Blood Vessel Diameter in Glaucoma

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Summary

Glaucoma is a leading cause of visual disability in the UK and major referral reason between high street optometry and hospital based ophthalmology. The standard optometric tests used to determine necessity of referral are currently leading to a high false positive burden on glaucoma clinics. The disease of glaucoma is considered to be multifactorial in the reasons for its onset and progression. An increasing body of research proposes a vascular dysregulation hypothesis, and retinal artery diameter reduction, as a recognisable risk factor for both the onset and progression of glaucoma. The Heidelberg Retina Tomograph (HRT) is a commercially available laser scanning ophthalmoscope designed principally for the detection of glaucoma by evaluation of the optic disc neuroretinal rim. An additional ability of the HRT is to measure, via an interactive window, the blood vessels of the scanned image without the need for export of the image or magnification to view them in detail. This thesis contributes to the field of early glaucoma detection by measurement of artery diameter via the interactive window on the HRT machine. The volunteers were divided into three groups normal, glaucoma and ocular hypertensive (OHT) and followed over a period of one year to determine if vessel diameter changed in relation to visual field or neuroretinal rim parameters. The main results in this thesis show that artery diameter does change with glaucoma onset and that the HRT machine is a valid instrument for collection of this data.

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Key Words

Glaucoma, Heidelberg Retina Tomograph (HRT), Blood Vessel Diameter, Artery, Vein, Manual Measurement.

List of Abbreviations

BP	Blood pressure
CCT	Central corneal thickness
CPSD	Corrected pattern standard deviation
CRAE	Central retinal artery equivalent
CRVE	Central retinal vein equivalent
CSFP	Cerebrospinal fluid pressure
DBP	Diastolic blood pressure
GPS	Global probability score
IOP	Intraocular pressure
HRQoL	Health Related Quality of Life
HRT	Heidelberg retinal tomograph
MD	Mean deviation
MOPP	Mean ocular perfusion pressure
MPHSD	Mean pixel height standard deviation
MRA	Moorfields regression analysis
OCT	Optical coherence tomography
OHT	Ocular hypertension
ONH	Optic nerve head
OPP	Ocular perfusion pressure
POAG	Primary open angle glaucoma
PACG	Primary angle closure glaucoma
PCA	Posterior ciliary artery
PSD	Pattern standard deviation
RGC	Retinal ganglion cell
SAP	Standard automated perimetry
SBP	Systolic blood pressure
SD-OCT	Spectral domain optical coherence tomography
SE	Standard Error
SF	Short term fluctuation
SWAP	Short wavelength automated perimetry

Equations

(1) C-Curve = (horizontal + vertical K-reading)/2

(2) Branching Coefficient = $(w^2_1 + w^2_2)/W^2$

(3) Arteries W= $0.88*(w^2_1 + w^2_2)^{1/2}$

(4) Venules W= $0.95*(w^2_1 + w^2_2)^{1/2}$

(5) Mean Ocular Perfusion Pressure= 2/3 (DBP+1/3(SBP-DBP))-IOP

DBP= diastolic blood pressure SBP= systolic blood pressure IOP=intraocular pressure

(6) Systolic Ocular Perfusion Pressure= Systolic Blood Pressure-IOP

(7) Diastolic Ocular Perfusion Pressure=Diastolic Blood Pressure-IOP

(8) $\mu_g = \sqrt[n]{a_1 a_2 a_3 \dots a_n}$ Geographic Mean

(9) $\mu_a = {}^{a_1+a_2+a_3+\cdots a_n}/\mathrm{n}$ Arithmetic Mean

1.0 Introduction

Glaucoma is an optic neuropathy of unknown origin. Current research has suggested vascular factors being involved in the pathogenesis of the disease. One of the measurable components of this vascular pathology is blood vessel diameter change. The Heidelberg Retinal Tomographer's (HRT) primary role is detection of changes in the neuro-retinal bundle indicating glaucoma onset, or, progression of an established disease. The HRT has an interactive tab button that can be used to measure diameter of vessels to 0.012mm without the need for additional computer software. This research used the HRT to measure the diameter of blood vessels, and relate any change in optic disc neuro-retinal rim to glaucoma onset, or, progression of established primary open angle glaucoma. Volunteers were divided into three groups, normal (n=32, mean age 67), ocular hypertensive (n=27, mean age 67) and open angle glaucoma (n=16, mean age 72). Results collected after one year (sd 135 days) showed the artery diameter reduced in both glaucoma (RE 0.009mm t=2.175 p=0.0039, LE 0.01mm t=3.114 p=0.004) and ocular hypertensive group (LE 0.001mm t=3.079 p=0.008). These results were consistent with other published work and showed the HRT to be a valid instrument for vessel analysis.

1.1 Definition of Glaucoma

Glaucoma can be defined as "*a group of progressive optic neuropathies that have in common a slow progressive degeneration of retinal ganglion cells and their axons resulting in a distinct appearance of the optic disc and a concomitant visual field loss*" (Weinreb & Khaw 2004). This definition is not universally accepted and variations on the parameters to define glaucoma are normally stated before a consideration of any prevalence survey (Foster 2002).

It is a well stated statistic that the prevalence for glaucoma for the over 40 age group, in the UK, is 2%(Quigley 1996). In a recent study in Sweden the prevalence increased with age, from 0.55% at 55-59 years to 2.73 between 75-79. Of significance was the increase of undiagnosed glaucoma with age (Heijl, Bengtsson, et al. 2013). To put this in context within the UK population for the same over 40 age group, both Diabetes Mellitus and coronary heart disease between the ages of 40-50 have a prevalence of 2% although this rises significantly with age; Cancer has a prevalence of 2% between 50-70 years, chronic obstructive pulmonary disease has a prevalence of 2% between the ages of 60-80 years. Diseases within Northern

Ireland have a similar distribution, cancer has a prevalence of 2%, chronic obstructive pulmonary disorder 1.9% and strokes/transient ischaemic attacks are 1.8% (Fibrillation 2014).

Intraocular pressure was first described as a significant factor in an optic neuropathy causing blindness approximately 100 years ago (Investigators 2000) For many years the definition of glaucoma was synonymous with elevated intraocular pressure (Hollows & Graham 1966). The study of glaucoma has lead to the discovery of progression in eyes where the IOP is well controlled (Öhnell, Heijl, et al. 2016; Spry & Johnson 2002) with the increased prevalence of this phenomenon glaucoma is now considered a multifactorial disease with inadequate methods of treatment (Tielsch 1996). Thus much effort is now directed towards identifying other risk factors.

It is difficult to establish causality in the study of glaucoma in humans since one cannot isolate a specific trait (Coleman & Miglior 2008). It is also unclear as to whether the disease, or the risk factor came first, a problem known as “temporal ambiguity”.

The search for risk factors have uncovered a variety of influences on the probability of glaucoma onset or progression, some of these are, age (Leske, Heijl, et al. 2007), race (Kass 2002), BMI (Leske 1995), central corneal thickness (Brandt, Beiser, et al. 2004), ischemia (Nickells 1996), vascular dysregulation (Grieshaber, Mozaffarieh, et al. 2007), and low ocular perfusion pressure (Leske 2009).

The current treatment of glaucoma is based on reducing the only modifiable risk factor namely intraocular pressure, this has been shown to reduce the progression of visual field defects attributed to glaucoma (Goldberg 2003).

1.2 Epidemiology of Glaucoma

Epidemiology is the study of causes and spread of a disease within a population.

Worldwide, glaucoma has been estimated to effect 60.5 million people in 2010 under its two main forms, primary open angle glaucoma (POAG) and primary angle closure glaucoma (PACG)(Quigley & Broman 2006). In 2013 this estimate has increased to 64.3 million and is projected to continue to rise to 76 million by 2020,

118.8 million by 2040 (Tham, Li, et al. 2014). Of the total number diagnosed with glaucoma, fourteen per cent will eventually be registered blind. The vast majority of this increase will be in Africa and Asia with only a small incremental rise posted in Europe, North America and Oceania until 2040.

The Department of Health Social Services and Public Safety in Northern Ireland (DHSSPS NI) conducted a sight test survey to gauge overall activity and outcomes with optometrists across Northern Ireland (Curran & Dooley 2014). The total referral rate was 4.6%, but the percentage within those numbers referred for glaucoma was 13.3% (table 1).

Referral Category	Count	% Of all referrals
Anterior Eye	29	16.1%
Cataract	42	23.3%
Contact Lens Service	0	0.0%
Eye Casualty	1	0.6%
Glaucoma Service	24	13.3%
GP	9	5.0%
Laser	2	1.1%
Low Vision Service	2	1.1%
Neurological	3	1.7%
Orthoptics	6	3.3%
Paediatric	2	1.1%
Retinal	36	20.0%
Visual Loss	12	6.7%
No Answer Provided	12	6.7%
TOTAL	**180**	**100.0%**

Table 1 Northern Ireland referral survey

The percentage of patients attending for an eye exam already diagnosed with glaucoma was 1.6%, and this would in line with the national average of glaucoma prevalence. Of the 180 referrals to ophthalmology 24 patients have been referred to the glaucoma service, given a prevalence of 1.6% this would suggest only 3 have glaucoma and the others are false positives.

Although it is an accepted statistic that approximately 2% of the population has glaucoma, between 50-67% of these patients go undiagnosed (Quigley & Broman 2006; Burr, Mowatt, et al. 2007). Without detection and treatment glaucoma disease

eventually causes blindness. Quigley et al estimated the disease progression by extrapolating the data of two random controlled trials for glaucoma progression and estimated the time, without intervention, to progress to blindness. For a single eye it was 23 years, but with treatment this was extended to 35 years.

Jay et al in 1993 looked at the estimated the mean rate of visual field loss by comparing the mean age of presentation of patients with early relative field loss with those who presented with absolute field loss within five degrees of fixation at the Glasgow infirmary (Jay & Murdoch 1993). Analysis of 177 patients showed similar prognosis to reach blindness with treatment to Quigley of 38 years, the rest were divided into groups depending on level of presenting IOP. Those with IOP 25-30mmHg progression to blindness was 6.5 years, whereas below 25 it was 14.4 years but IOP above 30mmHg blindness was predicted in 2.9 years.

The immediate conclusion for Optometry is that with the UK population at 63.6 million, and the workforce survey of optometry 2005/6 estimating that there were 17.5 million examinations per year (Sight Test and Workforce Survey), many cases of glaucoma are likely to go undetected.

1.3 Systemic Associations of Glaucoma

In a study of 180 patients with primary open angle glaucoma the most prevalent concomitant disease was systemic hypertension (Salim & Shields 2010) with a total of 73% of patients, the next highest was hypercholesterolemia with a significant, but, much reduced 47%. The question has to be asked if these conditions are linked or is the comorbidity factor simply one of age and therefore of no early consequence in diagnosis?

There is also a suggested relationship between glaucoma and neurodegenerative diseases such as Alzheimer's, Alzheimer's disease being the most common form of dementia. The classic findings are personality changes, progressive cognitive dysfunction, and loss of ability to perform everyday tasks. Visual impairment is common and appears relative to disease severity. Although there appears to be a greater percentage of Alzheimer's patients who have a comorbidity of glaucoma, the question is one of whether this is a causal relationship (Wostyn, Audenaert, et al. 2009). The suggestion by Wostyn (2009) was that, it is known that cerebrospinal fluid pressure is lower in dementia patients, and glaucoma patients, therefore, this lower pressure made the difference either side of the lamina cribrosa and leave the

retinal ganglion cells more susceptible to damage. This hypothesis was refuted in 2010 by case records (Phillips 2010) where cerebrospinal flow had been operated on and the optic discs did not develop cupping as expected. A longitudinal analysis over 24 years investigating if primary open angle glaucoma was associated with increased risk of developing Alzheimer's disease concluded no increase risk (Kessing, Lopez, et al. 2007). A recent study using a cohort of 87,658 patients diagnosed with primary open angle glaucoma, 217,302 with vascular dementia and 251,703 with Alzheimer's disease compared to a reference cohort of >2.5 million, concluded that the risk of Alzheimer's following diagnosis of primary open angle glaucoma, was not elevated, and the risk for vascular dementia was only modestly raised (Keenan, Goldacre, et al. 2015).

Diabetes is very often mentioned as a risk factor in glaucoma but the research is split on any correlation. Recent research by Coudrillier et al (Coudrillier, Pijanka, et al. 2015) suggested the link was scleral stiffness with diabetic eyes demonstrating an accelerated scleral stiffness compared to age matched controls. The age related increase in scleral stiffness was 87% larger in diabetic specimens compared to non-diabetic controls ($p=0.06$), the study suggested the age related increase in scleral stiffness was accelerated in eyes with diabetes which may have important implications for glaucoma. A recent meta analysis has suggested a definite link with diabetes, diabetes duration and fasting glucose levels being a significant increased risk factor for glaucoma, and additionally diabetes and fasting glucose levels being associated with slightly higher levels of IOP although this was a much weaker correlation (Zhao, Cho, et al. 2015). The suggested reason for the connection was vascular perfusion and reduced oxygen levels effecting vascular autoregulation. It should also be considered that diabetic patients have eye examination more frequently than the normal population so with this increased professional evaluation it is likely that glaucoma will be detected earlier.

1.4 Impact of Glaucoma on Everyday Life

Glaucoma in its final stage causes blindness. The World Health Organisation (WHO) definition of the health related quality of life (HRQoL) involves specific activities such as mobility, reading and driving. An additional aspect is psychosocial functioning, which is described as the psychological, social and emotional burdens of glaucoma. These aspects represent a growing interest from WHO and are

recognised as a distinctly different burden than ability to perform vision related tasks, but instead is a clinical consequence of chronic medical illness.

In an interesting piece of research by Chan (Chan, Chiang, et al. 2015) considered the psychosocial aspects of deteriorating field and acuity. Of the 192 glaucoma participants all acuity in the better eye was averaging at 6/9. This is good vision and would normally be ignored in the standard glaucoma clinic as a factor effecting practical life i.e. HRQoL. But, what the researchers found was that the chronic nature of the condition increased their anxiety by 63%, they also exhibited a 71% lower self-image and general reduced psychological well being (38%). Although there may be very little an eye care provider can do in the limited time spent examining a patient for glaucoma deterioration and medication compliance, it is worth noting that the patients psychological robustness is not as resilient as one might expect given the patients good vision, IOP under control and only mildly deteriorated fields.

The majority of patients diagnosed with glaucoma are not registered blind, but, in the UK glaucoma is the third largest cause for blindness registration (Green, Siddall, et al. 2002).

1.5 Glaucoma Referral from Optometric Practice

Optometry, with its presence on the high street, ease of immediate access, and general availability of diagnostic equipment create an optimum position for early detection of glaucoma with obvious benefits for the public. The benefits for individual patients could be described as

- Appointment availability
- Ease of premises access
- Diagnostic equipment
- Immediacy of appointments

The difficulty for optometrists is that only two in every hundred patients have glaucoma, so, the habit of seeing normal people makes the abnormal easy to detect, but, glaucoma in its infancy masquerades as normal.

Optometrists are responsible for 90% of glaucoma referrals in the UK and patients with primary open angle glaucoma contribute 25% of the workload in the hospital eye service (Gilchrist 2000). Unfortunately the other headline figure is that

undetected glaucoma cases in the community are estimated to be between 50-67% (Quigley 1996; Weih 2001; Burr, Mowatt, et al. 2007). A survey of the outcomes of referrals by UK community optometrist over a ten year period showed only 18% as positive referrals and 45.8% discharged at first review (Bowling, Chen, et al. 2005).

For Optometry this leads to a dichotomy of statistics suggesting on one hand a majority stake hold in referral to glaucoma clinics, but yielding high false positive rate, and therefore excessive use of ophthalmology resources. The clinical effectiveness an cost-effectiveness of screening for open angle glaucoma was assessed in the UK 2007 (Burr, Mowatt, et al. 2007). They assumed that with treatment the time to blindness in at least one eye was 23 years but with treatment this was extended to 35 years. Their statistics suggest that general screening of the population at any age is not cost-effective. Selecting groups with a higher prevalence of glaucoma e.g. family history, black ethnicity did appear to be worthwhile but this only included 6% of the population. Extending the groups further to cover other at-risk cohorts e.g. myopia reduced the prevalence and again was not cost effective. The economic model proposed has considerable parameter uncertainties and should either the cost of visual impairment rise or the rate of progression of glaucoma increase then the statistics for cost-effectiveness for screening become more favourable.

For the high street optometrist there is a pressure not to miss any pathology, set against an almost zero negative outcome for a high false positive referral rate. There is no disincentive to ‘not’ refer, and from a business reputation perspective there is a pressure to err on the side of caution.

A survey of optometry practices in N.Ireland assessed their readiness to take part in glaucoma co-management (Willis, Rankin, et al. 2000). The conclusion was that the equipment needed, namely tonometer, visual field instrument and some method of disc evaluation were present, but that more training was needed to effectively monitor an active condition. A UK wide survey of equipment found that most optometrists were well equipped for glaucoma detection but that the type of machines used was varied (Myint, Edgar, et al. 2011). The real problem is not the machines being used but the optometric knowledge applied to the patient and interpretation of results. An insightful comparison of survey results and actual patient experience was carried out in 2012 and found a poor correlation between self-reported clinical tests and routine tests performed on actual patients (Theodossiades, Myint, et al. 2012). This suggestion of below acceptable standard,

despite the high standard of equipment available, becomes manifest when referring, and the requirement to put medical diagnosis on paper. A survey of optometry referrals concluded that only 49% were of acceptable quality (Scully, Chu, et al. 2009).

The temptation is that one type of instrumentation becomes a panacea for glaucoma diagnosis, but as research has shown (Ng, Zangwill, et al. 2006) agreement between the visual field and the optic nerve head imaged by the HRT over a three month time period was only fair. The suggestion is that structural and functional tests are complimentary, and should be used in combination, for early detection of glaucoma. The only feasible way to improve glaucoma detection and referral is to raise the knowledge level of high street optometrists.

If it was shown that artery/vein diameter reduction was consistent in prediction of glaucoma diagnosis, then this could become a simple fourth measurement to reduce false positive referrals to the hospital system. It would only be with considerable further research that the weighting factor of this change could be compared to the known changes in IOP, disc changes and visual field loss.

2.0 Materials and Methods

2.1 Optic Nerve Head Anatomy

There are several parameters used to describe the optic nerve head (ONH)

- Size and shape of disc
- Size and shape of neuroretinal rim
- Size, shape and depth of optic cup in relation to size of disc
- Position of blood vessels in relation to neuroretinal rim
- Presence and position of peripapillary atrophy

There is considerable variability between individuals in a normal optic nerve. The ONH can vary in area from 0.8mm^2 to 5.6mm^2, with the average for Caucasians being in the range 1.74mm^2 to 2.47mm^2 and neuroretinal rim area ranging from 0.8mm^{2}to 4.66mm^2 (Jonas, Gusek, et al. 1988; Hoffmann, Zangwill, et al. 2007). It was also noted in this study that the horizontal cup/disc ratio was larger in 93.2% discs compared to vertical, thereby describing a vertically oval optic disc with a horizontally oval optic cup. The optic disc size does not vary significantly between males and females (Zangwill, Weinreb, et al. 2004) and the disc ceases to grow around the age 3-10 years old (Garway-Heath, Rudnicka, et al. 1998).
The degree of refractive error is correlated with disc size and is significantly smaller in eyes with a refraction of >+5.00 dioptres and larger in eyes >-8.00 dioptres compared to emmetropic eyes. However between the range +5.00>-5.00 optic disc size does not correlate with refraction (Jonas, Gusek, et al. 1988).

Although not applicable to the ethnic groups evaluated in this research, black individuals have significantly larger disc area compared to Caucasians and this must be taken into account when assessing the neuroretinal rim volume for glaucoma. The disc size smallest to largest appears to follow the trend Caucasian, Mexican, Asian and largest African-American The known increase in prevalence amongst African American is thought to be due to other comorbidities such as genetic susceptibility (Hoffmann, Zangwill, et al. 2007). The size of the disc is not effected by glaucoma (Caprioli 1992).

The optic nerve is the weak spot in an otherwise strong corneal-scleral envelope. The lamina cribrosa provides structural and functional support of the retinal ganglion

cells as they exit the relatively high-pressure ocular environment and enter the relatively low-pressure region in the retrobulbar space. The lamina cribrosa has developed into a complex structure to support the retinal ganglion cells and is nourished by the short posterior ciliary arteries. (Figure 1)

Figure 1 Vascular supply Optic Nerve Head

(https://classconnection.s3.amazonaws.com/68/flashcards/1756068/png/screen_shot_2013-02-08_at_121258_am1360300399719.png)

2.2 Ageing Changes

Death of the retinal ganglion cells and its associated loss of neuroretinal tissue observed at the optic nerve head is the hallmark of glaucomatous damage. There is considerable variation in the total number of retinal ganglion cells in the normal eye. The count varies between 600,000 - 1.2 million between different reports (Medeiros, Zangwill, et al. 2012; Medeiros, Lisboa, et al. 2013; Mikelberg, Drance, et al. 1989). Additional normal variability is found in the reduction of ganglion cell density with age (Repka & Quigley 1989), and this loss may accelerate after middle age (Jonas, Schmidt, et al. 1992). Jonas estimated that there was a mean loss of 4000 fibres annually after the age of 40 and this loss had the appearance of a bulk reduction in

volume rather than focal notching typical of glaucoma. Ageing also leads to a loss of retinal cells at an average rate of 0.3% across retinal photoreceptors (Panda-Jonas, Jonas, et al. 1995), retinal pigment epithelial cells (Panda-Jonas, Jonas, et al. 1995) and retinal ganglion cell axons (Repka & Quigley 1989).

2.3 Glaucomatous Changes

Loss of neuroretinal rim tissue with enlargement of the optic cup is the hallmark of glaucoma. This characteristic cupping of the optic nerve head is due to a combination of posterior bowing of the lamina cribrosa and apoptosis of retinal ganglion cells (RGC)(Xu, Weinreb, et al. 2014). The neuroretinal rim volume is affected in all quadrants but each sector involvement may be differentially effected depending on the stage of the disease (Jonas, Budde, et al. 1999). This pattern of change is not found in other causes of RGC death.

The pattern of glaucoma neuroretinal rim loss appears to start in the inferotemporal disc region and then progress to superotemporal, temporal horizontal, inferior nasal and finally superior nasal (Jonas, Fernández, et al. 1993).

A well-documented clinical feature of glaucoma is an optic disc haemorrhage (Suh & Park 2014). Disc haemorrhage is more commonly detected in open angle glaucoma with normal tension than in open angle glaucoma with high tension. The prevalence of disc haemorrhage in normal tension glaucoma is between 20.5% and 33.3%, but in high-tension glaucoma only between 8.3% and 17.6%. The prevalence of disc haemorrhage was found to be independent of gender (Suh & Park 2011).

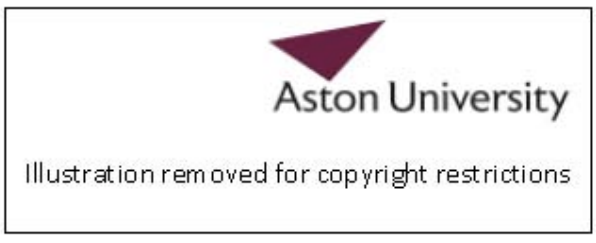


Figure 2 Four pressures known to impact on optic disc (Berdahl, Allingham et al 2008)

There are four pressures that are thought to interact and effect glaucoma progression (Figure 2) Cerebrospinal fluid (CSF) surrounding the optic nerve is a balance between the anterior force of the CSF pressure and the posterior force of the IOP in the area of the optic disc called the lamina cribrosa. The concern is that variations in this pressure difference can damage the optic disc (Berdahl, Allingham, et al. 2008). Blood pressure is linked with glaucoma in the form of ocular perfusion pressure (Costa, Arcieri, et al. 2009). This is a mathematical equation, which takes into account diastolic blood pressure and systolic blood pressure alongside IOP. It is considered in more detail in section 2.8.

2.4 Measurement of Blood Vessels

The first reference to using the HRT as a method of measuring blood vessel diameters was in 1997 (Joos, Singleton, et al. 1997), the scan from the HRT was uploaded to Photoshop™ software to improve the image quality and the measurement of vessels diameters compared between the two results. The HRT was found to be capable of measuring diameters although doubt was cast on the ability to detect vessel edges without photo enhancement.

The parameters of blood vessel change in diameter must be consistent across all refractive errors in a named ethnicity. Heidelberg has considered this potential liability when calculating for neurofibre rim volume. The HRT makes key assumptions about the eye involving refractive index of aqueous and vitreous humour and equivalent power of the cornea. These assumptions are then modified with the known value of the C-Curve (mm), which is the average value of the K readings.

(EQ1) C-Curve = (horizontal + vertical K-reading)/2

The keratometer readings were entered for every patient before scans were taken so that accuracy of measurements was maximised.

This approximation of eye parameters can be made more accurate by the measurement of axial length but the difference when this extra measurement was used in calculation compared to the HRT method was found to be insignificant (Garway-Heath, Rudnicka, et al. 1998).

The measurements for this research were carried out according to previously established procedures (Knudtson, Lee, et al. 2003), explained below.

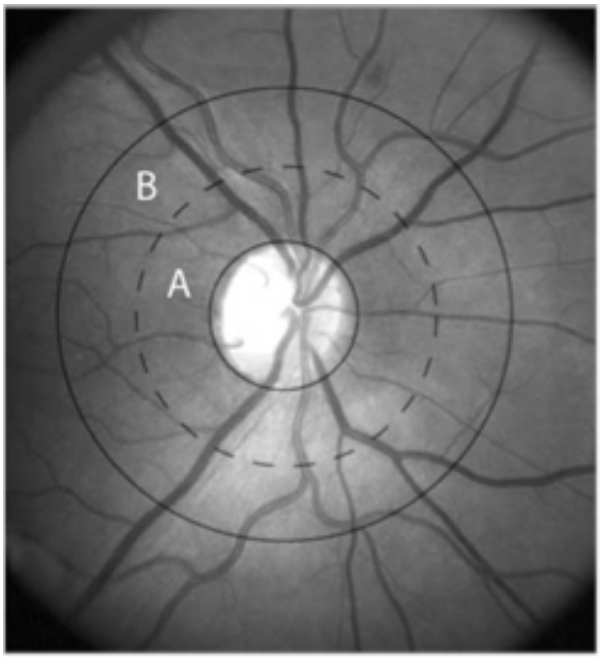


Figure 3 Zone A and Zone B placement for measurement of vessel diameter (Knudtson et al)

Zone A is one half disc diameter from the optic disc margin and zone B is between half disc diameter and one and one half disc diameter from optic disc margin. All retinal vessel measurements were taken in zone B (Figure 3).

The vertical length of the optic disc was measured by using the interactive measurements tab of the HRT 3 (version 1.5.10.0, 2006 Heidelberg Engineering GmbH). The horizontal line was placed at perpendicular to the edge of the neuroretinal rim and the y-axis number noted, the horizontal line was then moved down to the inferior edge of the neuroretinal rim and the vertical height calculated as the top number subtracting the bottom. Half of this vertical height was then added to the top and bottom of the disc edges to give the start of zone B. The edge of zone B was simply this edge plus half of the disc height.

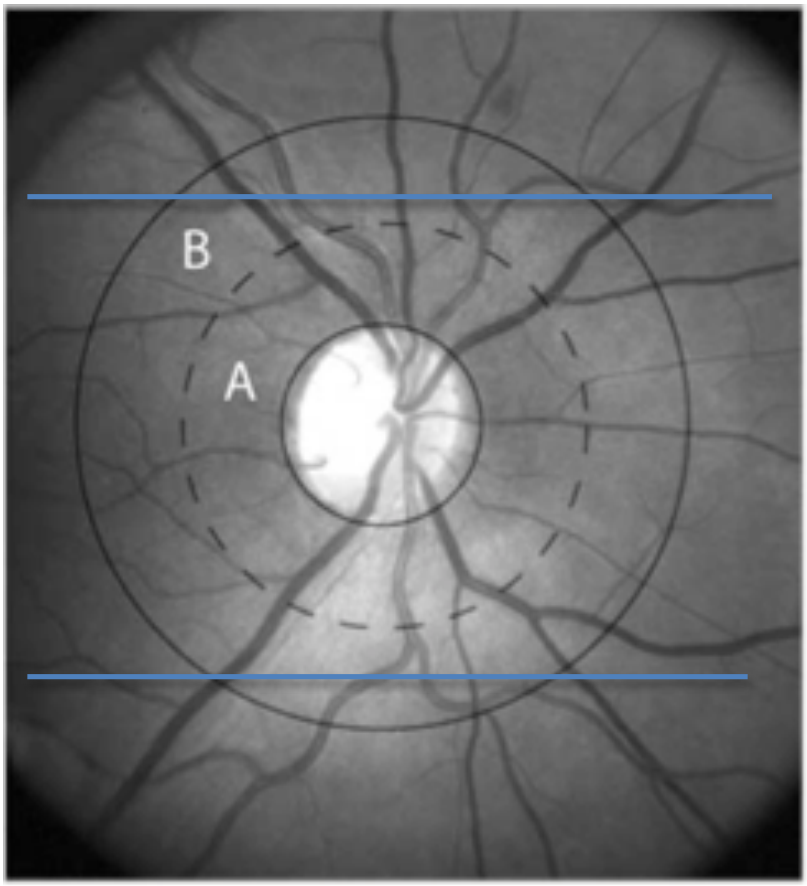


Figure 4 Measurements taken of BV diameter above and below on blue horizontal lines

The position of the horizontal line within zone B was determined as a best-fit line to cross as many vessels perpendicular to their direction as possible. Any vessels travelling parallel to the horizontal line in zone B were ignored, in some cases the defined measurement line would travel obliquely across a blood vessel. This oblique diameter would on many occasions be larger than the average diameter of either arteries or veins, but, the yearly follow-up measurement is taken along the exact horizontal line and it is the change in diameter that is correlated with glaucoma onset or progression.

The diameter of the blood vessel is the distance between vessel edges as crossed by the best-fit horizontal line. The HRT is able to measure to 0.001mm axially so in theory there was no minimum vessels diameter. An important practical, restricting parameter, was that the cursor only allowed movements of 0.011mm between two positions. Vessels that overlapped obscuring vessel edges could not be included, but these crossing were minimised by best-fit horizontal line.

The horizontal y-axis value was noted and used for the comparative measurements for that particular volunteer one-year later. Because the HRT aligned the second

visit scan to the same orientation as the first all blood vessels were at the same angle to the designated line and therefore all measurements were comparable.

2.5 Visual Field Measurement

Visual field for this research was performed exclusively on the Humphrey Visual Field Analyser (Carl Zeiss Meditech, Dublin, CA). The values used in comparative statistics were the mean deviation and pattern standard deviation values calculated by the Humphrey Statpac program. The visual field is classed as unreliable if the numbers of fixation losses exceed 33%. This is a figure that has been raised from the original accepted cut off point of 20%. The lower point was calculated by Sanabria et al (Sanabria, Feuer, et al. 1991), who had found that the sensitivity and specificity dropped off past this percentage of fixation losses. The accepted fixation loss percentage has since been raised to 33% because of research done that showed diagnosed glaucoma patients missed more points because the disease had a reduced field sensitivity and therefore made patient fixation less reliable (Katz, Gilbert, et al. 1997). In order to combat this inherent unreliability the number of field tests within two years has been raised to a recommend five so as to determine a reliable mean field defect (Artes & Chauhan 2005). The stimulus size for this research is the accepted value of Goldmann size III, this size was chosen because it has been shown to be more resistant to optical defocus (Atchison 1987), and lens opacities (Wood, Wild, et al. 1987).

2.6 Vessel Analyses

There have been two methods used in literature for summarising retinal vein measurements taken from digitised or digital retinal images;

1/ Methods for Evaluation of Retinal Micro vascular Abnormalities Associated with Hypertension/Sclerosis in the Arthrosclerosis Risk in Communities Study (Hubbard, Brothers, et al. 1999)

2/ Revised Formulas for summarising retinal vessel diameters (Knudtson, Lee, et al. 2003)

The Parr-Hubbard method was a combination of the methods developed by Parr et al and Hubbard et al (Parr & Spears 1974)(Hubbard, Brothers, et al. 1999) the

original Parr method attributed a different multiplication factor to each vein or artery depending on is level of branching from the main trunk. This is a very labour intensive exercise and is prone to errors because disc resolution did not always allow an exact determination of branching. The revised formula of Parr-Hubbard just combined the largest vessel with the smallest and disregarded branching continuing pairing and calculation until a central equivalent value was obtained.

This research will use the most common formula used in current literature, “Revised Formulas for summarising retinal vessel diameters”.

The revised formula is restricted to the six largest retinal arterioles and veins (Knudtson, Lee, et al. 2003). To compensate for the considerable variation in the number of bifurcations in an eye the relationship between trunks and branches was expressed in terms of an empirically derived branching coefficient.

(EQ2) Branching Coefficient = $(w^2_1 + w^2_2)/W^2$

Where w_1, w_2 and W are the widths of narrow branch, the wider branch and the parent trunk. The branching coefficient was estimated by Knudtson et al to be 1.28 for arteries and 1.11 for veins. When inserted into the above formula and solved for W it yields two approximate formals for vessel equivalents

(EQ3) Arteries $W= 0.88*(w^2_1 + w^2_2)^{1/2}$

(EQ4) Venules $W= 0.95*(w^2_1 + w^2_2)^{1/2}$

These formulas provide a more precise and consistent estimate of retinal vessel calibre and so these were chosen to calculate central retinal artery equivalent (CRAE) and central retinal vein equivalent (CRVE) for this research.

The mean of the arteries RE and LE were calculated. This value allowed comparison to past and current research measurements to assess if they were generally in agreement (Table 2).

	Arterial Diameter RE (µm)		
	Blue Mountains Study (2005)	Present Study	Beaver Eye Dam (2004)
	RE	RE	RE
Normal	194.0 (0.4)	301.5 (41.5)	202.3 (21.97)
Glaucoma	183.0 (2.6)	262.2 (20.47)	-----
OHT	195.0 (1.6)	271.9 (29.6)	-----

Table 2 Comparison of Current Research Artery Diameters RE with Present Research

The larger diameter measurement taken with the HRT interactive tab is explained by the axis of the dissecting line of the artery or vein. The horizontal line is fixed at a certain y-value; this allows continuity of position for measuring future vessel diameters. The line intercepts the arteries and veins at different angles, which are greater than a perpendicular bisect of the vein, increasing the measured diameter. The important measurement is not the measured diameter compared against a normative set of data, but instead the difference between two consecutive diameters, measured at the same position, one year apart. Although the average diameter does not correspond with the research normal it is the change of diameter, which is the important consideration and the results from this research do follow the accepted at risk groups. The larger value of the standard deviation is explained by the bisection of some blood vessels being perpendicular and others being closer to parallel.

The arteries and veins were measured and the average diameter at baseline and one year is shown (table 3&4)

	R&L arterial mean baseline and one year			
	Baseline		1 Year After Baseline	
	RE	LE	RE	LE
Normal	301.5 (41.5)	269.9 (60.97)	302.4 (38.6)	269.2 (59.38)
Glaucoma	262.2 (20.47)	246.3 (38.53)	257.8 (23.4)	236.6 (38.04)
OHT	271.9 (29.6)	260.0 (48.69)	262.8 (34.83)	249.6 (37.07)

Table 3 R&L arterial mean baseline and one year

	R&L Venous mean baseline and one year			
	Baseline		1 Year After Baseline	
	RE	LE	RE	LE
Normal	349.3 (45.29)	329.7 (72.71)	344.4 (41.39)	332.4 (72.56)
Glaucoma	315.8 (46.66)	285.4 (34.13)	310.0 (39.36)	282.7 (28.36)
OHT	344.2 (40.49)	326.8 (37.18)	330.3 (37.45)	322.4 (32.67)

Table 4 R&L Venous Mean baseline and one year

In the normal group using a paired sample t-test there was no significant difference between either the RE mean artery diameter baseline and one year (t=-0.322, df=31, p=0.75) or LE (t=0.210, df=31, t=0.84). There was a significant change in diameter in the Hypertensive group for both the RE (t=2.175, df=26, t=0.039) and LE (t=3.11, df=26, t=0.04). The final glaucoma group did not show a difference from baseline with the RE (t=1.083, df=15, p=0.296) but there was a significant change for the LE (t=3.079, df=15, t=0.08), (figure 5+6).

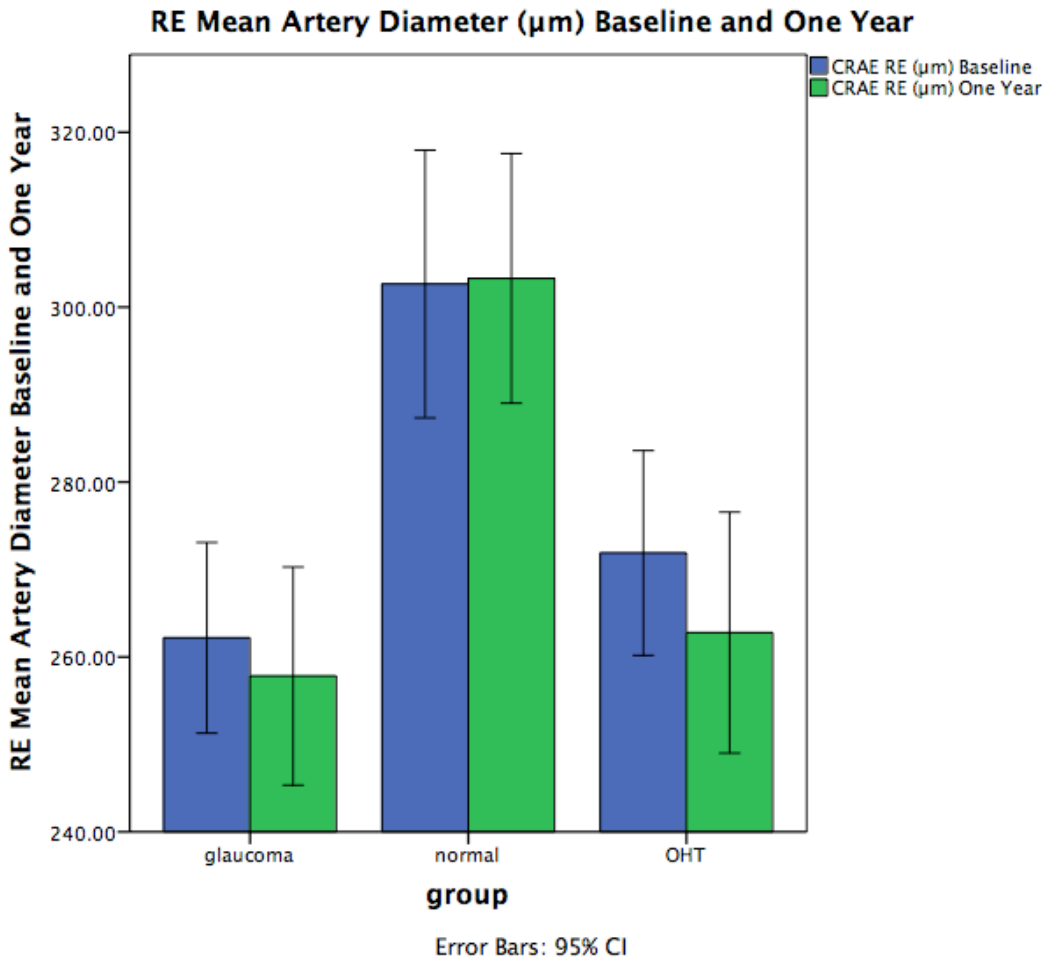


Figure 5 RE Mean Artery Baseline and One Year

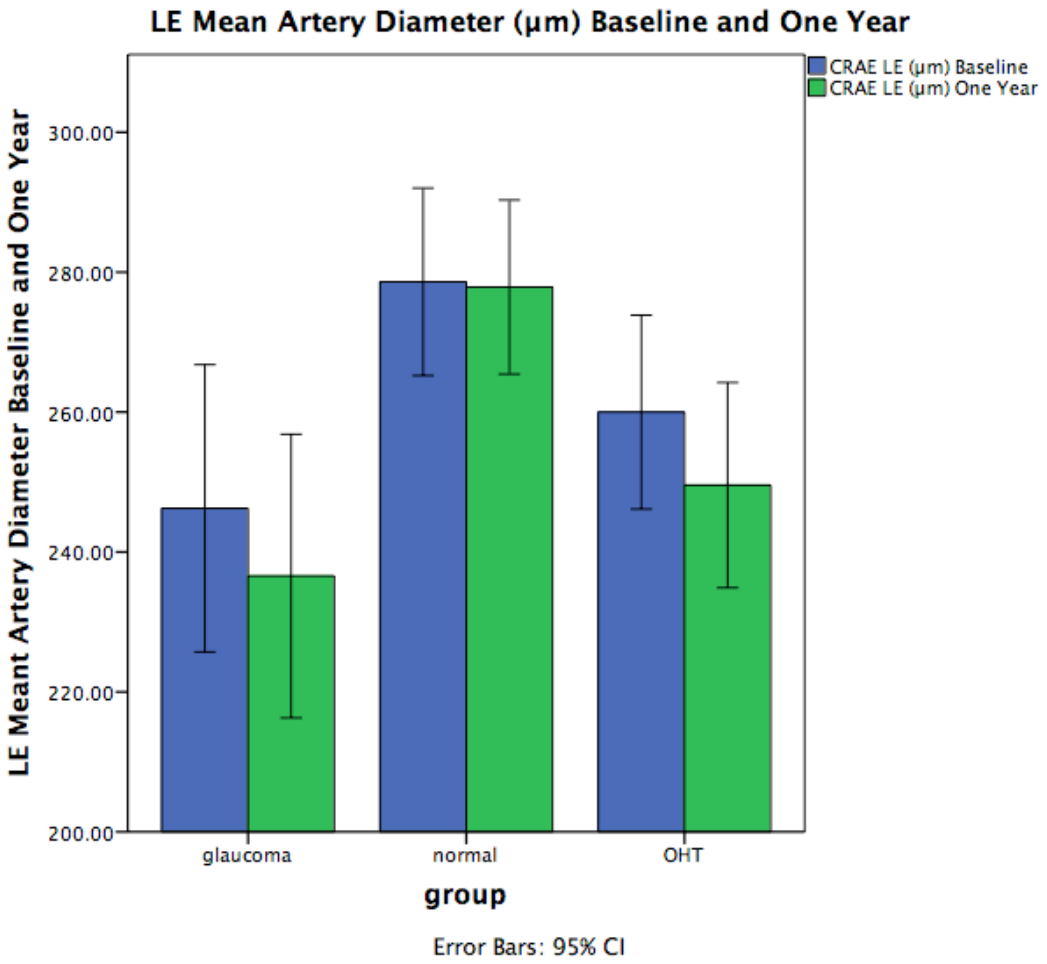


Figure 6 LE Mean Artery Baseline and One Year

Comparing the vein diameter change there was no significant difference in either the normal or glaucoma group, but, the Hypertensive group had a significant change in the RE (t=3.237, df=26, t=0.03) but not the LE (t=1.254, df=26, t=0.221).

2.7 Ocular Blood Supply

Figure 7 Anatomy of Ocular Blood Supply (http://www.retinareference.com/anatomy/arterial.jpg)

The common carotid artery provides circulation to the head and neck; this major artery divides into two, called the internal and external carotid. It is the internal carotid that subdivides into the ophthalmic artery and subsequently the central retinal artery and posterior ciliary arteries (figure 7). There is considerable variation in the exact pattern of branching from the ophthalmic artery, but, the central retinal artery is one of the first, and smallest of the branches leaving the main ophthalmic artery as it lies below the optic nerve and enters the sheath of the nerve about 10-12 mm behind the globe (Remington 2011). Within the optic nerve, collaterals come off the central retinal artery to supply the nerve itself, and pia mater. The central retinal artery passes through the lamina cribrosa approximately 8mm posterior to the globe and travels anteriorly central in the optic nerve until it enters the optic disc and divides into superior, inferior, nasal and temporal branches (Semmer, McLoon, et al. 2010; Bell, Severson, et al. 2009)(figure 8).

Figure 8 Blood Supply to the Optic Nerve Head

(http://clinicalgate.com/wp-content/uploads/2015/04/B9781437704341000153_f015-003-97814377043413.jpg)

The posterior ciliary artery (PCA) circulation is the major source of blood supply to the ocular structures and optic nerve head. After branching from the ophthalmic artery they travel along the optic nerve and further divide into multiple branches, which then pierce the sclera around the optic disc. The PCAs are the blood supply for the optic nerve head, peripapillary choroid circle of Zinn, Haller and choroid. These choriocapillaries exist as a single network of continuous capillaries located directly beneath the retinal pigment epithelium. This choroidal circulation accounts for 85% of the total ocular blood flow (Ehrlich, Harris, et al. 2010).

The major source of blood supply to the inner retina (extending from neuroretinal layer to inner nuclear layer) is the central retinal vein. The outer portion of the retina (outer section of inner nuclear layer to retinal pigment epithelium) receives its blood supply from the choriocapillaries.

Retinal circulation is characterised by a low level of flow and high level of oxygen extraction. It is autoregulated, but receives no autonomic innervation. Tight junctions exist between the capillary endothelial cells forming a blood retinal barrier similar to the blood brain barrier of the CNS (Hofman, Hoyng, et al. 2001). This barrier protects the tissues by only allowing metabolites through, but, the optic nerve head prelaminar portion has been shown to be not as efficient a barrier as the rest of the

retinal circulation and this may have some part to play in allowing a damaging permeability and effect regulation of blood flow (Hofman, Hoyng, et al. 2001).

2.7.1 Changes in a Normal Eye

The definition of a normal blood vessel diameter is difficult given the number of confounding factors namely, age (García-Ortiz, Recio-Rodríguez, et al. 2015), sex, ethnicity, optic disc size (Lee, Klein, et al. 2007), and systemic conditions (Sun, Wang, et al. 2009; Sherry, Wang, et al. 2002; Leung, Wang, et al. 2003). Older people are known to possess a narrower vascular calibre. Retinal arterioles are estimated to decrease by 1.8-4.8μm for each decade of increasing age independent of sex and hypertension (Leung, Wang, et al. 2003; Wong, Klein, et al. 2003). Retinal blood vessels are an everyday observation for the high street optometrist. The vessels are described on patient record cards in terms of tortuosity, artery/vein (A/V) ratio and any focal decrease of the vein diameter crossed over by an artery.

The historical reason for assessing blood vessel calibre is to predict the possibility of systemic blood pressure and refer for treatment. The prevalence of blood pressure over the age of 40 in the UK (taken at >160/90) is 10-15% of the population (Sharp, Chaturvedi, et al. 1995). The patients with systemic hypertension have an increased mortality rate compared to normotensive from stroke (Ikram, De Jong, et al. 2006), coronary artery disease (Wong, Kamineni, et al. 2006), and peripheral arterial disease (Rosendorff, Lackland, et al. 2015). It is with this background of blood pressure detection in the eye that all optometrists are trained to observe retinal vessel calibre.

The commonly quoted A/V ratio is used to describe artery narrowing as a comparison to vein diameter. Arteriolar narrowing is associated with prolonged raised blood pressure, particularly diastolic (Scheie 1953), and age (Fuchs, Maestri, et al. 1995). The other features used to describe artery changes linked with blood pressure are arteriolar reflex, arteriolar tortuosity and focal arteriolar calibre changes (Hurcomb, Wolffsohn, et al. 2001). It has been estimated that with each 10mmHg increase in arterial blood pressure the retinal arterioles reduced by 2.0-2.4μm (Mitchell, Cheung, et al. 2007). Unfortunately subtle changes of A/V ratio are difficult to reproduce in manual assessment, and consistency is variable with practitioners of different education and experience (Heitmar, Kalitzeos, et al. 2015).

Medication may also be a complicating factor in the clinical decision as to whether blood vessel diameter has changed due to glaucoma. The question of vessel diameter change due to systemic medication has been considered (Wong, Knudtson, et al. 2005), the retinal photographs in the Beaver Dam Eye Study were digitised and retinal vessels measured and compared to the participants current medication. There were no significant correlations other than patients already taking beta-blocker topical medication had narrower arterial and venous diameters, but notably those taking beta-blocker systemic medication did not.

Although this research did not include multi-ethnicity participants, measurement across a range of ethnic background reveals that black and Hispanic individuals have larger arteriolar and venular calibres than whites and Chinese (Tien, Islam, et al. 2006). Of greater significance was the association of smaller retinal arteries to higher diastolic and systolic blood pressure, current alcohol consumption, greater body mass index and higher levels of homocysteine. Larger retinal arteries were related to diabetes, current cigarette smoking and higher levels of plasma fibrinogen. Larger retinal veins were related to diabetes, current cigarette smoking, greater body mass index and higher levels of serum glucose.

Retinal vascular calibre is gathering an increased background of research to indicate validity in its measurement as a routine part of medical data collection as a prognostic factor for a patient without signs or symptoms of any ocular condition. The Blue Mountains Eye Study examined 3654 patients at baseline and 2461 patients at 5 and 10 years and concluded that retinal arteriolar narrowing was associated with long term risk of open angle glaucoma (Kawasaki, Wang, et al. 2013). The difficulty in determining change in blood vessel diameter is not in achieving a measurement of that change but in considering how normal the degree of change is when set alongside the range of other factors adding to vessel narrowing.

2.7.2 Retinal Vessel Measurement

Because vascular calibre is considered a valuable measurement for prediction of both cardiovascular and cerebrovascular disease (Sun, Wang, et al. 2009), and the retina facilitates non-invasive *in vivo* measurement of blood vessel diameter, the potential prognostic ability to be gained from accurate and repeatable measurement of artery and vein diameters has lead to a number of instruments being developed

to attain data, Fundus photography, Zeiss Retinal Vessel Analyser and the Dynamic Vessel Analyser.

Fundus photography is a standard machine in most high street optometrists. The evaluation of blood vessel diameter using these photos is based on the development of appropriate software, recent advances have shown good agreement with manual measurement (Bhuiyan, Kawasaki, et al. 2013).

The Zeiss Retinal Vessel Analyser^{tm} (RVA, Imedos GmbH) is a commercially available tool for assessment of retinal vessel diameter in relation to time. The RVA assesses retinal vessel diameter by using the brightness difference between blood vessels and surrounding retina to allow computer based software to make the calculation. Dilation is essential to achieve adequate image quality and a green filter is used to attain optimum contrast of the erythrocyte column flowing through the blood vessel against the surrounding retina (Garhofer, Bek, et al. 2010). The RVA can measure to 90μm. The limitations of the RVA are a requirement for dilation and a notable susceptibility to cloudy media e.g. cataract which degrades the image making computer analysis of image unreliable. The HRT can compensate for mild eye movement but the RVA needs good fixation to achieve quality image. In current research with this machine it appears to be an accurate system for measurement of blood vessels (Polak 2000).

The Dynamic Vessel Analyser is a commercially available tool for the assessment of retinal vessel diameter in relation to time. In principle it analyses the brightness profile of the vessel using video sequences obtained with a conventional camera. This machine allows stimulation of the blood vessels during image acquisition and may in the future be a better version of static measurement (Heitmar, Blann, et al. 2010). But this may be a difficult machine to bring to the high street optometrist given its very specific purpose.

2.8 Ocular Perfusion Pressure

Blood Pressure is a constant background factor in both the increased risk of developing glaucoma (Charlson, de Moraes, et al. 2014), and a potential indicator for glaucoma progression (Costa, Arcieri, et al. 2009). It is a known comorbidity variable in relation to glaucoma with increased frequency, in glaucoma patients, of migraine (Phelps & Corbett 1985), silent myocardial infarction (Kaiser, Flammer, et al. 1993), and cerebrovascular accidents (Feldman, Sweeney, et al. 1969). Blood

pressure reduces overnight, but a drop of more the 20% compared to the mean daytime arterial pressure has been shown to be an important finding in normal tension glaucoma patients who progress in visual field defects compared to patients who have a smaller daytime/night-time blood pressure disparity (Lee, Choi, et al. 2015).

Blood pressure can be converted in combination with IOP, to ocular perfusion pressure, and it is this variable, which is a more useful indicator in the detection glaucoma, or, it's prediction for progression.

In this research a battery operated Omron BP monitor was used to attain the blood pressure values. This was done via an arm cuff that was wrapped around the patient's bare left arm and the start button pressed on the machine. After inflation and deflation the machine produced two readings, diastolic pressure and systolic pressure. The systolic pressure is defined as the pressure in the arteries when the contraction of the heart forces blood into them at the height of pulsation. The diastolic pressure is the pressure maintained by the elastic recoil of the large arteries during cardiac relaxation.

The gold standard for BP measurement is the mercury sphygmomanometer. The Omron monitor (MIT3 HEM-7270-E, Omron Healthcare Europe B.V.) was chosen, it is well known in high street pharmacies. Measuring BP using the arm cuff has well documented instructions to reduce erroneous readings generally attributable to poor cuff positioning (Ramsey 1991). This type of automated instrument was compared to the gold standard and the comparative results were considered valid (Yacoub, Dettenborn, et al. 2005).

Ocular perfusion pressure (OPP) is defined as the difference between arterial and venous pressure. In the eye, venous pressure is considered to be the same or slightly higher than IOP. OPP can therefore be defined as the difference between IOP and BP. This can further be broken down into diastolic and systolic perfusion pressure. In the eye, the ocular perfusion pressure can be referred to as systolic (SOPP), diastolic (DOPP) and mean ocular perfusion pressure (MOPP). MOPP is calculated by the formula

(EQ5) Mean Ocular Perfusion Pressure= 2/3 (DBP+1/3(SBP-DBP))-IOP

DBP= diastolic blood pressure SBP= systolic blood pressure IOP=intraocular pressure

When recording MOPP over 24 hours two different equations were used for sitting and supine body positions. The supine position is calculated by multiplying by 115/130 and the sitting position uses the multiplier 95/140, this was used as the equation to calculate MOPP when the patient was monitored for 24 hours.

Further ocular perfusion pressure equations divide out systolic and diastolic values

(EQ6) Systolic Ocular Perfusion Pressure= Systolic Blood Pressure-IOP

(EQ7) Diastolic Ocular Perfusion Pressure=Diastolic Blood Pressure-IOP

OPP is complex because of auto regulatory processes that attempts to keep blood flow constant. Epidemiological studies have shown low OPP to be a risk factor in the prevalence, incidence and progression of glaucoma (Leske 2009). The Early Manifest Glaucoma Trial (EMGT) reported that patients with low systolic OPP showed almost a 50% higher risk for the progression of glaucoma (Leske, Heijl, et al. 2007). In contrast, the Rotterdam Eye study (Hulsman, Vingerling, et al. 2007) showed an increased risk of progression, with only diastolic ocular perfusion pressure. This obvious dichotomy between which perfusion pressure measurement is the significant predictor for glaucoma is further complicated by the results from Kidd et al (2008), who monitored ocular blood flow using a scanning laser Doppler flowmeter. This recorded blood volume, flow and velocity in the sitting position. Their results demonstrated that older patients had a reduced ocular blood flow at night despite MOPP remaining constant throughout the 24-hour cycle. This was in contrast to younger patients who had no change in ocular blood flow despite MOPP reducing significantly at night (Kida, Liu, et al. 2008). The conclusion was that age compromised the auto regulatory capacity present in younger patients to ensure adequate perfusion of ocular tissues. Glaucoma and a normal control group were compared for diurnal variation over 24 hours by Sehi et al, they concluded the variation was widest for glaucoma and the MOPP value was lowest at 7am, a time whenever IOP is reaching its peak and BP measurements are in a trough (Sehi, Flanagan, et al. 2005).

The control of the blood pressure, and consequently, ocular perfusion pressure, has been shown to reduced visual field progression in those receiving blood pressure medication (Hirooka, Baba, et al. 2006). Blood pressure is diurnal, but unlike IOP, it has its lowest point around 3am compared to an increasing IOP. This increased dip in blood pressure at night has been postulated to be responsible for visual field

progression in glaucoma sufferers with otherwise stable IOP (Graham & Drance 1999; Charlson, de Moraes, et al. 2014). The variation of IOP over 24 hours was estimated to be a risk factor for glaucoma if it varied by greater than 9.4mmHg (Hughes, Spry, et al. 2003).

The opposite IOP rhythm is difficult to explain since aqueous production is known to decrease at night (Reiss, Lee, et al. 1984), it has been postulated that this increase in IOP is due to reduced episcleral outflow.

Auto regulation is the ability of the capillary bed to regulate blood flow via resistance in the vein. This is necessary to allow a stable environment for the cells in normal blood pressure variation. In contrast to the extra ocular and choroidal vessels, retinal vessels have no neural innervation, therefore only local vascular mechanisms are responsible for regulating flow (Schmidl, Garhofer, et al. 2011). Glaucoma is considered to have impaired blood flow regulation, one study comparing resistance in the central retinal artery between normal and glaucoma subjects in the sitting and supine position showed an inability to adjust resistance in glaucoma patients (Feke & Pasquale 2008). Grieshaber et al have alluded to this abnormal auto regulation, in the form of vascular dysregulation. They suggested an underlying endothelium defect. The vascular endothelium cells are a confluent monolayer of flattened cells that line blood vessels. This layer acts to regulate vascular tone and when the complex chemical chain breaks down the result is an irregular vasospasm which effects ocular blood flow (Grieshaber, Mozaffarieh, et al. 2007). They named this phenomenon endothelium based defect primary vascular dysregulation and linked it to cold extremities and reduced desire to drink due to reduced reabsorption of salt in the kidneys. This link with sodium uptake and normal tension glaucoma has been noted before (Pechere-Bertschi, Sunaric-Megevand, et al. 2007). This background catalyst for glaucoma leads to the potential assertion of two distinct types of chronic open angle glaucoma, type one driven by vasospastic factors and not as easy to control with IOP lowering drugs and the other which responds well to reduction of IOP (Schulzer, Drance, et al. 1990).

Because of this weakness in glaucoma patients, then, Ocular Perfusion pressure when it is low becomes a risk factor for ischemia during changes in mean arterial pressure and diurnal spike in IOP.

As a confounding factor, both diurnal variation of BP and IOP are known to be more variable in primary open angle glaucoma with increased spikes and more

pronounced troughs generally indicating a greater probability of glaucoma (Quaranta, Katsanos, et al. 2013). The normal variation is shown in figure 9 & 10

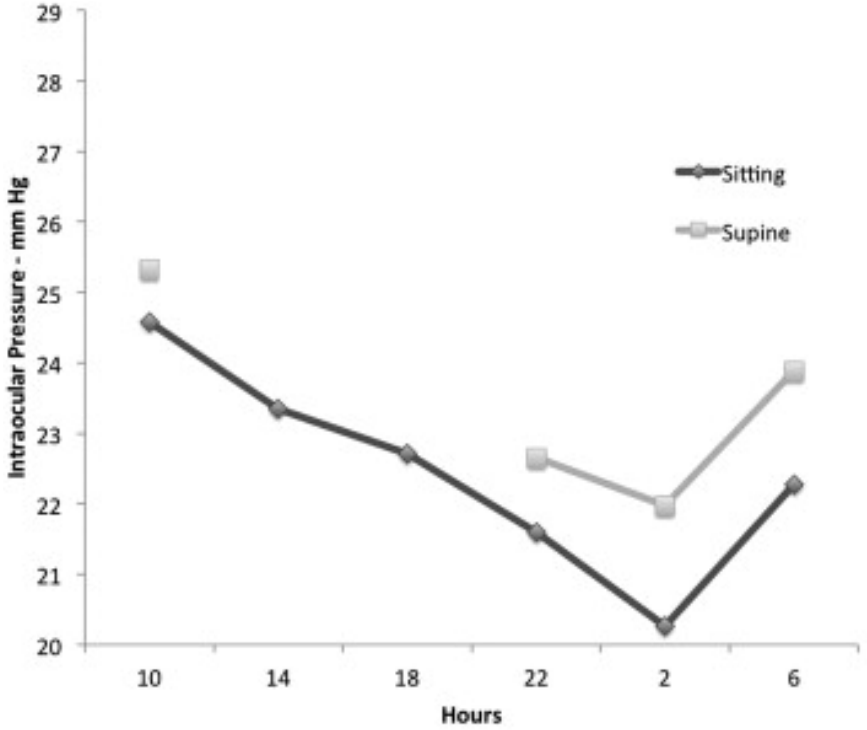


Figure 9 Normal IOP in sitting and supine position (Quaranta, Katsanos et al 2013)

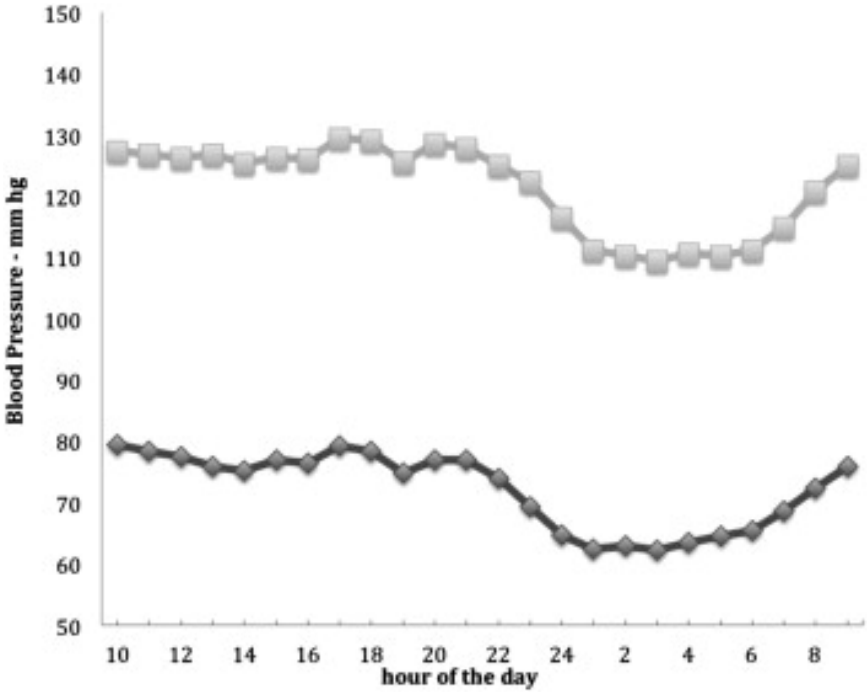


Figure 10 Diurnal BP of Systolic and Diastolic Blood Pressure (Quaranta, Katsanos et al 2013)

2.9 Imaging the Optic Nerve Head

Confocal Microscopy was invented by Professor Marvin Minsky in 1955 (Minsky 1988) . Professor Minsky's father was an ophthalmologist and Marvin had spent his childhood surrounded by optical instruments and lenses. His obsession with understanding the brain lead him to consider the basics of how cells connect to one another, and since this could not be done with simple staining techniques the pursuit of a machine to measure a single point of illumination took him in the direction of confocal microscopy. Initially he used zirconium arc as the light source and moved the sample in order to scan it, but it was the future invention of laser and the modification of scanning mirrors that made the confocal microscope practical in both use and production for retail sale.

The HRT machine was developed in the late 1980's with the first research paper written in 1988 describing the measurement of the optic nerve in vivo (Zinser, Wijnaendts-van-Resandt, et al. 1988).

2.9.1 The Heidelberg Retinal Tomograph (HRT)

The Heidelberg Retina Tomograph 3 (HRT 3) is a confocal scanning laser imaging system used for the analysis of three-dimensional images of the posterior segment of the eye, specifically the optic nerve head. The HRT has a focal adjustment range of -12D to +12D spherical (in 1.00D steps), with external magnetic, clip-on lenses, allowing correction of astigmatism in the range -6D to +6D (in 1.00DC steps). The image acquisition time is between 1-6 seconds with an optical resolution of 10µm/pixel transversal and 62µm/pixel longitudinal. The size 3D image acquired is 384*384*64 pixels.

2.9.2 Imaging Principles

A Confocal Optical System is defined as " *Measurement of light that can only reach the photo detector if it is reflected from a narrow area surrounding the set focal plane. Light reflected outside of the focal plane is highly suppressed. For this reason, a two dimensional confocal image may be regarded as an optical section through an examined object at the location of the focal plane."* (HRT2 & HRT3 Glaucoma Premium User Manual V3.2). A series of optical images are acquired for different positions along the optical axis giving the resultant three-dimensional image, this imaging technique is known as Scanning Laser Tomography.

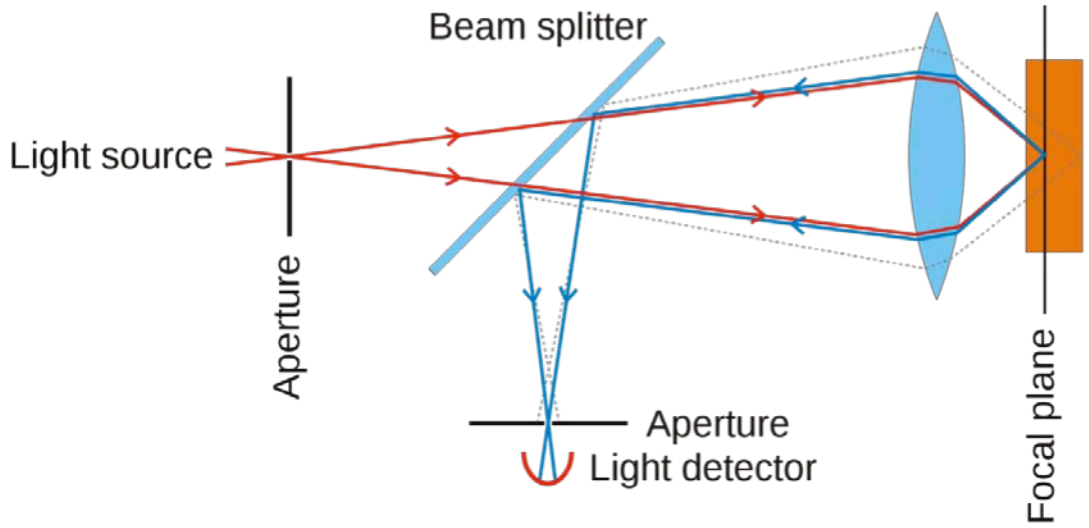


Figure 11 Principles of Confocal Scanning showing placement of pinholes in front of the laser and detector

(https://upload.wikimedia.org/wikipedia/commons/thumb/d/dc/Confocalprinciple_in_English.svg/1280px-Confocalprinciple_in_English.svg.png)

To acquire the optical section images, laser light is focused on the retina and is periodically deflected by oscillating mirrors so that a two dimensional section of the retina is scanned sequentially (Figure 11). The HRT moves the focal plane from anterior to posterior along the optical axis. This is a build up of the 3D image by slices. The amount of light reflected at each point of the slice is measured using a light sensitive detector. From the distribution of the amount of light along the optical axis i.e. perpendicular to sequential scanned plane, the height of the retinal surface can be calculated. The end product is a confocal stack of between 16-64 optical slices; the number of slices is determined automatically by pre-forming a pre-scan which determines the optimum scanning depth for a particular optic nerve head. This mathematical data results in a three dimensional x, y, z matrix that can be displayed as a topographic map (Figure 12).

The z-profile (fig 12,b) of each point may be presented as a plot of reflectance intensity versus scan depth. The peak intensity from the z-profile plot is assumed to correspond to the location of the internal limiting membrane that overlies the retina and the optic disc

Figure 12 Principle of Confocal Scanning Laser Tomography (a) "stack" of images at incremental focal depths. Measurements in the z-axis are referred to as axial, and those of the x and y axis are named transverse. (b) The set of axial values at a given transverse coordinate (x^1, y^1) and is known as z-profile. (c) The axial location of each z-profile maximal reflectance at coordinate (x^1, y^1) (d) axial locations are mapped to topographic height image (e) confocal scanning three-dimensional representation of (d)

2.9.3 Image Acquisition and Quality

The instrument uses a diode laser with a wavelength of 670nm. A three-dimensional image is generated as a series of sequential scans, each taking 24 milliseconds starting above the retinal surface then capturing parallel images at increasing depths. This 24-millisecond scan per section is faster than involuntary eye movements since micro-saccades last for approximately 30-50 milliseconds (Poletti & Rucci 2015). Each scan is composed of 384x384 pixels scanning a 15 degree area of the retina to a depth of 1-4mm, depending on the determination algorithm of

the pre-scan for each patient. Each successive scan plane is set to measure 0.0625 deeper as scans are taken incrementally through the tissue. Thus, if the pre-scan determines that a 1mm scan depth is required, 16 image planes will be used, whereas if the depth is 4mm then the imaging planes are set to 64.Three sets of scans are automatically captured at each acquisition allowing for data redundancy. The stack of image sections is then aligned using Heidelberg's Trutrack^{tm} technology, which discards any scan outside acceptable quality standards. This quality is determined by a standard deviation calculation between the three sequential scans on each point this value is then assigned a cut off value of 50, above which the scan is deemed unacceptable scan quality.

The quality reduction may be due to blinking, or fixation shift, and faulty scans are automatically repeated to ensure three adequate scans are acquired for image analysis.

Three single topography images are acquired automatically by the HRT3 and these images are averaged to calculate a mean topography. Image resolution algorithms within the HRT software align the three images. This has been shown to be accurate and reproducible for topographic images of the optic nerve head (Chauhan, LeBlanc, et al. 1994).

The lens is positioned close to the eye but not touching the eyelashes, approximately 10mm from the cornea. The pupil does not need to be dilated unless it is less than 3mm diameter or there is sufficient cataract opacity reducing image quality. The most immediate effect on image quality is poor tear quality and the HRT scan must be done prior to fluorescein instillation for readings of Goldmann tonometer artificial tears can be used as a tear substitute for patients with meibomian gland dysfunction, but, this was not a required solution for these research participants.

The quality of the HRT image is a key factor in measuring blood vessels to 0.001mm. Heidelberg recommends this begins with choosing a suitable lens power from the magnetic clip on lenses, this is calculated as a best form lens from entering the volunteer's spectacle prescription into the patient details form and the HRT will suggest a best spherical lens. The HRT also has a magnetic clip-on astigmatic for prescriptions over +/- 1.00DC. The corneal curvature is entered so that magnification can be accounted for should the patient undergo any future corneal surgery. All volunteers in this research had refractions under +/- 4.00DS and less

than -2.00DC, with no history of corneal surgery or diagnosis of corneal dystrophy. This minimised any change in magnification effects on any blood vessel measurement. The optic disc was then aligned. The optic disc is centred by first directing the volunteer to fixate on a green flashing light inside the camera, the laser is then centred to enter the pupil, the live image should appear on screen. Any laser light falling on the iris should be avoided as this will lead to a crescent shaped shadow on the acquired image. The image is more finely tuned using the focusing dial; this value is automatically recorded with the scan and on subsequent images if this number changes by more than two dioptres, then a warning notice is flashed up. In practice we found patients more easily achieve the correct position by getting then to follow the fixation white light mounted on a movable stalk. The understanding of an instruction to “look at the white light” or “ follow the white light” so that the optic disc could be centred, provided clarity of instruction for the patient, leading to quicker image acquisition. We viewed and centred the optic disc placing it inside a green target ring superimposed on the live video image.

The image bar gave an immediate estimate of quality based on numerical number (0-100) and colour with green indicating good, red is poor and yellow if image illumination or patient fixation is only adequate (Figure 13).

Figure 13 Image Quality bar during HRT image acquisition (image courtesy of Heidelberg)

The image quality of a HRT topography scan is measured by a number of criteria. This assessment of image attributes allows the interpretation of the HRT scan to be repeatable over time.

The first check is the standard deviation in the analysis centre. Heidelberg recommends that images with a standard deviation above 40µm be repeated to improve quality. Heidelberg goes further to suggest that a standard deviation above 50µm should be interpreted with caution. This standard deviation is calculated as a geographical mean. The normal arithmetic mean is a simple calculation of sum of the parts divided by number of entries;

The mean μ_a is calculated as follows:

(EQ8) $\mu_a = {}^{(a1 - a2 + a3 + \cdots an)/n}$,

Whereas the geometric mean is calculated as follows:

(EQ9) $\mu_g = \sqrt[n]{a_1} a_2 a_3 \ldots . a_{1t}$

The arithmetic mean considers that each value has no impact on the previous or following values, but the geometric mean takes into account that each value relates to one another. This is a much better mean of the topographic results.
The quality of the image is summarised in the “Qualitative Image Assessment” window. The image quality score gives as assessment of mean standard deviation value of the topography. The imaging quality score gives an overall assessment of the variety of parameters that directly effect image definition (Figure 14).

Figure 14 Qualitative Image Assessment Window HRT (image courtesy of Heidelberg)

2.9.4 Interactive Analysis of the Image

The tab *interactive measurements* allow the display of the cross sections of the topography to perform height (x, y, z) or horizontal measurements (x, y) (figure 15). A single mouse click inside the large image superimposes a green coloured crosshair on the image. The horizontal and vertical cross sections show height variation of the retinal surface along the horizontal and vertical lines of the crosshairs respectively. The crosshairs are manually positioned by moving the mouse inside the large image, or alternatively, by using the cursor keys (arrow keys). The cursor keys move the crosshairs in increments of 0.011mm horizontally and vertically. The coordinates of the crosshairs are shown in the bottom right of the screen. There are four different coordinate systems within the HRT software. The default system, *relative and tilted*, was used for this research. In this system "*the x,y plane runs parallel to the retinal surface and the mean height of the z-axis is determined by the mean height of the peri-papillary retinal surface*" (definition taken from "The Essential HRT Primer" Fingeret, Flanagan & Liebmann).

Figure 15 Reflectance Image in interactive window we used for measuring blood vessel diameter (image courtesy of Heidelberg)

The image can be toggled between reflectance (Figure 15) and topographic (Figure 16). The reflectance image is a false colour image that appears similar to a photograph of the optic disc. The image should be clear and evenly illuminated, with sharp borders and visible margins of optic disc and retinal vessels.

Figure 16 Topographic image in interactive window we used for measuring blood vessel diameter (image courtesy of Heidelberg)

The reflectance image is the result of the summation of the two dimensional reflectance images and is presented as a 384 x 384 pixel map illustrating the degree of reflectance from regions in the optic disc and peripapillary retina. Lighter areas such as the base of the cup are areas of greatest reflectance. This does not equate to height measurement and is purely related to overall reflectance of the image. The reflectance image is useful in locating and drawing the contour line around the optic disc.

The topographic image in contrast to the reflectance image does relay information about contour height of the optic disc and retina. The image is false colour coded, but is based on the height measurement matrix constructed from the determination of the depth of maximal reflectance in the z-axis at each pixel (figure 8) Pixels that appear bright in the topographic image are deeper and darker pixels are elevated. Thus, the neuroretinal rim should appear darker than the surrounding retina and the base of the cup usually appears the lightest.

2.9.5 Drawing the Contour Line

The contour line is the outline of the scleral ring of Elschnig (Figure 17). This is estimated to be approximately at the optic disc border. The border is drawn by placements of a series of points at the external edge of the optic disc and the HRT

machine will draw an automatically derived circle. The diameter and contour of the circle can be adjusted to match the individual disc. The scleral ring frequently appears as a pale band in the reflectance image, the contour line is then placed on the inner edge of this ring. The interactive height contour is very useful since the scleral ring is displayed as a depression. The contour line is then drawn on the maximum height inside of this depression. The contour line, once accepted, is fixed throughout the lifetime of scans for that patient. If, in the future it is decided to redraw the contour line, then all measurements for neuro retinal rim volume would be recalculated. Because of its manual input, the accuracy of mapping the scleral ring of Elschnig has been compared to optical coherence tomography (OCT). Research has shown there to be a high correlation (r=0.93 to 0.98) between OCT and HRT identification of the optic disc margin (Schuman, Wollstein, et al. 2003). This has recently been queried by research based on the increased detail available to assess optic disc anatomy with the Heidelberg Spectralis OCT. The original Bruch's membrane opening, thought to define the optic cup margin and subsequently the neuro retinal volume, has been reassessed using the Spectralis OCT (Chauhan & Burgoyne 2013). If proved to be correct, and accepted as the gold standard for optic disc assessment, this new value of rim volume would render the HRT version out of synch with average neuro retinal rim volume.

Figure 17 Anatomical Position of Elschnig's Ring (permission from Heidelberg Images)

2.9.6 The Standard Reference Plane

The mean peripapillary retinal surface height is found from topographic height values within a peripheral *reference ring* shown in figure 18 & 19.

Figure 18 HRT position of reference plane (permission from Heidelberg images)

(http://www.oculist.net/downaton502/prof/ebook/duanes/graphics/figures/v3/048a/003f.gif)

The reference plane is calculated by the HRT after the contour line is drawn. This plane is parallel to and below the peripapillary retinal surface and is used to divide the optic disc into neuroretinal rim and cup (Burk, Vihanninjoki, et al. 2000).

Figure 19 Reference Ring Schematic for HRT (permission Heidelberg images)

The HRT3 has built in software for assessing the normality or an optic disc. This software uses an expanded normative database compared to HRT2 and HRT-

classic. It now includes 733 healthy eyes and 215 healthy African-American eyes (HRT glaucoma module operating instructions software version 3.0 Heidelberg Engineering). With this normal population the software equations were modified to improve accuracy.

In this research the glaucoma software results were not included in the statistics. The main focus of this research was to correlate vessel diameter change with neurofiber volume loss and visual field defects. The software included with the HRT is used to aid diagnosis of glaucoma based on probability of the disease set against a normal database or statistically abnormal change typical of glaucoma.

The important results obtained from the HRT were in the stereometric parameters tab which were calculated by the HRT after the contour line was drawn and reference plane set.

2.9.7 Moorfields Regression Analysis (MRA)

This algorithm takes a different approach to the normal derivation of software by supplying a machine with two sets of data, one normal and one defined as glaucoma then allowing the statistics, via linear discrimination, to determine the best parameters to distinguish the two groups. Instead, Moorfields Regression Analysis (MRA) was born of knowledge ‘ a priori’ i.e. the dependence or neuroretinal rim area on disc size, the possibility that neuroretinal rim area may decline with age, and knowledge of the glaucomatous process. The results are then presented as a bar chart as to whether the individual sectors are within, or outside normal limits. The decision as to whether this represents glaucomatous change is left with the clinician.

2.9.8 Global Probability Score (GPS)

The Global Probability Score (GPS) is a feature of the HRT3. It was introduced as an operator independent algorithm since it did not require the contour line. GPA differentiates between normal and glaucomatous eyes by generating a three dimensional shape, as glaucoma develops the GPS model assumes that the retinal nerve fibre layer (RNFL) changes such that its curvature flattens as it thins and the cup enlarges and consequently, the slope of the neuroretinal rim steepens. The parameters from the best 3-dimensional model, which fits from the HRT normative database, then gives a probability of the individual disc being outside the normal range. The data produced has been extensively research in current literature with a

general consensus that it is at least as effective as MRA in prediction of glaucoma (Moreno-Montañés, Antón, et al. 2008), and is slightly better at identifying a normal disc (Strouthidis, Demirel, et al. 2010). But the GPS value, as a standalone predictor of glaucoma, is recommended to be interpreted with caution (Ferreras, Pajarín, et al. 2007).

2.9.9 Topographic Change Analysis (TCA)

The Topographic Change Analysis (TCA) analysis of HRT data evaluates the significance of the observed changes in baseline and follow-up, with reference only to the individual patients variance at a particular location, and not to values derived from the normative population data set. It is a statistical method to compare the topographic values in discrete areas of the image called super pixels, which contain 4x4 (16 pixels) at two points in time. TCA does not require a contour line, or reference plane. TCA analysis is performed on the raw topography data and computes at each super pixel the probability of the difference in the two height values occurring by chance alone. When the TCA image is access placing the cursor at a point on the image gives a height difference and a probability score (p=) that the change could be down to chance. The variability of height measurements is greater at the edge of the optic cup and along blood vessels so the height variation is set to a higher value in the algorithm at these points.

2.9.10 Assessing Visual Field Progression

Visual field evaluation is a psychophysical test. It is designed to investigate the relationship between physical stimuli and perception of those stimuli. Like all psychophysical tests it is subject to a degree of variability.

The visual field is a measurement of all that is available to one eye at a given time. It has been likened to a *hill of vision surrounded by a sea of blindness*. The visual field plot is an attempt to draw this hill of vision and assess any change. The algorithms applied to the visual field endeavours to attribute visual field loss either to a normal ageing change or suggest the probability of an alternative diagnosis.

Standard Automated Perimetry (SAP) measures light sensitivity across various locations across the visual field. It is fundamental in the diagnosis and follow-up of glaucoma. Because SAP is not selective for a particular type of ganglion cell, and any ganglion cell may respond to the light stimulus, a substantial number of retinal

ganglion cells must die before development of visual field loss (Harwerth & Quigley 2006). A 50% loss is often quoted before the onset of detectable visual field damage (Quigley 1982). This has since been review downwards to 25-35% death of retinal ganglion cells before evident field loss (Kerrigan–Baumrind, Quigley, et al. 2000). The reason for this large number of ganglion cells being lost before functional visual field loss is because the relationship is logarithmic, and a relatively large number of retinal ganglion cells must be lost before the threshold measurement detects the defect as outside normal limits. The variation of these two correlations may well be due to the difficult task of comparing recent visual fields, to post-mortem retinal tissue in patients.

Study of post mortem retinas in glaucoma patients that compared retinal ganglion cell densities to static perimetric thresholds on a point-by-point basis showed that, on average, statistically significant abnormalities of visual sensitivity required neural loss of between 20-50% to occur, depending on retinal eccentricity. For any given level of neural loss there was a very large range of visual defects (Quigley, Dunkelberger, et al. 1989).
A more recent correlation of retinal ganglion cells (RGC) utilised an estimate of RGC using SAP sensitivity thresholds and retinal nerve fibre layer thickness measurements obtained from spectral domain optical coherence tomography (SD-OCT). The average RGC count for eyes with visual field defects were 652,057 ± 115,829 which is significantly lower than the healthy eye count of 910,584 ± 142,412 ($P<0.001$) which is an average loss of 28% (Medeiros, Lisboa, et al. 2013). This is a closer estimate to the lower value of Kerrigan who 25% ganglion cell loss with a 5dB loss in visual field sensitivity (Kerrigan–Baumrind, Quigley, et al. 2000). Medeiros went on to estimate that a minimum percentage loss of 17% was needed to cause a consistent visual field defect. This compared to a loss of 52% in an advanced glaucomatous field.

Glaucoma is not consistent in its perimetric loss, and patients are not consistent in their attention to the perimetric test (Gillespie, Musch, et al. 2003). This is compounded by increasing age and other comorbidity's e.g. cataract and macular degeneration. The problem with the diagnosis or assessment of glaucoma based on a patients' visual field examination is the variability of both the pattern of visual field loss and their subjective responses. Despite this perimetry remains the third, in the core triad of diagnosis and monitoring of glaucoma disease. This triad of IOP, perimetry and optic disc assessment is the gold standard for all research involving glaucoma (Öhnell, Heijl, et al. 2016).

Glaucomatous visual field loss defined by the Hodapp classification (Hodapp, Parrish, et al. 1993) was adopted by the NICE guidelines in 2009. Field loss was divided into three areas, early, moderate and advanced. The evolution of glaucoma through these criteria was based on increase in mean defect and 5% probability of a defect in an increasing number of tested points. It is evident from the literature that no consensus exists regarding the best method for differentiating between stable visual field defects and variable field loss from progressive defects. Furthermore the relationship between structural and functional loss is not fully understood.

Studies of laser induced, experimental glaucoma on monkeys have shown a much smaller variability in structure-function relationships when the criteria of static visual field testing, timing of collection and processing of retinal tissue was more tightly controlled (Harwerth, Crawford, et al. 2002) The general relationship suggested that when retinal ganglion cell losses were less than 50% there were small reductions in sensitivity but that functional and structural loss were not proportional. Thus these results suggested, that when translating retinal ganglion cell loss to visual field loss, other factors need to be included eg. redundancy and incomplete apoptosis.

The purpose of perimetry is to indicate the site and extent of damage to the visual pathway. The key factor in glaucoma treatment is whether this field loss is advancing and therefore the potential adverse reactions from the treatment is worthwhile, or, alternatively, the field loss is static and no treatment is required. In 1997 Joann Katz et al(Katz, Gilbert, et al. 1997) suggested that only one in three eyes with glaucoma had any progressive field loss and that average changes in threshold sensitivities of less than 1dB/Year could not be detected with seven fields acquired over six years. Their advice that increased frequency of field tests would need to occur to detect small changes probably does not work in a busy glaucoma practice.

A more sensitive visual field test in the form of blue target on yellow background (SWAP) may allow more accurate determination of progression, but, although this has been shown to be predictive of glaucoma damage (Johnson 1993) the increased inter-individual normal variability of SWAP limited its ability to be reliable in the practical decision of lifelong drug treatment by an ophthalmologist (Wild, Cubbidge, et al. 1998) The main reason for this unreliability is the blue stimulus is absorbed by the ocular media, mostly the crystalline lens. This absorption increases

with age and cataract formation; unfortunately it is age that is a known risk factor in glaucoma epidemiology.

Senescence has proved to be a difficult factor to mitigate for in visual field loss (Spry & Johnson 2001). Spry et al found that age exerted an increasing effect on perimetric sensitivity. This has implications for determining a correcting factor for the normal visual field based on age and although a nonlinear function proved the best fit devices currently using a linear model would underestimate aging changes for older subjects and thus classify aging changes as pathological.

The risk factors for glaucoma progression were summarised in an evidence based review paper published in 2013 (Ernest, Schouten, et al. 2013). The only two definite factors linked with glaucoma progression were age and neuroretinal rim disc haemorrhage. Although baseline visual field was a probable risk factor, it is listed alongside other equally weighted elements such as intraocular pressure and central corneal thickness.

The key to detecting progression is the frequency of visual field measurement (Chauhan, Garway-Heath, et al. 2008), but the frequency varies significantly amongst hospitals and practitioners (Hertzog, Albrecht, et al. 1996), and often falls markedly below recommendations (Friedman, Nordstrom, et al. 2005). The recommendation set out by Chauhan was that six visual examinations should be carried out in the first two years after diagnosis (Chauhan, Garway-Heath, et al. 2008).

In a recent survey of glaucoma specialists within the UK, the problem of 1 million appointments for glaucoma was considered against the NICE and research recommended six visual field examinations in the first two years (Malik, Baker, et al. 2013). The survey was replied to by half of the glaucoma specialists currently practising in UK hospitals. Current decision making of visual field testing is decided by the clinician's estimate of speed of glaucoma progression. It is acknowledged that six visual fields would yield useful data to estimate glaucoma progression but there are practical time and money barriers to implementing this as the accepted gold standard.

2.9.11 Interpretation of Visual Field Results

This research exclusively used the Humphrey Visual Field Analyser and the StatPac statistics program to interpret results from consecutive fields.

The Humphrey Stat Pac program performs three important functions
" *1/ it can point out suspicious areas that otherwise might not be evident until subsequent testing*
2/ it can identify areas that look suspicious but which, in fact, compare favourably with normal data
3/ using results from a series of tests, Stat Pac can provide a highly sensitive and informative analysis of changes in the patient's visual field over time " (The Field Analyser Primer, Humphrey systems, Dublin, California).

Visual fields are inherently noisy. The known difficulty of consistency of patient fixation is considered by the Humphrey Visual Field machine to be an excessive fixation loss when the blind spot tested has moved 20% of the time, but a research paper in 1990 concluded this was too high demand and that 33% was a more realistic value (Sanabria, Feuer, et al. 1991). This research has taken the value of 33% and any field exam with fixation above this was discarded and repeated within two weeks.

For interpretation of visual field the Hodapp et al glaucoma grading scale was used (see appendix)(Hodapp, Parrish, et al. 1993).

2.9.12 Mean Deviation (MD)

Mean Deviation represents the overall elevation, or depression, of patients overall field compared with the normal reference field. If the value is significantly outside the normal field for that patient's age, then a p value is given. The p value suggests that less than this percentage of the normal population shows an MD larger than the value calculated. A significant MD suggests the patient has an overall depression, or, that there is a very deep or widespread loss in part of the visual field and not others. Unfortunately this variable is prone to influence by other factors and typically a cataract will reduce the MD value as it increases in density and reduces the overall sensitivity of the field.

2.9.13 Pattern Standard Deviation

The Pattern Standard Deviation (PSD) is a measurement of the degree to which the shape of the patients measured field differs from the normal age corrected reference field. A low PSD indicates a smooth hill of vision and a high PSD suggests an irregular hill. To indicate the statistical significance of the calculated PSD a p value is assigned, as with MD this indicated the percentage of the population having a PSD numerical value larger than the printed result. The Pattern Standard Deviation (PSD) is a better statistical analysis for tracking localised field loss.

2.9.14 Correlation of the HRT with the Visual Field

The Heidelberg Retina Tomography is advertised as " *a proven essential tool for detecting and managing glaucoma, especially for assisting in the identification of pre-perimetric disease and tracking progression"* (Heidelberg Engineering UK website www.heidelbergengineering.co.uk). It is thought that retinal ganglion cell death with subsequent change at the optic nerve head precedes the onset of glaucomatous field loss (Sommer 1991). The basis of the HRT scanner is to detect this change before a functional loss of vision, which is detectable with a visual field exam. The ability to recognise loss in the retinal ganglion cells separates the structure function relationship with glaucoma. The clinical decision to start a lifetime of treatment for glaucoma based on this measurement has not been accepted by the majority of ophthalmologists, and therefore, the reliance on a coincident visual field defect returns the HRT to a structure function connection.

The HRT has been shown to detect neuroretinal tissue loss attributable to perimetric glaucoma (Wollstein, Garway-Heath, et al. 2000; Alencar, Bowd, et al. 2008). The effectiveness of the HRT measurements in prediction of glaucoma when compared to the gold standard of stereophotography has been shown to be equal but not superior to expert analysis (Weinreb, Zangwill, et al. 2010). The correlation of visual field to the HRT has been shown to be statistically significant with visual field MD value and neuroretinal rim volume (Tole, Edwards, et al. 1998). More recent studies have shown only a more moderate comorbidity with visual field and two specific stereometric HRT values, rim area and rim volume (Danesh-Meyer, Ku, et al. 2006). This reduced relationship between visual field indices and optic nerve head parameters could be explained by the research suggesting that retinal ganglion cell losses when less than 50% had only a small reduction in sensitivity and this reduction in sensitivity was not proportional to the structural loss (Harwerth, Carter-

Dawson, et al. 2004). They went on state that with greater degrees of neuropathy the structure function relationship was more systematic although with considerable variability. This would suggest a large degree of redundancy in retinal ganglion cell structure making a linear relationship between HRT and visual field exam an unreliable statistic. This reduced correlation between optic nerve head topography and visual field was shown for both angle closure and open angle glaucoma (Boland, Zhang, et al. 2008).

	Current Research Correlation of HRT parameters with Visual Fields (MD)	
	Regional Correlation of Structure and Function in Glaucoma Correlation Coefficient (P value)	HRT and OCT in Normal, OHT and Glaucomatous Eyes correlation Coefficient (P value)
Rim Area (mm^2)	0.35 (0.0018)	0.49 (0.0001)
Rim Volume (mm^3)	0.28 (0.0030)	0.43 (0.0001)

Table 5 Current Research Correlation of HRT parameters and Visual Field (MD)

The literature found the best correlation of HRT parameters with global visual field damage to be rim area and rim volume (Danesh-Meyer, Ku, et al. 2006; Mistlberger, Liebmann, et al. 1999).

3.0 Blood Vessel Measurement in Glaucoma

3.1 Aim of Study

There are three questions that guided this research:
1) how reliable are retinal vessel measurements with the HRT (assessed using Bland-Altman analysis)
2) Assess vessel diameter differences between controls and patients with glaucoma and ocular hypertension
3) Determine whether there is a change in the groups after a one year period

The HRT has an interactive tab within the computer software then enables a cursor to be place on the image acquired and measurements taken between any two points. This is a new method for measuring the diameter of blood vessels. This software is included within the HRT analysis program and is accessed by simply pressing the interactive tab GPS classification tab at the top of the screen. I hypothesise that the diameters of retinal blood vessels alter in glaucoma.

Early diagnosis of glaucoma is critical to reduce the risk of permanent structural damage and irreversible vision loss occurring (Garway-Heath 2008). Currently, the diagnosis of glaucoma is made via a triad of tests; optic disc assessment, intra-ocular pressure (IOP) and visual field examination (Anon 2009). These tests are looking for typical glaucoma traits in the form of nerve tissue loss at the optic disc, raised IOP with the potential to cause harm and a pattern of visual field loss typical of glaucoma. There are two other parameters not normally taken in a high street practice that can aid glaucoma diagnosis; central corneal thickness (CCT) and ocular perfusion pressure (OPP).

OPP is defined as the difference between arterial and venous pressure. In the eye, venous pressure is equal to, or slightly greater than IOP. OPP can therefore be defined as the difference between blood pressure (BP) and IOP (Costa, Arcieri, et al. 2009). These values are gaining increasing significance in glaucoma literature; helping to explain why lowering of IOP past the assigned target level does not slow glaucoma progression (Quaranta, Katsanos, et al. 2013). In the Early Manifest Glaucoma Trial low systolic perfusion pressure, low systolic blood pressure and cardiovascular disease were predictors of glaucoma (Leske, Heijl, et al. 2007).

3.2 Rationale

The retinal vessels provide a unique opportunity to monitor microvascular changes by direct non-invasive visualisation. This simplicity of observation is enhanced by electronic methods of scanning and magnification, and because the vessels share similar anatomical and physiological characteristics with vessels in the brain and heart (Wong, Klein, et al. 2001), correlations can be drawn by observation of diameter and corresponding changes in either disease onset or progression. Generalised artery narrowing is an early feature of hypertensive retinopathy (Wong, Klein, et al. 2001; McClintic, McClintic, et al. 2010) narrowing is hypothesised to predict cardiovascular disease, mortality (Wong, Klein, et al. 2003; Gillum 1991) and even knee replacement (Hussain, Wang, et al. 2015). However, retinal arteriolar

narrowing is imprecisely quantified from direct ophthalmoscopy (Maestri, Fuchs, et al. 2007).

The arterial supply of the adult optic nerve is derived completely from branches of the ophthalmic artery (chapter 2.1) The vascular theory of glaucoma proposes that optic nerve head blood flow is compromised (Flammer, Orgül, et al. 2002). This resultant ischemia can interfere with axonal nutrition and axoplasmic flow (Osborne, Ugarte, et al. 1999). A possible reason for this ischemia is an abnormality in blood vessel autoregulation, this would effect the ability of vessels to control ocular perfusion pressure (Sehi, Flanagan, et al. 2005).

The normal optic disc vasculature does not leak; this is easily demonstrated with a fluorescein angiogram (Figure 20).

Figure 20 Fluorescein Angiography normal Optic Disc and fundus, dark lines-veins, white lines-arterys

(http://www.medicine.uiowa.edu/uploadedImages/Departments/Ophthalmology/Content/Patient_Care/Imaging_Services/FA_Fig2(1).jpg)

The perfusion of any organ is dependant on, perfusion pressure, vascular resistance and local blood viscosities. An alteration in the diameter of blood vessels physiologically regulates the vascular resistance. There are two principle ways in which vessel diameter is changed metabolically and myogenically. Both of these mechanisms have been shown to be present in the retina (Bill 1985).

Current evidence suggests that an endothelium dependant mechanism is a key element in hypoxia-induced vasodilation. The most likely mediators being prostaglandins (Busse, Forstermann, Matsuda, & Pohl, 1984; Busse, Pohl, Kellner, & Klemm, 1983). This would suggest that the prostaglandin Xalatan could influence vessel diameter in addition to the lowering of IOP. Thus it can be hypothesised that in patients developing glaucoma, their retinal blood vessels should decrease in diameter and in patients being treated for glaucoma with Xalatan, blood vessel diameter constancy would suggest disease stability.

The second factor of myogenic regulation is affected by blood pressure and that although the exact mechanism is still under investigation the physical contraction is, at least partly, mediated by endothelial factors (Davies & Tripathi 1993). The irregular diurnal variation of IOP in a glaucomatous eye might then be affected by a corresponding variation in BP over 24 hours. This large diurnal variation in IOP has been shown to be an independent risk factor for glaucoma (Asrani, Zeimer, et al. 2000). By making an assessment of the ocular health of the patients eye alongside the retinal vessel diameter our research aims to determine if there is a retinal vascular element that is measurable in a high street optometry practice, in the hope this will further aid glaucoma diagnosis. Scanning laser tomography will be used to quantify blood vessel diameter change longitudinally in addition to estimation of ocular perfusion pressure.

3.3 Methods

This research took place at an independent Optometrists in Northern Ireland. Each volunteer was required to attend high street practice four times. The patient was examined with an extended eye examination on their first visit involving prescription determination, keratometer readings, IOP with a Goldman tonometer, stereoscopic fundus examination using a volk lens, gonioscopy to evaluate the anterior angle, slit-lamp exam of the anterior eye, central corneal thickness measurement using an ultrasound pachymeter, blood pressure measurement, scanning laser tomography

of the optic nerve head using the HRT and finally a full threshold field examination (see Section 2.9.10 for more details).

A second visit took place one week after the first appointment to repeat the initial visual fields. The third and fourth visits repeated the protocols of the first and second visits respectively and took place one year after initial two visits. The study was conducted in adherence to the tenets of the Declaration of Helsinki and was approved by the Aston University Ethics Committee.

3.4 Sample

The volunteer participants were recruited from a high street optometrist patient base, presently attending for routine eye care. Three patients groups were targeted for recruitment (40 volunteers in each group) aged between 30-70 since this is the group most prone to glaucoma. Assuming normally distributed data and a blood vessel variability of 30μm the sample size required to demonstrate differences in blood vessel diameter would be 16. Assuming for drop out in the longitudinal study, 40 patients were aimed initially recruited in each group (See Appendix; Audiology & Optometry Ethics Form).

3.5 Sample Categorisation

The volunteers were divided into three groups

- **Normal**; this group was defined as participants without any general health comorbidity that may affect vessel diameter (diabetes, unstable blood pressure, Raynaud's syndrome), visual field (epilepsy, history of anterior ischaemic neuropathy) or neuroretinal rim changes at the optic disc (Multiple sclerosis, dementia) In addition, their ocular history was without incident that may indicate instability of vision or visual field (recent artery or vein occlusion, active dry or wet macula degeneration, significant cataract effecting mean deviation or best corrected visual acuity) Against this stable ocular and general health history their IOP consistently measured below 21mmHg at any point during daytime waking hours
- **Ocular Hypertensive**; this group had the same criteria as the normal group except for the dividing factor that the IOP should measure above 21mmHg during daytime hours, meeting the classification requirements of the NICE guidelines of an Ocular Hypertensive

- **Glaucoma**; the diagnosis of primary open angle glaucoma by an ophthalmologist with a specialist interest in glaucoma and considered to be stable on the specific medication of Latanoprost (Xalatan 0.005%) The condition of stability was accepted if the patient was on routine recall of one year and the medication had not been changed in the last two years.

3.6 Inclusion/Exclusion Criteria

The recruited patients were placed into three categories;

1/ Normal: defined as a patient with no glaucomatous visual field loss, optic disc changes suggestive of glaucoma and IOP<21mmHg

2/ Ocular Hypertensive: defined as a patient with no glaucomatous visual field loss, no optic disc changes suggestive of glaucoma and IOP>21mmHg

3/ Glaucoma; defined as a patient diagnosed with primary open angle glaucoma by an ophthalmologist, currently under review, and being treated exclusively with Latanoprost (Xalatan (0.005%), a prostaglandin analogue) inserting one drop at night.

The NICE directive made a requirement for all optometrists to refer patients who measured IOP over 21mmHg (no instrument specified) (Edgar, Romanay, et al. 2010). N.Ireland has put in place a referral refinement scheme whereby a patient with an IOP greater than 21mmHg was asked to return and the IOP repeated with a Goldmann tonometer. If the reading was still over 21mmHg then they are referred into the hospital eye service for screening. All patients in the hypertensive group recruited for this research followed this protocol, and had either been seen and discharged, or were under review by an ophthalmologist.

Ethnicity could conceivably be a complicating factor in blood vessel diameter change; research has suggested different progression rates of glaucoma despite similar IOP with the suggested explanation relating to its vascular aetiology. To reduce the possibility of ethnic variability, all patients recruited for this research were Caucasian, which was also the dominating ethnicity in the recruitment practices, 98.58% (Curran & Dooley 2014).

General exclusion criteria included

- Refractive correction greater than +/- 4.00DS and 2.00DC
- Medication known to influence the visual field eg Vigabatrin-used for treatment epilepsy, or Hydroxychloroquine which has the potential to cause paracentral scotomas
- Medication known to influence the visual field eg Vigabatrin-used for treatment epilepsy, or Hydroxychloroquine which has the potential to cause paracentral scotomas
- Ocular Disease known to effect visual field eg. Retinitis Pigmentosa, Chorioretinitis
- Diabetic patients or other retinal disease
- Patients with significant lens opacity the Lens Opacities Classification System III was considered (Tan, Loon, et al. 2008) but in practice a best corrected visual acuity of 6/12 was considered the minimal vision required
- Closed angle glaucoma and individuals with anterior angles are at risk of closure, the angles were evaluated using a Goniscopy lens and the Shaffer system

The inclusion criteria relating to the glaucoma group was that they were being treated with Latanoprost (Xalatan (0.005%), a prostaglandin analogue).

All glaucoma patients were outside the normal limits on the glaucoma hemifield test, whilst all normal and OHT were within normal limits. The glaucoma group were all under the care of an ophthalmic surgeon with a special interest in glaucoma. All were being treated with Xalatan only, and inserted one drop in each eye at night. All glaucoma patients were under one year review for glaucoma and considered stable at their last review. They were diagnosed and referred for glaucoma over ten years prior to baseline field for this research and since the practice is only ten years old there are no records to define the patient at initial diagnosis as high, or low tension glaucoma. There were no patients with iridotomy holes indicating treatment for closed angle glaucoma. The severity of the glaucoma, defined as functional loss of the visual field is illustrated using the Hodapp-Parrish-Anderson classification system based on the Humphrey Visual Field analyser score and one other criteria (Hodapp, Parrish, et al. 1993) (see Appendix Glaucoma Grading Scale Hodapp-Parrish-Anderson). Using this scale and considering both the MD and PSD values together the glaucoma group could be defined as early glaucoma, which is stable.

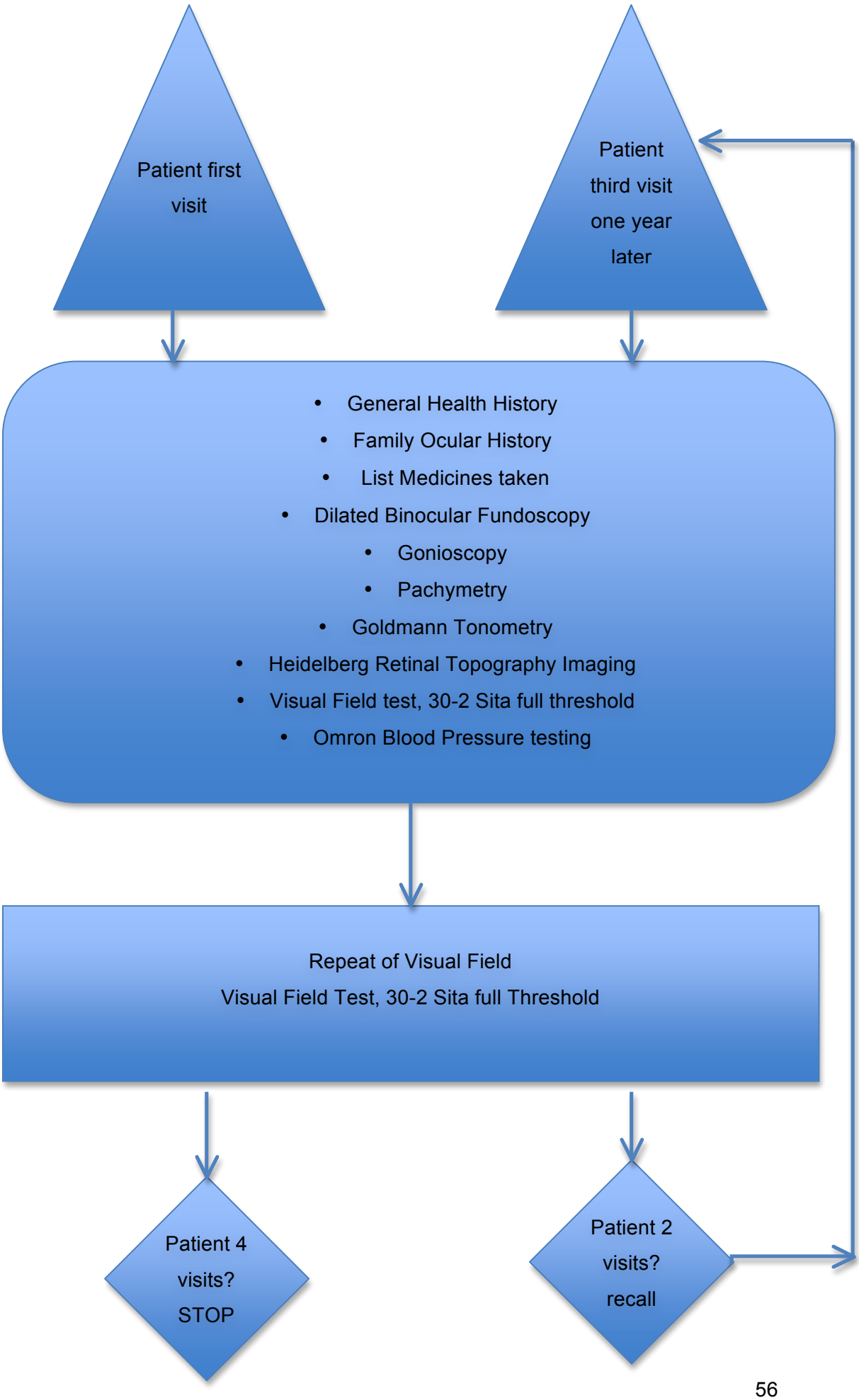
The treating ophthalmologist was notified if any significant glaucoma progression occurred in any of the volunteers during the duration of the research.

3.7 Informed Consent

See Appendix; informed consent sheet

3.8 Recruitment and sequence of visits

Each volunteer was required to attend four times. The patient was examined with an extended eye examination on their first visit involving prescription determination, keratometer readings, IOP with Goldmann, stereoscopic fundus examination using volk lens, gonioscopy to evaluate anterior angle, slit-lamp exam on anterior eye, central corneal thickness measurement using an ultrasound pachymeter, blood pressure measurement, scanning laser retinal tomography of the optic nerve head using HRT and finally a full threshold visual field examination. The second visit took place approximately one week after the first visit during which the visual field examination was repeated in order to reduce the learning effect of perimetry.


Patient first visit
Patient third visit one year later
General Health History
Family Ocular History
List Medicines taken
Dilated Binocular Fundoscopy
Gonioscopy
Pachymetry
Goldmann Tonometry
Heidelberg Retinal Topography Imaging
Visual Field test, 30-2 Sita full threshold
Omron Blood Pressure testing
Repeat of Visual Field
Visual Field Test, 30-2 Sita full Threshold
Patient 4 visits? STOP
Patient 2 visits? recall

3.9 Capture HRT Image

The quality of the HRT image is a key factor in measuring blood vessels to 0.011mm. Heidelberg recommends this begins with choosing a suitable lens power from the magnetic clip on lenses, this is calculated as a best form lens from entering the volunteer's spectacle prescription into the patient details form and the HRT will suggest a best spherical lens. The HRT also has a magnetic clip-on astigmatic for prescriptions over +/- 1.00DC. The corneal curvature is entered so that magnification can be accounted for should the patient undergo any future corneal surgery. All volunteers in this research had refractions under +/- 4.00 dioptres spherical and less than -2.00D cylinder, with no history of corneal surgery or diagnosis of corneal dystrophy. This minimised any change in magnification effects on blood vessel measurement. The optic disc was then aligned. The optic disc is centred by first directing the volunteer to fixate on a green flashing light inside the camera, the laser is then centred to enter the pupil, the live image should appear on screen. Any laser light falling on the iris should be avoided as this will lead to a crescent shaped shadow on the acquired image. The image is more finely tuned using the focusing dial; this value is automatically recorded with the scan and on subsequent images if this number changes by more than two dioptres, then a warning notice is flashed up. During this data collection for this research patients were more readily setup with correct position by getting then to follow and focus on the fixation white light attached to the HRT machine and mounted on a movable stalk. The understanding of an instruction to "look at the white light" or "follow the white light" as the optic disc image was centred left no room for misinterpretation by the patient, this lead to quicker image acquisition. The optic disc was centred inside a green target area superimposed on the live image. The line topography at the base of the scan gave an indicator of vessel edge start and finish points. The reflection of the vessel wall allow determination of grouping, either vein or artery.

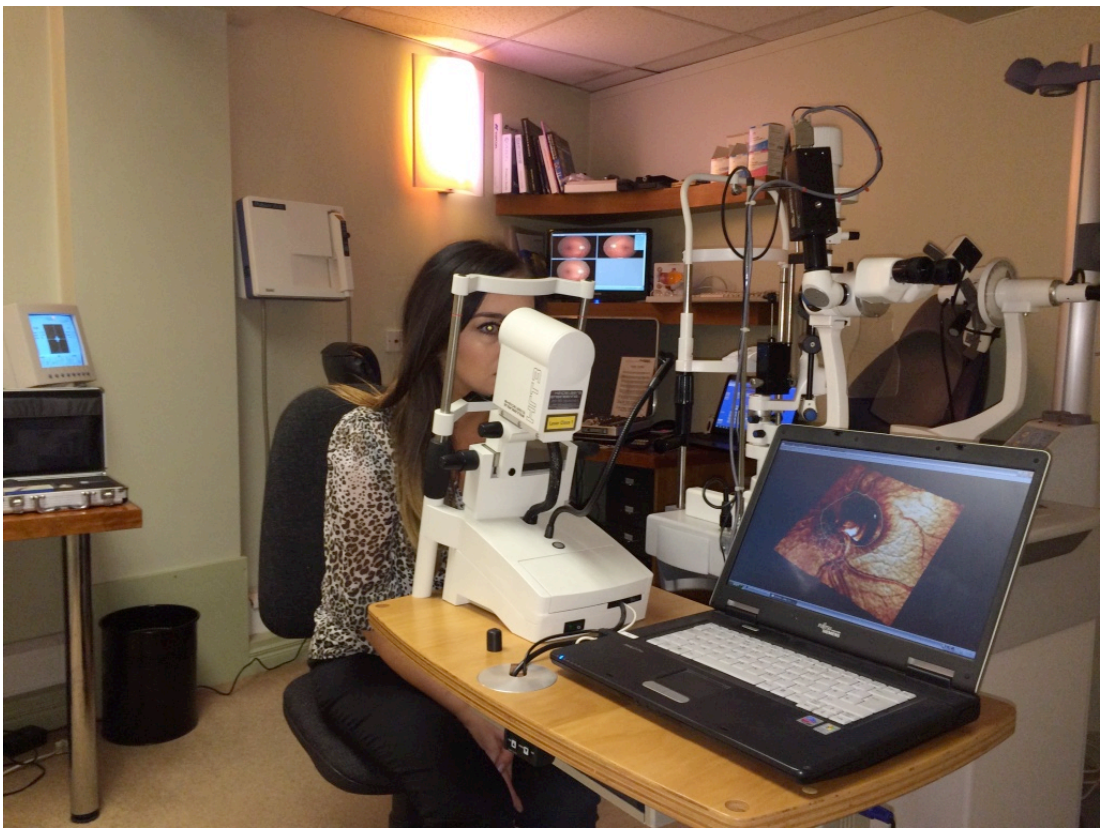


Figure 21 HRT Image Capture

3.10 Statistical Analysis

Statistical analysis was done on IBM SPSS version 22.

3.12 Sample Characteristics

Across all groups, the average time between appointments was 360 days with a standard deviation of 135 days.

Before applying statistical tests to examine for differences between and within groups, the data was tested for normality using Shapiro Wilk test. When the output is significant >0.05, then the data distribution is considered to be normal. The α level was set to $\alpha = 0.05$. The retinal vessel analyses are the primary outcome of this research and Shapiro Wilk testing of superior and inferior CRAE and CRVE showed normal distribution. Consequently, parametric statistics were used to explore for differences between and within the normal, glaucoma and ocular hypertensive groups.

A Shapiro-Wilk test was done for for the central retinal artery RE baseline reading. All three groups were to be normally distributed (p>.05); a visual inspection of the histogram and Q-Q plots also showed the results to be normally distributed. Skewness and Kurtosis are shown in table 6.

Right Eye	Skewness (standard error)	Kurtosis (standard error)
Normal	0.764 (0.421)	0.286(0.821)
Glaucoma	0.548(0.564)	-0.002(1.091)
OHT	0.467(0.448)	0.172(0.872)

Table 6 Skewness and Kurtosis of CRAE RE Baseline Reading

A Shapiro-Wilk test was done for the central retinal artery LE baseline reading. All three groups were normally distributed (p>.05): a visual inspection of the histogram and Q-Q plots showed the results to be normally distributed. Skewness and Kurtosis are shown in table 7.

Left Eye	Skewness (standard error)	Kurtosis (standard error)
Normal	-0.279(0.564)	-0.099(1.091)
Glaucoma	0.387 (0.421)	-0.34 (0.821)
OHT	0.253(0.448)	-0.741(0.872)

Table 7 Skewness and Kurtosis of CRAE LE Baseline reading

In all tests using the one-way ANOVA calculation, homogeneity of variances was preformed to determine normal sampling of data and p confirmed at p>.05 before accepting the result from the ANOVA table.

103 subjects were recruited with 74 completing the second visit one year after first collection of data. The age distribution is shown in table 8.

	Mean Age	N	Standard Deviation
Normal	67.4	32	8.72
Glaucoma	72.2	16	5.44
OHT	67.48	27	6.63

Table 8 Average Age of Volunteers in this research

The distribution of males (n=34) and female (n=40) was tested for normality. Shapiro-Wilk test (p<0.05) showed the distribution of male/female was not normal, but both groups fell within the z-value of -1.96-+1.96. The skewness for male -0.435 (standard error 0.403) and kurtosis -0.863 (standard error 0.788) were normal and for female wqs normal with a skewness -0.89 (standard error 0.374) and kurtosis -1.337 (standard error 0.733).

4.0 Results

4.1 Continuity of Measurement, Bland-Altman Plot

The gold standard in data research is a blind, randomised collection of data. All the patients in this research were known, and although measurements were taken of vessel diameter without reference to previous year, the assessment of ocular health for the patient could bring a bias to vessel measurement. There was no potential for a second observer to rule out this bias. Taking a sample of ten volunteers from both the normal and glaucoma group, a second measurement of artery diameter was recorded. This artery measurement was then compared to original measurement and a Bland Altman graph plotted, this would determine if there was a significant difference and therefore lack of continuity in vessel diameter.

	Masked Artery Measurement, Repeatability Comparison n=10			
	Original Measurements		Comparison Measurements	
	RE	LE	RE	LE
Normal	301.5 (41.5)	269.9 (60.97)	301.6 (22.41)	273.9 (35.27)

Table 9 Masked Artery Measurement, Repeatability Comparison, Normal group

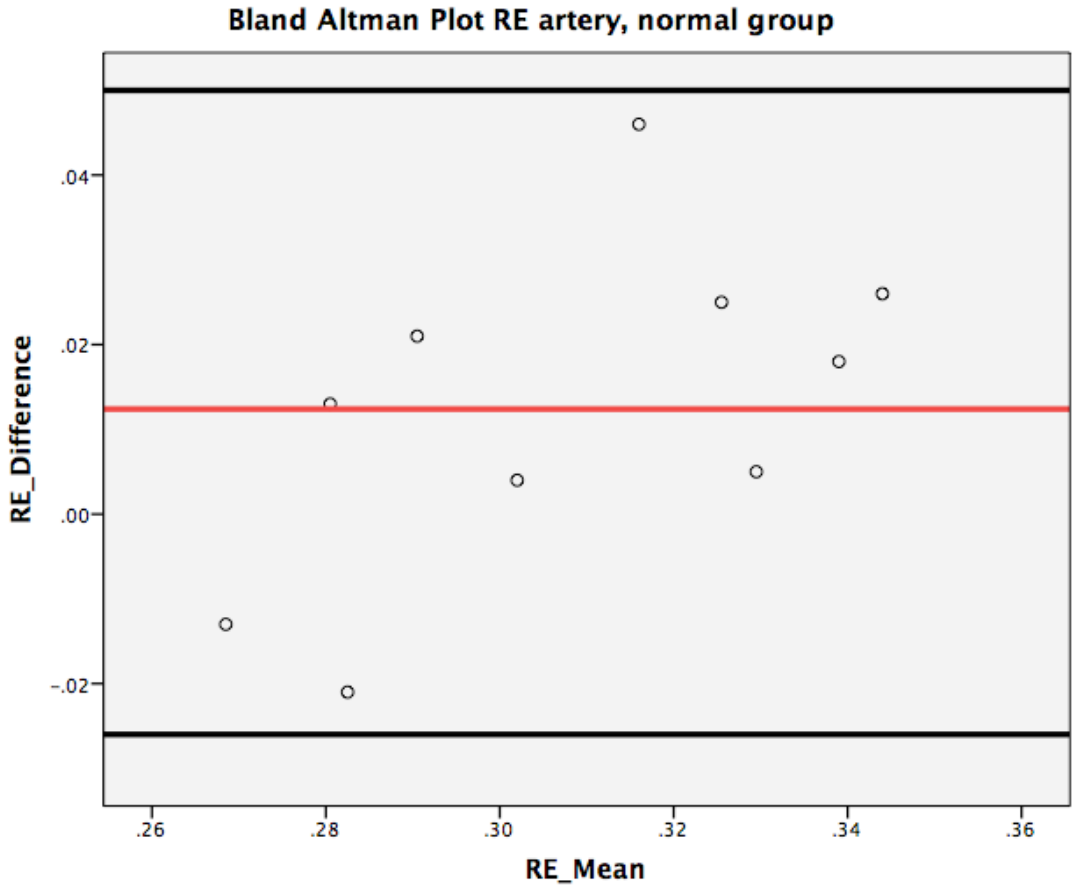


Figure 22 Bland Altman plot RE artery, normal group, red line mean, black lines upper and lower 95% confidence

A one sample t-test for the RE artery diameter difference between a second blind re-measurement of a sample gave the values t=1.999, df=9 and a non significant p=0.077. This would demonstrate that there is no significant difference between the diameter measured at baseline and a second measurement of the baseline to determine if there was difference attributable to observer error. The scatter plot showed all values lying within the 95% confidence limits. A linear regression calculation showed t=2.015 and t=0.079 showing there was no statistical significant proportional bias.

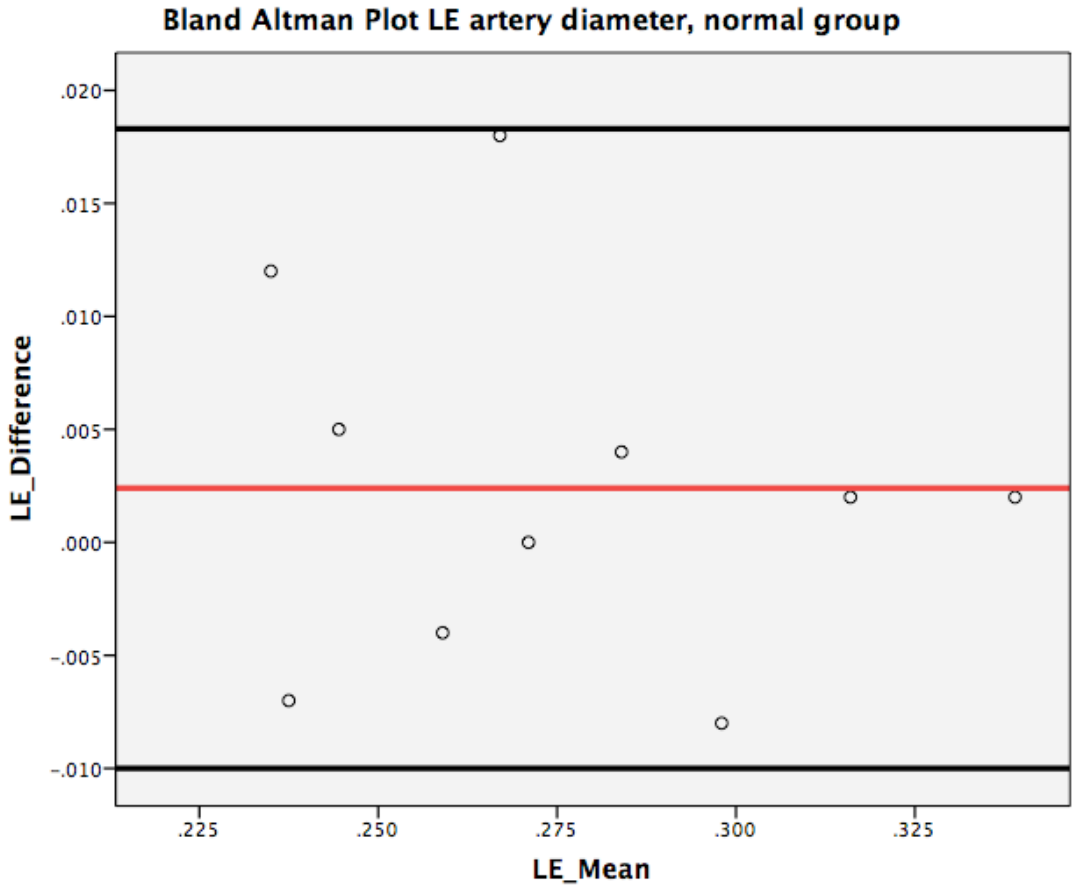


Figure 23 Bland Altman plot LE artery diameter red line is mean, black lines upper an lower 95% confidence limits

A one sample t-test for the LE artery diameter difference gave a t=0.939, df=9 and p=0.372 indicating there is no significant difference between the baseline measurement and a second measurement taken to assess observer error. The scatter plot shows all values lying within the 95% confidence limits. A linear regression calculation showed t=-0.446 and a non-significant p=0.667 indicating no statistical proportional bias.

	Masked Artery Measurement, Repeatability Comparison n=10			
	Original Measurements		Comparison Measurements	
	RE	LE	RE	LE
Glaucoma	262.19 (20.46)	246.25 (38.53)	262.7 (21.71)	246.0 (42.64)

Table 10 Masked Artery Measurement, Repeatability Comparison Glaucoma group

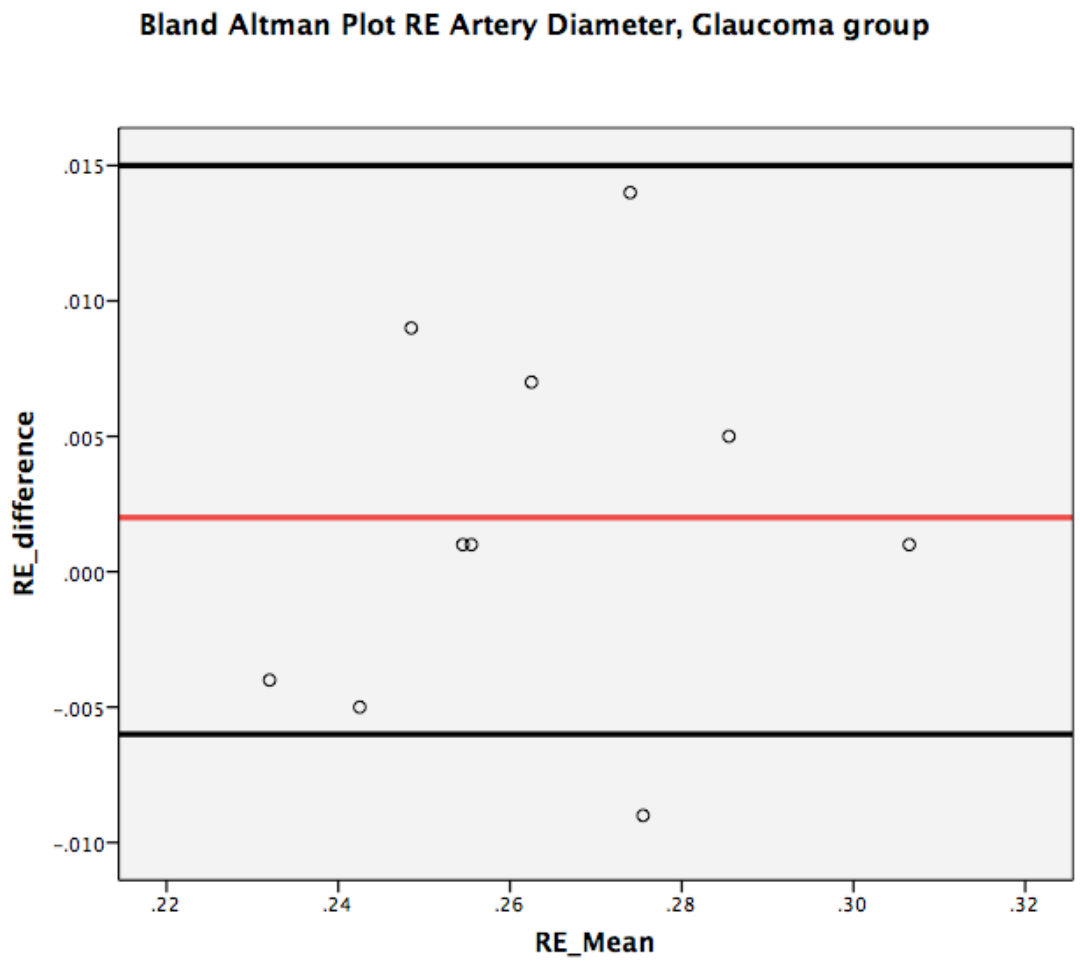


Figure 24 Bland Altman plot RE artery, glaucoma group, red line mean, black lines upper and lower 95% confidence

A one sample t-test for the RE artery diameter difference gave t=0.909, df=9 and p=0.387 indicating there is no significant difference between baseline measurement and a second baseline measurement taken to assess observer error. A linear regression calculation showed t=-.541 and a non-significant p=0.603 indicating no proportional bias.

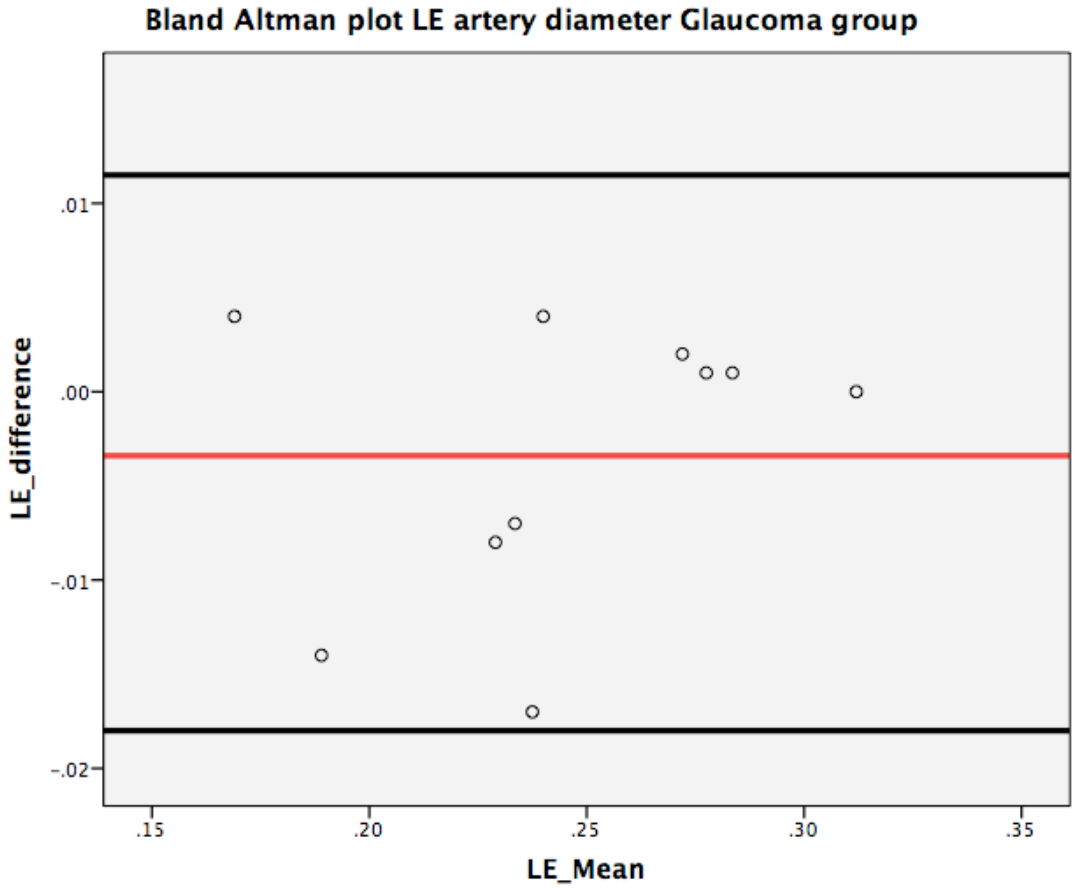


Figure 25 Bland Altman plot LE artery diameter Glaucoma group red line mean, black lines upper and lower 95% confidence limits

A one sample t-test for the RE artery diameter difference gave t=-1.414, df=9 and p=0.0191 indicating there is no significant difference between baseline measurement and a second baseline measurement taken to assess observer error. A linear regression calculation showed t=0.891 and a non-significant p=0.399 indicating no proportional bias.

4.2 Artery Diameter Comparison

In glaucoma the superior visual field is more frequently affected compared to inferior. The interactive tab on the HRT software makes the separation of these two hemispheres relatively easy. This research then expanded the normal consideration of circumferential vascular analysis to a more detailed consideration of whether diameter changes were more pronounced in the superior vascular network compared to the inferior.

The group mean superior and inferior CRAE values are shown for each group at baseline and one year later for right (Table 11) and left (Table 12) eyes.

	RE Group Mean superior and inferior CRAE (µm)			
	Baseline		1 Year After Baseline	
	Superior	Inferior	Superior	Inferior
Normal	212.7 (35.60)	199.9 (30.84)	207.3 (31.05)	201.7 (20.71)
Glaucoma	165.8 (30.64)	184.6 (21.26)	161.5 (31.32)	182.6 (21.26)
OHT	186.1 (23.81)	183.4 (23.41)	178.1 (24.89)	179.3 (25.79)

Table 11 Group Mean superior and inferior CRAE for RE baseline and one year later

In comparison between superior and inferior at baseline there was no significant difference in any groups (normal p=0.07, glaucoma p=0.09, OHT p=0.63). Using paired t-test, after one year there was no significant difference between superior and inferior (normal p=0.42, OHT p=0.83, glaucoma p=0.06).

At baseline the RE superior artery glaucoma group mean showed a reduced diameter of 46.91µm compared to the normal group (p=0.009) and 20.30µm compared to the OHT group (p=0.04). The OHT group also showed a smaller diameter compared to normal group of 26.61µm ($p=0.001$) For the RE inferior artery glaucoma group there was a significant difference between smaller diameter arteries OHT group, to normal, of 16.49µm (p=0.02), no other significant differences between groups (figure 26).

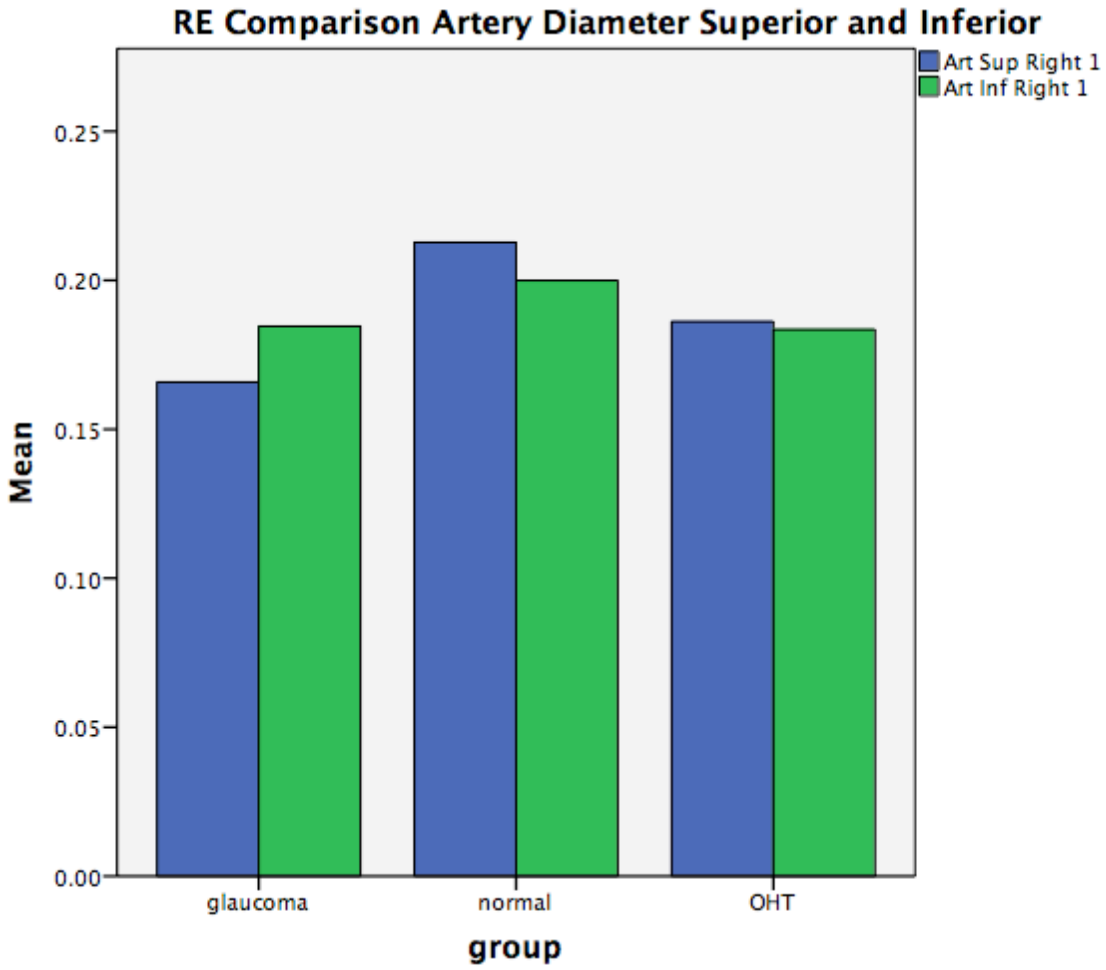


Figure 26 RE Comparison Artery Diameter Superior and Inferior at baseline

	LE Group Mean superior and inferior CRAE (µm)			
	Baseline		1 Year After Baseline	
	Superior	Inferior	Superior	Inferior
Normal	190.6(34.04)	190.1(31.43)	186.0(35.55)	193.5(21.56)
Glaucoma	156.7(23.42)	156.1(26.36)	147.5(23.45)	156.1(27.64)
OHT	174.9(33.08)	173.3(28.21)	167.6(35.96)	169.9(25.88)

Table 12 Group Mean superior and inferior CRAE for the LE at baseline and after one year

Using paired t-test, in comparison between superior and inferior LE there were no significant differences between any of the groups (normal p=0.95, glaucoma p=0.15, OHT p=0.85), table 12.

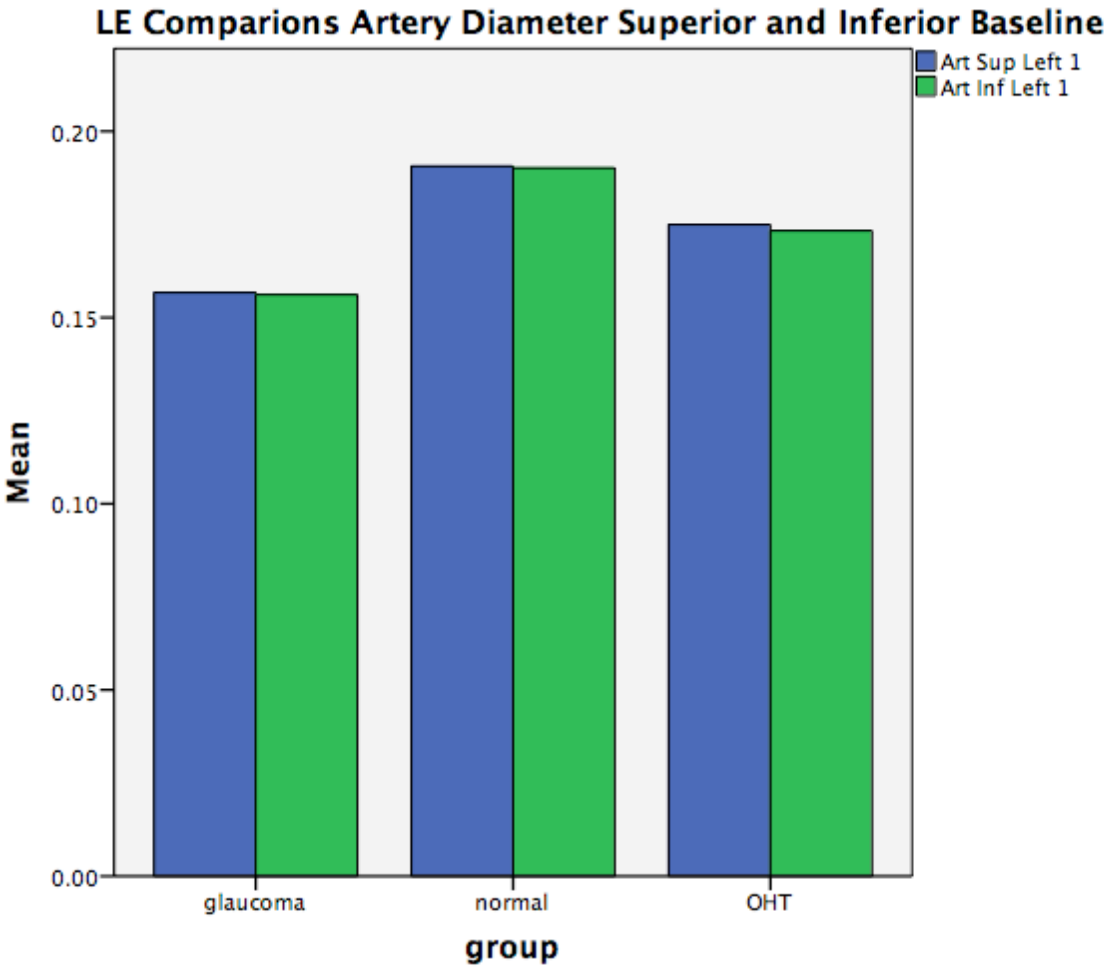


Figure 27 LE Comparison Artery Diameter Superior and Inferior at baseline

Using one way ANOVA to compare between groups in the LE, at baseline the superior glaucoma artery had the smallest diameter, compared to the larger OHT this was not significant (18.24 p=0.07), but, the normal compared to glaucoma was larger by a mean of 33.93µm, and this was significant (p=0.001). The OHT was smaller compared to the normal but this was not significant (15.69µm p=0.06). The inferior artery also had the glaucoma group as the smallest, this was only significant compared to normal (38.47µm p=0.00) but not the OHT group (20.06µm p=0.06) In contrast to the superior, the inferior artery, had a significantly smaller diameter in the OHT group, compared to normal (18.41 p=0.04)

	Paired Differences t-Test Mean (SD, p= µm)			
	Baseline CRAE compared to 1 Year After Baseline			
	RE Superior	LE Superior	RE Inferior	LE Inferior
Normal df (31)	5.43(21.20,0.16)	4.65(17.07,0.14)	-1.75(20.18,0.63)	-3.32(16.5,0.27)
Glaucoma df16	4.31(17.73,0.35)	9.19(14.19,0.02)	1.94(13.68,0.58)	0.01(13.54,0.98)
OHT df (27)	7.96 (21.3,0.06)	7.37 (13.8,0.01)	4.11(17.25,0.23)	6.44(14.89,0.03

Table 13 Paired t-test differences R&L, Superior & Inferior CRAE

Using table 12 to summarise an overall t-test comparison between baseline and one year for both right and left CRAE diameter measurements, there are three significant values; For glaucoma, the LE superior artery has reduced in diameter, but for the LE OHT group, both inferior and superior arteries have reduced in diameter.

Using an independent t-test to compare baseline RE with baseline LE, this showed not significant differences between right and left eye at baseline (table 14)

	Independent t-test compare RE & LE CRAE (µm)	
	Baseline (df,t,p=)	
	Superior	Inferior
Normal	(61)(t=2.517 p=0.517)	(60)(t=0.058 p=0.861)
Glaucoma	(30)(t=0.946 p=0.443)	(30)(t=3.359 p=0.535)
OHT	(52)(t=1.497 p=0.247)	(52)(t=1.439 p=0.445)

Table 14 Independent t-test to compare RE & LE CRAE

The veins have not carried any weighting in current research to determine a connection with glaucoma onset and progression. The veins diameters were measured in this research, as with the artery diameters, to determine if the results followed the generally non-significant values from reports into vein diameter change aiding glaucoma diagnosis and prognosis.

	RE Group Mean Superior and Inferior CRVE (µm)			
	Baseline		1 Year Later	
	Superior	Inferior	Superior	Inferior
Normal	250.6 (45.58)	230.3 (41.26)	250.09 (44.39)	227.4 (39.24)
Glaucoma	231.6 (35.49)	210.7 (46.82)	216.8 (23.25)	215.9 (41.84)
OHT	247.1 (34.81)	228.6 (47.78)	236.3 (28.64)	220.8 (48.09)

Table 15 RE Group Mean Superior and Inferior CRVE

The glaucoma group RE superior vein reduced in diameter, and this was shown to be significant (14.8 p=0.05), the other two groups had no significant change in the superior vein diameter. The RE inferior also reduced in diameter (7.85 p=0.01) but both the normal and OHT had no significant change. Between groups in the RE superior vein only the glaucoma being smaller in diameter than the normal showed a significant statistical difference (34.06µm p=0.02).

	LE Group Mean Superior and Inferior CRVE (µm)			
	Baseline		1 Year After Baseline	
	Superior	Inferior	Superior	Inferior
Normal	244.0 (44.59)	229.8 (25.81)	248.4 (16.71)	230.0 (25.96)
Glaucoma	203.0 (36.25)	195.9 (27.03)	202.1 (28.03)	194.7 (24.17)
OHT	230.4 (40.86)	218.0 (35.79)	224.1 (38.63)	217.7 (35.29)

Table 16 LE Group Mean Superior and Inferior CRVE

The LE superior and inferior baseline diameter showed no statistical difference between groups.

4.3 Global Artery Comparison

	Global Artery Comparison Baseline and One Year			
	Baseline µm (SD)		1 Year After Baseline µm (SD)	
	RE	LE	RE	LE
Normal	302.65 (41.74)	278.61 (36.53)	303.29 (38.91)	277.87 (33.92)
Glaucoma	262.19 (20.47)	246.25 (38.53)	257.81 (23.40)	236.56 (38.04)
OHT	271.89 (29.60)	260.00 (35.03)	262.78 (34.83)	249.56 (37.07)

Table 17 Global Artery Change Baseline and One Year

	Global Artery Change Baseline and One Year µm (t=, p=)	
	RE	LE
Normal	-0.001 (-0.322, 0.75)	0.001 (0.210, 0.83)
Glaucoma	0.004 (1.083, 0.296)	0.01 (3.079, 0.008)
OHT	0.009 (2.175, 0.039)	0.01 (3.114, 0.004)

Table 18 Paired T-test on Change of Artery Diameter Baseline and One Year

A paired sample t-test on global artery measurement change between baseline and one year showed the expected no statistical significance normal group. The Glaucoma group was also stable RE, but the Hypertensive group and LE Glaucoma group showed statistical significant change in artery diameter change.

5.0 HRT Parameters

Although optic disc size is not linked with glaucoma disease, the area of the disc is a confounding factor in judgement of neuroretinal rim loss and determination of either disease beginning or progression. This research reduced the effect of optical factors by excluding high spherical powers and high cylinders. It is useful to have a baseline mean to observe disc area in each group (table 19)

	RE Mean Disc Area (mm^2)(sd)	LE Mean Disc Area (mm^2)(sd)	N
Normal	1.84 (0.43)	1.82 (0.46)	32
Glaucoma	1.66 (0.35)	1.63 (0.26)	16
OHT	1.85 (0.37)	1.76 (0.38)	27

Table 19 Group Mean Disc Area (mm^2)

Although the glaucoma group was the smallest disc area in both right and left eyes compared to normal and OHT, one way ANOVA showed no significant difference between groups (glaucoma/normal p=0.112, glaucoma/OHT p=0.102, normal/OHT p=0.91) It was noted that the normal had the greatest standard variation in area, with glaucoma the smallest, which as a cross-section of optic discs, fits with the difficulty of judging neuroretinal rim normality across a range of disc areas.

The HRT machine divides up the optic nerve head into six segments. Both the Nasal and Temporal segment are approximately 100'deg each. The remaining four sections totalling 160'deg are split evenly between superior temporal, superior nasal, inferior temporal and inferior nasal. This is shown in fig.28 & 29

Figure 28 Segments of optic disc corresponding to visual field plot (Danesh-Meyer, Ku, et al. 2006)

Figure 29 Map of Visual field RE relating to Optic Nerve Head (Danesh-Meyer, Ku, et al. 2006)

For the purposes of research the superior hemifield in blood vessel measurement is taken to correspond to superior temporal and superior nasal with the inferior hemifield mapping to inferior temporal and inferior temporal.

The HRT machine produces volume values assigned to each of these sections. For the purpose of glaucoma in this research the nasal and temporal sections are have not been considered. This cross-sectional relationship is a useful first approximation for the longitudinal relationship between structure and function in patients.

	RE Group Mean Volume Superior and Inferior			
	Baseline (μm^3)		1 Year Later (μm^3)	
	Superior	Inferior	Superior	Inferior
Normal	84.7 (31.62)	93.8 (45.21)	80.3 (33.26)	90.9 (42.23)
Glaucoma	63.1 (29.15)	80.6 (42.81)	63.8 (23.06)	75.0 (37.24)
OHT	98.1 (35.63)	95.9 (42.47)	100.0 (37.42)	91.9 (37.63)

Table 20 RE Mean Volume Superior and Inferior Optic Disc

The glaucoma group was smaller in RE rim neuroretinal rim volume for both superior and inferior aspects. This reduced volume was only statistically significant in comparison between glaucoma and OHT superior (36.25μmm^3 p=0.001). The normal group rim volume was smaller than the OHT group and this was significant in the superior (19.69μmm^3 p=0.02). The inferior rim was not significant in any comparison combination. Paired t-test statistics comparing baseline and one year later showed a no significant difference in normal group rim volume RE either superior (p=0.114) or inferior (p=0.202). In the glaucoma group there was no significant change in rim volume RE superior (p=0.860) or inferior (p=0.057). The

OHT group showed no significant difference RE superior (p=0.593) or inferior (p=0.133).

	LE Group Mean Volume Superior and Inferior			
	Baseline (μm^3)		1 Year Later (μm^3)	
	Superior	Inferior	Superior	Inferior
Normal	83.1 (46.59)	101.9 (46.59)	78.1 (39.63)	90.9 (44.31)
Glaucoma	78.1 (39.63)	90.0 (44.31)	76.3 (36.96)	72.5 (57.91)
OHT	99.3 (39.90)	96.7 (33.51)	100.0 (37.42)	91.9 (37.63)

Table 21 LE Mean Volume Superior and Inferior Optic Disc

The LE glaucoma group had the smallest volume of the three groups at baseline; in a one way ANOVA between groups at baseline there was a significant difference when comparing glaucoma to OHT (34.49 p=0.005) and in comparing OHT to normal (22.62 p=0.026) but no significant difference when in comparing glaucoma to normal. Using a paired t-test to compare between baseline and one year later there was no significant change in LE normal group superior (p=0.306) but there was a noted change decrease in volume inferiorly (10.94μmm^3 p=0.004). In the glaucoma group LE neither the superior (p=0.222) or inferior (p=0.784) had any change. The OHT group had no significant change in either rim volume superior (p=0.694) or inferior (p=0.593) aspects.

A total neuroretinal rim volume was calculated to see if any change was approximately equal to superior or inferior rim volume in order to justify the segment comparison to artery diameter change.

	Total Neuroretinal Rim Volume			
	Baseline (μm^3)(sd)		1 Year Later (μm^3)(sd)	
	RE	LE	RE	LE
Normal	310.6 (112.48)	338.4 (113.79)	297.2 (109.4)	306.8 (100.91)
Glaucoma	263.1 (100.61)	245.6 (141.47)	255.5 (85.56)	235.6 (109.48)
OHT	335.9 (116.16)	331.1 (119.11)	330.7 (108.74)	336.1 (107.55)

Table 22 Total Mean Volume Optic Disc μmm^3 baseline and one year later

When comparing between groups at baseline there was a significant reduction in volume of the glaucoma group compared to the OHT group in both RE (72.80 p=0.04) and LE (85.49 p=0.02). In both eyes the other group comparisons were not significant. Performing a paired t-test between baseline and one year later the glaucoma group had no significant difference in either RE (p=0.41) or LE p=0.63).

The OHT group also showed no change RE (p=0.68) LE (p=0.59) The normal group was unchanged in the RE (p=0.11) but the LE did show a statistically significant reduction in volume (p=0.01).

	Group Mean Rim Area (μm^2)(sd)			
	Baseline		1 Year Later	
	RE	LE	RE	LE
Normal	1217.8(264.83)	1243.9(249.37)	1215.0(281.33)	1266.1(259.03)
Glaucoma	1215.0(264.83)	965.0(266.91)	1045.0(201.86)	948.1(274.23)
OHT	1270.4(207.5)	1207.4(250.02)	1262.6(200.57)	1203.7(248.68)

Table 23 Group Mean Rim Area (μm^2)

Using one-way ANOVA between groups at baseline the RE glaucoma was significantly smaller than the OHT (225.37 p=0.003) and normal (172.81 0.018) but not significant for normal compared to OHT (525.6 p=0.391). For the LE glaucoma was significantly reduced in area compared to both the normal (278.87 p=0.001) and OHT (242.41 p=0.003) but the OHT was not significant different to the normal (36.46 p=0.586) In comparing rim area with a paired t-test there was no significant difference between baseline and one year in any of the groups.

6.0 How do all the Parameters Link?

This research was based on determining the change in vessel diameter over time with the hypothesis that this change was related to glaucoma. In order to compare any relationship between the different variables measured a linear regression calculation was carried out.

All of the calculations were based on change between baseline and one year. Using linear regression the results of artery diameter change were compared between the major parameters of IOP, MD, PSD and Neuroretinal rim volume.

Because the participant numbers were relatively low, then heteroscedasticity was tested for each group to determine normal distribution and additionally a scatter plot was done to check for homogeneity of variance.

6.1 Neuroretinal Rim Volume and Artery Diameter

Pearson's correlation showed that there was no correlation between RE rim volume loss and artery diameter reduction for the glaucoma group. There was a normal distribution of data (Fig 30). The change of neuroretinal rim volume RE was not statistically significant when comparing to artery diameter reduction (F=0.68 t=0.825 p=0.423).

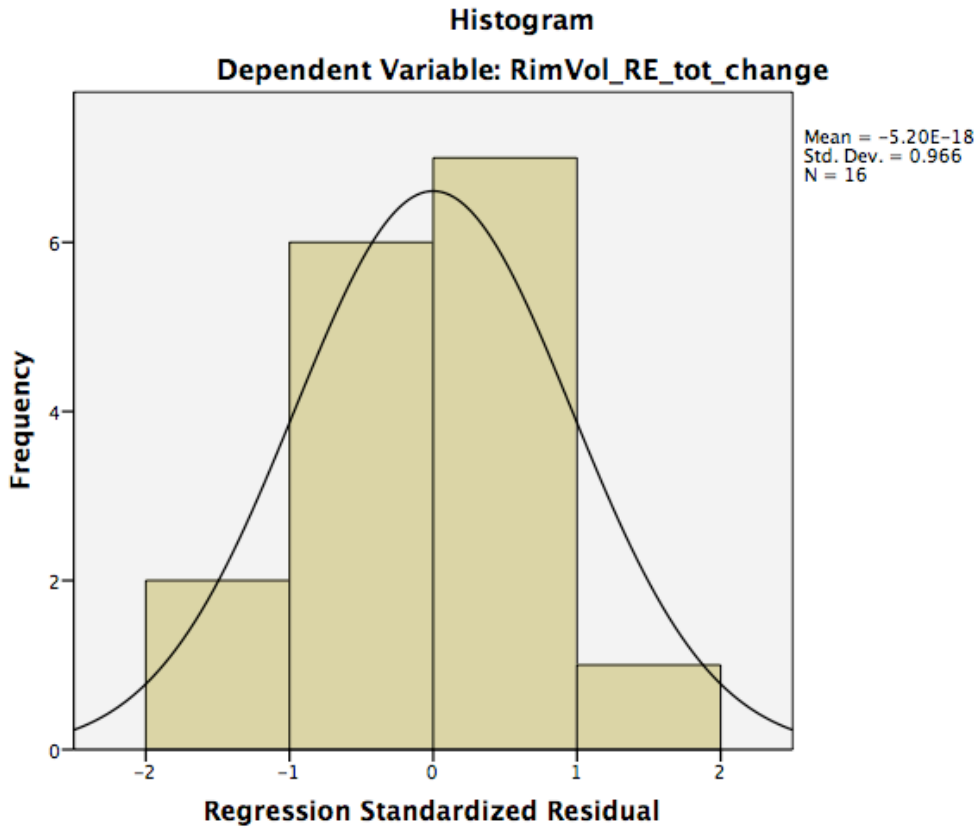


Figure 30 Normal Distribution of linear regression comparing Rim Volume Change to Artery Diameter change RE

The scatter plot showed a bird's nest pattern showing data was randomly distributed (fig 31).

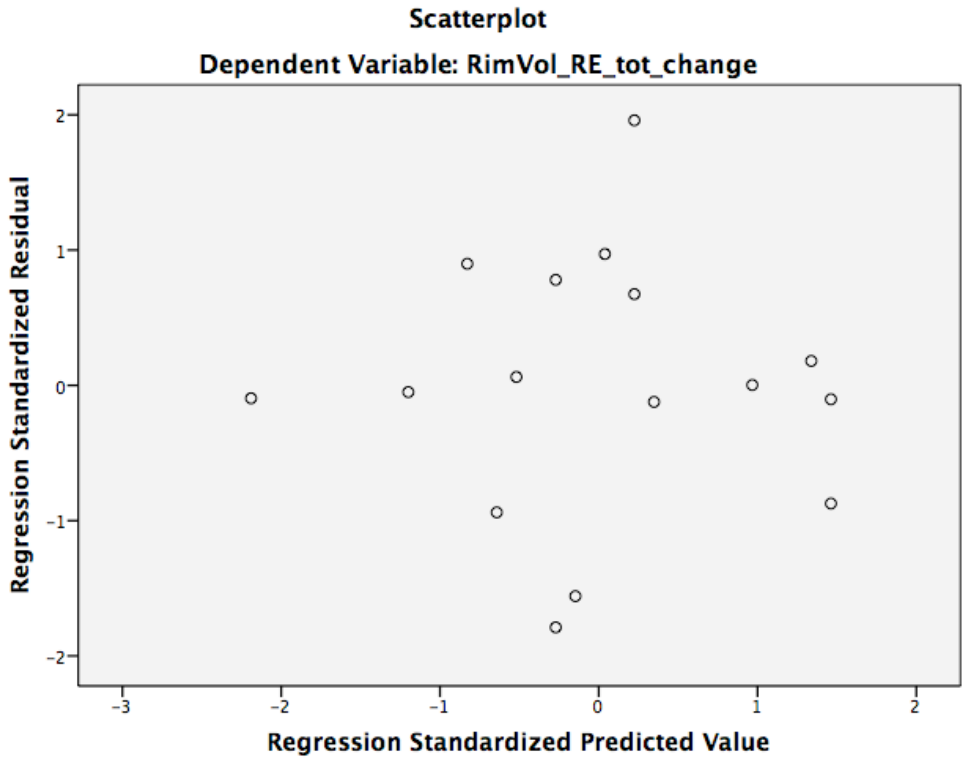


Figure 31 Scatterplot of Rim Volume RE change against RE artery diameter change to show data randomly distributed

Pearson's correlation showed no correlation between LE rim volume loss and artery diameter reduction. There was a normal distribution of data (fig 32). The change of neuroretinal rim volume LE was not statistically significant when comparing to artery diameter reduction (F=0.932 t=0.965 p=0.351). The scatter plot showed a birds nest pattern showing data was randomly distributed (fig 33).

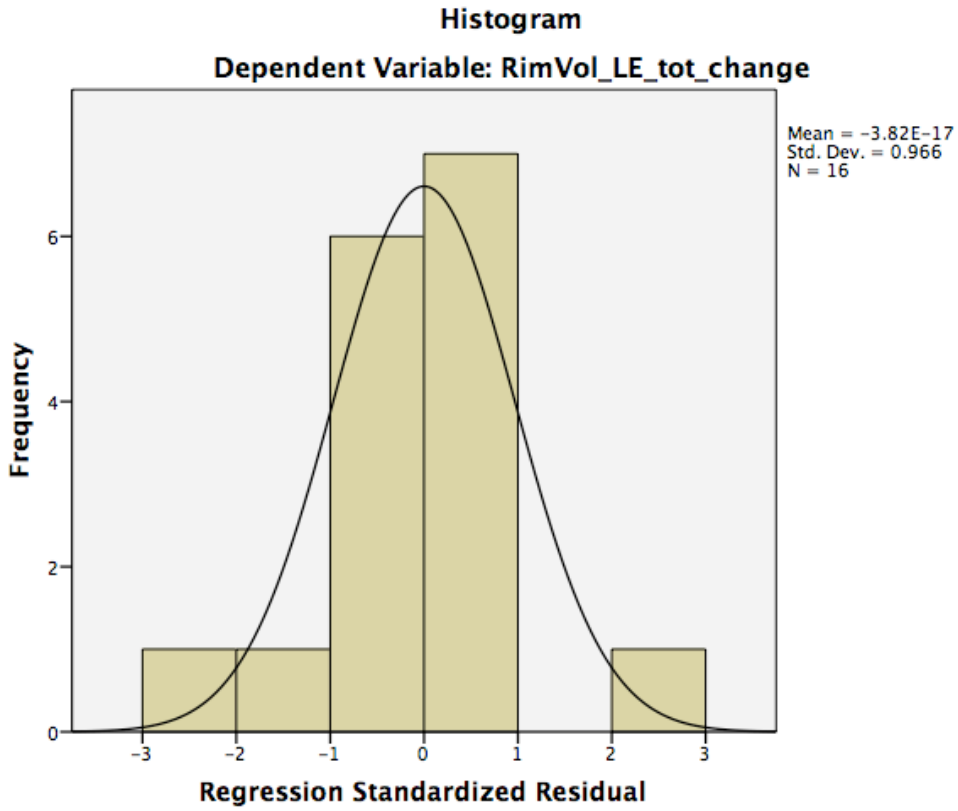


Figure 32 Normal Distribution of data for LE rim volume change compared to artery diameter change Glaucoma group

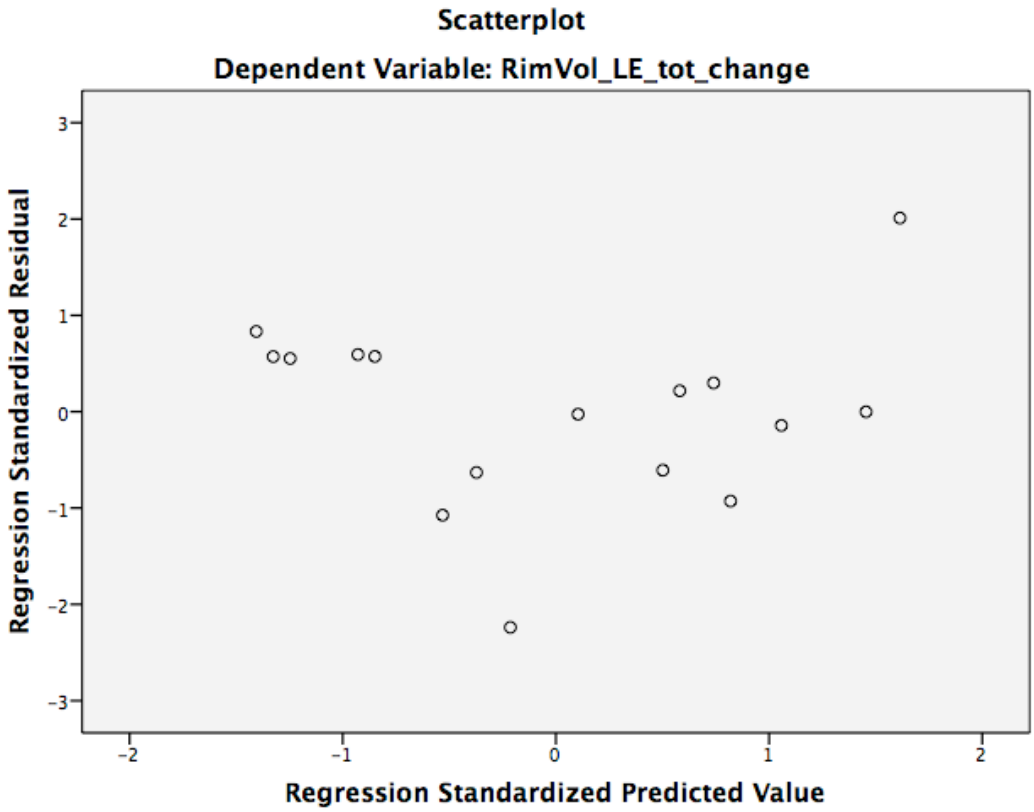


Figure 33 Scatter plot showing random distribution of change in neurofiber rim volume compared to artery diameter change LE Glaucoma group

Pearson's correlation was not significant between neurofiber rim volume loss and

artery diameter reduction RE normal group, there was normal distribution of data, and a scatterplot showed the data to be randomly distributed. The change of the neurofiber rim volume was not statistically significant (F=2.687 t=1.639 p=0.112).

Pearson's correlation for the normal group was significant between neurofiber rim volume loss and artery diameter reduction LE (t= 2.258 p=0.032). There was normal distribution of data, and a scatterplot showed the data to be randomly distributed, the P-P line showed good adherence to the regression line (fig 31). Although the rim volume change appeared to be connected to the reduction in artery diameter the confidence limits were large (CI lower 0.115 to upper 2.329).

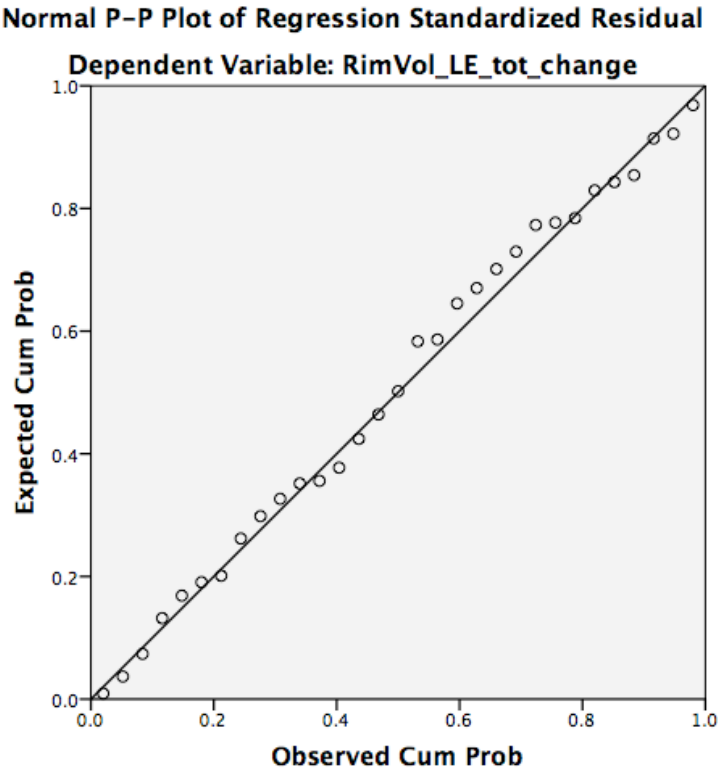


Figure 34 P-P plot showing good adherence to regression line for LE change in artery diameter compared to rim volume for Normal Group

Pearson's correlation for the Hypertensive group RE was significant in neurofiber rim volume reduction when compared to artery diameter reduction RE (p=0.05). There was normal distribution of data when plotted as a histogram (fig 34) and a scatter plot showed data randomly distributed. The change of neurofiber rim showed a statistically significant result (F=3.99 t=-1.998 p=0.05) but the confidence limits were large (CI lower -2.244 to upper 0.34).

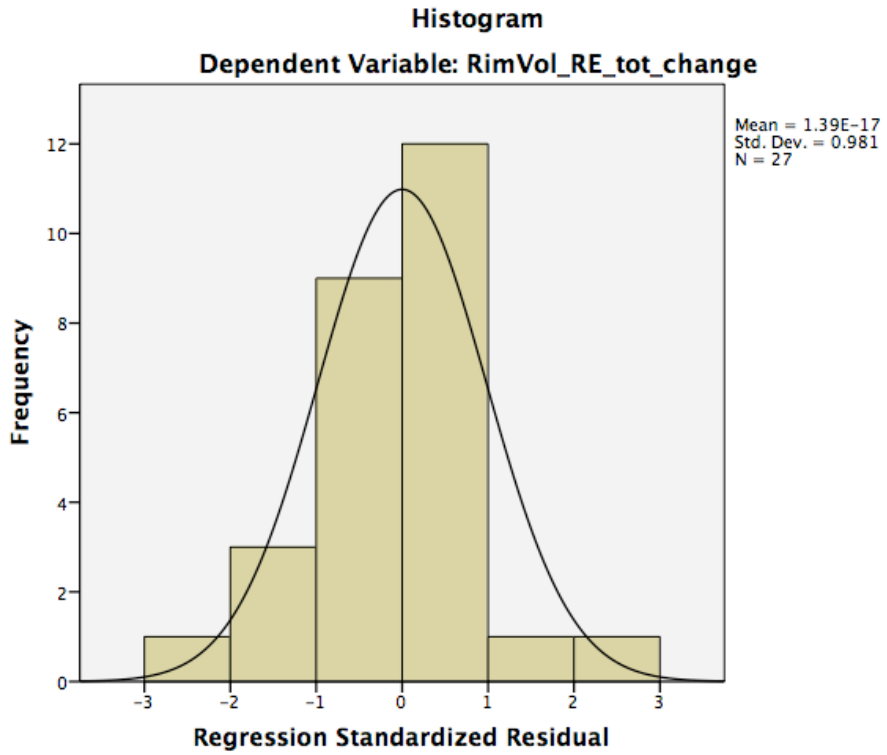


Figure 35 Histogram showing normal distribution of data in Hypertensive group comparing rim volume change against artery diameter reduction RE

Pearson's correlation was significant for LE for the Hypertensive group (p=0.05). There was normal distribution of data and a scatterplot showed the data to be randomly distributed. The rim volume change was statistically significant (F=4.072 t=2.018 p=0.05) the confidence limits were large (CI lower -0.22 to upper 2.188).

6.2 Comparison MD, PSD and Artery Diameter

	Comparison MD, PSD and Artery Diameter (F value, t value)(p value)			
	Mean Deviation		Pattern Standard Deviation	
	RE	LE	RE	LE
Normal	0.014, 0.959 (0.906)	1.317, 0.804 (0.26)	7.481, -1.143 (0.01)	2.053,0.541 (0.162)
Glaucoma	2.713, -1.647 (0.122)	1.184, 1.088 (0.295)	2.689,1.64 (0.123)	1.097,0.56 (0.313)
OHT	0.049,0.221 (0.827)	0.297,0.543 (0.591)	1.008,-1.004 (0.325)	2,241, -1.497 (0.147)

Figure 36 Comparison MD,PSD and Artery Diameter

Linear regression in the glaucoma group showed normal distribution both R&L eye. The RE in the glaucoma group r-value suggested a moderate correlation of diameter change being attributable to 16% of MD change. The LE r-value suggested only a small correlation of 7% MD change attributable to diameter change.

Linear regression in the normal group showed a normal distribution both R&L eye. The RE in the normal group r-value suggested no correlation of artery diameter attributable to MD change. The LE in the normal group r-value suggested a small correlation of 20% MD attributable to diameter change.

Linear regression in the OHT group showed normal distribution both R&L eye. The RE in the OHT group r-value showed no correlation of diameter change with MD. The LE r-value showed a small correlation of diameter change being attributable to 11% of MD.

Linear regression in the glaucoma group showed normal distribution both R&L eye. The RE in the glaucoma group, r-value, suggested a moderate correlation of diameter change being attributable to 16% of PSD change. The LE r-value showed a small correlation of 27% of PSD attributable to artery diameter change.

Linear regression in the normal group showed normal distribution both R&L eye. The RE in the normal group r-value showed a significantly correlated moderate change of 45% PSD attributable to diameter change. The LE r-value showed a small correlation of 25% of PSD attributable to artery diameter change.

The linear regression in the OHT group showed normal distribution in both R&L eye. The RE in the OHT group showed a small correlation of diameter change being attributable to 20% of PSD change. The LE showed a small correlation of diameter change being attributable to 28% of PSD change.

6.3 The Predictive Value of MD,PSD and Neuroretinal Rim volume on Artery Diameter

A multiple linear regression was done to see if the neurofiber rim volume change combined with the PSD change and MD changes were able to predict the change in artery diameter.

	Multiple Linear Regression Predictive Value of Neuroretinal rim volume, PSD and MD change to predict Artery Diameter Change	
	RE (F=, adjusted r square,p=)	LE (F=, adjusted r-square,p=)
Normal	3.999,0.225,0.017	3.513,0.201,0.029
Glaucoma	2,264,0,202,0.133	0.47, -0.119, 0.709
OHT	1.709, 0.076, 0.193	1.922, 0.096, 0.154

Table 24 Predictive Value of MD,PSD and NF rim volume on Artery Diameter

The adjusted r-squared value was small in all three groups, making any predictive value meaningless. The reason for the adjusted r-squared value being so small is the small sample size.

The significant correlation in the predictive value of change of PSD,MD and neurofiber rim volume in the normal group is a good indicator of the specificity of artery diameter change for correctly identifying normals.

6.4 Functional Analyses

6.4.1 AGE

The age distribution for each group was tested by Shapiro-Wilk for normality. Both the Glaucoma and Ocular hypertensive group were normally distributed (p>.05) but the normal group had a significant value (p=0.017), any statistics using age as a confounding parameter will use non-parametric tests. Visual inspection of the histogram and Q-Q plot showed normal distribution for all three groups and the skewness and kurtosis values are shown table 25.

AGE	Skewness (standard error)	Kurtosis (standard error)
Normal	-0.813(0.421)	-0.271(0.821)
Glaucoma	0.107(0.564)	-1.007(1.091)
OHT	-0.953(0.448)	2.051(0.872)

Table 25 Values of Skewness and Kurtosis for Age Distribution

6.4.2 IOP

The group mean IOP for each of the examined groups at baseline and after one year is shown in Table 26.

	Mean (SD) IOP (mmHg)			
	Baseline		1 Year After Baseline	
	RE	LE	RE	LE
Normal	16.6 (3.38)	16.3 (3.44)	16.0 (3.21)	15.2 (3.08)
Glaucoma	19.1 (5.01)	18.8 (4.37)	17.2 (2.32)	16.0 (3.54)
OHT	22.5 (3.31)	20.6 (2.83)	20.4 (3.39)	19.7 (3.37)

Table 26 Group mean IOP for each of the examined groups

The group mean IOP at baseline was significantly higher in the OHT group compared to the glaucoma group (RE 3.36mmHg greater p=0.01, LE 1.88 greater p=0.09) and normal group (RE 5.85mHg higher p=0.01, LE 4.37mmHg higher p=0.01), analysis of variance using repeated measures showed this difference to be significant for normal in both right and left eyes, but for the glaucoma group it was only significant in the right eye. In a paired t-test comparison between baseline and one year later there was no significant difference in normal right and left eyes (RE p=0.31, LE p=0.07), OHT patients had a small, but significant reduction right (RE 2.87 p=0.01) but not in the left eye (LE p=0.17) With glaucoma, the right eye had no

significant difference (RE p=0.08), but, the left eye was statistically reduced (LE 2.38 p=0.03).

6.4.3 Visual Field

All visual fields were within the reliability criteria of <33% false positive and negative and <33% fixation losses. This is in agreement with the accepted normal for a reliable visual field. The visual field global indices of mean deviation (MD) and pattern standard deviation (PSD) were measured at baseline and one year later, shown in Table 27,28

	Group Mean (SD) Mean Deviation (dB)			
	Baseline		1 Year After Baseline	
	RE	LE	RE	LE
Normal	-0.05 (1.56)	-0.27 (1.59)	-0.27 (2.02)	-0.45 (1.73)
Glaucoma	-0.50 (1.46)	-3.08 (2.46)	-0.69 (1.36)	-3.86 (2.72)
OHT	-0.43 (1.67)	-0.89 (1.92)	-0.44 (1.95)	-0.86 (2.15)

Table 27 Global Mean Deviation Indices at baseline and after one year

	Group Mean (SD) Pattern Standard Deviation (dB)			
	Baseline		1 Year After Baseline	
	RE	LE	RE	LE
Normal	1.93 (0.46)	1.96 (0.60)	2.05 (0.97)	1.89 (0.53)
Glaucoma	3.09 (1.59)	4.75 (3.19)	2.79 (0.86)	4.79 (0.86)
OHT	2.26 (0.67)	2.50 (1.45)	2.27 (0.82)	2.47 (1.95)

Table 28 Global Visual Field PSD at baseline and one year later

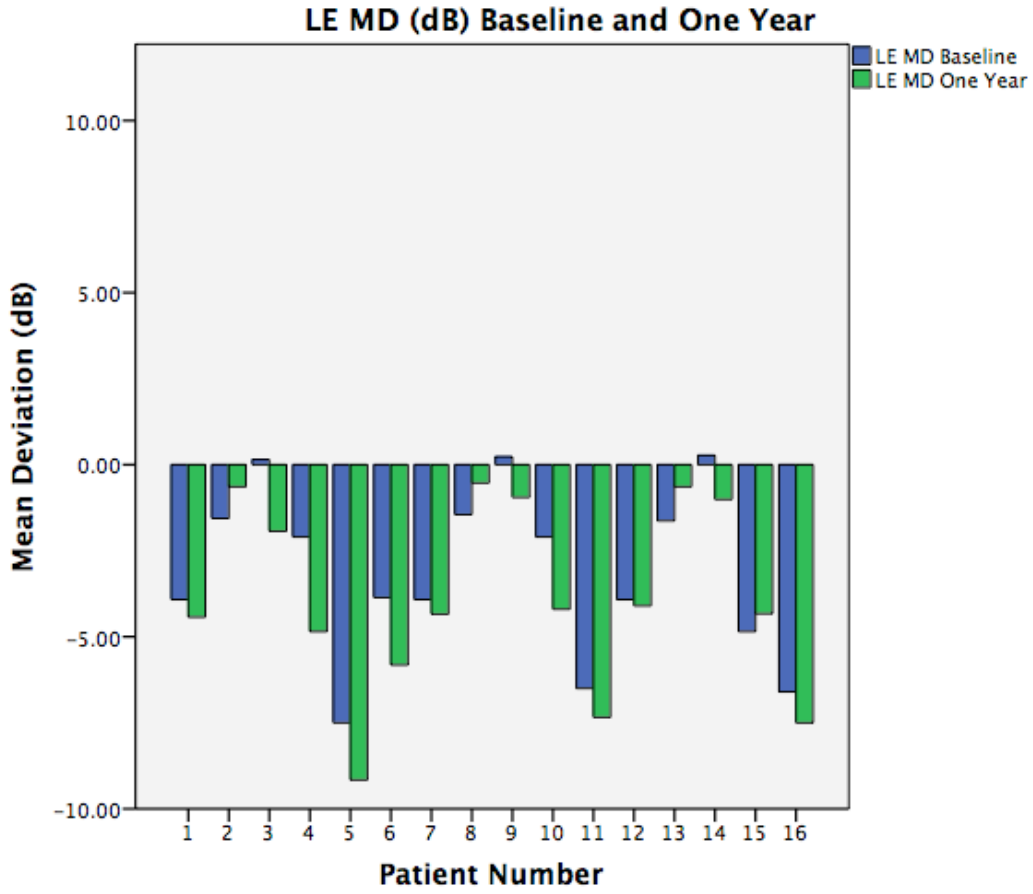


Figure 37 LE MD Baseline and One Year Later

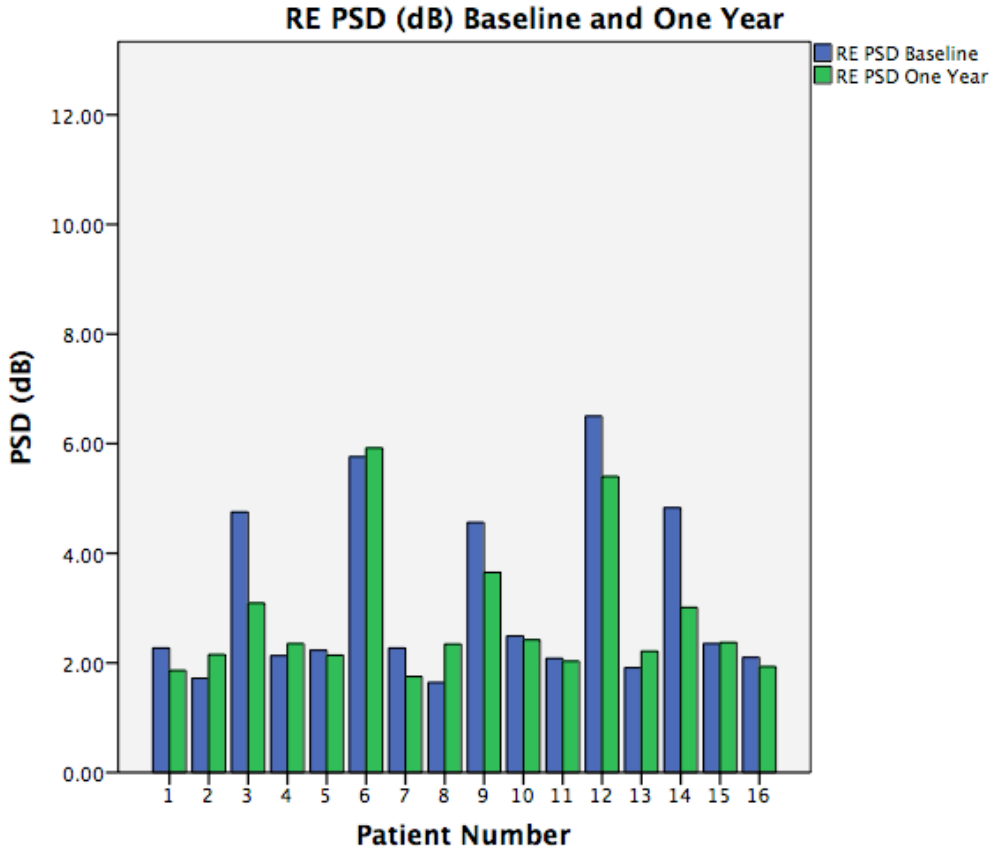


Figure 38 RE PSD (dB) Baseline and One Year

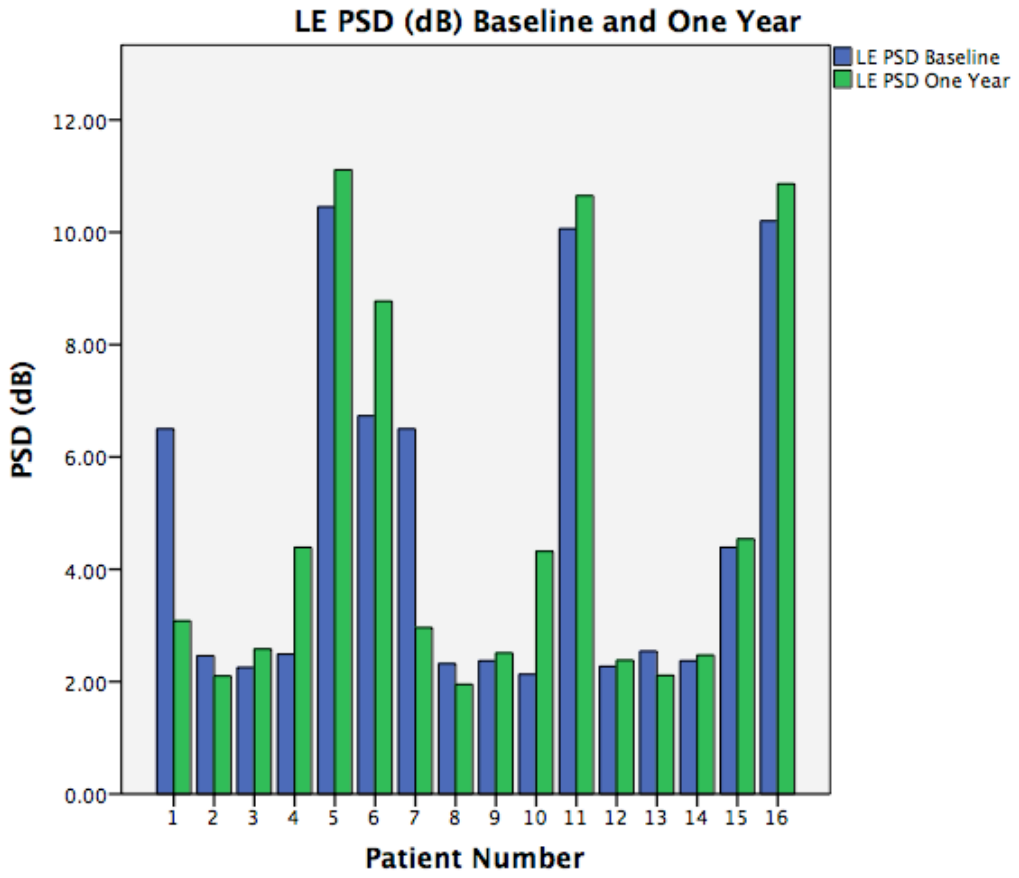


Figure 39 LE PSD (dB) Baseline and One Year

In comparison with paired t-test of mean deviation values both the OHT group and the normal group had no significant change between baseline and one year, OHT (RE 0.07 p=0.94, LE -0.12 p=0.91), normal (RE 0.97 p=0.34, LE 0.74 p=0.47). The glaucoma group did show a statistically significant worsening MD in the LE (RE 1.30 p=0.21, LE 2.64 p=0.02).

Using the t-test to compare PSD between baseline and one year there was no significant difference in all three groups, OHT (RE -0.30 p=0.98, LE 0.05 p=0.96), glaucoma (RE 1.71 p=0.11, LE -0.12 p=0.91) and normal (RE -0.89 p=0.38, LE 0.59 p=0.56)

Since there was no significant change in visual field indices between baseline and one year, all groups can be considered as stable and not progressing. The LE change in the MD of the LE for the glaucoma group did not have a corresponding change in PSD LE.

6.4.5 Blood Pressure

Table 28 illustrates blood pressure in terms of group mean diastolic and systolic pressures measured at baseline and one year later for each of the examined groups.

	Group Mean Diastolic and Systolic Pressures (mmHg)			
	Baseline		1 Year After Baseline	
	Diastolic	Systolic	Diastolic	Systolic
Normal	139.56 (17.31)	80.50 (6.75)	138.56 (17.05)	80.03 (5.72)
Glaucoma	145.25 (24.07)	79.56 (6.24)	140.06 (19.39)	78.31 (3.89)
OHT	135.93 (13.91)	82.48 (5.77)	139.19 (11.45)	81.44 (4.41)

Table 29 Group mean Diastolic and Systolic Pressures baseline and one year later

The mean group diastolic blood pressure at baseline was highest in the glaucoma group, but the differences between groups was not statistically significant. The mean systolic reading in the glaucoma group had become the lowest, but again at baseline between groups this was not significant. Using a paired t-test to assess significance between baseline and one year there was no significance in the normal group (diastolic 0.92 p=0.36, systolic 0.48 p=0.64). The OHT patients showed a significant increase in diastolic pressure but not systolic (diastolic -3.49 p=0.002, systolic 0.988 p=0.33). The glaucoma group had a significant reduction in diastolic but not systolic (diastolic 3.21 p=0.006, systolic 0.801 p=0.44).

6.4.6 Ocular Perfusion Pressure

Table 30 shows the group mean ocular perfusion pressure for each of the examined groups at baseline and one year later

	Group Mean Ocular Perfusion Pressure (mmHg)			
	Baseline		1 Year After Baseline	
	RE	LE	RE	LE
Normal	63.39 (7.49)	63.67 (7.03)	63.36 (7.75)	64.15 (7.67)
Glaucoma	63.10 (8.99)	63.40 (8.91)	62.46 (7.88)	63.65 (9.12)
OHT	56.26 (8.40)	58.11 (7.90)	59.59 (6.40)	60.26 (6.20)

Table 30 Group Mean Ocular perfusion pressure baseline and one year later

In comparison between mean ocular perfusion pressure the OHT group had the lowest value this was statistically significant compared to the other two groups, glaucoma (RE -6.85mmHg p=0.01, LE -5.37mmHg p=0.03) normal (RE -7.03mmHg p=0.002, LE -5.56mmHg p=0.008) The normal group was not significantly different compared to glaucoma. Using t-test to compare baseline to one year, neither the normal nor glaucoma group were changed significantly, but, the OHT group did have a significant increase (RE 3.38 p=0.02, LE 2.6 p=0.02).

6.4.7 Corneal Thickness

	Group Mean Central Corneal Thickness (µm)			
	Baseline		1 Year Later	
	RE	LE	RE	LE
Normal	579.34 (31.25)	580.66 (35.99)	578.28 (32.47)	581.09 (37.84)
Glaucoma	572.94 (29.04)	568.87 (25.28)	579.5 (13.37)	568.5 (14.96)
OHT	596.78 (65.88)	604.22 (84.69)	577.59 (33.52)	580.78 (35.56)

Table 31 Group mean Central Corneal Thickness Baseline and One Year (µm)

The cornea should not change in thickness without a pathological background, which was an exclusion criterion for entrance into this research. Therefore the central corneal thickness measurement can be simply treated as a baseline characteristic for each group. There was no significant difference between groups at baseline and no significant change in corneal thickness in any group between baseline and one year.

7.0 Discussion

The research objective was to measure blood vessel diameter using the HRT. The interactive measurements tab in the software was used to measure blood vessel diameter to within 0.011mm. The results when comparing the separate hemispheres, superior and inferior, follow generally accepted values of vessel diameter (Wong, Shankar, et al. 2004; Lee, Kim, et al. 2014; Joos, Singleton, et al. 1997). The artery diameter was larger when both hemispheres were combined. The initial reason for the increase in artery width was the fixed horizontal line that was used to consistently measure the same position in the artery between two visits. This line bisected the artery at an angle that was greater than the perpendicular and therefore the value was naturally greater. This increase in diameter was then mathematically inflated when entering the value into the correction equation for vessel diameter. The important value is the diameter change over time; as a consequence the average diameter being larger was of no relevance to statistical outcomes. This variation of bisection of blood vessels being perpendicular for some vessels but almost parallel for others explains the much larger standard deviation compared to other research. But, the trend for glaucoma arteries to be smaller in diameter than normal arteries held (Lee, Kim, et al. 2014); this decrease in diameter is thought to be a predictor for glaucoma onset.
The basis of glaucoma diagnosis is separating structural and functional changes relative to the normal eye. The aim of the research was to evaluate whether a correlation exists between functional change defined by the visual field status and retinal vessel diameter change with a normal group acting as the control, the glaucoma group being a known disease variable and the OHT group acting as an “at risk’ population under observation.

The IOP diurnal variation has been compared between right and left eyes (Liu, Sit, et al. 2005) and not found to be different. This agrees with these results in both the normal and glaucoma group, which would be expected in a stable population. The OHT group did show reduction in IOP in the right eye, but the standard deviation was relatively high at 3.39mmHg so this finding could be a statistical outlier rather one of clinical significance. The same outlier argument could be used for the reduction in IOP left eye since again the standard deviation of the group was relatively high at 3.54mmHg. The majority of volunteers were statistically similar in measurement of IOP between baseline and one year, as a group statistic any one

individual would need to change by a large amount to show up as significant against a stable majority.

The difference between right and left eye vessel diameters for the 75 volunteers at baseline was compared. There was no difference in any group, and this would agree with the revised formula research of (Knudtson, Lee, et al. 2003) who did not differentiate between right and left eye vessel diameters when using their researching their central retinal vessel equivalent calculation. The method of retinal vessel calculation using the revised formulas was central to this research, and post 2003, was overwhelmingly the calculation of choice among more experienced researchers (Wong 2004; Kawasaki, Wang, et al. 2013). The lack of significant differences between right and left eyes for vessel diameter reduces the possibility of the left eye being influenced by its proximity to the heart and a higher systolic blood pressure.

The central retinal artery/vein equivalent formula is now an accepted standard in research, but there is no accepted framework for how to measure vessel diameters using manual or automated methods. Nor is there a framework for the assessment of inferior or superior vessel diameters. This research measured these parameters as separate values, and compared them directly to other known indices of glaucomatous change i.e. IOP and visual field global indices. Uniquely, this research took advantage of the interactive tab on the HRT machine software to measure blood vessels using the movable cursor over a fixed retinal image without need for magnification correction or exporting an image to a separate computer program for analysis.

Previously with manual measurement of blood vessels was carried out by taking the retinal image and projecting onto a wall, then physically measuring at a known point followed by a mathematical calculation accounting for the magnification, and converting the value into millimetres. The most difficult part in this task was measuring at exactly the same point one year later. The HRT has inbuilt software to align the optic disc, allowing calculation of neuroretinal rim volume, and attributing change to sectors on the disc which are then used in its progression software to deduce if there has been a statistical change suggestive of glaucoma. This means that the optic disc, and subsequently the blood vessels are always in the same position and the X, Y placement values determined for each measurement at baseline can be returned to at any point thereafter. One of the difficulties discussed in other research about taking manual measurements was the determination of

blood vessel edge (Rassam, Patel, et al. 1994). This was also a confounding variable whilst using the HRT. It was alleviated somewhat by the interactive tab having two additional line graphs showing a 2D topography of the vertical and horizontal retina. When taking the measurements these were utilised to determine the beginning of the vessel edge, the sudden change in height visible on contour line set against a relatively flat retinal background. There was generally a slight dip in traveling over the vessel due to the lumen but the edges were identified because of their height in comparison to surrounding contours. Where this identification was difficult, measurements were avoided in order to reduce repeatability errors in the data. Because there is no option to magnify the image, then small diameter vessels <0.012mm were not measured because edges could not be identified. The identification of blood vessels as either artery or vein was sometimes unknown and these vessels were also not included in data collection. This is an unfortunate consequence, since it is the smaller diameter arteries that are thought to be affected first in the glaucoma disease process (Sun, Wang, et al. 2009), and therefore this data was lost to analysis. One way to improve this data collection would be to increase the scanning accuracy of the confocal laser and add a magnification option to the interactive tab, but, this is a difficult option because the confocal system has a physical limit of the moving mirror and aperture setup (figure 11) so the improvement is directed towards the machine hardware as opposed to software interpretation of already gathered data. The measurement of blood vessels has a learning curve for the user, and a specific weakness in this research was the absence of any consistency check of diameter data collected. For each volunteer there was no anonymity or masking of patient grouping, but each data collection was carried out immediately after scanning without reference to other volunteers or previous measurements for that individual. A Bland-Altman plot was done on one group of 10 participants from both the normal and glaucoma volunteers. This involved a second measurement done on the baseline data and compared to the original data to determine continuity of measurement. The plot, kurtosis and skewness showed a normal distribution and any differences to be within accepted values. Although this is not ideal for collection of data the aim of this research was to determine if this vessel diameter variable could be determined by the HRT and therefore used as a reliable criteria for glaucoma detection in a high street optometric practice. The research data collection did not have the statistical robustness of a random control trial, but instead was based in the real world scenario of a high street optometrist screening for glaucoma.

The difficulty in assessing change of blood vessel diameter in the group of 75 volunteers is that the disease pattern is often stable and therefore no change would be expected. The general expected baseline results did concur with current research but any change in vessel diameter may have been masked and consequently did not show up in the statistical analysis. The general data did show a decrease in the left eye superior artery diameter, but this was not corroborated by a change in left eye the MD or PSD value in the visual field, and so was probably an outlying statistic. The OHT group did show a reduction in artery diameter for both inferior and superior in the left eye, but this did not manifest as a change in visual field and neither did it manifest as a reduction in neuroretinal rim volume. When the data from the four suspect volunteers were separated from the stable data the typical glaucomatous change in visual field was noted in both MD and PSD with a much greater change in the left eye and this would explain its statistical presence even combined with the stable data. All of the glaucoma suspects developed a greater visual field defect in the superior visual field, and this correlated well with the reduced volume in inferior neuroretinal rim. When comparing the artery diameter, both right and left superior and inferior had diameters had reduced, but the inferior diameter had reduced by a greater amount in comparison to superior diameter and to a greater extent in the left eye compared to right eye which is consistent with the visual field outcome and also current research findings which suggest that artery diameter change being is linked with glaucoma. The vein diameter also decreased between baseline and one year but not by the same degree as artery diameter. The difficulty posed when measuring blood vessel diameter would suggest that the current viewpoint of changes in vein diameter not being significant is probably correct.

Three of the OHT group and one in the normal group were suspect for conversion to glaucoma and have subsequently been referred with all relevant data to be assessed by an ophthalmologist. When this group data was separated out and the same t-test and one-way ANOVA performed there was no significant change between baseline and one year. It is assumed that the reason for a result of no statistical significance is due to the very small sample size. It was noted that when simply observing the results the IOP and ocular perfusion pressure were almost static but the predicted results of visual field indices (MD & PSD), optic disc rim volume (total, superior & inferior), optic disc rim area, and artery diameter (superior and inferior) all changed as expected for a positive glaucomatous diagnosis. This would suggest a larger sample size would allow a statistical significance to prove a clinical observation.

Blood vessel diameter is known to reduce with age by approximately 4.8µm for CRAE and 4.1µm for CRVE every increase of 10 years (Leung, Wang, et al. 2003). This value was significantly less than the diameter change found in the OHT group over only one year. This natural decrease in diameter for both artery diameter and vein diameter is a useful known variable for assessing abnormality over time in suspect groups.

If blood vessel diameter is considered as a risk factor alongside all other variables then it may be advisable to bracket the diameters into different groups, allowing for a range of diameters within a set range in any one group. This would highlight the importance of an individual moving between groups. The Blue Mountains Eye Study did exactly this when assessing risk of glaucoma over ten years for a standard deviation decrease in artery diameter giving an odds ratio of 1.77 (95% CI 1.11-2.78). This odds ratio increased as incidence of glaucoma persisted with IOP less than 20mmHg (Kawasaki, Wang, et al. 2013). They divided up their patient base into four groups of artery diameters; this grouping would allow a higher sensitivity but a lower specificity. In our research the individual measurements allow a higher specificity but the repeatability of the measurements need to be high. The manual aspect of vessel measurement would probably be more suited to placing the patient in a certain diameter group on baseline measurement allowing this variable to act more as a risk factor for glaucoma onset or progression as opposed to an absolute change defining the beginning of glaucoma.

There are two questions to consider when accepting the reduction of artery diameter reported by this research, with regards to glaucoma onset and whether it could be considered as an important variable in glaucoma progression.

1) Why does the artery change in diameter?
2) Does the artery change before ganglion cell death occurs?

Blood vessels are composed of two interacting cell types. Endothelial cells form the inner lining of the vessel wall and pericytes envelope the surface of the vascular tube. Pericytes are the supporting cells of the microvasculature. Elevated BP produces changes in the pericytes in the retinal capillary walls (Wallow, Bindley, et al. 1993) Pericytes are functionally significant; when a vessel loses a pericyte they become haemorrhagic and hyperdilated (Bergers & Song 2005).

Figure 40 Pericyte Function on Blood Vessels (Google images)

http://www.nature.com/neuro/journal/v14/n11/images/nn.2946-F4.jpg

It has been shown that oxygen levels regulate pericyte contraction and that pericyte density is dependent on blood pressure levels with the highest density of pericytes found in the retina, and analysis of structure in diabetic retinopathy showing micro aneurysms coincident with pericyte dropout (Bergers & Song 2005). This pericyte susceptibility to BP and IOP and their role in vascular stability may mean they are the initiating factor in artery diameter reduction and subsequently retinal ganglion cell death.

The initiating factor in dysregulation of ocular blood flow is coincident with endothelium-1 (ET-1)(Grieshaber, Mozaffarieh, et al. 2007). This increased level of ET-1 lead to a reduction in blood flow in both the choroid and the optic nerve head. There is estimated to be four times as many pericytes in the retina compared to a similar area in the brain, which is the only other vascular bed with equivalent properties to that of the retina. This increased density, is also known to be combined with a turnover rate, which is slower than any other vascular bed. ET-1 is a very potent vasoconstrictor. ET-1 and nitric oxide are important mediators of cellular signalling within the retinal microvasculature, ET-1 has been shown to cause retinal pericyte contraction (Chakravarthy 1999).

The retinal blood vessels are known to lie in the nerve and ganglion cell layer from examination of OCT scans of the 3D retina topography (Zhu, Tong, et al. 2014). The weight of evidence is building for the role of vascular dysregulation in glaucoma with known factors such as poor auto regulation in glaucoma sufferers (Leske 2009), migraine associated with glaucoma (Phelps & Corbett 1985) and blood pressure (Zhao, Cho, et al. 2014). The suggestion of Topouzis et al that BP alone has been

shown to effect optic disc cupping is suggestive of ganglion cell death (Topouzis, Coleman, et al. 2006). Furthermore, the finding that ACE inhibitors have an inhibitory effect on glaucoma progression by controlling BP by dilating blood vessels (Hirooka, Baba, et al. 2006) adds to the weight of there being significant vascular factors involved in the development and progression of glaucoma.

Up to 50% of the ganglion cells need to die to give a reliable glaucomatous field loss (Kerrigan–Baumrind, Quigley, et al. 2000; Katz, Gilbert, et al. 1997), it is difficult to ascertain at what point narrow arteries catalyse or initiate ganglion cell death. The determination of when glaucoma starts to develop point is still fraught with difficulties, and although vessel diameter is an increasingly important variable, the interpretation of diameter as a weighting factor when set alongside well researched parameters needs careful consideration.

8.0 Implications for Optometry Practice

When considering Glaucoma as a disease, the raised IOP in congenital, angle closure and secondary glaucoma's demonstrate that IOP can be the sole factor causing the condition. Conversely, the findings of glaucomatous cupping, loss of neurfibers at the optic disc and repeatable defects in the visual field show glaucoma in patients who have normal IOP. These two, polar opposite IOP cardinal signs, suggest there are other significant weighting factors in the transformation of a healthy eye to glaucoma.

In the early diagnosis of glaucoma, optometrists in the UK rely on the triad of IOP, visual field and optic disc assessment. The measurement of IOP is variable, because of the diurnal factor, corneal thickness, instrument used and glaucoma as a condition makes the IOP more variable between troughs and peaks. The field exam is variable, not only because of subjective considerations, but also because the redundancy in retinal ganglion cells does not translate into consistent visual field defects. The final part, in this longstanding triad of glaucoma diagnosis, is optic disc assessment. The optometrist is attempting to describe a 3D object in a 2D picture, adding the important detail of change, in a $3mm^2$ circle containing 1.2 million nerves, to finally come to a conclusion of normality, in the 3 minutes allocated to fundoscopy during an eye exam.

Measurement of retinal arteries would add an objective, independent factor to aid diagnosis in glaucoma in its many permutations.

Glaucoma appears to follow a pattern of blood vessel diameter change, followed by neuroretinal rim loss leading to visual field loss. All other factors act as a catalyst for each of these sequenced events.
With further software development it may be possible to automate measurement of blood vessel diameters and calculate the percentage change considered to be outside of normal limits with respect to a large robust normally distributed population. With an increasing database then the predictive power of artery diameter change would become a dependable factor in glaucoma diagnosis.

The primary function of the HRT is a machine for assessing neuroretinal rim volume and determining change over time. The added value of its ability to assess blood vessel diameter, would allow a high street optometrist to add this as a weighting factor in their glaucoma decision.

But, the manual aspect of fine repeatable measurements, make this a time consuming task, with no definite diagnosis of glaucoma or normality at the end. Until larger scale studies are carried out to determine the clinical value of blood vessel diameter measurement in glaucoma diagnosis, its use is limited.

8.1 Future Work

The most important development for this work would be expansion it to a much larger clinical population of normal, and then comparison a larger range of glaucoma patients, stratified precisely for degree of glaucoma severity. A randomised control study would need to confirm the repeatability, of vessel diameter measurements.

The HRT has inbuilt software for predicting glaucoma or the probability of glaucomatous damage. This research did not utilise any available data from these programs. The HRT software has already been shown in research to be predictive of glaucoma. Because the change in artery diameter is also considered a pre-perimetric change then using the progressor software and following over an extended period of time, has the potential to corroborate early diameter narrowing with HRT values and visual field loss.

With the increasing availability of the spectral-domain optical coherence tomography (SD-OCT) and its acceptance as a normal purchase for a high street optometry practice, the extension of the scan to volume data collection of retinal artery and vein diameters may become a valuable resource of general health indicators for cardiology, general practice and ophthalmology. A recent study using SD-OCT technology showed reliable, repeat measurements of vascular structure (Zhu, Tong, et al. 2014), and this will only increase in accuracy with improving scan speed, depth and measurable anatomical change. The automatic identification of veins and arteries would allow for computerised diameter measurement, this would allow utilisation of this additional criteria to be added as a further weighting factor in early glaucoma detection.

9.0 References

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10.0 Appendix

10.1 Volunteer Information Sheet

Researchers, School and subject area responsible
Principal Investigator: Mr Colum Rooney, 5 Ballynahinch Street, Hillsborough, Northern Ireland

Project Supervisor: Dr Robert Cubbidge, Optometry, Life and Health Sciences, Vision Sciences, Aston University

Project Title **The influence of blood vessel diameter on glaucoma progression**

Invitation
You are being invited to take part in a research study. Before you agree it is important for you to understand why the research is being carried out and what it will involve. Please take time to read the following information carefully.

What is the purpose of this study?
When an optometrist looks into the back of your eye they can see the small blood vessels which supply your retina. The purpose of this investigation is to assess the retinal blood vessels in order to determine whether they change in size with glaucoma.

There are no personal benefits to participation in this research and all of the tests carried are available if necessary in your normal eye examination.

If blood vessels are found to alter in size in glaucoma, this finding may become an aid in the diagnosis of glaucoma.

Why have I been chosen?
You have been asked to participate in this study because you fall into one of three pre-determined groups;
1/ diagnosed glaucoma 2/ the pressure of the liquid inside your eye is higher than in the normal population but is not damaging your eye 3/ you have no detectable risk of glaucoma.

The study aims to recruit 120 subjects.

What will happen to me if I take part?
If you agree to participate, you will be asked to attend the practice in Hillsborough on four separate occasions. The first visit should take about 30 minutes to complete and is an extended eye examination; this will involve the normal eye examination plus a measurement of your blood pressure. An image of the back of your eye will then be taken using an instrument called a Heidelberg Retinal Tomograph. You will be asked to place your chin on a rest and look at a steady flashing red light. The image takes a few seconds to acquire and you will see a bright red light from a low powered laser. This imaging procedure is commonly used in hospital eye clinics and is known to be safe.

There are two additional measurements not normally taken in an optometric practice, these are, corneal thickness (the clear window of your eye) and gonioscopy. A single drop of local anaesthetic will be placed in your eyes, the effects of which will wear off after about 30 minutes. Two procedures will be performed on each eye. In the first procedure, a contact lens type instrument called a gonioscope placed on your eye to observe the structures inside your eye where liquid normally drains into the blood stream. Secondly, a plastic pen-like instrument will lightly touch the front surface of your eye (the cornea) to measure its thickness.

The second visit will take place one week later when you will be asked to repeat the visual field test completed during the first visit, this is a peripheral field exam where you click a button when you see lights at the corner of your vision, this will only take ten minutes and does not require any anaesthetic drops.

After one year you will be recalled for a repeat of the first visit, and your fourth and final visit will, once again, repeat the visual field measurement.

Should any of the tests reveal an eye problem, you will be referred to an ophthalmologist.

Should the blood pressure measurement show a high reading, you will be referred to your GP for further investigation.

Are there any potential risks in taking part in the study?

There is a very small risk that the topical anaesthetic Benoxinate Hydrochloride may cause a toxic reaction which causes corneal inflammation. If this occurs, your vision will be monitored closely and if necessary you will be referred urgently to an ophthalmologist.

The gonioscope and instrument which measures corneal thickness could cause an abrasion to the corneal surface. This does not occur often and in most cases, the regenerative ability of the cornea leads to healing within about an hour. Your eyes will be checked for abrasion after these procedures have been carried out and if it an abrasion is found to be severe, which occurs very rarely, you will be referred to an ophthalmologist for treatment.

Do I have to take part?

No, you do not have to participate and you are also free to withdraw from the study at any time after agreeing to take part without any giving a reason. Your normal eye care will not be affected by not participating.

Expenses and payments?

No expenses or payments can be offered for participating in this research.

Will my taking part be kept confidential?

In accordance with the Data Protection Act, Mr Colum Rooney will be the only investigator who has access to your name. Any database used for analysis of the results will not contain your name. Your name will be replaced by an identification number which will only be known by Mr Rooney. All data used for analysis will be encrypted electronically and stored in a secure location.

You will not be able to be identified in any future publication of results.

What will happen to the results of the research study?

The results from this research may be published in a thesis and peer reviewed journal. You will not be identifiable in any of these publications.

Who is organising and funding the research?

This project and purchase of additional equipment is fully funded by Colum Rooney.

Who has reviewed the study?

The research has been approved by the Audiology and Optometry Research Ethics Committee (AROC) at Aston University

Who do I contact if something goes wrong, or I need further information?

In the first instance, you should contact Mr Colum Rooney, 5 Ballynahinch Street, Hillsborough, BT26 6AW, (028) 9268 8881

Who do I contact if I wish to make a complaint about the way in which the research is conducted?

If you have any concerns about the way in which the study has been conducted then you should contact the Audiology and Optometry Research Ethics Committee (AROC) at Aston University, l.n.davies@aston.ac.uk 0121 204 4152

10.2 Volunteer Consent Form

Aston University
Life & Health Sciences

Aston Triangle
Birmingham, B4 7ET, UK
Phone: +44 (0) 121 2043853
Fax: +44 (0) 121 2043892

Personal Identification Number:
_____

CONSENT FORM

Title of Study :The influence of blood vessel diameter on glaucoma progression

Research Venue: 5 Ballynahinch Street, Hillsborough, Northern Ireland

Investigators' names: Mr. Colum Rooney, 5 Ballynahinch Street, Hillsborough, Northern Ireland
Dr. R.Cubbidge, School of Life & Health Sciences, Aston University

Please tick boxes as appropriate

1. I confirm that I have read and understood the information sheet dated (version .....) for the above study. ☐

2. I have had the opportunity to ask questions about the study and I was given a minimum of 24hrs to make a decision on participation. ☐

3. I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason and without my legal rights being affected. ☐

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

_____	_____	
Full name of Research Participant	Date (dd/mm/yyyy)	Signature
_____	_____	
Full name of Researcher	Date (dd/mm/yyyy)	Signature

10.3 Audiology & Optometry Ethics Approval

Aston University
Life & Health Sciences

AUDIOLOGY & OPTOMETRY ETHICS FORM

All parts of the *Ethics Application* must be written concisely using terminology that would be understandable to an educated lay person on an ethics committee.

Title: The influence of blood vessel diameter on glaucoma progression

Principal Investigator: Mr Colum Rooney

Contact Details: 5 Ballynahinch Street, Hillsborough, Northern Ireland

Other Staff / Students involved: Dr Robert Cubbidge, Optometry, School of Life & Health Sciences, Aston University, Birmingham B4 7ET, r.p.cubbidge@aston.ac.uk

A. PROJECT OBJECTIVES / BACKGROUND

A1. What are the primary research questions / objective?

Purpose and Justification:

The purpose of this study is to correlate either progression in glaucoma, or conversion to glaucoma, against blood vessel diameter changes measured using the Heidelberg Retinal Tomograph 3(HRT). We hypothesise that the diameter of retinal blood vessels is altered in glaucoma.

Early diagnosis of glaucoma is critical to prevent permanent structural damage and irreversible vision loss. Currently, the diagnosis of glaucoma is made via a triad of tests; optic disc assessment, intra-ocular pressure (IOP) and visual field examination. These tests are looking for typical glaucoma traits in the form of ganglion cell loss on the optic disc, raised IOP with the potential to cause harm and a pattern of visual field loss typical of glaucoma. There are two other parameters not normally taken in a high street optometry practice aiding glaucoma diagnosis, central corneal thickness (CCT) and ocular perfusion pressure (OPP).

OPP is defined as the difference between arterial and venous pressure, in the eye, venous pressure is equal to, or slightly greater than IOP. OPP can therefore be defined as the difference between blood pressure (BP) and IOP. OPP can be further broken down to diastolic perfusion pressure and systolic perfusion pressure. These values are gaining increasing significance in glaucoma literature, helping to explain why lowering of IOP past the assigned target level does not slow glaucoma progression. In the Early Manifest Glaucoma Trial low systolic perfusion pressure, low systolic blood pressure and cardiovascular disease were predictors of glaucoma.

Significant reductions in flow velocities, and increases in resistance indices, of blood vessels in both primary open angle glaucoma, and normal tension glaucoma, in comparison to normal control subjects have been demonstrated using scanning laser Doppler flowmetry.

By making an assessment of visual field status, vascular status and retinal blood vessel diameter, this research aims to determine if there is a retinal vascular element that is measureable in a high street optometry practice, in the hope that it will further aid glaucoma diagnosis. Scanning laser tomography will be used to quantify blood vessel diameter change longitudinally and cross-sectionally in addition to estimation of ocular perfusion pressure.

A2. Where will the study take place?

Practice based: 5 Ballynahinch Street, Hillsborough, Northern Ireland

A3. Describe the statistical methods and/or other relevant methodological approaches to be used in the analysis of the results *(e.g. methods of masking / randomization)*

The protocol will be randomised between volunteers in order to minimise any order effects.

A4. List the clinical techniques to be conducted on patients as part of the study and indicate whether they fall within the scope of normal professional practice of the individual to perform them

Each volunteer will be required to attend for four times. The patient will be examined with an extended eye iexamination on their first visit involving prescription determination, keratometer readings, IOP with Goldmann, stereoscopic fundus examination using a volk lens, gonioscopy to evaluate the anterior angle, slit-lamp exam on anterior eye, central corneal thickness measurement using an ultrasound pachymeter, blood pressure measurement, scanning laser retinal tomography of the optic nerve head using the HRT and finally a full threshold visual field examination. At the second visit which will take place one week after the first visit, the visual field examination will be repeated in order to reduce the perimetric learning effect.

Visits 3 and 4 are a repeat of visits 1 and 2 and will take place one year after they have attended for the initial visits.

B. RESEARCH PARTICIPANTS

B1. How many participants will be recruited? Please provide justification (power analysis software available from http://www.psycho.uni-duesseldorf.de/abteilungen/aap/gpower3/)

Using the diameter of artery and vein measurements reported in the Blue Mountains Eye Study, and knowing that a two tailed parametric test will be performed on the data, allows the GPower program to estimate the sample set that will be required. This estimate varies between 7-44 depending on whether vein or artery diameter is used. Based on this estimation forty volunteers per group will be recruited. In total 120 volunteers will be recruited for this research.

B2. What restrictions will there be on participation (age, gender, language comprehension etc)?

Three patient groups will be recruited (40 volunteers in each group). The research participants will be between the ages of 30-70 since this is the group most prone to glaucoma.

The research will involve a selection of patients from a normal, high street optometrists. These will be selected, and placed into three categories of patients;

-1/ Normal: defined as a patient with no glaucomatous visual field loss, no optic disc changes suggestive of glaucoma and IOP<21mmHg

-2/ Ocular Hypertensive: defined as a patient with no glaucomatous visual field loss, no optic disc changes suggestive of glaucoma and an IOP>21mmHg

-3/ Glaucoma: defined as a patient diagnosed with glaucoma by an ophthalmologist and currently under review by them.

Because of the NICE directive, and guidelines from the College of Optometrists all hypertensive patients will have been referred and discharged by the hospital, or, are presently waiting to be seen.

Ethnicity may be a complicating factor in blood vessel diameter change, research has suggested different progression rates of glaucoma despite similar IOP with the suggested explanation relating to its vascular etiology. The demographics of N.Ireland, and in particular the patient sample area will heavily skew the ethnicity towards Caucasian. This will help reduce the variables for statistical analysis.

EXCLUSION CRITERIA
I intend to limit the glaucoma group to only those being treated with Latanoprost (Xalatan, a prostaglandin analogue) This will remove the potential complicating factor of beta-blockers as a treating agent for glaucoma and its potential for effecting blood vessel diameter

I will not include any participants who have a significant general health history, significant ocular history or a notable mental health history so as to attempt to normalise variables between the three research groups.

General Exclusion criteria will include

- Refractive correction greater than +/- 4.00DS and 2.00 DC
- Medication known to influence the visual field
- Diabetic patients
- Patients with significant lens opacity
- Closed angle glaucoma and volunteers who's anterior angles are at risk of closure

Specific Inclusion criteria relating to the glaucoma group

- Glaucoma will be treated with Latanoprost (Xalatan, a prostaglandin analogue) This will remove the potential complicating factor of beta-blockers as a treating agent for glaucoma and its potential for effecting blood vessel diameter

The NICE guidelines will be followed for any patient found to become glaucoma suspect in either the normal or ocular hypertensive group, and referral to an ophthalmologist arranged; whilst any progression in the glaucoma group will be noted and a copy sent to their treating ophthalmologist for consideration in their complete care regime.

B3. How will potential research participants in the study be (i) identified, (ii) approached and (iii) recruited? *If research participants will be recruited via advertisement then attach a copy of the advertisement in the appendix of the ethics report.*

The volunteers will be recruited from the patients attending Mr Colum Rooney's Optometry practice.

B4. Will the participants be from any of the following groups? *Tick as appropriate and justify any affirmative answers.*

Children under 16:	☐
Adults with learning disabilities:	☐
Adults who are unconscious or very severely ill:	☐
Adults who have a terminal illness:	☐
Adults in emergency situations:	☐
Adults with mental illness (particularly if detained under Mental Health Legislation):	☐
Adults suffering from dementia:	☐
Prisoners:	☐
Young Offenders:	☐
Healthy volunteers:	☒
Those who could be considered to have a particularly dependent relationship with the investigator, e.g. those in care homes, audiology students:	☐
Other vulnerable groups:	☐

Justification for healthy volunteers is outlined above in section B2.

B5. What is the expected total duration of participation in the study for each participant?

12 months

B6. Will the activity of the volunteer be restricted in any way either before or after the procedure (e.g. diet or ability to drive)? *If so then give details.*

no

B7. What is the potential for pain, discomfort, distress, inconvenience or changes to life-style for research participants during and after the study?

No additional precautions are required before or after the visit other than advice following the instillation of benoxinate hydrochloride local anaesthetic drops.

B8. What levels of risk are involved with participation and how will they be minimized?

Hazards

The participant may become distressed by one of the procedures, or during the study we may find a new eye condition.

There is a very small risk that the topical anaesthetic Benoxinate Hydrochloride may cause a toxic reaction which causes corneal inflammation. If this occurs vision may become blurry and gritty pain may follow the effect of the anaesthetic wearing off. Patients who exhibit a toxic reaction to the anaesthetic will be monitored closely and if necessary referred urgently to an ophthalmologist.

The gonioscope and instrument which measures corneal thickness could cause an abrasion to the corneal surface. This does not occur often and in most cases, the regenerative ability of the cornea leads to healing within about an hour. Slit lamp examination of the cornea after the procedures will check for abrasion and if found to be severe, which occurs very rarely, the volunteer will be referred to an ophthalmologist for treatment.

Gonioscopy and measurement of corneal thickness involves direct contact of probe with the surface of the cornea. If the intraocular pressure is found to be above 21 mmHg through the screening process, then the volunteer will undergo further investigations in accordance with the NICE guidelines for referral of glaucoma and ocular hypertension to determine the presence of either condition. If necessary, the optometrist referral pathway for these conditions will be followed.

B9. What is the potential for benefit for research participants?

No direct benefit.

B10. If your research involves individual or group interviews/questionnaires, what topics or issues might be sensitive, embarrassing or upsetting? Is it possible that criminal or other disclosures requiring action could take place during the study?

N/A

B11. How will participants be de-briefed after the study? [See separate guidance notes on designing a De-briefing Sheet]

Volunteers will be de-briefed verbally. Information sheets supplied to volunteers express the opportunity for participants to request copies of any associated published material

B12. Describe any arrangement that have been made for indemnity insurance, if you have reason to think it is not already covered by the University's policy. [NOTE: We have been advised by the Risk & Insurance officer that research on Aston campus, or undertaken by Aston OD students, is covered by the University's professional indemnity policy, currently provided by Zurich Municipal Insurance with a £10 million limit.]

Covered by Aston University policy

C. CONSENT

C1. Will a signed record of informed consent be obtained from the research participants? *If consent is not to be obtained, please explain why not.*

Yes

C2. Who will take consent and how it will be done?

Prospective volunteers will be supplied with information sheet. An investigator and prospective volunteer will convene at least 24 hours after to discuss any issues raised by the prospective volunteer before signing of consent form by both investigator and volunteer.

The participant will be informed before they enter the study both in writing and orally that they may leave the study at any time. If we discover a new eye condition we will ensure that they receive the correct advice and referral, consistent with the statutory regulatory framework of optometric practice.

C3. How long will the participant have to decide whether to take part in the research? *Justify your answer.*

24 hours. Volunteers will be supplied with an information sheet. This is the minimum period of time recognized by the NHS National Research Ethics Service guidance for participation to qualify as informed consent.

C4. What arrangements are in place to ensure participants receive any information that becomes available during the course of the research that may be relevant to their continued participation?

If we discover a new eye condition we will ensure that they receive the correct advice and referral, consistent with the statutory regulatory framework of optometric practice

C5. Will individual research participants receive any *payments/reimbursements* or any other *incentives* or *benefits* for taking part in this research*? If so, then indicate how much and on what basis this has been decided?*

No

C6. How will the results of research be made available to research participants and communities from which they are drawn?

Information sheets supplied to volunteers express the opportunity for participants to request copies of any associated published material

D. DATA PROTECTION

D1. Will the research involve any of the following activities? *Delete as appropriate and justify any affirmative answers.*

Examination of medical records by those outside the NHS, or within the NHS by those who would not normally have access: ☐

Electronic transfer of data by e-mail:	☐
Sharing of data with other organizations:	☐
Use of personal addresses, postcodes, faxes, emails or telephone numbers:	☐
Publication of direct quotations from respondents:	☐
Publication of data that might allow identification of individuals:	☐
Use of audio/visual recording devices:	☐

D2. Will data be stored in any of the following ways? *Delete as appropriate and justify any affirmative answers.*

Manual files:	☒
Home or other computers:	☒
University computers:	☐

Data will be accessible only from local computers. Access to data is password protected.

D3. What measures have been put in place to ensure confidentiality of personal data? *Give details of whether any encryption or other anonymisation procedures will be used, and at what stage.*

All volunteers will be assigned a personal identification number. Only the personal identification number will be used for storing data.

D4. If the data are not anonymised, where will the analysis of the data from the study take place and by whom will it be undertaken?

N/A

D5. Other than the study staff, who will have access to the data generated by the study?

None

D6. Who will have control of, and act as the custodian for, the data generated by the study?

Mr Colum Rooney

D7. For how long will data from the study be stored [minimum 5 years]? *Give details of where and how the data will be stored.*

Data will be stored for at least 7 years. Data will be stored on computer machines. Rooms are locked and require key access. Access to data on machines will be password protected.

E. GENERAL ETHICAL CONSIDERATIONS

E1. What do you consider to be the main ethical issues or problems that may arise with the proposed study, and what steps will be taken to address these?

The primary ethical considerations relate to hazards associated with the investigative techniques that are employed. As outlined in section B8 above, the degree of risk associated with these risks is either very low, and where possible, procedures will be implemented to further minimize any risks.

Stage	Humphrey MD score	Additional Criteria at least 1 of the listed criteria must apply)
Stage 0: No or Minimal Defect		
Stage 1: Early Defect	≥ -6.00 dB	- a cluster of ≥ 3 points on the pattern deviation plot in an expected location of the visual field depressed below the 5% level, at least one of which is depressed below the 1% level - CPSD/PSD significant at P<00.5 - GHT Outside Normal Limits""
Stage 2: Moderate Defect	≥ -6.00 to -12.00 dB	- ≥ 25% but <50% of points on the attern deviation plot depressed below the 5% level, and ≥15% but <25% of points deprese below the 1% level - at least 1 point within the central 5° with sensitivity of <15 dB but no points in the central 5° with sensitivity of <0 dB - only 1 hemifield containing a point with sensitivity <15 dB within 5° of fixation
Stage 3: Advanced Defect	≥ -12.01 to -20.00 dB	- ≥ 50% but <75% of points on pattern deviation plot depressed below the 5% level and ≥25% but <50% of points depressed below the 1% level - any point within the central 5° with sensitivity <0 dB - both hemifields containing a point(s) with sensitivity <15 dB within 5° of fixation
Stage 4: Severe Defect	≥ -20.00 dB	- ≥ 75% of points on pattern deviation plot depressed below the 5% level and ≥50% but <50% of points depressed below the 1% level - at least 50% of points within the central 5° with sensitivity <0 dB - both hemifields containing >50% of points with sensitivity <15 dB within 5° of fixation
Stage 5: End-Stage Disease		Unable to perform HVFA in worst eye due to central scotoma or worst eye VA 6/60 or worse due to POAG. Fellow eye may be at any stage

PSD=Pattern Standard Deviation, POAG=Primary Open Angle Glaucoma

Hodapp E, Parrish RK II, Anderson DR. Clinical decisions in glaucoma.

St Louis: The CV Mosby Co; 1993. pp. 52–61.