PHYSIOLOGICAL AND PATHOLOGICAL HUMAN OCULAR PERFUSION CHARACTERISTICS

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Non-invasive techniques to investigate ocular perfusion of the human eye *in-vivo* have provided greater understanding of both the physiological and pathological haemodynamic status. Furthermore, they offer an alternative clinical indicator for the detection and monitoring of the vascular status in ocular disease.

There were three principle aims to this thesis. Firstly, the acquisition protocols of clinical blood flow apparatus were investigated in order to optimise them for both cross-sectional and longitudinal application. Secondly, the effects of physiological factors including age and systemic circulation on ocular blood flow were investigated. Finally, the ocular perfusion characteristics of patients diagnosed with ocular diseases considered to be of a vascular origin were investigated.

Two procedures were applied in order to explore different intraocular vascular beds. Firstly, the scanning laser Doppler flowmeter was used to explore circulation in the retinal microvasculature. Secondly, the ocular blood flow analyser was used to provide a global measure of ocular pulsatility, being predominantly choroidal in nature.

The principle findings of this work are: -

1) Optimisation of clinical investigations

Photodiode sensitivity of the scanning laser Doppler flowmeter should be kept within a range of 70-150 DC when acquiring images of the retina and optic nerve head in order to optimise the reproducibility of capillary blood flow measures.

Account of the physiological spatial variation in retinal blood flow measures can be made using standard analysis protocols of the scanning laser Doppler flowmeter combined with a local search strategy.

Measurements of pulsatile ocular blood flow using the ocular blood flow analyser are reproducible, however this reproducibility can be improved when consecutive intraocular pressure pulses are used to calculate pulsatile ocular blood flow.

Spectral analysis of the intraocular pressure pulse-wave is viable and identifies the first four harmonic components of the waveform.

2) Physiological variation in ocular perfusion

Age results in a significant reduction in perfusion of the retinal microcirculation, which is not evident in larger vessel beds such as the choroid.

Despite known asymmetry in the systemic vasculature, no evidence of interocular asymmetry in ocular perfusion is apparent.

3) Pathological variation in ocular perfusion

In primary open angle glaucoma, perfusion is reduced in the retinal microcirculation of patients classified as having early to moderate visual field defects. However, ocular pulsatility defects are masked when patients and subjects are matched for systemic variables (pulse rate and mean arterial pressure); differentiation is facilitated by the application of waveform analysis to the continuous intraocular pressure curve even in the early stages of disease.

Diabetic patients with adequate glycaemic control, exhibit maintenance of macula blood flow, macula topography and visual function following phacoemulsification.

Keywords

Blood flow, Heidelberg retina flowmeter, ocular blood flow analyzer, age, glaucoma, diabetes

For my Mother

Rosemary Jane Embleton

16th March 1951 – 8th February 2002

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TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

CHAPTER 1
INTRODUCTION

1.1 Introduction

Ocular perfusion characteristics of the normal human eye are known to alter with physiological factors such as age (Groh, Michelson, Langhans, *et al.*, 1996), refractive error (Mori, Hikichi, Yamaguchi, *et al.*, 2001), sex (Centofanti, Bonini, Manni, *et al.*, 2000), and pulse rate (Trew & Smith, 1991a; Yang, Hulbert, Batterbury, *et al.*, 1997). The role of altered ocular haemodynamics as a causative mechanism in the pathogenesis of eye disease has been investigated extensively, as clinical tools for the non-invasive investigation of ocular perfusion characteristics *in-vivo* have become more widely available as diasgnostic or monitoring techniques.

Glaucoma and diabetic retinopathy are diseases, which reflect both local (ocular) and systemic insufficiencies in vascular perfusion. Specifically, reductions in blood flow have been identified in the retina (Michelson, Langhans & Groh, 1996a) and lamina cribrosa (Nicolela, Hnik & Drance, 1996) of glaucoma patients. In the diabetic eye conflicting arguments exist; some authors suggest increases in ocular blood flow prior to the onset of retinopathy (Grunwald, DuPont & Riva,1996), while others have demonstrated a reduction (Yoshida, Feke, Morales-Stoppello, *et al.*, 1983; Grunwald, Riva, Sinclair, *et al.*, 1986; Schmetterer & Wolzt, 1999).

A number of techniques have been developed which enable the differential assessment of the three vascular beds (retrobulbar, choroidal and retinal). Of these, the Heidelberg Retina Flowmeter (Heidelberg Engineering, Heidelberg, Germany) facilitates the measurement of microvascular perfusion at the retina and optic nerve head, while the ocular blood flow analyser (OBFA, Paradigm Medical Industries Inc., Utah, USA) gives a measure of the pulsatile component of ocular blood flow, which although influenced by all three vascular beds, is predominantly choroidal in origin.

The following review will highlight the vascular characteristics of both normal and diseased eyes together with the principles and applications of the HRF and OBFA. For the purpose of comparative assessment, topography and visual function techniques

will be outlined. The need for further investigations into the modus operandi of these non-invasive perfusion techniques, and further consideration of both physiological and pathological studies of perfusion will be highlighted and, form the basis of the work for this thesis.

1.2 Normal ocular haemodynamics

The arteries and veins of the eye consist of three layers surrounding a central lumen. The innermost layer, the tunica interna, is comprised of the endothelium, which provides a smooth surface to minimise friction as blood travels through the vessel lumen. The central layer, the tunica media, comprises mainly muscle cells, which are controlled by the vasomotor nerve fibres of the sympathetic system resulting in either vasoconstriction or vasodilation, dependent upon the requirement of the adjacent tissues. This layer is more substantial in arteries compared to veins. The outer protective layer of a blood vessel wall is significantly thicker in the veins than it is in the arteries. The capillaries consist of an endothelial layer only to promote diffusion of nutrients to, and waste products from the surrounding tissues.

The eye consists of two distinct vascular systems: the retinal vasculature (see section 1.2.1) and the uveal vasculature (see section 1.2.2). Approximately 95% of the ocular circulation is choroidal (Alm & Bill, 1973; Claridge & Smith, 1994). Both the uvea and retinal vascular beds are supplied by the retrobulbar ophthalmic vessels, which originate from the ophthalmic artery, the only branch of the internal carotid artery outside the cranium (see figure 1.1) (Hayreh, 1962). The ophthalmic artery enters the orbit via the optic canal, which runs parallel to the optic nerve. The first part of the ophthalmic artery lies below and lateral to the nerve, the second part crosses the nerve, usually above it; and the third part lies nasal to the nerve.

1.2.1 Retinal blood supply

The retina is predominantly supplied with blood by the central retinal artery, which originates from the ophthalmic artery (Hayreh, 1962). The central retinal artery enters the optic nerve from below and approximately 10mm behind the globe and pierces the sclera through the lamina cribrosa alongside the central retinal vein. After entering the back of the eye it divides into two branches that further divide into superior, inferior,

nasal and temporal branches, each supplying blood to one quadrant of the retina (Snell & Lemp, 1998). From these branches, arterioles and capillaries develop which lie within the nerve fibre layer of the retina and supply the inner layers of the retina with the nutrients required. The photoreceptor layer of the retina is supplied by the choroid and is avascular (Bill, 1981).

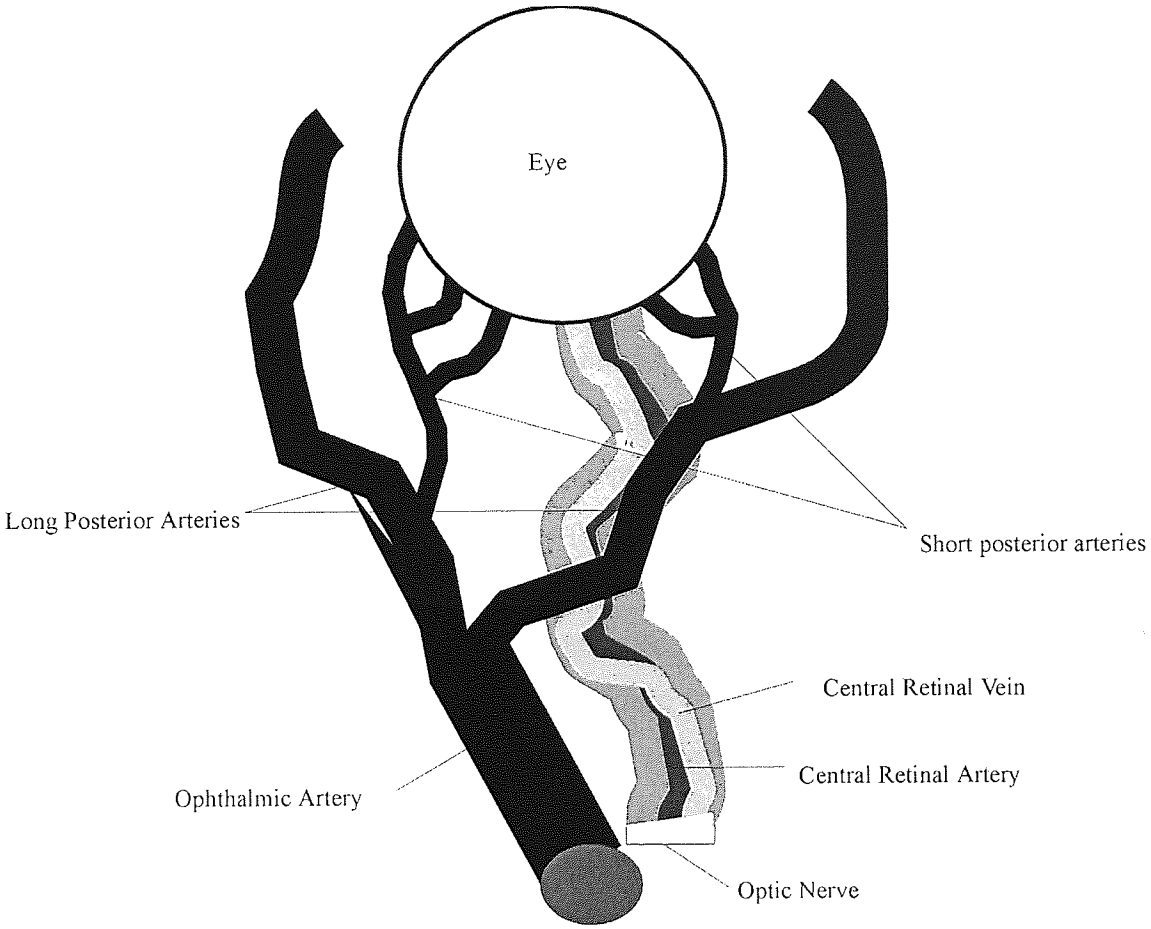

Figure 1.1. Diagram of the blood supply to the orbit (adapted from Harris, *et al.*, 1998b)

1.2.2 The uveal blood supply

The uvea consists of the ciliary body, iris and choroid. These structures receive their blood supply from the posterior ciliary arteries (Bloom & Migdal, 1995). The trunk posterior ciliary arteries originate from the ophthalmic artery and divide into 2 long and 6 – 20 short posterior arteries (Cioffi & Van Buskirk, 1994; Onda, Cioffi, Bacon, *et al.*, 1995; Harris, Cuilla, Chung, *et al.*, 1998a). The long posterior ciliary arteries supply the iris, ciliary body and anterior portion of the choriocapillaris (Bill, 1981). Some of the short posterior arteries supply the anterior optic nerve head whilst the

remainder go on to form the choriocapillaris of the choroid. The arteries become capillaries relatively quickly after piercing the sclera at the posterior pole to form the choriocapillaris.

The veins of the eye and orbit do not have valves and their contents are drained directly into the cavernous sinus. The central retinal vein communicates with the superior ophthalmic vein and the cavernous sinus, into which its contents are emptied (Snell & Lemp, 1998). The choroidal blood supply leaves the eye primarily via the vortex vein (Bill, 1981).

1.2.3 Anterior optic nerve

The superficial layers of the optic nerve head receive blood from branches of the central retinal artery and the cilioretinal artery if present (Onda, *et al.*, 1995). However, only a small percentage of the population has a cilioretinal artery, which travels from the temporal side of the optic nerve head and extends towards the macular (Bill, 1981). The prelaminar region of the optic nerve and the lamina cribrosa receive blood from the short posterior arteries (Hayreh, 1978) and from indirect branches from the circle of Zinn-Haller. In some instances blood is also supplied to these areas by the arterioles of the choroid (Cioffi & Van Buskirk, 1994). The retroocular region of the optic nerve obtains its nutrients from the central retinal artery and the pial blood vessels. The pial blood supply, situated in the pia mater (figure 1.2), consists of a network of capillaries that arise from the circle of Zinn-Haller and occasionally the short posterior ciliary arteries. Branches from this system together with branches from the central retinal artery extend to feed the optic nerve head axons with the necessary nutrients.

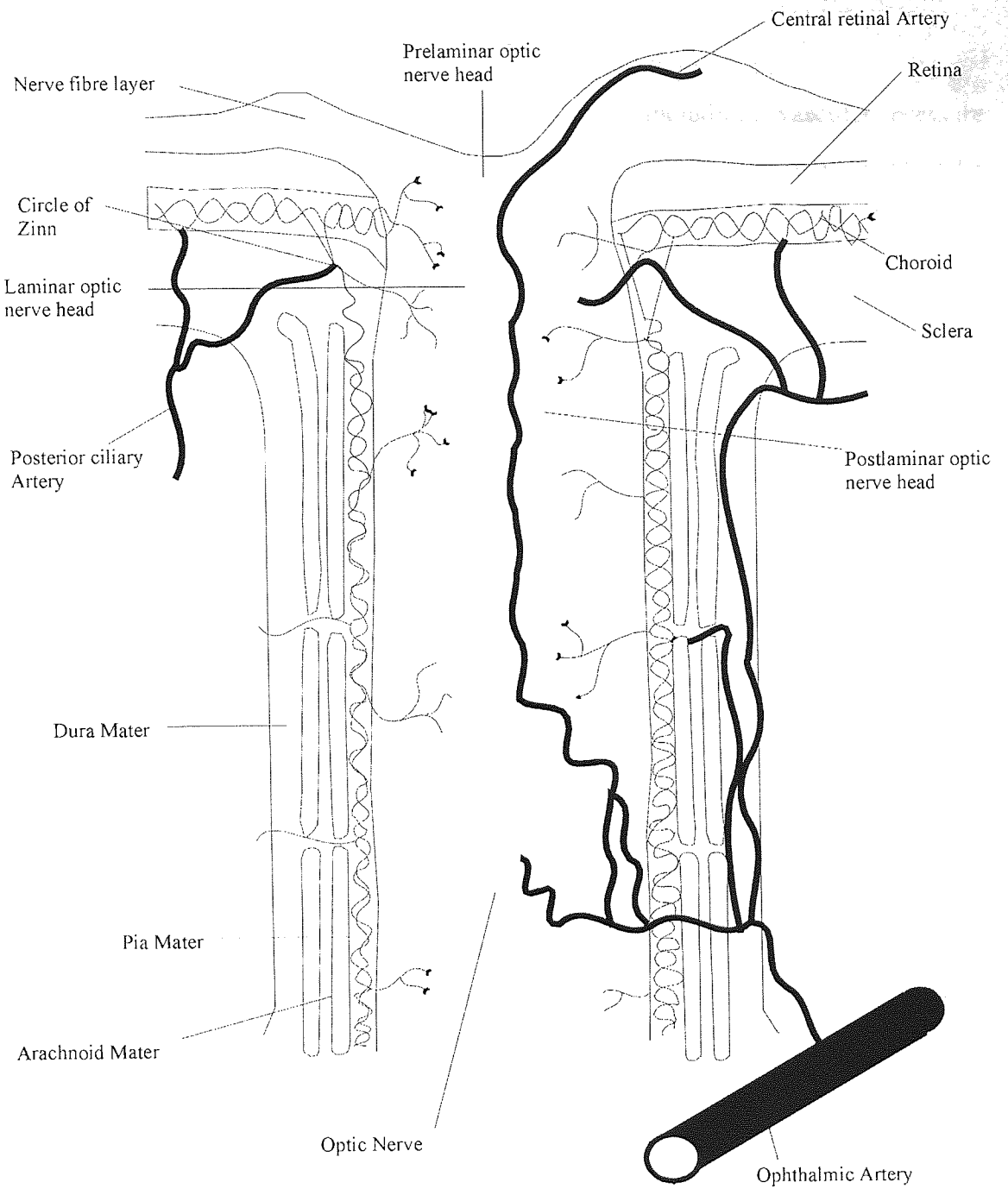

Figure 1.2. **Diagram of the optic nerve head anatomy and blood supply (adapted from Hayes, 1997)**

1.3 Ocular blood flow regulation

Blood flow is influenced by a variety of factors including, vascular pressure (Robinson, Riva, Grunwald, *et al.*, 1986; Hayreh, 1997), muscle tone, induced by vasoactive nerves (Bill, 1981), exogenous and endogenous vasoactive substances (Harris, *et al.*, 1998a), and metabolic activity (Bill & Sperber, 1990). Autoregulation may be defined as the ability of a tissue to maintain constant blood flow, capillary pressure and nutrient supply during changes in perfusion pressure (Hayreh, 1997). Autoregulation of blood flow is achieved by altering the tone of the blood vessels and hence the resistance to blood flow (Hayreh, 1997). Generally, tissues achieve vascular autoregulation via myogenic and/or metabolic mechanisms.

In myogenically controlled tissues, cells within the tissue structure, known as pericytes, detect pressure changes. The vascular endothelium regulates vascular tone, platelet activity and vascular permeability by releasing vasoactive substances that result in either vasodilation (nitric oxide) or vasoconstriction (endothelin). This results in alterations in the arteriolar tone to maintain a constant blood flow (Bill, 1981).

In metabolically controlled tissues vascular resistance is altered to maintain a constant pH, which is determined from the level of oxygen present in the tissue (Bill, 1981). Increasing levels of carbon dioxide result in a more acidic environment thereby causing the blood vessels to dilate to increase blood flow and hence oxygen levels to maintain a more alkaline pH level. The eye utilizes both myogenic and metabolic mechanisms to maintain constant blood flow (Harris, *et al.*, 1998a).

1.3.1 Ocular perfusion pressure

Perfusion pressure is the pressure that promotes blood flow through a tissue and is defined as the difference between the pressure in the arteries entering the tissue (P_a) and that of the veins leaving it (P_v) (Bill, 1981). The relationship between blood flow (BF), perfusion pressure and vascular resistance (R) is given by: -

$$BF = \frac{Pa - Pv}{R} \qquad \text{Equation 1.1}$$

Ocular perfusion pressure is defined as the ocular arterial pressure minus the intraocular pressure (Harris, *et al.*, 1998a) or two thirds of the mean arterial pressure minus the intraocular pressure (Riva, Sinclair & Grunwald, 1981). Ocular perfusion pressure can be reduced by decreasing arterial pressure or by increasing intraocular pressure, the outcome of which is reduced ocular blood flow unless vascular resistance is modified. Changes in the positioning of the body will also cause alterations in ocular perfusion pressure since arterial blood pressure reduces during periods of recumbency (Bill & Sperber, 1990). In an upright position the average pressure in the arteries entering the eye is between 60 and 70mmHg; this increases when the supine position is adopted. The pressure of the veins leaving the eye is approximately equal to the intraocular pressure (Bill, 1963). If this pressure increases, venous congestion and capillary leakage ensue.

Although the process of autoregulation controls ocular blood flow, such that it is not influenced by small changes in blood pressure, if the change is sufficiently large (40% increase or more) it falls outside the levels of autoregulatory capacity and the ocular blood flow will increase (Robinson, *et al.*, 1986).

The mechanisms of autoregulation for the retina, optic nerve head and choroid are discussed in the following sections (1.3.2 – 1.3.4).

1.3.2 Autoregulation of retinal blood flow

Retinal blood flow is autoregulated through changes in ocular perfusion pressure and this is done by altering the vascular resistance of the blood vessels within the eye to maintain a constant supply of blood (Robinson, *et al.*, 1986; Hayreh, 1997; Harris, *et al.*, 1998a). This autoregulation however, only holds true for intraocular pressures up to approximately 30mmHg (Riva, Grunwald & Petrig, 1986). Using laser Doppler velocimetry Grunwald, Riva & Kozart, (1988) showed that retinal blood flow in a patient with an intraocular pressure of 47 mmHg was reduced threefold when compared to a healthy subject with an intraocular pressure within normal limits. Riva *et al* (1981) investigated the effect increasing intraocular pressure has on retinal blood flow using the blue field entoptic phenomenon. It was found that retinal blood flow was reduced when intraocular pressure was increased to above 30mmHg. These results suggest that there is a limit to the autoregulatory capacity of the retina and that

autoregulation fails when intraocular pressure rises above a critical level; it has been suggested that this upper intraocular pressure limit lies around 30mmHg (Riva, *et al.*, 1981).

Metabolic autoregulation also influences the blood supply to tissues within the retina (Bill & Sperber, 1990). As the requirement of oxygen to the tissues increases the perfusion alters to maintain constant levels of carbon dioxide and oxygen. Retinal blood flow has been shown to increase in monkeys during episodes of flicker stimulation when metabolic demands increase (Bill & Sperber, 1990). Illumination has a metabolic effect and is known to influence blood flow; during episodes of darkness the retinal photoreceptors utilize more oxygen due to the regeneration processes that occur and retinal blood flow has been reported to increase during this period (Linseimer, 1986). More recently this has been disputed with no alteration in retinal blood flow being found during dark adaptation (Riva, Logean, Petrig, *et al.*, 2000). However immediately after dark adaptation, blood flow increased, thought to be induced by the transition from dark to light (Riva, *et al.*, 2000).

Studies carried out to investigate the effects of carbon dioxide and oxygen on retinal blood flow have added evidence that metabolic autoregulation exists within the eye. Increasing oxygen concentration (hyperoxia) has been shown to reduce retinal blood flow through vasoconstriction (Riva, Grunwald & Sinclair, 1983; Harris, Arend, Kopecky, *et al.*, 1994a; Strenn, Menapace, Rainer, *et al.*, 1997; Lietz, Hendrickson, Flammer, *et al.*, 1998). In contrast, increasing levels of carbon dioxide (hypercapnia) results in vasodilation and consequently retinal blood flow increases (Roff, Harris, Chung, *et al.*, 1999). The importance of carbon dioxide as a vasodilatory catalyst has been highlighted in investigations where vascular perfusion has been found to preferentially increase during carbogen breathing (carbon dioxide 6%, oxygen 94%) (Arend, Harris, Martin, *et al.*, 1994; Lietz, *et al.*, 1998). These findings emphasise the potent metabolic effect that carbon dioxide has.

It appears that blood flow rate is influenced by the regional distribution of vessels. Using laser Doppler flowmetry Feke, Tagawa, Deupree, *et al.*, (1989) reported an average total ocular blood flow of 80µl/min across all vessels; the rate in the temporal retinal vessels was significantly greater than in nasal vessels. Feke *et al.;* (1989) also

measured the diameter of the vessels and found that this difference in blood flow rate was accounted for by differences in the vessel diameters located in the nasal and temporal areas of the retina, the larger vessels being found temporally. This anatomical asymmetry in retinal vasculature may be due to the metabolic demand of the different areas of the retina. In particular the macula region, which is situated in the temporal retina, may require more blood due to the increased number of ganglion cells present in this area, which will result in a greater metabolic demand. A recent study by Roff *et al.*, (1999) found that under conditions of hypercapnia retinal blood flow increased and that this increase was significantly greater in the superior temporal quadrant of the peripapillary retina. This finding suggests that autoregulation of this quadrant of the retina is more efficient than that demonstrated elsewhere.

1.3.3 Autoregulation of optic nerve head blood flow

Blood flow of the optic nerve head is also autoregulated, remaining constant during changes in perfusion pressure (Pillunat, Anderson, Knighton, *et al.*, 1997; Riva, Hero, Titze, *et al.*, 1997a). As for the retina, this autoregulation breaks down when intraocular pressures exceed approximately 45mmHg (Pillunat, *et al.*, 1997). Furthermore, blood flow at the optic nerve head has been shown to decrease in response to hyperoxia (Harris, Anderson, Pillunat, *et al.*, 1996a; Langham, Michelson & Groh, 1997), and increase during episodes of hypercapnia (Harris, *et al.*, 1996a).

1.3.4 Autoregulation of choroidal blood flow

The question of whether choroidal blood flow is autoregulated is controversial. It has been suggested by some authors that little autoregulation exists within the choroid (Bill & Sperber, 1990), however this is not the general consensus. Early studies, employing animal models, demonstrated that metabolic autoregulation exists in the choroid as elevated carbon dioxide levels resulted in increased blood flow and oxygen levels resulted in reduced blood flow (Trokel, 1965). Experiments carried out on rabbits showed that blood flow in the choroid remains stable during changes in the mean arterial pressure (Kiel & Van Heuven, 1995; Kiel & Maldonado, 1997). During periods of hyperoxia fundus pulsations at the macular decrease, and these increase during episodes of hypercapnia (Schmetterer, Wolzt, Lexer, *et al.*, 1995), implying the existence of metabolic autoregulatory mechanisms within the choroid. More recent

studies on human subjects have shown that breathing of carbon dioxide results in increases in the pulsatile component of ocular blood flow (Kergoat & Faucher, 1999; Schmetterer, Dallinger, Findl, *et al.*, 2000), although no changes were observed during periods of hypoxia. Conversely, a study conducted by Roff, *et al.*, (1999) found no changes in pulsatile ocular blood flow during episodes of hypercapnia.

Myogenic autoregulation within the choroid has been demonstrated, whereby an increase in intraocular pressure results in a decrease in the pulsatile component of ocular blood flow (Quaranta, Manni, Donato, *et al.*, 1994). A more recent study by Riva, Titze, Hero, *et al.*, (1997c) demonstrated that some autoregulatory capacity of the choroid existed as choroidal blood flow remained constant when intraocular pressure was increased from 12 to 29mmHg. Furthermore, choroidal blood flow has been shown to remain constant during changes in perfusion pressure of up to 67%, but reduces when it is further increased (Riva, Titze, Hero, *et al.*, 1997b), implying autoregulatory capabilities of the choroid.

1.4 Physiological factors affecting ocular blood flow measurements

When comparing ocular blood flow values between subjects it is important to consider a number of physiological variables, these include sex (Centofanti, *et al.*, 2000), pulse rate (Trew & Smith, 1991a; Yang, *et al.*, 1997), scleral rigidity (Price, Gray & Button, 1999), axial length (Price, *et al.*, 1999; Mori, *et al.*, 2001), refractive error (Kothe, Vachon & Woo, 1992), posture (Kotche, 1994) and age (Groh, *et al.*, 1996; Ravalico, Toffoli, Pastori, *et al.*, 1996).

1.4.1 Gender

In a comparison of pulsatile ocular blood flow (POBF) measures between males and females collectively; it was found that females have a higher ocular blood flow value than males (Yang, *et al.*, 1997; Fontana, Poinoosawmy, Bunce, *et al.*, 1998). Values have been quoted as being approximately 841.9$\mu lmin^{-1}$ for females and approximately 669.9$\mu lmin^{-1}$ for males (Yang, *et al.*, 1997). One reason for this apparent difference in blood flow may be that females have, on average, a higher heart rate than their male counterparts (Yang, *et al.*, 1997). This gender-related difference in measures of POBF

has been reported in females below the age of 55 years; however, it was not present in subjects over the age of 55 years (Centofanti, *et al.*, 2000). It was suggested that the differences observed in younger subjects was due to hormonal status and that with the onset of menopause and reduction in oestrogen, blood flow in females reduces (Centofanti, *et al.*, 2000). More recently it has been found that this difference in POBF between males and females is still apparent after correction for age, refraction, intraocular pressure, pulse rate and blood pressure (Gekkieva, Orgul, Gherghel, *et al.*, 2001).

1.4.2 Biometric parameters

The effect of axial length on measures of POBF using the ocular blood flow analyser (OBFA, Paradigm Medical Industries Inc., Utah, USA) has also been investigated and it has been reported that with increases in axial length (as myopia increases), blood flow decreases (Massey, O'Brien & Crowhurst, 1996; Ravalico, Pastori, Crose, *et al.*, 1997; Mori, *et al.*, 2001; Lam, Wong, Lam, *et al.*, 2002). It has been suggested that this is not a true difference in blood flow between the two groups but a mathematical error (James & Smith, 1991). A standard axial length is used to calculate POBF measures, which is based on Gullstrands model eye (Silver & Geyer, 2000). Since eye volume is given by equation 1.2, it may be more appropriate to change this value according to the axial length of each individual's eye; carrying out ultrasound biometry prior to measurements and entering the axial length into the equation could easily achieve this.

$$\text{Volume} = 4/3\pi*(a/2)^3 \qquad \text{Equation 1.2}$$

Where a = axial length

Blood flow in the posterior ciliary arteries and central retinal artery has been assessed in patients with degenerative myopia using color Doppler imaging (Akyol, Kukner, Ozdemir, *et al.*, 1996). Similarly to measures of POBF, reduced blood velocity was reported in the myopic individuals with increases in the resistivity indexes; however, these findings may reflect the progressive nature of this class of myopia with it resulting in degenerative changes in the retina.

1.4.3 Posture

When subjects move from a sitting to a supine position, a number of vascular-related changes take place, intraocular pressure increases and POBF decreases (James & Smith, 1991; Trew & Smith, 1991a; Trew & Smith, 1991b; Kotche, 1994; Ravalico, *et al.*, 1996). This change in ocular blood flow has been demonstrated in normal, healthy subjects (Kotche, 1994), ocular hypertensives (Trew & Smith, 1991a), normal tension glaucoma patients (James & Smith, 1991) and primary open angle glaucoma patients (Trew & Smith, 1991b). When adopting a supine position from a sitting position, heart rate and systemic blood pressure decrease (James & Smith, 1991; Trew & Smith, 1991a). A decrease in heart rate results in an increase in the diastolic phase of the ocular pulse and the non-pulsatile component of blood flow thereby causing a reduction in POBF (Vachon & Kothe, 1992). It would seem reasonable therefore, to presume that POBF falls at night during periods of supine rest. However, a study on ocular hypertensive and primary open angle glaucoma patients, that investigated the diurnal variation of POBF, found that during the night intraocular pressure, pulse amplitude, blood pressure and heart rate significantly decrease but POBF remains stable (Claridge & Smith, 1994). It was hypothesized that changes in the nocturnal intraocular pressure, heart rate and systemic blood pressure in addition to other autoregulatory mechanisms; result in preservation of POBF during long periods of recumbency (Claridge & Smith, 1994).

1.4.4 Age

A number of studies have demonstrated an age-related decline in measures of ocular blood flow (Groh, *et al.*, 1996; Ravalico, *et al.*, 1996). Using the HRF reductions in the microcirculation of the retina have been reported with increasing age (Groh, *et al.*, 1996). Using the OBFA the effect of age on POBF has been investigated in the supine and upright positions (Ravalico, *et al.*, 1996). It was found that blood flow measured, with the patients sitting upright, significantly decreased with advancing age. When repeated on supine subjects the pulsatile ocular blood flow was unaffected up to the age of 50 years and then reduced with further increases in age. All participants in this study were required to be emmetropic, an important factor to consider since hypermetropia increases with advancing age and this has been shown to result in

higher ocular blood flow readings when compared to myopic patients (Massey, *et al.*, 1996).

It is known with advancing age the incidence of atherosclerosis increases and the compliance of the vasculature reduces which may explain the findings of reduced retinal and choroidal blood flow in the aged population. The reduction in POBF observed when adopting the supine position in subjects greater than 50 years of age may be explained by less efficient autoregulation of the circulatory system with increasing age and higher arterial pressures (Ravalico, *et al.*, 1996).

A more recent study did not confirm these findings of reduced POBF with advancing age (Mori, *et al.*, 2001). Unlike Ravalico *et al* (1996) multiple regressions were conducted taking blood pressure, intraocular pressure, refractive error and axial length into consideration and may explain the discrepancies found in the two studies.

1.5 Ocular diseases of vascular origin

1.5.1 Glaucoma and the eye

Primary open-angle glaucoma (POAG) is a disease in which the retinal ganglion cells and nerve fibre axons are compromised resulting in characteristic optic nerve head appearances and visual field defects (Quigley, 1993).

1.5.1.1 Risk factors in POAG

There are a number of risk factors associated with the development of POAG including: -

(1) Age – The incidence of POAG increases with advancing age (Hollows & Graham, 1966; Bankes, Perkins, Tsolakis, *et al.*, 1968). Individuals are considered to be at more risk after 40 years of age as the incidence of glaucoma is in excess of 2% in this age range of the population (Nuzzi & Cerruti, 1995). Furthermore, this risk increases with further advances in age (Bankes, *et al.*, 1968).

(2) Race – It is known that Afro-Caribbean individuals are at a greater risk from developing glaucoma when compared to Caucasians and the onset of it is more likely to occur at an earlier age (Tielsch, Sommer, Katz, *et al.*, 1991).

(3) Inheritance – There is a link between the incidence of POAG and the family history of the disorder (Tielsch, Katz, Sommer, *et al.*, 1994; Haefliger & Flammer, 1997). The odds ratio for developing glaucoma is 3.69 for those who have siblings with the disease and 2.17 for those with parents diagnosed with it (Tielsch, *et al.*, 1994).

(4) Diabetes – It is thought that diabetic patients are more likely to develop glaucoma when compared to healthy individuals (Klein, Klein & Jenson, 1994), although a more recent study was unable to confirm this theory (Ellis, Evans, Ruta, *et al.*, 2000)

(5) Hypertension – This is another systemic risk for glaucoma. A positive correlation has been found between systemic blood pressure and intraocular pressure (Carel, Korczyn, Rock, *et al.*, 1984).

(6) Hypotension – Low blood pressure is a risk factor for POAG (Hayreh, Zimmerman, Podhajsky, *et al.*, 1994a), particularly normal-tension glaucoma (Hayreh, *et al.*, 1994a). It has been suggested that in patients with underlying vascular insufficiencies the nocturnal dip in blood pressure may contribute to optic nerve head damage (Hayreh, *et al.*, 1994a).

(7) Gender – It has been reported that normal tension glaucoma is more prevalent in females compared with males (Orgul, Flammer & Gasser, 1995). In addition, intraocular pressure measurements of females are, on average, greater than those of males (Armaly, 1965; Hollows & Graham, 1966; Bankes, *et al.*, 1968).

(8) Refractive Error – It has been found that myopic individuals are at a greater risk of developing POAG (Grodum, Heijl & Bengtsson, 2001).

POAG is a chronic, slowly progressive, usually bilateral disease. Most patients with it are asymptomatic until the late stages when a significant proportion of the visual field has been compromised.

POAG is sub-divided further into two groups: High-tension glaucoma (HTG) and normal- or low-tension glaucoma (NTG/LTG).

1.5.1.2 High-tension glaucoma

HTG is defined as glaucomatous optic neuropathy in the presence of high intraocular pressure (Geijssen & Greve, 1995). The clinical features of HTG include: -

(1) Optic nerve head changes, with decreases in the size of the neuroretinal rim (Geijssen & Greve, 1995)

(2) Raised intraocular pressure (Geijssen & Greve, 1995)

(3) Abnormal fluctuations in diurnal variations of intraocular pressure, a fluctuation of 7mmHg or more is deemed highly suspicious (Katavisto, 1964).

(4) Characteristic visual field defects (Hoddapp, Parrish & Anderson, 1993).

1.5.1.3 Normal or low tension glaucoma

Normal tension glaucoma (NTG) is defined as glaucomatous optic neuropathy resulting in visual field loss with intraocular pressure being within normal limits (<22mmHg) (Hoddapp, *et al.*, 1993; Geijssen & Greve, 1995).

It has been suggested that a decrease in vascular perfusion to the optic nerve head occurs in NTG (Flammer, Haefliger, Orgul, *et al.*, 1999), resulting in damage to the optic nerve fibres, this continues, eventually leading to death of the nerve fibres with associated visual field losses.

It is known that people who suffer with vasospastic tendencies such as migraine headaches are at an increased risk of developing glaucoma, in particular NTG (Cursiefen, Wisse, Cursiefen, *et al.*, 2000). Additionally there is evidence to suggest that a large proportion of patients with NTG suffer from vasospastic reactions (Phelps & Corbett, 1985; Drance, Douglas, Wysman, *et al.*, 1988; Rojanapongpun, Drance & Morrison, 1993; Harris, *et al.*, 1994a; Broadway, Drance, Parfitt, *et al.*, 1998). Patients with vasospastic tendencies exhibit autoregulatory dysfunction where blood vessels either constrict or dilate disproportionately to the stimulus applied e.g. hot or cold, and take longer to recover to their normal state when compared to non-vasospastic individuals (Flammer, 1996). Chronic episodes of vasospasm could result in decreased perfusion to the optic nerve head resulting in nerve fibre loss (Harris, Sergott, Spaeth, *et al.*, 1994b).

Diagnosis of NTG is more difficult than HTG. Clinical features include: -

(1) Neuroretinal Rim thinning, especially inferiotemporally (Broadway & Drance, 1998; Broadway, Nicolela & Drance, 1999).

(2) Acquired optic disc pits (Broadway, *et al.*, 1999).

(3) Peripapillary crescents (Broadway & Drance, 1998; Broadway, *et al.*, 1999).

(4) Flame shaped haemorrhages near or on the optic nerve head (Broadway, *et al.*, 1999; Graham, 1999)

(5) Pallor (Broadway, *et al.*, 1999)

(6) Visual field defects (Lee, Bathija & Weinreb, 1998).

It is worth noting that visual field defects in POAG are only clinically detectable once approximately 50% of the nerve fibres have been damaged (Sommer, Katz, Quigley, *et al.*, 1991). As HTG sufferers have characteristically higher intraocular pressures diagnosis is usually achieved at an earlier stage and for this reason NTG tend to present with more advanced optic nerve fibre damage and visual field loss.

1.5.1.4 Pathogenesis of glaucoma

Two main theories exist concerning the pathogenesis of glaucomatous damage. These are outlined in the following two sections

1.5.1.5 The direct mechanical theory

In this theory raised intraocular pressure results in direct damage to the retinal nerve fibres that pass through the lamina cribrosa of the optic nerve head. The increase in intraocular pressure results in a misalignment of the lamina cribrosa pores, which interferes with axonal transport (Quigley, Guy & Anderson, 1979). Studies carried out on glaucoma patients have examined the structure of the lamina cribrosa and findings from these studies include: -

(1) Patients with moderate visual field loss have compression of the cribiform plates (Quigley *et al* 1983)

(2) Patients with advanced glaucomatous damage have bowing and posterior rotation of the lamina cribrosa (Quigley, Hohman, Addicks, *et al.*, 1983)

(3) Glaucomatous patients demonstrate a reduction in the number of collagen fibres, an increase in the number of glial cells, thickening of the basement membrane and alterations to the elastic fibres (Hernandez, 1982).

(4) Finally alterations in the lamina cribrosa pores have been reported in patients with glaucomatous optic neuropathy (Miller & Quigley, 1988)

In normal, healthy individuals the nerve fibres pass through the pores of the lamina cribrosa unhindered (Quigley, *et al.*, 1979; Quigley, Flower, Addicks, *et al.*, 1980). It is unknown whether the primary cause of damage is a result of pressure being exerted on the optic nerve axons directly or due to a decrease in the blood supply reaching the nerve fibres (Quigley, Hohman, Sanchez, *et al.*, 1985).

1.5.1.6 The vascular theory

Patients with raised intraocular pressure can continue to develop further optic nerve damage even when their intraocular pressure is within normal limits (Werner, Drance & Schulzer, 1977; Minckler & Spaeth, 1981). In addition patients suffering from NTG exhibit glaucomatous damage although their intraocular pressures are within the accepted boundaries of normal (Drance, 1992). This suggests that in addition to the mechanical theory there is another mechanism, which, can contribute to nerve fibre loss (Drance, 1992; Flammer, *et al.*, 1999).

The vascular theory for glaucomatous damage depicts that a decrease in the perfusion to the optic nerve results in a lack of nutrients reaching the nerve fibres, which in turn leads to the eventual death of the nerve fibres. It is not known whether this dysfunction in perfusion to the optic nerve is secondary to increases in intraocular pressure or is itself a primary cause of nerve fibre loss.

1.5.1.7 Ischaemia due to elevated intraocular pressure

The vascular theory may be mechanically related; an increase in intraocular pressure may result in a reduction in ocular perfusion pressure leading to alterations in the extracellular matrix and compromised optic nerve head microcirculation (Quigley, 1995). At the lamina cribrosa capillaries are situated within the connective tissue cribiform plates; independent movement of these plates, which may occur due to

increased intraocular pressure and mechanical stress, results in their misalignment and disruption and kinking of the capillaries passing through them (Quigley, 1995). The result is reduced blood flow and disruption of retrograde and orthograde axoplasmic flow of the optic nerve fibres (Quigley, 1995).

1.5.1.8 Ischaemia due to autoregulatory dysfunction

It has also been suggested that perfusion to the optic nerve fibres in glaucoma patients is compromised as a result of autoregulatory dysfunction (Alm & Bill, 1973; Grunwald, Riva, Stone, *et al.*, 1984; Pillunat, Stodtmeister, Wilmanns, *et al.*, 1985; Tielsch, Katz, Sommer, *et al.*, 1995; Evans, Harris, Garret, *et al.*, 1999a).

The autoregulatory capacity of the normal eye has been compared to that of glaucomatous eyes with the use of stress tests, these include the use of intraocular pressure modification. Pillunat *et al* (1985) showed that an autoregulatory response exists in healthy subjects when their intraocular pressure was artificially increased but that no such response was observed in the patients with glaucoma. Liu, Chou, Chou, *et al.*, (1997) investigated blood flow velocities in glaucoma patients and normal subjects with spontaneously raised intraocular pressure, and reported decreased flow velocities and increased resistivity indices in the diseased group, indicating a faulty autoregulatory response in this group to increases in intraocular pressure.

A recent study utilized color Doppler imaging to assess the retrobulbar haemodynamics of normal subjects, stable glaucoma patients and patients with progressive glaucomatous visual field defects (Gherghel, Orgul, Gugleta, *et al.*, 2000). It was found that the patients with progressive glaucomatous damage had low retrobulbar blood flow and high ocular resistance to flow when ocular perfusion pressure was low, a finding not exhibited by the stable glaucoma group and normal subjects. It was suggested that vascular dysregulation existed in the patients with progressive visual field loss resulting in nerve fibre loss (Gherghel, *et al.*, 2000).

1.5.2 Diabetes mellitus and the eye

Diabetes Mellitus is defined as a metabolic disorder in which the ability to oxidase carbohydrates is either reduced or absent, usually due to faulty pancreatic activity, in

particular the islets of Langerhans, and this results in disturbance of normal insulin mechanisms (Stogdale, 1986). There are two forms of this disease: -

1) Insulin Dependent Diabetes Mellitus (IDDM)

This subclass of diabetes is also known as type 1 diabetes. It usually manifests itself between the ages of 10 to 20 years of age although elderly patients can also acquire this form of the disease (Cudworth, 1978; Alberti & Zimmer, 1998).

2) Non-Insulin Dependent Diabetes Mellitus (NIDDM)

This second subclass of diabetes mellitus is also known as type 2 diabetes. This form of the disease is an acquired form of the disease, which usually develops between the ages of 50 to 70 years (Alberti & Zimmer, 1998).

Diabetes is known to affect the eye in the form of diabetic retinopathy. IDDM patients are at an increased risk of developing diabetic retinopathy (40%) when compared to NIDDM patients (20%). Diabetic retinopathy is the most common cause of blindness in the working population (Ferris & Patz, 1984).

1.5.2.1 Risk factors for diabetic retinopathy

1) Duration of diabetes - This is the most important factor known to influence the occurrence of diabetic retinopathy. After having the disease for fifteen years up to 98% of patients with diabetes mellitus have some degree of non-proliferative retinopathy, and after 35 years 67% progress to have proliferative diabetic retinopathy (Harper, O'Day & Taylor, 1995). This risk factor increases in patients suffering from IDDM (Cerutti, Sacchetti, Vigo, *et al.*, 1989).
2) Metabolic Control – It has been suggested that diabetic patients that exhibit poor metabolic control are morely likely to develop diabetic retinopathy (Ferris, 1993; Porta, 1993; Yoshida, Hagura, Hara, *et al.*, 2001).
3) Other Factors – The onset of diabetic retinopathy can also be influenced by pregnancy, systemic hypertension, renal disease and anaemia.

1.5.2.2 Classification of diabetic retinopathy

Diabetic retinopathy is classed as one of three types: background, preproliferative and proliferative diabetic retinopathy.

1.5.2.3 Mild, nonproliferative retinopathy

(1) Microaneurysms – are the first signs of background diabetic retinopathy. They are situated within the inner nuclear layer of the retina.

(2) Intraretinal haemorrhages – are another sign of background diabetic retinopathy and can be in either dot or blot formation. They originate from the venous end of the capillaries and are located within the middle layers of the retina

(3) Hard Exudates – have a yellow, waxy appearance and are usually arranged in a circinate or radial pattern surrounding an area of chronic focal leakage, which usually occurs from a microaneurysm. The exudates are situated between the inner plexiform layer and inner nuclear layer of the retina.

(4) Retinal/Macular Oedema – In its early stages retinal oedema is situated between the outer plexiform and inner nuclear layers, with time the inner plexiform and nerve fibre layers may become involved eventually leading to all the retinal layers becoming oedematous. Retinal oedema is characterised by retinal thickening, which obscures the underlying retinal pigment epithelium and choroid and is usually diagnosed with fluorescein angiography. Diabetic patients with retinal oedema are at risk from developing macular oedema where fluid accumulates beneath the fovea and the area assumes a convex or cystoid form (Ferris & Patz, 1984). Clinically significant macular oedema is defined as the presence of any retinal thickening or hard exudates within one disc diameter (approximately 1500μm) from the centre of the fovea (Ferris & Patz, 1984). Patients with clinically significant macular oedema may be treated using laser photocoagulation, which is thought to reduce the risk of further visual impairment (Ferris & Patz, 1984).

1.5.2.4 Moderate to severe nonproliferative retinopathy

This stage of diabetic retinopathy results from retinal ischaemia and the clinical signs of it include: -

(1) Changes to the venous vasculature – these alterations include beading, looping and segmentation of the venous blood vessels in a 'sausage like formation' (Ferris, 1993).

(2) Blot haemorrhages – these haemorrhages are dark red in colour and represent haemorrhagic retinal infarcts. They may be present with or without microaneurysms (Ferris, 1993).

(3) Intraretinal Microvascular Abnormalities – Capillary closure occurs in the retinal nerve fibre layer and results in the formation of cotton wool spots, further to this formation of intraretinal microvascular abnormalities (IRMA) in adjacent areas of the retina can occur (Ferris, 1993).

1.5.2.5 Proliferative Diabetic Retinopathy

This is advanced diabetic retinopathy. Approximately 5 to 10% of the diabetic population are affected by it (Ferris, 1993), and of these patients the majority are insulin dependent diabetics.

Clinical features of proliferative diabetic retinopathy include: -

(1) Neovascularisation – this may occur at the optic nerve head (NVD = new vessels at disc), within one disc diameter of the optic nerve head (Ferris, 1993) or along the course of the major temporal vascular arcades (NVE = new vessels elsewhere), greater than one disc diameter away from the optic nerve head (Ferris, 1993). The new vessels usually originate from veins and pass through the internal limiting membrane to the vitreoretinal space (Ferris, 1993). The new vessels are prone to haemorrhage that can fill the vitreous cavity (Ferris, 1993).

(2) Vitreous detachment – the fibrous new vessels within the vitreoretinal space can adhere to the posterior vitreous and leak plasma into the adjacent vitreous gel. This can lead to partial tractional vitreous detachment (Ferris, 1993).

(3) Haemorrhage – this can occur into the vitreous gel to form an intragel haemorrhage or more frequently, into the retrohyaloid space to form a preretinal haemorrhage.

1.5.2.6 Pathogenesis of diabetic retinopathy

Diabetic retinopathy is a microangiopathy, which affects the retinal precapillary arterioles, capillaries and venuoles. The features of diabetic retinopathy are of microvascular occlusion and leakage

1.5.2.7 Microvascular occlusion

It has been postulated that microvascular occlusion is caused by vessel compression due to swollen extravascular cells, changes in blood including aggregation of erythrocytes and excessive platelet production, and changes in the vessel wall and its cells (Engermann, 1989). These changes result in non-perfusion of the retinal capillary bed, which leads to retinal ischaemia and causes retinal hypoxia. Retinal hypoxia results in arteriovenous shunts and neovascularisation (Engermann, 1989). The formation of arteriovenous shunts occurs in response to the closure of capillaries and these run from the venules to the arterioles and are known as intraretinal microvascular abnormalities (IRMA). Neovascularisation occurs in response to hypoxia of the retina and is activated by a vasoformative substance in an attempt to revascularise hypoxic areas of the retina. Neovascularisation can occur on the retina and optic nerve head (proliferative diabetic retinopathy) and on the iris (rubeosis iridis) (Ferris, 1993).

1.5.2.8 Microvascular Leakage

Retinal capillaries are made up of endothelial cells and pericytes (Bron, Tripathi & Tripathi, 1997). The tight junctions of the endothelial cells make up the inner blood-retinal barrier. The pericytes surround the capillaries and are thought to be responsible for the structural integrity of the blood vessel wall (Frank, 1991). In normal, healthy individuals each endothelial cell contains a pericyte, however in patients with diabetes mellitus there is a decrease in the number of pericytes (Engermann, 1989; Frank, 1991). This reduction in the number of pericytes results in distension of the capillary walls and microaneurysms form (Engermann, 1989), these can either leak or become thrombosed.

1.6 Measurement of ocular blood flow

Choroidal, retinal and optic nerve blood flow can be measured using a variety of techniques. An interpretation of choroidal blood flow can be obtained indirectly using the ocular blood flow analyser (OBFA; Paradigm Medical Industries Inc., Utah, USA). Microvascular retinal and optic nerve head blood flow can be assessed

invasively using Fluorescein Angiography and measured non-invasively using the Heidelberg Retina Flowmeter (HRF; Heidelberg Engineering, Germany). Color Doppler Imaging (CDI) can be used to assess blood flow in the retrobulbar and large retinal arteries. The research presented in this thesis is based on measurements obtained using the OBFA and HRF; therefore discussion will be focused on these techniques.

1.6.1 Ocular blood flow tonometry

Blood flow into the eye comprises pulsatile and non-pulsatile components arising from the systolic and diastolic parts of the ocular pulse. Blood flow during systole increases and decreases during diastole. In the OBFA outflow is assumed to be constant (Riva, Grunwald, Sinclair, *et al.*, 1985). Blood reaching the optic nerve head only accounts for a small percentage of the total ocular blood flow. The measurement of pulsatile flow is important as any decrease in total ocular blood flow will include that to the optic nerve head, resulting in low perfusion of the nerve head (Spry, 1996). Furthermore POBF is an index of choroidal flow; the posterior ciliary arteries supply the choroidal circulation and these are the main blood supply to the anterior optic nerve (Onda, *et al.*, 1995). The total amount of blood flow to the eye is in the region of 900μl/min (Langham, Farrell, O'Brien, *et al.*, 1989a) and the pulsatile component of this has been quoted as varying between 590μl/min (Silver, Farrell, Langhan, *et al.*, 1989) and 724μl/min (Langham, Farrell, O'Brien, *et al.*, 1989b).

The OBFA is a contact tonometer that utilizes air to applanate the cornea and derives a measurement of the pulsatile component of ocular blood flow. This is achieved using a mathematical model of the relationship between pressure and volume (Krakau, 1995). Given that approximately 95% of the ocular circulation is choroidal (Alm & Bill, 1973; Claridge & Smith, 1994), the OBFA is said to predominantly measure choroidal blood flow (Langham, *et al.*, 1989a), with a minor influence from the retinal and optic nerve head circulation.

The instrument comprises a sensor which is covered with a silastic membrane, behind which is a central chamber filled with pressurized air. The air exerts force on the membrane and deforms the cornea (Silver & Farrell, 1994). The amount of pressure

required to deform the cornea varies according to the elastic properties of the cornea (Silver & Farrell, 1994). When the area of corneal contact equals the cross-sectional area of the central chamber of the sensor, an initial inflection is recorded; this inflection reflects intraocular pressure and the force required to deform the cornea. As the corneal contact area enlarges, the bending force is transferred to the face of the nozzle and the pressure readings fall to a trough; it is this value that is recorded as the intraocular pressure.

The OBFA may be used to measure intraocular pressure continuously (Shields, 1998). A single recording with the OBFA takes approximately 7 to 10 seconds encompassing several complete cardiac cycles. During this time, over 200 individual intraocular pressure measurements are obtained each second, resulting in a waveform. The intraocular pulse amplitude is related to that of the arterial pulse and gives a measure of the influx of blood into the eye with each heartbeat. The eye's rigidity and resistance to expansion gives rise to a cyclic variation in intraocular pressure otherwise known as the ocular pulse. The relationship between the change in ocular volume and ocular pulse at a given heart rate facilitates an estimation of the pulsatile component of ocular blood flow (Langham, *et al.*, 1989a; Silver, *et al.*, 1989). Consequently the amplitude and shape of the intraocular pulse reflects the pulsatile component of ocular blood flow.

Certain assumptions are made in order to calculate POBF, which include: -

(1) The change in intraocular pressure is related to the change in volume induced by the flow of blood into the eye with each pulse (Silver & Farrell, 1994; Williamson & Harris, 1994).

(2) Retrograde blood flow does not occur (Silver & Farrell, 1994; Williamson & Harris, 1994).

(3) The outflow of blood is constant and non-pulsatile (Silver & Farrell, 1994; Williamson & Harris, 1994; Krakau, 1995).

(4) The formulas for the calculation of pulsatile blood flow from the pressure changes are valid and assume that blood vessels do not collapse and are elastic (Silver & Farrell, 1994; Williamson & Harris, 1994).

(5) The inflow into the eye over a stroke period is equal to the outflow (Michelson & Groh, 1994).

(6) The relationship between the change in ocular volume and change in intraocular pressure is described by a logarithmic relation; because the variation in intraocular pressure over a pulse cycle is very small, the approximation is made that the relation is linear in this small range (Michelson & Groh, 1994).

(7) The pulsatile component of ocular blood flow is zero at some point in the pulsation (Michelson & Groh, 1994).

There are two methods available to capture ocular pulse information using the OBFA. The first method calculates the average blood flow value from five adequate pulses rated from 1 to 5 dependent on their quality. This is the most common way in which clinical data is acquired. This means that the pulses can be separated by time and spread over a twenty-second period (figure 1.3). Using the second method, pulses are recorded continuously, over a ten second period and intraocular pressure, pulse amplitude, pulse volume and POBF measures are provided for each individual wave captured.

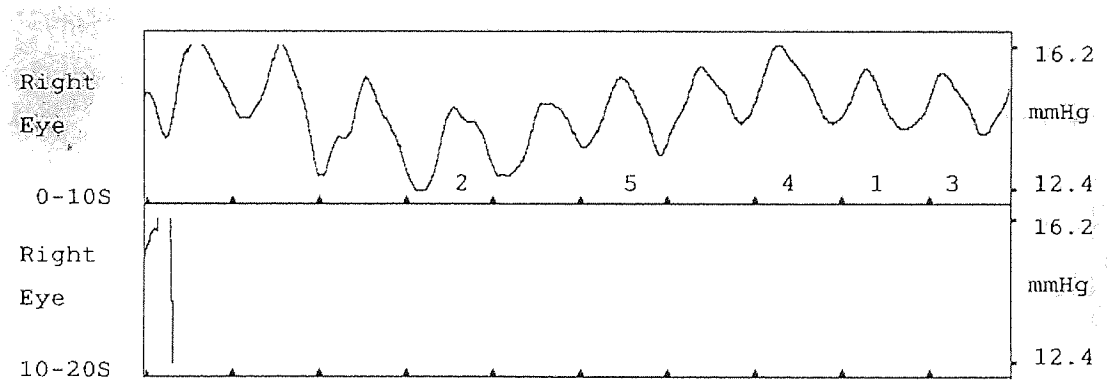

Figure 1.3. **Figure of the intraocular pressure wave measured using the OBFA**

1.6.1.1 Validity of the OBFA

POBF is a theoretical measurement of the pulsatile component of ocular blood flow and therefore direct measures of its validity are impossible. POBF is, however, determined directly from intraocular pressure and this can be evaluated. Studies have investigated the measurement of intraocular pressure using the OBFA and Goldmann

tonometer (the Gold standard for intraocular pressure measurement) and have found that they compare favourably with one another (Pianka, Geyer, Naftaliev, *et al.*, 1998; Spraul, Lang, Ronzani, *et al.*, 1998). Changes in the volume of the eye relate to changes in intraocular pressure and it is this pressure volume relationship that forms the basis for the calculation of pulsatile ocular blood flow. Manometric studies have resulted in the validation of this pressure-volume equation (Eisenlohr, Langham & Maumenee, 1962). Finally the validity of the formula used to calculate values of POBF has also been confirmed (Silver & Farrell, 1994). It would therefore seem that the measurement of ocular blood flow using the OBFA is theoretically sound, subject to artefacts that may be induced due to assumed biometric constants used for the determination of eye volume.

1.6.1.2 The pressure/volume relationship and calculation of pulsatile ocular blood flow

During the cardiac cycle the volume of the eye alters and these volume changes relate to fluctuations in intraocular pressure (Friedenwald, 1937; Eisenlohr, *et al.*, 1962). This pressure/volume relationship is described by the equation below (Friedenwald, 1937)

$$P_1 = P_0 e^{k\Delta V} \qquad \text{Equation 1.3}$$

Where:

P_1 = Final pressure

P_0 = Initial Pressure

ΔV = Change in Volume

k = Constant proportional to the average intraocular pressure

When log P_1 is plotted against ΔV a linear relationship results that has been validated on human living eyes (Eisenlohr, *et al.*, 1962). It was suggested that in living eyes this relationship was not linear but infact curved due to variations in ocular rigidity that occur with changes in intraocular pressure (Eisenlohr, *et al.*, 1962). POBF is calculated from very small changes in intraocular pressure over a given pulse cycle and therefore the pressure/volume relationship is assumed to be linear (Kraukau, 1992) and is described as:

$$(V_1 - V_2) = k(IOP_1 - IOP_2)$$ Equation 1.4

Where:

V_1 = Initial ocular volume

V_2 = Final ocular volume

k = constant

IOP_1 = Initial intraocular pressure

IOP_2 = Final intraocular pressure

This equation enables changes in ocular volume to be determined from variations in intraocular pressure, which are directly measured by the OBFA. POBF is then calculated using the following equation:

$$POBF = k \times f \times (\Delta V)$$ Equation 1.5

Where:

k = constant related to the length of time and period of the pulse

f = pulse rate

ΔV = Change in volume

1.6.1.3 Reproducibility of the OBFA

The reproducibility of the OBFA has been validated by a number of investigators (Butt & O'Brien, 1995; Yang, *et al.*, 1997; Spraul, *et al.*, 1998). The intra-visit reproducibility has been reported as varying between 92% and 83% (Yang, *et al.*, 1997; Spraul, *et al.*, 1998). The inter-visit reproducibility has been reported as being 70% (Spraul, *et al.*, 1998). Yang and associates (1997) stated that the OBFA makes reproducible measurements over short periods of time, but found that there was a high interindividual variation in healthy subjects making comparisons between subjects difficult. This may have implications when trying to identify blood flow differences between subject groups, as a considerable overlap in blood flow measures exists between subject groups.

1.6.2 The Heidelberg Retina Flowmeter

The Heidelberg retina flowmeter (HRF) is a scanning laser Doppler flowmeter (SLDF) that allows the non-invasive measurement of retinal capillary blood flow, volume and velocity.

1.6.2.1 Principles of the HRF

This SLDF uses the optical Doppler effect (Michelson, Schmauss, Langhans, *et al.*, 1996b). The Doppler effect is the shift in frequency of waves reflected from moving objects such as moving red blood cells. Light emitted by an infrared laser is directed onto the retina and is reflected or scattered by moving red blood cells. The reflected light is frequency shifted due to the optical Doppler effect (Bonner & Nossal, 1990; Nicolela, Hnik, Schulzer, *et al.*, 1997) and interferes with light reflected by stationary objects, which maintains the same frequency as the original source. This results in temporal variations of the reflected light intensity that can then be measured and used to calculate flow, volume and velocity at a given point. The shift in frequency that results from the moving blood cells is proportional to the velocity of the moving object relative to the speed of the wave. The Doppler shift can be calculated using the following equation: -

$$\Delta F = (2\pi/\lambda)(K_s - K_i) \times V \qquad \text{Equation 1.6}$$

Where:

ΔF = Frequency shift

λ = Wavelength of the incident light

K_i and Ks = vectors for the incident and scattered light respectively

V = velocity vector of the moving red blood cell

(Riva, Harino, Petrig, *et al.*, 1992)

The red blood cell velocity is a very small fraction of the speed of light and therefore the resulting Doppler shift cannot be measured directly. The frequency of light reflected off of stationary objects and the light reflected off of the moving blood cells, which is frequency shifted are combined and the resultant waveform demonstrates a

beat frequency. The frequency of the resultant waveform is equal to the difference between the two light components and hence the Doppler frequency shift of the moving blood corpuscles. A photodiode detector records the intensity of the beat frequency (figure 1.4).

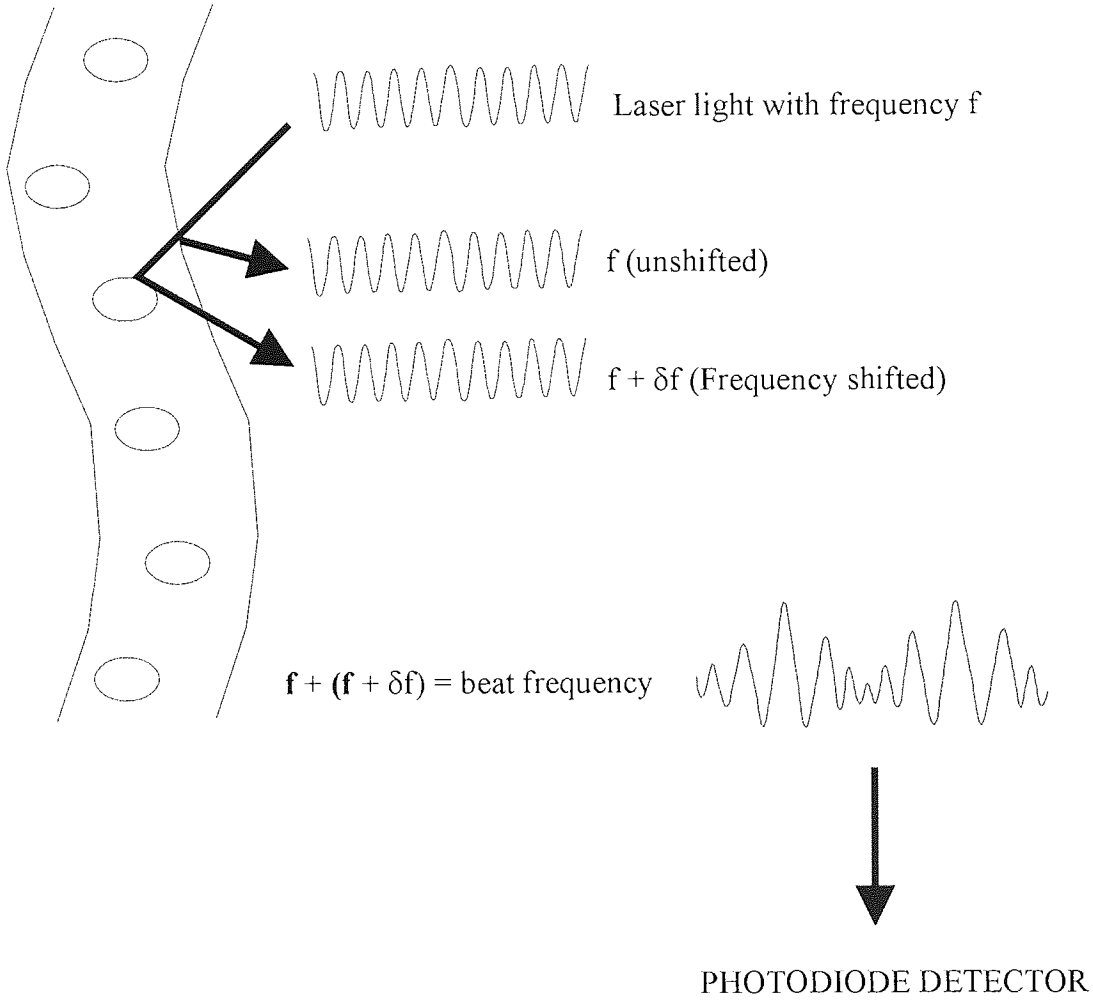

Figure 1.4. **Schematic of the Doppler shift utilized by the Heidelberg Retina Flowmeter to obtain measures of blood flow, volume and velocity (adapted from Roff, 1999)**

The parameters of flow, volume and velocity are determined from the Doppler shifted power spectrum and are calculated using the following equations: -

$$w = \frac{2\pi\left(\sum_{125}^{2000\,Hz} fP(f)df\right)}{P(f=0)}$$

Equation 1.7

Where:

w = flow

P(*f*)*df* = power of the photodiode detector current associated with a frequency range *df* about f (Hz)

P(*f*=0) = DC power in the detected signal

$$x = \frac{2\pi\left(\sum_{125}^{2000\,Hz} P(f)df\right)}{P(f=0)}$$ Equation 1.8

Where:

x = volume

P(*f*)*df* = power of the photodiode detector current associated with a frequency range *df* about f (Hz)

P(*f*=0) = DC power in the detected signal

$$y = w/x$$ Equation 1.9

where:

y = velocity

w = flow

x = volume

(Michelson & Schmauss, 1995)

The values of blood flow, volume and velocity are in arbitrary units.

The size of the retinal field measured can be varied from 10 × 2.5 degrees to 20 × 5 degrees. Measurement of the temporal variation occurs for 64 rows of 256 pixels in the field (Michelson, Welsenbach, Pal, *et al.*, 1998b) and each row is scanned 128 times with a scan frequency of 4000 Hz. Two-dimensional perfusion maps are

obtained, from which values of velocity, flow and volume can be extracted (figure 1.5). The time taken to take any one measurement is two seconds (Michelson & Schmauss, 1995) and pupil dilation is not required (Nicolela, *et al.*, 1996). Every value of perfusion is expressed as a colour, for each pixel location, the lighter the colour the faster the blood corpuscles move at that retinal point. Measurements of blood flow parameters using the HRF are restricted to those of the retinal capillaries. This is because the line sampling frequency is 4000 Hz, which limits the detection of Doppler frequencies to less than 2000 Hz and blood velocity measurements in the order of 1mm/sec in the direction of measurement. Frequencies of less than 125 Hz are excluded from the analysis of the perfusion images, known as zero-offset, to limit the effects due to influence of movement such as breathing and the cardiac cycle (Michelson & Schmauss, 1995).

The utilization of the confocal scanning laser system enables the laser beam to be focused on superficial or deep layers in the prelaminar area of the optic nerve head. An imaged layer has a thickness of 300μm and therefore only light reflected from 300μm of this imaged layer is detected (Michelson & Schmauss, 1995). This is due to the optical properties of both the eye and the HRF. The two-dimensional perfusion map that results reveals the intricate capillary network that is normally not observable in a standard fundus photograph. Following fast Fourier transformation the computer displays six images, including a reflectivity image and perfusion images for flow, volume and velocity (figure 1.5).

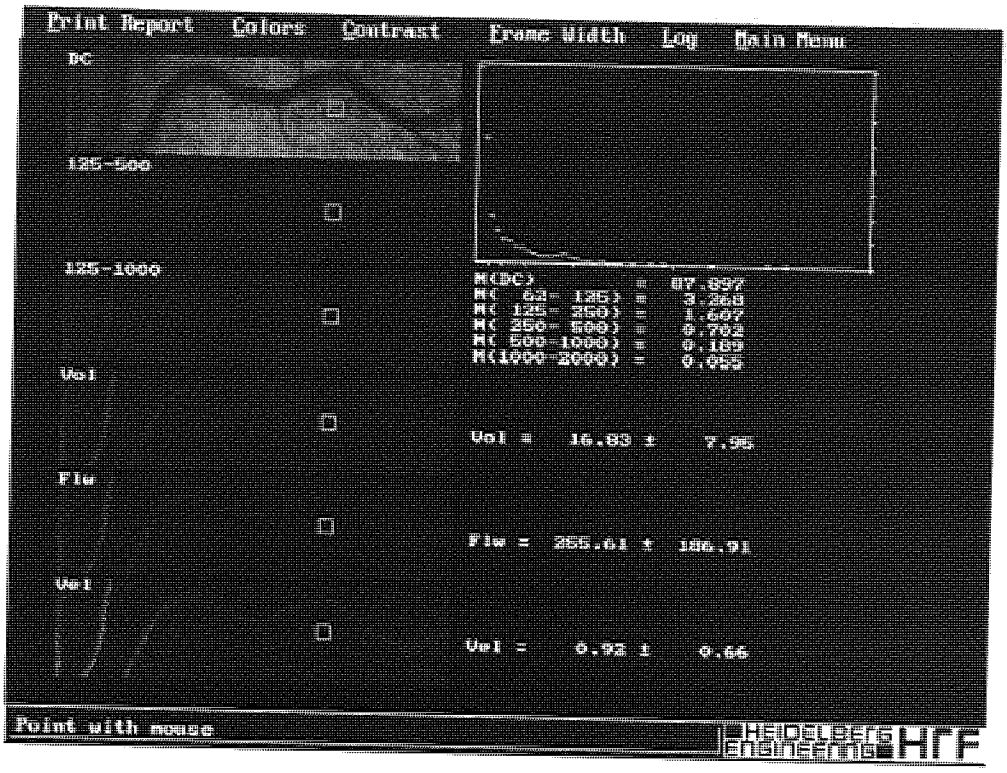

Figure 1.5. **Typical perfusion map displayed by the Heidelberg retina flowmeter**

To obtain values of blood flow, volume and velocity an area of interest has to be chosen by the operator. The area chosen can vary from 1 × 1 pixel (10 μm^2) to 50 × 50 pixels (2500μm^2) and is located according to the area of interest using a cursor. It has been recommended that the optimal measurement frame size is 10 × 10 pixels (approximately 100μm^2) as this size allows avoidance of avoids large vessels which would result in unrealistically high measures of blood flow (Michelson & Schmauss, 1995). When obtaining values of blood flow, volume and velocity it is very important that the large blood vessels are avoided and in addition, that placement of the measurement frame is free from any micro saccades that may occur during image acquisition. Once the measurement frame has been placed at the desired location the mean value ± standard deviation is given for the three blood flow parameters in arbitrary values. It is important to note that the standard deviation values given reflect the pulsatile nature of blood flow and are not mathematical derivations. In addition a DC or reflectance value is given, it has been recommended to only use images that have a DC value of between 80 and 150 (Michelson & Schmauss, 1995), more recently this recommended range has been confirmed as being between 70 and 150 (Hosking, Embleton, Kagemann, *et al.*, 2001b). It is important to control for the DC

value as changes in photodiode sensitivity may mimic or mask true changes in ocular haemodynamic indices measured using the HRF (Hosking, *et al.*, 2001b).

1.6.2.2 Validity of the HRF

Many investigators have justified the validity of the HRF. It has been investigated in an *in-vitro* model by Chauhan & Smith (1997). They found that the operational velocity range was between 0.8 to 1.0 mm/sec, which is in agreement with the restricted Doppler frequency range accepted for analysis as stated by Michelson & Schmauss (1995).

The effects of hypoxia and hypercapnia on retinal blood flow using the HRF have also been investigated (Strenn, *et al.*, 1997; Lietz, *et al.*, 1998). It was found that blood flow measurements using the HRF responded to changes in gas perturbation such that reduced retinal blood flow measures were observed during hyperoxia and increased measures during hypercapnia. Langham *et al.*, (1997) conducted a similar study on smokers and non-smokers and comparable results were found.

1.6.2.3 Reproducibility of the HRF

The reproducibility of the HRF has been investigated. Michelson & Schmauss (1995) assessed the intra-visit reliability of the HRF and it was found that the coefficients of reliability for retinal flow, volume and velocity were 0.84, 0.85 and 0.84 respectively. Chauhan & Smith (1997) conducted a similar experiment and reported the variability in blood flow measures using the HRF to be slightly higher with coefficient of reliability ranging from 0.89 to 0.86.

In addition to the intra-visit reproducibility it is important to consider the inter-visit reproducibility of the HRF; this is especially important for long-term follow up. Surprisingly, a study that assessed inter-visit reproducibility over a five-day period reported similar values to the intra-visit reproducibility, with reliability coefficients for retinal flow, volume and velocity being 0.82, 0.81 and 0.83 respectively (Michelson, *et al.*, 1996b). Nicolela *et al.*, (1997) investigated the inter-visit reproducibility of SLDF measurements over three visits on normal and glaucomatous patients using three different anatomical areas, the retina, neuroretinal rim and lamina

cribrosa and found the variability to range between 18.8% to 25.22% with the least amount of variability being exhibited at the retina. A more recent study by Kagemann, Harris, Chung, *et al.*, (1998a) investigated both the intra- and inter-visit reproducibility of HRF measures and found that the variability in inter-visit reproducibility was greater than that for the intra-visit reproducibility, with the former being 30% and the latter 7%. This finding is more expected than the previous values reported on intervisit reproducibility; one would expect variability between visits to exceed that of within visits.

There are a number of factors that affect measurements of blood flow using the SLDF. These include eye movements during image acquisition, which result in micro saccades in the perfusion map, and hence unstable blood flow readings (Michelson & Schmauss, 1995). In addition, it is important that the distance between the camera and the patient is maintained at a constant distance as this has been shown to affect reproducibility of HRF measurements (Kagemann, *et al.*, 1998a). Other factors that are known to effect HRF measures include cardiac cycle (Sullivan, Cioffi, Wang, *et al.*, 1999), zero-offset (Chauhan & Smith, 1997) and positioning of the measurement frame (Kagemann, *et al.*, 1998a; Michelson, *et al.*, 1998b).

During the cardiac cycle blood flows in and out of the eye, this results in a non-uniform or pulsatile flow of blood in the eye over time. Image acquisition using the HRF takes approximately 2 seconds and incorporates between one and two cardiac cycles; therefore two different images taken at different points in the cardiac cycle are likely to contain variable blood flow readings. The incorporation of pulse synchronisation has been shown to minimise this variability (Michelson, *et al.*, 1998b). It has been found that the alternating bright and dark bands observed in any one-perfusion map reflect the pulsatile nature of capillary blood flow (Sullivan, *et al.*, 1999). Hence pulse synchronisation will ensure that in subsequent images the positioning of these bands remains constant, thereby reducing fluctuations in measurements that may occur due to physiological alterations in blood flow.

Another potential source of variability in HRF measurements is the zero-offset. *In-vitro* models have shown that when stationary particles are measured using the HRF the velocity is not equal to zero (Chauhan & Smith, 1997); this is due to background

noise (Michelson & Schmauss, 1995). It is impossible to measure the zero-offset, but fortunately this does not pose a problem when comparing measurements for the same patient; it may however result in increased variability when comparing blood flow values between patients and should therefore be considered (Chauhan & Smith, 1997).

The illumination level of the HRF, which can be altered manually on the control panel, can also add an element of variability to blood flow measures using the HRF (Hosking, *et al.*, 2001b; Jonescu-Cuypers, Chung, Kagemann, *et al.*, 2001; Kagemann, Harris, Chung, *et al.*, 2001). Kagemann *et al.*, (1998a) showed that low illumination levels, which lead to underexposed images, result in an underestimation of blood flow parameters. This variability can be kept to a minimum by keeping images within and between patients at a constant DC level; a range of between 70 to 150 DC has been recommended (Hosking, *et al.*, 2001b).

It is very important that the repositioning of the measurement frame is placed at the same anatomical area in subsequent images of the same patient to minimise variability (Michelson, *et al.*, 1998b). In practice this is difficult as the capillary bed is not stationary and pulsates with the cardiac cycle. A landmark is usually chosen such as a bifurcation of a blood vessel to keep this variability to a minimum (Michelson & Schmauss, 1995; Langham, *et al.*, 1997; Nicolela, *et al.*, 1997). One way of eliminating this source of variability is by using a different method of analysis, which includes every pixel within the image (Michelson, *et al.*, 1998b; Chung, Harris, Kagemann, *et al.*, 1999). This method incorporates software in which blood measurements from the entire image are only included if they have a DC value within a specified range and are located in areas free from micro saccades and large vessels (Michelson, *et al.*, 1998b).

There are many ocular diseases in which ocular blood flow is affected, including age related macular degeneration (Remsch, Lang & Lang, 1999; Remsch, Spraul, Lang, *et al.*, 2000) and proliferative diabetic retinopathy (Findl, Dallinger, Ransi, *et al.*, 2000a). Both of these conditions cause changes in ocular blood vessel formation, and therefore in ocular blood flow. The investigation of blood flow is of interest in relation to the diagnosis, progression, recovery, and treatment of many diseases.

1.6.3 Clinical Application of the OBFA and HRF in disease

1.6.3.1 Glaucoma

The OBFA has been utilized to assess POBF in HTG patients (Trew & Smith, 1991b), NTG glaucoma patients (James & Smith, 1991; Quaranta, *et al.*, 1994; Ravalico, Pastori, Toffoli, *et al.*, 1994; Fontana, *et al.*, 1998) and ocular hypertensives (Trew & Smith, 1991a). Overall POBF values were found to be lower in patients with HTG patients when compared to ocular hypertensives (Trew & Smith, 1991a), and significantly lower in NTG patients when compared to age matched normal subjects (James & Smith, 1991; Ravalico, *et al.*, 1994; Fontana, *et al.*, 1998).

Furthermore, it has been shown that increases in intraocular pressure result in greater reductions in ocular blood flow in NTG patients than are observed in normal, healthy subjects (Quaranta, *et al.*, 1994), suggesting autoregulatory dysfunction in these patients in response to alterations in intraocular pressure. A study conducted by Ravalico *et al.*, (1994) reported lower POBF values in NTG patients when compared to normal controls. However, a 30mmHg increase in the intraocular pressure resulted in a similar drop in blood flow values for the two groups (Ravalico, *et al.*, 1994). If the lower blood flow observed in NTG patients was due to a dysfunctional autoregulatory system, one would expect the drop in POBF seen with increases in intraocular pressure to be more pronounced in the diseased patient group than with the healthy subjects. The absence of this result may be due to the increase in intraocular pressure of 30mmHg being outside the autoregulatory capacity for both groups, thus resulting in similar drops in the recorded ocular blood flow values (Quaranta, *et al.*, 1994).

Michelson *et al.*, (1996a) compared the retinal and optic nerve head blood flow of glaucoma patients and age-matched normal subjects using the HRF. They found reduced blood flow in both the juxtapapillary retina and neuroretinal rim of glaucomatous patients when compared with the control group. An additional finding was that measures of the cup: disc ratio and optic nerve head blood flow were inversely proportional. Other investigators have also found reduced ocular blood flow at the neuroretinal rim of glaucomatous patients (Riva, *et al.*, 1992; Bohdanecka, Orgul, Meyer, *et al.*, 1999). Nicolela *et al.*, (1996) compared blood flow measures in

glaucoma patients and normal subjects using the HRF. Similarly to Michelson *et al.*, (1996a) a reduction in the peripapillary retinal blood flow was found in addition to a decrease in the lamina cribrosa blood flow, however, no reduction in blood flow was apparent at the neuroretinal rim of the glaucomatous subject group.

More recently investigations utilizing the scanning laser Doppler flowmeter (SLDF) have revealed blood flow deficits at the neuroretinal rim and cup of primary open-angle patients compared to age-matched normal subjects (Findl, Rainer, Dallinger, *et al.*, 2000b), the finding of reduced neuroretinal blood flow was confirmed using laser interferometry. In addition to these findings choroidal blood flow was reported as being reduced at the macula of the POAG patients using laser interferometry (Findl, *et al.*, 2000b). The reductions in blood flow were found to correlate with the degree of visual field defects (Findl, *et al.*, 2000b), a finding that contradicts those made by Michelson, Langhans, Harazny, *et al.* (1998a).

Investigations using the HRF have shown that artificial elevation of intraocular pressure with the utilization of a suction cap, results in reductions in retinal blood flow (Pillunat, *et al.*, 1985; Lietz, *et al.*, 1998). This helps to explain the reduction in ocular perfusion pressure observed in patients with POAG with raised intraocular pressure. However, blood flow reductions have also been observed in normal tension glaucoma patients where no elevation in intraocular pressure exists (Chung, *et al.*, 1999), suggesting a possible autoregulatory dysfunction in the aetiology of normal tension glaucoma.

1.6.3.2 Diabetes

Diabetes is a vascular disease, which primarily affects the small blood vessels of the body and some of the smallest blood vessels are found in the eye.

Geyer, Neudorfer, Snir, *et al.*, (1999) used the OBFA to measure POBF in normal subjects and patients with varying degrees of diabetic retinopathy. They found that in patients with no diabetic retinopathy, POBF was reduced when compared to that of normal subjects. As the degree of retinopathy increased the POBF also increased proportionately. In contrast, Neuforfer, Goldstein, Snir, *et al.*, (1998) found no

significant difference in POBF between normal subjects and diabetic patients with no diabetic retinopathy and mild non-proliferative diabetic retinopathy. However, in agreement with Geyer *et al* (1999) they found the POBF to significantly increase in patients with severe non-proliferative and proliferative diabetic retinopathy, suggesting that choroidal blood flow increases with advances in the severity of the retinopathy. These findings are not in agreement with other authors (Savage, Nash & Wilkinson, 1996; Becker, Schmidt, Ruckman, *et al.*, 1997). Becker *et al.*, (1997) found no significant difference in POBF between diabetic and normal subjects, suggesting functional stability of choroidal perfusion in diabetes mellitus. Savage *et al.*, (1996) reported that ocular blood flow was unaffected in early diabetic retinopathy, increased in patients with moderate to severe non-proliferative diabetic retinopathy and reduced in patients with proliferative diabetic retinopathy.

Grunwald, Dupont & Riva, (1996) investigated the effects diabetes mellitus has on retinal blood flow and found that in insulin-dependent diabetic patients retinal blood flow is increased before signs of diabetic retinopathy become evident. Thus adding evidence to the theory that increased retinal blood flow may play an important role in the development of diabetic retinopathy (Kohner, Patel & Rassam, 1995). In contrast, maintenance of retinal blood flow has been reported in patients with background diabetic retinopathy when compared to aretinopathic diabetic patients (Findl, *et al.*, 2000a).

Polak, Dallinger, Polska, *et al.*, (2000) investigated the effects of insulin, known to have ocular vasodilatory effects, on the retinal and choroidal blood flow. They found that the administration of insulin resulted in an increase in the POBF with no apparent effect on retinal blood flow. Perrott, North, Drasdo, *et al.*, (2001b) investigated the effects conversion from oral hypoglycaemic agents to insulin has on POBF. They found that in the patients with previously high values of POBF, implementation of strict metabolic control resulted in decreases in blood flow and diabetic retinopathy progression. Furthermore six months into the treatment regime the diabetic patients who had low values of POBF prior to conversion to insulin, demonstrated increases in blood flow and no retinopathy development (Perrott, *et al.*, 2001b). Another study conducted by Perrott, North, Drasdo, *et al.*, (2001a) found that POBF increased as

plasma glucose levels rose and suggested that this was due to uncontrolled hyperglycaemia.

1.7 Measurement of Retinal Topography

There are a variety of different methods available that enable the measurement of retinal and optic nerve head topography; these include the Heidelberg Retina Tomograph (HRT, Heidelberg Engineering, Germany), the Rodenstock nerve fibre layer analyser and the Humphrey optical coherence tomograph. The investigations carried out throughout the duration of this PhD utilized the HRT and for this reason discussions will be limited to the mechanisms and theory behind this instrument.

1.7.1 The Heidelberg Retina Tomograph

The HRT is a confocal scanning laser ophthalmoscope (cSLO) that enables three-dimensional mapping and topographic assessment of the posterior segment of the eye. The HRT utilizes the confocal optical principle. It is particularly useful in monitoring change in the parameters of the optic nerve head (Rohrschneider, Burk, Kruse, *et al.*, 1994; Topouzis, Peng, Kotas-Neumann, *et al.*, 1999) and therefore can aid early recognition of structural damage and progression of glaucoma (Mikelberg, Parfitt, Swindale, *et al.*, 1995; Uchida, Brigatti & Caprioli, 1996; Cucevic, Brooks, Strang, *et al.*, 1997; Wollstein, Garway-Heath & Hitchings, 1998; Iester, Defarri & Zanini, 1999). The HRT can also be used in the management and follow-up of patients with cystoid macular oedema (Hudson, Flanagan, Turner, *et al.*, 1998; Zambarakji, Butler & Vernon, 1999), central serous retinopathy, age related macular degeneration (Malinovsky, 1996; Wu, Karbassi, Mui, *et al.*, 2001b), and macular holes (Hudson, Charles, Flanagan, *et al.*, 1997).

1.7.2 Principles of the HRT

Topographic measurements obtained using the HRT, would normally be influenced by scattering of the light of the laser beam from beyond the point of focus. This is overcome with the incorporation of a pinhole aperture located in front of the photodiode detector, conjugate to the focal plane (figure 1.6) (Thomson, 1994;

Hudson, *et al.*, 1998). The confocal scanning laser system uses a light source (class I laser beam) with a wavelength of 670nm. This is scanned over the anatomical area of interest with only one point illuminated at any one time. The illuminating laser is periodically deflected in two directions (x and y), using mirrors or a beam splitter (prism), 90 degrees to the optical axis. A lens then condenses this beam in order to obtain an optical section through the object at the location of the focal plane. The two dimensional images are obtained at different depths by altering the focal plane of the live image (Thomson, 1994). The light is reflected off the desired layer and the reflectance intensity is calculated as a function of scan depth for each pixel within the image where:

$$I_{rel} = (I_i / I_{max}) \times 100 \qquad \text{Equation 1.10}$$

Where

I_{rel} = The relative Intensity

I_i = The measured digitised intensity

I_{max} = The maximum digitised intensity

(Hudson, *et al.*, 1998)

A photodiode detector receives this reflectance information and the light energy is converted into electrical energy in video signal form. The computer system records the video signals and derives a z profile that is a plot of reflectance intensity against scan depth. The depth of the vitreous/internal limiting membrane is taken to be the peak intensity of the z profile (Kruse, R.O, Volcker, *et al.*, 1989; Hudson, *et al.*, 1998).

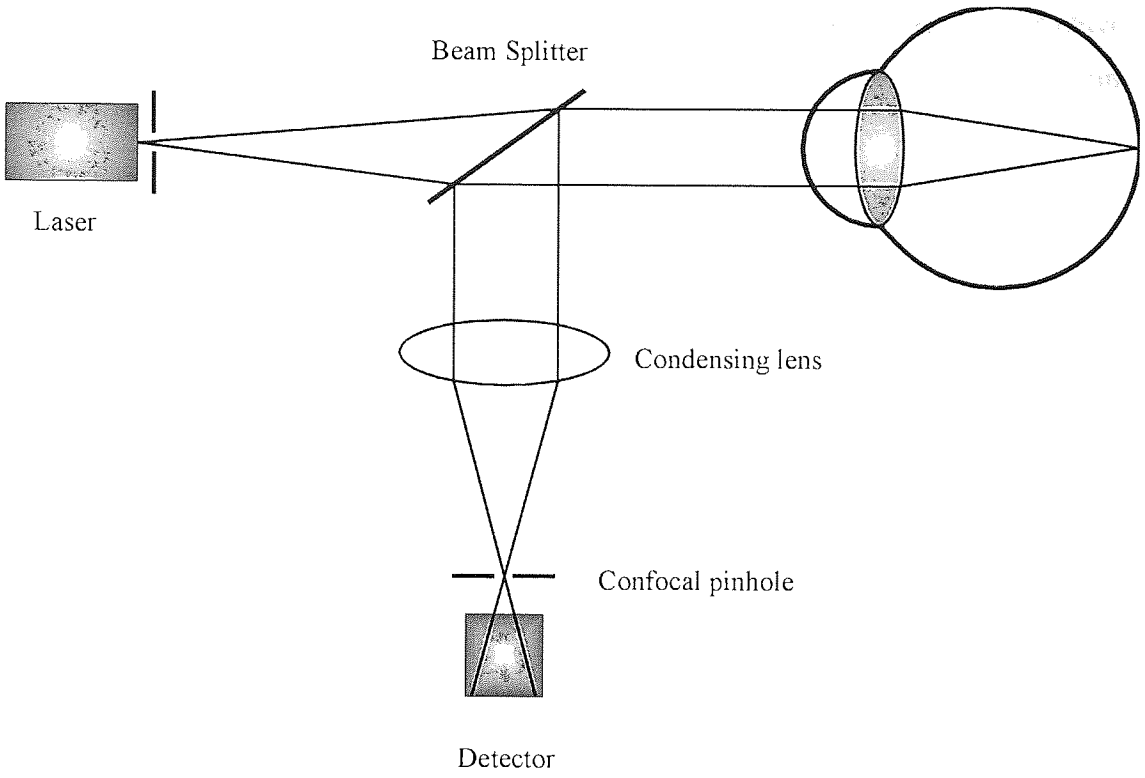

Figure 1.6. **Schematic diagram of the optics of the Heidelberg Retina Tomograph**

The instrument assumes the eye under examination is of average dimensions as determined from Gullstrands model II eye (Emsley, 1969) and a model of the crystalline lens with a gradient index is also utilized (Blaker, 1980). The only variables that are required are the anterior radius of the cornea, which is determined using keratometry and can be input manually by the operator prior to image acquisition, and ametropia, which is calculated by the instrument from the divergence of the laser beam.

1.7.3 Image Acquisition

The image series that results consists of 32 equidistant confocal section images along the z-axis, each located at a different focal plane. The first image of the series is located at the focal plane of the vitreous and this image appears dark due to the other structures being out of focus. As the focal plane is moved posteriorly towards the

retina, by means of a step motor within the camera head, the images become brighter (Thomson, 1994). The brightest image is achieved when the focal plane is located on the surface of the anatomical area of interest. With further posterior movement of the focal plane the image darkens once again as the structures go out of focus. The topography of the light-reflecting surface can then be calculated.

Although the HRT is designed to obtain images through pupils as small as 1mm (Lusky, Bosem & Weinreb, 1993; Thomson, 1994) image quality increases when pupils are dilated (Zangwill, Irak, Berry, *et al.*, 1997). This is because the signal-to-noise ratio increases as more light reflected from the fundus is detected (Zangwill, *et al.*, 1997). The distance between the patient and the instrument should be set to approximately 15 mm (Heidelberg, 1997). The size of the field of view can be set to 10x10, 15x15 or 20x20 degrees. For topographic examination of the optic nerve head a scan area of 10 degrees is recommended (Heidelberg, 1997). The resultant image consists of 256 x 256 independent height measurements and at each point the HRT can measure height to within 0.02mm (Thomson, 1994). Therefore in any one HRT image there are 65,536 individual points and each one of these points can be analysed (Thomson, 1994). The location of the focal plane can be adjusted from +12.00 to – 12.00 dioptres in 0.25 dioptre steps. The depth of the image series can be adjusted on the control panel from 0.5mm to 4.0mm in intervals of 0.5mm, thus enabling imaging of different structures such as the optic nerve head and macula. Data acquisition for one image takes approximately 1.6 seconds and during this time a three-dimensional image is acquired as a series of 32 sectional images at 32 equally spaced two-dimensional optical sections perpendicular to the optical axis (figure 1.7).

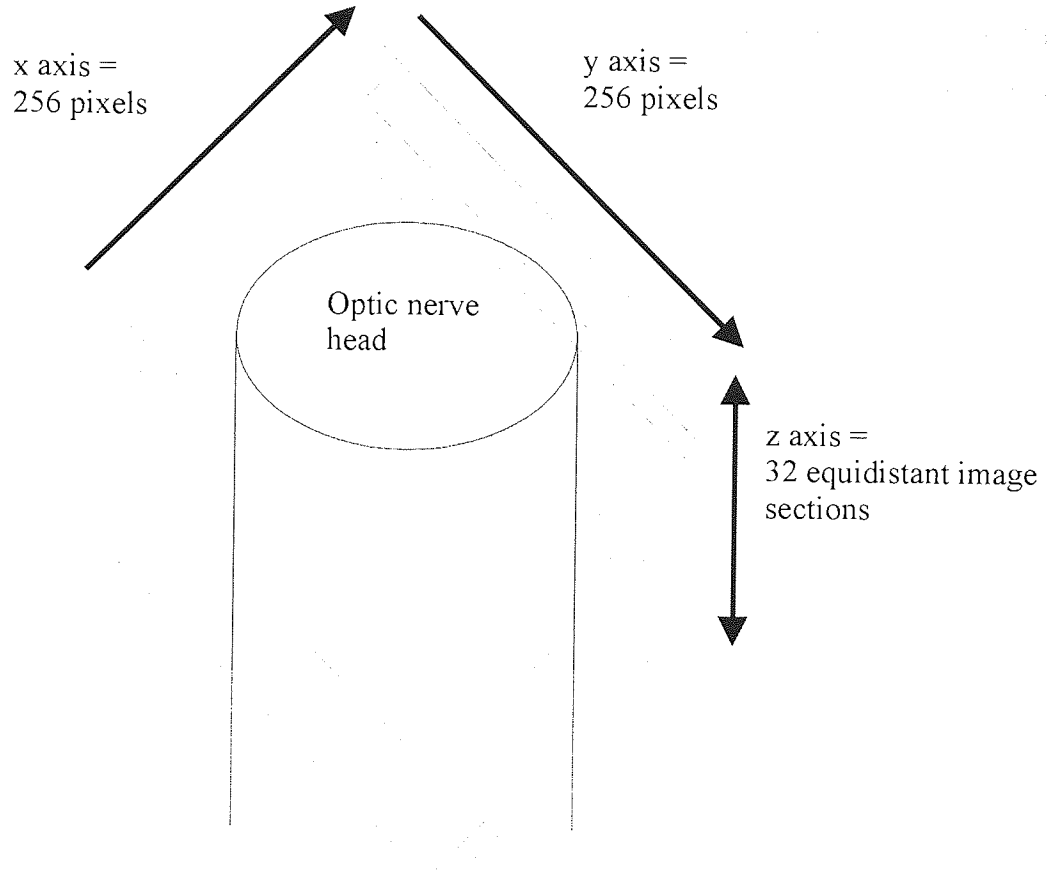

Figure 1.7. **Schematic of the confocal imaging of the Heidelberg Retina Tomograph adapted from (Thomson, 1994)**

On completion of image acquisition the HRT determines the topography images from the acquired two-dimensional image sections, and compensates for any small eye movements that may have occurred during data acquisition (Thomson, 1994; Chauhan, 1996). The resolution of the depth measurement is limited to 300μm due to the radius of the confocal pinhole and the intrinsic aberrations of the refractive components of the eye (Zinser, Wijnaendts-van-Resandt, Dreher, *et al.*, 1989). Using the topographic and reflectance image for reference, the operator is required to outline the area of the image to be analysed, (i.e. the optic nerve head). This outline is termed the contour line, which is then corrected for crossing blood vessels. Two further points of reference are then determined: the reference plane and the curved surface. The reference plane lies parallel to the peripapillary retinal surface 50μm posterior to the retinal surface of the contour line in the segment (Chauhan, 1996). This location is chosen, as during the progression of glaucoma the nerve fibres at the papillomacular bundle remain intact for the longest period of time and is therefore thought to provide the most stability (Chauhan, 1996). In this area of the retina the nerve fibre layer is

approximately 50μm thick (Malinovsky, 1996). If the reference plane is situated just below the nerve fibre layer then any structure situated below the reference plane can be considered as being cup and structures above the plane and within the contour line, neuroretinal rim. The HRT is able to give volumetric values of the optic nerve head. In order to do this the software uses the two depth-wise reference planes, the curved surface and the reference plane (figure 1.8). The curved surface is bound by the contour line and its central point is equal to the mean height of the contour line. As already stated, the reference plane is situated 50 μm posterior to the mean height of the contour line in the inferior temporal quadrant, within the papillo macular bundle (Burk, Tuulonen & Airaksinen, 1998). Using these reference planes stereometric analysis can be carried out yielding two- and three-dimensional parameters such as cup area, cup volume, mean and maximum cup depth, rim area, rim volume, a measurement for three-dimensional shape of the cup and an estimation of the average nerve fibre layer thickness along the contour line. In addition to stereometric analysis, topographic images can be analysed interactively. In this case height and distance measures are used from a cross-section of the topographic image. This form of analysis is useful for measuring localised areas of increased retinal thickness as seen, for instance with macular oedema or macular holes (Malinovsky, 1996).

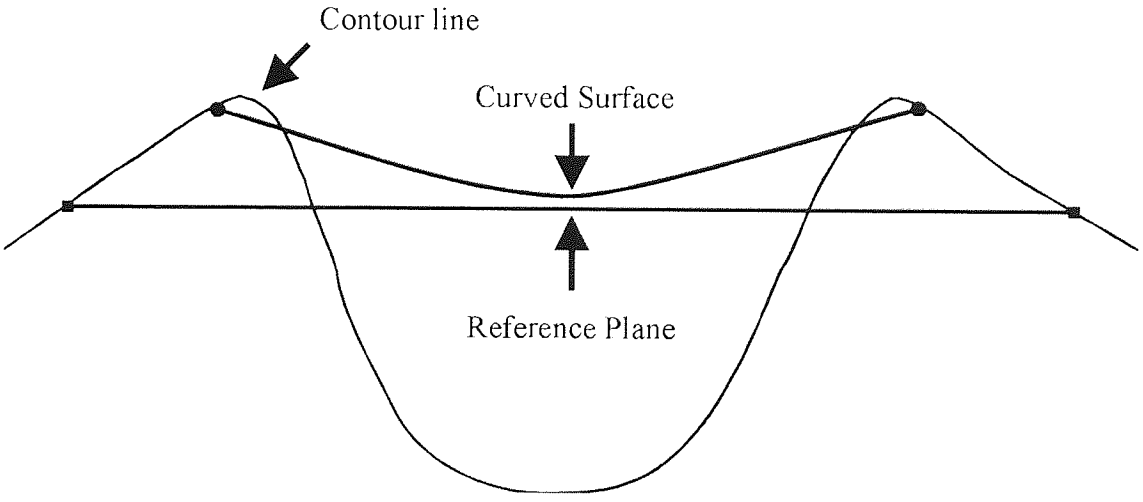

Figure 1.8. **Schematic diagram of the reference plane, contour line and curved surface**

A number of stereometric parameters are available for measurement of the optic nerve head. Appendix 1 outlines these parameters and gives a brief description of them as given by the HRT manual (version 2.01)

1.7.4 Validity of the HRT

Traditionally changes in the topography of the optic nerve head have been quantified using ophthalmoscopy by defining the cup-disc ratio (Armaly & Sayegh, 1969). However there is a large inter-individual variation in the clinical estimation of cup-disc ratios, even among glaucoma experts (Lichter, 1976). The cSLO however, has been shown to be an objective, reproducible, and sensitive method for measuring optic nerve head parameters (Lusky, *et al.*, 1993; Mikelberg, Wijsman & Schulzer, 1993; Rohrschneider, *et al.*, 1994; Azuara-Blanco, Harris & Cantor, 1998; Wollstein, Garway-Heath, Fontana, *et al.*, 2000).

1.7.5 Reproducibility of the HRT

The reproducibility of the HRT has been investigated in both normal and glaucomatous eyes (Lusky, *et al.*, 1993; Chauhan, LeBlanc, McCormick, *et al.*, 1994; Rohrschneider, *et al.*, 1994; Miglior, Albe, Guareschi, *et al.*, 2001a). Variability in measurements obtained by the HRT has been reported to be similar in normal subjects and glaucomatous patients (Chauhan, *et al.*, 1994), and it has been reported that age has no effect on the variability of results (Dreher, Tao & Weinreb, 1991). In order to maximise the ability of the HRT to detect change over time in individuals the reproducibility of the acquired images must be optimised.

Orgül, Cioffi, Bacon, *et al.*, (1996) stated that the reproducibility of HRT measures could be optimised by taking several images at each visit thereby reducing the variability caused by misalignment between the laser scanner and the eye being imaged. Furthermore, Weinreb, Lusky, Bartsch, *et al.*, (1993) recommended that three topographic HRT measurements should be taken of the desired structure and the mean topographic values for each parameter calculated.

Hosking & Flanagan (1996) investigated the effect that altering focus setting has on images and found that focus must be kept the same for consecutive images. In addition, scan area should also be kept constant for subsequent images of the same patient (Jonescu-Cuypers, Thumann, Hilgers, *et al.*, 1998). A more recent study investigated the effects that uncorrected astigmatism and changes in working distance have on HRT images and found that astigmatism up to 2.5 DC and alterations in working distance from 15 to 25 mm had no significant effect on optic disc parameters as measured using the HRT (Sheen, Aldridge, Drasdo, *et al.*, 2001).

Although the HRT is said to be able to acquire reproducible topographic images through an undilated pupil as small as 1mm (Thomson, 1994), it has been shown that variability decreases when pupil dilation is carried out (Zangwill, *et al.*, 1997). It has been hypothesized that this may be due to the cycloplegic effect dilating drugs have on the ciliary muscles resulting in a loss of accommodation and thereby increasing the efficacy of image acquisition. In addition dilation of the pupil can be beneficial in the longitudinal follow-up of patients where the development of media opacities can reduce the image quality (Zangwill, *et al.*, 1997).

Reproducibility of optic nerve head topography measurements was investigated by Cioffi, Robin, Eastman, *et al.*, (1993); they found that topographic measurements had a 75 percent confidence interval, a value considered reproducible. Variability was found to be greatest where the neuroretinal rim sloped at the edge of the cup and around the blood vessels, and lowest in the peripapillary retinal area. Rohrschneider *et al.*, (1994) investigated the reproducibility of topography measurements in normal subjects, glaucoma suspects and glaucomatous patients and concluded that scanning laser ophthalmoloscopy permits highly reproducible topographic measurements. Coefficient of variability values for the normal subjects, glaucoma suspects and glaucomatous patients were 2.9%, 5.0% and 3.4% respectively for cup area; 4.9%, 4.6% and 4.6% respectively for cup volume; 5.2%, 3.8% and 3.3% respectively for mean cup depth; and 5.2%, 4.1% and 4.0% respectively for maximal cup depth.

Mikelberg *et al.*, (1993) investigated the reproducibility of optic nerve head parameters on normal and glaucomatous subjects using the HRT. Values of reproducibility coefficients ranged from 73.7% to 99.6% for the two subject groups for all HRT measures, with the exception of the measure of height variation (60.5% for the normal subjects and 62% for the glaucoma patients). The HRT height variation parameter represents the range of height measurements along the contour line and is strongly influenced by a reading difference of as little as one pixel; its poor reproducibility was therefore expected. It was concluded that the HRT provides reproducible topographic measurements of the optic nerve head (Mikelberg, *et al.*, 1993). A more recent study by Azura-Blanco *et al.*, (1998) found that topographic measures of the optic nerve head in normal subjects had reproducibility values of between 80.6% and 98.2%, with the most reproducible measures being volume below surface (99.3%), mean depth in contour (98.8%) and effective area (98.5%)

Chauhan *et al.*, (1994) investigated the test-retest variability of the HRT in both normal and glaucomatous eyes and it was found that the variability was greater in the diseased group compared to the normal subject group and that variability increased with advancing age; a finding not in agreement with other investigators (Dreher, *et al.*, 1991). It was hypothesized that the increased variability observed with age may have been due to media opacities present in the older subjects (Chauhan, *et al.*, 1994). Furthermore, Chauhan & MacDonald, (1995) evaluated the influence of different time separations between images on test-retest variability and it was found that no discrepancy in variability existed between long and short-term time separations in HRT measurements. Variability was found to be at a maximum at the border of the cup and around the blood vessels (Chauhan, *et al.*, 1994), areas where sloping is at a maximum, this is in agreement with the findings made by Cioffi *et al.*, (1993).

Miglior, Albe, Guareschi, *et al.*, (2001b) investigated the inter- and intra-observer reproducibility of the HRT and found the optic disc stereometric parameters to have a high reproducibility. Using Bland & Altman analysis (Bland & Altman, 1986) variability of inter-image evaluation was found to be greater than that of intra-image evaluation.

Tsai, Zangwill, Gonzalez, *et al.*, (1995a) investigated the effect ethnic origin has on topographic parameters and reported that disc area, cup volume, maximum cup depth and vertical cup-disc ratio were greatest in Afro-Caribbean individuals and smallest in Caucasians, interestingly, rim volume and area were not significantly different between groups. These findings are important when evaluating individuals suspected of having glaucoma and other optic neuropathies. It was concluded that in the diagnosis of glaucoma, rim volume could be the most important clinical sign, as this parameter is not affected by ethnic origin (Tsai, *et al.*, 1995a).

Reproducibility of topographic images using the HRT is influenced by the cardiac cycle (Chauhan & McCormick, 1995). During the cardiac cycle blood enters and then leaves the eye, this change in the volume of the eye is known as the ocular pulse (Langham, 1975). The vascular bed of the eye is not stationary and pulsates in time with the ocular pulse. This results in changes in the appearance of the retinal vascular bed. It has been found that if images are acquired at the same point of the cardiac cycle using pulse synchronisation with an electrocardiographic signal, image variability significantly decreases (Chauhan & McCormick, 1995). Variability in HRT measures were found to decrease by 13.62% for the entire image, 12.26% for the optic nerve head and 18.51% for the peripapillary retina when cardiac synchronisation was introduced, all of these values were highly significant (Chauhan & McCormick, 1995).

1.7.6 Clinical application of the HRT in disease

1.7.6.1 Glaucoma

The nerve fibres of the retinal nerve fibre layer radiate in an arcuate pattern inferior and superior to the macula area of the retina (figure 1.9).

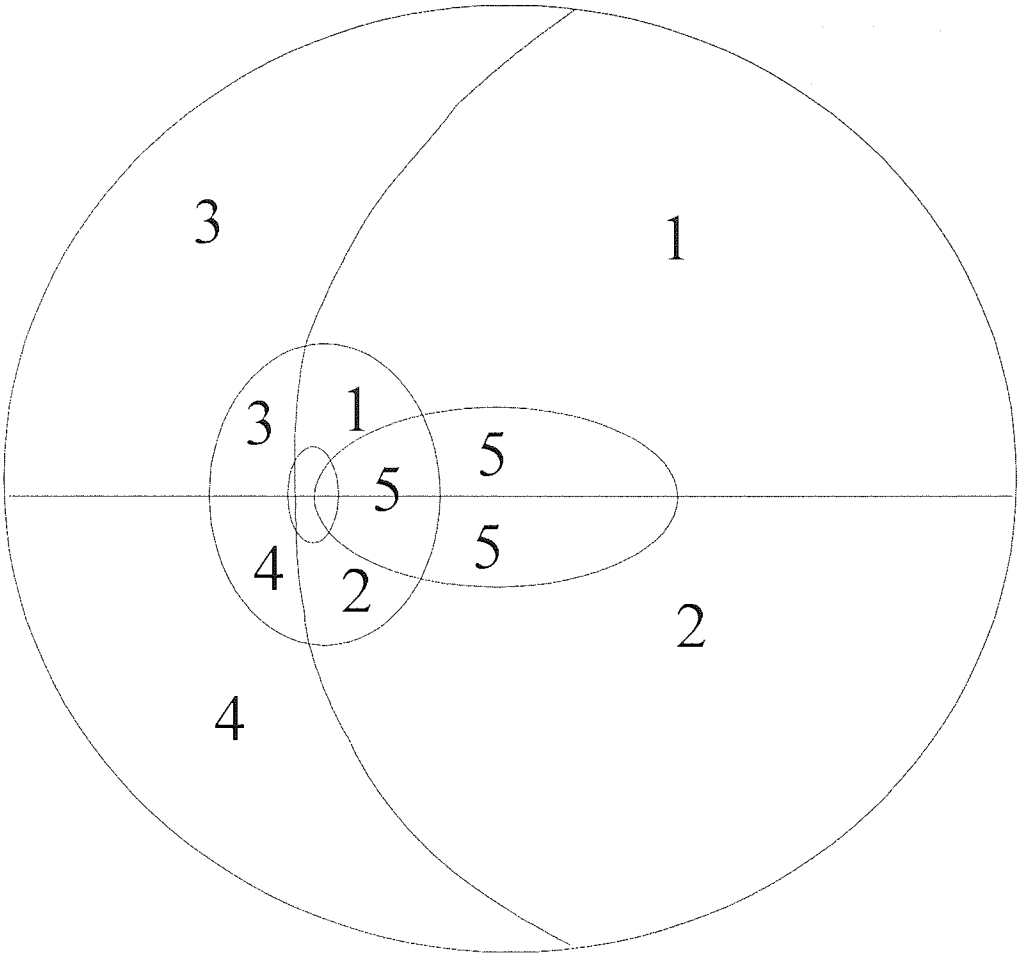

Figure 1.9. **Schematic representation of the topography of the nerve fibres. Section 1: Superior arcuate fibres. Section 2: Inferior arcuate fibres. Section 3: Superior radiating fibres. Section 4: Inferior radiating fibres. Section 5: Papillomacular bundle.**

The nerve fibre layer is most dense superiorly and inferiorly (Varma, Staf & Barron, 1996) and it is in these areas that glaucomatous damage most commonly occurs. The retinal nerve fibre layer is slightly opaque in a healthy individual, when areas of damage are present they appear darker and less striated (DeMaio, Schweitzer, Werner, *et al.*, 1992). The number of ganglion cells and nerve fibres present in the retina of normal subjects is influenced by factors such as age (Mikelberg, Drance, Schulzer, *et al.*, 1989; Jonas, Schmidt, Muller-Bergh, *et al.*, 1992), and optic nerve head size (Jonas, *et al.*, 1992) which result in variations in the make-up of the normal peripapillary nerve fibre layer.

The HRT enables the measurement of retinal nerve fibre layer thickness. Glaucoma results in the death of retinal ganglion cells which occurs prior to the development of

visual field defects. Loss in the number of the retinal ganglion cells results in a decrease in the thickness of the retinal nerve fibre layer, especially in the superior and inferior regions of the retina (Hoyt, Frisen & Newman, 1973). Retinal nerve fibre layer defects have been found in patients with diagnosed glaucoma (Brigatti & Caprioli, 1995; Eid, Spaeth, Katz, *et al.*, 1997; Anton, Yamagishi, Zangwill, *et al.*, 1998), and it has been hypothesized that changes in the nerve fibre layer may occur up to four years prior to the development of visual field defects (Sommer, *et al.*, 1991; Quigley, Katz, Derick, *et al.*, 1992).

A number of studies have utilized the HRT to investigate optic nerve head and retinal parameters in healthy subjects and patients with both high and low tension glaucoma. Differences have been identified between normal and glaucomatous patients at the optic nerve head using the HRT (Mikelberg, *et al.*, 1995; Wollstein, *et al.*, 1998; Iester, *et al.*, 1999; King, Bolton, Aspinall, *et al.*, 2000). Mikelberg *et al.*, (1995) identified the parameters, third moment (cup shape measure), volume above reference and height variation in contour, as being important for identifying glaucoma patients using the HRT. They found that these measures resulted in a diagnosis of glaucomatous optic neuropathy with sensitivity of 87% and specificity of 84% (Mikelberg, *et al.*, 1995). Wollstein *et al.*, (1998) also found significant differences between normal subjects and glaucoma patients in all HRT measures except disc area. More recently King *et al.*, (2000) used the HRT to identify differences between glaucoma patients and normal subjects. Significant differences in retinal nerve fibre layer volume were found between the normal subjects and the patients with both high and low tension glaucoma.

Some parameters identified using the HRT correlate with visual field defects found in glaucomatous patients (Brigatti & Caprioli, 1995; Tsai, Zangwill, Sample, *et al.*, 1995b; Iester, Mikelberg, Courtright, *et al.*, 1997). Tsai *et al.*, (1995b) identified an association between the peripapillary retinal height and visual field sensitivity, which was evident in both normal subjects and glaucomatous patients. Eid *et al.*, (1997) compared topographic measures obtained with the HRT between normal subjects and glaucomatous patients and found that the retinal nerve fibre layer height and cross sectional area in the glaucoma group was significantly reduced when compared to normal subjects. Additionally, it was found that retinal nerve fibre layer height

correlated strongly with measures of rim volume, rim area and cup disc area ratio. In this study visual function was assessed using the Humphrey visual field analyser, program 24-2 and visual field defects were found to correlate strongly with retinal nerve fibre layer thickness measures and cup disc area ratio. These results have implications regarding the clinical utilization of the HRT in glaucoma diagnosis prior to the development of visual field defects. A similar study by Brigatti & Caprioli, (1995) investigated the correlation between visual field defects and HRT measures in glaucoma patients. They found a strong correlation between the visual field defect and the third central moment of the frequency distribution of the depth values for the optic nerve head structure. The third moment gave an indicator of the degree of optic nerve head damage. Another study by Anton *et al.,* (1998) examined the relationship between structural damage and functional loss in POAG patients using the HRT and HFA. They found a positive correlation between visual field defects and topographic parameters, a finding in agreement to those made by other investigators (Weber, Dannheim & Dannheim, 1990; Yamagishi, Anton, Sample, *et al.*, 1997).

Broadway *et al.,* (1998) investigated the ability of the HRT to identify glaucoma patients with different characteristic glaucoma optic disc appearances. Subjects were divided into four groups: focal ischaemic, where notching was apparent; myopic glaucomatous; senile sclerotic and glaucomatous with generalised enlargement of the optic cup. They found that the sensitivity was at its optimum when classifying focal ischaemic discs (93.2%) followed by myopic discs (81.6%), then discs with generalised enlargement of the optic cup (78.6%), with senile sclerotic discs having the lowest specificity of 66.7%. This highlights reduced efficacy of the HRT to consider variations in pathological optic nerve head appearances with its ability to identify some glaucomatous optic nerve heads being very good (focal ischaemic) when compared to others (senile sclerotic).

Zangwill, Horn, Lima, *et al.*, (1996) investigated the topographic parameters of the disc area, cup disc area ratio, cup shape, height in contour, rim area, rim volume, maximum cup depth, cup area, cup volume, retinal height and retinal cross sectional area in primary open angle glaucoma patients, ocular hypertensives and normal, healthy subjects. They found the HRT measurements of ocular hypertensive eyes were significantly different from those of the glaucomatous eyes with the exception of

height of reference plane. Also, significant differences were found between ocular hypertensive and normal eyes for height in contour, rim area, rim volume, disc area and reference plane height. There was overlap in the topographic measures between all three-subject groups.

A recent study by Mistlberger, Liebmann, Greenfield, *et al.*, (1999) investigated optic nerve head parameters and retinal nerve fibre layer thickness in normal subjects, ocular hypertensives and glaucoma patients. They found significant differences between the glaucoma patients compared with the normal and ocular hypertensive subjects in all the evaluated parameters using the HRT. No difference was found between the ocular hypertensive and normal eyes, a finding not in agreement with Zangwill, *et al.*, (1996).

Iester, *et al.*, (1999) combined two methods of topographic analysis (double-cup and contour-line area) to determine the presence or absence of glaucomatous optic nerve damage. Combining these two methods of analysis resulted in a sensitivity of 80% and a specificity of 100% in detecting glaucomatous optic neuropathy.

The HRT has been used to assess the topography of the optic nerve head following trabeculectomy over time (Topouzis, *et al.*, 1999). Topouzis *et al* (1999) acquired images preoperatively and two weeks, four months and eight months postoperatively. Changes were found in the topography of the optic nerve head two weeks after surgery that were accompanied with an expected decrease in intraocular pressure (Topouzis, *et al.*, 1999). After four months the only optic nerve head parameter to remain different from preoperative topographic measures was cup shape and this returned to preoperative values after eight months (Topouzis, *et al.*, 1999). This is different to the results found by Irak, Zangwill, Garden, *et al.*, (1996) who reported significant changes in several topographic measures of the optic nerve head four and a half months post operatively and also Raitta, Tomita, Vesti, *et al.*, (1996) who reported significant change twelve months after trabeculectomy surgery.

1.7.6.2 Diabetes

Diabetic macular oedema is one of the major causes of visual impairment in patients with diabetic retinopathy (Giovannini, Amato, Mariotti, *et al.*, 2000). Diabetic macular oedema can be classed into three different types according to its pattern; these types are focal, diffuse and cystoid. The occurrence of diabetic macular oedema results in thickening of the retinal layers in the macular area. Currently retinal thickening is assessed subjectively using methods such Volk lens fundus biomicroscopy and early retinal thickening can be difficult to distinguish from normal (Ferris & Patz, 1984). In addition, subjective assessment of retinal thickness makes quantification of the degree of thickening over time difficult (Hudson, *et al.*, 1998). Early diagnosis of diabetic macular oedema is important, as it has been shown that laser photocoagulation treatment can be beneficial in the early stages (ETDRS, 1985). Laser photocoagulation prevents further loss of visual function by inhibiting the progression of fluid accumulation within the macular retinal layers (Ferris, 1993).

The HRT provides a potentially useful technique for the measurement of clinically significant diabetic macular oedema. Menezes, Giunta, Chrisholm, *et al.*, (1995) investigated the reproducibility of HRT measures at the macula of ten healthy subjects. One operator acquired five images for each subject firstly with the subject undilated and then with them dilated. Increased variability of HRT measurements was found at the macula in undilated pupils, compared to dilated eyes. Overall reproducibility of the absolute height measurements at the macula was good with pooled standard deviation values being between 47.4 and 36 μm for undilated and dilated eyes respectively. The standard deviation of 36 μm for measurements on dilated eyes is similar to values of the optic nerve head reported by other authors (Dreher, *et al.*, 1991; Weinreb, *et al.*, 1993). This comparison must be viewed with caution as in these studies individual pixel height measures were examined whereas Menezes *et al.*, (1995) evaluated the reproducibility of the mean height of contour line measurements. In addition Menezes *et al.*, (1995) reported excellent reproducibility when the HRT software calculated the relative difference between the height of the perimeter of the contour line and the height of the area inside the contour line. This latter finding indicates that the HRT may be useful in monitoring patients with macular lesions over time, as in patients with diabetic macular oedema.

Hudson *et al.*, (1998) conducted a study to investigate the relationship between retinal thickness and z-profile signal width on normal, healthy volunteers and diabetic patients with macular thickening. Three patients with macular thickening were recruited, one with widespread diabetic macular oedema, one with localised diabetic macular oedema and the last with a macular hole. It was reported that the z-profile of patients with macular thickening was much greater when compared to that of healthy subjects. The increase in z-profile was attributed to two underlying mechanisms:

(1) An increase in retinal thickness which results in an increased depth that the reflectance intensity can be measured over

(2) A decrease in the reflectance index of the internal limiting membrane and retina, which results in a reduction in the maximum reflectance intensity.

The latter mechanism results in an increase in the z-profile even when no retinal thickening is evident. Consequently, the utilization of z-profile analysis may aid diagnosis of early macular oedema before significant changes in the retinal structure have occurred (Hudson, *et al.*, 1998).

Zambarakji *et al.*, (1999) investigated the role of the HRT in identifying macular oedema in 81 diabetic patients and compared these results with those from 20 age-matched controls. It was found that the HRT's volumetric analysis (volume above the reference plane) identified patients without diabetic macular oedema successfully, but only identified between 50 to 60 % of patients with macular oedema, thus, suggesting that the technique has high specificity but poor sensitivity. It was therefore suggested that the HRT may be a useful addition to the yearly assessment of diabetic patients and may be important in reducing the number of falsely identified diabetic patients referred for suspect diabetic macular oedema, and, in identifying patients that require urgent macular treatment.

More recently a similar study conducted by Ang, Tong & Vernon, (2000) utilized the HRT to obtain volumetric measurements of the macular in diabetic patients with macular oedema and clinically healthy subjects. They found the coefficient of variability of the volume above reference to be much lower than that found by Zamburakji *et al.*, (1999) varying between 7 to 13 % compared with 20 to 31%. The

improvement in repeatability is likely to be associated with the method used to relocate the contour line for subsequent images. Ang *et al.*, (2000) positioned the centre of the contour line with respect to the major arcadic vessels within the retina, which were presumed not to vary between scans and found that a change in the volume above reference of $0.036mm^2$ or more within the contour line over time represented a significant change. They concluded that this method of topographic assessment would be useful in identifying early cases of clinically significant diabetic macular oedema, particularly in detecting small increases in macular volume not detectable using traditional methods such as slit lamp biomicrospcopy.

More recent studies have confirmed the ability of the HRT in detecting diabetic macular oedema; with changes in x axis values (Wu, Karbassi, Mui, *et al.*, 2001a) and edema index (Hudson, McCreesh, Quinn, *et al.*, 2001a) in diabetic patients with clinically significant macula oedema.

1.8 Assessment of visual function

1.8.1 Anatomical factors in visual function

There are two different types of photoreceptors in the human retina: these are the rods and cones. These receptors contain four photopigments. Rhodopsin is found in the rods and comprises approximately 95% of all photopigment; the remaining three photopigments are found in the cones. These three types of cones are known as long, medium and short wavelength photoreceptors according to the wavelength of their peak absorption. The difference in the absorption spectra of each of the cone photopigments enables the differentiation of colour (Bron, *et al.*, 1997).

The fovea is free from rods (Spooner, 1957; Bron, *et al.*, 1997), and the cones situated in this area have a one-to-one relationship with the ganglion cells (Hirsch & Curcio, 1989). In the retinal periphery the rods predominate with several rods converging onto a single ganglion cell. The rods result in poor acuity but high sensitivity whilst the cones are able to detect fine detail such as edges, shape, texture and colour. The arrangement of the photoreceptor cells that project onto a ganglion cell determines the receptive field of that retinal ganglion cell (Schwiegerling, 2000).

Visual function can be assessed using several different methods; these include visual acuity, contrast sensitivity, colour vision assessment, white-on-white perimetry and short wavelength automated perimetry.

1.8.2 Visual acuity

Visual acuity is defined as the resolving power of the eye (Eskridge, Amos & Bartlett, 1991). Snellen visual acuity is the most widely used clinical test to measure visual function and measures visual spatial processing (O'Leary, 1988). It uses high contrast letters on a white background that range in size from large to small, the observer is required to read the smallest letters that he or she can resolve. It is a limited test however, as it only measures a small part of the visual system's spatial resolution capabilities (Johnson & Casson, 1995).

Repeatability of visual acuity measurements using Snellen letter charts have been shown to be poor (Lovie-Kitchin, 1988) and it is thought that this could be due to the design of the chart (Ferris, Kassoff, Bresnick, *et al.*, 1982).

A useful alternative to Snellen visual acuity is the logMar vision chart, which gives values of acuity as a number and is available at different contrast levels (Bailey & Lovie, 1976). LogMar visual acuity is measured using a letter chart placed 20 feet from the patient. There are several charts available and they are either high (90%) or low (10%) contrast charts. Visual acuity is scored as the logarithm of the minimum angle of resolution and letter size decreases from the top of the chart downwards in a logarithmic progression (logMar 1 to logMar –0.3). On each line there are equal numbers of letters, optotypes are of similar legibility and the spacing between each line and each letter is proportional to the letter size (Raasch, Bailey & Bullimore, 1998). Use of the logMar visual acuity chart has been shown to improve repeatability of visual acuity measurements (Bailey & Lovie, 1976; Lovie-Kitcin, 1988). The log MAR visual acuity chart has been shown to be repeatable with 95% confidence within ± 0.1 log units or ± 1 line on a log Mar chart (Arditi & Cagenello, 1993). Furthermore, a real change in visual acuity is said to have occurred between visits when there is a difference of at least ± 0.15 log MAR or 8 letters (Siderov & Tiu, 1999) (each individual letter has a value of logMAR 0.02).

1.8.3 Contrast sensitivity

Contrast is defined as the difference in luminance between brightest (L_1) and dimmest (L_2) areas divided by their sum as defined by the equation below: -

$$\frac{L_1 - L_2}{L_1 + L_2}$$ Equation 1.11

In its simplest form, contrast is the degree of blackness to whiteness of a target (Arden, 1978). This equation describes the difference in average luminance between two areas. The contrast threshold is known as the smallest detectable difference in luminance between two tested areas that is recognized 50% of the time and the ability to detect this difference is called the contrast sensitivity, which is inversely proportional to the contrast threshold (O'Leary, 1988). Contrast sensitivity measures the sensitivity to luminance contrast at various spatial frequencies. It measures spatial visual processing over the visible range of sinusoidal spatial frequencies. Contrast sensitivity is governed by both optical and neural factors and represents the resolving capability of the human visual system for every contrast level and spatial frequency. In the normal eye contrast sensitivity is at a minimum at low and high spatial frequencies with a peak sensitivity being evident from 3 to 6 cycles per degree (for temporal contrast sensitivity, optimum performance is obtained with moving gratings of between 10 and 25 Hz). A typical contrast sensitivity curve is similar to the Gaussian distribution profile. The maximal spatial resolution achieved at high contrast is equal to the visual acuity. Figure 1.10 shows a typical spatial contrast sensitivity curve.

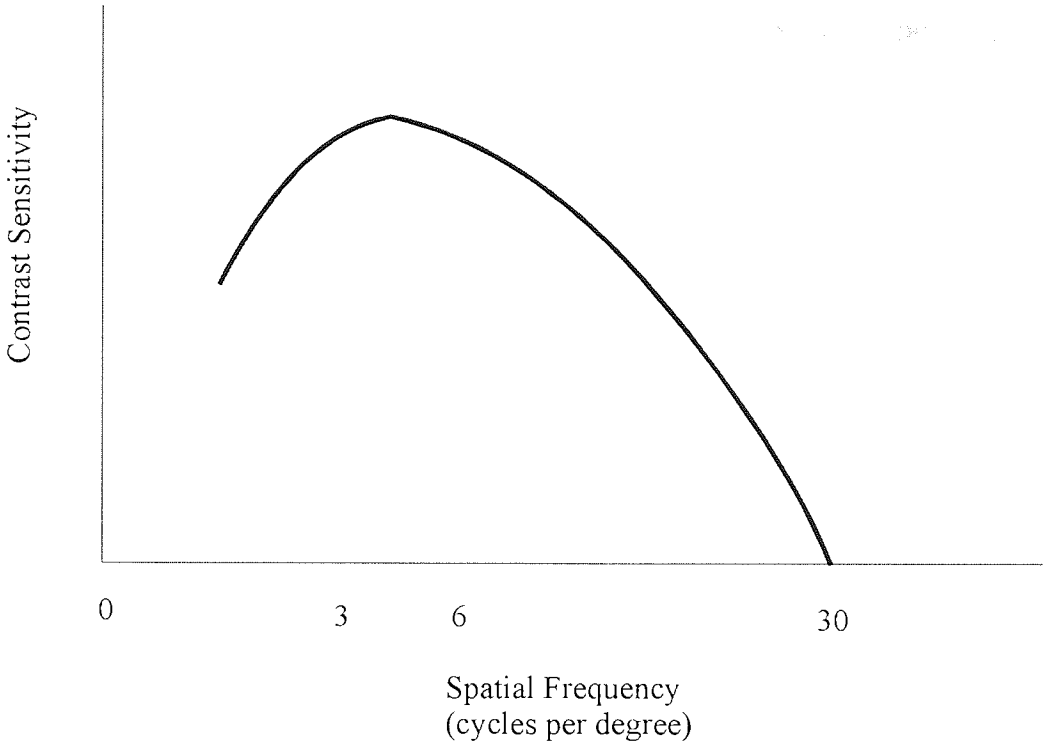

Figure 1.10. **Typical human contrast sensitivity function**

If the contrast sensitivity is tested as a function of space where two areas are adjacent to one another it is known as spatial contrast sensitivity (O'Leary, 1988). If, however, the visual areas occur sequentially in time it is known as temporal contrast sensitivity (O'Leary, 1988). The frequency is the number of cycles (bars or stripes) per degree of visual angle, or the number of flickering frames per second (O'Leary, 1988). Consequently, units are given in cycles per degree for spatial frequency and in hertz (Hz) for temporal frequency. When measuring spatial contrast sensitivity, the thinner the stripes are the higher the spatial frequency is.

Contrast sensitivity can be measured clinically in a number of ways usually employing either letter or sinusoidal wave grating form. Contrast sensitivity charts include the Pelli-Robson chart, Vistech charts and the high and low contrast logMar charts. A more recent addition to the battery of tests available to test the contrast sensitivity function is the CSV-1000 contrast sensitivity test chart (Pomerance & Evans, 1994).

The CSV-1000 contrast test is a sinusoidal grating contrast sensitivity test that is used at a distance of eight feet from the subject. It houses its own illumination system, and this can be calibrated to the required illumination using the lightmeter provided. The

chart presents four spatial frequencies of 3, 6, 12, and 18 cycles per degree. Each spatial frequency is presented on a separate row and on each row there are 17 circular patches, which are 1.5 inches or 3.75 cms in diameter. In every row the first patch or sample patch shows a high contrast grating of the spatial frequency of that row, the remaining 16 patches appear in 8 columns with one patch above another. In each column one patch comprises of a grating and the other patch is blank. Across any of the rows the patches comprising the grating have a decreasing contrast from the left of the row to the right. The patient is shown the sample patch and is then asked to state whether the grating is in the top, the bottom, or if no grating is present in either of the circles. It is important to emphasize to the patient that they should not guess where the patch is due to the high probability of guessing correctly. The contrast level of the last correct response is said to be the contrast threshold. The differences in contrast between the patches are set in log units. Between the sample patch and column 1 there is a 0.15 log unit change in contrast; between columns 1 through to column 3 there is a decrease in contrast of 0.17 log units and from columns 3 through to 8 a reduction in contrast of 0.15 log units occurs between each patch. For the 3, 6, 12 and 18 cycle per degree rows the contrast sensitivity levels range from 0.70 to 2.08, 0.91 to 2.29, 0.61 to 1.99 and 0.17 to 1.55 log units respectively (Pomerance & Evans, 1994). The reliability of this contrast sensitivity-testing device has been validated by Pomerance & Evans, (1994) where they found that the coefficient of reliability of the instrument was comparable to other contrast sensitivity tests.

1.8.3.1 Factors affecting reproducibility of contrast sensitivity

There are a number of factors that are known to affect measurements of contrast sensitivity. Contrast sensitivity reduces with increasing age (Lovie-Kitchin & Brown, 2000; Nio, Jansonius, Fidler, *et al.*, 2000); a decline that begins at around 50 years of age (Nio, *et al.*, 2000). This decline may occur earlier if juvenile media opacities develop, and is more prominent at higher spatial frequencies (Nio, *et al.*, 2000). An average contrast sensitivity loss of approximately 0.1 to 0.2 log units per decade has been reported in older adults with no evidence of ocular pathology (Burton, Owsley & Sloane, 1993), although a large overlap in results was found between the distribution of young and mature contrast sensitivity functions. A more recent study found the loss in contrast sensitivity observed with advancing age to be much lower with only a 0.017 log unit decrease per decade using the low contrast LogMar testing unit (Lovie-

Kitchin & Brown, 2000). The loss in contrast sensitivity can be attributed to both optical and neural changes that occur with advancing age (Burton, *et al.*, 1993).

Surround luminance is also known to affect contrast sensitivity measurements. It has been found that increasing surround luminance results in an increase in contrast sensitivity measurements using the Pelli-Robson letter contrast sensitivity chart (Cox, Norman & Norman, 1999). This is thought to be due to the mechanism of pupil miosis (Cox, *et al.*, 1999), which results in the reduction of spherical aberration. Using computer generated sinewave gratings it was found that much lower luminance levels gave optimum results (Cox, *et al.*, 1999). From these findings a surround luminance of between 10% and 30% of the mean target luminance was recommended and it was suggested that these levels should be adjusted when computer generated sinewave gratings are used to evaluate contrast sensitivity (Cox, *et al.*, 1999).

1.8.4 Visual fields

The visual field is defined as that area in space within which the eye can perceive a visual stimulus without altering its position of gaze. It has maximum normal limits of 65 degrees superiorly, 75 degrees inferiorly, 60 degrees nasally and 95 degrees temporally from the visual axis (Henson, 1993). A physiological blind spot, corresponding to where the optic nerve exits the eye, exists, which is devoid of any photoreceptors (McClure, 1988). The blind spot is positioned in the temporal field; it is vertically oval and is approximately 8.5 degrees by 5.5 degrees. One fifth of the blind spot lies above and four fifths lies below the horizontal midline. The point of fixation which results in maximum visual acuity is called the macular, all other areas of the retina perceive detail to a lesser degree. The visual field of any one individual can be described as a hill of vision with the peak of the hill corresponding to the point of fixation of each eye (see figure 1.11). The sensitivity of the retina is greatest at the point of fixation, at the macular, and this sensitivity reduces as the distance from this anatomical area increases.

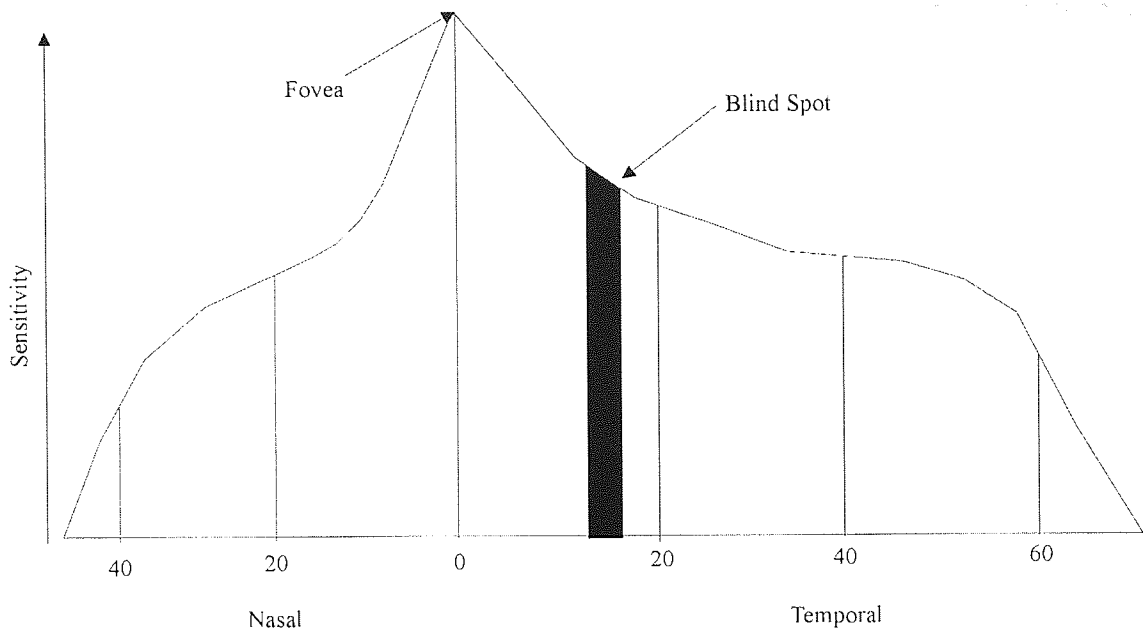

Figure 1.11. Schematic of the typical hill of vision

A variety of factors are known to influence the normal visual field. These include variations in facial contours; a person with deeply set eyes for example will exhibit a significantly smaller visual field when compared to an individual with exophthalmus (McClure, 1988; Henson, 1993). Superior visual field defects may result from ptosis or overhanging brows (Henson, Dix & Oborne, 1984; McClure, 1988; Henson, 1993). With increases in myopia the size of the physiological blind spot increases whilst aphakia results in a reduction in the overall size of the visual field (McClure, 1988; Henson, 1993). Other causes of visual field reductions include miosis, media opacities and increasing age (McClure, 1988).

There are two types of visual field testing, kinetic and static. Kinetic perimetry predominantly tests the sensitivity of the magnocellular neurons whilst static perimetry tests the sensitivity of the parvocellular neurons. Static perimetry can be carried out using a white stimulus on a white background or using a blue stimulus on a yellow background (Short wavelength automated perimetry, SWAP). In both techniques the lowest light level the stimulus is perceived at is found at various retinal locations, this is known as threshold perimetry. The former technique (white on white perimetry) examines the sensitivity of all the photoreceptors whilst the latter technique (SWAP) is more specific and examines the sensitivity of the short wavelength

sensitive cones of the retina, it is these cones that are thought to be more susceptible to glaucomatous damage (Demirel & Johnson, 1996).

There are several instruments available which reliably test the sensitivity of the visual field (Henson, 1993). In kinetic visual field testing the sensitivity of the retina is evaluated by changing the size or colour of the stimulus. The stimulus is moved from a non-seeing area towards the point of fixation and the visual field is plotted. Static visual field testing enables the depth of any scotoma present to be evaluated and is able to monitor the recovery or regression of visual function. In addition this method of perimetric assessment eliminates successive lateral summation, which is a major cause of fixation loss in kinetic perimetry and is therefore a more successful method of visual field examination in uncooperative patients (McClure, 1988). During our investigations we used static perimetry in the form of the Humphrey visual field analyser (HFA) and this is discussed in the following section.

1.8.4.1 The Humphrey visual field analyser

The Humphrey visual field analyser (HFA) is a bowl perimeter which projects lights of varying sensitivities onto the bowl; this is different to the light emitting diodes employed by other visual field analysers such as the Henson perimeter. The patient is instructed to maintain their fixation on a central target and press a button to indicate when a white light is seen.

The HFA has full threshold programmes that are able to assess the central 24 degrees (24-2) and 30 degrees (30-2) of the visual field. It utilizes a staircase strategy to find the threshold of individual points of the retina. Initially the threshold for four central points, known as primary points, are determined and then used as starting levels for neighbouring points. The values determined for neighbouring, or secondary points, are then used as starting levels for subsequent points. A stimulus is presented at a level that the patient is expected to see; its intensity is then reduced in 4-decibel (dB) steps until it is no longer seen. The intensity is then increased in 2 dB steps to find the exact threshold of each retinal location tested. If the threshold of a point is 5 dB or more

below it's expected value then it is retested and both the first and repeated value are presented on the print out (Humphrey Instruments, 1987).

The HFA has inbuilt mechanisms to test the patient's fixation; detectors ensure that contact is maintained throughout the testing period between the chin rest, forehead rest and patient, in addition the blind spot is mapped out at the start of the examination and this is checked regularly throughout the program to verify that fixation is maintained (Humphrey Instruments, 1987). Approximately 5 % of the total numbers of stimuli presented to the patient are located within this area (Humphrey Instruments, 1987). A test is deemed unreliable if the number of fixation losses exceeds 20 % (Humphrey Instruments, 1987). In addition to monitoring fixation losses false positive and false negative trials are presented to the patient. During a false positive presentation no stimulus is presented to the patient. During a false negative presentation the stimulus is presented to the patient at a location where the threshold sensitivity has previously been determined, the stimulus presented is at a much brighter intensity than threshold. Reliability is said to be poor if false positive or false negative errors exceed 15 % (Humphrey Instruments, 1987).

1.8.4.2 Analysis of the visual field using the Humphrey field analyser (HFA)

The HFA uses Statpac to analyse the visual results of any one individual. The statistical package is based on a model of the visual field developed through testing of numerous normal visual fields (Humphrey Instruments, 1987). A grey scale representation of the visual field is given where a 5 dB change in sensitivity is shown as a different shade of grey. A numerical plot shows the sensitivity found at every retinal location tested. Two total deviation plots are also given; one being numerical and the other being a grey scale representative (Humphrey Instruments, 1987). These total deviation plots compare the patient's values with those of an age corrected normal. Finally a pattern deviation plot is given which corrects the data for overall changes in sensitivity caused for example, by media opacities such as cataracts resulting in an overall decrease in the hill of vision. On each print out the instrument indicates whether any one field is within normal limits, borderline or outside normal

limits. In addition it states whether there is a general reduction in results or if they are better than are expected (Humphrey Instruments, 1987).

Global indices are given to enable the examiner to assess the visual field plots as a whole (Humphrey Instruments, 1987). The global indices given are:

(1) **Mean Deviation (MD):** This is the average elevation or depression of the individuals visual field compared to a normal reference.

(2) **Pattern Standard Deviation (PSD):** This is a measurement of the amount in which the shape of the visual field differs to one of an age-matched normal.

(3) **Short-term Fluctuation (SF):** This gives a value of the consistency of the patient's responses throughout the test and is an index of the reliability of the results.

(4) **Corrected Pattern Standard Deviation (CPSD):** This is a measure of the extent in which the total hill of vision differs from a normal hill of vision corrected for age and intra-test variability (SF).

1.8.4.3 Reproducibility of the HFA

It has been found that with increasing age the sensitivity of each retinal location measured with the HFA decreases in the central 30^{o} of the visual field (Katz & Sommer, 1986). It was found that age-related changes were greatest in the superior quadrant, from 16 to 30 degrees of the central visual field. Katz & Sommer (1986) concluded that if the observed reduction in the superior visual field was solely due to senile ptosis, then only the peripheral zone of the superior field (25 to 30 degrees) should be affected. The normal visual field is known to decrease in sensitivity with increasing eccentricity (Jaffe, Alvarado & Juster, 1986), with advancing age the threshold sensitivity decreases and the standard error of this decrease increases with eccentricity (Jaffe, *et al.*, 1986). Haas, Flammer & Schneider, (1986) also investigated the age-related decline in visual field sensitivity and similarly found that the sensitivity of the superior visual field decreases to greater extent than the inferior

hemifield, and that the periphery and centre are more influenced than the pericentric area (Haas, *et al.*, 1986).

When using the HFA a significant learning effect has been identified with repeated testing (Heiji, Lindgren & Olsson, 1989). The largest changes occur in the mid-periphery whilst the area closest to fixation remains the most stable (Heiji, *et al.*, 1989). Average sensitivity increases by approximately 1 to 2 dB between the first few tests and inter-individual variability decreases with repeated visual field testing (Heiji, *et al.*, 1989). Fatigue is also known to influence visual field results (Hudson, Wild & O'Neill, 1994; Johnson, Adams & Lewis, 1998), with increases in the visual field indices mean defect and loss variance in a time-related way (Hudson, *et al.*, 1994)

1.8.4.4 Short Wavelength Automated Perimetry (SWAP)

Short wavelength automated perimetry (SWAP) assesses the sensitivity of the short wavelength sensitive pathway of the retina (Sample & Weinreb, 1990). Utilization of a two-colour increment threshold procedure allows the assessment of the sensitivity of the blue-yellow chromatic channel. A large blue stimulus with a wavelength of approximately 440 nm is presented on a bright yellow background, which inhibits the medium and long wavelength sensitive photoreceptors (Demirel & Johnson, 1996; Wild, 2001).

Retinal sensitivity in normal subjects to SWAP is known to differ between the superior and inferior hemispheres, with the superior retina being less sensitive to short wavelength stimuli than the inferior retina (Sample, Irak, Martinez, *et al.*, 1997). Furthermore, the difference in sensitivity between the superior and inferior fields significantly increases with eccentricity. This difference in sensitivity is not observed between the temporal and nasal hemifields (Sample, *et al.*, 1997).

A study investigating SWAP in diabetic patients reported that the short wavelength sensitive cones are more susceptible to damage than other cone types (Nork, Wang & Poulson, 1994). It has been suggested that this susceptibility may be responsible for the reduction of short wavelength sensitivity in disorders involving the outer retina. It is thought that glaucomatous visual field defects are more readily detected using

SWAP as the ganglion cells responsible for blue-yellow opponency are preferentially damaged in this disease (Demirel & Johnson, 1996).

A study by Blumenthal, Sample, Zangwill, *et al.*, (2000) investigated the variability in visual field assessment using both standard and short-wavelength automated perimetry in 25 stable glaucoma patients. Results showed that with SWAP, variability was greater when compared to that of standard perimetry and that this variability increased for both methods when the point under examination was further away from fixation.

1.8.4.5 Reproducibility of SWAP

As with white-on-white perimetry a learning effect is observed in repeated testing of the visual field using SWAP, and it has been suggested that fluctuations observed due to the learning effect may not be eliminated after two examinations (Rossetti, Miglior, Invernizzi, *et al.*, 1999). It is also likely that fatigue plays a major part in fluctuations in the visual field using SWAP as the average examination time is 15% to 17% greater than standard white-on-white perimetry (Wild, Cubbidge, Pacey, *et al.*, 1998), and fatigue is known to have an affect on results using standard white-on-white perimetry (Marra & Flammer, 1991; Hudson, *et al.*, 1994; Johnson, *et al.*, 1998). Blumenthal, *et al.*, (2000) assessed the long-term variability for standard and short-wavelength automated perimetry in stable glaucoma patients and found the variability was 0.55 dB higher for SWAP than for standard visual fields.

SWAP is influenced by the incidence of lens yellowing which results in a reduction in the transmission of short wavelength light (Demirel & Johnson, 1996). In addition to the normal lens yellowing that occurs with age the development of cataract is also known to have a significant effect on SWAP results (Moss, Wild & Whitaker, 1995). Furthermore, it has been found that posterior subcapsular cataracts have a greater affect on SWAP sensitivity than anterior cortical cataracts (Moss, *et al.*, 1995); the reverse of this is true for standard automated perimetry (Moss, *et al.*, 1995). This can be explained from the effect background illuminance has on pupil diameter. With SWAP the background illuminance is brighter, causing the pupil to constrict, thereby increasing the effect of posterior subcapsular cataract, which is usually situated on the optical axis. Anterior cortical cataracts are situated peripherally and will therefore have more of an effect when the pupil is large as seen during standard automated

perimetry when the background luminance is lower (Moss, *et al.*, 1995). As well as the affects from ocular media SWAP is influenced by macular pigment, with the blue-yellow field being attenuated by approximately 0.80 log units at the fovea (Wild & Hudson, 1995).

A study carried out by Wild, Kim, North, *et al.*, (2001) investigated the effect of stimulus size on the variability of SWAP results. They found that the variability in SWAP results using stimulus sizes V and VI were far greater than that exhibited using stimulus sizes III, V and VI in standard white on white perimetry. Optimum results were obtained using a stimulus size of VI in SWAP (Wild, *et al.*, 2001), although group mean standard deviations for SWAP size VI were up to four times greater than those found with white on white perimetry stimulus size III.

1.8.5 Application of visual function measurements in ocular disease

1.8.5.1 Contrast sensitivity and glaucoma

A number of studies have investigated visual function in glaucoma patients using contrast sensitivity charts (Arden, 1978; Lundh, 1983; Ross, Bron & Clarke, 1984; Adams, Heon & Husted, 1987; Sponsel, DePaul, Marone, *et al.*, 1991; Mantyjarvi & Terasvirta, 1993). There have been discrepancies in the results found from these studies with some showing decreases in the contrast sensitivity function with glaucoma (Arden, 1978; Ross, *et al.*, 1984) while others show no change in contrast sensitivity in glaucoma patients (Lundh, 1983). These differences may be due to variations in the test device used to assess contrast sensitivity, age differences between patient groups or media opacities present.

1.8.5.2 Contrast sensitivity and diabetes

Contrast sensitivity is a useful technique for the detection of early macular dysfunction (Verrotti, Lobefalo, Petitti, *et al.*, 1998). A number of studies have investigated central foveal function in diabetic patients using contrast sensitivity (Ghafour, Foulds, Allan, *et al.*, 1982; Dosso, Bonvin, Morel, *et al.*, 1996; Arend, Remky, Evans, *et al.*, 1997). Verrotti *et al.*, (1998) investigated the use of contrast sensitivity on diabetic patients to assess metabolic control and the severity of the

diabetic retinopathy. Contrast sensitivity was tested using the CSV-1000 with a background illuminance of 85cd/m^2. It was found that reductions in contrast sensitivity occurred prior to the development of diabetic retinopathy and preceded a decrease in visual acuity. This loss in contrast sensitivity was more pronounced in patients with preproliferative or proliferative diabetic retinopathy suggesting that contrast sensitivity values correlate with the degree of retinal involvement in diabetic patients (Verrotti, *et al.*, 1998). Furthermore, it was reported that contrast sensitivity improved in patients without retinopathy and with background diabetic retinopathy as metabolic control improved. This was not replicated in patients with more severe degrees of diabetic retinopathy, suggesting that the decrease in contrast sensitivity observed in these patients may be a marker of irreversible visual loss (Verrotti, *et al.*, 1998). Arend *et al.*, (1997) reported a loss in contrast sensitivity at 6 and 12 cycles per degree using the CSV-1000 in diabetic patients when visual acuity levels were within normal limits. The loss in contrast sensitivity was directly related to the size of the foveal avascular zone and perifoveal intercapillary area; sensitivity decreased as the size of these areas increased. The loss in contrast sensitivity observed in diabetic patients suggests that it is a more sensitive indicator than visual acuity assessment in detecting early function reductions secondary to changes in retinal vasculature (Arend, *et al.*, 1997). This is in agreement with other studies where the assessment of visual acuity has been critisized as being an insensitive tool for measuring early alterations in retinal function in diabetic patients (Regan & Neima, 1984).

Arend, *et al.*, (1997) found the greatest reduction in contrast sensitivity at 12 cycles per degree in the diabetic patients; these findings are similar to those reported by Sokel, Moskowitz, Skarf, *et al.*, (1985) who found contrast sensitivity to be at a minimum at 11.4 cycles per degree. Furthermore, Harris, Arend, Danis, *et al.*, (1996b) identified significant reductions in contrast sensitivity at 12 and 18 cycles per degree in diabetic patients with no or early diabetic retinopathy. It was found that under hypoxic conditions contrast sensitivity significantly increased at 12 cycles per degree in these diabetic patients (Harris, *et al.*, 1996b). This suggests that tissue hypoxia results in visual and vascular dysfunction in diabetic patients.

Dosso *et al.*, (1996) also noted reductions in contrast sensitivity in diabetic patients and found that the severity of the contrast sensitivity deficits positively correlated with advancing age, systolic blood pressure and degree of nephropathy.

A recent study by Gartaganis, Psyrojannis, Koliopoulos, *et al.*, (2001) using the CSV-1000 contrast test chart, observed a significant reduction in contrast sensitivity in patients with impaired oral glucose tolerance test compared to normal subjects. These results suggest early functional damage of the tissues in the optical pathway in early diabetes and support the theory that contrast sensitivity is affected by faulty glycaemic control.

1.8.5.3 Visual fields and glaucoma

One of the characteristic signs of glaucoma is visual field loss resulting from the death of ganglion cell nerve fibres. The majority of glaucomatous visual field loss occurs within the central 30 degrees of the visual field (Armaly, 1971), usually in the form of small and isolated scotomas. As the disease progresses the scomata enlarge and join to form an arcuate shaped field defect which extends from the optic nerve head nasally or vice versa; this is a characteristic visual field defect of glaucoma that may occur superiorly or inferiorly.

Hoddapp, *et al.*, (1993) characterise glaucomatous visual field loss as: -

- Glaucoma hemifield test outside normal limits on at least two fields, or
- A cluster of three or more non-edge points in a location typical for glaucoma, all of which are depressed on the pattern deviation plot at a $p<0.05$ level and one of which is depressed at a $p<0.01$ level on two consecutive fields, or a corrected pattern standard deviation that occurs in less than 5% of normal fields on two consecutive visits.

Intraocular pressure is known to influence the rate at which visual field loss develops, with higher intraocular pressures resulting in a more rapid rate of visual field defect progression (Vogel, Crick, Newson, *et al.*, 1990). Visual field examination is therefore of importance when diagnosing or monitoring a patients condition. Stabilization of a glaucoma patient's visual field can be taken to imply adequate control of the disease. It is therefore important to have a common standard, which denotes when true visual

field change has occurred. Werner, Petrig, Krupin, *et al.*, (1989) reported variability in visual field results in clinically stable glaucoma patients with a one-year interval between tests; this variability was approximately 2.8 dB.

There are a variety of different ways to determine whether visual field progression has actually occurred (Hoddapp, *et al.*, 1993). The Humphrey visual field analyser has a glaucoma change probability program incorporated into its system that identifies whether any visual field defect progression has occurred. Follow-up fields are compared with the average values from two baseline fields. Each point of examination is analysed on each visual field plot and is compared across visits. The software takes into account the variability associated with repeated visual fields then identifies areas of the visual field that have significantly changed. Morgan, Feuer & Anderson, (1991) have shown that this method of analysis satisfactorily identifies visual field defect progression.

A number of investigations have used the HRT to assess structural damage in combination with visual field examinations to assess functional damage (Brigatti & Caprioli, 1995; Mikelberg, *et al.*, 1995; Eid, *et al.*, 1997; Anton, *et al.*, 1998). It has been found that strong correlations exist between nerve fibre layer height losses and visual field defects (see section 1.7.6.1).

1.8.5.4 Visual fields and diabetes

It has been shown that diabetic patients, with and without early diabetic retinopathy, have reduced colour discrimination to short wavelength stimuli (Utku & Atmaca, 1992; Kurtenbach, Wagner, Neu, *et al.*, 1994). In these studies, the tests carried out to examine the short wavelength sensitive cones concentrated on foveal function, with the utilization of colour vision tests such as the Farnsworth-Munsell D15 and 100 Hue.

Studies investigating the effect of diabetes on colour vision discrimination have shown the existence of colour vision defects in patients before the development of diabetic retinopathy (Bresnick, Condit & Palta, 1985; Green, Ghafour, Allan, *et al.*, 1985). The ganglion cells are made up of parvocellular and magnocellular cells, which are characterised, by their morphology and functionality. The parvocellular system

enables the resolution of high spatial frequencies and is responsible for the processing of chromatic information. The finding of colour vision defects and contrast sensitivity loss at high spatial frequencies indicates the involvement of the parvocellular system during the early stages of ocular involvement in diabetic patients.

It should be taken into account that patients with diabetes are at an increased risk of developing cataracts compared to normal subjects (Klein, Klein & Moss, 1985). Thus, diabetic patients are more prone to yellowing of the crystalline lens and increased absorption of the lens to short wavelength light. A recent study by Kessel, Alsing & Larsen, (1999) which investigated colour vision in diabetic and normal patients after cataract surgery, found no significant difference in colour vision between the subject groups. They concluded that the colour vision defects observed in diabetic patients are primarily due to accelerated lens aging.

SWAP enables the assessment of short wavelength sensitive receptors throughout the retina. Remky, Arend & Hendricks, (2000) found that SWAP enabled the identification of diabetic patients with early ischaemic diabetic maculopathy. These results are in agreement with those made by McCreesh, Hudson, Silvestri, *et al.*, (2000) and Hudson, McCreesh, Quinn, *et al.*, (2001b) who found SWAP to be superior to standard automated white on white perimetry in the discrimination of patients with clinically significant macula oedema. Nomura, Terasaki, Hirose, *et al.*, (2000) carried out SWAP on two cohorts of diabetic patients with simple background diabetic retinopathy and no diabetic retinopathy and found that with advancing diabetic retinopathy, short wavelength sensitivity decreased. An outcome from the study identified the greater loss in sensitivity to be in the upper visual field, a reduction of the foveal short wavelength sensitivity was also noted but to a lesser, (non-significant), degree. These results suggest that in diabetic patients the short wavelength sensitive system in the lower paracentral area of the retina is more vulnerable to diabetic retinal damage.

1.9 Research Rationale

1.9.1 Introduction

There are a number of techniques available that enable the measurement of ocular blood flow. Fluorescein angiography and indocyanine green are commonly used to assess retinal and choroidal blood flow in patients with suspected vascular abnormalities, however the invasive nature of these techniques prohibits their wider clinical application. The development of relatively non-invasive techniques such as scanning laser Doppler flowmetry and pneumotonometry allows clinical measurements of retinal and choroidal blood flow to be obtained easily with little or no risk to patients.

Ocular diseases such as glaucoma and diabetic retinopathy are considered to be at least partially vascular in origin. Detection of these abnormalities would help in identifying disease at an earlier stage and may be useful for monitoring progression.

1.9.2 Research aims

There were three principle aims to this thesis:

1. To optimise the acquisition protocols of the HRF and OBFA for cross sectional and longitudinal application.
2. To investigate the effects of age and interocular symmetry on ocular blood flow measures in normal, healthy individuals using the HRF and OBFA.
3. To investigate the ocular perfusion characteristics of patients diagnosed with glaucoma and diabetes.

1.9.3 Optimisation of data acquisition protocols for ocular blood flow measurement.

1.9.3.1 Influence of detector sensitivity on measures of neuroretinal rim and retinal capillary blood flow

For subtle changes in blood flow to be detected over time, the reproducibility of the HRF must be optimized. Reproducibility of the HRF is influenced by various factors including eye movement (Michelson & Schmauss, 1995), the cardiac cycle (Sullivan,

et al., 1999), positioning of the laser (Kagemann, *et al.*, 1998a) and zero offset (Chauhan & Smith, 1997).

The purpose of this study was to investigate the effect photodiode sensitivity has on the reproducibility of blood flow measures using scanning laser Doppler flowmetry. Recommendations have been made that images should not be too dark or bright with them being within a range of between 80 and 150 DC (Michelson & Schmauss, 1995). However the effect of DC value on the different tissue types of the peripapillary retina and neuroretinal rim has not been investigated. In order to investigate this a comparative study of images from 15 young and 15 mature, healthy subjects and 15 patients with primary open-angle glaucoma were explored.

1.9.3.2 Application of a local search strategy to improve detection of blood flow deficits using the HRF.

Using the scanning laser Doppler flowmeter, significant reductions in blood flow measures have been demonstrated in the retina (Michelson, *et al.*, 1996a) and lamina cribrosa (Nicolela, *et al.*, 1996) of primary open angle glaucoma patients and in the peripapillary retina (Chung, *et al.*, 1999) in patients with normal tension glaucoma. At the neuroretinal rim, blood flow measurements have been reported as being lower by some authors (Michelson, *et al.*, 1996a), however this has not been a universal finding (Nicolela, *et al.*, 1996). It is possible that that some of these reported differences are due to the effects of a local physiologic variability, most likely arising from vascular pulsation, which is not accounted for during conventional analysis.

The purpose of this study was to determine whether blood flow deficits are identified more easily when a search strategy is applied. In order to investigate this, ocular perfusion maps acquired using the HRF, were analysed by repositioning the standard 10×10 pixel box within a 15×15 pixel grid and determining the highest and lowest local flow values for each image. A cross-sectional analysis of images from 15 mature, healthy subjects and 15 glaucoma patients was performed.

1.9.3.3 Consecutive versus non-consecutive intraocular pressure pulses in the analysis of ocular pulsatility

In order to calculate measures of POBF, the OBFA requires 5 good quality pulses captured within a twenty second period. This means that the pulses used for analysis may be separated by time or captured consecutively. If the pulses are acquired over a greater time lapse, as with the non-consecutive method, then factors such as changes in intraocular pressure due to respiration or any tonometric effect are more likely to have an influence on the data, possibly resulting in larger variability in measures of pulsatility.

Spraul *et al.*, (1998) reported that the majority of the variance found in ocular blood flow measures was due to between-subject variability, but that the remaining 30 to 40% of variability was due to measurement error of the instrument. In order for the OBFA to be of clinical use for the long-term follow-up of individuals and for the assessment of ocular blood flow pre- and post-treatment, within-subject error needs to minimised.

The purpose of this study was to compare the reproducibility of ocular blood flow measures using consecutive and non-consecutive intraocular pressure pulses in a sample of twenty young, healthy subjects.

1.9.3.4 Spectral analysis of the intraocular pressure pulse.

The ocular pulse is a periodic function and may therefore be resolved into its component sinusoidal waves of different frequencies (Best & Rogers, 1974). The Fourier components of the ocular pulse have been assessed previously in rabbits (Best, Rogers, York; 1974) to assess the sensitivity of spectral analysis in detecting different degrees of carotid stenosis. Fourier analysis of the waveform was more sensitive to small degrees of carotid stenosis compared with standard graphical techniques of waveform analysis.

If dysfunctional ocular blood flow contributes to the pathogenesis of glaucoma and the Fourier harmonics of the pressure pulse wave are related to the mechanical status of

the vessels, then it is possible that these measures differ between normal subjects and glaucomatous patients.

The purpose of this study was firstly to determine whether the higher harmonic frequency components of the intraocular pressure pulse could be resolved using Fourier analysis. Secondly, using a sample of 10 healthy subjects and 10 patients with early glaucoma, the differential properties of this power analysis was investigated.

1.9.4 Influence of normal physiological factors on ocular perfusion characteristics.

Age is known to effect blood vessel histology with decreases in vascular elasticity occurring with advancing age (Wadsworth, 1990; Wei, 1992). It would appear logical therefore that these changes in blood vessel morphology would have a corresponding effect on ocular blood flow. Ocular pulsatility, which is derived from the pulsatile component of blood inflow during cardiac systole and is influenced by, among other things, vascular elasticity, has been shown to reduce with advancing age (Ravalico, *et al.*, 1996; Massey, Geyer & Silver, 1999). Reductions in blood flow have also been demonstrated in the retina with advancing age (Groh, *et al.*, 1996), although these findings were not replicated at the neuroretinal rim.

1.9.4.1 Age and systemic circulation on ocular pulsatility.

The OBFA measures the pulsatile component of ocular blood flow. Reductions in POBF have been observed with advancing age (Ravalico, *et al.*, 1996) and in glaucoma patients (James & Smith, 1991; Trew & Smith, 1991b; Fontana, *et al.*, 1998).

Interarm differences in systolic and diastolic blood pressure have been reported in subjects (Frank, Norris, Christopherson, *et al.*, 1993; Singer & Hollander, 1996; Cassidy & Jones, 2001), and these differences have been greater in patients with vascular disease (Frank, *et al.*, 1993; Singer & Hollander, 1996). The carotid artery follows a pathway that perfuses the left ophthalmic artery before the right, and it has been suggested that the left eye is more vulnerable to vasoactive stimuli (Kagemann,

Harris, Evans, *et al.*, 1998b), and hence more susceptible to ocular diseases with haemodynamic involvement.

With advancing age the composition of the blood vessels alters resulting in reduced blood vessel elasticity and vascular compliance (Wadsworth, 1990; Wei, 1992).

The purpose of this study was two fold. Firstly, to assess whether interocular differences in POBF are present in a normal, age stratified population and, secondly, to examine the influence of systemic circulation and age on measures of ocular pulsatility.

The sample consisted of 240 eyes of 120 healthy volunteers banded twenty per decade (20 to 80 years) with 10 males and 10 females being in each group.

1.9.4.2 Effect of age on intraocular capillary perfusion.

The lamina cribrosa is an elastic structure composed of perforated cribriform plates through which the nerve fibres and the central retinal artery pass. It receives its blood supply from the choroidal arteries and the short posterior ciliary arteries (Hayreh, 1978). With advancing age the components of the cribriform plates alter (Albon, Karwatowski, Avery, *et al.*, 1995); this results in a less pliable structure with reduced mechanical compliance (Albon, Purslow, Karwatowski, *et al.*, 2000). It has been suggested that these changes are likely to make the ageing eye more susceptible to retinal ganglion cell axon damage especially when combined with fluctuations in intraocular pressure as seen with primary open angle glaucoma (Albon, *et al.*, 2000).

In addition to the known vascular changes associated with age it is possible that a decrease in the elasticity of the lamina cribrosa may have an effect on the blood flow of the vessels that supply the lamina cribrosa with nutrients. This has been postulated as a cause of optic nerve damage in glaucoma, but has not previously been investigated in normal, healthy eyes.

The purpose of this study was to determine the effect of increasing age on ocular blood flow in the retina, neuroretinal rim and lamina cribrosa. A comparative study of

perfusion images obtained with the HRF was performed using a subject sample of 15 young and 15 mature, healthy subjects.

1.9.5 Pathological variation in ocular perfusion.

1.9.5.1 Capillary blood flow of the retina and neuroretinal rim of glaucoma patients

See section 1.9.3.2

1.9.5.2 Ocular pulsatility in patients with primary open angle glaucoma.

See section 1.9.3.4

1.9.5.3 Ocular perfusion in the macula of diabetic patients following cataract surgery.

In diabetic retinopathy the development of diabetic macular oedema results in a significant reduction in vision, and in its early stages laser photocoagulation is thought to aid visual prognosis. It has been reported that cataract surgery in diabetic patients results in further progression of diabetic retinopathy, including clinically significant macula oedema, and that its progression is associated with poor visual prognosis (Pollack, Dotan & Oliver, 1991; Pollack, Leiba, Bukelman, *et al.*, 1992b). More recently the visual improvement in non-diabetic patients following phacoemulsification was found to be superior than that attained by diabetic patients (Sadiq, Sleep & Amoaku, 1999).

The purpose of this study was to determine whether measures of macula blood flow, by scanning laser Doppler flowmetry, and topography, by confocal scanning laser tomography, can be used to differentiate diabetic patients at risk of developing clinically significant diabetic macula oedema following phacoemulsification. Further, the effect that plasma glucose and glycosated haemoglobin levels have on these parameters was investigated. The sample comprised seventeen normal subjects and diabetic patients in a three-month follow up study.

For comparative analysis visual performance was also investigated. Snellen visual acuity is often used to assess functional capability but contrast sensitivity has been shown to be a more sensitive indicator of functional ability in diabetic patients, with

decreases in contrast sensitivity being found in individuals with background diabetic retinopathy (Sokol, *et al.*, 1985), whilst visual acuity remains unchanged. Furthermore, short wavelength automated perimetry provides early detection of visual function loss in diabetic patients (Nomura, *et al.*, 2000; Remky, *et al.*, 2000). These additional performance indicators were included in the study.

CHAPTER 2:

The influence of detector sensitivity on blood flow measures of the retina and neuroretinal rim using scanning laser Doppler flowmetry

2.1 Abstract

The Heidelberg Retina Flowmeter (HRF) is a scanning laser Doppler flowmeter that enables the measurement of retinal capillary perfusion. It can be used to assess blood flow deficits between subject groups and to monitor blood flow of patients over time. The photodiode sensitivity can be altered manually on the control panel to obtain images of varying brightness's. It has been recommended that images should be collected with a DC value of between 80 and 150 (Michelson & Schmauss; 1995). The purpose of this study was to determine whether blood flow measures of different tissue types are influenced by DC value and whether age and disease has further effects on these measures.

2.2 Background

The scanning laser Doppler flowmeter (SLDF) is used for the non-invasive *in-vivo* assessment of ocular blood flow, volume and velocity of the microvasculature of the retina and optic nerve head. The combination of laser scanning ophthalmoscopy and laser Doppler flowmetry offered by the Heidelberg Retina Flowmeter (HRF, Heidelberg Engineering, Germany) enables two-dimensional mapping of retinal perfusion.

Blood flow measures obtained using the HRF yield valid and reproducible measurements of retinal blood flow (Michelson & Schmauss, 1995; Michelson, *et al.*, 1996b; Nicolela, *et al.*, 1997). Application of the HRF has resulted in findings of reduced ocular blood flow in ocular diseases such as branch retinal vein occlusion (Avila, Bartsch, Bitner, *et al.*, 1998) and glaucoma (Michelson, *et al.*, 1996a; Nicolela, *et al.*, 1996; Chung, *et al.*, 1999). However in diabetes, while differences in ocular blood flow have been identified between subjects groups using other techniques

(Geyer, *et al.*, 1999; Findl, *et al.*, 2000a), investigations using the HRF revealed no significant alterations in measures of retinal blood flow (Findl, *et al.*, 2000a).

Ocular blood flow values measured by HRF are affected by a number of optical and physiological factors such as incidence luminance (Kagemann, *et al.*, 1998a) and tear film stability. In an *in-vitro* study, Tsang, Harris, Kagemann, *et al.*, (1999) showed that darker backgrounds resulted in higher measured blood flow velocities. This finding suggests that HRF blood flow values may be altered by image brightness.

In the HRF, the sensitivity of the photodiode detector can be altered using a control panel. This results in images of varying brightness, a measure of which is derived from the direct current (DC) recorded at the detector. This value can be determined within any selected window in the processed blood flow map. Images may be obtained for DC values ranging from approximately 30 to 230; at a DC of 30 images appear dull, whilst at a DC of 230 they are very bright, or even saturated.

2.3 Hypothesis

The sensitivity setting of the photodiode detector on images acquired using the HRF influences measures of perfusion in the retinal microcirculation.

2.4 Aims and Objectives

The purpose of this study was to investigate the effect of changing photodiode sensitivity on blood flow measurements obtained using the HRF in young and mature healthy subjects and in patients suffering from primary open angle glaucoma.

2.5 Materials and Methods

2.5.1 Subject Sample

The sample consisted of one eye of each of fifteen young, healthy subjects subjects, fifteen mature, healthy subjects and fifteen primary open angle glaucoma patients. Details of the subject's samples are given in table 2.1.

Subject Group	Gender		Test Eye		Mean Age ± SD (range) (years)
	Male	Female	Right	Left	
Young Subjects	7	8	8	7	27.8 ± 6.14 (20-37)
Mature Subjects	7	8	8	7	65.2 ± 13.7 (48-82)
Glaucoma Patients	8	7	8	7	69.1 ± 6.6 (58-82)

Table 2.1. Details of the subject samples used in the study.

2.5.2 Inclusion Criteria

2.5.2.1 Young and mature healthy subjects

The young healthy subjects were recruited from members of the undergraduate optometry course, whilst the mature healthy subjects were recruited from the University eye clinic, spouses of patients or were members of staff from the hospital. In order to be categorised as healthy, all subjects in both groups exhibited: -

- Visual acuity of 6/9 or better in each eye, assessed using Snellen visual acuity charts.
- Less than 1 dioptre of astigmatism and 8 dioptres of spherical ametropia.
- Intraocular pressures below 22mmHg in each eye as measured using Goldmann tonometry.
- Normal optic nerve head appearance confirmed by ocular examination.
- Open anterior chamber angles ascertained from Van-Hericks slit lamp biomicroscopy technique.
- No history of ocular trauma or surgery.
- No diabetes mellitus or hypertension.
- No family history of glaucoma.
- No systemic or ocular medication that may affect blood flow.

2.5.2.2 Primary Open angle glaucoma patients

Patients were recruited from the ophthalmology department of Birmingham Heartlands hospital. They were required to have:

- Snellen visual acuity of 6/9 or better in each eye

- Refraction of less than 8 dioptres spherical ametropia and 1 dioptre astigmatism
- No history of ocular trauma or surgery
- No diabetes mellitus
- Repeatable mild to moderate visual field defects as defined by Hodapp, *et al.*, (1993), assessed using the Humphrey visual field analyzer and the 24-2 threshold program.
- Confirmed optic nerve head cupping consistent with a diagnosis of glaucoma.

At the time of the study all glaucoma patients were on ocular hypotensive medications with mean intraocular pressures of 15.8±3.8mmHg.

2.5.3 Ethical approval and informed consent

Ethical approval was obtained from the ethical committee boards of Birmingham Heartlands and Solihull NHS Trust and Aston University. Approval conformed to the tenets of the declaration of Helsinki. Informed consent was obtained from all subjects and patients.

2.5.4 Preliminary Tests

The personal and family general and ocular health of every subject and patient was obtained. Following this assessment of the posterior segment was conducted by a consultant ophthalmologist at Birmingham Heartlands and Solihull hospital, to ensure that no ocular pathology was evident in each of the healthy subjects and to verfy the existence of glaucomatous optic neuropathy in each of the glaucoma patients.

Measurements of Snellen visual acuity, refraction, anterior chamber angle depth (Van Herrick's method), and keratometry readings (measurement of anterior corneal curvature in mm) using the Bausch & Lomb Keratometer were acquired.

2.5.5 Dilation

One drop of 1% tropicamide was instilled to the test eye. After twenty minutes the pupillary light reflex was tested to ensure maximal mydriasis and imaging of the retinal vasculature began.

2.5.6 HRF image acquisition

Prior to image acquisition subject details were entered into the HRF database, this included mean spherical refractive error and corneal curvature. The subjects were instructed to place their chin on the chin-rest and fixate on a cross target placed approximately 2 metres from the patient. One eye was alternatively assigned for consecutive subjects within each group. The HRF was positioned approximately 1.5 cm from the subject's cornea. The light source of the laser was centred on the pupil and the angle of the laser head manipulated until the desired anatomical area of interest was in view. Using the subjects refraction as a starting point the laser was focused onto the retina or neuroretinal rim and adjusted until optimal focus was obtained using the coarse and fine focusing dials situated on the control panel. The intensity was set to maximum and a uniformly lit image acquired.

Three HRF images were taken of the superior temporal retina. Three additional images were acquired of the superior temporal rim. In each case images were acquired in five different DC bands resulting in three images within each band for each fundus location. Movements and blinking are observed in the image series as either skewed or blank sections; image series obtained were only accepted when eye movements were minimal. The sensitivity of the HRF was altered manually on the control panel in order to acquire images with different DC values. The superior temporal area of the optic nerve head and retina was chosen for data collection as it has been shown that measurements taken here exhibit the least amount of variability (Bohdanecka, Orgul, Prunte, *et al.*, 1998), thus variability due to DC changes would be more clearly manifested. The minimum DC value used was 30 and the maximum 230; representing the darkest to brightest range of values which provide visible microvasculature. Five DC bands were used; 30 to 70 (band 1), 70 to 110 (band 2), 110 to 150 (band 3), 150 to 190 (band 4) and 190 to 230 (band 5). Throughout the examination each subject was requested to keep his or her chin on the rest. This ensured the distance between

the laser and the eye remained constant, thereby increasing the reproducibility of image acquisition (Kagemann, *et al.*, 1998a).

Fast Fourier transformation was used to derive maps of retinal and neuroretinal rim perfusion. Values of blood flow, volume and velocity (arbitrary units) were determined for each image using a 10 by 10 pixel square grid located at a pre-determined position on the retina and rim of each subject. This pixel frame size was chosen, as it is small enough to allow avoidance of the larger blood vessels (Michelson & Schmauss, 1995). The corresponding DC values were measured within the pixel frames for each subject in both locations within each DC band. Acetate sheets were used to trace over the retinal vasculature of each fundus and a blood vessel landmark, such as a vessel bifurcation, was identified to ensure that the same retinal and neuroretinal rim locations were used for each image. Every effort was made to keep the retinal and neuroretinal rim locations constant within subject image series. Large vessels and image areas interrupted by movement saccades were avoided

2.6 Statistical Analysis

The mean and standard deviation of each blood flow parameter at both locations was determined for each eye examined. Group-mean blood flow, volume and velocity measures and group-mean standard deviations were determined for the retina and neuroretinal rim locations for each subject group; such that: -

$$Mean = \sum_{i=1}^{N} Xi \qquad \text{Equation 2.1}$$

where: $\sum X_i$ = The sum of values

N = number of subjects

and

$$\text{Standard Deviation (s)} = v\frac{\sum x^2}{N} \qquad \text{Equation 2.2}$$

where:

$\sum x^2$ = The sum of the squared mean deviation

(Norman & Streiner, 1994)

Repeated measures analysis of variance (ANOVA) was then used to test for significance at the 95% level, with DC value as the dependent variable. *Post hoc* analysis was used to identify outliers.

A repeated measures ANOVA studies the effect of nominal independent variables (DC value) on a continuous response variable within sucessive measurements (measures of blood flow, volume and velocity); and determines whether there are any significant effects. *Post hoc* analysis is used to identify which of the mean dependent variable values are different from each other.

2.7 Results

Analysis of variance revealed significant reductions in retinal blood flow, volume and velocity in the mature normal and glaucomatous subject group and in retinal flow and velocity only in the young, healthy subject group with increasing DC levels. Neuroretinal rim blood flow, volume and velocity measures significantly decreased using a higher DC value to acquire images in all the subject groups. Table 2.2 shows p-values for the blood flow parameters with changing DC level for the three different subject groups.

Blood Flow Parameter	Young Subjects		Mature Subjects		Glaucoma Patients	
	Retina	Rim	Retina	Rim	Retina	Rim
Flow (A.U)	0.035	0.016	0.002	0.0153	0.0004	<0.0001
Volume (A.U)	NS	0.003	0.0003	0.0003	0.0087	0.0056
Velocity (A.U)	0.049	0.026	0.001	0.0113	0.0003	<0.0001

Table 2.2. **Summary table showing ANOVA (p-values) for change in measured perfusion parameters as a function of changing DC values for the three subject groups. NS = not significant.**

Blood flow, volume and velocity measures for different sensitivity settings are shown for the retina (Figures 2.1, 2.2 and 2.3 respectively) and neuroretinal rim (Figures 2.4, 2.5 and 2.6 respectively). *Post hoc* analysis showed that perfusion values were shifted in DC band 5 as indicated on the graphs by an asterisk for the young subjects, a circle for the mature subjects and a square for the glaucoma patients; where $p<0.05$ in each case.

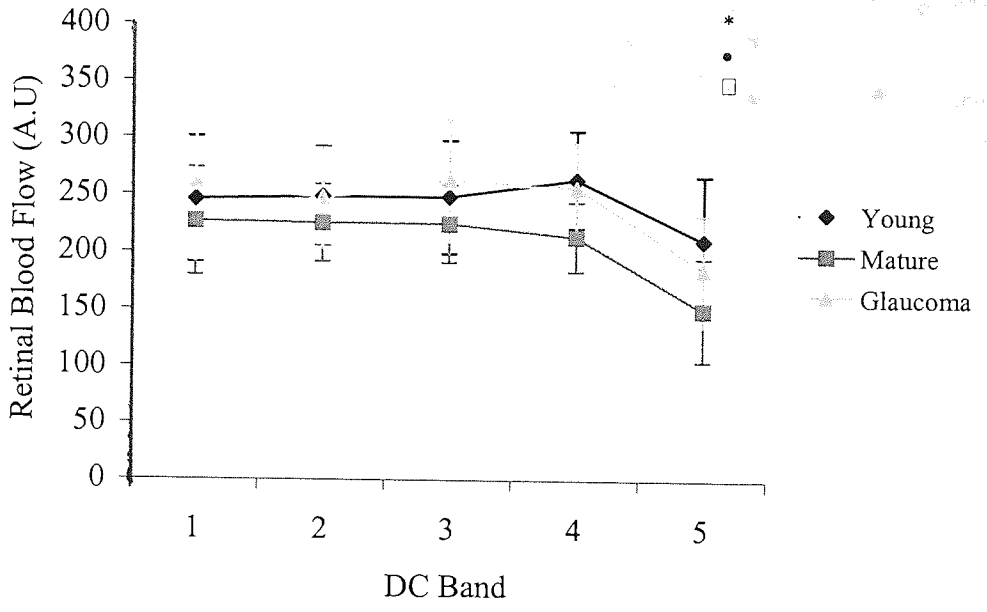

Figure 2.1. **Measures of retinal blood flow (A.U) ± standard deviation, with changes in DC level. *Post hoc* analysis revealed significant reductions in retinal blood flow when the DC level was between 190 and 230 in young normal subjects (*), mature, healthy subjects (•) and glaucoma patients (□).**

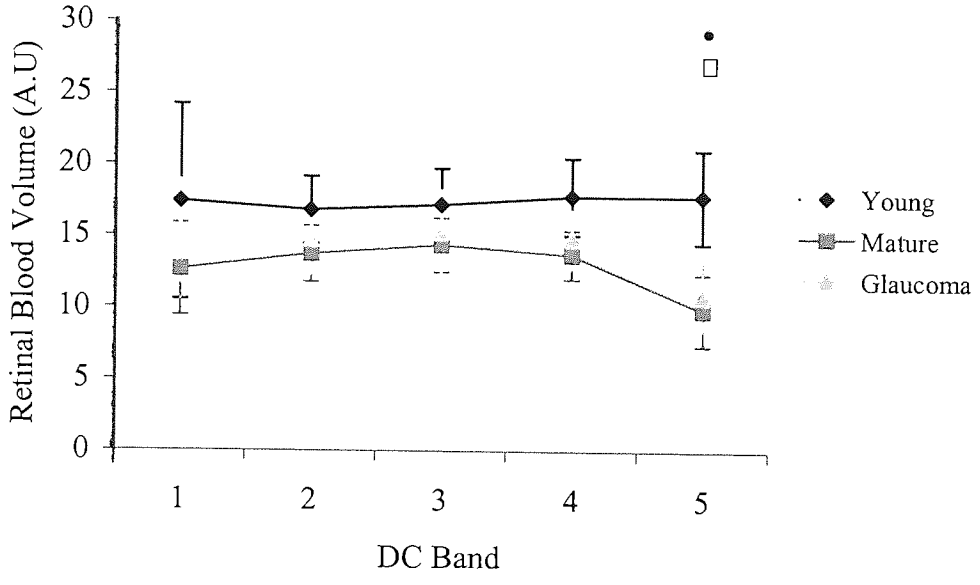

Figure 2.2. **Measures of retinal blood volume (A.U) ± standard deviation, with changes in DC level. *Post hoc* analysis revealed significant reductions in retinal blood volume when the DC level was between 190 and 230 in mature, healthy subjects (•) and glaucoma patients (□).**

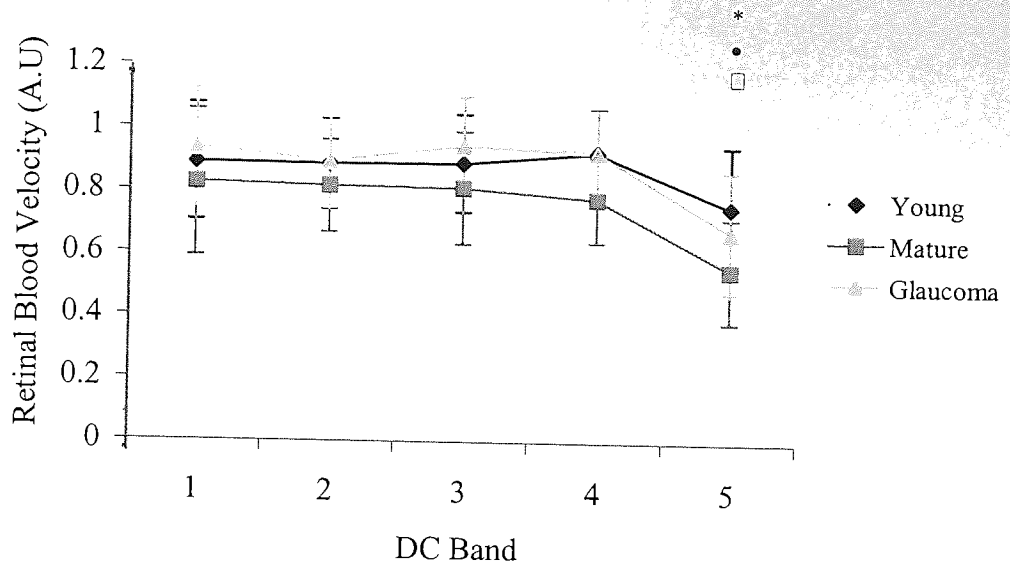

Figure 2.3. **Measures of retinal blood velocity (A.U) ± standard deviation, with changes in DC level. *Post hoc* analysis revealed significant reductions in retinal blood velocity when the DC level was between 190 and 230 in young normal subjects (*), mature, healthy subjects (•) and glaucoma patients (□).**

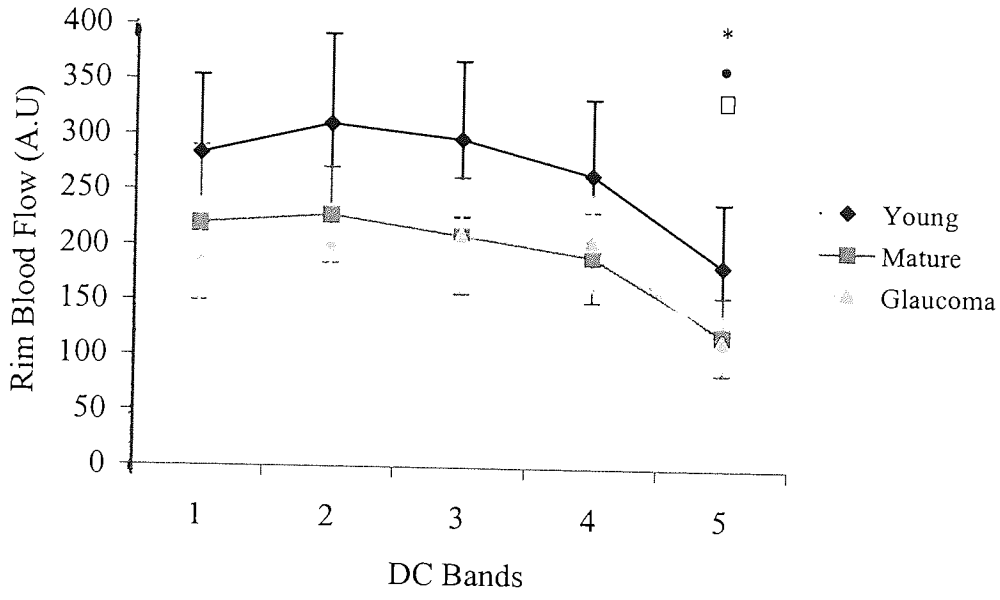

Figure 2.4. **Measures of neuroretinal rim blood flow (A.U) ± standard deviation, with changes in DC level. *Post hoc* analysis revealed significant reductions in neuroretinal rim blood flow when the DC level was between 190 and 230 in young normal subjects (*), mature, healthy subjects (•) and glaucoma patients (□).**

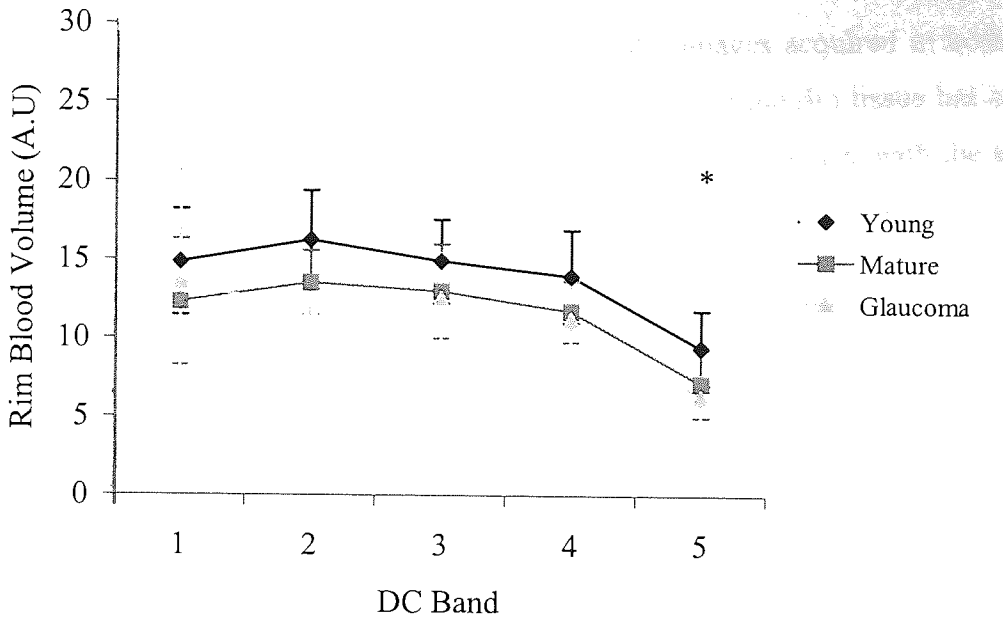

Figure 2.5. **Measures of neuroretinal rim blood volume (A.U) ± standard deviation, with changes in DC level. *Post hoc* analysis revealed significant reductions in neuroretinal blood volume when the DC level was between 190 and 230 in young normal subjects (*), mature, healthy subjects ( ) and glaucoma patients ( ).**

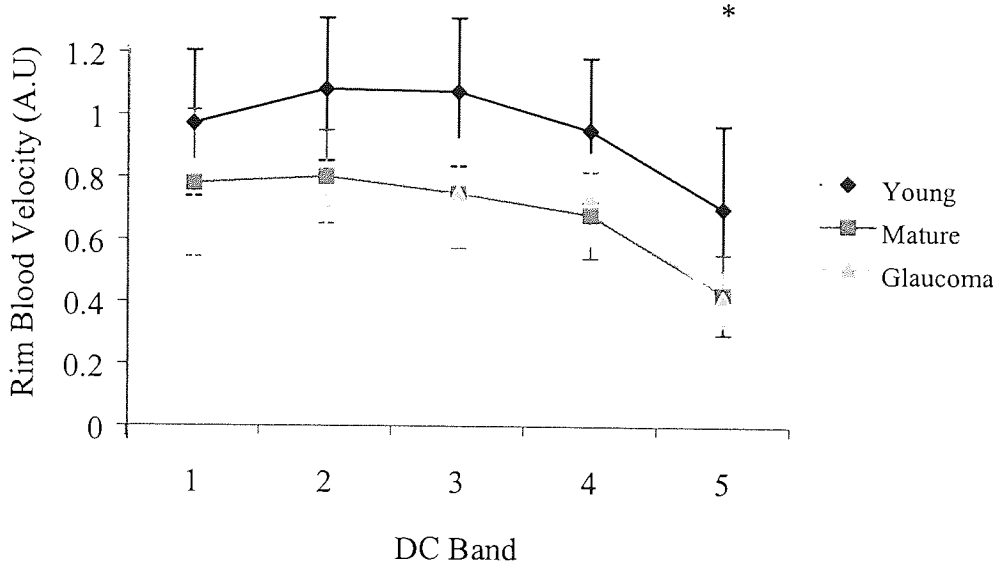

Figure 2.6. **Measures of neuroretinal rim blood velocity (A.U) ± standard deviation, with changes in DC level. *Post hoc* analysis revealed significant reductions in neuroretinal blood velocity when the DC level was between 190 and 230 in young normal subjects (*), mature, healthy subjects ( ) and glaucoma patients ( ).**

2.8 Discussion

A linear increase in the DC value was achieved for the images acquired in both the retina and rim tissues. It is worth noting that images in which the rim tissue has a DC value of, for example 70, would have to be much brighter than images with the same DC value in the retina. For this reason, images of the retina and neuroretinal rim were collected entirely separately.

Measured values of retinal blood flow and velocity were significantly lower in band 5 compared to other DC bands in all the subject groups, and for retinal blood volume in the mature normal and glaucoma patient subject groups. This is illustrated in Figure 2.1 for retinal blood flow in which it can be seen that measured values remain relatively stable throughout DC bands 1 to 4, with a decrease in values in DC band 5. In the young, healthy subject group retinal blood volume was not significantly altered by changes in photodiode sensitivity, although there was a trend for blood volume values to decrease in DC band 5. These results indicate that for measures at the retina, HRF flow values are relatively stable until DC values exceed 190. These results are in agreement with the findings from a study conducted by Kagemann *et al.*, (2001); where measures of retinal blood flow using the HRF were artificially low when photodiode detector sensitivity was too high.

In the neuroretinal rim, values of blood flow, volume and velocity were also significantly reduced in DC band 5 when compared to values recorded in all other DC bands ($p < 0.05$). In all the subject groups all parameters showed a peak blood flow value in DC bands 2 and 3 (DC 70 to 150), as shown in Figure 2.4 for the blood flow values in the neuroretinal rim. Although this latter finding is not a statistically significant increase, it has implications for optimising the sensitivity and specificity in studies of a cross-sectional or longitudinal nature, in that it may mimic or mask subtle differences between groups or subtle change, and should be considered when acquiring and analyzing data.

Changes to the measured DC values arise predominantly from alterations in photodiode detector sensitivity. It is unclear why measured blood flow values in tissue

should differ with DC value in this way. It is possible that there is a sampling effect in which changes in detector sensitivity result in altered sensitivity of the photodiode detector to blood corpuscles moving with different velocities. A bias towards low velocity particles for example, would result in both the number and mean rate of measured corpuscles recorded being lower, resulting in reduced blood flow, volume and velocity as seen in the data from DC band 5 in this study. Conversely, increased sensitivity to rapidly moving particles may explain the slight peak in measured flow seen in band 2 and 3 for measures at the neuroretinal rim (Figure 2.4).

Previous studies have shown blood flow differences between normal volunteers and glaucoma patients (Michelson, *et al.*, 1996a; Nicolela, *et al.*, 1996), and between ocular hypertensives and glaucoma patients (Kerr, Nelson & O'Brien, 1998). Michelson and associates (1996a) investigated blood flow at the peripapillary retina and neuroretinal rim in primary open angle glaucoma patients. In the retina, compared to normal subjects significant reductions in measured blood flow, volume and velocity were found in the order of 226 ± 119, 13.6 ± 8.5 and 0.7 ± 0.34 (arbitrary units, A.U) respectively, and 381 ± 173, 17.8 ± 10.2 and 1.2 ± 0.57 (A.U) respectively in the neuroretinal rim. Nicolela *et al.*, (1996) also reported lower blood flow measures in primary open angle glaucoma patients compared to normal subjects in both the superior temporal retina and lamina cribrosa, which were slightly less marked than in Michelson's study and were not confirmed at the neuroretinal rim. In our study differences in blood flow measures were induced by changes in DC value alone. From figures 2.1 and 2.4 it can be seen that the range of mean flow values were altered by as much as 170 A.U. at the retina and 260 A.U. at the neuroretinal rim. This could offer an explanation for the discrepancy in results obtained by different authors, particularly at the neuroretinal rim (Michelson, *et al.*, 1996a; Nicolela, *et al.*, 1996). Investigators are more likely to detect subtle changes when DC values are carefully controlled between groups and over time as variation in blood flow values due to sampling of the photodiode detector will be kept to a minimum.

2.8. Summary

In conclusion, we observed DC-dependent alterations in each blood flow measure of flow, volume and velocity; for each parameter the fluctuation in values with change in

DC was greater in the neuroretinal rim than in the retina in all the subject groups. Furthermore, an increase in blood flow measures was observed in the neuroretinal rim when the DC value was between 70 and 150, this increase was not statistically significant but has implications for studies investigating neuroretinal rim blood flow. Our results suggest that if the DC value is not carefully controlled, changes in photodiode sensitivity may mimic or mask true changes in ocular haemodynamic indices measured with the HRF. When acquiring data for cross-sectional or longitudinal evaluation, we recommend that data should be analysed from areas where the DC value is in the range 70-150. This finding is in agreement with Michelson and Schmauss (1995) who recommend a DC value of between 80 and 150 and more recently by Kagemann *et al.,* (2001), who recommend neither too high or too low photodiode detector sensitivity settings when acquiring images using the HRF. This recommendation of using a setting of between 70 and 150 DC will optimise both sensitivity and specificity of data acquired using the HRF.

CHAPTER 3:

Investigation to determine the effectiveness of a local search strategy in detecting capillary blood flow deficits in patients with glaucomatous optic neuropathy.

3.1 Abstract

Blood flow measures acquired using the scanning laser Doppler flowmeter (SLDF) are known to be highly susceptible to spatial and temporal variations of physiological origin. The purpose of this study was to evaluate a local search strategy intended to overcome these intrinsic variations, thereby improving the detection of blood flow defects resulting from glaucoma.

3.2 Background

Investigations using the Heidelberg Retina Flowmeter (HRF, Heidelberg Engineering, Germany) have shown that it yields reproducible values of retinal perfusion (Michelson & Schmauss, 1995; Michelson, *et al.*, 1996b; Nicolela, *et al.*, 1997). Measures of capillary blood flow, volume and velocity are acquired by placing a 10×10 pixel grid at an area of interest. A particular difficulty with this method is that when comparing blood flow values between images for a patient, even when using a carefully selected pre-determined location, measurements can vary significantly. Large variations are often noted when the measurement box is moved by as little as 1 or 2 pixels. This is due, at least in part, to normal physiological variations in blood flow arising from the cardiac cycle, which may influence the actual positioning of the capillary bed, or the phase of the pulsation cycle during which data is acquired. The incorporation of pulse synchronisation during data acquisition has been shown to reduce the consequences of the spatial and temporal variability induced by systemic circulatory variation (Michelson, *et al.*, 1998b). The manufacturers recommend that blood flow measures are obtained by choosing a location on the retina or neuroretinal rim and measuring blood flow parameters within a 10x10 pixel grid (Michelson & Schmauss, 1995). If the vascular network is not stationary relative to the retina retracing of the same location using a mapping technique can be difficult. This will result in variability in measured flow, which is difficult to account for.

The HRF has been used to investigate blood flow deficits in patients with normal tension and primary open angle glaucoma (Michelson, *et al.*, 1996a; Nicolela, *et al.*, 1996; Kerr, *et al.*, 1998; Chung, *et al.*, 1999). Significant reductions in blood flow measures have been reported in patients with primary open angle glaucoma in the retina (Michelson, *et al.*, 1996a) and lamina cribrosa (Nicolela, *et al.*, 1996) and for normal tension glaucoma patients in the peripapillary retina (Chung, *et al.*, 1999). At the neuroretinal rim, blood flow measurements have been reported as being lower by some authors (Michelson, *et al.*, 1996a), however this has not been a universal finding (Nicolela, *et al.*, 1996). It is possible that the effects of a local physiologic variability, which is not accounted for during the analysis, can explain some of these differences in reported findings.

3.3 Hypothesis

The application of a local search strategy to identify the highest and lowest values of blood flow will reduce the variability due to vascular pulsation and improve the detection of group-wise differences in retinal microcirculation of normal and glaucomatous eyes.

3.4 Aims and Objectives

The purpose of this study was: -

- To establish the effects of primary open angle glaucoma on blood flow at the neuroretinal rim and peripapillary retina using the HRF.
- To optimise the detection of blood flow differences between subject groups using a new search strategy with the standard software.

3.5 Materials and Methods

3.5.1 Subject Sample

The sample consisted of one eye of each of fifteen mature, healthy subjects and fifteen primary open angle glaucoma patients. Details of the subject samples are given in table 3.1.

Subject Group	Gender		Eye		Mean Age ± SD (range) (years)
	Male	Female	Right	Left	
Normal Subjects	7	8	8	7	65.2 ± 13.7 (48-82)
Glaucoma Subjects	8	7	8	7	69.1 ± 6.6 (58-82)

Table 3.1. **Table showing the distribution of gender, study eye and age for the normal and primary open-angle glaucoma subjects.**

3.5.2 Inclusion Criteria

3.5.2.1 Mature healthy subjects

Normal subjects were recruited from the university eye clinic, spouses of patients or were members of staff from the hospital. All subjects were required to have: -

- Visual acuity of 6/9 or better in each eye
- Spherical ametropia of less than 8 dioptres and astigmatism of less than 1 dioptre
- Intraocular pressures below 22mmHg in each eye
- Normal optic nerve head appearance confirmed by ocular examination
- Open anterior chamber angles (grade 2 or above) as assessed using Van Herricks technique
- No history of ocular trauma or surgery
- No diabetes mellitus or hypertension
- No family history of glaucoma
- No systemic or ocular medication that may affect blood flow.

3.5.5.2 Primary Open angle glaucoma patients

Patients were recruited from the ophthalmology department of Heartlands Hospital. They were required to have: -

- Visual acuity of 6/9 or better in each eye
- Refraction of less than 8 dioptres mean sphere
- No history of ocular trauma or surgery
- No diabetes mellitus

- Repeatable mild to moderate visual field defects as defined by Hodapp, Parrish and Anderson (1993), using the 24-2 threshold program on the Humphrey visual field analyzer.
- confirmed optic nerve head cupping consistent with a diagnosis of glaucoma.

At the time of the study all glaucoma patients were on ocular hypotensive medications with mean intraocular pressures of 15.8±3.8mmHg.

3.5.6 Ethical approval and informed consent

Ethical approval was obtained from the ethical committee boards of Birmingham Heartlands and Solihull NHS Trust and Aston University. Approval conformed to the tenets of the declaration of Helsinki. Signed consent was obtained from all subjects and patients prior to commencement of the study.

3.5.7 Preliminary Tests

Details of each patients and subjects personal and family general and ocular health were obtained. Measurements of visual acuity, refraction, anterior chamber angle depth (Van Herrick's method) and keratometry readings (measurement of anterior corneal curvature in mm) were acquired. Following this each subject was assessed by a consultant ophthalmologist at Birmingham Heartlands and Solihull hospital, to ensure that no ocular pathology was evident in each of the mature, healthy subjects and to verify the existence of glaucomatous optic neuropathy in each of the glaucoma patients.

3.5.8 Dilation

One drop of 1% tropicamide was instilled to the test eye and after twenty minutes the pupillary light reflex was tested to ensure maximal mydriasis

3.5.9 HRF image acquisition

Before HRF images were acquired details of the subjects refraction and corneal curvature were entered into the HRF database and the subject was instructed to look at the target ahead of them. Images of the superior temporal retina and neuroretinal rim in each mature subject and glaucoma patient were then collected according to the methodology outlined in section 2.5.6.

The test eye was alternatively assigned so that approximately equal numbers of right and left eyes were included, as indicated in Table 3.1. Three ten-degree images were taken of the superior temporal neuroretinal rim, and three of the superio-temporal peripapillary retina, using the HRF. Retinal analysis was undertaken within 1.5 disc diameters of the rim, superior and temporal to the disc and avoiding the larger blood vessels. The superior temporal area of the retina and neuroretinal rim were chosen for image acquisition as these areas have been shown to exhibit less variability in blood flow measures when using the HRF (Bohdanecka, *et al.*, 1998). Efforts were made to optimize the reproducibility of blood flow measurements acquired by obtaining separate HRF images of the different fundus locations, and ensuring that the DC level of the area under examination remained consistently within the range 70 to150 A.U (Hosking, *et al.*, 2001b). In addition each subject was asked to keep their chin on the rest throughout the examination to ensure that the camera-eye distance remained constant, thus improving the reproducibility of blood flow measures (Kagemann, *et al.*, 1998a). As with the other HRF studies conducted, images were only accepted where no or very little eye movement had occurred during image acquisition, this ensured that only perfusion maps with no or very few movement saccades were used for analysis.

Using the HRF software (version 1.02) fast Fourier transformation was used to derive perfusion images. With the standard method, blood flow, volume and velocity (arbitrary units) were determined for each image using a 10×10 pixel square grid located at a pre-determined position on the retina and neuroretinal rim for each mature subject and glaucomatous patient. This was done using acetate sheets to map out the vascular network of the retina and neuroretinal rim in each patient and including the

position of the 10×10-pixel frame. This frame could then be repositioned in each subject and patient at the same location on the retina and neuroretinal rim in subsequent images to obtain blood flow measures.

Using the search strategy the 10×10-pixel frame was systematically re-positioned within a 15x15 pixel window located at a similar position on the retina or neuroretinal rim (figure 3.1). The highest and lowest local values of blood flow, volume and velocity were identified and recorded.

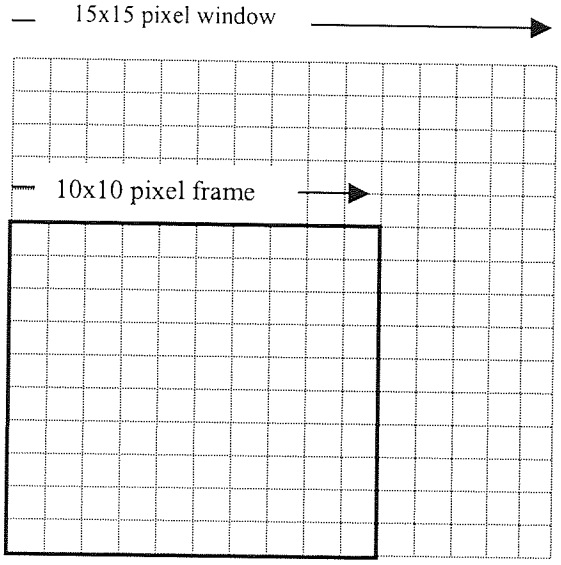

Figure 3.1. **Diagram illustrating the search strategy, showing a 10 × 10 pixel frame for analysis within a 15 × 15 pixel window.**

3.6 Statistical Analysis:

The mean and standard deviations of blood flow, volume and velocity were determined at the retina and neuroretinal rim for both the glaucoma patients and normal subjects using the standard strategy and the search strategy (highest and lowest values) for each eye (see section 2.6). Group-mean values and standard deviations were determined for the retina and rim locations for both methods. Student's unpaired *t*-tests were used to identify significant differences in blood flow, volume and velocity between the two groups at the retina and neuroretinal rim using both methods. To use the t-test to compare values between groups the group mean and standard deviation of

differences was calculated (see section 2.6). The standard error of the differences is calculated using the following formula:

$$S.E._d = \frac{(s_1^2 + s_2^2)}{n} \qquad \text{Equation 3.1}$$

Where:

$S.E._d$ = Standard Error difference

s_1 = Mean of sample 1

s_2 = Mean of sample 2

n = number of subjects

The *t*-test is then obtained by determining the signal-to-noise ratio such that: -

$$t = \frac{(X_1 - X_2)}{S.E._d} \qquad \text{Equation 3.2}$$

Where:

t = *t*-test value

$X_{1/2}$ = Mean of sample 1 / Mean of sample 2

3.7 Results

- There was no significant difference between the two groups in age.
- The glaucoma patients exhibited a mean of -5.44 ± 2.3 dB for the visual field mean deviation.
- At the retina, no significant difference was found between the two groups for blood flow, volume and velocity ($p > 0.05$) using either method. Blood flow, volume and velocity values are given in table 3.2.
- At the neuroretinal rim the standard technique revealed no significant difference between the two groups for blood flow, volume or velocity. Using the search strategy, significant perfusion reductions were found in the glaucoma group for the highest measured blood flow, volume and velocity values ($p=0.002$, $p=0.02$ and $p=0.002$ respectively). When the lowest blood flow, volume and velocity measures were evaluated the difference was less

marked, reaching significance for blood flow and velocity (p=0.023 and p= 0.021 respectively) but not for blood volume. Figures 3.2 to 3.4 show neuroretinal rim blood flow, volume and velocity measured using the two strategies for both the glaucoma and normal subject groups. Blood flow, volume and velocity values at the neuroretinal rim are given in table 3.2.

		Blood Flow (A.U) ± Standard error		Blood Volume (A.U) ± Standard error		Blood Velocity (A.U) ± Standard error	
		Retina	Neuroretinal Rim	Retina	Neuroretinal Rim	Retina	Neuroretinal Rim
Normal Subjects	Standard Values	224.98 ± 8.71	210.32 ± 21.73	14.35 ± 0.77	13.01 ± 0.90	0.81 ± 0.06	0.75 ± 0.08
	High Values	253.44 ± 14.22	294.74 ± 25.52	15.71 ± 0.74	16.34 ± 1.24	0.91 ± 0.05	0.99 ± 0.09
	Low Values	189.74 ± 11.58	201.81 ± 22.08	12.89 ± 0.60	12.49 ± 1.10	0.69 ± 0.04	0.64 ± 0.08
Glaucoma Patients	Standard Values	262.61 ± 13.62	210.27 ± 15.06	15.27 ± 1.01	12.49 ± 0.85	0.94 ± 0.06	0.73 ± 0.05
	High Values	266.60 ± 15.87	210.58 ± 10.44	16.33 ± 0.85	12.74 ± 0.94	0.96 ± 0.05	0.73 ± 0.03
	Low Values	206.60 ± 12.81	147.75 ± 10.27	13.77 ± 0.77	10.04 ± 0.84	0.75 ± 0.04	0.53 ± 0.04

Table 3.2. **Table showing the standard, high and low blood flow, volume and velocity measures (Arbitrary units) ± standard error at the retina and neuroretinal rim for the glaucoma patients and normal subjects.**

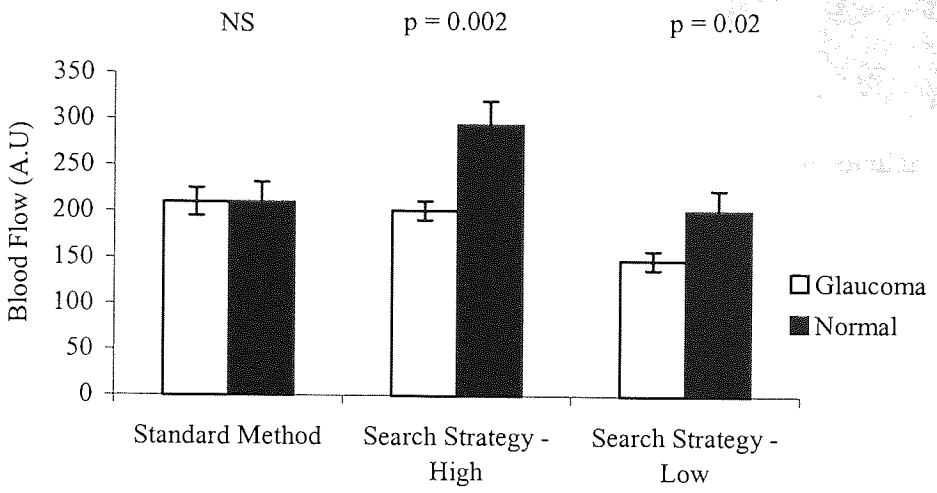

Figure 3.2. **Blood flow measures at the neuroretinal rim. No significant difference (NS) was found between the two groups using the standard strategy, while the search strategy revealed reduced flow in the glaucoma group using high (p=0.002) and low (p=0.02) values.**

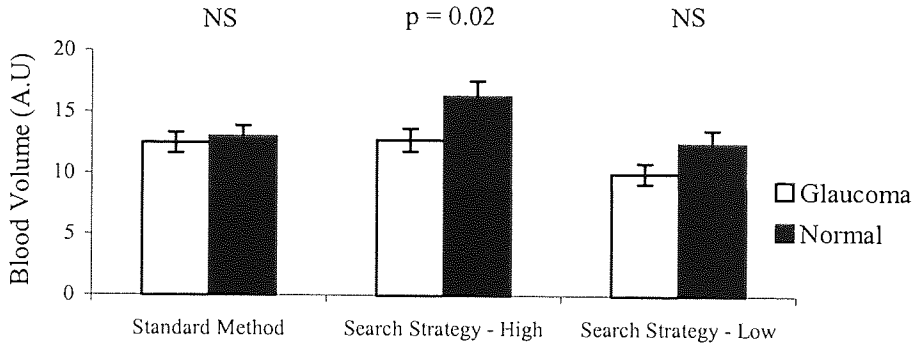

Figure 3.3. **Blood volume measures at the neuroretinal rim. No significant difference (NS) was found between the two groups using the standard technique, while the search strategy revealed reduced volume in the glaucoma group using high values only (p=0.02).**

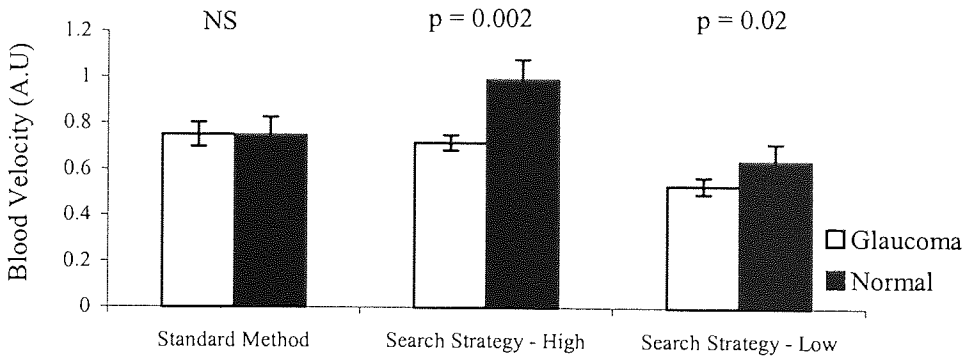

Figure 3.4. **Blood velocity measures at the neuroretinal rim. No significant difference (NS) was found between the two groups using the standard technique, while the search strategy revealed reduced blood velocity in the glaucoma group using high (p=0.002) and low (p=0.02) values.**

3.8 Discussion

Using the standard (static) method to acquire measures of ocular perfusion no significant difference was found between the two groups at either the retina or neuroretinal rim for any of the blood flow parameters. Using the search strategy, glaucoma patients exhibited significantly lower blood flow, volume and velocity in the neuroretinal rim when compared to age-matched normal subjects, however this difference was not replicated in the retina. These differences in blood flow were more marked when highest blood flow readings were used to compare between the two groups. For the lowest blood flow values, only the blood flow and velocity were significantly reduced in the diseased group.

The apparent variability in blood flow measures seen in any one image is largely due to the physiological pulsation of the retinal capillary bed which results in sizeable differences in blood flow measures with movement of the pixel frame by only one or two pixels (Michelson, *et al.*, 1998b; Sullivan, *et al.*, 1999). Analysis of ocular perfusion using the HRF technique and a standard 10x10 pixel grid samples data over a fixed area of interest and over a fixed period of time, thereby sampling velocities in both the spatial and temporal domains. The flow of blood corpuscles will vary both in velocity and number of cells, providing a distribution of values of blood flow for the area. We therefore suggest that the sampling process yields a range of velocities with varying frequency, and that these distributions may differ with factors such as age (Groh, *et al.*, 1996) or in the diseased compared to normal eye (Nicolela, *et al.*, 1996; Chung, *et al.*, 1999). Figure 3.5 shows a schematic frequency histogram that illustrates a possible model for the total blood flow defect in glaucoma patients compared to normal subjects, with a reduction in both the mean velocity and number of moving corpuscles. In this study, using the standard technique, no blood flow defects were identified, a finding that is at odds with most other reported literature (Michelson, *et al.*, 1996a; Nicolela, *et al.*, 1996). It is possible that this difference arises from the stage of glaucoma, which in our study was only mild to moderate based on visual field classifications, and may have been earlier than in previous studies. Alternatively there may be a sampling error such that blood flow differences were masked between the groups because the small sampling window does not allow for the inherent variations

in flow within the local vascular neighbourhood. For example, some patients may have been assessed in an area of relatively high local flow whilst others were assessed in an area of low flow, thereby masking subtle differences between the two groups. In other words, a small measuring area such as the 10x10 pixel window, may sample with a particular spatial bias resulting in particularly high or low velocity and flow. By using the search strategy, the local peaks and troughs of the velocity histogram can be compared between subject groups.

The model of flow deficits in glaucoma that we propose is shown in Figure 3.5, and would suggest that reductions in flow in glaucoma patients are greater in the higher velocity range of the spectrum, with more subtle differences evident at lower velocities. This model (figure 3.5) is consistent with the absence in our data of a significant difference in the low values of blood velocity and the generally smaller absolute reductions and significance levels of blood flow, volume and velocity found when the low flow values are used to compare between groups. With the high flow values, as can been seen from the graphs in Figure 3.2 to 3.4, the absolute level of change is much greater for all parameters. This model may not be applicable in other diseases or in glaucoma assessed at later stages of damage.

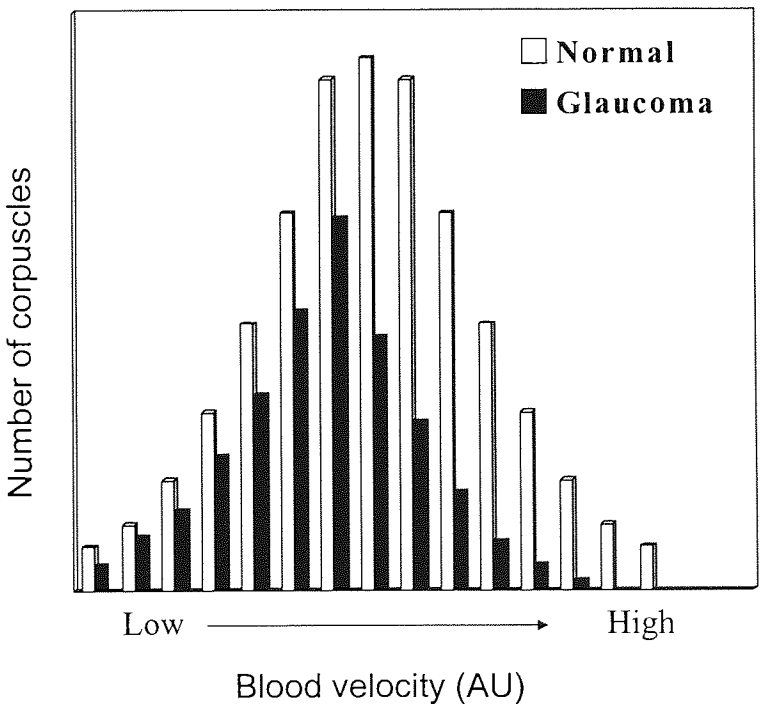

Figure 3.5. **Schematic model for reduced blood flow in glaucoma. The histogram shows a normal distribution of velocity values representing the total number of blood corpuscles moving in the retina over a period of time. The proposed model suggests that the mean velocity and number of cells is reduced in glaucoma with more profound loss in the higher velocity range.**

In a study by Chung *et al.*, (1999) two different methods of image analysis were employed, the first using a 10x10 pixel frame and the other in which the entire image was analyzed to obtain blood flow measures. Findings using the standard 10×10 pixel window showed no difference between the glaucoma patients and normal subjects, a result, which is in agreement with our own data. However using their own pointwise analysis involving a larger sampling area, significant differences in blood flow measures existed between the two subject groups. These findings concur with our own observations that a small (10 × 10) pixel frame does not use a large enough sampling window to overcome the physiological variability in blood flow across the retina. While the pointwise analysis overcomes the local temporal and spatial variability in flow by using a larger window, this may not be appropriate in all investigations, particularly those in which smaller areas of the fundus are to be considered.

Previous studies have shown that blood velocity is lower in glaucoma patients when compared to age matched normal subjects in the retina (Wolf, Arend, Sponsel, *et al.*, 1993; Michelson, *et al.*, 1996a; Nicolela, *et al.*, 1996), optic nerve head (Michelson, *et al.*, 1996a), choroid (Duijm, VandenBerg & Greve, 1997), and ophthalmic artery (Rojanapongpun, *et al.*, 1993). The results of our study offer some explanation for the discrepancy in findings obtained by different authors when determining blood flow deficits in glaucoma. For example, blood flow deficits have been reported in the retina (Michelson, *et al.*, 1996a; Chung, *et al.*, 1999), neuroretinal rim (Michelson, *et al.*, 1996a) and lamina cribrosa (Nicolela, *et al.*, 1996) of glaucoma patients. In some studies blood flow appeared to be normal in the neuroretinal rim (Nicolela, *et al.*, 1996; Hollo, 1997) or peripapillary retina (Hollo, Greve, VandenBerg, *et al.*, 1997). Although the possibility of age effects may account for the absence of defects in some studies (Hollo, 1997), a sampling error may offer a more reliable explanation.

The results from our study have implications for investigations in which blood flow in glaucoma patients and normal subjects are compared, or in longitudinal studies in which blood flow changes are monitored over time. In cross-sectional studies, differences in blood flow between subject groups may not be identified due to averaging of blood flow measures between individuals. In addition, when attempting to identify changes in blood flow measures in patients over time, as in longitudinal

studies, alterations may not be detected due to noise, resulting from the unintentional, randomized identification of high and low blood flow measures between visits. Our results suggest that the standard strategy resulted in a sampling error which is induced because too small an area of tissue has been evaluated without due account of local variations in velocity, and the local tissue variations in flow mask real changes resulting from the disease process. The result of this was that no significant differences in blood flow parameters were observed at the retina and neuroretinal rim. If the local physiological variation in flow measures is not accounted for then false positive or false negative findings of change could be reported.

3.8 Summary

Our data and the data of others (Chung, *et al.*, 1999) suggest that blood flow deficits are more consistently and more readily identified when a larger sample area is considered, or when account is taken of the local physiological variation in blood flow. Our study suggests that this can be achieved using the standard software by adopting a local search strategy and finding the lowest or highest values of blood flow, volume and velocity to compare blood flow measures between subject groups. Our data further suggests that in glaucoma, a greater flow deficit is observed in the higher range of velocities, resulting in reduced mean velocity and volumetric flow.

CHAPTER 4:

Repeatability of the Ocular Blood flow Analyser: Consecutive verses non-consecutive pulses

4.1 Abstract

The repeatability of pulsatile ocular blood flow (POBF) measures using the ocular blood flow analyser (OBFA; Paradigm Medical Industries Inc., Utah, USA) has been investigated previously. In order to calculate POBF the OBFA software selects the five optimum intraocular pressure pulses and as a result these pulses can be separated over time. The purpose of this study was to determine if repeatability of blood flow measures could be improved by using five good consecutive pulses.

4.2 Background

The repeatability of the OBFA has been evaluated by several investigators (Yang, *et al.*, 1997; Spraul, *et al.*, 1998). Yang *et al.*, (1997) stated that the OBFA provides reproducible measurements of POBF over short periods of time with the coefficient of repeatability being 92%, but reported that there was a high interindividual variation in healthy subjects making comparisons between individuals difficult. Spraul *et al.*, (1998) reported the coefficient of reliability to be 0.88, a lower value than that found by Yang *et al.*, (1997). This lower value may be due to the measurements of ocular blood flow being taken on consecutive days rather than within a short period of time on the same day. Spraul *et al.*, (1998) also reported that the majority of the variance found in ocular blood flow measures was due to between-subject variability, however they stated that the remaining 30 to 40% of variability was due to measurement error of the instrument.

In order to calculate measures of ocular blood flow, the OBFA requires at least five pulses of adequate quality and ranks them from 1 to 5, 1 being of superior calibre to 5. These "adequate" pulses need not be consecutive and may be separated in time over a twenty second period. If the pulses are acquired over a greater time lapse in this way, factors such as changes in intraocular pressure due to respiration or the tonometric

effect may influence the measured values, possibly resulting in larger standard deviations of blood flow values. The instrument attempts to correct for this by selecting the five most similar pulses. The validity of this approach remains unproven.

In order for the OBFA to be of clinical use for the long-term follow-up of individuals and for the assessment of ocular blood flow pre- and post-treatment, within-subject error needs to minimised. It is therefore of importance to eliminate error by optimising repeatability of blood flow measures using the OBFA.

4.3 Hypothesis

Repetability of OBFA data is improved using consecutive as opposed to separated (non-consecutive) pulses.

4.4 Aims and Objectives

The purpose of this study was to determine if the current method of selecting and matching the five best pulses from a larger data sample is sufficiently robust to provide optimal repeatability when compared to data acquired with five good consecutive pulses.

4.5 Materials and Methods

4.5.1 Subject Sample

The subject sample consisted of both eyes of twenty normal volunteers; eight males and twelve females, with a mean age of 27.9 ± 6 (age range 19 – 42 years).

4.5.2 Exclusion Criteria

Subjects were excluded if they exhibited:

- A history of previous ocular trauma or surgery
- Diabetes mellitus
- Hypertension
- Any medication known to affect ocular blood flow

- A family history of glaucoma
- Anisometropia greater than 1DS/DC

4.5.3 Inclusion Criteria

For inclusion into the study, subjects demonstrated:

- Equal visual acuity in both eyes of LogMar 0.3 or better
- Intraocular pressure of less than 21mmHg
- Normal ocular exam

4.5.4 Ethical approval and informed consent

Ethical approval was obtained from the ethical committee board of the University of Alabama, Birmingham, US for all the experimental procedures carried out. Approval conformed to the tenets of the declaration of Helsinki. Each subject was required to give informed consent prior to the initiation of experimental procedures.

4.5.5 Preliminary investigations

Details of ocular, general medical and family history were acquired and an ocular exam performed to ensure all subjects met the exclusion and inclusion criteria. Visual acuity was measured using a LogMar 90% contrast chart for each eye. Corneal integrity of each subject was assessed using slit lamp biomicroscopy to ensure the cornea of each eye was intact and exhibited no staining.

4.5.6 Measures of ocular blood flow

Each subject attended two research visits with no more than a two-week time separation between them. Each visit was performed at the same time of the day ± one hour to avoid diurnal fluctuations in intraocular pressure (Zeimer, 1989) and the possible diurnal variation in POBF (Claridge & Smith, 1994). Ocular blood flow measurements were carried out using the slit-lamp mounted OBFA with all subjects in an upright, sitting position to avoid changes in blood flow due to alterations in posture (Trew & Smith, 1991a; Trew & Smith, 1991b; Kotche, 1994).

One drop of proparacaine 0.5% was instilled into each eye and patients were instructed to look straight ahead during testing. The eyelid was held open with care taken not to exert any pressure onto the globe and measures of POBF were taken using a sterile OBFA probe.

At each visit, two sets of readings were acquired using the slit-lamp mounted OBFA. Set one consisted of five consecutive pulses. Set two consisted of five non-consecutive pulses that were separated by time and other intraocular pressure waves of inadequate quality. The average number of pulses used to acquire blood flow measures using the non-consecutive technique was 11.2 ± 3.1 (range 6 to 20 pulses). Measures of POBF were repeated until datasets with 5 consecutive and 5 non-consecutive pulses were acquired. In 18 of the eyes tested the consecutive data set was obtained first, and in the remaining 22 eyes the non-consecutive pulses were collected first. The average number of attempts to obtain both data sets was 3.6 ± 1.33. The eye tested first was randomised between subjects.

4.6 Statistical Analysis

The mean ± standard deviation for POBF, intraocular pressure (IOP), pulse volume (PV) and pulse amplitude (PA) for the group was calculated for each visit using both the consecutive and non-consecutive methods of data acquisition (table 4.1). Section 2.6 gives details of the calculations used to obtain the mean and standard deviation of data sets.

Student's paired *t*-tests were used to identify significant inter-visit differences between any of the measured parameters for the two separate techniques. The coefficient of variability was calculated for measures of POBF, IOP, PV and PA using both techniques of data acquisition (equation 4.1).

Coefficient of Variability (CoV) = Standard deviation / mean Equation 4.1

The mean of the difference and 95% limits of agreement between the two methods were determined for the first visit to assess differences in POBF, IOP, PV and PA using the two methods of data acquisition (Bland & Altman, 1986). The difference

was assigned a positive value when the consecutively measured parameters were greater or a negative value when the non-consecutive values were greater. Therefore the mean of the difference is equal to zero if there is no mean difference in the parameters using the two different methods, a positive value if greater using the consecutive method and a negative value if the non-consecutive method yields the highest results.

The mean of the difference and 95% limits of agreement of the data were determined for each parameter for both the consecutive and non-consecutive methods for the two visits (Bland & Altman, 1986), table 4.2. Intra-class correlation coefficients (ICC) were then calculated for POBF only to enable comparison with other studies. The ICC gives a measure of the variability between the two methods. To determine the ICC a one-way factorial analysis of variance (ANOVA) was determined using a statistical software package (Statview). In each case, sample size was assigned as the factor and pulsatile ocular blood flow measurement as the continuous variable. Separate ANOVAs were carried out for the two ocular blood flow acquisition methods. Using the ANOVA analysis, the total sums of squares and the sums of squares between subjects were calculated to determine the ICC for each method using equation 4.2.

$$r_I = \frac{mSS_B - SS_T}{(m-1) * SS_T}$$

Equation 4.2

Where:

r_I = ICC (given as a percentage)

m = The observations per subject

SS_T = Total sum of squares

SS_B = Sums of squares between subjects

(Bland & ltman, 1986)

4.7 Results

4.7.1 Coefficient of Variability (CoV)

Table 4.1 gives the mean POBF, IOP, PV and PA values ± standard deviation and the CoV for POBF measures only for the consecutive and non-consecutive methods for each visit. No significant difference in any of the measured parameters existed between the two visits using either method of pulse capture ($p>0.05$)

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		Visit 1	Visit 2
Consecutive	**POBF (µl/min) {CoV}**	1143.4 ± 330.7 {29%}	1163.98 ± 327.7 {28%}
	Intraocular Pressure (mmHg) {CoV}	13.74 ± 3.4 {25%}	12.80 ± 4.11 {32%}
	Pulse volume (µl) {CoV}	8.27 ± 2.75 {33%}	8.43 ± 2.62 {31%}
	Pulse amplitude (mmHg) {CoV}	3.35 ± 1.03 {31%}	3.19 ± 0.76 {24%}
Non-Consecutive	**POBF (µl/min) {CoV}**	1140.4 ± 367.8 {32%}	1190.8 ± 400 {34%}
	Intraocular Pressure (mmHg) {CoV}	13.81 ± 3.24 {24%}	13.32 ± 3.76 {28%}
	Pulse volume (µl) {CoV}	8.38 ± 3.06 {36%}	8.22 ± 2.34 {29%}
	Pulse amplitude (mmHg) {CoV}	3.36 ± 0.97 {29%}	3.24 ± 0.71 {22%}

Table 4.1. **Mean values of pulsatile ocular blood flow, intraocular pressure, pulse volume and pulse amplitude at each visit for the two methods. Coefficient of variability for all measures is given in brackets. No significant inter-visit differences in measures obtained using the OBFA were found using either the consecutive or non-consecutive technique ($p>0.05$).**

4.7.2 Repeatability: within visit comparison of methods

Table 4.2 shows the mean of the difference ± standard deviation for the first visit using both methods of pulse capture for measures of POBF, IOP, PV and PA.

Parameter	Consecutive verses non-consecutive pulses Mean of Difference ± 95% limits of agreement
POBF (µl/min)	2.93 ± 352.57
Intraocular Pressure (mmHg)	-0.11 ± 3.57
Pulse Volume (µl)	-0.12 ± 2.27
Pulse Amplitude (mmHg)	-0.005 ± 1.14

Table 4.2. **Values of the mean of the difference and the 95% limits of agreement for visit 1 for measures of POBF, intraocular pressure, pulse volume and pulse amplitude. The positive sign for POBF indicates higher values of blood flow using the consecutive method; the negative signs for IOP, PV and PA indicate larger values for these measures using the non-consecutive method.**

Figures 4.1 to 4.4 show the repeatability of POBF, IOP, PV and PA for the first visit using each method (Bland and Altman, 1986). The solid line represents the mean of the difference between the two methods and the two dotted lines are the 95% limits of agreement between the methods. The coefficient of repeatability (the difference divided by the mean) is shown as a scatter plot within each graph (Bland and Altman, 1986).

For measures of POBF the mean of the difference was 2.93 ± 352.57 µl/min, indicating that blood flow measurements using the consecutive method were on average slightly higher than those obtained using the non-consecutive method. Two of the data points lie outside the limits of agreement indicating a large difference in blood flow measures between the two methods for these subjects.

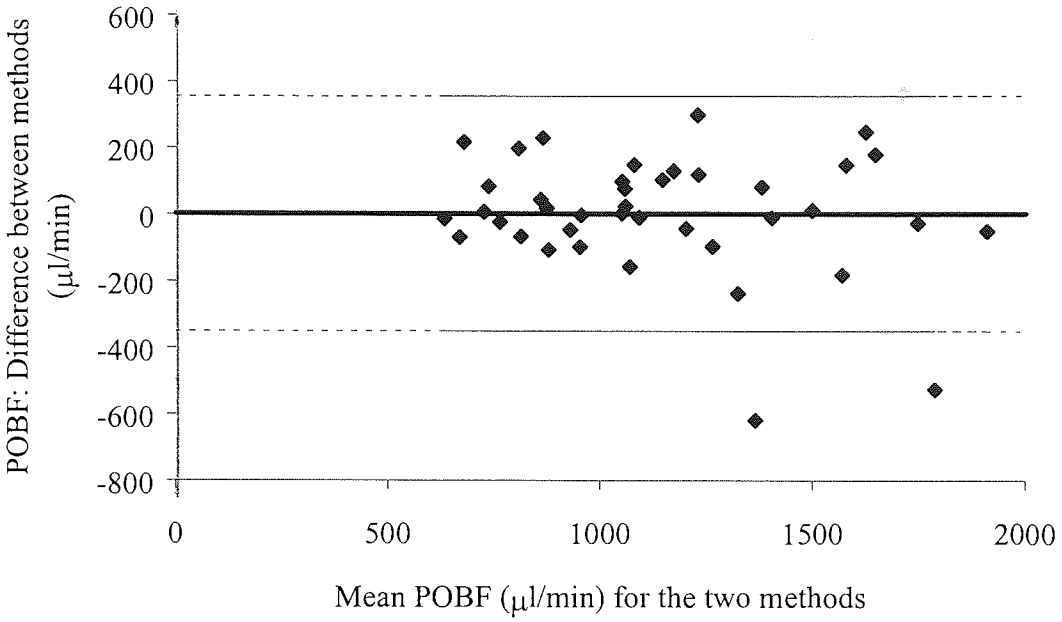

Figure 4.1. **Graph showing difference in pulsatile ocular blood flow between the consecutive and non-consecutive acquisition methods. The central solid line represents the mean of the difference between the methods (2.93 μl/min) with the dotted lines representing the 95% limits of agreement (± 352.57 μl/min).**

Figure 4.2 shows the repeatability for measures of IOP for the two methods. The mean of the difference was close to zero, being –0.11 ± 3.57 mmHg, indicating slightly higher values of IOP for the non-consecutive method. All points lie within the 95% limits of agreement.

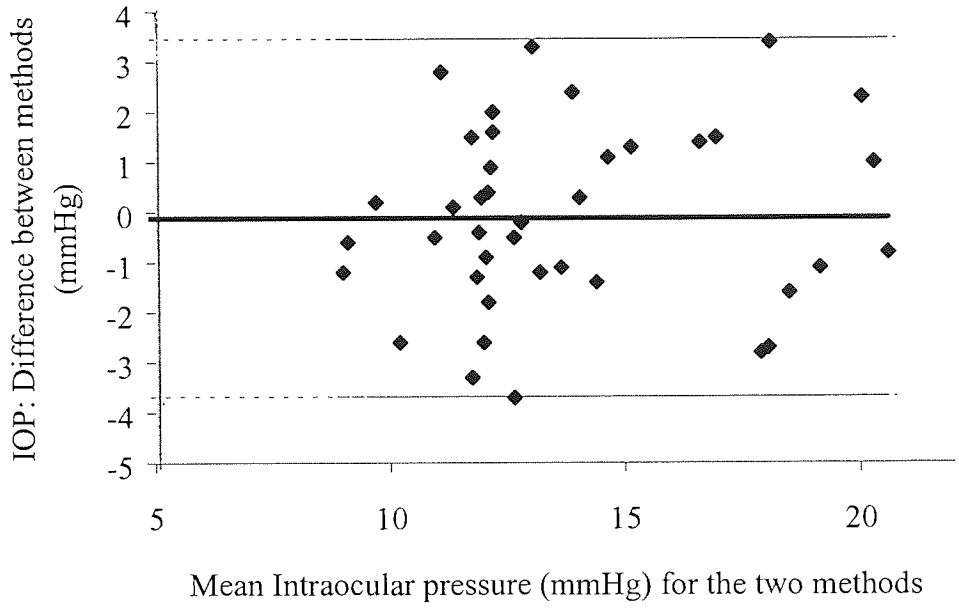

Figure 4.2. **Graph showing difference in intraocular pressure between the consecutive and non-consecutive acquisition methods. The mean of the difference and 95% limits of agreement were –0.11 ± 3.57 mmHg.**

The repeatability of PV measures is shown in figure 4.3. The mean of the difference and 95% limits of agreement were –0.12 ± 2.27 μl, indicating slightly higher values using the non-consective method. There are two outliers, suggesting a large difference in PV measures between the two methods for these subjects.

Figure 4.4 shows the repeatability for PA. The mean of the difference and 95% limits of agreement were –0.005 ± 1.14 mmHg, indicating, on average, negligible difference in measures of PA between the two methods. Three of the data points lie outside the limits of agreement.

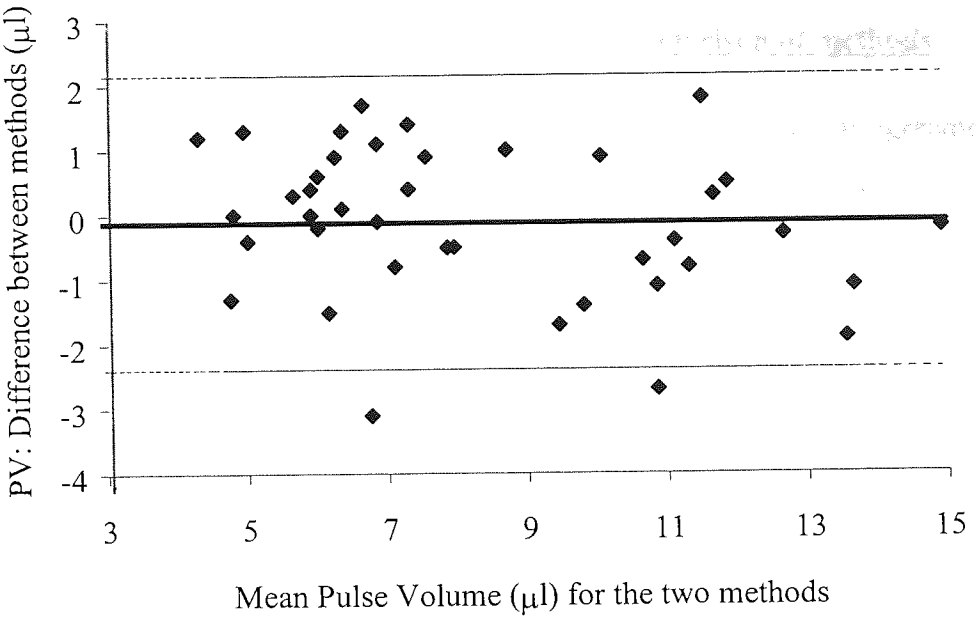

Figure 4.3. **Graph showing difference in pulse volume measures between the consecutive and non-consecutive acquisition methods. The mean of the difference and 95% limits of agreement were –0.12 ± 2.27 µl.**

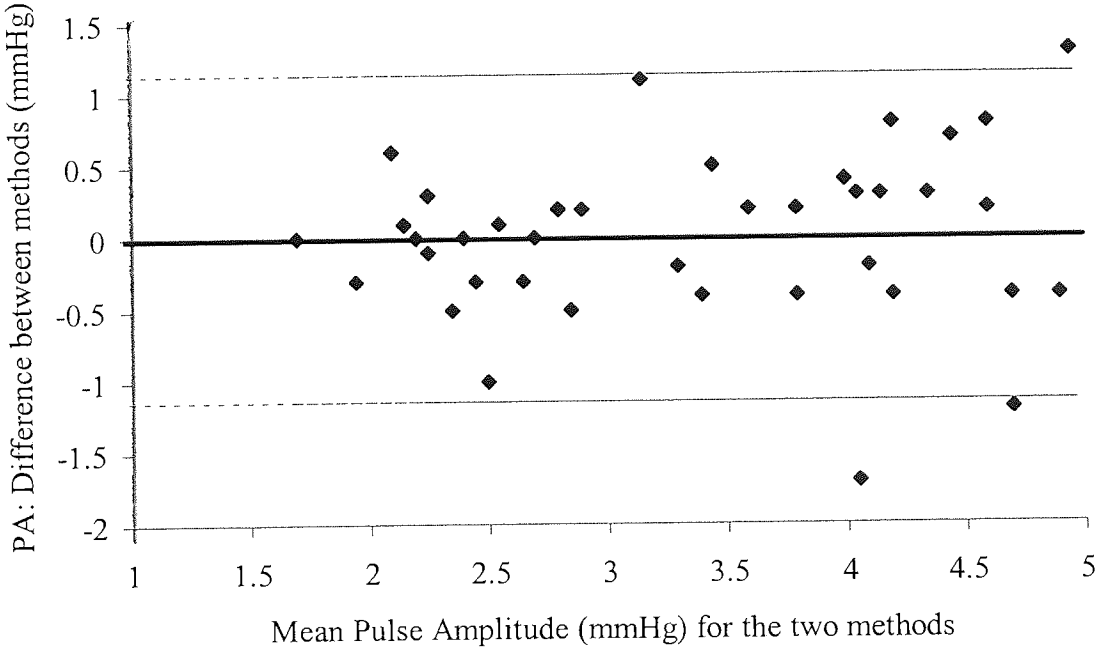

Figure 4.4. **Graph showing difference in pulse amplitude measures between the consecutive and non-consecutive acquisition methods. The mean of the difference and 95% limits of agreement were –0.005 ± 1.14 mmHg.**

4.7.3 Repeatability: between visit comparison of methods

Table 4.3 shows the mean of the difference and 95% limits of agreement for all the measured parameters for the two techniques to assess repeatability between visits. Figures 4.5 to 4.12 show this repeatability graphically (Bland and Altman, 1986).

		Mean of difference ± 95% limits of agreement
Consecutive Method	**POBF (µl/min)**	-20.63 ± 395.87
	Intraocular pressure (mmHg)	0.61 ± 6.35
	Pulse Volume (µl)	-0.16 ± 3.58
	Pulse Amplitude (mmHg)	0.16 ± 1.48
Non-Consecutive Method	**POBF (µl/min)**	-50.38 ± 489.83
	Intraocular pressure (mmHg)	0.50 ± 5.31
	Pulse Volume (µl)	0.08 ± 3.46
	Pulse Amplitude (mmHg)	0.08 ± 1.29

Table 4.3. **Values of the mean of the differences ± 95% limits of agreement for POBF, intraocular pressure, pulse volume and pulse amplitude to assess repeatability between visits for the two methods. A positive value indicates higher measures of ocular pulsatility at the first visit whilst a negative value indicates higher values at the second visit.**

For measures of POBF (figures 4.5 and 4.6) the mean of the difference and 95% limits of agreement were –20.63 ± 395.87 for the consecutive method and –50.38 ± 489.83 for the non-consecutive method of waveform capture. The smaller confidence limit obtained using the consecutive method of data acquisition indicates superiority in the repeatability of POBF measures using consecutive pulses.

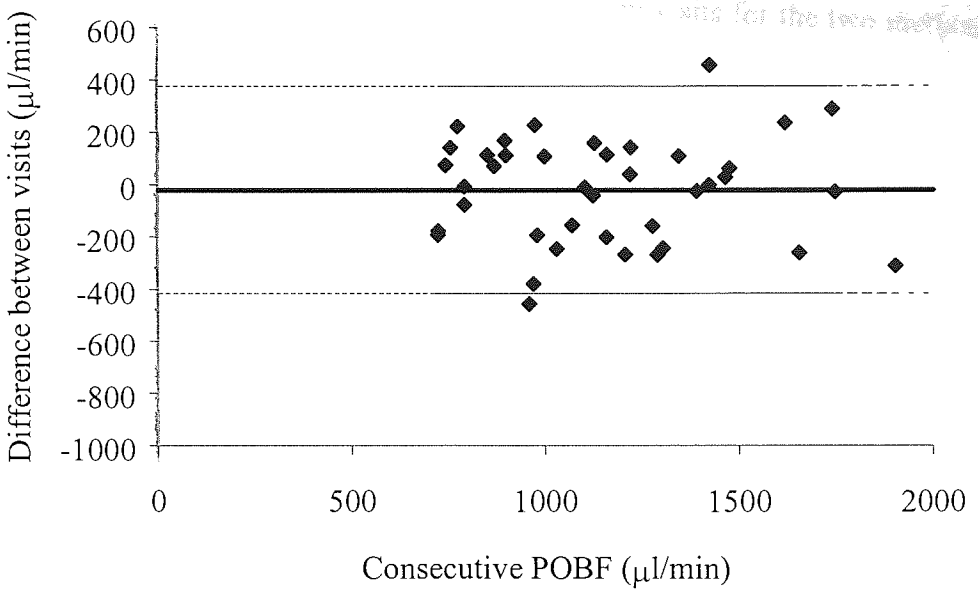

Figure 4.5. **Graph showing difference in pulsatile ocular blood flow between visits 1 and 2 for the consecutive acquisition method. The mean of the difference and 95% limits of agreement were –20.63 ± 395.87 µl/min.**

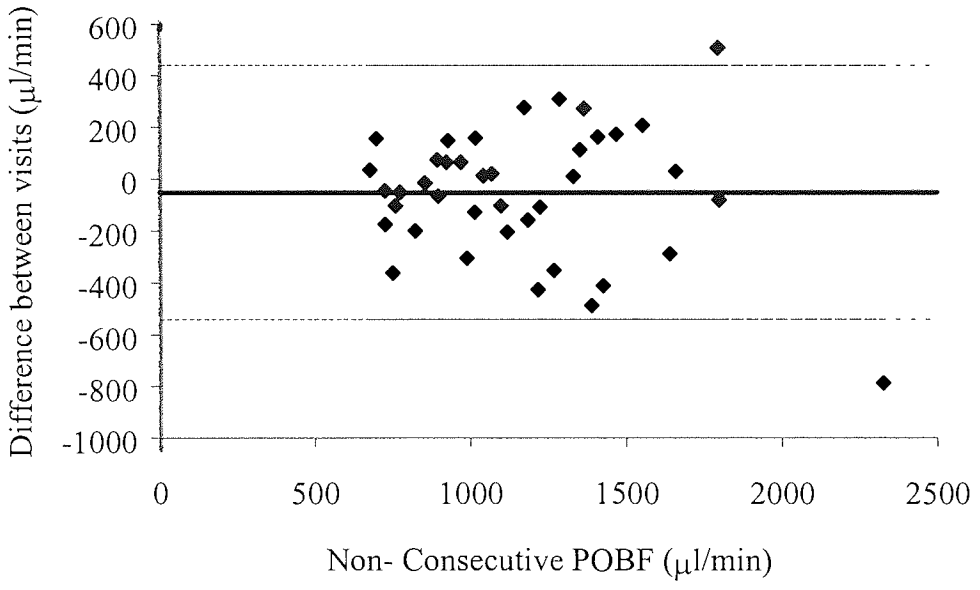

Figure 4.6. **Graph showing difference in pulsatile ocular blood flow between visits 1 and 2 for the non-consecutive acquisition method. The mean of the difference and 95% limits of agreement were –50.38 ± 489.83 µl/min.**

Figures 4.6 and 4.7 show the repeatability between visits for the two methods of pulse capture for IOP.

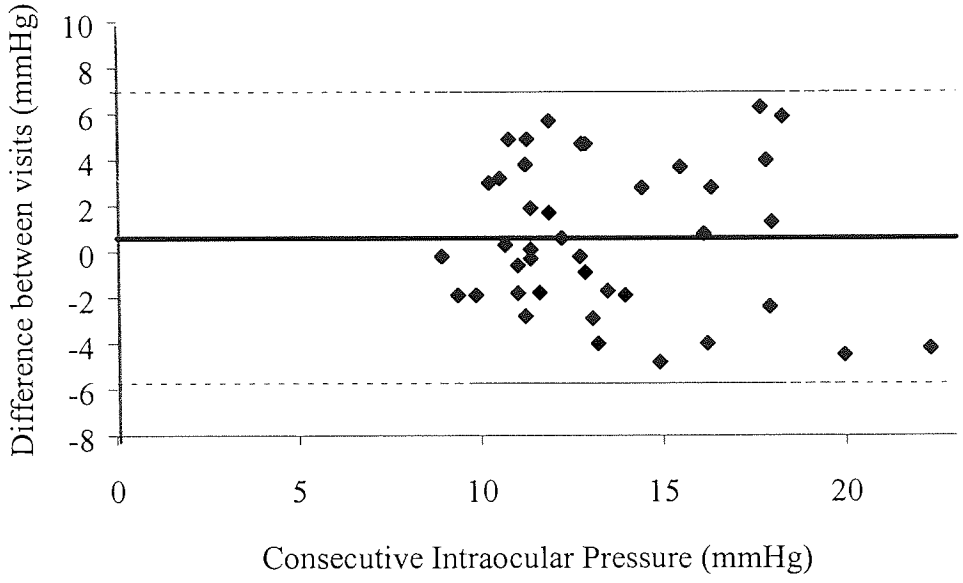

Figure 4.7. **Graph showing difference in intraocular pressure between visits 1 and 2 for consecutive pulses. The mean of the difference and 95% limits of agreement were 0.61 ± 6.35 mmHg.**

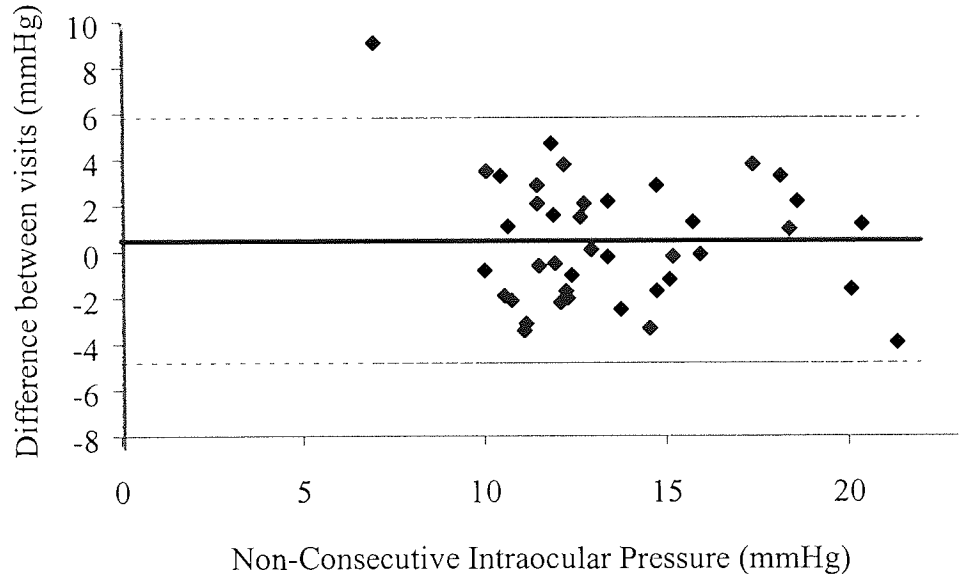

Figure 4.8. **Graph showing difference in intraocular pressure between visits 1 and 2 for non-consecutive pulses. The mean of the difference and 95% limits of agreement were 0.50 ± 5.31 mmHg.**

Using consecutive pulses the mean of the difference and 95% limits of agreement were 0.61 ± 6.35 mmHg and for non-consecutive pulses were 0.50 ± 5.31 mmHg.

Repeatability of measures of PV are shown in figures 4.9 and 4.10. Consecutive pulse capture resulted in a mean of difference and 95% limits of agreement of –0.16 ± 3.58 μl whilst for the non-consecutive pulses was 0.08 ± 3.46 μl.

Figures 4.11 and 4.12 show the repeatability for measures of PA between visits for both methods. The consecutive method resulted in a mean of difference and 95% limits of agreement of 0.16 ± 1.48 mmHg and for the pulses separated over time, 0.08 ± 1.29 mmHg. There was one outlier using each different type of pulse capture.

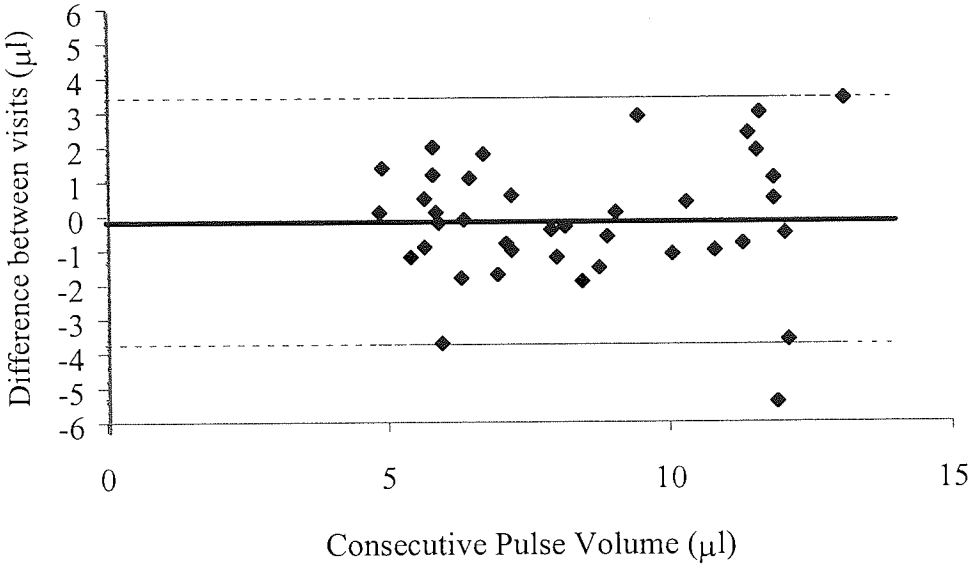

Figure 4.9. **Graph showing difference in pulse volume between visits using consecutive pulses. The mean of the difference and 95% limits of agreement were –0.16 ± 3.58 µl.**

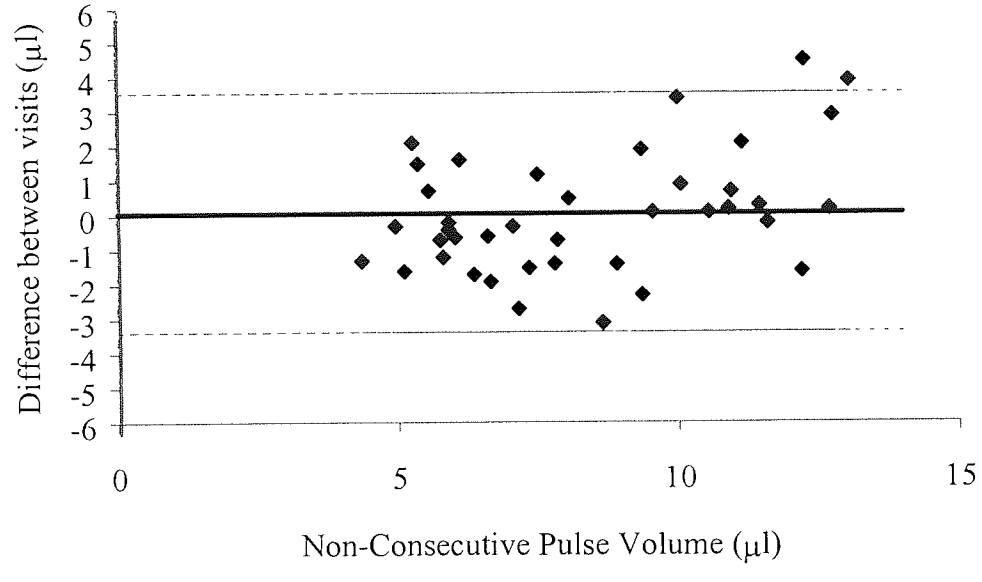

Figure 4.10. **Graph showing difference in pulse volume between visits using non-consecutive pulses. The mean of the difference and 95% limits of agreement were 0.08 ± 3.46µl.**

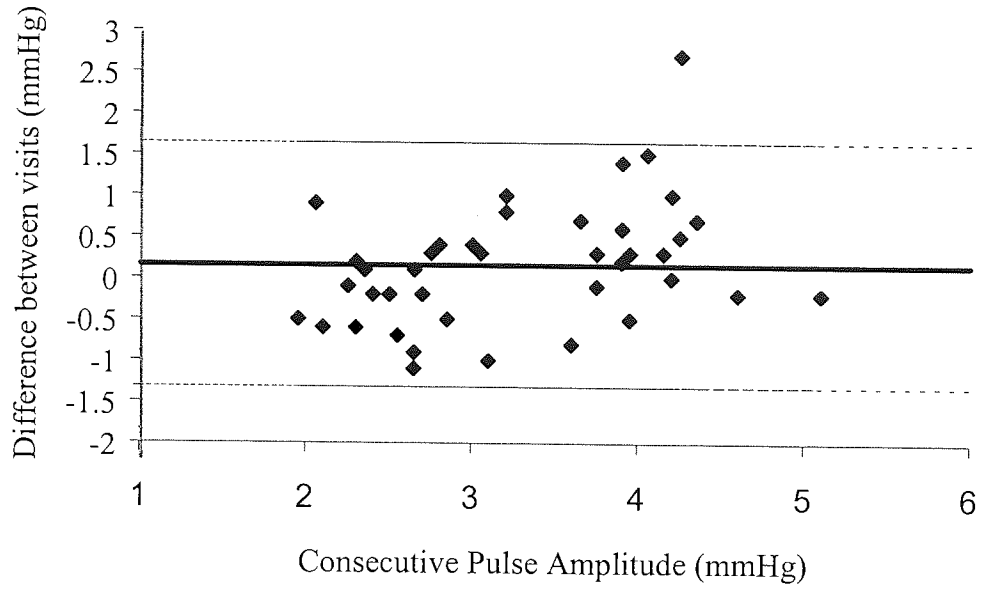

Figure 4.11. **Graph showing difference in pulse amplitude between the two visits using consecutive pulses. The mean of the difference and 95% limits of agreement were 0.16 ± 1.48 mmHg.**

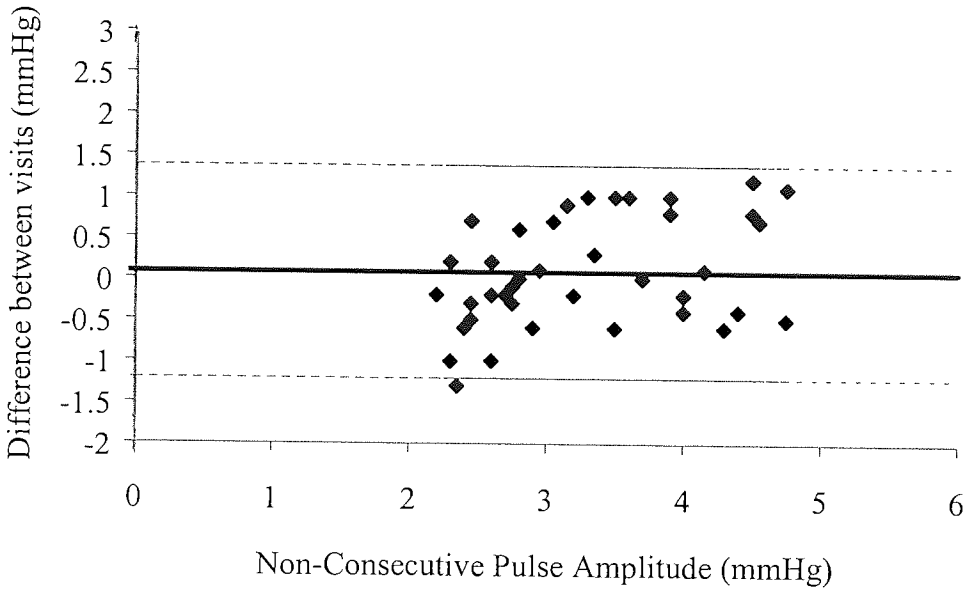

Figure 4.12. **Graph showing difference in pulse amplitude between the two visits using pulses separated over time. The mean of the difference and 95% limits of agreement were 0.08 ± 1.29 mmHg.**

The ICC of POBF for the consecutive method was 81% and for the non-consecutive method was 78%.

4.7 Discussion

The purpose of this study was to assess the repeatability of blood flow measures using consecutive and non-consecutive intraocular pressure waves. The coefficient of variability for measures of POBF was only slightly better for the consecutive method being 29% and 28% for the two visits, compared to 32% and 34% using the non-consecutive technique. This suggests that blood flow results obtained were reliable for both methods however using the non-consecutive method were slightly more variable than those obtained using the consecutive method. Unsurprisingly the coefficient of variability was slighly greater using consecutive method for measures of IOP (25% and 32% compared with 24% and 28% using the non-consecutive pulses) and PA (31% and 24% compared with 29% and 22% for the non-consecutive dataset), this is likely to be due to the increased number of attempts required in obtaining the consecutive pulse dataset and may reflect a slight tonographic effect. For measures of PV variability was greater at the first visit using the non-consecutive pulses (consecutive = 33%, non-consecutive = 36%) but at the second visit the reverse was true (consecutive = 31%, non-consecutive = 29%).

In the Bland & Altman analysis shown in figures 4.1 to 4.4, the closer the mean of the differences is to zero, the more similar the methods are in providing comparable measures of each parameter for the group. A narrow confidence interval indicates a lower variation in measures of ocular pulsatility between the two techniques. For measures of POBF the mean of the difference was 2.93 µl/min, indicating that blood flow measurements using the consecutive method were on average slightly higher but similar to those obtained using the non-consecutive method. The 95% limits of agreement were ± 179.89 µl/min and two points were not within these 95% limits, indicating a large difference in blood flow readings between the two methods in these subjects. Interestingly both of these subjects demonstrated significantly lower measures of POBF using the consecutive method of data acquisiton. Obtaining consecutive pulses for calculation of POBF proves more difficult for the operator and an average of 3.66 ± 1.33 attempts were made to obtain these pulses. Repeating measures of POBF could result in a tonometric effect, thereby affecting measures of POBF. Assessing intraocular pressure repeatability (figure 4.2) revealed a mean of

difference of -0.11 mmHg indicating slightly higher values for the non-consecutive method, but all points lay within the 95% limits of agreement suggesting comparable results between the two methods and an insignificant tonometric effect. Indeed, if a tonometric effect did have a significant effect on blood flow measures, POBF would be expected to be significantly higher for the consecutive pulses since lower intraocular pressures have been associated with higher values of POBF (Quaranta, *et al.*, 1994).

Figures 4.5 and 4.6 show the repeatability of each method of data acquisition for measures of POBF. The consecutive method resulted in a mean of difference of -20.63 µl/min with the limits of agreement being ± 395.87 µl/min and for the non-consecutive method these values were –50.38 ± 489.83 µl/min. The negative value for the mean of the difference for both methods indicates that measures of POBF were higher at the second visit and the larger value obtained using the pulses separated over time indicates greater discrepency in the results between visits. The smaller confidence level found with the consecutive method indicates that this method resulted in less variability in measures of pulsatile ocular blood flow suggesting higher repeatability than that achieved using non-consecutive pulses.

Figures 4.7 to 4.12 show the repeatability for the consecutive and non-consecutive pulses for measures of IOP, PV and PA. For intraocular pressure the mean of the difference and 95% limits of agreement for the consecutive pulses was 0.61 ± 6.35 mmHg and for non-consecutive pulses was 0.50 ± 5.31 mmHg. The larger confidence limit found using consecutive pulses is not surprising given the greater number of attempts made in collecting this data set and indicates a slight tonographic effect using the consecutive method of pulse capture. However all points lie within the 95% limits of agreement for the consecutive pulses whereas one lies outside these limits for the non-consecutive method. This result indicates that outliers are more common using the non-consecutive method and has implications for evaluating change. One possible explanation for this finding is that the variability resulting from the use of intraocular pressure pulses separated over time is greater than any tonometric effect caused by repeated measures in collecting the consecutive pulse data. For measures of pulse volume the consecutive method of pulse capture resulted in a mean of difference and 95% limits of agreement of –0.16 ± 3.58 µl whilst for the non-consecutive pulses was

0.08 ± 3.46 µl. This indicates that using the consecutive method of data acquisition, values of pulse volume were, on average, slightly higher at the second visit, hence the negative value of the mean of the difference. Using the consecutive pulses there was one outlier and for the non-consecutive pulses there were two. The mean of the difference and 95% limits of agreement for measures of pulse amplitude were 0.16 ± 1.48 mmHg using the consecutive pulses and 0.08 ± 1.29 for the non-consecutive pulses. This result suggests slightly higher variability in repeated measures of pulse amplitude using consecutive pulses, similarly to measures of intraocular pressure and may be due to the increased number of attempts made in collecting this dataset.

As the heart contracts, blood flows into the eye resulting in an increase in the IOP and with relaxation of the cardiac tissue outflow of blood ensues with a reduction in IOP. The combination of these processes results in the formation of the IOP wave that continuously alters as a function of heart rate. A small variation in IOP also occurs during respiration. If blood flow measures were influenced by small fluctuations in intraocular pressure then one would expect repeatability to be compromised when non-consecutive pulses are used to calculate blood flow due to the larger standard deviation in blood flow measures induced. The results from this study confirm this theory. Bland and Altman analysis revealed slightly superior repeatability in blood flow measures when consecutive pulses are used compared to non-consecutive pulses, with the confidence level being smaller with the consecutive method. Intraclass coefficient analysis revealed a slightly higher reliability coefficient of 81% compared with 78% for the non-consecutive method.

The repeatability of the OBFA has been investigated previously (Yang, *et al.*, 1997; Spraul, *et al.*, 1998). The two studies reported the intra-visit reproducibility of the OBFA to be 88% (Spraul *et al.*, 1998) and 92% (Yang *et al.*, 1997). When assessing the inter-visit reproducibility of the OBFA a reliability coefficient of 70% was reported (Spraul *et al.*, 1998), this value is lower than that found in our study using both consecutive (81%) and non-consecutive (78%) intraocular pressure pulses and may be accounted for by the smaller subject sample used in the study.

4.8 Summary

This study verifies the findings from other investigators that ocular blood flow measurements using the OBFA are repeatable, however, this repeatability can be slightly improved by using consecutive pulses to calculate measures of pulsatile ocular blood flow and this may be of particular importance when assessing change.

CHAPTER 5:

Investigation of waveform analysis on ocular pulsatility data: glaucoma patients and normal subjects

5.1 Abstract

Many studies have found that blood flow measures obtained from the intraocular pressure pulse are lower in glaucomatous populations than in normal groups. An alternative method of analysis, commonly used in the investigation of arterial blood pressure, is to reduce the pulse waveform to its component Fourier parts. The purpose of this study, therefore, was to determine whether such a technique is applicable to the intraocular pressure pulse and to investigate whether it was superior at differentiating diseased from healthy eyes.

5.2 Background

Glaucoma is a disease of unknown origin (Coleman, 1999). While elevated intraocular pressure (IOP) remains the most prevalent risk factor (Wolfs, Borger, Ramrattan, *et al.*, 2000), evidence now suggests that abnormal ocular blood flow may contribute to the pathogenesis in some patients (Harris, *et al.*, 1994b). The small variation in IOP associated with each heartbeat is a manifestation of the intraocular vasculature pulsating during the cardiac cycle (Bynke & Schele, 1967). This IOP pulse wave has been used as a means of assessing the ocular vascular status in glaucoma patients. Numerous studies have demonstrated reduced IOP pulse amplitudes and reduced pulsatile ocular blood flow in glaucoma patients compared with control subjects (James & Smith, 1991; Trew & Smith, 1991b; Ravalico, *et al.*, 1994; Schmidt, Ruckmann, Mittag, *et al.*, 1997; Fontana, *et al.*, 1998; Schmidt, Ruckmann & Mittag, 1998).

In the systemic circulation, the blood-pressure pulse wave has also been subject to much detailed research (Finkelstein, Collins & Cohn, 1988; McVeigh, Brennan, Hayes, *et al.*, 1993; Cohn, Finkelstein, McVeigh, *et al.*, 1995). Many of these studies have focused on investigating the blood-pressure pulse as a waveform through Fourier

analysis (Nichols & O'Rourke, 1998). In brief, such analysis uses the principle that all periodic functions, such as the repeating arterial pressure pulse, can be constructed by adding sinusoidal waveforms of varying amplitude and phase: Fourier analysis reduces such a waveform or signal into its component sinewave functions (Pugh, Eadie, Winn, *et al.*, 1987). These functions are frequently displayed by plotting their amplitude against their frequency, and this technique is known as representing the waveform in its frequency, or spectral, domain, compared with the waveform being represented in its original time domain (Bloch, 2000). The frequency that contains the spectral component of highest power is known as the fundamental frequency, and for the arterial pulse this is the frequency associated with the heart rate. Waveform analysis of the arterial pressure pulse has demonstrated that the principal spectral components below the fundamental frequency are related to the respiratory and vasomotive cycles of the cardiovascular system: 0.15Hz and 0.1 Hz respectively (Akselrod, Gordon, Madwed, *et al.*, 1985). Of greater cardiovascular research interest, however, is that the spectral components at successive orders of frequency above the fundamental, known as the harmonic components, which have been found to be associated with the vessel's vascular impedance and wave reflections from the distal arterial tree (Nichols & O'Rourke, 1998).

Returning to the eye, it would be of interest to establish whether a similar technique can be applied to the IOP pulse. If the harmonic components of the IOP pulse are related to some similar vascular measure of the eye, then waveform analysis by Fourier transformation may provide a novel approach to investigating ocular conditions of a vascular nature.

5.3 Hypothesis

Fourier harmonics of the IOP pulse wave can be determined, and differ between normal subjects and glaucomatous patients.

5.4 Aims and Objectives

The purpose of this study was twofold:

1) To determine whether the harmonic frequency components can be readily discerned from Fourier analysis of the IOP pulse wave.
2) To compare the harmonic components of the IOP pulse wave in glaucoma patients with those in normal subjects.

5.5 Materials and Methods

5.5.1 Subject Sample

The sample consisted of one eye from each of ten normal, healthy subjects and ten untreated primary open-angle glaucoma (POAG) patients, recruited from the clinic population at the University of Alabama at Birmingham (UAB) School of Optometry.

Diagnosis of glaucoma was made on the basis of visual field analysis and optic disc appearance in accordance with established clinical tenets (Hoddapp, *et al.*, 1993). No limitation was placed on the minimum level of IOP for study inclusion. The eye from each patient with the more repeatable visual field loss was selected for evaluation (left=6: right=4).

The control group was chosen so as to match the patient group for age, gender, blood pressure and heart rate. Details of the subject samples are given in table 5.1.

Parameter	Normal Subjects	Glaucoma patients
Age ± SD (years)	58 ± 10	53.5 ± 9
Race	5 white: 5 afrocarribean	3 white: 7 afrocarribean
Gender	5 male: 5 female	4 male: 6 female
Heart Rate (Bpm ± SD)	72.8 ± 8.42	77.1 ± 6.2
Mean Arterial Pressure ± SD (mmHg)	100.5 ± 5.9	102.4 ± 3.63
Intraocular pressure (mmHg)	*13.2 ± 3.9	*19.7 ± 6.8

Table 5.1. Details of the subject sample. * Glaucoma patients had significantly higher intraocular pressure than normal subjects ($p<0.01$)

Medication for systemic hypertension was taken by four of the glaucoma patients (two diuretic, one ACE inhibitor and one calcium channel blocker) and two of the control subjects (two diuretic). One patient and one subject had non-insulin diabetes but neither had any retinopathy.

5.5.2 Inclusion Criteria

5.5.2.1 Normal Subjects:

All the normal subjects were required to have:

- LogMar visual acuity better than 0.2.
- Normal visual fields in both eyes.
- No ocular abnormalities, verified from ophthalmic examination.
- Intraocular pressure of less than 22 mmHg in each eye.
- No history of any previous ocular trauma or surgery.

5.5.2.2 Glaucoma patients:

The glaucoma patients were required to exhibit:

- LogMar visual acuity better than 0.2.
- Reproducible visual field loss consistent with a diagnosis of glaucoma as described by Hoddap *et al.*, (1993). The Humphrey visual field analyser and the full threshold 24-2 programme was utilized to assess this.
- Optic nerve heads characteristic of glaucomatous optic neuropathy, confirmed by indirect ophthalmoscopy.
- No other ocular abnormality.

5.5.3 Ethical approval and informed consent

All participants reviewed and signed informed consent statements, in accordance with the 1964 Declaration of Helsinki, before participation in the study, and the experimental protocol and procedures were approved by the UAB Institutional Review Board.

5.5.4 Preliminary Investigations

The corneal integrity of each subject was checked using slit-lamp biomicroscopy. Blood pressure was evaluated using sphygmomanometry and the heart rate assessed by palpation.

5.5.5 Data Acquisition

Prior to acquisition of the ocular pressure pulse using the OBFA, the corneal surface of each participant was anaesthetized with proparacaine 0.5% and positioned in the slit lamp chin rest. The standard measurement technique was used to obtain measures of intraocular pressure, pulse amplitude, pulse volume and pulsatile ocular blood flow. Following this the instrument was switched to the "data-only" mode to record the intraocular pressure waveform over a ten second period. Data collection for continuous intraocular pressure was considered acceptable if eight consecutive pulses were acquired in which a clear audible note, representing the heartbeat, was heard and in which the intraocular pressure pulse wave appeared, from visual inspection, to be periodic and symmetric. If these criteria were not met, the patient was retested.

5.5.6 Fourier Analysis

Any repeating waveform can be represented by a series of sine and cosine waves of appropriate amplitudes at the repetition frequency of the waveform and integral multiples of it. In the case of a simple indefinite sine wave (figure 5.1) only one component is required to represent the data and this is known as the fundamental frequency (figure 5.2). In the case of blood pressure, this frequency would be the heart rate.

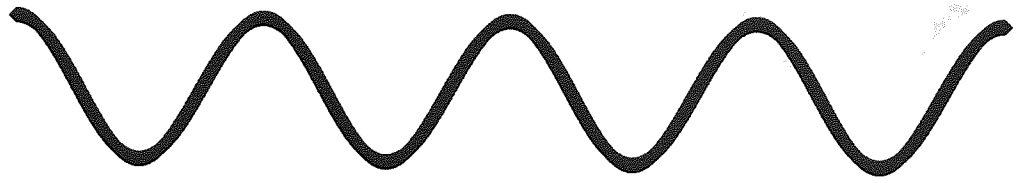

Figure 5.1. **Schematic representation of a mathematically derived sine wave**

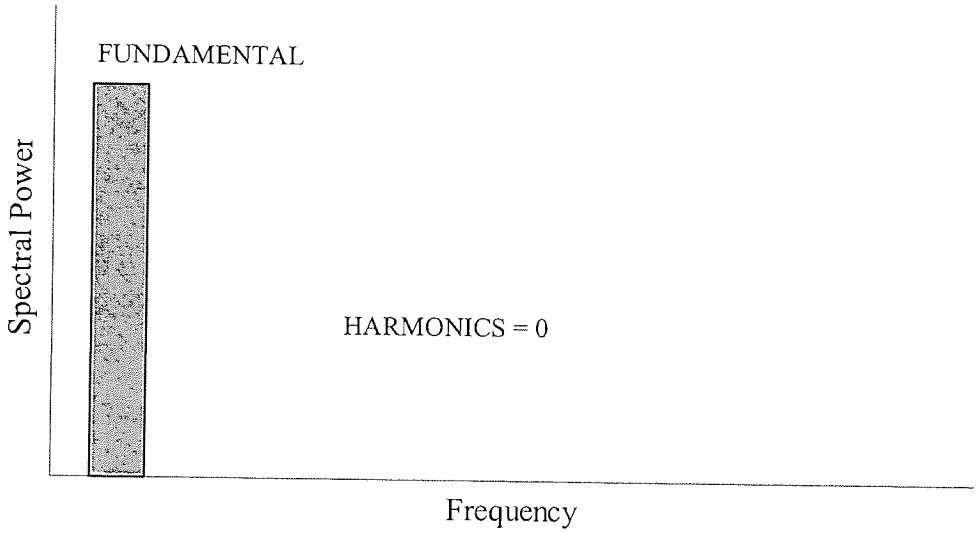

Figure 5.2. **Fourier transformation of the continuous wave shown in figure 5.1.**

For more complex waves, such as the IOP pulse (figure 5.3), the fundamental frequency contains the majority of the power of the spectra and also represents the heart rate of the patient. This is accompanied by a series of higher-frequency waves known as the harmonic components, each being a multiple of the fundamental frequency or heart rate (figure 5.4).

Figure 5.3. **The intraocular pressure curve of a patient with early glaucoma, showing a steep rise and shallow fall in pressure.**

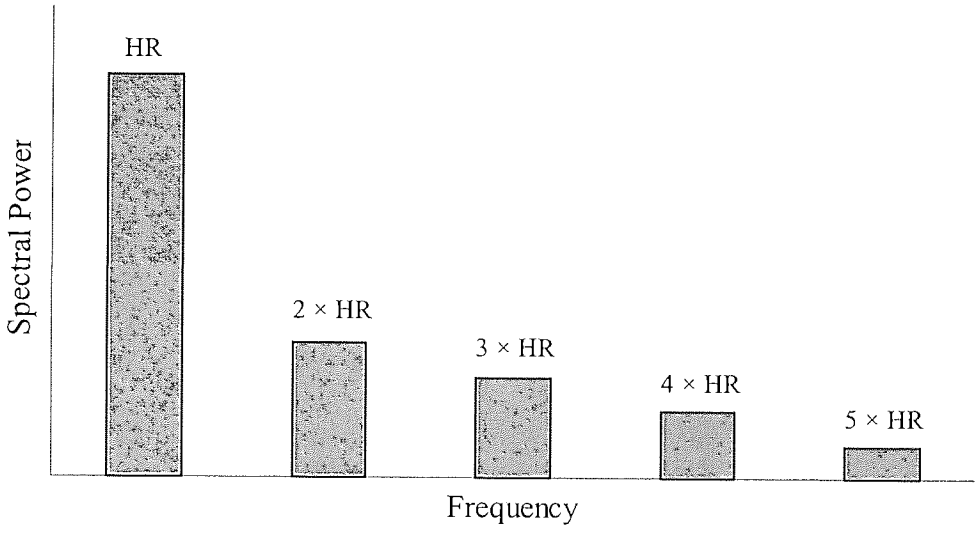

Figure 5.4. **Fourier analysis of the intraocular pressure curve showing the fundamental frequency resulting from the heart rate (HR) and four harmonic components representing the higher frequencies of the intraocular pressure wave as a multiple of HR.**

The OBFA samples IOP at 200 hertz, which, combined with a 10 second capture time, provides 2000 IOP datum points available for analysis. Fourier analysis, using the FFT algorithm (Cooley & Tukey, 1965), requires that the data sample size is equal to 2^n (where n=1,2,3,4,5,6...). As such, the sample must contain one of the following number of datum points; 2, 4, 8, 16, ...512, 1024 or 2048, etc. To provide the highest resolution to discern the Fourier frequency components, the largest possible number of

sampled data points should be used. The sample size of 1024 data was chosen for analysis because it provided the greatest number of usable data points that could be captured during the 10-second sample period.

After capture with the OBFA, the IOP data were downloaded to a computer and analysed using a spreadsheet (Microsoft Excel). Three different, but overlapping, 1024-data-point sections of the IOP pulse wave were then evaluated using the FFT. Specifically, the section succeeding the first peak (or trough) of the pulse wave, the section preceding the final peak (or trough) of the pulse wave and a section midway between these two endpoints were evaluated (figure 5.5). After the FFT was conducted, the percentage of power represented in the fundamental frequency and in the first, second, third, fourth and fifth harmonics were calculated by dividing the power in each frequency component by the total power. These percentages were averaged across the three data sections to obtain the final value for each harmonic.

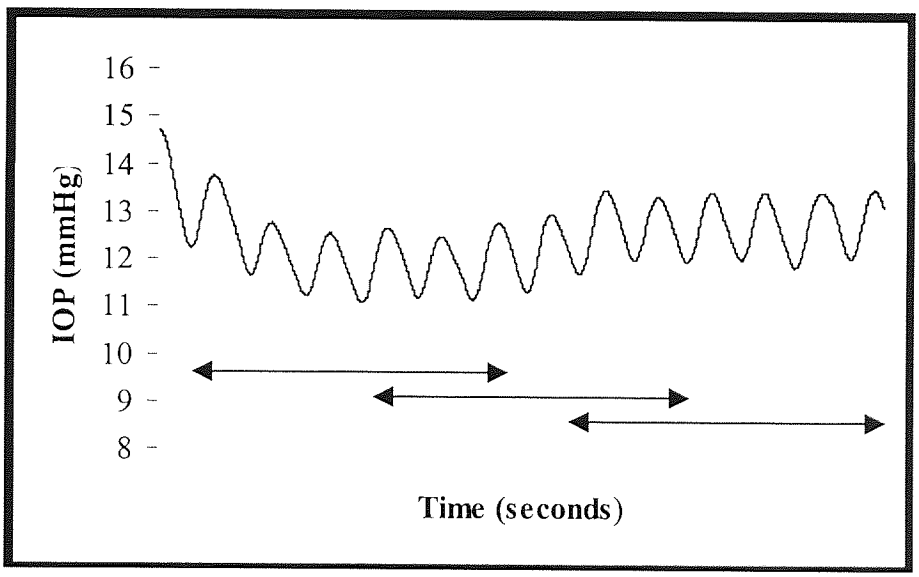

Figure 5.5. **Intraocular pressure pulse variation showing three overlapping sectors for analysis.**

5.6 Statistical Analysis

Unpaired Student's *t*-tests were used to compare differences between the normal subjects and glaucoma patients for all the measured variables (see section 3.6), significance was achieved if $p<0.05$. Pearson's product-moment correlation analysis was used to ascertain any significant association between variables. The analysis

describes the linear relationship that exists between variables and is denoted in equation 5.1 below.

$$r = \frac{\sum xy}{\sqrt{\sum x^2 \sum y^2}} \qquad \text{Equation 5.1}$$

In this equation the denominator is made up of the sums of the squares for x and y, and the numerator is the product of the deviation or variance of x and y for their respective means.

For the statistical tests carried out a p-value of less than 0.05 was considered statistically significant.

5.7 Results

5.7.1 Baseline data

Patients and subjects were similar for age and gender distribution, heart rate and blood pressure (table 5.1).

Patients exhibited significantly higher IOP than normal subjects in both eyes ($p<0.01$, table 5.1).

Glaucoma patients had lower pulse amplitude, pulse volume and pulsatile ocular blood flow than normal subjects, but these differences did not reach significance ($p > 0.05$) (Table 5.2).

Parameter	Normal Group	Glaucoma Group	Difference ($p<0.05$)
Pulse Amplitude (mmHg)	2.69 ± 0.9	2.44 ± 0.7	0.24
Pulse Volume (µl)	6.84 ± 2.6	4.98 ± 2.6	0.07
POBF ($\mu l min^{-1}$)	1081 ± 370	842 ± 426	0.09

Table 5.2. **Standard IOP pulse parameter measures for glaucoma patients and normal subjects. Measures of pulse amplitude, pulse volume and POBF were lower in the glaucoma patients but these differences were not significant ($p>0.05$).**

5.7.2 Spectral Components

A ten second continuous IOP measurement was successfully recorded from each patient and subject, an example of which is shown in figure 5.6.

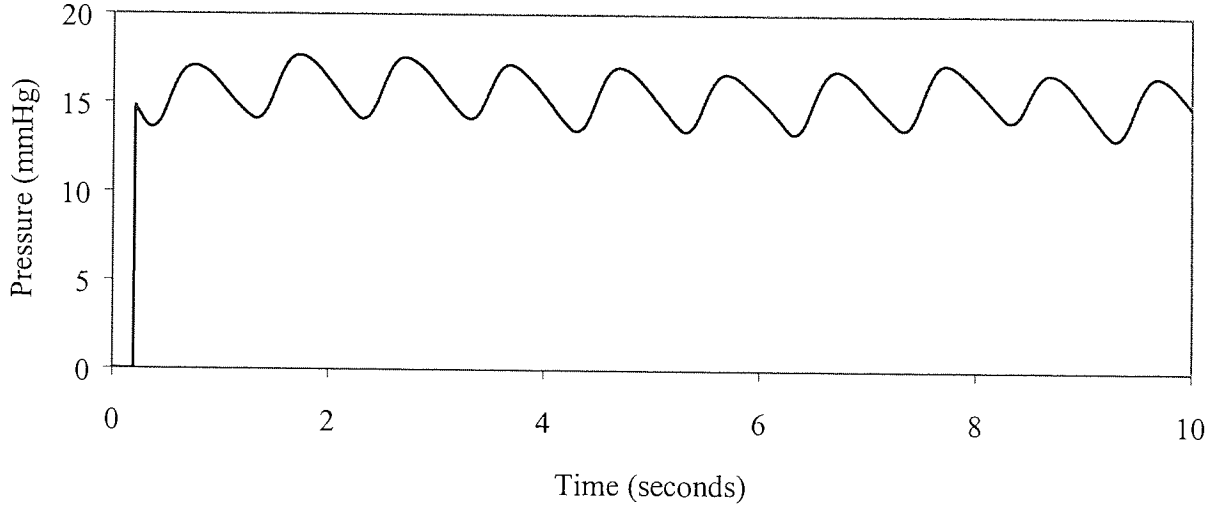

Figure 5.6. Example of a 10 second recording of a normal subjects intraocular pressure.

Analysis of the IOP data by FFT found that the higher spectral components of the pulse wave could be discerned up to the fourth harmonic: a typical example of the spectral components from an intraocular pressure waveform is shown in figure 5.7. As this is a new measure that has not been previously accomplished in glaucoma patients, one concern is the variability differences between groups. The mean subject coefficients of variation for the fundamental and the first, second, third and fourth harmonics were 21.4%, 25.3%, 30.0%, 34.6% and 28.7% for the normal subjects and 22.7%, 26.3%, 25.8%, 22.9% and 28.1% for the glaucoma patients. For the groups, the coefficients of variation for the fundamental and the first, second, third and fourth harmonics were 38.1%, 21.9%, 34.1%, 12.0% and 10.4% for the normals and 38.8%, 38.5%, 27.5%, 39.0% and 33.0% for glaucoma, suggesting that the spread of data was similar between groups. The similarity of the coefficients of variation within and between groups suggests that the testing methodology provides similar variability for glaucoma patients and age-matched normal subjects.

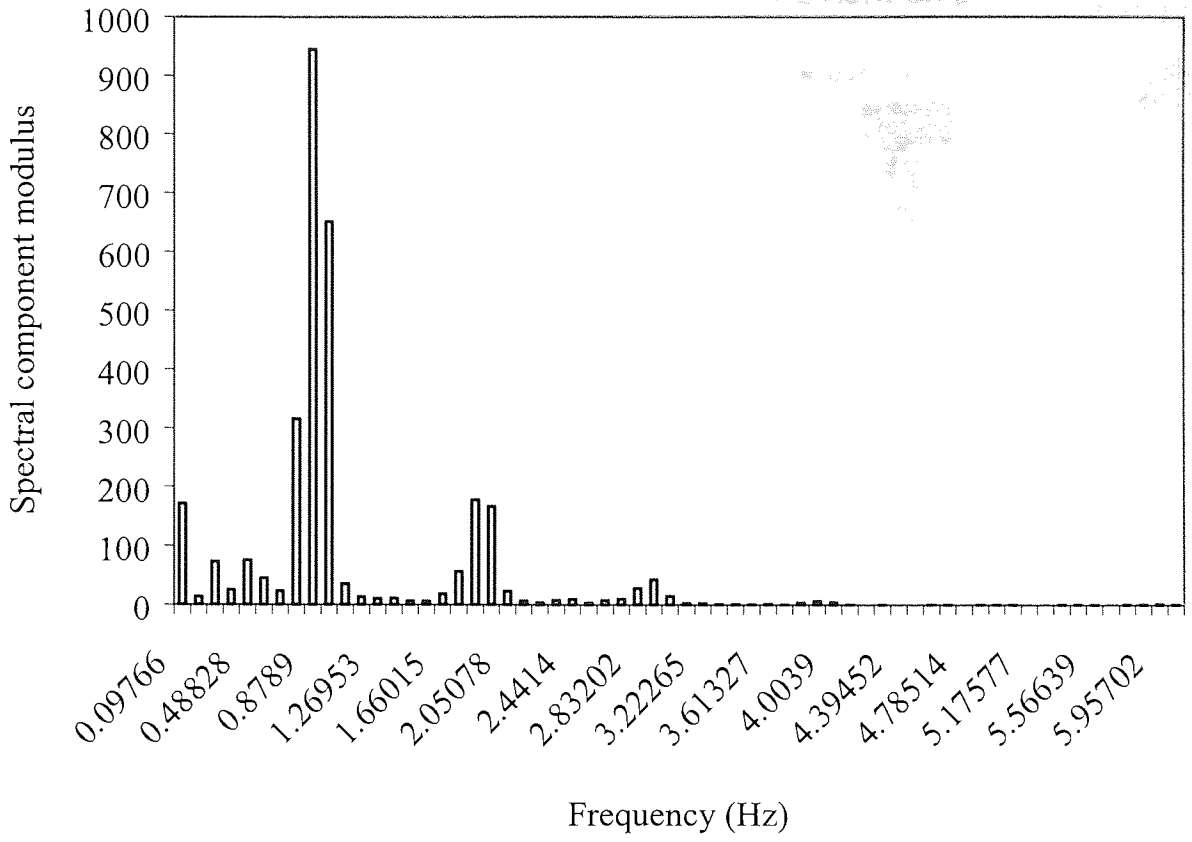

Figure 5.7. **Example of the actual spectral components from a normal subjects intraocular pressure recording after transformation by Fourier analysis.**

Glaucoma patients had lower percentage power than normal subjects for the second ($p=0.034$), third ($p=0.015$) and fourth ($p=0.013$) harmonics (figure 5.8). Correlation analysis demonstrated no significant correlations between any systemic measures, intraocular pressure or Fourier component.

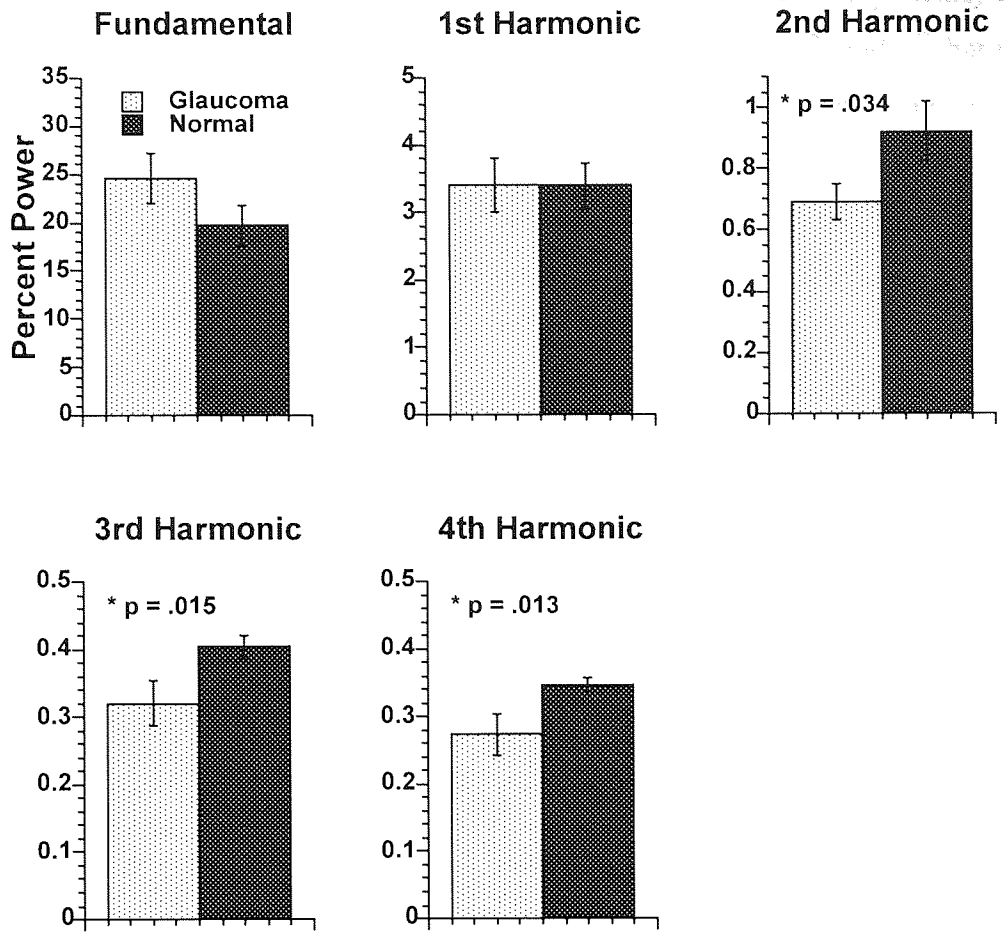

Figure 5.8. **Percentage power (standard deviation) of the fundamental and harmonic components of the intraocular pressure pulse for the normal and glaucomatous groups.**

5.8 Discussion

Firstly, this study shows that the IOP pulse can be readily analysed in the frequency domain and that its spectral components can be determined up to the fourth harmonic. This method of analysis provides a novel and exciting avenue of research. Best and Rogers (1974) used Fourier analysis to investigate it's ability in detecting carotid artery stenosis and found that calculation of the first three harmonics of the intraocular pressure pulse was superior to measuring pulse amplitude for detecting low grades of stenosis.

Secondly, our results indicate that previously untreated glaucoma patients, compared with normal subjects, demonstrate lower power in the 2nd, 3rd and 4th harmonics of their IOP pulse when analysed in the frequency domain. These same patients and subjects did not show significant differences for the measures of pulse amplitude, pulse volume and pulsatile ocular blood flow. This is likely to be due in part to the relatively small sample in this study and perhaps more importantly to the fact that the patient and subject groups were matched for heart rate, which has a major impact on ocular pulsatility measures (Yang, *et al.*, 1997). This is particularly pertinent to the interpretation of our findings as it highlights the greater ability to differentiate the groups when this more sensitive analysis is used, even when the influence of heart rate is removed.

The higher harmonics in the arterial pressure pulse can be used as a measure of vascular impedance. Vascular impedance is dependent on the dimensions and the viscoelasticity of the artery involved, on the physical properties of the blood and on waves reflected from more distal parts of the arterial tree (Nichols, Conti, Walker, *et al.*, 1977). Vascular impedance is closely related to vascular resistance, and is defined as the resistance to flow in an oscillatory system. It is a frequency-dependent quantity, rather than a time-dependent quantity (Nichols & O'Rourke, 1998). Resistance, with which impedance is often confused, is confined to non-oscillatory or steady motions. Resistance may therefore be considered as impedance at zero frequency. Considering such an explanation for the findings in this study, some support may be gleaned from colour Doppler ultrasound investigations that have found some glaucoma patients to have increased resistivity indices for the ophthalmic artery (Harris, *et al.*, 1994b), central retinal artery (Evans, Harris, Chung, *et al.*, 1999b) and the short posterior ciliary arteries (Evans, *et al.*, 1999a) in certain situations. Therefore one may postulate that the reduced harmonics found in this study's glaucoma patients are associated with some difference in vascular status.

However, it is important to note that the pressure pulse under analysis here is not directly that of the eye's internal vasculature. The pulsation, although initially originating from the intraocular vessels, is dependent on: the volume change of those vessels and not their pressure change; on the dimensions of the eye; and on the elasticity of the ocular coat (Silver & Geyer, 2000). Therefore the differences found in

this study, may be due to one or more of these factors, such as a difference in scleral rigidity.

Another explanation for these findings is that artifacts are introduced by the chosen method of analysis. The spectral components of the Fourier transform can be affected by truncation of the signal, that is, by inclusion of a partial waveform in the data set. If the sampled data does not include an exact number of cycles, the introduction of partial waveforms can induce spurious harmonics that may not be present in the spectra of the true waveform. Since the data collection for this study occurred over a short period of ten seconds and there was a variance in heart rate (cycle length) between patients, each data set invariably contained partial cycle lengths. The resulting truncation could have potentially biased the data by differentially influencing the percentage of power appearing in the harmonics for each group. However, as patients and subjects were similar for heart rate, it can be argued that truncation was not the cause of these differences. Since the heart rate determines the number of IOP pulse waves that occur during any ten second time period, both groups, on average, would be expected to exhibit the same number of cycles within the sampling period and thus the same level of truncation. Of course, if the heart rate were not normally distributed in each group, then it is possible that some members in each group had heart rates that closely matched the sampling period. Such clustering of patients could affect the level of truncation and thus differentially effect the overall power level in the harmonics of that group. A review of the data indicates that this is unlikely since the heart rate distribution within each group appears to be normally distributed, and as such, the level of truncation would not be clustered within a group, but would also be normally distributed.

Significant differences have been found between glaucoma patients and normal subjects in measures of ocular pulse volume, pulse amplitude and pulsatile ocular blood flow (James & Smith, 1991; Trew & Smith, 1991b; Ravalico, *et al.*, 1994; Fontana, *et al.*, 1998; Schmidt, *et al.*, 1998). In contrast, patients and subjects in this study were not found to differ significantly for these measures. One dissimilarity between this study and the previous results is that in our study the groups were matched for heart rate. Heart rate has been shown to significantly correlate with measures of the IOP pulse wave such that a higher heart rate corresponds to higher

values of pulsatile ocular blood flow (Yang, *et al.*, 1997); with the groups matched for heart rate, however, this factor is eliminated. Our results suggest that when patients and subjects are matched for systemic factors that affect the intraocular pressure pulse wave, the harmonic content of the IOP pulse wave may be a better indicator for abnormalities in the ocular blood flow of glaucoma patients than pulse volume, pulse amplitude or pulsatile ocular blood flow. Furthermore, our results imply that systemic factors must not be ignored when utilizing the IOP pulse wave as a measure of ocular blood flow. Specifically, a normal subject with a low heart rate may present with low pulsatile blood flow simply due to the heart rate and not due to the presence of an ocular blood flow abnormality.

5.9 Summary

Our results show that it is possible to measure the spectral components of the intraocular pressure pulse up to the fourth harmonic. The differences noted in the harmonic amplitudes between groups in this study suggest that some component of the IOP pulse (be it blood-vessel resistance or some other factor) varies in glaucoma. Measurement of the IOP's harmonics may be an additional parameter, possibly more sensitive than those of pulse amplitude, pulse volume and pulsatile ocular blood flow, that enable identification of patients suffering from diseases with a vascular aetiology. Further studies are required to determine the effect of altering confounding variables on the outcome of the analysis, and whether this method of IOP pulse investigation will have physiological and clinical utility in such areas as glaucoma.

CHAPTER 6

Investigation of age, gender and systemic blood pressure on the ocular pulsatility of the normal human eye.

6.1 Abstract

The ocular blood flow analyser (OBFA; Paradigm Medical Industries Inc., Utah, USA) measures the pulsatile component of ocular blood flow (POBF). Alterations in POBF have been observed with advancing age (Ravalico, *et al.*, 1996) and in diseases such as glaucoma (James & Smith, 1991; Trew & Smith, 1991b; Fontana, *et al.*, 1998) and diabetes (MacKinnon, O'Brien, Swa, *et al.*, 1997; Geyer, *et al.*, 1999). The carotid artery follows a pathway that perfuses the left ophthalmic artery before the right. It has been suggested that the left eye is more vulnerable to vasoactive stimuli (Kagemann, *et al.*, 1998b) and hence more susceptible to ocular diseases with haemodynamic involvement.

The purpose of this investigation is to explore the effect age, laterality, gender, systemic blood pressure and biometric parameters have on POBF analysed using standard measures and those which have undergone waveform analysis.

6.2 Background

The OBFA measures intraocular pressure continuously during the cardiac cycle. The resulting intraocular pressure waveform is then used to derive measures of ocular pulse amplitude, pulse volume and POBF (Kraukau, 1992). The pulsatile component of ocular blood flow accounts for 75 to 85% of the total blood flow to the eye and is predominantly derived from the choroidal circulation (95%) with negligible contribution from retinal and optic nerve head vasculature (Langham, *et al.*, 1989a). The choroid is predominantly perfused by the short posterior ciliary arteries, which are also major providers of the vascular supply of the anterior optic nerve head (Onda, *et al.*, 1995), and as such, measures of choroidal perfusion may be of value in determining ocular health.

A number of studies have used the OBFA to investigate POBF measures in normal eyes. Gender-related differences have been reported for POBF, with females having higher values than males (Yang, *et al.*, 1997; Fontana, *et al.*, 1998). Reductions in POBF have also been observed with advancing age (Ravalico, *et al.*, 1996; Fontana, *et al.*, 1998), increasing intraocular pressure (Fontana, *et al.*, 1998) and increasing myopic refractive error (Fontana, *et al.*, 1998; Massey, *et al.*, 1999; Mori, *et al.*, 2001).

The OBFA has been used to assess ocular pulsatility in ocular diseases such as glaucoma. Overall, POBF values are lower in patients with HTG compared to ocular hypertensives (Trew & Smith, 1991a), and lower in NTG patients compared to age matched normal subjects (James & Smith, 1991; Quaranta, *et al.*, 1994; Ravalico, *et al.*, 1994; Fontana, *et al.*, 1998). In addition, differences in POBF have been reported between diabetic patients and normal subjects, with increases in POBF being found with advancing retinopathy (MacKinnon, *et al.*, 1997; Geyer, *et al.*, 1999).

In the systemic circulation, the carotid artery originates from the aortic arch on the left side of the heart and arises as a branch of the right brachiocephalic trunk on the right side of the heart, reaching the left eye prior to the right (Bron, *et al.*, 1997; Rawji & Flanagan, 2001). Interarm differences in systolic and diastolic blood pressure have been reported, with some investigators reporting significantly higher values of systolic and diastolic blood pressure in the right arm (Cassidy & Jones, 2001) and others finding no consistency in the arm demonstrating the higher blood pressure (Frank, *et al.*, 1993; Singer & Hollander, 1996). These interarm differences have been found to be greater in patients with vascular disease (Frank, *et al.*, 1993; Singer & Hollander, 1996). In the eye, a study conducted by Kagemann *et al.*, (1998b) investigated the effects of hypercapnia on retinal blood flow to the right and left eyes of normal, healthy subjects. They found that the blood flow to the left eye was more sensitive to changes in carbon dioxide concentration than that of the right eye. It was suggested that the left eye might be more vulnerable to stimuli which result in reduced ocular blood flow. In a retrospective cross-sectional study Poinoosawmy, Fontana, Wu, *et al.*, (1998) investigated visual field defects in both normal- and high-tension glaucoma patients. They found the degree of functional loss to be greater in the left eye when compared with the right eye in normal-tension glaucoma patients, thereby supporting

the theory made by Kagemann *et al.,* (1998b) that the left eye may be more susceptible to damage. It is possible therefore that intereye differences in POBF exist in normal, healthy subjects either due to anatomical or autoregulatory discrepencies between the two eyes.

The choroid receives its nutrition via blood flow from the posterior ciliary arteries (Bill, 1981). Over time, the structure of the arteries changes and the incidence of atherosclerosis increases resulting in reduced arterial distensibility (Wadsworth, 1990). In addition, the structure of endothelial cells alters with advancing age and these play a crucial role in vascular tone and regulation (Wei, 1992). These alterations may result in reduced ocular pulsatility over time.

Fourier analysis of the intraocular pressure pulse reveals its spectral components up to the 4^{th} harmonic (Evans, *et al.*, 2002, and chapter 5) and has been used to identify patients with carotid artery stenosis (Best & Rogers, 1974) and glaucoma (Evans, *et al.*, 2002). It has been suggested that the Fourier harmonics of the intraocular pressure waveform are related to the vascular status of the blood vessels (Evans, *et al.*, 2002). Since vascular tone and reactivity alters with age, Fourier harmonics may demonstrate subtle age effects in a normal population.

6.3 Hypotheses

1) POBF reduces with age.

2) In patients with normal systemic vascular physiology adequate regulation exists, resulting in the maintenance of interocular symmetric flow.

3) Fourier analysis can be used to resolve the first four harmonics of the intraocular pressure pulse and provides further information regarding the vascular status of the ocular vessels with advancing age.

6.4 Aims and Objectives

The purpose of this study was to examine the influence of age, laterality, gender and systemic circulation on measures of POBF.

6.5 Materials and Methods

6.5.1 Subject Sample

120 healthy subjects (60 males and 60 females) were recruited. The subjects were between the ages of 20 and 80 years and were split into six age-differentiated groups with ten females and ten males in each (subject sample characteristics are shown in table 6.1).

Age Band (years)	Mean age ± SD (yrs)	Right mean sphere ± SD (Dioptres)	Left mean sphere ± SD (Dioptres)	Right K readings ± SD (mm)	Left K readings ± SD (mm)
20 to 30	23.4 ± 3.4	-1.8 ± 2.1	-1.9 ± 2.2	7.69 ± 0.16	7.71 ± 0.18
30 to 40	35.1 ± 2.9	-0.8 ± 2.5	-1.0 ± 2.4	7.76 ± 0.23	7.75 ± 0.23
40 to 50	44.8 ± 2.7	-0.3 ± 1	-0.37 ± 1.4	7.82 ± 0.24	7.80 ± 0.23
50 to 60	54.6 ± 2.6	+0.95 ± 3.01	+0.86 ± 3.47	7.86 ± 0.19	7.87 ± 0.19
60 to 70	65.2 ± 2.6	+1.5 ± 1.34	+1.43 ± 1.21	7.89 ± 0.23	7.90 ± 0.23
70 to 80	74.4 ± 2.9	+1.33 ± 1.4	+1.38 ± 1.5	7.87 ± 0.14	7.86 ± 0.14

Table 6.1. **Subject sample characteristics showing average ages for each subject band, mean refractive error and mean keratometry readings of the right and left eyes. ANOVA revealed no differences between the right and left eyes for refraction or anterior corneal curvature ($p>0.05$).**

6.5.2 Inclusion Criteria

Subjects were recruited from members of the undergraduate optometry course at Aston University, members of staff from the Neuroscience Research Institute and patients from the optometry clinics.

In order to be classified as normal all subjects were required to have:

- Snellen visual acuity of 6/9 or better in each eye

- Refractive error (mean sphere) of less than ±8 dioptres, with less than 2 dioptres of anisometropia. All subjects were required to have less than 1.5 dioptres of corneal astigmatism.
- Normal optic nerve head appearance confirmed by ocular examination
- Open anterior chamber angles (grade 2 to 4), assessed using Van Herricks technique
- No history of ocular trauma or surgery
- No diabetes mellitus
- No hypertension
- No family history of glaucoma
- No systemic medication that may affect blood flow.
- No ocular medication

6.5.3 Ethical Approval and Informed Consent

Prior to commencement of the study ethical approval was obtained from the ethical research committee of Aston University. Approval conformed to the 1965 tenets of the declaration of Helsinki. Prior to data acquisition signed consent was acquired from every subject.

6.5.4 Investigations

Initially a series of questions were asked to ascertain each subject's personal and family general and ocular health. Preliminary measurements of anterior corneal curvature (Bausch and Lomb Keratometer), as indicated by measures of corneal radius (note that these measures are inversely proportional) and ocular refraction were obtained together with an assessment of corneal integrity using slit-lamp biomicroscopy.

6.5.5 Data Acquisition

Blood pressure measurement was taken on each arm using an automated sphygmomanometer prior to obtaining measures of POBF. The test sequence was alternated by gender within age bands so that equal numbers of right and left eyes

were tested first within each band; five females right first, five males right first, five females left first and five males left first.

For measurements of POBF one drop of 0.4% benoxinate hydrochloride was administered to each eye. Each subject was instructed to fixate on a distant target, and the eyelid was gently held open with care taken not to exert any pressure on the globe. Using the OBFA, three measures of pulse volume, pulse amplitude, intraocular pressure and POBF were taken for each eye and the mean for each measured parameter was calculated.

Finally, the intraocular pressure pulse was captured over a 10 second period using the data only mode on the OBFA. Methodology for this is outlined in sections 5.5.5 and 5.5.6. In order to explore any age effect, the Fourier components of the intraocular pressure pulse were calculated for the youngest (20-30) and oldest (70-80) age groups.

6.5.6 Mathematical derivation of Mean arterial pressure (MAP)

Mean arterial pressure (MAP) was calculated using the following formula: -

MAP = 2/3 systolic blood pressure + 1/3 diastolic blood pressure Equation 6.1

6.6 Statistical Analysis

Analysis of variance (ANOVA) was used to investigate systemic differences between the right and left sides for MAP, systolic and diastolic blood pressure and systemic pulse amplitude. Gender-related differences in pulse rate, POBF, pulse volume and pulse amplitude were investigated using ANOVA. Interocular differences in refraction, anterior corneal curvature, pulse volume, pulse amplitude, intraocular pressure, POBF and the harmonic components of the intraocular pressure pulse were also explored.

Multiple regression analysis was used to investigate whether MAP, systolic and diastolic blood pressure, systemic pulse amplitude, systemic and ocular pulse rate,

ametropia, anterior corneal curvature, intraocular pressure and blood flow measures altered with advancing age. Analysis of variance was used to determine whether any alterations to the harmonic components of the intraocular pressure pulse occurred between the first and last age bands.

Pearson's product moment correlation analysis was used to ascertain any significant association between systemic and ocular variables (see section 5.6 for equation).

6.7 Results

6.7.1 Systemic Data

Table 6.2 gives details of the systolic and diastolic blood pressure, MAP, the systemic pulse amplitude (systolic minus diastolic blood pressure) and the pulse rate for the right and left arms.

None of the measured systemic parameters demonstrated any interarm differences ($p>0.05$).

Regression analysis revealed a significant positive trend between diastolic blood pressure ($r^2=0.031$, $p=0.007$; figure 6.1), MAP ($r^2=0.095$, $p<0.001$; Figure 6.2) and systemic pulse amplitude ($r^2=0.035$, $p=0.004$; figure 6.3) with advancing age.

Age (years)	Pulse rate ± SD		Systolic Blood Pressure ± SD (mmHg)		Diastolic Blood Pressure ± SD (mmHg)		Mean Arterial Pressure ± SD (mmHg)		Systemic Pulse Amplitude ± SD (mmHg)	
	Right	Left	Right	Left	Right	Left	Right	Left	Right	Left
20-30	75.25 ± 9.86	73.85 ± 10.84	122.85 ± 13.13	118.6 ± 13.95	73.85 ± 8.20	73.40 ± 8.38	90.85 ± 12.56	90.82 ± 12.82	49.65 ± 12.71	52.70 ± 16.67
30-40	72.0 ± 11.61	70.84 ± 11.58	124.79 ± 12.33	120.00 ± 17.07	79.16 ± 9.60	78.11 ± 8.03	88.28 ± 5.91	90.91 ± 9.03	45.21 ± 10.96	44.26 ± 12.11
40-50	74.84 ± 11.28	73.32 ± 10.49	144.95 ± 28.50	133.53 ± 23.99	81.63 ± 9.35	81.21 ± 9.55	92.3 ± 9.46	91.7 ± 7.71	42.74 ± 9.94	40.79 ± 9.87
50-60	74.16 ± 11.6	74.42 ± 11.96	121.47 ± 16.50	128.32 ± 20.97	74.00 ± 10.51	75.79 ± 10.63	95.12 ± 9.79	93.81 ± 11.54	45.68 ± 9.84	42.05 ± 12.87
60-70	74.95 ± 15.78	72.0 ± 12.56	119.70 ± 14.73	120.30 ± 15.07	78.25 ± 7.96	79.95 ± 6.89	100.55 ± 13.98	97.68 ± 11.47	52.80 ± 20.12	48.40 ± 12.76
70-80	70.85 ± 12.17	72.55 ± 12.36	132.75 ± 23.46	135.65 ± 18.99	80.15 ± 11.92	81.15 ± 8.71	99.2 ± 14.76	100.03 ± 12.54	62.25 ± 22.15	58.00 ± 18.33

Table 6.2. **Pulse rate, systolic and diastolic blood pressure, MAP and systemic pulse amplitude for the subject sample. ANOVA revealed no interarm differences ($p>0.05$). Regression analysis revealed a significant increase in diastolic blood pressure, MAP and systemic pulse amplitude with advancing age.**

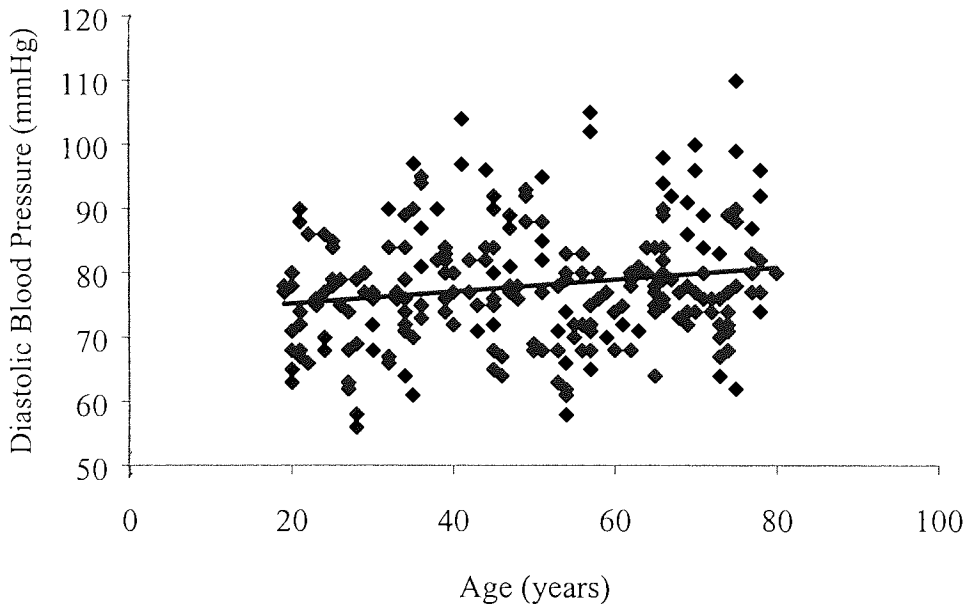

Figure 6.1. **Scatterplot showing the trend between diastolic blood pressure and age ($r^2=0.031$, $p=0.007$).**

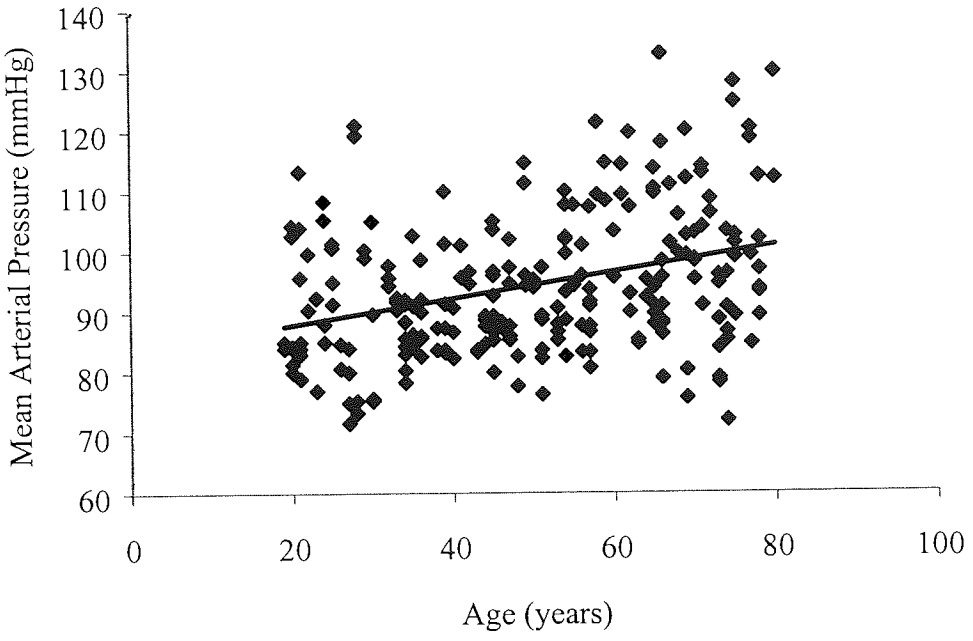

Figure 6.2. **Scatterplot showing the trend between MAP and age (r^2=0.095, p<0.001).**

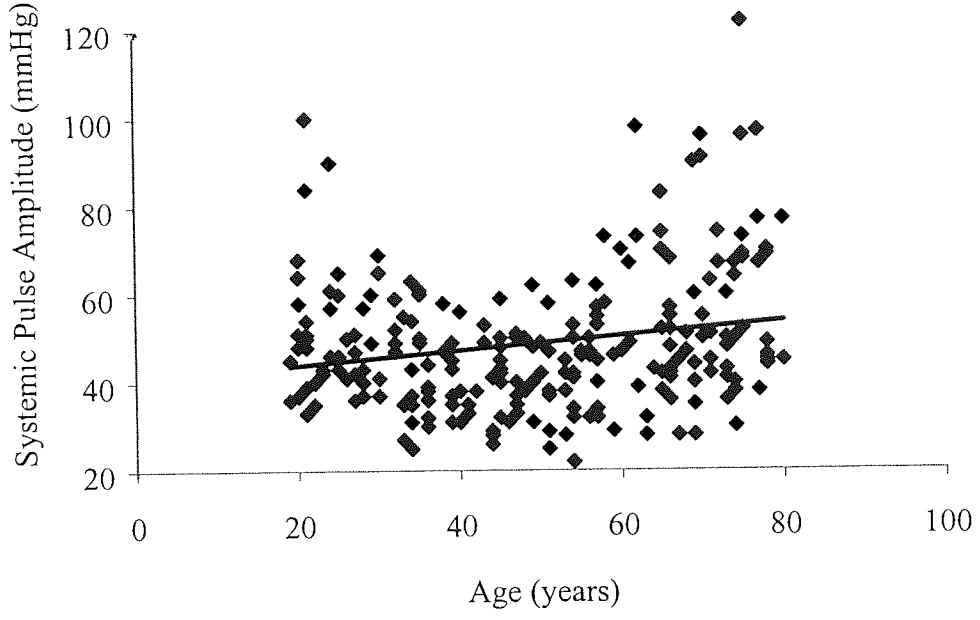

Figure 6.3. **Scatterplot showing the trend between systemic pulse amplitude and age (r^2=0.035, p=0.004).**

6.7.2 Ocular Parameters

Analysis of variance revealed no gender-related differences in measures of ocular pulse rate, POBF, IOP, pulse volume or pulse amplitude ($p>0.05$).

There were no significant interocular differences for refraction, keratometry, or any of the measured OBFA parameters, including the harmonic components of the intraocular pressure pulse ($p>0.05$).

Table 6.3 gives the mean intraocular pressure and blood flow measures for the six age groups.

There was a significant increase in the degree of hypermetropia ($r^2=0.26$, $p< 0.0001$; Figure 6.4) and flattening of the anterior corneal curvature ($r^2=0.09$, $p< 0.0001$; figure 6.5) with advancing age.

Age Band	POBF ± SD (µl/sec)		Pulse Volume ± SD		Pulse amplitude (mmHg)		Intraocular Pressure ± SD (mmHg)	
	Right	Left	Right	Left	Right	Left	Right	Left
20-30	19.13 ± 5.76	19.64 ± 6.93	7.73 ± 2.49	7.90 ± 2.90	3.06 ± 0.94	3.06 ± 0.89	11.7 ± 2.3	11.7 ± 2.98
30-40	19.02 ± 5.16	19.03 ± 4.45	9.10 ± 3.68	8.69 ± 2.99	3.42 ± 1.08	3.51 ± 1.10	11.1 ± 2.6	11.7 ± 2.6
40-50	17.66 ± 5.89	18.60 ± 7.10	7.61 ± 2.66	8.09 ± 3.42	3.21 ± 0.89	3.26 ± 0.89	13.0 ± 4.0	12.7 ± 4.5
50-60	19.06 ± 4.93	18.44 ± 4.27	8.31 ± 2.96	8.10 ± 2.64	3.36 ± 1.16	3.39 ± 1.09	12.0 ± 3.0	12.9 ± 3.5
60-70	21.01 ± 6.18	21.29 ± 6.83	9.01 ± 2.97	9.07 ± 2.80	3.45 ± 1.11	3.47 ± 1.05	11.5 ± 4.1	11.3 ± 4.0
70-80	19.42 ± 5.55	19.20 ± 5.60	8.40 ± 2.41	8.44 ± 2.34	3.07 ± 0.79	3.15 ± 0.84	10.7 ± 2.7	10.9 ± 3.1

Table 6.3. **Table showing measures of ocular pulsatility and intraocular pressure data. There was no interocular asymmetry ($p>0.05$) for any parameter.**

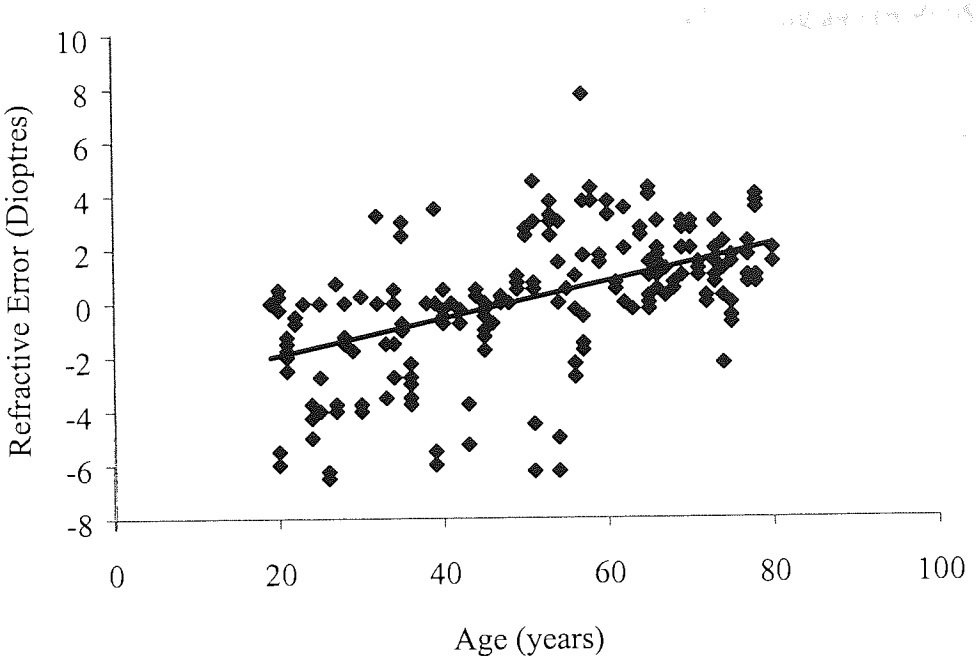

Figure 6.4. **Scatterplot showing increasing hypermetropia with advancing age (r^2=0.26, p < 0.0001).**

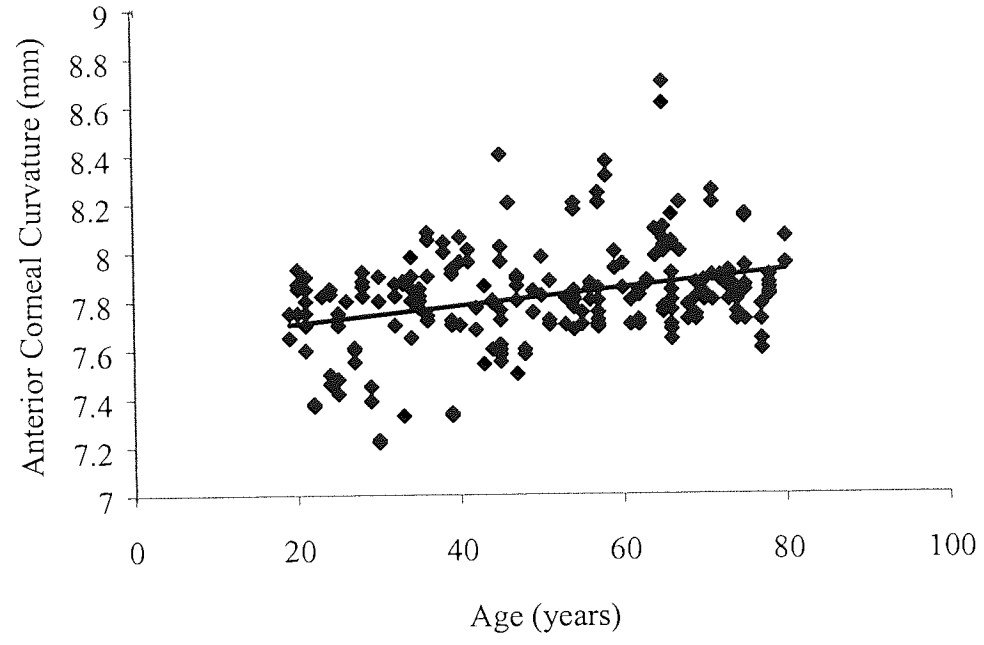

Figure 6.5. **Scatterplot showing increasing anterior corneal radius, denoting a decrease in corneal curvature, with age (r^2=0.09, p < 0.0001).**

Regression analysis revealed no significant alteration in measures of intraocular pressure, POBF, pulse volume and pulse amplitude with advancing age ($p>0.05$).

Spectral analysis of the intraocular pressure pulse revealed up to the fourth harmonic in all subjects. However, ANOVA revealed no differences in the harmonic components of the waveform between the youngest and oldest age groups ($p>0.05$).

6.7.3 Correlation between Systemic and Ocular Parameters

Pearson's product moment analysis revealed significant trends between the systemic and ocular pulse rate ($p<0.0001$, $r^2=0.73$; figure 6.6), MAP and ocular pulse amplitude ($p=0.045$, $r^2=0.011$; Figure 6.7), systolic blood pressure and intraocular pressure ($p=0.008$, $r^2=0.030$; figure 6.8), diastolic blood pressure and intraocular pressure ($p=0.003$, $r^2=0.031$; figure 6.9) and systolic and ocular pulse amplitude ($p=0.008$, $r^2=0.030$; figure 6.10).

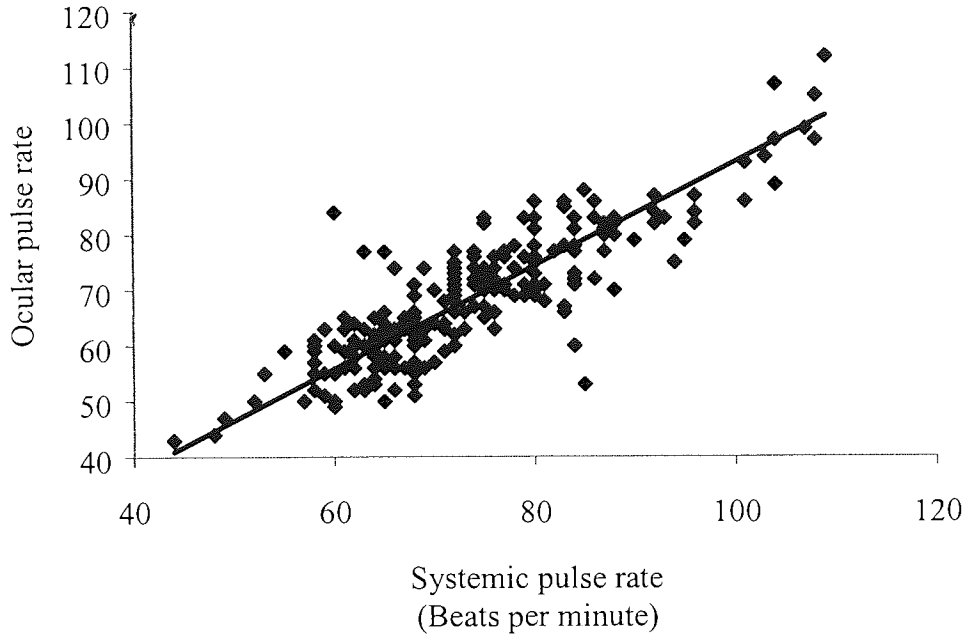

Figure 6.6. **Scatterplot showing the correlation between the systemic and ocular pulse rate ($r^2=0.73$; $p<0.0001$).**

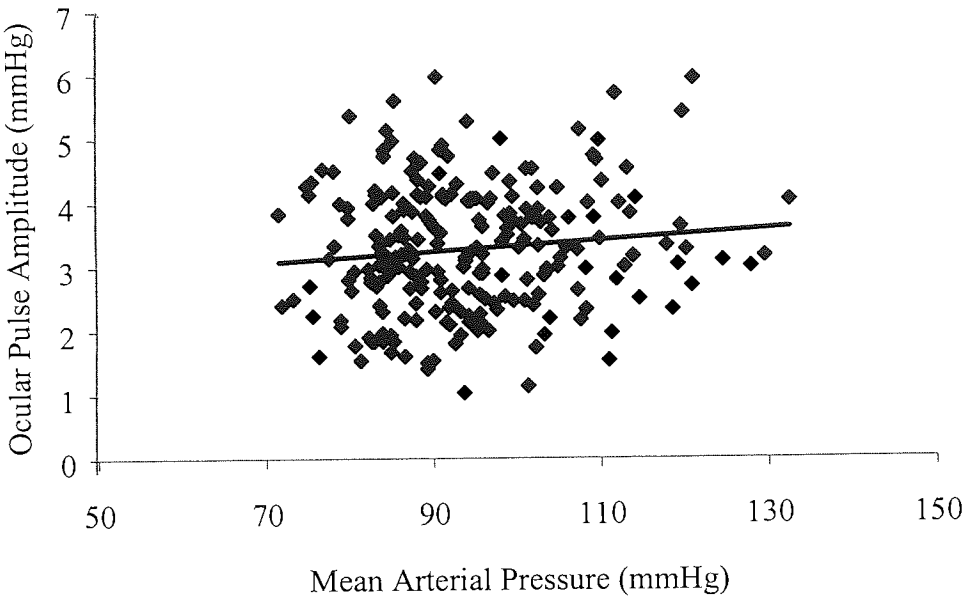

Figure 6.7. **Scatterplot showing the correlation between MAP and ocular pulse amplitude (r^2=0.011; p=0.045).**

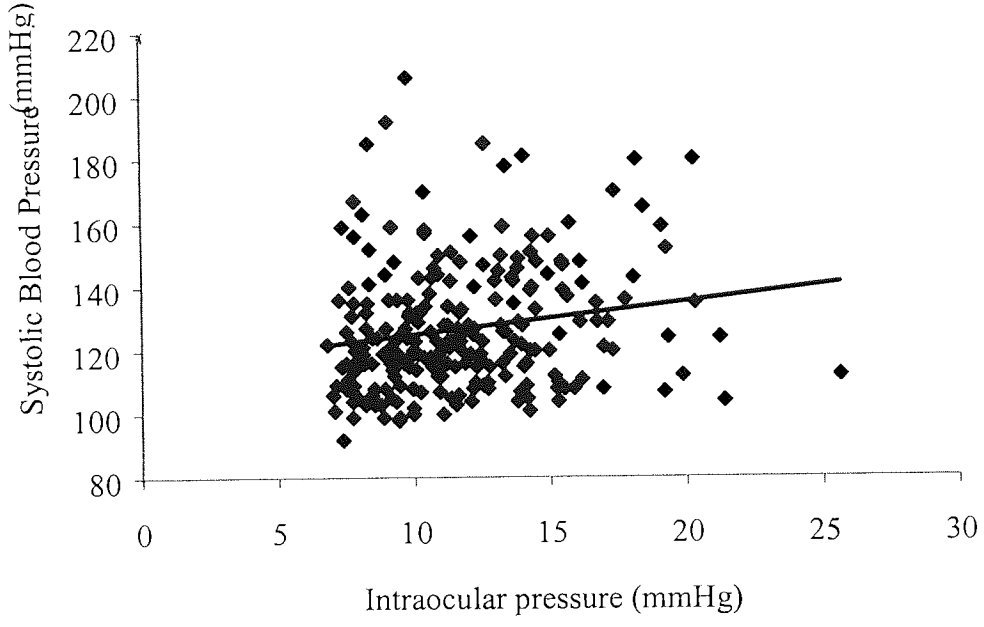

Figure 6.8. **Scatterplot showing the correlation between intraocular pressure and systolic blood pressure (r^2=0.030; p=0.008).**

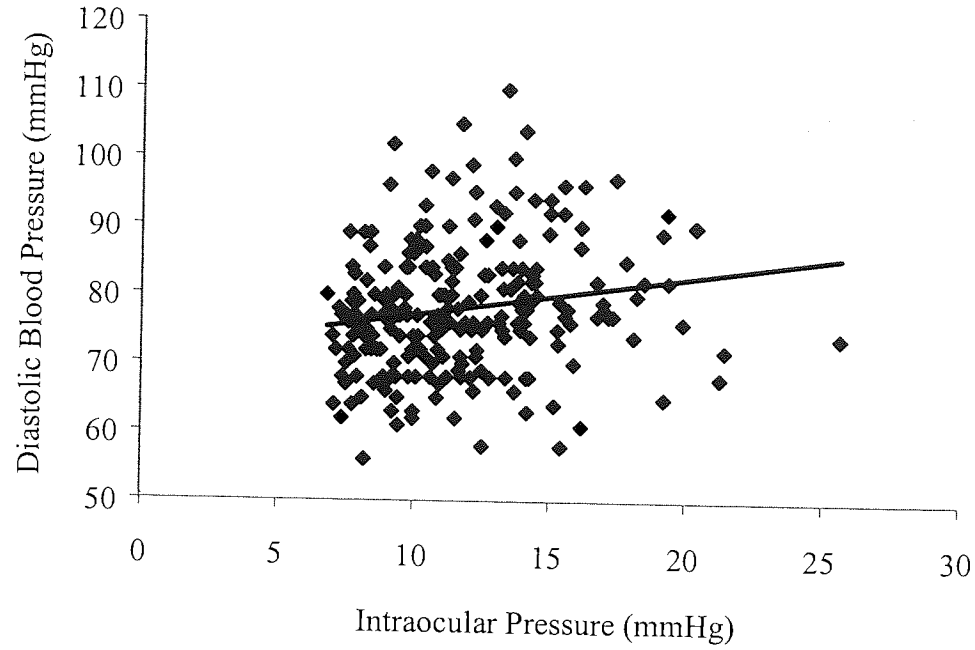

Figure 6.9. **Scatterplot showing the correlation between intraocular pressure and diastolic blood pressure (r^2=0.031; p=0.003).**

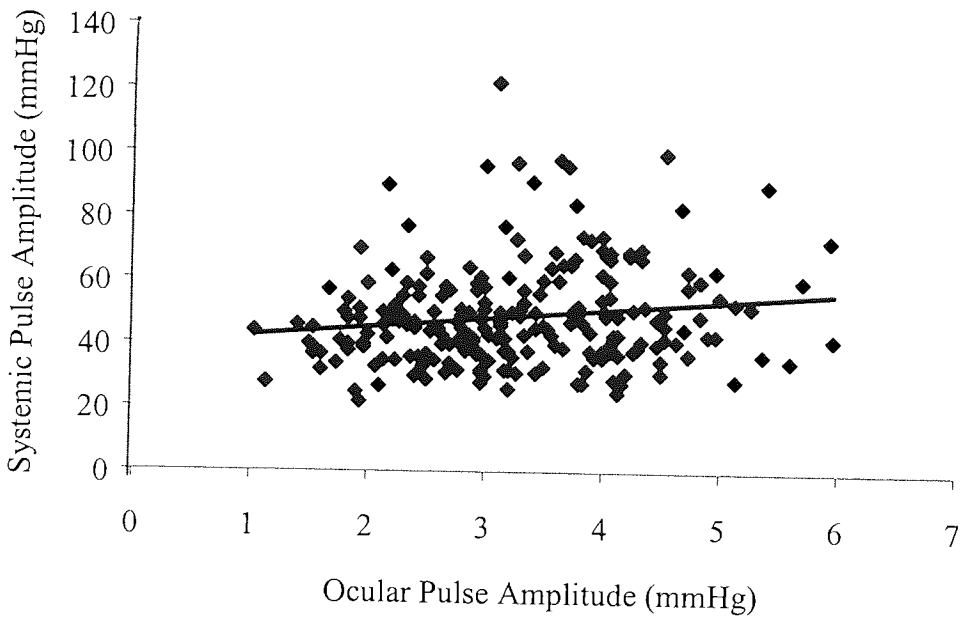

Figure 6.10. **Scatterplot showing the correlation between ocular and systemic pulse amplitude (r^2=0.030; p=0.008).**

6.8 Discussion

6.8.1 Principle findings

The study revealed interarm symmetry for all the measured systemic parameters for this subject group. Significant increases were observed for diastolic blood pressure, MAP and systemic pulse amplitude with advancing age.

Similarly, there were no interocular or gender differences for any of the ocular parameters. Pulse volume, pulse amplitude, intraocular pressure and POBF did not alter with age while both hypermetropia and anterior corneal radius significantly increased. Fourier analysis of the intraocular pressure pulse did not manifest changes to its spectral components when comparing the youngest and oldest age groups.

Significant relationships existed between systemic and ocular pulse rate, MAP and ocular pulse amplitude, systemic and ocular pulse amplitude, systolic blood pressure and intraocular pressure, diastolic blood pressure and intraocular pressure.

6.8.2 Systemic blood pressure

There is some controversy over the existence of inter-arm differences in blood pressure. Cassidy & Jones (2001) reported that both systolic and diastolic blood pressure was significantly higher in the right arm compared with the left. Other studies have found interarm discrepencies but with no consistency in the arm demonstrating the higher blood pressure (Frank, *et al.*, 1993; Singer & Hollander, 1996), however, patients with vascular disease had a higher incidence and magnitude of interarm differences in blood pressure measures when compared to healthy subjects (Frank, *et al.*, 1993; Singer & Hollander, 1996). In this study, no interarm differences in systolic or diastolic blood pressure, MAP or systemic pulse amplitude were found. This finding may reflect our strict inclusion criteria, with all our subjects being free from vascular disease.

6.8.3 Ocular Pulsatility

No difference in measures of POBF, pulse volume, pulse amplitude or pulse rate were found between male and female subjects. This finding contradicts those made by Yang, *et al.*, (1997) and Fontana, *et al.*, (1998) who reported higher values for POBF in females, which was attributed to higher heart rates in females than males. This would explain the absence of any gender differences in this study, in which no significant difference in pulse rate was evident between males and females.

The carotid artery perfuses the left ophthalmic artery prior to the right but this difference has been found to have no impact on blood flow to the ophthalmic and central retinal arteries of the two eyes (Greenfield, Heggerisk & Hedges, 1995; Williamson, Lowe & Baxter, 1995), a finding that is in agreement with those made in this study. A more recent study by Rawji & Flanagan (2001) investigated interocular differences in retinal capillary blood flow in normal eyes using the HRF and found no significant differences in blood flow between the two eyes. These findings together with those made in this study suggest that symmetry in ocular blood flow is maintained in subjects free from ocular and vascular disease throughout life.

Previous studies have identified a significant decrease in measures of ocular pulsatility with advancing age (Ravalico, *et al.*, 1996; Fontana, *et al.*, 1998). It is important to note that there is a shift in the refractive properties of the eye with age, resulting in an increase in hypermetropia (Attebo & Ivers, 1999). This implies that previously myopic individuals will become more emmetropic with advancing age as the refractive indices of the ocular media change. By insisting on emmetropic subjects to examine the effect of age, the sample used by Ravalico, *et al.*, (1996) is likely to have been atypical, in that to maintain emmetropia in the older subjects, eyes of longer axial length would have to be selected to conteract the normal hypermetropic shift that occurs with age (figure 6.11). This suggests that the older eyes were larger. As a result the reduction in POBF observed could have been due to increases in eye volume and not due to changes in vascular resistance with increasing age, since reductions in POBF have been documented in eyes of longer axial length (Fontana, *et al.*, 1998; Mori, *et al.*, 2001). Our subject sample demonstrated a typical refractive hypermetropic shift, which fits with the increase in anterior corneal radius (corneal

flattening) found. Therefore, there is no reason to assume axial length changes (figure 6.11), suggesting preservation of POBF throughout life in normal, healthy individuals.

If we were to consider the hypermetropic shift in our subject sample as being axial in nature, then eyes would have to shorten with age (resulting in the increase in hypermetropia; figure 6.11). Pulse volume and POBF can be recalculated to correct for axial ametropia using refractive error and anterior corneal curvature (for methodology see appendix 2). Age-related reductions in POBF would be masked, with the older subjects exhibiting artificially higher values of POBF. When POBF measures were corrected for presumed axial hypermetropia they exhibited a significant decline with advancing age (r^2=0.024, p=0.03; figure 6.12). Table 6.4 gives values for POBF and pulse volume corrected for refractive error and anterior corneal curvature.

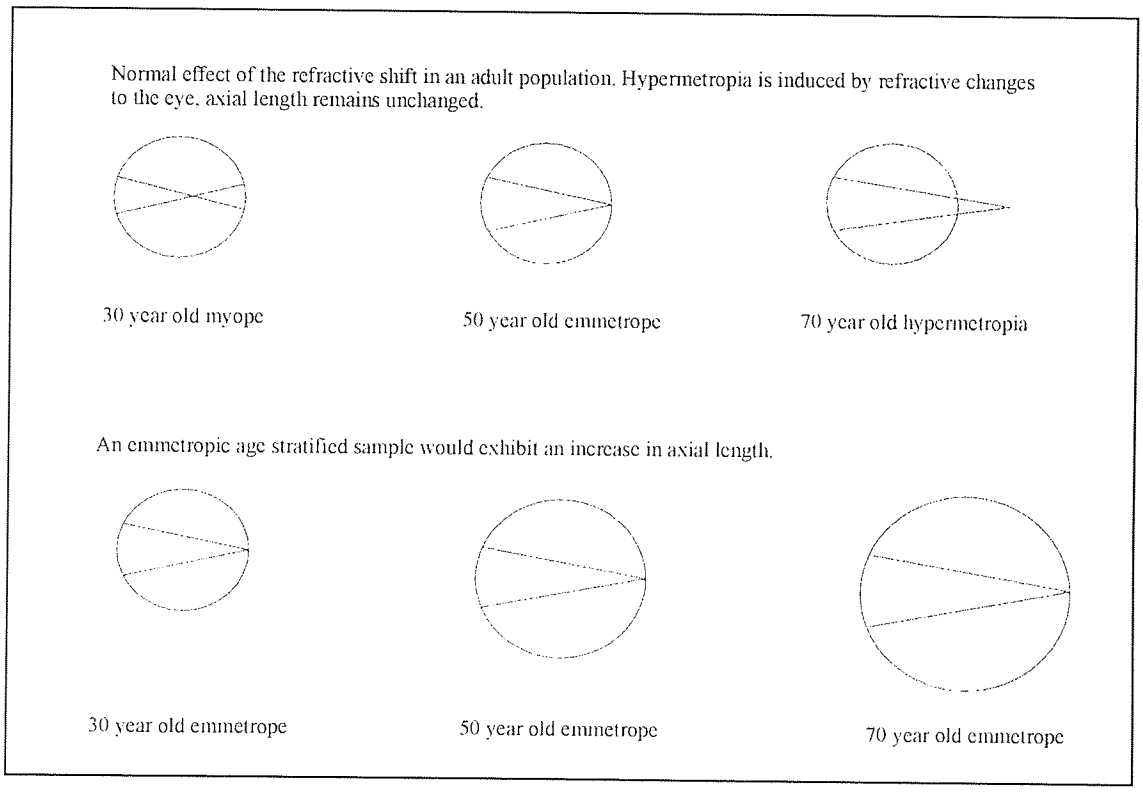

Figure 6.11. **Figure demonstrating the hypermetropic shift with advancing age. To maintain emmetropia in an ageing subject sample axial length would have to increase.**

Age Band (years)	Corrected POBF ± standard deviation (µl/sec)		Corrected Pulse Volume ± SD (µl)	
	Right	Left	Right	Left
20-30	22.17 ± 5.81	22.76 ± 6.49	9.87 ± 3.42	10.10 ± 3.51
30-40	21.43 ± 5.52	21.49 ± 4.20	11.70 ± 5.51	11.06 ± 4.06
40-50	19.42 ± 6.67	20.24 ± 7.07	9.25 ± 4.14	9.94 ± 4.68
50-60	20.04 ± 5.09	19.45 ± 4.21	9.74 ± 3.94	9.13 ± 3.07
60-70	21.15 ± 6.03	21.65 ± 7.09	10.28 ± 3.95	10.72 ± 4.53
70-80	19.72 ± 5.20	19.48 ± 5.09	9.50 ± 2.89	9.44 ± 2.69

Table 6.4. **Table to show corrected measures of POBF and pulse volume for refractive error and anterior corneal curvature, assuming an axial hypermetropic shift. A significant decrease in corrected measures of POBF was induced with advancing age ($p=0.03$). There was no significant change in pulse volume with advancing age ($p>0.05$).**

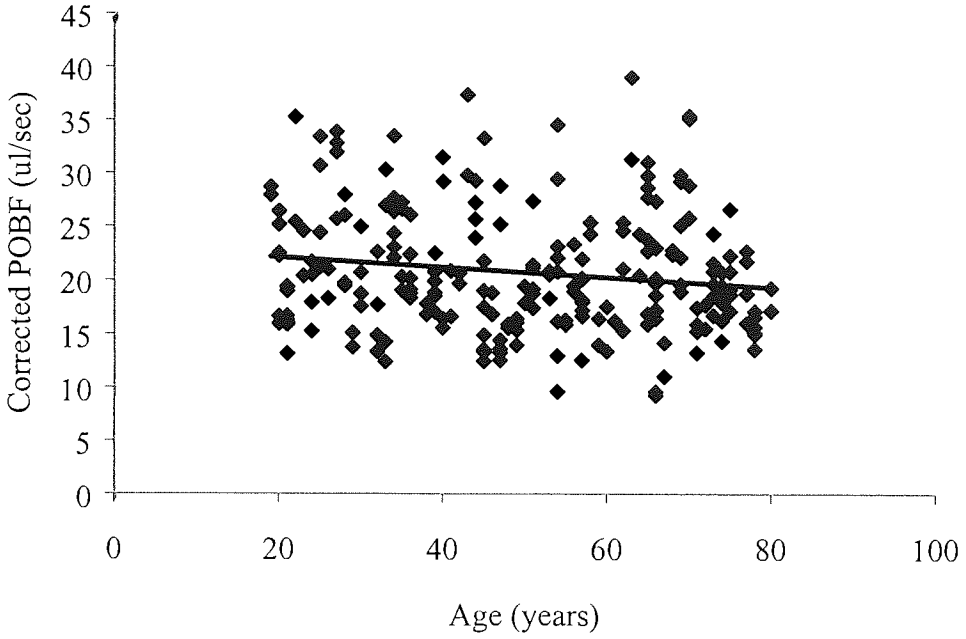

Figure 6.12. **Scatterplot showing an induced decrease in pulsatile ocular blood flow with advancing age when corrected for presumed axial hypermetropia ($r^2=0.024$, $p=0.03$).**

The structure of the blood vessels alters with age such that there is a reduction in the elasticity of the blood vessels (Wadsworth, 1990) and alterations in the endothelial cell structure (Wei, 1992). These structural changes may result in an increase in blood vessel resistivity and decreased compliance. Given this and the histology of the vascular supply to the choroid, one might expect that increasing age would correlate with decreased choroidal blood flow and consequently with measures of POBF. Indeed, reductions in micro-vascular perfusion have been reported with increasing age in the retina (Groh, *et al.*, 1996) and neuroretinal rim (Embleton, Hosking, Roff

Hilton, *et al.*, 2002) using scanning laser Doppler flowmetry, and in the central retinal artery (Groh, *et al.*, 1996) using pulsed Doppler sonography. In this study, diastolic blood pressure and MAP significantly increased with age and these increases were accompanied by a significant elevation in systemic pulse amplitude, which in turn, correlated positively with measures of ocular pulse amplitude. The ocular pulse amplitude reflects the choroidal vascular capacity and an increase indicates an increase in pulse volume and hence higher choroidal perfusion (Mittag, Serle, Schumer, *et al.*, 1994). The correlation between systemic and ocular pulse amplitude, suggests that increases in systemic pulse amplitude translate directly to increases in pulse amplitude in the eye. As such, physiological reductions in vascular compliance due to age are conteracted by blood pressure elevations.

The higher harmonics of the systemic arterial pressure pulse have been found to correspond to vascular impedence (Nichols, *et al.*, 1977), which is closely related to vascular resistance. Spectral analysis of the intraocular pressure pulse has enabled the early detection of carotid artery stenosis (Best & Rogers, 1974) and differentiation between normal subjects and glaucoma patients (Evans, *et al.*, 2002). Given the vascular changes that occur with age (Wadsworth, 1990; Wei, 1992), and the observed increase in MAP in our ageing subject sample, it seems reasonable to suggest that vascular resistivity also increases with age. If the higher harmonics of the intraocular pressure pulse are influenced by vascular resistivity then alterations in these may exist between young and mature subjects. Fourier analysis of the intraocular pressure pulse revealed no differences between the youngest and oldest subject groups. This finding may reflect insensitivites in this method of waveform analysis such as errors induced by waveform truncation (as discussed in Chapter 5). Alternatively there may be preservation of the harmonic components of the intraocular pressure pulse with advancing age in healthy individuals, supporting the theory that POBF remains stable throughout life.

6.8.4 Relationship of systemic to ocular pulsatility

Correlation analysis between systemic and ocular variables revealed a significant trend between systemic and ocular pulse rate and systemic and ocular pulse amplitude, suggesting that the systemic cardiovascular cycle translates directly to the eye in normal, healthy individuals. Furthermore, correlations were found between MAP and

ocular pulse amplitude, systolic blood pressure and intraocular pressure and diastolic blood pressure and intraocular pressure. These findings indicate that the systemic vascular status has, as one would expect, a direct influence on the intraocular pressure pulse and confirms the findings made by Carel, *et al.*, (1984). Increases in systolic and diastolic blood pressure are related to elevation in the intraocular pressure of the eye and as the MAP increases ocular pulse amplitude increases, suggesting that systemic blood pressure responses influence the IOP and it's pulse directly. These results indicate that patients with high systolic and diastolic blood pressure are at an increased risk of elevated intraocular pressure, providing further evidence that hypertension is a risk factor for the development of high tension glaucoma.

6.9 Summary

Results from this study show that symmetry in systemic and ocular blood flow measures exist and are maintained throughout life. Furthermore, systemic blood pressure directly influences the IOP pulse, with increases in blood pressure resulting in higher values of IOP and ocular pulse amplitude. Measures of POBF remain stable throughout life and it is possible that this preservation is due to subtle increases in the ocular pulse amplitude that occur with advancing age.

CHAPTER 7

Investigation of the effect of senescence on ocular blood flow in the retina, neuroretinal rim and lamina cribrosa, using scanning laser Doppler flowmetry

7.1 Abstract

Reductions in blood flow have been reported with increasing in age in the retina, choroid and posterior arteries. Furthermore it is known that changes in the elasticity of the lamina cribrosa occur with advancing age. However, it has not been investigated whether blood flow at the lamina cribrosa, through which the retinal nerve fibres travel, decreases with age.

The purpose of this study was to investigate the effects of age on retinal, neuroretinal and lamina cribrosa blood flow using the Heidelberg, Retina Flowmeter (HRF).

7.2 Background

Many of the changes associated with reduced blood flow in ocular disease are known to become more prevalent with age, which is a known risk factor in glaucoma (Garway-Heath, Wollstein & Hitchings, 1997; Hayreh, 1999). Blood flow deficits identified using the HRF have been reported in both normal-tension glaucoma (Chung, *et al.*, 1999) and primary open angle glaucoma (Michelson, *et al.*, 1996a; Nicolela, *et al.*, 1996) in the retina, neuroretinal rim and lamina cribrosa. Since blood flow is known to decrease with increasing age (Groh, *et al.*, 1996; Ravalico, *et al.*, 1996; Fontana, *et al.*, 1998; Massey, *et al.*, 1999) then this may be a confounding factor in the development of or the progression of glaucoma.

The optic nerve head is supplied by branches of the posterior ciliary arteries (Neetens, 1994), with influence from the central retinal artery in the superficial layers (Buchi, 1996). The choroid receives its nutrients from the posterior ciliary arteries (Bill, 1981). Over time the structure of the arteries change, culminating in a decrease in the elasticity of blood vessels (Wadsworth, 1990). In addition the structure of endothelial cells alters with increases in age and these play a crucial role in vascular tone

regulation (Wei, 1992). One might predict, therefore, that these alterations result in reduced retinal blood flow. Reductions in blood velocity and flow with advancing age have been demonstrated using pulsed Doppler sonography and scanning laser Doppler flowmetry (Groh, *et al.*, 1996). In addition, it has been demonstrated that ocular pulsatility, derived from blood inflow during cardiac systole and influenced by, among other things, vascular elasticity, reduces with age (Ravalico, *et al.*, 1996; Massey, *et al.*, 1999).

The lamina cribrosa is situated in the central region of the optic nerve head. It is an elastic structure composed of perforated cribiform plates through which nerve fibres and the central retinal artery pass. The lamina cribrosa receives its blood supply from the choroidal arteries and the short posterior ciliary arteries (Hayreh, 1978). It is known that with age the components of the cribiform plates alter (Albon, *et al.*, 1995) resulting in a stiffer structure as its mechanical compliance decreases (Albon, *et al.*, 2000). It has been hypothesized that these changes are likely to make the ageing eye more susceptible to retinal ganglion cell axon damage especially when coupled with fluctuations in intraocular pressure as seen in glaucoma (Albon, *et al.*, 2000). The cribiform plates are in very close proximity to one another together with the nerve fibre axons, which pass through them. During changes in intraocular pressure the cribriform plates are rearranged, and in turn these exert force on the nerve fibre axons. In a more rigid structure these forces are likely to have a greater detrimental effect on the nerve fibres. In addition to the known vascular changes associated with age it is possible that a decrease in elasticity of the lamina cribrosa may have an effect on the blood flow of the vessels that supply the lamina cribrosa with nutrients. This has been postulated as a cause of optic nerve damage in glaucoma, but has not previously been investigated in normal, healthy eyes.

7.3 Hypothesis

Ocular blood flow at the lamina cribrosa, as measured using scanning laser Doppler flowmetry, reduces with age.

7.4 Aims and Objectives

The purpose of this study was to determine the effect of increasing age on blood flow, volume and velocity in the retina, neuroretinal rim and lamina cribrosa.

7.5 Materials and Methods

7.5.1 Subject Sample

The subject sample consisted of one eye each of 15 mature and 15 young healthy volunteers. Good quality images were obtained for the neuroretinal rim and peripapillary retina for each subject. For the images located at the lamina cribrosa, 12 of the 15 subjects provided images of sufficient quality for analysis. Details of the subject samples used for each anatomical area of interest are given in Table 7.1.

Area	Group	Gender		Eye		Age ± SD (years)	Age Range (years)
		Male	Female	Right	Left		
Retina and Neuroretinal Rim	Young	7	8	8	7	27.8 ± 6.2	20 - 38
	Mature	7	8	8	7	65.2 ± 13.7	48 – 82
Lamina Cribrosa	Young	6	6	6	6	27.1± 6.3	20 - 38
	Mature	7	5	6	6	64.8± 13.2	48 – 82

Table 7.1. Details of the subject samples used in the study for the retina, neuroretinal rim and lamina cribrosa.

7.5.2 Inclusion Criteria

Young subjects were required to be under 40 years of age and were students from the undergraduate optometry course at Aston University. Mature subjects were over 45 years of age, with no upper age limit and were volunteers recruited from the University eye clinic. All subjects were required to have: -

- Normal optic nerve head appearance confirmed by ocular examination
- Open anterior chamber angles, assesed using a slit-lamp biomicroscope and Van-Herricks grading system (grade 2 to 4).

7.5.3 Exclusion Criteria

Exclusion from the study was made if any of the subjects had: -

- Snellen visual acuity worse than 6/9 in each eye.
- Spherical refractive error of more than 8 dioptres or astigmatism greater than 1 dioptre.
- Intraocular pressures equal to or greater than 22mmHg in each eye ascertained from Goldmann tonometry
- A history of ocular trauma or surgery
- Diabetes mellitus, systemic hypertension or hypotension
- Family history of glaucoma
- Any systemic or ocular medication that may affect blood flow.

7.5.4 Ethical approval and informed consent

Ethical approval was obtained from the ethical committee boards of Birmingham Heartlands and Solihull NHS Trust and Aston University. Approval conformed to the tenets of the declaration of Helsinki. Informed consent was attained from every subject.

7.5.5 Preliminary Tests

Preliminary investigations wer carried out as described in section 2.5.4.

7.5.6 Dilation

All images were acquired through a dilated pupil. One drop of 1% tropicamide was instilled to the test eye and after twenty minutes the pupillary light reflex was tested to ensure maximal mydriasis.

7.5.7 HRF image acquisition

Prior to image acquisition details of each subjects refractive error and anterior corneal curvature were entered into the HRF database and the subject was instructed to look at

the target ahead of them. Images were then acquired using the method outlined in section 2.5.6. Additional images of the lamina cribrosa were taken in all eyes in which a satisfactory area of the lamina cribrosa could be viewed (12 in each group).

One eye was chosen at random for each subject and nine HRF images were acquired: three located in the superior temporal retina, three located in the superior temporal neuroretinal rim and three located in the lamina cribrosa. In order to overcome physiological variations in blood measures occurring due to the cardiac cycle, mean values of blood flow, volume and velocity were obtained from the three images taken at each anatomical area.

The superior temporal area of the retina and neuroretinal rim were chosen for image acquisition as these areas have been shown to exhibit the least amount of variability in blood flow measures when using the HRF (Bohdanecka, *et al.*, 1998). Using the HRF software (version 1.02) fast Fourier transformation was used to derive perfusion images. Blood flow, volume and velocity (arbitrary units) were determined for each image using a 10×10 pixel square grid located at a pre-determined position on the retina, neuroretinal rim and lamina cribrosa for each subject. Acetate sheets were utilized to trace the retinal and neuroretinal rim vasculature for each fundus, and a blood vessel landmark, such as a vessel bifurcation, was identified to ensure that the same retinal, neuroretinal rim and lamina cribrosa locations were used for each image. Every effort was made to keep the locations constant within each subject image series. In addition, efforts were made to optimize the reproducibility of blood flow measurements acquired by obtaining separate HRF images of the different fundus locations, and ensuring that the DC level of the area under examination remained consistently within in the range 110 to150 A.U (Hosking, *et al.*, 2001b). In addition each subject was asked to keep their chin on the rest throughout the examination to ensure that the camera-eye distance remained constant, thus improving the reproducibility of blood flow measures (Kagemann, *et al.*, 1998a). Images were only excepted where no or very little eye movement had occurred during image acquisition, thus ensuring perfusion maps had minimal movement saccades.

7.6 Statistical Analysis

Student's two-tailed unpaired *t*-tests were used to test for significance at the 95% level between the two groups for each blood flow parameter at the retina, rim and lamina cribrosa (see section 3.6). A p-value of less than 0.05 was considered significant.

Pearson's correlation coefficient was used to investigate a linear correlation between age and blood flow parameters. Equations for type of statistical anlysis are given in section 5.6.

7.7 Results

Student's two-tailed unpaired *t*-tests revealed a significant reduction with age in retinal blood volume (p=0.01), neuroretinal rim blood flow (p=0.02), neuroretinal rim blood velocity (p=0.01), lamina cribrosa blood flow (p=0.008) and lamina cribrosa blood velocity (p=0.01) (Table 7.2). No significant difference was found for retinal blood flow or velocity or neuroretinal rim and lamina cribrosa blood volume between the two groups, although the trend was for blood flow parameters to decrease with increasing age (Table 7.2).

	Flow (A.U)			Volume (A.U)			Velocity (A.U)		
	Retina	NRR	Lam Crib	Retina	NRR	Lam Crib	Retina	NRR	Lam Crib
Mature Group	224.98 ± 69.23	210.32 ± 84.15	187.21 ± 57.02	14.35 ± 2.96	13.01 ± 3.48	11.47 ± 5.31	0.812 ± 0.024	0.75 ± 0.30	0.67 ± 0.2
Young Group	248.62 ± 77.94	298.06 ± 106.21	261.53 ± 67.22	17.16 ± 2.7	14.92 ± 4.59	15.03 ± 4.31	0.89 ± 0.27	1.08 ± 0.30	0.897 ± 0.21
p-values	NS	0.02	0.008	0.01	NS	NS	NS	0.01	0.01
r-values (p-values)	-0.084 NS	-0.31 NS	-0.374 NS	-0.455 <0.05	-0.172 NS	-0.475 <0.05	-0.071 NS	-0.359 <0.05	-0.327 NS

Table 7.2. **Mean values (arbitrary units ± SD) for blood flow, volume and velocity measured at the retina, neuroretinal rim (NRR) and lamina cribrosa (lam crib) for the two subject groups with corresponding p and r values.**

Figure 7.1 shows the mean blood flow values measured at the retina, neuroretinal rim and lamina cribrosa for the two subject groups, while figures 7.2 and 7.3 show the mean blood volume and velocity values measured at the retina, neuroretinal rim and lamina cribrosa. Figures 7.4 to 7.6 show the linear correlation (r) for retinal blood volume, neuroretinal rim blood velocity and lamina cribrosa blood volume respectively. Measures of blood flow, volume and velocity at all three of the anatomical areas demonstrated a reduction with increasing age. Significant negative correlations were obtained for retinal blood volume ($r = -0.455$, $p < 0.05$), neuroretinal rim blood velocity ($r = -0.359$, $p < 0.05$) and lamina cribrosa blood volume ($r = -0.475$, $p < 0.05$). No significance was found for neuroretinal rim blood flow and lamina cribrosa blood flow and velocity.

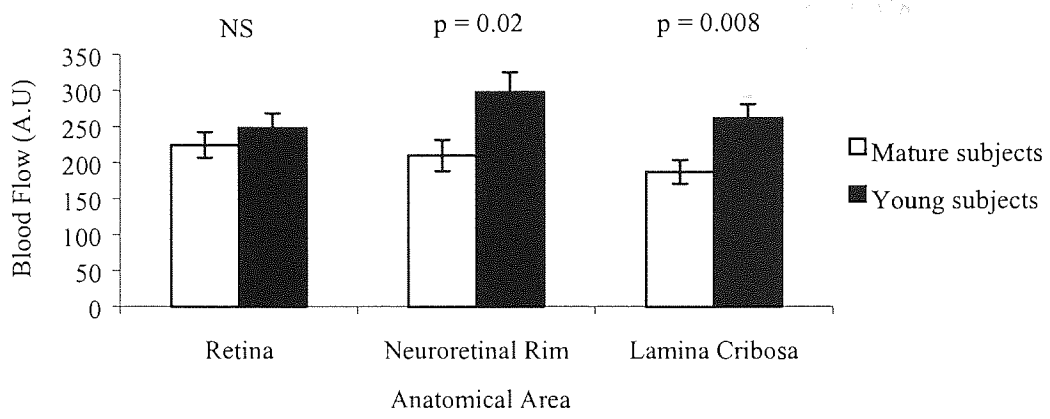

Figure 7.1. **Histogram showing blood flow measures (arbitrary units) for the two groups at the retina, neuroretinal rim and lamina cribrosa. Significant differences were found between the groups at the neuroretinal rim and lamina cribrosa (p = 0.02 and p=0.008 respectively).**

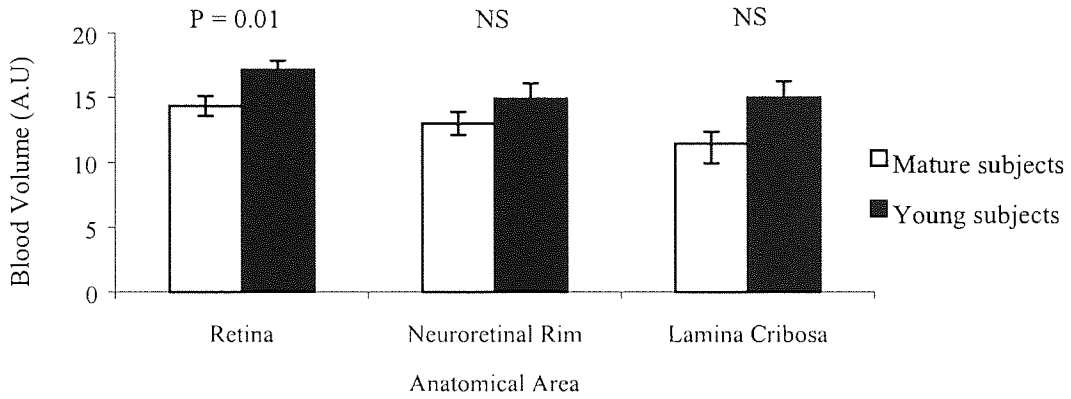

Figure 7.2. **Histogram showing blood volume measures (arbitrary units) for the two groups at the retina, neuroretinal rim and lamina cribrosa. A significant difference was found between the groups at the retina (p = 0.01).**

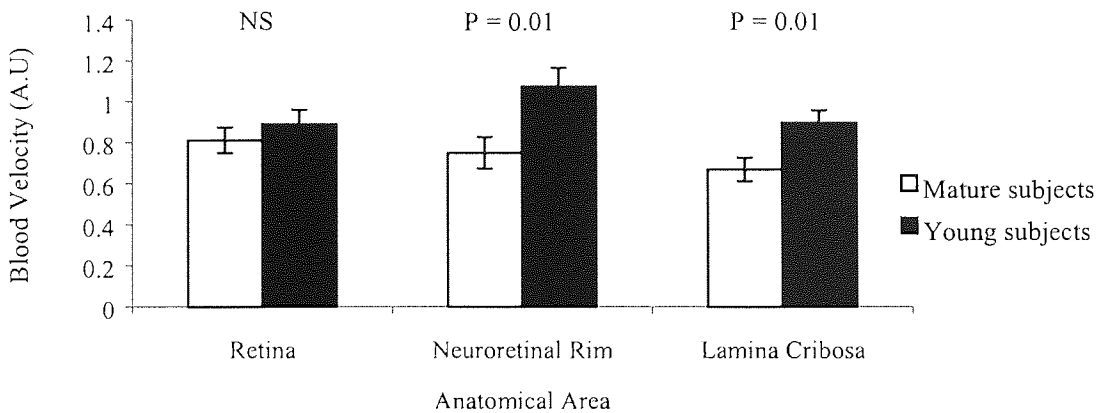

Figure 7.3. **Histogram showing blood velocity measures (arbitrary units) for the two groups at the retina, neuroretinal rim and lamina cribrosa. Significant differences were found between the groups at the neuroretinal rim and lamina cribrosa (p = 0.01 for both areas).**

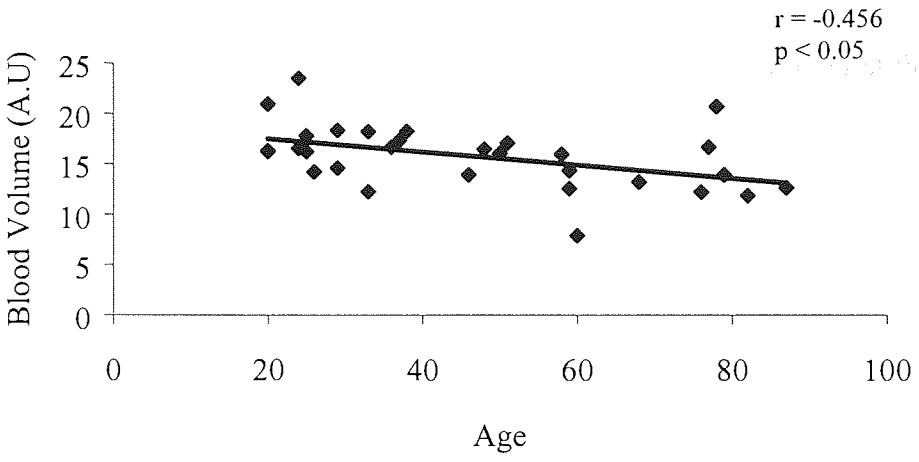

Figure 7.4. **Scatterplot showing significant correlation between retinal blood volume and age (r = - 0.456, p < 0.05). Retinal blood flow significantly reduced with advancing age.**

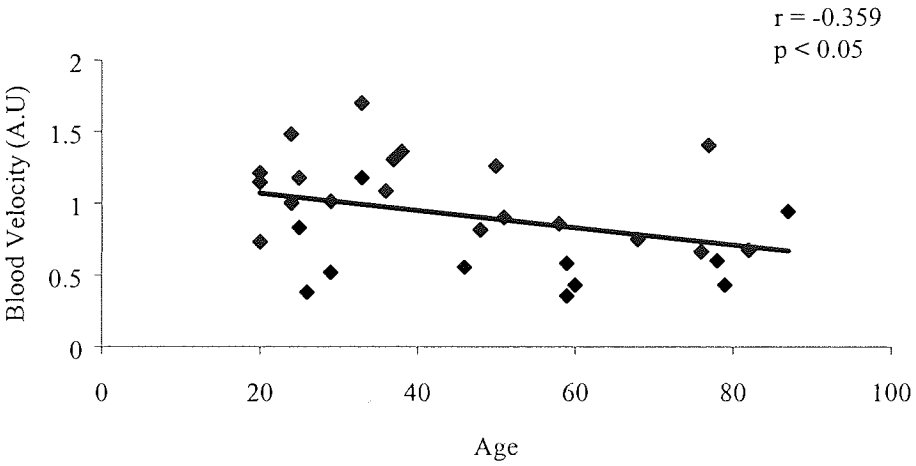

Figure 7.5. **Scatterplot showing the significant negative correlation between neuroretinal rim blood velocity and age (r = - 0.359, p < 0.05).**

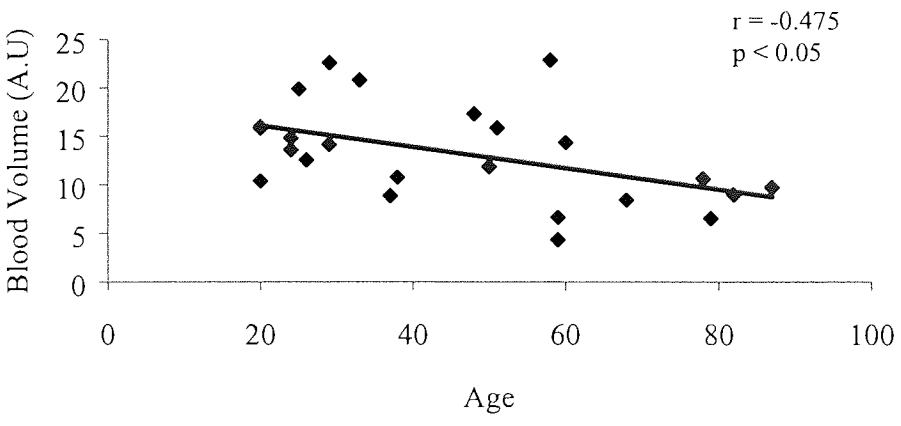

Figure 7.6. **Scatterplot showing the significant negative correlation found between lamina cribrosa blood volume and against age (r=-0.475, p < 0.05)**

7.8 Discussion

The results from this study suggest that capillary blood flow measured at the superior temporal retina, superior temporal neuroretinal rim and at the lamina cribrosa decreases with increasing age. A comparison of group-mean values identified significant reductions in blood volume measured at the retina and in blood flow and velocity measured at the neuroretinal rim and lamina cribrosa. Regression analysis revealed a significant negative correlation between advancing age and retinal blood volume, neuroretinal rim blood velocity and lamina cribrosa blood volume. The absence of any significant change in blood flow and velocity at the lamina cribrosa by regression analysis may be the result of the slightly smaller sample size in this group. The overall finding in this study that ocular blood flow decreases with advancing age is in agreement with previous investigations (Groh, *et al.*, 1996; Ravalico, *et al.*, 1996; Dallinger, Findl, Strenn, *et al.*, 1998; Grunwald, Hariprasad & DuPont, 1998).

It is known that the lamina cribrosa changes throughout life to become a stiffer, less elastic structure (Albon, *et al.*, 2000) and our results revealed, for the first time, that a significant reduction in blood flow and velocity occurs with age in the lamina cribrosa. This supports the theory that, with age, blood flow in the choroidal arteries and short posterior ciliary arteries that supply the lamina cribrosa, decreases. This observed reduction in blood flow at the lamina cribrosa might be due to the combined effects of reduced vascular and mechanical compliance with advancing age. Furthermore, it is known that with the onset of some ocular diseases the rigidity of the lamina cribrosa increases (Zeimer & Ogura, 1989) thus potentially contributing further to reductions in flow. With reference to glaucoma, reduced ocular blood flow has been directly implicated as a contributing factor in its pathogenesis. Nicolela *et al.*, (1996) found that in glaucoma patients the blood flow, volume and velocity in the lamina cribrosa were significantly lower when compared to age matched normals.

Groh *et al.*, (1996) investigated the effect of age on the microcirculation of the retina and neuroretinal rim using the HRF. Consistent with our findings, blood flow measured at the retina significantly reduced with advancing age; unlike the results from our study, the blood flow of the neuroretinal rim was not found to be significantly influenced by age. This discrepancy in results may be due to differences

in the average ages of the subject groups used in each of the studies. Another contributing factor could be the DC level at which the perfusion images were obtained, this was controlled for in our study but average DC values may have been much lower in the study of Groh *et al.*, (1996) as blood flow values for the retina and optic nerve head were taken from the same image. Groh *et al.*, (1996) also investigated the macrocirculation of the central retinal artery, using pulsed Doppler sonography, and reported that significant decreases in blood velocity occur with advancing age. This finding is supported by other investigators, (Harris, Harris, Biller, *et al.*, 2000; Straubhaar, Orgul, Gugleta, *et al.*, 2000) who have found reduced retrobulbar and choroidal blood velocity with advancing age.

Anatomically, the optic nerve head is supplied by both the posterior ciliary arteries and the central retinal artery (in the more superficial layers), whereas the choroid is supplied solely by the posterior ciliary arteries. If blood flow is reduced in the central retinal artery, as Groh *et al.*, (1996) suggests, then one would expect this to have implications on both retinal and neuroretinal rim perfusion. The observed decrease in both neuroretinal rim and retinal blood flow in this study serves to support this. Furthermore, the finding that blood flow is reduced in the lamina cribrosa suggests the presence of decreased blood flow in the posterior ciliary arteries. Alternatively, the reduction in microvascular flow in the lamina cribrosa may be restricted to the capillaries. A reduction in posterior ciliary blood flow may also have implications for choroidal perfusion. Ravalico *et al.*, (1996) reported a reduction in the pulsatile component of ocular blood flow with increasing age, it is known that pulsatile ocular blood flow is primarily choroidal in origin (James, 1998) thus providing indirect support for reduced choroidal flow. Further studies are required to determine the involvement of the posterior ciliary arteries on the perfusion of the ageing eye.

7.8 Summary

Age has a significant effect on neuroretinal rim and lamina cribrosa blood flow and velocity and retinal volume. Morphological changes associated with age, such as reductions in retinal ganglion cells and their axons, have previously been reported (Jonas, Nguyen & Naumann, 1989; Gao & Hollyfield, 1992; Jonas, *et al.*, 1992). This depletion in nerve fibre numbers may result in a reduced requirement for ocular

perfusion, or alternatively reduced perfusion may itself result in the depletion of retinal ganglion cell axons. Blood flow measurements may fall due to increases in vascular resistance following changes in the capillary vessel structure with age (Lee, Blass & Shaw, 1987). It is known that with age the incidence of atherosclerosis increases, which reduces arterial distensibility (Wadsworth, 1990). It therefore follows that with advancing age the compliance of the retrobulbar arteries, including the posterior ciliary arteries and the central retinal artery, are likely to diminish and in turn will result in a decrease in the retinal and neuroretinal rim microcirculation. Alternatively, the blood flow reductions observed might be due to loss of autoregulatory processes (Hayreh, Bill & Sperber, 1994b) that are strongly influenced by the endothelium, which is known to alter with increasing age (Wei, 1992). While the exact basis for reduced blood flow is uncertain, what remains clear is that advancing age results in lower ocular blood flow. It is likely that multiple factors are involved in the process of blood flow diminution with age, and such reduction may be of significance in the aetiology of some age related eye diseases, or indeed exacerbated by them.

CHAPTER 8:

Effect of cataract surgery on visual function, retinal structure and retinal blood flow in the macula of diabetic patients.

8.1 Abstract

Diabetic patients are at an increased risk of developing lenticular opacities. Cataract extraction in patients with diabetes mellitus increases the progression of diabetic retinopathy in some patients including the development of clinically significant macula oedema. Diagnosis of diabetic macula oedema is made by subjective evaluation and in its early stages proves difficult.

The purpose of this study was to determine whether measures of macula blood flow and topography can be used to differentiate diabetic patients at risk of developing clinically significant diabetic macula oedema.

8.2 Background

Diabetes is a major risk factor for the development of cataracts with the incidence increasing with severity and duration of the disease (Leske, Chylack & Wu, 1991; Harding, Egerton, Van Heyningen, *et al.*, 1993; Delcourt, Cristol, Tessier, *et al.*, 2000), and with age (Klein, *et al.*, 1985). It has been reported that cataract surgery in diabetic patients results in the progression of diabetic retinopathy, including clinically significant macular oedema, and that this progression is associated with poor visual prognosis (Pollack, *et al.*, 1991; Pollack, *et al.*, 1992b). In contrast, Henricsson, Heijl & Janzon, (1996) assessed the visual acuity and degree of diabetic retinopathy in diabetic patients before and after cataract surgery and found these patients to have a good visual prognosis following cataract surgery; the progression of diabetic retinopathy was associated with poor glycaemic control. More recently Sadiq *et al.*, (1999) found that the visual improvement in diabetic patients following cataract surgery did not reach the same level as that attained by non-diabetic patients. Furthermore, diabetic patients were found to be five times more likely to suffer from visual deterioration following surgery (Sadiq, *et al.*, 1999). In this study, the degree of

diabetic retinopathy prior to phacoemulsification was a major indicator for the level of post operative visual acuity; 84% of patients without diabetic retinopathy achieved a post operative vision of 6/12 or better, compared to 56% of those with background diabetic retinopathy and 50% of those with clinically significant macular oedema (Sadiq, *et al.*, 1999). These findings are in agreement with those made by Zacrek, Olivestedt & Zetterstrom, (1999) who found advances in the degree of diabetic retinopathy as early as 1-week post phacoemulsification, assesed from fundus photographs and fluorescein angiograms.

In all these studies visual acuity was used to assess visual function before and after surgery. Contrast sensitivity has been shown to be a more sensitive indicator of functional ability in diabetic patients, with reduced contrast sensitivity featuring in patients with background diabetic retinopathy (Sokol, *et al.*, 1985), in whom visual acuity remains unchanged. Furthermore, short wavelength automated perimetry (SWAP) provides early detection of visual loss in diabetic patients (McCreesh, *et al.*, 2000; Nomura, *et al.*, 2000; Remky, *et al.*, 2000; Hudson, *et al.*, 2001b).

Retinal blood flow has been reported as being increased in diabetic patients prior to the development of diabetic retinopathy (Grunwald, *et al.*, 1996), although this is not a universal finding, with reductions in blood flow before the onset of retinopathy being reported in some studies (Yoshida, *et al.*, 1983; Grunwald, *et al.*, 1986; Schmetterer & Wolzt, 1999). Blood flow measures using the OBFA have demonstrated increased blood flow in patients with severe non-proliferative and proliferative diabetic retinopathy (Neuforfer, *et al.*, 1998; Geyer, *et al.*, 1999) and with increasing plasma glucose levels (Perrott, *et al.*, 2001a). Findl, *et al.*, (2000a) assessed the fundus pulsation amplitude (a measure of pulsatile ocular blood flow) and retinal blood flow in patients with insulin-dependent diabetes mellitus. Increases in the fundus pulsation amplitude were found with progression of diabetic retinopathy but measures of retinal blood flow remained unchanged and did not differ from that of healthy control subjects.

The detection of sight threatening diabetic macula oedema is currently made with the assessment of retinal elevation by subjective clinical evaluation making early detection difficult (Ferris & Patz, 1984). The HRT has been used to successfully

identify patients with established macula oedema (Hudson, *et al.*, 1998; Zambarakji, *et al.*, 1999; Ang, *et al.*, 2000; Hudson, *et al.*, 2001b) but its ability to detect early alterations in retinal thickness has not previously been investigated.

8.3 Hypothesis

Quantitive ocular blood flow and retinal topography measures can be used to identify and monitor macula defects that occur following cataract surgery in diabetic patients.

8.4 Aims and Objectives

The purpose of this study was to explore the effects of phacoemulsification on macula topography, blood flow and visual function in diabetic patients compared to normal subjects.

8.5 Materials and Methods

8.5.1 Subject Sample

The sample comprised 17 normal subjects and 17 diabetic patients. Table 8.1 shows the age and gender distribution of the two groups.

	Age ± standard deviation (years)	Sex (Male: Female)
Diabetic Patients (n=17)	73.06 ± 9.17	11: 6
Normal Subjects (n=17)	72.1 ± 13.1	9:8

Table 8.1. Table showing age and gender distribution for the two subject groups.

8.5.2 Inclusion Criteria

All subjects and patients were recruited from the ophthalmology departments of Birmingham Heartlands and Solihull hospitals and were already listed for phacoemulsification on one eye.

i) Normal subjects were required to have: -

- No diabetes mellitus – confirmed by analysis of blood glucose levels

- No previous ocular trauma or surgery
- No existing retinal problems such as age-related macular degeneration
- No glaucoma and intraocular pressure of less than 21mmHg in each eye

ii) Diabetic patients were required to have: -

- No diabetic maculopathy
- No age-related macular degeneration
- No previous ocular surgery or trauma
- No glaucoma and intraocular pressure of less than 21mmHg in each eye

Six of the diabetic patients and nine of the normal subjects suffered from systemic hypertension and were taking antihypertensive medication.

Ophthalmological records for both the normal subjects and diabetic patients were assessed prior to recruitment to the study and suitable individuals were selected from this information. Since all the subjects and patients had significant lenticular opacification, confirmation that all the inclusion criteria were met could not be fully assessed until their first postoperative visit.

8.5.3 Ethical Approval and informed consent

Ethical approval was sought from the ethical committee of Birmingham Heartlands and Solihull NHS trust prior to commencement of the study. This conformed to the tenets of the declaration of Helsinki. Informed consent was acquired from each subject and patient prior to commencing data collection.

8.5.4 Baseline Investigations

Each subject/patient was seen during the two-week interval preceding surgery and full details of their general health were obtained, together with measurements of their blood pressure and heart rate, using an automated sphygnomanometer (Omron HEM 708). The study eye was examined and a full refraction carried out. Details of each participant's visual function was acquired using LogMar visual acuity charts (90% and 10% contrast) and the CSV-1000 contrast sensitivity charts (Vector Vision,

Dayton, Ohio). Finally, blood samples were taken and tested to provide details of blood glucose, cholesterol and glycosated haemoglobin levels. Blood glucose levels were considered normal if between 3.6 to 7.8 g/dl and between 3.6 to 6.5 g/dl for cholesterol. Levels of glycosated haemoglobin (HbA_1C) were measured in order to assess glucose metabolism; the normal range lies between 4.3 to 6.0 g/dl (Bertram, Wolf, Fiehofer, *et al.*, 1991)

8.5.5 Post-Operative Investigations

Each diabetic patient and normal subject was asked to attend the ophthalmology department of Birmingham Heartlands hospital one, seven, thirty and ninety days following surgery. Anterior corneal curvature was measured using a keratometer (Bausch and Lomb) and slit lamp biomicroscopy was performed to determine the presence of any cells or flare in the anterior chamber; this was graded according to Hogan's grading scheme using a 1×1mm slit beam. Presence of any anterior chamber cells or flare resulted in exclusion of the subject or patient from the study since anterior segment pathology would have a detrimental effect on visual function results.

Measures of blood pressure and heart rate were also taken using an automated sphygmomanometer (Omron HEM 706).

8.5.5.1 Investigation of visual function

At each visit the study eye was refracted and measures of visual acuity, using a logMar 90% contrast chart, and contrast sensitivity, using a logMar 10% contrast chart and a CSV-1000 contrast sensitivity chart, were performed. The CSV-1000 contrast sensitivity unit assesses contrast sensitivity to sinusoidal gratings at four spatial frequencies: 3, 6, 12 and 18 cycles per degree (Pomerance & Evans, 1994).

Central visual function was also assessed by short-wavelength automated perimetry (SWAP), using the full threshold 10-2 programme on the Humphrey visual field analyser. The mean deviation for 10-2 SWAP was calculated using the methodology outlined in appendix 3 and Cubbidge, Hosking & Embleton (2002).

8.5.5.2 Pupil dilation

One drop of 1.0% tropicamide was inserted into the eye of interest and patients were asked to rest for twenty minutes to ensure maximal mydriasis. During this resting period the diabetic patients were taken to the haematology department at Birmingham Heartlands Hospital where blood was collected by qualified nurses and analysed for the parameters outlined in section 8.5.5.3.

8.5.5.3 Haematological analysis

Post-operatively, details of the blood glucose, cholesterol and glycosated haemoglobin levels were acquired from all of the diabetic patients at each of the visits. Post-operative blood analysis was not performed in the normal subject group.

8.5.5.4 Diabetic Retinopathy grading

Following full mydriasis indirect ophthalmoscopy was performed using a +90D volk lens and slit lamp to ascertain whether any diabetic retinopathy was present. The diabetic retinopathy was graded according to the modified ETDRS grading scheme such that : -

Grade 1 = No diabetic retinopathy

Grade 2 = Mild non-proliferative diabetic retinopathy: at least one retinal microaneurysm, but haemorrhages and microaneurysms are less than those seen in the ETDRS standard photograph 2a.

Grade 3 = Moderate/Severe non-proliferative diabetic retinopathy:

i) Moderate - haemorrhages and microaneurysms greater than those seen in ETDRS standard photograph 2a. Cotton wool spots, venous beading and intra-retinal microvascular abnormalities (IRMA) are present to a minor degree

ii) Severe – characterised by any one of the following lesions

a) Haemorrhages and microaneurysms greater than those seen in ETDRS standard photograph 2A in all four quadrants
b) Venous beading in two or more quadrants
c) IRMA's greater than those seen in ETDRS photograph 8a in at least one quadrant

Grade 4 = Proliferative diabetic retinopathy comprising:

i) New vessels at the disc or new vessels elsewhere
ii) Pre-retinal or vitreous haemorrhage
iii) Fibrous tissue formation

8.5.5.5 Blood flow measurements

Using the Heidelberg Retina Flowmeter (HRF), measurements of blood flow, volume and velocity were taken at the macula of each subject and patient. Details of the image acquistion methodology are described in full in section 2.5.6, but in brief, subjects and patients were asked to fixate on a white fixation light whilst three images of the macula vasculature were taken, keeping the DC level between 70 and 110 to improve reproducibility of blood measures (Hosking, *et al.*, 2001b). Images acquired with saccades present were discarded and another image taken.

Using the HRF software (version 1.02) fast Fourier transformation was used to derive perfusion images. Blood flow, volume and velocity (arbitrary units) were determined for each image using a 10×10 pixel square grid located at a pre-determined position at the macula for each normal subject and diabetic patient. As with previous HRF analysis, acetate sheets were utilised to trace the vascular network of the macula of each subject and patient and the position of the 10×10 pixel frame was marked on the sheet to facilitate good repositioning in each image for both areas. Using the search strategy (described in full detail in chapter 3 and Hosking, Embleton & Cunliffe, 2001a) the 10×10 pixel frame was systematically re-positioned within a 15x15 pixel window located at a similar position on subsequent macula images. The highest and lowest local values of blood flow, volume and velocity were identified and recorded.

8.5.5.6 Topographic measurements

Using the Heidelberg Retina Tomograph (HRT; Heidelberg Engineering, Germany) measurements of macula topography were obtained. Patients were asked to fixate on a white fixation light and keep their chin on the chin rest between image acquisitions. Three topographic images were acquired and these were accepted if acquisition parameters were said to be acceptable ("OK") by the HRT software, and no eye movements were evident, determined by viewing the movie mode of the image acquired.

A mean topographic image was obtained from the three individual images of the macula using the HRT software (version 2.01). Following this the signal width intensity was calculated to enable analysis of the z-profile. The z-profile represents the summation of specular reflection from the internal limiting membrane and retinal pigment epithelium, and diffuse reflection from the intraretinal layers (Hudson, *et al.*, 1998) and provides an index of retinal thickness. As a result, an increase in retinal thickness, as seen in clinically significant diabetic macular oedema, will result in a greater depth over which reflectance intensity can be measured. In normal subjects the z-profile is narrow and symmetrically distributed at the fovea but becomes asymmetric and wider in other macula areas as the retina becomes thicker (Hudson, *et al.*, 1998).

8.5.5.7 Z-profile Analysis

A HRT image is a three-dimensional image consisting of 32 sequential two-dimensional images, acquired at different locations along the focal plane. At each point along the image plane the three-dimensional image contains the distribution of the measured reflected light intensity or reflectance and is known as the z-profile. The z-profiles are analysed to measure the location of the light-reflecting surface at each point. Determining the position of peak reflectance within the z-profile provides information on the shape of the examined structure.

For each mean topographic file generated z-profile signal width analysis was carried out using menu driven custom software (Heidelberg Engineering, Germany). The

software applied a polynomial of the 16th degree to each z-profile signal width file obtained. The z-profile signal width was defined as the width of the polynomial at 50% of the maximum relative intensity.

Acetate sheets were fixed onto the monitor and the retinal vasculature of the macula was mapped out. Five cells were chosen, as depicted in figure 8.1. Each cell contains 16 by 16 pixel locations, representing 1.25 × 1.25 degrees. These were drawn onto the acetate sheets. The cells were chosen so that cell 1 included the foveal pit with cells 2, 3, 4 and 5 being directly adjacent to it in order to provide a distribution of retinal thickness. This sheet was used on subsequent mean topographic files at all of the post-operative visits to determine whether any alterations in macula topography occurred following phacoemulsification in the normal subjects and diabetic patients. The average of these five cells was calculated for each subject and patient and these values were used to compare alterations in macula topography over time between the two groups.

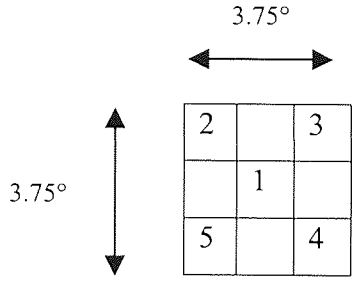

Figure 8.1. **Schematic diagram showing the areas chosen at the macula for z-profile analysis. Area 1 represents the foveal region and each cell represents 1.25 × 1.25 degrees of the macula region.**

8.6 Statistical Analysis

Analysis of variance (ANOVA) was used to determine whether there were any differences between the two groups for any of the measured blood parameters at the preoperative visit (blood glucose, cholesterol and glycosated haemoglobin levels).

To assess the difference between subject groups over time in all the measured parameters (mean arterial pressure, visual acuity, contrast sensitivity, SWAP, macula blood flow and macula topography) repeated measure ANOVA's were obtained using

the statistical package STATVIEW (SAS Institute Inc.). This analysis was also used to assess whether the blood characteristics of the diabetic patients (blood glucose, cholesterol and glycosated haemoglobin) altered over time. The diabetic patients with diabetic retinopathy were analysed individually using ANOVA to determine whether any alteration in visual function, macula blood flow, macula topography and blood chemistry occurred in these patients following phacoemulsification. A p-value of less than 0.05 was considered significant.

Pearson's product moment correlation analysis was used to determine whether alterations in blood glucose and glycosated haemoglobin related to changes in visual function, retinal blood flow and retinal topography over time in the diabetic subject cohort (section 5.6 gives details of the equations used for this type of statistical analysis). Further, correlations between measures of mean arterial pressure, visual function, retinal blood flow and topography in the two subject samples were investigated.

8.7 Results

8.7.1 Sample

All subjects and patients underwent phacoemulsification and had an intraocular lens implant inserted. The average age for the diabetic group was 73.06 ± 9.17 years and for the normal subjects was 72.1 ± 13.1 years. There was no significant difference between the two groups for age ($p>0.05$).

Table 8.2 shows the average mean arterial pressure (MAP) for the two subject groups at each post-operative visit. There was no significant difference in the MAP between the groups ($p>0.05$).

	Mean arterial Pressure (mmHg) ± standard deviation	
	Diabetic patients	Normal subjects
1 day	109.69 ± 35.18	112.67 ± 45.34
7 days	122.19 ± 14.94	126.55 ± 21.52
30 days	119.56 ± 15.23	119.53 ± 12.62
90 days	121.87 ± 14.27	119.43 ± 13.63

Table 8.2. **Table showing the mean arterial pressure (MAP) ± standard deviation for the diabetic patients and normal subjects at each of the post-operative visits. There was no significant difference between the two groups ($p>0.05$).**

8.7.2 Visual Acuity

Table 8.3 shows the LogMar visual acuity for both subject groups at all the post-operative visits. Figure 8.2 shows the change in LogMar visual acuity post-operatively for both groups. Visual acuity values were significantly better for the normal subjects compared to the diabetic patients at thirty (p=0.03) and ninety days (p=0.01) post-operatively. There was no significant difference between the groups in the pattern of visual recovery following phacoemulsification (p> 0.05).

	Post-operative LogMar Visual Acuity (Log units) ± standard deviation			
	1 day	7 days	30 days	90 days
Diabetic Patients	0.25 ± 0.18	0.17 ± 0.23	0.13 ± 0.19	0.12 ± 0.16
Normal Subjects	0.15 ± 0.20	0.06 ± 0.11	0.02 ± 0.08 *	0.01 ± 0.08 *

Table 8.3. **Table showing average values of LogMar visual acuity at all post-operative visits for the diabetic patients and normal subjects. Visual acuity was significantly better in the normal subjects 30 days (p=0.03) and 90 days (p=0.01) following phacoemulsification (*).**

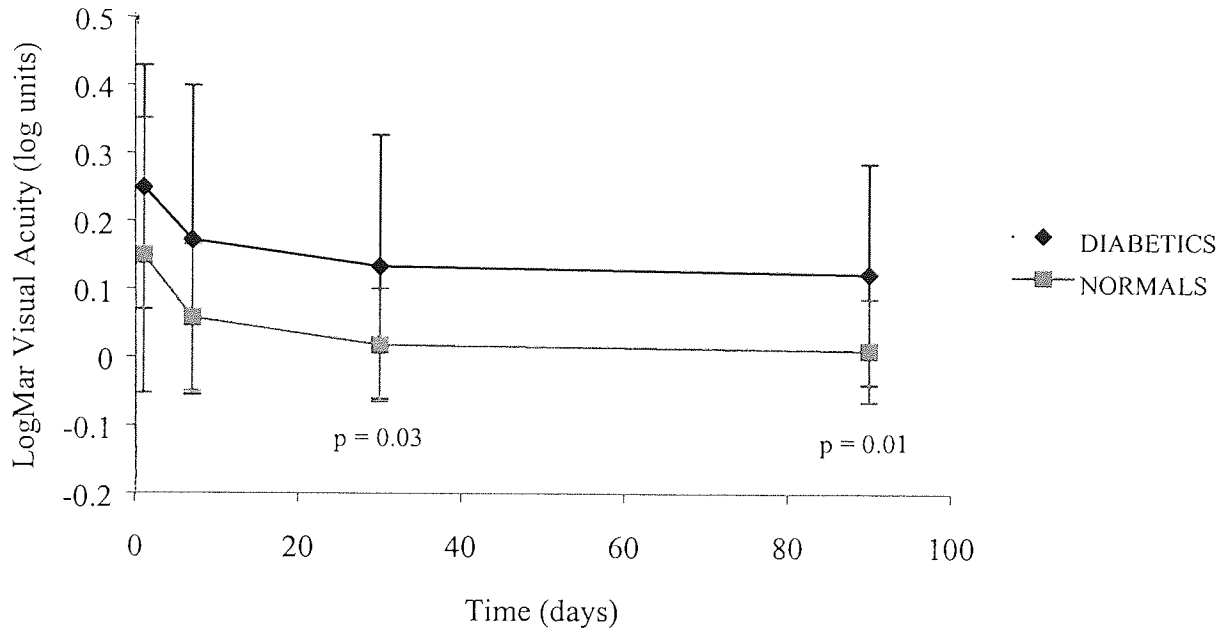

Figure 8.2. **Graph showing post-operative change in LogMar visual acuity (± standard deviation) for the normal subjects and diabetic patients. Values were significantly better for the normal subjects at 30 days (p=0.03) and 90 days (p=0.01). There was no difference in the rate or extent of visual acuity over time between the two groups (p>0.05).**

8.7.3 Contrast Sensitivity

Table 8.4 shows the average contrast sensitivity acquired using the LogMar 10% chart for the diabetic patients and normal subjects. Contrast sensitivity was significantly better for the normal subjects compared to the diabetic patients 1 day (p=0.02), 7 days (p=0.03), 30 days (p=0.02) and 90 days (p=0.03) following phacoemulsification. Table 8.5 shows the mean contrast sensitivity using sinusoidal gratings at 3, 6, 12 and 18 cycles per degree for the two subject groups. At all the measured spatial frequencies the contrast sensitivity was better for normal subjects compared to diabetic patients at all the post-operative visits (p<0.05).

	LogMar Contrast Sensitivity 10% (cycles per degree) ± standard deviation (log units)			
	1 day	7 days	30 days	90 days
Diabetic Patients	0.52 ± 0.20	0.43 ± 0.23	0.37 ± 0.23	0.35 ± 0.23
Normal Subjects	0.35 ± 0.13	0.28 ± 0.14	0.22 ± 0.12	0.20 ± 0.10

Table 8.4. **Average values of 10% LogMar contrast sensitivity at all the post-operative visits for the diabetic patients and normal subjects. Contrast sensitivity was significantly better for the normal subjects compared to the diabetic patients 1 day (p=0.02), 7 days (p=0.03), 30 days (p=0.02) and 90 days (p=0.03) following phacoemulsification.**

	Visit	Diabetic patients	Normal Subjects	p-values
Contrast Sensitvity at 3 cycles per degree ± standard deviation (log units)	**1 day**	1.11 ± 0.35	1.43 ± 0.31	0.02
	7 days	1.29 ± 0.32	1.50 ± 0.18	0.02
	30 days	1.38 ± 0.20	1.62 ± 0.21	0.002
	90 days	1.40 ± 0.22	1.62 ± 0.19	0.0003
Contrast Sensitvity at 6 cycles per degree ± standard deviation (log units)	**1 day**	1.28 ± 0.36	1.57 ± 0.29	0.03
	7 days	1.39 ± 0.36	1.64 ± 0.19	0.02
	30 days	1.51 ± 0.33	1.76 ± 0.19	0.01
	90 days	1.53 ± 0.22	1.78 ± 0.16	0.0003
Contrast Sensitvity at 12 cycles per degree ± standard deviation (log units)	**1 day**	0.65 ± 0.39	1.09 ± 0.28	0.003
	7 days	0.91 ± 0.43	1.29 ± 0.29	0.004
	30 days	1.01 ± 0.42	1.38 ± 0.23	0.003
	90 days	1.04 ± 0.37	1.44 ± 0.20	0.0003
Contrast Sensitvity at 18 cycles per degree ± standard deviation (log units)	**1 day**	0.11 ± 0.35	0.60 ± 0.28	0.0004
	7 days	0.34 ± 0.42	0.84 ± 0.35	0.0005
	30 days	0.55 ± 0.43	0.98 ± 0.19	0.0006
	90 days	0.56 ± 0.35	1.01 ± 0.23	<0.0001

Table 8.5. **Table showing the mean ± standard deviation contrast sensitivity at 3, 6, 12 and 18 cpd at each post-operative visit for diabetic patients and normal subjects. At all the measured spatial frequencies the contrast sensitivity was significantly better for normal subjects compared to diabetic patients at all the post-operative visits (p<0.05).**

Figure 8.3 shows the change in logMar 10% contrast sensitivity post-operatively for both groups. LogMar contrast sensitivity was significantly better in the normal subject group at one day (p=0.02), seven days (p=0.03), 30 days (p=0.02) and ninety days (p=0.03) post operatively compared to the diabetic patients. The pattern of change in LogMar contrast sensitivity over time was not significantly different between the groups (p>0.05).

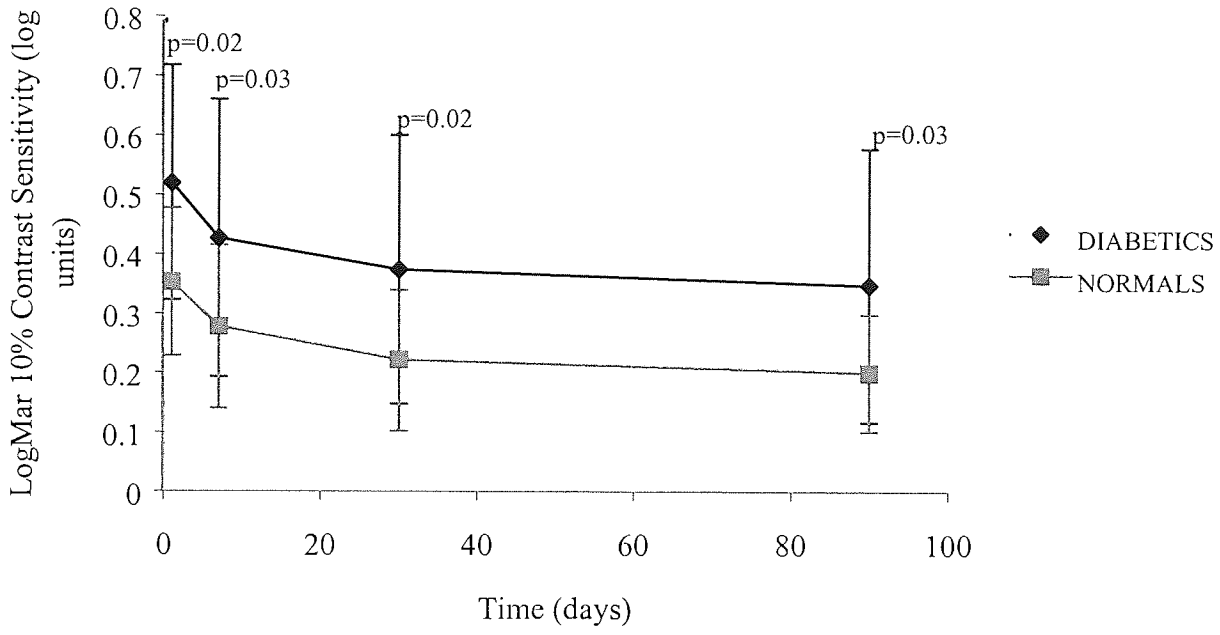

Figure 8.3. **Graph showing post-operative 10% LogMar contrast sensitivty (± standard deviation) for normal subjects and diabetic patients. Values were significantly better for normal subjects at 1 day (p=0.02), 7 days (p=0.03), 30 days (p=0.02) and 90 days (p=0.03). There was no difference in the rate or extent of contrast sensitivity improvement post-operatively between the groups (p>0.05)**

Figures 8.4 to 8.7 show the change in contrast sensitivity at 3, 6, 12 and 18 cycles per degree (cpd) for the normal subjects and diabetic patients. At all the measured spatial frequencies for all visits the contrast sensitivity was significantly worse in the diabetic group compared to the normal subjects. The groups were not significantly different for the pattern of change over-time post-operatively (p>0.05).

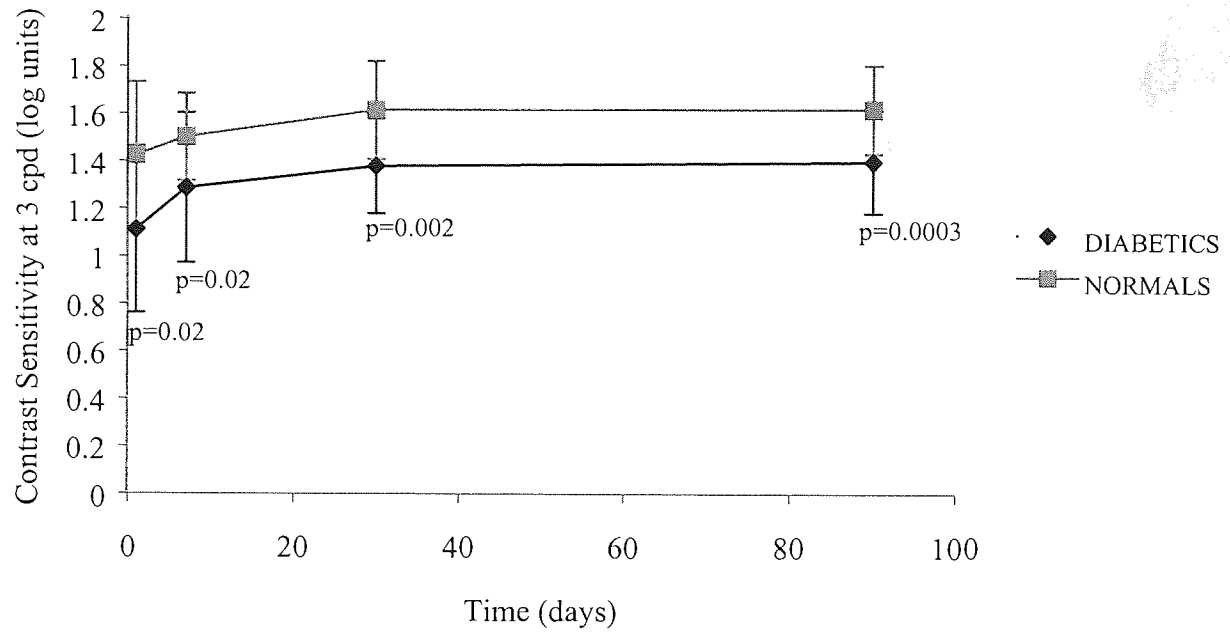

Figure 8.4. **Graph showing post-operative change in contrast sensitivity (± standard deviation) at 3 cycles per degree (cpd) for normal subjects and diabetic patients. Values were significantly better for normal subjects at 1 day (p=0.02), 7 days (p=0.02), 30 days (p=0.002) and 90 days (p=0.0003). There was no difference in the rate or extent of contrast sensitivity improvement post-operatively between the groups (p>0.05)**

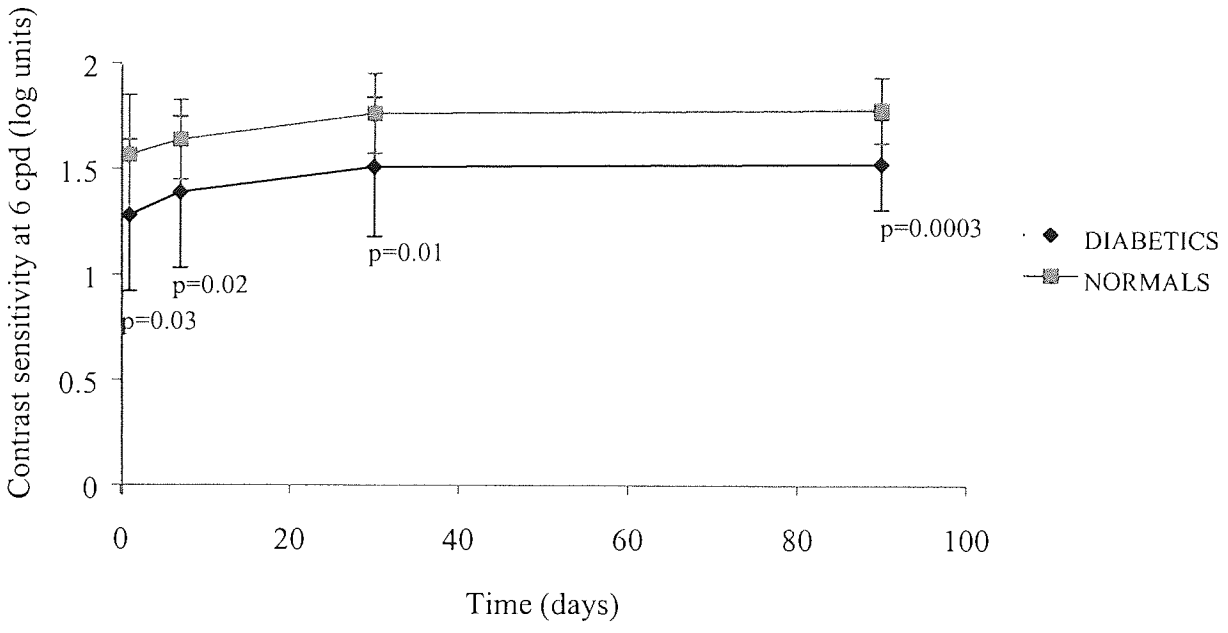

Figure 8.5. **Graph showing post-operative change in contrast sensitivity (± standard deviation) at 6 cycles per degree (cpd) for normal subjects and diabetic patients. Values were significantly better for normal subjects at 1 day (p=0.03), 7 days (p=0.02), 30 days (p=0.01) and 90 days (p=0.0003). There was no difference in the rate or extent of contrast sensitivity improvement post-operatively between the groups (p>0.05)**

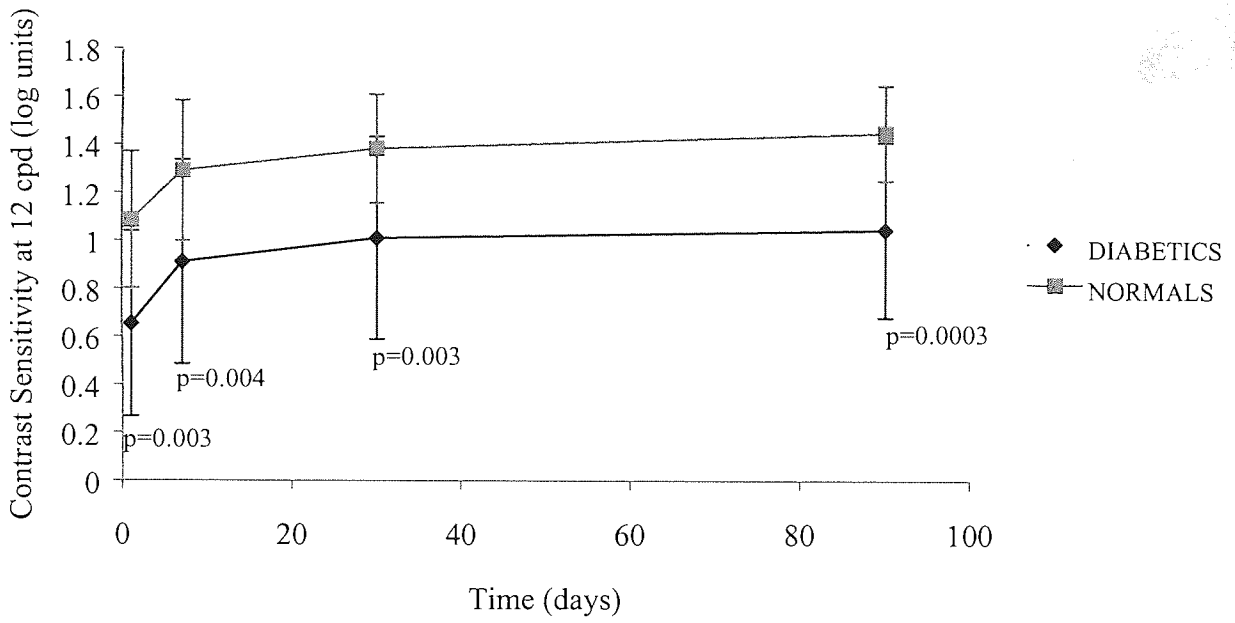

Figure 8.6. **Graph showing post-operative change in contrast sensitivity (± standard deviation) at 12 cycles per degree (cpd) for normal subjects and diabetic patients. Values were significantly better for normal subjects at 1 day (p=0.003), 7 days (p=0.004), 30 days (p=0.003) and 90 days (p=0.0003). There was no difference in the rate or extent of contrast sensitivity improvement post-operatively between the groups (p>0.05)**

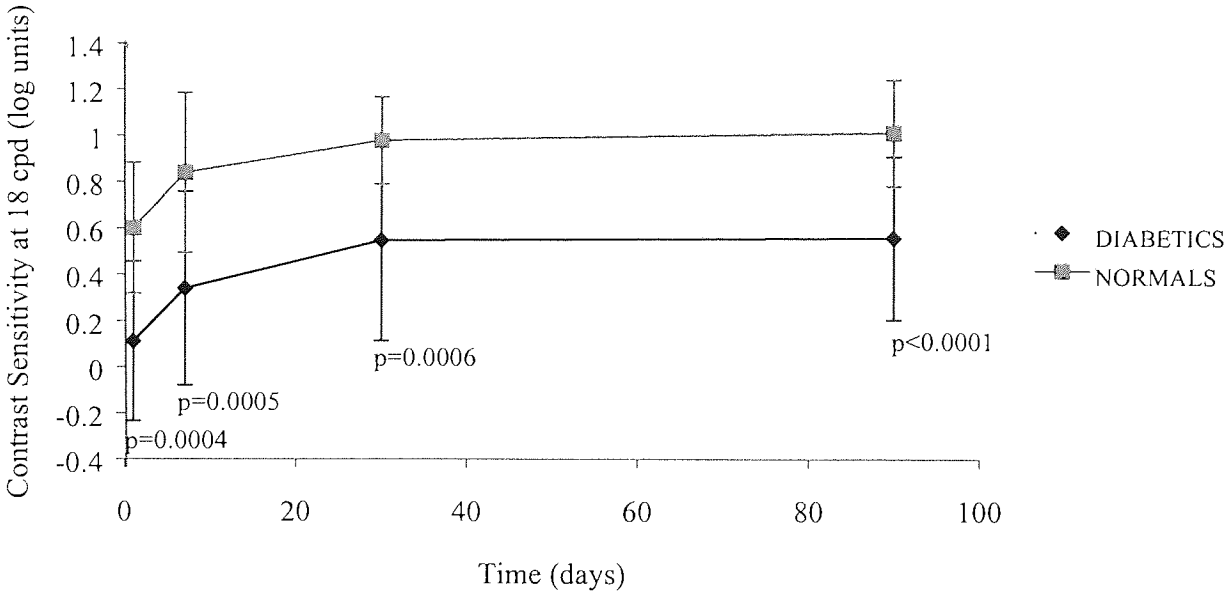

Figure 8.7. **Graph showing post-operative change in contrast sensitivity (± standard deviation) at 18 cycles per degree (cpd) for normal subjects and diabetic patients. Values were significantly better for normal subjects at 1 day (p=0.0004), 7 days (p=0.0005), 30 days (p=0.0006) and 90 days (p<0.0001). There was no difference in the rate or extent of contrast sensitivity improvement post-operatively between the groups (p>0.05)**

8.7.4 SWAP

Table 8.6 shows the average mean deviation (dB) of the 10-2 SWAP attained by the diabetic patients and normal subjects at all of the post-operative visits.

	Mean Deviation (dB)			
	1 day	**7 days**	**30 days**	**90 days**
Diabetic Patients	-2.01 ± 4.47	-1.66 ± 5.74	-0.64 ± 5.58	-1.09 ± 5.60
Normal Subjects	-0.09 ± 5.71	0.89 ± 5.25	2.37 ± 4.34	2.31 ± 4.49

Table 8.6. **Table showing average values of the SWAP mean deviation values obtained by the diabetic patients and normal subjects. There were no significant differences between the two groups at any of the post-operative visits ($p>0.05$).**

Figure 8.8 shows the change in the mean deviation post-operatively for the normal subjects and diabetic patients. There was no significant improvement in the mean deviation over time ($p>0.05$) for either group. There was no significant difference between the two groups in the mean deviation at any of the post-operative visits and this may be due, in part, to the large standard deviation present between groups. Furthermore, the way in which the mean deviation altered over time was not significantly different between the two groups ($p>0.5$).

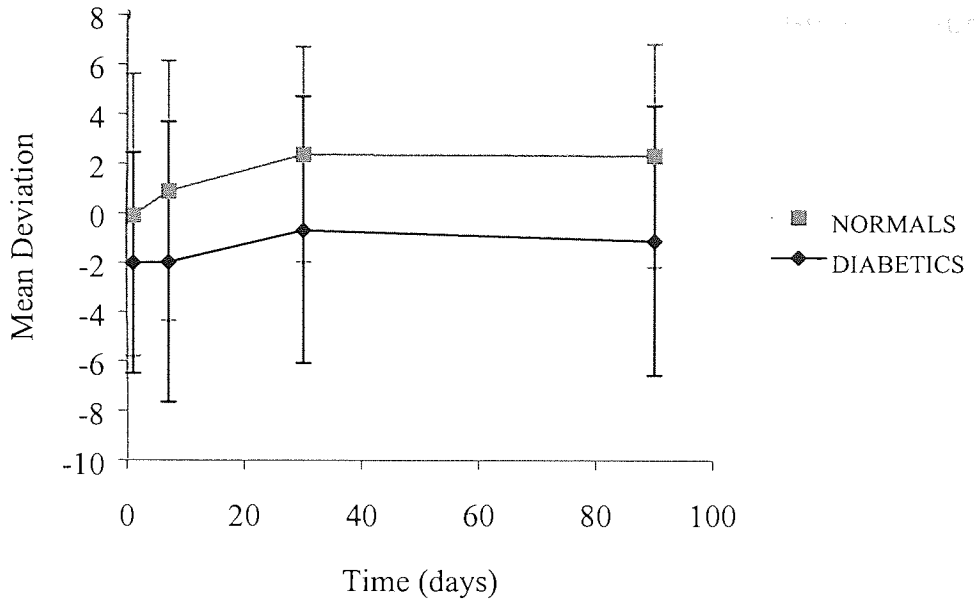

Figure 8.8. **Graph showing change in the mean deviation post operatively for the two groups. Statistical analysis revealed no significant differences between the normal subjects and diabetic patients at any of the postoperative visits ($p>0.05$). There was no difference in the rate or extent of improvement in the mean deviation post-operatively between the groups ($p>0.05$)**

8.7.5 Haematology Results

Table 8.7 summarises the blood glucose, cholesterol and glycosated haemoglobin concentrations for the normal subjects at the pre-operative visit only and for the diabetic patients at all visits. Analysis of the blood glucose levels pre-operatively revealed that the diabetic group exhibited significantly higher levels of blood glucose ($p=0.006$) and glycosated haemoglobin ($p<0.0001$) compared to the normal subject group. Cholesterol levels were significantly higher in the normal subject group compared to the diabetic patients ($p=0.05$). Postoperatively in the diabetic group none of the measured blood parameters significantly altered over time ($p>0.05$).

		Glucose (g/dl) ± standard deviation	Cholesterol (g/dl) ± standard deviation	Glycosated Haemoglobin (g/dl) ± standard deviation
Normal Subjects	**Pre-operative visit**	5.48 ± 1.02	5.43 ± 1.11	4.57 ± 0.52
Diabetic Patients	**Pre-operative visit**	10.29 ± 5.44	4.63 ± 0.53	6.92 ± 0.94
	1 day	8.03 ± 1.84	4.30 ± 0.46	6.80 ± 0.86
	7 days	8.32 ± 2.62	4.52 ± 0.74	6.53 ± 1.26
	30 days	7.03 ± 2.38	4.69 ± 0.75	6.55 ± 1.32
	90 days	9.74 ± 4.00	4.56 ± 0.83	6.34 ± 1.17

Table 8.7. **Table giving average values of blood glucose, cholesterol and glycosated haemoglobin concentrations for the normal subjects (pre-operative visit only) and diabetic patients (all visits). Statistical analysis revealed that glucose and glycosated haemoglobin levels were significantly higher in the diabetic group ($p=0.006$ and $p<0.0001$ respectively) pre-operatively. Cholesterol levels were significantly higher in the normal subjects pre-operatively ($p=0.05$).**

8.7.6 Retinal status of diabetic patients

Due to the presence of lenticular opacities assessment of retinal status was not performed pre-operatively. According to the ophthalmological records of the diabetic patients fourteen had no diabetic retinopathy pre-operatively and the three remaining patients had grade 2 diabetic retinopathy (according to the modified ETDRS grading scheme) and were included in the study.

At the first post-operative visit (1 day) a dilated fundus examination was carried out to confirm the diabetic retinopathy status of each patient. This revealed that the same fourteen patients had no evidence of any diabetic retinopathy with the three other diabetic patients having diabetic retinopathy of grade 2. Thirteen patients exhibited no diabetic retinopathy throughout the duration of the study; all of these patients had non-insulin dependent diabetes mellitus. One insulin-dependent diabetic patient with no evidence of any retinopathy pre-operatively or at 1 day, exhibited grade 2 retinopathy at 1 week with no further progression. Two patients (1 non-insulin dependent and 1 insulin dependent) with grade 2 retinopathy pre-operatively and at day 1, demonstrated no further progression. The final patient (non-insulin dependent diabetic) exhibited diabetic retinopathy of grade 2 at day 1 which progressed to grade 3 after 1 week, with no further changes.

8.7.7 Ocular blood flow measures

Table 8.8 shows the high and low values of macula blood flow, volume and velocity for the diabetic patients and normal subjects at all the post-operative visits. There was no significant difference between the two groups at any of the visits ($p > 0.05$). Furthermore there were no alterations in blood flow over time in either of the groups ($p>0.05$).

			Diabetic patients	Normal Subjects
Macula Blood Flow (AU) ± standard deviation	High Values	1 day	357.24 ± 91.94	310.16 ± 59.87
		7 days	344.47 ± 81.14	296.07 ± 54.18
		30 days	339.75 ± 72.66	334.54 ± 96.50
		90 days	346.33 ± 67.40	337.02 ± 101.51
	Low Values	1 day	294.88 ± 80.50	250.76 ± 60.95
		7 days	276.55 ± 71.91	234.80 ± 59.37
		30 days	271.41 ± 65.31	264.52 ± 85.12
		90 days	286.19 ± 60.08	270.43 ± 87.50
Macula Blood Volume (AU) ± standard deviation	High Values	1 day	15.49 ± 1.65	14.87 ± 2.24
		7 days	15.34 ± 2.35	15.25 ± 2.00
		30 days	15.04 ± 2.74	15.88 ± 3.27
		90 days	15.28 ± 2.46	15.35 ± 3.65
	Low Values	1 day	13.28 ± 1.61	12.93 ± 2.18
		7 days	12.96 ± 2.49	12.52 ± 2.12
		30 days	12.90 ± 3.06	13.15 ± 3.03
		90 days	13.01 ± 2.59	12.97 ± 2.97
Macula Blood Velocity (AU) ± standard deviation	High Values	1 day	1.28 ± 0.31	1.12 ± 0.21
		7 days	1.24 ± 0.28	1.07 ± 0.19
		30 days	1.27 ± 0.25	1.20 ± 0.32
		90 days	1.25 ± 0.23	1.21 ± 0.33
	Low Values	1 day	1.06 ± 0.28	0.91 ± 0.22
		7 days	1.00 ± 0.25	0.85 ± 0.21
		30 days	0.98 ± 0.23	0.96 ± 0.29
		90 days	1.03 ± 0.20	0.98 ± 0.30

Table 8.8. **Table showing average high and low values of macula blood flow, volume and velocity (arbitrary units) for the glaucoma patients and normal subjects. There was no significant difference in blood flow measures between the two groups at any of the post-operative visits ($p>0.05$) and there was no significant alteration in them over time ($p>0.05$).**

8.7.8 Topographic Measures

Table 8.9 shows the average z-profile signal width for the mean of five cells analysed for the diabetic patients and normal subjects at all the post-operative visits. Topographic measurements were not significantly different between the two groups ($p>0.05$) at any of the post-operative visits. In addition the way in which the macula

topography altered over time was not significantly different between the normal subjects and diabetic patients (p>0.05).

	Z-profile signal width (mm)	
	Diabetic Patients	Normal subjects
Day 1	1.30 ± 0.45	1.05 ± 0.44
Day 7	1.16 ± 0.26	1.07 ± 0.39
Day 30	1.16 ± 0.39	1.14 ± 0.39
Day 90	1.14 ± 0.35	1.14 ± 0.37

Table 8.9. **Table showing average z-profile signal widths for diabetic patients and normal subjects at all the post-operative visits. There was no significant difference in values between the two subject groups at any of the visits following phacoemulsification (p>0.05) and there was no significant alteration in them over time (p>0.05).**

8.7.9 Diabetic retinopathy patients

Analysing the data from the four diabetic patients with diabetic retinopathy individually revealed no alteration in any of the visual function, blood flow or topographic parameters post-operatively. This result suggests that following phacoemulsification these patients did not develop any degree of diabetic macula oedema, although background changes were seen to increase.

8.7.10 Correlation Analysis

8.7.10.1 Normal Subjects

A significant negative trend between MAP and LogMar 10% contrast sensitivity existed such that as MAP increased for the sample, contrast sensitivity reduced (p=0.01; figure 8.9), Similarly, for CSV-1000 contrast sensitivity at 6 cycles per degree (p=0.05; figure 8.10) and at 18 cycles per degree (p=0.02; figure 8.11) a negative trend was evident with MAP. No other correlations were apparent in the normal subject group.

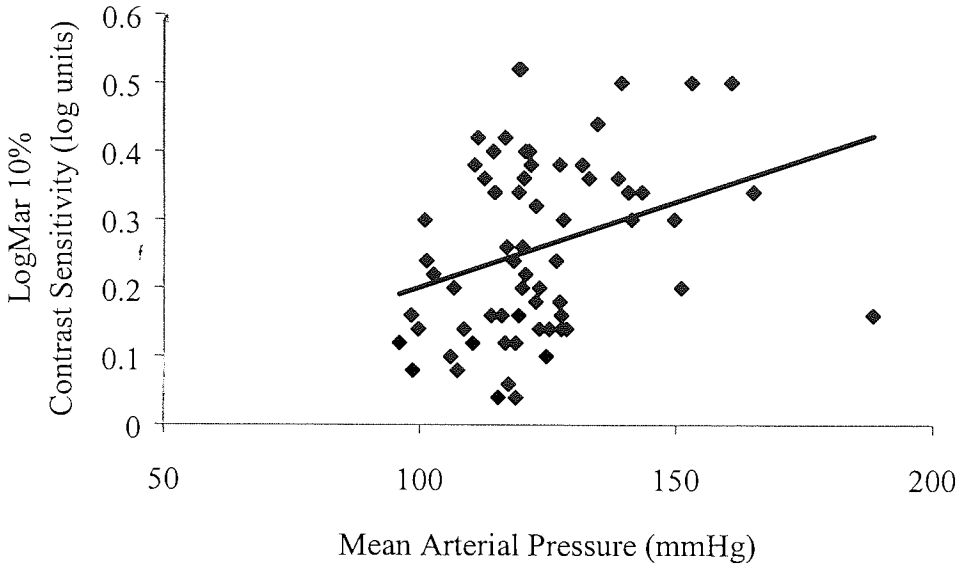

Figure 8.9. **Graph depicting trend between LogMar 10% contrast sensitivity and mean arterial pressure in the normal subjects. As the mean arterial pressure increased, contrast sensitivity significantly reduced ($p=0.01$, $r^2=0.10$).**

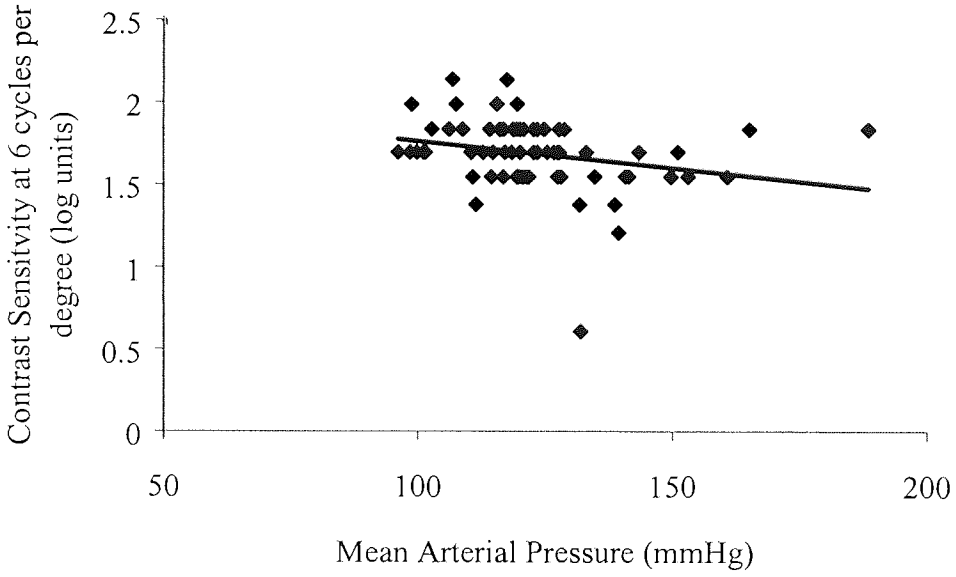

Figure 8.10. **Graph depicting trend between contrast sensitivity at 6 cycles per degree and mean arterial pressure in the normal subjects. As the mean arterial pressure increased, contrast sensitivity significantly reduced ($p=0.05$, $r^2=0.06$).**

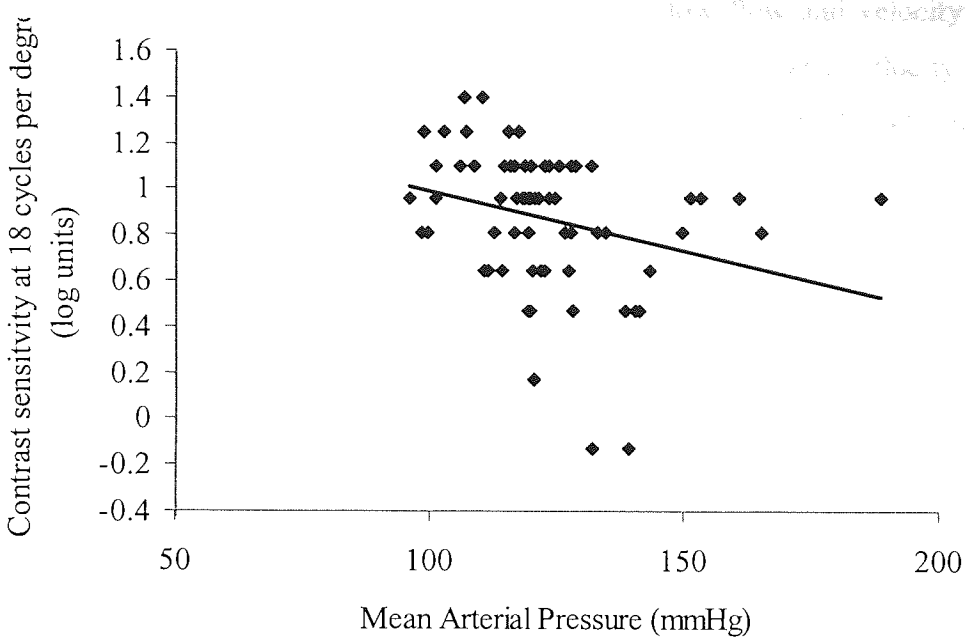

Figure 8.11. **Graph depicting trend between contrast sensitivity at 18 cycles per degree and mean arterial pressure in the normal subjects. As the mean arterial pressure increased, contrast sensitivity significantly reduced ($p=0.02$, $r^2=0.08$).**

8.7.10.2 Diabetic patients

Table 8.9 shows the r^2 and p-values for the correlations in the diabetic patient group. It was found that: -

1) MAP correlated inversely with LogMar visual acuity ($p=0.02$, $r^2=0.08$).
2) MAP correlated with the high values of blood volume ($p=0.01$, $r^2=0.13$).
3) MAP correlated with z-profile signal width ($p=0.02$, $r^2=0.09$).
4) Both high and low blood flow and velocity; and high blood volume correlate inversely with LogMar visual acuity (high flow $p=0.0003$, $r^2=0.22$; high volume $p=0.03$, $r^2=0.08$; high velocity $p=0.0006$, $r^2=0.20$; low flow and velocity $p=0.0003$, $r^2=0.22$).
5) Both high and low values of blood flow and velocity negatively correlate with SWAP mean deviation (high flow and velocity $p=0.04$, $r^2=0.07$; low flow and velocity $p=0.02$, $r^2=0.10$), LogMar 10% contrast sensitivity (high flow $p=0.004$, $r^2=0.14$; high velocity $p=0.008$, $r^2=0.12$; low flow and velocity $p=0.003$, $r^2=0.15$) and contrast sensitivity at 6 cycles per degree (high flow $p=0.02$, $r^2=0.10$; high velocity $p=0.02$, $r^2=0.08$; low flow and velocity $p=0.01$, $r^2=0.11$), 12 cycles per degree (high flow $p=0.005$,

r^2=0.13; high velocity p=0.008, r^2=0.12; low flow and velocity p=0.002, r^2=0.16) and 18 cycles per degree (high flow and velocity p=0.002, r^2=0.16; low flow and velocity p=0.001, r^2=0.18). At 3 cycles per degree only low values of blood flow and velocity were found to correlate with the reduction in contrast sensitivity (low flow p=0.04, r^2=0.07; low velocity p=0.03, r^2=0.07).

6) High values of blood flow and velocity and low values of blood flow, volume and velocity correlate with levels of glycosated haemoglobin (high flow p=0.002, r^2=0.20; high velocity p=0.001, r^2=0.24; low flow and velocity p=0.001, r^2=0.22; low volume p=0.02, r^2=0.12).

	Stats
MAP and LogMar visual acuity	r^2=0.08, p=0.02
MAP and high blood volume	r^2=0.13, p=0.01
MAP and z-profile signal width	r^2=0.09, p=0.02
High blood flow, volume and velocity and LogMar visual acuity	Flow r^2=0.22, p=0.0003; Volume r^2=0.08, p=0.03; Velocity r^2=0.20, p=0.0007
Low blood flow and velocity and LogMar visual acuity	r^2=0.22, p=0.0003
High blood flow and velocity and SWAP mean deviation	r^2=0.07, p=0.04
Low blood flow and velocity and SWAP mean deviation	r^2=0.10, p=0.02
High blood flow and velocity and LogMar 10% contrast sensitivity	Flow r^2=0.14, p=0.004; Velocity r^2=0.12, p=0.008
Low blood flow and velocity and LogMar 10% contrast sensitivity	r^2=0.15, p=0.003
Low blood flow and velocity and contrast sensitvity at 3 cycles per degree	Flow r^2=0.07, p=0.04; Velocity r^2=0.07, p=0.03
High blood flow and velocity and contrast sensitivity at 6 cycles per degree	Flow r^2=0.10, p=0.02, Velocity r^2=0.08, p=0.02
Low blood flow and velocity and contrast sensitivity at 6 cycles per degree	r^2=0.011, p=0.01
High blood flow and velocity and contrast sensitivity at 12 cycles per degree	Flow r^2=0.13, p=0.005; Velocity r^2=0.12, p=0.008
Low blood flow and velocity and contrast sensitivity at 12 cycles per degree	r^2=0.16, p=0.002
High blood flow and velocity and contrast sensitivity at 18 cycles per degree	r^2=0.16, p=0.002
Low blood flow and velocity and contrast sensitivity at 18 cycles per degree	r^2=0.18, p=0.001
High Blood flow and velocity and glycosated haemooglobin	Flow r^2=0.20, p=0.002; Velocity r^2=0.24, p=0.001
Low Blood flow, volume and velocity and glycosated haemooglobin	Flow r^2=0.22, p=0.001; Volume r^2=0.12, p=0.02; Velocity r^2=0.22, p=0.001

Table 8.10. **Table showing significant correlations evident in the diabetic patient group.**

8.8 Discussion:

Results from this study indicate that, following phacoemulsification there is no difference in the pattern of change in visual function, macula blood flow or macula topography over time in diabetic patients compared with normal subjects. Neither group exhibited any significant change in visual function, ocular blood flow or topographic parameters following phacoemulsification.

Correlation analysis revealed a significant negative trend between MAP and both LogMar 10% contrast sensitivity, and CSV-1000 contrast sensitivity at 6 and 18 cycles per degree in the normal subject group. These results indicate reductions in visual function correlate with increases in the MAP in normal subjects. Patients with systemic hypertension often complain of blurred vision (Bulpitt, Fletcher, Thijs, *et al.*, 1999), and our findings indicate sensitivites in the visual system at higher spatial frequencies to increases in MAP.

For the diabetic patients there was a significant negative relationship between MAP and LogMar visual acuity. Increases in the MAP also correlated with increases in measures of high blood volume and z-profile signal width. As blood flow measures increased an associated reduction in visual function was found (visual acuity, LogMar 10% contrast sensitivity, contrast sensitivity at 3, 6, 12 and 18 cycles per degree and SWAP mean deviation). Finally, increases in macula blood flow measures were found to correlate with increases in glycosated haemoglobin levels.

In this study there were seventeen diabetic patients, of which fourteen were non-insulin dependent and three were insulin dependent diabetics. Only four patients exhibited any evidence of diabetic retinopathy at any time and of these, only two (one insulin dependent and one non-insulin dependent) showed progression of the retinopathy following phacoemulsification.

It has been reported that cataract surgery can result in the progression of diabetic retinopathy with the development of new haemorrhages, exudates, and macula oedema and that this progression is associated with poor visual prognosis (Pollack,

Leiba, Bukelman, *et al.*, 1992a; Schatz, Atienza, McDonald, *et al.*, 1994), although this has not been a universal finding (Henricsson, *et al.*, 1996). The progression of retinopathy has been linked to the degree of glycaemic control before and after surgery, presence of retinopathy prior to surgery and duration of diabetes (Henricsson, *et al.*, 1996).

Visual function was assessed using logMar visual acuity at 90% and 10% contrast, the CSV-1000 contrast sensitivity unit and SWAP. Visual acuity was significantly better in the normal group compared to the diabetic group at 30 and 90 days post-operatively while contrast sensitivity was significantly better in the normal group at all post-operative visits. There was no difference between groups for the change in visual acuity or contrast sensitivity post-operatively. These results indicate that in diabetic patients the improvement in visual function following phacoemulsification does not reach the same level as that attained by normal, healthy subjects. This supports the findings of Sadiq, *et al.*, (1999) who reported visual function improvement in diabetic patients following cataract surgery but to a lesser degree than that observed in normal, healthy individuals. Dosso, *et al.*, (1996) investigated contrast sensitivity in phakic, aretinopathic diabetic patients and normal controls. The optical density of the crystalline lens was not different between groups but contrast sensitivity deficits were found in the diabetic group at 6, 15 and 27 cycles per degree, a finding in agreement with our study. It was suggested that contrast sensitivity might detect early changes in visual function, prior to the appearance of microvascular retinal damage (Dosso, *et al.*, 1996). A similar study conducted by Gartaganis, *et al.*, (2001) assessed contrast sensitivity in diabetic patients with no diabetic retinopathy and also found a reduction in contrast sensitivity at 3, 6, 12 and 18 cycles per degree. These deficits in contrast sensitivity have been reported in younger insulin dependent diabetic patients with and without retinopathy and were found to correlate with elevation in glycosated haemoglobin levels (North, Farrell, Banford, *et al.*, 1997). Since the reduction in contrast sensitivity was unable to differentiate between patients with and without retinopathy (North, *et al.*, 1997), the loss in visual function may reflect inferior glycaemic control rather than the presence of retinopathy.

SWAP revealed no differences between the two subject groups. Colour deficits have been observed in phakic diabetic patients (Kinnear, Aspinall & Lakowski, 1972).

Kessel, *et al.*, (1999) investigated colour vision in normal subjects and diabetic patients with varying degrees of diabetic retinopathy following cataract surgery and found no significant difference between the two groups. In addition, no correlation between the degree of diabetic retinopathy and the colour vision error score was found, a finding that is in agreement with those made by Kinnear *et al.*, (1972). Colour vision deficits in diabetic patients occur in the blue-yellow axis of the Farnsworth-Munsell 100 hue test and SWAP investigates the functional capabilities of the blue-yellow chromatic channel. Kessel, *et al.*, (1999) attributed the blue-yellow colour deficits observed in diabetic patients to the age-related yellowing of the crystalline lens that is known to be accelerated in diabetic patients (Bleeker, van Best, Virji, *et al.*, 1986). However, deficits in the blue-yellow chromatic channel have been observed under hypoxic conditions (Zisman & Adams, 1982), as would be the case in patients with clinically significant macula oedema. The absence of a difference between groups in the mean deviation of SWAP in our study suggests that SWAP is not sensitive in the detection of visual field decreases in pre-clinical maculopathy.

Measurements of macula blood flow, volume and velocity revealed no inter-group differences and no alterations in any parameters over time, as reported elsewhere (Arend, Wolf, Harris, *et al.*, 1995b). However, these results have been contradicted by other studies in which an increase in retinal blood flow has been reported in diabetic patients with background diabetic retinopathy (Cunha-vaz, Fonseca, De-Abreu, *et al.*, 1978; Kohner, 1993; Arend, Remky, Harris, *et al.*, 1995a) and in those with cystoid macula oedema (Arend, *et al.*, 1995a). Increases in retinal blood flow and reductions in volumetric flow have been found with advances in diabetic retinopathy and these alterations were found to correlate with increases in the blood vessel diameter (Grunwald, *et al.*, 1996). The absence of any blood flow differences between our two subject groups may be due to the majority of our diabetic patients being aretinopathic and may reflect maintenance of retinal blood flow in diabetic patients exhibiting good glycaemic control.

Topographic analysis of the macula was assessed post-operatively using the HRT to ascertain whether alterations in retinal z-profile signal width, an indicator of retinal thickness, occurred in the diabetic patients following phacoemulsification. Results indicated no difference in macula thickness between the two groups and no change in

retinal thickness over time in either of the groups. Grewing & Becker, (2000) utilized the optical coherence tomograph (OCT) to assess retinal thickness in normal subjects and similarly demonstrated no alteration in macula topography following phacoemulsification. A more recent study demonstrated increases in foveal thickness in diabetic patients with and without retinopathy using the OCT, although no differences were found in the adjacent areas in the patients without diabetic retinopathy or with non-proliferative diabetic retinopathy (Sanchez-Tocino, Alvarez-Vidal, Maldonado, *et al.*, 2002). The absence of any alterations in macula thickness found in our study suggests that none of the diabetic patients went on to develop any degree of diabetic macula oedema following cataract extraction; alternatively it could reflect insensitivities in this method of topographic analysis. More recently a new method of analysis using the de-convolution theory has been developed and may prove to be a more sensitive indicator for changes in retinal topography (Dr. N. Hutchings, Univeristy of Waterloo, Canada; personal communication and Hutchings, Flanagan, Hudson, *et al.*, 2000). Re-analysis of our data using this new technique may reveal more subtle differences in macula thickness.

In the normal subjects a significant negative correlation was found between measures of MAP and LogMar 10% contrast sensitivity and contrast sensitivity at 6 and 18 cycles per degree. In the diabetic patients as MAP increased measures of LogMar visual acuity reduced and z-profile signal width increased. Increases in measures of macula blood flow correlated with reductions in all measures of visual function. Finally, a significant positive correlation was evident in the diabetic patients between macula blood flow and glycosated haemoglobin levels. Measures of glycosated haemoglobin provide an indication of the degree of glycaemic control and the normal range is taken as being between 4.3 and 6.0 g/dl. Our findings indicate that as glycaemic control deteriorates, retinal blood flow increases and this is associated with a reduction in visual function. In addition, it would seem that mean arterial pressure influences visual function in both normal subjects and diabetic patients with increases in it resulting in a decrease in contrast sensitivity.

Cunha-vaz, *et al.*, (1978) and Kohner, (1993) measured retinal blood flow in diabetic patients and found that those with mild background retinopathy exhibited increased blood flow levels compared to normal controls. In this study there were no differences

in macula blood flow, volume and velocity between the two subject groups but the majority of the diabetic patients had no evidence of any diabetic retinopathy. Our results indicate that deteriorating glycaemic control, influences the development of diabetic retinopathy, and is associated with an increase in retinal blood flow, supporting the findings made by Cunha-vaz, *et al.*, (1978) and Kohner, (1993). In our study we found a signficant relationship between glycosated haemoglobin concentration and measures of macula blood flow; visual function was found to deteriorate with increases in blood flow. These findings are consistent with those made by Banford, North, Dolben *et al.*, (1994), who reported a significant negative correlation between levels of glycosated haemoglobin and contrast sensitvity at 6 and 12 cycles per degree. A possible explanation for the similarity in blood flow measures between groups in this study, and the absence of any macula involvement in the diabetic patients may be due to the maintenance of consistent glycosated haemoglobin levels over time. Although these levels were higher in the diabetic group they did not exceed levels thought to accelerate the development of retinopathy (Bonney, Hing, Fung, *et al.*, 1995).

8.9 Summary

In this study none of the diabetic patients went on to develop diabetic macula oedema following phacoemulsification. This explains why there were no differences between the groups in the way in which visual function, macula topography and macula blood flow changed over time. The absence of diabetic macula oedema in the diabetic group may be explained by the relatively small sample size used, with the majority of the diabetic patients having no evidence of diabetic retinopathy prior to phacoemulsification and adequate glycaemic control being maintained. The retinal status and glycaemic control are known to have a major influence on the development of diabetic retinopathy following cataract extraction (Henricsson, *et al.*, 1996). Finally, the follow-up period of three months was relatively short; a recent study found that the development of diabetic macula oedema following phacoemulsification occurred over three months post surgery (Flesner, Sander, Henning, *et al.*, 2002). Over a longer time frame and with alterations in glycosated haemoglobin and blood glucose levels, the diabetic patients may have gone on to develop clinically significant macula oedema.

CHAPTER 9:

Discussion

9.1 Influence of detector sensitivity on measures of neuroretinal rim and retinal capillary blood flow.

Factors affecting the reproducibility of perfusion images using the Heidelberg Retina Flowmeter (HRF; Heidelberg Engineering, Germany) were discussed in chapter 2.

This study demonstrated that for measures at the retina, HRF flow values are relatively stable until DC values exceed 190. These results are in agreement with the findings from a study conducted by Kagemann *et al.,* (2001), where measures of retinal blood flow using the HRF were artificially low when photodiode detector sensitivity was too high. In the neuroretinal rim, values of blood flow, volume and velocity were also significantly reduced when the photodiode sensitivity exceeded 190. Furthermore, this tissue type appeared to be more sensitive to alterations in photodiode sensitivity with all the neuroretinal rim parameters showing a peak blood flow value when the DC value was between 70 and 150. This latter finding was not statistically significant but it has implications for optimising the sensitivity and specificity in both cross-sectional and longitudinal studies as small alterations in photodiode sensitivity between images may limit the sensitivity of the HRF in detecting subtle differences in blood flow measures between groups.

In conclusion, we recommend that when acquiring HRF data for cross-sectional or longitudinal evaluation the DC value should be kept within a range of between 70-150 for both the retina and neuroretinal rim. This finding is in agreement with Michelson and Schmauss (1995) who recommend a DC value of between 80 and 150 and more recently by Kagemann *et al.,* (2001), who recommend neither too high or too low photodiode detector sensitivity settings when acquiring images using the HRF. For publication of this work see Appendix 4.1.

9.2 Application of a local search strategy to improve detection of blood flow deficits using the HRF.

In chapter 3 a new search strategy was employed to optimise detection of blood flow deficits at the neuroretinal rim and retina in patients with primary open angle glaucoma.

Using the standard (static) method to acquire measures of blood flow, no blood flow deficits were found in the glaucomatous patients at either the retina or neuroretinal rim. Using the search strategy, glaucoma patients exhibited significantly lower blood flow, volume and velocity in the neuroretinal rim when compared to age-matched normal healthy subjects. These differences in blood flow were more marked when the highest blood flow readings were used to compare the groups.

Our data suggests that blood flow deficits are more readily identified when a larger sample area is considered, or when account is taken of the local physiological variation in blood flow (e.g due to vascular pulsation). Our study suggests that this can be achieved using the standard software by adopting a local search strategy to identify the lowest or highest values of blood flow, volume and velocity. Our data further suggests that in glaucoma, a greater flow deficit is observed in the higher range of blood velocities, resulting in reduced mean velocity and volumetric flow. Publication of this work is shown in Appendix 4.2.

Further experimentation involving this analysis may aid identification of blood flow deficits in other diseases affecting the eye including diabetes and age related macula degeneration.

9.3 Consecutive verses non-consecutive intraocular pressure pulses in the analysis of ocular pulsatility.

In chapter 4 the repeatability of blood flow measures using the OBFA was investigated. The software selects and matches the five best independent pulses from a data sample to acquire measures of POBF. The purpose of this study was to determine

whether the repeatability of the OBFA could be improved by using consecutive IOP pulses (rather than those separated over time) to calculate measures of POBF, pulse volume and pulse amplitude.

The coefficient of variability for POBF was only slightly better for the consecutive method, suggesting a small improvement in the repeatability of ocular pulsatility measures when consecutive IOP pulses are captured. Conversely, the coefficient of variability was greater for measures of IOP and PA using the consecutive data set. This is unsurprising given the increased number of attempts required to obtain these pulses and may reflect a slight tonographic effect.

Bland and Altman analysis revealed a slightly higher mean of difference and 95% limits of agreement for the non-consecutive pulses between visits. This result indicates that for the pulses separated over time, more variability in measures of POBF exist and is substantiated with the ICC being slightly greater using the consecutive pulses.

As the heart contracts, blood enters the eye and IOP increases, this is followed by a reduction in IOP as the heart relaxes and outflow of blood ensues. This repetitive cycle results in the formation of the IOP wave, which alters as a function of heart rate. If POBF was influenced by small fluctuations in IOP, as seen with the respiratory cycle, then one might expect repeatability to be compromised when non-consecutive pulses are used to calculate blood flow due to the larger standard deviations induced.

The results from this study suggest ocular blood flow measurements using the OBFA are repeatable using both consecutive and non-consecutive intraocular waveforms; however, the repeatability can be improved by using consecutive pulses to calculate measures of POBF.

9.4 Spectral analysis of the intraocular pressure pulse

In chapter 5 the periodicity of the intraocular pressure pulse was investigated in glaucomatous patients and normal, healthy subjects. This work has been accepted for publication and is currently *in-press* (Appendix 4.4).

The intraocular pressure pulse was analysed in the frequency domain and its spectral components determined up to the fourth harmonic in all the subjects and patients. Results indicated that untreated glaucoma patients demonstrate lower power in the 2nd, 3rd and 4th harmonics of their intraocular pressure pulse when analysed in the frequency domain when compared to normal subjects. Conversely standard measures of ocular pulsatility were not significantly different between groups; this may be due to the normal subjects and glaucoma patients being matched for systemic variables including pulse rate, which are known to have a major influence on POBF (Yang, *et al.*, 1997).

The higher harmonics in the arterial pressure pulse can be used as a measure of vascular impedance, which is closely related to vascular resistance (Nichols & O'Rourke, 1998). Vascular impedance is defined as the resistance to flow in an oscillatory system, while resistance is confined to non-oscillatory motions (Nichols & O'Rourke, 1998). Colour Doppler ultrasound investigations have shown glaucoma patients exhibit increased resistivity indices in the ophthalmic artery (Harris, *et al.*, 1994b), central retinal artery (Evans, Harris, Chung, *et al.*, 1999b) and short posterior ciliary arteries (Evans, *et al.*, 1999a). It is therefore possible that the reduced harmonics found in this study are associated with alterations in the vascular status of glaucoma patients. It should be noted, however, that the pulsation under investigation is dependent upon a number of different factors including the dimensions and elasticity of the eye (Silver & Geyer, 2000) and therefore, the differences found here, may be due to one or more of these factors, such as a difference in scleral rigidity.

Fourier analysis of OBFA readings using the non-standard technique can be used to discern the higher frequency components of the intraocular pressure pulse wave, up to and including the fourth harmonic. The differences found between the groups in the harmonic amplitudes suggest that some component of the intraocular pressure pulse

varies in glaucoma. Calculation of the harmonics of the IOP pulse may be an additional parameter, possibly more sensitive than standard measures of ocular pulsatility, that enables identification of patients suffering from diseases with a vascular aetiology.

Further work is required to determine the exact meaning of the higher harmonics of the intraocular pressure wave and their relation to the vasculature of the intraocular blood vessels. With greater understanding, analysis of the intraocular pulse may aid diagnosis of diseases affecting the eye that have a vascular aetiology.

9.5 Effects of age on ocular pulsatility.

In chapter 6 symmetry of systemic and ocular blood measures and the effects age has on these were investigated. Results from this study showed that no asymmetry between the right and left sides exists in any of the systemic or ocular variables measured.

Differences in systemic blood pressure have been found between the right and left sides of the body (Frank, *et al.*, 1993; Singer & Hollander, 1996; Cassidy & Jones, 2001), with some authors reporting higher values in the right arm (Cassidy & Jones, 2001) and others finding no consistency in the side demonstrating the higher blood pressure (Frank, *et al.*, 1993; Singer & Hollander, 1996). In this study no interarm differences in systolic or diastolic blood pressure, MAP or systemic pulse amplitude were found. This finding may be due to our strict inclusion criteria and all our subjects being free from vascular disease since greater interarm differences in blood pressure measures have been found in patients with systemic vascular disease (Frank, *et al.*, 1993; Singer & Hollander, 1996).

Our finding of interocular symmetry in ocular blood flow measures is consistent with those reported by Greenfield, *et al.*, (1995) in retrobulbar blood flow and Rawji & Flanagan, (2001) in measures of retinal capillary blood flow.

Age had no effect on measures of ocular pulsatility in our cohort of normal, healthy individuals. This contradicts the findings of Ravalico, *et al.*, (1996) who reported

significant reductions in ocular blood flow with increasing age using the OBFA. Ravalico, *et al.*, (1996) recruited a group of emmetropic patients in an attempt to eliminate any blood flow reductions caused by increases in axial length (Massey, *et al.*, 1999; Mori, *et al.*, 2001). However, hypermetropia is known to increase with advancing age due to alterations in the refractive indices of the ocular media (Bennett & Rabetts, 1989; Attebo & Ivers, 1999). This would suggest that previously myopic individuals become more emmetropic with increases in age. Therefore the subject sample utilised by Ravalico, *et al.*, (1996) may have demonstrated increases in axial length (and eye volume) with age, and increases in axial length are known to be associated with reduced POBF (Mori, *et al.*, 2001).

From the results found is this study we suggest that blood flow measures using the OBFA demonstrate interocular symmetry and remain stable throughout life. Significant increases in diastolic blood pressure and MAP were found with advancing age and these were accompanied by a significant elevation in systemic pulse amplitude, which in turn, positively correlated with ocular pulse amplitude. The ocular pulse amplitude is an indirect measure of choroidal capacity and elevations in it indicate increases in pulse volume and higher choroidal perfusion (Mittag, Serle, Schumer, *et al.*, 1994). The correlation between systemic and ocular pulse amplitude, suggests that increases in the systemic pulse amplitude result in a corresponding increase to the ocular pulse amplitude. As such, the physiological reductions in vascular compliance due to age may be counteracted by increases in the ocular pulse amplitude.

Pearson's product correlation analysis was used to investigate the relationship between all the measured parameters. The r^2 value describes the linear relationship between variables. Diastolic blood pressure, MAP and systemic pulse amplitude significantly increased with advancing age, and the corresponding r^2 values were 0.031, 0.095 and 0.035 respectively. This means that 3.1% of the increase in diastolic blood pressure is explained by age. The remaining 96.9% is due to other factors and these are likely to include weight and height of the subjects; documentation of these at the time of the study may have provided stronger correlations.

Conflicting arguments exist on the effects of age on POBF, this study suggests maintenance throughout life but is not in agreement with the findings made by Ravalico, *et al.*, (1997). Clarification could be made by conducting a similar study with the inclusion of axial length measurement using A-scan ultrasonography. The results from such a study would determine whether the age-related reduction in POBF observed by Ravalico, *et al.*, (1997) was indeed due to reductions in vascular compliance or caused by the inclusion of smaller eyes in their older subject cohort.

9.6 Effect of age on intraocular capillary perfusion.

In chapter 7 the effect of increasing age on blood flow, volume and velocity in the retina, neuroretinal rim and lamina cribrosa was investigated. The results indicated a decrease in retinal, neuroretinal rim and lamina cribrosa blood flow with advancing age. This finding is in agreement with those made in previous investigations (Groh, *et al.*, 1996; Dallinger, *et al.*, 1998; Grunwald, *et al.*, 1998).

A comparison of group-mean values identified significant reductions in blood volume measured at the retina and in blood flow and velocity measured at the neuroretinal rim and lamina cribrosa. Regression analysis revealed a significant negative correlation between advancing age and retinal blood volume, neuroretinal rim blood velocity and lamina cribrosa blood volume.

A number of morphological changes occur with advancing age, such as reductions in the number of retinal ganglion cells and their axons (Jonas, *et al.*, 1989; Gao & Hollyfield, 1992; Jonas, *et al.*, 1992). This depletion in nerve fibre numbers may result in a reduced requirement for ocular perfusion, or alternatively reduced perfusion may itself cause this depletion in the number of retinal ganglion cell axons. Blood flow measurements may fall due to increases in vascular resistance following changes in the structure of capillary vessels with age (Lee, *et al.*, 1987). It is known that with age the incidence of atherosclerosis increases, which reduces arterial distensibility (Wadsworth, 1990). Alternatively, the blood flow reductions observed might be due to loss of autoregulatory processes (Hayreh, *et al.*, 1994b) that are strongly influenced by the endothelium, which changes with increasing age (Wei, 1992). The results from chapter 6 showed that POBF remained unchanged with advancing age contradicting

the findings of reduced capillary blood flow measures reported in this chapter. It is possible that the retinal microcirculation is more sensitive to the histological and autoregulatory alterations that occur with age, thus reductions in blood flow in the larger vessels may only become apparent with further deteriorations in the vascular system.

It is likely that multiple factors result in the observed reduction in capillary blood flow with increasing age, and this reduction may be of significance in the aetiology of some age-related eye diseases, or indeed be exacerbated by them. Publication of this data is in Appendix 4.3.

9.7 Ocular perfusion in the macula of diabetic patients following cataract surgery.

In chapter 8 the effects of phacoemulsification on retinopathy, macula topography, macula blood flow and visual function in diabetic patients was investigated.

The results indicated that following phacoemulsification there is no difference in the change in visual function, macula blood flow or macula topography over time in diabetic patients exhibiting good glycaemic control compared with normal subjects.

Only four of the patients exhibited any evidence of diabetic retinopathy and, of these, one insulin-dependent and one non-insulin dependent diabetic showed progression following phacoemulsification. It has been reported that cataract surgery can result in the progression of diabetic retinopathy (Pollack, *et al.*, 1992a; Schatz, *et al.*, 1994). Furthermore, the progression of diabetic retinopathy has been related to the degree of glycaemic control before and after surgery, the presence of retinopathy prior to surgery, the duration of diabetes, and the insulin treatment (Henricsson, *et al.*, 1996).

Visual acuity was significantly better in the normal group compared to the diabetic group at 30 and 90 days post-operatively, while contrast sensitivity was significantly better in the normal group at all post-operative visits. These results indicate that, in diabetic patients, the improvement in visual function following phacoemulsification does not reach the same level as that attained by normal healthy subjects, and supports

the findings made previously (Sadiq, *et al.*, 1999; Dosso, *et al.*, 1996; Gartaganis, *et al.*, 2001). It has been suggested that reductions in contrast sensitivity reflect deterioration of visual function prior to the development of microvascular damage in the eye (Dosso, *et al.*, 1996). More recently, contrast sensitivity deficits have been reported in young insulin-dependent diabetic patients. No relationship between the severity of retinopathy and visual function reduction was found, however; a negative correlation was evident with glycosated haemoglobin levels (North, *et al.*, 1997). These results suggest that contrast sensitivity can differentiate diabetic patients from normals, but cannot indicate the degree of diabetic retinopathy present. It is possible that the reduction in contrast sensitivity observed in diabetic patients may be caused by insufficiencies in glycaemic control rather than presence of retinopathy.

Measurements of macula topography were not different between the normal subjects and diabetic patients, which contradict the findings of Sanchez-Tocino, *et al.*, (2002). The absence of any topographic discrepancies between groups may be due to insensitivities in the calculation of the z-profile signal width. Since completion of this study the data has been sent to the University of Waterloo, Canada, for deconvolution analysis to be carried out. This type of analysis is thought to be more sensitive in identifying alterations in retinal thickness and may reveal more subtle differences between the groups.

A significant negative trend was evident between MAP, and both LogMar 10% contrast sensitivity and CSV-1000 contrast sensitivity at 6 and 18 cycles per degree in the normal subject group. These findings indicate sensitivities in the visual system at higher spatial frequencies, although only 10% of the reduction in contrast sensitivity could be accounted for by increased MAP ($r^2 = 0.1$, 0.06 and 0.08 respectively).

Correlation analysis in the diabetic patient cohort revealed significant negative trends between HRF measures and visual function. This was coupled with a positive correlation between macula capillary perfusion and glycosated haemoglobin levels. Measures of glycosated haemoglobin provide an indication of the degree of glycaemic control and these results suggest that as glycaemic control deteriorates, retinal blood flow increases, and increases in retinal perfusion was associated with a reduction in visual function.

Increases in retinal blood flow have been reported in diabetic patients with background diabetic retinopathy (Cunha-vaz, *et al.*, 1978; Kohner, 1993; Arend, *et al.*, 1995a) and in those with clinically significant diabetic macula oedema (Arend, *et al.*, 1995a). In this study there were no differences in measures of macula blood flow, volume and velocity between the two groups, and this could reflect maintenance of glycaemic control since no diabetic retinopathy was evident in the majority of patients. With deterioration of glycaemic control, diabetic retinopathy develops, which is in turn associated with an increase in retinal blood flow (Cunha-vaz, *et al.*, 1978; Kohner, 1993). In this study a significant relationship existed between glycosated haemoglobin concentration and measures of macula blood flow; in addition, visual function was found to deteriorate with increased blood flow. These findings are consistent with those made previously where a significant negative correlation was found between levels of glycosated haemoglobin and contrast sensitivity (Banford *et al.*, 1994). The similarity in blood flow measures found in the normal subjects and diabetic patients may be accounted for by maintenance of glycosated haemoglobin levels over time; which, although higher in the diseased group, did not exceed levels thought to accelerate the development of retinopathy (Bonney, *et al.*, 1995).

If the follow-up period of these patients had been extended, alterations in blood flow, topography and visual function may have been observed. In a very recent study, the development of diabetic macula oedema post-phacoemulsification was identified more than three months post-operatively (Flesner, *et al.*, 2002), and this was attributed to surgery. Arguably, findings from this thesis, suggests that progression in diabetic retinopathy is largely due to a deterioration in glycaemic control, and as such the importance of this should be emphasised to patients prior to surgery.

9.8 Conclusions

The aims of this work were three-fold:-

Firstly, the acquisition protocols of the HRF and OBFA were investigated in order to optimise them for both cross-sectional and longitudinal application. The findings were: -

1) When acquiring perfusion maps using the HRF the DC value should be kept within a range of between 70-150 for both the retina and neuroretinal rim.
2) Blood flow deficits are more readily identified when a larger sample area is considered. This can be achieved using the standard software using a local search strategy to identify the lowest or highest values of blood flow, volume and velocity.
3) Repeatability of ocular blood flow measures using the OBFA can be improved with the use of consecutive pulses rather than those separated over time.
4) Fourier analysis of the IOP pulse identifies the higher frequency components up to and including the fourth harmonic and these may reflect the vascular status of the ocular vessels.

In the second part of this work physiological variations in ocular perfusion were investigated. The results from these studies show: -

1) POBF remains stable throughout life and exhibits interocular symmetry in healthy subjects devoid of vascular disease.
2) The ocular microvasculature is more susceptible to autoregulatory and anatomical changes that occur with age and this results in a reduction of retinal capillary perfusion with advancing age.

Lastly, the ocular perfusion characteristics of patients diagnosed with ocular diseases considered to be of a vascular origin were explored. The following observations were made: -

1) Glaucoma patients have significantly lower blood flow, volume and velocity in the neuroretinal rim when compared to age-matched normal subjects.
2) Untreated glaucoma patients demonstrate lower power in the 2^{nd}, 3^{rd} and 4^{th} harmonics of their intraocular pressure pulse when analysed in the frequency domain when compared to normal subjects.
3) Diabetic patients exhibit maintenance of macula blood flow, topography and visual function following phacoemulsification, when glycaemic levels are controlled adequately.

APPENDICES

Appendix 1:

Description of HRT parameters (Software Version 2.01)

PARAMETER	DESCRIPTION
Disc Area (mm^2)	Total area within the contour line
Cup Area (mm^2)	Area below the reference plane
Cup: Disc Area Ratio	Cup area: disc area
Rim area (mm^2)	Area above reference plane
Height Variation Contour (mm)	Height variation of the retinal surface along the contour line. It is the difference between the most elevated and most depressed points of the contour line.
Cup Volume (mm^3)	Volume below the reference plane
Rim Volume (mm^3)	Volume above reference plane
Mean cup depth (mm)	Mean depth inside the contour relative to the curved surface
Maximum Cup depth (mm)	Maximum depth inside the contour relative to the curved surface
Cup shape measure	Measure for the three-dimensional shape of the cup
Mean RNFL thickness (mm)	Mean distance between the retinal surface along the contour line and the reference plane
RNFL cross sectional area (mm^2)	Mean distance between the retinal surface along the contour line and the reference plane, multiplied by the length of the contour line.
Effective Area (mm^2)	Area within the contour line and below the curved surface
Area below reference (mm^2)	Area within the contour line and below the reference plane
Mean radius (mm)	Mean radius of the contour line
Mean height of contour (mm)	Mean height (z-position) of the retinal surface along the contour line
Volume below reference (mm^3)	Volume within the contour line and below the reference plane
Volume above reference (mm^3)	Volume within the contour line and above the reference plane
Volume below surface (mm^3)	Volume within the contour line and below the curved surface
Volume above surface (mm^3)	Volume within the contour line and above the curved surface
Mean depth in contour (mm)	Mean depth within the contour line

Effective mean depth (mm)	Mean depth within the contour line and below the curved surface
Maximum depth in contour (mm)	Maximum depth within the contour line
Third moment in contour	Third central moment of the frequency distribution of the depth values within the contour line and below the curved surface
Mean variability (mm)	Mean standard deviation of the height measurements
Centre x/y/z (mm)	x-, y-, z-coordinates of the centre of gravity of the surface within the contour line

Appendix 2:

Mathematical correction of ocular blood flow for anterior corneal curvature and refractive error.

In order to determine a refractive error and anterior corneal curvature corrected measure of POBF the original POBF value had to be corrected for pulse volume using an approximation based on these measures. In this way:-

CORRECTED POBF = original POBF x (pulse volume/6200) **Equation A.2.1**

Where: 6200 = ocular volume constant assumed by OBFA, where

PULSE VOLUME = $\{V(-8.03 \times 10^{-3} + 4.87 \times 10^{-3}\ \text{lnIOPmax} + 3.90 \times 10^{-5}\text{IOPmax}) - V(-8.03 \times 10^{-3} + 4.87 \times 10^{-3}\ \text{lnIOPmin} + 3.90 \times 10^{-5}\ \text{IOPmin})\}$

(Silver & Geyer, 2000)

Equation A.2.2

Where,

V= eye volume= anterior-posterior AL x transverse AL x vertical AL x $\Pi/6$

(Silver & Geyer, 2000)

And an approximation for axial length was derived from: -

Axial length (AL) = anterior chamber depth (ACD) + posterior chamber depth (k')

(Garway-Heath, Rudnicka, Lowe, *et al.*, 1998)

Equation A.2.3

Where:

ACD = constant = 1.6mm

k' = 1/K'

And:

K' = 1.336 / (Refractive power of the eye + Ametropia)

Where: Refractive power of the eye = (300.3 / k)+ crystalline lens power

Where: crystalline lens power = 21.76

Appendix 3:

Interpolation of 10-2 SWAP normal values from 30-2 SWAP empirical data

A linear interpolation procedure was developed to enable calculation of normal SWAP sensitivity values for all the points presented in the 10-2 SWAP programe. This was determined from a database consisting of 51 age-stratified normal subjects.

A linear surface ($z= ax + bx + cy$) was fitted to stimulus locations of known sensitivity corresponding to the 30-2 spatial grid. The visual field sensitivity at the fovea and those locations that coincide with those presented in the 30-2 and 10-2 programmes were incorporated into the 10-2 normal database.

To obtain sensitivities of the remaining locations on the 10-2 grid the following methodology was employed: -

Consider a spatial grid: -

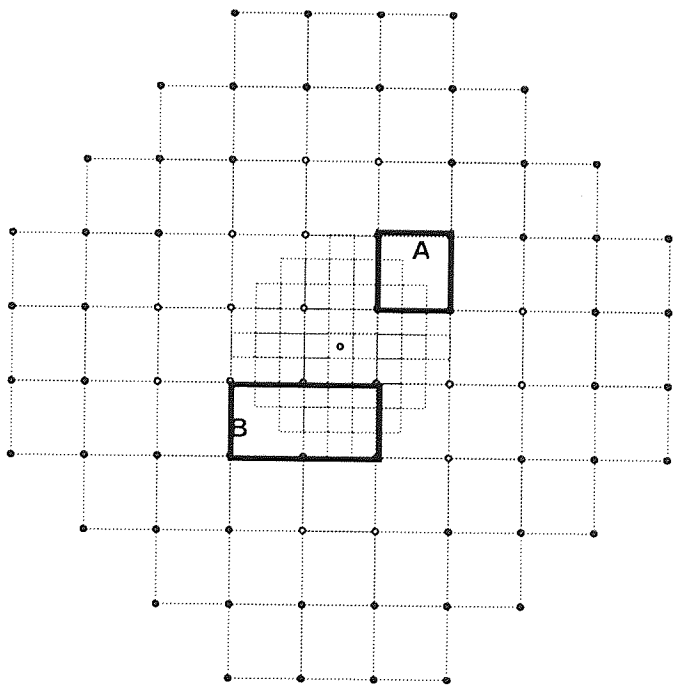

Figure A.3.1: Spatial grid

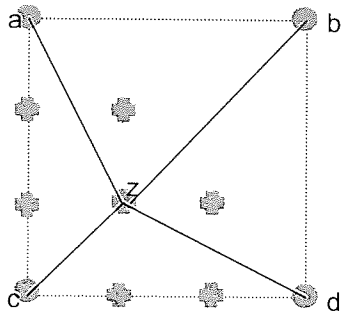

Figure A.3.2: Area A from figure 1.

Figure A.3.2 shows the stimulus locations a, b, c, d, which are existing 30-2 locations of known sensitivity. In figure 2 z' is a stimulus location of unknown sensitivity within the 10-2 grid.

For each subject, a linear surface was fitted to the spatial grid abcd:-

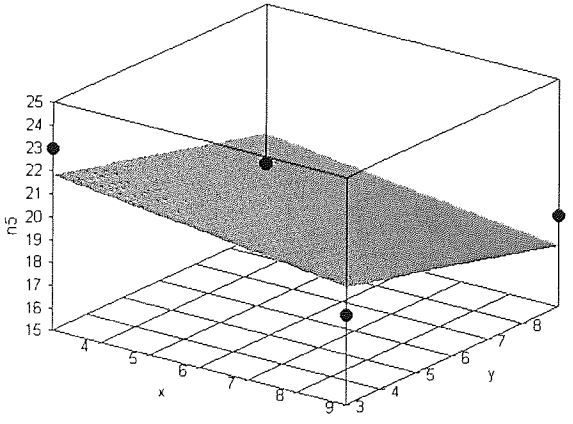

Figure A.3.3: Four-point interpolation figure. z= ax + bx +cy.

Substituting into the equation of the resulting surface yielded the interpolated sensitivity of the 10-2 stimulus locations.

If we now consider area B shown in figure A.3.1 and enlarged below:

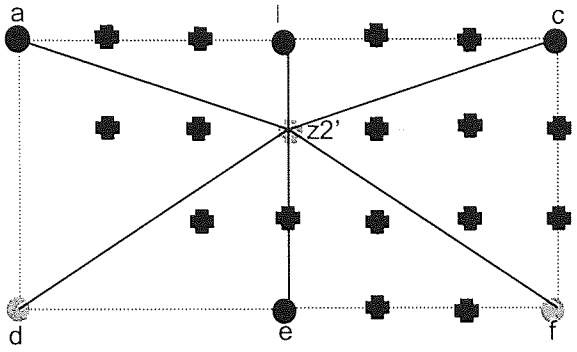

Figure A.3.4: Area B enlarged from figure 1.

Point z2' is closest to points c and d and points a, b, e and f are at equal distances from it. Therefore for such stimulus locations in the 10-2 spatial grid a six-point linear interpolation procedure was used.

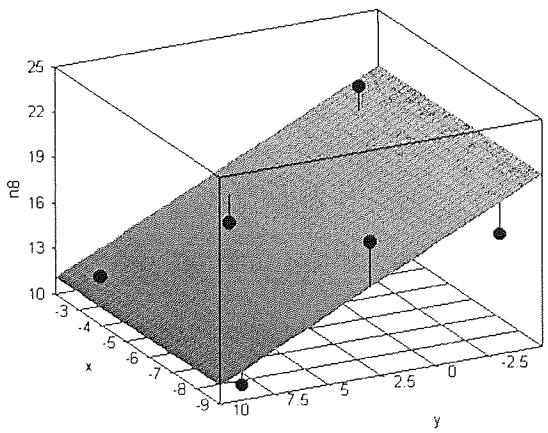

Figure A.3.5: Six-point interpolation diagram.

On completion of the 10-2 interpolation procedure for each subject, the intercept and gradient of the univariate linear regression of sensitivity with age was calculated for each of the 10-2 spatial grid locations. Substituting into the equation, $y = a + bx$, the sensitivity at each location as a functions of year (b = 15 to 95) was calculated and an extrapolated normal database for the 10-2 programe was derived.

Using these values it is possible to calculate the global perimetric indices: mean deviation, pattern standard deviation and short-term fluctuation. Furthermore the total deviation map can be calculated by subtracting the normal sensitivity from the measured sensitivity at each stimulus location.

Appendix 4:

Publications

A.4.1

Hosking, S.L.; Embleton, S.J.; Kagemann, L.; Chabra, A.; Jonecu-Cuypers, C.; Harris, A. (2001)

Detector sensitivity influences blood flow sampling in scanning laser Doppler flowmetry. *Grefes Archives of Clinical and Experimental Opthalmology.* 239. 407-410

A.4.2

Hosking, S.L.; Embleton, S.J.; Cunliffe, I.A. (2001)

Application of a local search strategy improves the detection of blood flow deficits in the neuroretinal rim of glaucoma patients using scanning laser Doppler flowmetry. *British Journal of Ophthalmology.* 85. 1298-1302

A.4.3

Embleton, S.J.; Hosking, S.L; Roff Hilton, E.J.; Cunliffe, I.A. (2002)

Effect of senescence on ocular blood in the retina, neuroretinal rim and lamina cribrosa, using scanning laser Doppler flowmetry. *Eye.* 16. 156-162

A.4.4

Evans, D.W.; Hosking, S.L.; Embleton, S.J.; Morgan, A.J.; Bartlett, J.D. (2002)

Spectral content of the intraocular pressure pulse wave: glaucoma patients versus normal subjects. *Grefes Archives of Clinical and Experimental Opthalmology. In press.*

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Abstract Purpose: To establish the effect of photodiode sensitivity on the DC (brightness) value and the resultant blood flow measurements of retina and rim tissue using a scanning laser Doppler flowmeter (SLDF). *Methods*: The sample consisted of one eye of each of 15 healthy subjects (mean age 27.8 ± 6.1 years). Using the Heidelberg Retina Flowmeter (HRF), three 10-deg images of the superior temporal retina and three further images of the superior temporal rim were acquired for each of five DC bands: band 1: 30–70; band 2: 70–110; band 3: 110–150; band 4 150–190; band 5: 190–230. Retinal blood volume, flow and velocity were determined for each image using a 10×10 pixel square grid located at a predetermined location on the retina and rim for each subject. Following image acquisition, the DC values corresponding to each pre-assigned retinal or rim location were determined. The mean and standard deviation were determined for the blood flow parameters within each DC band for each subject in both locations. Analysis of variance was used to identify significant change in the data as a function of the DC value ($P<0.05$). *Results*: Analysis of variance revealed that retinal blood flow measures acquired within DC band 5 resulted in significantly lower measures of blood flow and velocity ($P=0.035$ and $P=0.049$ respectively) than at lower DC values. Band 5 values of flow, volume and velocity in the neuroretinal rim were also significantly low ($P=0.016$, $P= 0.003$ and $P=0.026$ respectively). Peak neuroretinal rim blood flow was recorded when the DC value was between 70 and 110. For blood flow measurement at the retina and neuroretinal rim the DC value should not exceed 190. *Conclusion*: Photodiode sensitivity as indicated by the DC value affects measurements of ocular blood flow using the HRF.

Introduction

The scanning laser Doppler flowmeter (SLDF) is used for the non-invasive in vivo assessment of ocular blood flow, volume and velocity in the microvasculature of the retina and optic nerve head. The combination of laser scanning ophthalmoscopy and laser Doppler flowmetry offered by the Heidelberg Retina Flowmeter (HRF, Heidelberg Engineering, Heidelberg, Germany) enables two-dimensional mapping of fundus perfusion.

Blood flow measures obtained using the HRF yield valid and reproducible measurements of retinal blood flow [8, 9, 10, 12]. Application of the HRF has resulted in findings of reduced ocular blood flow in ocular diseases such as branch retinal vein occlusion [1] and glaucoma [3, 9, 10, 11, 13, 6]. However, in diabetes, while ocular blood flow has been shown to be reduced when using other techniques [4, 5, 14], investigations using the HRF revealed no significant differences in retinal blood flow [4].

Ocular blood flow values measured by HRF are affected by a number of optical and physiological factors such as incidence luminance and tear film stability. In an in vitro study, Tsang et al. [15] showed that darker backgrounds resulted in higher measured blood flow velocities. This finding suggests that HRF blood flow values may be altered by image brightness.

In the HRF, the sensitivity of the photodiode detector can be altered using a control panel. This results in images of varying brightness, a measure of which is derived from the detector current recorded, or DC value. This value can be determined within any selected window in the processed blood flow map. Images of acceptable quality may be obtained for DC values ranging from 30 to 230; at a DC of 30 images appear dull, whilst at a DC of 230 they are very bright, or even saturated. The purpose of our study was to investigate the effect of changing photodiode sensitivity on blood flow measurements obtained using the HRF.

Materials and methods

The HRF employs the optical Doppler effect to measure the number and rate of movement of red blood cells. The Doppler shift refers to the change in frequency of waves reflected from a moving object [13] (in this instance the red blood cells) and is proportional to the velocity of the moving object relative to the speed of the incident wave. The HRF emits light from an infrared laser (780 nm) directed onto the retina, where it is reflected from or scattered by moving red blood cells, resulting in a shift in frequency [8, 16]. Interference between the laser light reflected without frequency shift from stationary targets (in this case the blood vessel wall) and the laser light reflected from the moving target (in this case the blood corpuscles) results in a beat frequency. This beat frequency is in direct proportion to the velocity of the moving target, or blood cell, and can be measured using the photodiode detector. Multiple measurements at each pixel location facilitate the derivation of both the number and velocity of moving corpuscles over a fixed period of time. The total blood flow is the product of the number and rate of corpuscles flowing past each pixel location as a function of time.

Sample

The sample consisted of one eye of each of 15 normal subjects, eight females and seven males, with a mean age of 27.8±6.14 years (range 20–37 years).

Subjects were included if they had a visual acuity of 6/9 or better in each eye with a refractive error (mean sphere) of less than 8 dioptres. Intraocular pressures were below 22 mmHg in each eye, ocular examination confirmed normal optic nerve head appearance, and anterior chamber angles were open. There was no history of ocular trauma or surgery, no diabetes mellitus or hypertension, no family history of glaucoma and no systemic or ocular medication that may affect blood flow.

One eye was randomly selected from each patient and three HRF images were taken of the superior temporal retina. Three additional images were acquired of the superior temporal rim. In each case images were acquired in five different DC bands, resulting in three images within each band for each fundus location. The sensitivity of the HRF was altered manually on the control panel in order to acquire images with different DC values. The superior temporal area of the optic nerve head and retina was chosen for data collection as it has been shown that measurements taken here exhibit the least variability [2]; thus, variability due to DC changes will be more clearly manifested. The minimum DC value used was 30 and the maximum 230, representing the darkest to brightest range of values that provide visible microvasculature. Five DC bands were used; 30–70 (band 1), 70–110 (band 2), 110–150 (band 3), 150–190 (band 4) and 190–230 (band 5).

Fast Fourier transformation was used to derive perfusion images. Blood flow, volume and velocity (arbitrary units) were determined for each image using the 10×10 pixel square grid located at a predetermined position on the retina and rim of each subject. The corresponding DC values were measured within the pixel frames for each subject in both locations within each DC band.

Statistical analysis

The mean and standard deviation of each blood flow parameter at both locations was determined for each eye. Group-mean blood flow measures and the group-mean standard deviation were determined for the retina and rim locations for each blood flow parameter. Repeated-measures analysis of variance (ANOVA) was used to test for significance at the 95% level, with DC value as the dependent variable. Post hoc analysis was used to identify outliers.

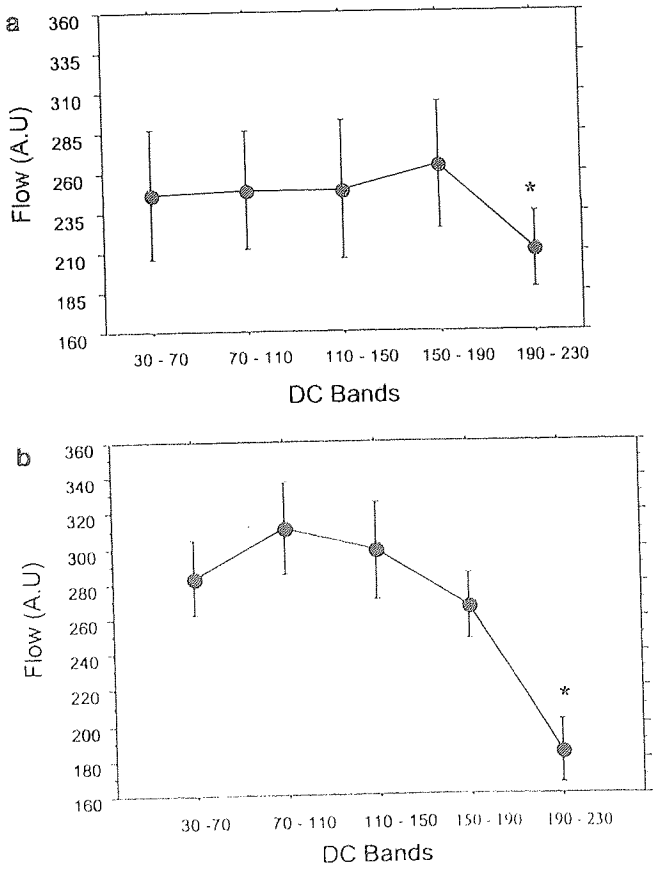

Fig. 1a, b Mean blood flow values for each DC band measured at a the retina and b the rim

Results

Analysis of variance revealed significant alterations to retinal flow and velocity (P=0.035 and 0.049 respectively) and rim flow, volume and velocity measures (P=0.016, 0.003, 0.026 respectively) with changing DC levels. Blood flow measures for different sensitivity settings are shown for the retina (Fig. 1a) and neuroretinal rim (Fig. 1b). Outliers were identified in each case by post hoc analysis at DC band 5 and are indicated on the graphs by an asterisk.

Discussion

A linear increase in the DC value was achieved for the images acquired in both the retina and rim tissues. It is noteworthy that images in which the rim tissue has a DC value of, for example 70, would have to be much brighter than images with the same DC value in the retina. For this reason, images of the retina and rim were collected entirely separately.

Measured values of retinal blood flow and velocity were significantly lower in band 5 than in other DC bands. This is illustrated for retinal blood flow in Fig. 1a, in which it can be seen that measured values remain relatively stable throughout DC bands 1–4, with a decrease in values in DC band 5. Of all the HRF haemodynamic parameters, retinal blood volume was not significantly altered by changes in photodiode sensitivity, although there was a trend for blood volume values to decrease in DC band 5. These results indicate that for measures at the retina, HRF flow values are relatively stable until DC values exceed 190.

In the neuroretinal rim, values of blood flow, volume and velocity were also significantly reduced in DC band 5 compared to values recorded in all other DC bands (P<0.05). All parameters showed a peak blood flow value in DC bands 2 and 3 (DC 70–150), as shown in Fig. 1b for the blood flow values in the neuro-retinal rim. Although this latter difference was not statistically significant, it has implications for optimising the sensitivity and specificity in studies of a cross-sectional or longitudinal nature, in that it may mimic or mask subtle differences between groups or subtle change, and should be considered when acquiring and analysing data.

Changes to the measured DC values arise predominantly from alterations in photodiode detector sensitivity. It is unclear why measured blood flow values in healthy tissue should differ with DC value in this way. It is possible that there is a sampling effect such that changes in detector sensitivity result in altered sensitivity to blood corpuscles moving with different velocities. A bias towards low-velocity particles, for example, would result in both the number and mean rate of measured corpuscles recorded being lower, resulting in reduced blood flow, volume and velocity as seen in the data from DC band 5 in this study. Conversely, increased sensitivity to rapidly moving particles may explain the slight peak in measured flow seen in band 2 for measures at the retina (Fig. 1a).

Previous studies have shown blood flow differences between normal volunteers and glaucoma patients [9, 11] and between ocular hypertensives and glaucoma patients [6]. Michelson and associates [9] investigated blood flow at the peripapillary retina and neuroretinal rim in patients with primary open-angle glaucoma. In the retina, compared to normal subjects significant reductions in measured blood flow, volume and velocity were found in the order of 226 ± 119, 13.6 ± 8.5 and 0.7 ± 0.34 (arbitrary units, AU) respectively, and 381 ± 173, 17.8 ± 10.2 and 1.2 ± 0.57 respectively in the neuroretinal rim. Nicolela et al. [11] also reported lower blood flow measures in primary open-angle glaucoma patients than in normal subjects in both the superior temporal retina and lamina cribrosa; the differences were slightly less marked than in Michelson's study and were not confirmed at the neuroretinal rim. In our study, differences in blood flow measures were induced by changes in DC value alone. From Fig. 1 it can be seen that the range of mean flow values was altered by as much as 160 AU at the retina and 260 AU at the neuroretinal rim. This could offer an explanation for the discrepancy in results obtained by different authors, particularly at the neuroretinal rim [9, 11]. Investigators are more likely to detect subtle changes when DC values are carefully controlled between groups and over time, as variation in blood flow values due to sampling of the photodiode detector will be kept to a minimum.

In conclusion, we observed DC-dependent alterations in volume, in velocity and in flow; for each parameter the fluctuation in values with change in DC was greater in the neuroretinal rim than in the retina. Our results suggest that if the DC value is not carefully controlled, changes in photodiode sensitivity may mimic or mask true changes in ocular haemodynamic indices measured with the HRF. When acquiring data for cross-sectional or longitudinal evaluation, we recommend that data be gathered from areas where the DC value is in the range 70–150; this is in agreement with Michelson and Schmauss, who recommend a DC value of between 80 and 150 [8]. This will optimise both sensitivity and specificity of data acquired using the Heidelberg retina flowmeter.

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Application of a local search strategy improves the detection of blood flow deficits in the neuroretinal rim of glaucoma patients using scanning laser Doppler flowmetry

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Abstract

Background/aim—Blood flow measures acquired using the scanning laser Doppler flowmeter (SLDF) are known to be highly susceptible to spatial and temporal variations of physiological origin. The purpose of this study was to evaluate a local search strategy intended to overcome these intrinsic variations, thereby improving the detection of blood flow defects resulting from glaucoma.

Methods—The sample consisted of one eye of each of 15 glaucoma patients (aged 69.1 (SD 6.6) years) and 15 normal subjects (aged 65.2 (13.7) years). Three 10 degree images of the superior temporal retina and three images of the superior temporal rim were acquired using the Heidelberg retina flowmeter (HRF). Standard analysis was performed using a 10 × 10 pixel frame. For the search strategy the same frame was located within a 15 × 15 pixel window and manually repositioned in order to identify the highest and lowest local values of blood flow. Student's paired *t* test was used to identify differences between groups for the two methods ($p<0.05$).

Results—The standard strategy revealed no significant differences in blood flow measures between the subjects at either the retina or neuroretinal rim. With the search strategy there was also no difference in blood flow measures at the retina. At the neuroretinal rim, the search strategy demonstrated that the highest measured blood flow, volume, and velocity values were significantly lower for the glaucoma patients ($p = 0.002$, 0.02, and 0.002 respectively) while comparison of the lowest flow values showed that glaucoma patients had lower blood flow and velocity only ($p = 0.023$ and 0.021 respectively).

Conclusions—Glaucoma patients exhibit reduced ocular blood flow at the neuroretinal rim, which seems to affect high velocity flow more profoundly than low velocity flow. When analysing perfusion images a local search strategy is recommend to identify the highest local blood flow values in order to optimise the ability to differentiate between subject groups.

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The scanning laser Doppler flowmeter (SLDF) measures blood flow, volume, and velocity in the retina and neuroretinal rim. It combines the optical Doppler effect with scanning laser flowmetry to give values of capillary blood flow, volume, and velocity measured in arbitrary units. Infrared light with a wavelength of 780 nm is emitted from the instrument and reflected from both stationary and moving objects. Interference occurs between light reflected from the relatively stationary background, such as the blood vessel walls, and that reflected from moving objects, such as the red blood cells. This interference results in a beat frequency, which is proportional to blood corpuscle velocity.[1 2] This beat frequency is detected and measured by a photodiode detector. Following fast Fourier transformation, blood flow measurements are obtained from two dimensional perfusion images. Values of blood flow, volume, and velocity are acquired by placing a 10 × 10 pixel frame at the neuroretinal rim or peripapillary retina.

Investigations using the SLDF, in this case, the Heidelberg retina flowmeter (HRF, Heidelberg Engineering, Germany) have shown that it yields reproducible values of retinal perfusion.[3–5] However, a particular difficulty with this method is that when comparing blood flow values between images for a patient, even when using a carefully selected predetermined location, measurements can vary significantly. Large variations are often noted when the measurement box is moved by as little as 1 or 2 pixels. This is the result of, at least in part, normal physiological variations in blood flow arising from the cardiac cycle, which may influence the actual positioning of the capillary bed or the phase of the pulsation cycle during which data are acquired. The incorporation of pulse synchronisation during data acquisition has been shown to reduce the consequences of the spatial and temporal variability induced by systemic circulatory variation.[6] The manufacturers recommend that blood flow measures be obtained by choosing a location on the retina or neuroretinal rim and measuring blood flow parameters within a 10 × 10 pixel grid.[3] If the vascular network is not stationary relative to the retina retracing of the same location using a mapping technique can be difficult. This will result in variability in measured flow, which is difficult to account for.

The HRF has been used to investigate blood flow deficits in patients with normal tension

and primary open angle glaucoma.[7-10] Significant reductions in blood flow measures have been reported in patients with primary open angle glaucoma in the retina[7] and lamina cribrosa[9] and for normal tension glaucoma patients in the peripapillary retina.[8] At the neuroretinal rim, blood flow measurements have been reported as being lower by some authors[7]; however, this has not been a universal finding.[9] It is possible that some of these differences in reported findings can be explained by the effects of a local physiological variability, which is not accounted for during the analysis.

The purpose of this study was to determine whether blood flow deficits are more readily detected when a search strategy, involving the lowest or highest local values of flow, is applied when compared. Further, the possibility of a preferential loss in the number or rate of moving corpuscles within a specific range of velocities has not previously been reported.

Methods

Fifteen glaucoma patients (mean age 69.1 (SD 6.6) years) and 15 age matched normal subjects (mean age 65.2 (13.7) years) were recruited. Glaucoma patients were recruited from outpatient clinics at Birmingham Heartlands Hospital; normal subjects were recruited from spouses of patients or were members of staff from the hospital. Both subject groups were required to have a visual acuity of 6/9 or better in each eye, a refraction of less than 8 dioptres mean sphere, no history of ocular trauma or surgery, and no diabetes mellitus. The normal subjects were required to have no family history of glaucoma. Ocular examination in the age matched normal subjects confirmed open anterior chamber angles and normal optic nerve head morphology. Intraocular pressures in the normal subject group were 13.7 (SD 3.3) mm Hg. Glaucoma patients exhibited repeatable mild to moderate Humphrey 24-2 visual field defects (average mean defect −5.44 (2.3) dB) as defined by Hodapp *et al*[11] and confirmed optic nerve head cupping consistent with a diagnosis of glaucoma. All glaucoma patients were taking ocular hypotensive medications with mean intraocular pressures of 15.8 (3.8) mm Hg.

One eye of each of the subjects alternately selected so that approximately equal numbers of right and left eyes were included. Three 10 degree images were taken of the neuroretinal rim, and three of the superotemporal retina, using the HRF. Retinal analysis was undertaken within 1.5 disc diameters of the rim, superior and temporal to the disc, and avoiding larger blood vessels. This area was chosen for image acquisition of the retina and neuroretinal rim as it has been shown to exhibit the least variability in blood flow measures.[12] Fast Fourier transformation was used to derive perfusion images. Images were included if the (direct current) DC value within the measurement area was between 110 and 150 arbitrary units (AU).

Blood flow, volume, and velocity (arbitrary units) were determined for each image using the 10 × 10 pixel square grid located at a predetermined position on the retina and rim of each subject. Using the standard method the 10 × 10 pixel frame was repositioned at the same location of the retina and neuroretinal rim in each image to obtain blood flow measures. Using acetate sheets the vascular network of the retina and neuroretinal rim was mapped out in each patient and the position of the 10 × 10 pixel frame was marked on the sheet to facilitate good repositioning in each image for both areas.

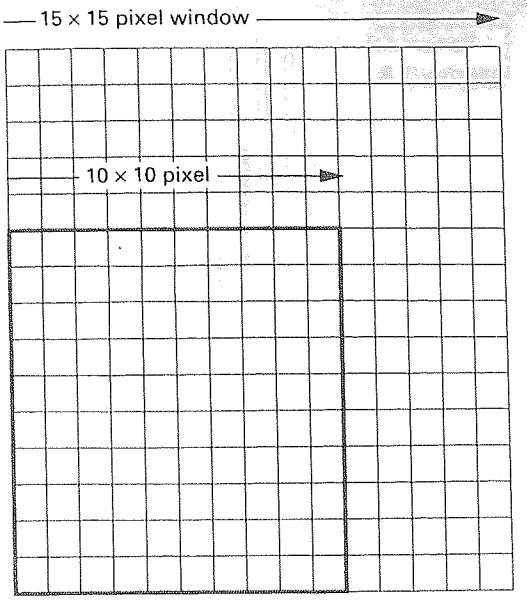

Figure 1 Diagram illustrating the search strategy, showing a 10 × 10 pixel frame for analysis within a 15 × 15 pixel window.

Using the search strategy the 10 × 10 pixel frame was systematically repositioned within a 15 × 15 pixel window located at a similar position on the retina or neuroretinal rim (Fig 1). The highest and lowest local values of blood flow, volume, and velocity were identified and recorded.

Full ethical approval was granted before the start of the study from the institutions involved and informed consent was obtained from each subject. All procedures conformed to the tenets of the Declaration of Helsinki.

STATISTICAL ANALYSIS

The mean and standard deviations of blood flow, volume, and velocity were determined for the standard strategy and search strategy (highest and lowest values) for each eye at the retina and neuroretinal rim for the glaucoma patients and the age matched normal subjects. Group mean values and standard deviations were determined for the retina and rim locations for both methods. Student's paired *t* tests were used to identify significant differences in blood flow, volume, and velocity for the two groups at the retina and neuroretinal rim using both methods.

Results

At the retina, no significant difference was found between the two groups for blood flow, volume, and velocity ($p > 0.05$) using either method.

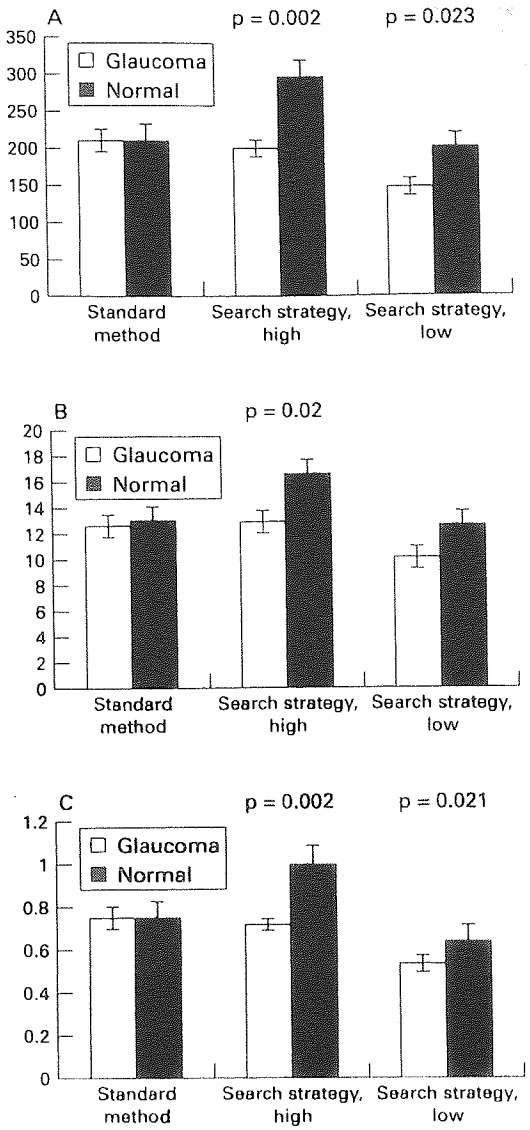

Figure 2 Graphs showing the normal and glaucoma group mean values for ocular blood (A) flow, (B) volume, and (C) velocity (arbitrary units) measured using the standard method and the search method. No differences were found between groups using the standard strategy. Using the search strategy, significant differences between groups were found for the highest values of blood flow ($p = 0.002$), volume ($p = 0.02$), and velocity ($p = 0.002$) and for the lowest flow ($p = 0.023$) and velocity ($p = 0.021$).

At the neuroretinal rim the standard technique revealed no significant difference between groups for blood flow, volume, or velocity. Using the search strategy, significant perfusion reductions were found in the glaucoma group for the highest measured blood flow, volume, and velocity values ($p = 0.002$, 0.02, and 0.002 respectively). When the lowest blood flow, volume, and velocity measures were evaluated the difference was less marked, reaching significance for blood flow and velocity ($p = 0.023$ and $p = 0.021$ respectively) but not for blood volume ($p = 0.07$). Figure 2 shows the neuroretinal rim blood flow, volume ,and velocity measured using the two strategies for both the glaucoma and normal subject groups.

Discussion

Using the standard (static) method to acquire measures of blood flow no significant difference was found between the two groups at either the retina or neuroretinal rim. Using the search strategy, glaucoma patients exhibited significantly lower blood flow, volume, and velocity in the neuroretinal rim when compared to age matched normal subjects; however, this difference was not replicated in the retina. These differences in blood flow were more marked when highest blood flow readings were used to compare between the two groups. For the lowest blood flow values, only the blood flow and velocity were significantly reduced in the diseased group.

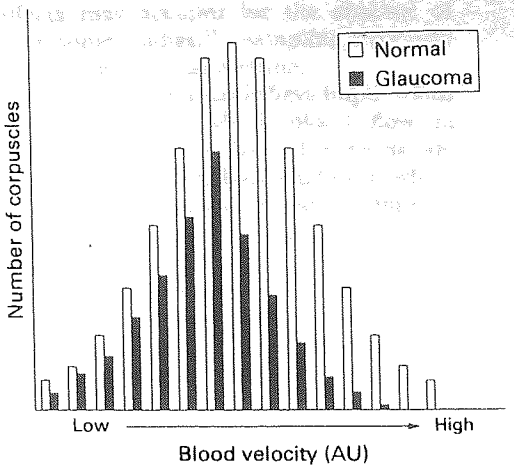

Figure 3 A proposed schematic model for reduced blood flow in glaucoma. The histogram shows a normal distribution of velocity values representing the total number of blood corpuscles moving in the retina over a period of time. The proposed model suggests that the mean velocity and number of cells is reduced in glaucoma with more profound loss in the higher velocity range.

The apparent variability in blood flow measures seen in any one image is largely due to the physiological pulsation of the retinal capillary bed which results in sizeable differences in blood flow measures with movement of the pixel frame by only one or two pixels.[6 13] Analysis of ocular perfusion using the HRF technique and a standard 10 × 10 pixel grid samples data over a fixed area of interest and over a fixed period of time thereby sampling velocities in both the spatial and temporal domains. The flow of blood corpuscles will vary in terms of both the number and velocity of cells, providing a distribution of values of blood flow for the area. We therefore suggest that the sampling process yields a range of velocities with varying frequency, and that these distributions may differ with factors such as age[14] or in the diseased eye compared to the normal eye.[7 8] Figure 3 shows a schematic frequency histogram that illustrates a possible model for the total blood flow defect in glaucoma patients compared to normal subjects, with an overall reduction in both the mean velocity and number of moving corpuscles.

In this study, using the standard technique, no blood flow defects were identified, which may suggest that there were no deficiencies in the volume and rate of flow in our glaucoma sample, a finding that is at odds with most other reported literature.[7 9] It is possible that this difference arises from the stage of glaucoma, which in our study was only mild to moderate based on visual field classifications, and may have been earlier than in previous studies. Alternatively there may be a sampling

error such that blood flow differences were masked between the groups because the small sampling window does not allow for the inherent variations in flow within the local vascular neighbourhood. For example, some patients may have been assessed in an area of relatively high local flow while others were assessed in an area of low flow, thereby masking subtle differences between the two groups. In other words, a small measuring area, such as the 10 × 10 pixel window, may sample with a particular spatial bias resulting in particularly high or low velocity and flow. By using the search strategy, the local peaks and troughs of the velocity histogram can be compared between subject groups. The model of flow deficits in glaucoma that we propose is shown in Figure 3, and would suggest that reductions in flow in glaucoma patients are greater in the higher velocity range of the spectrum, with more subtle differences evident at lower velocities. This model is consistent with the absence in our data of a significant difference in the low values of blood velocity and the generally smaller absolute reductions and significance levels of blood flow, volume, and velocity found when the low flow values are used to compare between groups. With the high flow values, as can been seen from the graphs in Figure 2, the absolute level of change is much greater for all parameters. This model may not be applicable in other diseases or in glaucoma assessed at later stages of damage.

In a study by Chung *et al*[8] two different methods of image analysis were employed, the first using a 10 × 10 pixel frame and the other in which the entire image was analysed to obtain blood flow measures. Findings using the standard 10 × 10 pixel window showed no difference between the glaucoma patients and normal subjects, a result which is in agreement with our own data. However, using their own pointwise analysis involving a larger sampling area, significant differences in blood flow measures existed between the two subject groups. These findings concur with our own observations that a small (10 × 10) pixel frame does not use a large enough sampling window to overcome the physiological variability in blood flow across the retina. While the pointwise analysis overcomes the local temporal and spatial variability in flow by using a larger window, this may not be appropriate in all investigations, particularly those in which smaller areas of the fundus are to be considered.

Previous studies have shown that blood velocity is lower in glaucoma patients when compared to age matched normal subjects in the retina,[7 9 15] optic nerve head,[7] choroid,[16] and ophthalmic artery.[17] The results of our study offer some explanation for the discrepancy in findings obtained by different authors when determining blood flow deficits in glaucoma. For example, blood flow deficits have been reported in the retina,[7 8] neuroretinal rim,[7] and lamina cribrosa[9] of glaucoma patients. In some studies blood flow appeared to be normal in the neuroretinal rim[9 18] or peripapillary retina.[19] Although the possibility of age effects may account for the absence of defects in some studies,[19] a sampling error may offer a more reliable explanation.

The results from our study have implications for investigations in which blood flow in glaucoma patients and normal subjects are compared, or in longitudinal studies in which blood flow changes are monitored over time. In cross sectional studies, differences in blood flow between subject groups may not be identified due to inappropriate sampling of blood flow measures between individuals. In addition, when attempting to identify changes in blood flow measures in patients over time, as in longitudinal studies, alterations may not be detected because of the noise, resulting from the unintentional, randomised identification of high and low blood flow measures between visits. Our results suggest that the standard strategy resulted in a sampling error which is induced because too small an area of tissue has been evaluated without due account of local variations in velocity and the local tissue variations in flow mask real changes resulting from the disease process. The result of this was that no significant difference in blood flow parameters at the retina and neuroretinal rim were observed. If the local physiological variation in flow measures is not accounted for then false positive or false negative findings of change could be reported.

In summary, our data and the data of others[8] suggest that blood flow deficits are more consistently and more readily identified when a larger sample area is considered, or when account is taken of the local physiological variation in blood flow. Our study suggests that this can be achieved using standard software by adopting a local search strategy and finding the lowest or highest values of blood flow, volume, and velocity to compare blood flow measures between subject groups.

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CLINICAL STUDY

Effect of senescence on ocular blood flow in the retina, neuroretinal rim and lamina cribrosa, using scanning laser Doppler flowmetry

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Abstract

Purpose To determine the effects of age on blood flow measurements obtained using the scanning laser Doppler flowmeter (SLDF).

Method Using SLDF (Heidelberg retina flowmeter, Heidelberg Engineering, Germany) three 10° images were taken of the superior temporal retina and three further images of the superior temporal neuroretinal rim in 15 young, healthy subjects (mean age 27.9 years ± 6.2 years) and 15 mature, healthy subjects (mean age 65.2 years ± 13.7 years). In addition, measurements were taken of the lamina cribrosa in 12 of the volunteers from each subject group (mean age 27.1 ± 6.3 years and 64.8 ± 13.2 years respectively). Using a 10 × 10 pixel measurement frame, blood flow readings were obtained at a predetermined position on the retina, neuroretinal rim and lamina cribrosa. Student's two-tailed unpaired *t*-tests were used to compare measures of blood flow, volume and velocity between the two subject groups ($P < 0.05$). In addition, linear regression analysis was used to assess the relationship between age and blood flow, volume and velocity at the retina, neuroretinal rim and lamina cribrosa.

Results Retinal blood volume measured at the retina was significantly lower in the mature compared with the young subject group ($P = 0.01$). Mature subjects also exhibited reduced blood flow and velocity at the neuroretinal rim ($P = 0.01$ for both parameters) and lamina cribrosa ($P = 0.008$ and $P = 0.01$ respectively). Regression analysis revealed negative trends for all blood flow parameters in each of the anatomical areas with advancing age.

Significant negative correlations were obtained for retinal blood volume ($r = -0.455$, $P < 0.05$), neuroretinal rim blood velocity ($r = -0.359$, $P < 0.05$) and lamina cribrosa blood volume ($r = -0.475$, $P < 0.05$).

Conclusion Capillary blood flow in the retina, neuroretinal rim and lamina cribrosa decreases with advancing age. This may be of consequence in the progression of chronic ocular diseases such as glaucoma, and should be considered in the longitudinal determination of change in disease monitoring.

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Introduction

The Heidelberg retina flowmeter (HRF; Heidelberg Engineering, Germany) is a scanning laser Doppler flowmeter (SLDF) recommended for the investigation of fundus blood flow *in vivo*. It combines the confocal scanning laser technique with Doppler flowmetry to generate two-dimensional maps of retinal perfusion.[1,2]

The principles of this technique have been described in detail elsewhere.[2] In brief, infrared light with a wavelength of 780 nm is used to scan the retina. Stationary targets, such as the blood vessel wall, and moving objects, such as the red blood cells, reflect the laser beam. The light reflected by the moving red blood cells is frequency-shifted due to the optical Doppler effect and this shift in

frequency is proportional to the rate of movement of the corpuscles. The frequency shift itself is too small to measure directly, but after interference between this and the light reflected from stationary targets, a beat frequency is generated. The beat frequency is measured at each point by a photodiode detector in the HRF.[2] Following fast Fourier transformation, the data are used to derive indices of blood flow, volume and velocity.[3] The number and velocity of moving corpuscles is obtained by multiple measurements at each pixel location over a fixed period of time. The total blood flow is the product of the number and rate of corpuscles flowing past each pixel location over time.

Many investigators have reported favourably on the reproducibility and validity of measurements obtained using the HRF.[4–7] Compromised ocular blood flow has been shown to underlie various ocular pathologies. Using the HRF, altered blood flow has previously been reported in diabetes,[8] age-related macular degeneration[9] and glaucoma.[1,7,10,11] Many of the changes associated with reduced blood flow in ocular disease are known to become more prevalent with age, which is a known risk factor in glaucoma.[12,13] Blood flow deficits identified using the HRF have been reported in both normal-tension glaucoma[14] and primary open angle glaucoma[1,11] in the retina, neuroretinal rim and lamina cribrosa. Since blood flow is known to decrease with increasing age,[15–17] this may be a confounding factor in the development or progression of glaucoma.

The optic nerve head is supplied by branches of the posterior ciliary arteries,[18] with influence from the central retinal artery in the superficial layers.[19] The choroid receives its nutrients from the posterior ciliary arteries.[20] Over time the structure of the arteries changes, culminating in a decrease in the elasticity of blood vessels.[21] In addition, the structure of endothelial cells alters with increasing age and these cells play a crucial role in vascular tone and regulation.[22] One might predict, therefore, that these alterations result in reduced retinal blood flow. Reductions in blood velocity and flow with advancing age have been demonstrated using pulsed Doppler sonography and scanning laser Doppler flowmetry.[17] In addition, it has been demonstrated that ocular pulsatility, derived from blood inflow during cardiac systole and influenced by, among other things, vascular elasticity, reduces with age.[15,16]

The lamina cribrosa is situated in the central region of the optic nerve head. It is an elastic structure composed of perforated cribriform plates through which nerve fibres and the central retinal artery pass. The lamina cribrosa receives its blood supply from the choroidal arteries and the short posterior ciliary arteries.[23] It is known that with age the components of the cribriform plates alter,[24] this results in a stiffer structure with decreased mechanical compliance.[25] It has been hypothesised that these changes are likely to make the ageing eye more susceptible to retinal ganglion cell axon damage, especially when coupled with fluctuations in intraocular pressure common to primary open angle glaucoma.[25] The cribiform plates are in very close proximity to one another, as are the nerve fibre axons which pass through them. During changes in intraocular pressure the cribriform plates are rearranged, and in turn exert force on the nerve fibre axons. In a more rigid structure these forces are likely to have a greater detrimental effect on the nerve fibres. In addition to the known vascular changes associated with age it is possible that a decrease in the elasticity of the lamina cribrosa may have an effect on the blood flow of the vessels that supply it with nutrients. This has been postulated as a cause of optic nerve damage in glaucoma, but has not previously been investigated in normal, healthy eyes.

The purpose of this study was to determine the effect of increasing age on ocular blood flow in the retina, neuroretinal rim and lamina cribrosa.

Materials and methods

The subject sample consisted of one eye each of 15 mature and 15 young healthy volunteers. Good-quality images were obtained for the neuroretinal rim and peripapillary retina for each subject. For the images located on the lamina cribrosa, 12 of the 15 subjects provided images of sufficient quality for analysis. Details of the subject samples used for each anatomical area of interest are given in Table 1.

Young subjects were required to be between the age of 18 and 40 years, whilst the mature subjects were all over 45 years with no upper age limit. Both subject groups were required to have a visual acuity of 6/9 or

Table 1 Details of the subject samples used in the study for the retina, neuroretinal rim (NRR) and lamina cribrosa

Area	*Group*	*Gender*		*Eye*		*Mean age ±SD (years)*	*Age range (years)*
		F	*M*	*R*	*L*		
Retina and NRR	Young	7	8	8	7	27.8 ± 6.2	20–38
Retina and NRR	Mature	7	8	8	7	65.2 ± 13.7	48–82
Lamina cribrosa	Young	6	6	6	6	27.1 ± 6.3	20–38
Lamina cribrosa	Mature	7	5	6	6	64.8 ± 13.2	48–82

better in each eye with a refractive error of less than 8 dioptres mean sphere. Intraocular pressures were less than 22 mmHg in both eyes, anterior angles were open and ocular examination confirmed a normal optic nerve head appearance. There was no history of ocular trauma or surgery, no diabetes, no systemic hypertension or hypotension and no family history of glaucoma. Furthermore, none of the subjects were receiving any systemic or ocular medications known to affect blood flow. Two subjects from each group were smokers.

Full ethical approval from the institutions involved was granted prior to commencement of the study and informed consent was obtained from every subject. All procedures conformed to the tenets of the Declaration of Helsinki.

Following pupil dilation, and a 20 min resting period to ensure maximal mydriasis, one eye was chosen at random for each subject and nine HRF images were acquired: three located in the superior temporal retina, three located in the superior temporal neuroretinal rim and three located in the lamina cribrosa. Three images were taken at each anatomical area in order to obtain mean values of blood flow, volume and velocity. This was done to overcome physiological variations in blood measures occurring due to the cardiac cycle. Of the subjects included in this study, three had optic nerve head vasculature that prevented positioning of the 10×10 pixel grid on the lamina cribrosa. This was due to either interference from the larger blood vessels or the absence of any visible cupping.

The superior temporal area of the retina and neuroretinal rim were chosen for image acquisition as these areas have been shown to exhibit less variability in blood flow measures when using the HRF.[26] Acetate sheets were utilised to trace the retinal and neuroretinal rim vasculature for each fundus, and a blood vessel landmark, such as a vessel bifurcation, was identified to ensure that the same retinal, neuroretinal rim and lamina cribrosa locations were used for each image. Every effort was made to keep the locations constant within each subject image series. In addition, efforts were made to optimise the reproducibility of blood flow measurements acquired by obtaining separate HRF images of the different fundus locations, thus ensuring that the DC level (direct current) of the area under examination remained consistently within the range of 110 to 150 DC.[27] Images were only accepted where no or very little eye movement had occurred during image acquisition, thus ensuring perfusion maps had minimal movement saccades.

Using the HRF software (version 1.02) fast Fourier transformation was used to derive perfusion images. Blood flow, volume and velocity (arbitrary units) were determined for each image using a 10×10 pixel square grid located at a predetermined position on the retina, neuroretinal rim and lamina cribrosa for each subject.

Statistical analysis

Student's two-tailed unpaired *t*-tests were used to test for significance between the two groups for each blood flow parameter at the retina, neuroretinal rim and lamina cribrosa. A *P*-value of less than 0.05 was considered significant.

Pearson's correlation coefficient was used to investigate a linear correlation between age and blood flow parameters.

Results

Students unpaired two-tailed *t*-tests revealed a significant reduction with age in retinal blood volume ($P = 0.01$), neuroretinal rim blood flow ($P = 0.02$), neuroretinal rim blood velocity ($P = 0.01$), lamina cribrosa blood flow ($P = 0.008$) and lamina cribrosa blood velocity ($P = 0.01$) (Table 2). No significant difference was found for retinal blood flow or velocity or neuroretinal rim and lamina cribrosa blood volume between the two groups, although the trend was for blood flow parameters to decrease with increasing age (Table 2).

Figure 1 shows the mean blood flow values measured at the retina, neuroretinal rim and lamina cribrosa for the two subject groups, while Figures 2 and 3 show the mean blood volume and velocity values measured at the retina, neuroretinal rim and lamina cribrosa. Figures 4 to 6 show the linear correlation (r) for retinal blood volume, neuroretinal rim blood velocity and lamina cribrosa blood volume respectively. Measures of blood flow, volume and velocity at three of the anatomical areas showed a negative trend with increasing age. Significant negative correlations were obtained for retinal blood volume ($r = -0.455$, $P = 0.01$), neuroretinal rim blood velocity ($r = -0.359$, $P = 0.01$) and lamina cribrosa blood volume ($r = -0.475$, $P = 0.008$). No significance was found for neuroretinal rim blood flow and lamina cribrosa blood flow and velocity.

Discussion

The results from this study suggest that capillary blood flow measured at the superior temporal retina, superior temporal neuroretinal rim and at the lamina cribrosa decreases with increasing age. A comparison

Table 2 Mean values (arbitrary units ±SD) for blood flow, volume and velocity measured at the retina, neuroretinal rim (NRR) and lamina cribrosa (LC) for the two subject groups with corresponding P and r values

	Flow			*Volume*			*Velocity*		
	Retina	*NRR*	*LC*	*Retina*	*NRR*	*LC*	*Retina*	*NRR*	*LC*
Mature subjects	224.98 ± 69.23	210.32 ± 84.15	187.21 ± 57.02	14.35 ± 2.96	13.01 ± 3.48	11.47 ± 5.31	0.812 ± 0.24	0.75 ± 0.30	0.67 ± 0.2
Young subjects	248.62 ± 77.94	298.06 ± 106.21	261.53 ± 67.22	17.16 ± 2.70	14.92 ± 4.59	15.03 ± 4.31	0.89 ± 0.27	1.08 ± 0.3	0.897 ± 0.21
P value	NS	0.01	0.008	0.01	NS	NS	NS	0.01	0.01
r value	−0.084	−0.31	−0.374	−0.455	−0.172	−0.475	−0.071	−0.359	−0.327
(P value)	NS	NS	NS	<0.05	NS	<0.05	NS	<0.05	NS

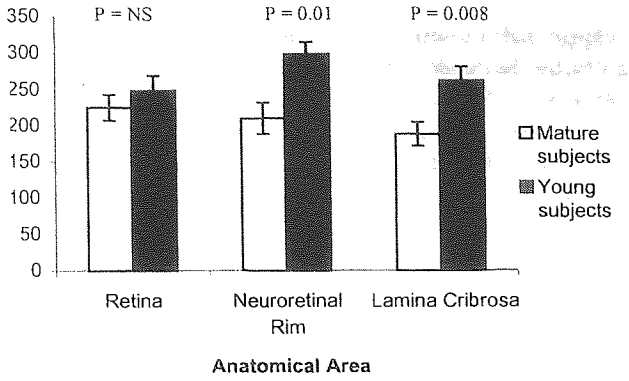

Figure 1 Mean values (± SE) for blood flow measured for the two groups at the retina, neuroretinal rim and lamina cribrosa.

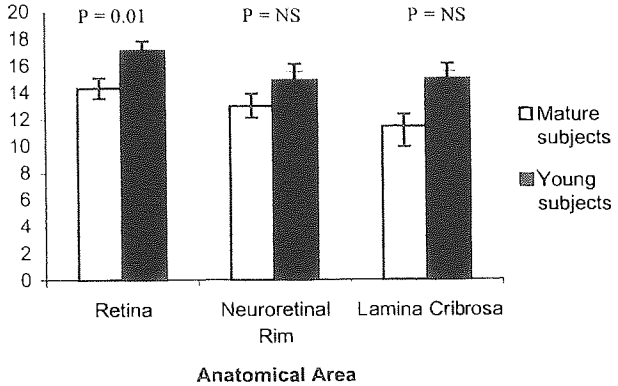

Figure 2 Mean values (± SE) for blood volume measured for the two groups at the retina, neuroretinal rim and lamina cribrosa.

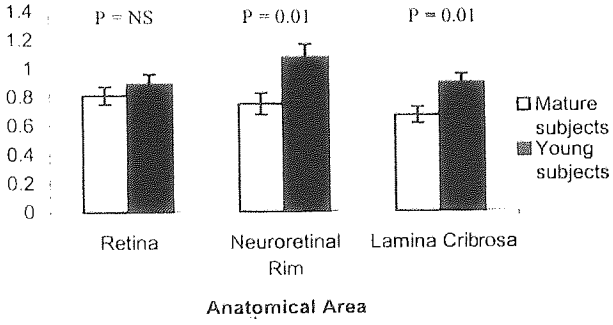

Figure 3 Mean values (± SE) for blood velocity measured for the two groups at the retina, neuroretinal rim and lamina cribrosa.

of group-mean values identified significant reductions in blood volume measured at the retina and in blood flow and velocity measured at the neuroretinal rim and lamina cribrosa. Regression analysis revealed a significant negative correlation between advancing age and retinal blood volume, neuroretinal rim blood velocity and lamina cribrosa blood volume. The

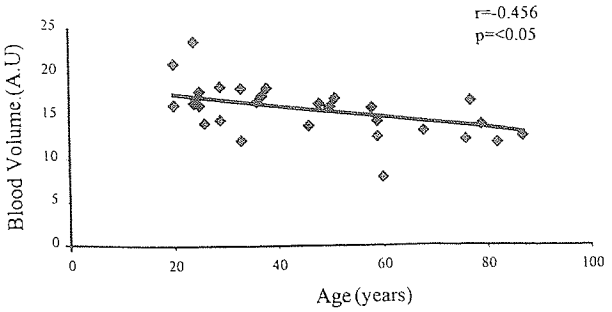

Figure 4 Effect of age on retinal blood volume.

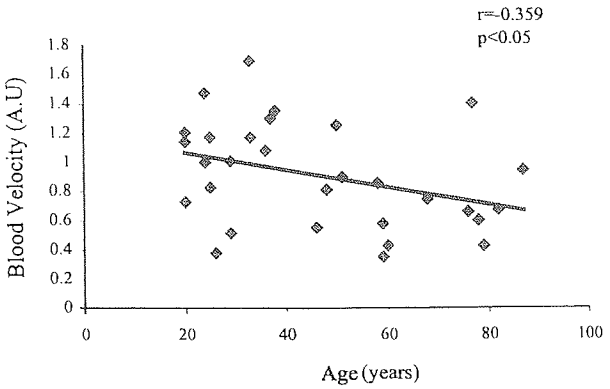

Figure 5 Effect of age on neuroretinal rim blood velocity.

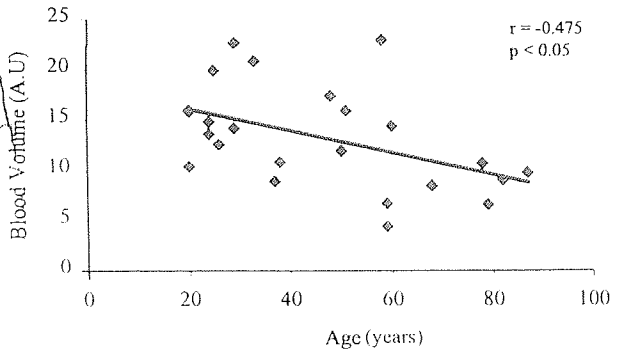

Figure 6 Effect of age on lamina cribrosa blood volume.

absence of any significant change in blood flow and velocity at the lamina cribrosa by regression analysis may be the result of the slightly smaller sample size in this group. The overall finding in this study that ocular blood flow decreases with advancing age is in agreement with previous investigations.[15,17,28,29]

It is known that the lamina cribrosa changes throughout life to become a stiffer, less elastic structure[25] and our results revealed, for the first time, that a significant reduction in blood flow and velocity occurs with age in the lamina cribrosa. This supports the theory that, with age, blood flow in the choroidal arteries and short posterior ciliary arteries that supply the lamina cribrosa, decreases. This observed reduction in blood flow at the lamina cribrosa may be due to the combined effects of reduced vascular and mechanical compliance with advancing age. Furthermore, it is known that with the onset of some ocular diseases the rigidity of the lamina cribrosa increases,[30] thus potentially contributing further to reductions in flow. With reference to glaucoma, reduced ocular blood flow has been directly implicated as a contributing factor in its pathogenesis. Nicolela *et al*[11] found that in glaucoma patients the blood flow, volume and velocity in the lamina cribrosa were significantly lower when compared with age-matched normals.

Groh *et al*[17] investigated the effect of age on the microcirculation of the retina and neuroretinal rim using the HRF. Consistent with our findings, blood flow measured at the retina significantly reduced with advancing age; however, unlike the results from our study, the blood flow of the neuroretinal rim was not found to be significantly influenced by age. This discrepancy in results may be due to differences in the average ages of the subject groups used in each of the studies. Another contributing factor could be the DC level at which the perfusion images were obtained; this was controlled for in our study but average DC values may have been much lower in the study of Groh *et al*[17] as blood flow values for the retina and optic nerve head were acquired from the same image. Groh *et al*[17] also investigated the macrocirculation of the central retinal artery, using pulsed Doppler sonography, and reported that significant decreases in blood velocity occur with advancing age. This finding is supported by other investigators,[31,32] who have found reduced retrobulbar and choroidal blood velocity with advancing age.

Anatomically, the optic nerve head is supplied by both the posterior ciliary arteries and the central retinal artery (in the more superficial layers), whereas the choroid is supplied solely by the posterior ciliary arteries. If blood flow is reduced in the central retinal artery, as Groh *et al*[17] suggest, then one would expect this to have implications for both retinal and neuroretinal rim perfusion. The observed decrease in both neuroretinal rim and retinal blood flow in this study serves to support this. Furthermore, the finding that blood flow is reduced in the lamina cribrosa suggests the presence of decreased blood flow in the posterior ciliary arteries. Alternatively, the reduction in microvascular flow in the lamina cribrosa may be restricted to the capillaries. A reduction in posterior ciliary blood flow may also have implications for choroidal perfusion. Ravalico *et al*[15] reported a reduction in the pulsatile component of ocular blood

flow with increasing age. It is known that pulsatile ocular blood flow is primarily choroidal in origin,[33] thus providing indirect support for reduced choroidal flow. Further studies are required to determine the involvement of the posterior ciliary arteries in the perfusion of the ageing eye.

To summarise, age has a significant effect on neuroretinal rim and lamina cribrosa blood flow and velocity and retinal volume. Morphological changes associated with age, such as reductions in retinal ganglion cells and their axons, have previously been reported.[34–36] This depletion in nerve fibre numbers may result in a reduced requirement for ocular perfusion, or alternatively reduced perfusion may itself result in the depletion of retinal ganglion cell axons. Blood flow measurements may fall due to increases in vascular resistance following changes in the capillary vessel structure with age.[37] It is known that with age the incidence of atherosclerosis increases, which reduces arterial distensibility.[21] It therefore follows that with advancing age the compliance of the retrobulbar arteries, including the posterior ciliary arteries and the central retinal artery, are likely to diminish and in turn will result in a decrease in the retinal and neuroretinal rim microcirculation. Alternatively, the blood flow reductions observed may be due to loss of autoregulatory processes[38] that are strongly influenced by the endothelium, and known to alter with increasing age.[22] While the exact basis for reduced blood flow is uncertain, what remains clear is that advancing age results in lower ocular blood flow. It is likely that multiple factors are involved in the process of blood flow diminution with age, and such reduction may be of significance in the aetiology of some age-related eye diseases, or indeed exacerbated by them.

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Abstract *Background:* Many studies have found that the intraocular pressure (IOP) is lower in glaucomatous populations than in normal groups. An alternative method of analysis, commonly used in the investigation of arterial blood pressure, is to reduce the pulse waveform to its component Fourier parts. The purpose of this study, therefore, was to determine whether such a technique is applicable to the IOP pulse and to investigate whether it was superior at differentiating diseased from healthy eyes. *Methods:* Using a pneumatonometer, continuous 10-s IOP recordings were taken from 10 glaucoma patients and 10 normal subjects. The IOP recordings were then analysed using the fast Fourier transform (FFT) to determine their spectral components. In addition, standard IOP parameters (pulse amplitude, pulse volume, and pulsatile ocular blood flow) were measured to allow comparison with the waveform analysis technique. *Results:* Analysis by FFT successfully determined the IOP pulse's higher spectral components up to the fourth harmonic. In addition, although standard measurements of the IOP pulse (such as pulse amplitude) were insignificant, the second (P=0.034), third (P=0.015) and fourth (P=0.013) harmonics of the waveform successfully differentiated between the glaucoma and normal groups. *Conclusion:* Spectral analysis of the IOP pulse appears to be a promising technique in the investigation of ocular vascular disease.

Introduction

Glaucoma is a disease of unknown origin [6]. While elevated intraocular pressure (IOP) remains the most prevalent risk factor [25], evidence now suggests that abnormal ocular blood flow may contribute to the pathogenesis in some patients [12]. The small variation in IOP associated with each heart-beat is a manifestation of the intraocular vasculature pulsating during the cardiac cycle [4]. This IOP pulse wave has been used as a means of assessing the ocular vascular status in glaucoma patients. Numerous studies have demonstrated reduced IOP pulse amplitudes and reduced pulsatile ocular blood flow in glaucoma patients compared with control subjects [11, 14, 15, 20, 21, 22, 24].

In the systemic circulation, the blood-pressure pulse wave has also been subject to much detailed research [5, 10, 16]. Many of these studies have focused on investigating the blood-pressure pulse as a waveform through Fourier analysis [18]. In brief, such analysis uses the principle that all periodic functions, such as the repeating arterial pressure pulse, can be constructed by adding sinusoidal waveforms of varying amplitude and phase: Fourier analysis reduces such a waveform or signal into its component sinewave functions [19]. These functions are frequently displayed by plotting their amplitude against their frequency, and this technique is known as representing the waveform in its frequency, or spectral, domain, compared with the waveform being represented in its original time domain [3]. The frequency that contains the spectral component of highest power is known as the fundamental frequency, and for the arterial pressure pulse this is the frequency associated with the heart-

beat. Waveform analysis of the arterial pressure pulse has demonstrated that the principal spectral components below the fundamental frequency are related to the respiratory and vasomotive cycles of the cardiovascular system: 0.15 Hz and 0.1 Hz respectively [1]. Of greater cardiovascular research interest, however, is that the spectral components at successive orders of frequency above the fundamental, known as the harmonic components, have been found to be associated with the vessel's vascular impedance and wave reflections from the distal arterial tree [17].

Returning to the eye, it would be of interest to establish whether a similar technique can be applied to the IOP pulse. If the harmonic components of the IOP pulse are related to some similar vascular measure of the eye, then waveform analysis by Fourier transformation may provide a novel approach to investigating ocular conditions of a vascular nature. The purpose of this study, therefore, was twofold: (1) to determine whether the harmonic components can be readily discerned from Fourier analysis of the IOP pulse wave and (2) to compare the harmonic components of the IOP pulse wave in glaucoma patients with those in normal subjects.

Methods

Ten previously untreated glaucoma patients and 10 control subjects were recruited for this study from the clinic population at the University of Alabama at Birmingham (UAB) School of Optometry. All participants reviewed and signed informed consent statements, in accordance with the 1964 Declaration of Helsinki, before participation in the study, and the experimental protocol and procedures were approved by the UAB Institutional Review Board. Diagnosis of glaucoma was made on visual field analysis and optic disc appearance in accordance with established clinical tenets [13]. No limitation was placed on the minimum level of IOP for study inclusion. The eye from each patient with the more advanced visual field loss was selected for evaluation (left=6: right=4). The control group was selected to be similar to the patient group for age, gender distribution, blood pressure and heart rate. Control subjects had no history of ophthalmic disorders and the results of an ophthalmic examination were normal. Medication for systemic hypertension was taken by four of the glaucoma patients (two diuretic, one ACE inhibitor and one calcium channel blocker) and two of the control subjects (two diuretic). One patient and one subject had non-insulin-dependent diabetes, but neither had retinopathy. The right eye of each patient in the control group was used for evaluation.

For each subject the following procedure was followed. First, blood pressure was evaluated using sphygmomanometry and the heart rate assessed by palpation. Second, IOP was measured using the O.B.F. Labs pneumatonometer (O.B.F. Labs, Wiltshire, UK). Before testing, the corneal surface was anaesthetized with proparacaine 0.5% and each participant was properly positioned in the slit-lamp chin rest. First, IOP, pulse amplitude, pulse volume and pulsatile ocular blood flow were determined using the "standard" measurement technique supplied with the O.B.F. pneumatonometer. Then the instrument was switched to "data-only" to record 10 continuous seconds of IOP. Data collection for continuous IOP was considered acceptable if eight consecutive pulses were acquired in which a clearly audible "squawk" (representing the heart-beat) was heard and in which the IOP pulse wave appeared, from visual inspection, to be periodic and symmetric. If these criteria were not met, the patient was retested.

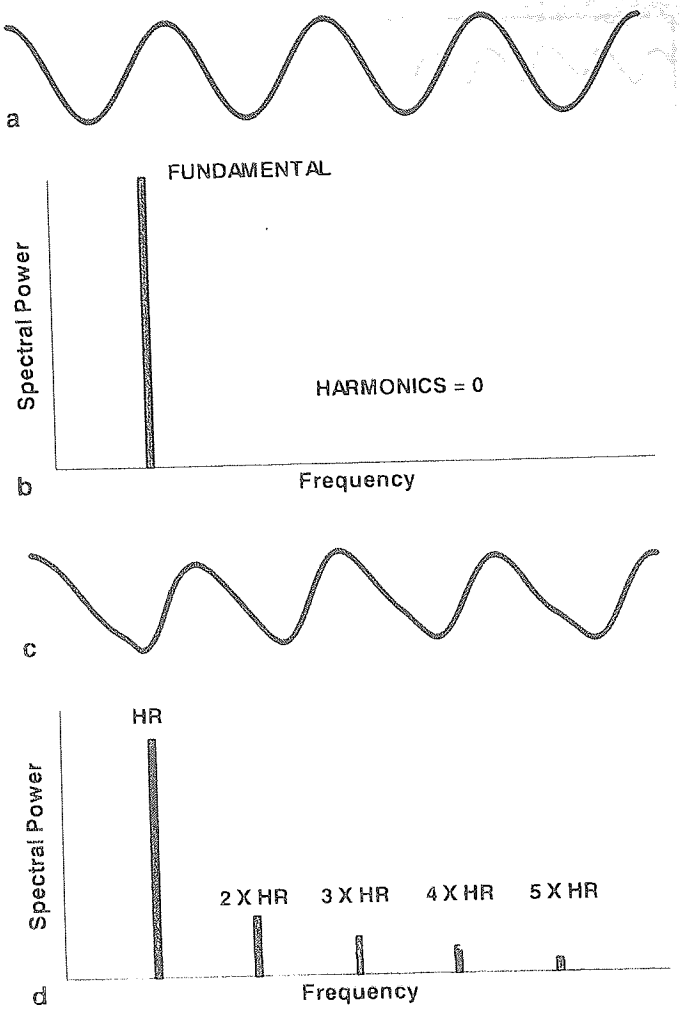

Fig. 1 Schematic representations of **a** a mathematically derived sine wave; **b** Fourier transformation of the continuous wave shown in **a**; **c** the IOP curve of a patient with early glaucoma, showing a steep rise and shallow fall in pressure; **d** Fourier analysis of the IOP curve showing the fundamental frequency resulting from the heart rate (HR) and four harmonic components representing the higher frequencies of the IOP wave as a multiple of HR

Fourier analysis

In 1807, Fourier proposed a theorem suggesting that a quantity that varies with time, such as sound pressure, could be expressed as the sum of sinusoidal waves, of constant amplitudes, which persist indefinitely. Any repeating waveform can be represented by a series of sine and cosine waves of appropriate amplitudes at the repetition frequency of the waveform and integral multiples of it. In the case of a simple indefinite sine wave (Fig. 1a), only one component is required to represent the data and this is known as the fundamental frequency (Fig. 1b). In the case of blood pressure, this frequency would be the heart rate. For more complex waves, such as the IOP pulse (Fig. 1c), the fundamental frequency contains the majority of the power of the spectra and also represents

the heart rate of the patient. This is accompanied by a series of higher-frequency waves known as the harmonic components, each being a multiple of the fundamental frequency or heart rate (Fig. 1d).

The O.B.F.A pneumatonometer samples IOP at 200 hertz, which, combined with a 10 s capture time, provides 2000 IOP datum points available for analysis. Fourier analysis, using the FFT algorithm [7], requires that the data sample size is equal to 2^n (where n=1, 2, 3, 4, 5, 6....). As such, the sample must contain one of the following number of datum points; 2, 4, 8, 16,.....512, 1024 or 2048, etc. To provide the highest resolution to discern the Fourier frequency components, the largest possible number of sampled data points should be used. The sample size of 1024 data was chosen for analysis because it provided the greatest number of usable data points that could be captured during the 10-s sample period.

After capture with the pneumatonometer, the IOP data were downloaded to a computer and analysed using a spreadsheet (Microsoft Excel). Three different, but overlapping, 1024-data-point sections of the IOP pulse wave were then evaluated using the FFT. Specifically, the section succeeding the first peak (or trough) of the pulse wave, the section preceding the final peak (or trough) of the pulse wave and a section midway between these two endpoints were evaluated. After the FFT was conducted, the percentage of power represented in the fundamental frequency and in the first, second, third, fourth and fifth harmonics were calculated by dividing the power in each frequency component by the total power. These percentages were averaged across the three data sections to obtain the final value for each harmonic.

Statistical analysis

Unpaired Student's t-tests were used to compare differences between groups for each condition. Pearson's product moment correlation analysis was used to ascertain any significant association between variables. A P value of <0.05 was considered statistically significant.

Results

Patients and subjects were similar for age, gender distribution, heart rate and blood pressure. Patients exhibited significantly higher IOP than normal subjects in both eyes (Table 1).

Glaucoma patients had lower pulse amplitude, pulse volume and pulsatile ocular blood flow than normal subjects, but these differences did not reach significance (Table 2).

A 10-s continuous IOP measurement was successfully recorded from each patient and subject, an example of which is shown in Fig. 2.

Analysis of the IOP data by FFT found that the higher spectral components of the pulse wave could be discerned up to the fourth harmonic: a typical example of the spectral components from an IOP waveform is shown is Fig. 3. As this is a new measure that has not been previously accomplished in glaucoma patients, one concern is the variability differences between groups. The mean subject coefficients of variation for the fundamental and the first, second, third and fourth harmonics were 21.4%, 25.3%, 30.0%, 34.6% and 28.7% for the normal subjects and 22.7%, 26.3%, 25.8%, 22.9% and 28.1% for the glaucoma patients. For the groups, the coefficients of variation for the fundamental and the first, second, third and fourth harmonics were 38.1%, 21.9%, 34.1%, 12% and 10.4% for the normals and 38.8%,

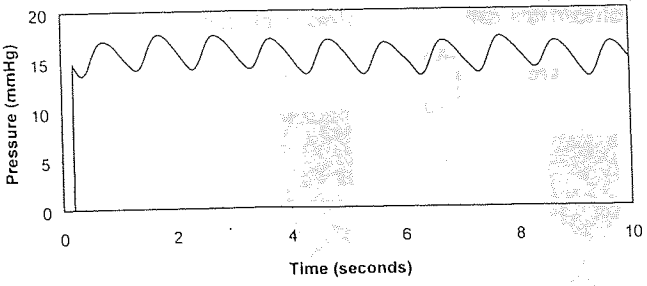

Fig. 2 Example of a 10-s recording of a normal subject's IOP

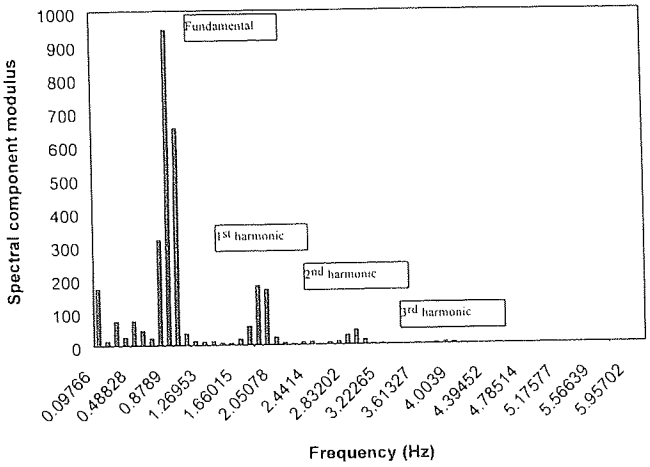

Fig. 3 Example of the actual spectral components from a normal subject's IOP recording after transformation by Fourier analysis

Table 1 The characteristics of glaucoma patients and normal subjects

Parameter	Glaucoma	Normal
Age(years)	53.5±9	58.0±10
Sex (F/M)	4/6	5/5
Mean arterial pressure (mmHg)	102.4±3.63	100.5±5.9
Heart rate(beats/min)	77.1±6.2	72.8±8.4
IOP (OD/OS; mmHg)	19.7±6.8*	13.2±3.9*

* Glaucoma patients had significantly higher IOP than normal subjects (P<0.01)

Table 2 Standard IOP pulse parameter measures for glaucoma patients and normal subjects

Parameter	Glaucoma	Normal	P value
Pulse amplitude (mmHg)	2.69±0.9	2.44±0.7	0.24
Pulse volume (µl)	4.98+2.6	6.84+2.6	0.07
POBF (µl/min)	842+426	1081+370	0.09

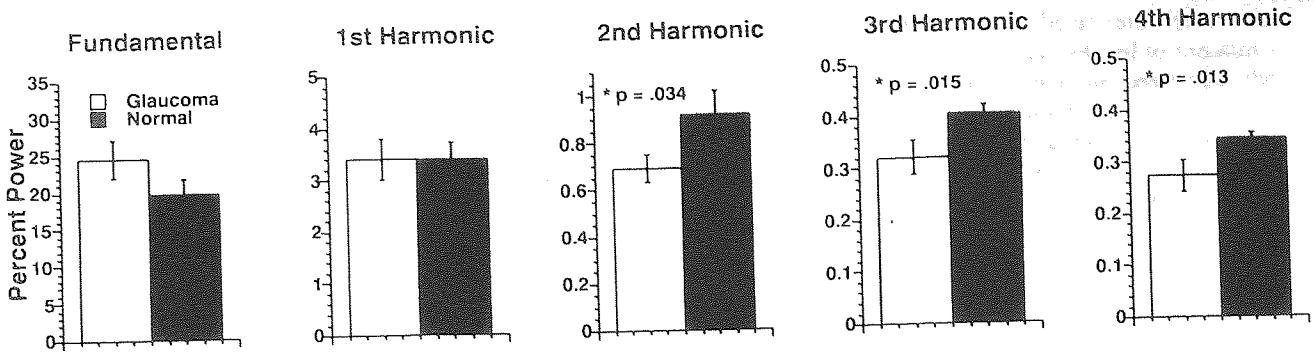

Fig. 4 Percentage power (± standard deviation) of the fundamental and harmonic components of the IOP pulse for the normal and glaucomatous groups

38.5%, 27.5%, 39% and 33% for glaucoma, suggesting that the spread of data was similar between groups. The similarity of the coefficients of variation within and between groups suggests that the testing methodology provides similar variability for glaucoma patients and age-matched normal subjects.

Glaucoma patients had lower percentage power than normal subjects for the second (P=0.034), third (P=0.015) and fourth (P=0.013) harmonics (Fig. 4). Correlation analysis demonstrated no significant correlations between any systemic measure, IOP or Fourier component.

Discussion

Firstly, this study shows that the IOP pulse can be readily analysed in the frequency domain and that its spectral components can be determined up to the fourth harmonic. This method of analysis provides a novel and exciting avenue of research. To the authors' knowledge, the only other published study to use Fourier analysis on the IOP pulse is by Best and Rogers [2]. They investigated the technique's ability to detect carotid artery stenosis and found that calculation of the first three harmonics of the IOP pulse was superior to measuring pulse amplitude for detecting low grades of stenosis.

Secondly, our results indicate that previously untreated glaucoma patients, compared with normal subjects, demonstrate lower power in the second, third and fourth harmonics of their IOP pulse when analysed in the frequency domain. These same patients and subjects did not show significant differences for the measures of pulse amplitude, pulse volume and pulsatile ocular blood flow. This is likely to be due in part to the relatively small sample in this study and perhaps more importantly to the fact that the patient and subject groups were matched for heart rate, which has a major impact on ocular pulsatility measures. This is particularly pertinent to the interpretation of our findings as it highlights the greater ability to differentiate the groups when this more sensitive analysis is used, even when the influence of heart rate is removed.

As discussed in the introduction, the higher harmonics in the arterial pressure pulse can be used to measure vascular impedance. Vascular impedance is dependent on the dimensions and the viscoelasticity of the artery involved, on the physical properties of the blood and on waves reflected from more distal parts of the arterial tree [17]. Vascular impedance is closely related to vascular resistance. Impedance is defined as the resistance to flow in an oscillatory system; for example, it is also found in the fields of acoustics and alternating electrical current. It is a frequency-dependent quantity, rather than a time-dependent quantity [18]. Resistance, with which impedance is often confused, is confined to non-oscillatory or steady motions. Resistance may thus be considered as impedance at zero frequency. Considering such an explanation for the findings in this study, some support may be gleaned from colour Doppler ultrasound investigations that have found some glaucoma patients to have increased resistivity indices for the ophthalmic artery [12], central retinal artery [9] and the short posterior ciliary arteries [8] in certain situations. Therefore one may postulate that the reduced harmonics found in this study's glaucoma patients are associated with some difference in vascular status.

However, it is important to note that the pressure pulse under analysis here is not directly that of the eye's internal vasculature. The pulsation, although initially originating from the intraocular vessels, is dependent on: the volume change of those vessels and not their pressure change; on the dimensions of the eye; and on the elasticity of the ocular coat [23]. Therefore the differences found in this study may be caused by one or more of these possible factors – such as a difference in scleral rigidity.

Another explanation for this study's findings is that they are artefacts of the chosen method of waveform analysis. The spectral components of the Fourier transform can be greatly affected by truncation of the signal, that is, by inclusion of a partial waveform in the data set. If the sampled data set does not include an exact number of cycles, the introduction of partial waveforms can in-

duce spurious harmonics that may not be present in the spectra of the true waveform. Since the data collection in our study occurred over a relatively short time, i.e. 10 s, and since many patients had different heart rates (cycle lengths), each data set invariably contained partial cycle lengths. The resulting truncation could have biased the data by differentially influencing the percentage of power appearing in the harmonics for each group. However, as patients and subjects were similar for heart rate, it can be argued that truncation was not the cause of the differences. Since the heart rate determines the number of IOP pulse waves that occur during any 10-s time period, both groups, on average, would be expected to exhibit the same number of cycles within the sampling period and thus the same level of truncation. Of course, if the heart rates were not normally distributed in each group, then it is possible that some members in each group had heart rates that more (or less) closely matched the sampling period. Such clustering of patients could affect the level of truncation and thus differentially effect the overall power level in the harmonics in that group. A review of the data indicates that this is unlikely since the heart rate distribution within each group appears to be normally distributed, and as such, the level of truncation would not be clustered within a group, but would also be normally distributed.

As noted above, previous studies have demonstrated significant differences between glaucoma patients and age-matched normal subjects in ocular pulse volume, pulse amplitude and pulsatile blood flow. In contrast, patients and subjects in this study were not found to differ significantly for these measures. One dissimilarity between this study and the previous results is that in our study the groups were matched for heart rate. Heart rate has been shown to be significantly correlated to measures of the IOP pulse wave, i.e. the higher the heart rate, the lower the pulse amplitude, pulse volume and pulsatile ocular blood flow; with the groups matched for heart rate, however, this factor is eliminated. This significant correlation occurs because, as heart rate increases, there is less time between heart-beats for the intraocular vasculature to reach maximal distension. Our results suggest that when patients and subjects are properly matched for systemic factors that affect the IOP pulse wave, the harmonic content of the IOP pulse wave may be a better indicator for abnormalities in the ocular blood flow of glaucoma patients than pulse volume, pulse amplitude or pulsatile blood flow. Further, our results imply that systemic factors must not be ignored when utilizing the IOP pulse wave as a measure of ocular blood flow. Specifically, a normal subject with high heart rate may present with low pulsatile ocular blood flow simply due to the heart rate and not due to an ocular blood flow abnormality.

In summary, our results show that it is possible to measure the spectral components of the IOP pulse up to the fourth harmonic. The differences noted in the harmonic amplitudes between groups in this study suggest that some component of the IOP pulse (be it blood-vessel resistance or some other factor) varies in glaucoma. Measurement of the IOP pulse's harmonics may be an additional parameter, possibly more sensitive than those of pulse amplitude, pulse volume and pulsatile ocular blood flow. Further studies are required to determine the effect of altering confounding variables on the outcome of the analysis, and whether this method of IOP pulse investigation will have physiological and clinical utility in such areas as glaucoma.

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