl-D-aspartate receptors in neurons and metabotropic receptors in astrocytes. The activations lead to accumulation of intercellular calcium ion, activating an arachidonic acid pathway that is associated with vasodilator synthesis.[290, 319] In addition, the NO in neurons also contribute to this hyperemic response.[1, 320]

Vasodilation by flicker stimulation was shown to be reduced in glaucoma.[321-323] Garhofer and co-workers[321] using square wave light of different temporal frequencies investigated vasodilation in early POAG and healthy controls using RVA. They observed a significantly smaller flicker-induced vasodilation in retinal veins of POAG patients compared to the controls. Observation on

the dynamics of change under three 20 seconds flicker stimulations with a break of 80seconds in between revealed a delayed response in POAG and OHT groups compared to healthy controls.[323]

1.13.5 Reactivity in response to cold stress test

The hypothesis of ANS dysregulation association with glaucoma has been tested by using the cold stress test. The test involves cold stimulation of the hand by immersing in cold water (0 to 15° Celsius) to invoke sympathetic system, while measuring the ocular haemodynamics. Other variations may include immersion of the upper limb in warm water of 40 degrees following by cooling,[324, 325], wearing a head-vest cooling garment[145] and by blowing cold air to the nailfold area (in nailfold capillaroscopy)[225]. The response from the cooling includes vasoconstriction of the arteriolar vessels, elevated BP, and reduction in peripheral blood flow.

An eye with intact autoregulation will not have blood flow changes upon cold provocation on the upper limbs. Gherghel and associates[301] reported a reduced temporal neuroretinal rim blood velocity after 1 minute of cold provocation in POAG patients, compared to the healthy controls which did not show any significant changes. The regulatory mechanism responsible to regulate blood flow during cold-provocation is not clear. However, plasma ET-1 level has been reported to increase among POAG patients after cold provocation test.[145] It was speculated that an increased level of plasma ET-1 in response to cold provocation may be involved in glaucoma pathology.

1.14 Conclusions

The retinal and ONH blood flow anatomy is well documented, although blood flow quantification on these structures is far from being perfect. Various methods have been utilized to measure aspects of haemodynamics in POAG with conflicting results, however, most of the studies indicated ocular haemodynamic reductions.

Among many methods developed to study haemodynamics, the BLDV-SVD and the prototype Doppler SD-OCT are the only instruments, to the best of our knowledge, which enable blood flow measurement in real units. The BLDV-SVD produces a flow value immediately after a scan, although it only allows measurement for one specific site on a given vessel at a time. The prototype Doppler SD-OCT enables total retinal blood flow from all visible venules that traverse through the ONH at one scan, although tedious post-scan analyses is required to produce flow values. Regardless, both instruments are suitable to study POAG, a disease that affects the ONH and retinal blood vessels. Previous published studies using BLDV-SVD indicated reduced vascular reactivity in POAG, while retinal reactivity using the prototype Doppler SD-OCT has not been studied as yet.

Pressure variables such as IOP, BP, and OPP are observed to follow circadian rhythms. IOP is at its peak during night time while BP normally reduces at the same time. These concomitant events in turn result in a decreased OPP which at extreme levels may lead to damaging effects, especially in patients with glaucoma. This has led to a thought that patients with glaucoma are most vulnerable at night, particularly in patients with extreme NBPR. A few studies have indicated that POAG patients with extreme NBPR progress faster than patients with normal or

low NBPR. An extreme nocturnal hypotension is thought to represent a vascular dysregulation that may be relevant to glaucoma. This hypothesis, however, has never been tested. A study examining the retinal vascular reactivity is thus warranted on POAG patients with extreme nocturnal hypotension.

2 Rationale and hypothesis

Vascular dysregulation has been associated with glaucomatous optic neuropathy (GON). Reduced retinal vascular reactivity to various provocations has been linked to failure of vascular regulation in primary open angle glaucoma (POAG). Systemic hypotension has also been associated with POAG, and patients with extreme nocturnal hypotension have been shown to progress more than those with normal nocturnal blood pressure reduction (NBPR) and reduced NBPR status.[1, 2] Reduced blood velocity in the retrobulbar vessels have been reported in POAG patients with high NBPR[3] but no study, to the best of our knowledge, has yet examined the retinal blood flow, or the retinal vascular reactivity response in POAG patients with differing nocturnal blood pressure (NBP) characteristics.

The global aim of this work was to evaluate retinal blood flow, and retinal vascular reactivity in POAG patients of differing NBP characteristics using a standardized and stable hypercapnic stimulus. A prototypeDoppler spectral domain optical coherence tomography (SD-OCT) and the bi-directional laser Doppler velocimetry with simultaneous vessel densitometry (BLDV-SVD) methodology were utilized to measure the retinal haemodynamics, while a standardized hypercapnic stimulus was achieved using a sequential gas delivery (SGD) breathing circuit and prospective gas targeting system, the RespirAct™. Using normoxic hypercapnia, the retinal vessels were stimulated to dilate, and the magnitude of retinal vascular reactivity was measured. It was anticipated that the study of vascular reactivity in POAG patients with differing NBP status would reveal a better understanding to the pathophysiology of glaucoma.

Considering the recent application of Doppler phenomenon into the SD-OCT methodology to study retinal haemodynamics, in particular the prototype circum-papillary double circular blood flow scan protocol, there is no normative data from a sufficient sample size study available in the literature (e.g. Wang et al.[4-6]). Thus, the first objective in this research project was to investigate the haemodynamics in healthy eyes as being assessed by the prototype Doppler SD-OCT (Chapter 3). Healthy participants of different ages, with an equal number of males and females, were recruited to define the retinal blood flow normative value as measured by the Doppler SD-OCT. After consideration of intraocular pressure (IOP), age is established strong risk factor for developing glaucoma,[7, 8] however, only a fraction of the elderly population develop the disease. Changes in haemodynamics are thought to be gradual, which in turn predispose the eye to dysfunction but does not necessarily result in disease. It is not clear how retinal haemodynamics change with age, thus this thesis was also aimed to examine the interaction between retinal haemodynamics with aging (Chapter 3). It was hypothesized that there was no difference in the haemodynamics of healthy participants following an increase age.

As mentioned earlier, POAG patients with marked drop in their NBP were reported to progress more than POAGs with normal and low NBPR.[1, 2] This subset of POAG patients were also reported to have reduced velocity in the retrobulbar vessels.[3] It was suggested that extreme NBPR may be a manifestation of systemic vascular dysregulation and may be relevant to vascular regulation in the POAG ocular vasculature.[3] To the best of our knowledge, there is no study that has yet, examined the retinal vasculature and its association to the NBPR. Using both BLDV-SVD and Doppler SD-OCT, retinal haemodynamics can be measured in POAG cohorts with differing NBP characteristics. Therefore, this thesis also aimed to elucidate the relationship between the retinal haemodynamics and the NBPR in patients with POAG (Chapter 4). In

addition, it was also aimed to determine the relationship between the magnitude of NBPR and retinal vascular reactivity using normoxic hypercapnic provocation in subjects with POAG (Chapter 6). It was hypothesized that POAG patients with extreme NBPR have compromised vascular function and a reduced retinal vascular reactivity compared to the healthy controls.

Glaucoma progresses by altering vision and the optic nerve head (ONH) structure. Standard automated perimetry (SAP) retinal sensitivity values and retinal nerve fiber layer (RNFL) thickness are lower in glaucoma compared to healthy eyes. While it is clear that severe glaucoma results in worse vision and thinner RNFL,[9] the associations between ONH structures, visual field indices, and retinal haemodynamics is currently uncertain. Previous haemodynamic studies using different technologies to assess retinal blood flow produced mix reports on the associations.[10-20] Histological studies and fundus photography, however, have shown an association between retinal vessels attenuation and structural loss in glaucoma which could be independent of IOP.[21, 22] Thus, it is interesting to investigate the relationship between retinal haemodynamics, RNFL thickness and visual field status in POAG patients (Chapter 5). The hypothesis was haemodynamics parameters are associated with neural structure and visual field status in susceptible glaucomatous eyes. It was expected that these parameters have a positive correlation, where reduced haemodynamics will be evident in thinner RNFL and worse visual field.

2.1 Methodology rationale

2.1.1 Doppler measurement methodologies

Studies using the established BLDV-SVD have showed that selected POAG patients have dysfunctional retinal haemodynamics, including reduced blood flow and retinal vascular reactivity to hypercapnia.[17, 23] A pilot study using the prototype Doppler SD-OCT also showed reduction in retinal blood flow in POAG with small samples of patients.[4] Both BLDV–SVD and Doppler SD-OCT measure absolute retinal blood flow but using completely different methodologies. The BLDV-SVD measures blood flow of a single retinal arteriole, while the prototype Doppler SD-OCT measures blood flow by adding flow from multiple venules around the ONH. It is informative to study retinal haemodynamics by analyzing the blood flow signal from a selected retinal point, as measured by the BLDV-SVD method, and the signal of a relatively global nature as measured by the prototype Doppler SD-OCT. In addition, there is a need to test the newer Doppler SD-OCT method to establish normative values in a healthy population, and also to investigate disease conditions in particular the POAG. Comparing data from these two methodologies may give a better understanding of glaucoma.

2.1.2 Provocation stimulus

Hypercapnia was chosen over hyperoxic stimulus to assess retinal vascular reactivity. A hyperoxic stimulus may be damaging to a compromised vascular bed such that in POAG patients. Vasoconstriction secondary to hyperoxia reduces blood flow and may further aggravate the glaucomatous disease process. It may not be ethically correct to use a provocation that similarly promotes vasoconstriction and could potentially cause harm on patients. Whereas

hypercapnia induces vasodilation, hence an increase in blood flow and thus is likely to be a safe stimulus to study vascular reactivity in POAG.

Blood vessels in compromised vascular beds such as in POAG may already be constricted compared to the normal eye. Using hyperoxia to constrict the retinal blood vessels may not cause substantial further vasoconstriction, and in turn this may reduce the signal-to-noise ratio of an effect that we wish to investigate.

2.1.3 Blood gas manipulation

The magnitude of hypercapnia required to produce extreme vasodilation of the retinal arterioles was achieved using the SGD breathing circuit and the prospective gas targeting system to produce an increase in carbon dioxide end-tidal partial pressure ($P_{ET}CO_2$) of 15% relative to baseline (i.e. approximately 6mmHg increase in $P_{ET}CO_2$). The system we used to manipulate the blood gas manages to control the level of desired oxygen end-tidal partial pressure ($P_{ET}O_2$) and $P_{ET}CO_2$ at precision. It has been demonstrated that using this gas sequencer (RespirAct^{TM}, Thornhill Research Inc., Toronto, Canada) together with SGD breathing circuit, the $P_{ET}CO_2$ is correlated with arterial partial pressure (Pa) of CO_2.[24, 25] The sequential rebreathing circuit is a mask with a clamping ability to maintain normoxia/normocapnia irrespective to minute ventilation changes. RespirAct^{TM} uses metabolic O_2 and CO_2 consumptions, resting P_{ET} and target P_{ET} data which has been pre-programmed in the software to determine the precise concentration of gas to be used to achieve desired stimulus.

Unlike most other vision research labs to date, we have utilized a novel technology that optimally stimulates vascular reactivity at much lower levels of $P_{ET}CO_2$ which in turn results in

minimal change in pulse rate, blood pressure, and $P_{ET}O_2$, and far greater comfort and sustainability for research participants. For example, many groups have used carbogen (gas mixture containing 4 to 7% CO_2 and 93 to 96 % O_2[26]) to induce vasodilation which would result in a highly variable increase of $PaCO_2$. Gas provocation studies in other labs may also have achieved $P_{ET}CO_2$ by patient rebreathing his exhaled gases[27] or by adding CO_2 manually to the breathing air.[28, 29] Such techniques may trigger concomitant change in the $P_{ET}O_2$, thus response in vascular reactivity may not purely from the $P_{ET}CO_2$ increase.

2.2 Summary

In summary, extreme NBPR has been associated with POAG patients. Literature indicates that POAG patients with extreme NBPR progress more and may have reduced ocular blood flow. Evidence concerning the effect of NBPR on retinal haemodynamics is still lacking, hence this thesis was aimed to investigate any difference in vascular reactivity in POAG as a function of the magnitude of reduction in NBP and in normal healthy controls. The established BLDV-SVD method, together with the prototype Doppler SD-OCT were used to measure retinal blood flow in absolute units. The SGD breathing circuit together with prospective gas targeting system were used to achieve precise $P_{ET}CO_2$ and $P_{ET}O_2$ levels in inducing normoxic hypercapnia. Hypercapnia was chosen as provocative stimulus to study the retinal vascular reactivity considering it is safer than hyperoxic stimulus which may aggravate glaucomatous disease process by means of constricting the blood vessels and reducing retinal blood flow. The concern about inducing vasoconstriction in a disease with vasoconstrictive component is not widely

discussed but it was a major issue for the research ethics committees that approved this study and for the glaucoma specialists involved in this study.

3 The Influence of Age on Doppler Spectral-Domain Optical Coherence Tomography Derived Retinal Blood Flow

	Concept/Design	**Recruitment**	**Acquisition of data**	**Analysis**	**Write-up**
Yusof, F	X	X	X	X	X
Hudson, C	X			X	X
Tayyari, F	X	X	X	X	
Vymyslicky, M			X		
Huang, D	X				
Tan, O	X				
Flanagan, JG	X				X

Table detailing role of each person (X denotes significant contribution).

Purpose: The aim of the study was to evaluate the association between age and retinal haemodynamics, as derived by a prototype Doppler spectral-domain optical coherence tomography (SD-OCT) system.

Methods: A total of 100 healthy participants were recruited (48 males), divided into 5 age groups of 20 participants each; 18-29 years (25.1±2.6 years, 10 males,), 30-39 years (33.0±2.6 years, 10 males,), 40-49 years (44.2±3.0 years, 10 males), 50-59 years (55.7±3.1 years, 9 males), and above 60 years of age (64.2±3.0 years, 9 males). One eye of each subject was randomly selected and dilated. A prototype system based upon the RTVue instrument (Optovue Inc., Freemont, CA, USA) was used to measure the retinal haemodynamics, including the parameters total retinal blood flow (TRBF), venous area, and velocity. The circum-papillary double circular scan protocol was employed and all measurements were centered on the optic nerve head (ONH). The flow was calculated using data from valid scans, by summing flow from all detectable venules, using Doppler OCT of Retinal Circulation (DOCTORC) software version 2.1.1.4 (Centre for Ophthalmic Optics and Lasers, CA, USA). Pearson correlation analysis was employed to find relationship between the retinal haemodynamics with age, and sex. One-way ANOVA was employed to compare values between groups.

Results: 85.96% of the images taken were deemed valid for the flow calculation. Mean TRBF was 43.84±10.47µL/min, while superior and inferior quadrant retinal blood flows were 22.30±6.49µL/min and 21.35±7.28µL/min, respectively. The venous area and blood velocity were 43.83±8.70(x10^{-3}) mm^2, and 14.64±3.01mm/s, respectively. One-way ANOVA showed no significant difference in the TRBF between age groups (p=0.30), between superior and inferior quadrant (p=0.33), and between the sexes (p=0.30). Correlation analysis showed no significant

association between age and; TRBF (r=0.06, p=0.96), venous area (r=-0.08, p=0.426) or velocity (r=0.09, p=0.38), indicating that retinal haemodynamics were independent of age, and sex.

Conclusions: The perfusion to the inner retina is not altered with increasing age, indicating that retinal perfusion in healthy eyes is maintained at an overall constant level despite aging.

3.1 Introduction

The impact of aging on the eye, in particular on the retinal vasculature, is important to understand age-associated ocular disease. It is well established that aging is a risk factor for ocular diseases such as macula degeneration, glaucoma, diabetic retinopathy.[1, 2] The prevalence of people with glaucoma increases 10 fold from 0.7% among the 40-49 year olds, to 7.7% among those aged 80 years or over.[3] Muraoka and associates,[4] found that the retinal vessel wall thickness were highly correlated with advancing age where the elderly had thicker vessel walls and consequently smaller lumen diameters, suggesting an increase in vascular resistance. However, aging is a physiological process that may, or may not lead to disease. Only a small percentage of the elderly develop ocular disease, demonstrating that aging changes vary on an individual basis.[1] The precise connection, if any, between a given age-induced change and the onset of disease remains uncertain.[5] Changes such as in haemodynamics and vascular reactivity are gradual, which in turn predispose the eye to disease, but do not necessarily result in disease.

The influence of sex on retinal haemodynamics is not well explored in the literature. Harris and co-workers[5] measured the retrobulbar perfusion using the colour Doppler imaging (CDI) technique and found a significant reduction among women compared to men in the ophthalmic artery end diastolic velocity, but not in the central retinal artery. In a different study using laser Doppler flowmetry (LDF), choroidal flow was reported to be lower among elderly women.[6] Hormonal differences in ocular blood flow are thought to cause the disparity between men and women.

Several methods have been developed to study retinal haemodynamics using Doppler imaging techniques, with each having its own advantages and limitations. The Doppler colour ultrasound imaging is limited to measure velocity in large retrobulbar vessels.[7] It lacks the ability to measure diameter, hence quantification of the blood flow is unattainable.[8] Laser Doppler velocimetry (LDV) enables volumetric measurements,[9] but is limited to one vessel of a certain size. In addition, to measure total retinal blood flow, all vessels need to be imaged separately and values are later be summed. LDF permits continuous perfusion measurements, but is limited to sampling a small tissue volume.[10] The technique also gives values in arbitrary units. Bi-directional LDV with simultaneous vessel densitometry (BLDV-SVD) in the Canon Laser Blood Flowmeter (CLBF) enables an absolute measurement of retinal blood flow, but permits only measurement of one specific vessel site at a time.[11] Recently, a prototype Doppler spectral domain optical coherence tomography (SD-OCT) has emerged as a tool capable of evaluating retinal haemodynamics in absolute units.[8]

The prototype Doppler SD-OCT allows safe, fast, and non-invasive quantitative volumetric measurement of retinal blood flow.[12] Wang and associates[13, 14] have developed a double circular scan pattern that measures the total retinal blood flow. The laser scan pattern is centred on the ONH and enables measurement of all blood vessels around the optic nerve head (ONH). This prototype Doppler imaging technique was reported to be repeatable with coefficient of variation (COV) of 10.9% in healthy group and 14.3% in diseased group.[8] The overall intra-class coefficient of variation (ICC) was 0.85. Our group recently found that the COV for younger subjects were at median of 7.5% and elderly at median 9.8% (Tayyari et al., in press). The aim of this current study was to investigate any association between age, sex and retinal haemodynamics, as derived by a prototype Doppler SD-OCT system.

3.2 Methods

3.2.1 Participants

An initial 107 healthy, medication-free, non-smoking participants were randomly recruited. However, 7 participants failed to complete the study. Written consent was obtained prior to data collection. All participants met the following inclusion criteria; log MAR visual acuity of 0.3 or better, refractive error within ±6.00DS and/or ±2.00DC, normal 24-2 Humphrey Field Analyzer (HFA) automated static perimetry, intraocular pressure (IOP) of ≤ 21 mmHg, no media opacities, no history of ocular disease or ocular surgery (with the exception of uneventful cataract surgery), no endocrine disease or uncontrolled systemic hypertension, and no history of central nervous system disorders. All participants were asked not to consume any caffeinated beverages and/or meals containing red meat 4 hours prior to the study as caffeine constricts ocular vessels,[15-17] while red meat has been reported to have a vasodilatory effect.[18] The study was approved by the Office of Research Ethics, University of Waterloo, Canada, and follows the tenets in the Declaration of Helsinki.

The sample size calculation used the mean r value of 4 prior studies [38, 39, 42, 43]. R was estimated at -0.30. The number of participants required to reach this r value and to be significant (using a power of 90% and a p of 0.05) was calculated to be 92. Based upon prior studies, it was felt that the age range of 20 to 70 years of age would be adequate to investigate the effect of blood flow with age.

3.2.2 Doppler SD-OCT

A prototype Doppler SD-OCT system was incorporated into the Optovue RTvue OCT (Optovue Inc, Freemont, CA, USA), which uses an 841nm infrared light source, with an axial resolution of 5.4μm in tissue, a transverse resolution of 20μm, and power incident on the eye of 750μW.[8, 14, 19] A circum-papillary double circular scan pattern with concentric radii (inner and outer diameters measured 3.4 and 3.75mm respectively) was used and centred on the ONH.[20, 21] The concentric scans transect all retinal arterioles and venules around the ONH in two locations (Figure 3.1C).[19] In a single blood flow scan, six dual circular frames were acquired consecutively over 2 seconds, and then were averaged.[21]

The principle of the Doppler effect and its application has been described in detail in Chapter 1 (section 1.11.6.1). Reflected light from a moving particle, such as blood, undergoes a shift in its frequency (Doppler shift, Δf), relative to the wave frequency prior to reflection (or relative to a non-moving structure such as the vessel wall). The shift is proportional to the velocity component as given by the formula:

$$\mathbf{\Delta f = \frac{2nV \sin\propto}{\lambda_0}} \qquad \textbf{Equation 3.1}$$

where V is the blood velocity, n is the refractive index of the medium, α is the Doppler angle (between scanning beam and the flow direction which is dependent upon the position that a given retinal blood vessel transects the inner and outer rings of the scan), and λ_0 is the center wavelength of the beam. The Δf introduces a phase shift in the interference pattern which is captured by the OCT camera. The shifting interference pattern between sequential axial scans is

then Fourier transformed to calculate the Δf. Rearranging Equation 3.1, the velocity of a moving blood can be calculated by:

$$\boldsymbol{V = \frac{\Delta f \,.\lambda_0}{2n\,.sin\propto.}} \qquad \textbf{Equation 3.2}$$

Subsequently, retinal blood flow is calculated by multiplying velocity to the vessel lumen area value, a factor determined by vessel diameter.[13]

3.2.3 Retinal haemodynamics analysis using the semi-automated Doppler OCT of Retinal Circulation (DOCTORC) software

Semi-automated Doppler OCT of retinal circulation (DOCTORC) software version 2.1.1.4 (Centre for Ophthalmic Optics and Lasers, CA, USA) was used for the retinal blood flow calculation. Doppler scans were automatically-processed to identify vessel type, location and the strength of the Doppler signal. The Doppler scans were registered using a color fundus image, the infrared scanning laser ophthalmoscope image (Figure 3.1A), and the OCT projection image. Deletion of vessels with extraneous influences and the addition of missing vessels were then performed manually. Vessel classification (arteriole or venule) was reviewed based on the colour fundus image. Each vessel was manually defined, as necessary, for location and diameter boundary using the Doppler B-scan images. Confidence scores were assigned for each scan (maximum of 5, minimum of zero), judged from their Doppler strength, distinctiveness of vessel boundary, size agreement between the outer and inner ring, and Doppler signal sign agreement between the outer and inner ring (Figure 3.1C). DOCTORC automatically computes the retinal haemodynamics giving values for total retinal blood flow (TRBF), venous area, and venous velocity. TRBF was calculated by summing all flow from valid venules and the estimated flow

of invalid venules. Velocity was computed by dividing flow in each valid venule by the cross sectional area of all valid venules.[20]

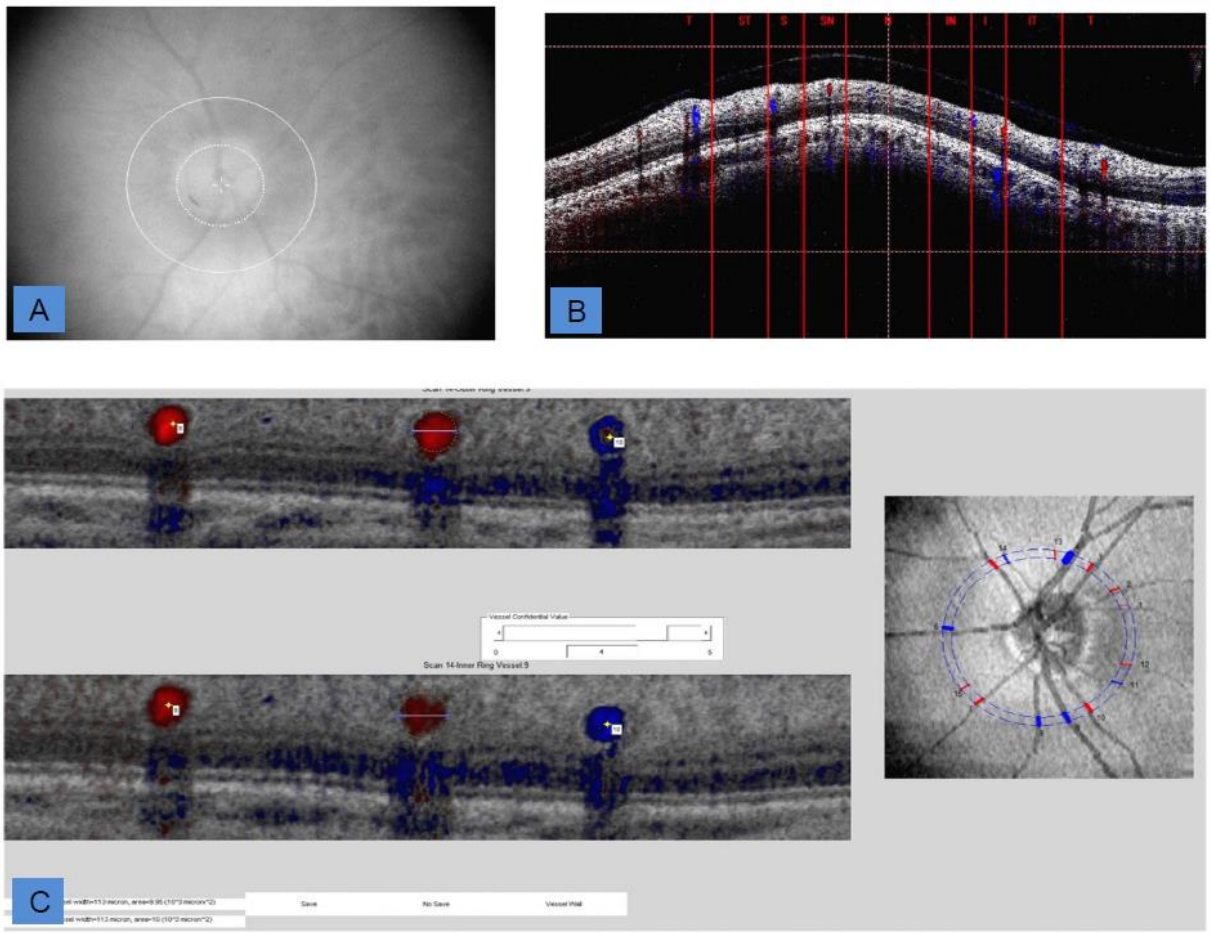

Figure 3.1: A) Scanning laser ophthalmoscope (SLO) image during the Doppler imaging, with the double para-papillary circular scan centered on the ONH. B) A still image of the real-time video of the ONH during the Doppler imaging, with the OCT beam positioned at the superonasal pupil location. C) A print-screen of the DOCTORC software during the analysis of one of the vessels, the top strip shows the vessels transected by the outer circular ring scan, and bottom strip for the inner circular ring scan. The smaller ONH image on the right shows the double circular scan transecting the retina in two different locations. The vessels were manually refined for their location, boundary and type (artery or vein). All these attributes, together with the Doppler signal strength and Doppler signal agreement were given a confidence score, following the guidelines in the DOCTORC grading protocol.

3.2.4 Procedures

Refraction, logMAR visual acuity, HFA automated static perimetry, Goldmann applanation tonometry, van Herick assessment of the anterior angle depth, and resting blood pressure measurement were determined for each volunteer. The HFA Analyzer (Carl Zeiss Meditec, Inc., Dublin, CA), SITA-standard 24–2 threshold test was used. Only subjects with <30% fixation losses, false positive and false negative responses, within normal limit glaucoma hemifield test (GHT) indices, and <5% pattern standard deviation (PSD) values were allowed into the study. One eye was randomly selected and dilated using 0.5% tropicamide (Alcon, Mississauga, Canada). Participants were then seated and rested for 10 minutes to stabilize baseline cardiovascular parameters. The circum-papillary double circular scan protocol was employed, and image acquisition was centered on the ONH. An average of 12 scans were acquired using two different beam alignments. Six scans were made with the beam passing through the superonasal pupil edge, and another 6 were made through the inferonasal pupil edge. Color fundus images and high definition 3D disc OCT scan images were also taken to aid blood flow grading. Three retinal nerve fiber layer (RNFL) thickness measurements were taken using RNFL scan protocol in the RTVue, and values were averaged. Systemic blood pressure (BP) was measured on the left arm, before and after Doppler imaging. Systemic mean arterial pressure (MAP) was calculated using the formula:[22-24]

$$\boldsymbol{MAP = Diastolic\ BP + \frac{1}{3}(Systolic\ BP - Diastolic\ BP)} \qquad \textbf{Equation 3.3}$$

The ocular perfusion pressure (OPP) was calculated using the formula:[22, 25]

$$OPP = \frac{2}{3} MAP - IOP$$ **Equation 3.4**

The Doppler scans were then extracted to be processed using DOCTORC.

3.2.5 Statistical analysis

All data were presented in mean ± standard deviation. Normality of data was tested using Wilk-Shapiro test. One-way analysis of variance (ANOVA) was employed to compare retinal haemodynamics values between groups, and comparison between males and females. The Tukey post-hoc analysis was employed to check for intragroup significance because it is generally regarded as not being overly conservative. To test for interaction between parameters, the Pearson product-moment correlation was used. The significance level was set at $p<0.05$. All statistical analyses were employed using STATISTICA (StatSoft Inc., Tulsa, OK, USA) version 11.0.

3.3 Results

3.3.1 Demographic data

Analysis included data from 100 eyes, from 100 healthy participants. Table 3.1 shows the demographics of all participants. The mean age for each decade group was 25.10±2.64, 33.01±3.16, 44.24±2.69, 55.71±3.06, and 64.24±3.02 years. No significant differences were recorded between the groups for IOP, MAP, and OPP. Although BP showed incremental change past the fourth decade, only diastolic BP showed a significant difference ($p=0.02$). However,

Tukey post-hoc analysis revealed no significant difference between groups, with only the 20-29 years versus 40-49 years group comparison achieving near significance (p=0.052, Figure 3.2). OPP values were not different across the age groups. The mean and the superior RNFL thickness were not significantly different across groups. The inferior RNFL thickness showed a significant difference across the groups (p=0.022) but intraclass analysis showed no significance (Figure 3.3).

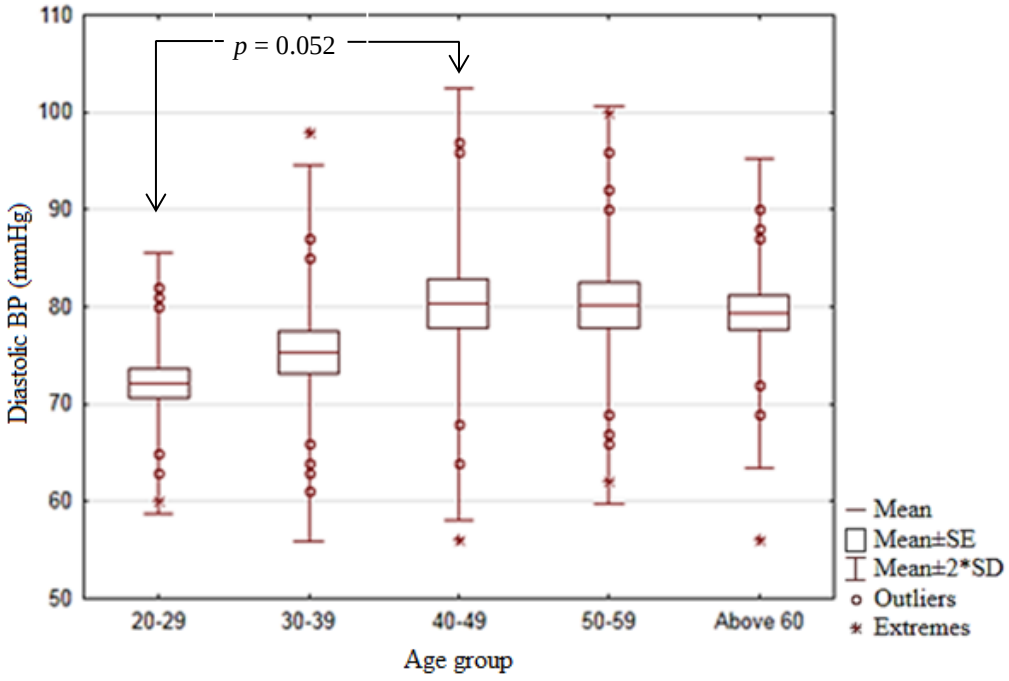

Figure 3.2: One-way ANOVA showed a significant difference in the diastolic pressure between the age groups (p=0.022). The Tukey post-hoc analysis however revealed no intergroup differences. Only the 20-29 vs 40-49 age group showed a nearly significant value (p = 0.052).

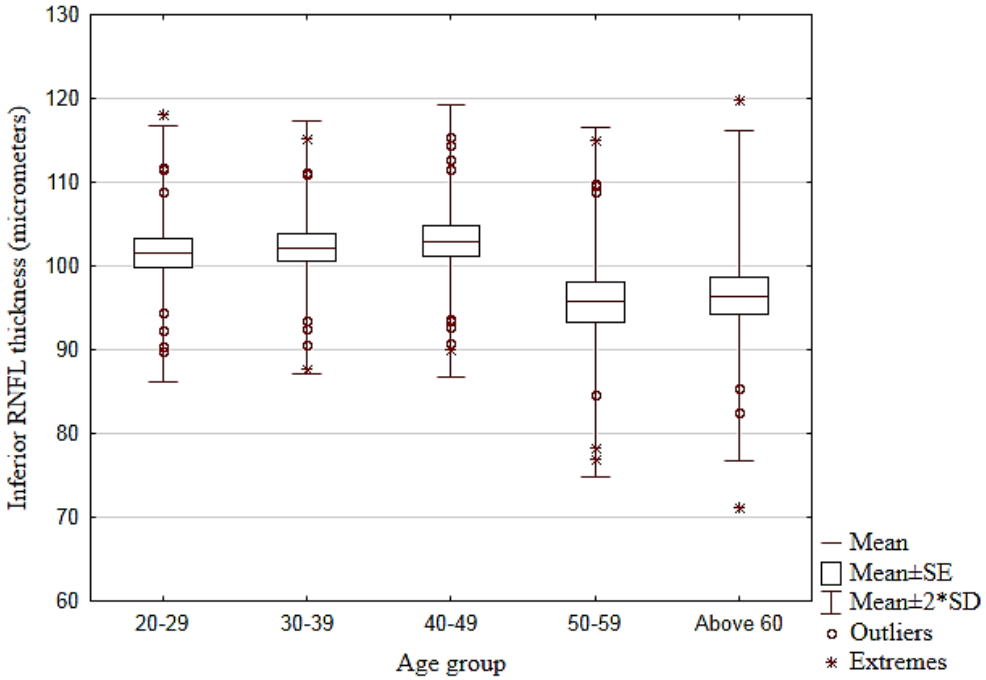

Figure 3.3: One-way ANOVA showed significant difference in the inferior retinal nerve fiber layer (RNFL) thickness between the age groups (p=0.022). Tukey post-hoc analysis however revealed no intergroup differences, with the 40-49 vs 50-59 age group showed p = 0.079.

Age group (Years)	20 -29	30-39	40-49	50-59	Above 60	p
Number	20	20	20	20	20	
Male : Female	10:10	10:10	10:10	9:11	9:11	
Systolic BP (mmHg)	123.85±15.50	123.70±17.30	128.45±17.54	127.20±15.99	131.14±13.95	0.547
Diastolic BP (mmHg)	72.20±6.69	75.30±9.67	80.30±11.12	80.20±10.21	79.37±7.97	**0.022**
IOP (mmHg)	13.80±3.16	15.07±2.58	13.37±2.91	14.18±2.81	14.28±1.84	0.364
MAP (mmHg)	89.42±8.20	91.43±12.00	96.35±12.08	95.87±11.42	96.63±7.89	0.104
OPP (mmHg)	47.01±6.41	45.79±8.23	50.46±8.67	49.73±8.26	50.12±5.31	0.193
Mean RNFL (µm)	101.85 8.65	99.88 7.25	102.55 6.79	96.86 10.09	97.33 9.75	0.138
Superior RNFL (µm)	100.88 10.66	97.10 9.77	102.17 7.42	97.84 11.31	97.20 10.61	0.372
Inferior RNFL (µm)	101.49 7.67	102.21 7.59	102.95 8.14	95.69 10.47	96.43 9.84	**0.022**

Table 3.1: Demographics including blood pressure (BP), intraocular pressure (IOP), mean arterial pressure (MAP), ocular perfusion pressure (OPP), mean retinal nerve fiber layer (RNFL) thickness, superior RNFL thickness, and inferior RNFL thickness of all participants, by age group.

3.3.2 Interaction between age and demographic data

Pearson correlation analysis showed weak significant correlation between age and; diastolic BP (r=0.31, p=0.002; Figure 3.4), MAP (r=0.28, p=0.004; Figure 3.5), OPP (r=0.24, p=0.014; Figure 3.6), mean RNFL thickness (r=-0.23, p=0.19; Figure 3.7), and inferior RNFL thickness (r=-0.31, p<0.001; Figure 3.7). Systolic BP showed a tendency to increase with age (r=0.192, p=0.056). IOP and superior RNFL thickness showed no relationship with age (r=-0.04, p=0.669; r=-0.12, p=0.230).

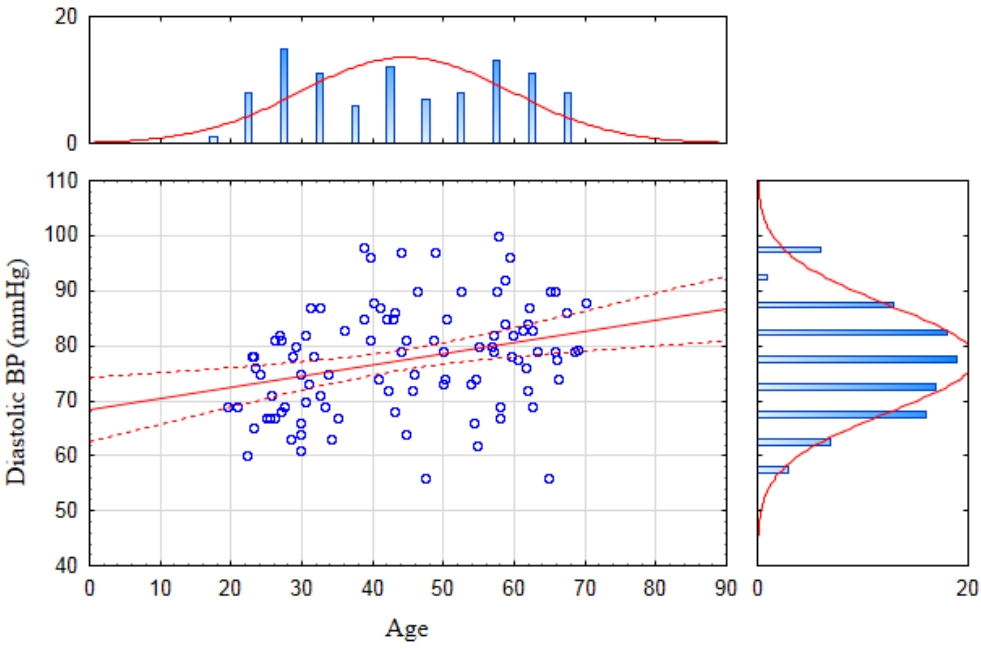

Figure 3.4: Scatterplot of diastolic blood pressure (BP) against age showing weak positive relationship (r=0.31, p=0.02).

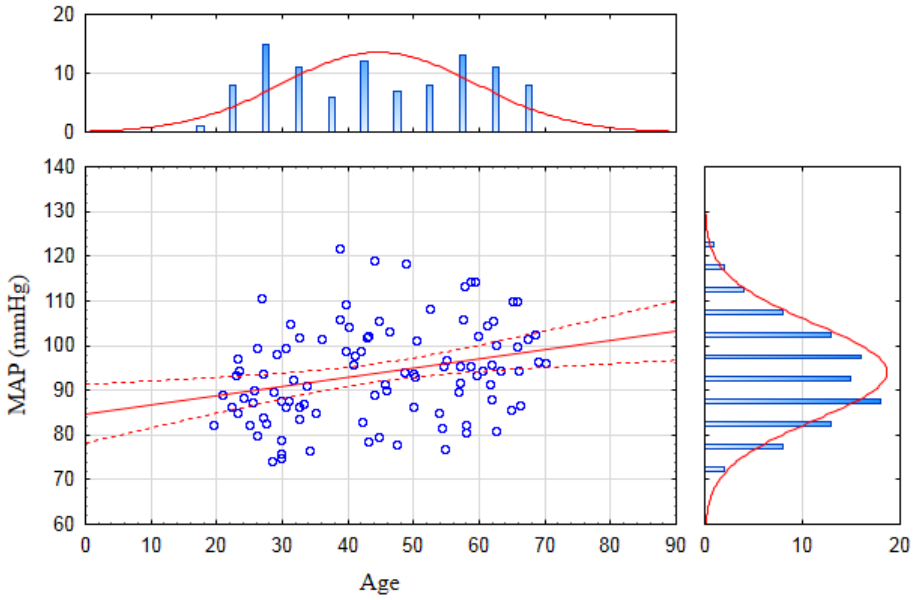

Figure 3.5 Scatterplot of mean arterial pressure (MAP) against age showing weak positive relationship (r=0.28, p=0.004).

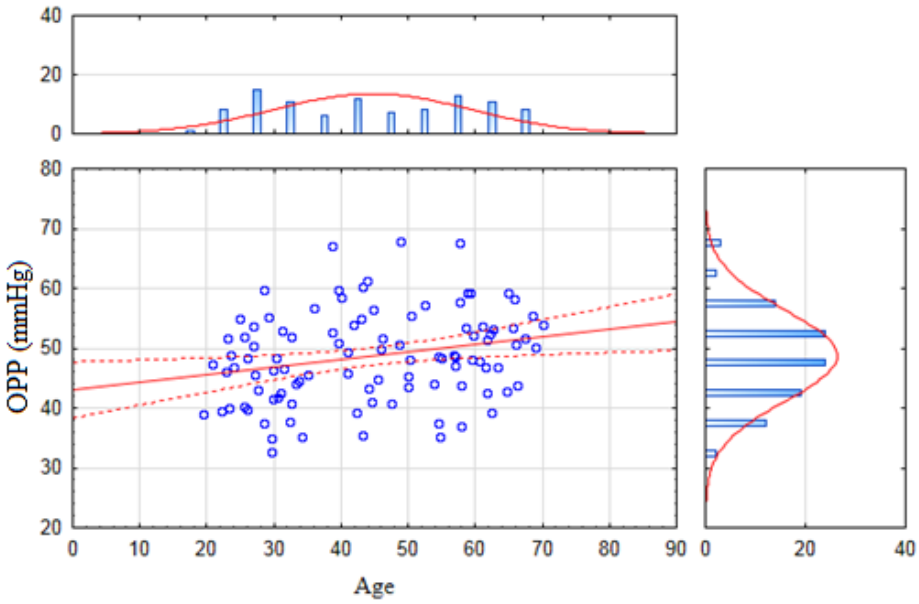

Figure 3.6 Scatterplot of ocular perfusion pressure (OPP) against age showing weak positive relationship (r=0.24, p=0.014).

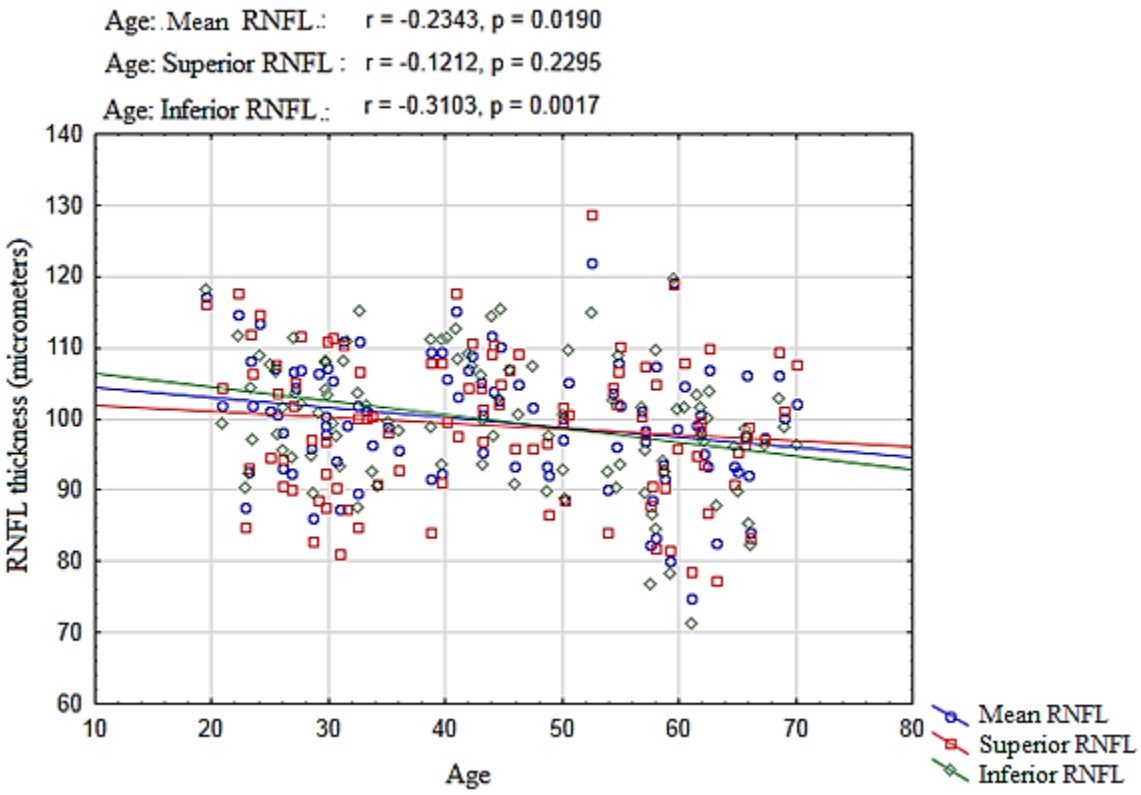

Figure 3.7: Scatterplot of retinal nerve fiber layer (RNFL) thicknesses against age. Mean RNFL thickness and inferior RNFL thickness showed weak correlation against age (r=-0.23 and r=-0.31, respectively). Superior RNFL thickness does not correlate with age.

3.3.3 Retinal haemodynamics

Table 3.2 shows the retinal blood flow parameters (mean ± standard deviation) based on age group. The average TRBF for all participants was 43.83±10.47µL/min. One-way ANOVA showed no significant difference in the TRBF between the age groups (p=0.693). Average venous area and velocity were 49.89±8.70(x10^{-3})mm^2 and 14.64±3.01mm/s, respectively, and

both showed no significant differences across the age groups (p=0.664 and p=0.679, respectively).

Age group (years)	Total Retinal Blood Flow (μL/min)	Venous Area (mm^2)	Velocity (mm/s)
20 -29	45.52±8.12	49.95±9.68($x10^{-3}$)	14.31±2.16
30-39	43.04±10.55	50.22±9.40($x10^{-3}$)	13.90±2.75
40-49	46.79±11.73	51.90±10.06($x10^{-3}$)	15.12±3.13
50-59	44.17±9.05	49.75±6.62($x10^{-3}$)	15.00±3.48
Above 60	42.65±12.91	47.63±7.81($x10^{-3}$)	14.88±3.52
Average value	**43.83±10.47**	**49.89±8.70 ($x10^{-3}$)**	**14.64±3.01**

Table 3.2: Retinal haemodynamics of all participants as a function of age group. The whole group average value for each parameter is noted in the bottom row. All parameters were not significantly different across age groups.

3.3.4 Interaction between retinal haemodynamics and age

Correlation analysis between TRBF and age showed no interaction (r=0.02, p=0.95; Figure 3.8). Venous area and venous velocity also showed no relationship against age (r=-0.08 and r=0.09, respectively).

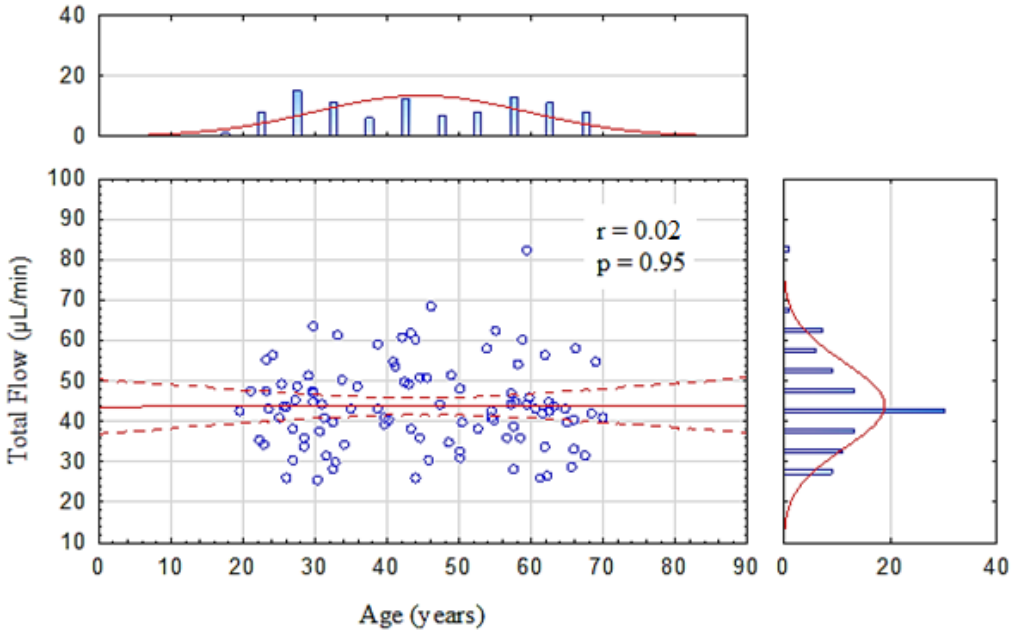

Figure 3.8: Scatterplot of total retinal blood flow against age showing no relationship (r=0.02)

3.3.5 Interaction between sex and retinal haemodynamics

Descriptive data segregated by sexare shown in Table 3.3. Fifty two females and 48 males were included in this analysis. The mean age for females was 45.39±14.73 years, and for males was 43.46±14.70 years (p=0.513). There was no significant difference in BP between males and females (systolic BP p=0.415, diastolic BP p=0.73). IOP was higher in males with values at 14.82±2.70mmHg compared to 13.51±2.57mmHg in females (p=0.015). No significant difference was found for all retinal haemodynamics. Figure 3.9 shows the box plot for TRBF as a function of sex.

Correlation analysis was conducted separately for each sex to analyze the relationship between retinal haemodynamics and age (Table 3.4). Only velocity in the male group was found to weak tendency for a relationship with age (r=0.28; p=0.052, Figure 3.10).

	Female	**Male**	**p**
Number	52	48	
Age (years)	45.39±14.73	43.46±14.70	0.513
IOP (mmHg)	13.51±2.57	14.82±2.70	0.015
Systolic BP (mmHg)	125.60±17.52	128.24±14.30	0.415
Diastolic BP (mmHg)	77.15±10.61	77.82±8.55	0.730
TRBF (µL/min)	44.88±10.72	42.70±14.70	0.303
Venous area (mm^2)	49.82±7.31($x10^{-3}$)	49.97±1.01($x10^{-3}$)	0.934
Velocity (mm/s)	14.99±2.64	14.26±3.38	0.231

Table 3.3: One way analysis of variance results between sexes. [IOP = intraocular pressure; BP = blood pressure; TRBF = total retinal blood flow]

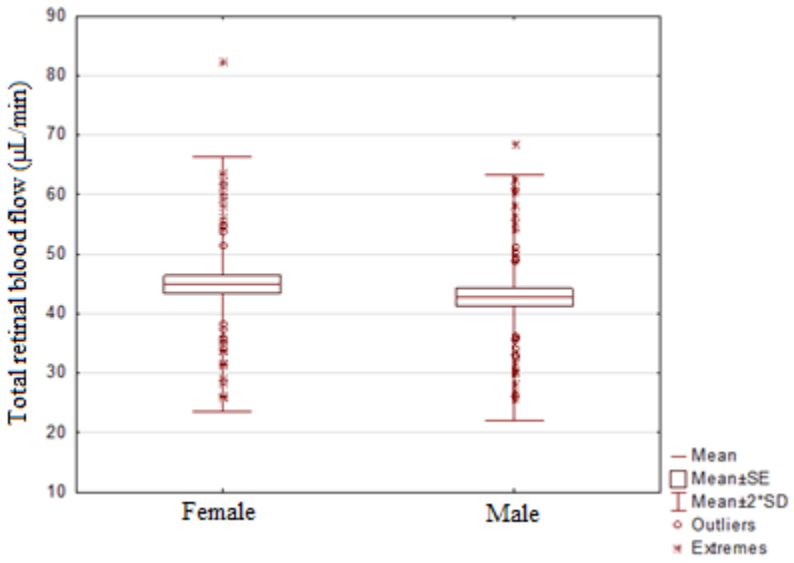

Figure 3.9: Box plot of the total retinal blood flow, segregated according to sex. One-way ANOVA revealed no significant difference between the gender groups (p=0.33).

Age vs.	Male		Female	
	r	**p**	**r**	**p**
Total Retinal Blood Flow (µL/min)	0.15	0.301	-0.14	0.334
Venous Area (mm^2)	-0.10	0.520	-0.06	0.655
Velocity (mm/s)	0.28	0.052	-0.15	0.283

Table 3.4: Pearson product-moment correlation coefficient (r) and significance value (p) separately for each sex, for correlation analysis between retinal haemodynamics and age.

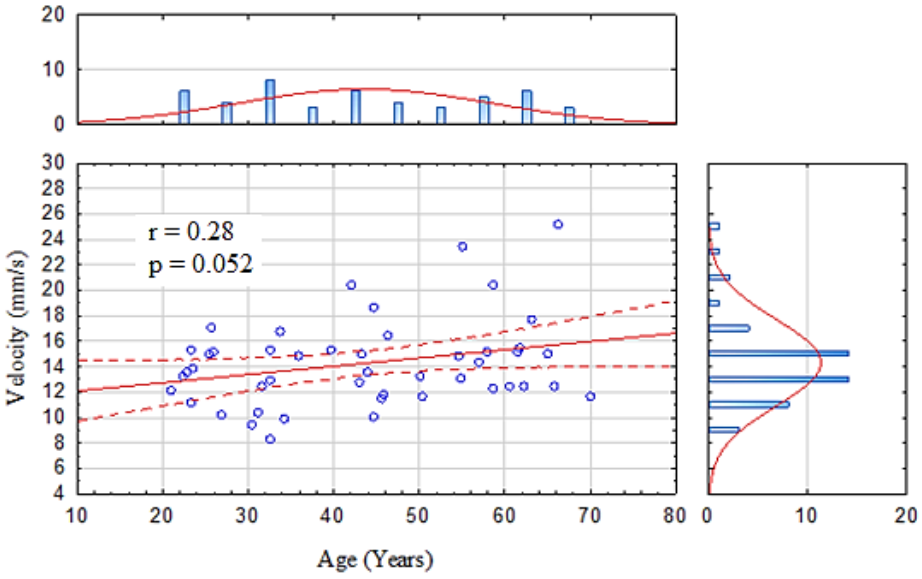

Figure 3.10: Scatterplot of velocity in male group against age. Pearson product-moment correlation coefficient shows a weak positive trend with age (r=0.28, p=0.052).

3.4 Discussion

The current work evaluated the effect of aging on retinal haemodynamics as measured by a prototype Doppler SD-OCT which enables the quantification of absolute retinal blood flow, along with venous area, and blood velocity. Flow measurement was performed by summing the flow from all venules that were transected by the circum-papillary double circular scan pattern. This flow value may be considered as the total inner retinal perfusion measurement, a novel value that has not been measured previously. Wang and co-workers[14] argued that the total venous flow can be taken as the total retinal flow considering the retina has a closed circulatory

system. The Doppler SD-OCT is also unique as it allows rapid, simultaneous measurement of blood velocity and vessel size in multiple vessels.

In this study, the average TRBF was 43.8±10.5μL/min, similar to previous reports.[14, 19] Wang and co-workers[14] have reported the mean TRBF of 8 healthy participants of 45.6±3.8μL/min. Wang and co-workers[19] repeated their measurements on an additional 20 healthy participants and reported a mean value of 47.6±5.4μL/min. The standard deviation reported in this study was higher than those by Wang and co-workers' but this study's sample age range was larger than theirs. The venous area and velocity reported in this study were also in the range of previous reports, that is venous area of $50\pm9(x10^{-3})mm^2$ versus $46\pm8(x10^{-3})mm^2$ and velocity of 14.6±3.0mm/s versus 17.7±3.1mm/s, respectively.[19] Using swept source Fourier domain OCT, Baumann and co-workers[26] reported the retinal arterial total flow of 49.15±2.46μL/min. Choi and co-workers[27] used a prototype Fourier domain OCT reported total retinal arterial flow of 43.2μL/min, while Dai and co-workers[28] recently reported total retinal venous flow of 49.19μL/min. The values they reported are in agreement with values in this study. There were studies using LDV that investigated the total retinal blood flow among healthy eyes. As LDV can only measure one vessel per time, individual vessel blood flow was summed to derive the total perfusion value. Riva and co-workers[29] reported the total retinal flow of 33μL/min while Garhofer and co-workers[30] reported value of 44μL/min. A few groups also have reported mean values in the range of 65 to 80μL/min.[31-33] The large discrepancy between these LDV reports may be due to the variability induced by a single vessel flow measurement. In the LDV studies, vessels were measured at locations which may have variable diameter and hence result in aberrant projected TRBF estimations. Also, multiple measurements over period of time to derive

the total flow, may suffer with bias stemming from changes that may occur during the measurement duration such as cardiac output or hormone level.

This current study did not observe changes in the retinal haemodynamics following age. This is in agreement with the work reported by Nagaoka and associates.[34] Using LDV with the simultaneous vessel densitometry technique, they compared 3 groups of young, middle-aged and elderly males. None of the hemodynamic parameters showed significant differences across the three groups. Their findings can be considered as the closest comparison to our study as the LDV technique measures the same vasculature in a single scan. The major disparity is that they measured only one major arteriole to generate all three hemodynamic parameters, whereas values in this current study were from many venules around the ONH. Nevertheless, the similarity of the Doppler calculated parameters and their relationship to aging was comparable. Yoshida and co-workers[32] studied 2 healthy groups; younger aged 25-38 years and older aged 54-58 years. They used the LDV without simultaneous diameter measurement and also found no significant difference in flow with age. It is interesting that we saw positive weak relationships in BP, MAP and OPP with age, but not in IOP and retinal haemodynamics. However, consideration needs to be given to the fact that the correlation coefficients for MAP and OPP were relatively weak and neither of these 2 factors were found to be significant for the ANOVA. The findings suggest that healthy eyes are regulated to maintain a constant blood flow in the inner retinal circulation, despite change in the OPP.

Other groups have investigated retrobulbar flow,[5, 35-37] choroidal flow,[38-42] ONH flow[37, 43] and perifoveal capillary flow[37, 44] with aging. This study did not found any relationship between the TRBF and age. Similar observations were reported by Lovasic and associates[38]. They

investigated sub-choroidal blood flow in 120 healthy participants, using the LDF technique. They recruited 20 participants for each age decade from 20 years to 80 years, and found no correlation with age (r=0.005). However, they found a significant negative relationship of age with velocity (r=-0.22), and positive significant correlation of age with scanned Doppler volume (r=0.22). Ambarki and co-workers[36] investigated the ophthalmic artery flow, using magnetic resonance imaging, and found no significant difference between groups of healthy young and healthy elderly adults. They did find a significant increase in the resistive index and arterial volume pulsitility in the elderly compared to the young groups, suggesting reduced vascular compliance as vessels age. A study of choroidal flow using indocyanine green angiography, investigated 35 healthy eyes and reported a reduction in arterial fluorescence intensity with increasing age, but interestingly, the same result was not observed for venous florescence intensity.[41] Dallinger and associates[42] used the laser interferometry method to measure pulsatile ocular blood flow (POBF), a measurement relating primarily to choroidal flow, on 130 healthy participants. They reported a negative relationship with age (r=-0.24). Ravalico and co-workers[39] also investigated the POBF in 105 healthy participants where they observed a negative correlation with age, in the sitting position (r=-0.36) and in the supine position with induced IOP increase (r=-0.28). Nevertheless, it is imperative to stress that pulsatile flow primarily measures the choroidal circulation with little contribution from the retinal flow. Moreover, the measurement is also highly related to scleral rigidity, a mechanical ocular property that may change with age. Reports on ONH flow and age were not unanimous. Groh and co-workers[37] used scanning laser Doppler flowmetry (SLDF), a method which gives arbitrary flow values, showed no relationship with age. Boehm and co-workers[43] on the contrary reported a weak negative relationship between flow and age for both temporal and nasal neuroretinal rim flow.

The female estrogen hormone has vasodilatory capability and this may influence the retinal haemodynamics. A few studies have reported an estrogen influence on the systemic circulation,[45, 46] as well as on the ocular circulation.[5, 6, 47] We compared our hemodynamic values between the sexes and our analysis showed no significant differences, in agreement with the preliminary findings of Wang and associates[19] using the same flow measurement technique. On the contrary, Harris and co-workers[5] reported a significant difference between men and women in the end-diastolic velocity for both the ophthalmic artery and short posterior arteries, with the female values reducing with advancing age. No differences were reported from the central retinal artery. Interestingly, their sample included post-menopausal women who had not received estrogen replacement therapy and it was speculated that this could increase vascular resistance. Kavroulaki and colleagues[6] used LDF to investigate the sub-macular choroidal flow between the sexes and reported that mean flow was highest in younger women when compared to older women, and male groups. In agreement to that finding was a study by Geyer and co-workers[47] which have observed the highest POBF values in younger women compared to elderly female, young male, and elderly male groups. Contradictory findings were reported in an Asian population study where the mean POBF value for women was significantly higher than men.[48] The different findings were speculated to be caused by a different sample population and race. In addition, that study on Asian population also did not find age influence on blood flow.

The results of this study can be interpreted in 2 ways. Firstly, the lack of any statistical relationship between retinal blood flow and age could be due to inadequate power to detect any effect. For this reason, we conducted a post-hoc power analysis. Using a power of 90% and a type I error of 0.05, the estimated sample size to determine a significant difference in total retinal blood flow was 715. However, an alternative interpreatation would be that there is no effect of

age on retinal blood flow. This is supported by the fact that the r and p values were remarkably unconvincing and also that the inspection of the plot of retinal blood flow as a function of age showed a strikingly flat regression line.

A potential criticism of our study relates to the fact that we used a cross-sectional experimental design. It would be preferable to measure retinal haemodynamics of the same individual over say 6 to 7 decades. Instead, this study cross-sectionally compared values from different individuals of different ages, in order to study any possible effect. Although correlation analysis is highly suggestive to a relationship between the selected parameters, a true temporal effect of aging may ideally be investigated longitudinally. Nonetheless, a longitudinal age study was obviously not feasible for this current study since it involves a follow-up over a number of years. Another limitation is that the age range of the sample was limited to between 20 to 70 years of age. Also, even our oldest participants were remarkably healthy, such that none of them were receiving systemic medications. In effect, this study focuses on healthy controls and is not typically representative of the general population. As a result, the study could be criticized on the basis that we were insvestigating changes in the retinal blood flow in a sample that had no health effect.

In conclusion, we found that the total retinal blood flow, as measured by a prototype Doppler SD-OCT system, is independent of age and sex. Venous area was also independent of age and sex, but blood velocity showed a weak, negative relationship with age in men.

4 Retinal blood flow in primary open angle glaucoma patients with differing nocturnal blood pressure profiles.

	Concept/Design	Recruitment	Acquisition of data	Analysis	Write-up
Yusof, F	X	X	X	X	X
Hudson, C	X			X	X
Cheng, R		X	X		
Espahbodi, N		X			
Buys, YM	X	X			X
Trope, GE	X	X			X
Flanagan, JG	X			X	X

Table detailing role of each person (X denotes significant contribution).

Purpose: To evaluate retinal blood flow (RBF) in patients with primary open angle glaucoma (POAG) and differing nocturnal blood pressure (NBP) profiles.

Methods: All participants underwent 48 hours of non-invasive ambulatory blood pressure monitoring (ABPM) and the magnitude of the NBP reduction (NBPR) was calculated using data from the second day. The participants included 17 healthy controls (mean age 61.9±6.8 years; NBPR 13.5±8.2%); 17 early POAG patients with normal NBPR (age 66.1±9.2 years; NBPR 10.9±4.7%) and 16 early POAG patients with high NBPR (age 63.5±7 years; NBPR 24.1±4.7%). RBF was measured using a prototype Doppler SD-OCT (Optovue Inc., Freemont, CA) and using the established bi-directional laser Doppler velocimetry with simultaneous vessel densitometry (BLDV-SVD) methodology in Canon Laser Blood Flowmeter (CLBF; Canon, Inc., Tokyo, Japan). Six Doppler SD-OCT scans were acquired using a circum-papillary double circular scan protocol in one eye. Six BLDV-SVD measurements of the superior temporal arteriole were taken.

Results: In general, RBF parameters were reduced in the early POAG groups, and particularly being lowest in the high NBPR group. Using the Doppler SD-OCT, the high NBPR group had the lowest flow of 29.57±8.930µL/min, followed by the normal NBPR group at 31.59±9.79µL/min, and the control group at 37.05±7.42µL/min (p=0.048). Post-hoc analysis showed a significant difference in RBF between the high NBPR group and the control group (p=0.047). The venous area was the lowest in the high NBPR group with $39.91\pm7.00(x10^{-3})mm^2$, compared to the normal NBPR group with 43.33 ± 8.66 $(x10^{-3})mm^2$ and the control group with 46.57 ± 6.60 $(x10^{-3})mm^2$ (p=0.047). Post-hoc analysis showed a significant difference in venous

area between the high NBPR group and the control group (p=0.037). The BLDV-SVD retinal blood flow parameters were not significant between groups.

Conclusions:

Patients with early POAG who exhibited an exaggerated nocturnal reduction in MAP also demonstrated lower RBF values as shown by the measurement of Doppler SD-OCT.

4.1 Introduction

A subset of treated primary open angle glaucoma (POAG) patients experience deteriorating vision despite well-controlled intra-ocular pressure (IOP), suggesting the involvement of other risk factors in the patho-physiology. The vascular theory of glaucoma advocated that optic neuropathy is a consequence of inadequate blood supply to the optic nerve head (ONH), which may be caused by either increased IOP or other causes such as vasospasm or abnormally reduced systemic blood pressure, or a combination of these factors.[1] The potential role of blood pressure (BP) in the pathogenesis of glaucoma has been a subject of interest for many investigators since it is clinically modifiable and therefore can potentially be used to modify IOP,[2] although this is still controversial. It is hypothesized that chronic hypertension will induce changes to small vessels and increase peripheral resistance, thus affecting the ONH perfusion. On the other hand, hypotension reduces perfusion pressure which may cause ischemia, oxidative stress or both. This can lead to ganglion cell death at the level of the ONH, particularly in the presence of coexisting IOP elevation or poor autoregulation.[2]

BP is known to have circadian variability with the values obtained at night, usually around 2 to 4 A.M.[3, 4] being much lower than those obtained during the day.[5, 6] It is hypothesized that an excessive systemic BP falls that occur nocturnally may result in optic nerve ischemia especially if the autoregulation of the vascular beds is compromised, as this leads to a reduced local ONH/peripapillary retina perfusion.[4, 7] The magnitude of the physiological nocturnal blood pressure reduction (NBPR) is generally a 10-20% reduction from the average daytime BP.[6, 8, 9] Two thirds of the normal population are believed to have a 10-20% NBPR.[4] A non-physiological

value has been showed to be a predictor of a compromised cardiovascular system and carries a higher risk of cardiovascular mortality.[4, 9]

The relationship of the systemic BP to the retinal perfusion pressure seems to be apparent. The ocular perfusion pressure (OPP) is the driving force for the retinal blood circulation and is defined as the difference between the systemic mean arterial pressure and the venous pressure, the value of which approximates to the IOP value. Therefore, it is important to determine whether an abnormal NBPR is an indicator for an abnormal ocular perfusion. Gherghel and co-workers[10] have investigated the relationship between the NBPR and retrobulbar blood flow using the colour Doppler imaging (CDI) method in patients with POAG. They showed that in the central retinal artery, high NBPR group exhibited a lower end-diastolic velocity (EDV), and higher resistivity index (RI) value, compared to control patients. That may suggest that the magnitude of the NBPR may be a relevant manifestation of the systemic vascular system for vascular dysregulation, at least among the POAG population. To the best of our knowledge, current literature has yet given attention to the retinal vascular bed and its association to the NBPR. Therefore, this study was sought to elucidate the relationship between the retinal blood flow and the NBPR in patients with POAG.

4.2 Methods

4.2.1 Participants

Approval for the study was granted by the Research Ethics Boards of the University Health Network, University of Toronto, and the Office of Research Ethics of the University of

Waterloo. The study abided by the Declaration of Helsinki. Written consent was obtained from all participants. Non-smoking participants with POAG and also age-matched healthy controls were recruited. POAG patients were recruited from the Toronto Western Hospital glaucoma clinic, and their diagnosis was made by a glaucoma specialist (either YMB or GET).

All participants had a corrected logMAR visual acuity (VA) of 0.3, or better, in each eye with ametropia less than ±6.00DS and ±2.50DC. All healthy controls had no immediate history of glaucoma or diabetes and all had normal visual field (VF) as assessed by program 24-2 SITA Standard VF of the Humphrey Visual Field Analyser (HFA). The HFA result was deemed valid when fixation loses, false positive and false negative responses were each less than 30%. None of the participants with POAG; had any history of other ocular pathology (other than POAG), any history of ocular surgery (with the exception of uneventful cataract surgery), history of stroke or chronic lung disease, poorly controlled hypertension, diabetes mellitus and any other endocrine diseases, ocular media opacities significant enough to hinder retinal imaging, pseudoexfoliation, pigment dispersion, closed iridocorneal angles, retinal/neuro-ophthalmological disease that could result in VF defects, and blood abnormality including hyperviscosity.

Participants refrained from consuming any caffeinated beverages and/or meals containing red meat four hours prior of the study. Several studies showed that caffeine consumption may constrict ocular blood vessels,[11-13] while red meat consumption has been reported to have a vasodilatory effect.[14] Participants with POAG were dichotomized into two groups based on the NBPR profile; normal NBPR group exhibited a NBPR<20%, and high NBPR group exhibited an NBPR≥20%. By calculation, the OPP is increased about 15mmHg when a person takes a supine

position to sleep due to hydrostatic column pressure.[9] Thus, the NBP should reduced more than 15mmHg, or more than 20% than the diurnal value to potentially cause an ischemic insult. During the Doppler measurements, the operators (FY and RC) were masked to the NBPR status of the subject.

Using the data from Wang and co-workers[26], the sample size estimation for studies involving POAG patients using prototype Doppler SD-OCT were calculated. Homeostatic blood flow for 20 healthy controls was 47.6 ±5.4µl/min and 16 glaucoma patients was 34.1±4.9µl/min. The effect size was calculated conservatively as two-third of the difference in homeostatic blood flow between controls and patients, i.e. 2/3*(47.6-34.1). The standardized effect size for blood flow = $2/3*(47.6-34.1)/((5.4^2 + 4.9^2) / 2)^{1/2} = 1.7$. Using a two-tailed $\alpha = 0.05$ and $\beta = 0.20$, the minimal required sample size is 9.

4.2.2 Ambulatory blood pressure monitoring (ABPM)

The A&D TM2430 ambulatory blood pressure recorder system (A&D Co. Ltd., Saitama, Japan) was utilized to record 48 hours of BP readings. The system is an oscillatory type sphygmomanometer. Typically, the pressure cuff was placed on the left arm, and attached to the recorder in a portable pouch which the participant wore around their waist. The measurement frequency was set at every half an hour for 48 hours. It has been suggested that sleep disturbance due to BP monitoring is minimal when the frequency of measurement is between 15 to 30 minutes.[4] All data were stored in the recorder's memory chip and then transferred for analysis. The A&D ABPM Data Analysis Software for windows (A&D Co. Ltd., Saitama, Japan) was

utilized. Parameters recorded by the monitor included diastolic blood pressure (DBP), systolic blood pressure (SBP) and pulse rate (PR). Mean arterial pressure (MAP) was calculated using the formula:[10, 15, 16]

$$\boldsymbol{MAP = DBP + \frac{1}{3}(SBP - DBP)} \qquad \textbf{Equation 4.1}$$

Non-physiological findings were excluded, i.e. (1) when the pulse pressure (SBP - DBP) was less than 10mmHg, (2) when pulse pressure was less than 10% of SBP with the SBP>100mmHg, or (3) if DBP was more than 160mmHg.[10, 17, 18] Faulty readings were detected automatically and the monitor would then initiate a new measurement to avoid missing data.

The magnitude of the NBPR was calculated from the BP readings of the second half of the 48 hours (i.e. day two). A few studies have suggested that the circadian rhythm is still adjusting on the first 24 hours of ABPM, hence artificial effects may bias the measurement during this period.[19, 20] NBPR was calculated using the formula:[21, 22]

$$\boldsymbol{NBPR = \frac{(Diurnal\ average\ MAP - Nocturnal\ average\ MAP)}{Diurnal\ average\ MAP} * 100} \qquad \textbf{Equation 4.2}$$

The ambulatory BP monitoring was done in the participants own home. With a familiar sleep arrangement, it was postulated that a better nocturnal reading was acquired. Besides, a hospital admission type of ABPM probably will not measure a true value due to controlled activities. Although the monitor is automatic and can function independently, participants were briefed on how the monitor works and were supplied with working instructions, i.e. manual deflation, cancelling measurement, missed readings, arms position, and machine location/fitting. Participants were advised to minimize movement when the measurement was actually being

acquired. An activity diary was given for participants to record any activity that may affect BP. Participants also noted the time they sleep and wake-up for a precise NBPR calculation.

4.2.3 Instrumentation

4.2.3.1 Retinal blood flow measurement using Doppler SD-OCT

Doppler imaging was performed using the Optovue RTvue OCT (Optovue Inc, Freemont, CA, USA). The optical build-up of this instrument has been discussed in detail in Chapter 1 (section 1.11.6.6) and Chapter 3 (section 3.2.2). Briefly, the infrared light source used by the OCT traverses through a beam splitter and diverged into both, eye (i.e. the sample arm) and reference mirror (i.e. reference arm). Both arms reflect the light causinginterference, and is read by a spectrometer. The interference pattern is Fourier-transformed to produce depth profile of the sample tissue (A-scan image). Different layer of the sample have a different reflectance properties, hence producing many A-scan images. The combination of these A-scans images produce a B-scan, a detailed image of the tissue. Using prototype software, the Doppler shift of the reflected light frequency was determined by the phase difference between sequential axial scans.[23]

The retinal blood flow imaging was performed using the circum-papillary double circular scan, centred on the ONH. The inner and outer concentric circular scans has radii of 3.4mm and 3.75mm, respectively.[24 -26] The concentric radii transected all retinal arterioles and venules around the ONH at two locations corresponding to where a given vessel crossed each circle. In a single blood flow scan, six dual circular frames were taken consecutively over 2 seconds and were averaged.[25]

The Doppler principle has been described in detail in Chapter 1 (section 1.11.6.1). In brief, reflected light from moving blood cells experiences a frequency shift (the Doppler shift), Δf, while the non-moving structures (such as the vessel wall) do not generate a change in frequency. Using the Doppler formula, Δf is proportional to the velocity of the moving particle; hence the moving blood velocity can be calculated if the Doppler shift is known. Blood flow can later be quantified by multiplying the calculated velocity by the vessel diameter, to produce flow value in µL/min.

4.2.3.2 Retinal haemodynamics analysis using the Semi-automated Doppler OCT of Retinal Circulation (DOCTORC) software

The blood flow scans taken by the Doppler SD-OCT were processed using the semi-automated DOCTORC software version 2.1.1.4 (Centre for Ophthalmic Optics and Lasers, CA, USA). The Doppler scans were pre-processed by the software to identify candidate vessels type, location and the Doppler signal. Deletion of vessels with extraneous influences and the addition of missing vessels were then performed manually. Vessels type was manually reviewed based on the colour fundus image. Each vessel was manually refined for its location and diameter boundary. A confidence score for each scan was assigned, by judging the Doppler signal strength, vessel boundary, size agreement between outer and inner ring, and Doppler signal sign agreement between outer and inner ring. Automatic computation was then initiated producing retinal haemodynamics values which included the total retinal blood flow (TRBF), venous cross-sectional area and the blood velocity. TRBF was calculated by summing all flow from valid venules and the estimated flow of invalid venules. Velocity was computed by dividing TRBF from all valid venules by the valid cross sectional area of all the venules.[24]

4.2.3.3 Bidirectional laser Doppler velocimetry with simultaneous vessel densitometry (BLDV-SVD)

BLDV-SVD is incorporated into the Canon laser blood flowmeter (CLBF). The CLBF utilizes the Doppler principle to measure retinal blood flow in absolute units. The instrument measures the Doppler shift produced by the fastest moving blood particles in the central part of a vessel lumen. The CLBF can only measure blood flow at a specific site of a single blood vessel at a time, hence the measurement site in this study was standardized at 1 to 2 disc diameters from the ONH along the superotemporal arteriole, on a relatively straight vessel segment distant from bifurcations.

The instrument comprises a fundus-camera plus a red laser for measuring Doppler shift and a green rectangular laser to measure vessel diameter. Light scatter by red blood cells at the illuminated site is detected simultaneously in two distinct directions separated by a known angle to detect the absolute Doppler shift, irrespective of the angle between the photodetectors and the moving red blood cells. This angle is termed as Doppler shift angle. Using this BLDV-SVD methodology, maximum frequency shift from the centreline of a blood vessel is calculated taking the difference in frequency shifts between the two photodetectors which are separated at a fixed angle (Δα). Maximum Doppler shift can be calculated using:

$$\Delta f = \delta f_2 - \delta f_1 = \frac{\Delta \alpha . v}{\lambda} \qquad \textbf{Equation 4.3}$$

The CLBF uses a red laser (675nm, 80μm x 50μm oval) to measure blood velocity every 0.02 seconds over 2 seconds measurement window. A green laser (543nm, 1500μm x 150μm rectangle) is used to measure vessel wall diameter using densitometric reflection analysis,

acquired every 4ms during the first and last 60ms of the 2s measurement window. RBF was derived from the velocity and diameter data.[27-29]

4.2.4 Procedures

Participants attended for 2 visits. During the first visit, participants were screened for eligibility. The following procedures were executed; refraction, log MAR visual acuity, automated static perimetry, Goldmann applanation tonometry, resting blood pressure, A-scan pachymetry and fundus imaging. Morphometric retinal scans were taken using the Doppler SD-OCT, with each scans repeated three times. The choice of selected eye was often driven by study recruitment criteria in the POAG groups, but was chosen randomly in the control group. Participants were set for the ABPM at the end of the first visit. After 48 hours, participants returned to the lab and the ambulatory BP readings were collected. The NBPR status for each participant was calculated last after finishing all blood flow measurement procedures.

The study eye was then dilated with tropicamide 0.5% (Mydriacyl 0.5%, Alcon Laboratories, Mississauga, Canada). Participants were then rested for 10 minutes, or longer if required, to stabilize the cardiovascular parameters. BP and heart rate were monitored using a rapid response critical care gas analyser (Cardiocap 5, Datex-Ohmeda, Helsinki, Finland). RBF measurement was undertaken using the Doppler SD-OCT and the BLDV-SVD at random order. Measurements consisted of six BLDV-SVD readings and six circum-papillary double-circular scan in the Doppler SD-OCT. IOP was reassessed after the blood flow scans.

4.2.5 Statistical analysis

All data were graphically presented showing the mean ± standard deviation. Normality of data was tested using Wilk-Shapiro test. One-way analysis of variance (ANOVA) was employed to compare retinal blood flow values between groups. The Tukey post-hoc analysis was employed to check for intra-groups significance. Statistical significance was taken at the $p<0.05$ level. All statistical analyses were employed using STATISTICA (StatSoft Inc., Tulsa, OK, USA) version 11.0.

4.3 Results

4.3.1 Demographic data

Seventeen POAG patients with normal NBPR, 16 POAG patients with high NBPR, and 18 healthy controls were recruited. In the healthy control group, one participant was excluded for the SD-OCT analysis, reducing the number of analyzed data sets to 17. A different participant was also excluded in the healthy control group for the BLDV-SVD, reducing the number of analyzed data sets to 17. Two POAG patients with normal NBPR and 1 POAG patient with high NBPR were excluded in the BLDV-SVD analysis, reducing the number to 15 in each group. The reason for exclusion was due to fatigue and inability to continue with the procedure.

Analyzing the Doppler SD-OCT participants demographic data (Table 4.1), there was a significant difference in the NBPR value between groups (ANOVA $p<0.001$) with the high NBPR group value significantly higher than the value in the normal NBPR and control groups

(Tukey HSD $p<0.001$; Figure 4.1). The mean RNFL thickness in the control group was significantly greater than both of the POAG groups but the RNFL thickness values between the POAG groups were not significantly different. The same observation was recorded for the superior and inferior RNFL thickness (Figure 4.2). There were no significant differences for age, VA, central corneal thickness (CCT), IOP, and MD between the groups ($p>0.05$). However, PSD was significantly different between groups ($p=0.038$), with the high NBPR POAG group have a borderline higher PSD than the control group (Tukey HSD $p=0.053$, Figure 4.3). In general, the visual field MD and PSD indices can be interpreted as representing an early POAG group. There were equal number of patients in each of the POAG groups who were taking topical beta-blockers for glaucoma ($p=0.784$). The number of patients using alternative, or additional, topical IOP-lowering medications were not large enough in total number per group to justify further analysis. There was no statistical difference in the number of participants who were taking systemic hypotensive medication between all groups.

The demographics for participants that completed the BLDV-SVD session showed a similar trend to Doppler SD-OCT (Table 4.2). The NBPR showed significant difference between groups (ANOVA $p<0.001$) with the high NBPR group value was significantly higher than the normal NBPR and control groups (Tukey HSD $p<0.001$). The healthy controls have the thickest RNFL and significantly different to both high NBPR and normal NBPR POAG groups, for mean RNFL, superior RNFL and inferior RNFL thickness. The PSD was significantly different between groups (ANOVA $p=0.038$), with the Tukey post-hoc test showed a near significant difference between the control and the normal NBPR group ($p=0.054$). No significant differences were noted for age, VA, CCT, IOP and MD. In general, the visual field MD and PSD indices can be interpreted as representing an early POAG group. There were equal number of patients in

each of the POAG groups who were taking beta-blockers for glaucoma (p=0.784). The number of patients using alternative, or additional, topical IOP-lowering medications were not large enough in total number per group to justify further analysis.There was no statistical difference in the number of participants who were taking systemic hypotensive medication between all groups.

Doppler SD-OCT	Controls	Normal NBPR	High NBPR	P (ANOVA)
Number	17	17	16	-
Male : Female	7 : 10	5 : 12	10 : 6	-
Age (years)	61.91±6.82	66.12±9.23	63.48±7.26	NS
VA (log)	0.03±0.07	0.06±0.08	0.03±0.07	NS
CCT (µm)	540.88±26.87	515.53±41.00	525.13±26.77	NS
IOP (mmHg)	14.76±3.23	14.68±3.07	12.88±2.60	NS
NBPR (%)	13.47±8.19	10.85±4.64	24.06±4.70%*	**<0.001**
Mean RNFL thickness (µm)	102.97±7.73 **	82.25±13.15	83.08±10.72	**<0.001**
Superior RNFL thickness (µm)	103.65±8.07**	85.14±13.77	84.47±9.84	**<0.001**
Inferior RNFL thickness (µm)	102.39±8.33**	80.34±14.20	81.68±13.83	**<0.001**
MD	-0.75±1.49	-2.82±4.59	-1.70±2.90	NS
PSD	1.64±0.72***	3.93±3.67	4.22±3.88	**0.038**
Patients taking β-blockers for glaucoma	-	12	12	**NS**
Participants taking systemic hypotension medication	2	5	1	**NS**

Table 4.1: Participants demographics and baseline data for the 3 groups, for Doppler SD-OCT. The values presented are as mean ± standard deviation. One-way ANOVA was undertaken to compare the parameters between groups, and the p-values are shown in the right hand column [VA = visual acuity, CCT = central corneal thickness, IOP = intraocular pressure, NBPR = nocturnal blood pressure reduction, RNFL = retinal nerve fiber layer, MD = mean deviation, PSD = pattern standard deviation, NS = not significant].

*Note: * denotes the group is significantly different to the healthy control group and normal NBPR group (Tukey's HSD post-hoc test). ** denotes the group is significantly different to both POAG groups. *** denotes the group is significantly different to the high NBPR group.*

BLDV-SVD	Controls	Normal NBPR	High NBPR	P (ANOVA)
Number	17	15	15	-
Male: Female	8 : 9	5 : 10	10 : 5	-
Age (years)	62.06±6.97	65.59±9.25	63.15±7.39	**NS**
VA (log)	0.04±0.08	0.05±0.08	0.02±0.03	**NS**
CCT (µm)	541.59±26.72	512.73±42.90	525.87±27.54	**NS**
IOP (mmHg)	14.35±3.62	15.17±2.93	12.80±2.68	**NS**
NBPR (%)	13.62±8.22	10.85±4.95	24.11±4.86*	**<0.001**
Mean RNFL thickness (µm)	102.61±7.61 **	82.51±14.02	82.36±10.68	**<0.001**
Superior RNFL thickness (µm)	103.39±8.02**	85.70±14.62	83.31±9.00	**<0.001**
Inferior RNFL thickness (µm)	101.95±8.17**	80.42±15.14	81.40±14.27	**<0.001**
MD	-0.74±1.49	-2.98±4.83	-1.72±3.00	**NS**
PSD	1.62±0.72***	4.25±3.81	3.97±3.88	**0.038**
Patients taking β-blockers for glaucoma	-	12	12	**NS**
Participants taking systemic hypotension medication	2	5	1	**NS**

Table 4.2: Participants demographics and baseline data for the 3 groups, for BLDV-SVD. The values presented are as mean ± standard deviation. One-way ANOVA was undertaken to compare the parameters between groups, and the p-values are shown in the right hand column [VA = visual acuity, CCT = central corneal thickness, IOP = intraocular pressure, NBPR = nocturnal blood pressure reduction, RNFL = retinal nerve fiber layer, MD = mean deviation, PSD = pattern standard deviation, NS = not significant].

*Note: * denotes the group is significantly different to the healthy control group and normal NBPR group (Tukey's HSD post-hoc test). ** denotes the group is significantly different to both POAG groups. *** denotes the group is significantly different to the normal NBPR group.*

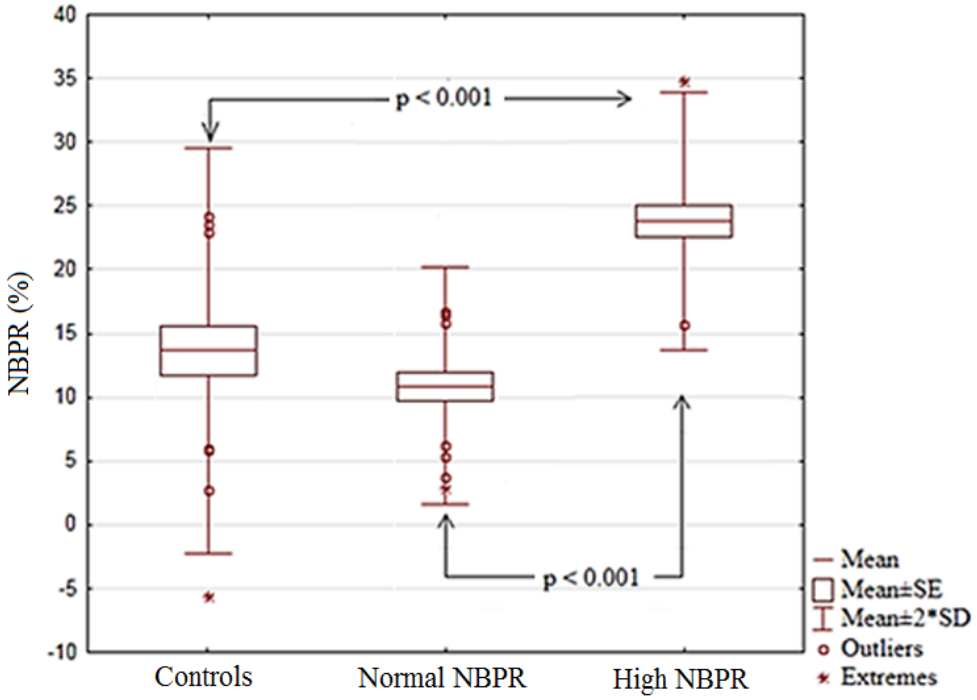

Figure 4.1: The box plot showing the group mean magnitude of the nocturnal blood pressure reduction (NBPR) for all groups. The p-values indicated significant interaction between groups, as computed by the Tukey post-hoc test. The analysis showed that both normal NBPR and control groups were different to the high NBPR group.

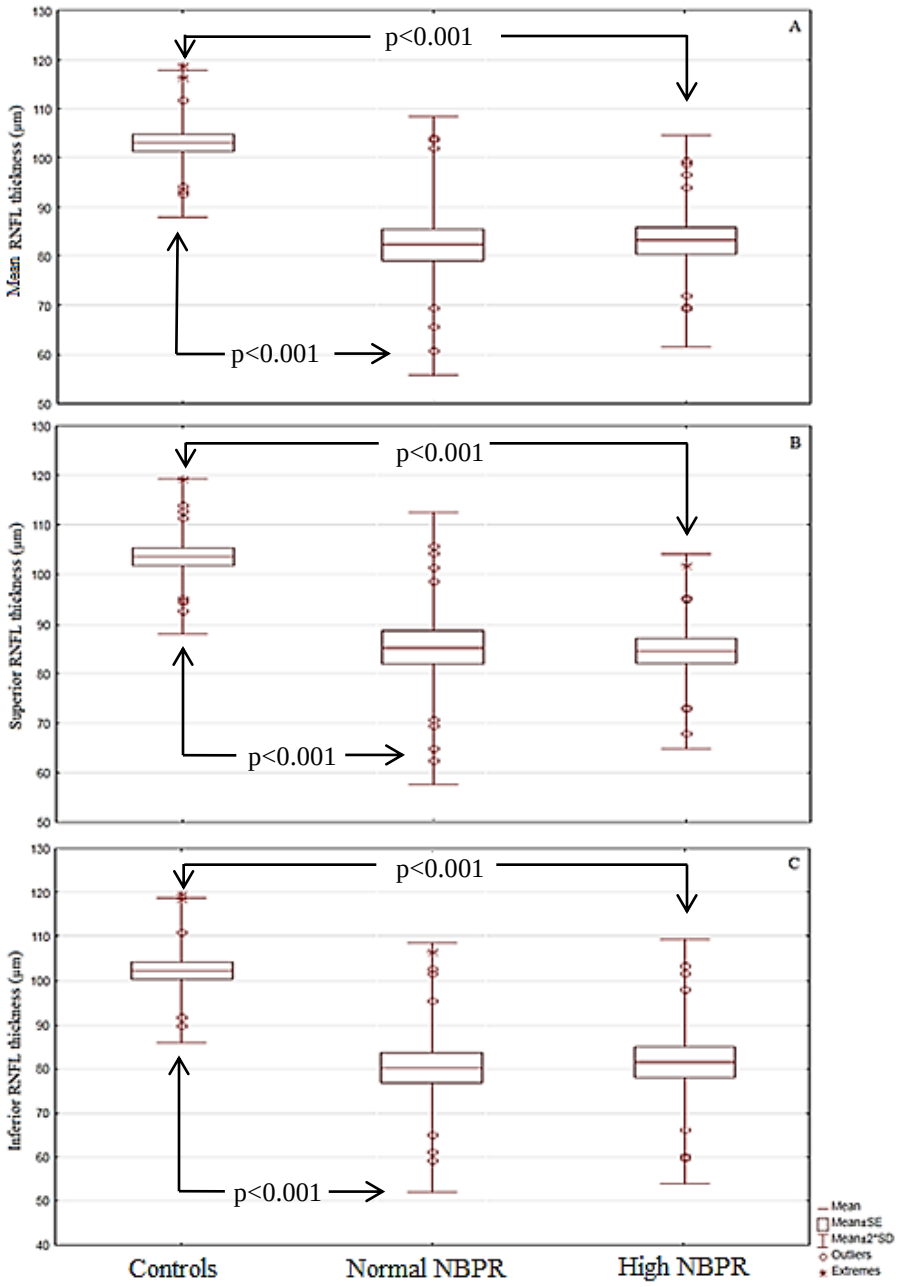

Figure 4.2: Retinal nerve fiber layer (RNFL) thickness box plots for each group; A) Mean RNFL thickness, B) Superior RNFL thickness, C) Inferior RNFL thickness. The p-values indicated significant differences between the groups (Tukey HSD test). The analysis showed that both of the POAG groups had a significantly lower RNFL thickness than the controls, but were not different from each other.

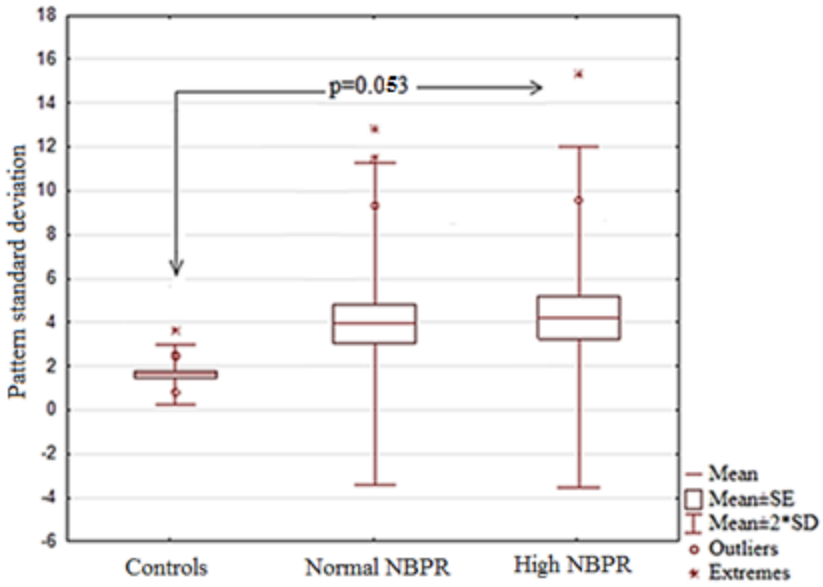

Figure 4.3: Box plot for pattern standard deviation (PSD) for each groupduring ther SD-OCT session. PSD values were recorded from the Humphrey Field Analyzer. The Tukey HSD test indicated a significantly higher PSD in the high NBPR group than in the control group.

4.3.2 Ambulatory blood pressure parameters

The blood pressure parameters are tabulated in Table 4.3. All of the diurnal parameters were not significant between the groups, except for the pulse rate (PR). The high NBPR group day PR (69.74±8.20beats/min) was significantly lower than the control's (77.84±12.00beats/min, Tukey HSD p=0.048). The mean diurnal BP values for all 3 groups were found to be in the normal clinical range. Conversely, all nocturnal parameters significantly different between groups, except for the PR. The high NBPR group exhibited a significantly lower systolic BP, diastolic BP and MAP (Figure 4.4). Although significance differences were recorded for the nocturnal ambulatory values, the BP was not different between groups during the measurement of RBF (Table 4.3).

	Controls	Normal NBPR	High NBPR	P (ANOVA)
Day SBP (mmHg)	127.34±11.29	124.87±11.38	131.95±20.22	NS
Day DBP (mmHg)	77.54±7.22	72.33±8.44	78.16±9.57	NS
Day MAP (mmHg)	94.14±8.30	89.83±8.90	96.00±12.34	NS
Day PR (beats/min)	77.84±12.00	71.06±7.62	69.74±8.20*	**0.040**
Night SBP (mmHg)	111.81±11.61	112.15±9.67	100.56±15.90**	**0.016**
Night DBP (mmHg)	65.80±7.58	64.40±6.60	58.53±7.97*	**0.016**
Night MAP (mmHg)	81.13±8.43	80.26±6.91	72.54±10.22**	**0.011**
Night PR (beats/min)	67.48±11.84	64.30±8.24	59.96±9.19	NS
SBP during Doppler SD-OCT (mmHg)	122.41±10.40	126.71±14.25	125.56±23.53	NS
DBP during Doppler SD-OCT (mmHg)	77.82±6.56	76.76±12.18	77.38±12.28	NS
PR during Doppler SD-OCT (beats/min)	66.68±8.71	66.15±10.23	63.49±6.71	NS
SBP during BLDV-SVD (mmHg)	124.12±12.50	129.36±18.33	128.87±24.64	NS
DBP during BLDV-SVD(mmHg)	79.65±8.12	77.00±12.23	78.67±11.73	NS
PR during BLDV-SVD (beats/min)	68.51±9.83	63.37±9.99	62.21±6.43	NS

Table 4.3: Blood pressure parameters for all participants presented as mean± standard deviation. One-way ANOVA was used to compare the parameters between groups [SBP = systolic blood pressure, DBP = diastolic blood pressure, MAP = mean arterial pressure, PR = pulse rate, NS = not significant].

*Note: * denotes the group is significantly different to the healthy control group (Tukey's HSD post-hoc test). ** denotes the group is significantly different to the normal NBPR group and to the control group. *** denotes the group is significantly different to the high NBPR group.*

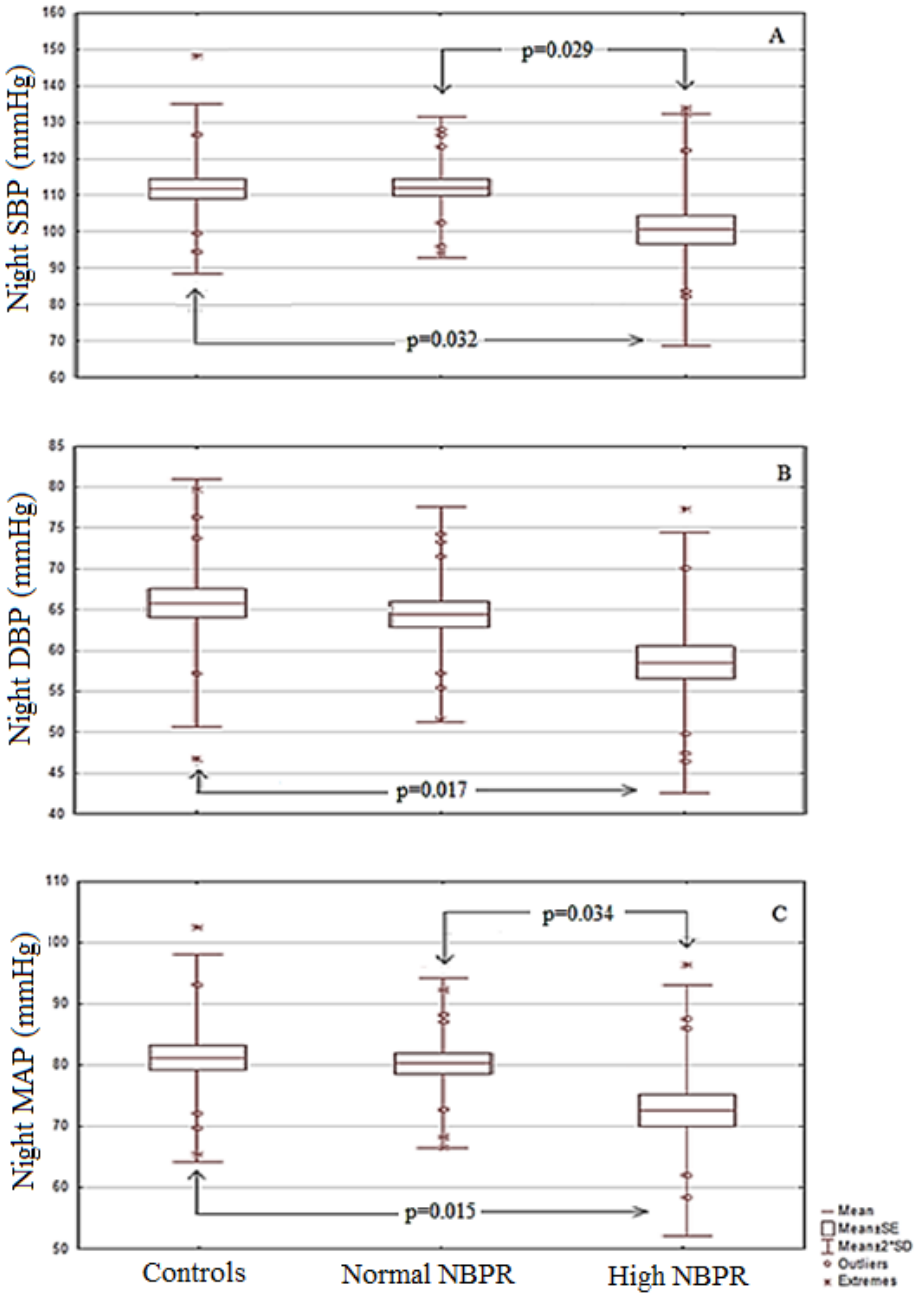

Figure 4.4: Box plot for night time blood pressure parameters for each group; A) night time systolic blood pressure (SBP), B) night time diastolic blood pressure (DBP), C) night time mean arterial blood pressure (MAP). The p-values indicate significant differences between the groups, as computed by the Tukey HSD test.

4.3.3 Doppler SD-OCT's retinal hemodynamic parameters

The retinal haemodynamics from the Doppler SD-OCT were compared between groups. The TRBF in the high NBPR group was found to be the lowest at 29.57±8.93µl/min, compared to 31.59±9.79µl/min in the normal NBPR group, and 37.05±7.42µl/min in the control group (p=0.048; Figure 4.5). The Tukey HSD test indicated that the high NBPR group had a significantly lower RBF than the controls (p=0.047). There were no significant differences for superior RBF, inferior RBF, and blood velocity between the groups. The venous area showed significant difference between groups (p=0.047). The Tukey HSD test indicated that high NBPR group have lower blood velocity than the controls (p=0.037; Figure 4.6). A summary is tabulated in Table 4.4.

	Controls	Normal NBPR	High NBPR	P (ANOVA)
Total Retinal Blood Flow (µl/min)	37.05±7.42	31.59±9.79	29.57±8.93*	**0.048**
Superior Retinal Blood Flow (µl/min)	18.45±4.78	16.81±6.18	14.24±5.72	0.105
Inferior Retinal Blood Flow (µl/min)	18.59±4.82	14.78±6.37	15.33±6.03	0.127
Venous area (mm^2)	46.57±6.60 ($x10^{-3}$)	43.33±8.66 (x10-3)	39.91±7.00 (x10-3)*	**0.047**
Velocity (mm/s)	13.23±2.21	12.05±2.16	12.19±2.54	0.274

Table 4.4 : The retinal haemodynamics as measured by the Doppler SD-OCT. Values presented by their means ± standard deviation. One-way ANOVA was done to compare the parameters between groups and the p-values are stated on the right hand column.

*Note: * denotes the group is significantly different to the healthy control group (Tukey's HSD test).*

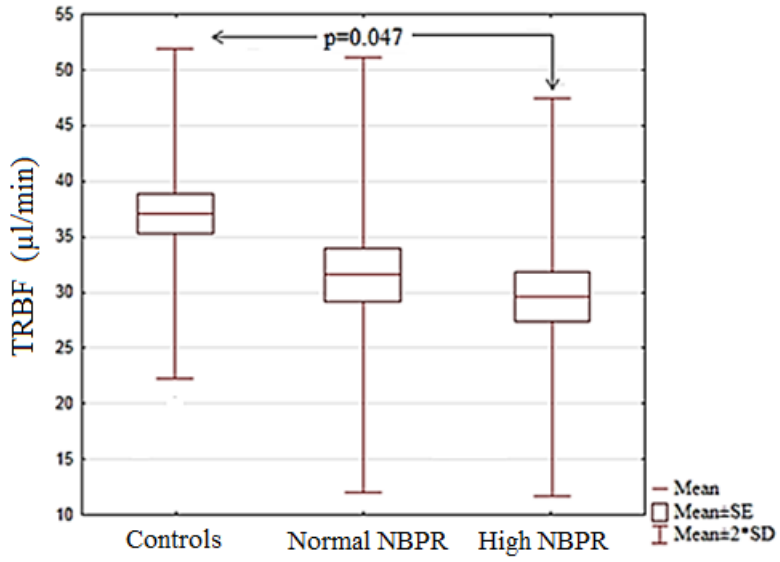

Figure 4.5 : Box plot for the total retinal blood flow (TRBF) for each group, as derived by the Doppler SD-OCT. The Tukey HSD test indicated a significant difference between the high NBPR group and the control group.

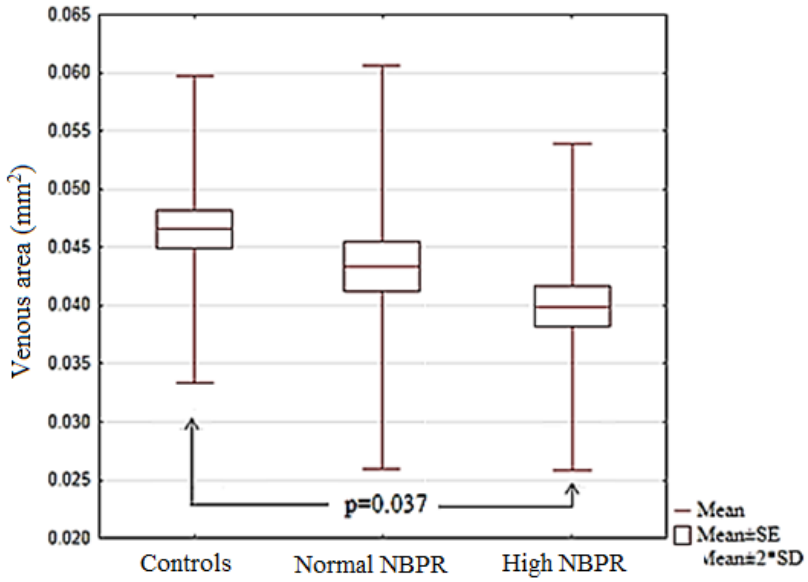

Figure 4.6 : Box plot for the venous area of each group, as derived by the Doppler SD-OCT. The Tukey HSD test indicated a significant difference between the high NBPR group and the control group.

4.3.4 BLDV-SVD's retinal hemodynamic parameters

The retinal blood flow parameters from the BLDV-SVD were compared between groups. Group mean values for all parameters were found to be generally lower among POAG patients in comparison to the control group, although the differences were not significant. In addition, most RBF values were lowest in the high NBPR group, although statistically insignificant. Table 4.5 summarizes the findings.

	Controls	**Normal NBPR**	**High NBPR**	**P (ANOVA)**
Diameter (µm)	107.84±16.11	103.35±15.29	101.00±13.26	0.604
Velocity (mm/s)	34.10±7.88	29.25±8.17	30.60±6.13	0.175
Flow (µL/min)	9.38±4.12	7.77±3.42	7.57±2.67	0.281

Table 4.5 : Retinal blood flow parameters measured using BLDV-SVD as a function of group. Values presented are group means ± standard deviation. One-way ANOVA was done to compare the parameters between groups, and the p-values are stated in the right hand column.

4.4 Discussion

There is little consensus regarding any possible relationship between NBPR and glaucoma. Some studies have found glaucoma to be associated with an exaggerated NBPR,[4, 16, 17, 30] while some have reported it to be associated with a lower NBPR[18, 21]. There are also studies which relate glaucoma to patients with both low and high NBP profiles,[3, 22] while several studies did not find any association with NBPR[31, 32]. It is interesting to note that even though the studies of Plange and co-workers[31] and of Riccadonna and co-workers[32] did not find any definitive association between glaucoma and NBP status, they reported that glaucoma patients experience a higher variability in NBP parameters, proposing that fluctuation in the perfusion pressure in POAG may be pathogenic. The inconsistency of the results between studies may be explained by: (1) variation in the definition, or the calculation of an NBPR, (2) the different frequencies and durations in the ABPM measurements, (3) the location of BP measurements, i.e. home versus hospital admission, and (4) the different characteristics of glaucoma being recruited.

The consequence of an excessive BP fall at night is a loss of perfusion to some of the organs and tissues in the body. Some authors have suggested that the greater NBP fall, may represent a form of vascular dysregulation.[10] In a compromised vascular bed, excessive systemic hypotension may initiate ischemic tissue damage. Many findings in the past have indicated that glaucoma patients with extreme NBPR may have a less favourable prognosis.[22, 30, 33-36] Grunwald and co-workers[37] assessed the ONH blood flow in glaucoma patients with and without systemic hypertension. It was found that patients without hypertension had a 26% reduction in the ONH flow, suggesting a higher BP provides a better perfusion to the eye. Krasinska and colleagues[33] showed that medication induced nocturnal hypotension lowers retrobulbar velocity. Their prospective study

was conducted on glaucoma patients at 6 months interval. These findings may implicate a negative effect of hypotension, and perhaps a benefit of hypertension, on ocular and particularly ONH perfusion.

The relationship of the NBPR and ONH/peri-papillary retinal perfusion is uncertain. To the best of our knowledge, no study has ever been conducted to investigate the relationship between NBPR and ONH/peri-papillary retinal perfusion. A few groups have reported a linkage between NBPR to the ocular perfusion among glaucoma patients but all used the color Doppler imaging (CDI) technology that measure haemodynamics in the retrobulbar area.[10, 30] The CDI is an ultrasound technique which measures the velocity profile of the retrobulbar arteries, usually the ophthalmic or the central retinal artery. A study by Pache and co-workers,[38] used nail-fold capillaroscopy to investigate the relationship of peripheral blood flow and the NBPR but this study also measured blood velocity rather than blood flow. In this current study, we used technologies that quantified retinal blood flow in absolute units, by measuring velocity and vessel diameter.

The response of blood pressure to nocturnal factors is complex, since activity levels are reduced and posture is horizontal rather than vertical. The immediate response of the retinal vasculature to adoption of a supine position is vasoconstriction in order to protect the terminal capillaries from the sudden rise in OPP which occurs due to elevation of the heart relative to the eye, i.e. hydrostatic column effect. However with longer adoption of supine position, vagus nerve responses are initiated which lower the blood pressure and as a result the retinal vessels subsequently dilate. This study essentially measures all aspects of these changes and takes into account the maximum and the minimum of the blood pressure over the 24-hour recording period,

irrespective of causality. In addition, the conditions experienced across the groups were equivalent, and therefore the results of valid irrespective of the role of posture upon NBPR.

An overall look at the prototype Doppler SD-OCT results suggested that early POAG patients with a high NBPR have an impaired retinal blood flow in comparison to healthy controls. Eventhough comparison to the normal NBPR POAG group yields no statistical significance, the values in the high NBPR group were usually lower. Our findings confirmed that the high NBPR group has a lower blood flow than the controls. At this time, we are unaware of any other studies that measure retinal perfusion among early POAG with different NBP characteristics. The previous haemodynamic studies pertaining to the NBPR only measured blood velocity, rather than flow, by means of the CDI technique. Nevertheless, flow is presumably reduced in a high NBPR group. Ramli and co-workers[39] demonstrated that their glaucoma patients have lower perfusion pressure (OPP) compared to healthy controls. They measured the diurnal and nocturnal OPP on 72 normal tension glaucoma and 55 healthy controls. The OPP for their glaucoma group was 55.5mmHg and 49.7mmHg for day and night, respectively, lower than the healthy groups which exhibited OPP values of 56.7mmHg at daytime and 53.2mmHg at night ($p=0.005$). Joe and co-workers[16] examined normal tension glaucoma (NTG) patients of Asian descent and showed that large reductions in NBP were correlated with the structural parameters that they measured using scanning laser polarimetry. Their linear regression analysis revealed that the high NBPR patients were associated with decreasing ONH anatomical score (i.e. TSNIT score), proposing that high NBPR patients have a thinner RNFL. A few studies have reported relationship between reduced haemodynamics and RNFL loss.[40-44] Taking the link between haemodynamics and RNFL thickness, together with the link between RNFL thickness and NBPR, it may be appropriate to deduce that POAG patients with excessive NBPR have reduced

blood flow. Our findings using Doppler SD-OCT confirms this. RBF values derived using BLDV-SVD method did not show a significant difference between groups, although the high NBPR group generally exhibited lower RBF parameters. A related study from our group also found no difference between baseline hemodynamic (homeostatic) parameters acquired using the BLDV-SVD in untreated POAG, progressive POAG and healthy control groups.[45] However, vascular reactivity was found to be significantly reduced in the POAG groups using a hypercapnic provocation. Whether a reduced vascular reactivity occurs in POAG patients with different NBPR profiles needs to be further investigated (see Chapter 6).

Any association of the NBPR and blood velocity is unclear. Gherghel and associates[10] reported reduced end diastolic velocity (EDV) and increased resistive index (RI) in the central retinal artery among glaucoma patients with high NBPR. They divided their glaucoma patients into three groups of NBPR<10%, NBPR 10-20% and NBPR>20% based on 24-hour ABPM measurement. Nevertheless, they did not find a significant difference in ophthalmic artery haemodynamic parameters between the groups. Krasinska and co-workers[30, 33] also published a series of reports utilizing CDI and observed a similar finding to those by Gherghel and co-workers.[10] In an earlier study, Krasinska and co-workers[30] found that POAG with the highest NBPR was associated to the lowest peak systolic velocity (PSV) and EDV in both the ophthalmic and central retinal arteries. Both arteries also showed a significantly higher RI in the high NBPR POAG group. Of note, they also observed greater visual field defect in the same group. Their subsequent study,[33] a prospective designed study, showed that increase reduction of the NBPR, by means of taking antihypertensive medication before sleeping resulted a significant reduction of PSV and EDV in the short posterior ciliary arteries relative to baseline 6 months earlier. These studies suggest that blood velocity is negatively correlated to the NBPR in

glaucomatous eyes. Conversely, Pache and co-workers[38] reported no differences in the nail-fold velocity across glaucoma groups with different NBPR characteristics. However, it is important to highlight that Pache and co-workers measured the blood velocity in the finger's vessels using a nail-fold capillaroscopy technique. A recent study by Karadag and colleagues[46] on healthy eyes using the CDI did not find a significant difference between normal NBPR (>10%) and low NBPR (<10%) subjects. Their result is somehow expected as only healthy eyes was considered, where autoregulation should still be intact thus maintaining stable perfusion. In the current study, we did not find any significant difference for the retinal blood velocity between the 3 groups, using both Doppler SD-OCT and BLDV-SVD. The different outcome may be due to the assessment of different vascular beds.

The venous area measured by the Doppler-SD OCT is a summed value of all detectable venules transected by the double circular scan pattern. Our study found that the high NBPR group had a significantly smaller venous area than the controls, but not significantly different to the normal NBPR POAG group. Using Doppler SD-OCT, Wang and co-workers[26] compared retinal haemodynamics of glaucoma patients to those of controls, plus patients with non-arteritic ischemic optic neuropathy (NAION) and with proliferative diabetic retinopathy (PDR). They did not observe any significant difference in the venous area in glaucoma and the controls, but positively larger venous area to those in the NAION and PDR group. The different observation in our findings may be caused by different selection criteria of glaucoma patients. Nevertheless, many studies investigating vessel's caliber size and its association with glaucoma indicated that narrower vessels are consistent with glaucoma.[47-50]

Our non-significant findings in the retinal blood flow assessed using the BLDV-SVD method may be due to the anatomical variability of the retina among participants. Despite our best effort to standardize the measurement site on a superotemporal retinal arteriole, different retinal vessel size may have reduced the signal-to-noise ratio. A previous study from our group did not find any difference in homeostatic RBF between glaucomatous and healthy groups using BLDV-SVD.[45] They reported significant changes only for the vascular reactivity experiment which used a hypercapnic provocation. The BLDV-SVD methodology detected significant change within the group whereas the SD-OCT detected differences between the groups. This implies that the methodology measuring the total RBF offers an improved signal-to-noise-ratio, evident from our significant findings from the Doppler SD-OCT. Doppler SD-OCT covers a much larger measurement area than the BLDV-SVD, enables a more comprehensive measurement. We suggest that the difference found between the two techniques, that is Doppler SD-OCT and BLDV-SVD, was most probably because the NBPR profiles is a global vascular dysregulatory indicator that generates a stronger signal-to-noise ratio as a result of a diffuse change of perfusion. A localized measurement, in BLDV-SVD case, may not be sensitive enough to differentiate a significant effect between the various NBPR groups. It has been advocated that vascular dysregulation may be transient in presentation.[7] Inhomogeneity of flow may exist in an ischemic retinal tissue, such as in glaucomatous eye, causing preferential perfusion.[10] However the difference in findings between the two techniques may be explained by possibility that the BLDV-SVD methodology was underpowered relative to SD-OCT Doppler. In this study, a post-hoc power analysis demonstrated that the sample of 18 POAG patients would yield a power of 80% for the detection of a difference in TRBF between the groups, while 39 subjects would be required to gain the same power using BLDV-SVD. In addition, longitudinal studies are required

to determine if reduced perfusion associated with exaggerated NBPR is a cause of POAG or an effect.

The ambulatory pressure parameters showed a striking difference between the groups, particularly during sleep. The high NBPR group in this study had the lowest value for all the nocturnal BP parameters and the highest diurnal BP parameters which resulted in a prominent NBP fall. This same trend was observed by Gherghel and co-workers[10], where they reported an average day to night MAP of 100:73mmHg for high NBPR patients (versus ours of 96:73mmHg). In their study, the diurnal value was not significantly different between groups, but the nocturnal parameters were significantly different, also in agreement with the findings of our study. Of note, Joe and co-workers[16] and Krasińska and co-workers[30] also recorded the same trend. The trend suggests that high NBPR patients have normal-like BP values if clinicians only consider the daytime readings. The reduced BP during sleep suggests a deficiency of systemic autoregulation in the cardiovascular system, and this may in turn reduce local perfusion. In our study, only the diurnal PR was found to be significantly different. A reduction of PR during the night is expected because the heart is responding to lack of physical activity. It is postulated that the reduction in the sympathetic activity causes the reduction in pulse, cardiac output, and peripheral vascular resistance.[51]

The ambulatory BP recordings in this study were acquired for 48 hours, instead of 24 hours as measured by the majority of ABPM studies.[3, 4, 10, 16-18, 30-32, 38, 52] A longer recording duration was selected because it has been reported that the circadian rhythm only stabilized in the 25th hour of ABPM recording.[19, 20] In consequence, our NBPR calculation using the last 24-hour recordings (from the two days interval) was expected to present a valid estimate of the NBPR value. We had

also conducted the ABPM measurements in the patients home instead of in a hospital settings with sleep lab facilities, as in most other studies.[3, 10, 16, 18, 30, 31, 38, 38, 52-54] Such a controlled setting of a hospital or laboratory may produce aberrant BP measurements.[55] Kashiwagi and colleagues[21] debated that daily data obtained in a hospital setting would probably reduce the diurnal BP because the patient was less active than when at home. Sleeping in an unfamiliar surrounding may also disturb sleep quality which may result in an abnormal low NBPR profile. Pikilidou and co-workers[55] studied the effect of hospitalization on the 24 hour ABP measurement in hypertensive adults with POAG. They reported that the daytime SBP during hospitalization was lower compared to those done at home. They observed a larger SBP reduction in the out-patients of 11%, compared to an 8% reduction during hospitalization ($p=0.02$). The inpatient setting probably produces a false diurnal reading, which may lead to underestimation of the BP and over diagnosis of low NBPR.

In this study, the NBPR was calculated based on the patient real sleeping time. An activity diary was supplied for each participant and they were asked to note their sleep time(s). We postulated that an activity diary provided a means to improve the validity of the NBPR. The majority of previous studies simplified the NBPR calculation by defining day time and night time periods. We assumed that sleep time may differ individually, hence the NBPR calculation in our study was based on true sleep time.

To conclude, early POAG patients with a high NBPR have a reduced venous flow and venous lumen area which may indicate impaired RBF. This may occur secondary to a systemic autoregulatory dysfunction of the cardiovascular system, exhibiting as abnormal circadian BP regulation. It may be a form of vascular dysregulation that affects the ocular circulation, at least

in glaucomatous eyes. Our retinal blood flow measurements were acquired outside the period of NBPR episode. Whether or not the same reduction of retinal perfusion occurs during the NBPR period needs to be further investigated.

5 The Relationship between Retinal Nerve Fiber Layer Thickness and Retinal Haemodynamics in Primary Open Angle Glaucoma

	Concept/Design	**Recruitment**	**Acquisition of data**	**Analysis**	**Write-up**
Yusof, F	X	X	X	X	X
Hudson, C	X			X	X
Tayyari. F	X	X	X		
Cheng, R		X	X		
Buys. YM	X	X			X
Trope, GE	X	X			X
Flanagan, JG	X			X	X

Table detailing role of each person (X denotes significant contribution).

Purpose: To investigate the relationship between Doppler SD-OCT derived retinal haemodynamics and the retinal nerve fiber layer (RNFL) thickness in patients with primary open angle glaucoma (POAG) and healthy age-matched controls.

Method: Thirty three POAG patients (age, 64.84±8.31 years; Humphrey Field Analyzer, HFA, mean deviation, MD, -2.28±3.85) and 33 healthy controls (age, 62.86±5.32 years; HFA MD -0.48±1.56) were recruited. The Doppler SD-OCT retinal blood flow measurement was taken using the prototype circum-papillary double circular scan protocol in the RTVue system (Optovue Inc., Freemont, CA, USA). A minimum of six retinal blood flow (RBF) scans were acquired. Peri-papillary RNFL thickness was measured using RTVue's RNFL scan protocol.

Results: The total RBF in the POAG group was significantly lower (30.61±9.29µL/min) than the control group (40.68±11.32µL/min; $p<0.001$). Superior and inferior RBF were also significantly lower in the POAG group (superior RBF $p=0.001$, inferior RBF $p=0.004$). The mean, superior, and inferior RNFL thickness were significantly lower in the POAG group ($p<0.001$). Pearson correlation analysis showed a significant positive relationship between the total RBF and mean RNFL thickness ($r=0.382$, $p=0.028$), between the superior RBF and superior RNFL thickness ($r=0.37$, $p=0.032$), between the mean RNFL thickness and venous area ($r=0.35$, $p=0.050$), and between the RNFL thickness and MD ($r=0.47$ to 0.7, $p\leq0.006$). In the control group, only the mean RNFL thickness showed a relationship with venous area ($r=0.58$, $p<0.001$).

Conclusion: The change in retinal circulation in POAG is related to reduced mean RNFL. Larger venous area is associated with thicker RNFL among controls.

5.1 Introduction

Glaucomatous optic neuropathy (GON) causes death to ganglion cells and leads to thinning of the retinal nerve fiber layer (RNFL), clinically apparent in the optic nerve head (ONH) as excavation of the cup. Study evidence has shown a strong relationship between the reductions of neuroretinal rim structure with deterioration of function, measured by the mean deviation (MD) index of the standard automated perimetry (SAP).[1-4]

Morphological changes in glaucomatous eyes were shown to be accompanied with capillary drop-out. Gottanka and associates[5] autopsied POAG eyes and concluded that the capillary count and capillary density was reduced in the region of ONH retro laminar area, relative to healthy eyes. In addition, various imaging studies suggested vascular narrowing in eyes with POAG.[6-8] It is however unsure whether capillary drop-out and vascular alterations in glaucoma are a primary cause, or a secondary phenomenon to the disease. Regardless, the alteration in vascular structure in POAG eyes may have an impact to vascular haemodynamics.

In the previous chapter, we demonstrated a significant reduction in venous flow and venous velocity in POAG patients (Chapter 4, section 4.3.3). We have also presented that the RNFL thickness in the POAG group was significantly thinner than the controls (Chapter 4, section 4.3.1). The relationship between retinal haemodynamics and the structural glaucomatous damage is still not clear. This study thus, was conducted to investigate the relationship between retinal haemodynamics and RNFL thickness in patients with POAG. It is hypothesized that the haemodynamics impairment is related to the RNFL loss.

5.2 Methods

5.2.1 Participants

The Research Ethics Boards of the University Health Network, University of Toronto, and the Office of Research Ethics of the University of Waterloo approved the study. The study follows the Declaration of Helsinki and written consent was obtained prior to data collection. Two groups were recruited comprising a POAG group and a group of age matched healthy controls. All healthy participants did not have any immediate history of glaucoma or diabetes, and had normal visual field (VF). All participants had a corrected log MAR visual acuity (VA) of 0.3 or better in each eye with ametropia less than ±6.00DS and ±2.50DC. Program 24-2 SITA Standard of the Humphrey Visual Field Analyzer (HFA; Carl Zeiss Meditec, Dublin, CA) was undertaken, and the field result was deemed valid when fixation loses, false positive and false negative responses were less than 30%. None of the participants with POAG; had any history of other ocular pathology, history of ocular surgery (exception to uneventful cataract surgery), history of stroke or chronic lung disease, poorly controlled hypertension, diabetes mellitus and other endocrine diseases, ocular media opacities significant enough to hinder retinal imaging, pseudoexfoliation, pigment dispersion, closed iridocorneal angles, retinal/neuro-ophthalmological disease that could result in VF defects, and blood abnormality including hyperviscosity. All participants were refrained from taking any caffeinated beverages and/or meals containing red meat four hours prior of the study to prevent bias in blood flow reading.

Using the data from Wang and co-workers[12], the sample size estimation for studies involving POAG patients using prototype Doppler SD-OCT were calculated. Homeostatic blood flow for

20 healthy controls was 47.6 ±5.4µL/min and 16 glaucoma patients was 34.1±4.9µL/min. The effect size was calculated conservatively as two-third of the difference in homeostatic blood flow between controls and patients, i.e. 2/3*(47.6-34.1). The standardized effect size for blood flow = $2/3*(47.6-34.1)/((5.4^2 + 4.9^2) / 2)^{1/2}$ =1.7. Using a two-tailed $\alpha = 0.05$ and $\beta = 0.20$, the minimal required sample size is 9.

5.2.2 Retinal blood flow measurement using Doppler SD-OCT

The Optovue RTvue OCT (Optovue Inc, Freemont, CA, USA) was utilized for the Doppler imaging. The optical principles of this instrument have been discussed in Chapter 1 (section 1.11.6.6), Chapter 3 (section 3.2.2) and Chapter 4 (section 4.2.3.1) of this thesis. Blood flow measurement was performed using the circum-papillary double circular blood flow scan, centered on the ONH. Doppler images were analyzed using DOCTORC software to give haemodynamic parameters that include total retinal blood flow (TRBF), venous area, and venous velocity.[9]

5.2.3 Para-papillary RNFL thickness measurement

Para-papillary RNFL thickness measurement was acquired using SD-OCT (RT-Vue, Optovue Inc., Freemont, CA) using the RNFL scan mode. RNFL scan mode measures nerve fiber layer thickness along a circular diameter of 3.45mm around the ONH centre. The OCT completes 4 circular scans in 0.16s and values are averaged and presented within the normative range. Three scan repetitions were obtained, and values from each scan were added and averaged. Only images with signal strength >40 were used in the analysis. The para-papillary RNFL sectors considered in this study were global (mean), superior quadrant, and inferior quadrant thickness.

The SD-OCT segmentation algorithm was manually checked and at least the best 3 scans were taken into the analysis.

5.2.4 Procedures

All participants underwent a standard assessment including refraction, log MAR VA, program 24-2 SITA-standard HFA, Goldmann tonometry, resting blood pressure measurement, and fundus imaging. The study eye was determined in the POAG group based upon the study criteria, but was chosen randomly in the control group. Selected eye was dilated using 0.5% tropicamide (Alcon, Mississauga, Canada). The participants were seated and rested for at least 10 minutes to stabilize baseline cardiovascular parameters. The participant's head was stabilized on the OCT's chin rest. Participant was then instructed to look at the internal fixation target. A real-time video display of the fundus was used to aid Doppler imaging. An average of 6 scans were taken at two different beam alignment; 3 scans were made with the beam passing through the superonasal pupil edge, and another 3 were made through inferonasal pupil edge. Three scans of the para-papillary RNFL thickness measurement were then executed using RNFL scan mode. Color fundus image and high definition 3D disc scan image were also taken to aid blood flow grading.

5.2.5 Statistical analysis

All data were presented in mean ± standard deviation. Normality of data was tested using Wilk-Shapiro test. One-way analysis of variance (ANOVA) was employed to compare demographic values between groups, RNFL thickness and retinal haemodynamics. Pearson product-moment correlation analysis was used to study correlation between RNFL thickness and haemodynamics, and between RNFL thickness and VF indices. Upon comparing haemodynamic parameters to

MD, the values were converted to decibel (dB) scale by 10 x $\log_{10}$ [value/(average value of the normal group)]. The significance level (p) was taken at <0.05. All statistical analyses were employed using STATISTICA (StatSoft Inc., Tulsa, OK, USA) version 11.0.

5.3 Results

5.3.1 Demographic data

The demographic of participants are shown in Table 5.1. Thirty three participants in each group were recruited with 13 males in the control and 15 males in the POAG group. No significant difference for age (p=0.253), VA (p=0.190), intraocular pressure (IOP; p=0.281), systolic blood pressure (SBP; p=0.912), diastolic blood pressure (DBP; p=0.876), and mean ocular perfusion pressure (MOPP; p=0.704) were noted between groups. The controls had thicker cornea (539.48±33.23µm) compared to POAG (520.18±34.65µm; p=0.024). The POAG group showed worse MD (p=0.016) and pattern standard deviation (PSD; p<0.001) compared to the controls (Figure 5.1). In general, the visual field MD and PSD indices can be interpreted as representing an early POAG group.

In the control group, 2 participants were taking systemic hypertensive-lowering medications, while 6 patients were on the same medications. The number of patients in the POAG group who were taking beta-blockers for glaucoma was 12.

	Controls	POAG	p (ANOVA)
Number	33	33	-
Male : Female	13 : 20	15 : 18	-
Age (Years)	62.86±5.32	64.84±8.31	0.253
VA (log MAR)	0.03±0.07	0.05±0.08	0.190
IOP (mmHg)	14.54±2.50	13.80±2.95	0.281
SBP	125.72±11.91	126.15±19.01	0.912
DBP	77.44±6.74	77.06±12.04	0.876
MOPP	47.81±5.05	48.48±8.77	0.704
CCT (µm)	539.48±33.23	520.18±34.65	0.024
MD (dB)	-0.52±1.44	-2.28±3.85	0.016
PSD (dB)	1.80±0.68	4.07±3.72	<0.001
Patients taking β-blockers for glaucoma	-	12	-
Participants taking systemic hypotension medication	2	6	-

Table 5.1: Participants' demographic values presented as mean ± standard deviation. One-way ANOVA was used to compare parameters between groups.

[VA = visual acuity, IOP = intraocular pressure, SBP = systolic blood pressure, DBP = diastolic blood pressure, MOPP = mean ocular perfusion pressure, CCT = central corneal thickness, MD = mean deviation, PSD = pattern standard deviation]

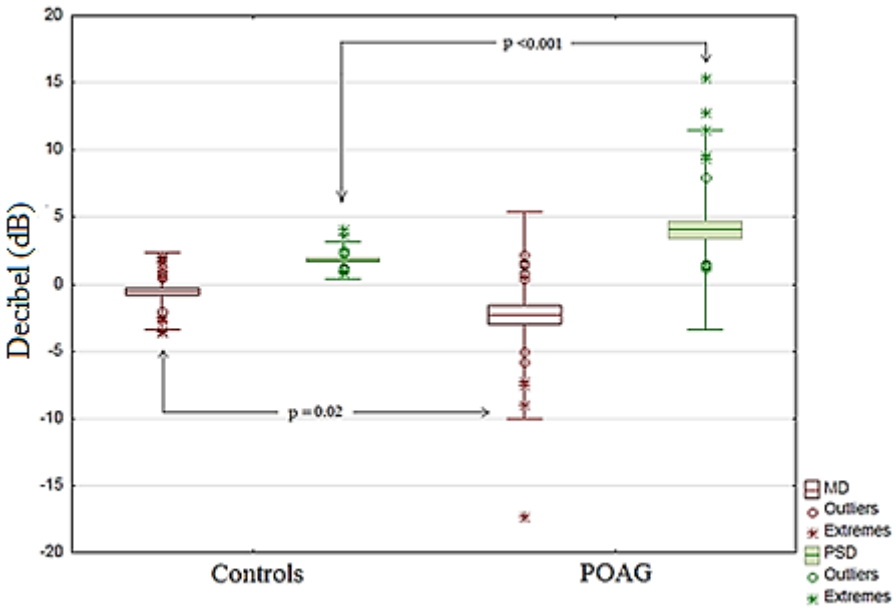

Figure 5.1: Box plot of mean deviation (MD) and pattern standard deviation (PSD) of each group measured using Humphrey Visual Field Analyzer. One-way ANOVA showed that the POAG group has worse visual field indices compared to the controls.

5.3.2 RNFL thickness profile

Table 5.2 shows the RNFL profiles for both groups. The mean, superior and inferior RNFL thickness in the POAG group are significantly thinner than the control group ($p<0.001$, Figure 5.2).

RNFL (μm)	Controls	POAG	p (ANOVA)
Mean thickness	100.04±9.58	82.65±11.85	<0.001
Superior quadrant	100.36±10.36	84.81±11.84	<0.001
Inferior quadrant	99.65±9.95	80.99±13.82	<0.001

Table 5.2: The retinal nerve fiber layer (RNFL) thickness values presented as mean ± standard deviation. One-way ANOVA was used to compare parameters between groups. [RNFL = retinal nerve fiber layer, POAG = primary open angle glaucoma]

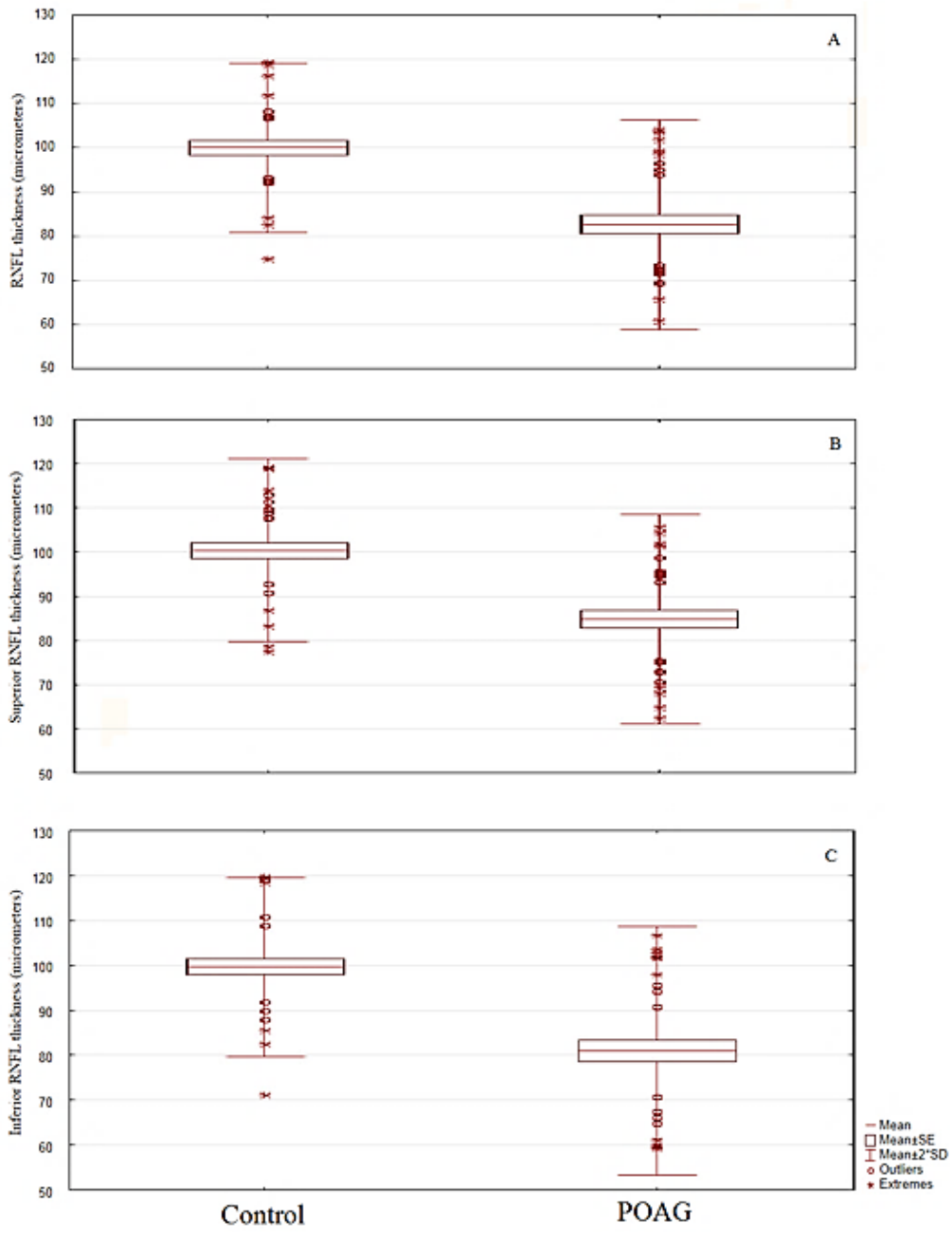

Figure 5.2: Box plot for the retinal nerve fibre layer (RNFL) thickness profiles for the control group and the primary open angle glaucoma (POAG) group; A) mean RNFL thickness, B) superior RNFL thickness, and C) inferior RNFL thickness. One-way ANOVA revealed that the POAG group has thinner RNFL for all RNFL profiles ($p<0.001$).

5.3.3 Retinal Haemodynamics

Table 5.3 shows the retinal haemodynamics for the control and POAG groups. In general, the POAG group showed significantly lower values compared to the healthy control group. TRBF was 30.61±9.29µL/min in POAG, compared to 40.68±11.32µL/min in controls ($p<0.001$, Figure 5.3A). The superior RBF and inferior RBF were also significantly lower in POAG ($p=0.001$, and $p=0.004$ respectively, Figure 5.3B and Figure 5.3C). The venous area in the control group was larger by approximately 14% than the POAG group ($p=0.003$, Figure 5.4). The venous velocity was also reduced in the POAG group with values lower by approximately -18% than those in the control group ($p=0.003$, Figure 5.5).

Retinal haemodynamics	Controls	POAG	p (ANOVA)
TRBF (µL/min)	40.68±11.32	30.61±9.29	<0.001
Superior RBF (µL/min)	20.38±5.30	15.56±6.01	0.001
Inferior RBF (µL/min)	20.30±8.17	15.05±6.12	0.004
Venous area (mm^2)	47.31±7.06 ($x10^{-3}$)	41.67±7.97 ($x10^{-3}$)	0.003
Venous velocity (mm/s)	14.28±3.19	12.12±2.31	0.003

Table 5.3: Retinal haemodynamics values presented as mean ± standard deviation. One-way ANOVA was used to compare parameters between groups. [TRBF = total retinal blood flow, RBF = retinal blood flow, POAG = primary open angle glaucoma]

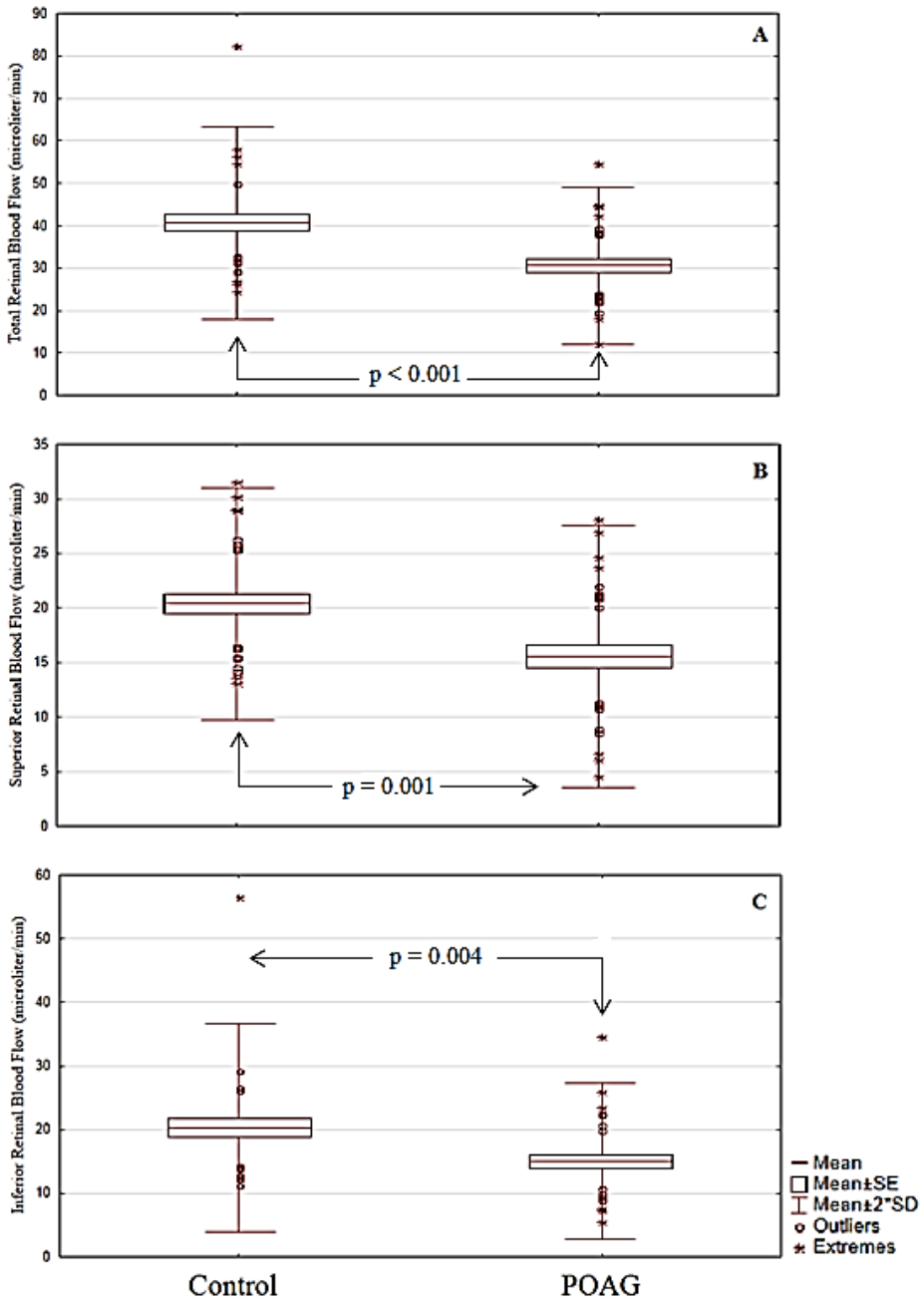

Figure 5.3: Box plot for retinal blood flow (RBF) profiles for the control group and the primary open angle glaucoma (POAG) group; A) total retinal blood flow, B) superior retinal blood flow, C) inferior retinal blood flow. One-way ANOVA revealed that the POAG group has lower values for all the retinal blood flow parameters ($p \leq 0.004$).

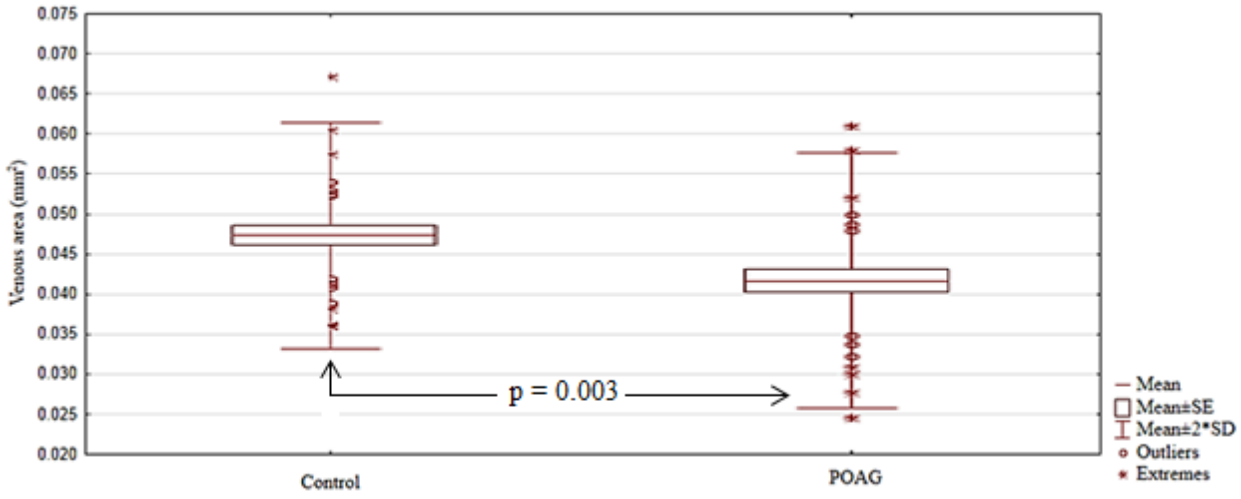

Figure 5.4: Box plot for venous area for the control group and the primary open angle glaucoma (POAG) group. One-way ANOVA revealed POAG has a significantly smaller venous area than the controls (p=0.003).

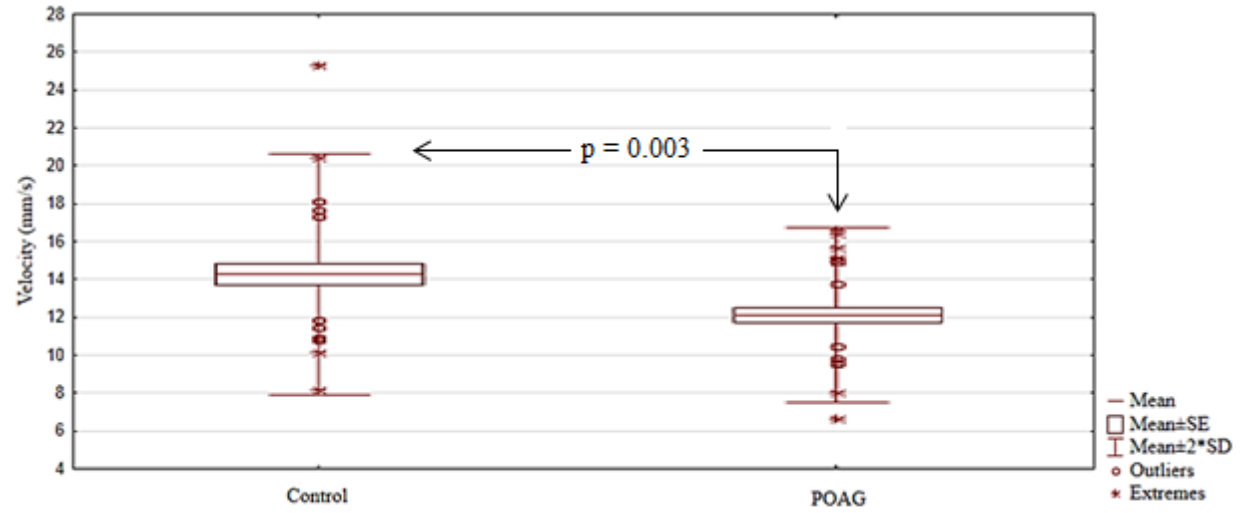

Figure 5.5: Box plot for venous velocity for the controlgroup and the primary open angle glaucoma (POAG) group. One-way ANOVA revealed that the POAG group has significantly slower venous velocity than the controls (p=0.003).

5.3.4 Correlation analysis between retinal haemodynamics, RNFL thickness profile, and visual field sensitivity

5.3.4.1 Control group

Table 5.4 shows the correlation analysis between RNFL thickness, haemodynamics, and visual field sensitivity in the control group. The relationship between mean RNFL thickness and venous area was significant ($r=0.58$, $p<0.001$; Figure 5.6). The other retinal haemodynamic parameters did not show a relationship with RNFL thickness, although TRBF has a tendency to be higher with increase in mean RNFL ($r=0.30$, $p=0.09$). The RNFL thickness and MD did not show any significant association.

Correlation analysis between retinal haemodynamic parameters and MD did not show any association.

Correlation between		r	p
Mean RNFL thickness	**TRBF**	0.30	0.089
Superior RNFL thickness	**Superior RBF**	0.23	0.204
Inferior RNFL thickness	**Inferior RBF**	0.28	0.113
Mean RNFL thickness	**Venous area**	0.58	<0.001
Mean RNFL thickness	**Venous velocity**	-0.11	0.529
Mean RNFL thickness (dB)	**MD**	0.06	0.761
Superior RNFL thickness (dB)	**MD**	0.12	0.49
Inferior RNFL thickness (dB)	**MD**	-0.01	0.94

Table 5.4: Pearson product-moment correlation coefficient (r) and significance value (p) for correlation analysis between retinal nerve fiber layer thickness, retinal haemodynamics, and visual field sensitivity in the healthy control group. [RNFL = retinal nerve fiber layer, TRBF = total retinal blood flow, RBF = retinal blood flow, MD = mean deviation]

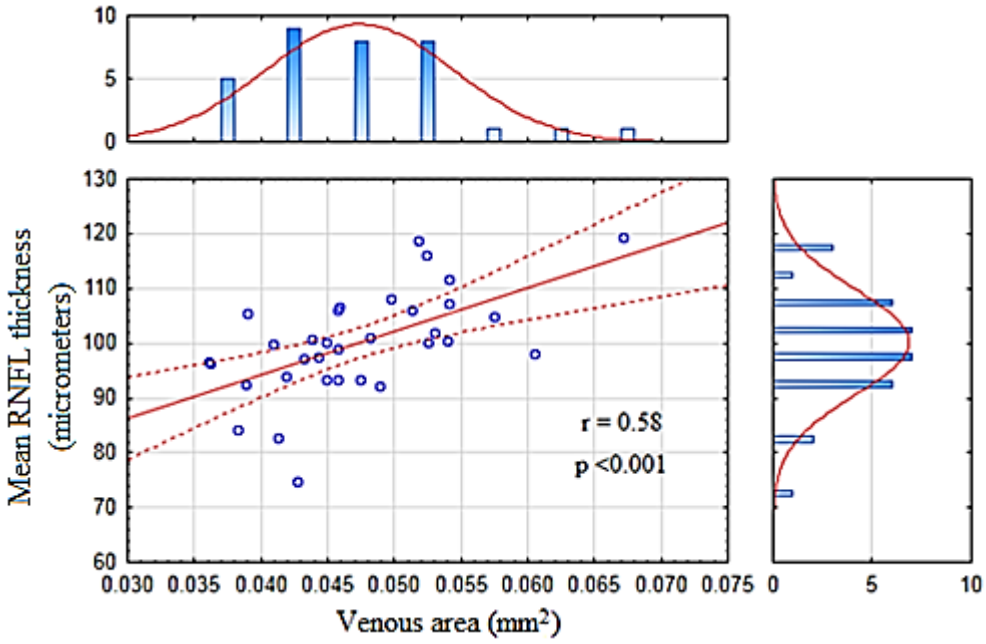

Figure 5.6: Scatterplot of mean RNFL against venous area in the control group. Pearson product-moment correlation coefficient shows a medium positive relationship, r=0.58 (p<0.001).

5.3.4.2 POAG group

Table 5.5 shows the correlation analysis between RNFL thickness, haemodynamics, and visual field sensitivity in the POAG group. Mean RNFL was observed to have a moderate correlation with TRBF (r=0.38, p=0.03; Figure 5.7). Superior RBF was also found to have a moderate positive relationship with superior RNFL thickness (r=0.37, p=0.03; Figure 5.8). Inferior RBF had a tendency to be higher in thicker inferior RNFL (r=0.31, p=0.09). Venous area showed a moderate positive correlation with mean RNFL (r=0.35, p=0.05; Figure 5.9). No relationship was observed between venous velocity and mean RNFL thickness in POAG group (r=0.25, p=0.15). The structure-function relationship between mean RNFL thickness and MD showed a strong

positive relationship (r=0.63, p<0.001; Figure 5.10). The superior and inferior RNFL thickness also showed correlation with MD (r=0.47, p=0.006; r=0.70, p<0.001, respectively).

Correlation analysis between retinal haemodynamics and MD did not show any association.

Correlation between		r	p
Mean RNFL thickness	**TRBF**	0.38	**0.028**
Superior RNFL thickness	**Superior RBF**	0.37	**0.032**
Inferior RNFL thickness	**Inferior RBF**	0.30	0.085
Mean RNFL thickness	**Venous area**	0.35	**0.050**
Mean RNFL thickness	**Venous velocity**	0.25	0.154
Mean RNFL thickness (dB)	**MD**	0.63	**<0.001**
Superior RNFL thickness (dB)	**MD**	0.47	**0.006**
Inferior RNFL thickness (dB)	**MD**	0.70	**<0.001**

Table 5.5: Pearson product-moment correlation coefficient (r) and significance value (p) for correlation analysis between the retinal nerve fiber layer thickness and the retinal haemodynamics, and visual field sensitivity in the POAG group. [RNFL = retinal nerve fiber layer, TRBF = total retinal blood flow, RBF = retinal blood flow, MD = mean deviation]

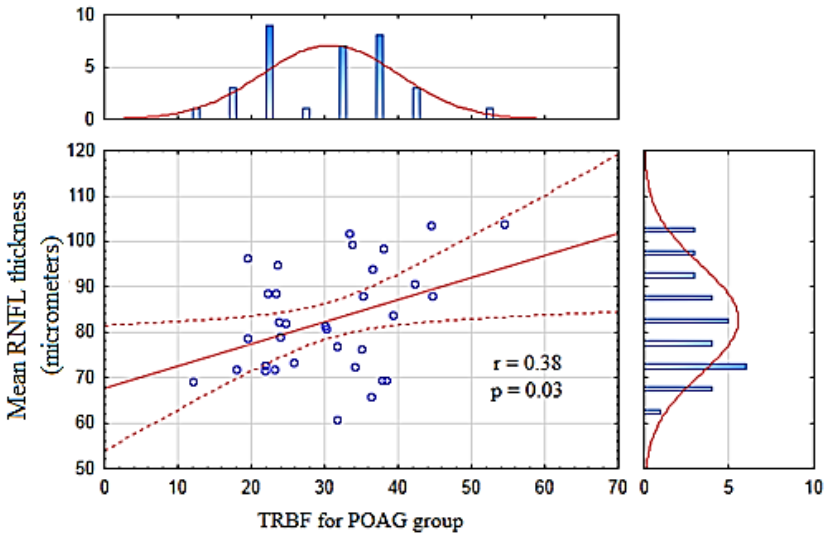

Figure 5.7: Scatterplot of mean RNFL against total retinal blood flow in the POAG group. Pearson product-moment correlation coefficient shows a medium positive relationship (r=0.38, p=0.03).

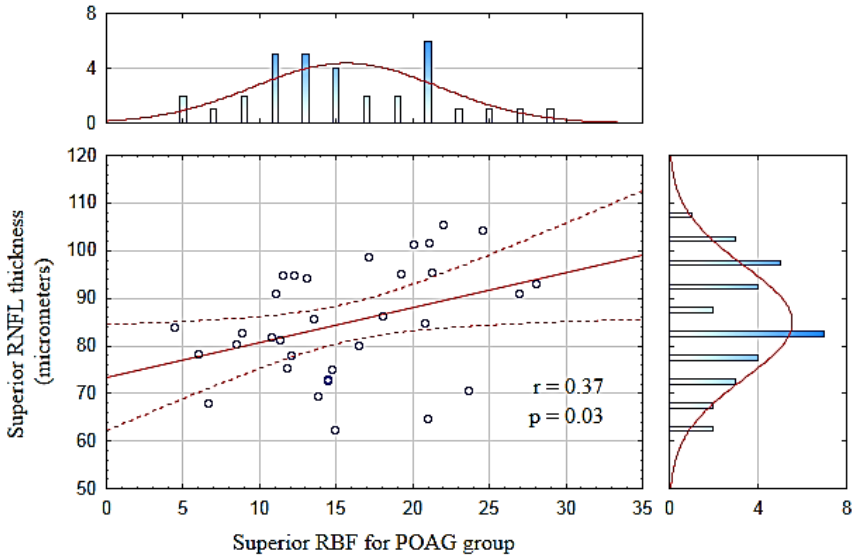

Figure 5.8: Scatterplot of superior RNFL against superior quadrant retinal blood flow in the POAG group. Pearson product-moment correlation coefficient shows a medium positive relationship, r=0.37 (p=0.03).

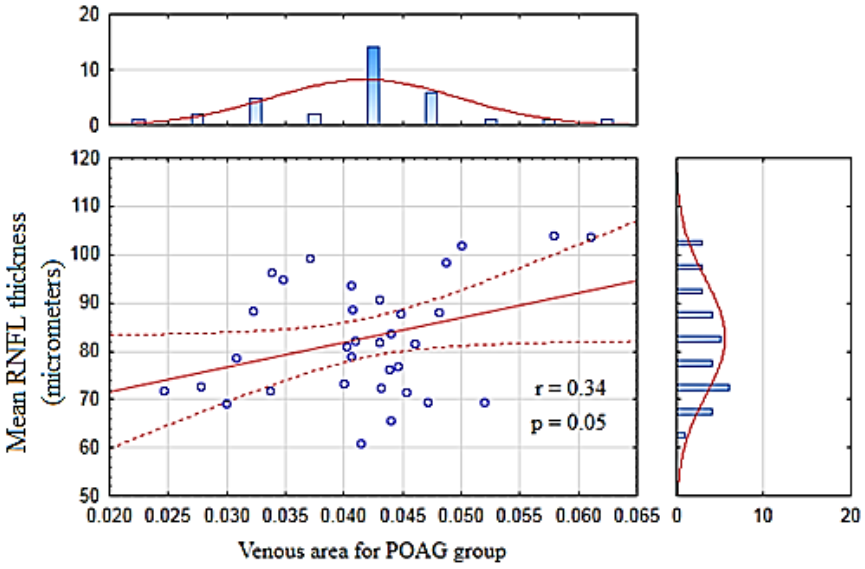

Figure 5.9: Scatterplot of mean RNFL against venous area in the POAG group. Pearson product-moment correlation coefficient shows a medium positive relationship, r=0.34 (p=0.05).

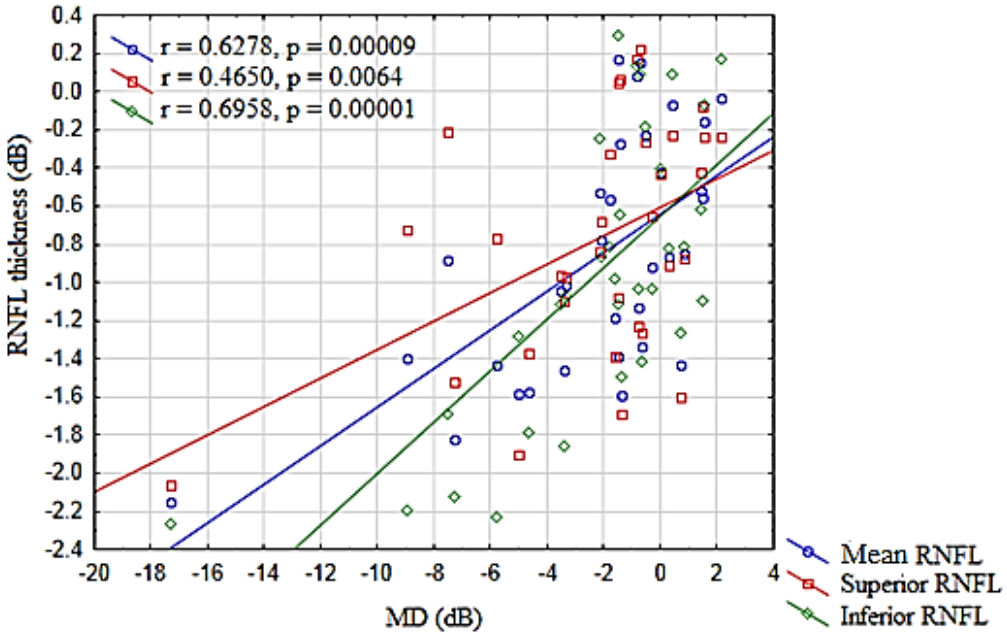

Figure 5.10: Scatterplot of mean (blue), superior (red), and inferior (green) RNFL thickness (in decibel) against MD in the POAG group. Pearson product-moment correlation coefficient shows a strong relationship for all RNFL profile. [RNFL=retinal nerve fiber layer, MD=mean deviation, dB=decibel, POAG=primary open angle glaucoma]

5.4 Discussion

As expected, POAG patients showed a significant reduction in the RNFL thickness, relative to healthy eyes. All three RNFL profiles, namely the mean, superior and inferior RNFL were valued around 83μm in glaucoma group, in comparison to 100μm in the control group. Retinal flow was lower in the glaucoma patients, with venous total retinal blood flow recorded at 31μL/min, compared to 41μL/min in the healthy group. The glaucoma group also recorded a significantly smaller venous area and venous blood velocity compared to the healthy controls. This observation is in agreement with other reports in the literature which have used the same flow measuring methodology.[10-12] The lower haemodynamic values in the glaucoma group indicates vascular components in its pathogenesis. Previous work has shown that there is an association between structural changes in the retinal vessel wall and neural loss in glaucoma, which could be independent to IOP.[5, 13]

Retinal haemodynamic parameters are significantly correlated with RNFL loss in the early POAG group. In this current study, our analysis indicated that the total RBF is reduced in thinner mean RNFL, and superior RBF being lower in thinner superior RNFL. In addition, lower venous velocity is correlated with a thinner mean RNFL. The significant correlations, in general, suggest a vascular component in the early POAG pathology. Reduction in blood flow or blood velocity may primarily promote the disease, or, a secondary effect as a result of the RNFL loss. Regardless, the association confirms perfusion impairment in POAG. We also saw significant associations between RNFL loss and visual field deterioration, although we did not find associations between retinal haemodynamics and field loss. Nevertheless, since haemodynamics reduction is correlated with RNFL loss, it may be possible that the haemodynamic impairment

may lead to field deterioration, although this may need further investigation. To confirm this hypothesis, it is suggested that in the future, retinal blood flow and RNFL thickness is monitored over time.

The association between ocular haemodynamics and RNFL structure was a subject of interest of many groups, and studies have been conducted using various methods on different ocular vessels. Association between RNFL thickness and vascular parameters were evident in retrobulbar,[14, 15] ONH,[16-22] retinal[10, 17, 23] and choroidal blood vessels[17]. Some studies showed no relation between these parameters,[10, 17, 19] but most have reported relationships between reduced haemodynamics and RNFL loss.[14, 15, 17, 18, 20] Hwang and associates[10] using Doppler SD-OCT on subjects with perimetric glaucoma cohort saw associations between field loss with reduced retinal flow and reduced RNFL thickness. They failed to see any relationship between reduced blood flow and RNFL loss, which lead them to conclude that impaired flow and structural loss are independent determinant of field deterioration. It is not clear why we observed a different result, but we speculated that it may be attributed to the different patient composition. The POAG subjects in the prior work had more severe field loss than ours, with lower MD of -4.39dB and PSD of 6.54 dB, compared to our sample with -2.28dB and 4.07dB, respectively. Of note, the same group published an earlier pilot data where they reported a strong correlation between flow with visual field loss in 19 perimetric glaucoma patients ($r=0.83$, $p=0.003$).[12]

A few studies reported a different observation with an inverse association between RNFL structure and retinal flow in POAG.[16, 23] They argued that neural layer thinning in GON is associated with an increase in flow as a result of compensatory response attributed to autoregulation. It was further speculated that the relationship between flow and progressing

glaucoma is bimodal, where an up-regulation in flow occurs in the beginning of the disease until a certain critical point, and afterwards will gradually decrease with progression.[23] Nevertheless, this theory cannot be clarified and a longitudinal study measuring blood flow over the course of glaucoma progression needs to be undertaken. A longitudinal study on macaque eyes, was in agreement with this hypothesis of bimodal flow alteration.[24] Following experimental glaucoma for an mean of 8 months, the ONH flow as measured using laser speckle flowgraphy was observed to increase during 10% of RNFL loss, which later reduced when nerve fiber layer further deteriorated. The authors speculated that induction of experimental glaucoma, by means of increasing IOP, caused vascular dysregulation which leads to altered perfusion level. They further theorized that the increment in flow may also be justified to an increased metabolism due to energy demand up-take caused by ONH gliotic alterations or lamina cribosa remodeling.

A lower flow value in POAG and its association with neural loss, and vision loss, can be explained in a number of ways. The simplest explanation may be attributed to the supply and demand theorem whereby neural loss reduces metabolic needs,[25] which eventually results in lower perfusion. At the same time, the neural loss will affect the visual field. Alternatively, vascular deficits may exist before alteration to neural count or field sensitivity.[26] Ischaemia as a result to vascular dysregulation, in combination with other factors such as elevated pressure may impact the neural layer, leading to RNFL loss and eventually field loss. Another possible explanation is that flow reduction may cause field loss independently to neural loss, as well as neural loss can cause field loss independently to impaired flow.[10] Cherecheanu and colleagues[27] proposed a model to explain vascular involvement in the development and progression of GON. They suggested two levels of attack to the neural beds which dampen the retinal ganglion cell axons. Primary insult occurs in the ONH region, taking form in an increase of IOP and ischaemia

of the post-laminar nerve bundle. Cerebrospinal fluid pressure, together with surrounding tissues' biomechanical properties play their role to modulate the primary insult. This initial insult causes ganglion cells to function at a reduced level resulting in increased vulnerability to further damage. Secondary insults may occur when perfusion pressure falls beyond the autoregulation limit, or failure of neurovascular coupling (perfusional needs following neural activity).

It has been suggested by Kochkorov and co-workers[28] that a low level reduction in perfusion will result in tissue atrophy over the long term but will avoid clinically visible infarction. Fluctuation of perfusion, however, is claimed to be damaging as it promotes oxidative stress to the tissue bed. Based on the ischaemia-perfusion damage concept, free oxygen radicals are abundant during reperfusion after an ischaemic attack.[28] Varying perfusion levels prompt a varying oxygen supply, hence, triggering a cascade of reperfusion damage stemming from oxidative stress. Erratic perfusion has been associated with glaucomatous damage.[29, 30] Kochkorov and co-workers[28] reported that the POAG patients have a significantly higher variability in blood pressure and choroidal blood flow compared to controls and ocular hypertensives. In a different study, it was reported that POAG patients with more severe field defect have higher variability in para-papillary blood flow, compared to POAG patient with less field defect, and controls.[19]

The regular clinical assessment to monitor glaucoma progression is usually comprises the assessment of neural morphology and visual function. Additionally, improved diagnosis can be made by combining structural and functional discriminant functions, rather than making diagnosis based on function or neural structure alone.[31] On top of this, pairing the assessment of structural features with ocular perfusion evaluation allows scientists to discriminate other glaucoma risk factors independent to IOP. A clinical risk factor of vascular origin should be

taken into consideration, especially in cases where progression exists despite well controlled IOP. Nevertheless, the clinical role for retinal haemodynamic measurement and the relevance of vascular alterations are still ambiguous. Further study is needed.This study has some limitations. First, the POAG patients recruited are confined to early POAG with relatively small field sensitivity depression. It would be interesting to see the relationship between haemodynamics, neural structure, and field loss, in more advanced glaucoma. However, Doppler imaging may be difficult in patients with poor vision as they may experience difficulty to fixate which is pivotal for reliable, valid, scans. Secondly, a cross-sectional design study like this can only identify associations rather than to identify causations. Although correlation analysis is highly suggestive, yet debatable, of causation, further investigation using different study designs is needed to equate the link between perfusion, neural structure and visual function, in glaucoma.

To summarize, the reduction of venous blood flow and blood velocity among early POAG patients is associated with neural structure loss. Impaired haemodynamics in early POAG group, together with non-correlating parameters in the control group signifies vascular dysfunction in our early POAG group. Whether this is primary or secondary of the disease is equivocal. Prospective longitudinal clinical trial studies are warranted to test the prognostic value of impaired retinal haemodynamics in POAG.

6 Vascular reactivity in primary open angle glaucoma patients with differing nocturnal blood pressure status

	Concept/Design	Recruitment	Acquisition of data	Analysis	Write-up
Yusof, F	X	X	X	X	X
Hudson, C	X			X	X
Cheng, R		X	X		
Buys, YM	X	X			X
Trope, GE	X	X			X
Flanagan, JG	X			X	X

Table detailing role of each person (X denotes significant contribution).

Purpose: To evaluate the vascular reactivity in patients with primary open angle glaucoma (POAG) with different nocturnal blood pressure (NBP) profiles using normoxic hypercapnia.

Methods: Nocturnal and diurnal blood pressure data was collected using 48 hours ambulatory blood pressure monitoring (ABPM). The NBP reduction (NBPR) magnitude was calculated by taking the difference between mean arterial pressure (MAP) during the day and night while awake and asleep, respectively. The study included 17 healthy controls (group mean age 61.9±6.8 years; group mean NBPR 13.5±8.2%); 17 POAG with normal NBPR (age 66.1±9.2 years; NBPR 10.9±4.7%); and 16 POAG with high NBPR (age 63.5±7 years; NBPR 24.1±4.0%). An automated gas sequencer (Respiract™, Thornhill Research Inc., Toronto, Canada) was used to achieve normoxic hypercapnia (15% increase in the end-tidal carbon dioxide partial pressure, $P_{ET}CO_2$, relative to homeostatic baseline). Retinal haemodynamics were measured using the Doppler SD-OCT in the RTVue system (Optovue Inc., Freemont, CA), and bi-directional Laser Doppler Velocimetry with simultaneous vessel densitometry (BLDV-SVD) in the Canon Laser Blood Flowmeter 100 (CLBF; Canon, Inc., Tokyo, Japan). Six blood flow scans were acquired for each instrument during baseline, normoxic hypercapnia, and recovery period.

Results: Using Doppler SD-OCT, there was no change in retinal haemodynamics in response to normoxic hypercapnia in the high NBPR POAG group. Normal NBPR POAG group showed a significant increase in the TRBF (3.82±4.71µl/min; reANOVA $p=0.01$). The control group demonstrated a significant increase in the TRBF (8.20±10.75µl/min, $p<0.01$) and velocity (2.41±3.33mm/s, $p<0.01$). Comparison between normal NBPR, high NBPR and control groups showed that velocity change in response to hypercapnia was significantly smaller in the high

NBPR group (-0.58±3.10mm/s) than the controls (2.41±3.33mm/s; Tukey HSD p=0.01). TRBF change in the high NBPR group was significantly lower (0.95±8.64µL/min), in comparison to the controls (8.20±10.75µL/min; Tukey HSD p=0.04). The BLDV-SVD did not show any significant vascular hemodynamic differences between POAG with normal NBPR, POAG with high NBPR and control groups.

Conclusions: Patients with POAG who exhibited an exaggerated nocturnal reduction in MAP also demonstrated vascular dysregulation.

6.1 Introduction

Glaucoma is a multifactorial disease of the optic nerve resulting in progressive loss of ganglion cells, distinctive changes to the optic nerve head (ONH) structure and visual field defects. Glaucoma has increasingly been accepted as a manifestation of both ocular and systemic risk factors. Multiple studies have suggested that glaucomatous optic neuropathy (GON) seems to be associated with changes in ocular haemodynamics in the ONH,[1] retina,[2, 3] choroid[4] and retrobulbar area[5]. Some studies have even found reduced blood flow in the brain[6] and peripheral vasculatures of patients with glaucoma[7]. Although those reports indicated peculiarities in the vascular system, it is still unknown whether haemodynamic changes cause nerve degeneration, or if they occur secondary to the disease itself. It is hypothesized that the involvement of vascular risk factors in glaucoma may be due to vasospasm or dysregulation, or both.[8, 9]

Autoregulation is defined as the ability of a vascular tissue to maintain stable blood supply despite changes to perfusion pressure.[10] Vascular reactivity is the measurement of change in the retinal blood flow, to provocation, designed to drive the retinal vascular system to the extremes. Our group has previously investigated retinal vascular reactivity using mixtures of oxygen (O_2) and carbon dioxide (CO_2) in healthy[11-15, 49] and diseased cohorts.[3, 16, 17]

O_2 is known as a potent vasoconstrictor while CO_2 is a potent vasodilator.[8, 15, 18] An increase in the arterial partial pressure (Pa) of CO_2, termed as hypercapnia, can be indirectly measured from the peak concentration of CO_2 during expiration, as indicated by the parameter end-tidal partial pressure (P_{ET}). It has been demonstrated that hypercapnia causes vasodilation in healthy retinal vessels, raising the retinal blood flow.[14] Thus, the absence of reactivity in lieu with the CO_2

increase represent deficient vascular reactivity which, in turn, may suggest an impaired autoregulation in the vascular bed. Using hypercapnia stimulus, Venkataraman and associates[3] have shown that untreated primary open angle glaucoma (POAG) patients did not experience significant change in their haemodynamics as measured using bi-directional laser Doppler velocimetry with simultaneous vessel densitometry (BLDV-SVD). However, treated POAG patients and healthy controls were observed to have a significant increase for their arteriolar diameter, blood velocity and blood flow.

The cardiovascular system exhibits variability following the circadian rhythm and experiences a slow down during sleep. The nocturnal blood pressure (NBP) reduces normally 10-20%, or 15mmHg from its diurnal value.[19, 20] It has been advocated that the decline at night is due to the physiological attenuation in the sympathetic tone.[21] An exaggerated nocturnal hypotensive condition, in theory, may cause ischemia to organs such as the eye, especially in people with impaired autoregulation.[19, 22, 23] Graham and co-workers[19] in their study have reported an association between POAG and excessive NBP reduction (NBPR). They had observed lower values in the nocturnal BP parameters among POAG patients with progressive field defect, in comparison to POAG patients with stable field. Gherghel and co-workers[24] have evaluated the relationship between NBPR and retrobulbar blood flow using colour Doppler imaging (CDI) on patients with POAG. They reported lower blood velocity in the central retinal artery in the POAG group with high NBPR compared to POAG groups with normal and lower NBPR. We observed a similar finding in the retinal vessels, as reported in chapter 4 (Section 4.3.3). Caprioli & Coleman[20] calculated that ocular perfusion pressure (OPP) is increased about 15mmHg, due to the position of the hydrostatic column pressure, when a person sleeps in a typical supine position. Thus, the NBP should fall more than 15mmHg for the OPP to cause an ischemic insult.

Reduction of such value is equal to an NBP fall of more than 20%, i.e. in patient with high NBPR.

In chapter 4, we have demonstrated that glaucoma patient whom exhibits extreme NBPR have a significantly lower homeostatic retinal blood flow and smaller venous area, compared to the healthy controls. It is not clear whether a differing characteristic of the NBPR would also exhibit different vascular reactivity. To the best of our knowledge, there has been no study that has addressed this and related questions. Hence, this study was aimed to determine the relationship between NBPR status and vascular reactivity in subjects with POAG, using normoxic (homeostatic level oxygen partial pressure, PaO_2) hypercapnia provocation. It is interesting to determine whether increased NBPR is truly a manifestation of vascular dysregulation in a POAG cohort.

6.2 Methods

6.2.1 Participants

The study was approved by the Research Ethics Boards of the University Health Network, University of Toronto and the Office of Research Ethics of the University of Waterloo. Written informed consent was obtained prior to the commencement of the Procedures with participants being explained to the nature and probable consequences of the study according to the Declaration of Helsinki. Non-smoking, treated patients with POAG and age-matched healthy controls were recruited. POAG patients were recruited from the Toronto Western Hospital glaucoma clinic, and their diagnosis was made by a glaucoma specialist (either YMB or GET).

All participants had a corrected log MAR VA of 0.3, or better, in each eye with ametropia less than ±6.00DS and ±2.50DC. Automated perimetry comprising Humphrey Visual Field Analyser (HFA) program 24-2 with SITA Standard thresholding was undertaken on all participants and the result was deemed valid when fixation losses, false positive and false negative responses were less than 30%. All healthy participants did not have any immediate history of glaucoma or diabetes and had a normal visual field (VF). None of the participants with POAG; had any history of other ocular pathology, history of ocular surgery (exception to uneventful cataract surgery), history of stroke and chronic lung disease, poorly controlled hypertension, diabetes mellitus and other endocrine diseases, ocular media opacities significant enough to hinder retinal imaging, pseudoexfoliation, pigment dispersion, closed iridocorneal angles, retinal/neuro-ophthalmological disease and blood abnormalities including hyperviscosity. All participants were refrained from taking any caffeinated beverages and/or meals containing red meat four hours prior of the study to prevent the potential impact of diet related extraneous factors that may impact flow readings. Caffeine consumption has been shown to constrict ocular vessels,[25-27] while red meat induces vasodilatation.[28] Participants with POAG were further divided into groups based on their NBPR profile; normal NBPR of <20%, and high NBPR of ≥20%). By calculation, the OPP is increased about 15mmHg when a person takes a supine position to sleep due to hydrostatic column pressure.[20] Thus, the NBP should reduced more than 15mmHg, or more than 20% than the diurnal value to potentially cause an ischemic insult. During the Doppler measurements, the operators (FY and RC) were masked to the NBPR status of the participants.

Using data from Venkataraman and co-workers[3], arteriolar blood flow change from baseline to hypercapnia for treated POAG group was ~25±11%). Assuming that retinal blood flow changed is reduced by 50% in the group with an exaggerated NBPR, the standardized effect size was

calculated as (0.5*25%)/11=1.14. Using a two-tailed $\alpha = 0.05$ and $\beta = 0.20$, the minimal required sample size is 15.

6.2.2 Ambulatory blood pressure monitoring (ABPM)

The A&D TM2430 ambulatory blood pressure recorder system (A&D Co. Ltd., Saitama, Japan) was used in the study. All participants were monitored for 48 hours, and the measurement frequency was set at every half an hour. The ambulatory BP monitoring was done in the participants own home. The A&D ABPM Data Analysis Software for windows (A&D Co. Ltd., Saitama, Japan) was utilized for analysis. Mean arterial pressure (MAP) was used to calculate the NBPR profile. Explanation of the formula to calculate MAP and NBPR profile was fully explained in Chapter 1(section 1.8.1.1) and Chapter 4 (section 4.2.2).

6.2.3 Gas delivery system and provocation

A sequential gas delivery (SGD) breathing circuit (HiOx, Viasys Healthcare Inc, Yorba Linda, California) was used together with an automated gas sequencer (RespirAct™, Thornhill Research Inc., Toronto, Canada). The SGD circuit comprised of two gas reservoirs and a face mask (Figure 6.1). The automatic gas sequencer supplies fresh gas into the inspiratory reservoir, while breathed gas fills the expiratory reservoirs. Each reservoir is connected to the mask with a separate one-way valve. The two reservoirs are interconnected by a positive end-expiratory pressure (PEEP) valve, which enables participants to rebreathe the exhaled gas in situations where fresh gas reservoir is emptied. Participant's values of metabolic O_2 consumption (VO_2) and metabolic CO_2 production (VCO_2), together with their basal $P_{ET}O_2$ and $P_{ET}CO_2$, are entered in the RespirAct™ program in order for the software to compute the needed gas concentrations

to achieve the targeted PET value. The novelty of the breathing system with the SGD circuit and the automated gas sequencer is its efficiency to target the desired PET concentrations and to maintain the stability of the PET concentrations.[11-14] The system is able to manipulate O_2 or CO_2 PET concentration independently, irrespective of minute ventilation.[29]

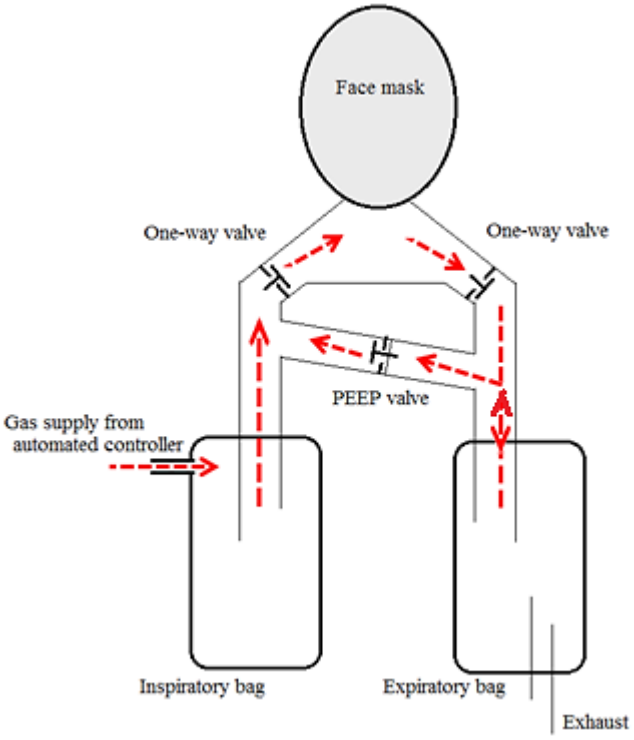

Figure 6.1: Schematic diagram of sequential gas delivery (SGD) circuit. [PEEP = positive end-expiratory pressure]

6.2.4 Instrumentation

6.2.4.1 Retinal blood flow measurement using Doppler SD-OCT

The Optovue RTvue OCT (Optovue Inc, Freemont, CA, USA) was utilized for the prototype Doppler imaging. The optical set-up of this instrument has been discussed in detail previously in Chapter 1 (section 1.11.6.6), Chapter 3 (section 3.2.2) and Chapter 4 (section 4.2.3.1). Blood flow measurement was performed using the circum-papillary double circular blood flow scan, centred on the ONH. The concentric scan transects all retinal arterioles and venules around the ONH using two concentric circles.[30] In a single blood flow scan, six dual circular frames were taken consecutively over 2 seconds, and then were averaged.[31] Doppler images taken were then analysed and processed externally using software to generate haemodynamic parameters which included the total retinal blood flow, venous area, and blood velocity.

6.2.4.2 Bidirectional laser Doppler velocimetry with simultaneous vessel densitometry (BLDV-SVD) in the Canon Laser Blood Flowmeter (CLBF)

The CLBF utilizes the Doppler principle to assess blood flow in absolute unit. The instrument measures the Doppler shift produced by the fastest moving blood particles in the central part of a vessel. It measures blood velocity and vessel diameter simultaneously to calculate the rate of retinal blood flow.[3, 11, 32] The CLBF can only measure one blood vessel at a time, hence the measurement site for this study was standardized at the superotemporal retina, on a straight segment of an arteriole that lies within one to two disc diameter to ONH. Detailed explanation on the instrument has been described in Chapter 1 (section 1.11.6.5) and Chapter 4 (section 4.2.3.3).

6.2.5 Procedures

Each participant attended two visits. During visit 1 after testing for eligibility, morphometric retinal scans were acquired using the RTVue Doppler SD-OCT, with each scan being repeated three times. The choice of selected eye was often driven by study recruitment criteria in the POAG groups, but was chosen randomly in the controls group. The ABPM device was attached to the upper arm of every participant at the end of the first visit. Patients were instructed in the appropriate response to obvious instrument malfunction and instrument induced discomfort.

Participants returned to the lab after 48 hours and the ambulatory BP readings were collected. The study eye was then dilated with tropicamide 0.5% (Mydriacyl 0.5%, Alcon Laboratories, Mississauga, Canada). Participants were then rested for a period of time, often 10 to 15 minutes, until their cardiovascular parameters stabilized.

Using Tegaderm^{TM} adhesive tape (3M Health Care, St Paul, Minnesota), the face mask (HiOx, Viasys Healthcare Inc, Yorba Linda, California) which connected to the automated gas flow controller was then attached to the participants face. The absolute integrity of the mask fitting to the face was then inspected so that it provided a 100% seal and so that it covered both the mouth and nose. Inspired and expired gas concentrations were monitored and sampled continuously. Blood pressure, heart rate, and oxygen saturation were monitored using a rapid response critical care gas analyser (Cardiocap 5, Datex-Ohmeda, Helsinki, Finland) throughout the procedures. The retinal hemodynamic that was measurement consisted of six BLDV-SVD readings and 6 circum-papillary double-circular scan protocol in the Doppler SD-OCT, in each of the following 3 breathing conditions;

i) Baseline homeostatic breathing. Participants breathed room air. The retinal blood flow measurements were taken to establish baseline hemodynamic parameters.

ii) Normoxic hypercapnia. The P_{ET} CO_2 was increased 15% relative to the homeostatic baseline while P_{ET} O_2 was kept at homeostatic level.

iii) Recovery. The P_{ET} CO_2 was reduced to original homeostatic level.

For each breathing condition, a minimum of 5 minutes stabilization time was given prior to retinal blood flow imaging.

6.2.6 Analysis

6.2.6.1 Doppler SD-OCT blood flow analysis

The Doppler images were processed using the semi-automated Doppler OCT of retinal circulation (DOCTORC) software version 2.1.1.4 (Centre for Ophthalmic Optics and Lasers, CA, USA). A description of the grading procedure has been explained in detail in Chapter 3 (section 3.2.3). The Doppler image of each vessel was manually refined for its location and diameter boundary. A confidence score for each scan was assigned, by judging the Doppler strength, vessel boundary, size agreement between outer and inner rings, and the Doppler signal sign agreement between the outer and inner rings. An automated processing algorithm calculated values which included the total retinal blood flow (TRBF), venous area, and velocity. TRBF was calculated by summing all flow from valid venules and the estimated flow of invalid venules. Velocity was computed by dividing flow in valid venules to the valid cross sectional area of all venules.[33] To calculate changes that resulted from the provocation (i.e. vascular reactivity values), values during normoxic hypercapnia were subtracted to its baseline.

6.2.6.2 CLBF velocity waveform analysis

The CLBF analysis software was employed to analyse each velocity waveform. As the waveform could be affected by eye movement, tear film break-up, or improper measurement laser tracking, a standardized protocol was performed to eliminate aberrant waveforms.[32] At least one complete waveform (trough-to-trough or peak-to-peak, a complete cycle) was considered as a valid waveform. The maximum number of acceptable waveforms in each measurement was made for analysis purposes. To calculate vascular reactivity, values during normoxic hypercapnia were subtracted to their baseline value.

6.2.6.3 Statistical analysis

All data were presented in mean ± standard deviation. Normality of data was tested using the Shapiro-Wilk test. Repeated measures Analysis of Variance (reANOVA) was performed in each group (i.e. normal NBPR, high NBPR, and healthy controls) to detect significance of change for each hemodynamic variable during homeostatic baseline, normoxic hypercapnia and recovery. The dependent variables for Doppler SD-OCT were change (Δ) in the total retinal venous blood flow (TRBF), Δ in the venous area, and Δ in the venous blood velocity, whereas for the BLDV-SVD the variables were Δ in the arteriolar diameter, Δ in the arteriolar blood velocity, and Δ in the arteriolar blood flow. A separate reANOVA was executed to analyze possible changes for $P_{ET}CO_2$, $P_{ET}O_2$, respiration rate, pulse rate, systolic and diastolic BP, and oxygen saturation. Tukey honestly significant difference (HSD) post-hoc test was performed for any significant findings by reANOVA to determine the inter-condition (i.e. during baseline, hypercapnia, and recovery) significance.

One-way analysis of variance (ANOVA) was employed to compare (between groups) the amount of change for each haemodynamics. Tukey HSD post-hoc test was performed for any significant findings by ANOVA to determine the inter-group significance (i.e. normal NBPR, high NBPR, and healthy controls). Significance was set at $p \leq 0.05$.

Pearson product-moment correlation analysis was used to study correlation between retinal vascular reactivity parameters, RNFL thickness, MD and NBPR.

6.3 Results

6.3.1 Demographic data

Table 6.1 summarizes the demographic of the participants for both sessions. Seventeen POAG patients with normal NBPR, 16 POAG patients with high NBPR, and 18 healthy controls were recruited. In the healthy control group, one participant was excluded for the SD-OCT, reducing the number of analyzed data sets to 17. A different participant was also excluded in the healthy control group for the BLDV-SVD, reducing the number of analyzed data sets to 17. Two POAG patients with normal NBPR and 1 POAG patient with high NBPR were excluded in the BLDV-SVD analysis, reducing the number to 15 in each group. The reason for exclusion was these participants were fatigued and were unable to continue with the procedure.

Participants who completed the Doppler SD-OCT showed mean NBPR of 24.06±4.07% in the high NBPR POAG group, 10.85±4.64% in the normal NBPR POAG group, and 13.47±8.19% in the control group (ANOVA $p<0.01$). There was a significant difference in NBPR between the high NBPR POAG group and the normal NBPR POAG group (Tukey HSD $p<0.011$) and also

between the control group (Tukey HSD $p<0.01$).There was no difference for the age, IOP, and MD between groups. PSD was significantly different between groups (ANOVA $p =0.04$) with the POAG group with high NBPR showed a higher PSD value compared to the healthy control group (Tukey HSD $p=0.05$). In general, the visual field MD and PSD indices can be interpreted as representing an early POAG group. Both normal and high NBPR POAG groups exhibited a lower mean RNFL thickness in comparison to the healthy controls (Tukey HSD $p<0.01$).

The demographics for participants who completed the BLDV-SVD showed that there was a significant difference in the NBPR value between the high NBPR POAG group and the normal NBPR POAG group (Tukey HSD $p<0.001$) and also the control group (Tukey HSD $p<0.01$). Both normal and high NBPR POAG groups exhibited a lower mean RNFL thickness in comparison to the healthy controls (Tukey HSD $p<0.01$). No significant difference was noted between groups for the age, IOP and MD. PSD showed significant difference between groups (ANOVA $p=0.04$) with the control group was lower than the normal NBPR POAG group (Tukey HSD $p=0.05$). In general, the visual field MD and PSD indices can be interpreted as representing an early POAG group.

	Controls	Normal NBPR	High NBPR	p (ANOVA)
Doppler SD-OCT				
Number	17	17	16	-
Age (years)	61.91±6.82	66.12±9.23	63.48±7.26	NS
NBPR (%)	13.47±8.19	10.85±4.64	24.06±4.07 *	**<0.01**
IOP (mmHg)	14.76±3.23	14.68±3.07	12.88±2.60	NS
Mean RNFL thickness (µm)	102.97±7.73 *	82.25±13.15	83.08±10.72	**<0.01**
MD	-0.75±1.49	-2.82±4.59	-1.70±2.90	NS
PSD	1.64±0.72	3.93±3.67	4.22±3.88 **	**0.04**
BLDV-SVD				
Number	17	15	15	-
Age (years)	62.06±6.97	65.59±9.25	63.15±7.39	NS
NBPR(%)	13.62±8.22	10.85±4.95	24.11±4.86 *	**<0.01**
IOP (mmHg)	14.35±3.62	15.17±2.93	12.80±2.68	NS
Mean RNFL thickness (µm)	102.61±7.61 *	82.51±14.02	82.36±10.68	**<0.01**
MD	-0.74±1.49	-2.98±4.83	-1.72±3.00	NS
PSD	1.62±0.72	4.25±3.81**	3.97±3.88	**0.04**

Table.6.1: Participants' demographics and the values presented are as mean ± standard deviation. P-value denotes significant across groups using one-way ANOVA. [IOP = intraocular pressure, NBPR = nocturnal blood pressure reduction, RNFL = retinal nerve fiber layer, MD = mean deviation, PSD = pattern standard deviation, NS = not significant]

*Note: * denotes the group is significantly different to other groups, and **denotes the group is significantly to the healthy control group (Tukey HSD post-hoc test).*

6.3.2 Retinal vascular reactivity assessed by Doppler SD-OCT

Table 6.2 shows the magnitude of retinal vascular reactivity from baseline to normoxic hypercapnia for all groups, measured using Doppler SD-OCT. Figures 6.2A, 6.2B and 6.2C illustrate the retinal vascular reactivity response to normoxic hypercapnia for TRBF, venous area, and blood velocity, respectively. Using reANOVA, there was no retinal vascular reactivity observed in the high NBPR POAG group in terms of TRBF, venous area, and velocity (Figure 6.2). The POAG with normal NBPR group exhibited an increase in their TRBF of 3.82±4.71µl/min ($p=0.02$; Figure 6.2A), whereas the healthy controls exhibited an increase in TRBF of 8.20±10.75µl/min ($p<0.01$; Figure 6.2A) and an increase in blood velocity of 2.41±3.33mm/s ($p<0.01$; Figure 6.2C). There was no retinal vascular reactivity in terms of the venous area was observed in any group.

The magnitude of vascular reactivity in terms of blood velocity response was significantly different between the groups (one-way ANOVA $p=0.02$; Table 6.2). The Tukey HSD test revealed that the POAG group with high NBPR showed a significantly lower magnitude of reactivity in the blood velocity compared to the control group ($p=0.01$; Figure 6.2C). The magnitude of reactivity in the TRBF was significantly different between the groups (one-way ANOVA $p=0.05$) and the post-hoc analysis showed that the high NBPR group exhibited a lower magnitude of retinal vascular reactivity in terms of total retinal blood flow response than the healthy controls ($p=0.04$; Figure 6.2A).

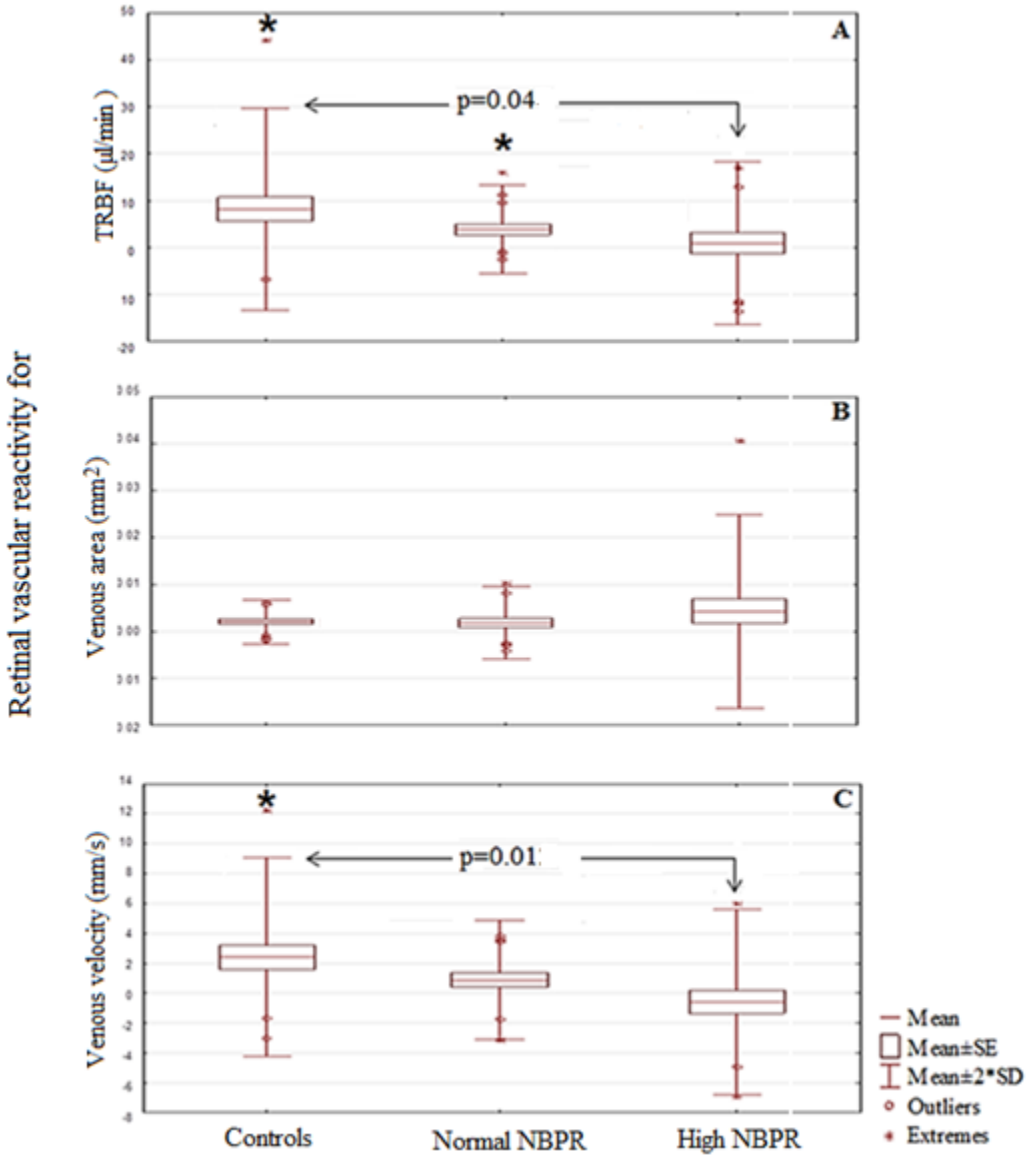

Figure 6.2 The magnitude of change measured using Doppler SD-OCT, in A) total retinal blood flow; B) venous area; and C) blood velocity, in response to normoxic hypercapnia. P-value denotes significance using Tukey HSD post-hoc test.

*Note: * indicates significance when compared across the three breathing conditions (baseline, normoxic hypercapnia and recovery) within the groups at the level of p≤0.05 (ReANOVA).*

	Controls	Normal NBPR	High NBPR	p (ANOVA)
Δ Total Retinal Blood Flow (µl/min)	8.20±10.75	3.82±4.71	0.95±8.64 **	0.05
Δ Superior Retinal Blood Flow (µl/min)	4.21±4.16	1.74±3.21	1.57±6.93	0.24
Δ Inferior Retinal Blood Flow (µl/min)	3.99±8.51	2.09±3.70	-0.63±4.17	0.09
Δ Venous area (mm^2)	2.09±2.34 ($x10^{-3}$)	1.82±3.86 ($x10^{-3}$)	4.32±10.32 ($x10^{-3}$)	0.48
Δ Velocity (mm/s)	2.41±3.33	0.88±2.00	-0.58±3.10 **	**0.02**

Table 6.2: The magnitude of reactivity from baseline to normoxic hypercapnia measured using Doppler SD-OCT. Values are presented by their means ± standard deviation. P-value denotes significant across groups using one-way ANOVA.

*Note: ** denotes the group is significantly different to the healthy control group (Tukey's HSD post-hoc test).*

6.3.3 Retinal vascular reactivity assessed by BLDV-SVD

Table 6.3 shows the magnitude of retinal vascular reactivity from baseline to normoxic hypercapnia for all groups, measured using the BLDV-SVD technique. Figure 6.3A, 6.3B and 6.3C illustrate the retinal vascular reactivity response to normoxic hypercapnia for arterial diameter, blood velocity, and blood flow, respectively. In the healthy control group, the retinal vascular reactivity for diameter was 1.79±13.86µm (ReANOVA p=0.01; Figure 3A), for blood velocity it was 3.01±4.98mm/s ($p<0.01$; Figure 6.2B), and for retinal blood flow it was 1.29±2.05 µl/min ($p<0.01$; Figure 6.3C). The normal NBPR POAG group exhibited a retinal vascular reactivity for velocity of 3.86±7.13mm/s (p=0.02; Figure 6.3B) and for retinal blood flow of 1.00±1.79 µl/min (p=0.02; Figure 6.3C). In the high NBPR POAG group, the retinal vascular reactivity for diameter was 1.39±2.83µm (p=0.03; Figure 6.3A), and RBF it was 1.27±2.16µl/min (p=0.02; Figure 6.3C).

There was no significant difference in the retinal vascular reactivity response to normoxic hypercapnia between the normal NBPR POAG group, the high NBPR POAG group, and the healthy control group (Table 6.3).

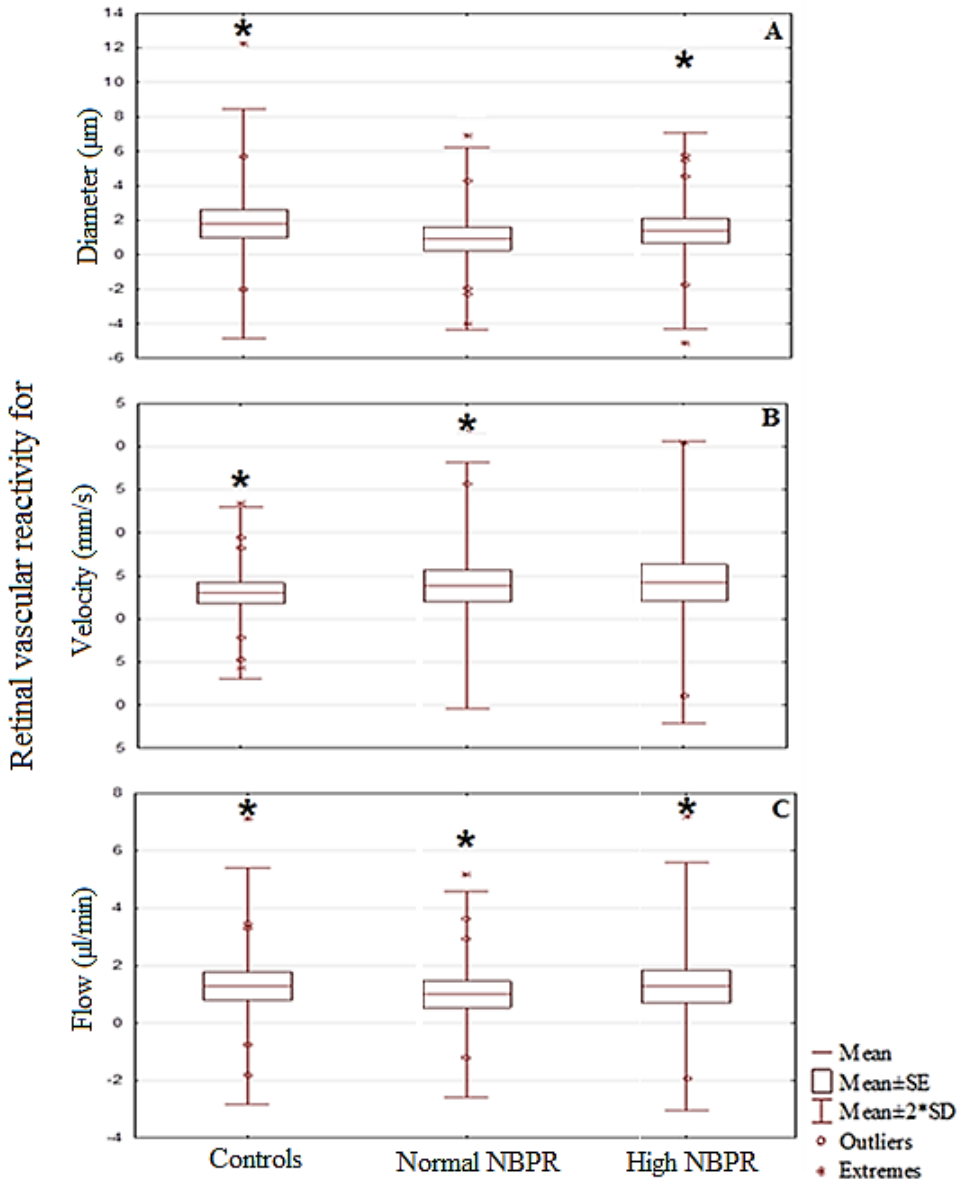

Figure 6.3 The magnitude of change measured using BLDV-SVD, in A) total retinal blood flow; B) venous area; and C) blood velocity, in response to normoxic hypercapnia in POAG patients with normal NBPR, POAG patients with high NBPR, and healthy controls.

*Note: * indicates significance when compared across the three breathing conditions (baseline, normoxic hypercapnia and recovery) within the groups at the level of $p \leq 0.05$ (ReANOVA).*

	Controls	Normal NBPR	High NBPR	p (ANOVA)
Δ Diameter (μm)	1.79±13.86	0.93±2.65	1.39±2.83	0.71
Δ Velocity (mm/s)	3.01±4.98	3.86±7.13	4.23±8.18	0.87
Δ Flow (μl/min)	1.29±2.05	1.00±1.79	1.27±2.16	0.91

Table 6.3: Magnitude of change in the retinal haemodynamics measured by the BLDV-SVD. Values presented by their means ± standard deviation. P-value denotes significant across groups using one-way ANOVA.

6.3.4 Correlation analysis between retinal vascular reactivity and RNFL thickness

Table 6.4 shows the Pearson product-moment correlation coefficient and the p values for analysis between retinal vascular reactivity and RNFL thickness, for the POAG with normal NBPR, POAG with high NBPR and healthy control groups. No significant association was evident in any parameters.

Table 6.5 shows the Pearson product-moment correlation coefficient and the p values for analysis between controls and POAG groups; between retinal vascular reactivity parameters, RNFL thickness, MD, and NBPR. Only the control group shows medium correlation strength to the total retinal blood flow (TRBF) change (r=-0.50, p=0.04; Figure 6.4). No significant association was evident for other parameters.

	Controls (n = 17)		Normal NBPR (n = 17)		High NBPR (n = 16)	
	r	**p**	**r**	**p**	**r**	**p**
Δ TRBF vs Mean RNFL	0.33	0.20	0.34	0.18	0.34	0.20
Δ Venous area vs Mean RNFL	0.43	0.09	0.09	0.73	0.45	0.08
Δ Vein velocity vs Mean RNFL	0.27	0.30	0.17	0.51	0.01	0.98
	Controls (n = 17)		**Normal NBPR (n = 15)**		**High NBPR (n = 15)**	
	r	**p**	**r**	**p**	**r**	**p**
Δ Arterial flow vs Mean RNFL	-0.43	0.09	-0.11	0.69	0.02	0.96
Δ Arterial velocity vs Mean RNFL	-0.23	0.37	-0.23	0.41	0.03	0.91
Δ Arterial diameter vs Mean RNFL	-0.31	0.23	0.22	0.43	-0.40	0.14

Table 6.4: Pearson product-moment correlation coefficient (r) and significance value (p) for correlation analysis between retinal nerve fiber layer (RNFL) thickness and retinal vascular reactivity parameters, in the control, POAG with normal NBPR, and POAG with high NBPR groups. [Δ = change]

	Controls (n = 17)		POAG (n = 30)	
	r	**p**	**r**	**p**
Δ TRBF vs Mean RNFL	0.33	0.20	0.30	0.09
Δ Venous area vs Mean RNFL	0.43	0.09	0.29	0.11
Δ Venous velocity vs Mean RNFL	0.27	0.30	0.07	0.72
Δ TRBF vs MD	-0.17	0.52	0.07	0.70
Δ Venous area vs MD	-0.13	0.61	0.25	0.16
Δ Venous velocity vs MD	-0.14	0.60	-0.11	0.54
Δ TRBF vs NBPR	**-0.50**	**0.04**	-0.11	0.54
Δ Venous area vs NBPR	-0.33	0.20	0.09	0.62
Δ Venous velocity vs NBPR	-0.47	0.06	-0.16	0.38
Δ Arterial flow vs Mean RNFL	-0.43	0.09	-0.05	0.79
Δ Arterial velocity vs Mean RNFL	-0.23	0.37	-0.11	0.58
Δ Arterial diameter vs Mean RNFL	-0.31	0.23	-0.06	0.76
Δ Arterial flow vs MD	-0.33	0.20	0.12	0.54
Δ Arterial velocity vs MD	-0.23	0.38	0.19	0.32
Δ Arterial diameter vs MD	-0.13	0.62	-0.25	0.18
Δ Arterial flow vs NBPR	-0.20	0.45	0.04	0.82
Δ Arterial velocity vs NBPR	-0.02	0.95	-0.06	0.76
Δ Arterial diameter vs NBPR	-0.28	0.28	0.05	0.79

Table 6.5: Pearson product-moment correlation coefficient (r) and significance value (p) for correlation analysis between retinal vascular reactivity parameters, retinal nerve fiber layer (RNFL) thickness, mean deviation (MD) and nocturnal blood pressure reduction (NBPR), in healthy control group and POAG group. [Δ = change]

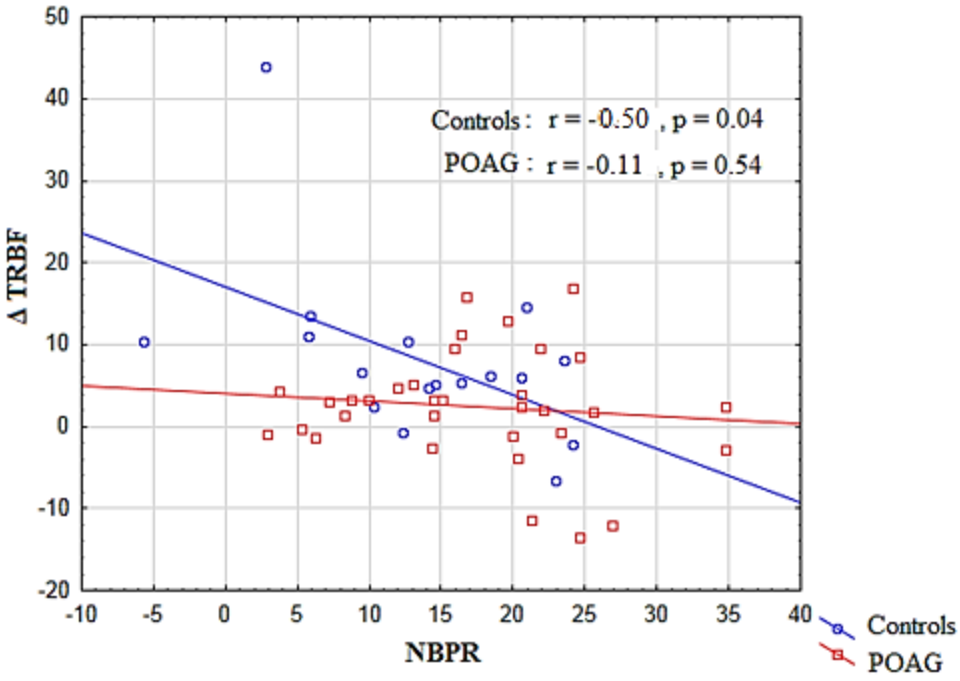

Figure 6.4 : Scatter plot of retinal vascular reactivity in terms of total retinal blood flow (TRBF) against nocturnal blood pressure reduction (NBPR). Pearson product-moment correlation coefficient shows the control group a medium negative relationship, r = -0.50 (p = 0.04).

6.3.5 Breathing and relevant systemic parameters

Table 6.6 and Table 6.7 show detailed breathing and relevant systemic parameters during the retinal haemodynamic assessment using Doppler SD-OCT and BLDV-SVD, respectively for all groups.

In the POAG group with normal NBPR, the mean percentage increase in $P_{ET}CO_2$ relative to baseline during hypercapnia was 14.26±2.10% ($p<0.01$) with a concomitant increase in mean $P_{ET}O_2$ of 3.21±2.05% ($p<0.01$) during the Doppler SD-OCT session. During the BLDV-SVD

session, there was 14.99±2.46% increase in mean $P_{ET}CO_2$ ($p<0.01$) relative to baseline with a non-significant concomitant increase in $P_{ET}O_2$ by 2.59±4.36%.

In the POAG group with high NBPR, the mean $P_{ET}CO_2$ increment during hypercapnia was 15.36±3.55% ($p<0.01$) with a concomitant increase in mean $P_{ET}O_2$ of 2.79±4.35 ($p=0.03$) during the Doppler SD-OCT session. During the BLDV-SVD session, there was 16.18±3.91% increase in $P_{ET}CO_2$ ($p<0.01$) relative to baseline with non-significant concomitant increase in mean $P_{ET}O_2$ (2.00±3.71%).

The healthy control group mean $P_{ET}CO_2$ was increased by 14.92±2.91% ($p<0.01$) relative to baseline with non-significant concomitant increase in mean $P_{ET}O_2$ (1.49±5.22%) during the Doppler SD-OCT session. During the BLDV-SVD session, there was 16.41±3.75% increase in $P_{ET}CO_2$ ($p<0.01$) with a concomitant increase in mean $P_{ET}O_2$ of 1.04±2.97% ($p=0.02$).

Using one-way ANOVA, all of the parameters at baseline were deemed not significant between the groups in both sessions.

Breathing and Relevant Systemic Parameters	Baseline	Hypercapnia	Recovery	P (ReANOVA)
Healthy controls				
$P_{ET}CO_2$ (mmHg)	37.34±1.74	42.89±1.63	37.10±1.94	<0.01
$P_{ET}O_2$ (mmHg)	112.05±5.20	113.59±5.62	112.32±5.45	NS
Respiration rate (breaths/min)	17.23±4.31	18.80±4.35	18.48±3.40	0.01
Pulse rate (beats/min)	66.68±8.71	67.35±9.15	66.00±9.49	NS
Systolic BP (mmHg)	122.41±10.40	124.59±11.21	122.76±10.46	NS
Diastolic BP (mmHg)	77.82±6.56	80.31±7.35	77.65±7.08	<0.01
O_2 saturation (%)	97.67±0.94	97.95±0.92	97.75±1.04	NS
POAG with normal NBPR				
$P_{ET}CO_2$ (mmHg)	37.49±3.34	42.80±3.41	37.32±3.29	<0.01
$P_{ET}O_2$ (mmHg)	115.59±6.8	119.21±5.65	115.90±6.81	<0.01
Respiration rate (breaths/min)	16.69±3.36	17.27±3.87	15.76±2.97	<0.01
Pulse rate (beats/min)	66.15±10.23	66.00±10.52	65.84±10.32	NS
Systolic BP (mmHg)	126.71±14.25	129.82±15.70	126.24±15.81	0.03
Diastolic BP (mmHg)	76.76±12.18	78.76±11.93	75.59±10.85	0.01
O_2 saturation (%)	97.36±1.37	97.66±1.16	97.49±1.20	NS
POAG with high NBPR				
$P_{ET}CO_2$ (mmHg)	37.23±3.59	42.86±3.47	36.99±3.65	<0.01
$P_{ET}O_2$ (mmHg)	110.55±8.39	113.35±5.24	111.41±7.02	0.03
Respiration rate (breaths/min)	15.81±3.39	16.20±3.16	16.13±3.48	NS
Pulse rate (beats/min)	63.49±6.71	63.33±7.66	63.18±6.87	NS
Systolic BP (mmHg)	125.56±23.53	129.31±23.78	126.00±23.82	0.05
Diastolic BP (mmHg)	77.38±12.28	78.81±11.17	75.88±11.70	0.04
O_2 saturation (%)	97.25±1.01	97.64±0.80	97.69±0.86	0.04

Table 6.6: The group mean ± SD of breathing and relevant systemic parameters in normal NBPR POAG group, high NBPR POAG group, and healthy control group during measurement using Doppler SD-OCT. The p-value denotes significance across three breathing conditions using reANOVA ($p \leq 0.05$). [$P_{ET}CO_2$ = partial pressure of end-tidal carbon dioxide, $P_{ET}O_2$ = partial pressure of end-tidal oxygen, BP = blood pressure, O_2 saturation = oxygen saturation NS = not significant].

Breathing and Relevant Systemic Parameters	Baseline	Hypercapnia	Recovery	p (ReANOVA)
Healthy controls				
$P_{ET}CO_2$ (mmHg)	36.55±2.25	42.51±2.16	36.72±2.29	<0.01
$P_{ET}O_2$ (mmHg)	112.82±5.8	113.95±6.03	111.56±6.36	0.02
Respiration rate (breaths/min)	18.34±4.06	19.43±3.62	19.49±4.22	NS
Pulse rate (beats/min)	68.51±9.83	67.27±9.05	67.56±9.17	NS
Systolic BP (mmHg)	124.12±12.50	126.98±12.83	125.65±11.25	NS
Diastolic BP (mmHg)	79.65±8.12	80.92±7.62	80.94±6.17	NS
O_2 saturation (%)	98.01±1.18	98.11±0.91	98.26±0.85	NS
POAG with normal NBPR				
$P_{ET}CO_2$ (mmHg)	37.18±3.52	42.68±3.41	37.11±3.59	<0.01
$P_{ET}O_2$ (mmHg)	113.34±7.49	116.09±6.24	113.16±6.08	NS
Respiration rate (breaths/min)	16.01±2.55	16.90±2.95	15.83±2.68	0.01
Pulse rate (beats/min)	63.37±9.99	64.38±11.23	64.26±11.85	NS
Systolic BP (mmHg)	129.36±18.33	132.21±19.07	128.93±18.55	<0.01
Diastolic BP (mmHg)	77.00±12.23	77.36±12.43	75.50±11.98	0.04
O_2 saturation (%)	97.65±1.23	97.70±1.08	97.65±0.23	NS
POAG with high NBPR				
$P_{ET}CO_2$ (mmHg)	36.92±3.40	42.79±3.08	37.05±3.35	<0.01
$P_{ET}O_2$ (mmHg)	110.36±5.36	112.45±4.25	110.56±4.43	NS
Respiration rate (breaths/min)	16.51±4.52	16.41±3.37	16.49±3.57	NS
Pulse rate (beats/min)	62.21±6.43	63.27±7.12	63.13±6.69	NS
Systolic BP (mmHg)	128.87±24.64	133.67±22.40	129.93±23.31	<0.01
Diastolic BP (mmHg)	78.67±11.73	81.20±10.26	78.67±11.39	<0.01
O_2 saturation (%)	97.60±0.99	97.88±0.64	97.87±0.90	NS

Table 6.7: The group mean ± SD of breathing and relevant systemic parameters in normal NBPR POAG group, high NBPR POAG group, and healthy controls group during measurement using BLDV-SVD. The p-value denotes significance across three breathing conditions using reANOVA ($p \leq 0.05$). [$P_{ET}CO_2$ = partial pressure of end-tidal carbon dioxide, $P_{ET}O_2$ = partial pressure of end-tidal oxygen, BP = blood pressure, O_2 saturation = oxygen saturation, NS = not significant].

6.4 Discussion

In this current study, we are the first to report evidence of vascular dysregulation, by means of using hypercapnic provocation, in early POAG patients with an extreme nocturnal hypotension. Early POAG patients with an NBPR of ≥20% showed no significant reactivity upon 15% increment of $P_{ET}CO_2$ relative from the baseline (while keeping $P_{ET}O_2$ at homeostatic level) in all retinal haemodynamics, as measured using Doppler SD-OCT. On the other hand, early POAG patients with a normal NBPR and healthy controls showed a significant change in the TRBF, with the normal NBPR POAG group's magnitude being lower than the control group, although not statistically significant. We also observed the retinal vascular reactivity in terms of venous blood velocity in the high NBPR POAG group being significantly lower than the control group. The reduced retinal vascular reactivity in all venular blood flow parameters upon hypercapnic provocation in the high NBPR POAG cohort indicated vascular dysregulation in this group. The inefficiency in regulating the cardiovascular system at night, translated into an excessive NBPR, possibly leads to abnormal retinal vascular regulatory function.[24, 34] No study, to the best of our knowledge, has ever investigated vascular dysregulation among early POAG patients with different NBP profiles using a hypercapnic stimulus. It is difficult to directly compare our findings with the literature considering the differences in the technology we used to quantify retinal vascular reactivity and the specific selection of our NBPR groups.

The response of blood pressure to nocturnal factors is complex, since activity levels are reduced and posture is horizontal rather than vertical. The immediate response of the retinal vasculature to adoption of a supine position is vasoconstriction in order to protect the terminal capillaries from the sudden rise in OPP which occurs due to elevation of the heart relative to the eye, i.e.

hydrostatic column effect. However with longer adoption of supine position, vagus nerve responses are initiated which lower the blood pressure and as a result the retinal vessels subsequently dilate. This study essentially measures all aspects of these changes and takes into account the maximum and the minimum of the blood pressure over the 24-hour recording period, irrespective of causality. In addition, the conditions experienced across the groups were equivalent, and therefore the results of valid irrespective of the role of posture upon NBPR.

Our findings in the arterial retinal vessels showed no significant difference in the magnitude of change between the groups. However, most of the haemodynamics measured by the BLDV-SVD showed significant change upon hypercapnia within its individual group. The control group showed significant vascular reactivity upon hypercapnic provocation in all retinal blood flow parameters, while early POAG with high NBPR group showed only significant reactivity in diameter and blood flow upon hypercapnic provocation. The early POAG with normal NBPR on the other hand showed significant increase in arterial velocity and blood flow upon hypercapnia. Our colleagues previously reported an increase of +3% in arterial diameter, +18% in velocity, and +22% in the arterial flow among their control group upon hypercapnia.[3] In addition, the progressive POAG group only showed +9% increase in flow, while untreated POAG showed no significant change for all haemodynamics upon hypercapnia.

The results from the BLDV-SVD method suggest it has advantages in detecting significant retinal vascular reactivity within a group, over the Doppler SD-OCT method. On the other hand, the Doppler SD-OCT showed better identification of different retinal vascular reactivity between the groups. These different observations between the two instruments may be advocated to the nature of the each instrument's Doppler measurement. The BLDV-SVD measures the retinal

blood flow of a single retinal arteriole, while Doppler SD-OCT measures total retinal blood flow by summing flows from multiple venules around the ONH. A fine local signal as measured by the BLDV-SVD is sensitive to detect changes introduced by a provocation, but may lacking the signal-to-noise ratio upon effects originating from a global signal such as the signal that might be associated with diseased induced vascular dysregulation. Hence, comparison of vascular reactivity between the groups yields no significance since BLDV-SVD measurements may have missed the strongest signal in another retinal location. A measurement that is derived from the global retinal area such as in the Doppler SD-OCT contains information from a large number of observations. Based upon the Central Limit Theorem,[35] the average of this global signal will be distributed according to the normal distribution curve, and any difference in peak values between groups will be evident. The signal-to-noise ratio when comparing local effects within the group following provocation may not alter the peak value large enough to be detected globally as significantly different.

In this study, retinal vascular reactivity was investigated using a hypercapnic stimulus. As hypercapnia induces vasodilation, it may be safer to be used in POAG patients as it increases blood flow. In a compromised vascular bed such as that in POAG, a hyperoxic stimulus may be damaging as it induces vasoconstriction. Vasoconstriction secondary to hyperoxia reduces blood flow and may further aggravate the glaucomatous disease process. Relative to a normal eye, it is also thought that the vasculature in a diseased eye may already be relatively constricted, and thus, hyperoxia may not cause substantial further vasoconstriction which in turn may reduce the signal-to-noise ratio effect that we wish to investigate.

Gas provocation studies in other labs may have achieved hypercapnia by patients' rebreathing their exhaled gases[36] or by adding CO_2 manually to breathing air.[8, 37] Such techniques may trigger concomitant change in the $P_{ET}O_2$, thus response in vascular reactivity may not be purely from the $P_{ET}CO_2$ increase. In addition, those techniques that aimed to induce hypercapnia introduce relatively large amounts of inhaled CO_2 to achieve a highly variable level of $P_{ET}CO_2$[38] and therefore cause uncertain magnitude of hypercapnia which will significantly increase pulse rate and blood pressure. Our lab uses the automatic gas sequencer in the Respiract™ system, together with the SGD breathing circuit to produce stable, standardized and accurate targeted $P_{ET}CO_2$ and $P_{ET}O_2$ levels. In this study, an increase in $P_{ET}CO_2$ of 15% relative to participants' homeostatic baseline (i.e. approximately 6mmHg increase in $P_{ET}CO_2$) was achieved to induce hypercapnia. The magnitude of hypercapnia in this study is much lower than what has been used in other gas perturbation methods, resulting to minimal change in pulse rate, blood pressure and $P_{ET}O_2$. It has been demonstrated that the $P_{ET}CO_2$ is correlated with the CO_2 arterial partial pressure ($PaCO_2$) using the technique that we use.[29, 39] The SGD breathing circuit is a mask with series of valves, tubes and reservoirs that provide the ability to clamp or maintain normoxia/ normocapnia irrespective to minute ventilation changes. Our technique is unique as it enables a precise targeting of the $PaCO_2$ and PaO_2 at the same time. Venkataraman and co-workers[14] has shown that the technique we used in the current study is highly repeatable in achieving desired $P_{ET}CO_2$, whilst maintaining normoxia.

A recent study in the Hudson lab used the Doppler SD-OCT to assess vascular reactivity, using normoxic hypercapnia stimulus on young healthy- smokers and non-smokers.[40] The findings were comparable to this current study whereby an increase in the venous area, venous velocity and TRBF by +7%, +18%, and +26%, respectively, were reported. In this current study, the

magnitude of retinal vascular reactivity that we observed among our control group was +4.5%, +18%, and +21% in the venous area, venous velocity, and TRBF, respectively. Our smaller magnitude change in venous area may have been caused by the fact that our sample population was elderly. Confounding factors such as atherosclerosis may lessen the retinal vascular reactivity response to hypercapnia. Also, the power of the study needs to be considered when interpreting these results. Post-hoc power analysis of our results demonstrated a requirement to attained 21 POAG patients to achieve a power of 80% with a type I error of $p \leq 0.05$ when using Doppler SD-OCT, while the BLDV-SVD methodology required a sample of 432 to achieve an equivalent power. In addition, longitudinal studies are required to determine if reduced vascular reactivity is associated with exaggerated NBPR is a cause of POAG or an effect.

Other studies using a hypercapnic stimulus on glaucoma patients reported a mixed results as various techniques were used on various vascular beds. Using BLDV-SVD, our lab previously measured the retinal vascular reactivity upon normoxic hypercapnia provocation on untreated and progressive POAG group which found that the retinal arterial vascular reactivity was significantly reduced compared to the healthy controls.[3] The untreated POAG group did not show any reactivity to normoxic hypercapnia while the progressive POAG only showed reactivity in terms of the retinal blood flow response. In the same study, capillary vascular reactivity in response to normoxic hypercapnia in the ONH showed that the untreated POAG and progressive POAG groups have a smaller magnitude of vascular reactivity for the blood velocity and blood flow compared to healthy controls. Using carbogen as provocation stimulus, Gugleta and co-workers[41] used the LDF technique on vasospastic non-glaucomatous healthy patients to measure the ONH neuroretinal rim and subfoveal choroidal vascular reactivity. Interestingly, they reported an increase in blood flow in the vasospastic patients and decrease in flow in the

non-vasospastic patients to carbogen breathing. Using the CDI method, Harris and co-workers[37] reported an increase velocity in the central retinal artery (CRA), short posterior ciliary artery (SPCA), and ophthalmic artery (OA) in the normal tension glaucoma group, whereas the control group only experienced increase velocity in the CRA during hypercapnia. Hosking and colleagues[42] measured the retrobulbar vascular reactivity to 15% increase of $P_{ET}CO_2$ above baseline. They found that glaucoma group showed an increased velocity in the short posterior ciliary artery (SPCA) and central retinal artery (CRA), in comparison to the controls which showed only increase in the CRA. These results are completely contrary to our findings and the work of others. Sines and associates[43] also used the CDI technique to compare patients with POAG and ocular hypertensive (OH). They reported a significant velocity increase in the CRA but decrease velocity in the ophthalmic artery (OA) in the POAG group, while the OH group showed normal vasodilatory response during hypercapnia. The disparate reports suggest variability in the haemodynamic response to hypercapnia across different vascular beds. It was hypothesized that the vascular bed response to a stress situation, such as increment in $PaCO_2$, may vary from one location to another.[22]

A few studies have inspected the values of haemodynamics during sleep. Osusky and associates[44] used laser Doppler flowmetry (LDF) in healthy eyes, to investigate whether ONH perfusion drops nocturnally. They reported that the mean LDF-flow and LDF-volume decreases from daytime to midnight, whereas the velocity did not change. Of note, they took the LDF measurement at 0800, 1200, 1600, 2000, 2400 and 0800 hours. Whether perfusion reduction was present during sleep is equivocal. Claridge and Smith[45] used the pulsatile ocular blood flow (POBF) method on OH, POAG and healthy controls, to investigate the diurnal variation of the ocular haemodynamics. Their measurement was repeated every three hours during diurnal and

nocturnal hours. They failed to show a significant difference between the diurnal and nocturnal haemodynamic values in all groups, but noticed a large individual variation. In a different study, Harris and co-workers,[46] measured haemodynamics in retrobulbar and cranial arteries using the CDI and transcranial Doppler, respectively, among stable POAG patients, in the evening (baseline) and during the NBPR episode. The BP measurement was set at 15 minutes frequency, and the nocturnal haemodynamics measurement was initiated once the MAP fell 10% or more below baseline. Their findings indicated that blood velocity in the POAG group was unaltered during nocturnal hypotension periods compared to baseline. Their control group showed a reduction in the SPCA only, and the analysis between POAG and control groups indicated no significant difference. Results from sleep studies with experimental intervention during sleep need to be interpreted with care. With the current technological capability, it may be impossible to monitor nocturnal haemodynamic without disturbing patient's sleep.

A few limitations exist in this study. First, there was a significant $P_{ET}O_2$ increase during hypercapnia, in all early POAG groups during Doppler SD-OCT measurement and in the healthy control group during BLDV-SVD measurement. By calculation, those increment were, at maximum, amounting to 3.6mmHg (or 3%) extra relative to the baseline $P_{ET}O_2$. Although statistically significant, such a small amount of $P_{ET}O_2$ increase is not strong enough to influence the reactivity induced by hypercapnia. Unpublished data from our group indicated that 1mmHg increase of $P_{ET}O_2$ will only decrease flow by 0.1% (Cheng et al., unpublished)[48]. Second, this study also showed a significant increase in systolic BP in the normal and high NBPR groups during both Doppler SD-OCT and BLDV-SVD sessions. A similar increment was noted for diastolic BP in all groups during Doppler SD-OCT, and in the normal and high NBPR groups during BLDV-SVD session. By calculation, these increments were only between 0.4 to 4.8

mmHg (0.5% to 3.7%) relative to the BP baseline value. Such a small amount will not alter OPP and cause a significant effect to retinal perfusion. Third, the subjective element in the Doppler SD-OCT blood flow analysis grading may result in bias in the total venous blood flow value. The Doppler image of each vessel was manually refined for its location and diameter boundary using the semi-automatic DOCTORC software. This element of subjectivity may be the reason for insignificance observations in the retinal vascular reactivity pertaining to the venous area, in all intra- and intergroup analysis. However, one may speculate that the insignificant observation in the venous area upon hypercapnia provocation may also stemmed from the fact that venules are less reactive to CO_2 compared to arterioles.[47] Fourth, our protocol was a little bit long, which took more than two days to complete (48 hours of ambulatory BP monitoring and extra few hours for haemodynamics measurements). We saw a few participants dropout due to this. Fifth, the participants' daily activity during ABPM was conducted manually using activity diary which may not be accurate compared to by using an electronic actigraph system.

To conclude, normoxic-hypercapnia provocation did not stimulate significant venular retinal vascular reactivity in the early POAG with high NBPR group, in comparison to the early POAG with normal NBPR group response and the healthy control group response. The early POAG patients with extreme NBPR showed reduced venular reactivity compared to other groups. This observation suggests vascular dysregulation in this particular group of early POAG patients. Our vascular reactivity investigation was done outside the nocturnal sleeping time, whether similar finding is observed at night is unsure.

7 General discussion

Although nocturnal blood pressure reduction (NBPR) is normal physiological phenomenon, an excessive magnitude of NBPR may be dangerous to susceptible tissues such that occurs in glaucoma. Excessive ocular perfusion pressure (OPP) reduction may cause ischaemia to the optic nerve head (ONH) and an unstable OPP may trigger reperfusion injury. These two separate events have possibly been advocated to contribute to glaucomatous optic neuropathy (GON). While many have linked systemic hypotension to primary open angle glaucoma (POAG), a report pertaining to retinal haemodynamics and its relationship to systemic blood pressure is still lacking. The work in this thesis may be the first covering all aspects in retinal blood flow in early POAG cohorts with different NBPR profiles. In addition, this is the first study to examine the retinal vascular reactivity among early POAG patients with different NBPR using a standardized normoxic hypercapnic provocation. No study has directly measured the effect of the NBPR on retinal haemodynamics. In the literature, two studies have investigated POAG patients with differing NBPR profiles, one using color Doppler imaging (CDI)[1] and the other using nail-fold capillaroscopy[2] to measure peripheral blood flow. The work in this thesis thus provided an assessment of retinal vascular haemodynamics at sites that encompassed total retinal blood flow in glaucoma patients with different NBPR.

The Doppler methodologies used in this thesis measure the retinal blood flow in absolute units. Unlike other methodologies reported in the literature, the circum-papillary double circular blood flow in the prototype Doppler SD-OCT and the established bi-directional laser Doppler velocimetry with simultaneous vessel densitometry (BLDV-SVD) derived retinal blood flow in actual flow values (µL/min), apart from other parameters such as blood velocity (mm/s) and

vessel area/diameter ($mm^2/\mu m$). The prototype Doppler SD-OCT measures total retinal blood flow (TRBF) from all valid venules traversing through the ONH, while the BLDV-SVD measures retinal arteriolar blood flow at one specific retinal location. Considering the different nature of measurement that each instrument provides, the retinal haemodynamics that we have studied focused on local and global aspects of retinal haemodynamics. By doing so, this thesis enable a better understanding of retinal haemodynamics, especially in POAG cohort with special characteristics of NBPR.

A normoxic hypercapnic stimulus was used in this thesis to study the retinal vascular reactivity. The novel methodology included an automatic gas sequencer together with a sequential gas delivery (SGD) breathing circuit. This allowed a precise and stable targeting of end-tidal partial pressure (P_{ET}) of carbon dioxide (CO_2) and oxygen (O_2). Normoxic hypercapnia in this thesis was set at an increment of 15% in $P_{ET}CO_2$ relative to baseline while maintaining the $P_{ET}O_2$ at homeostatic level.

In Chapter 3, the normative data of retinal blood flow were gathered on 100 participants consisting of healthy, medication free, participants of different ages and an equal number of males and females. The retinal blood flow was assessed using the circum-papillary double circular blood flow scan protocol in the prototype Doppler SD-OCT. Our retinal blood flow parameters were comparable to other small sample/pilot studies. The correlation analysis showed no relationship of age to any retinal blood flow parameters, although the systemic blood pressure (BP) increased with age and the RNFL thickness reduced with age. These findings suggest that in the healthy population perfusion is constant regardless of aging. Analysis between sexes also revealed no significant difference in the haemodynamics. The haemodynamics correlation

analyses against age in each sex also showed no association. Overall, it is concluded that the retinal blood flow, as measured by a prototype Doppler SD-OCT is independent of age and gender. This experiment also advocated the Doppler SD-OCT as a promising tool in measuring the retinal haemodynamics.

Chapter 4 details the retinal haemodynamics in early POAG patients with different characteristics of NBPR. BLDV-SVD and Doppler SD-OCT were used. POAG patients were divided by NBPR status, after following a 48-hours ambulatory blood pressure monitoring (ABPM) assessment. There was a marked difference in the TRBF and venous area in early POAG patients with high NBPR compared to the healthy controls. Retinal blood flow measurement using the BLDV-SVD, however, did not show any significant difference between groups. It was thought that BLDV-SVD results were equivalent between groups due to its measurement at a fine retinal location. Anatomical variation between patients may have given different homeostatic values, despite effort to standardize measurement site for all participants. The work in this chapter is the first to study homeostatic retinal blood flow in early POAG patients with different NBPR. The findings concluded that POAG patients with high NBPR have impairment in the retinal blood flow compared to healthy people. An abnormal circadian BP regulation may represent a form of systemic vascular dysregulation that is relevant to the retinal vasculature, at least in glaucomatous eyes. Since the retinal blood flow measurement was done outside the nocturnal period, it is unknown whether the same blood flow reduction happens during the real nocturnal hypotension event. This experiment also warranted the ABPM assessment in managing POAG cases. Knowing a patient's BP circadian variation may give an idea of the extent of OPP variation throughout the day. It has also been suggested to treat

hypertension in POAG patients cautiously as overtreatment may set the cardiovascular system into extreme hypotension that may jeopardize the ocular perfusion.

The experiment in Chapter 5 describes the relationship between retinal blood flow parameters as assessed with Doppler SD-OCT, ONH structure (i.e. retinal nerve fiber layer, RNFL thickness profiles) and visual function (i.e. the Humphrey field analyzer, HFA, mean deviation, MD values). There was a positive correlation between TRBF and mean RNFL thickness in the early POAG group while no relationship was observed in the control group. In addition, positive relationships between RNFL thickness profiles and the MD were present in the early POAG group only. Correlation analysis of MD and retinal haemodynamics did not show significance in both early POAG and control groups. These results indicated that reduction of retinal blood flow among early POAG patients is associated with neural structure loss, but to a lesser extent than with visual function disruption. It was speculated that the failure to see relationship between haemodynamics and MD were caused by the non-extreme early POAG sample that were recruited. Considering together the non-correlating blood flow parameters in the control group, the situation signifies vascular dysfunction in POAG. It is however unknown whether the retinal blood reduction occurred primarily, or secondary to the structural loss. A prospective longitudinal clinical trial study is warranted to test prognostic value of impaired retinal blood flow in POAG.

In Chapter 6, the retinal vascular reactivity of early POAG patients with different NBPR characteristics were tested. A standardized normoxic-hypercapnic provocation was achieved by means of increasing $P_{ET}CO_2$ to 15% relative to the baseline, while keeping $P_{ET}O_2$ at homeostatic levels. Retinal vascular reactivity was taken by measuring the change in the retinal blood flow

parameters during hypercapnia relative to its homeostatic value. This experiment is the first of its kind to measure retinal vascular reactivity in a sample consisting patients with early POAG of different NBPR characteristics. It was observed that early POAG patients with high NBPR, in general, exhibited reduced vascular reactivity, with TRBF and venous velocity change magnitude being significantly smaller than the controls. No significant difference was noted between groups in magnitude of change measured by the BLDV-SVD method. These results point to a vascular dysregulation in the early POAG patient group with excessive nocturnal hypotension. This experiment was conducted, however, outside the nocturnal sleeping time. Whether or not similar findings will be observed at night is equivocal.

One general proviso to consider about the overall aspects of this work relates to the method of recruitment of normal controls. POAG patients were recruited in the Glaucoma Clinics of the Toronto Western Hospital by YMB and GET, whereas healthy controls were recruited by leaflet and poster exposure. The glaucoma patients are therefore motivated by health concerns while the healthy controls maybe motivated by different factors. We are unable to conclude whether or not this is a significant bias.

7.1 Future work

The work in this thesis has raised a number of questions for future investigations:

- POAG patients with low NBPR were not included in the studies. It would be interesting to know how nocturnal hypertension may affect haemodynamics and reactivity. In a

susceptible eye such as glaucoma, high OPP may provide a better perfusion, which is possibly of protective in nature to the neural tissue.

- Reperfusion injury has been suggested as a mechanism in glaucoma pathology. Variation in OPP leading to blood flow variability may trigger the formation of reactive oxygen species (ROS) and oxidative injury. Analysis of nocturnal variability in the haemodynamics, or pressures value such as OPP, BP and IOP should be considered in future studies of POAG patients with different NBPR status. In addition the vascular reactivity assessment should incorporate the retinal oximetry measurements to judge the degree of impairment in each glaucoma group.
- Considering that NBPR is closely related to the function of the autonomic nervous system, one may hypothesize that an abnormal NBPR may occur due to autonomic dysfunction. It is interesting to study the involvement of autonomic dysfunction in POAG patients, in particular among POAG patients with different characteristics of NBPR. This may be possible by including biochemical studies to measure certain blood markers indicating disturbed autonomic activity such as catecholamines levels[3] or plasma cortisol.[4] However longitudinal studies are required to determine if reduced perfusion and vascular reactivity due to exaggerated NBPR is associated with POAG or an effect.
- The observed haemodynamics and reactivity reduction in this study were conducted outside the nocturnal hypotension event(s). Given current technological capabilities, it is impossible to measure haemodynamics without disrupting sleep. Keenly with technological advancement, haemodynamics study while patient is sleeping would benefit to better understand the pathophysiology of GON.

- NBPR may change depending on diurnal activities,[5] and sleep quality.[6, 7] In addition, lifestyle has been reported to influence positively to higher OPP.[8] It is interesting to know how POAG patients with different activities and sleep patterns, as well as having active or passive lifestyles, behave in terms of retinal haemodynamics assessment.

7.2 Summary

Perfusion to the retina was not altered with increasing age among healthy poeple. Patients with early POAG who exhibited an exaggerated nocturnal reduction in mean arterial pressure (MAP) also demonstrated lower RBF values as shown by the measurement of Doppler SD-OCT. The change in retinal circulation in early POAG is related to reduced RNFL thickness. A larger venous area may be associated with thicker RNFL among controls. Patients with early POAG who exhibited an exaggerated nocturnal reduction in MAP also demonstrated disturbance of retinal vascular reactivity.

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Chapter 2

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Chapter 7

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