OCULAR BLOOD FLOW IN UNTREATED OCULAR HYPERTENSION AND PRIMARY OPEN ANGLE GLAUCOMA

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MD Thesis

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for my family

Declaration

I, Dr Jan Kerr MBChB(Ed) FRCOphth(Lon) declare that this thesis submitted for the degree of Doctor of Medicine to the University of Edinburgh was composed by me and that the work comprising it is my own. It has not been submitted in candidature for any other degree, diploma or other professional qualification.

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Table of Contents

Contents

Contents

Contents

Appendices

Index of Tables

Tables

Index of Figures

Abstract

Introduction: Primary open angle glaucoma (POAG) is a potentially blinding disease which for most of its course is asymptomatic. Raised intraocular pressure (IOP) is a risk factor for developing glaucoma and is used to screen for at risk individuals. Up to 5% of the population have raised IOP but only a small proportion of these ocular hypertensives (OHT) will develop POAG. Intraocular pressure therefore performs poorly as a screening test for glaucoma and other factors are suspected to play a role in the aetiology of the disease.

Purpose: To compare ocular and systemic circulation and haematological factors affecting perfusion in groups of untreated ocular hypertensives (OHT) and primary open angle glaucoma patients (POAG) matched for IOP.

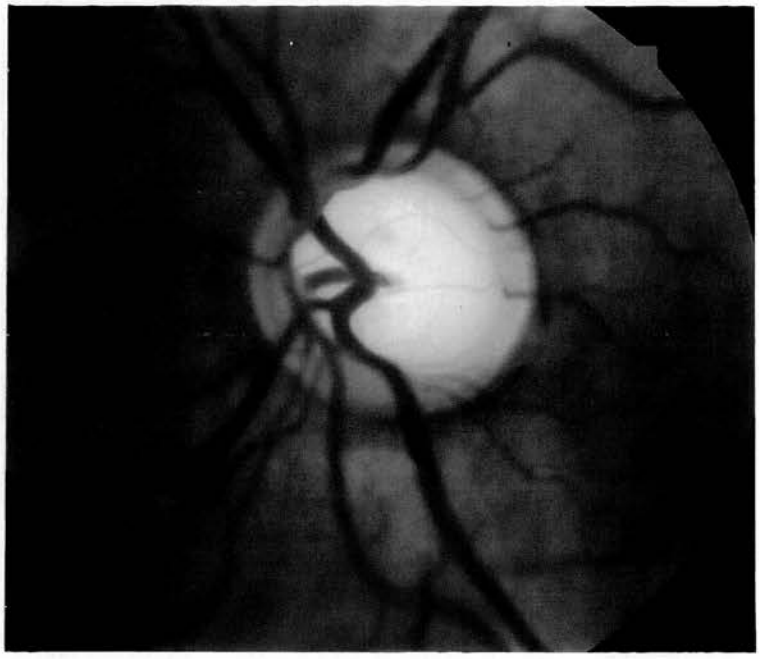
Methods: This was a prospective observational study. Twenty seven high risk OHT (IOP>25mmHg), 24 low risk OHT (IOP<26mmHg), 24 POAG patients and 23 normal subjects were recruited. Subjects were admitted for a morning during which the following measurements were made; intraocular pressure, visual field assessment, sitting, standing and supine pulsatile ocular blood flow, scanning laser Doppler retinal blood flow and finger tip laser doppler blood flow. Venous blood was taken for the following; full blood count, manual fibrinogen, D-dimer, prothrombin fragments F1 and 2, and beta-thromboglobulin.

Results: Pulsatile ocular blood flow: High risk ocular hypertensives (HROHT) were similar to POAG subjects in terms of their pulsatile ocular blood flow and both groups had reduced POBF compared to normal. POAG had a smaller fall in POBF on lying down than the other 3 groups. Scanning laser doppler flow: POAG had reduced blood flow at the optic cup and increased blood flow in the temporal retina compared to HROHT matched for IOP. Finger tip laser doppler flow: No difference in fingertip blood flow was found between HROHT and POAG. Haematology: A small but significant increase in platelets and fibrinogen was seen in POAG compared to normals. There was no difference between HROHT and POAG.

Conclusions: Ocular hypertensives with IOP above 25mmHg have levels of POBF similar to POAG. Differences in the response of POBF to changing posture and the distribution of blood flow in the retina in POAG compared to OHT point to a failure of control of ocular blood flow as a possible factor in the aetiology of glaucoma.

Abbreviations

POAG	Primary Open Angle Glaucoma
NTG	Normal tension glaucoma
HROHT	High risk ocular hypertension
LROHT	Low risk ocular hypertension
IOP	Intraocular pressure
LDF	Laser Doppler Flowmeter (finger tip)
POBF	Pulsatile ocular blood flow
SLDF	Scanning Laser Doppler Flowmeter (retina)
HRF	Heidelberg Retinal Flowmeter
CDI	Colour Doppler Imaging



SECTION I

Introduction and Review of Literature

1.1

Introduction

The role of vascular factors in the pathogenesis of glaucoma has been a subject of debate since the end of the 19th century. The glaucomatous optic disc was first described in the 1850's following the invention of the direct ophthalmoscope. In 1857 von Graefe proposed what would become the mechanical theory of glaucoma pathogenesis suggesting that optic nerve cupping arose secondary to backward pressure on the lamina cribrosa in eyes with raised intraocular pressure (IOP)[1]. The following year Jaeger suggested that deficient circulation in the short posterior ciliary arteries was responsible for glaucomatous optic atrophy[2], a proposal which has become known as the vascular theory of glaucoma damage. Over the following 100 years researchers continued to debate the aetiology of the disease. Other risk factors were identified and it became clear that open angle glaucoma was a disease produced by the interaction of several factors of which intraocular pressure was one. Additional evidence that glaucoma was more than simply a mechanical response to raised IOP was seen in the existence of a large group of people with raised intraocular pressure who never developed glaucoma. The difficult clinical problem of how to best manage these patients, known as ocular hypertensives, has been a subject for discussion in recent years.

There is a wealth of published research describing changes in ocular blood flow in glaucoma but it is difficult to determine how these changes relate to raised IOP. Is

reduced blood flow simply a consequence of increased pressure in the eye or is there a primary fault in the control of both IOP and ocular circulation in open angle glaucoma? In addition to this the results of many studies are difficult to interpret due to the past or concurrent use of drugs to lower intraocular pressure in the patient groups.
The purpose of this study is to examine the ocular circulation and factors affecting it such as clotting function and peripheral vasoreactivity in glaucoma patients using ocular hypertensive subjects as a comparison group. This allows the groups to be matched for IOP exposing any differences in blood flow which may be attributed to the presence of disease. It may also provide information about ocular hypertension which could help to answer questions about the most appropriate way to manage people with raised intraocular pressure.

Section I reviews the literature relating to primary open angle glaucoma and ocular hypertension. The epidemiology of both is outlined. The literature relating to intraocular pressure and its value as a predictor of POAG is reviewed and normal ocular circulation is described. This is followed by a summary of investigations into other risk factors for glaucoma and OHT with emphasis on vascular factors, broadly divided into ocular blood flow measurement, peripheral vascular reactivity and haematological investigations. Section II outlines the study protocol and subject recruitment. Results of the study are presented and discussed and conclusions are drawn.

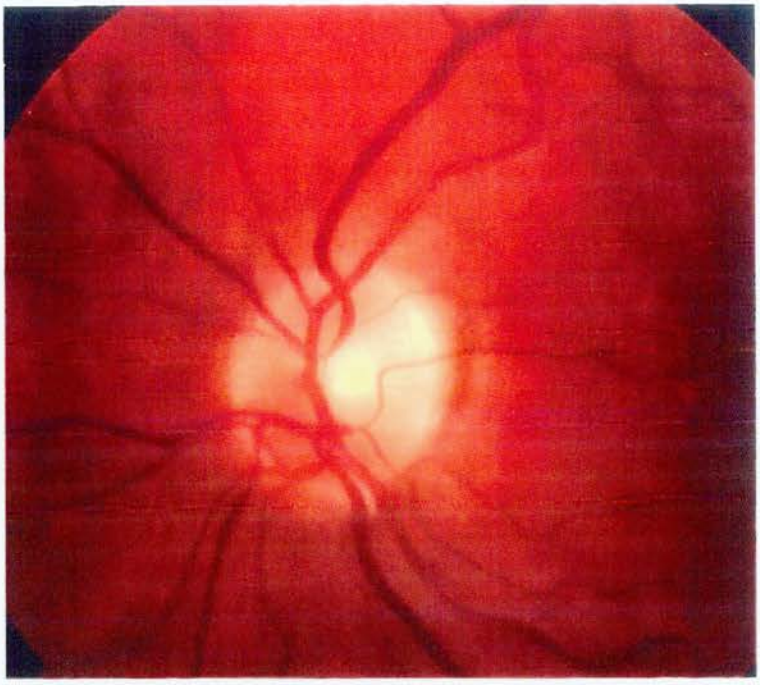
1.2

Glaucoma

Definition: Primary open angle glaucoma is an optic neuropathy with characteristic visual field loss and optic disc changes usually associated with raised intra ocular pressure.

Glaucoma is the name given to a collection of diseases which together represent one of the most important causes of blindness and visual impairment in the Western world [3]. Evans in 1995 stated that glaucoma was the second most prevalent cause of registered blindness in the UK after age related macular degeneration, accounting for 12% of registrations[4], and Quigley in 1996 estimated that 66.8 million people world-wide would suffer from glaucoma by the year 2000, 6.7 million of those being bilaterally blind[5]. Glaucoma can be broadly classified as chronic or acute. Primary open angle glaucoma (POAG) is the most prevalent form of chronic glaucoma. Diagnosis of POAG is based on the presence of raised intraocular pressure (IOP), cupping of the optic disc (figure 1) and characteristic visual field loss in an arcuate pattern between 10 and 20 degrees of fixation (figure 2) in the presence of open drainage angles. However, approximately 1/3 of chronic glaucoma patients have intraocular pressure within the normal range [6-8]. This variant of the disease is known as normal tension glaucoma (NTG).

(a)



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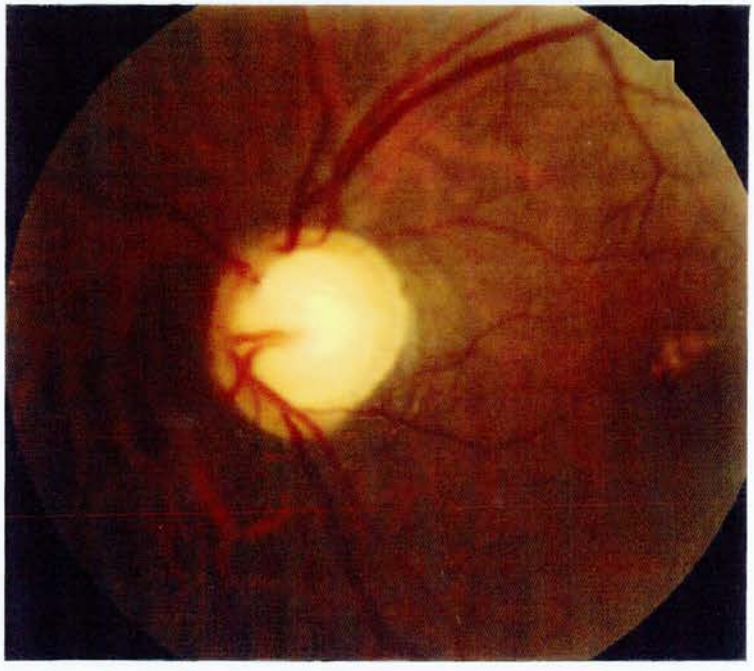
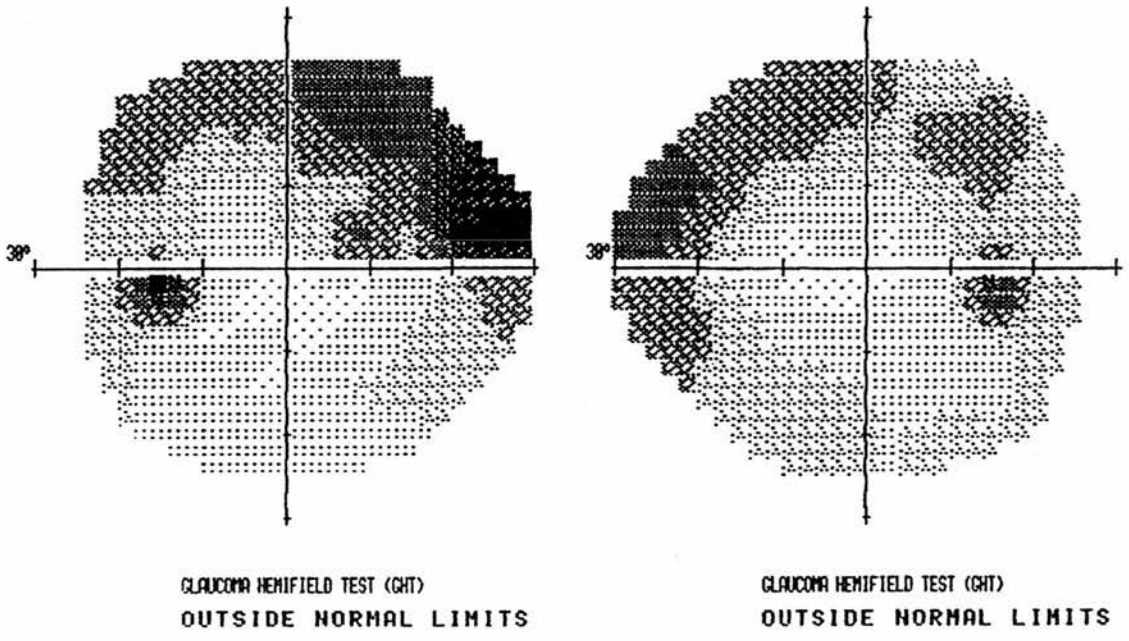


Figure 1: Colour photographs of the optic disc:

(a) healthy and (b) glaucomatous



Left Eye

Right Eye

Figure 2: Glaucomatous visual field loss

(Humphrey visual field analyser program 24-2)

A small proportion of chronic glaucomas are due to congenital abnormalities in the structure of the eye or occur secondary to damage to or blockage of the trabecular meshwork (angle recession, pigment dispersion, pseudoexfoliation, uveitis and chronic angle closure). In the initial stages of the disease chronic open angle glaucoma is almost invariably asymptomatic and population studies estimate that less than 50% of those with glaucomatous field loss have received an appropriate diagnosis or treatment[9,10]. The work reported here relates to patients with primary open angle glaucoma.

Structural changes identified in the glaucomatous eye include optic disc cupping with posterior movement of the lamina cribrosa and loss of axons at the optic nerve head, a decreased photoreceptor count over the entire retina and loss of RPE cells particularly in the peripapillary area [11]. In postmortem glaucoma eyes Quigley et al found preservation of normal capillaries and astrocytic glial cells at the optic nerve head. He concluded that structural weakness in the lamina cribrosa was the primary problem in POAG leading to impaired axonal transport in the presence of raised IOP[12]. In contrast to Quigley's finding of no vascular changes at the glaucomatous optic disc, a study by Kubota et al in 1993 identified significant choroidal thinning with reduced choroidal vessel diameter most marked at the optic disc border in enucleated eyes with chronic angle closure glaucoma secondary to malignant ciliary body melanoma. The control group comprised enucleated eyes with choroidal melanoma and normal intraocular pressure [13].

Studies looking at the prevalence of chronic glaucoma (Table 1) have given fairly consistent results although in the past a clear distinction has not always been made between ocular hypertension (raised pressure in a healthy eye) and glaucoma. The prevalence of primary open angle glaucoma and normal tension glaucoma combined is approximately 2% in caucasian populations over the age of 40.

TABLE 1: Prevalence of Glaucoma

Authors, Location	Age (n)	Diagnostic criteria	Prevalence
Bonomi et al, Italy[14]	>40yrs (4297)	IOP>22, VFD, ODC IOP<21,VFD, ODC IOP>22	POAG 1.4% NTG 0.6% OHT 2.1%
Klein et al, Beaver Dam[15]	>43yrs (4926)	VFD and ODC IOP not specified	POAG+NTG 2.1%
Davanger et al, Norway[16]	>65yrs (1887)	IOP>24, vCDR>0.8, VFD 2 of 3 present	POAG+NTG 6.4%
Tielsch et al, Baltimore[17]	>40yrs 2395 black 2913 white	VFD and ODC IOP not specified	POAG+NTG Black 4.7% White 1.3%
Hollows et al, Wales[6]	40-74yrs (4231)	VFD and ODC NTG iop<21mmHg POAG+OHT iop>20	POAG+NTG 0.5% OHT 9.1%
Khan et al, Framingham[18]	52-85yrs (2631)	IOP>21mmHg	POAG+NTG 1.4% OHT 6.9%
Coffey et al, Ireland[19]	>50yrs (2186)	IOP>21mmHg, VFD ODC not specified	POAG+NTG 1.9% OHT 4.2%

KEY: VFD = visual field defect
ODC = optic disc cupping
VCDR = vertical cup/disc ratio
NTG = normal tension glaucoma

It has been shown that the prevalence of POAG increases with age [7,19,20]. The Framingham study found an increased incidence in men[18], although other studies have found no gender difference[21]. Chronic open angle glaucoma has also been found to be significantly more common in blacks than in Caucasians[17]. There is a higher incidence of POAG among diabetics than in the general population[22,23]. Some studies have found an association between POAG and myopia [24] and also between POAG and higher systolic blood pressure[25]. The use of these associations to help to identify OHT patients at risk of progressing to POAG is common in clinical practice and has been addressed recently in the Ocular Hypertension Treatment Study[26] which is covered in more detail in the next chapter.

1.3

Ocular Hypertension

Definition: Intraocular pressure consistently raised above 21mmHg in a healthy eye with full visual fields, normal optic disc appearance and open drainage angles.

The distribution of IOP in the general population is not normal but is skewed to the right with a preponderance of higher IOPs (figure 3).

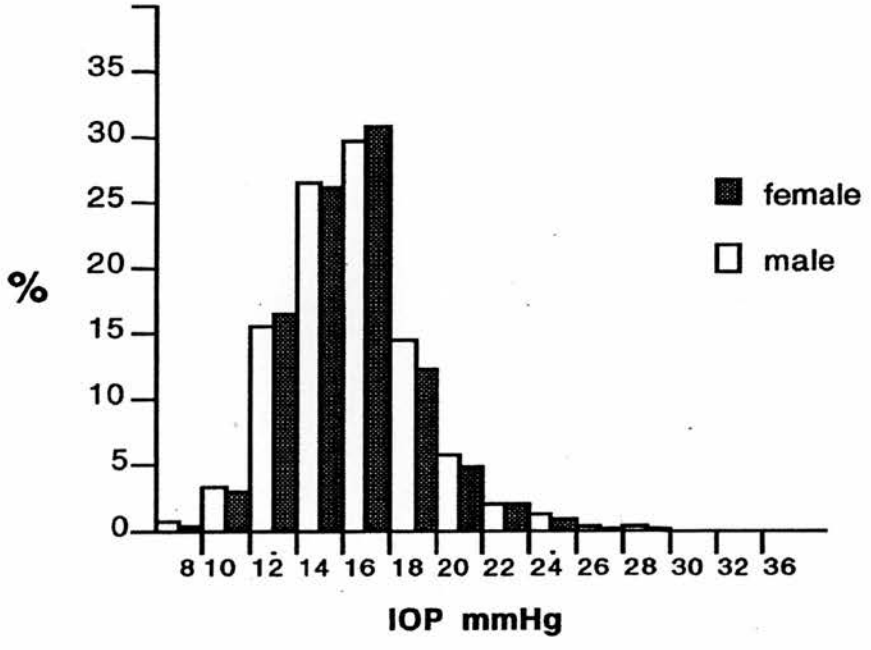


Figure 3: Distribution of IOP in the population

Data taken from right eyes of 4297 subjects, Bonomi et al[14]

The concept of raised IOP has arisen from a desire to identify individuals at the greatest risk of developing glaucoma. It has been established that high IOP has a strong positive association with glaucoma risk, the higher the pressure the higher the risk [8,27,28]. In the past it was felt the most effective way to identify future glaucoma patients was to concentrate on those members of the population with IOPs in excess of two standard deviations above the population mean. According to the known distribution of pressures this group of people will have an IOP of over 21 mmHg. Thus the pressure of 21mmHg has become the level above which people are labelled 'ocular hypertensive' and are assumed to be at increased risk of glaucoma.

Studies have shown that approximately 5% of the population have an IOP of over 21mmHg[29] although this proportion changes with age and may also be affected by race[17]. Only a small proportion of ocular hypertensives develop glaucoma. Longterm follow-up of cohorts of ocular hypertensives has given varying results but the majority of studies put the incidence of conversion to glaucoma at about 2% per year (Table 2).

Table 2: Incidence of conversion from OHT to POAG

Study	n	Endpoint	Time of follow-up	Treatment effect	% per year
Lundberg[30]	41	field loss	20y	not studied	1.7
Schappert-Kimmijser[31]	94	field loss	5yr	not studied	2.6
Schultzer[32]	143	field loss disc haem incr. cupping*	6yrs	Timolol made no difference	4.9 (3.4% with field loss)
Epstein[33]	107	field loss IOP>32 incr. cupping*	2-6yrs	Timolol reduced risk of POAG	Rx 2.6 no Rx 6.2
Kass[34]	62	field loss	5yrs+	Timolol reduced risk	6.8 (more untreated eyes progressed p=0.04) †

*Any one of these representing progression or failure of treatment
† Cumulative probability over 5 years
Rx = treatment no Rx = no treatment

Problems have arisen in clinical studies because there is no consensus of opinion on the diagnostic criteria for glaucoma and ocular hypertension, in addition because the conversion rate is low large cohorts of ocular hypertensives must be followed up for many years to obtain accurate data. As seen in table 2 there has often been no agreement between studies as to what constitutes progression to glaucoma. The Ocular Hypertension Treatment Study (OHTTS)[26] ran from 1994 to 1999 with the aim of addressing these problems. The major finding of the study was that treatment of 'higher risk' ocular hypertensives (with IOP of 24-32mmHg) producing a 20% fall in IOP reduced the risk of conversion to glaucoma from 9.5% to 4.4% over 5 years. Conversion to glaucoma was defined as development of reproducible field loss or optic disc deterioration.

Visual field assessment

Many methods have been devised for assessing visual fields. Visual field testing is one of the mainstays of glaucoma screening and characteristic deterioration in visual field is generally taken to indicate conversion from OHT to glaucoma. There are recognised difficulties in obtaining reliable and reproducible results from a group of patients many of whom are elderly and have coexistent eye disease that may interfere with visual acuity. Recent advances in automated perimetry have produced tests that are quicker and easier for patients to perform such as the Swedish Interactive Threshold Algorithm (SITA fast) and Frequency Doubling Technology (FDT). Short Wavelength Automated Perimetry (SWAP) may identify very early glaucomatous damage and have some

predictive value in glaucoma screening and the multifocal electroretinogram (mfERG) can provide an objective measurement of the visual field although it is not commonly used in routine screening [35]. Large, multicentre studies such as the OHTTS and the Advanced Glaucoma Intervention Study (AGIS) have devised methods for assessing field progression as measured with automated perimetry with the aim of achieving better reliability and objectivity [36]. A recent paper by Vesti et al reviewed seven such methods and found that those with high specificity such as that used by AGIS had lower sensitivity and classed fewer cases as having progressed than the other methods reviewed [37].

Clinical importance of ocular hypertension

One of the difficult problems in glaucoma management is how best to look after ocular hypertensives. It is known that two thirds of glaucoma patients are in this group but that only a small proportion of ocular hypertensives will develop the disease. Raised IOP continues to be used as the main indicator of future glaucoma risk and IOP is targeted by all current treatment methods. Many studies have pointed out the inefficiency of tonometry as a screening measurement [7,15,38] confirming that although IOP has a strong positive association with glaucoma [8,28] for most patients it is a poor predictor of disease.

Until the publication of the findings of the Ocular Hypertension Treatment Study there was no clear evidence that reducing IOP held any benefit in preventing glaucoma. In addition to this important finding the OHTTS confirmed an association of several

baseline factors with a higher risk of developing POAG in ocular hypertension[39]. On univariate analysis older age, African race, male sex, heart disease, and thinner central corneal measurement were all associated with a higher risk of conversion to POAG in addition to larger vertical or horizontal cup to disc ratio, higher IOP and areas of reduced sensitivity (greater CPSD) on visual field testing. However the best predictors of conversion as identified with multivariate analysis were baseline age, cup to disc ratio, pattern standard deviation on field testing and high intraocular pressure. Central corneal thickness was found to be a powerful predictor of POAG, a thinner cornea being associated with higher risk. The authors postulate that corneal thickness influences IOP measurement and patients with thicker corneas have a true IOP lower than the measured IOP[26]. Subgroups of OHT patients were identified with a significantly higher risk of progression than the 0.5% to 1% risk found by the study overall. Baseline IOP of over 25.75mmHg combined with a thin cornea (<556μm) carried a 36% risk of conversion over 5 years, whilst a cup to disc ratio of over 0.3 combined with a thin cornea was associated with a 24% risk.

Despite this significant advance in our understanding of the clinical history of ocular hypertension and risk factors for progression to glaucoma, treatment of the high risk groups as defined by the OHTTS would still result in the majority of patients receiving unnecessary treatment for at least 5 years. This emphasises the fact that our knowledge of the underlying factors producing glaucoma is far from complete and that there is still no accurate method for identifying the preglaucomatous state in the ocular hypertensive population.

1.4

Intraocular pressure

There follows a review of the literature relating to intraocular pressure with respect to visual function and glaucoma and the effects of drugs designed to lower it.

Intraocular pressure is the most universally used indicator of glaucoma risk. As stated previously the traditional assumption that raised pressure is the primary cause of glaucoma is controversial. The majority of ophthalmologists will decide whether to treat a patient with raised intraocular pressure based on the level of IOP in the context of the person's age, family history and other ophthalmic findings.

Intraocular pressure is known to vary naturally with time. Several studies have examined this tendency both in populations and in cohorts of patients. Schwartz and Talusan [40] looked at spontaneous trends in IOP in a group of untreated ocular hypertensives with IOP>21mmHg over 3-4 years. They found that the majority of ocular hypertensives had stable IOP as did a group of normal controls. A proportion (13%) of OHTs had a slight increase in IOP over time. Thirty seven percent of OHTs also showed cyclical changes in pressure over 1 to 2 year periods. On average the total population showed a small decrease in IOP over time, and they suggest that the group of OHTs showing a tendency to increasing IOP may be the group that go on to develop glaucoma. They do not provide longterm evidence to support this theory however and none of their subjects developed field loss during the course of the study. Linner [41] found a slight decrease in

IOP when following a group of ocular hypertensives with IOP 21-25mmHg for 10 years. He postulates that this fall is secondary to reduced aqueous production by the ageing ciliary body. Sorensen et al[42] followed a group of 39 untreated ocular hypertensives for 15 years and found 20 had reduced IOP over time while the remainder had stable IOP. None of this group developed field loss. Shiose [43] reported on a very large population study in Japan in which 192,138 people were examined over a period of 10 years. It is not clear from the report whether any of the subjects had more than one visit during the period of the study but the data appears to cross sectional rather than longitudinal in nature. A lower average IOP was found in older age groups compared to younger subjects. It was found that the presence of obesity and hypertension were positively associated with higher IOP and that the prevalence of both was increased in the older subjects but not to the same extent as in a Western population. This possible confounding effect of systemic factors may explain the conflicting results found by longitudinal studies performed on small groups of selected patients and cross-sectional population studies in the West.

Intraocular pressure has also been found to change in individuals in a cyclical fashion in relation to the seasons, the highest IOP being found in winter. This is a finding which is difficult to explain but it has been confirmed by different observers[40,44,45]. It has also been recognised for many years that IOP shows diurnal variation in the healthy eye with highest pressures present in the mornings [46]. Glaucoma patients[47] and high myopes[48] have been found to have patterns of IOP throughout the day and night which differ

significantly from those recorded in normals. Liu et al measured IOP in newly diagnosed glaucoma patients and normal controls. They found that in addition to higher diurnal IOP glaucoma patients showed a smaller increase in nocturnal (supine) IOP and an increase in IOP on wakening which was not seen in normals [47].

The response of IOP to changing position has also been investigated. Most reports identify an increase in IOP in normal subjects on changing position from sitting to supine[49-51]. Linder et al investigated the effect of inverted body position on IOP in normal subjects[52]. They found that placing a subject in positions ranging from supine (0°) to upside down (–90°) produced very significant increases in IOP of up to three times baseline level. Associated with this increase was a reduction in visual function as assessed by pattern reversal ERG and VEP. The authors propose a reduction in ganglion cell function associated with head down posture as an explanation for their findings. Many studies have identified a larger postural change in IOP (increase on lying down) in some conditions including primary open angle glaucoma[50,53], ocular hypertension[54], normal tension glaucoma[53], and non arteritic anterior ischaemic optic neuropathy[51].

In an interesting study by Schuman and associates[55] an increase in IOP was identified in musicians playing high resistance wind instruments associated with a transient increase in uveal thickness as measured with ultrasound biomicroscopy. The magnitude of IOP rise was dependant on the resistance provided by the particular instrument. High resistance wind instrument players had a small but significantly greater incidence of visual field defects than other musicians which was related to life hours of playing. This

suggests that even healthy eyes when repeatedly subjected to large variations in IOP may suffer loss of visual function.

There is little doubt that very high levels of IOP produce optic nerve damage. This has been demonstrated experimentally in primates [56] and also in humans. Drance performed clinical experiments with people on whom a suction cup was used to temporarily increase IOP. Characteristic scotomata developed in Bjerrum's area with elevated IOP and those with established POAG developed field abnormalities at lower levels of IOP than normal subjects[57].

It is clear from the preceding studies that while raised intraocular pressure plays an important role in the development of glaucoma there are physiological influences on IOP including age, the time of day and the time of year which may make the task of obtaining a meaningful measurement in an individual at a given time quite difficult.

The effect of short term reduction in IOP

Current treatment of glaucoma is aimed at lowering the intraocular pressure.

A number of studies have looked at the effect of an experimental reduction in IOP on visual function. Flammer and Drance looked at the effect of lowering IOP by administering oral acetazolamide to 9 patients with POAG and 16 with OHT. The mean pre-treatment pressure was 25mmHg in both groups and a mean reduction of 8mmHg was achieved, a small reduction in blood pressure also occurred. A significant

improvement in visual field in POAG subjects was found on automated testing. There was no change in the OHT group. The degree of improvement in visual field did not correlate with the magnitude of pressure reduction and they suggest that improvement produced by acetazolamide may be due to an acidosis-induced increase in choroidal blood flow[58]. Other investigators have also found an improvement in visual field after acetazolamide[59]. The effect of other IOP reducing medications has been explored in the acute situation . Flammer and Drance [60] used a masked randomised study to compare the effects of topical pindolol with placebo. They found no significant effect on visual fields with either preparation. They also looked at the effect of epinephrine and timolol and found a small nonsignificant reduction in visual field after both. Several factors must be taken into account when considering the above studies. Bias in both patient and observer is a potential problem particularly when manual perimetry is used. Learning effects producing improvement over time may also confound results. Flammer and Drance used experienced patients in their study in an attempt to reduce this effect.

Effect of longterm reduction in IOP

The effect of longterm reduction in IOP has been investigated in the context of glaucoma and glaucoma risk. Progression of visual field loss, and in some cases an increase in optic nerve head cupping and pallor is taken to indicate failure of treatment. Problems with compliance and long term follow-up particularly in ocular hypertensives can blur the distinction between failure of treatment to prevent glaucoma and failure of

treatment to lower IOP. Reports in the literature in the past have given conflicting results. Some have found no benefit in reducing the IOP in ocular hypertensives [28,32]. Other studies have found that lowering the IOP in ocular hypertension reduces the incidence of progression to glaucoma [33,34,61]. In most of these studies numbers were small and problems were encountered with poor compliance and high dropout rates among ocular hypertensives. The recently published findings of the ocular hypertension treatment study add considerable weight to the view that lowering the intraocular pressure in ocular hypertension reduces the risk of progressive optic disc damage and visual field loss[26].

At the outset of this study the benefit of reducing IOP in established glaucoma had still not been definitely confirmed. Prospective randomised trials of treatment vs. no treatment were difficult to justify for ethical reasons and were therefore non-existent. The recent publication of the findings of two important studies has added greatly to our understanding of the role of IOP in disease progression. The Advanced Glaucoma Intervention Study provides good evidence that visual field loss in eyes with lower intraocular pressure progresses significantly more slowly[62]. In addition the Early Manifest Glaucoma Trial found that the risk of progressive visual field loss is closely linked to reduction of IOP, subjects treated with argon laser trabeculoplasty and betaxolol having half the risk of progression of control patients in the study[63].

As the preceding review demonstrates the relationship between IOP and glaucoma is not straightforward. There is a definite link between increased levels of IOP and risk of

developing open angle glaucoma but for levels below 30 mmHg a clear causal relationship has not been proved. In addition the reduction of IOP in both ocular hypertension and established glaucoma has been shown to have a beneficial effect on visual field preservation. There is a natural tendency for IOP to vary with time in an individual and the physiological mechanisms governing this are not well understood. These facts contribute to the poor performance of IOP as a screening test for glaucoma as confirmed by clinical studies that find while raised IOP is a significant risk factor for the development of glaucoma it is a poor predictor of disease [28,30,31]. When IOP is used to detect the population at risk of developing glaucoma the group labelled 'ocular hypertensive' is produced. This group which comprises approximately 5% of the population contains only a small number who will go on to develop glaucoma. The search for better markers of the pre-glaucomatous state has continued in the hope that patients may be identified and treated before visual field loss occurs.

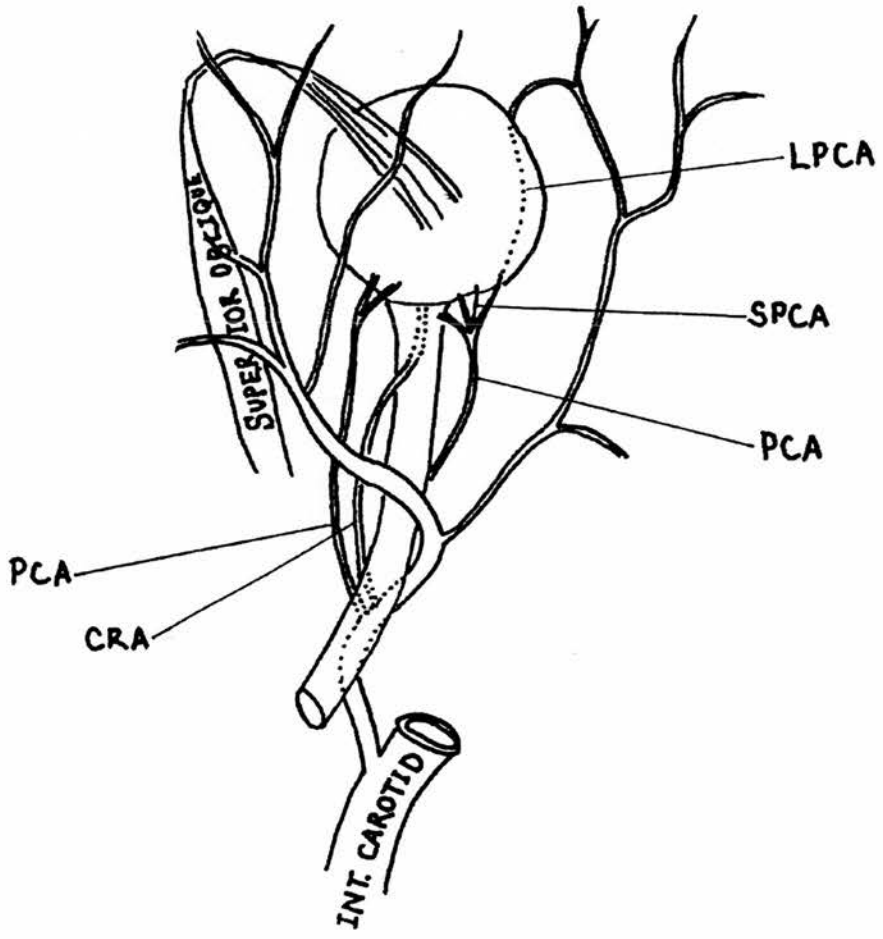
1.5

Ocular circulation

Anatomy

Two separate vascular systems supply the eye; the retinal circulation and the choroidal circulation. Both are derived from the ophthalmic artery, the first branch of the internal carotid. Within the orbit the ophthalmic artery divides into the central retinal artery, between 1 and 5 posterior ciliary arteries and several anterior ciliary arteries (figure 4). The central retinal artery enters the optic nerve 10 to 15mm behind the globe and lies beside the central retinal vein in the anterior nerve before emerging into the fundus where its branches lie within the nerve fibre layer and supply the inner 2/3 of the retina. Retinal circulation accounts for 5 to 10% of total ocular blood flow.

The ciliary vessels divide into 10 to 20 short posterior ciliary arteries (SPCAs) which enter the posterior globe and in many cases contribute to an anastomotic circle around the optic nerve called the circle of Zinn-Haller. Two long posterior ciliary arteries pass anteriorly to supply the anterior portion of the globe. The SPCAs empty into the choroid which supplies the outer retinal layers including the photoreceptors. Choroidal blood flow is of major importance in supplying nutrients to the retina, up to 95% of the total volume of ocular blood flow is through the choroid and in animal studies 65% of the O_2 required by the retina comes from choroidal blood.



LPCA	=	Long Posterior Ciliary Artery
SPCA	=	Short Posterior Ciliary Artery
PCA	=	Posterior Ciliary Artery
CRA	=	Central Retinal Artery

Figure 4: Branches of the Ophthalmic Artery

(adapted from Grants Atlas of Anatomy 9th Edition[64])

The head of the optic nerve can be divided into 4 distinct anatomical areas. The superficial nerve fibre layer is supplied by branches of the central retinal artery. The prelaminar area and the lamina cribrosa are supplied by branches of the short posterior ciliary arteries often via the circle of Zinn Haller[65]. The retrolaminar region is supplied by the central retinal artery and the pial network of vessels[66] (Figure 5). A study by Anderson in 1974 using monkeys found very little damage to the optic nerve after occlusion of the short posterior ciliary arteries demonstrating the presence of a very effective anastomosis with the pial vessels via the circle of Zinn Haller. He cautions against the assumption that optic nerve head blood flow behaves in the same way as that in the choroid or that it is dependant on the integrity of the SPCAs [67] (figure 5).

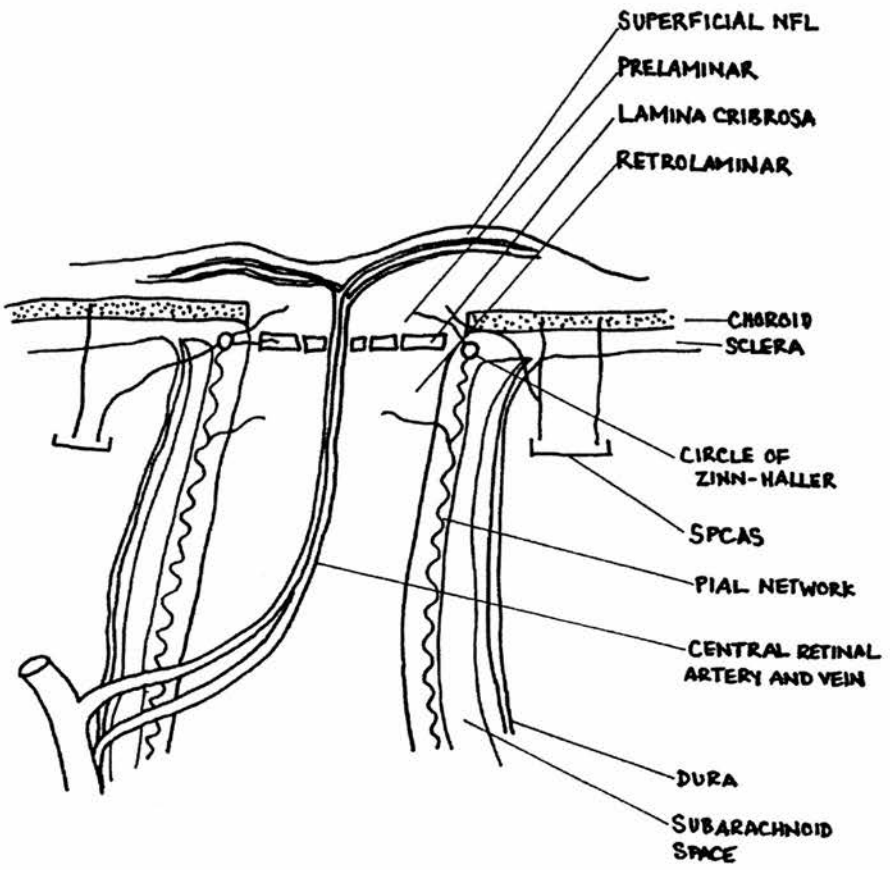


Figure 5: Blood Supply of the Optic Nerve Head

(adapted from Snell[68] and Harris[66])

Physiological controls on ocular blood flow

The ocular perfusion pressure (OPP) is the local arterial blood pressure in the vessels entering the eye. This is calculated as 2/3 of mean arterial pressure (MAP) in a seated subject due to the elevated position of the eye relative to the heart, minus the intraocular pressure (IOP).

$$OPP = 2/3\ MAP\text{-}IOP^{69}$$

Both local and systemic factors act to maintain optimal perfusion of the tissues by alterations in vessel diameter. The terminology in the literature relating to this of type of blood flow modulation is confusing. For the purpose of this review autoregulation is taken to mean a change in local vascular resistance or arteriolar calibre occurring in response to an alteration in either local or systemic requirements. The local changes in blood vessel diameter may be mediated by three mechanisms; myogenic, neurogenic or metabolic. Vascular smooth muscle may respond to stretch or to factors released by the vascular endothelium (myogenic). Changes may occur in response to local levels of pO_2, pCO_2 and pH (metabolic), and blood flow distribution may also be altered by the autonomic nervous system (neurogenic). The relative importance of these different influences on ocular blood flow has been investigated.

The autonomic nerve supply to the eye is distributed only within the uvea and the extra ocular parts of the retinal vessels. Parasympathetic vasodilator nerves secreting VIP and sympathetic αreceptors have been found in the choroid suggesting that autonomic input may play a part in the regulation of choroidal flow.

Stjernschantz et al investigated intracranial stimulation of the oculomotor nerve (containing parasympathetic fibres) in rabbits. They found that it produced sustained choroidal vasodilation in addition to a transient vasoconstriction in the iris and ciliary body and pupil miosis. The effect on the anterior uvea was potentiated by inhibition of prostaglandin synthesis with indomethacin. [70].

Michelson et al documented alterations in ocular blood flow associated with exercise induced changes in blood pressure in humans. Using transcranial Doppler and laser Doppler flowmetry they demonstrated an increase in vascular resistance of the branches of the ophthalmic artery and the iris vessels associated with an increase in blood pressure. The blood velocity in these vessels was unaffected and they conclude that a sympathetic mechanism exists which protects the eye from overperfusion in a 'fight or flight' situation.[71]

A disturbance in autonomic function has been suggested in the past as a possible etiological factor in glaucoma[72]. However investigation of autonomic function in glaucoma patients has not been extensively reported in the literature. In 2002 Brown and associates noted impaired cardiovascular responses to baroreflex stimulation using sinusoidal neck suction in open angle and normal pressure glaucoma patients[73].

Kashiwagi et al reported a disturbance in the circadian rhythm of the autonomic nervous system in glaucoma as measured with ambulatory electrocardiography over 48 hours[74].

These very recent studies may herald a resurgence of interest in the autonomic system in relation to glaucoma.

Studies investigating autoregulation in glaucoma

Animal studies have produced evidence of efficient autoregulation in the retina but conflicting results regarding the optic nerve head. The mechanism is assumed to be metabolic or myogenic as autonomic nerves have not been found in the retina. Alm and Bill, using monkeys, found that choroidal and retrolaminar blood flow reduced by 30% in eyes exposed to an experimental increase in IOP while retinal blood flow remained unchanged [75]. The blood vessels at the optic nerve head resemble retinal and central nervous system capillaries and it is reasonable to assume that they could show autoregulation in a similar way. Quigley et al using tritiated iodopyrine in monkeys found no alteration in optic nerve head blood flow in response to raised intraocular pressure [76]. Bill and Sperber used the deoxyglucose method in monkeys and found that inner retinal blood flow increased in response to flicker light while choroidal flow remained unchanged [77]. Anderson suggested in 1974 that at normal intraocular pressures choroidal circulation is far in excess of requirements. Alterations in choroidal perfusion secondary to raised IOP can therefore be accommodated by the eye without loss of function. The retinal circulation in contrast is more exactly matched to the local needs of the tissue and therefore displays greater constancy due to autoregulation [67]. Pillunat presented evidence to support the presence of autoregulation in human eyes. Intraocular pressure was increased using a suction cup in glaucoma subjects and in normal controls. Retinal and ciliary perfusion pressure were measured with oculooscillodynamography. Visual evoked potential (VEP) was recorded at each

increase in pressure. The results demonstrated a plateau phase in the reduction of VEP with increasing IOP in the graphs produced by normals which was not present in POAG patients. The authors suggest that this finding supports the existence of autoregulatory mechanisms governing blood flow at the optic nerve head which are reduced or absent in glaucoma[78].

Grunwald et al used the blue field entopic phenomenon (BFE), which allows a subject to see leukocytes moving in their own macular capillaries, and a suction cup to artificially increase intraocular pressure. They found that POAG patients could not maintain their retinal circulation when mean IOP was over 24.2mmHg whereas normal and OHT subjects could maintain perfusion to much higher levels of IOP (30.8mmHg and 29.9mmHg respectively). After reduction of IOP back to resting levels OHT and normal subjects showed a hyperaemic response suggesting that vasodilation had occurred during the period of increased IOP. This hyperaemia was not present in POAG. In this study both POAG and OHT subjects were using topical treatment to lower the IOP and more POAG subjects than OHTs were on timoptol (7 and 1 respectively) which may well have affected the results [72].

Visual Function and Blood Flow

Some studies have identified changes in visual function associated with alterations in blood flow.

Paris et al found a significant improvement in contrast sensitivity in normal adults after taking sildenafil associated with a 30% increase in pulsatile ocular blood flow and no change in intraocular pressure [79].

Ginko biloba extract (GBE) has been found to increase end diastolic velocity in the ophthalmic artery on CDI without changing IOP, systemic blood pressure or pulse rate [80]. It is known to reduce vasospasm, and serum viscosity in addition to having an inhibitory effect on apoptosis and platelet activation [81]. Quaranta gave GBE to normal tension glaucoma patients in a double blind cross-over study and found a significant improvement in visual field performance (MD and CPSD, $p<0.0001$). No change in IOP, blood pressure or pulse rate was noted [82]

1.6

Blood Flow Studies

in Glaucoma and Ocular Hypertension

A. Pulsatile Ocular Blood Flow

Continuous measurement of intraocular pressure reveals a pulsatile variation synchronous with the systemic pulse (figure 6). It is possible to calculate the volume of blood responsible for the pressure increase during each cardiac systole using a formula derived from experimental data (see chapter 2.2). This calculated change in volume over time is known as the pulsatile ocular blood flow (POBF) and is expressed in microlitres per minute. Because choroidal filling is thought to account for 85 to 90% of the ocular pulse volume POBF is taken as an indirect measure of choroidal blood flow.

Several assumptions are made in the calculation of POBF. Most importantly it is assumed that outflow from the eye is constant, that there is no reflux and that the pressure-volume relationship (ocular rigidity) derived from Langhams living eye experiments in the 1960's can be applied to all eyes[83].

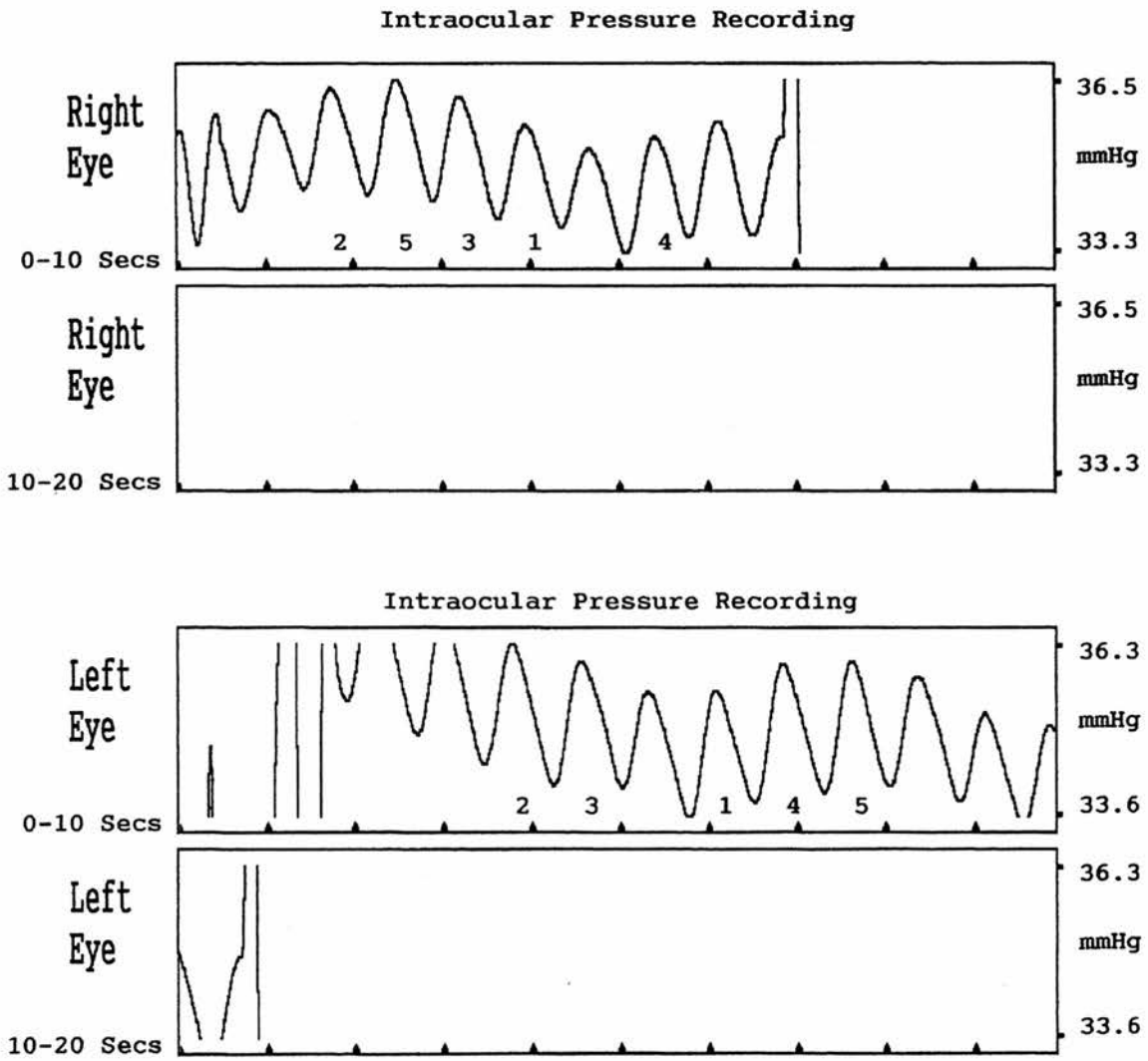


Figure 6: POBF Machine printout

Colour Doppler studies have measured diastolic blood flow in the ophthalmic artery which increases in the supine position. This suggests the presence of non pulsatile component to ocular blood flow [84]. Measurement of POBF gives no information about this non-pulsatile component of ocular perfusion and the ratio of pulsatile to non-pulsatile flow which may be altered in an individual by changes in heart rate or ocular perfusion pressure.

There have been numerous studies looking at pulsatile ocular blood flow in glaucoma and ocular hypertension over the last twenty years. The results of these studies can be difficult to interpret due to variations in diagnostic criteria for POAG and OHT and the frequent presence of topical treatment. However, many studies have identified a reduction in POBF in glaucoma patients compared to normal subjects. There is less agreement in the literature regarding findings in OHT.

In 1981 E.S. Perkins recorded the cyclical changes in IOP with an adapted Goldman tonometer. He found increased pulse amplitude in OHT and POAG subjects compared to normal. Ocular hypertensives were found to have higher pulse amplitude than POAG patients. Pulse amplitude was also affected by refractive error being lower in myopia [85].

In 1987 Nicastro et al compared groups matched for heart rate, systemic pulse pressure and arterial pressure. They found that ocular hypertensives had higher pulse amplitude than either normals or POAG patients but, in contrast to Perkins, they found that POAG patients had lower pulse amplitude than normals [86].

The device used by Perkins was adapted by Langham to produce a pneumotonometer [87] [88]. Subsequent studies have used this device and more recently its descendant developed by OBF Labs UK Ltd [89] for calculation of pulsatile ocular blood flow.

Fontana et al looked at POBF measurements from a group of 777 normal subjects. They found mean values of 886μl/min for females (mean age 53) and 731μl/min for males (mean age 54)[90]. Yang et al found similar normal values and calculated a reliability coefficient of 0.92 for the measurements produced by the machine[91]. Trew and Smith used the Langham OBF system to look at the effect of posture in untreated OHT subjects compared to normals[92]. They found that POBF fell significantly in both groups on lying down and was accompanied by a rise in IOP which was more marked in OHT. They also found increased pulse amplitude in ocular hypertensives in the supine position but no significant difference in POBF between the groups in either position. The mean IOP of the OHTs in this study was only 22.7mmHg. In a second paper comparing treated POAG patients on timoptol with OHT the same authors found reduced pulse amplitude ($p<0.01$) and POBF ($p<0.05$) in POAG. Both groups had a significant fall in POBF associated with a rise in IOP on lying down. Treatment was withdrawn from POAG subjects for 2 weeks resulting in a significant increase in IOP (from 18.8mmHg to 23.2mmHg) but no change occurred in POBF or in the postural response compared to the treated phase of the study. The authors conclude that POBF is reduced in glaucoma compared to ocular hypertension and that it is not increased by the use of timoptol[93].

There have been some studies examining the influence of other factors on POBF. James et al [94] found that POBF decreases with increasing axial length most probably due to the increased volume of myopic eyes, a finding confirmed by others[95]. Ravalico reported a reduction in POBF and pulse amplitude (PA) with age, the relationship being more marked in subjects over the age of 50 and in the supine position[96].

The influence of IOP on POBF has also been explored, James et al [94] found no relationship between IOP and POBF in a group of 34 normal subjects, a finding confirmed by Yang with a group of 163 normals [91] However Massey and O'Brien found a strong correlation ($p<0.001$) between IOP and POBF in their study of 664 normal subjects[95].

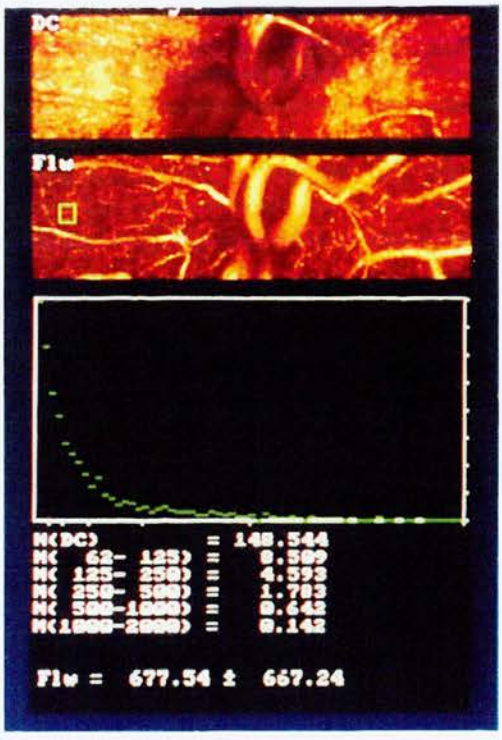
B. Scanning Laser Doppler Flowmetry

A relatively new method of measuring retinal blood flow was developed shortly before this study began. Scanning laser Doppler flowmetry (SLDF) quantifies blood flow to the superficial layers of the optic nerve head and retina [97,98]. The method combines laser Doppler flowmetry with a scanning laser system to produce pictures of the area under investigation. The image produced is a two dimensional map of the retina with 256x64 points representing retinal perfusion which yields information about 'flow', 'velocity', and 'volume' of red cells within a selected 'region of interest'. Further details of this method can be found in Section 2.2.

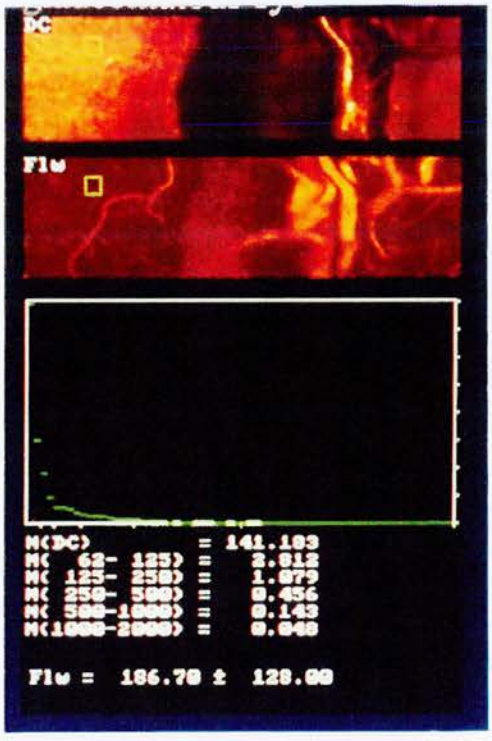
Michelson and associates[99] compared patients with primary open angle glaucoma with normal subjects and found reduced blood flow at the lamina cribrosa and nasal and temporal retina in the glaucoma patients. Nicolela and associates [100] using scanning laser Doppler flowmetry also found reduced blood flow at the lamina cribrosa and temporal retina in POAG compared to normals. The subjects in Nicolela's study were a heterogeneous group that included normal tension glaucoma patients, two thirds of the group were also using antiglaucoma medication .

Groh et al used SLDF to investigate changes in retinal and optic nerve head blood flow with age. They found a significant reduction in retinal microcirculation of 6-11% per decade in normal subjects. They did not detect any age related changes in optic nerve head blood flow[101]. At the time of the investigation reported here there were no

published studies looking at retinal blood flow measured with SLDF in ocular hypertension.



Normal optic disc



Glaucomatous optic disc

Figure 7: Images of the optic disc taken with the Heidelberg Retinal Flowmeter

C. Colour Doppler Imaging

Colour Doppler ultrasound imaging (CDI) is a noninvasive technique that has been used to examine the pulsatile blood velocity profiles in various blood vessels in the orbit including the ophthalmic artery, central retinal artery and vein, posterior ciliary arteries and the orbital veins. The technique uses simultaneous B-scan ultrasound and colour Doppler imaging to locate and investigate the blood vessels. The resolution of the ultrasound prevents accurate measurement of vessel diameter therefore the volume of blood flow cannot be calculated with this method. Pulsed Doppler sonography is a slightly different method which allows velocity measurements to be made at defined tissue depths. Pulsatile ocular blood flow measurement has been found to correlate most strongly with the CDI parameter peak systolic velocity (PSV) in the central retinal artery[102].

Investigation of glaucoma patients using this method has consistently found reduced flow velocities in the ophthalmic artery compared to normal subjects.

Rojanapongpun et al measured supine ophthalmic artery flow velocities with 2MHz pulsed Doppler ultrasound in 60 POAG, 42 NTG and 35 normal subjects. They found significantly reduced peak, mean and diastolic flow velocities in both glaucoma groups compared to normals, to a greater extent in NTG. The authors address the possible confounding effect of treatment in the NTG group and conclude that the use of topical or systemic treatment by half the patients did not affect their results. No comment is made about the use of topical treatment or previous surgery in the POAG group[103].

Michelson confirmed these findings in advanced treated glaucoma reporting increased vascular resistance and reduced blood flow velocity in the ophthalmic artery compared to normals[104].

Nicolela et al compared subjects with ocular hypertension and open angle glaucoma and examined blood flow velocities and resistance in the central retinal artery, temporal short posterior ciliary artery, and the ophthalmic artery. They found reduced peak systolic velocity and end diastolic velocity in the central retinal artery in POAG compared to ocular hypertensives. There were no differences found in the ophthalmic artery. However several of their subjects were using topical betablockers and though matched for highest recorded IOP the groups were not matched for IOP at the time of investigation, the OHT group having the higher pressure[105].

Butt et al examined groups of untreated POAG, NTG and normals using CDI. They found an increased resistance to blood flow in the central retinal artery and ophthalmic artery and increased peak systolic velocity (PSV) in the ophthalmic artery of patients with POAG compared to normals. Ophthalmic artery PSV was related to intraocular pressure but there was significantly more vascular disease and higher systolic blood pressure in the POAG subjects compared to the normal group[106] which may have explained the unexpected finding of higher PSV in glaucoma.

Williamson et al examined 95 healthy volunteers to determine the effect of age, systemic blood pressure, smoking and blood viscosity on orbital blood velocities as measured with CDI. They found that systolic blood pressure correlated with peak systolic velocity, smoking was associated with lower velocities, higher haematocrit and viscosity

correlated with increased resistance to flow proximal to the ophthalmic artery and red cell rigidity negatively correlated with pulsatility of flow in the retinal vein. Increasing age was associated with reduced blood velocity in the ophthalmic artery and increased resistance to flow in the retinal circulation[107]. Rojanapongpun also found a decrease in ophthalmic artery velocities with increasing age[108]. Harris et al used CDI to investigate the effect of acute IOP elevation in healthy eyes. They found no change in ophthalmic artery blood flow up to an IOP of 45mmHg but central retinal artery velocities were highly IOP dependant[109].

D. Fluorescein Angiography

Fluorescein angiography is the mainstay of ocular vascular investigation in the clinical setting. It has also been widely used in research and various methods have been developed to gather additional information about retinal and choroidal circulation from the images. The time taken for the dye to pass from a retinal arteriole to a venule may be measured using dye dilution curves and this information gives an indication of the arteriovenous passage time in the retina. Scanning laser ophthalmoscopy combined with fluorescein angiography enables measurement of arm-retina time, arteriovenous passage time and mean dye velocity. The pattern of flow in the choriocapillaris may also be observed using videoangiography or indocyanine green (ICG) angiography.

Wolf et al used SLO combined with fluorescein angiography to examine 51 patients with POAG. They found an 11% reduction in mean dye velocity and 41% prolongation of arteriovenous passage time in POAG compared to normals[110]. Schwartz et al found that with increasing age there was a reduction in the rate of filling of retinal arterioles at the optic disc and peripapillary choroid. In addition higher diastolic blood pressure was associated with slower filling of the peripapillary choroid. In normal and OHT eyes as systemic pulse pressure increased less time was needed to fill the retinal veins due to a presumed faster intraretinal transit time. This pattern was reversed in POAG with a longer filling time for the retinal veins associated with higher pulse pressure. Comparing OHT with POAG the same study reported significantly slower rates of filling of the retinal arteries and peripapillary choroid in OHT. The use of medication in these patients

is not discussed however the median IOP of 20mmHg for POAG and 22mmHg for OHT suggests that the patients in this study were on treatment[111].

Best and Toyofuku investigated circulatory changes in response to artificial elevation of IOP with a suction cup in normal eyes. They found that small elevations of IOP (up to 40mmHg) produced more effect on choroidal than on retinal circulation. The velocity of blood flow was reduced in both choroid and retina but fluorescein appearance time was delayed to a greater extent in the choroid[112].

E. Blue Field Entopic Phenomenon

This method utilises the fact that it is possible for a subject to see leukocytes moving within their own paramacular retinal capillaries when looking at a diffuse blue light. A blue light of wavelength 430nm is presented to one eye and white cells are seen as multiple comma shaped flecks crossing the paracentral visual field. The density and velocity of these is matched by the subject to the density and velocity of spots on a VDU screen which is observed with the other eye.

Grunwald et al used this method to examine the response of POAG, OHT and normal eyes to increased intraocular pressure. They found that paramacular circulation in POAG eyes reduced in response to raised intraocular pressure at a lower pressure than that in OHT or normal eyes and attributed this difference to faulty autoregulation of retinal blood flow in glaucoma[72].

Sponsel et al found a correlation of better visual function with higher retinal leukocyte velocity in a mixed group comprising 4 glaucoma patients and 8 ocular hypertensives 3 of whom had suspicious discs or fields. Eyes with higher leukocyte velocity had better visual field ($p<0.05$) and much better contrast sensitivity ($p<0.001$)[113].

F. Animal Studies

Other more invasive methods of studying blood flow have been employed in animal studies. For example radiolabelled or unlabelled microspheres may be injected into the circulation and postmortem measurement of the density of trapped microspheres in the tissues allows an estimation of blood flow. This technique has been used to study ocular blood flow in cats[115], rabbits[70], dogs[114] and monkeys[75].

Alm and Bill in 1973 used radiolabelled microspheres to investigate the effect of an experimental increase in IOP on ocular blood flow in Macaque monkeys[75]. They found that an increase of IOP to a mean of 41mmHg in one eye led to a 30% reduction in choroidal and prelaminar blood flow in that eye when compared to the normotensive fellow eye. No change was seen in retinal blood flow and the authors suggest that this provides evidence for protective autoregulation in the retinal circulation. Sebag et al measured blood flow in experimental optic atrophy in cats and found good agreement between fluorescein angiography and the radiolabelled microsphere method [115]

Anderson et al performed surgical occlusion of the short posterior ciliary arteries in one eye of squirrel monkeys. They found that this led to areas of geographic RPE atrophy and outer retinal damage but caused only minimal damage to the optic nerve. They proposed that the optic nerve is protected by an effective anastomotic supply from the pial vessels and emphasised that the behaviour of optic nerve head blood flow cannot be reliably predicted from choroidal or short posterior ciliary artery blood flow [67].

Another method using tritiated iodoantipyrine was used by Quigley to investigate the effect of long and short term increase in IOP in monkeys. He found no difference in blood flow in the retina, optic nerve head or retrobulbar optic nerve unless IOP exceeded 75mmHg. Above this level a progressive decrease in optic nerve head blood flow was seen [76]. Weinstein et al used this method to investigate optic nerve head blood flow with alteration of mean arterial blood pressure in cats. They found evidence to support the presence of autoregulation of blood flow at the optic nerve head [116].

Sperber in 1985 used 2-deoxyglucose, a substance taken up by cells but which cannot be metabolised, to determine ocular blood flow in monkeys subjected to increasing IOP. He confirmed the presence of partial ischaemia of the optic nerve head and retina in eyes with raised IOP [117]. Bill and Sperber used the same method to provide evidence supporting the existence of retinal vascular autoregulation in response to increased demand in monkeys. They demonstrated an increase in inner retinal blood flow in eyes exposed to flicker light [77].

Khoobehi described an alternative method using heat labile liposomes containing dye which may be injected and then the dye released using laser light[118]. Retinal blood flow in individual vessels has been examined in primates using this method [119].

Funk et al used microendoscopy and spectroscopy to investigate the effect of intra-arterial injection of adrenaline on iris and ciliary body vessels in the rabbit. They found prolonged vasoconstriction followed by vasodilation suggesting the possible presence of sympathetic receptors in these uveal vessels [120].

Prior to this Stjernschantz demonstrated that intracranial stimulation of the oculomotor nerve, containing parasympathetic fibres, in rabbits produced an increase in choroidal blood flow [70]

Chapter 1.7

Peripheral vasoreactivity

In 1985 Phelps and Corbett published results of a study which found that 47% of a group of 54 patients with normal tension glaucoma suffered from migraine[121]. This compares with an incidence in the general population of 20 to 30%[122] and raised the possibility that an increased vasospastic tendency may have a role to play in causing visual field loss in this type of glaucoma.

Drance et al in 1988 measured fingertip blood flow in groups of NTG patients and normal controls both with and without a history of migraine. They found that 65% of NTG patients with no history of classic migraine had a vasospastic tendency seen as reduced baseline blood flow and prolonged recovery time after immersion of the hand in cold water. This incidence was very similar to that seen in control subjects with a history of migraine (65%) but differed significantly from that in controls without migraine (26%)[123].

There is a wealth of literature reporting similar findings in support of the theory that vasospasm plays a role in the development of field loss in normal pressure glaucoma[124]. There is also evidence that vasospasm plays a role in the progression of POAG. Broadway and Drance looked at the incidence of vasospasm in four previously described clinical subtypes of primary open angle glaucoma (optic disc types 'focal ischaemic', 'myopic glaucomatous', 'senile sclerotic and 'generalised cup

enlargement'). They found a significantly higher incidence of peripheral vasospasm (p=0.01) as measured with a laser Doppler flowmeter in the subgroup with 'focal ischaemic' disc appearance associated with a higher reported incidence of migraine (p=0.02) and cold extremities (p=0.00003), suggesting that vasospasm may play a role in the development of field loss in some patients with primary open angle glaucoma[125]. Orgul et al looked at a mixed group of 11 NTG and 9 POAG selected on the basis of continuing field loss despite controlled IOP. The implication was that the POAG group had progressing damage due to compromised ocular circulation rather than pressure related disease, they were therefore a highly selective subgroup of POAG patients. The study found that the majority of subjects (13/20) demonstrated a vasospastic tendency as shown by prolonged blood flow stand-still in nailfold capillaries after cold provocation. Systemic blood pressure however was lower in the nonvasospastic group and the authors suggest that both vasospasm and systemic hypotension may play a role independently to produce progressive damage in glaucoma patients with field loss that does not respond to pressure lowering treatment[126].

Systemic or ocular vasospasm has not been identified as a feature of ocular hypertension although it has only been in recent years that a consensus has been reached on the diagnostic criteria for this group. Many studies in the literature refer to 'glaucoma suspects' and the lack of consistent diagnostic criteria make it difficult to draw conclusions from the results. The ocular hypertension treatment study did not identify a significant association between a history of migraine and the risk of progression to glaucoma[39].

Endothelin and Nitric Oxide

The vascular endothelium is an important functional unit in the regulation of vascular smooth muscle tone. It has a diverse functions including control of permeability and the activation and inactivation of hormones. It also affects the coagulation system, platelet function and fibrinolysis and can release vasoactive substances which relax smooth muscle e.g. nitric oxide, or produce vasoconstriction e.g. endothelin-1.

Endothelin and nitric oxide have been found to have an effect on ocular circulation and also on intraocular pressure.

Endothelial dysfunction is implicated in the pathophysiology of vasospastic disorders and both nitric oxide and endothelin-1 have been studied in the context of vascular insufficiency in glaucoma. Endothelin-1 converting enzyme (ECE-1) produces endothelin-1 from a biologically inactive substrate 'big endothelin' and has been identified immuno-histochemically in the endothelium and vascular smooth muscle of the blood vessels of the retina, optic nerve and choroid[127]. An endothelin–1 receptor agonist sarafotoxin has been found to lower intraocular pressure in rabbits by regulating aqueous humour dynamics[128]. Sugiyama et al found elevated plasma endothelin concentration in normal tension glaucoma[129]. On the basis of these and other studies selective antagonists of endothelin-1 have been proposed as possible therapeutic agents in glaucoma[130].

Calcium channel blockers selectively inhibit endothelin-1 induced contraction of porcine ciliary artery in vitro[131] and calcium channel blockers such as nifedepine may have a

therapeutic role in glaucoma patients with vasospastic tendency. Gaspar et al found that nifedepine improved visual field in NTG[132], and Tomita et al found that it produced a significant reduction in orbital vascular resistance and an increase in optic nerve head blood flow [133]. Magnesium is a physiological calcium channel blocker and it has been found to improve peripheral circulation and visual field in glaucoma patients with a vasospastic tendency[134].

Nitric oxide is involved in the control of basal blood flow in the choroid, optic nerve and retina. In addition it performs several other roles; it mediates vasodilation in ocular vessels in response to numerous agonists including acetylcholine, substance P and insulin. It is involved in hypercapnia induced vasodilation in the choroid and is a modulator of autoregulation in this vascular bed[135]. Reduced levels of nitric oxide have been found in the aqueous humour of POAG patients undergoing cataract surgery compared to patients with cataract alone and, as with endothelin, the nitric oxide pathway in the eye has been proposed as a possible target for future glaucoma treatments[136].

1.8

Blood Rheology and Glaucoma

Blood flow through a tissue is dependant on the perfusion pressure and the vascular resistance in that tissue. Factors influencing the viscosity of the blood will have an effect on perfusion by altering resistance to flow. For this reason investigators have looked for haematological changes in glaucoma in an attempt to better understand the aetiology of the disease.

Coagulation

Drance and associates in 1973 [137] identified a 'greater tendency to thrombosis' in 30 of 45 patients with normal pressure glaucoma (NPG) due to increased platelet adhesiveness and abnormal euglobulin lysis time. This introduced the idea that a hypercoagulable state may be a contributing factor in the development of glaucoma. Subsequent studies produced mixed results. Joist et al found no difference in NTG subjects compared to normals when they measured blood coagulability, platelet function, and fibrinolysis[138]. Klaver et al also found no significant differences in fibrinogen comparing groups of NTG, POAG and normals [139].

Previous published findings from this department[140] showed a significant increase in prothrombin fragments 1 and 2 and increased levels of D-dimer in untreated POAG and NTG compared to normals suggesting activation of the clotting cascade in those groups.

The POAG group had a higher incidence of systemic vascular disease and higher blood pressure than the other two groups which may have contributed to the findings.

Plasma viscosity

Klaver et al identified increased whole blood and plasma viscosity in NTG compared to normals. Whole blood viscosity and packed cell volume were also found to be significantly increased in POAG subjects but this may have been due to higher levels of smoking in this group[139].

Trope et al measured blood viscosity, haematocrit and plasma fibrinogen in 27 POAG subjects and 18 controls matched for sex, mean arterial blood pressure and smoking habits. They found significantly increased blood viscosity in POAG but no difference in the other two parameters and they suggest raised plasma viscosity, increased levels of plasma proteins or changes in erythrocyte deformability as possible causes for this finding [141].

This theory was supported by the findings of Wolf and associates who recorded increased plasma viscosity in POAG associated with increased retinal arteriovenous passage time and reduced mean dye velocity compared to normals using fluorescein angiography. In addition to this Wolf also found no significant difference between normal and POAG in haematocrit or erythrocyte aggregation [110].

In contrast Carter et al in a prospective study of lipid profiles and blood rheology including coagulation and blood viscosity, found no significant differences between groups of 46 NTG, 69 POAG and 47 normal subjects. There was however a

nonsignificant trend towards increased blood viscosity in glaucoma in this study and the authors state that they used a different method to measure viscosity to that used in previous studies [142].

A problem encountered with all the above studies is the difficulty of controlling for other factors which influence viscosity such as age, cardiovascular disease and smoking in the elderly glaucoma population. In addition the possible confounding effect of concurrent treatment to lower intraocular pressure is seldom addressed.

Erythrocyte abnormalities

In 1993 Mary et al reported an investigation into erythrocyte deformability and aggregability, plasma fibrinogen and haematocrit in 21 POAG and 18 age matched control subjects. They found significantly reduced erythrocyte deformability in POAG but no difference in any of the other parameters. They propose that this may explain the previous finding of increased blood viscosity in glaucoma[143]. Reduced red cell deformability may be induced by platelet activation and oxygen free radical generation both of which have been implicated in glaucoma.

The following year Hamard et al published their findings of increased erythrocyte aggregation in POAG [144].

Platelets

Hoyng et al found a higher incidence of in vitro platelet aggregation in a mixed group of NTG and POAG patients with progressive field loss compared to glaucoma subjects with stable fields or ocular hypertensives. They suggested that aggregations of platelets at the optic nerve head may cause microinfarctions leading to splinter haemorrhages and field loss [145].

Hayreh et al published results of a study looking at the effect of serotonin, which is released when platelets aggregate, on the retinal and posterior ciliary circulation in monkeys fed on an 'atherogenic' diet high in cholesterol. They found that in the presence of atherosclerotic lesions serotonin can cause transient, complete or partial occlusion in the central retinal artery and posterior ciliary arteries. This serotonin induced vasoconstriction was reduced or abolished by discontinuing the atherogenic diet[146].

1.9

Other Risk Factors in Glaucoma and Ocular Hypertension

In addition to the topics already covered in this review there have been numerous studies searching for other possible risk factors associated with field loss in glaucoma and ocular hypertension.

Systemic Haemodynamic Factors

The relationship between systemic blood pressure and glaucoma is not straightforward and there are conflicting reports in the literature regarding the influence of blood pressure on the risk of visual field deterioration.

Arterial Hypertension

Many studies have reported an association between high systemic blood pressure and raised intraocular pressure[147,148].

The Framingham Eye Study[25] found that subjects with IOP above 21mmHg in at least one eye had an increased prevalence of systemic hypertension and diabetes, and in multiple regression analysis systolic blood pressure was the variable most related to IOP.

However, no association was found between IOP and cardiovascular disease (defined as the presence of coronary heart disease, atherothrombotic brain infarction, intermittent claudication, or congestive heart failure). In addition they found that while subjects without visual field defects demonstrated an association between IOP and blood pressure (higher blood pressure being associated with higher intraocular pressure) those*with* field defects did not. This resulted in a lower ratio of blood pressure to intraocular pressure in the group with field loss giving support to the vascular hypothesis of optic nerve damage.

Wilson et al also found an association between high IOP and systemic hypertension in a case control study to investigate risk factors in glaucoma and OHT. They found that black race and untreated systolic hypertension were the most important risk factors for having glaucoma in their group of 83 POAG, 121 OHT and 237 controls. They also found 'suggestive' associations between glaucoma and cigarette smoking, family history of glaucoma, definite or borderline diabetes and myopia. Similar but weaker associations were noted for the OHT group[148].

The relationship between systemic hypertension and IOP was explored by Williams et al in 1984. They found that normal subjects with untreated systemic hypertension had a significantly elevated mean level and variance of IOP in the morning and a greater increase in IOP on lying down in comparison to normotensive individuals[149].

Despite the acknowledged association of high systemic blood pressure with glaucoma it is not a good predictor of future field loss in ocular hypertensives[21,150]. The Ocular

Hypertension Treatment Study (OHTTS) failed to find any association between high or low systemic blood pressure and the risk of developing POAG[39].

Arterial Hypotension

Heyreh et al measured 24-hour ambulatory blood pressure in a mixed group of 166 patients with 'ocular ischaemic disorders' including NTG (n=67) and POAG (n=21). They found a significant reduction in systolic and diastolic blood pressure at night. Patients with treated arterial hypertension showed a significant association between progressive field loss and nocturnal hypotension. There was no correlation found between IOP and field loss in this study. The authors postulate that ocular ischaemia secondary to reduced perfusion pressure causes field loss and that subjects with a history of systemic hypertension are particularly susceptible to this due to hypertensive changes in the ocular blood vessels[151].

These findings were confirmed by Kaiser and associates when they found that POAG patients with progressing field loss despite controlled IOP had significantly lower systolic blood pressure both day and night than normal controls[152].

Meyer et al studied a group of 20 normal tension glaucoma patients. They found that NTG patients had slightly lower blood pressure than controls overall (non significant) but that they had significantly greater nocturnal blood pressure dips ($p<0.001$)[153].

Systemic vascular disease

The OHTTS identified heart disease as a predictive factor for the development of field loss in ocular hypertension on univariate analysis[39].

The Framingham eye study found no association between high IOP and history of cardiovascular disease but did find an association with important risk factors for vascular disease; systemic hypertension and diabetes[25].

Walker et al found a higher incidence of systemic vascular disease in POAG (n=50) and OHT (n=45) than in normals but there was also a significantly higher number of smokers in these groups[154].

Optic Disc Haemorrhages

For many years there has been a known association between splinter haemorrhages at the neuroretinal rim and glaucoma damage. In a long term study of 1270 patients optic disc haemorrhages were seen in 20% of POAG patients and were found to be a precursor of glaucomatous disc changes and associated visual field defects. This finding was seen independent of IOP and the authors suggest that the finding supports the vascular theory of damage in POAG [155].

Non Vascular Risk Factors

Age

The effects of age on IOP are discussed in detail in chapter 1.4.

Many studies have identified an increasing incidence of POAG with age[156]. Quigley described the prevalence of POAG in a European-derived population as following an exponential curve sharply increasing above the age of 75. Prevalence among African and Asian derived people follows a linear function increasing with age with a steeper gradient on the line for Africans[5].

Hart et al in a retrospective study of 92 OHT patients 33 of whom developed field loss over 5 years found that the strongest predictors of future field loss were age, mean IOP, vertical cup to disc ratio, and a positive family history of glaucoma[150].

The OHTTS also found that age was an important risk factor for the development of field loss both on univariate and multivariate analysis[39].

Optic disc and nerve fibre layer appearance

Unsurprisingly optic cup enlargement and nerve fibre layer defects have been found to be associated with increased risk of future field loss. Studies have shown that a significant proportion of nerve fibres are lost in glaucoma before detectable field loss appears[157]. Patients with observed disc or nerve fibre layer changes may in fact have very early disease.

Quigley et al followed a group of 647 ocular hypertensives (40% of whom were receiving treatment) for 12 years. Sixty eight subjects developed field loss during followup. Moderate or severe nerve fibre layer atrophy at baseline was associated with a 7 to 8 times greater risk of field loss. Other attributes significantly associated with field loss were larger cup to disc ratio, smaller rim to disc area ratio, greater optic cup asymmetry, presence of optic disc crescent, older age and higher IOP[21].

The association between smaller area of neuroretinal rim, larger area of peripapillary atrophy and risk of progression of glaucomatous field loss was confirmed by Jonas et al in a prospective observational study of 257 glaucoma patients [158].

Budde and Jonas also investigated whether the presence of a cilioretinal artery influenced the pattern of neuroretinal rim loss and parapapillary atrophy in glaucoma. They found that the presence and position of cilioretinal arteries did not appear to have an effect on the pattern of progressive disc changes [159].

Myopia

The Blue Mountains Eye Study [24] found an increased incidence of glaucoma in myopic eyes. Glaucoma was present in 4.2% of eyes with –1 to –3 dioptres of myopia, 4.4% of eyes with over 3 dioptres myopia and only 1.5% of non myopic eyes. The odds ratio after adjusting for known glaucoma risk factors was 2.3 for low myopia and 3.3 for moderate to high myopia. There was a borderline association between myopia and ocular hypertension (odds ratio 1.8 for low myopia and 0.9 for high myopia).

The association between myopia and open angle glaucoma has been confirmed by other authors[148] although the presence of myopia in ocular hypertensives was not found to hold any predictive value for progression to field loss in the OHTTS.

Diabetes mellitus

Dielemans et al reported the findings of the Rotterdam Study which included 4178 individuals over the age of 55yrs. They found that newly diagnosed diabetes and high levels of blood glucose were associated with ocular hypertension and glaucoma[22]. This finding has been confirmed by others[25,148].

Conversely diabetes was found to have a protective effect against developing POAG in the OHTTS. This may be explained by the exclusion of patients with diabetic retinopathy from the study leading to an unrepresentative, healthier group of diabetics[25]. The conflicting results from studies looking at the relationship between POAG and diabetes may be explained by the coexistence of a number of confounding factors in diabetics. They may have large or small vessel disease, autonomic neuropathy, systemic hypertension, diabetic retinopathy and optic neuropathy, all of which may have an effect on visual function and ocular blood flow. In addition to this diabetic subjects with severe diabetic retinopathy are often excluded from ophthalmic studies. Finally diabetics as a group are exposed to routine eye examination including dilated fundoscopy more frequently than the general population and this may make it more likely for a diabetic

with POAG to be identified while a higher proportion of non-diabetic glaucoma patients remain undiagnosed.

Race

African derived people tend to develop POAG at a younger age and the disease runs a more aggressive course than in European or Asian derived populations[5].

The Baltimore Eye Survey found that age adjusted prevalence rates for glaucoma in blacks were 4 to 5 times higher than in whites[17].

Wilson et al also found that black race was one of the strongest predictors of field loss in their study of OHT, POAG and normals[148].

Genetics/Family history

A family history of glaucoma or ocular hypertension has been identified in many studies as a risk factor for developing the disease[150].

In 1997 Stone and associates identified a gene (TIGR) associated with juvenile open angle glaucoma and screening of adults with POAG found that 4% of this group also carried a mutation in the coding region for this gene[160]. Since then many more mutations in the TIGR gene sequence (now replaced with the term myocilin, abbreviated to MYOC) have been identified in patients with glaucoma. Other genes related to specific conditions associated with glaucoma such as PITX2 in Rieger syndrome[161],

FOXC1 in Axenfeld-Rieger anomaly[162], and CYP1B1 in autosomal recessive congenital glaucoma[163] have also been identified.

The Glaucoma Inheritance Study in Tasmania identified eight pedigrees with the most common MYOC gene mutation. However within those eight families only half of those with POAG or OHT carried the mutation and not all of those with the mutation had evidence of POAG or ocular hypertension[164]. This highlights the fact that although myocilin mutations are the most commonly found genetic abnormality implicated in glaucoma they account for a very small proportion of glaucoma cases overall.

Studies have also looked at genetically based mechanisms through which vascular endothelial cells undergo changes in structure and function in response to laminar shear stress. It has been found that positive and negative transcriptional regulatory elements combined with shear-induced transcription factors can provide a mechanism for coordinated patterns of response of otherwise unrelated genes in the endothelial cell[165]. In other words the expression of genes in the vascular endothelium is regulated in part by haemodynamic factors.

Gender

The Framingham eye study found that men were more than twice as likely as women to have POAG[166], an earlier Swedish study found that women were at higher risk than men[6], but the majority of studies have found no consistent gender difference in the incidence of POAG [5,7].

Alcohol and Smoking

A weak association between smoking and POAG has been noted in the literature[148]. However colour Doppler studies of orbital blood flow in smokers have identified increased blood flow velocities particularly in the ophthalmic artery in the absence of increased resistance. This is thought to be due to an increased sensitivity to endogenous vasodilators in healthy smokers[167]. A similar effect has been recorded in non smokers given a low dose of nicotine via chewing gum[168]. The relationship between smoking and glaucoma may be due to their common association with systemic vascular disease in later life.

Summary of Section I

Section I introduces the topics covered by this investigation and reviews the literature on blood flow in primary open angle glaucoma and ocular hypertension.

Primary open angle glaucoma and ocular hypertension are defined. Epidemiological studies related to the conditions are reviewed. A review of the literature on normal IOP variation and control is followed by a review of investigations into the effects of lowering IOP in the long or short term in OHT and POAG. Normal ocular circulation is described and the results of investigations into physiological controls on blood flow to the eye are related. The reported effects of alterations in IOP on the ocular circulation are reviewed. In chapter 1.6 different methods used to investigate blood flow are described and studies comparing blood flow in OHT and POAG are reviewed. Peripheral vasoreactivity in glaucoma is discussed in chapter 1.7 and studies of haematological changes in glaucoma are reviewed in chapter 1.8. The final chapter of Section I lists other vascular and non-vascular risk factors which have been identified as playing a possible role in the development of glaucoma.

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SECTION II

Investigations and Results

Aims and Objectives

1. To determine if differences in ocular blood flow exist between POAG and OHT which can be attributed to the presence of disease rather than intraocular pressure or treatment.

2. To compare groups of prospectively recruited, untreated POAG and OHT patients matched for intraocular pressure.

3. To investigate choroidal and retinal blood flow in POAG and OHT.

4. To investigate clotting parameters including markers of platelet activation in POAG and OHT.

5. To investigate peripheral vasoreactivity in POAG and OHT.

2.1

Patient Recruitment and Study Protocol

Ethical approval

Ethical approval was granted for the study by Lothian Research Ethics Committee and informed consent was obtained from all subjects. (See copy of consent form and information sheet, appendix B)

Subject Selection

Patients were recruited prospectively over a fourteen month period from glaucoma clinics at the Princess Alexandra Eye Pavilion Edinburgh. This institution is the sole referral centre for ophthalmology for Edinburgh City, East Lothian and Midlothian districts. The estimated population served by the hospital was 617,030 in the first year of this study, of whom 94,546 were aged 65 and above. All new patients seen in our clinic who met study criteria and were able to travel to and from the hospital without difficulty were recruited. All those who were asked to participate agreed to do so, all were Caucasian.

Inclusion Criteria

Glaucoma patients

Glaucoma patients had an intraocular pressure on presentation greater than 21 mmHg, open drainage angles on gonioscopy, optic disc cupping and characteristic visual field defects on testing with the Humphrey visual field analyser (24-2 threshold program). Clinical diagnosis was confirmed in all cases by an experienced glaucoma specialist (COB).

Ocular Hypertensives

Ocular hypertensive patients had intraocular pressure of over 21mmHg and full visual fields as assessed with the Humphrey visual field analyser 24-2 threshold program. Optic disc appearance in ocular hypertensive subjects was noted but was not part of the inclusion criteria. A decision was made not to include optic disc appearance in the criteria for diagnosis of POAG and OHT. This was because of the difficulty in obtaining a truly objective assessment of glaucomatous damage based on the appearance of the disc at a single examination. It was expected that the OHT group would contain some preglaucoma subjects both in terms of both optic disc changes and blood flow. No attempt was made to identify these patients at the recruitment stage. The ocular hypertensive group was subdivided into 'high risk ocular hypertensive' (HROHT) and 'low risk ocular hypertensive' (LROHT) on the basis of intraocular pressure. A

maximum intraocular pressure less than or equal to 25mmHg was considered 'low risk', a maximum pressure of 26mmHg or above was 'high risk'.

All subjects had central visual acuity of 6/9 or better in the study eye. All measurements were made on both eyes, the eye with the worse visual field was taken as the study eye in glaucoma subjects. For normal subjects and ocular hypertensives the eye with the higher IOP was selected or, if there was no difference between the eyes one eye was chosen at randomly tossing a coin.

Normal control subjects

Normal subjects were friends or spouses of patients. They had normal intraocular pressure and no signs of ocular disease on examination. They had no previous history of eye disease or ophthalmic surgery. As in the patient groups, normal subjects had a visual acuity of 6/9 or better in the study eye and full visual fields on testing with Humphrey visual field analyser (Armaly programme).

Fifty-one ocular hypertensives, 24 glaucoma patients and 23 normal subjects were recruited to the study over the course of fourteen months.

Exclusions

Exclusion criteria were as follows:

A history of use of topical antiglaucoma medication or topical steroid to the eye.

A history of eye disease or previous eye surgery.

The concurrent use of systemic vasoactive medications e.g. systemic nitrates, betablockers or calcium channel blockers.

An inability to sit at the slit lamp or difficulty attending the hospital due to physical disability.

Cup to disc ratio greater than 0.8 in ocular hypertensives. (No subjects were excluded on this basis).

Investigations

Arrangements were made for the subjects to attend the hospital for one day during which the following protocol was observed.

1. Consent and questionnaire
2. Full ocular examination including Goldmann tonometry and gonioscopy.
3. Venepuncture
4. Pulsatile ocular blood flow measurement in sitting, standing and supine positions.
5. Laser Doppler finger capillary blood flow with temperature stress test
6. Heidelberg laser Doppler retinal blood flow measurement
7. Visual field testing; Humphrey 24-2 programme for POAG and OHT subjects, Armaly visual fields for normal subjects.
8. Axial length (Ocuscan biometry machine, Alcon Labs UK Ltd)
9. Intraocular pressure measurement at 9am, 11am and 1pm.

Results of the measurements were recorded on a standard form (see example results form, Appendix C)

Consent and questionnaire

Patients and controls were asked to attend the hospital at 8.30 am. On arrival they were given the patient information form to read and informed consent was obtained. The questionnaire covering past medical and ocular history (see appendix B) was completed before commencing the investigations.

Ocular examination

Best corrected Snellen visual acuity was recorded. Anterior segments of both eyes were examined with the patient seated at the slitlamp. Intraocular pressure was measured with a Goldmann tonometer and gonioscopy was performed with a contact lens following tonometry.

Venepuncture

Blood was taken for the following; full blood count, beta-thromboglobulin, fibrinogen, prothrombin fragments F 1 and 2 (PTF1&2) and D-dimer. All blood tubes were kept on ice, venepuncture was performed with as little trauma as possible using a 19 gauge needle, one 5ml syringe and two 10ml syringes. Blood was drawn without compression on the vein and the first 5ml drawn were used for the full blood count. Particular care was taken in the handling of the sample for beta thromboglobulin. All samples were transported immediately on ice to the haematology lab where the full blood count was recorded and the remainder of the samples were spun down and frozen (-70 °C).

Pulsatile ocular blood flow

Following venepuncture and after a 15 minute break, subjects were seated at the slit lamp for 5 minutes. Blood pressure and pulse were measured with an automatic cuff (Criticon Dynamap, Tampa, Fla). Pulsatile ocular blood flow, pulse amplitude and pulse volume were measured with the pneumotonometer mounted on a slit lamp. We used the ocular blood flow system developed by OBF labs UK (System 3000, Version 14.4, Revision 4). The subject was then asked to stand for 5 minutes. The blood pressure, pulse and POBF measurements were repeated with the pneumotonometer hand-held by the examiner. Finally the subject was placed in a supine position for 5 minutes and the measurements were repeated. The pneumotonometer was again hand-held for this measurement.

Laser Doppler finger capillary blood flow

For measuring changes in fingertip capillary blood flow we used the Periflux laser Doppler flow meter for microvascular perfusion produced by Perimed KB (PO Box 5607, Stockholm, Sweden)

After POBF measurement subjects were seated for a further 5 minutes. Ambient room temperature was noted. The probe for the laser doppler flow meter was applied to the middle finger of the left hand and a baseline blood flow reading was taken. The left hand was then immersed in a water bath at 40 °C for 2 minutes, on removal the hand was wrapped in a towel to limit heat loss and peak blood flow was recorded from the finger tip. Blood flow was observed returning to baseline level and then the left hand was

placed in a mixture of ice and water at –4°C for 10 seconds. The minimum flow was noted and the time taken for the blood flow to return to normal was recorded.

Heidelberg laser Doppler retinal blood flow measurement

The Heidelberg retinal flowmeter was used to take flow images of the study eye. Images were taken in the late morning in a windowless room with consistent background lighting. A 2.5°x 10° frame was used centred on the optic disc and focused on the retinal vessels. Pupils were undilated and ambient lighting was standardised. The images taken were stored and reviewed at a later date as detailed in chapter 2.2.

Intraocular pressure measurement

Intraocular pressure was measured with a Goldman tonometer with the subject seated at the slit lamp at 2 hourly intervals during the course of the visit (phasing). In addition to this, maximum and minimum IOP readings recorded by the OBF pneumotonometer in sitting and supine positions were noted.

Review visit

Subjects were started on antiglaucoma medication after the study visit if required, and all were reviewed in the outpatient clinic in the usual way. Fourteen glaucoma patients (6 female 8 male) and eleven high risk ocular hypertensives (5 female 6 male) were recalled for repeat study measurements after commencing anti glaucoma medication.

The average time interval between investigations was 15.8 months. The treatment for each patient was selected on an individual basis and was therefore not standardised. Twenty three patients were on betaxolol 0.5% bd (Alcon), seven required a second topical drug in addition to betaxolol and of these four were on dorzolamide 2% tds (MSD), one was on latanoprost 0.005% once daily (Pharmacia), one was on brimonidine tartrate 0.2% bd (Allergan) and one on pilocarpine 1% qds. Three were unable to tolerate betablockers and were therefore started on dorzolamide 2% tid (MSD). See tables 3 and 4.

Table 3: Numbers of patients reviewed

	Male	Female	total
Treated POAG	6	8	14
Untreated POAG	0	0	0
Treated HROHT	5	6	11
Untreated HROHT	1	1	2
Treated LROHT	0	1	1
Untreated LROHT	3	4	7

Table 4: Details of treatment and follow-up

Patient id.	diagnosis	Treatment	Time to follow-up (months)	Systemic Rx on follow-up
3	hroht	B	24	no
5	LROHT	B	26	no
8	POAG	B	23	no
13	POAG	B L	22	no
14	hroht	B	25	no
19	POAG	B	24	no
20	POAG	B	12	no
21	POAG	dz	12	no
24	hroht	B	21	no
25	hroht	B	23	no
27	hroht	B	23	no
30	hroht	B dz	12	no
32	POAG	B α	14	no
33	hroht	B	17	no
37	POAG	B dz	17	no
42	hroht	B	16	bendrofl.
46	POAG	B	5	no
47	hroht	dz	15	aspirin
50	hroht	B	17	no
55	POAG	B p	15	no
56	POAG	B dz	4	no
64	POAG	B dz	7	no
68	POAG	B	7	no
70	hroht	B	11	no
71	POAG	dz	11	no
74	POAG	B	10	no

Key: B = betaxolol
dz = dorzolomide
α = alphagan (brimonidine)
L = latanoprost
p = pilocarpine

Statistical methods

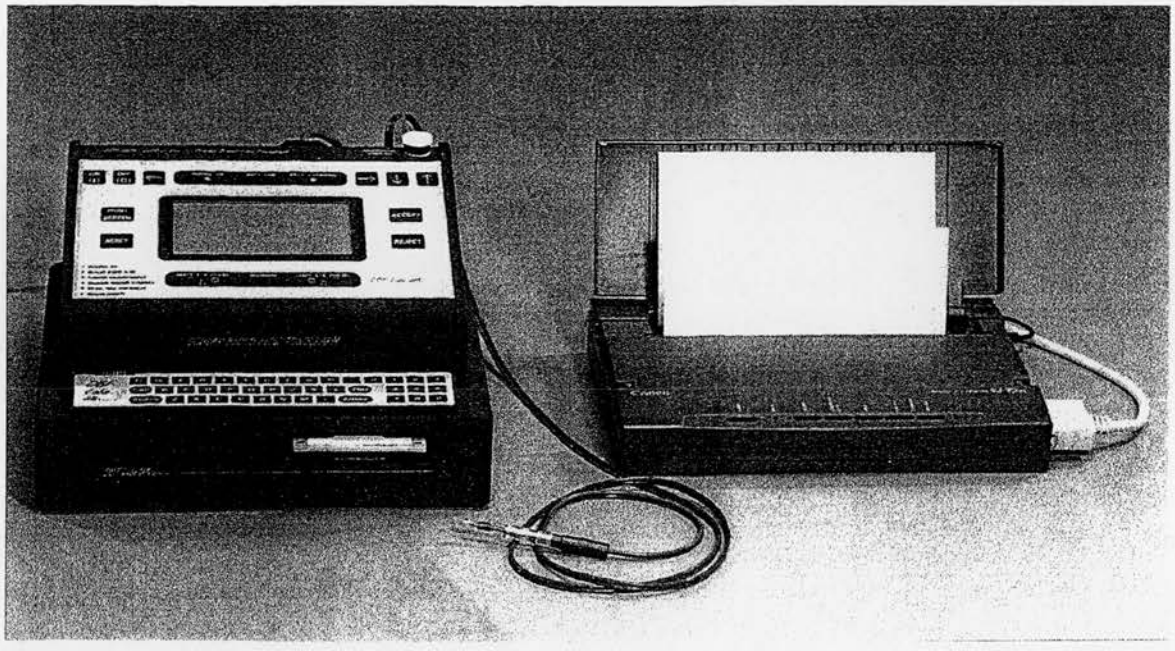
In general when data was normally distributed comparisons across four groups were made using a two way ANOVA with Bonferroni correction and comparisons between two groups were made using the unpaired Student t test. The chi squared test was used for categorical data. Data was checked for skewdness and if not normally distributed was examined using non-parametric tests. The Mann Whitney U test was used for paired comparisons and the Kruskall Wallis ANOVA was used for comparisons across 4 groups. Statistical significance was set at $p \leq 0.05$. Correction was made for age when necessary using partial correlation when continuous data was normally distributed or multiway logistic regression if not. All statistical calculations were made using SPSS software. Details of the statistical methods used for each set of results are given in chapter 2.2.

2.2

Methods and Statistical Analysis

A. Pulsatile Ocular Blood Flow

The POBF machine used in this investigation was developed by OBF Labs UK Ltd. It consists of a pneumatic probe linked to an internal compressor which supplies filtered air to the tip and a computer which displays the blood flow parameters (figure 8). The face of the pneumatic probe consists of a thin membrane. It is kept in contact with the cornea by a light flow of air within the tip which gently holds the sensor against the eye with a constant force. As the pressure in the eye changes the pressure required to applanate the cornea varies. These subtle changes in pressure are continuously monitored by the POBF machine. During the test the IOP is monitored 200 times per second. The computer filters this signal using real time analysis and constructs an image of each pulse. The 5 pulse wave forms that are closest to each other in IOP variation with time are automatically averaged by the computer software and used in the calculation of the ocular pulse characteristics and the POBF. A digital display provides information about the IOP (mmHg), pulse amplitude (mmHg), pulse volume (μl), systolic and diastolic time (seconds), pulse rate (beats per minute), and POBF (μl /min). The information displayed by the computer is stored on a floppy disc and may be printed out in a standard format (figure 9).



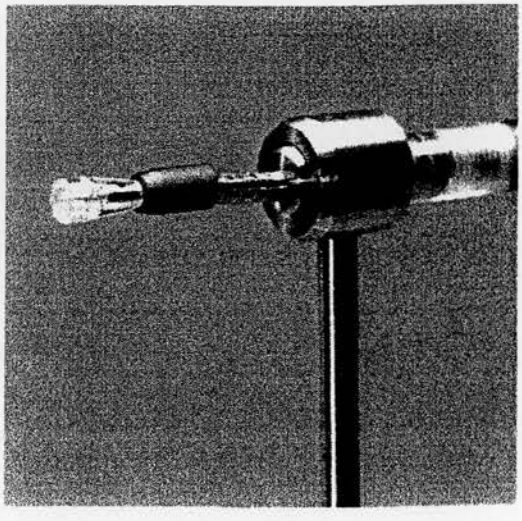


Figure 8: OBF machine and pneumatic tip

standing

P.A.E.P.

Chalmers Street Edinburgh EH3 0666 822269

OBF Test recorded using OBF Labs (UK) Ltd Tonograph, Copyright 1992.

The test was carried out with the patient at a slit lamp
The right eye was recorded first.

Name :
Date : 21.2.1996
Time : 11:49
Test No. : MC2001-112-129

Patient No.: 311026
D.o.B. : 31.10.26
Sex :

Right Eye	Pulse:	1	2	3	4	5	Average
Minimum Intraocular Pressure	[mmHg]	32.0	32.4	31.8	33.8	32.0	32.4
Maximum Intraocular Pressure	[mmHg]	34.0	34.5	33.6	35.9	34.1	34.4
Average Intraocular Pressure	[mmHg]	33.0	33.4	32.7	34.9	33.0	33.4
Pulse Amplitude	[mmHg]	2.0	2.1	1.8	2.1	2.1	2.0
Pulse volume	[µl]	2.3	2.4	2.1	2.3	2.4	2.3
Systolic Time	[sec]	0.28	0.30	0.31	0.29	0.32	0.30
Diastolic Time	[sec]	0.39	0.34	0.32	0.37	0.35	0.35
Pulse Rate	[/min]	89	93	95	90	89	92
Pulsatile Ocular Blood flow	[µl/min]	365	453	470	491	527	461
OBF % Standard Deviation							12
MNI : 813	PEQ : 2.8			IDR : 46			

Left Eye	Pulse:	1	2	3	4	5	Average
Minimum Intraocular Pressure	[mmHg]	31.7	32.0	31.8	32.0	32.1	31.9
Maximum Intraocular Pressure	[mmHg]	33.7	33.8	34.0	34.2	34.2	34.0
Average Intraocular Pressure	[mmHg]	32.7	32.9	32.9	33.1	33.2	33.0
Pulse Amplitude	[mmHg]	2.0	1.9	2.2	2.3	2.1	2.1
Pulse volume	[µl]	2.2	2.1	2.5	2.6	2.4	2.4
Systolic Time	[sec]	0.24	0.26	0.30	0.25	0.30	0.27
Diastolic Time	[sec]	0.41	0.37	0.40	0.38	0.33	0.37
Pulse Rate	[/min]	92	95	85	95	95	93
Pulsatile Ocular Blood flow	[µl/min]	379	413	450	505	526	454
OBF % Standard Deviation							13
MNI : 910	PEQ : 3.0			IDR : 42			

Comments :-

The right eye recordings were repeated tests.
The left and right eye IOP seems to be high.

Figure 9: Data sheet produced by OBF machine

POBF is automatically calculated by the machine from an analysis of the IOP pulse waveform, pulse rate and the ocular pulse amplitude. The calculations and underlying assumptions used to derive POBF are described in a paper by D. Silver[1]. In summary, for the purpose of POBF calculation the eye is represented as a chamber with elastic walls with a pulsatile incoming flow of incompressible fluid and a steady outgoing flow. It is assumed that outflow from the eye remains constant, that there is no reflux and that the pressure volume relationship (ocular rigidity) derived from Langham's living eye experiments in the 1960s can be applied to all eyes[2].

Repeatability studies using this machine were carried out by Z. Butt prior to the investigation reported here[3] using intraclass correlations of five repeated POBF measurements on 20 patients. Intraclass correlation is derived from the equation;

$$\frac{\text{Patient's variance}}{\text{Total variance}}$$

It is therefore an assessment of the proportion of total variance that can be attributed to the patients. For this investigation the result was not significant ($p=0.15$) signifying good repeatability.

Other studies have looked at the reliability of measurements with this machine. Yang et al found an acceptable reliability coefficient of 0.92 for POBF values from 290 µl/min to 2196µl/min [4].

All measurements in this study were performed by one, experienced operator (JK), following the technique described by Butt.

Axial length was measured in each subject using a biometry machine.

Statistical analysis of group comparisons was performed using ANOVA with the Bonferroni correction for pairwise comparisons when the overall f test was significant. i.e. if a significant difference was found to exist across the 4 groups the Bonferroni correction identified between which groups the significance lay. Results were adjusted for age. Paired comparisons (the effect of treatment and the effect of posture) were made using the Student t test. The chi-squared test was used for categorical data. Partial correlations were performed for males and female separately correcting for age.

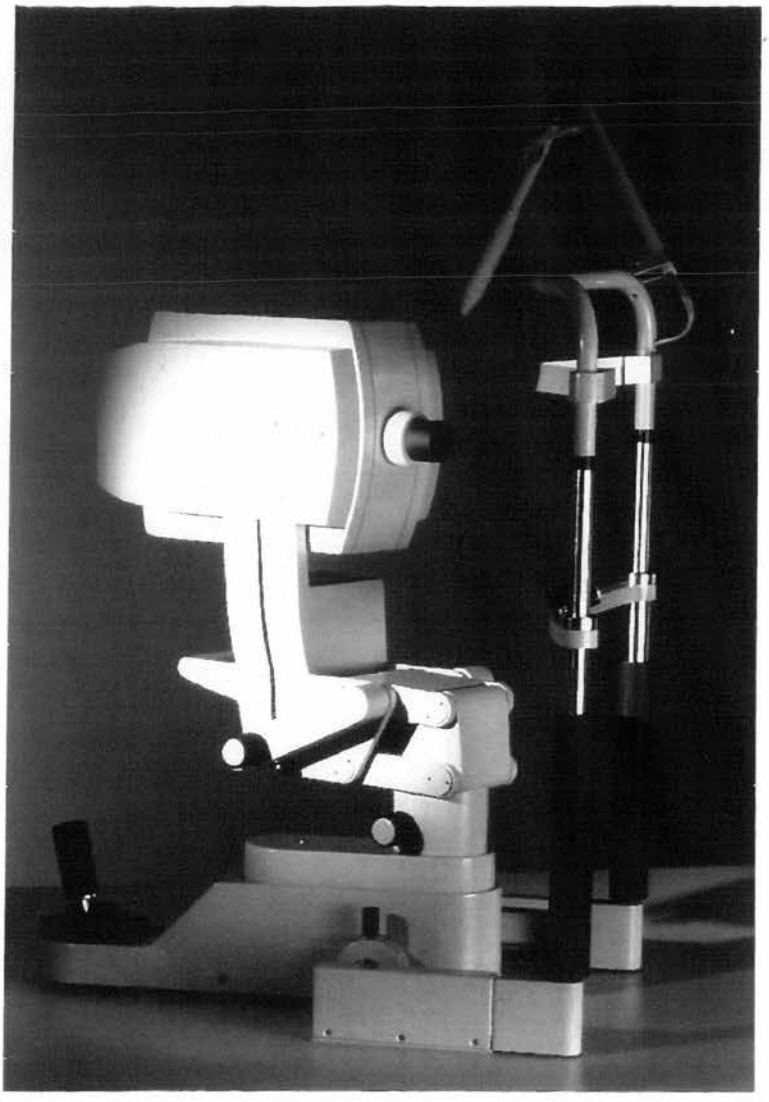


Figure 10: Heidelberg Retinal Flowmeter

B. Retinal Blood Flow: Scanning Laser Doppler Flowmetry

Laser Doppler flowmetry developed as a non-invasive technique for measuring blood flow in the retina and optic nerve head. In this study we used the Heidelberg Retinal Flowmeter (HRF, Heidelberg Engineering Germany), a machine which combines a confocal laser scanning system with laser Doppler flowmetry described in 1995 by Michelson and Schmauss [5].

The measurement of blood flow by laser is based on the optical Doppler effect. Laser light reflected by a moving particle is shifted in frequency by an amount Δf according to the equation:

$$\Delta f=(1/2\pi)(k_{sc}-k_I)\underline{v}$$

Where k_{sc} is the wave vector of the scattered light, k_I is the wave vector of the incident light and $\underline{v}$ represents the velocity vector of the incident light. Using an infrared laser (wavelength 780nm) the HRF scans the retina two-dimensionally. Light reflected or scattered by moving red blood cells is frequency shifted due to the optical Doppler effect. It interferes with light reflected by stationary objects resulting in a characteristic temporal variation of the reflected light intensity. The HRF measures this temporal variation at each point in a two dimensional field by multiple scanning. The frequency shift of the reflected light is computed from the acquired data and used to quantify the local amount and velocity of moving red blood cells. The size of the measurement field on the retina is from 10 by 2.5 degrees up to 20 by 5 degrees. Within this field the temporal variation of the reflected or scattered light is measured at 256x64 points. Total

data acquisition time is 2 seconds. Perfusion maps are produced from the acquired data in which the brightness of each point or pixel is coded by the value of the Doppler shift of laser light reflected from that point. The observer acquires numerical information about "flow" (the distance travelled by all moving red blood cells inside the sample volume per unit time), "volume" (the number of moving red blood cells), and "velocity" (mean red blood cell speed) by placing a "region of interest" square on the image to select a sample volume which is 2,560 x 640 x 400 μm in size.

We used a 2.5 x 10 degree frame centred on the optic disc and focused on the retinal vessels. Images were taken in the late morning. Pupils were undilated and ambient lighting was standardised. For the purpose of analysis we divided the flowmeter image into five areas; nasal retina, nasal neuroretinal rim, optic cup, temporal neuroretinal rim and temporal retina (Figure 8).

One problem with the use of the SLDF is the potential bias produced by subjective positioning of the sample square on the image. It is possible to obtain very different results within a given anatomical area simply by moving the sample square a small distance, this is the case even when visible blood vessels are avoided and occurs presumably because the capillary network is not uniform. We attempted to reduce potential bias by increasing the number of samples taken in each area and recording the maximum and minimum sets of results obtained (volume, velocity and flow). Measurements were made using a 10x10 pixel square and up to 10 samples were taken in each area. Areas of peripapillary atrophy and visible blood vessels were avoided when placing the square.

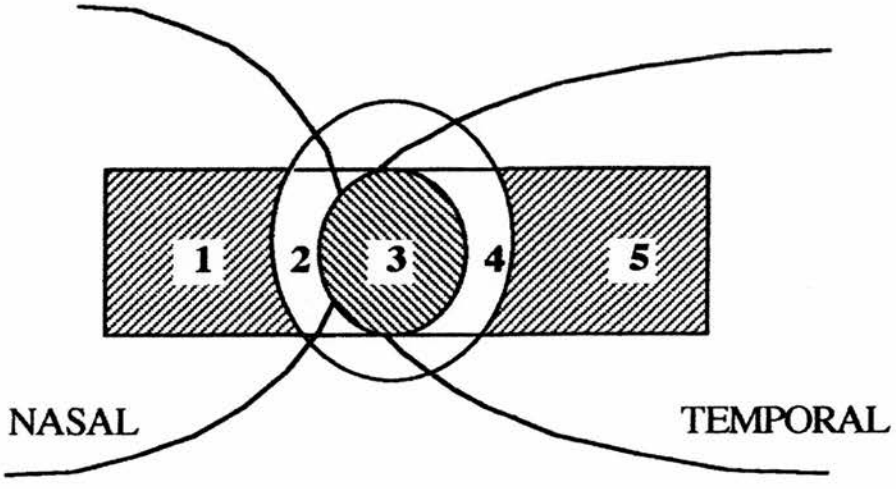


Figure 11: Schematic representation of the optic nerve head and peripapillary retina.

The area of the retinal flowmeter image is within the shaded box. Numbers indicate the different areas of measurement as discussed in the text.

1= nasal retina, 2= nasal neuroretinal rim, 3= optic cup, 4= temporal neuroretinal rim, 5= temporal retina.

Reliability coefficients for 'flow', 'volume' and 'velocity' were found to be 0.84, 0.85 and 0.84 respectively in the paper by Michelson which looked at 5 consecutive measurements in the same retinal area in one eye of 12 individuals. The validity of the method was investigated in the same paper and a significant linear relationship was found between SLDF flow and the ocular perfusion pressure when the latter was varied with a suction cup. It was also found that there was a linear relationship between the SLDF measurement and a pre-existing laser Doppler flowmeter suggesting that the two methods measure the same thing[6].

For statistical analysis of the SLDF results the Kruskal-Wallis ANOVA was used and comparison was made maximum with maximum and minimum with minimum results between the groups in each of the five areas. Comparison between OHT and POAG was performed using the Mann Whitney U test.

C: Haematological investigations

Blood samples were taken from each patient according to the protocol described in chapter 2.1. Blood was transferred to one EDTA tube (3.5ml for full blood count), one BTG tube (5ml) and two sodium citrate tubes (3ml each, for fibrinogen, prothrombin fragments 1 and 2, and D-dimer). The following methods were used for analysis of the samples.

B-thromboglobulin

The BTG sample was spun at 2000rpm for 30 minutes and stored at -70°C. β-thromboglobulin levels were measured using a radioimmunoassay kit (Kodak Clinical Diagnostics Ltd, Amersham UK). Betathromboglobulin is a platelet specific protein released into the circulation when the blood platelets are activated. The protein was isolated, characterised and named in 1975 by Moore, Pepper and Cash [7]. It is the most abundant platelet specific protein and is located in the α-granules of the platelets. Elevated levels have been found in patients with subacute deep vein thrombosis, pre eclampsia and in some infections. Normal mean values supplied by the manufacturers of the assay kit are within the range 24-28ng/ml. They report occasional high readings in normal individuals due to problems with the technical processing of the sample which must be taken atraumatically, cooled quickly and treated gently to prevent platelet degranulation prior to assay.

Full Blood Count

Full blood count and differential were measured in the standard way using a Sysmex 8000 FBC counter.

Clotting Factors

Blood from the sodium citrate tubes was centrifuged at 4000rpm for 10 minutes.

Fibrinogen was measured using the Clauss method.

Prothrombin fragment F1 and 2 was assayed by ELISA (Enzygnost, Dade Behring Marburg GmBH, Germany).

The conversion of prothrombin to thrombin is a key event within the coagulation cascade. Quantification of prothrombin fragment F 1 and 2 gives information about the amount of thrombin formed. Elevated levels are found in patients with thrombosis, pulmonary embolism, DIC, polytrauma and septicaemia and in the plasma of patients with hereditary protein C or protein S deficiency. Levels are reduced in patients taking oral anticoagulants.

D-dimer was also assayed using ELISA (Coa test, Chromagenix AB, Sweden).

Blood samples were collected and plasma prepared for these investigations with appropriate precautions as recommended by the manufacturers of the assay kits.

Statistical analysis of blood results

Comparison of results across the four diagnostic groups was made using Kruskall Wallis ANOVA because some of the data was not normally distributed. Demographic variables were normally distributed and were compared using one-way ANOVA. Comparison of HROHT with POAG was made using the non-parametric Mann Whitney test after examination of the data showed a skew distribution. Correlations were performed for men and women separately and correction was made for age.

D: Peripheral Blood Flow: Laser Doppler Flowmetry

To investigate peripheral blood flow this study used the Periflux PF2 laser Doppler flowmeter (Perimed, Sweden) with a 2mW HeNe laser of wavelength 632.8nm. This machine utilises the Doppler shift to measure peripheral microvascular perfusion. As explained in the previous section when a beam of laser light strikes a moving object, such as a red blood cell, it undergoes a change in frequency known as the Doppler shift. The magnitude of this shift is used to calculate blood flow velocity. The instrument then calculates the blood cell flux in arbitrary units by multiplying the number of red blood cells moving in the measured volume of tissue by the mean cell velocity. This flux represents the blood cell flow through the microvasculature from the arterial to the venous side. It is integrated over the entire measured volume of tissue - a hemisphere with a radius of approximately 1mm.

The probe of the LDF was placed against the pulp of the middle finger avoiding undue pressure and kept in position so that the signal indicator remained green. The baseline flow (flux) was recorded once a steady recording was seen on the printout. The hand was then immersed in warm water at 40 °C for 2 minutes, wrapped in a towel to prevent heat loss and the flow monitored until a maximum value was noted. After approximately 10 minutes the hand was placed in ice cold water at 4 °C for 10 seconds and the flow again monitored until a return to baseline. The following velocity parameters were determined; baseline flow, maximum flow(warm), minimum flow(cold), time to recover

to baseline flow after cold immersion, and ratios of max:min, max:baseline, and min:baseline following the protocol described by Drance[8].

For statistical analysis of the peripheral blood flow data we compared the results across 4 groups using Kruskal Wallis ANOVA because the data was not normally distributed.

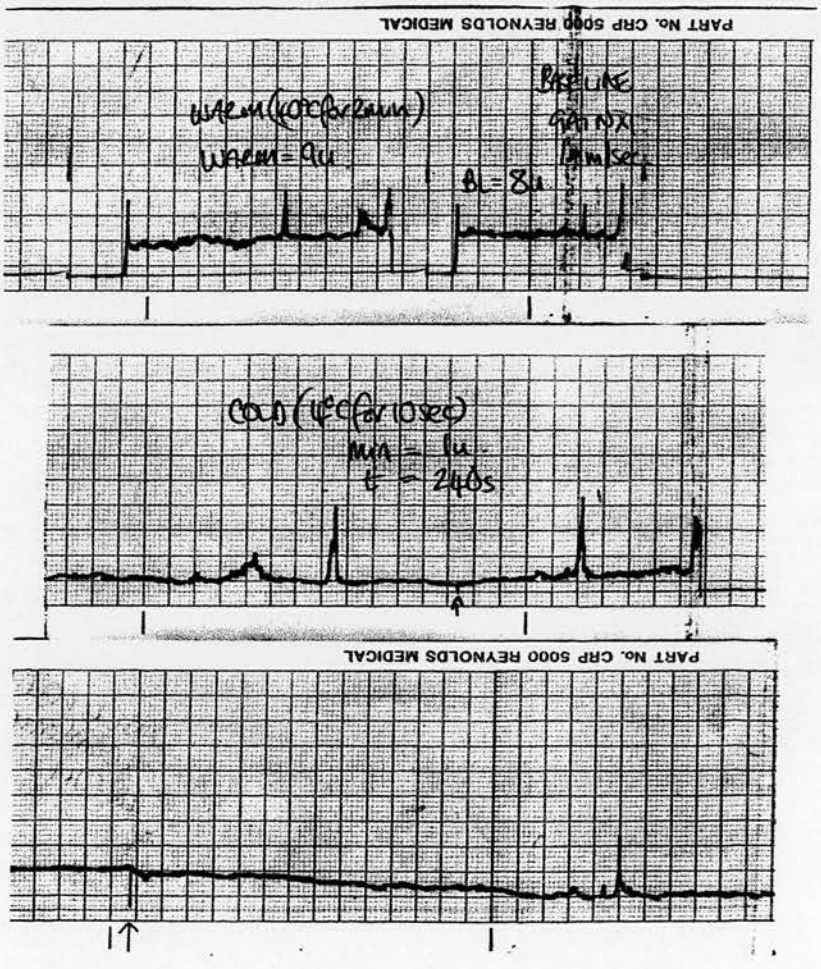


Figure 12: Printout from Laser Doppler Flowmeter (fingertip)

Read from right to left.

2.3

Results

A: Pulsatile ocular blood flow

Demographic data for all subjects are shown in tables 5 and 5a. A history of hypertension, vasospasm (migraine or Raynaud's disease), smoking and the presence of a family history of glaucoma was recorded based on the answers given by the subjects to the questionnaire (see appendix C). There was no significant difference between the groups in gender, axial length or refractive error (one-way ANOVA). Patients were asked about history of smoking, vasospastic diseases (Raynauds disease and migraine) or hypertension and no significant difference was found between the groups in these variables. There was a significant difference in age ($p=0.02$) and the Bonferroni test indicated that this lay between the glaucoma patients and low risk OHT, glaucoma patients being significantly older. Adjustment was therefore made for age in group comparisons using partial correlation. Glaucoma patients and high risk OHT had very similar levels of intraocular pressure (average seated IOP 28.6mmHg and 28.5mmHg respectively). Perfusion pressure (2/3MAP-Max IOP) was significantly lower in both POAG and high risk ocular hypertension (HROHT) than in normals ($p=0.001$). This was due to higher IOP in POAG and HROHT, mean arterial pressure (MAP) being no different between the groups.

Table 5 Demographic data: mean values(SD)

Variable	POAG	HROHT	LROHT	normal	p value
	n=24	n=27	n=24	n=23	
Age (years)	68.8(10)	64.8(10)	60.0(11)	64.7(8)	**0.02**
IOPmax (mmHg)	29.9(4.3)	29.4(3.2)	23.4(1.6)	16.2(2.3)	**<0.001**
IOPav (mmHg)	28.6(4.2)	28.5(3.0)	22.3(1.5)	16.0(2.3)	**<0.001**
AxLength (mm)	22.9(0.9)	22.7(1.0)	23.0(1.2)	22.6(0.8)	0.35
SphEquiv (DS)	0.54(2.3)	1.1(2.1)	0.44(2.4)	0.76(1.5)	0.72
Sitting perfusion pressure(mmHg)	47.7(13.2)	48.8(13.6)	53.3(8.9)	59.3(10.9)	**0.004**
Lying perfusion pressure(mmHg)	88.0(18.8)	86.3(20.6)	88.6(13.6)	96.9(15.6)	0.16
MD (dB)	-14.0(9.3)	-1.25(2.3)	-0.78(2.3)		**<0.001**
CPSD (dB)	7.6(3.8)	1.9(1.1)	1.56(1.3)		**<0.001**
Sex(F/M)	12/12	8/19	14/10	15/8	0.06
Hypertension (N/Y)	22/2	22/5	19/5	21/2	0.47
Vasospasm (N/Y)	19/5	20/7	20/4	16/7	0.70
FamHx (N/Y)	15/9	18/9	16/8	18/5	0.68
Smoker (N/Y)	19/5	20/7	20/4	21/2	0.46

Table 5a Significant differences in Table 1

Bonferroni test

	POAG vs. HROhT	POAG vs. normal	POAG vs. LROhT	HROHT vs. normal	HROHT vs. LROHT	LROHT vs. normal	p
Age			*				0.02
IOPav		*	*	*	*	*	<0.001
IOPmax		*	*	*	*	*	<0.001
MD	*	*	*				<0.001
CPSD	*	*	*				<0.001
sitPP		*		*			0.004

* signifies a significant difference lies between the groups shown .

KEY: Table 5 and 5a

IOPmax	maximum intraocular pressure on phasing
IOPav	average IOP on phasing
AxLength	axial length
MD	mean deviation on field testing with Humphrey 24-2
CPSD	corrected pattern standard deviation with Humphrey 24-2
F/M	female/male
N/Y	no/yes
sitPP	Sitting perfusion pressure = 2/3 mean arterial pressure - maximum IOP

SphEquiv (DS)= spherical equivalent refractive error (dioptres sphere)

Sitting, standing and lying POBF (Table 6)

Table 6 shows results of a one-way ANOVA comparing pulsatile ocular blood flow parameters and blood pressure readings across the four groups. There were significant differences between the groups in all positions (table 6a). In general normals had significantly higher mean POBF and pulse volume (PV) when compared to high risk ocular hypertensives and glaucoma patients. Glaucoma patients had the lowest POBF and PV in all positions. There were no significant differences in pulsatile ocular blood flow between POAG patients and high risk ocular hypertensives.

Table 6 Sitting, lying and standing POBF (mean and SD)

Variable	POAG	High risk OHT	Low risk OHT	Normals	p value
	n=24	n=27	n=24	n=23	
SitPOBF(μl/min)	491(174)	551(250)	732(227)	846(284)	**<0.001**
SitPA(mmHg)	2.4(0.8)	2.9(1.4)	3.3(1.1)	2.9(1.2)	0.08
SitPV((l)	3.3(1.1)	3.9(2.0)	5.1(1.5)	6.4(2.5)	**<0.001**
SitSBP(mmHg)	156(25)	157(28)	152(16)	155(22)	0.86
SitDBP(mmHg)	85(10)	88(14)	86(9)	85(13)	0.87
Sitpls	70(10)	70(12)	70(8)	66(10)	0.36
SitMAP(mmHg)	114(17)	116(21)	113(12)	113(15)	0.91
StdPOBF	501(151)	568(201)	683(203)	848(283)	**<0.001**
StdPA	2.3(0.7)	2.7(1.2)	2.9(0.9)	2.5(1.2)	0.19
StdPV	3.1(1.1)	3.7(1.7)	4.6(1.4)	5.9(2.6)	**<0.001**
StdSBP	151(23)	156(30)	150(20)	150(22)	0.74
StdDBP	88(10)	92(13)	89(9)	90(13)	0.64
Stdpls	76(12)	74(11)	76(10)	72(12)	0.58
StdMAP	116(14)	117(21)	114(13)	117(21)	0.93
LyPOBF	471(156)	499(190)	631(203)	777(272)	**<0.001**
LyPA	2.5(0.8)	2.9(1.2)	3.1(1.6)	2.8(1.2)	0.22
LyPV	3.4(1.2)	3.8(1.5)	4.8(1.6)	6.5(2.8)	**<0.001**
LySBP	153(21)	155(28)	146(16)	146(22)	0.37
LyDBP	85(11)	86(12)	81(11)	82(14)	0.35
Lypls	66(9)	64(10)	66(9)	62(9)	0.29
LyMAP	111(15)	113(21)	105(12)	106(19)	0.41

Table 6a Significant differences in Table 6

Bonferroni test

	POAG vs HROHT	POAG vs Normal	POAG vs LROHT	HROHT vs Normal	HROHT vs LROHT	LROHT vs Normal	p
Sit POBF		*	*	*	*		<0.001
Sit PV		*	*	*			<0.001
Std POBF		*	*	*		*	<0.001
Std PV		*		*			<0.001
Ly POBF		*		*			<0.001
Ly PV		*		*		*	<0.001

KEY: Table 6 and 6a

Prefixes: Sit = sitting Std = standing Ly = lying (supine)

POBF	pulsatile ocular blood flow
PA	pulse amplitude
PV	pulse volume
SBP	systolic blood pressure
DBP	diastolic blood pressure
MAP	mean arterial blood pressure
Pls	radial pulse, beats per minute

Gender and POBF (Table 7 and Figure 13)

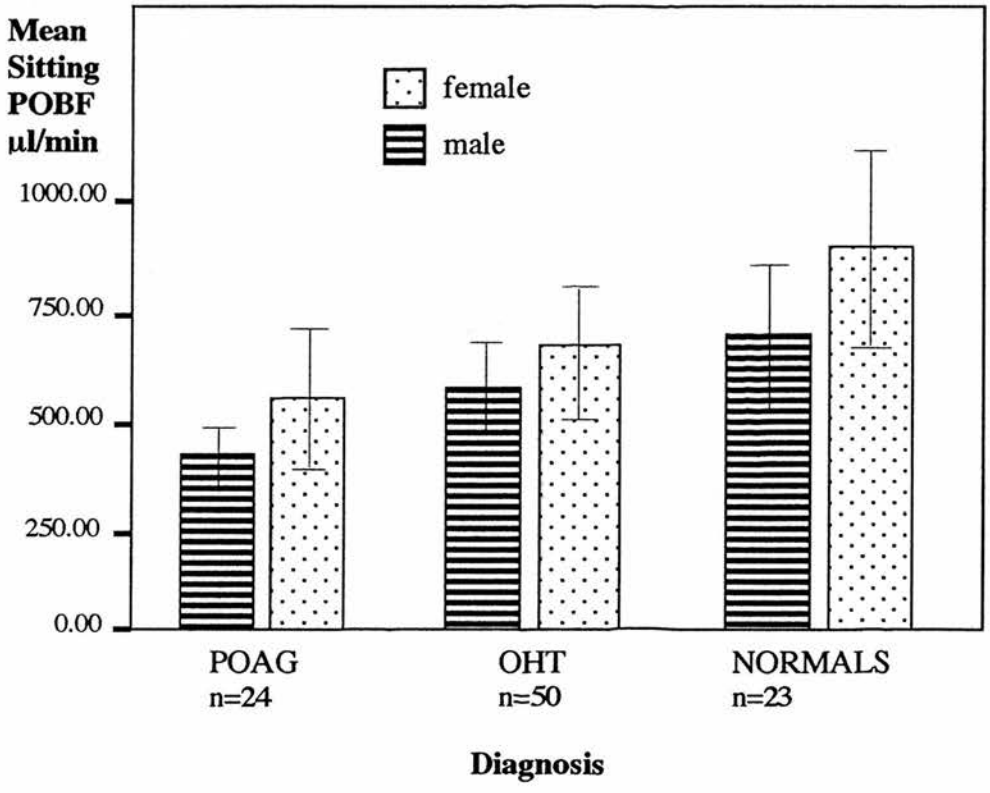
Females had significantly higher POBF than males (male:female difference after adjusting for group p=0.009, two-way ANOVA). This is shown in table 7 and figure 13 and has been noted by others [4,9,10]. Gender differences present in our groups which may have contributed to this finding were a higher pulse rate in females in all groups, significant only in POAG subjects (p=0.02), and a higher mean level of IOP in males in all groups (again this difference was significant only in the POAG group, p=0.02).

Table 7 Gender differences in POBF(μl/min): mean(SD)

	Normal	LROHT	HROHT	POAG
Female	925(288) N=15	750(235) N=14	619(380) N=8	559(204) N=12
Male	697(218) N=8	707(226) N=10	521(178) N=19	423(108) N=12

Male/ female difference overall p=0.009
(Two way ANOVA corrected for group)

Figure 13: Gender and POBF



Error bars show 95% CI of mean
Male/Female difference after correcting for group, p=0.009 (2 way ANOVA)

Postural change in POBF (Table 8)

In normal subjects it has been observed that the POBF falls on changing position from sitting to lying down [11]. This was found to be the case generally with our subjects but there was a difference between the groups in the magnitude of change.

In table 8 the postural change in POBF is expressed as a percentage of the sitting value in each group. Primary open angle glaucoma patients showed the smallest reduction in mean POBF (3.8%) while low risk ocular hypertensives had the largest (13.7%). Using a paired Student's t-test the difference in POBF from sitting to supine position was significant ($p<0.05$) in the normal, low risk and high risk OHT patients but not in the POAG group.

Table 8: Postural change in POBF (µl/min) mean(SD)

	POBF sitting	POBF supine	Difference	% difference*	P value for difference†
POAG n=24	490(174)	471(156)	-19	-3.8	0.30
HROHT n=27	550(251)	499(370)	-51	-9.2	**0.03**
LROHT n=24	732(227)	631(203)	-101	-13.7	**0.001**
Normals n=23	846(283)	777(272)	-69	-8.1	**0.02**

* change expressed as a percentage of sitting value
† paired students t-test comparing sitting POBF with lying POBF

Postural change in IOP and blood pressure (Tables 9, 10 and 11)

Maximum and minimum intraocular pressure were recorded in sitting and supine positions with the POBF machine. These readings were used to examine postural change in maximum IOP. Results are shown in table 9. Primary open angle glaucoma and HROHT subjects showed a small increase in maximum intraocular pressure on lying down (+2.2% and +0.7% respectively). Low risk ocular hypertensives showed no change and normals had a small reduction in max IOP on lying down (-3.5%). None of these differences were statistically significant, however the result is supported by numerous reports in the literature describing similar findings; i.e. a larger increase in IOP on lying down in POAG and ocular hypertension than in normals[12-14].

Table 9: Postural change in IOP (mmHg): mean (SD)

	max IOP sitting	max IOP supine	Difference mmHg	% difference*	P value for difference†
POAG n=22	27.3(3.6)	27.9(4.3)	+0.6	+2.2%	0.31
HROHT n=27	28.7(2.5)	28.9(2.8)	+0.2	+0.69%	0.66
LROHT n=24	24.5(2.9)	24.5(2.4)	0	0	0.89
Normals n=23	16.7(2.8)	16.1(2.8)	-0.6	-3.5%	0.07

* change expressed as a percentage of sitting value
† paired students t-test comparing sitting IOP with lying IOP

NB: IOP values from OBF pneumotonometer

Mean arterial blood pressure did not differ significantly between the groups in any position (table 2). All groups showed a small decrease in MAP on lying down. This fall was slightly larger in LROHT and normals (7% and 5.5%) than in HROHT and POAG (2.6% and 3.1%) (table 10).

Table 10:
Postural change in mean arterial blood pressure
mmHg mean (SD)

	Sitting MAP	Lying MAP	Difference	%	p
POAG	114(17)	111(15)	-3.5(13.9)	-3.1%	0.227
HROHT	116(21)	113(20)	-3.0(10.6)	-2.6%	0.156
LROHT	113(12)	105(12)	-8.0(9.7)	-7%	0.001
Normals	113(15)	107(19)	-6.2(10.7)	-5.5%	0.011

The perfusion pressure was calculated for sitting and supine positions for each group as follows:

Sitting perfusion pressure was calculated as 2/3 sitting MAP –sitting IOP.

Supine perfusion pressure was calculated as supine MAP -supine IOP.

Brachial MAP is reduced by one third in the calculation of sitting perfusion pressure to allow for a reduction in ophthalmic artery pressure due to the elevated position of the eye relative to the arm when seated. All groups had a moderately large increase in ocular perfusion pressure on lying down. The increase was approximately 38mmHg in all groups. Proportionally this change ranged from 65.5% in normals to 79.6% in high risk OHT.

Table 11:
Postural change in perfusion pressure
mmHg mean (SD)

	Sitting PP (2/3MAP-IOP)	Lying PP (MAP-IOP)	Difference	%	p
POAG N=22	49.9(12.5)	88.0(18.8)	38.1(6.8)	+76%	<0.0001
HROHT N=27	48.0(13.9)	86.3(20.6)	38.2(6.9)	+79.6%	<0.0001
LROHT N=24	50.7(9.5)	88.6(13.6)	37.9(4.5)	+74.7%	<0.0001
Normals N=23	58.6(10.1)	96.9(15.6)	38.3(5.8)	+65.3%	<0.0001

NB: maximum IOP reading from OBF machine used for calculation of perfusion pressure in both positions.

Comparing the absolute levels of postural change in IOP, POBF and MAP there was no significant difference across the four groups although the trend for a larger positive change in IOP and smaller postural change in POBF and MAP in glaucoma was evident (table 12).

Table 12: Group comparison of postural change: Mean(SD)

One way ANOVA

	POAG n=22	HROHT n=27	LROHT n=24	Normals n=23	p
Minimum sitting IOP	24.9(3.3)	25.8(2.4)	21.6(2.0)	13.7(2.4)	<0.001
Maximum sitting IOP	27.3(3.6)	28.7(2.4)	24.7(2.7)	16.7(2.8)	<0.001
Minimum lying IOP	25.4(4.2)	26.0(2.6)	21.3(2.1)	13.3(2.5)	<0.001
Maximum lying IOP	27.9(4.3)	28.9(2.8)	24.5(2.5)	16.1(2.8)	<0.001
ΔIOP (min)	0.45(2.2)	0.19(1.65)	-0.23(1.3)	-0.51(1.37)	0.239
ΔIOP (max)	0.58(2.5)	1.15 (1.79)	-0.18(1.5)	-0.63(1.60)	0.189
ΔMAP	-3.5(13.9)	-3.0(10.6)	-8.0(9.7)	-6.3(10.7)	0.366
ΔPOBF	19.67(90.4)	51.2(116.9)	101.2(134)	69.3(129.9)	0.124

Key: Δ = supine value –sitting value

Units: IOP = mmHg
MAP = mmHg
POBF = μl/min

Correlations (tables 13 to 17)

Although the gender distribution across the 4 diagnostic groups did not differ significantly there was a preponderance of women in the normal group and an excess of men in the high risk OHT group (table 5). Because of this and the finding of higher POBF in women (table 7) correlations were undertaken for men and women separately to avoid skewing of the result.

Correlations with POBF were very similar for both sexes.

In men (table 14) sitting and lying pulse amplitude and pulse volume correlated with radial pulse rate in both positions. Lying POBF correlated with sitting perfusion pressure ($c=0.33$, $p=0.02$).

In women (table 13) the same correlations were present with the addition of significant negative correlation between axial length and POBF and PA in both positions. The same negative association was present in men but not to a significant extent.

Table 13
POBF Correlations by gender

Females n=49

	Axlength	Sitting SBP	Sitting DBP	Sitting radial pulse
sitting POBF	c=-0.30 **p=0.03**	0.18 0.21	-0.09 0.51	0.06 0.68
sitting PA	-0.31 **0.03**	-0.01 0.90	-0.15 0.29	-0.29 **0.04**
sitting PV	-0.25 0.08	0.03 0.79	-0.14 0.34	-0.37 **0.008**
lying POBF	-0.28 **0.05**	0.26 0.07	-0.06 0.65	-0.12 0.41
lying PA	-0.32 **0.02**	0.05 0.91	-0.15 0.31	-0.32 **0.02**
lying PV	-0.25 0.08	0.07 0.60	-0.14 0.35	-0.43 **0.002**

	Lying SBP	Lying DBP	Lying radial pulse	Sitting perf. pressure
sitting POBF	0.02 0.86	-0.18 0.21	-0.04 0.79	0.22 0.12
sitting PA	-0.13 0.38	-0.22 0.12	-0.27 0.06	-0.03 0.83
sitting PV	-0.18 0.21	-0.26 0.07	-0.34 **0.02**	0.15 0.31
lying POBF	0.01 0.95	-0.14 0.32	-0.10 0.49	0.29 **0.04**
lying PA	0.01 0.92	-0.17 0.25	-0.35 **0.01**	-0.01 0.93
lying PV	0.10 0.51	-0.22 0.13	-0.42 **0.002**	0.19 0.18

c= Pearson's correlation coefficient
p= p value, shown bold if < 0.05

Table 14
POBF Correlations by gender

Males n=49

	Axlength	Sitting SBP	Sitting DBP	Sitting radial pulse
sitting POBF	c=-0.01 p=0.97	0.16 0.28	0.01 0.95	0.06 0.69
sitting PA	-0.310 0.48	-0.01 0.92	-0.07 0.63	-0.39 **0.005**
sitting PV	-0.07 0.64	0.01 0.91	-0.09 0.52	-0.42 **0.003**
lying POBF	-0.10 0.51	0.23 0.11	-0.07 0.63	-0.06 0.68
lying PA	-0.25 0.08	0.12 0.39	-0.02 0.91	-0.30 **0.03**
lying PV	-0.17 0.23	0.08 0.56	-0.03 0.84	-0.33 **0.01**

	Lying SBP	Lying DBP	Lying radial pulse	Sitting perf. pressure
sitting POBF	0.08 0.59	-0.11 0.44	-0.05 0.71	0.27 0.06
sitting PA	-0.03 0.83	-0.21 0.16	-0.38 **0.008**	-0.03 0.85
sitting PV	-0.07 0.62	-0.20 0.16	-0.43 **0.002**	0.15 0.29
lying POBF	0.24 0.09	-0.01 0.93	-0.04 0.79	0.33 **0.02**
lying PA	0.15 0.29	-0.07 0.63	-0.24 0.09	-0.06 0.65
lying PV	0.08 0.56	-0.09 0.53	-0.29 **0.04**	0.26 0.07

Table 15
Correlations

Partial correlation corrected for age

All subjects

	Axial length	Sitting SBP	Sitting DBP	Sitting MAP	Sitting radial pulse
sitting POBF	c=-0.23 **p=0.02**	0.20 **0.05**	-0.11 0.29	0.03 0.73	0.02 0.87
sitting PA	-0.27 **0.007**	0.04 0.68	-0.18 0.07	-0.07 0.51	-027 **0.009**
sitting PV	-0.23 **0.02**	0.06 0.51	-0.19 0.06	-0.07 0.49	-0.29 **0.004**
lying POBF	-0.25 **0.01**	0.25 **0.01**	-0.05 0.60	0.09 0.35	-0.03 0.76
lying PA	-0.33 **0.001**	0.10 0.33	-0.13 0.20	0.01 0.86	-0.25 **0.01**
lying PV	-0.26 **0.01**	0.11 0.27	-0.14 0.17	0.00 0.9	-0.30 **0.003**

	Lying SBP	Lying DBP	Lying MAP	Sitting PP
sitting POBF	0.05 0.65	-0.17 0.10	-0.07 0.46	0.26 **0.01**
sitting PA	0.01 0.95	-0.22 **0.03**	-0.12 0.25	-0.01 0.94
sitting PV	-0.01 0.59	-0.24 0.02	-0.16 0.11	0.12 0.08
lying POBF	0.10 0.30	-0.12 0.24	-0.01 0.94	0.32 **0.002**
lying PA	0.14 0.16	-0.13 0.21	0.00 0.98	0.04 0.69
lying PV	0.02 0.80	-0.17 0.09	-0.08 0.42	0.24 **0.02**

Taking all subjects together (table 15) there was a strengthening of the correlations seen in tables 13 and 14. In addition the positive association between sitting systolic blood pressure and POBF became significant (c=0.20, p=0.05) and a negative correlation was seen between lying diastolic BP and sitting PA and PV. There was a significant positive correlation between perfusion pressure and sitting POBF, lying POBF and lying PV. This may reflect a relationship between IOP and POBF since no significant correlation was seen between MAP and POBF. Increasing axial length was associated with reductions in all POBF parameters in the full group.

Separating the groups by diagnosis (table 16) a significant correlation was seen between Goldman IOP and seated POBF in the POAG group (r=-0.43, p=0.03) but not in the other groups. The magnitude of postural change in POBF (ΔPOBF) was found to correlate with sitting POBF in the three groups with raised intraocular pressure but not in normals. No significant association was found between visual field result (MD and CPSD) and sitting POBF in POAG or ocular hypertension.

The magnitude of the postural change in maximum IOP was related to average IOP, sitting POBF and to the change in POBF (table 17). In other words higher IOP and lower POBF were associated with a bigger increase in IOP on lying down across all subjects.

Table 16

Correlation with sitting POBF by diagnosis

	POAG sit POBF (n=24)	**HROHT** sit POBF (n=27)	**LROHT** sit POBF (n=24)	**Normal** sit POBF (n=23)
IOPav	c=-0.43 **p=0.03**	-0.14 0.47	0.24 0.25	-0.25 0.25
ΔPOBF	0.45 **0.03**	0.69 **<0.001**	0.46 **0.02**	0.31 0.14
MD	0.084 0.698	0.141 0.482	0.165 0.441	-
CPSD	-0.068 0.753	-0.032 0.874	0.057 0.793	-

IOPav = average IOP on phasing

ΔPOBF = postural difference in POBF

Table 17

Correlations with postural change in IOP (ΔIOP)

Partial correlation corrected for age and gender

All subjects

	ΔIOP minimum	ΔIOP maximum
IOPav*	0.56 **0.001**	0.058 **<0.001**
ΔPOBF	-0.12 0.51	-0.39 **0.02**
Axial length	-0.29 0.09	-0.32 0.07
Sitting POBF	-0.22 0.21	-0.37 **0.04**
Sitting PV	-0.18 0.32	-0.32 0.07
Lying PA	-0.02 0.89	-0.01 0.96

*IOP measured with Goldmann tonometer

NB: Postural change calculated using sitting and

lying IOP (maximum and minimum) from OBF machine

Treatment and POBF (Table 18)

Details of topical treatment used in the reviewed subjects are in tables 3 and 4 (chapter 2.1). There was a significant reduction in IOP in treated subjects. Treated POAG patients (n=14) had a mean seated pre-treatment IOP (Goldman tonometry) of 29.1mmHg falling to 21.1mmHg on treatment (p=<0.001). Ocular hypertensive subjects (n=11) had a mean pre-treatment pressure of 27.5mmHg falling to 21.4mmHg on treatment (p=<0.001). Treated POAG patients showed a significant fall in seated mean arterial pressure (112mmHg to 99mmHg, p=0.03) and also in supine pulse amplitude (2.6μl to 2.2μl, p=0.002). Ocular hypertensive subjects showed the same pattern with significant drops in both sitting MAP (120mmHg to 113mmHg, p=0.05) and supine pulse amplitude (3.3μl to 2.8μl, p=0.008). Both OHT and POAG subjects showed a non significant increase in POBF after treatment. Results from OHT and POAG groups were combined as numbers were small and the groups were alike in the pattern of changes. In the combined group (table 18) there was a significant increase in mean POBF on treatment (589.9μl/min to 670.8μl/min; p=0.02), an increase in mean sitting pulse volume (4.01μl to 4.77μl, p=0.008) and the decrease in mean supine pulse amplitude became more significant (2.97μl to 2.48μl, p<0.001).

Table 18

Effect of treatment on POBF

OHT and POBF combined; 25 pairs

	Pre-treatment	Post-treatment	P value*
Average IOP	28.6(3.9)	21.2(3.5)	**0.029**
Seated perfusion pressure	48.5(14.3)	48.6(15.5)	0.927
Sitting POBF	595.4(265.7)	671.5(208.3)	**0.029**
Sitting PA	3.0(1.3)	2.8(0.8)	0.228
Sitting PV	4.0(1.8)	4.8(1.4)	**0.009**
Sitting pulse	72.6(15.9)	72.4(15.9)	0.960
Sitting SBP	156.8(29.5)	149.4(29.7)	**0.021**
Sitting DBP	87.2(13.3)	84.4(14.0)	0.061
Sitting MAP	116.6(20.1)	106.6(23.9)	**0.008**

Table 18 (cont.)

Lying POBF	543.6(202.3)	553.1(178.9)	0.715
Lying PA	3.01(1.1)	2.5(1.0)	**<0.001**
Lying PV	3.9(1.6)	4.2(1.6)	0.339
Lying pulse	67.7(0.4)	64.7(8.9)	0.146
Lying SBP	154.4(28.1)	147.7(25.9)	**0.010**
Lying DBP	84.8(13.0)	83.4(11.50	0.418
Lying MAP	110.8(20.3)	107.5(10.9)	0.167

Postural changes after treatment (tables 19-21)

In treated subjects with pharmacologically lowered IOP the magnitude of change in POBF on lying down increased and became significant in POAG and of increased significance in OHT ($p<0.0001$, table 19) although the mean seated POBF was still lower than that seen in the normal group at the initial visit (table 6).

The postural change in IOP remained insignificant in both groups after treatment (table 20). When subjects with treated IOP below 21mmHg were selected (mean post treatment IOP =18mmHg, table 21) mean values suggested possible conversion to a small decrease in IOP on lying down as seen in normals (table 9) but numbers were to small to draw any conclusions from this.

Table 19

Postural changes in POBF: Subjects on treatment

	sittingPOBF (µl/ml)	lying POBF (µl/ml)	difference	%	p*
pretreatment POAG n=24	490(174)	471(156)	-19	-3.8	0.03
Treated POAG n=14	607(175)	512(164)	-95	-15.6	<0.001
pretreatment HROHT n=27	550(251)	499(370)	-51	-9.2	0.03
Treated HROHT n=11	753(225)	605(191)	-148	-19.6	<0.001

*paired Student t test

Table 20

Postural changes in IOP: Subjects on treatment

	maximum sitting IOP (mmHg)	maximum lying IOP (mmHg)	Difference	%	p*
pretreatment POAG n=22	27.3(3.6)	27.9(4.3)	0.6	2.2	0.31
Treated POAG n=14	21.0(3.02)	21.4(3.57)	0.4	1.9	0.18
pretreatment HROHT n=27	28.7(2.5)	28.9(2.8)	0.2	0.69	0.66
Treated HROHT n=11	22.6(3.2)	22.5(5.1)	-0.1	-0.004	0.92

*paired Student t test

Table 21

Effect of treatment on postural change in IOP

(cases with treated IOP <21)

N=9 (6 POAG and 3 HROHT)

	Max sitting IOP (mmHg)	Max lying IOP (mmHg)	Difference	% of sitting IOP	p
Pretreatment (27.33)*	27.4(2.2)	27.6(3.2)	+0.27(2.3)	+0.98%	0.738
Post treatment (18.00)*	19.4(1.8)	18.8(3.5)	-0.64(3.1)	-3.0%	0.550

Student t test

*average Goldman IOP for the group (mmHg)

B: Retinal Scanning Laser Doppler Flowmetry

All groups (table 22 A-E)

Significant technical difficulties were encountered when trying to obtain clear images of the optic disc and retina for analysis. Many patients were unable to maintain steady fixation for the required length of time and images from approximately one third of subjects had to be discarded for this reason. Demographic details were rechecked for the reduced groups and no significant differences were found. Mean ages of the groups were POAG 66.6(10.9) yrs, HROHT 61.6(8.9) yrs, LROHT 57.9(10.0) yrs, and normal 63.4(8.3)yrs, (p=0.12).

The images for analysis were divided into 5 areas (see fig 11, page 125). Nasal retina (area 1), nasal neuroretinal rim (area 2), optic cup (area 3), temporal neuroretinal rim (area 4), and temporal retina (area 5).

In the nasal retina minimum velocity (p=0.031) and minimum flow (p=0.044) were both significantly lower in LROHT than in HROHT (22A).

There were no significant differences between the groups in blood flow at the nasal neuroretinal rim (22B) although difficulties obtaining reliable measurements from this area in several images due to crowding of large blood vessels led to further reduction in group size.

At the optic cup (22C) measurements of minimum velocity and minimum volume were both significantly lower in POAG than in HROHT ($p=0.051$ and $p=0.033$).

At the temporal neuroretinal rim (22D) POAG subjects again had significantly lower maximum volume than HROHT ($p=0.042$).

In the temporal retina (22E) minimum velocity was significantly higher in normals than in LROHT ($p=0.021$) and maximum and minimum flow were both significantly higher in POAG than in LROHT ($p=0.09$ and $p=0.020$).

Table 22

Scanning Laser Doppler Flowmetry Results (All subjects)

Kruskall Wallis ANOVA

A: Nasal Retina

	POAG (15)	HROHT (17)	LROHT (13)	Normal (19)	P
Max volume	25.45 (6.02)	25.95 (5.95)	26.61 (5.9)	27.87 (6.65)	0.478
Min volume	14.43 (3.58)	16.84 (3.19)	15.12 (3.1)	16.48 (4.66)	0.277
Max velocity	1.84 (0.50)	1.80 (0.48)	1.71 (0.50)	1.79 (0.37)	0.486
Min velocity	1.01 (0.31)	1.12 (0.29)	0.84 (0.22)	0.98 (0.25)	**0.031**
Max flow	523.73 (147.7)	530.89 (151.9)	495.71 (162.2)	525.68 (123.1)	0.554
Min flow	290.49 (98.6)	316.08 (85.6)	239.52 (64.6)	276.01 (74.9)	**0.044**

B: Nasal Rim

	POAG (11)	HROHT (14)	LROHT (10)	Normal (14)	P
Max volume	16.76 (5.14)	24.41 (11.2)	16.62 (8.3)	19.27 (4.98)	0.125
Min volume	10.22 (3.23)	15.62 (6.36)	11.21(4.9)	13.25 (3.06)	0.075
Max velocity	1.77 (0.63)	2.07 (1.03)	1.53 (0.79)	1.62 (0.40)	0.496
Min velocity	1.09 (0.38)	1.37 (0.70)	1.10 (0.52)	1.12 (0.34)	0.888
Max flow	512.68 (189.7)	630.40 (347.5)	431.42 (249.0)	474.98 (126.2)	0.425
Min flow	308.84 (110.4)	396.98 (214.5)	306.55 (155.8)	325.23 (102.3)	0.887

Table 22 cont.

C: Optic Cup

	POAG (14)	HROHT (16)	LROHT (12)	Normal (16)	P
Max volume	15.12 (5.32)	23.72 (12.7)	20.20 (6.81)	20.37 (7.68)	0.100
Min volume	7.49 (1.98)	12.41 (8.22)	8.67 (2.14)	11.09 (5.28)	**0.033**
Max velocity	1.08 (0.32)	1.57 (0.66)	1.26 (0.42)	1.21 (0.36)	0.196
Min velocity	0.59 (0.25)	0.98 (0.48)	0.68 (0.18)	0.82 (0.33)	**0.051**
Max flow	295.87 (125.9)	446.34 (200.5)	371.97 (153.7)	340.81 (140.9)	0.240
Min flow	165.26 (70.4)	270.99 (127.8)	211.79 (86.7)	230.23 (93.8)	0.077

D: Temporal Rim

	POAG (14)	HROHT (17)	LROHT (13)	Normal (16)	P
Max volume	15.99 (4.15)	21.46 (5.75)	15.27(1.46)	20.16 (6.48)	**0.042**
Min volume	12.11 (2.35)	14.72 (94.79)	13.77 (2.47)	13.07 (4.17)	0.232
Max velocity	1.42 (0.49)	1.76 (0.59)	1.52 (0.32)	1.55 (0.42)	0.403
Min velocity	1.14 (0.32)	1.15 (0.51)	1.16(0.31)	1.03 (0.36)	0.715
Max flow	407.78 (147.7)	521.25 (187.4)	439.23 (100.8)	453.61 (137.1)	0.322
Min flow	321.11 (92.8)	343.71 (161.4)	317.47 (91.7)	294.95 (104.7)	0.821

Table 22 cont.

E: Temporal Retina

	POAG (15)	HROHT (17)	LROHT (12)	Normal (19)	P value
Max volume	26.74 (8.82)	25.06 (4.25)	23.59(2.80)	26.65 (4.92)	0.191
Min volume	15.57 (6.19)	15.52 (5.23)	14.2(2.78)	15.09 (3.80)	0.700
Max velocity	1.94 (0.78)	1.63 (0.23)	1.45(0.18)	1.66 (0.41)	0.108
Min velocity	1.07 (0.26)	0.85 (0.29)	0.79 (0.21)	1.18 (1.30)	**0.021**
Max flow	571.23 (248.5)	474.92 (79.9)	394.77 (85.3)	488.25 (126.9)	**0.049**
Min flow	302.12 (77.7)	243.18 (90.4)	216.65 (59.5)	241.73 (74.3)	**0.020**

Flow = distance travelled by all moving red blood cells

Volume = number of moving red blood cells

Velocity = mean red cell speed

POAG vs HROHT (Table 23)

In comparing POAG with HROHT most differences were noted at the optic cup where all readings were higher in HROHT. A significant difference was seen in maximum flow (p=0.03), minimum volume (p=0.016), maximum velocity (p=0.045), minimum velocity (p=0.016) and minimum flow (p=0.016).

At the neuroretinal rim (NRR) readings were again higher in HROHT. Significant results were seen for maximum volume (p=0.043) and minimum volume (p=0.029) at the nasal NRR and for maximum volume (p=0.010) at the temporal NRR.

In contrast significantly higher readings were seen for POAG in the temporal retina, minimum velocity (p=0.014) and minimum flow (p=0.022) were both higher in POAG than in HROHT and all other parameters in this area were increased to a nonsignificant extent in POAG compared to HROHT.

Supplementary analysis of correlation of SLDF results with other parameters is recorded in Appendix B (page 221)

Table 23

Scanning Laser Doppler Flowmetry

POAG vs. HROHT

Mann Whitney test

Nasal retina

	POAG (15)	HROHT (17)	P value
Max volume	25.45 (6.02)	25.95 (5.95)	0.806
Min volume	14.43 (3.58)	16.84 (3.19)	0.109
Max velocity	1.84 (0.50)	1.80 (0.48)	0.678
Min velocity	1.01 (0.31)	1.12 (0.29)	0.317
Max flow	523.73(147.7)	530.89 151.9)	0.955
Min flow	290.49 (98.6)	316.08 (85.6)	0.355

Nasal rim

	POAG (11)	HROHT (14)	P value
Max volume	16.76 (5.14)	24.41 (11.20)	**0.043**
Min volume	10.22 (3.23)	15.62 (6.36)	**0.029**
Max velocity	1.77 (0.63)	2.07 (1.03)	0.565
Min velocity	1.09 (0.38)	1.37 (0.70)	0.443
Max flow	512.68 (189.7)	630.40 (347.5)	0.547
Min flow	308.84 (110.4)	396.98 (214.5)	0.511

Optic cup

	POAG (14)	HROHT (16)	P value
Max volume	15.12 (5.32)	23.72 (12.70)	**0.034**
Min volume	7.49 (1.98)	12.41 (8.22)	**0.016**
Max velocity	1.08 (0.32)	1.57 (0.66)	**0.045**
Min velocity	0.59 (0.25)	0.98 (0.48)	**0.016**
Max flow	295.87 (125.9)	446.34 (200.5)	0.056
Min flow	165.26 (70.4)	270.99 (127.8)	**0.016**

Table 23 (cont.)

Temporal rim

	POAG (14)	HROHT (17)	P value
Max volume	15.99 (4.15)	21.46 (5.75)	**0.010**
Min volume	12.11 (2.35)	14.72 94.79)	0.088
Max velocity	1.42 (0.49)	1.76 (0.59)	0.121
Min velocity	1.14 (0.32)	1.15 (0.51)	0.889
Max flow	407.78 (147.7)	521.25 (187.4)	0.088
Min flow	321.11 (92.8)	343.71 (161.4)	0.905

Temporal retina

Temp. retina	POAG (15)	HROHT (17)	P value
Max volume	26.74 (8.82)	25.06 (4.25)	0.925
Min volume	15.57 (6.19)	15.52 (5.23)	0.509
Max velocity	1.94 (0.78)	1.63 (0.23)	0.361
Min velocity	1.07 (0.26)	0.85 (0.29)	**0.014**
Max flow	571.23 (248.5)	474.92 (79.9)	0.361
Min flow	302.12 (77.7)	243.18 (90.4)	**0.022**

C: Haematology

All groups (Table 24)

Kruskall Wallis ANOVA was used to compare haematology results across the four diagnostic groups. Beta-thromboglobulin, D-dimer and prothrombin fragment F 1&2 did not vary significantly between the groups.

Manual fibrinogen was highest in LROHT (2.85±0.51g/l), intermediate in POAG and HROHT and lowest in normals (2.47±0.43g/l, p=0.048). LROHT differed significantly from the normal group. Haematocrit and platelets were highest in POAG and lowest in normal subjects. This difference was significant for platelets (p=0.027) and of borderline significance for haematocrit (p=0.056). All values other than prothrombin fragment F1&2 (PF1&2) were within the normal range for all groups. PF1&2 was elevated in POAG and LROHT and at the upper limit of normal in HROHT and normals.

Demographic data was reviewed for the appropriate groups. A significant difference in age ($p<0.05$) was seen between LROHT (59yrs+/-10)) and POAG (69yrs+/-10). Neither POAG nor LROHT differed significantly from the other two groups with respect to age. Correction was made for age in the statistical analysis of haematology results.

There were no other significant demographic differences between the groups other than the expected ones for IOP and visual field result (MD and CPSD).

Table 24

Haematology Results, 4 groups

Mean (standard deviation)

Normal range in italics

	POAG	HROHT	LROHT	Normal	P value
βtg *<50ngms/ml*	45.27(31.06) n=22	44.19(37.33) n=26	44.71(38.91) n=21	36.28(21.83) n=21	0.851
Manual Fibrinogen *1.5-4g/l*	2.79(0.55) n=24	2.64(0.56) n=25	2.85(0.51) n=23	2.47(0.43) n=20	**0.048**
Ddimer *<400 ngms/ml*	364.47 (126.21) n=23	312.66 (145.37) n=27	305.22 (150.73) n=23	329.62 (175.34) n=21	0.294
PF 1&2 *0.44-1.1 nmols/l*	1.44(0.66) n=23	1.13(0.29) n=27	1.29(0.53) n=23	1.19(0.35) n=21	0.225
Platelets *150-350x10/l*	273.08(95.8) n=23	239.7(107.3) n=26	249.43(48.1) n=23	218.75(64.2) n=20	**0.027**
Haematocrit *0.37-0.47*	0.415(0.02) n=22	0.412(0.03) n=26	0.414(0.04) n=23	0.389(0.03) n=20	**0.056**

Marginally non linear data; Kruskal Wallis non parametric test used

Table 25

Haematology: missing cases

	POAG	HROHT	LROHT	normal
Total cases	**24**	**27**	**24**	**23**
Beta-tg	2	1	3	2
fibrinogen	0	2	1	3
Ddimer	1	0	1	2
PF 1&2	1	0	1	2
Platelets	1	1	1	3
Hct	2	1	1	3

Notes

Beta tg: Results were excluded if level was excessively high (>150) indicating activation of platelets secondary to traumatic venepuncture or transport.

Clotting: One LROHT and one POAG had very high results suggestive of artefact or problems with processing, 2 HROHT had no fibrinogen result returned from the lab for unknown reasons.

Full blood count: One HROHT and one POAG had no FBC taken, one LROHT had clotted FBC, and one POAG had very high haematocrit which appeared to be artifactual as repeat testing one month later gave a normal result.

Normals: Two normal subjects had no blood taken and one additional full blood count result was missing from the lab report for unknown reasons.

A number of haematology results were missing from the analysis, the reasons for this are shown in table 25.

POAG vs HROHT (Table 26)

No significant differences were found between HROHT and POAG although all results were higher in POAG than in HROHT and borderline significance (p=0.056) was seen for the difference in platelets and PF 1&2.

Supplementary analysis of the correlation of haematology results with other parameters is shown in Appendix B (page 225).

Table 26

Haematology Results

POAG vs HROHT

Mann Whitney test

	POAG	HROHT	P value
βtg ***<50ngms/ml***	45.27(31.06) n=22	44.19(37.33) n=26	0.836
Manual Fibrinogen ***1.5-4g/l***	2.79(0.55) n=24	2.64(0.56) n=25	0.225
Ddimer ***<400ngms/ml***	364.47(126.21) n=23	312.66(145.37) n=27	0.088
PF 1&2 ***0.44-1.1nmols/l***	1.44(0.66) n=23	1.13(0.29) n=27	0.056
Platelets ***150-350x10/l***	273.08(95.85) n=23	239.7(107.3) n=26	0.056
Haematocrit ***0.37-0.47***	0.415 n=22	0.412 n=26	0.835

D: Laser Doppler finger blood flow

All groups (table 27)

Analysis of the demographic data for the groups found no significant differences other than that in age (p=0.02) between POAG (68.4 +/- 10yrs) and LROHT (59.6 +/- 11yrs). Comparing all subject groups it was found that low risk ocular hypertensives had significantly higher baseline blood flow than normals (p=0.010). There were no significant differences in the level of fingertip blood flow after immersion in warm or cold water, or in the length of time taken for recovery to baseline flow after cold immersion.

POAG vs HROHT (table 28)

There were no significant differences between POAG and HROHT in the measurements of fingertip blood flow.

Correlations

No correlation was found between the parameters of fingertip blood flow and other measurements such as IOP, systolic and diastolic blood pressure and pulsatile ocular blood flow.

Table 27

Laser Doppler finger blood flow (All groups)

Arbitrary units; mean (SD)

Kruskall Wallis ANOVA

	POAG N=23	HROHT N=26	LROHT N=23	Normal N=22	P value
Baseline	5.67 (3.05)	6.63 (2.62)	7.71 (3.06)	5.02 (2.26)	**0.010**
Warm	11.80 (3.92)	11.98 (3.09)	12.87 (3.49)	9.86 (4.34)	0.106
Cold	2.27 (1.67)	2.19 (1.33)	2.60 (1.96)	1.88 (1.25)	0.534
Time	106.86 (146.55)	121.53 (88.72)	93.04 (109.12)	73.86 (83.41)	0.076
Hot/cold	7.51 (5.47)	7.12 (4.05)	7.03 (4.17)	6.85 (4.41)	0.987
Hot/ baseline	2.64 (1.63)	2.00 (0.87)	2.08 (1.84)	1.98 (0.74)	0.185

Missing cases:

1 HROHT due to lost data
1 LROHT due to unreadable tracing
1 POAG due to patient having to leave early
1 normal measurement not done

Table 28

Laser Doppler finger blood flow
POAG vs HROHT

Mann Whitney t test

	POAG N=23	**HROHT** N=26	**P value**
Baseline	5.67 (3.05)	6.63 (2.62)	0.164
Warm	11.80 (3.92)	11.98 (3.09)	0.627
Cold	2.27 (1.67)	2.19 (1.33)	0.764
Time	106.86 (146.55)	121.53 (88.72)	0.083
Hot/cold	7.51 (5.47)	7.12 (4.05)	0.904
Hot/ baseline	2.64 (1.63)	2.00 (0.87)	0.214

2.4

Discussion

A: Pulsatile ocular blood flow

Summary of results

This investigation has found that in the untreated state POAG patients and ocular hypertensives with IOP above 25mmHg have significantly reduced POBF and pulse volume compared to ocular hypertensives with an IOP of 25mmHg and below or normal subjects. We found no significant difference in pulsatile ocular blood flow parameters between POAG and high risk ocular hypertensives. Primary open angle glaucoma and high risk OHT patients had a significantly lower ocular perfusion pressure than normals. Glaucoma patients differed from the other three groups in showing an insignificant postural change in POBF (reduction in POBF on lying down). There was an inverse correlation between pulsatile ocular blood flow and axial length, and between pulse amplitude (PA), pulse volume (PV) and radial pulse rate. There was positive correlation between seated perfusion pressure and POBF in sitting and supine positions. A previously recognised finding was confirmed in recording higher POBF in women than in men across all the groups. Finally, in a group of patients who received topical treatment, predominantly with betaxolol, a significant increase in POBF was seen

associated with a significant fall in IOP and seated mean arterial blood pressure and no change in perfusion pressure. The fall in POBF occurring when changing position from sitting to supine became more marked in patients on treatment. In POAG the difference became statistically significant, in OHT the change showed increased significance.

POBF and visual function

The theory and assumptions underlying pulsatile ocular blood flow measurement have been explained in Chapter 2.2. Pulsatile ocular blood flow is the calculated change in ocular volume over time derived from continuous measurement of the ocular pressure pulse at the corneal surface. It is generally assumed that POBF is an indirect measure of the volume of blood entering the choroid during systole because the choroidal circulation accommodates up to 90% of the blood arriving at the eye in each cardiac cycle[2]. In support of this it has been found that POBF is reduced in conditions associated with choroidal atrophy such as high myopia[15] and retinitis pigmentosa[16]. Yin et al demonstrated choroidal atrophy in post mortem specimens of eyes with primary open angle glaucoma [17]so we might also expect to find reduced POBF in this condition.

There is evidence that POBF is related to visual function. Paris et al recently found a significant increase (approx. 30%) in both POBF and contrast sensitivity with no associated change in IOP in a group of normal adults after taking the phosphodiesterase 5 inhibitor sildenafil [18]. Sildenafil (Viagra) augments the action of nitric oxide by

preventing degradation of cyclic GMP, producing vasodilation. Reduced POBF has been found in normal tension glaucoma[9], and in anterior ischaemic optic neuropathy[12] the reduction has been noted to be more marked in the worse affected eye in both conditions.

In this study peripheral retinal function was assessed by standard visual field analysis and central Snellen acuity was recorded. Only the POAG subjects had visual field defects as defined by the inclusion criteria and it was therefore not possible to assess more subtle differences in visual function in the other subjects. No correlation was found between visual field loss (MD and CPSD) and POBF in the patients in this study (table 16).

Sources of Error

It has been established that POBF is influenced by many factors.

Age, intraocular pressure, axial length, heart rate and gender have all been shown to have some effect on POBF measurements [4,9,16,19,20]. Treatment has also been shown to influence POBF although published results are conflicting [21-24]. None of the patients in this study were using, or had ever used topical antiglaucoma medication at their initial visit. The groups were well matched for heart rate, blood pressure, axial length and refractive error and correction was made for age. High risk ocular hypertensives and POAG patients were well matched for IOP. Although there was no significant difference in gender distribution across the four diagnostic groups there was a preponderance of

females in the normal group and of males in the HROHT group and for this reason correlations were calculated separately for men and women.

Results from the Ocular Hypertension Treatment Study have highlighted the importance of central corneal thickness as a confounding factor in the measurement of IOP[79]. Central corneal thickness was not measured in this study and differences in CCT may have influenced the results presented here in two ways. Firstly in the IOP dependant categorisation of patients into groups: OHT subjects have been shown to have on average a thicker central cornea than normal producing an artifactual increase in measured IOP. The true IOP of the OHT subjects in this study may therefore be slightly lower than that recorded. The difference in mean CCT measurements between OHT and normals has been found to be approximately 30 microns by Herman et al [76]. Singh and associates [77] reported similar findings with no difference in CCT between POAG and normals. Bhan et al[78] calculated that CCT could account for an increase of 0.23mmHg intraocular pressure per 10 microns corneal thickness over a wide range of IOPs. Using the above values as a guide the mean IOP of the OHT groups in this study might be reduced by approximately 1mmHg after correcting for CCT. While knowledge of CCT appears to be important in assessing an individual's risk of developing glaucoma it is unlikely that the results of the group comparisons presented here are greatly influenced by the absence of this data. Secondly, it is to be expected that POBF may be influenced by CCT in the same way as IOP because POBF data is derived from corneal applanation. A thicker cornea may be more rigid and give rise to artificially low POBF

readings in OHT. In summary, in the absence of detailed information about CCT it may be that the OHT groups presented here have a true IOP slightly lower and POBF slightly higher than that recorded, and are therefore less 'glaucoma like' than they appear. Based on data available in the literature the magnitude of this error would appear to be small. Oestrogen is thought to increase POBF[80] and hormone replacement therapy (HRT) has been found to reduce vascular resistance in the ophthalmic artery[81]. The patients in this study were not specifically asked about HRT so it is not known what proportion of patients in each of the groups were taking HRT and to what extent it may have influenced the results if at all. There was however a preponderance of men in the HROHT group in comparison to the POAG group which had equal numbers of men and women. This may have led to a comparatively lower average POBF in the HROHT group. Other non-prescription drugs which have an effect on blood flow such as ginko biloba may also have been taken by the subjects in this study but, as with HRT, patients were not specifically asked about this type of medication and the effect on the results is not known.

Another possible source of error in POBF measurement is operator variability. In an attempt to reduce this all measurements were performed by one experienced operator (JK) following the same protocol and using the same machine as was used by Butt in her reproducibility study[3].

The Effect of Treatment

Twenty three of the twenty six patients reviewed were using the $beta_1$ selective antagonist betaxolol. The three patients in whom betablockers were contraindicated were using dorzolomide. Seven patients were on a second topical treatment in addition to betaxolol (4 dorzolomide, 1 latanoprost, 1 alphagan and 1 pilocarpine).

The literature regarding the effects of betaxolol on ocular blood flow is inconclusive. $Beta_2$ receptors have been identified in the optic nerve head and anterior optic nerve in addition to the trabecular meshwork and ciliary body where non selective beta blockers such as timolol exert their maximum effect[83]. Stimulation of beta receptors associated with blood vessels generally causes vasodilation and antagonists such as timoptol cause vasoconstriction. Some authors have suggested that the $beta_1$ selective action of betaxolol may have a beneficial effect on optic nerve head blood flow in comparison with non selective beta blockers[84]. In addition to this, in vitro experiments using bovine ciliary arteries have shown a direct vasorelaxant effect produced by beta blockers which is not mediated by beta receptors and thought to be due to inhibition of calcium entry into the cell. Some authors have claimed that this effect is more marked for betaxolol than for other beta blockers[91]. However Harris, in a recent review of the literature concluded that there was no convincing evidence to support a clinically significant effect of beta blockers on ocular blood flow distinct from the IOP effect[85].

Dorzolamide is a carbonic anhydrase inhibitor and it is known to produce vasodilation in addition to lowering IOP. Several studies have found increased ocular blood flow after

topical dorzolamide as measured with CDI[86] and scanning laser ophthalmoscopy[87]. Schmidt et al found increased ocular pulse amplitude in POAG and normals after dorzolamide[88]. Pilocarpine and Latanoprost have not been found to have any consistent effect on ocular blood flow although one study found hyperaemia of the anterior structures of the eye in rabbits after latanoprost application[89]. Brimonidine is an alpha agonist and as such might be expected to produce vasoconstriction. Although systemic vasoconstrictive effects have been measured studies have not found any significant alteration in ocular blood flow after brimonidine[90]. The studies referenced above have examined retrobulbar and optic nerve head blood flow after administration of IOP-lowering drugs and have largely given inconclusive results. In recent years there have been some publications looking at the effect of antiglaucoma medication on POBF. Shaikh and Mars found a significant increase in POBF after pilocarpine in ocular hypertensive subjects[92]. Geyer et al reported similar findings in normal subjects after latanoprost[93].

How these results compare with published findings

The normal results reported here fall within the normal range described by Fontana et al in 1998[9]. Pulse volume and POBF were found to be significantly reduced in both POAG and high risk OHT compared to normals and low risk OHT. This result concurs with that of Trew and Smith [11] who found no difference in POBF between OHTs with mean IOP 22.6mmHg (similar to that of the LROHT group) and normals. In comparing glaucoma

patients with ocular hypertensives Trew and Smith found significantly lower POBF and pulse amplitude in POAG. However the mean IOP of their OHT group was lower than that of our low risk OHTs and their POAG patients were treated with timolol prior to the study which may have had a bearing on the results [25]. In a published paper based on the research presented here, reduced POBF was found in POAG compared with untreated OHT subjects [26]. The patient groups in that study differed in that the OHT group consisted of a mixture of high risk and low risk subjects and the POAG patients had more severe mean visual field loss than the group under consideration here. Mean values reported here for POBF parameters are consistently lower in POAG than in high risk OHT although the differences are not significant. It may be that there is a genuine difference between OHT and POAG which becomes smaller when high risk OHT are selected i.e. OHT subjects become more glaucoma-like in terms of their POBF as the IOP rises.

Bergstrand and associates [27] recently compared blood flow in untreated OHT and POAG matched for IOP using color Doppler imaging (CDI). They found increased resistive index and reduced end diastolic velocity in the central retinal artery in untreated POAG compared to OHT. POBF has been found to correlate most strongly with the CDI parameter peak systolic velocity (PSV) in the central retinal artery[28]. Bergstrand found no difference between the groups in this parameter which concurs with the finding presented here of no difference in POBF between HROHT and POAG.

One of the most striking differences in POAG subjects found by this investigation was in the postural change in POBF. While normal subjects and ocular hypertensives demonstrated a fall in POBF on lying down of between 8.1% and 13.7% of the sitting value ($p<0.05$), POAG patients had an insignificant reduction of only 3.8% (table 8). This picture changed to one resembling normal after topical treatment to lower IOP with the treated postural change increasing to 15.6% in POAG (table 19). The sitting POBF in treated POAG did not increase to normal levels but the mean treated IOP in the group remained relatively high in this study (21.0mmHg).

The OBF machine records details of maximum and minimum IOP in addition to POBF parameters. These measurements of IOP have been found by other researchers to correlate well with Goldmann tonometry and were used in this study to calculate sitting and lying perfusion pressure for the purpose of investigating postural changes.

Very small postural changes in mean IOP were found. There was a slight increase in IOP in POAGs on lying down and a slight decrease in normals. These findings are in agreement with those published by others[13,29] but were not significant in this study.

The magnitude of change in maximum IOP on lying down (ΔIOPmax) was found to correlate strongly with seated Goldman IOP and also with sitting POBF. In subjects on treatment to lower IOP the increase in IOP on lying down became smaller although once again the change was not significant (tables 20 and 21).

All groups showed an increase in perfusion pressure on lying down. This was proportionally more in the groups with higher IOP due to their lower initial perfusion pressure but did not differ significantly between the groups.

Blood pressure and IOP

A significant fall in blood pressure was seen in treated subjects. Systolic blood pressure was significantly lower in patients on treatment in seated and supine positions and diastolic blood pressure fell to a lesser (nonsignificant) degree. It is not known whether these changes are the result of a systemic effect of topical beta blockers, the consequence of reduced anxiety in subjects attending for follow-up, or if they represent an interactive relationship between IOP and blood pressure.

Betablockers are effective antihypertensives when given systemically but their mode of action is not understood. They reduce cardiac output, alter baroreceptor reflex sensitivity and block peripheral adrenoceptors. Some block plasma renin secretion and they may also exert a central effect. Some systemic absorption of betaxolol might be expected to occur when it is administered topically to the conjunctival sac and the expected consequence of this would be lowering of blood pressure and slowing of the heart rate[94]. However, Harris et al in a study investigating the effect of betaxolol on perimacular blood flow found no change in brachial blood pressure after administration of topical betaxolol in healthy subjects[87].

The third possibility has been proposed in the past and there is evidence in the literature to suggest that IOP may indeed exert some influence over blood pressure.

If IOP is elevated an increase in blood pressure will maintain a constant ocular perfusion pressure according to the equation:

Seated perfusion pressure = 2/3 mean arterial pressure -IOP

Because the formula for supine perfusion pressure is as follows:

Supine perfusion pressure = mean arterial pressure –IOP,

a larger increase in blood pressure is required in the sitting position to maintain the status quo. After treatment the group presented here had a larger fall in sitting MAP(p=0.008) than in supine MAP(p=0.167), a finding which tends to support the theory that there is a link between blood pressure and IOP. Other researchers have identified higher blood pressure in POAG patients. Wilson et al identified systolic hypertension associated with POAG[30] and systolic blood pressure was 'the variable most related to IOP' in the Framingham Eye Study[31]. Low blood pressure has been identified as a risk factor for visual field progression in POAG[32] but particularly in NTG[33] possibly representing a failure of the balance between BP and IOP. Wagner et al found evidence of angiotensin II synthesis in the choroid, RPE and neural retina[34]. Angiotensin II is a potent vasoconstrictor and stimulates aldosterone release, its production by the eye is one possible mechanism through which the eye may influence systemic blood pressure.

Very recently Polak et al published a paper reporting a significant association between increased choroidal blood flow and higher blood pressure in a group of 318 healthy men with normal or slightly elevated BP. Choroidal blood flow was assessed using fundus pulsation amplitude and blood flow velocities in the posterior ciliary arteries[35]. This raises the possibility that increased systemic blood pressure may be a response to reduced choroidal perfusion possibly triggered by the relatively ischaemic glaucomatous

eye. The measured fall in blood pressure associated with lowering of IOP and increase in choroidal blood flow could be explained by this hypothesis. However, as mentioned before, in this study systemic effects of the topical β-blocker betaxolol could also have caused a reduction in blood pressure and it is not possible to draw conclusions about the origin of the change from the data presented here.

The relationship between POBF and IOP

Some previous studies have failed to find a relationship between IOP and POBF in normal eyes[4,16]. In 1996, Harris presented CDI data supporting the existence of autoregulatory control of ocular blood flow in the presence of changing IOP in normal subjects[36]. Subsequent studies using POBF[9] and CDI[27] have identified a loss of this control in glaucoma patients.

In this study IOP was excluded from correlations across the four groups because it was one of the variables restricted by the inclusion criteria, i.e. no glaucoma patients with IOP below 21mmHg were included. Normal pressure glaucoma comprises up to 30% of the glaucoma population and NTG subjects are known to have low POBF. The effect of excluding this group would be to artificially emphasise the association between high IOP and low POBF and for this reason IOP correlations were examined for each diagnostic group separately.

Looking at the diagnostic groups separately a significant negative correlation was seen between IOP and POBF in POAG but not in the other groups (table 16). This finding tends to support the theory that there is a loss of autoregulatory control of blood flow in

glaucoma and that IOP has a greater influence on choroidal blood flow in glaucomatous eyes than in normals eyes. This theory is supported by the finding of James and Smith who found a correlation between POBF and IOP in NTG patients which was not present in a normal group matched for IOP[82].

In studies on rabbits Gual et al demonstrated that an intact autonomic supply to the eye helps to buffer IOP elevations induced by water loading or cephalic venous stasis. They propose that this is through autonomic input to the ocular vasculature[37].

One possible interpretation of the results presented here is that the choroid may perform a central function in accommodating changes in ocular perfusion pressure in the normal eye, not only in fight or flight situations as proposed by others[38] but also on lying down. The increase in ocular perfusion pressure which occurs on lying down will lead to an increase in the nonpulsatile component of ocular blood flow, reducing the volume of choroid available for pulsatile perfusion. The choroidal capacity in a healthy eye may be enough to accommodate this significant reduction in pulsatile flow without compromising function although postural studies have shown some reduction in retinal function does occur on lying down which becomes more marked if a subject is tilted into a head down position[39]. If the potential choroidal volume is effectively reduced by raised intraocular pressure which increases resistance to choroidal filling[40], by vasospasm, or by the effects of ageing and atherosclerosis the ability of the eye to cope with changes in perfusion pressure may be compromised. In glaucomatous eyes an

already significantly reduced POBF is reduced further when the patient lies down. It may be that this reduction is not enough to fully accommodate the increase in nonpulsatile flow and a small increase in IOP ensues further compromising perfusion. Regarding the differences between ocular hypertensives and glaucoma patients, ocular hypertensives with IOP above 25mmHg show changes in pulsatile ocular blood flow in the same direction as the glaucoma subjects but not to the same extent. The only difference found between the two groups was in the response of POBF to changing posture, the HROHTs having a larger and more 'normal ' reduction in POBF on lying down than the glaucoma patients perhaps suggesting that their choroid retains the capacity to accommodate changes in perfusion pressure despite their elevated IOP.

B: Scanning Laser Doppler Flowmetry

Summary of results

This investigation has found alterations in retinal blood flow in POAG and HROHT as measured with scanning laser Doppler flowmetry (Heidelberg retinal flowmeter).
In the nasal retina the trend was for higher SLDF readings of velocity and flow in high risk OHT. There was a significant difference between HROHT and LROHT in minimum velocity and minimum flow in this area. At the nasal neuroretinal rim the trend once again was for highest readings in HROHT although there were no significant differences between the groups. At the optic cup the highest readings were seen in HROHT and the lowest in POAG. This difference was significant for minimum volume and minimum velocity. At the temporal neuroretinal rim HROHT had the highest readings for all parameters and was significantly higher than POAG for maximum volume. In the temporal retina there was a change in the pattern of results with a trend for highest readings in POAG subjects in this area. POAG patients had significantly higher readings than LROHT for minimum velocity, maximum and minimum flow in the temporal retina. Comparing POAG with HROHT the same pattern was seen with higher readings in OHT at the nasal rim, optic cup and temporal rim. POAG subjects had higher levels of blood flow in the temporal retina. The difference between some parameters in each area was statistically significant.
Taking all subjects together significant positive correlation was seen between systolic BP and SLDF parameters at the nasal neuroretinal rim (maximum and minimum

volume). For the reasons mentioned in the previous section correlations with IOP were not carried out for the full patient group because of the restriction of IOP in the inclusion criteria and the exclusion of glaucoma subjects with IOP less than 21 mmHg. There was also positive correlation between age and SLDF in the nasal retina (maximum volume) and in the temporal retina (minimum flow). There was no significant correlation between SLDF and POBF or pulse. Taking all OHT and POAG subjects together a more negative MD was significantly correlated with lower SLDF particularly at the optic cup, there was a similar relationship between more positive CPSD and lower SLDF. In other words worse visual field parameters were associated with lower SLDF readings at the optic cup. Looking at POAG subjects alone no significant correlations were found. This may be because the group size was small (n=15).

These results show evidence of reduced blood flow at the lamina cribrosa and temporal neuroretinal rim and of increased flow in the temporal retina in untreated POAG compared to HROHT subjects with a similar level of IOP.

Comparison with other studies

At the time of this investigation there was very little published data comparing OHT and POAG and existing studies referred to normal subjects for comparison with OHT and POAG. Michelson and associates compared POAG patients with normals and found reduced blood flow at the lamina cribrosa and nasal and temporal retina in POAG [41]. Nicolela and associates, using SLDF, also found reduced blood flow at the lamina cribrosa in POAG compared to normals with a non-significant increase in blood flow at

the temporal neuroretinal rim in POAG. They found reduced flow at sites on the temporal retina in POAG compared to normals. The subjects in their study were a heterogeneous group including normal pressure glaucoma patients and two thirds of the group were also on topical antiglaucoma medication[42].

Bohdanecka et al found a highly significant correlation between the Heidelberg retinal flowmeter parameter 'volume' at the optic nerve head and blood flow velocities in the retrobulbar vessels measured with colour Doppler imaging (CDI), suggesting that patients with altered blood flow in the retrobulbar vessels are likely to also have alteration in their optic nerve blood flow as measured with SLDF. Many CDI studies have found reduced flow velocities in the retrobulbar vessels in glaucoma[43,44].

The study reported here found no significant differences between normal controls and POAG although mean results for POAG were consistently lower than the normal group at the lamina and higher than normal at the temporal retina. It might be that with larger groups this difference would become significant. The results presented here concur to some extent with those previously published by others in that the group with normal visual function (HROHT) had higher blood flow at the optic nerve head than the glaucoma group.

The finding in this study of increased temporal retinal blood flow in glaucoma has not been previously reported. Of all the 5 areas measured in this study (figure 11) the retina was least likely to present problems due to limited space or large blood vessels, in addition the images were taken focussed on the retinal vessels so lack of focus should not have been a major source of error. Areas of peripapillary atrophy (PPA) were

avoided when placing the sample square which may have produced higher flow readings than those recorded in other studies. Blood flow was noted to be markedly reduced in these areas and it may be that an average blood flow measurement for the entire area including PPA would be reduced in POAG compared to normal, or that measurements taken at a set distance from the disc would include some peripapillary atrophy more frequently in POAG than in normals.

In 1999 Roff et al reported a significant increase in blood volume, flow and velocity measured with SLDF in the superior temporal quadrant of the peripapillary retina during isoxic hypercapnia in normal subjects[45]. This raises the possibility that the findings presented here represent an autoregulatory increase in blood flow in the glaucomatous retina in response to e.g. an increase in pCO2.

This study has found a correlation between worse visual field parameters and reduced blood flow at the optic cup. This is in agreement with a recent study by Ciancaglini et al who found a correlation between both MD and CPSD and SLDF parameters measured at the lamina cribrosa in glaucoma patients[46].

In contrast to previous studies the subjects in this investigation were not on any form of topical or systemic vasoactive medication. While some authors have found that topical treatment does not make a significant difference to ocular blood flow[41,47], others have demonstrated changes in blood flow produced by topical antiglaucoma medication [22,24,48]. The possibility that treatment may produce changes in local distribution of

blood flow or autoregulation remains and may account for the differences in the findings of this study and those of others in which treatment was not excluded.

Reduced retinal blood flow may be a function of increased IOP [36]. In previous studies POAG patients have been compared with normal subjects and the groups have not been matched for IOP[41,49,50]. The intention in this investigation was to reduce the confounding effect of this variable by comparing two groups matched for IOP and the results may therefore reflect a truer, IOP independent, assessment of blood flow in glaucoma.

Sources of error

We encountered several technical problems in our use of the SLDF. It was difficult to obtain well focussed images without movement artefact particularly in elderly patients with poor vision in the contralateral eye which interfered with fixation. The group sizes for this investigation are therefore small and the results must be viewed with this in mind. The most common reasons for omission of an image from the analysis were movement artefact in the picture or poor focus. In the nasal and temporal neuroretinal rim areas there were also problems obtaining reliable measurements due to crowding of large blood vessels. In some cases it was not possible to place the enquiry square without including a portion of a large vessel and in these cases measurements at the neuroretinal rim were omitted. Nicolela and associates reported similar problems in their study using the same machine[42]. These problems have been addressed in recent years

with the development of image analyser software which combines optic disc topography with SLDF perfusion images to give 'mean flow' results for selected areas[51].

In taking measurements from the images the placement of a 10 x 10 pixel square on the temporal rim unavoidably includes an area of optic cup when the rim is thin. This produces a tendency for results at the temporal neuroretinal rim to be artificially lower in POAG than in subjects with a healthy optic disc. The 10 pixel square was used in this study because previous researchers had used this size of sample area [41,42,52] and reliability and reproducibility studies relate to a 10 x 10 pixel square[6,53]. A similar problem was encountered when taking measurements from the optic cup in OHT subjects i.e. the sample area included neuroretinal rim if the cup was small and there was a risk of artificially elevated blood flow readings in OHT. Images of OHT subjects with an optic cup too small to allow placement of the sample square were excluded in an attempt to avoid this problem. Finally when focussing on the retinal vessels the floor of the optic cup in POAG may be deeper and therefore further from the focal plane in POAG than in OHT. This would tend to produce lower results in POAG at the optic cup.

In comparing POAG with OHT it is important to remember that OHTs are a heterogeneous group containing some 'pre-glaucoma' patients. This study has not attempted to differentiate between types of OHT but has simply compared two groups with similar IOP one of which, the POAGs, have optic nerve damage producing demonstrable visual field loss. It is therefore likely that the changes in blood flow identified in this study relate to the damaged state of the optic nerve in POAG. It is also possible that the difference between the two groups is reduced by the presence of

preglaucoma patients in the OHT group as it is known that structural changes may occur at the optic nerve head in ocular hypertension before the appearance of measurable field loss.

Interpretation of results

The results presented here suggest that the choroidal and retinal circulation respond in different ways in POAG with a decrease in choroidal blood flow as demonstrated by reduced SLDF parameters at the lamina cribrosa in the glaucoma group, and an apparent increase in retinal circulation as shown by the increased minimum velocity and flow in the temporal retina.

The blood supply of the retina and choroid are anatomically different; the choroid derives its supply from the short posterior ciliary arteries which also supply the lamina cribrosa, and the retinal supply comes from the central retinal artery. There is also a difference in the control mechanisms of these two vascular beds. The choroid receives an autonomic nerve supply and studies have demonstrated increased choroidal blood flow in response to parasympathetic stimulation [54] and reduced flow in response to stimulation of the cervical sympathetic chain[38]. In contrast the retinal vessels do not have an autonomic supply and blood flow is autoregulated in response to local tissue demand and accumulation of metabolites[55].

These anatomical and physiological differences may help to explain the findings. A relative reduction in parasympathetic activity in the choroid could produce reduced choroidal blood flow due to unopposed sympathetic action leading to retinal ischaemia.

In addition to causing damage to the photoreceptors and visual field loss this ischaemia may also lead to a reactive autoregulatory vasodilation in the retinal vessels in response to hypoxia or hypercapnia[45].

Autonomic dysfunction has been suggested before as a possible underlying abnormality in glaucoma [56]. Jordan and associates demonstrated parasympathetic denervation hypersensitivity to dilute pilocarpine in glaucoma patients [57] and several studies have found reductions in both sympathetic and parasympathetic activity on autonomic testing in both NTG[58] and POAG patients[59 60]. One study reports an increase in sympathetic activity and a decrease in parasympathetic activity in closed angle glaucoma[61].

Autonomic neuropathy has also been suggested as an explanation for the reported increased incidence of POAG found in diabetic patients[62,63].

Review of patients on treatment

SLDF images were taken on the review visit but the relatively small number of patients reviewed combined with the difficulty obtaining good images and non-uniform treatment make it impossible to draw any reliable conclusions from the data.

C: Haematology

Summary of results

This study has identified some significant differences in haematological parameters between the groups examined. Manual fibrinogen was found to be slightly higher in ocular hypertension and POAG than in normals (P=0.048). The platelet count was also higher in POAG and OHT than in normals (p=0.027) and there was borderline significance for the difference between the groups in haematocrit (p=0.056) with higher levels again seen in POAG and OHT. Other parameters measured (betathromboglobulin (βtg), D-dimer, and prothrombin fragment 1 and 2 (PF1&2)) did not differ significantly between the groups. The mean level of prothrombin fragment 1&2 in the POAG group was found to be elevated above the upper limit of the normal range, all other results were within normal limits. Comparing POAG with HROHT mean levels of βtg, manual fibrinogen , D-dimer, PF1&2, platelets and haematocrit were all higher in POAG. This difference was not significant for any measurement although borderline significance (p=0.056) was seen for the difference in platelets and PF1&2.

Correlations were examined for males and females separately and correction was made for age. In men a positive correlation was found for haematocrit with diastolic blood pressure and for platelets with systolic BP. No relationship between blood pressure and haematological parameters was found in females.

Coagulation

There has been much research in recent years into the possible role of a hypercoagulable state in the pathogenesis of glaucoma. Drance in 1973 reported an increased thrombotic tendency in normal tension glaucoma (NTG) due to increased platelet adhesiveness or abnormal euglobulin lysis time[64]. Conflicting findings were published by Joist et al 3 years later when they reported no difference in platelet function, blood coagulability or fibrinolytic activity between NTG and normals matched for age and systemic vascular disease[65]. Drance's team also failed to reproduce their original findings in a later study[66]. O'Brien et al published findings from an investigation comparing clotting parameters in POAG, NTG and normals[67]. They found a significant ($p<0.001$) elevation in prothrombin fragments 1&2 and D-dimer in POAG compared to both NTG and normals, however the POAG group had higher systemic blood pressure and both POAG and NTG had significantly more systemic vascular disease than normals. Of the parameters measured in this investigation fibrinogen, D-dimer and PF1&2 are indicators of coagulation.

In the coagulation pathway PF1&2 are released when prothrombin is converted into thrombin. Thrombin acts on fibrinogen to produce fibrin. D-dimer is produced as the terminal fragment in fibrin degradation following secondary fibrinolysis (figure 14). Elevated levels of D-dimer and PF1&2 are found in hypercoagulable states[68,69].

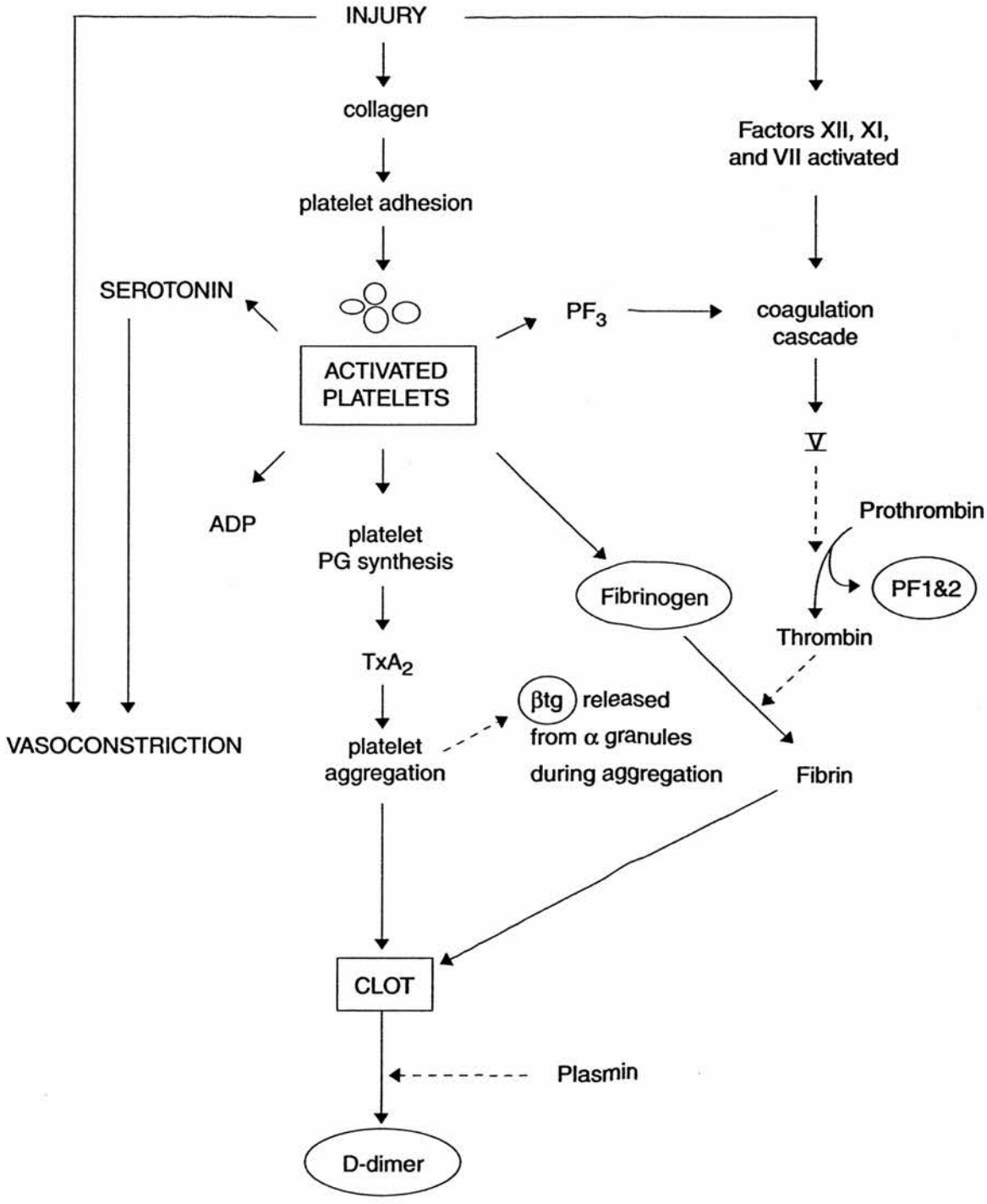


Figure 14: Platelet function and the clotting cascade

In this study the groups under investigation do not differ significantly in their mean systemic blood pressure, in the incidence of events signifying systemic vascular disease or in the number of smokers in each group. The low risk ocular hypertensives were significantly younger than the POAG subjects and there was a difference in gender distribution between the groups although this difference was not statistically significant . Differences in parameters which normally rise with age or are higher in men than in women such as haematocrit and platelets may be accentuated with higher levels seen in HROHT and reduced levels seen in normals (due to higher proportion of women) and LROHT (due to younger age). A trend for higher levels of all parameters in POAG was seen with a significant difference between the four groups in fibrinogen levels ($p=0.048$). Although they did not differ from normals in this study POAG subjects had a mean level of PF1&2 which was elevated above the normal range.

The subjects in this study were selected prospectively over 18 months with no specific age or gender requirements. The exclusion of patients on vasoactive medication will have produced a selection bias for patients with less systemic vascular disease. It is not known whether a larger proportion of POAG subjects were excluded for this reason because recruitment was initiated by several different doctors. Awareness of the inclusion criteria for the study meant that only patients not on systemic medication were brought to the attention of the investigator. It is possible that if patients had been selected with no restrictions related to vasoactive medication the difference between

normal and POAG may have been more marked. There is known to be an increased prevalence of systemic hypertension among subjects with raised IOP[31].

This investigation has therefore examined what is probably a subgroup of POAG and OHT with relatively little systemic vascular disease. The slight increase seen in markers of coagulation in POAG is in agreement with other studies[67] and has the additional benefit that the observations relate to groups that do not differ significantly in blood pressure, IOP, or reported systemic vascular disease. Based on the results presented here it seems likely that POAG is associated with mildly increased activation of the clotting system such as is seen with increasing age or atherosclerosis.

Platelet function

Abnormal platelet function has also been identified as a possible contributing factor in glaucoma. There have been numerous studies examining platelet function both in vitro and in vivo. Hoygn et al reported an increased incidence of in vitro spontaneous platelet aggregation in deteriorating glaucoma (POAG and NTG) compared to subjects with stable glaucoma or ocular hypertension[70]. This finding was recently confirmed by Matsumoto who found a higher incidence of abnormal platelet aggregation in both POAG and NTG, more marked in the latter[71].

In this study plasma levels of beta thromboglobulin (βtg) were measured as an indicator of in vivo platelet activity. Betathromboglobulin is contained in the alpha granules of platelets and is released when platelets are activated. Elevated levels have been found in

DVT, preeclampsia and some infections. At the time of this investigation no studies had looked at levels of this protein in untreated POAG or OHT. No difference in plasma βtg level was found in this study between glaucoma or OHT and normals however platelets were significantly more numerous in the groups with higher intraocular pressure.

Viscosity

Plasma and whole blood viscosity have been found to be elevated in POAG by some authors [50,72,73], but not others[66]. Results can be difficult to compare due to the high prevalence of confounding variables such as cardiovascular disease in the glaucoma population and the use of different techniques to measure viscosity.

No specific investigation into viscosity was made in this study however two components of whole blood which contribute to viscosity are haematocrit and fibrinogen. Fibrinogen was found to be significantly elevated in POAG and LROHT in this study and a higher haematocrit in POAG was of borderline significance.

Treatment effect

A number of glaucoma and ocular hypertensive subjects who were started on treatment to lower IOP had repeat blood samples taken at a review visit (see appendix). No significant differences were found either between HROHT and POAG post treatment or in the measurements for the group before and after treatment although there was a borderline reduction in platelets ($p=0.06$)

In summary the findings presented here tend to support those already published in the literature. Differences between POAG and normal may have been reduced by the exclusion of subjects on systemic vasoactive medication as an unrestricted sample of POAG may have contained a larger proportion of people with systemic vascular disease than the normal group. Limited conclusions can be drawn from the results relating to the treated group because treatment was not uniform, the mean treated IOP for the group remained at the upper limit of normal and treatment was associated with a significant drop in systemic blood pressure.

It may be that untreated glaucoma and ocular hypertensive subjects have mild activation of the clotting cascade and a tendency to increased blood viscosity to an extent compatible with increasing age or mild atherosclerosis. Topical treatment to lower intraocular pressure would not be expected to have any great effect on rheology however the attendant reduction in blood pressure whether due to systemic effects of betablockers or a response to lower IOP may have a beneficial influence reflected in a non-significant reduction in clotting parameters. This theory is supported by the correlation of platelets and haematocrit with systolic and diastolic blood pressure respectively in men in this study.

D: Peripheral vasoreactivity

Summary of results

This study found significantly increased baseline fingertip blood flow in low risk OHT compared to normals. No significant differences were found between the groups in blood flow after warm or cold provocation nor in the time taken to recover from immersion in ice cold water.

The laser Doppler flowmeter used to measure peripheral blood flow in this study is straightforward to use. Possible error may arise if the finger pulp is compressed by the probe during measurement. Care was taken to ensure that this did not occur and all measurements were performed by one experienced operator (JK). Another factor which may affect peripheral blood flow is ambient temperature. All measurements were made in the same windowless room and the room temperature was recorded. There was no variation in temperature in excess of $1^{o}C$.

As mentioned before the exclusion of subjects on vasoactive medication will have led to the selection of a group with less systemic vascular disease and it is not known if more glaucoma patients than ocular hypertensives were excluded on these grounds. In addition no information is available about the possible confounding presence of non-prescribed drugs such as ginko biloba which may have a profound effect on peripheral blood flow and vasospasm.

Many previous studies have identified an increased vasospastic tendency in patients with normal pressure glaucoma[8]. Others have found colder peripheries without frank vasospasm in POAG. At the time of this study there were no publications investigating peripheral blood flow in untreated ocular hypertension. The finding of increased baseline blood flow in LROHT is unexpected and may simply relate to the younger age of the LROHT subjects. In addition to this the incidence of reported vasospastic symptoms (migraine or Raynauds) was higher in the normal group (43%) than in the other groups (POAG 26%, HROHT 35% and LROHT 20%). Although this difference was not statistically significant across the four groups it may have had a bearing on the results (Table 5). Another possible interpretation of the result is that ocular hypertensives show relative peripheral vasodilation which occurs in response to a reduction in ocular perfusion secondary to raised IOP. This ability to compensate for reduced ocular perfusion pressure may be reduced or lost in subjects with higher IOP or established glaucoma.

Vascular changes have been demonstrated in the eye in response to stresses imposed on the peripheral vasculature[74,75] and it is possible that a generalised vascular response may result from changes in ocular perfusion.

The findings presented here are difficult to interpret due to the confounding variables of age and differing incidence of vasospastic disorders between the groups, however it raises interesting questions about the behaviour of the peripheral circulation in ocular hypertension which deserve further investigation.

2.5

Summary and Conclusions

The main aim of this study was to compare ocular blood flow and related factors influencing perfusion in groups of untreated ocular hypertensives and glaucoma patients matched for IOP.

Differences between OHT and POAG which may be related to the presence of disease in the glaucoma group were identified. There was a trend for lower POBF in glaucoma compared to OHT although this was not statistically significant in this study it may be that with larger numbers it would become so. Both POAG and HROHT had significantly lower POBF than normals. A difference was evident between HROHT and POAG in the response of POBF to changing posture and in the distribution of blood flow in the retina and optic nerve head as measured with scanning laser Doppler flowmetry. A significantly higher temporal retinal blood flow was found in POAG suggesting possible differences in the autoregulatory response between the two groups.

No difference was found between OHT and POAG with respect to clotting factors or peripheral vasoreactivity. POAG had a small but significant increase in clotting parameters and platelet count compared to normals and significantly increased peripheral perfusion was found in low risk ocular hypertensives. The latter result may be attributable to a younger mean age in the LROHT group.

The findings of this study point to a disturbance in choroidal perfusion in POAG which is at least in part related to IOP and present to a lesser extent in OHT subjects with IOP over 26mmHg. The findings also suggest that there may be some degree of decompensation of autoregulatory mechanisms in glaucoma which is not present in OHT with a similar level of IOP.

Further research should look at the role of the choroid as a buffer accommodating changes in ocular perfusion pressure and IOP. The possibility that the choroid is the common endpoint at which intraocular pressure, vasospasm, atherosclerosis, autonomic dysfunction and age exert their effect to produce glaucomatous optic atrophy deserves further investigation and there may be therapeutic potential in drugs which modulate choroidal perfusion in addition to those which act on intraocular pressure.

2.6

Suggestions for future work

1. The role of the choroid as a buffer of fluctuations in IOP could be explored by examining patients with damaged or reduced choroid in one eye. Comparison of postural IOP fluctuations in fellow eyes of patients who have undergone treatment for choroidal malignant melanoma in one eye may give information about whether the choroid is involved in IOP homeostasis.

2. Follow-up studies of the ocular hypertensive group examined here may give information about the number converting to POAG and allow retrospective examination of blood flow results to determine if any differences existed in blood flow in these subjects prior to the appearance of glaucomatous field loss.

3. Peripheral blood flow in ocular hypertension: Larger groups of ocular hypertensives could be examined to determine whether they have a genuine increase in peripheral blood flow associated with raised IOP.

4. Studies examining the shape of the ocular pulse in ocular hypertension may give additional information about ocular elasticity and the ability of an eye to withstand raised IOP. In this and in many other studies ocular hypertensive subjects were found to

consistently have higher pulse amplitude than glaucoma patients. The significance of this is not clear.

Section II References

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Appendix A

Publications and Presentations arising from this thesis

'Pulsatile ocular blood flow in ocular hypertension and primary open angle glaucoma'
Kerr J, Nelson P, O'Brien C: Am J Ophthalmol 2003: 136:

'A comparison of ocular blood flow in untreated primary open angle glaucoma and ocular hypertension.'
Kerr J, Nelson P, O'Brien C: Am J Ophthalmol 1998: 126:42-51

'Optic nerve and retinal capillary blood flow in in untreated primary open angle glaucoma (POAG) and ocular hypertension (OHT)'
Kerr J M, O'Brien C: IOVS, March 15 1997, Vol 38, No 4, page s276

'Pulsatile ocular blood flow in untreated ocular hypertension and primary open angle glaucoma'
Glaucoma Society 18th Annual Meeting, November 1997
Kerr J, O'Brien C. (presentation)

'Ocular blood flow in untreated primary open angle glaucoma and ocular hypertension'
Royal College of Ophthalmology Annual Meeting, May 1997
Kerr J, O'Brien C. (poster)

'A comparison of Heidelberg retinal flowmeter results in untreated primary open angle glaucoma and ocular hypertension'
Scottish Ophthalmological Club Meeting, March 1997
Kerr J, O'Brien C. (presentation)

'A comparison of Heidelberg retinal flowmeter results in untreated primary open angle glaucoma and ocular hypertension'
Glaucoma Society 17th Annual Meeting, November 1996
Kerr J, O'Brien C. (presentation)

Appendix B

Additional Tables

SLDF Correlations (table A)

Data was examined for correlation between SLDF, POBF, visual fields, and systemic haemodynamic measurements. In the full group of subjects comprising normals, OHT and POAG, significant correlation was seen between seated systolic BP and maximum volume at the nasal NRR (c=0.321, p=0.024, n=49) and minimum volume at the nasal neuro retinal rim (c=0.319, p=0.025, n=49). Age correlated with maximum velocity in the nasal retina (c=0.285, p=0.022, n=64), and minimum flow in the temporal retina (c=0.289, p=0.022, n=63).

Correlation with visual field was examined for OHT and POAG together. Results are shown in table A. Mean deviation (MD) of visual field was positively related to blood flow at the optic cup and negatively related to blood flow in the temporal retina. Corrected pattern standard deviation (CPSD) was positively correlated with minimum velocity and minimum flow in the temporal retina. No significant correlations were found for SLDF with other parameters in POAG alone (n=15). POBF showed positive correlation with minimum velocity in temporal retina and negative correlation with minimum flow in nasal retina (table A).

These results must be viewed with caution due to the large number of variables compared. It is expected that some significant correlations may arise by chance. A correction may be applied to the results to allow for this (Bonferroni) and it sets the level of true significance at p=0.005. At this level there is no significant correlation found between SLDF and the other variables measured.

Table A

Scanning Laser Doppler Flowmetry Correlations (All subjects)

Area 1	Mean deviation	CPSD	Sitting POBF
Maximum volume	c=0.186 p=0.221 n=45	0.019 0.902 n=45	0.206 0.103 n=64
Minimum volume	**0.319** **0.032** n=45	-0.236 0.119 n=45	0.188 0.136 n=64
Maximum velocity	-0.061 0.689 n=45	0.087 0.572 n=45	-0.114 0.369 n=64
Minimum velocity	-0.021 0.894 n=45	0.050 0.744 n=45	-0.236 0.063 n=63
Maximum flow	0.053 0.729 n=45	0.097 0.527 n=45	-0.067 0.600 n=63
Minimum flow	-0.075 0.624 n=45	0.036 0.816 n=45	**-0.253** **0.044** n=64

Area 2	Mean deviation	CPSD	Sitting POBF
Maximum volume	0.130 0.458 n=35	-0.127 0.467 n=35	0.055 0.708 n=49
Minimum volume	0.219 0.206 n=35	-0.192 0.269 n=35	0.001 0.995 n=49
Maximum velocity	-0.030 0.862 n=35	0.037 0.831 n=35	-0.071 0.630 n=49
Minimum velocity	0.056 0.750 n=35	-0.009 0.957 n=35	-0.119 0.417 n=49
Maximum flow	0.002 0.990 n=35	0.011 0.950 n=35	-0.051 0.726 n=49
Minimum flow	0.055 0.753 n=35	-0.015 0.930 n=35	-0.107 0.466 n=49

Area 3	Mean deviation	CPSD	Sitting POBF
Maximum volume	**0.365** **0.018** n=42	-0.282 0.070 n=42	0.008 0.953 n=58
Minimum volume	**0.316** **0.041** n=42	-0.202 0.200 n=42	-0.008 0.952 n=58
Maximum velocity	**0.341** **0.029** n=41	**-0.305** **0.052** n=41	-0.046 0.736 n=57
Minumum velocity	0.293 0.060 n=42	0.285 0.068 n=42	-0.098 0.466 n=58
Maximum flow	**0.307** **0.048** n=42	-0.255 0.103 n=42	0.083 0.534 n=58
Minimum flow	**0.325** **0.038** n=41	-0.285 0.071 n=41	-0.045 0.742 n=57

Area 4	Mean deviation	CPSD	Sitting POBF
Maximum volume	**0.396** **0.008** n=44	-0.273 0.072 n=44	0.100 0.447 n=60
Minimum volume	0.214 0.162 n=44	-0.164 0.287 n=44	-0.042 0.749 n=60
Maximum velocity	0.183 0.235 n=44	-0.090 0.562 n=44	-0.092 0.484 n=60
Minumum velocity	-0.025 0.873 n=44	0.071 0.645 n=44	-0.222 0.089 n=60
Maximum flow	0.200 0.194 n=44	-0.103 0.505 n=44	-0.079 0.548 n=60
Minimum flow	0.009 0.953 n=44	0.044 0.778 n=44	-0.209 0.110 n=60

Area 5	Mean deviation	CPSD	Sitting POBF
Maximum volume	-0.031 0.819 n=44	0.092 0.552 n=44	0.235 0.063 n=63
Minimum volume	0.033 0.832 n=44	-0.018 0.905 n=44	0.102 0.426 n=63
Maximum velocity	**-0.345** **0.024** n=43	0.278 0.071 n=43	0.079 0.544 n=62
Minimum velocity	**-0.410** **0.006** n=44	**0.337** **0.025** n=44	**0.278** **0.028** n=63
Maximum flow	**-0.329** **0.031** n=43	0.288 0.061 n=43	0.110 0.393 n=62
Minimum flow	**-0.375** **0.012** n=44	**0.314** **0.038** n=44	-0.147 0.251 n=63

Note
Significant correlations ($p<0.05$) are shown bold.

Haematology Correlations (Tables B and C)

Partial correlations were performed with correction for age examining the relationship of haematocrit, platelets, fibrinogen and PF1&2, to other measured parameters. The results are shown in tables B and C. Normal haematological reference values for men and women differ and there was an imbalance in gender distribution between the study groups as noted before (there was a higher proportion of females in the groups with lower IOP). For this reason results from men and women were examined separately. In men systolic blood pressure correlated with platelets (c=0.32, p=0.052) and haematocrit (c=0.371, p=0.024). There were no other significant correlations involving haematological measurements.

Table B

Haematology results

Correlations corrected for age

Males (n=35)

	fibrinogen	platelets	haematocrit
Average IOP	C=0.247 P=0.140	0.091 0.592	0.041 0.808
Maximum IOP	0.297 0.075	0.091 0.593	0.520 0.759
Systolic BP	0.083 0.622	0.322 **0.052**	0.219 0.192
Diastolic BP	0.003 0.984	0.243 0.147	0.371 **0.024**
Sitting POBF	-0.072 0.674	0.178 0.292	-0.065 0.700
Sitting Pulse volume	-0.080 0.638	0.213 0.205	-0.292 0.079

Table C

Haematology results

Correlations controlled for age

Females (n=40)

	fibrinogen	platelets	haematocrit
Average IOP	C=0.097 0.540	0.254 0.104	0.165 0.295
Maximum IOP	0.128 0.418	0.232 0.138	0.213 0.175
Systolic BP	-0.020 0.895	0.034 0.831	-0.145 0.359
Diastolic BP	0.007 0.963	-0.078 0.622	-0.823 0.602
Sitting POBF	-0.045 0.779	-0.162 0.306	-0.087 0.583
Sitting Pulse volume	-0.075 0.635	-0.176 0.264	-0.122 0.441

There was no significant change found in haematological parameters in treated subjects.

Table D

Effect of Treatment on Rheology

Mean (standard deviation)

HROHT (n=10) and POAG (n=14) combined (24 pairs)

Parameter *Normal value*	**Pre-treatment**	**Post-treatment**	**P value**
βtg ***<50ngms/ml***	49.88(42.2)	48.64(26.6)	0.86
Manual Fibrinogen ***1.5-4g/l***	2.83(0.57)	2.75(0.79)	0.66
Ddimer ***<400 ngms/ml***	341.6(160.7)	335.2(202.9)	0.84
PF 1&2 ***0.44-1.1 nmols/l***	1.37(0.63)	1.43(0.84)	0.71
Platelets ***150-350x10/l***	295.9(134.3)	269.4(92.3)	0.06
Haematocrit ***0.37-0.47***	0.41(0.003)	0.42(0.004)	0.13

Appendix C

Forms and documents relating to the study

SUBJECT INFORMATION SHEET

OCULAR BLOOD FLOW IN OCULAR HYPERTENSION AND PRIMARY OPEN ANGLE GLAUCOMA . THE EFFECT OF TREATMENT

Glaucoma is generally associated with raised pressure in the eyes. However there are a number of people with raised pressure who do not suffer from glaucoma and never develop damage to the nerve of sight (optic nerve). This group of people are called "ocular hypertensives" and a slightly high pressure is normal for them. In this group there is a small number of people who are at risk of developing glaucoma.At present there is no way of telling which people are healthy ocular hypertensives at no risk of glaucoma and which people are likely to develop the disease. It is possible that if the people at risk of developing glaucoma could be identified and started on treatment to lower the pressure in their eyes damage to their sight from glaucoma may be reduced or avoided.

Recent research has suggested that faulty blood flow to the back of the eye may play a role in producing damage to the optic nerve in glaucoma. We can now get detailed information about the blood flow to the eye using ultrasound. This is a painless, entirely safe method of investigation which has been in use for many years. It uses sound waves to "look" at blood flow. In this study we will be using ultrasound and other tests to look at factors affecting blood flow to the eyes. We hope to identify abnormalities in glaucoma patients which are also present in those ocular hypertensives at risk of developing glaucoma allowing early identification and treatment of this group.

We have three main objectives;

Firstly to compare people with normal eye pressure with people who have glaucoma.

Secondly to look at ocular hypertensives to see whether it is possible to identify those people who may develop glaucoma. It may be that these people have a similar blood flow pattern to people with established glaucoma.

Thirdly we will ask people diagnosed as having glaucoma to come back for repeat tests 3 months after they have started their treatment to see whether the treatment has any effect on bloodflow in addition to lowering the eye pressure.

If you agree to take part in this study you will be asked to come to the Eye Pavilion for one day from 9am to 4pm (two separate days if you have glaucoma). In addition to the bloodflow test on the eyes we will also measure the blood flow in your hand and will take some photographs of the back of your eyes. We will take a blood sample from your arm to check for the levels of several substances which can alter bloodflow. During the day the pressure in your eyes will be checked at regular intervals. There is no surgery involved in this study and the only procedure which you may find uncomfortable is the blood test.

We would be very grateful if you would agree to take part. The study will involve 150 people; 90 ocular hypertensives, 30 people with glaucoma and 30 healthy volunteers with normal eye pressure. Clinical data relating to you will be held in strict confidence and your name and other identifiable particulars will not appear in any report.

Your General Practitioner will be informed about your participation in this study if you agree to take part .

The doctor involved with the study will discuss it with you in detail and you are free to ask any questions prior to taking part. Unfortunately we are not able to give you travelling expenses unless without this help you would be unable to take part in the study.

Participation in this study is entirely voluntary and if you do not wish to take part or wish to withdraw while taking part, you may do so without giving any explanation and also without in any way affecting your usual standard of care.

The main doctor involved in this study is Dr Jan Kerr and she will be supervised by consultant surgeon Dr Colm O'Brien. If there are any points you wish to discuss or any further questions please do not hesitate to contact Dr Kerr at the Eye Pavilion on (0131) 536 4056.

Subject Consent Form

CONFIDENTIAL

Ocular Blood Flow in Ocular Hypertensives
and Primary Open Angle Glaucoma.
The Effect of Treatment.

Subject number .....

SUBJECT INFORMED CONSENT

I, ..., understand what I have been told by Dr ..... about the above study. I have read and understood the Subject Information Sheet about this study (a copy of which has been given to me) and have had the chance to ask any questions about it. I understand and accept the answers that were given. I confirm that I have been given time to think about and have freely agreed to participate in this study.

I know that I can at any time;

a. ask for more information from the doctor and
b. stop taking part in this study without this affecting my usual medical care in any way.

I also know that if I wish to stop taking part in this study I do not need to give any reason.

I agree to my General Practitioner being informed about my taking part in this study as well as him being asked about my present and / or past illness and treatment.

I confirm that I have told the responsible doctor about all known past illnesses, operations and medication.

Ocular bloodflow in ocular hypertensives and primary open angle glaucoma. The effect of treatment.

Complete if the patient is providing written informed consent.

I, the undersigned, consent to participation in the above study.

Signature of patient .....

Patients name (print) .....

Date of consent .....

I, being the undersigned research doctor , confirm that I have fully explained the nature and purpose of the study to the above patient and that he / she has read and kept a copy of the Subject Information Sheet. He / she has freely agreed to participate in the study.

Signature of doctor .....

Doctors name (print) .....

Date of consent .....

Patient Data Sheet

SUBJECT NO:

AGE:

DIAGNOSIS:

STUDY EYE:

DATE:

HRF	
Visual Fields	
Doppler	

Tel.

PMH

	Y	N
Hypertension		
Angina / MI		
Diabetes		
COAD		
Migraine		
Raynauds		

Other:

POH

FHx

EYE DROPS

SHx smoker/ nonsmoker

SYSTEMIC MEDS

Asprin Y/N

B-blockers Y/N

VA

correction

/ @ / @

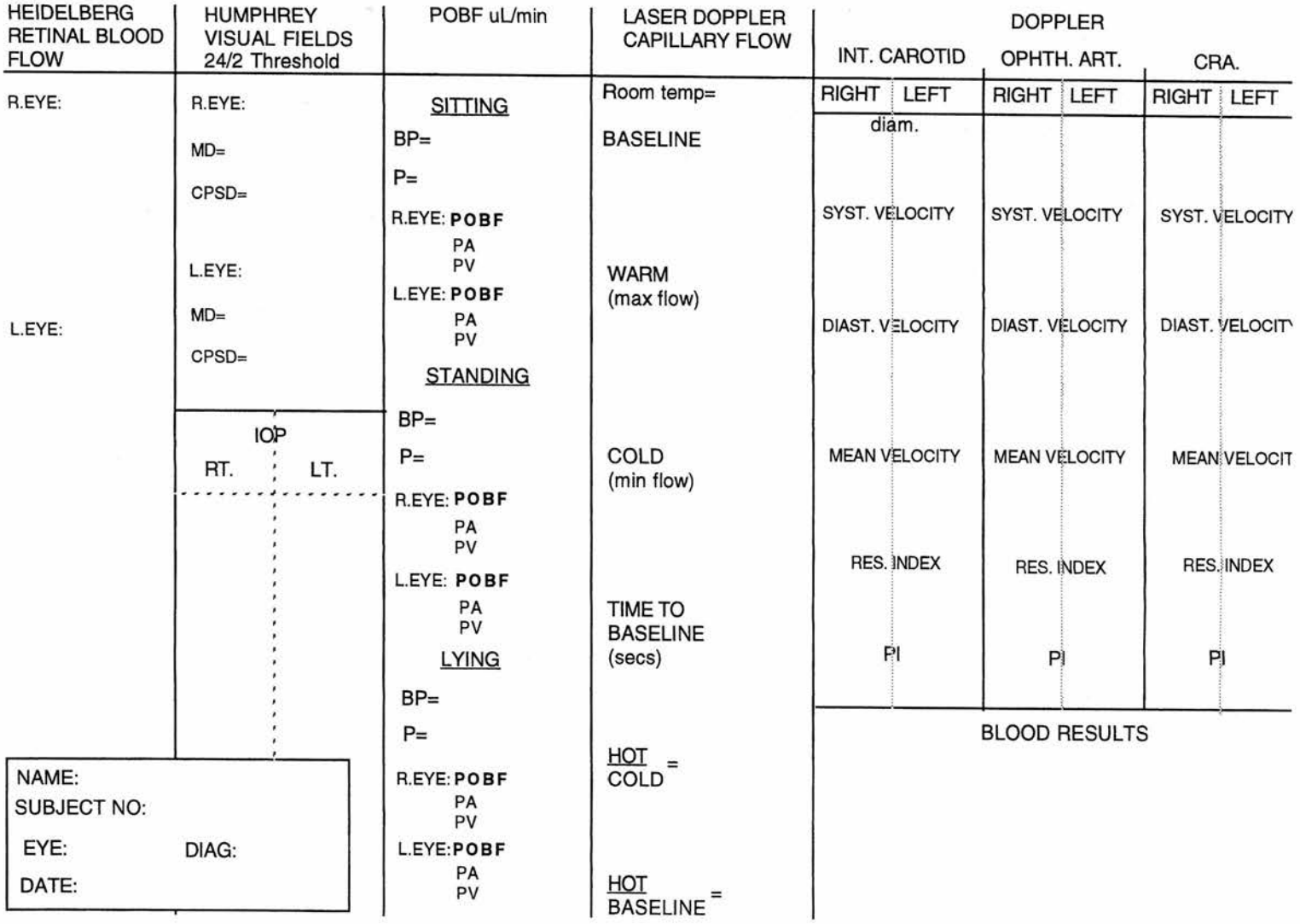
AC

gonio

T

discs

mm biometry mm


HEIDELBERG RETINAL BLOOD FLOW
R.EYE:
L.EYE:
HUMPHREY VISUAL FIELDS 24/2 Threshold
R.EYE:
MD=
CPSD=
L.EYE:
MD=
CPSD=
IOP
RT.
LT.
NAME:
SUBJECT NO:
EYE:
DIAG:
DATE:
POBF uL/min
SITTING
BP=
P=
R.EYE: POBF
PA
PV
L.EYE: POBF
PA
PV
STANDING
BP=
P=
R.EYE: POBF
PA
PV
L.EYE: POBF
PA
PV
LYING
BP=
P=
R.EYE: POBF
PA
PV
L.EYE: POBF
PA
PV
LASER DOPPLER CAPILLARY FLOW
Room temp=
BASELINE
WARM (max flow)
COLD (min flow)
TIME TO BASELINE (secs)
HOT / COLD =
HOT / BASELINE =
DOPPLER
INT. CAROTID
OPHTH. ART.
CRA.
RIGHT
LEFT
diam.
SYST. VELOCITY
DIAST. VELOCITY
MEAN VELOCITY
RES. INDEX
PI
BLOOD RESULTS