ANALYSIS OF THE INTRAOCULAR PRESSURE PULSE

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Synopsis

The pulsation in intraocular pressure (IOP) which occurs with each heart beat presents a unique haemodynamic parameter that contains both cardiovascular and ocular properties. This IOP pulse may offer a readily observable sign that contributes to both the understanding of ocular vascular physiology and the detection and monitoring of sight-threatening diseases where haemodynamic malfunction is known or suspected. This thesis was concerned with investigating methods of improving the IOP pulse's potential as a measure of clinical utility. There were three principal sections to the work.

1. Optimisation of measurement and analysis of the IOP pulse
A literature review, covering the years 1960 – 2002 and other relevant scientific publications, provided a knowledge base on the IOP pulse. Initial studies investigated suitable instrumentation and measurement techniques. Fourier transformation was identified as a promising method of analysing the IOP pulse and this technique was developed. Specific findings were:

- The Ocular Blood Flow Analyzer (OBFA) pneumatonometer is superior to the Dynamic Observing Tonometer both in terms of accuracy of IOP measurement and in precision of pulse amplitude measurement,
- There is a period of learning in which a new operator masters measurements taken with an OBFA pneumatonometer,
- Repeated applications of a pneumatonometer result in a tonographic effect which influences both IOP and pulse amplitude measurements,
- A previously described spectral analysis technique, for calculating the waveform components of an IOP pulse, was improved upon by two alternate described methods in terms of both within- and between-visit reliability.

2. Investigation of ocular and systemic variables that affect IOP pulse measurements
In order to recognise clinically important change in IOP pulse measurement, studies were performed to identify influencing factors. Fourier analysis was tested against traditional parameters in order to assess its ability to detect differences in IOP pulse. In addition, it had been speculated that the waveform components of the IOP pulse contained vascular characteristics analogous to those components found in arterial pulse waves. Validation studies to test this hypothesis were attempted. Specific findings were:

- Corneal thickness affects IOP measurements made with the OBFA pneumatonometer but not pulse amplitude measurements. In contrast, both axial length and mean corneal radius influence the measurement of pulse amplitude but not IOP,
- The discovery that waveform components of the IOP pulse are highly frequency specific. This highlights the importance of comparing components by frequency rather than the previously established comparison of harmonic number,
- Compared to pulse amplitude, Fourier analysis gives greater confidence in detecting change; no evidence however was found to support the hypothesis that the waveform moduli contain additional vascular characteristics analogous to those found in arterial pulse waves.

3. The nature of the intraocular pressure pulse in health and disease and its relation to systemic cardiovascular variables
Fourier analysis and traditional parameters were applied to the IOP pulse measurements taken on diseased and healthy eyes. Only the derived parameter, pulsatile ocular blood flow (POBF) detected differences in diseased groups. The use of an ocular pressure-volume relationship may have improved the POBF measure's variance in comparison to the measurement of the pulse's amplitude or Fourier components. Specific findings were:

- Inferring total ocular blood flow changes from the behaviour of the IOP pulse may be erroneous due to shifts in the quotient of pulsatile to non-pulsatile flow,
- The POBF measure, indicative of the pulsatile fraction of total ocular blood flow, is reduced in patients with normal-tension glaucoma,
- The POBF measure is abnormally high in diabetic patients with background retinopathy and may be related to their excessive arterial pressure pulse values.

Finally, the importance of the driving force of pulsatile blood flow, the arterial pressure pulse, is highlighted. A method of combining the measurements of pulsatile blood flow and pulsatile blood pressure to create a measure of ocular vascular impedance is described along with its advantages for future studies.

Keywords: intraocular pressure pulse; pneumatonometer; Fourier analysis; glaucoma; diabetes mellitus

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I am forever grateful to my wife Lisa for her unfailing support and my two sons Rowan and Ned for helping keep life in perspective.

Finally I would like to dedicate this work to the subjects and patients who freely gave up their time to make this project possible.

List of Contents

List of Tables

List of Figures

List of Equations

Statement of authenticity

This thesis represents the work of Mr AJ Morgan during a 3 year research fellowship, sponsored by the College of Optometrists, at the Neurosciences Research Institute, University of Aston University in Birmingham, UK. Five of the studies were performed in collaboration with other researchers. The work on glaucoma and the Dynamic Observing Tonometer was performed with assistance from Mr JF Salmon (Consultant Ophthalmologist, Oxford Eye Hospital) who performed clinical diagnosis and Goldmann tonometry measurements. The work on diabetes was performed with assistance from Mr J Gibson (Consultant ophthalmologist, Birmingham Heartlands Hospital), and Dr P Dodson (Consultant physician, Birmingham Heartlands Hospital), who both performed clinical diagnosis. The work on corneal thickness was performed with the assistance of Dr J Harper who took the original Scheimpflug images. The pneumatonometric data used to compare age differences in IOP pulse harmonic components was supplied by Dr S Embleton. The work on the IOP pulse during hypercapnia was performed with the assistance of Dr E Roff-Hilton.

The author has no proprietary interest in the equipment used in this work.

List of abbreviations

IOP	Intraocular pressure
PA	Intraocular pressure pulse amplitude (peak to trough)
POBF	Pulsatile ocular blood flow
OBFA	Ocular Blood Flow Analyzer pneumatonometer
DOT	Dynamic Observing Tonometer (SmartLens®)
SBP	Systolic blood pressure
DBP	Diastolic blood pressure
APP	Arterial pulse pressure (SBP-DBP)
MAP	Mean arterial blood pressure (DBP + (1/3 * APP))
OPP	Ocular perfusion pressure ((2/3*MAP)-IOP)
SPCA	Short posterior ciliary artery
CRA	Central retinal artery
CCT	Central corneal thickness
POAG	Primary open-angle glaucoma
NTG	Normal-tension glaucoma
LDF	Laser Doppler flowmetry
NS	No significance
SD	Standard deviation
CoR	Coefficient of reliability

"The information which the pulse affords is of so great importance and so often consulted, surely it must be to our advantage to appreciate fully all it tells us, and to draw from it every detail that it is capable of imparting"

Frederick A. Mahomed, 1872.

"Jam observivamus ordinatam oscillationum aquae manometri, cum systole et diastole isochronicarum, musculis oculi non dissectis."

"*Now we have observed the sequence of pressure change in the aqueous fluid, synchronised with systole and diastole, when the muscles of the eye have been removed.*"

Carl Weber, 1850.

1. Introduction: The Intraocular Pressure Pulse

1.1 *Background*

The rhythmic variation in intraocular pressure (IOP) that occurs with each heart beat has been studied for over a century and a half (Weber, 1850). This IOP pulse provides a unique measure of the physiological and pathological interplay between vasculature and ocular structure. Many factors have the potential to influence the IOP pulse: from its origin at the heart to its final measurement at the eye. By detailing those influential factors and assessing relevant studies, the purpose of this review is to provide the necessary background to interpret this small perturbation in IOP.

The review defines the IOP pulse and its relation to other cyclic IOP changes. A brief historical synopsis details early fundamental observations. The origins of the IOP pulse start at the heart and the subsequent wave of distension that spreads out across the arterial tree with each contraction. Important cardiovascular components responsible for the pulsatile quality of blood flow in the human body include stroke volume, heart rate, and arterial anatomy. How investigators have modelled the flow of blood in arteries (haemodynamics) is reviewed and the contour of the arterial pressure pulse examined. The level of blood flow to an end-arterial bed (in this case, the eye) is dependent on, in addition to the arterial pressure, the resistance (or impedance) of that vascular bed. The major determinant of that vascular resistance is through the regulation of the lumen diameter of the small arterial vessels.

Having arrived at the eye, the relevant ocular vascular anatomy and physiology is covered. The circulations of the retina and optic nerve head are described but particular attention is given to the vascular bed that is the major contributor to the IOP pulse: the tunica uvea. The control of blood flow to the eye, both systemically and at a local level is described.

How a change in IOP arises from the pulsatile influx of blood volume to the eye's internal vasculature needs to be understood. This ocular pressure-volume relationship has been studied from a number of theoretical and experimental observations. Studies on the IOP

pulse are reliant on the accuracy and validity of the instrumentation and methods of analysis used. Current techniques to measure and analyse the IOP pulse are therefore reviewed.

This background information will provide the reader with the necessary basic theory to best interpret the numerous studies on the IOP pulse that are reviewed. These studies can be categorised as observations on the IOP pulse associated with a physiological or pathological change, or medical intervention.

1.1.1. Cyclic variations in intraocular pressure

IOP displays a number of cyclic variations of both long and short duration (Schottenstein, 1996). The slowest rhythmic change occurs annually with IOP rising in the winter, by the order of 2 mmHg, compared to that in the summer (Hart, 1992; Shiose, 1990). Reports of monthly IOP variations in women, synchronous with the menstrual cycle, are controversial and are not supported by large scale studies (Guttridge, 1994; Qureshi, 1997). Diurnal change in IOP has been extensively studied. The majority of studies show that peak IOP occurs in the early hours of the morning, often prior to waking and falling to its nadir in early evening (Hart, 1992). A peak to trough amplitude of up to 5 mmHg is the expected normal change in a 24 hour period (Zeimer, 1996). The most physiologically satisfactory study of diurnal IOP variation has been made by Liu, Kripke, Twa *et al.* (1999b). Whereas many studies measured nocturnal IOP by waking a patient and sitting them at a slit-lamp, Liu and co-workers took supine IOP measurements using a pneumatonometer. In healthy elderly subjects, the trough of IOP appeared at the end of the light/wake period and the peak at the beginning of the dark period. The main factor in night-time IOP elevation appeared to be the shift to a supine position.

Shorter term IOP variations, of an amplitude approximately 10 to 20% that of the IOP and a cycle length of 10 to 60 seconds, have been described in tonographic and manometric studies (Becker & Friedenwald, 1953; Krishna & Botelho, 1963). As they are in synchrony with diastolic arterial blood pressure, their origin has been attributed to the vasomotor activity in the body's arterial network. Some authors refer to them as Trau*ße*-Hering waves (Leydhecker, 1976). The respiratory cycle is associated with an approximate 4 mmHg

change in IOP (Leydhecker, 1976): inspiration producing a rise in IOP and expiration, a fall.

The rhythmic variation in IOP that has attracted the most interest in research and clinical investigation is that associated with the cardiac cycle. This small pulsation in IOP, of the order of 15% of the IOP, is influenced by numerous physiological and pathological factors and is the subject of the remainder of the review.

1.1.2. Historical perspective

In 1850, Weber, whilst taking measurements on live animal eyes as they accommodated, first noted the IOP pulse and attributed it to the changes in pressure of the choroidal and ciliary vasculature. The first quantified measurements of the IOP pulse were made by Bellarminoff (1886). He again recorded IOP manometrically on a number of living animals and found the IOP pulse to be in the range of 1 to 2 mmHg. By the close of the 19th Century the IOP pulse was a known cause of variation in the IOP amongst ophthalmologists (Smith, 1891a). Wessely (1908) studied a number of influences on the IOP pulse in animals and was the first to report that, as IOP increased, the IOP pulse increased until, when IOP surpassed arterial blood pressure values, the pulse became extinguished. He also showed that the waveform is normally the same in right and left eyes in amplitude, frequency and phase and that stimulation of the vagus nerve produced a fall in IOP pulse frequency.

At the start of 20th century, although the IOP pulse was readily discernable as a cyclic variation in the plunger of an indentation tonometer, further investigation was limited due to the inability to record the event accurately (Schiotz, 1920). Grant (1950) introduced an electronically recording tonometer based on the Schiotz design in 1950 and successfully recorded the cyclical variations in IOP over a number of minutes (Becker *et al.*, 1953). Similar electronic indentation tonometers soon followed (Bynke & Krakau, 1964; Hørven, 1968; Maurice, 1958). All indentation tonometers displaced a not insignificant amount of aqueous fluid over time and it was only with the introduction of continuously recording applanation tonometers that the accurate study of the IOP pulse with minimal disturbance to the living human eye could be performed (Perkins, Edwards & Saxena, 1977; Thorburn, 1972; Tønjum, 1972).

1.2. *Cardiovascular anatomy and physiology*

The origins of the IOP pulse start at the heart. Cardiac influence on the wave of arterial distension that travels from the heart to the eye affects the character of the IOP pulse.

On contraction of the heart's left ventricle (cardiac systole) a new bolus of blood enters the proximal aorta producing the wave of distension and pressure that radiates throughout the arterial network (Levick, 2000d). Immediately before this ejection of blood, the left ventricle is primed with freshly oxygenated blood from the left atrium. On commencing contraction, the left ventricle causes a steep rise in its chamber's pressure which forces the fibrous valve between atrium and ventricle (mitral valve) closed (Figure 1.1). At the peak of systolic contraction, when ventricular blood pressure surpasses aortic blood pressure, the aortic valve opens and a bolus of blood enters the aorta. As the ventricular contraction wanes, the pressure gradient reverses across the threshold of the aorta and the outflow valve closes. The sudden closure of the aortic valve produces a distinctive kink in the pressure pulse of the body's larger arteries known as the dicrotic notch or incisura (Noble, 1968).

1.2.1. Stroke volume

The volume of blood ejected per contraction is defined as the stroke volume and, in a normal resting adult, is 70 – 80 ml (Berne & Levy, 1997b). Stroke volume is not fixed and is governed by three factors:

Pre-contractile stretch in cardiac muscle fibres

The amount of stretch cardiac muscle fibres (myocytes) experience before contraction, determines their contractile force. This is an ability also found in skeletal muscle fibres and is described by the Frank-Starling law: 'The energy of contraction of a cardiac muscle fibre, like that of a skeletal muscle fibre, is proportional to the initial fibre-length at rest'. The mechanism for this increase in contractility is due to the improved efficiency of the contractile elements (actin-myosin cross bridges) within the muscle filaments (sarcomeres) and their increased sensitivity to the depolarising agent (intracellular Ca^{2+}) that initiates contraction (Allen & Kentish, 1985).

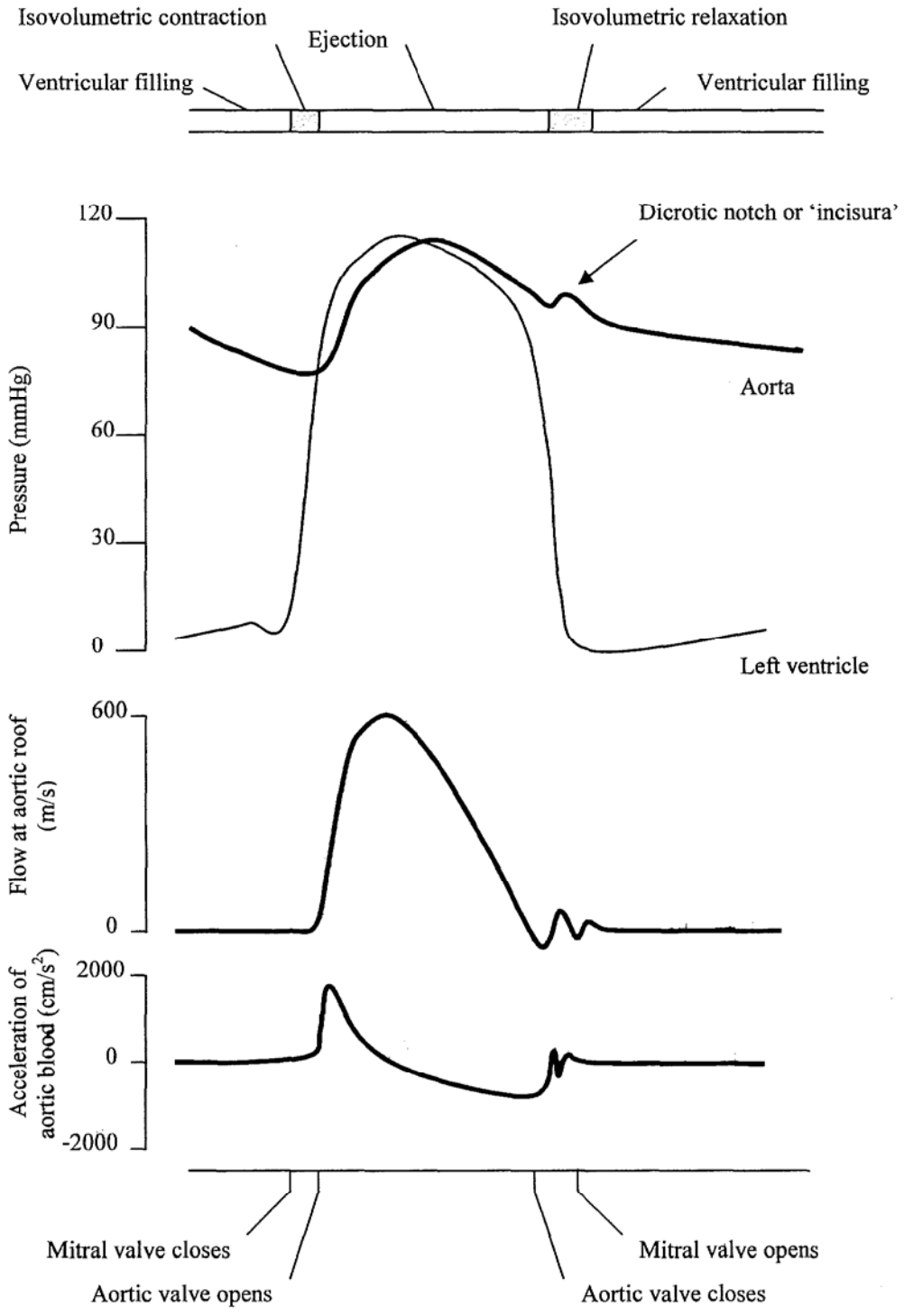

Figure 1.1 **Blood pressure, flow and acceleration relationships in the left ventricle and aorta. Adapted from Noble (1968).**

The consequence of the Frank-Starling law is that the amount of stretch or preload in the ventricle before systole determines the subsequent force of contraction. Any change in the amount of blood in the ventricle before contraction (the end-diastolic volume) will therefore change stroke volume. Matching ventricular contractile force to end-diastolic volume, helps the heart self-regulate changes in blood arriving from the venous side of the circulation.

Common physiological changes in end-diastolic volume are due to respiration, exercise and a shift in posture. For example, when lying down from a standing position, venous blood (that was previously pooled in the lower limbs) redistributes throughout the body and increases left ventricular volume and pressure. Were it not for the Frank-Starling mechanism, the heart's ventricles would become dangerously engorged from the increased volume of blood not being pumped on (Braunwald & Ross, 1979).

The influence of extrinsic factors on the contractility of myocytes

The contractile energy of a myocyte, independent of its pre-contractile stretch length, is known as its inotropic state and is influenced by a number of extrinsic regulators. In man, the most important of these inotropic factors is the amount of noradrenaline released from sympathetic nerve endings in the walls of the ventricle. Noradrenaline increases the amount of free intracellular Ca^{2+} in the myocytes which produces a shorter, more powerful contraction. Other circulating inotropic factors include adrenaline and angiotensin II. Although parasympathetic fibres innervate the heart, in man they are predominantly connected with the pacemaker (the Sino-Atrial node) and conduction system (Atrio-Ventricular node and Purkinje fibres) and therefore only contribute to slowing heart rate rather than affect contractility of the myocardium (Levick, 2000a).

Opposing arterial pressure

During systole, left ventricular pressure has to surpass aortic pressure before a quantity of blood can be ejected (Figure 1.1). As aortic arterial pressure rises, the volume of blood that can be ejected by the heart becomes smaller (Berne *et al.*, 1997b).

Alteration in stroke volume, coupled with the ability to vary the number of ventricular contractions per minute (heart rate), allows total cardiac output in a normal healthy adult to vary from 5 to 25 litres per minute (Nichols & O'Rourke, 1998c).

1.2.2. Heart rate

An increase in heart rate influences diastole more than systole, as the duration of ventricular ejection increases relative to the period of diastole (Levick, 2000a). A higher heart rate produces a more symmetric arterial pressure pulse contour. Heart arrhythmia will influence stroke volume and arterial pressure pulse from one beat to the next. Sinus arrhythmia, a physiologically normal arrhythmia found commonly in healthy young adults, is a change in heart rate and stroke volume that accompanies the respiratory rhythm (Figure 1.2). On inspiration, thoracic pressure increases and retards venous inflow into the left ventricle. This reduces stroke volume and to compensate, heart rate increases (tachycardia). On expiration, the drop in thoracic pressure causes an influx of blood into the ventricles. Stroke volume increases and heart rate falls (bradycardia) respectively (Waldo & Wit, 1993).

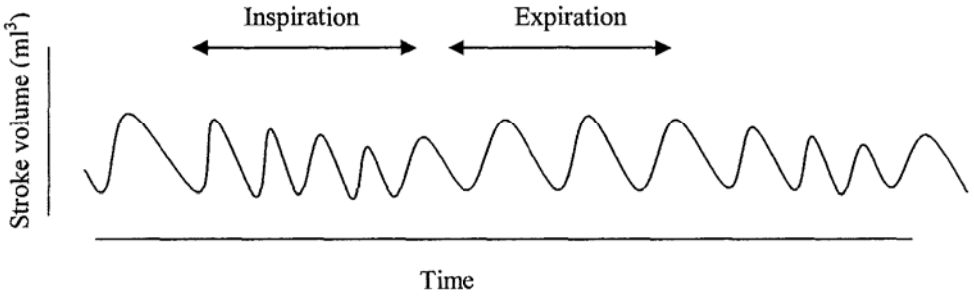

Figure 1.2 **Schematic of a typical recording of ejected blood volume with each heart beat (stroke volume) exhibiting sinus arrhythmia. Adapted from Waldo *et al.* (1993)**

Pathological arrhythmias similarly influence stroke volume due to the irregular effect on left ventricular filling time and myocyte contraction. For example ectopic beats produce a premature ejection with a smaller stroke volume. This is then followed, after an abnormally long diastolic period (a 'skipped' beat) by an exaggerated stroke volume due to the long filling time. Other cardiac abnormalities, for example aortic stenosis and aortic incompetence, produce characteristic changes in the profile of the resultant arterial pressure pulse (Mills, Gabe, Gault *et al.*, 1970): Figure 1.3.

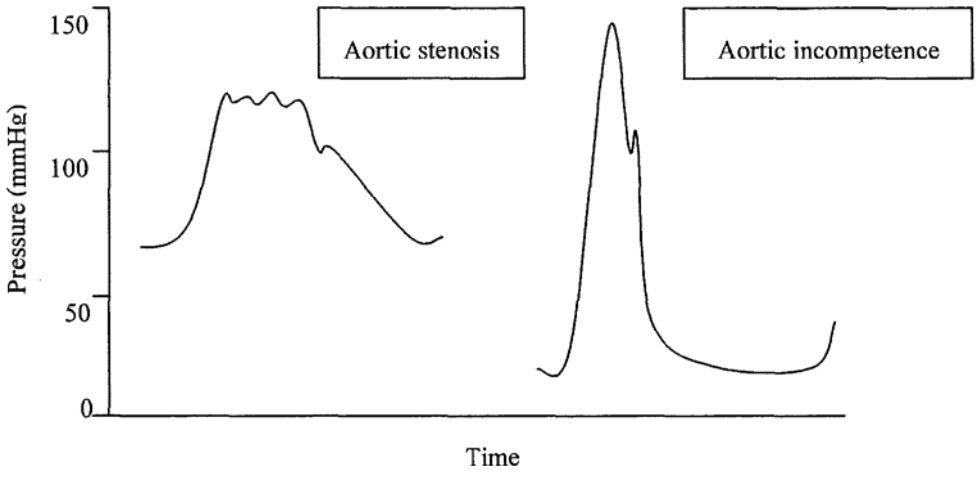

Figure 1.3 Examples of abnormal arterial pressure pulse contours arising from cardiac disease. Adapted from Mills *et al.* (1970)

1.2.3. Arterial anatomy and physiology

Artery walls are composed of a trilaminar structure (Nicholls & O'Rourke, 1998b):

Tunica intima

An innermost layer composed of endothelium cells lining a basement membrane.

Tunica media

A central layer of smooth muscle cells, elastin and collagen. Elastin, which is six times more extensible than rubber, provides the artery with the ability to distend under pressure. Collagen, being 100 times stiffer than elastin, prevents the artery exceeding its elastic point. It is the contraction of smooth muscle fibres that produces a narrowing of the vessel's lumen (vasoconstriction) whilst their relaxation, coupled with the force from the blood pressure, increases lumen diameter (vasodilatation).

Tunica adventitia

An external layer that comprises of connective tissue that tethers the artery to its surrounds and, in the larger arteries, contains its own vascular network (vasa vasorum).

Arteries progressively diminish in diameter and increase in number as they progress distally from the heart. Arteries can be functionally divided in to the following (Levick, 2000d):

Elastic arteries

Of 1-2 cm in diameter, elastic arteries have very distensible walls to allow them to accommodate the large change in volume on receiving the stroke volume of blood. These arteries (such as the aorta and iliac) have a high percentage (40%) of elastin in their medial layer and a relatively small amount of smooth muscle (25%).

Conduit arteries

Arteries, such as the carotid, are 0.1 – 1 cm in diameter and have a thicker wall in relation to their lumen than elastic arteries. This prevents the arteries from collapsing at sharp bends in the body's anatomy.

Resistance arteries

Due to their limited number and narrow lumens, the terminal arteries (100 to 1000 μm in diameter) and arterioles (40 to 100 μm) produce the greatest resistance to the body's circulating blood. It is also here that blood experiences its greatest drop in pressure. The vessel wall's composition now comprises approximately 10% elastin and 60% smooth muscle. The muscular vessel wall is also relatively thick compared to the lumen diameter (ratio of 1:1) and is highly innervated by the autonomic nervous system. Vasoconstriction and vasodilatation of these vessels allow efficient regulation of down stream blood flow.

1.2.4. Arterial compliance

How much an arterial wall expands due to a change in blood pressure is a measure of its distensibility or compliance (Berne & Levy, 1997a). Compliance can be defined as shown in Equation 1.1.

$$\text{Compliance} = \frac{\text{Increase in volume}}{\text{Increase in presssure}}$$

Equation 1.1 Relationship describing compliance of a vessel.

A number of factors affect the compliance of an arterial wall (Nicholls *et al.*, 1998b):

- The relative proportion of elastin and collagen in the tunica media.
- The age of the artery. Elastin fibres become fragmented and degenerated with time and there is a concomitant rise in the amount of collagen.
- The level of mean arterial pressure and the amplitude of the pressure pulse. In large elastic arteries, high mean blood pressure and pulse pressure stretches the arterial wall to its elastic limit producing a smaller volume change per unit change in pressure.
- High ejection velocities reduce compliance because the arterial wall, having viscoelastic properties, requires time to expand.
- The level of contraction in the vessel wall's smooth muscle. The effect of a change in smooth muscle tone is not uniform amongst artery types. In the smaller arteries and arterioles, a relaxation in smooth muscle fibres produces a more distensible vessel. However in a large central elastic artery, such as the aorta, a relaxation in smooth muscle tone may produce an increase in wall stiffness due to the increased vessel diameter stretching the fibres to their elastic limit.

Due to the changing nature of an arterial wall's compliance, many investigators use an alternative expression, the incremental elastic modulus (Peterson, Jensen & Parnell, 1960):Equation 1.2.

$$E_p = \frac{D_d(P_s - P_d)}{D_s - D_d}$$

E_p = incremental elastic modulus at a particular mean arterial pressure

D_d = arterial diameter at diastolic pressure

D_s = arterial diameter at systolic pressure

P_d = diastolic pressure

P_s = systolic pressure

Equation 1.2 An incremental elastic modulus expression for an arterial vessel (Peterson *et al.*, 1960).

Although both describing a measure of arterial distensibility, compliance and elastic modulus are inversely related. For example, a highly distensible artery would be said to have high compliance but a low elasticity modulus. A synonym for elasticity therefore, in its scientific sense, is the degree of material 'stiffness'.

1.3. *Vascular haemodynamics*

Haemodynamics describe the mechanical properties of blood in terms of its pressure and flow (Levick, 2000c).

1.3.1. Models of flow and pressure haemodynamics

A simple model for blood flow in an artery is that of Poiseuille's law of flow in a single tube (Pappenheimer, 1984): Equation 1.3.

$$Q = \frac{\pi r^4 (P_a - P_v)}{8nl}$$

Q = flow

Pa = arterial blood pressure

Pv = venous blood pressure

l = vessel length

r = vessel radius

n = blood viscosity

Equation 1.3 Poiseuille's law of flow.

One can see from Poiseuille's law that flow, as well as being linearly related to the pressure differential along a tube, is extremely sensitive to the radius of the vessel. A mere 16% reduction in vessel radius halves the flow. Although Poiseuille's law is a useful relationship to describe blood flow it is dependent on a number of assumptions which, as will be seen, are not truly appropriate for arterial blood flow (Nichols & O'Rourke, 1998b):

- The liquid behaves as a homogenous fluid that does not alter its viscosity. Although this assumption holds for large arteries, at the level of smaller arterioles the particulate nature of blood produces variations in viscosity.
- The rate of flow is 'steady' and is not subjected to acceleration or deceleration. This is clearly not true for arteries as blood flow, down to the level of the capillaries, is pulsatile in nature.
- The tube is rigid and the diameter does not vary with the internal pressure. Again due to the viscoelastic nature of arterial walls, this assumption is invalid.

Due to these inherent limitations of using Poiseuille's law for arterial blood flow, Womersley (1955) formulated an expression relating flow for pulsatile pressure (Equation 1.4). Of importance is that the expression states that pulsatile flow is dependent on the gradient of the pressure pulse, rather than its amplitude. That is, it is the steepness of the arterial pressure pulse upslope that drives arterial blood flow forward.

$$Q = \frac{\pi R^4 M}{\mu} \frac{M'_{10}}{\alpha^2} \sin(\omega t - \phi + \varepsilon_{10})$$

Q =	Flow	R =	Vessel radius
μ =	Fluid viscosity	M =	The 'real' part of the pressure gradient
ω =	Frequency of the oscillatory cycle	t =	Time

α = A non-dimensional function of the radius, frequency and kinematic viscosity

M' = The modulus of the 'imaginary' component of the pressure gradient

ϕ = The phase of the 'imaginary' component of the pressure gradient

ε_{10} = A phase adjustment factor that is dependent upon heart frequency

Equation 1.4 The relation of flow to an oscillatory pressure gradient (Womersley, 1955).

1.3.2. Arterial pressure pulse and flow contour

The rapid introduction of blood volume into the elastic aorta causes a wave of arterial distension (radial pulsation) and pressure change (pressure pulsation) to course up the arterial tree and spread peripherally to the smaller arteries and arterioles. This pulse wave travels at a velocity of between 2 and 10 m/s and is of an order of velocity higher than mean blood flow: the approximate mean blood velocity in the aorta, 0.2 m/s (Levick, 2000c). The velocity of pulse travel in an artery is dependent upon its degree of distensibility and can be calculated from the Moens-Korteweg equation (Nicholls & O'Rourke, 1998c): Equation 1.5.

$$C_0 = \sqrt{Eh / 2R\rho}$$

C_0	= velocity of wave propagation	E	= elasticity modulus
h	= thickness of the arterial wall	R	= radius of the artery
ρ	= blood density		

Equation 1.5 The Moens-Korteweg equation for velocity of a pressure pulse wave in an artery.

The Moens-Korteweg equation shows that as the elasticity of an artery increases (that is the artery wall becomes stiffer), the velocity of the wave propagation increases. This explains the observed increase in arterial pulse speed seen with age; pulse velocity increases from approximately 2 ms-1 in a healthy adolescent to 10 ms-1 in the elderly. An example of a normal pressure pulse and its corresponding flow wave taken from the aorta of a young human adult (approximate age 20 years) is shown in Figure 1.4 (Nicholls & O'Rourke, 1998a).

The initial systolic phase of the pressure pulse induces the majority of aortic blood flow. Due to the closure of the mitral valve, an inflection occurs in the pressure pulse (the dicrotic notch) with a brief reversal in flow. The peak and trough of the pressure pulse waveform are the systolic and diastolic blood pressure values. The systolic to diastolic arterial pressure range is known as the arterial pulse pressure (APP): Equation 1.6.

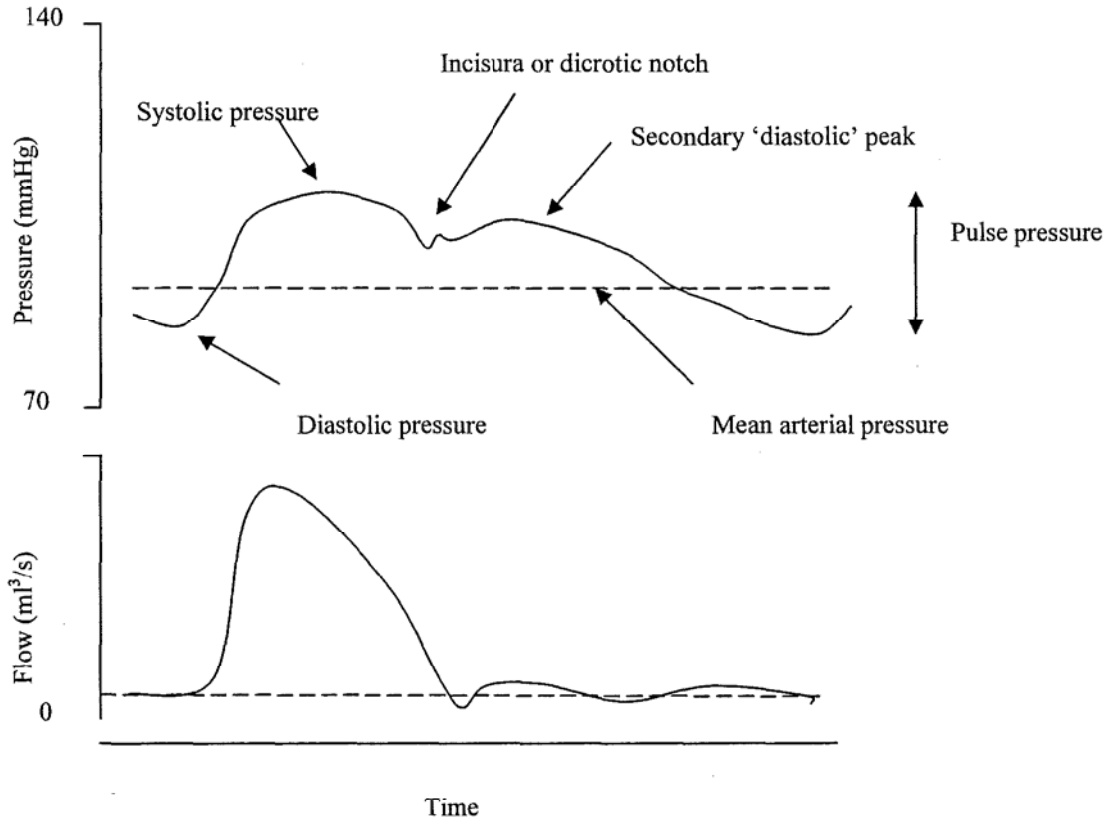

Figure 1.4 **An example of an arterial pressure pulse and its flow wave from an aorta of a young healthy adult. Adapted from Nicholls *et al.* (1998a)**

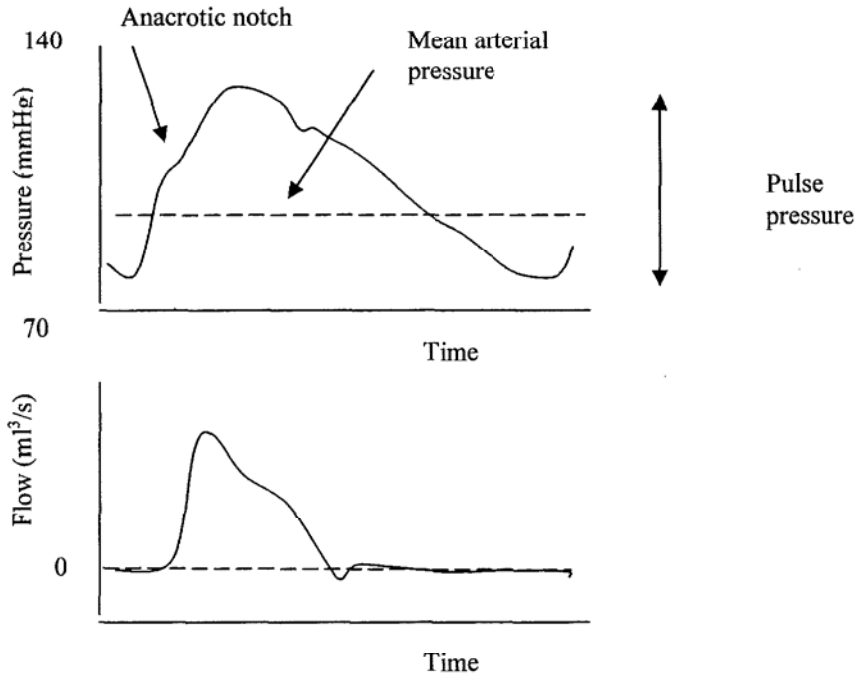

Figure 1.5 **An example of an arterial pressure pulse and its flow wave from an aorta of a middle aged subject. Adapted from Nicholls *et al.* (1998a)**

Arterial Pressure Pulse	=	Systolic Blood Pressure – Diastolic Blood Pressure

Equation 1.6 Calculation of arterial pulse pressure.

Mean arterial blood pressure (MAP) can be calculated by integrating the area under the pressure wave ($\int P.dt$). Or, as a rule of thumb, mean arterial pressure approximates to a third of the pressure pulse added to the diastolic value (Levick, 2000c): Equation 1.7.

Mean arterial blood pressure = 1/3 arterial pulse pressure + diastolic blood pressure

Equation 1.7 Clinical estimate of mean arterial blood pressure.

The arterial pressure pulse contour is also influenced by reflected pressure waves. Reflections in pressure or flow waves occur at any discontinuity in the arterial tree, be it a branching of an artery, an area of alteration in arterial distensibility, and, very importantly, the high-resistance arterioles (Nichols & O'Rourke, 1998h). These reflections can either add to (a 'positive' reflection) or subtract from (a 'negative' reflection) the incident pressure pulse. A positive reflection occurs at a partial closure of the arterial system, for example the high-resistance arterioles, and a negative reflection occurs at an opening, such as an arteriole sinus. Studies using peripheral vasodilating medications have shown that wave reflections are almost entirely due to arteriolar tone in the peripheral vascular bed (Westerhof, Sipkema, Van den Bos *et al.*, 1972).

In the young adult, due to their relatively slow pulse velocity, a pressure wave reflection occurs in the diastolic portion of the pressure pulse producing a characteristic diastolic peak (Figure 1.4). This causes little change in the flow wave. With advancing age, and increasing wave velocity, the wave reflection advances forward along the pulse contour (Nicholls *et*

al., 1998a): Figure 1.5. By middle age the reflected pressure wave coincides with the systolic portion of the pulse, augmenting its profile and producing a notch on its ascending limb (the anacrotic notch).

This early reflection component in an older subject's pressure pulse impedes ventricular ejection and produces a depression in the outflow pulse (the concavity in the descending limb of the flow pulse). In the elderly and hypertensive, the reflected pressure continues to influence the pressure pulse profile by augmenting the systolic peak and producing a subsequent rapid drop off in pressure during the diastolic phase (Black, Kuller, O'Rourke *et al.*, 1999). Such changes in the pressure and flow contours occur in all major arteries and depend on the local characteristics of the arteries and wave reflections present in that portion of the body (O'Rourke, 1999b).

The shape of the pressure pulse changes as it travels out through the arterial tree. Due to the increase in elastic modulus of the smaller arteries, the pressure pulse increases distally from the heart. For example, the pulse pressure of the radial artery is 50% greater than that of the aorta. Further wave travel into the smaller arteries and arterioles progressively dampens out the pulsations due to the viscous properties of blood and artery wall. Oscillations in pressure are practically extinct at the capillaries (Berne *et al.*, 1997a).

1.3.3. Vascular resistance

The degree of opposition, or resistance, blood flow experiences in an artery or network can be expressed in an analogous form to Ohm's law for an electrical current (Equation 1.8). In fluid dynamics, this relationship is known as Darcy's law of flow and, from Poiseuille's law (Equation 1.3), it can be seen that resistance is equal to Equation 1.9. Resistance to blood flow is therefore proportional to the tube length and blood viscosity and inversely proportional to the fourth power of the vessel radius.

$$Q = \frac{P_1 - P_2}{R}$$

Q = Flow $\quad$ $P_1 - P_2$ = Pressure gradient

R = Resistance

Equation 1.8 Darcy's law of flow.

$$R = \frac{8nl}{\pi r^4}$$

R = resistance $\quad$ l = vessel length

r = vessel radius $\quad$ n = blood viscosity

Equation 1.9 Resistance to fluid flow in a rigid vessel.

This simple description of opposition to flow does not however describe the behaviour of flow in a pulsatile system. A more exact description can be made, again taken from the analogous behaviour of electrical current flow, by calculating the impedance of a vascular system.

1.3.4. Vascular Impedance

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 (Nichols, Conti, Walker *et al.*, 1977). 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. The measurement of impedance (strictly known as input impedance) at a certain site of an artery represents the impedance for that vascular bed supplied by that artery. Impedance is the ratio of the harmonic terms of pressure to the corresponding harmonic terms of flow (Equation 1.10).

$$\text{Vascular impedance (at a particular frequency)} = \frac{\text{Harmonic of pressure at that frequency}}{\text{Harmonic of flow at that frequency}}$$

Equation 1.10 Vascular impedance.

Figure 1.6 illustrates the calculation of impedance in a typical large artery. (O'Rourke & Taylor, 1966) Blood pressure and blood flow measured at a point in an artery are deconstructed, via Fourier analysis (Appendix 2), into mean values and a series of harmonic components that fall at multiples of the heart rate frequency. Impedance is commonly described by its moduli components (modulus of pressure (P) divided by modulus of flow (Q) at each frequency), and their phases (the delay between each pressure and flow harmonic component). The calculation of impedance for a given artery provides a method of describing the degree of opposition pulsatile blood flow experiences from a downstream vascular bed. The impedance of a vascular bed is primarily determined by the degree of distensibility (or capacitance) of the end-arterial vessels. Figure 1.7 shows the effect of a vasodilatation and vasoconstriction upon the impedance spectra of a femoral artery (O'Rourke, 1981).

Vasodilatation of the peripheral bed flattens the spectra of impedance moduli and shifts the lowest point to the left. The movement of the profile's lowest point represents a reduction in wave reflections from the increased dampening on the pulse wave as it impacts upon the more dilated and compliant end-arterioles. Conversely vasoconstriction increases impedance moduli and their nadir shifts to the right as the end-arterioles contract and produce a more resonant terminus for the pulse waves. The effect on phase shift produces a similar response: vasodilatation sees a greater synchrony of pressure and flow waves and vasoconstriction produces a greater delay in pressure harmonics compared to flow. The amount of impedance change induced by a vasoactive agent in a particular artery depends upon the usual degree of smooth muscle tonus of the end-arterioles. Most vascular beds, having a high level of tonus, have a greater response to a vasodilating agent than a vasoconstrictor. Other vascular beds, such as renal, are in greater state of vasodilatation and a large impedance shift is seen on vasocontraction (Nichols & O'Rourke, 1998g).

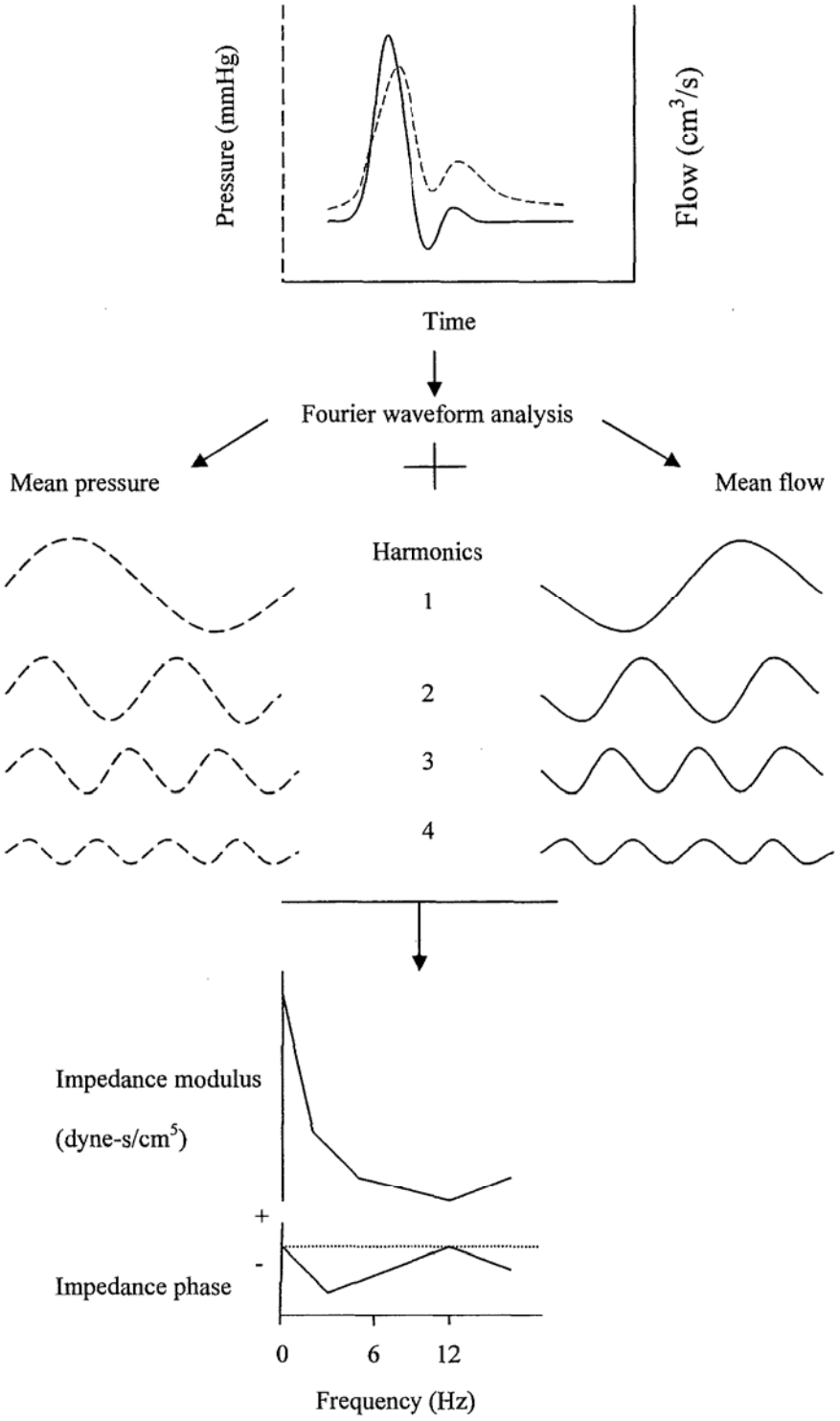

Figure 1.6 **The use of Fourier analysis to determine vascular impedance. Pressure (dotted line) and flow (solid line) measured in an artery are decomposed into mean values and a series of harmonic waves at multiples of heart rate frequency. Impedance modulus (top line of lower panel) is the modulus of pressure divided by the modulus of flow (P/Q) at the different frequencies, and phase (bottom line of lower panel) is the delay between pressure and flow harmonics. Adapted from O'Rourke & Taylor (1966).**

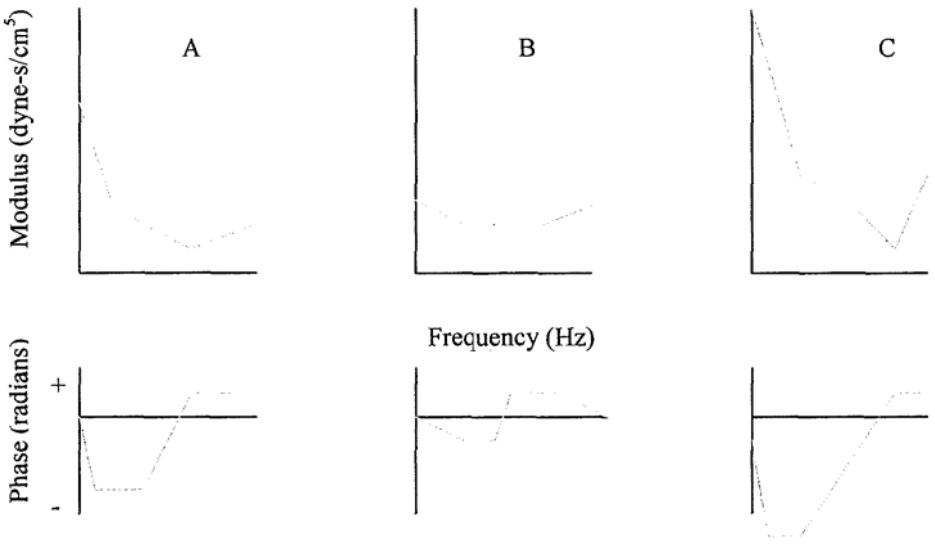

Figure 1.7 **Experimentally determined values of impedance modulus (above) and phase (below) in the dog femoral artery under: A, control conditions; B, following intra-arterial injection of acetylcholine (vasodilator); and C, norepinephrine (vasoconstrictor). Adapted from O'Rourke (1981).**

1.3.5. Control of arterial blood flow: local mechanisms

The supply of blood to a particular organ depends on the interplay of local and external factors on the tissue's vessels and the cardiovascular system as a whole (Levick, 2000d). General principles of regulation of blood flow will be covered here and those controlling factors specific to the eye will be covered later (1.4.6).

The cardiovascular system provides arterial blood at high pressure throughout the body in order that individual organs can tap off the necessary blood they require. It is through the control of an arterial vessels' radius that the tap mechanism functions. In most tissues, the location of these critical vessels occurs at the small arteries and arterioles where their small lumens and relatively sparseness combine to produce the highest site of resistance in the cardiovascular system (Berne *et al.*, 1997a). Some tissues, such as the skin, have bands of smooth muscle surrounding the entrance to a capillary bed and these are known as precapillary sphincters (Braverman, 1997). The exact amount of blood tapped by an individual organ is controlled by a number of mechanisms:

- Those operating wholly within the organ itself and known as local or intrinsic control mechanisms.

- Those that originate outside the organ and are known as extrinsic mechanisms or regulators.

The Bayliss myogenic response

Most arterioles and many cerebral arteries exhibit the phenomenon of vasoconstriction following a rise in pressure across the vessel wall (Levick, 2000b). This increase in transmural pressure may occur either as a rise in blood pressure or as a drop in extravascular tissue pressure. Stretch sensitive ion channels, found in the myocytes, are the main mechanism responsible for this phenomenon. An increase in blood pressure at first distends an arteriole and stretches the myocytes in the tunica media. Stretch-activated cation channels allow an increase in cytosolic Ca^{2+} which increases contractility (Meininger & Davis, 1992).

Endothelial vasoactive secretions

The endothelium produces both vasodilator (e.g. prostacyclin, PGI_2) and vasoconstrictor (e.g. endothelin or ET1) agents that act on the adjacent myocytes. Of these, the vasodilator nitric oxide (NO) is the most important (Palmer, Ashton & Moncada, 1988). The main physiological stimulant for NO production is shear stress on the endothelial wall produced by the flow of blood. Vasodilatation, induced by an increase in blood velocity, provides the important mechanism for feeder arteries to satisfy their vascular beds: that is, as blood demand at an organ rises, velocity increases in the up-stream artery which in turn produces vasodilatation that helps match blood supply (Melkumyants, Balashov & Khayutin, 1995).

Metabolic vasoactive factors

In the majority of tissues metabolic demand is tightly matched by blood supply. Metabolic activity produces numerous chemical agents that have a vasodilatory affect on nearby arterial walls, particularly those of the resistance arteries and arterioles. Cerebral vessels, for example, are particularly sensitive to the level of carbon dioxide (CO_2) and potassium ions in the interstitial fluid (Lassen, 1964).

Autocoids

A tissue may also produce a number of vasoactive agents in response to a pathological event. The majority of these local hormones (autocoids), such as histamine and bradykinin, have a vasodilatory effect in addition to their other roles in the inflammatory quartet (Renkin, 1984).

The above mechanisms combine to produce an important role in a tissue's blood supply. Taking metabolic demand to be constant, blood flow to an organ is remarkably stable over a wide range of perfusion pressures. This ability, which is in apparent defiance of Poiseuille's law, is known as **autoregulation**. Autoregulation can be defined as 'the ability of a vascular bed to keep blood flow constant despite changes in perfusion pressure' (Johnson, 1964). It is brought about by a combination of the Bayliss myogenic response and the degree of 'wash-out' of the various vasoactive chemicals in the blood stream. For example, a rise in arterial blood pressure produces both an increase in transmural pressure and a flushing out of NO which both lead to vasoconstriction. This balances the level of resistance to that of perfusion pressure in order to produce constant blood flow (Levick, 2000b).

1.3.6. Control of arterial blood flow: extrinsic mechanisms

The main external regulator on arterial wall calibre is the autonomic nervous system (Berne & Levy, 1997c). Vasoactive nerve endings richly innervate the resistance vessels of the body. The sympathetic arm of the autonomic nervous system dominates (Jänig, 1988) and produces vasoconstriction in those arteries with α adrenoreceptors (found in the majority of arterioles such as skin and intestine). Its effects are mediated by the classic sympathetic neurotransmitter, noradrenaline, and other adrenergic neurotransmitters such as adenosine triphosphate and Neuropeptide Y. Sympathetic vasoconstrictor nerves continually discharge producing a tonic level of vasoconstriction in an artery. In contrast to the ubiquitous sympathetic vasoconstrictor fibres, parasympathetic innervation of arteries is limited to select tissues such as the cerebral and coronary arteries (Neal, 1992). They are not tonically active and on stimulation cause vasodilatation. The neurotransmitters used include acetylcholine, vasoactive intestinal polypeptide and nitric oxide. Some vasodilatation is mediated by sensory nerves. For example when the skin is scratched, vasodilatory mediators (substance P and calcitonin-gene related peptide) are released from adjacent

sensory fibres which produce a rapid reddening around the area of trauma (Lynn & Cotsell, 1991).

Circulating hormones are also responsible for extrinsic control on the body's arteries (Renkin, 1984). Adrenaline and noradrenaline, produced from the adrenal gland, cause vasoconstriction in arteries with α receptors and vasodilatation in those with β_2 receptors (for example, found in skeletal and heart muscle). Other vasoactive circulating hormones include the oestrogens (vasodilators), insulin (vasodilator) and angiotensin II (vasoconstrictor).

1.3.7. Summary of the cardiovascular elements pertinent to the IOP pulse

The IOP pulse originates at the heart during systolic contraction. Stroke volume and heart rate are the primary cardiac factors that contribute to the characteristics of the bolus of blood that enters the aortic arch. This sudden influx of arterial blood produces a wave of pressure and flow that spreads distally through ever-narrowing arteries. The pulse contours of arterial pressure and flow become modified by the physical properties of lumen walls and end-arterial beds. The opposition of blood flow in a vessel is described by its resistance or, more accurately for a pulsatile arterial system, its impedance. The body greatly controls this opposition to flow through a number of intrinsic and extrinsic factors which, ideally, provide the end-organ with its physiological requirements.

The arterial pulse wave, its character having been modified by the above cardiovascular properties, now enters the eye. Before manifesting as the IOP pulse, a number of ocular factors contribute to its generation.

1.4. *Ocular anatomy and physiology*

1.4.1. Ocular volume

The internal ocular volume of a healthy adult human eye is approximately 6200 µl of which blood volume accounts for 4% (Bron, Tripathi & Tripathi, 1997b; Ridley, 1930). Male eyes are larger on average than female with estimated internal volumes of 6500 µl and 5900 µl

respectively (Silver & Geyer, 2000). Increased ocular volume has been found in high myopia (Cheng, Singh, Kwong *et al.*, 1992).

1.4.2. The arterial pathway to the eye

The arterial pathway from heart to eye starts at the aorta (Bron, Tripathi & Tripathi, 1997c). After a short distance, the right and left common carotid arteries branch off at the apex of the aortic arch. Each carotid artery rises with the spine and, before reaching the base of the skull, bifurcates to form the external carotid, responsible for the arteries to the face, and the internal carotid, that enters the skull. Upon entering the cerebral cavity, the internal carotid sends out a number of arterial branches one of which is the ophthalmic that follows the optic nerve through the optic canal into the orbit. The ophthalmic artery is responsible for blood flow to the orbit and some of the surrounding scalp. The eye receives its blood supply from two sources of the ophthalmic artery: the central retinal artery and the ciliary arteries (Figure 1.8).

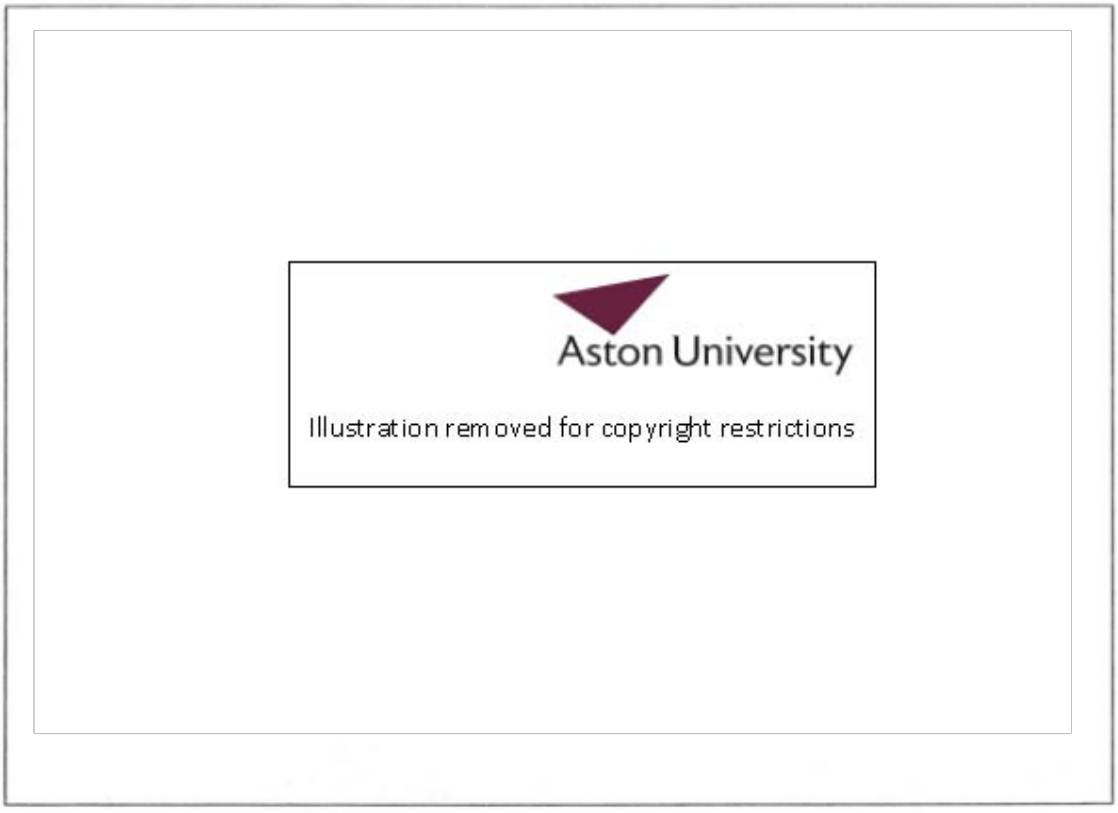

Figure 1.8. Retrobulbar vascular anatomy.

1.4.3. Retinal circulation

The central retinal artery (CRA) enters the optic nerve approximately 10 mm behind the globe and travels at its core before appearing at the surface of the disc (Alm, 1992; Anderson, 1989). There it branches to form four retinal arterioles, one for each quadrant of the retina. Occasionally the retinal blood supply is supplemented by an offshoot from the choroid, at the temporal disc margin, known as a cilioretinal artery. The arterioles traverse the fundus within the nerve fibre layer and supply a capillary network to the inner two thirds of the retina. The retinal capillaries are sparsely arranged with a narrow calibre demonstrating the restriction of supplying the high metabolic neural tissue without obstructing visual function (Funk, 1997).

Extensive tight junctions exist between retinal capillary endothelial cells. This creates a blood-retinal barrier to prevent leakage of proteins, lipid molecules and even small water soluble metabolic substrates. Like cerebral capillaries, the endothelial cells of the retina are surrounded by a single layer of muscular pericytes (Haefliger & Anderson, 1996b). These provide an alternative location and mechanism to alter vascular resistance in addition to the muscular arteries and arterioles (Funk, 1997). Retinal venous return is via a central vein that returns along the path of the artery and drains into the cavernous sinus.

1.4.4. The uveal circulation

The uveal circulation, mainly comprised of the choroid, dwarfs that of the retina (Table 1.1), and accounts for 95% of the intraocular blood volume (Chao & Bettman, 1957). It is supplied by the posterior ciliary arteries, that have branched from the ophthalmic artery, and anterior ciliary arteries that are supplied from arteries within the recti muscles. The posterior ciliary arteries, numbering 1 to 5, are usually grouped into medial and lateral bunches either side of the optic nerve and are occasionally supplemented by a superior branch (Figure 1.8). (Hayreh, 1990) They divide in to numerous (10 to 20) small arteries known as short posterior ciliary arteries (SPCAs). The majority of SPCAs pierce the posterior of the globe as two bundles, one medial to the optic nerve and one in the region posterior to the macula. (Olver, 1990) Two branches continue forward on either side of the globe. These long posterior ciliary arteries provide a supply, along with the anterior ciliary arteries, to the anterior uvea. The anterior ciliary arteries insert themselves into the globe below each rectus

	Retinal circulation	Choroidal circulation	
		Posterior pole	Equatorial zone
Capillary lumen diameter	5 to 6 μm[a]	6 to 10 μm[b] 16 to 20 μm[c]	20 to 50 μm[c]
Blood flow	35 μl/min[f] 80 μl/min[g]	900 μl/min[e]	

Table 1.1. **Vascular characteristics of the retinal and choroidal circulations. Sources: a, (Alm, 1992) b, (Ramrattan, van der Schaft, Mooy *et al.*, 1994) c, (Olver, 1990) d, (Funk, 1997) e, (Langham, Farrell, O'Brien *et al.*, 1989a) f, (Riva, Grunwald, Sinclair *et al.*, 1985) g. (Feke, Tagawa, Deupree *et al.*, 1989)**

muscle tendon and supply the ciliary muscle, iris and episclera with some recurrent arteries to the equatorial choroid.

The largest uveal tissue, the choroid, is approximately 900 μm in thickness posteriorly and 500 μm anteriorly (Cheng *et al.*, 1992; Coleman & Lizzi, 1979). The choroid changes with age (Ramrattan *et al.*, 1994) and over ten decades the choroidal thickness reduces by 57% and the choriocapillaris lumen narrows by 34%. Myopia and glaucoma have been associated with a thinning of the posterior choroid (Cheng *et al.*, 1992; Kubota, Jonas & Naumann, 1993). Once within the globe the SPCAs (up to 300 μm in diameter) radiate within the outer layer of the choroid (Haller's layer), divide into arterioles (25 to 40 μm in diameter) in the middle layer (Sattler's layer) and finally supply the inner choriocapillaris (Lieberman, Maumanee & Green, 1976). Unlike the retinal circulation there is an abrupt transition between arteriole and capillary (Fryczkowski, Sherman & Walker, 1991). Posterior choroidal arterioles enter the choriocapillaris at right angles and are up to 70 μm in diameter. The interstitial components of the choroid consist of melanocytes, fibroblasts, and a musculo-elastic system that connects with the ciliary processes anteriorly (Flügel-Koch, May & Lütjen-Drecoll, 1996).

The choriocapillaris network is functionally organised into a lobular arrangement with usually one feeding arteriole and a number of draining venules (Olver, 1990). These lobules

are polygonal in shape posteriorly, and become more elongated towards the ora serrata (Fryczkowski *et al.*, 1991). The lobular arrangement is difficult to distinguish in the submacular and peripapillary zones due to the large number of interconnections. Flower, Fryczkowski & McLeod, (1995) have shown, using indocyanine green angiography, that these lobules correspond to local pressure gradients rather than exact anatomical demarcations. Furthermore the exact position and shape of a lobule, although relatively stable short term (weeks), changes with time (months).

The choriocapillaris is likened to a network of wide-bored fenestrated capillaries which are oval in cross-section. An incomplete covering of pericytes is found only on the external surface of the choriocapillaris and this would suggest that the choriocapillaris, unlike the retinal capillaries, is incapable of altering its internal diameter. The inner surface of the choriocapillaris, abutting the retinal pigment epithelium, is highly fenestrated. This provides high permeability to large plasma molecules (for example, retinol-binding protein) which supply the overlying retina with necessary metabolites (for example, vitamin A) (Foulds, 1990). Having passed the uveal capillary beds, venous blood drains from the eye via the anterior ciliary and vortex veins.

1.4.5. Optic nerve head circulation

Figure 1.9 illustrates the anterior portion of the optic nerve and its vascular supply. The intraorbital portion of the optic nerve, between the CRA entry-point and the globe, is supplied by pial arteries surrounding the nerve (centripetal branches) and by the CRA at its core (centrifugal arteries). The vascular distribution of the intraocular portion of the nerve is more complex and can vary considerably in individuals (Hayreh, 1989). The vascular supply can be divided into three portions: the laminar, the prelaminar and the surface nerve fibre layer (Lieberman *et al.*, 1976):

- The laminar portion is primarily supplied by the short posterior ciliary arteries, either directly from recurrent branches through the choroid, or indirectly from the circle of Zinn-Haller (Bill, 1993). The circle of Zinn-Haller is formed by superior and inferior anastomoses between the medial and lateral para-optic SPCAs (Olver, Spalton & McCartney, 1990). This anastomatic ring is estimated to be a complete

circle in over half the population and an incomplete series of corollaries in the remainder (Cioffi & Buskirk, 1996). A minor vascular supply to this layer arises from the pial arteries and the peripapillary choroid (Olver, 1990).

Figure 1.9 Vascular anatomy of the anterior optic nerve. Adapted from. (Hayreh, 1989)

- The prelaminar portion is supplied by SPCAs and recurrent arteries of the choroid although their relative contribution is disputed (Cioffi *et al.*, 1996). Hayreh (1990) maintains that angiographic studies indicate the majority of supply comes from centripetal branches of the peripapillary choroid and that there is a strict sectorial distribution. In contrast, Liebermann *et al.* (1976) emphasise the role of SPCA branches (that arise from the scleral layer) in supplying the prelaminar optic nerve head.

- The surface nerve fibre portion is supplied by arterioles from the central retinal artery and occasionally, on the temporal side, by a cilioretinal artery.

Venous drainage of the optic nerve, in marked contrast to the arterial supply, is almost completely performed by the central retinal vein: a small part of the pre-laminar venous blood returns to the peripapillary choroid (Hayreh, 1995). This produces a dichotomous drainage system in which the choroid is drained by the vortex veins, and the nerve by the central retinal vein: a situation unique to primates (Cioffi *et al.*, 1996).

1.4.6. Intraocular blood flow

Total intraocular blood flow in humans has been estimated to be approximately 1000 μl/min of which retinal flow has been measured to be between 34 and 80 μl/min (Feke *et al.*, 1989; Langham *et al.*, 1989a; Riva *et al.*, 1985). Schlegel and Lawrence (1969) calculated the total blood flow from the vortex veins in rabbits to be 1300 μl/min. Choroidal blood flow is extremely high (Table 1.1) and far surpasses its own metabolic requirements (Alm, 1992). A number of reasons have been proposed for this excessive flow (Delaey & Van de Voorde, 2000):

- The excessive blood flow provides a steep diffusion gradient between choriocapillaris and outer retina which is reliant on the choroidal circulation for its metabolic demands (Foulds, 1990). For example, oxygen extraction from choroidal blood is only 3-4% (compared to 40% in retinal blood) which maintains a high diffusion pressure to drive such metabolites across the relatively large avascular space (Bill & Sperber, 1990).
- As up to a third of light entering the eye is absorbed by the retinal pigment epithelium, a high choroidal blood flow may have a role in regulating tissue temperature. Parver, Auker & Carpenter)1980) provided evidence for such a function by exposing the retinae of monkeys to different levels of incident light and taking temperature measurements within the retina and choroid.
- By providing a protective volume buffer. The sponge-like tissue of the choroid may cushion abrupt rises in IOP induced, for example, by rubbing the eyes (Bron, Tripathi & Tripathi, 1997a).

- High uveal blood flow helps provide the necessary filtration pressure for production of aqueous fluid (Delaey *et al.*, 2000).

Intraocular blood flow is pulsatile down to the level of the choriocapillaris lobules and the retinal capillaries (Flower & Klein, 1990; Sullivan, Cioffi, Wang *et al.*, 1999). The proportion of blood flow to the eye that is pulsatile has been estimated to be between 33% (Krakau, 1995) and 80% (Langham *et al.*, 1989a). Riva, Cranstoun, Grunwald *et al.*, (1994) measured blood flow in the subfoveal choroid by laser Doppler flowmetry and found the pulsatile proportion to be 23% of total flow in humans. Grunwald, Hariprasad & DuPont (1998a) again using laser Doppler flowmetry at the fovea, found choroidal blood flow to decrease with age but noted no change in the pulsatility. Blood flow in the retinal and vortex veins is constant although a periodic collapse can occur, usually in synchrony with IOP pulse, at the point of exit from the globe (Hedges, Baron, Hedges *et al.*, 1994; Michelson & Harazny, 1997; Riva *et al.*, 1985).

1.4.7. Ocular Perfusion pressure

As previously shown, the driving force of blood flow to an organ is its perfusion pressure (the numerator of Darcy's law of flow – Equation 1.8). In the eye this is equivalent to the difference between the arterial pressure entering the globe and that of the intraocular veins. In healthy young adult humans, *in vivo* studies have shown the ophthalmic arterial pressure to have a peak systolic pressure of approximately 90 mmHg and a diastolic trough of 50 mmHg (Jennings, 1991; Langham & To'mey, 1978). Clinically the mean ophthalmic artery pressure is often taken as two-thirds of the mean arterial brachial blood pressure and a common estimate of mean ocular perfusion pressure is shown in Equation 1.11 (Harris, Kagemann & Cioffi, 1998b).

Mean Ocular Perfusion Pressure	=	2/3 Mean Arterial Blood Pressure	–	Intraocular Pressure

Equation 1.11 Clinical estimate of mean ocular perfusion pressure.

Retinal artery perfusion pressure is higher than that of the ciliary arteries. (Ulrich, 1996) Direct micropuncture measurements have found the blood pressure in the anterior ciliary arteries of monkeys to be 55 mmHg and in the choriocapillaris of rabbits to be 23 mmHg (Mäepea, 1992); the choriocapillaris lobuli of the posterior pole having a higher perfusion pressure than those found at the periphery (Flower *et al.*, 1995).

The anatomical distribution of the SPCAs into temporal and medial bundles produces a zone between them that is susceptible to reduced perfusion when arterial pressure drops in one or both bundles. This area, known as a watershed zone, has been extensively studied by Hayreh and lies in a vertical band anywhere between the optic disc and fovea (Hayreh, 1990; Hayreh, 1995).

Intraocular venous pressure is usually assumed to be equivalent to IOP (Haefliger & Anderson, 1996a). While this may be correct in uveal veins, direct measurements of venous pressure in the retinal circulation have questioned this assumption (Bill, 1985; Glucksberg & Dunn, 1993). Micropuncture measurements on retinal veins in cats found venous pressure to be significantly above (>7 mmHg) IOP and is similar to that found in the choriocapillaris (Mäepea, 1992). Outside the eye, venous pressure falls to approximately 6 mmHg in the episcleral and central retinal veins (Morgan, Yu, Cooper *et al.*, 1995). At the junction between intraocular and extraocular venous pressure there is a waterfall phenomenon caused by the sudden drop in pressure (Bill, 1993).

For vessels above the capillary level, blood viscosity and vascular bed length can be considered constant and the main determinant of vascular resistance in the eye is vessel diameter (Haefliger *et al.*, 1996a): Equation 1.9.

1.4.8. Intrinsic determinants of intraocular vessel diameter

Classically, autoregulation (1.3.5) has been thought to be an ability of the retinal and anterior uveal circulations but not of the choroid (Michelson, Groh & Gründler, 1994; Schlegel *et al.*, 1969). This opinion stemmed from the work of Alm and Bill (1972) who found, in the presence of perfusion pressure changes in cats, retinal and iris blood flow remained stable but choroidal flow varied linearly to the arterio-venous pressure difference.

This was interpreted as the retinal and anterior ciliary arteries and arterioles having the necessary autoregulative mechanisms (Bayliss myogenic stretch receptors and sensitivity to local vasoactive metabolites and molecules). The ability of human retinal vessels to autoregulate in response to a rise in blood pressure has recently been illustrated by Nagaoka, Mori & Yoshida (2002). Retinal flow stabilised approximately two minutes after subjects placed their hands in ice-cold water (cold-pressor test) which, the authors suggest, is characteristic of a myogenic type autoregulation. The human retinal circulation has been shown to autoregulate down to perfusion pressures of approximately 30 mmHg (Grunwald, Sinclair & Riva, 1982; Riva, Sinclair & Grunwald, 1981). Lack of autoregulation in the choroid was explained as being due to its very high and, for the choroid's own nutritional needs, superfluous blood flow. That is, changes in perfusion pressure did not affect choroidal metabolism due to the excessive blood flow providing a buffer to its requirements. This classical view of intraocular autoregulation has however recently been questioned. Flower *et al.* (1995) and Kiel (1999) have both shown choroidal blood flow to demonstrate stability of flow over a limited range of perfusion pressure. Whether these latter studies demonstrate true local autoregulation or evidence of an extrinsic neural influence has yet to be clarified (Riva, Titze, Hero *et al.*, 1997c).

In the optic nerve head, as the majority of vessels derive from the SPCAs and choroid (1.4.4), no local regulation of flow was suspected (Alm, 1989). However autoregulation of optic nerve head blood vessels has been demonstrated in animals and humans (Geijer & Bill, 1979; Pillunat, Anderson, Knighton *et al.*, 1997; Riva, Hero, Titze *et al.*, 1997a). It is suspected that the autoregulatory mechanism lies in the optic nerve capillary bed (in particular, the pericytes) rather than the feeder arterioles (Haefliger *et al.*, 1996a). In healthy eyes, blood flow in the optic nerve remains normal with perfusion pressures down to about 30 mmHg (Bill, 1993).

Change in retinal blood flow from metabolic demand has been demonstrated during dark adaptation and during 'flicker' stimulation (Kondo, Wang & Bill, 1997; Wang & Bill, 1997). In contrast the choroidal circulation has not been shown to be influenced by retinal metabolism. This again has been explained by the choroid's excessive blood flow and the barrier imposed on retinal metabolic by-products by the retinal pigment epithelium (Delaey *et al.*, 2000).

The vasoactive mediators produced from the arterial endothelia have an important role in the ocular circulation (Haefliger, Flammer & Lüscher, 1992). Nitric oxide (NO) has been demonstrated as an important mediator of vasodilatation in the retina (Kondo *et al.*, 1997) and choroid (Mann, Riva, Stone *et al.*, 1995). A basal level of vasodilatation is maintained in both these vascular beds through a tonic production of endothelial NO (Brown & Jampol, 1996). Endothelin-1 contributes to the retinal vasoconstriction that occurs during hyperoxia (Dallinger, Dorner, Wenzel *et al.*, 2000) and is a powerful vasoconstrictor of choroidal vessels (Kiel, 2000; Schmetterer, Findl, Strenn *et al.*, 1997b).

1.4.9. Extrinsic determinants of intraocular vessel diameter

Neural control

Whilst nerve endings associated with the retinal circulation extend only as far as the lamina cribrosa, the uveal circulation is richly innervated by sympathetic, parasympathetic and sensory nervous systems (Delaey *et al.*, 2000). Evidence of neural control in intraocular blood vessels comes from histological and direct stimulation studies in animals and the findings are summarised in Table 1.2. It has been postulated that the rich sympathetic innervation of the choroid helps protect it from damaging high systemic arterial pressure during sympathetically driven 'fight or flight' conditions (Bill, 1985). Baroreceptors in the aortic arch and carotid sinus would detect a rise in arterial blood pressure and trigger, via the sympathetic efferents, choroidal vasoconstriction to buffer the increase in perfusion pressure (Chapleau, 1999). The role of vasodilatory nerve fibres in the eye has been associated with the possible control of ocular tissue temperature (Parver, Auker, Carpenter *et al.*, 1982).

Blood gases

The intraocular vessels are extremely sensitive to the partial pressure of blood oxygen and carbon dioxide (Friedman & Chandra, 1972; Harris, Ciulla, Chung *et al.*, 1998a). Breathing 100% oxygen produces strong vasoconstriction in retinal arteries and mild constriction in the choroid (Flower *et al.*, 1995; Harris, Anderson, Pillunat *et al.*, 1996). Hypercapnia produces a prominent vasodilatation in both retinal and choroidal vessels (Flower *et al.*, 1990; Roff, Harris, Chung *et al.*, 1999). Such sensitivity to the level of oxygen and carbon dioxide is similar to that found in cranial arteries (Orgül, Gugleta & Flammer, 1999).

Nerve Type	Nerve supply	Vascular bed supplied	Neurotransmitters	Effect of direct stimulation
Sympathetic	Superior Cervical Ganglion	All uveal beds	1. Noradrenaline 2. Neuropeptide Y	Vasoconstriction
Parasympathetic	Facial	Mainly choroid	1. Acetylcholine 2. Vasoactive intestinal polypeptide	1. Moderate vasodilatation in anterior uvea 2. Marked vasodilatation in choroid
	Oculomotor	Mainly anterior uvea	Acetylcholine	Increased blood flow in iris and ciliary body (except reduced blood flow in ciliary body of rabbits)
Sensory	Trigeminal	Mainly ciliary body and choroid	1. Substance P 2. Calcitonin gene-related peptide	Vasodilatation in the anterior uvea

Table 1.2 Summary of the innervation of the uveal circulation (Alm, 1992; Chandra & Friedman, 1972; Ruskell, 1971).

Humoral control

Although not taking a major role in the regulation of ocular blood flow, there is evidence that some circulating hormones influence intraocular vessel diameter (Alm, 1992). Angiotensin II, insulin and the oestrogens have all been implicated in affecting intraocular blood vessels (Centofanti, Zarfati, Manni *et al.*, 2000b; Polak, Dallinger, Polska *et al.*, 2000; Rockwood, Fantes, Davis *et al.*, 1987).

1.5. *The ocular pressure-volume relationship*

Any change in volume of an incompressible fluid within a distensible shell, such as the eye, will give rise to a new internal pressure; the internal pressure is balanced by the new tension created in the walls of the shell. The relationship between a certain intraocular volume change and the subsequent intraocular pressure change is known as the ocular pressure-volume relationship and is of importance in the understanding of the IOP pulse. The

simplest form of the ocular pressure-volume relationship is shown in Equation 1.12 (Friedenwald, 1937; Friedland, 1983):

$$\frac{\Delta P}{IOP} = k\frac{\Delta V}{V}$$

IOP = intraocular pressure (mmHg) ΔP = change in intraocular pressure (mmHg)

V = intraocular volume (μl) ΔV = change in intraocular volume (μl)

k = ocular elasticity

Equation 1.12 Basic ocular pressure-volume relationship.

For a given intraocular pressure (IOP) and ocular volume (V), a change in intraocular volume (ΔV) produces a subsequent change in pressure (ΔP) which is dependent on a constant (k) that represents the degree of elasticity in the ocular shell. One can see that this is an analogous relationship to that for describing arterial compliance: the eye has been described as having low compliance or high volumetric stiffness because a small volume change (0.1%) causes a high pressure change (4.5%) (Greene, 1985).

Friedenwald (1937) was the first investigator to produce a simplified equation to characterise the pressure-volume relationship in the human eye. Friedenwald's relationship showed that the pressure-volume relationships of earlier investigators (Ridley, 1930) became linear once the log of the pressure was plotted against volume (Equation 1.13).

$$\Delta V = (\log_{10} P_2 - \log_{10} P_1)/K$$

ΔV = change in intraocular volume (μl)

P_1 = original IOP (mmHg)

K = ocular rigidity

P_2 = IOP after intraocular volume change (mmHg)

Equation 1.13 Friedenwald's ocular pressure-volume relationship (Friedenwald, 1937).

Friedenwald's relationship makes the assumption that ocular volume (V) is so great in comparison to any foreseen change in volume (ΔV), that it can be incorporated with ocular elasticity (k) to form a new constant (K): a constant he termed **ocular rigidity**. Ocular rigidity, stated Friedenwald, is a measure of the resistance the eye exerts to a distending force. It is apparent from these relationships that, for the same intraocular volume change, a greater pressure change occurs as IOP increases.

Later investigators found imperfections in Friedenwald's pressure-volume relationship and a series of alternative mathematical formulae, derived either theoretically or empirically, have been created in subsequent years (Holland, Madison & Bean, 1960; McBain, 1958; Silver *et al.*, 2000; van der Werff, 1981): Table 1.3.

Author	**Pressure-Volume Relationship**
Friedenwald (1937)	$\Delta V = \frac{1}{K}(\log P_2 - \log P_1)$
McBain (1958)	$\Delta V = a\left(P_2^{0.36} - P_1^{0.36}\right)$
Holland *et al.* (1960)	$\Delta V = \frac{P_2 - P_1}{b(P_1 - c)^a}$
van der Werff (1981)	$\Delta V = \frac{1}{b}\left(P_2^{1/3} - P_1^{1/3}\right)$
Silver & Geyer (2000)	$\Delta V = V(c + d\ln P + eP)$

Table 1.3 **Ocular pressure-volume relationships: ΔV is the predicted change in intraocular volume (μl); P_1 and P_2 are the limits of measured change in intraocular pressure (mmHg); V is the original ocular volume (μl): a to e and K are coefficients (K being the familiar coefficient of ocular rigidity).**

Some investigators have attempted to describe the pressure-volume relationship in terms of a mechanical parameter such as the modulus of elasticity (Greene, 1985; Purslow & Karwatowski, 1996). They concluded that, for the eye, such a simple parameter cannot be defined but rather an incremental modulus of elasticity, that is highly dependent on internal volume and pressure, must be used. This again is analogous to the mechanical behaviour of artery walls (Equation 1.2). Another reason for the ocular shell to not follow simple laws of

elasticity is that any distension of its wall contains an element of viscous flow as well as elastic stretch (Perkins, 1981b). Such materials are described as viscoelastic and have their own characteristic biomechanical properties (Nichols & O'Rourke, 1998f). There is great inter-subject variability in ocular pressure-volume relationships. This is demonstrated by the range in normal ocular rigidity values: $K = 0.002$ to 0.055 (Friedenwald, 1937).

The ocular pressure-volume relationship of the living eye has been shown to be influenced by a number of factors:

1.5.1. Intraocular pressure

From first principles (Equation 1.12) it can be seen that any ocular pressure-volume relationship is dependent upon baseline IOP. Eisenlohr, Langham & Maumenee (1962b) described how the ocular pressure-volume relationship varies in living human eyes with any given IOP (Figure 1.10).

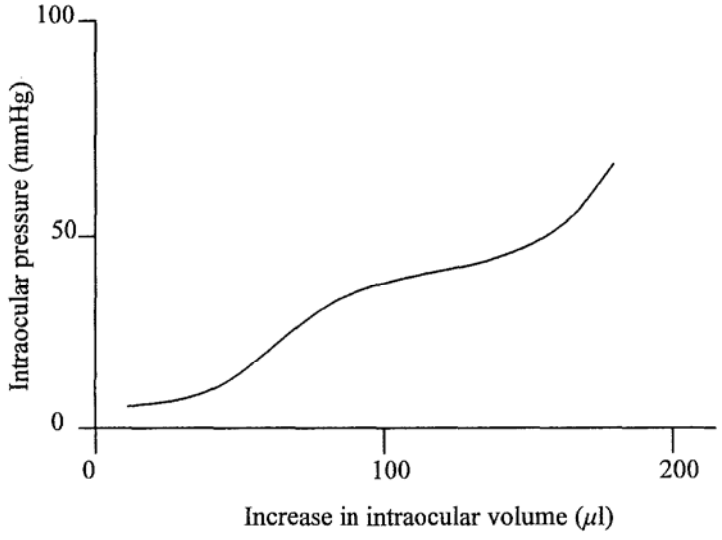

Figure 1.10 The ocular pressure-volume relationship in living human eyes. Adapted from Eisenlohr *et al.* (1962b)

The shape of the relationship is sigmoid in nature: starting with a convex rise, the relationship increases until, at approximately 50 mmHg, the slope flattens to a plateau stage; with further increase in IOP, the relationship steepens again and, at IOPs above around 80 mmHg, resembles that of the cadaver eye. The authors attributed the flattening of the curve

to the expulsion of blood from the eye as the IOP surpassed diastolic blood pressure; reasonably arguing that any further increase in IOP caused a reduction in intraocular blood volume with total ocular volume remaining relatively stable. The latter increase in the pressure-volume relationship signified that IOP was greater than systolic blood pressure and, having expressed all ocular blood the eye, behaved like that of a dead eye (Eisenlohr & Langham, 1962a). In the normal IOP range, the relationship is less complicated and a number of attempts have been made to describe it mathematically (Table 1.3).

1.5.2. Ocular volume

From Equation 1.12, it is to be expected that a pressure-volume relationship depends on original ocular volume. A major criticism of Friedenwald's relationship, is that his coefficient 'ocular rigidity' incorporates the original ocular volume (Silver *et al.*, 2000). Perkins (1981b)clearly showed, by injecting known quantities of fluid into enucleated human eyes, that larger eyes produced smaller pressure changes for the same increase in intraocular volume.

1.5.3. Refractive error

Friedenwald (1937) found that the pressure-volume relationship was correlated with the degree of ametropia: myopic eyes producing a smaller pressure change for a given volume change than hyperopic eyes. Perkins (1981b) attributed the correlation to the associated difference in ocular volume with ametropia (Cheng *et al.*, 1992). The latest ocular pressure-volume relationship, proposed by Silver and Geyer, (2000) incorporates a variable for the eye's original ocular volume. The authors' speculate that, by removing such an important source of variance from the pressure-volume relationship, the correlation with refractive error will be lost.

1.5.4. Blood pressure

Kiel (1995)studied the effect of systemic arterial blood pressure on the pressure-volume relationship in the rabbit. In addition to confirming the plateau phase found by Eisenlohr *et al.* (1962b), he found that the gradient of the initial slope correlated with mean arterial blood pressure (Figure 1.11). The change in pressure-volume relationship with blood

pressure is supported by earlier work that found ocular rigidity to be influenced by the volume of blood in the eye (Perkins & Gloster, 1957; Ytteborg, 1960). Kiel(1992) attributed the change in gradient to the efficacy of the choroidal vasculature to autoregulate under different perfusion pressures.

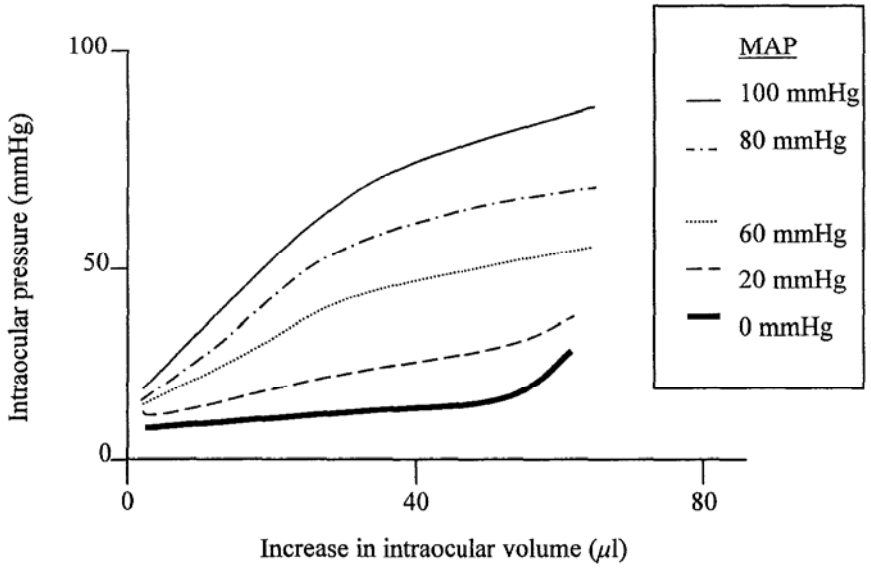

Figure 1.11. Pressure-volume relationships at different mean arterial pressures (MAP) in the rabbit. Adapted from Kiel (1995)

1.5.5. Age

Friedenwald (1937)and Perkins (1981b) found the ocular pressure-volume relationship to steepen with age. Friedenwald postulated that this may be due to a change in the distensibility of the sclera and the intraocular vascular bed from lipoid infiltration and arteriosclerosis respectively.

1.5.6. Corneal and scleral thickness

Although the thickness of the ocular envelope should theoretically influence the pressure-volume relationship, there is little experimental evidence in support of this (Purslow *et al.*, 1996). Neither scleral thickness nor corneal thickness have been shown to correlate with ocular rigidity (Ehlers, Hansen & Aasved, 1975; Perkins, 1981b). The thinner corneas of keratoconic patients however are associated with a flatter pressure-volume relationship;

although the abnormal elasticity of the keratoconic cornea may be a confounding variable (Brooks, Robertson & Mahoney, 1984; Edmund, 1988).

1.6. *Methods of recording the IOP pulse*

1.6.1. Manometry

The 'gold-standard' measurement of the IOP pulse is to take a direct high fidelity measure of IOP in the anterior chamber. This is usually only possible in experimental animals or human eyes undergoing concomitant surgery (Eisenlohr *et al.*, 1962b). Recently, investigators have looked at implanting electronic pressure transducers within the living eye (Draeger, Michelson & Rumberger, 2000; Rizq, Choi, Eilers *et al.*, 2001). The pressure transducer, usually designed as part of an intraocular lens implant, is then capable of telemetrically sending real-time IOP measurements to a receiving unit outside the eye (Walter, Schnakenberg, vom Bogel *et al.*, 2000).

1.6.2. Goldmann applanation tonometry

In a crude, yet remarkably simple technique, Ruiz-Barranco (1960) showed that it was possible to measure the IOP pulse with a normal Goldmann tonometer. It is well recognised that the IOP pulse is visible in the cyclic variation of the Goldmann prism mires (Goldman & Schmidt, 1957). By aligning the semicircular mires, first at the minimum value and then at the maximum value of their variation, a measure of the IOP pulse is possible. Ruiz-Barranco (1960) measured an oscillation in IOP in 96% of his subjects with this technique.

1.6.3. Ocular pneumoplethysmography

Ocular pneumoplethysmography and its sesquipedalian cousin, oculo-oscillodynamography, do not truly measure the IOP pulse (Gee & Reed, 1999; Ulrich, 1996). However as they are commonly described as techniques for measuring the 'ocular pulse' a brief description is necessary in order to differentiate them (Best, Kelly & Galin, 1970). These techniques involve attaching a suction cup to the perilimbal sclera of the eye. An air tube runs from the cup to a base unit which, as well as measuring the air pressure, maintains a necessary vacuum to attach the cup to the eye. Oscillations in the scleral coat with each heart beat produce small volumetric changes under the suction cup. These fluctuations in air pressure

within the tube are then recorded. The procedure usually followed in these techniques is to first increase the level of suction to raise the IOP above that of the ocular arterial pressures. All ocular pulsations associated with the cardiac rhythm then cease. Suction pressure is then slowly decreased back to a minimal level. At some point during this reduction, small oscillations in the scleral radius are detected by the corresponding oscillations in air pressure beneath the suction cup (Suzuki, 1962). The oscillations increase in amplitude as suction pressure decreases further. The point at which pulsations are first recorded equates to when IOP equals the systolic pressure of the ophthalmic artery. Ulrich and Ulrich (1985) have further advanced this technique to the point where it is possible to detect the individual systolic pressure points of both central retinal artery and posterior ciliary arteries and even, the authors claim, the diastolic arterial blood pressure (Ulrich, Ulrich & Walther, 1989). The technique requires a conversion between suction pressure and IOP, which can either be obtained by a calibration trial beforehand or by reliance upon standardised tables.

Because the techniques of ocular pneumoplethysmography and oculo-oscillodynamography measure fluctuations in scleral shape, the pulsations recorded are volumetric pulses rather than pressure pulses. The IOP pulse and the volumetric pulse recorded with these techniques are obviously related but are distinctly different (Lester, 1966). For example, in an eye with a high intraocular pressure and experiencing the same variation in *intraocular* volume, the recorded pressure pulse will be high but the *external* volumetric pulse will be low. A complete review of ocular pneumoplethysmography has been made by Gee(1985) and will not be discussed further.

1.6.4. Dynamic Observing Tonometer

A number of instruments use the technique of applanating a small area of the cornea with a probe that contains, at its centre, a borehole covered by an elastic membrane. A continuous measure of IOP is calculated either from the deflection of the central membrane, or by measuring the pressure required behind the membrane to retain a plane surface (Krakau, Lindberg & Havelius, 1995; Perkins *et al.*, 1977; Thorburn, 1972). Two currently available instruments, examples of each method, will be discussed further. The first instrument is the Dynamic Observing Tonometer (DOT).

The DOT resembles a traditional diagnostic contact lens and weighs approximately 25g (Dekker, Robert, Kanngiesser *et al.*, 1999). The lens' contact surface (radius: 8.50 mm and diameter: 11 mm) has, at its centre, a Mylar membrane-covered bore hole that provides a 2.5 mm diameter applanation zone (Figure 1.12).

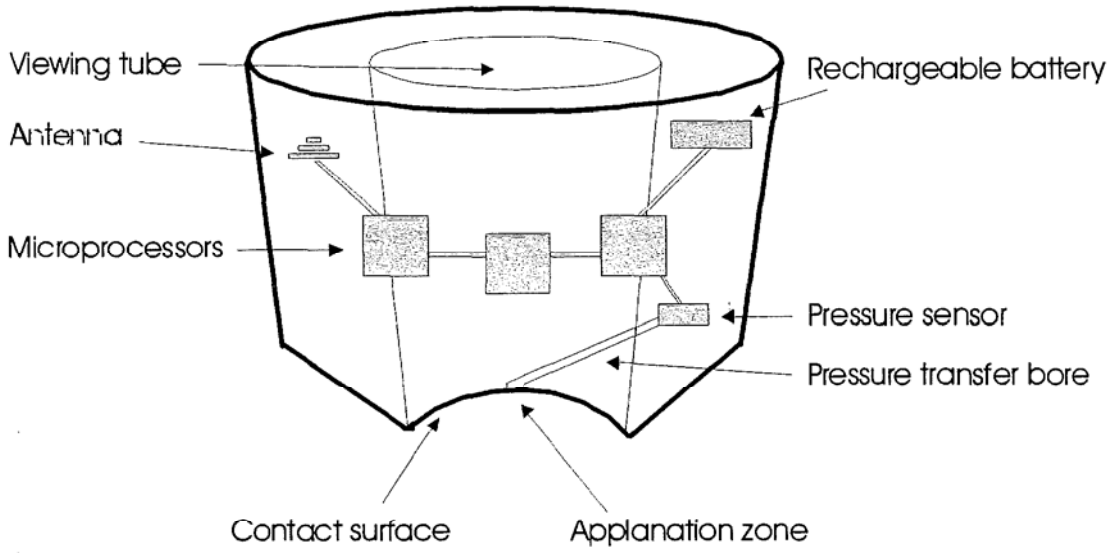

Figure 1.12 Schematic diagram of the Dynamic Observing Tonometer, adapted from an illustration provided courtesy of ODC AG, Zürich, Switzerland.

The cavity behind the membrane is filled with silicon oil and is continuous with a piezo-electric pressure transducer that is offset in the housing of the lens. The clear silicon oil provides an unobstructed optical pathway. A measure of IOP occurs through the transference of pressure in the oil to the transducer when the applanation membrane is displaced during its contact with the cornea (Entenmann, Robert, Pirani *et al.*, 1997). In order to avoid thermal-expansion pressure artefacts, as the silicon oil heats up or cools, the lens is preheated to body temperature before use. Pressure recordings by the lens are sampled at a frequency of 100 Hz and are transmitted to a base unit telemetrically (via radio waves) where the data are stored. The DOT is capable of recording up to four minutes of continuous IOP data which can then be downloaded to a personal computer for further analysis.

There is little research on the reproducibility and validity of the measurements made with the DOT. Dekker *et al.*, (1999) using an early prototype of the DOT, investigated the instrument's accuracy by comparing the measurement of IOP on enucleated eyes against

their manometric value. Pearson's correlation coefficient, over an IOP range of 0 to 60 mmHg, was +0.95. Entenmann *et al.* (1997) compared the DOT's IOP measurements to those of a Goldmann tonometer in normal healthy subjects. Pearson's correlation coefficient between the two instruments was +0.70. Recognising the lack of clinical utility these two early publications gave, Troost, Vogel, Beck *et al.* (2001) reported the 95% limits of agreement between DOT and Goldmann tonometers for a mixed group of normal and glaucoma patients. The DOT significantly overestimated IOP by a mean difference of 5.6 mmHg (95% limits of agreement: -5.4 mmHg to +16.6 mmHg) and the authors concluded that DOT was not an adequate substitute to Goldmann tonometry. The within- and between-observer repeatability of the DOT's IOP and pulse amplitude measurements has been studied by Vogel, Beck, Schwenn *et al.* (2001) Five repeated measurements of each parameter were taken on the same ten healthy subjects by three different observers. The intraobserver coefficients of variation for IOP and pulse amplitude measurements were 9.6% and 14.5% respectively. The interobserver coefficients of variation for IOP and pulse amplitude were 10.2% and 14.0% respectively. As one of the three groups of measurements was significantly different (one-way ANOVA), the instrument was deemed not to be observer independent.

1.6.5. The Pneumatic Tonometer

Durham, Bigliano & Masino (1965) developed the pneumatonometer as part of their search for a non-invasive method of measuring blood pressure in an artery. The instrument consists of an applanating probe with a central aperture encircled by a second exhaust aperture. Both apertures are covered by a thin elastic membrane which is cemented only to the outer wall of the probe (Tønjum, 1973): Figure 1.13. A continuous flow of gas, usually air, is forced down the central aperture causing the elastic film to deflect forward. The gas escapes through the side exhaust channels via the minute gap between aperture wall and elastic film (Langham & McCarthy, 1968). When the probe is placed against a cornea, gas pressure within the central aperture rises until it again creates an escape gap. To allow the tonometer to continually record variations in IOP, the tonometer head is borne on a frictionless bearing by part of the gas pressure (A_b in Figure 1.13) and has an applanating force of approximately 2g (Walker & Langham, 1975b).

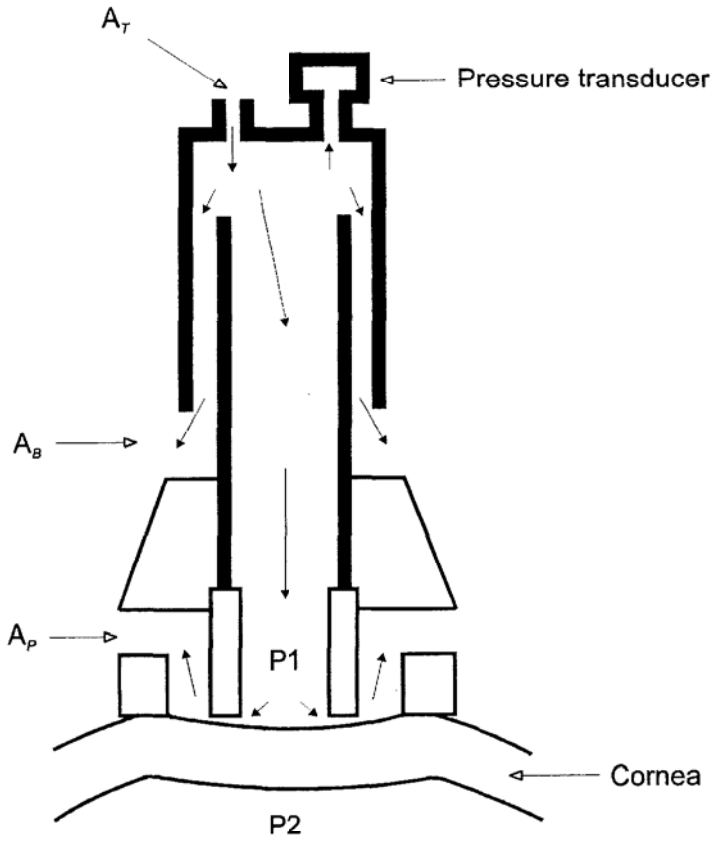

Figure 1.13. Schematic illustration showing a pneumatonometer probe in contact with a cornea: P1, air pressure within probe; P2, intraocular pressure; A_T, total air flow entering probe; A_B, proportion of air flow escaping from the 'air bearing'; A_P, proportion of air flow escaping from the probe head. Adapted from Tønjum (1973).

The accuracy of a number of early pneumatonometers (including the Digilab pneumatonograph, Block Engineering, Cambridge, MA and the Model 30 Classic, Mentor, Norwell, MA) has been investigated by comparing their measurements to manometric values. Langham *et al.* (1968) described graphically the close correlation between pneumatonometer IOP measurement and that of the manometer in recently enucleated human eyes. They also showed that pneumatonometer measurements taken on the sclera were of similar accuracy to those taken on the cornea. Comparisons indicate that pneumatonometric IOP measurements have regression coefficients of less than unity with the manometric value; typically, true IOP is overestimated at low levels and vice versa (Eisenberg, Sherman, McKeown *et al.*, 1998; Moses & Grodzki, 1979). Only one study compared measurements of the IOP pulse by manometer and pneumatonometer (Tønjum, 1972). IOP pulse amplitudes were measured by both techniques in live animals and one human. The average difference in pneumatonometer IOP pulse amplitude was 6.7% in both

animals and humans. In addition, the change in manometric IOP pulse amplitude due to pneumatonometer apposition on the eye was approximately ± 6%.

The currently available pneumatonometer, the Ocular Blood Flow Analyzer (OBFA pneumatonometer, Paradigm Medical Ind., Utah, USA), has only had the accuracy of its IOP measurements assessed by comparison to Goldmann tonometry. Spraul, Lang, Ronzani *et al.* (1998) found a Pearson correlation coefficient of +0.77 between the two tonometers and a regression formula of $IOP_{pneumatonometer} = 0.79 \times IOP_{Goldmann} + 5.8$. Yang, Illango, Cook *et al.* (2000) found a Pearson correlation coefficient of +0.94 ($IOP_{pneumatonometer} = 0.84 \times IOP_{Goldmann} + 3.5$) with the 95% limits of agreement to be -4.35 to +4.87 mmHg.

Lam, Chan, Fan *et al.* (1999) found no significant difference in intra- and inter-observer measurements of IOP and IOP pulse amplitude taken with the OBF pneumatonometer. Spraul *et al.* (1998) reported the coefficients of reliability for intra-observer IOP and IOP pulse amplitude measurements to be +0.90 and +0.86 respectively and inter-observer measurements to be +0.76 and +0.66.

In order for the pneumatic tonometer to measure IOP pulsations variations confidently it is necessary to determine the response of the tonometer to IOP variations of different frequency. This has been studied in theory by Walker, Compton & Langham (1975a) and reassessed by Silver & Farrell (1994). The frequency response of the pneumatic tonometer is characterised by its dynamic amplification factor and phase lag.

Dynamic amplification factor

The level of amplification a pressure measurement is subject to at a particular frequency is known as its dynamic amplification factor. For typical pneumatonometer parameters this can be calculated from Equation 1.14 (Silver *et al.*, 1994).

$$\frac{P_1}{P_2} = \frac{1}{\sqrt{1 + (f / f_0)^2}}$$

P_1/P_2 = the ratio of probe pressure to intraocular pressure

f = the frequency of interest

f_0 = the fundamental vibration frequency of the membrane beneath the tonometer (typically 150 Hz)

Equation 1.14 Formula for calculating the dynamic amplification factor for typical pneumatonometers (Silver *et al.*, 1994).

Phase lag

The amount of delay between the driving variation in pressure and the recorded variation at a particular frequency is known as phase lag. For a typical pneumatonometer this can be calculated from Equation 1.15 (Silver *et al.*, 1994). Silver *et al.* (1994) concluded that, for the specifications of the OBFA pneumatonometer, the dynamic amplification factor (P1/P2) for most IOPs is approximately 2 and phase lag less than 1% for frequencies up to 10 Hz. (Yang *et al.* (2000) made a limited investigation of the frequency response by comparing the OBF pneumatonometer's measurement of pulse rate to that of an ECG. There was good correlation between the two instruments (r = 0.98, y = 0.93x + 4.04). Hendrickson (1999) investigated the OBFA pneumatonometer's measurements of IOP pulse in vitro using a mechanical eye and found the slightest changes in IOP pulse parameter were closely detected by the pneumatonometer. Unfortunately a full frequency response of the instrument was not performed (Hendrickson, personal communication).

$$\theta = \tan^{-1}(f / f_0)$$

θ = phase lag

f = the frequency of interest

f_0 = the fundamental vibration frequency of the membrane beneath the tonometer (typically 150 Hz)

Equation 1.15 Formula for calculating the phase lag of typical pneumatonometers (Silver *et al.*, 1994).

Note on terminology: the pneumatonometer is also referred to as the pneumatic tonometer, pneumatic tonograph, and pneumotonometer. The etymologically more correct term pneumatonometer will be used in this work.

1.7. *Methods of analysing IOP pulse measurements*

1.7.1. Pulse Amplitude

The IOP pulse is most simply and classically measured as the difference between the systolic peak to the diastolic trough of the IOP waveform (Figure 1.14). It is commonly referred to as the IOP pulse amplitude (PA) and is measured in mmHg. PA values have been measured manometrically in the human eye: Tønjum (1972) reported an average amplitude of 2.4 mmHg at an IOP of 15 mmHg. However the eyes were commonly destined for enucleation due to an intraocular neoplasia and the values cannot be taken as representative of a normal range. Larger normative data exist for human IOP pulse amplitudes measured with a variety of applanation-type tonometers (Table 1.4).

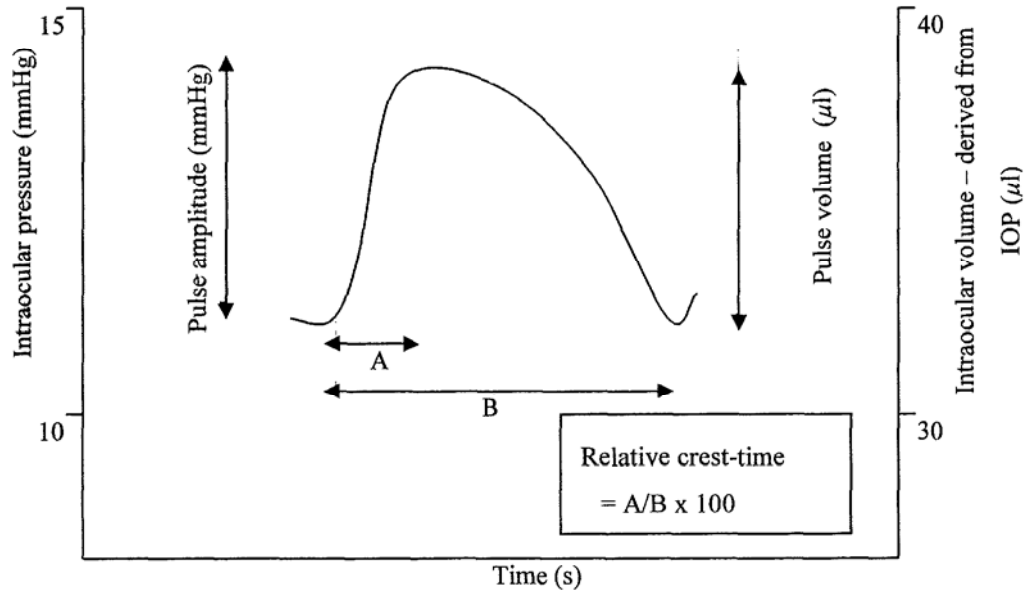

Figure 1.14. Analysis parameters of the IOP pulse.

Author	Tonometer type	Subject number	Age range (years)	IOP pulse amplitude (mmHg)
Ruiz-Barranco (1960)	Goldmann tonometer	150 eyes (77 subjects)	Not known	2.1 (range 0.3 to 4.0)
Perkins (1981)	Modified Goldmann	112 eyes	Not known	2.84 (1.16)
Langham *et al.* (1976)	Digilab pneumatonometer	85 eyes	20 to 45 years	1.5 (1.0)
Jain and Marmion (1976)	Digilab pneumatonometer	40 eyes (20 subjects)	50 to 70 years.	3.25 (0.25)
Buchanan and Williams (1985)	Digilab pneumatonometer	58 eyes	21 to 25 years	1.77 (0.67)
Tonjum (1972)	Digilab pneumatonometer	20 eyes	20 eyes (15 to 72 years)	2.9 (0.9)
Gekkieva *et al.* (2001)	Ocular Blood Flow Analyzer pneumatonometer	155 eyes	44.2 (16 to 78)	Male: 2.0 (0.6) Female: 2.3 (0.7)

Table 1.4. Normative values of intraocular pressure (IOP) pulse amplitude for a number of applanation-type tonometers. IOP pulse amplitude values are presented as mean (SD) unless specified differently (Buchanan & Williams, 1985; Gekkieva, Orgül, Gherghel *et al.*, 2001; Jain & Marmion, 1976; Langham, Leydhecker, Krieglstein *et al.*, 1976; Perkins, 1981a; Ruiz Barranco, 1960; Tønjum, 1972).

1.7.2. Pulse volume

The variation in IOP pulse may be converted to an estimate of the change in intraocular volume by using an appropriate pressure-volume relationship (Figure 1.14) (Langham, 1987). This is known as a derived ocular pulse volume.

1.7.3. Crest Time Evaluation

Adapting an analysis technique used in arterial occlusive disease, Hørven & Nornes (1971) described a method of analysing the position of the systolic peak in comparison to the IOP pulse duration. This was referred to as crest-time evaluation of the pulse and is calculated by dividing the time from the start of the pulse to systolic peak by the duration of the complete pulse (Figure 1.14). It is usually expressed as a percentage. A normal range was reported to be 35.5% to 47.5% (mean 41.5%).

1.7.4. Pulsatile ocular blood flow

In 1989, Langham, Farrell, O'Brien *et al.* (1989b) described a method of calculating the pulsatile component of ocular blood flow (POBF) from measurement of the IOP pulse. The theory has been described as follows (Silver *et al.*, 1994; Silver, Farrell, Langham *et al.*, 1989).

The theory of calculating POBF

The eye is taken to be an end-arterial bed where total blood flow consists of the following components: blood inflow comprised of pulsatile flow and non-pulsatile flow; and blood outflow which is steady and non-pulsatile but can be thought of as comprising two components each equalling pulsatile and non-pulsatile inflow (Figure 1.15).

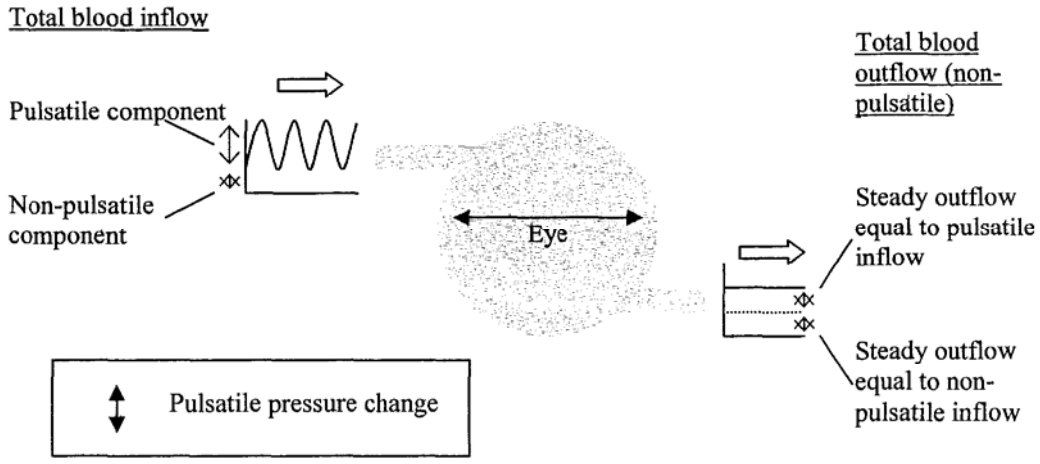

Figure 1.15 Schematic representing the components of total ocular blood flow.

The shape of the IOP pulse represents the change in intraocular volume as these components of flow vary over the period of one heart beat. Specifically, the increase in IOP from its diastolic trough to its systolic peak (A to B in Figure 1.16) represents an increase in intraocular volume as net blood inflow exceeds net blood outflow from the eye. The IOP pulse cycle can estimate gross flow through its conversion to a volume pulse, with the appropriate use of a pressure-volume relationship, and then by differentiating its change in volume over time (dV/dt):Figure 1.16.

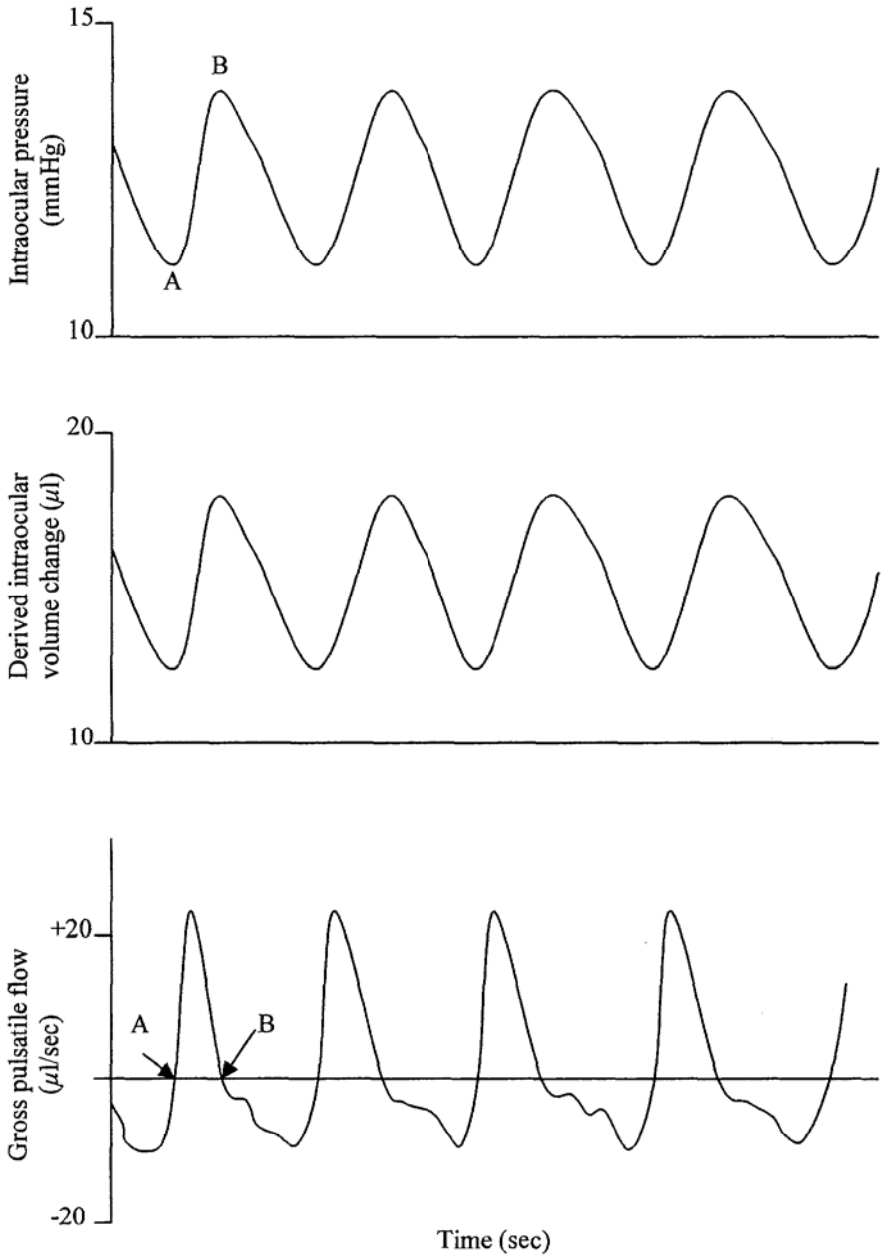

Figure 1.16 **Stages in the derivation of gross pulsatile ocular blood flow from the IOP pulse wave. Top figure, Original IOP pulse; middle figure, estimated intraocular volume change (calculated from a pressure-volume relationship equation); bottom figure, calculated gross pulsatile intraocular flow (differential of volume change).**

Any one point on the gross pulsatile flow graph corresponds to a combination of the two net flows: a time dependent amount of net pulsatile inflow and a steady component of net outflow that balances pulsatile inflow over time. While it is not possible to extract the net pulsatile inflow component, it is possible to find the steady outflow component that balances the pulsatile inflow. This corresponds to the lowest point on the flow graph: the point where net pulsatile inflow is zero and only steady outflow remains. As net pulsatile outflow must balance net pulsatile inflow over time, the reciprocal of this point corresponds to net pulsatile inflow. In order to find the minimum point in the gross flow chart, a mathematical curve is fitted which comprises a linear equation to fit the steep increase in flow and an exponential function for the descending portion. The intersection of these functions is taken as the minimum flow. An approximate value of POBF can also be calculated from the formula in Equation 1.16 (Silver *et al.*, 1989; Spraul *et al.*, 1998).

$$POBF = c * f * \left[\frac{dV(t_B)}{dt_B} - \frac{dV(t_A)}{dt_A} \right]$$

$POBF$ = pulsatile ocular blood flow (μl/min)

c = a constant c (which is related to the systolic duration of the pulse)

f = pulse rate (beats/min)

dV/dt = incremental change in volume at either point B or A on the pulse profile Figure 1.16 (μl/s)

Equation 1.16 Calculation of pulsatile ocular blood flow (Silver *et al.*, 1989; Spraul *et al.*, 1998).

The calculation of POBF is dependent on a number of assumptions. These have been discussed by Krakau (1992) and James (1998):

- Total inflow during a pulse period must be the same as total outflow. This can be taken for granted especially if a number of pulses are averaged (Krakau *et al.*, 1995).
- Pulsatile blood inflow has a point or time where it is at a minimum and does not reverse direction: i.e. there is no retrograde flow. This assumption is supported by ultrasound studies of arterial blood velocity in the ocular vessels of normal adults (Baxter, Williamson, McKillop *et al.*, 1992).

- There is a faithful conversion of IOP change to volume change. This is dependent on the pressure-volume relationship. Due to the large inter-individual variation in pressure-volume relationships, it is difficult to define an absolute POBF value for an individual subject (James, 1993). However, a comparison of average group values may be possible as well as a comparison between right and left eyes of an individual as long as the two eyes can be taken as similar (no marked anisometropia, for example).
- The pressure variations are recorded with high fidelity. This will be dependent on the nature of the tonometer used.
- Total blood outflow is steady and non-pulsatile. This appears to be justified from flow studies on the central retinal vein and vortex veins (Riva *et al.*, 1985; Schlegel *et al.*, 1969). At the exit point of the intraocular veins from the eye there is some collapse of the vessel which may constitute a limited amount of pulsatile outflow (Michelson *et al.*, 1997).
- That any pulsatile change in aqueous outflow is negligible

The non-pulsatile component of blood inflow has no influence on the IOP pulse and therefore cannot be estimated. Although Langham (1989a) has claimed that the non-pulsatile component of blood inflow is negligible, evidence from Doppler ultrasound studies and theoretical models indicate that a substantial component of blood inflow is steady (Baxter *et al.*, 1992; Krakau, 1995).

Average POBF values taken in the sitting position on a normal population are reported to be between 648 μl/min and 740 μl/min (Gekkieva *et al.*, 2001; Langham, Farrell, Krakau *et al.*, 1991a; Langham, Grebe, Hopkins *et al.*, 1991b; Quaranta, Manni, Donato *et al.*, 1994; Ravalico, Pastori, Toffoli *et al.*, 1994; Ravalico, Toffoli, Pastori *et al.*, 1996). Mean POBF values in oriental populations are lower: 593 μl/min to 661 μl/min (Lam *et al.*, 1999; Mori, Konno, Hikichi *et al.*, 2001a).

Fontana, Poinoosawmy, Bunce *et al.* (1998) conducted the largest study on normative POBF values. POBF measurements were taken on 777 subjects with a mean age of 53 ± 14 years that had been screened using a questionnaire to exclude ocular pathology and

cardiovascular disease. The distribution of POBF values had a positive skew with a significant difference between male and female populations. The results were therefore reported as median and interquartile ranges for each sex: male 731 (605; 913) μl/min and female 886 (696; 1089) μl/min.

1.7.5. Spectral analysis

Best & Rogers (1974), and more recently, Evans, Hosking, Embleton *et al.* (2002) have found deconstructing the IOP pulse, by Fourier analysis (Appendix 2), superior at detecting abnormal populations than more traditional IOP pulse parameters, such as PA and POBF. (Evans *et al.* (2002) speculate that the higher order components of the IOP pulse waveform may represent characteristics of the intraocular vascular bed (such as compliance) analogous to those found in the arterial pulse (1.3.3).

1.8. *Physiological studies of the IOP pulse*

1.8.1. General characteristics of the IOP pulse

The IOP pulse is a rhythmic oscillation in the IOP which is in phase with the cardiac cycle. The rising phase occupies approximately one third of the cycle time. In some recordings the descending phase can show what has been described as a dicrotic notch (Best *et al.*, 1970; Perkins, 1981a).

1.8.2. The influence of intraocular pressure

A number of studies have looked at the association between a human subject's IOP and their IOP pulse amplitude using applanation-type tonometers. The majority of studies have shown a strong positive correlation between the two (Buchanan *et al.*, 1985; Claridge & Smith, 1994b; Phillips, Tsukahara, Hosaka *et al.*, 1992): the higher a subject's IOP, the higher the IOP pulse amplitude. Two studies have found no significant association between the two (Gekkieva *et al.*, 2001; Perkins, 1981a).

The relationship between IOP pulse amplitude and mean IOP level has also been investigated by direct manipulation of the IOP. Lawrence & Schlegel (1966) directly

cannulated the anterior chamber of anaesthetised living rabbits in order to influence IOP and also measure the amplitude of the IOP pulse. In addition, they encircled the globe with a fine mercury filled latex tube which measured the concomitant pulse change in ocular volume. The change in both PA and ocular volume pulse could then be described as IOP changed. The results of these experiments can be summarised as follows (Figure 1.17).

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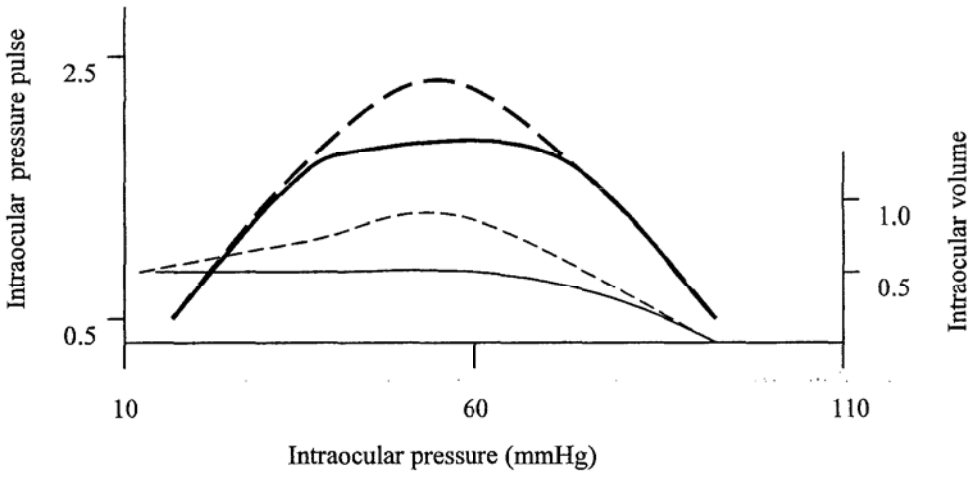


Figure 1.17 **The relationship between the spontaneous change in intraocular pressure (IOP pulse amplitude) and volume as a function of intraocular pressure: Bold curves, IOP pulse amplitude: Fine line curves, volume pulse; continuous lines denote relationships on first raising intraocular pressure and broken lines denote relationships on subsequent reduction of IOP. Adapted from Lawrence and Schlegel (1966).**

On first raising the IOP, the change in ocular volume with each heart beat remained stationary and the IOP pulse amplitude steadily increased. On reaching an IOP level just below the diastolic value of the femoral arterial blood pressure, the IOP pulse amplitude plateaued. Further increase in IOP saw both IOP pulse amplitude and volume pulse collapse until they were both extinguished at the level of the systolic arterial pressure. An interesting finding, on subsequent reduction of IOP, was the significant overshoot in IOP pulse amplitude and volume pulse (dashed lines in Figure 1.17). The authors attributed this increased ocular pulse to a change in the eye's vasculature, such as a metabolic hyperaemic response, induced by the ischaemia of the initial IOP rise. Bynke (1968) and Akazawa, Tokoro & Funata (1994) have confirmed this bell shaped relationship between IOP pulse amplitude and IOP in rabbits, although the plateau stage has been disputed (Akazawa *et al.*, 1994).

The direct manometric measurement of the IOP pulse as IOP changes in man appears not to have been directly reported (Eisenlohr *et al.*, 1962b). Instead, a number of investigators have measured the human IOP pulse, using a pneumatonometer, whilst manipulating IOP using a scleral suction cup (see 1.6.3). Surprisingly, the results differ substantially from the above manometric data in rabbits. Langham *et al.* (1978) reported the following response of the IOP pulse amplitude to a scleral cup-induced rise in IOP: an initial rapid fall; a plateau phase; and a final loss of pulse at suprasystolic pressures (Figure 1.18).

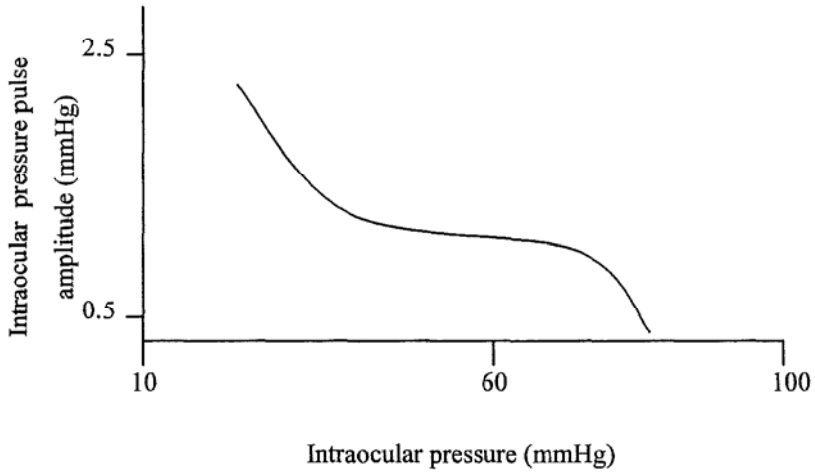


Figure 1.18. The mean IOP pulse amplitude as a function of IOP (induced by change in negative pressure of a scleral suction cup). Adapted from (Langham *et al.*, 1978).

A comparison of Figure 1.18 to the manometrically found relationship in rabbits (Figure 1.17) shows a conflicting response of the IOP pulse to the initial experimental rise in IOP: a rise in amplitude in rabbits and a fall in amplitude in humans. This contradictory reduction in IOP pulse amplitude to a scleral cup-induced rise in IOP in humans has been confirmed by Ravalico (Ravalico *et al.*, 1996). Either the human response of the IOP pulse to an experimental level of IOP differs from that predicted in theory and in animal studies, or a methodological difference exists between the studies (Akazawa *et al.*, 1994; Bellarminoff, 1886; Bynke, 1968; Friedland, 1983; Lawrence *et al.*, 1966). Bynke (1968; 1969) concluded that excessive force from instrumentation on the eye may deform the ocular shell or kink the retrobulbar vessels and diminish ocular pulse measurements. The use of scleral suction-cups in the human studies may have induced such a side-effect.

The relationship between POBF measurements and the level of IOP has similarly been studied. In addition to the misgivings raised about the use of scleral suction-cups to manipulate IOP discussed above, as POBF is derived from a change in IOP at a particular IOP level, such results are also highly dependent on the accuracy of the pressure-volume relationship used. Having noted these reservations, POBF values have been shown to fall as IOP rises in both suction-cup experiments and in cross-sectional population studies (Fontana *et al.*, 1998; Gekkieva *et al.*, 2001; Kergoat, 1997; Lam *et al.*, 1999; Ravalico *et al.*, 1996).

1.8.3. Refractive error

Myopic eyes have consistently been found to have significantly lower PA and lower POBF values than emmetropic and hyperopic eyes (Isambert, 1971; Perkins, 1981a; Ravalico, Pastori, Crocè *et al.*, 1997; Shih, Horng, Yang *et al.*, 1991; To'mey, Faris, Jalkh *et al.*, 1981). Furthermore there is a strong correlation between the degree of myopia and the amplitude of the IOP pulse or the POBF value (Lam *et al.*, 1999; Perkins, 1981a; Ravalico *et al.*, 1997; Shih *et al.*, 1991). This finding has most recently been reiterated by Lam, Wong, Lam *et al.* (2002) A number of reasons have been given for this reduction of PA and POBF values with increasing myopia (Ravalico *et al.*, 1997):

- Change in the elasticity of the ocular shell. Microscopic evidence in humans and histological studies on animals have shown that the sclera of myopic eyes, particularly the posterior sclera, is associated with a change in fibre patterns and elasticity (Curtin, Iwamoto & Renaldo, 1979; Phillips & McBrien, 1995). A more distensible ocular shell would, as predicted by the pressure-volume relationship (1.5), produce a smaller pressure change for a given change in volume.
- Variation in ocular volume. Myopic eyes are associated with larger ocular volumes (Cheng *et al.*, 1992). A larger eye would again, from the ocular pressure-volume relationship (1.5), produce a relatively smaller IOP pulse.
- Isambert (1971) suggested the smaller IOP pulse amplitude in myopes is due to a smaller choroidal volume. This is supported by MRI studies that have found choroidal beds to be thinner in myopes and the presence of choroidal degeneration found in severe myopia (Cheng *et al.*, 1992).

The substantial prevalence of myopia in oriental populations has been given as a reason for their low mean POBF value (1.7) (Goh & Lam, 1994; Lam *et al.*, 1999).

1.8.4. Axial length

James, Trew, Clark *et al.* (1991c) investigated the theory that variations in ocular volume was responsible for the correlation found between refractive error and ocular pulse values. Axial length measurements, as taken using A-scan ultrasonography, were correlated with IOP pulse amplitude and POBF values. A strong correlation was found that accounted for 37.4% and 58.6% of the total variance in PA and POBF measurements respectively. Although acknowledging that reduced elasticity or choroidal volume could still be a factor, the authors concluded that the strong correlation between IOP pulse values and refractive error primarily arose as an artefact from the pressure-volume relationship. The strong correlation with axial length measurements has subsequently been reconfirmed by Ravalico *et al.* (1997) and Mori *et al.* (2001a).

1.8.5. Elasticity of the ocular shell

Although theoretically an influencing factor upon IOP pulse measurements, lack of a non-invasive technique to measure ocular elasticity has hindered such a study. Kothe & Vachon (1992) have described a strong correlation between ocular rigidity and POBF. However as Friedenwald's coefficient of ocular rigidity does not dissociate the effect of ocular volume from ocular elasticity (1.5), the result may have arisen from the variation in eye volume rather than differing stiffness in the ocular shell (Perkins, 1981b).

1.8.6. Diurnal variation

IOP is well known for its change over a 24 hour cycle (1.1.1): most commonly peaking in the morning and falling to a trough in the evening (Zeimer, 1996). Claridge *et al.* (1994b) investigated whether the IOP pulse was subject to a similar daily cycle. In normal subjects, while PA value roughly followed IOP value (both highest in the morning), POBF remained stable. Although this may represent constancy of blood flow, it is dependent on the assumptions that the proportion of non-pulsatile flow is stable and that the pressure-volume relationship used to calculate POBF is precise.

1.8.7. Age

Although Perkins (1981a) and Gekkieva *et al.* (2001) found no correlation between either PA or POBF and age, larger studies demonstrated a small yet significant reduction in both measures in the older generations (Fontana *et al.*, 1998; Ravalico *et al.*, 1996).

1.8.8. Cardiovascular functions

A number of investigators have studied the IOP pulse during or immediately after exercise (Mittag, Serle, Schumer *et al.*, 1994; Schmetterer, Dallinger, Findl *et al.*, 1998; Schmidt, Mittag, Pavlovic *et al.*, 1996). The cardiovascular response to exercise in healthy subjects is to increase both heart rate and stroke volume (Levick, 1995). In isometric exercise (for example, when performing a sustained handgrip), mean arterial blood pressure rises significantly but in dynamic exercise (for example, using an exercise bike), although systolic blood pressure increases, diastolic blood pressure is stationary or may fall due to the decrease in peripheral vascular resistance. Schmetterer *et al.* (1998) found no change in PA or POBF value when subjects underwent isometric exercise. Colour Doppler ultrasound measurements were taken concurrently and indicated a shift in the proportion of pulsatile to non-pulsatile ocular blood flow occurred. The authors concluded that, although IOP pulse parameters were stationary, total ocular blood flow had increased during exercise. Schmidt *et al.* (1996) and Mittag *et al.* (1994) used an exercise bike in their studies. Although heart rate and APP increased more than 60%, no significant change was found in PA value. Schmidt *et al.* (1996) concluded that isolated functional autoregulation minimised the change in the IOP pulse and confirmed the premise that sympathetic vasoconstriction occurs during times of increased perfusion pressure (Bill *et al.*, 1990).

In a novel study, Trew, James, Thomas *et al.* (1991a) isolated the influence of heart rate on the IOP pulse without the usual concomitant physiological changes found, for example, in strenuous exercise. Using subjects who had previously been fitted with pacemakers, IOP pulse measurements were made as subjects' heart rates were varied from 70 to 120 beats per minute (bpm). The POBF value was found to be maximal at around a heart rate of 90 bpm and decreased at lower and higher frequencies. The measure of PA, pulse volume and stroke volume decreased linearly as heart rate increased.

In population studies, a subject's resting heart rate is correlated with PA value; a higher pulse rate producing a smaller amplitude (James *et al.*, 1991c; Ravalico *et al.*, 1996; Gekkieva *et al.*, 2001). In contrast, a weak although significant rise in POBF with increasing heart rate has been reported (Fontana *et al.*, 1998), James *et al.* (1991c) using multiple regression, found heart rate to account for approximately 5% of the variance in PA measurements.

When a subject moves from an upright to a supine position, stroke volume, heart rate and mean arterial blood pressure all decrease (Levick, 1995). In contrast, IOP is known to rise a few millimetres of mercury, possibly as a result of the engorgement of the intraocular vasculature as blood redistributes about the body (Kothe, 1994). The POBF value has been found to be consistently lower in the supine position (Ravalico *et al.*, 1996; Trew & Smith, 1991b). The effect on IOP pulse amplitude with posture change is less marked: Trew *et al.* (1991b) and Ravalico *et al.* (1996) finding no change but Buchanan *et al.* (1985) finding it falls. It has been speculated that the fall in IOP pulse values on lying down indicate a drop in ocular blood flow and reveal a potential ischaemic risk (Ravalico *et al.*, 1994). However as colour ultrasonography studies show a decrease in the proportion of pulsatile to steady blood flow in the retrobulbar vessels on lying down, the IOP pulse results may reflect just a shift in pulsatility (Canning & Restori, 1988). Kothe (1994) has stated that other reasons for a posture-related fall in IOP pulse values may include the associated fall in heart rate and the rise in episcleral venous pressure increasing resistance to flow.

Cross-sectional studies have not demonstrated a correlation between systolic, diastolic or mean arterial blood pressure and either the PA or POBF value (Gekkieva *et al.*, 2001; Mori *et al.*, 2001a; Ravalico *et al.*, 1996). A correlation between PA and APP has been reported by Bron, Knox & Gaasterland (1967) and Knox (1973) but not by others (James *et al.*, 1991c; To'mey *et al.*, 1981).

Schmetterer *et al.* (1998) investigated the influence of the Valsalva maneuver on the IOP pulse. The Valsalva maneuver occurs whenever a subject expires against an enforced pressure: such as when blowing up a balloon or when expiring against a closed glottis. During the Valsalva maneuver, intrathoracic pressure rises temporarily and reduces the volume of venous blood draining in to the heart. This produces a fall in stroke volume and,

to maintain body blood flow, an increase in heart rate. Schmetterer *et al.* (1998) found both PA and POBF to decrease significantly during the Valsalva maneuver: by 16% and 7% respectively. The authors concluded the change in IOP pulse reflected the fall in stroke volume. The fall in IOP pulse values during the Valsalva manoeuvre has recently been confirmed by Khan, Hughes, Tom *et al.* (2002).

1.8.9. Gender

Both PA and POBF values have consistently been found to be higher in the female gender (Fontana *et al.*, 1998; Gekkieva *et al.*, 2001; Perkins, 1981a). A smaller average ocular volume and a higher average heart rate have been given as possible explanations for this gender difference in IOP pulse. The possible vasoactive role of oestrogens as a reason for the higher IOP pulse values in females has also been investigated (Centofanti, Bonini, Manni *et al.*, 2000a). Noting that pre-menopausal women had significantly higher POBF and PA values than a post-menopausal group and a group of age matched males, Centofanti *et al.* (2000b) compared the effect of Hormone Replacement Therapy (HRT) on the IOP pulse. Both PA and POBF measures were significantly higher in a post-menopausal group whilst they were taking HRT compared to when they were off medication. The investigators concluded that the change in IOP pulse is a measure of the vasodilatory effect of oestrogen on an end-arterial bed. The increased level of oestrogen during pregnancy has similarly been given as the reason why women in their first and second trimester have higher values of POBF (Centofanti, Migliardi, Bonini *et al.*, 2002).

1.8.10. Blood gases

Knowing the sensitivity of ocular vessels to blood oxygen and carbon dioxide (CO_2), the response of the IOP pulse to hyperoxic and hypercapnic conditions has been studied (Kergoat & Faucher, 1999; Roff *et al.*, 1999; Schmetterer, Dallinger, Findl *et al.*, 2000b). Breathing 100% oxygen did not influence the PA or the POBF measure (Kergoat *et al.*, 1999; Roff *et al.*, 1999; Schmetterer *et al.*, 2000b). Subjects breathing either 5% CO_2 mixed with room air or 5% CO_2 mixed with 95% oxygen (Carbogen) had an increase in both the amplitude of the IOP pulse and the POBF measure (Kergoat *et al.*, 1999; Schmetterer *et al.*, 2000b). In studying a number of techniques to measure ocular blood flow, Roff *et al.* (1999) raised their subjects' end-tidal respiratory CO_2 level by approximately 15%. Whilst the

blood flow parameters found with colour Doppler ultrasonography and scanning laser flowmetry exhibited change, the POBF values was not significantly different during hypercapnia.

1.9. *Studies of the IOP pulse in pathology*

1.9.1. Carotid artery disease

The measurement of the IOP pulse pre-empted the use of Doppler ultrasonography as a non-invasive method of detecting carotid artery disease and proved moderately successful at detecting carotid artery stenoses and carotid arteriovenous fistulas (Best, Plechaty, Harris *et al.*, 1971b).

The carotid artery, particularly at its site of bifurcation into internal and external carotid, is prone to stenosis from atheromatous plaques (Figure 1.19). Reduction in PA value was associated with stenoses of the carotid where the lumenal cross sectional area had reduced to less than 50% (Galin & Best, 1971; Galin, Best, Plechaty *et al.*, 1972). Detection of lesser degrees of carotid narrowing, using the IOP pulse, was not deemed possible because blood flow in a carotid remains approximately normal until 50% of the artery is occluded (Best, Keyes, Plechaty *et al.*, 1971a; Best, Pola, Plechaty *et al.*, 1971c). Some investigators have claimed greater sensitivity at detecting early carotid stenosis with IOP pulse measurement. Langham & Preziosi (1984) found plotting PA value against suction cup-induced rises in IOP could detect more subtle stenoses (Schilder, 1989). Best *et al.* (1974) claimed even 20% carotid stenoses induced a detectable change in the Fourier components of the IOP pulse wave in rabbits.

In contrast, carotid arteriovenous fistulas (abnormal connections between carotid artery and cavernous sinus) have been associated with abnormally high IOP pulse values (Knox, 1973). Golnik & Miller (1992) found the PA to range from 3 to 10 mmHg in eyes whose subjects had ipsilateral carotid fistulas. The rise in IOP pulse amplitude is believed to occur from both the APP being augmented through the venous circulation and the associated rise in IOP due to increased episcleral venous resistance (Galin & Harris, 1966).

Figure 1.19 Example of an angiography of an internal carotid stenosis: provided courtesy of TBVM Study Group.

In order to detect or screen for carotid artery disease, a number of criteria for anomalous PA values have been suggested (Table 1.5) (Bron *et al.*, 1967; Golnik *et al.*, 1992; Perkins, 1985). Using these criteria, approximately 70 to 90% of the studies' subjects with carotid artery disease were detected. Golnik *et al.* (1992) stated the screening specificity of their criterion (a difference of more than 1.6 mmHg in amplitude between eyes) to be 100%.

Author	Absolute IOP pulse amplitude	Between eye difference in IOP pulse amplitude
Perkins (1985)	< 1.5 mmHg (for emmetropes and hyperopes) < 1.0 mmHg (for myopes)	> 0.5 mmHg
Bron *et al.* (1967)		> 25%
Golnik and Millar (1992)		> 1.6 mmHg

Table 1.5 Diagnostic IOP amplitude criteria used in the detection of carotid artery disease (Bron *et al.*, 1967; Golnik *et al.*, 1992; Perkins, 1985).

Having found that brachial APP influenced PA value (Bron *et al.*, 1967), Knox (1973) claimed better differentiation of diseased from normal eyes could be obtained by the ratio of the two; patients with carotid fistulas having abnormally large PA to brachial APP ratios and patients with carotid stenoses having low ratios.

A variant examination, using carotid artery compression, to investigate for disease was developed by Bron (1967). In a normal subject ipsilateral compression of the carotid artery (low down on the neck so as to avoid stimulating the vagal centre of the carotid sinus) produced an initial fall in IOP and PA followed by a quick recovery when compression was released. This represented falling blood supply to the eye, as its ipsilateral route was blocked, and is known as a positive ipsilateral compression response. In a subject with severe unilateral carotid stenosis the response may be opposite to normal. On compression of the ipsilateral carotid, no change may be seen in the IOP pulse (negative ipsilateral compression response) but may diminish on compression of the contralateral carotid. This represents the situation where one carotid is so severely occluded that the other carotid has replaced it as a collateral supply.

Castren & Lavikainen (1963) described how the IOP pulse on the ipsilateral side of a carotid occlusion was delayed in onset compared to the contralateral pulse. They concluded that the delay represented the longer path taken by the collateral arterial pressure pulse wave.

The exact site of the carotid stenosis influences the effect on the IOP pulse. Schilder (1994) described a case where a complete stenosis occurred above the point where the ophthalmic artery branched off. The IOP pulse amplitude was atypically large on the side of the stenosis. The author concluded the anomalously high PA value was due to the shunting of blood, previously supplying the brain, to the ocular circulation. Kaufmann, Fierz, Kollias *et al.* (2002) recently described a case report of asymmetric PA and brachial blood pressure values in a patient with a severe stenosis of the proximal brachiocephalic trunk.

The asymmetry in IOP pulse values in carotid artery disease is not fixed. There is evidence that over time the collateral supply from other large arteries of the neck take over and the

PA measure recovers (Best *et al.*, 1971a; Schilder, 1994). In addition, recovery of PA and POBF values are often seen once surgical removal of the stenosis has occurred (Claridge & James, 1994a).

Amaurosis fugax is a common symptom in carotid artery disease. McKibbin and Verma (1999c) found that PA values were lower in eyes of patients who complained of recurrent amaurosis fugax even though no significant carotid stenosis was found by Doppler ultrasound. The authors concluded that transient losses in vision were due to more local drops in ocular perfusion pressure arising from retrobulbar vessel disease.

1.9.2. Glaucoma: background

It is estimated that glaucoma affects 66.8 million people worldwide and as many as 6.7 million suffer bilateral blindness from the condition (Quigley, 1996). In the spectrum of degenerative optic neuropathies that the term glaucoma covers, primary open-angle glaucoma (POAG) is the most prevalent form in the western world. In primarily Caucasian populations, the prevalence in those aged over 40 years is 1.2% (Tuck & Crick, 1998). POAG causes degeneration of the optic nerve head through progressive retinal ganglion cell death and is characterised by distinctive changes in disc morphology and pathognomonic loss of visual field. Raised IOP is the leading risk factor (Table 1.6)(Sommer, 1996), but approximately one third of POAG patients have IOP values consistently within the normal range; these patients are notionally referred to as having normal-tension glaucoma (NTG) (Quigley, 1993). For the nomenclature of this thesis, patients with POAG are defined as having an abnormally high IOP and patients whose IOP is within the normal range described as having NTG.

The known increased risk (Table 1.6) of developing POAG if a direct member of the family has the disease, or if the subject is of African descent, indicates a hereditary component to POAG (WuDunn, 2002). A number of genetic loci have now been associated with various forms of glaucoma and the first protein to be discovered as coded from one of these sites (the myocilin protein) is found in the trabecular meshwork, optic nerve and retina (Alward, 2000).

	Risk Factor	Study	Year	Sample
Consistent risk factors *	Intraocular Pressure	Framingham Eye Study	1980	Population study (2,433 persons)
		Baltimore Eye Survey	1991	Population study (5,308 persons)
		Barbados Eye Study	1995	Population study (4709 persons)
		Rotterdam Study	2000	Population study (6760 persons)
		CNTG Study Group	1998	Prospective trial (145 patients)
	Age	Barbados Eye Study	1995	Population study (4709 persons)
		Rotterdam Study	2000	Population study (6760 persons)
	African descent	Baltimore Eye Survey	1991	Population study (5,308 persons)
	Family history	Barbados Eye Study	1995	Population study (4709 persons)
		Baltimore Eye Survey	1991	Population study (5,308 persons)
		Rotterdam Study	1998	Population study (6760 persons)
Possible risk factors #	Diabetes Mellitus	Beaver Dam Eye Study	1994	Population study (4926 persons)
		Blue Mountains Study	1997	Population study (3,654 persons)
	Myopia	Blue Mountains Study	1997	Population study (3,654 persons)
		Malmo Eye Survey	2001	Survey (32,918 persons)
	Migraine	CNTG Study Group	2001	Prospective trial (160 patients)
		Phelps & Corbett	1985	Case-control study (54 patients)
	Low blood pressure	Kaiser *et al.*	1993	Case-control study (149 patients)
		Graham *et al.*	1995	Retrospective study (84 patients)
	High blood pressure	Kashiwagi *et al.*	2001	Case-control study (43 patients)
		The Rotterdam Study	1995	Population study (4187 persons)
		Baltimore Eye Survey	1996	Population study (5,308 persons)

Table 1.6 **Risk factors in primary open-angle glaucoma: * Consistent risk factors found in large scale population epidemiological surveys and prospective longitudinal studies; # Less consistent risk factors found in some population studies and clinic-based studies (CNTGSG, 1998b; Dielemans, Vingerling, Algra *et al.*, 1995; Dielemans, Vingerling, Wolfs *et al.*, 1994; Drance, Anderson & Schulzer, 2001; Graham & Drance, 1999; Grødum, Heijl & Bengtsson, 2001; Kahn & Milton, 1980; Kaiser, Flammer, Graf *et al.*, 1993; Kashiwagi, Hosaka, Kashiwagi *et al.*, 2001; Klein, Klein & Jensen, 1994; Leske, Connell, Wu *et al.*, 1995; Mitchell, Hourihan, Sandbach *et al.*, 1999; Mitchell, Smith, Chey *et al.*, 1997; Sommer, Tielsch, Katz *et al.*, 1991; Varma, Hilton, Tielsch *et al.*, 1995; Wolfs, Borger, Ramrattan *et al.*, 2000; Wolfs, Klaver, Ramrattan *et al.*, 1998).**

The exact mechanism of glaucomatous degeneration is not completely known and evidence exists for a number of pathways that contribute and interact to produce final ganglion cell damage (Nickells, 1996). The primary location of the degenerative changes seen in POAG

is at the level of the lamina cribrosa in the optic nerve head. Retinal ganglion cell death in this region appears to occur through an apoptotic, rather than necrotic, mechanism. Apoptosis is described as programmed cell death and is not associated with the inflammatory signs seen in necrosis. Two principal theories of POAG pathogenesis, mechanical and vasogenic, remain viable explanations to the observed damage. Each theory feeds into a final common pathway of cell death (Figure 1.20).

1.9.3. Mechanisms in the pathophysiology of primary open-angle glaucoma

Intraocular pressure

Raised IOP is a consistent risk factor to the cause of POAG and, at present, is the only conventional method (through IOP reduction) of treatment. Excessive IOP at the level of the lamina cribrosa is thought to cause damage either through biomechanical disruption, ischaemia through reduced perfusion pressure, or a combination of the two. Numerous animal models have shown that abnormally raised IOP produces optic nerve degeneration that is anatomically and functionally equivalent to that observed in POAG (Dandona, Hendrickson & Quigley, 1991; Harwerth, Crawford, Frishman *et al.*, 2002; Quigley & Addicks, 1980).

Neurotrophic factor deprivation

Most neurons, including retinal ganglion cells, depend on certain biochemical signals expressed at synaptic connections. Prolonged withdrawal of these trophic signals travelling along the cell's axon leads to the initiation of cell death through apoptosis. A possible insult to axoplasmic transport in glaucoma, leading to neurotrophin withdrawal, could arise either from mechanical axon compression or local ischaemia (Nickells, 1996). Artificial application of neurotrophic factors following either raised IOP or optic nerve axotomy, significantly prevents ganglion cell degeneration (Osborne, Ugarte, Chao *et al.*, 1999).

Direct mechanical disruption

Quigley, Hohman, Addicks *et al.* (1983) have shown that glaucomatous optic nerve heads, even in the earliest stages of the disease, are associated with compression of the connective tissue sheets that compose the lamina cribrosa and support the axonal bundles.

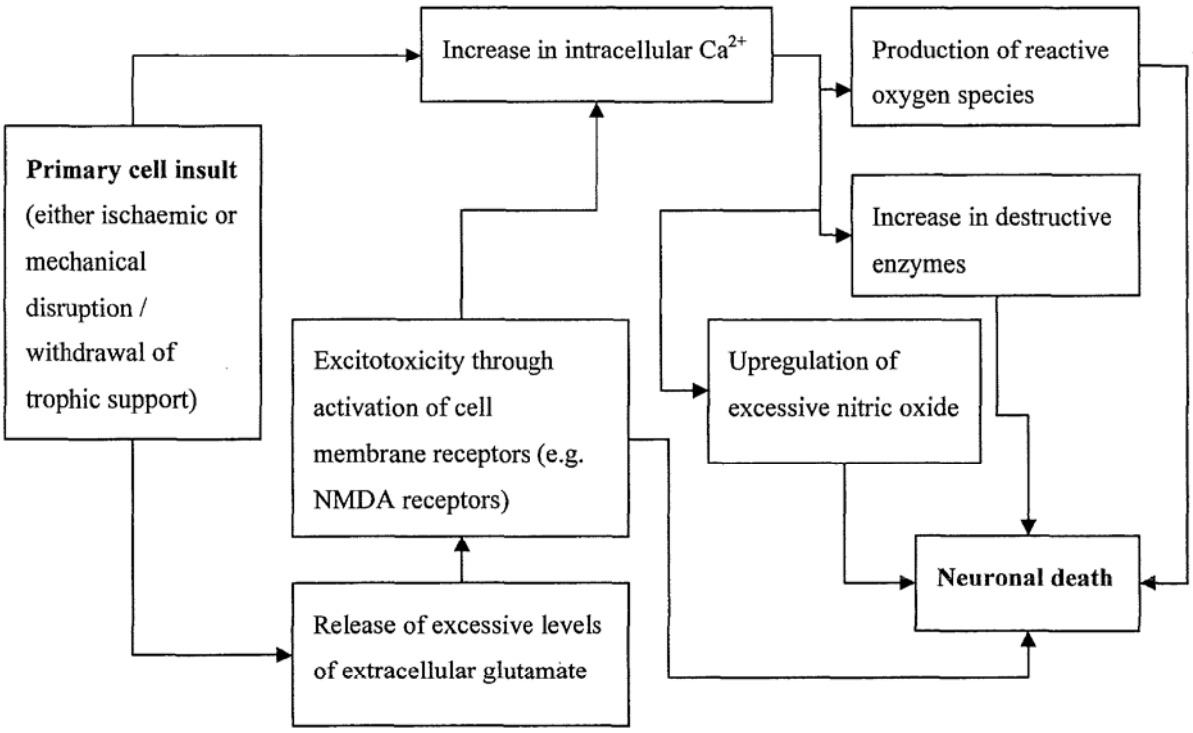


Figure 1.20 Possible pathophysiological pathway in Primary Open-Angle Glaucoma: adapted from (Osborne *et al.*, 1999).

Experimentally-induced glaucoma in primates is associated with interruption of axonal transport at the level of the lamina cribrosa which leads rapidly to ganglion cell degeneration (Quigley *et al.*, 1980; Quigley & Anderson, 1976). Yan, Coloma, Metheetrairut *et al.* (1994) proposed that shear stress at the disc edge, produced by the cribrosal plate bowing backwards under pressure, was the dominant disruptive force to ganglion cell axons. The inferior and superior poles of the lamina cribrosa appear particularly vulnerable to deformation due to the thinner collagen beams and larger pores in this region (Miller & Quigley, 1988).

Neurotoxicity

It has been speculated that excessive levels of neurotransmitter chemicals, such as glutamate, may play a role in retinal ganglion cell death. Although it is not known whether excessive glutamate production could be causative or secondary to glaucoma, its neurotoxic effects are likely to contribute to ganglion cell death (Sucher, Lipton & Dreyer, 1997). The membrane receptor, N-methyl-D-aspartate (NMDA), has been implicated as the mediator for such excitotoxicity (Siliprandi, Canella, Carmignoto *et al.*, 1992). Toxic levels of

glutamate or other neural amino acids have not been confirmed in rats with experimental glaucoma (Levkovitch-Verbin, Martin, Quigley *et al.*, 2002).

Excessive oxidative reactive species

Circulating oxygen free radicals could potentially damage retinal ganglion cells (Haefliger, Dettman, Liu *et al.*, 1999; Haefliger, Fleischhauer & Flammer, 2000). These reactive molecules, such as the peroxynitrate anion ($OONO^-$) or hydroxide radical (OH^-), denature proteins, phospholipids and nucleic acids. It is not known whether they may occur as a primary or secondary (arising from apoptotic or necrotic cell death) cause of POAG. Optic nerve head astrocytes play an important supporting role to passing ganglion cell axons and express a pathological form of NO synthase (NOS-2) when stimulated by inflammatory cytokines and possibly elevated hydrostatic pressure (Liu & Neufeld, 2000). Excessive NO is cytotoxic to neighbouring cells by either inhibiting mitochondrial respiration or binding with passing superoxide molecules (O_2^-) to form peroxynitrate.

The immune system

The possibility that immune-related disease is a factor in the pathogenesis of glaucoma, particularly the NTG form, was raised by Cartwright, Grajewski, Friedberg *et al.* (1992). The authors made a retrospective study of their NTG patients and found 30% had one or more immune-related disease(s) compared to 8% in a matched comparison group. In addition, Wax (2000) proposed that glaucoma has an autoimmune element in its pathogenesis based on the finding that patients exhibit increased numbers of auto-antibodies. In contrast Schwartz (2001) has found evidence that the immune system may play a role in limiting the extent of secondary cell damage in POAG and has termed this protective autoimmunity (Bakalash, Kipnis, Yolcs *et al.*, 2002; Moalem, Leibowitz-Amit, Yoles *et al.*, 1999).

Ischaemia: introduction

The hypothesis that reduced ocular blood flow is responsible as a direct or contributory cause of glaucoma is not new (Smith, 1891b), and has received much attention (Flammer, Orgul, Costa *et al.*, 2002). The presence of NTG, and the fact that many patients with abnormally raised IOP do not develop POAG (Kass, Heuer, Higginbotham *et al.*, 2002), has

fuelled speculation that ischaemia is the missing factor in the pathogenesis of POAG (Jay, 1992; Weinreb, 1992). Evidence of reduced ocular blood flow in glaucoma is not lacking. Deficient haemodynamic values in both high- and normal-tension glaucoma have been reported for the ophthalmic artery (Butt, O'Brien, McKillop *et al.*, 1997; Cellini, Possati, Profazio *et al.*, 1997; Galassi, Nuzzaci, Sodi *et al.*, 1994; Harris, Sergott, Spaeth *et al.*, 1994; Rojanapongpun, Drance & Morrison, 1993a). posterior ciliary arteries (Cellini *et al.*, 1997; Liu, Chiou, Chiang *et al.*, 1999a; Pillunat, Stodtmeister, Marquardt *et al.*, 1989; Rankin, 1999), central retinal artery (Ates, Uretmen, Killi *et al.*, 2000; Butt *et al.*, 1997; Liu *et al.*, 1999a; Rankin, 1999), and the circulations of optic nerve head (Grunwald, Piltz, Hariprasad *et al.*, 1998b; Kerr, Nelson & O'Brien, 1998; Michelson, Langhans & Groh, 1996; Nicolela, Hnik & Drance, 1996; Schwartz, 1994; Tuulonen, Nagin, Schwartz *et al.*, 1987), retina (Duijm, van den Berg & Greve, 1997; Findl, Rainer, Dallinger *et al.*, 2000b; Kerr *et al.*, 1998; Michelson *et al.*, 1996; Németh, Michelson & Harazny, 2001; Nicolela *et al.*, 1996; Schwartz, 1994; Wolf, Arend, Sponsel *et al.*, 1993), and choroid (Findl *et al.*, 2000b). Whether these reductions in blood flow parameters are causatory or secondary to the disease process, is a matter of debate (Iwata, Shirakashi, Fukuchi *et al.*, 1991; Prünte, Orgül & Flammer, 1998; Quigley, 1989).

Ischaemia: proposed model of pathophysiology

A model for the pathogenic role of insufficient blood flow to the optic nerve and its interaction with IOP has been proposed by Flammer (1996): Figure 1.21.

Optic nerve head ischaemia could arise from either increased resistance to blood flow or from a perfusion pressure that falls below the autoregulatory ability of the vascular bed. There is some evidence to support both these causatory aspects in POAG:

Ischaemia: reduced blood flow through increased resistance

Increased opposition to blood flow may arise (Equation 1.3) from raised blood viscosity or excessively narrow lumens of the end-arteries. Schulzer, Drance, Carter *et al.* (1990) described how POAG patients may be composed of two separate populations, one of which is characterised by disturbed blood clotting and biochemical measures. Moreover, rheological status has been speculated as the reason why diabetes mellitus may be a risk

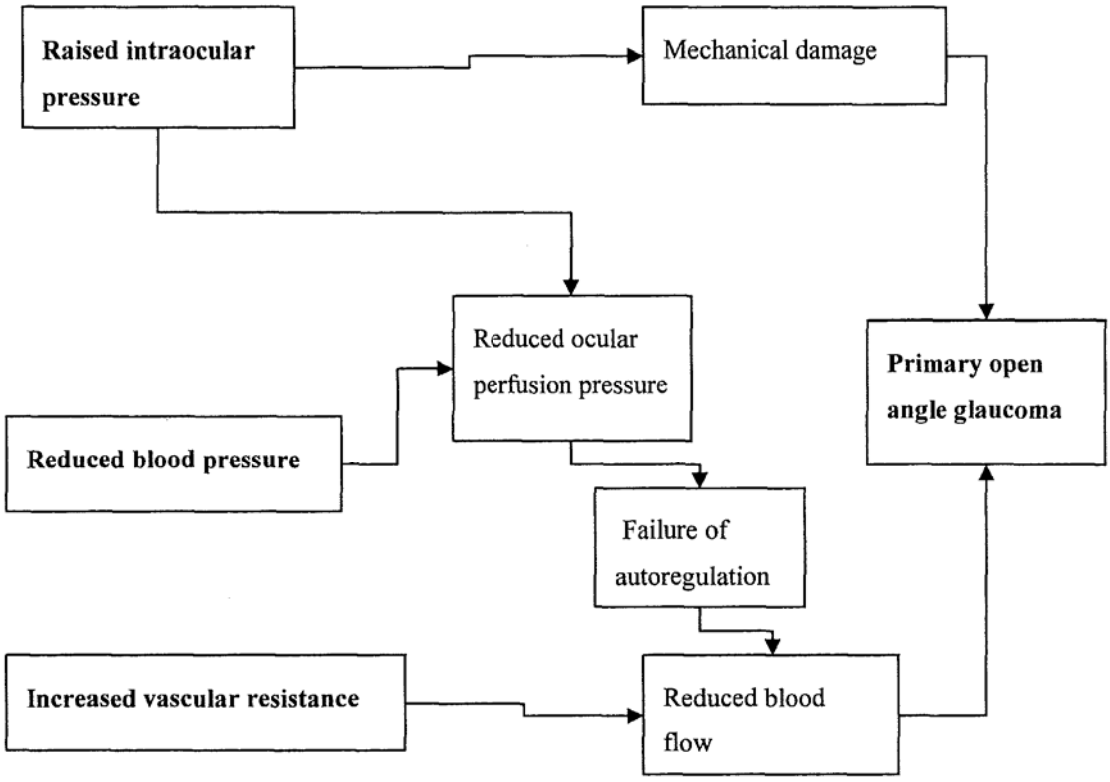


Figure 1.21 A proposed pathway for the role of ischaemia in the pathogenesis of primary open angle glaucoma. Adapted from (Flammer, 1996)

factor in POAG (Becker, 1971). Altered blood viscosity in POAG has not been confirmed by other investigators (Carter, Brooks, Doyle *et al.*, 1990), and the association between diabetes and POAG has not been supported by some large-scale population studies (Leske *et al.*, 1995; Tielsch, Katz, Quigley *et al.*, 1995a).

Although there is little evidence of a generalised increase in arterial resistance (arteriosclerosis) in POAG, the proposal that some patients exhibit a localised opposition to flow has received some support (Flammer, Haefliger, Orgul *et al.*, 1999). With the reported association between migraine and NTG (Phelps & Corbett, 1985), and the finding that some ordinary subjects exhibited glaucomatous-like field defects when their hands were placed in ice-cold water (Gasser & Flammer, 1987), the notion of a localised ocular **vasospasm** was proposed (Flammer, Guthauser & Mahler, 1987).

Vasospasm can be defined as an excessive vasoconstriction of an artery or arteriole to a physiological stimulus. Vasospasm can arise either as a primary condition or secondary to a medication or disease. Primary vasospastic syndrome is characterised by a tendency towards cold hands and sometimes feet, low blood pressure, slower sleep onset and a female gender (Flammer, Pache & Resink, 2001; Pache, Kräuchi, Cajochen *et al.*, 2001). Raynaud's disease is a special form of secondary vasospastic syndrome which involves mostly, though not exclusively, the hands. Migraine sufferers frequently exhibit vasospastic syndrome but the two conditions are not synonymous (Hegyalijai, Meienberg, Dubler *et al.*, 1997). Reports of visual field improvement in normal and NTG patients who took oral calcium channel blockers to improve peripheral circulation, appeared to support the concept of a localised ocular vasospasm (Flammer *et al.*, 1987; Guthauser, Flammer & Mahler, 1988; Kitazawa, Shirai & Go, 1989). Subsequent studies have found that glaucoma patients, both POAG and NTG, exhibit abnormally high levels of acral vasospasm, as demonstrated using nailfold capillary microscopy or cutaneous laser Doppler flowmetry, during cold provocation (Broadway & Drance, 1998; Gasser & Flammer, 1991; O'Brien & Butt, 1999). Recently Gherghel, Orgul, Dubler *et al.* (1999) found that those otherwise healthy subjects who exhibit acral vasospasm demonstrate altered blood flow regulation in the central retinal artery compared to subjects with normal finger capillary blood flow. According to Broadway, Nicolela & Drance (1999), a history of a vasospastic tendency, such as migraine or cold hands, is associated with focal-type neuroretinal rim loss in POAG.

The primary role of endothelin-1 in vasospasm has generated a number of investigations into whether this endothelial mediator has a role in POAG (Flammer *et al.*, 2001). Abnormally high levels of endothelin-1 have been found in the blood plasma of NTG patients and the aqueous of POAG patients (Cellini *et al.*, 1997; Tezel, Kass, Kolker *et al.*, 1997). Buckley, Hadoke, Henry *et al.* (2002) have shown that small resistance arteries taken from gluteal biopsies of NTG patients respond in an enhanced manner to endothelin-1 and 5-hydroxytryptamine in comparison to arteries of control subjects. An endothelin-1-induced vasospastic mechanism for the development of POAG is supported by animal models. Oku, Sugiyama, Kojima *et al.* (1999) have shown that chronic exposure to endothelin-1 in the vitreous cavity of rabbits produces an atrophic degeneration of the optic nerve head morphologically characteristic of glaucoma even though IOP remained within normal limits.

The association between a vasospastic tendency and POAG has been questioned by some investigators. Usui & Iwata (1992) failed to demonstrate any difference in finger blood flow between glaucoma patients and normal subjects. Improvements in the visual field performance of NTG patients, with either calcium channel blocker or carbonic anhydrase medication, were not found by Lumme, Tuulonen, Airaksinen *et al.* (1991) and not all POAG patient groups exhibit raised plasma endothelin-1 levels (Holló, Lakatos & Farkas, 1998). Large scale population studies and prospective cohort studies have not found migraine to be a risk factor in POAG (Bonomi, Marchini, Marraffa *et al.*, 2000; Gordon, Beiser, Brandt *et al.*, 2002; Klein, Klein, Meuer *et al.*, 1993).

Ischaemia: reduced blood flow through low ocular perfusion pressure

In addition to localised ocular vascular resistance, optic disc ischaemia may result where ocular perfusion pressure is so low that it is beyond autoregulation (Flammer, 1994). The clinical impression that haemodynamic crises are a causative factor in NTG (Chumbley & Brubaker, 1976), and that excessive nocturnal dips in blood pressure occur in glaucoma patients with progressive field loss (Graham, Drance, Wijsman *et al.*, 1995; Hayreh, Zimmerman, Podhajsky *et al.*, 1994), supports such a hypothesis in the development of glaucoma. POAG patients that exhibit overdipping in nocturnal blood pressure also demonstrate altered haemodynamic features of the ophthalmic artery (Gherghel, Orgul, Gugleta *et al.*, 2001). Pillunat *et al.* (1989) demonstrated lower posterior ciliary artery perfusion pressures in NTG patients compared to other glaucoma groups and normal controls. An increased prevalence of postural hypotension has been reported in patients with NTG and reduced ocular perfusion pressure has been identified as a risk factor for POAG in two population studies (Demailly, Cambien, Plouin *et al.*, 1984; Tielsch, Katz, Sommer *et al.*, 1995b). Reduced ocular perfusion pressure was not found to be a risk factor in NTG and this may indicate that the association arises through the influence of IOP on the perfusion pressure calculation (Bonomi *et al.*, 2000). Contrary to Western studies, Kashiwagi *et al.* (2001) found Japanese NTG patients to exhibit an increased perfusion pressure and an abnormally smaller dip in nocturnal blood pressure compared to healthy control subjects.

Ischaemia: reduced blood flow through faulty optic nerve head autoregulation

Where ocular perfusion pressure is normal, optic nerve head ischaemia may still occur if local autoregulation is faulty. Autoregulatory dysfunction in glaucoma patients has been

demonstrated in the central retinal artery (Evans, Harris, Garrett *et al.*, 1999), the macular retinal (Grunwald, Riva, Stone *et al.*, 1984), and optic nerve head circulations (Pillunat, Stodtmeister & Wilmanns, 1987; Ulrich, Ulrich & Bohne, 1986).

1.9.4. Glaucoma and IOP pulse studies

Anomalies in the amplitude of the IOP pulse of POAG patients have been noted for some time (Hørven, 1970a; Langham *et al.*, 1976; Perkins, 1981a; To'mey *et al.*, 1981). Initially, it was not known whether these anomalies were specific to POAG or arose, indirectly, because of the generally higher IOP. Nicastro, Requa, Campo *et al.* (1987) investigated further and, by controlling for important physiological variables (heart rate, IOP and arterial pulse pressure), were the first to conclude that, relative to their IOP, POAG patients had low PA values. Studies have since confirmed that POAG patients have lower PA and POBF mean values than healthy subjects with similar IOP (ocular hypertensive subjects) (Kerr *et al.*, 1998; Schmidt, von Ruckmann & Mittag, 1998a; Trew & Smith, 1991c). In the same way, NTG patients have lower IOP pulse values than matched control groups (Fontana *et al.*, 1998; James & Smith, 1991b; Lambrou, Sindhunata, van den Berg *et al.*, 1989; Mittag *et al.*, 1994; Ravalico *et al.*, 1994). The reduction in PA value found in NTG patients has most recently been confirmed by Schwenn, Troost, Vogel *et al.* (2002) using the DOT dynamic tonometer. Due to the large variability in IOP pulse values in the normal population, the ability of the PA or POBF value to differentiate between POAG patients and normal subjects is poor (Lambrou *et al.*, 1989).

Reduced IOP pulse values in POAG patients have given rise to much speculation that they represent a sign of a vascular pathogenic factor (Fontana *et al.*, 1998; James *et al.*, 1991b). Unlike the above group control studies, and perhaps of more value in investigating whether IOP pulse values are a causative sign, Hitchings (1998) presented evidence from a longitudinal study on the predictive value of the IOP pulse in POAG. Out of 100 POAG patients who were followed for 5 years, those with a lower POBF value taken at the start of the study demonstrated greater progression in visual field loss. The behaviour of IOP pulse values in POAG patients has also been examined for any evidence of a vascular autoregulatory dysfunction. Quaranta *et al.* (1994) studied the effect of suction cup-induced IOP increments on POBF in NTG patients. They found that, as expected (1.8.2), POBF values fell with increasing IOP but that NTG patients' POBF values fell significantly faster

than those of a matched control group. The authors concluded that in NTG a satisfactory level of perfusion cannot be maintained during small increments in IOP. This anomalous drop in glaucomatous eyes' POBF values, with suction cup-induced rises in IOP, has not been confirmed by others (Langham *et al.*, 1991a; Ravalico *et al.*, 1994).

Although extensively studied (James *et al.*, 1991b; Trew *et al.*, 1991b; Trew *et al.*, 1991c), the measurement of IOP pulse values in different postures has provided no clinical utility in the diagnosis of POAG, or shown no evidence of an autoregulatory dysfunction in POAG (Kothe, 1994). The amplitude of the IOP pulse has been investigated as an index of arterial vasospasm in the eye. Schmidt, Ruckmann, Mittag *et al.* (1997) confirmed that the amplitude in NTG patients was lower than a matched control group but was no different between NTG patients with and without a demonstrable vasospastic reaction to the nailfold capillary blood flow test. Evidence of a reversible ocular vasospastic tendency has been shown in other pulse studies: POAG and NTG patients that demonstrate a vasospastic tendency, and are treated with oral calcium channel blockers or topical carbonic anhydrase inhibitors, increased their PA levels to a normal level (Schmidt, von Ruckmann & Pillunat, 1998b; Schmidt *et al.*, 1996).

1.9.5. Diabetes mellitus: an introduction

Diabetes mellitus is a group of metabolic disorders characterised by chronic hyperglycaemia due to relative insulin deficiency, or resistance or both (Gale & Anderson, 1994). Complications from diabetes arise in many organs of the body and the eye is no exception (Ariffin, Hill & Leigh, 1992). As many as two million people suffer from the disease in the United Kingdom, of whom approximately 2% are registered blind (Evans, 1995). Visual loss arises primarily from retinal microangiopathy that produces ischaemia and leakage at the macular (diabetic maculopathy) and vitreal haemorrhage and retinal detachment from the subsequent proliferation of fragile new vessels and their sequelae (proliferative retinopathy) (Vafidis, 2000). In addition to the changes in the retinal vasculature, there is evidence that similar diabetic lesions in the choroid contribute to visual loss (Dimitrova, Kato, Tamaki *et al.*, 2001; Hidayat & Fine, 1985; McLeod & Lutty, 1994).

Owing to the vascular nature of diabetic retinopathy, blood flow in the diabetic eye has been studied in order to investigate the development and progression of the disease. The majority of studies have naturally concentrated on the retinal circulation but, indocyanine green angiography, indicates similar changes in flow occur in the choroid (Weinberger, Kramer, Priel *et al.*, 1998). Early studies, using fluorescein angiography to determine retinal circulation times, found that retinal blood flow progressively increased as retinopathy advanced and then fell back to normal levels at the proliferative end-stage (Blair, Feke, Morales-Stoppello *et al.*, 1982; Cunha-Vaz, Fonseca, de Abreu *et al.*, 1978; Kohner, 1976; Yoshida, Feke, Morales-Stoppello *et al.*, 1983). Subsequent studies have shown that blood flow in diabetic eye disease is more complex. Fallon, Chowincyzk & Kohner (1986) investigated the effect of retinopathy on the macular circulation by measuring leukocyte density and velocity with a blue-field entoptoscope: lowest flow values were found at the pre-proliferative stage. Improved fluorescein angiography studies indicated that progression in retinopathy was associated with a fall in blood flow (Bertram, Wolf, Fiehöfer *et al.*, 1991; Bursell, Clermont, Kinsley *et al.*, 1996). Konno, Feke, Yoshida *et al.* (1996) followed a group of diabetic patients for approximately four years and described the retinal blood flow changes that occurred during this time. Retinal blood flow values were calculated by combining bidirectional laser Doppler to measure arterial velocity and vessel cross-sectional area found from fundus photographs. Compared to normal subjects, who exhibited only a very shallow decline in retinal blood flow with age (a 10% reduction between the ages of 30 and 56 years), the diabetic group exhibited a bimodal pattern that related to their disease duration. Those most recently diagnosed diabetics exhibited abnormally low retinal flow values that decreased with time. At approximately 20 years duration of diabetes, flow values were at their lowest and diabetic retinopathy most severe. Further, duration of diabetes was subsequently associated with rising retinal blood flow values.

There may be a number of reasons for the large variation, and often contradictory, results found in retinal blood flow studies in diabetes. Diabetic eye disease is associated with both the generation of capillary drop-out, which increases blood flow through the formation of arteriovenous shunts, and increased blood viscosity, which decreases blood flow (Cunha-Vaz *et al.*, 1978; Lawrenson, 2000). The level of blood flow measured may therefore depend on the exact location chosen. The effect of glycaemia on retinal blood flow is important. Retinal blood flow is directly proportional to the level of glycaemia in diabetics

(Bursell *et al.*, 1996; Findl, Dallinger, Rami *et al.*, 2000a), and appears to be due to the increased metabolism generated by excess glucose (Tiedeman, Kirk, Srinivas *et al.*, 1998). The level of measured blood flow may therefore depend on the glycaemic status of the subjects in the study. In addition, although retinal blood velocity may fall in diabetes, overall flow may remain stable from the increase in diabetic vessel diameters (Feke, Buzney, Ogasawara *et al.*, 1994).

Defects in the autoregulatory ability of diabetic patients' ocular blood flow have been recorded. Evans, Harris, Danis *et al.* (1997) by measuring retrobulbar blood velocities with colour Doppler ultrasound, demonstrated that diabetics with minimal retinopathy exhibited an abnormal response to breathing 100% oxygen. Instead of the resistance index of the central retinal artery rising, as in normal subjects, diabetic patients showed a decline in retinal resistance during hyperoxia. Poor control of diabetic retinal blood flow, in response to a rise in ocular perfusion pressure, has been demonstrated using both a cold-pressor test and isometric exercise (Dumskyj & Kohner, 1999; Osei, Fields, Cataland *et al.*, 1985). Impaired protection of the ocular vascular beds to surges in blood pressure would possibly explain the correlation between systemic hypertension and diabetic eye disease (Rassam, Patel & Kohner, 1995).

1.9.6. Diabetes and IOP pulse studies

As with the above ocular blood flow studies, investigations using the IOP pulse in diabetes have found similarly confusing results. Langham *et al.* (1991b) found increasing severity of retinopathy to be associated with a fall in POBF value. In contrast, MacKinnon, O'Brien, Swa *et al.* (1997) described an opposite relationship: increasing POBF values with increasing grade of diabetic retinopathy. The authors speculated that the difference in results lay in the fact that previous investigators had measured POBF in the supine position. A third study showed POBF to be below normal values in the early stages of retinopathy and to increase above normal values in pre-proliferative and proliferative stages (Geyer, Neudorfer, Snir *et al.*, 1999). The authors proposed that their results were comparable to the bimodal shift in retinal blood flow found by Konno *et al.* (1996). Most recently, Schmidt, von Ruckmann, Kemkes-Matthes *et al.* (2000) found no difference in PA value when young type I diabetic subjects were grouped by retinopathy grade.

It appears likely that IOP pulse studies suffer from the same variance effects found in other diabetic retinal blood flow studies. Perrott, North, Drasdo *et al.* (2001) demonstrated how POBF values in Type II diabetics closely follow changes in blood glucose concentration. The level of blood pressure appears to influence IOP pulse values in diabetics. Esgin, Alimgil & Erda (2001) found a group of diabetic subjects, with an undefined level of untreated retinopathy and normal blood pressure, to demonstrate lower than normal values for POBF and PA. A comparison group of diabetic patients with raised blood pressure exhibited IOP pulse values within the normal range.

1.9.7. The IOP pulse in other disease

Retinitis pigmentosa

Both POBF and PA values were reduced in eyes with retinitis pigmentosa (Langham & Kramer, 1990; Schmidt, Pillunat, Kohler *et al.*, 2001). Langham *et al.* (1990) speculated that choroidal perfusion played a part in the progression of retinitis pigmentosa as the pattern of field loss followed the gradient of ocular perfusion: an equatorial ring scotoma expanding until only macular vision is left. Schmidt *et al.* (2001) found that PA reduction followed visual field loss rather than predicted it and proposed that the diminished pulse represented a secondary choroidal degeneration arising from reduction in metabolic demand from the retina.

Choroidal melanoma

Malignant choroidal melanoma is associated with raised IOP pulse values. Hørven (1969) found that 11 out of 15 patients with diagnosed melanomas exhibited an PA value that was 15%, or more, greater in the affected compared to the healthy eye. A 15% inter-eye difference was chosen as it related to the 95% limits of normal inter-eye IOP pulse amplitude variation. Yang, Kent, Fenerty *et al.* (1997b) although not finding any difference in PA value, found the POBF value to be significantly greater in eyes with choroidal melanomas. Both groups of investigators attributed the increased IOP pulse values to the increase in intraocular vascular volume that the tumour produces.

Uveal and other eye diseases

The effect of occlusive and degenerative uveal disorders on the IOP pulse supports the assumption on the origins of the IOP pulse. Central retinal occlusion has been shown to produce only a minor (10%) decrease in amplitude compared to an almost complete loss of pulsation in atrophic choroidal disease (Bynke & Schele, 1967; Suzuki, 1962). Obliteration of the IOP pulse has been reported in amaurotic eyes suffering from arteritic anterior ischaemic optic neuropathy (AION) and is consistent with the lumenal closure of the posterior ciliary and ophthalmic arteries found in this inflammatory disease (Bienfang, 1989; Hørven, 1970b). Investigators have not found similar reductions in IOP pulse values for eyes with non-arteritic AION and concluded that, in this non-inflammatory form of the disease, ischaemia occurs from an acute drop in systemic perfusion pressure rather than through local occlusion (Bienfang, 1989; James & Smith, 1991a). Occlusion of choroidal vessels has been proposed as a possible cause for abnormal POBF values in exudative age-related macular degeneration. Abnormally low POBF values were found in patients with exudative AMD and choroidal neovascular scarring, whilst POBF values were higher in patients with active choroidal new vessels (Chen, Cheng, Lee *et al.*, 2001; Mori, Konno, Hikichu *et al.*, 2001b). McKibbin, Cassidy, Dabbs *et al.* (1999a) found measurement of POBF to be of no value in detecting retinopathy of prematurity.

Single reports exist on anomalous IOP pulse values associated with cluster-type headaches, Grave's disease and cataract (Alimgil, Benian, Esgin *et al.*, 1999; Hopkins, Grebe & Langham, 1989; Hørven & Sjaastad, 1977). The relevance of these findings is not known.

1.10. *Pharmacological and Surgical Associations*

1.10.1. Topical ophthalmic medications

The measurement of IOP pulse values has allowed a relatively non-invasive method of determining the effect of topical and systemic medications on human ocular blood flow. In particular, the theory that POAG may have a vasogenic aetiology has focussed many studies on the haemodynamic properties of existing and prospective glaucoma medications.

Non-selective and selective beta-adrenoceptor blocking agents are currently a mainstay of topical POAG treatment. β_2-adrenoceptors are present in choroidal vessels (Grajewski, Ferrari-Dileo, Feuer *et al.*, 1991) and their blockade, in theory, may induce vasoconstriction through α-adrenoceptor predominance. Conversely some beta-blocking agents demonstrate some agonistic properties which could lead to vasodilatation (Harris & Jonescu-Cuypers, 2001). Topical timolol 0.5% has been reported to cause a significant fall of between 13% to 50% in POBF value in normal subjects and POAG patients (Boles Carenini, Sibour & Boles Carenini, 1994; Kitaya, Yoshida, Ishiko *et al.*, 1997; Yoshida, Feke, Ogasawara *et al.*, 1991). Grajewski *et al.* (1991) compared IOP pulse volume measurements in normal subjects taking either topical or oral timolol. They concluded, on the basis that only oral timolol decreased IOP pulse volume measurements, that topical timolol produced insufficient vasoactive concentration at the level of the uveal vascular beds. These previous findings may have an alternative explanation. Timolol is a non-selective beta-blocker and its systemic absorption produces a negative chronotropic and inotropic cardiac response. Other investigators, some who were careful to minimise systemic absorption through lacrimal duct occlusion, reported no change in POBF values with topical timolol use in normal subjects and POAG patients (Morsman, Bosem, Lusky *et al.*, 1995; Sponsel, Mensah, Kiel *et al.*, 2000a; Trew *et al.*, 1991c). Topical use of betaxolol 0.5%, a selective beta-blocker that avoids cardiac side-effects, has no effect on POBF values in POAG patients (Boles Carenini *et al.*, 1994; Morsman *et al.*, 1995). Topical use of carteolol HCl 1.0% and levobunolol HCl 0.5%, both non-selective beta-blockers, have been associated with modest rises in IOP pulse volume and POBF values respectively in normal subjects and POAG patients (Bosem, Lusky & Weinreb, 1992; Morsman *et al.*, 1995; Yamazaki & Baba, 1993).

The muscarinic agonist, pilocarpine, theoretically could induce increased ocular blood flow through parasympathetic induced vasodilatation. Whereas Claridge (1993) reported no association in POBF value with topical 1% to 4% pilocarpine use in POAG patients, Shaikh & Mars (2001) found a significant increase of approximately 25% in POBF value in ocular hypertensive subjects using 2% pilocarpine.

Alpha-adrenergic agonists are a recent addition to the range of topical ocular hypotensive agents and act through suppressing aqueous production and increasing uveoscleral outflow.

In addition, agents such as brimonidine tartrate 0.2%, may have a vasoconstrictive effect through stimulating a vessel's α_2-adrenoceptors or a vasodilative effect by stimulating vascular endothelial cells to produce smooth muscle relaxing factors (Harris *et al.*, 2001). POBF value studies have not shown a consistent response to the use of topical 0.2% brimonidine tartrate: Vetrugno, Maino, Cantatore *et al.* (2001) reported POBF values to rise with the start of treatment in a small group of POAG patients; Sponsel, Paris, Trigo *et al.* (2002b) found no significant change in POBF values in a trial using normal subjects. Trew, Wright & Smith (1991d) investigated the vasodilative properties of a prospective new topical ocular hypotensive agent, bunazosin 0.3% (an adrenoceptor *antagonist*). Although IOP showed significant reduction in a group of normal subjects, POBF remained stable.

The prostaglandin analogues are recent topical glaucoma agents which produce substantial therapeutic reductions in IOP. Of these, the use of topical latanoprost 0.005% has been associated with an increase in POBF value of between 18% and 28% in normal subjects and NTG patients (Geyer, Man, Weintraub *et al.*, 2001; McKibbin & Menage, 1999b; Sponsel *et al.*, 2000a). The authors concluded that the increase was due to the subsequent rise in perfusion pressure or a direct vasodilative effect on the uveal vasculature. Sponsel, Paris, Trigo *et al.* (2002a) recently compared the effect of topical latanoprost 0.005% in one eye and unoprostone 0.15% (the latest prostaglandin analogue) in the other eye on POBF values in a group of POAG patients. Latanoprost produced a significantly greater increase in POBF value (+30%) over unoprostone (+18%).

Schmidt *et al.* (1998b) reported the effect of a topical carbonic anhydrase inhibitor (dorzolamide 2%) on the PA value in POAG patients and normal control subjects. In both groups IOP level dropped by approximately 20% whilst amplitudes rose by approximately 20%. The authors interpreted the results as an increase in ocular blood flow either occurring directly from the medication on choroidal vessels or through the increase in ocular perfusion pressure.

The above studies suffer from a serious possible weakness: the concomitant fall in IOP value that an ocular hypotensive drug induces. The known effect of IOP on IOP pulse (1.8.2) makes comparisons of IOP pulse amplitude dubious and pulse volume or POBF values reliant on the veracity of the ocular pressure-volume relationship that is used. Some

investigators have attempted to account for this confounding variable. Sponsel *et al.*, (2000a) compared POBF values in a subgroup of 9 normal subjects on either timolol-XE or latanoprost who experienced similar falls in IOP. The latanoprost treated eyes experienced a significant increase in POBF value (+16.7%) whereas those on the beta-blocker showed no significant change. The authors maintained that systemic variables were unaltered with either treatment. Liu, Ko, Cheng *et al.* (2002) adjusted for the fall in IOP in their comparison study of latanoprost and brimonidine on POBF values using multivariate analysis. After IOP was adjusted for, neither drug showed a significant increase in POBF value and the investigators concluded that the drugs either did not exert a direct vasomotor effect or that the effects were too trivial for the pneumatonometer to detect. Recently, Poinoosawmy, Indar, Bunce *et al.* (2002) used a novel vector method to characterise the combined change in IOP and POBF measurements following treatment with latanoprost, betaxalol or brimonidine. The results indicated that changes in POBF value were drug specific rather than IOP related with latanoprost showing the greatest rise in POBF value.

1.10.2. Non-topical medications

Oral medications

Some systemic medications have been reported to affect IOP pulse values. Schmidt *et al.* (1996) reported that oral nifedipine, a calcium channel blocker, increased the IOP pulse amplitude only in those NTG patients whose nailfold capillaries exhibited a vasospastic reaction to cold water provocation. As reported above (1.9.2), the authors interpreted the finding as evidence of ocular vasospasm existing amongst some NTG patients. Oral acetazolamide (a carbonic anhydrase inhibitor) produces cerebral vasodilatation, through the increase in local tissue acidosis, as well as a local ocular hypotensive effect (Sponsel & Shipman, 1997). Kerty, Hørven, Dahl *et al.* (1994) found oral acetazolamide to be associated with a fall in IOP pulse volume in a group of healthy subjects and speculated that a shift in pulsatile to non-pulsatile ocular blood flow may have occurred to explain the unexpected findings. Finally, POBF values increased by approximately 29% with the use of oral sildenafil (Sponsel, Paris, Sandoval *et al.*, 2000b): raising the potential of this medication to new heights.

Regional anaesthesia

Cataract surgery involving regional anaesthesia can result in ischaemic complications including central retinal vascular occlusion and anterior ischaemic optic neuropathy (Klein, Jampol, Condon *et al.*, 1982). Such sight-threatening complications have prompted investigators to explore the haemodynamic response, through measurement of the IOP pulse, to local ocular anaesthesia. Lidocaine injections in the retrobulbar, or peribulbar, space were associated with falls in POBF and PA values of the order of 25% to 50% of the pre-operative value (Chang, Hee, Ling *et al.*, 2000; Coupland, Deschenes & Hamilton, 2001; Pianka, Weintraub-Padova, Lazar *et al.*, 2001; Rich & James, 1989; Watkins, Beigi, Yates *et al.*, 2001). Lidocaine injections frequently contain supplementary pharmacologically active substances and these additives appear to contribute to the IOP pulse response. The addition of both adrenaline and hyaluronadase to lidocaine have been reported to increase the fall in IOP pulse values (Hulbert, 1998; Syrdalen & Hørven, 1970). As cardiovascular parameters were unaltered, and minimal change in IOP occurred during these investigations, the authors conclude the response arises from a direct pharmacological interaction between the anaesthesia and its additives on the ocular blood vessels. This conclusion is supported by laboratory investigations that have shown local anaesthetic drugs reduce endothelium-dependent smooth muscle relaxation in ciliary arteries (Meyer, Flammer & Lüscher, 1993). Subconjunctival injection on lidocaine has not been associated with a change in IOP pulse value (Chang *et al.*, 2000).

General anaesthesia

Two reports exist on the effect of general anaesthesia on the IOP pulse. Hørven & Syrdalen (1969) reported the substantial fall in IOP pulse amplitude in the fellow eyes of those undergoing common surgical procedures. The fall in IOP pulse amplitude coincided with the onset of patient sleep using either ether or Halothane and decreased to approximately 33% of the premedicated value. The authors found no change in blood pressure (heart rate was not reported) and concluded the response demonstrated a fall in blood flow to the eye. Robinson, White, McCann *et al.* (1991) reported their experiences of measuring POBF values during general anaesthesia and endotracheal intubation. POBF values significantly fell on sleep induction, using suxamethonium as anaesthetic, followed by a significant rise during intubation. The authors concluded that the POBF rise occurred from the stress response, as shown by an acute rise in blood pressure, to intubation.

1.10.3. Ocular surgery

Trabeculectomy is the procedure of choice in glaucoma management where medical management is insufficient. James (1994) has reported the mean POBF value to increase by 29% for glaucoma patients undergoing trabeculectomy. As with the above cautionary note on ocular hypotensive medication and their effect on the IOP pulse, it is necessary to interpret the POBF response in relation to the dramatic fall in IOP level (Yang & Hulbert, 1995). A comparative study by Poinoosawmy *et al.* (2002) suggests that the POBF value increase is not solely attributable to the IOP level because, although trabeculectomy produced the greatest fall in IOP, the ocular hypotensive medication latanoprost produced the greatest increase in POBF value.

Scleral buckling is a well-established surgical technique for treating rhegmatogenous retinal detachment. IOP pulse amplitude values have been reported to be significantly lower in those eyes with scleral buckling compared to their fellow eyes (Yoshida, Hirokawa, Ishiko *et al.*, 1992). As no pre-surgical values of the IOP pulse amplitude were given and IOP levels not reported, the interpretation of this study is difficult. The effect of cataract surgery has been reported not to affect IOP pulse values (James, 1994).

1.11. *Summary*

Measurement of the IOP pulse represents a relatively accessible method of determining the pulsatile nature of the intraocular vascular beds. Observational studies agree with anatomical knowledge that the IOP pulse represents the dilation and contraction of primarily the choroidal vasculature during the cardiac cycle. The IOP pulse is susceptible to many ocular and systemic variables which need to be carefully evaluated before a study can attempt to draw inferences from its behaviour. This review highlighted those important variables. Numerous clinical studies have found tantalising associations between IOP pulse values and pathological, pharmacological or surgical conditions. Conclusions are not always possible, due to the difficulty in interpreting what a change in IOP pulse represents. The challenge remains to extract from this small perturbation in IOP, a measure that allows clearer inferences to be drawn on the eye's vascular status.

2. Research Rationale

2.1. *Introduction*

Measurement of the IOP pulse presents a unique parameter that represents the interaction of the cardiovascular system with the eye. The IOP pulse is an easily accessible, relatively non-invasive, parameter that can be measured in the clinical setting. Although numerous changes in the IOP pulse have been demonstrated in association with physiological and pathological conditions, its lack of specificity limits its use. Whilst providing a possible insight into the vascular status of the eye, the relevance of an IOP pulse measurement is clouded by numerous influencing factors that obscure its potential. For the IOP pulse to be of clinical value, the challenge remains to extract the maximum information from, and differentiate the many nebulous factors that affect, this small fluctuation in intraocular pressure. The principal purpose of this work was to investigate methods of improving the IOP pulse's potential as a measure of clinical utility.

2.2. *Research Aims*

The main aims of this thesis were threefold:

- To optimise the measurement and analysis of the IOP pulse
- To investigate ocular and systemic variables that affect IOP pulse measurements
- To explore the nature of the pulse in health and disease and its relation to systemic cardiovascular variables

The components of this work are outlined below:

2.3. *Optimisation of the measurement and analysis of the IOP pulse*

2.3.1. A clinical comparison of the Dynamic Observing Tonometer and Ocular Blood Flow Analyzer pneumatonometer

The introduction of a new commercially available dynamic tonometer may provide the

researcher with a superior instrument with which to measure the IOP pulse. Possible advantages of the Dynamic Observing Tonometer (DOT) include the long data capture time (up to 4 minutes) and the ability to inspect the internal structure of the eye during recording (Entenmann *et al.*, 1997). It is a priority that the tonometric measurements made with the DOT are valid in terms of accuracy and precision. The purpose of this work was to assess the DOT's measurements against clinically accepted reference standards: Goldmann applanation tonometry for IOP measurements; and the OBFA pneumatonometer for PA measurements.

2.3.2. OBFA pneumatonometer reproducibility: the effect of operator experience and mode of application

For further studies to be performed with the OBFA pneumatonometer, confidence is required in the instrument's measurements. Commercial literature (Crowhurst & Massey, 1996) states that measurements taken with the pneumatonometer are as precise when used by a novice as they are with an experienced operator. In addition, measurements can be taken with the instrument hand-held rather than in its traditional slit-lamp mount. If measurements are reliable, a hand-held pneumatonometer may provide new opportunities in research studies. It is also necessary to determine how many recordings of the pneumatonometer are required to reduce measurement error to a minimum. The purpose of this work was: to investigate any learning effect on measurements taken with the pneumatonometer; to determine whether hand-held measurements differ significantly from those taken when slit-lamp mounted; and to establish the optimum number of pneumatonometer recordings to take on an eye.

2.3.3. The intraocular pressure pulse: a comparison of spectral analysis techniques

Previous authors have indicated that measurement of the analysis of the IOP pulse in the frequency domain provides greater differentiation between diseased and non-diseased eyes (Best *et al.*, 1974; Evans *et al.*, 2002). In addition, one group has speculated that the higher spectral components of the intraocular pressure (IOP) pulse waveform are characteristic of the eye's internal vascular compliance (Evans *et al.*, 2002). As part of the hypothesis

validation, this study investigated the test-retest variability of three techniques in obtaining the harmonic components of the IOP pulse.

2.4. *Investigation of ocular and systemic variables on IOP pulse measurements*

2.4.1. The effect of corneal thickness and corneal curvature on pneumatonometer measurements

There is confusion over whether IOP measurements taken with a pneumatonometer are, like those of the Goldmann tonometer, influenced by central corneal thickness (CCT) (Abbasoglu, Bowman, Cavanagh *et al.*, 1998; Singh, Goldberg, Graham *et al.*, 2001; Zadok, Tran, Twa *et al.*, 1999). In addition, and of importance to the present work, it is not known whether CCT similarly affects IOP pulse measurements. The purpose of this study was to determine the effect of CCT on IOP and PA measurements made with the pneumatonometer.

2.4.2. Low level hypercapnia and intraocular pressure pulse parameters

The inhalation of hypercapnic air has been shown to be an effective vasodilator on the ocular circulations (Harris *et al.*, 1998b). Previous results documenting the influence of breathing hypercapnic air on the IOP pulse are not consistent (Kergoat *et al.*, 1999; Roff *et al.*, 1999; Schmetterer *et al.*, 2000b). Comparison of IOP pulse measurements made under hypercapnic and normocapnic conditions provides an opportunity to investigate the effect of a known ocular vasodilator on this haemodynamic measure. The purpose of this study was to determine the effect of hypercapnia on the IOP pulse in a group of young healthy subjects.

2.5. *The nature of the IOP pulse in health and disease and its relation to systemic cardiovascular variables*

2.5.1. Spectral analysis of the intraocular pressure pulse: validatory investigations

Spectral analysis of the arterial pressure pulse has proved a valuable tool in cardiovascular research (Nichols & O'Rourke, 1998e). In particular the use of Fourier transformation to calculate vascular impedance has provided valuable information about vascular beds. The purpose of this work was to determine whether spectral analysis of the IOP pulse provides a greater ability to detect differences in groups of subjects with physiological and pathological vascular characteristics over traditional IOP pulse parameters. In addition, the higher spectral components of the IOP pulse were calculated for any evidence of vascular characteristics analogous to those of impedance found in arterial waveforms.

2.5.2. IOP pulse parameters and systemic vascular characteristics in glaucoma

A large body of evidence suggests that glaucoma has a vascular factor as part of its aetiology (Flammer *et al.*, 2002). The purpose of this study was to investigate both traditional and newly devised methods of IOP pulse analysis in separating groups of high-tension and normal-tension primary open-angle glaucoma patients from their respective control groups. In addition, as it has been proposed that the level of ocular perfusion pressure, and/or systemic tendency to vasospasm, may be factors in an ischaemic model of glaucomatous optic neuropathy, measures of systemic blood pressure and cutaneous finger blood flow were compared between glaucoma and control groups and any association with IOP pulse explored.

2.5.3. IOP pulse parameters and systemic vascular characteristics in diabetes mellitus

Ocular haemodynamic abnormalities in diabetes are widely reported (Schmetterer & Wolzt, 1999). The purpose of this study was to investigate both traditional and newly devised methods of IOP pulse analysis on groups of diabetic eyes (with varying severity of retinopathy) and in normal controls. Ocular haemodynamic values in diabetics patients have been previously shown to be influenced by systemic vascular properties (Esgin *et al.*, 2001;

Findl *et al.*, 2000a; Perrott *et al.*, 2001). To explore these associations further, measures of systemic blood pressure, glycaemic control and cutaneous blood flow were taken and their associations with group and IOP pulse investigated.

3. A Clinical Comparison of the Dynamic Observing Tonometer and Ocular Blood Flow Analyzer Pneumatonometer

3.1. *Abstract*

Purpose: The Dynamic Observing Tonometer (DOT) is a new instrument that allows continuous measurement of IOP. The purpose of this study was to determine the accuracy and repeatability of this new tonometer's IOP and IOP pulse measurements in comparison to accepted reference instruments.

Method: The IOP was measured by Goldmann applanation tonometry in one randomly chosen eye of 40 subjects (median age 66 yrs, range 21 to 77 yrs) who were considered to be clinically normal but with an unrestricted IOP range: 21.8 ± 4.5 mmHg. The IOP and PA measurements were then taken by both the DOT and a pneumatonometer. The accuracy of IOP and PA measurements of the DOT was determined by calculating the mean bias and 95% limits of agreement compared to Goldmann IOP and pneumatonometer PA respectively. The repeatability of the measurements was assessed in terms of the standard deviation of the distribution of differences between two repeated consecutive measurements.

Results: Analysis of the difference in IOP measurements found that the DOT had a mean bias of +2.1mmHg (95% limits of agreement: –4.0 to +8.2 mmHg) compared to Goldmann tonometry. There was a reasonable correlation between Goldmann and DOT IOP readings (r = +0.78, p <0.01). In measuring PA, DOT was found to have a mean bias of +0.4 mmHg (95% limits of agreement: –1.6 to +2.3 mmHg) compared to the pneumatonometer (r = +0.78, p <0.01). In assessing the repeatability of the DOT's IOP measurements, the first reading was on average 0.4 mmHg higher than the second (95% limits of agreement: –3.8 to +4.6 mmHg) with a coefficient of reliability of 0.91. For PA readings, the first DOT reading was on average 0.1 mmHg lower than the second (95% limits of agreement: –1.4 to +1.2 mmHg) with a coefficient of reliability of 0.90.

Conclusion: The mean bias and large range of differences in IOP determined by DOT measurement, indicate that the DOT is unlikely to replace Goldmann tonometry in a clinical setting.

3.2. *Introduction*

The Diagnostic Observing Tonometer (DOT, Ophthalmic Development Company AG, Zürich, Switzerland) is a diagnostic contact lens that can measure and record IOP in addition to allowing the practitioner a view of the posterior pole and anterior chamber angle (Dekker *et al.*, 1999). The DOT (marketed as the SmartLens®) therefore provides a number of novel investigative opportunities (Entenmann *et al.*, 1997). Firstly, as the DOT records IOP continuously, the PA associated with each heart-beat can be measured. Secondly, a continuous IOP recording provides the opportunity to analyse the pressure data as a waveform and recent research suggests that spectral analysis of the IOP pulse may provide a more sensitive technique over standard IOP pulse parameters in detecting ocular and systemic pathology such as glaucoma (Hosking, Evans, Embleton *et al.*, 2000). Thirdly, by manipulating the amount of appositional force exerted by the DOT against the eye, the investigator can influence the IOP and observe and record any changes to the IOP pulse or internal vasculature.

3.3. *Aims*

Only three previous publications have reported results on the validity of the DOT measurements and two of these used early prototype models of the instrument (Dekker *et al.*, 1999; Entenmann *et al.*, 1997; Troost *et al.*, 2001). The purpose of this study was to assess the DOT by determining its accuracy through comparison to present clinical reference instruments (Goldmann applanation tonometry for IOP and pneumatonometry for PA) and by assessing the repeatability of measurement by calculating the amount of difference in two repeated measurements and comparing such differences to the performance of a pneumatonometer.

3.4. *Materials and methods*

3.4.1. Experimental design

This was a validation study that compared measurements made at a single visit to accepted reference measurements (a measure of the instrument's accuracy) and the repeatability of those measurements (a measure of the instrument's precision).

3.4.2. Ethical approval

All experimental procedures of the study conformed to the tenets of the declaration of Helsinki and were approved by the Oxfordshire Research & Ethics Committee and the Aston University Human Science Ethical Committee. Written informed consent was obtained from all subjects willing to participate in the study.

3.4.3. Subject sample

Forty subjects (median age 66 years, range 21 to 77; 21 male and 19 female) were recruited from a glaucoma screening clinic at the Oxford Eye Hospital, UK. All subjects had been referred from primary care as glaucoma suspects and only those who were not subsequently diagnosed as having POAG were invited onto the study. The criteria used for the exclusion of POAG are found in Table 3.1. This subject population was chosen for the study in order to generate a wide range of IOP measurements. Potential research volunteers were also subject to the exclusion criteria found in Table 3.2.

1. An ophthalmoscopically normal optic nerve head as defined by:
    - The proportion of neuroretinal rim thickness obeying Jonas' ISNT rule
    - A vertical cup to disc ratio of less than 0.7
    - Absence of focal narrowing of the neuroretinal rim
    - A healthy coloured neuroretinal rim
    - Absence of large cribrosal pores in the optic cup
    - Absence of disc haemorrhages
    - Absence of focal or diffuse optic disc arteriole narrowing
    - Absence of slit defects in the retinal nerve fibre layer on red-free ophthalmoscopy
    - Absence of parapapillary chorioretinal atrophy
    - Absence of any other sign indicative of optic disc pathology

2. Normal visual fields as defined by automated visual field examination (SITA 24-2 program, Zeiss-Humphrey Visual Field Analyser).

Table 3.1 **Criteria used to define absence of primary open-angle glaucoma in an eye.. (Alexander, 1994; Hodapp, Parrish II & Anderson, 1993; Jonas, Budde & Panda-Jonas, 1999)**

- Corneal abnormalities
- Narrow anterior chamber angles (grade 2 or less, Shaffer gonioscopy grading system)
- Concurrent or previous eye disease
- Current ocular medication
- Previous ocular surgery
- Physical or mental incapacity to perform experimental procedures

Table 3.2 Dynamic Observing Tonometer study exclusion criteria

3.4.4. Instruments

The Dynamic Observing Tonometer (DOT)

The DOT has been previously described (1.6.4). The instrument's base unit automatically records the lowest pressure measurement during the recording as the subject's IOP and this theoretically corresponds to the end-expiratory diastolic value. If adequate repeatable IOP pulses have been captured during this time the base unit calculates a mean PA value. In addition, continuous sections of IOP data can be downloaded from the base unit to a personal computer where further analysis can be performed.

The Goldmann tonometer

The Goldmann applanation tonometer (Haag-Streit, Bern, Switzerland) is the present clinical gold standard for measuring IOP and has been adequately described elsewhere (Gilchrist, 1996; Moses, 1958).

Ocular Blood Flow Analyzer (OBFA) pneumatonometer

This instrument has been previously described (1.6.4). The Ocular Blood Flow Analyzer, formerly known as the Ocular Blood Flow Tonograph, consists of a pneumatonometer linked to a base unit. The pneumatonometer automatically calculates a mean value of IOP and PA from 5 IOP pulses that its software detects as being sufficiently repeatable from a recording of up to 20 seconds.

3.4.5. Experimental Procedures

All measurements were taken at a slit-lamp with the subject in a seated position. Measurements were taken from one randomly chosen eye to ensure independence of data. The randomisation method used, was the sealed envelope technique. In brief, a number of sealed envelopes are created before the study containing equal numbers of the two procedures that need to be randomised. For example, in this study 40 envelopes were used: 20 containing instructions to measure the right eye and 20 to measure the left.

Following instillation of a combination drop of Proxymetacaine 0.5% and Fluorescein 0.25% (Minims, Chauvin pharmaceuticals), subjects first had their IOP measured by Goldmann tonometry by an experienced ophthalmologist. Before each measurement, the drum was set to an initial position of 10 mmHg and the instrument was calibrated before each clinic, according to the manufacturers instructions, using the supplied weighted lever.

After a 10 minute rest, in order to minimise the tonographic effects of applanation tonometry (Recep, Hasiripi, Vayisoglu *et al.*, 1998), pairs of IOP and PA measurements were taken with both the DOT and the pneumatonometer. The sequence of presentation of tonometer type was randomised between patients: for example, two IOP and PA measurements would be taken with the DOT followed by two IOP and PA measurements with the pneumatonometer or vice versa. The study sequencing is summarised in Table 3.3.

3.4.6. Statistical Analysis

The accuracy of the DOT's IOP and PA measurements, with respect to their reference standards, were expressed in terms of their mean bias and 95% limits of agreement (Bland & Altman, 1986). In brief, the difference in measurement between DOT and the chosen reference instrument was plotted against the reference instrument's value. The level of agreement between the two instruments can then be expressed as the mean difference (the average of all the measurement differences) and the 95% levels of agreement (calculated as +/- two standard deviations of the measurement differences).

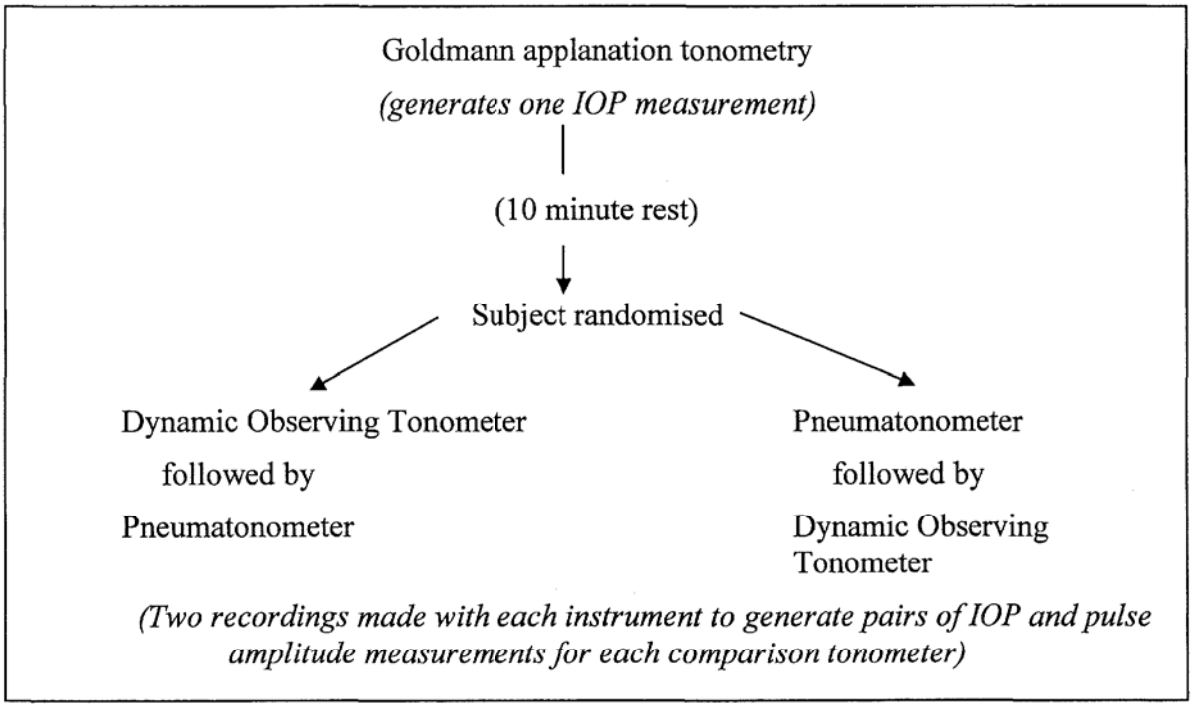


Table 3.3 Summary of tonometer sequencing.

The significance of the difference between the instruments' measurements was tested using paired *t*-tests taking $p < 0.05$ to be significant. Pearson correlation coefficients were calculated in order to make comparisons with other studies. Repeatability of the DOT and pneumatonometer pairs of IOP and PA measurements was expressed as the variation between the repeated measurements plotted against the mean of the two values (Bland *et al.*, 1986). In brief, this produces graphs similar to those for instrument accuracy but plot the difference in an instrument's two repeated measurements against the average of those measurements. Again the level of repeatability can be expressed as the mean and 95% limits of agreement.

The repeatability of the two instruments was alternatively expressed as a coefficient of reliability (Bland & Altman, 1996). The coefficient of reliability estimates the average correlation between all possible pairs of observations. For repeated measurements, as in this study, the coefficient was calculated via a one-way ANOVA as shown in Equation 3.1.

$$CR = \frac{(kSSb - SSt)}{(k-1)SSt}$$

SSb = the sums of squares between subjects

SSb = the total sums of squares

k = the number of measurements taken

Equation 3.1 Coefficient of reliability (CR) calculation

The coefficient of reliability also provides a measure of the percentage variance of disagreement between repeated measures by calculating (1-CR) x 100%. That is, measurements are perfectly repeatable when the coefficient of reliability is 1 and the percentage variance is 0%. The coefficient of reliability is sometimes referred to as the intraclass correlation coefficient.

3.5. *Results*

3.5.1. DOT accuracy

IOP measurements

The difference between Goldmann IOP and the first DOT IOP reading is plotted against the Goldmann measurement in Figure 3.1. DOT IOP values were on average 2.1 mmHg higher than Goldmann (mean value in Figure 3.1) and 95% were within the range of 4.0 mmHg below and 8.2 mmHg above the Goldmann value. Although there appeared to be a visible negative trend in the relationship between the agreement and IOP ($y = -0.11x + 4.49$), this was not statistically significant. For comparison the difference between Goldmann IOP and the first pneumatonometer IOP reading is plotted against Goldmann value in Figure 3.2. IOP readings made with the pneumatonometer were on average 1.3 mmHg lower than the Goldmann with 95% falling between 6.6 mmHg below and 4.0 mmHg above than the Goldmann value.

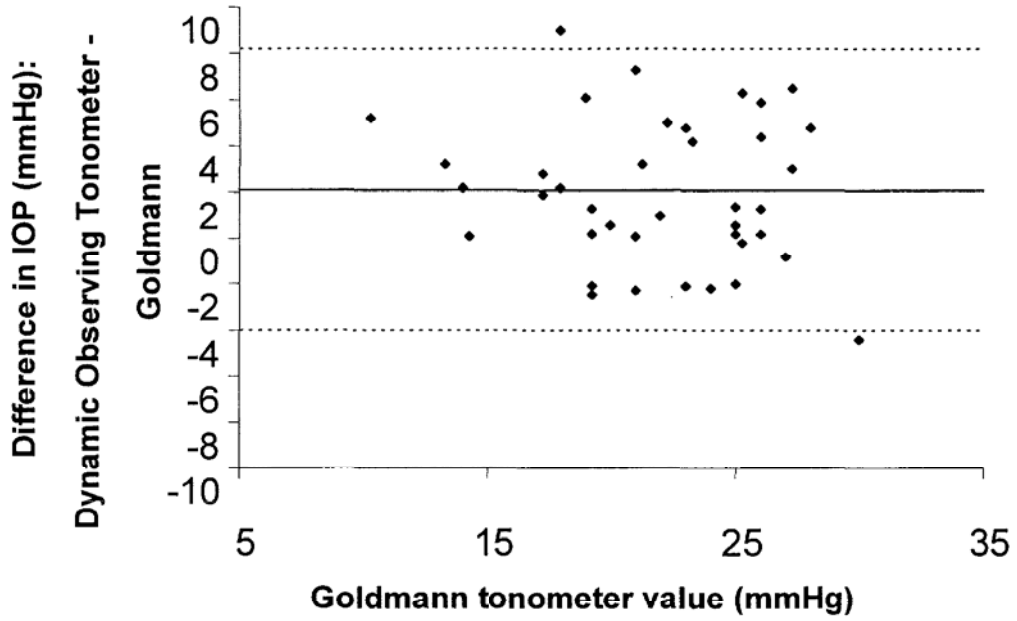


Figure 3.1 **Difference in Dynamic Observing Tonometer intraocular pressure (IOP) values versus Goldmann IOP values: —— = mean difference between tonometer values; ----- = two standard deviation limits of agreement.**

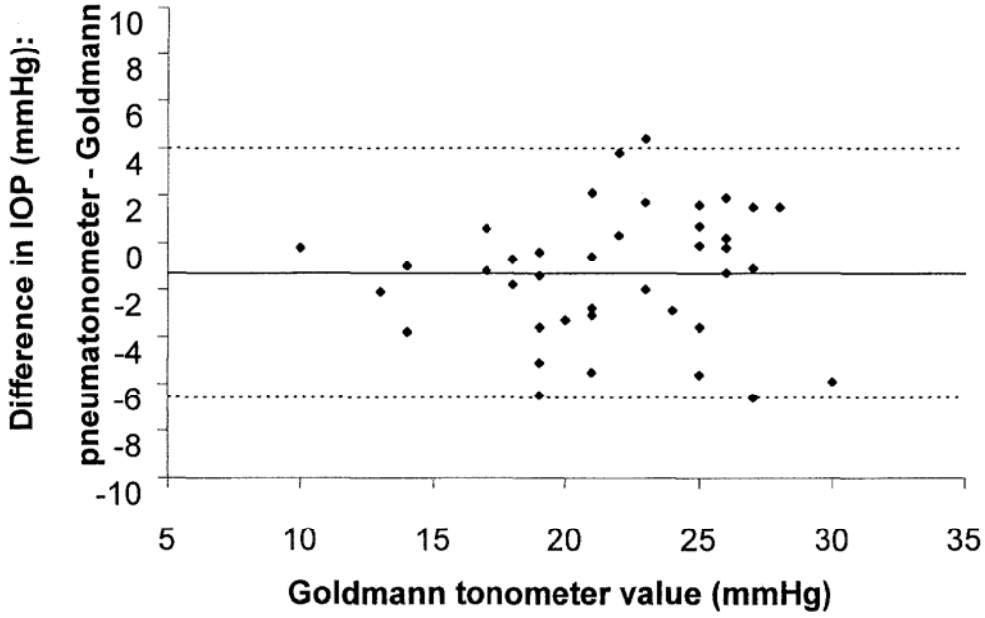


Figure 3.2 **Difference in pneumatonometer intraocular pressure (IOP) values versus Goldmann IOP values: —— = mean difference between tonometer values; ---- = two standard deviation limits of agreement.**

PA measurements

Figure 3.3 shows the differences found for PA between the first DOT and first pneumatonometer measurement. PA, as measured with the SmartLens, was 0.4 mmHg higher on average than the pneumatonometer with 95% of amplitudes falling between 1.6 mmHg below and 2.3 mmHg above the pneumatonometer's value.

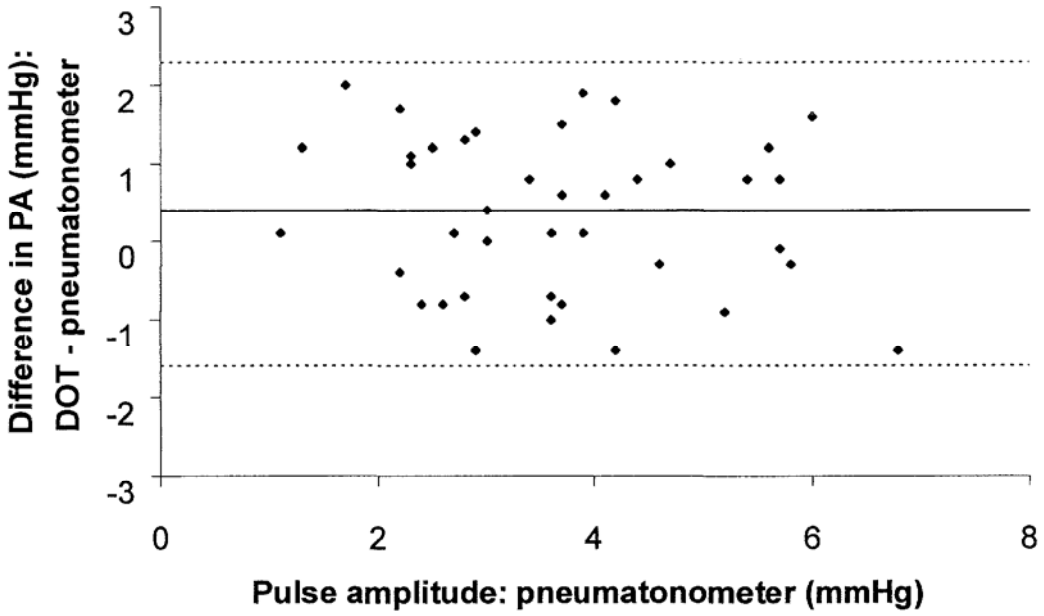


Figure 3.3 **Difference in PA values (Dynamic Observing Tonometer - pneumatonometer) against pneumatonometer PA values: —— = mean difference between tonometer values; ---- = two standard deviation limits of agreement.**

The mean differences between the instruments' IOP and PA measurements and their level of association (expressed as Pearson's correlation coefficient) are shown in Table 3.4.

	Mean difference (SD) in tonometer measurements (mmHg)	Significance of measurement differences (paired t-test)	Pearson's correlation coefficient
Dynamic Observing Tonometer minus Goldmann (IOP)	2.1 (3.1)	$p = 0.0001$	0.78 ($p < 0.001$)
Pneumatonometer minus Goldmann (IOP)	-1.3 (2.7)	$p = 0.004$	0.97 ($p < 0.001$)
Dynamic Observing Tonometer minus pneumatonometer (PA)	0.4 (1.0)	$p = 0.032$	0.78 ($p < 0.001$)

Table 3.4 **A comparison of the mean differences and Pearson's correlation coefficients, for intraocular pressure (IOP) and pulse amplitude (PA), of the comparison tonometers.**

3.5.2. DOT repeatability

IOP measurements

The repeatability of DOT IOP measurement is shown in Figure 3.4. The first IOP measurement was on average 0.4 mmHg higher than the second and 95% of values fell within 4.2 mmHg either side of this figure. The coefficient of reliability for DOT IOP measurements was 0.91. In comparison, the first IOP measurement made with the pneumatonometer was, on average, 0.6 mmHg higher than the second and 95% of readings fell within 2.0 mmHg either side of this figure (Figure 3.5). The coefficient of reliability for the pneumatonometer's IOP measurements was 0.98.

PA measurements

The repeatability of the DOT PA measurement is shown in Figure 3.6. The first PA value was, on average, 0.1 mmHg lower than the second and 95% of PA values fell within 1.3 mmHg either side of this figure. The coefficient of reliability for DOT PA measurements was 0.90. In comparison, the first PA measurement made with the pneumatonometer differed, on average, less than 0.1 mmHg from the second and 95% of values fell within 0.9 mmHg either side of this figure (Figure 3.7). The coefficient of reliability for the pneumatonometer PA measurements was 0.94.

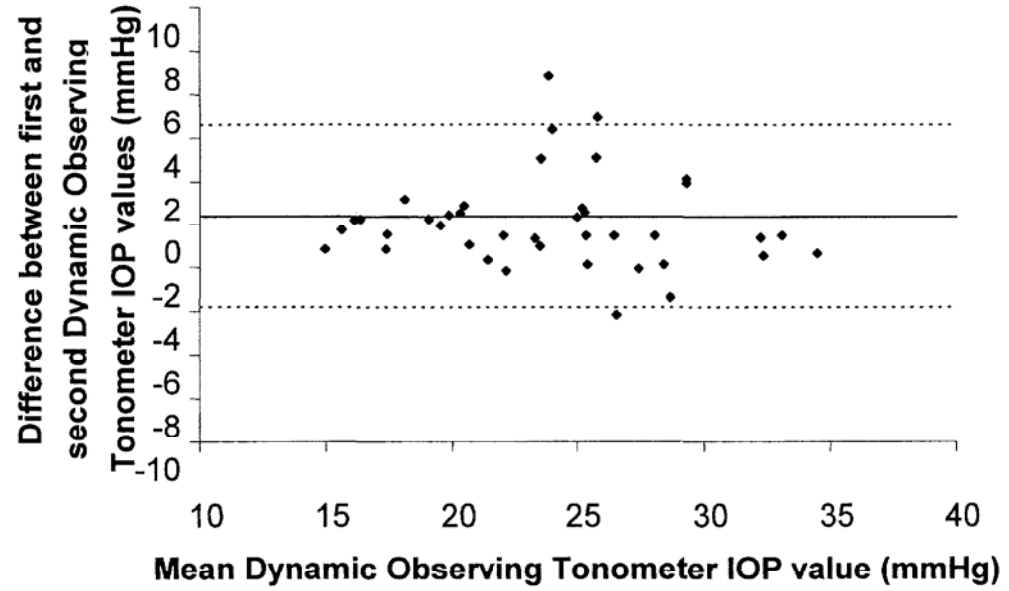


Figure 3.4 **Repeated-measures differences (IOP_1 - IOP_2) against mean measurement ([IOP_1 + IOP_2]/2) for the Dynamic Observing Tonometer: —— = mean difference between repeated measures; ---- = two standard deviation limits of agreement.**

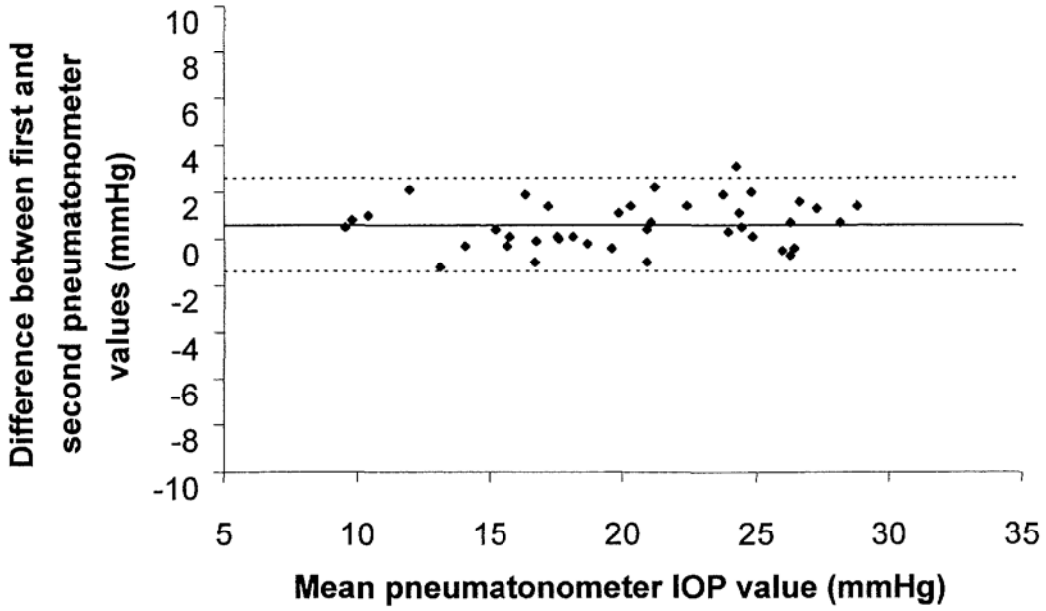


Figure 3.5 **Repeated-measures differences (IOP_1 - IOP_2) against mean measurement ([IOP_1 + IOP_2]/2) for the pneumatonometer: —— = mean difference between repeated measures; ---- = two standard deviation limits of agreement.**

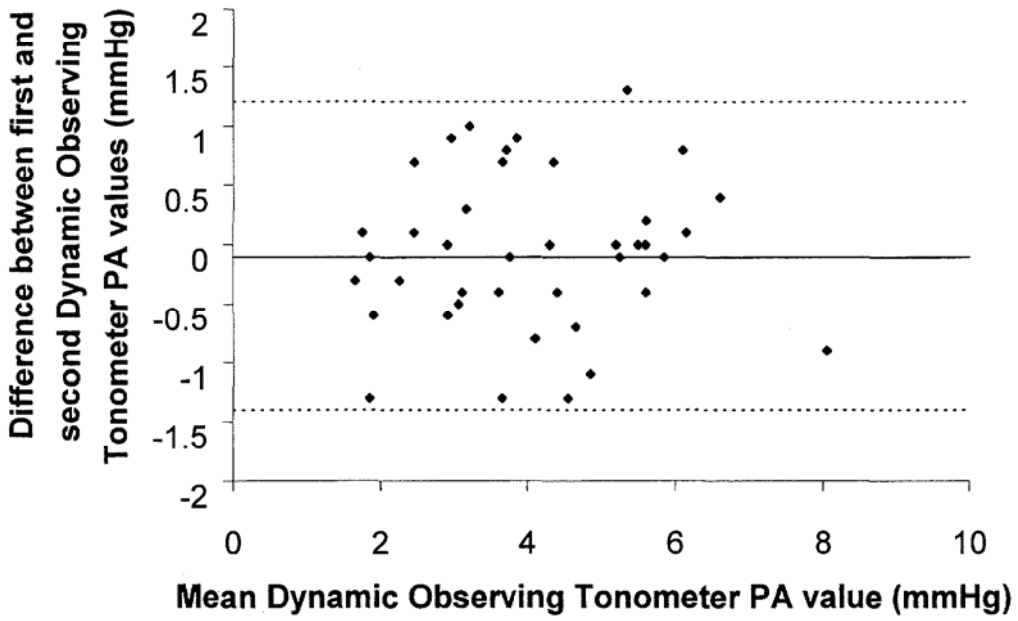


Figure 3.6 **Repeated-measures differences for the intraocular pressure pulse amplitude (PA1 - PA2) against mean measurement ([PA1 + PA2]/2) for the Dynamic Observing Tonometer: — = mean difference between repeated measures; ---- = two standard deviation limits of agreement.**

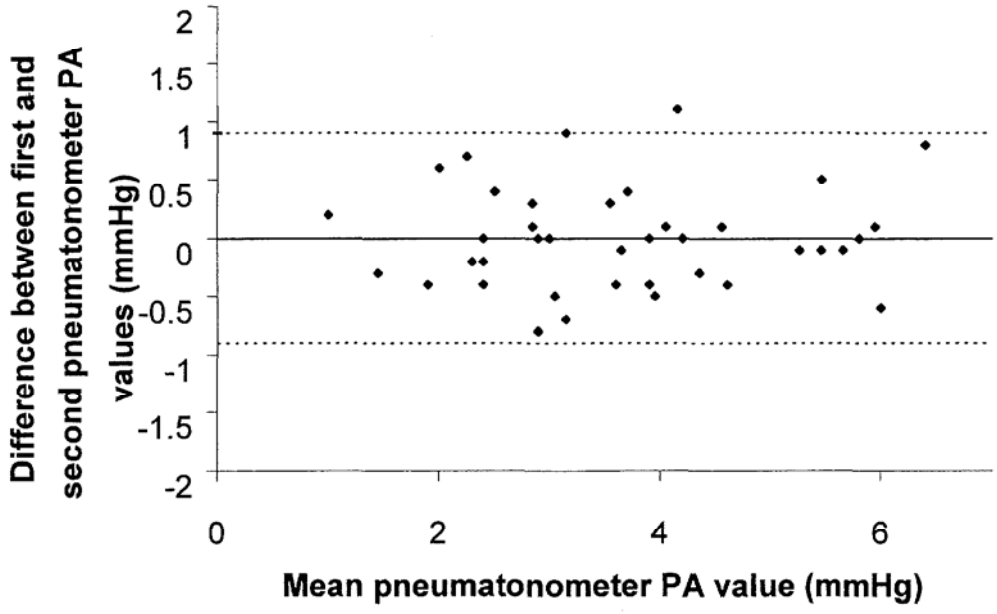


Figure 3.7 **Repeated-measures differences for the intraocular pressure pulse amplitude (PA_1 - PA_2) against mean measurement ([PA_1 + PA_2]/2) for the pneumatonometer: — = mean difference between repeated measures; ---- = two standard deviation limits of agreement.**

3.6. *Discussion*

3.6.1. Accuracy of IOP measurements

Measurement of IOP is a core procedure in the investigation of glaucoma. True accuracy of the IOP measured by a tonometer can only be assessed by comparison to invasive manometry. Dekker *et al.* (1999) investigated an early prototype of the DOT by comparing its readings to five freshly-enucleated eyes that had their intraocular pressures varied between 0 and 60 mmHg. They reported the level of agreement of the DOT's measurements as a Pearson correlation coefficient of +0.98. Unfortunately limits of agreement were not expressed and, as Pearson correlation coefficients are influenced by the range of measurements taken, the high *r* value may be a reflection of the wide (60 mmHg) IOP range (Bland *et al.*, 1986). Interestingly the calculated trendline for this manometric study ($y = 0.93x + 2.87$) had a very similar off-set value (+2.87) to the mean bias found in our results (+2.10 mmHg). The authors suggested that this off-set was due to electronic amplification in order to avoid negative values (Dekker *et al.*, 1999). If this amplification is still present in the current model, it may need to be re-evaluated.

In our study, accuracy of DOT IOP measurements was gauged by comparison to the present clinically accepted reference standard of Goldmann applanation tonometry. The DOT, on average, overestimated IOP by 2.1 mmHg and the 95% range of IOP differences between DOT and Goldmann was wide: –4.0 to + 8.2 mmHg. However this level of agreement is closer than that found by Troost *et al.* (2001) who reported that the DOT overestimated IOP with a mean bias of +5.6 mmHg and 95% limits of agreement ranging from -5.4 to 16.6 mmHg. Another study that compared the mean of three DOT IOP measurements to Goldmann tonometry, although not stating limits of agreement, reported a slightly poorer Pearson correlation coefficient ($r = 0.70$) to that found in the present study ($r = 0.78$) (Entenmann *et al.*, 1997). The level of discrepancy found in our study is similar to that found in other clinical tonometers when compared to Goldmann: 95% limits of agreement for the Tono-Pen have been reported as –5.37 to +6.99 mmHg and –9.09 to +7.91 mmHg; and 95% limits of agreement for the Pulsair 2000 reported to be –6.08 to +8.16 mmHg (Bafa, Lambrinakis, Dayan *et al.*, 2001; Frenkel, Hong & Shin, 1988; Mackie, Jay, Ackerley *et al.*, 1996).

The DOT was not however as accurate as the comparison pneumatonometer used in this study. The close agreement of the pneumatonometer to Goldmann tonometry has been reported by Yang *et al.* (2000) who found its 95% limits of agreement to be –4.35 to +4.87 mmHg which is similar to the this study (95% limits of agreement of –6.6 mmHg to +4.00 mmHg).

3.6.2. Accuracy of PA measurements

The accuracy of PA measurement is a necessary requirement for the investigations in this project. Without a true manometric comparison, the pneumatonometer was used as a clinical reference standard to assess the accuracy of the DOT's PA measurements. Pneumatonometer PA measurements have been validated against those taken manometrically in human and animal eyes (Tønjum, 1972). In addition, the pneumatonometer is the commonly used instrument for IOP pulse research studies (Lam *et al.*, 2002; Schmidt *et al.*, 2001). The present study found that the DOT overestimated PA on average by 0.4 mmHg, compared to the pneumatonometer, with 95% limits of agreement of –1.6 to +2.3 mmHg. In a scientific poster, Feucht, Pillunat, Mueller *et al.* (2001) compared PA measurements found with the OBF and DOT tonometers and, although limits of agreement were not reported, they found a Pearson correlation coefficient of 0.5: compared to $r = 0.78$ reported here. They attributed the poor correlation to a large tonographic effect caused by the DOT.

3.6.3. Repeatability of IOP and PA measurements

In investigating the repeatability of the DOT measurements, the instrument performed worse than the pneumatonometer in both the parameters of IOP and PA measurement. It is likely that a great deal of variability originated from the inherent rigid contact of the DOT against the eye. DOT is therefore very susceptible to artefacts arising from movements originating from the observer's hand or the subject's eye and head. The pneumatonometer, in contrast, gently apposes the eye by means of a pressurised air-piston (Chidlow, Nash, Crowhurst *et al.*, 1996; Spraul *et al.*, 1998). Our results are consistent with another study that investigated DOT repeatability. Vogel *et al.* (2001) looked at the intraobserver variability of IOP and PA with the DOT by taking 5 measurements on 10 healthy eyes. They reported coefficients of variation (standard deviation / mean x 100) for IOP and PA

values to be 9.57% and 14.5% respectively. Although poorer than the pneumatonometer, the DOT's repeatability is similar to that found with other clinical tonometers: 95% of differences between repeated IOP measurements have been reported to be within ± 5 mmHg for the Pulsair 2000 and within ± 3.5 mmHg for the American Optical MkII non-contact tonometers (Mackie *et al.*, 1996; Vernon, 1995).

When comparing repeatability of IOP measurements it is worth noting that even the present clinical gold standard of Goldmann applanation tonometry exhibits a significant degree of variability. Thorburn (1978) reported 95% limits of agreement between first and second Goldmann measurements to be –2.3 to 1.2 mmHg for a single observer and Phelps & Phelps (1976) found limits of –4.8 to +5.6 mmHg for two observers. Some variation in IOP measurement may be attributable to the tonographic effect associated with repeated applanations (Whitacre & Stein, 1993a). To avoid this tonographic effect influencing such studies, a mimimum time between measurements (Recep *et al.*, 1998) and/or randomisation of tonometer sequence is required.

On reflection, an improvement to the present study could have been made by taking two IOP measurements with Goldmann applanation tonometry in order to compare its repeatability. Unfortunately the necessity of requiring a third investigator, to ensure the recording of Goldmann applanation measurements were blinded to the operator, ruled out this option.

3.7. *Conclusion*

In conclusion, due to its wide range of agreement in comparison to Goldmann tonometry, the DOT is unlikely to be accepted as the sole instrument for measuring IOP in a clinical setting. In addition the DOT was less repeatable in taking IOP and PA measurements than the comparison pneumatonometer used in this study. Further investigations on the IOP pulse in this work will be performed with the OBFA pneumatonometer.

The findings of this study have been recently published and are shown in Appendix 4.1 (Morgan, Hosking & Salmon, 2002b).

4. Ocular Blood Flow Analyzer Pneumatonometer Repeatability: The Effect of Operator Experience and Mode of Application

4.1. *Abstract*

Purpose: The OBFA pneumatonometer has been promoted as a flexible device to measure the pulsatile component of the ocular circulation derived from continuous measures of IOP. Practically, it can be used either slit-lamp mounted or hand-held. Although a number of studies have validated the pneumatonometer's reproducibility when mounted on a slit-lamp, it is not known whether hand-held measurements are influenced by operator experience or if they are equally reliable. The purpose of this study was to investigate whether the effect of learning and the reproducibility of measurements varied between the two methods of application.

Method: Five consecutive measurements by each method were taken at one examination session on one eye of 35 healthy adults (mean age 36.6 years and range 17 to 84 years). The subjects were seated for all measures and the sequence of pneumatonometer method was randomised between subjects.

Results: A new operator does exhibit a significant learning effect and this learning effect is of a greater magnitude for the hand-held technique. No significant difference was found between methods for POBF, IOP, PA and heart rate. Both methods of application had a high coefficient of reliability for POBF: 0.91 for slit-lamp and 0.89 for hand-held.

Conclusions: We conclude that results from both methods of pneumatonometer application are reliable and interchangeable within studies. However it is important that new operators gain sufficient experience with the pneumatonometer to avoid initial operator-induced variability. It is recommended for further studies that a maximum of two repeated measurements with the pneumatonometer minimise measurement variation whilst avoiding undue disruption to the level of IOP and/or corneal integrity.

4.2. *Introduction*

The OBFA pneumatonometer in addition to providing a measure of IOP and its PA, calculates a measure of POBF. The concept and methodology of calculating POBF has been previously reviewed (1.7). The currently available instrument can be used either mounted on a slit-lamp or in a hand-held device. By freeing the tonometer from its slit-lamp mounting, testing can be made more convenient for the patient and examiner, while additional investigations, such as the effect of posture, made possible. It is therefore of great importance that the instrument's measurements are not influenced by the different method of application. Potential sources of variation between the two methods include operator proficiency and factors such as hand movement and patient anxiety.

A study, published by the pneumatonometer's manufacturers (Crowhurst *et al.*, 1996), concluded that the pneumatonometer is as reliable when used by a novice operator. Numerous studies have shown that POBF measurements are reliable when the pneumatonometer is mounted on a slit-lamp (Butt & O'Brien, 1995; Spraul *et al.*, 1998; Yang, Hulbert, Batterbury *et al.*, 1997a), with the most recent study showing good inter-observer agreement for POBF measurements (Lam *et al.*, 1999). In addition, pneumatonometers may be superior at measuring IOP because they are less influenced by corneal thickness (Abbasoglu *et al.*, 1998). However, no equivalent reproducibility studies have been performed with the OBF tonometer in its hand-held method of application.

Like most biological measures it is expected that some inherent variability exists when a measurement is repeated under the same conditions (Campbell & Machin, 1999). The number of repeated measures needed to provide the most reproducible estimate of a variable is relevant for research and clinical practice. The outputs from the pneumatonometer (POBF, IOP, PA and heart rate) are calculated automatically as the mean of five representative pulses selected by the system's computer. It would be of use to investigate if this is sufficient for intra-test reliability or whether repeated acquisitions are recommended.

4.3. *Aims*

The purposes of this study were therefore to:

- investigate the possibility of a learning effect with a new operator,
- compare the reliability between methods of tonometer application,
- evaluate the number of repeated measurements required for optimum reproducibility.

4.4. *Method*

4.4.1. Experimental design

This was a validation study. The null hypotheses were: that operator experience did not effect results; that there was no significant difference between the two application methods; and that there was no increase in standard deviation with repeated measurements. Results were taken as significant when the probability was less than 5%.

In order to recruit sufficient subjects, a power calculation was performed (Equation 4.1) (Norman & Streiner, 2000a).

$$n = \left[\frac{(Z_\alpha + Z_\beta)S}{\Delta}\right]^2$$

n	= required sample number
Z_α	= critical value to avoid a Type I error in standard errors
Z_β	= critical value to avoid a Type II error in standard errors
S	= standard deviation
Δ	= estimated difference between the two samples

Equation 4.1 Sample size estimation calculation

Yang *et al.* (1997a) found that repeated POBF measurements, at a single visit, had a percentage variance of 8%. Therefore with a percentage difference detectable between slit-lamp and hand-held techniques taken as 8% or more, the standard deviation of an individual subject's measurements as 8%, the chance of making a type I error as 5% (equivalent to

1.96 standard errors) and the chance of making a type II error as 10% (equivalent to 1.28 standard errors) the number of replicate subjects is predicted to be a minimum of 11.

4.4.2. Ethical approval

All experimental procedures of the study conformed to the tenets of the declaration of Helsinki and were approved by the Aston University Human Science Ethical Committee. Written informed consent was obtained from all subjects willing to participate in the study.

4.4.3. Subject sample

35 subjects were recruited from either staff at Aston University, Birmingham, UK or patients from optometric practice for the study. All volunteers had a full eye examination prior to being accepted onto the study. Exclusion criteria for the study are listed in Table 4.1. Subjects comprised of 12 males and 23 females with a mean age of 36.6 years (range 17 to 84 years).

- Reported history of present or past eye disease
- Reported history of cardiovascular disease or current medication with known cardiovascular properties
- Corrected visual acuity worse than 6/9
- Corneal or anterior chamber abnormalities as detected by slit-lamp examination
- Posterior segment abnormalities as detected by slit-lamp binocular indirect ophthalmoscopy
- Intraocular pressure greater than 21 mmHg
- Physical or mental incapacity to perform experimental procedures

Table 4.1 Subject's exclusion criteria for slit-lamp versus hand-held pneumatonometer study.

4.4.4. Instruments

The same OBFA pneumatonometer was used for all measurements either mounted on a Haag-Streit slit-lamp or in a purpose-built hand-held device (Morgan & Hosking, 2003).

The OBFA pneumatonometer and its parameter calculations have been previously described (1.5.5, 1.5.6 and 3.4.4).

4.4.5. Experimental procedures

Before measurements began, each subject was seated and allowed to rest for 10 minutes in order to minimise cardiac variability. All measurements were made by the same observer and on the same eye of each subject. Only one eye of each subject was measured and this was randomly chosen using a sealed envelope technique. Both eyes were anaesthetised with topical 0.4% benoxinate eye drops (Minims, Chauvin pharmaceuticals); the unexamined eye was also anaesthetised because, given the relatively long measurement time, it helped reduce the sensation of drying and therefore the tendency to blink. This is also consistent with normal clinical practice in which both eyes are anaesthetised and measured. Each subject was examined with both methods at one session.

Five consecutive measurements were taken with each method of application and the initial method was randomised between subjects (sealed envelope technique). For example, for each subject, either five recordings were taken with the pneumatonometer in the slit-lamp followed by five recordings with the pneumatonometer in its hand-held device or vice versa. There was no more than a five minute interval between each measurement. Occasionally an artificial tear drop (0.9% sodium chloride, Minims, Chauvin pharmaceuticals) was instilled, between measures, to ensure good tonometer contact and prevent excessive epithelial drying. Sodium chloride 0.9% eye drops were chosen because, being of low viscosity, they do not influence tonometric measurements (Dekker, Kanngiesser & Robert, 1996). To maintain corneal position during recordings the subject fixated a detailed object at a distance of two metres.

4.4.6. Statistical analysis

In order to investigate any operator learning effect, the standard deviations (SD) of each subjects' POBF values for each method were plotted sequentially in the order of subject seen. Significant regression slopes of SD values were taken to indicate a change in operator performance. To examine whether method of POBF measurement was a cause of variation, a factorial analysis of variance (ANOVA) was calculated. Method of tonometer application

(2 levels) and the number of consecutive measurements (5 levels) were taken as the 2 factors. The advantage of this analysis is that as well as application method being examined as a source of variance, the effect of consecutive measures and any interaction between measures and method can be ascertained (Norman & Streiner, 1993). For example, it is well known that successive Goldmann tonometer measurements reduce IOP. This method of analysis accounts for this factor as a source of variability.

To further examine the agreement between the two methods, the mean differences between methods were plotted against their average values. This method of analysis is preferred over the use of simple correlation coefficients, as it graphically depicts how one method is likely to differ from the other (Bland *et al.*, 1986). The lack of agreement between individual measurements of each method can then be summarised by the mean difference, or instrument bias, and the two standard deviation limits of the differences. In a study using repeated measurements, the corrected standard deviation of differences is calculated from Equation 4.2 (Bland *et al.*, 1986).

$$Sc = \sqrt{(S_D^2 + \tfrac{1}{4}S_1^2 + \tfrac{1}{4}S_2^2)}$$

Sc	= corrected standard deviation of differences
S_D	= standard deviation of the differences between the means of each method
S_1 and S_2	= the standard deviation of the differences between repeated measures for each method

Equation 4.2 Corrected standard deviation of differences calculation (Bland *et al.*, 1986).

In addition, to summarise the test-retest variation of each method the coefficients of reliability were calculated as previously described (3.4.6) (Bland *et al.*, 1996).

The number of repeated measurements needed to allow for within-test variation was gauged by examining how standard deviation changed with additional measurements. The optimum number was taken as the point at which SD levelled out. This plateau-point indicates the

number of measures sufficient to encapsulate the intra-test variation (Weinreb, Lusky, Bartsch *et al.*, 1993).

4.5. *Results*

4.5.1. Learning Effect

Figure 4.1 shows each subject's standard deviation, for the five OBF measurements of each application method, in the order they were seen during the study. It is apparent from the graph that there is a learning effect. This learning trend was shown to be significant for both the hand-held ($p = 0.001$) and slit-lamp methods ($p = 0.015$): the hand-held method showing greater improvement with experience ($r = 0.640$) compared to the slit-lamp method ($r = 0.424$). It is for this latter finding that the remaining analysis was performed on the final 16 subjects: where the learning effect had levelled out.

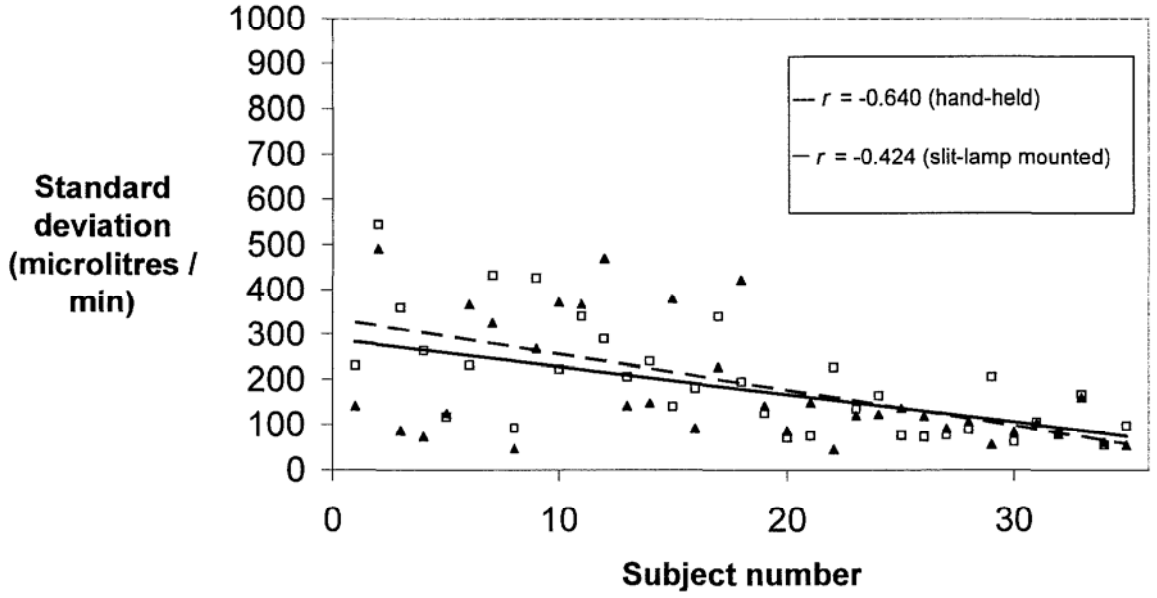


Figure 4.1 **Plot of each subject's standard deviation of their pulsatile ocular blood flow measurements in the order of the subjects examined: □ hand-held method; ▲slit-lamp method.**

4.5.2. Between-method variation

Table 4.2 shows the mean values of POBF, IOP, PA, and heart rate for both application methods. Factorial ANOVA showed that there was no significant difference between methods of tonometer application for any of the parameters.

Appendix 1 shows the between-method differences for POBF, IOP, PA, and heart rate measurements as a function of their mean value (Figures A.1.1 to A.1.4). Only IOP measurements showed a significant correlation of the between-methods differences and their mean values ($r = 0.63, p = 0.009$). Table 4.3 shows the mean differences between methods and the corrected standard deviations of the differences.

	POBF (μl/min)	**IOP (mmHg)**	**Pulse amplitude (mmHg)**	**Heart rate (beats / min)**
Slit-lamp method values: mean (SD)	1198 (353)	11.7 (3.1)	3.1 (0.9)	65 (9)
Hand-held method values: mean (SD)	1263 (378)	10.9 (2.5)	3.3 (0.9)	61 (6)
Significance of difference between methods (p value)	0.110	0.128	0.254	0.105

Table 4.2 **Mean values and standard deviations (SD) for both methods of tonometer application.**

	POBF (μl/min)	**IOP (mmHg)**	**Pulse amplitude (mmHg)**	**Heart rate (beats / min)**
Mean difference between methods (instrument bias)	-54	0.8	-0.2	4
One corrected standard deviation of the differences between methods	133	2.1	0.6	8

Table 4.3 **Mean and corrected standard deviations of the differences between slit-lamp mounted and hand-held methods of pneumatonometer presentation.**

4.5.3. Coefficients of reliability

Table 4.4 shows the coefficients of reliability for all four outputs with each application technique.

	POBF	IOP	Pulse amplitude	Heart rate
Slit-lamp method	0.91	0.51	0.78	0.56
Hand-held method	0.89	0.73	0.81	0.53

Table 4.4 Coefficients of reliability for both methods of tonometer application.

4.5.4. Consecutive tonometer applications

For both methods of application, no significant variation was caused by successive tonometer applanations on POBF or heart rate. However IOP and PA were significantly affected by successive applanations ($p = 0.004$ and $p = 0.017$ respectively). Figure 4.2 and Figure 4.3 show how mean IOP and PA varied over 5 successive applanations.

There were no significant interactions between methods and number of measurements for all four outputs: POBF, IOP, PA and heart rate (factorial ANOVA).

4.5.5. The affect of repeated measurements on parameter standard deviation

Figures A.1.5 to A.1.8 (Appendix 1) show how the mean standard deviations for measurements changed as the number of repeated measurements increased from 2 to 5.

4.6. *Discussion*

4.6.1. Effect of operator experience on POBF measurements

The reduction in SD of POBF values with the number of subject examined indicated that operator proficiency improved with experience. The hand-held pneumatonometer measurements had the steeper correlation with subject number, indicating this method has a greater operator learning effect. These results suggest that both slit-lamp and hand-held

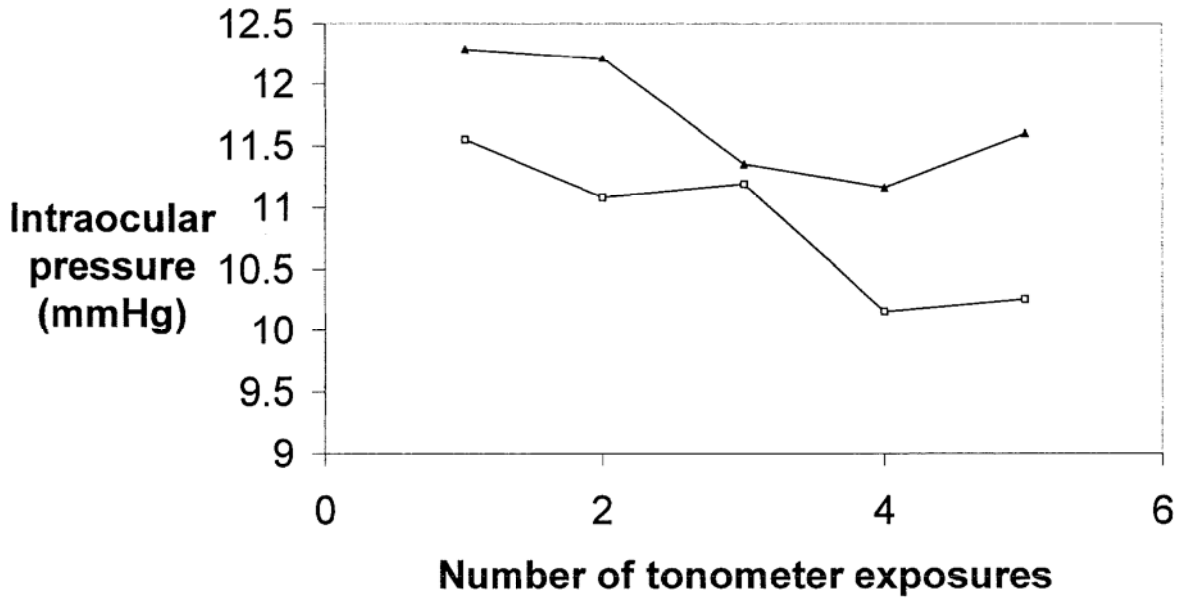


Figure 4.2 **Plot of mean IOP against repeated tonometer exposures: ▲slit-lamp method; □ hand-held method.**

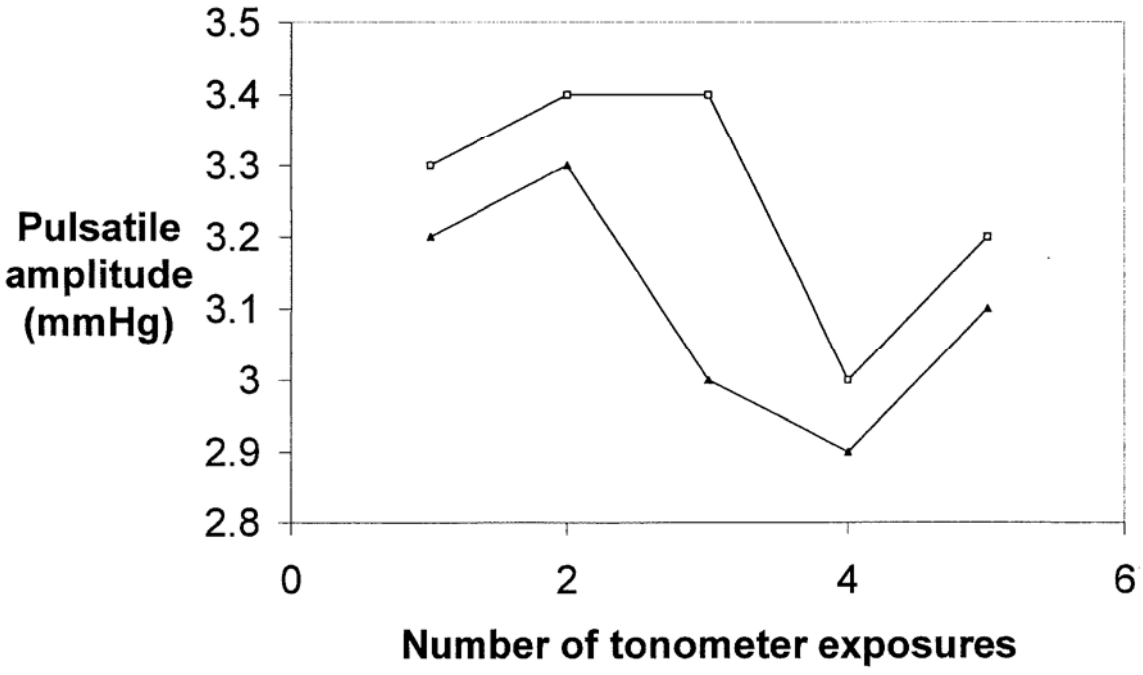


Figure 4.3 **Plot of mean pulse amplitude against repeated tonometer exposures: ▲slit-lamp method; □ hand-held method.**

methods need to be mastered before they are applied; otherwise significant errors will be introduced. Our study found that up to twenty subjects needed to be examined before sufficient experience was gained; although the number to attain adequate experience may vary between observers. This is contrary to the pneumatonometer manufacturer's own research that found novice operators were equally proficient as experienced ones.

4.6.2. Instrument accuracy and precision

Ideally, when an instrument is evaluated, both its accuracy and precision are assessed (Hulley & Cummings, 1988). Clinically, the terms accuracy and precision are often referred to as validity and repeatability respectively. A new instrument's accuracy is usually defined as its performance against the gold standard for that measure. No such direct comparative gold standard in humans is possible for POBF. The closest such direct comparison demonstrated that the pulsatile component of blood flow represents a large proportion of total ocular blood flow in the anaesthetised rabbit (Grebe, Hopkins & Langham, 1990). In contrast, the precision of all the OBFA tonometer's outputs can be investigated by examining their repeatability.

4.6.3. Repeatability between methods of tonometer application

No variability in the tonometer measurements was attributable to the method of application. The coefficients of reliability were similar for both methods of POBF measurement and compared well with those reported by Yang *et al.* (1997a)(coefficient of reliability: 0.92) and Spraul *et al.* (1998)(coefficient of reliability: between 0.70 and 0.90). This provides evidence for researchers and clinicians that both slit-lamp and hand-held methods can be used interchangeably. In a similar five-repeated measurements study, with the pneumatonometer mounted on a slit-lamp, Butt *et al.* (1995) reported that the POBF measurements did not vary significantly. They did not state the coefficient of reliability for POBF but recommended selecting the middle three measurements to improve its precision. Applying this recommendation to our data, we found that the coefficients of reliability for POBF improved to 0.96 and 0.95 for slit-lamp and hand-held methods respectively.

The range between the two corrected standard deviations of the differences in figures A.1.1 to A.1.4 can be used to give an approximate indication of the discrepancy required between

individual measurements before they can be considered significant (Bland *et al.*, 1986). For example, according to our data, the difference between two single POBF measurements would need to be over 532 μl/min before one could be confident of a real difference between them.

In examining whether differences between methods varied with mean value, only IOP had a significant positive correlation ($r = 0.629, p = 0.009$): that is, the higher the IOP, the higher the discrepancy between slit-lamp and hand-held measurements. From Figure A.1.2, it appears that the slit-lamp method records higher IOPs at higher mean values than the hand-held method. A possible explanation could be that successive measurements with the slit-lamp method induced a greater fall in high IOP than with the hand-held method; in other words, the hand-held method may have had a gentler and less tonographic influence on the eye. Alternatively this finding may be due to a sampling error and the fact that the data is concentrated around a narrow IOP range.

It has long been known that repeated tonometer applanations reduce IOP (Whitacre *et al.*, 1993a), and this effect is borne out by our results: both IOP and PA measurements diminished with repeated applanations (Figure 4.2 and Figure 4.3). A similar fall in PA value to that of IOP would be expected from the ocular pressure-volume relationship (1.4.10). That is, for the same volume of blood entering the eye the subsequent pressure change (PA) would decrease as IOP fell. Furthermore our study supported the findings of others (Yang *et al.*, 1997a) that demonstrated POBF, unlike IOP and PA, was not influenced by repeated measurements. This finding, that POBF remains constant while IOP falls with successive applanations, is a possible indication that the pressure-volume relationship from which the measure is derived is robust.

The fact that IOP decreases with successive applanations highlights the need to design studies appropriately. The above mentioned study of Lam *et al.* (1999) that compared the pneumatonometer to the Goldmann, did not randomise the sequence of tonometers and the result that the pneumatonometer measured on average 1.02 mmHg higher than the Goldmann may well be another example of this applanation-induced IOP reduction. An alternative solution to randomising the tonometers would be to use an appropriate time interval between applanations. Recep *et al.* (1998) compared successive IOP measurements,

using a Goldmann tonometer, with intervals of 1, 2, 3, 4, 5 and 10 minutes and found that no significant difference existed using a 10 minute interval.

4.6.4. Number of repeated measurements required for optimum reproducibility

The final purpose of this study was to examine whether one recording with the OBFA tonometer was adequate to account for within-test variation. The plots of standard deviation against accumulated measurements were reasonably flat indicating that the tonometer's software successfully reduces variation by analysing five acceptable pulses during one recording. For measuring POBF, repeating beyond 2 measurements, where the standard deviation plateaus out, appears unnecessary. In measuring IOP or PA, because of the influence of successive applanations on IOP, taking only one measurement, or leaving an interval of 10 minutes or more between additional measurements, is suggested. In addition, during the course of the study, it was noted that some subjects exhibited mild corneal epithelial disturbance from the repeated applanations with the pneumatonometer. In all cases these epithelial erosions resolved within 24 hours. It is therefore recommended that, given the flat levels of standard deviation, the potential tonographic effect and the risk of corneal damage with repeated measurements, a maximum of two pneumatonometer recordings of an eye are recommended for research purposes.

4.7. *Conclusion*

Both methods of pneumatonometer application are subject to an operator learning effect. The results of this study indicate that this particularly applies to when the pneumatonometer is hand-held. It is imperative that new operators make allowances for this before embarking on clinical or research work. Once experience is acquired, measurements of IOP, POBF and PA are as precise when they are taken with the pneumatonometer hand-held as when they are taken with it slit-lamp mounted. This allows practitioners to use this more flexible method with the confidence that the readings are not inherently different. It is recommended for further studies that a maximum of two repeated measurements with the pneumatonometer minimise measurement variation whilst avoiding undue disruption to the level of IOP and/or corneal integrity. The findings of this study have recently been published and are shown in Appendix A.4.2 (Morgan & Hosking, 2001).

5. The effect of corneal thickness and corneal curvature on pneumatonometer measurements

5.1. *Abstract*

Purpose: The aim of this study was to investigate the influence of corneal topography and thickness on IOP and PA as measured using the OBFA pneumatonometer.

Methods: 47 university students volunteered for this cross-sectional study: mean age 20.4 yrs, range 18 to 28 yrs; 23 male, 24 female. Only the measurements from the right eye of each participant were used. Central corneal thickness and mean corneal radius were measured using Scheimpflug biometry and corneal topographic imaging respectively. IOP and PA measurements were made with the pneumatonometer. Axial length was measured using A-scan ultrasound, due to its known correlation with these corneal parameters. Stepwise multiple regression analysis was used to identify those components that contributed significant variance to the independent variables of IOP and PA.

Results: The mean IOP and PA measurements were 13.1 (SD 3.3) mmHg and 3.0 (SD 1.2) mmHg respectively. IOP measurements made with the pneumatonometer correlated significantly with central corneal thickness ($r = +0.374$, $p = 0.010$), such that a 10 μm change in CCT was equivalent to a 0.30 mmHg change in measured IOP. PA measurements correlated significantly with axial length (part correlate = -0.651, $p < 0.001$) and mean corneal radius (part correlate = +0.459, $p < 0.001$) but not corneal thickness.

Conclusions: IOP measurements taken with the pneumatonometer are correlated with corneal thickness, but not axial length or corneal curvature. Conversely, PA measurements are unaffected by corneal thickness, but correlated with axial length and corneal radius. These parameters should be taken into consideration when interpreting IOP and PA measurements made with the pneumatonometer.

5.2. *Introduction*

The measurement of IOP is of clinical importance in the detection and monitoring of primary open-angle glaucoma (Control of Normal-Tension Glaucoma Study Group, 1998a; Sommer, 1996). The influence of corneal topography on the measurement of IOP by Goldmann applanation tonometry is well documented (Whitacre *et al.*, 1993a) and IOP measurements are known to be influenced by corneal thickness (Doughty & Zaman, 2000) and corneal curvature (Mark, 1973). These influences are of clinical concern as erroneous IOP measurements may lead to mislabelling of glaucoma patients and healthy subjects (Brubaker, 1999). Tomlinson & Leighton (1972) found the mean corneal radius to be flatter in patients with NTG than those with POAG or normal patients. Copt, Thomas & Mermoud (1999) on correcting IOP for corneal thickness, calculated that 31% of their patients with NTG would be reclassified as having POAG and that 56% of their ocular hypertensive patients would be classified as normal.

IOP measurements taken with pneumatonometers are used clinically (Bafa *et al.*, 2001) and PA measurements are principally used in research (1.6 to 1.8). However there have been few reports on the influence of corneal dimensions on these measurements. Only the association between corneal thickness and IOP measurements by pneumatonometry has been investigated and results have been mixed: some investigators conclude that IOP measurements are significantly influenced by corneal thickness (Singh *et al.*, 2001) and others that they are not (Abbasoglu *et al.*, 1998; Zadok *et al.*, 1999).

5.3. *Aims*

The purpose of this study was to investigate the influence of corneal topography and thickness on the pneumatonometric measurements of IOP and PA.

5.4. *Methods*

5.4.1. Experimental design

This was a cross-sectional study investigating sources of variance in the dependent parameters of IOP and PA taken with the pneumatonometer.

5.4.2. Ethical approval

All experimental procedures of the study conformed to the tenets of the declaration of Helsinki and were approved by the Aston University Human Science Ethical Committee. Written informed consent was obtained from all subjects willing to participate in the study.

5.4.3. Subject sample

47 university students volunteered for this study: mean age 20.4 yrs, range 18 to 28 yrs; 23 male, 24 female. All prospective volunteers underwent a full eye examination. Exclusion criteria for the potential study subjects are found in Table 5.1.

- Reported history of present or past eye disease
- Reported history of systemic disease, or current medication, with known cardiovascular properties
- Reported history of wearing hard or rigid contact lenses
- Astigmatism greater than 2.00 dioptres cylindrical
- Corrected visual acuity of less than 6/6
- Corneal or anterior chamber abnormalities as detected by slit-lamp examination or computerised corneal topography
- Posterior segment abnormalities as detected by slit-lamp binocular indirect ophthalmoscopy
- Intraocular pressure greater than 21 mmHg
- Physical or mental incapacity of perform experimental procedures

Table 5.1 Subjects' exclusion criteria for corneal topography study

As one of the purposes of this study was to investigate the relationship between pneumatonometer measurements and mean corneal curvature, it was felt that high degrees of corneal astigmatism or causes of corneal distortion could be a source of error. Subjects were therefore excluded from the study if they had greater than 2.00 dioptres of astigmatism, had evidence of corneal distortion on video keratoscopy, or had a history of

wearing hard or rigid contact lenses. All measurements were taken with the subject in a seated position, between the hours of 10.00 am and 4.00 pm, by the same investigator.

5.4.4. Instrumentation

The pneumatonometer used in this study was the Ocular Blood Flow Analyzer (OBFA; Paradigm Medical Industries, Utah, USA) which has been previously described (1.5.5). After anaesthetising the cornea with 0.4% benoxinate hydrochloride (*Minims*®, Chauvin, UK), average IOP and PA measurements were automatically calculated from one continuous IOP recording (approximate recording time 5-10 seconds) that was sufficient to encapsulate five similar IOP pulses. Mean corneal radius was measured with a computerised corneal topographer (*EyeSys 2000* Corneal Analysis System, Spectrum Ophthalmic, UK) by taking the average of three readings. A measure of central corneal thickness (CCT) was calculated by taking the average of three corneal width images produced from a Scheimpflug camera system (*CASE-S*, Marcher Enterprises, Hereford, England). In addition, because of the known correlations between corneal radius with axial length (Goss, Van Veen, Rainey *et al.*, 1997) and axial length with pulse amplitude (James *et al.*, 1991c), axial length measurements were taken on all subjects. Axial length was calculated automatically as the average of 10 A-scan ultrasound readings (Storz *Omega* Compu-Scan Biometric Ruler, Storz International, St Louis, USA). In order to assess repeatability of measurements, coefficients of reliability for each parameter were calculated and are shown in Table 5.2. The equation for calculating coefficients of reliability has previously been described (Equation 3.1).

	Intraocular pressure measurement	**Pulse amplitude**	**Mean corneal radius**	**Central corneal thickness**	**Axial length**
Coefficient of Reliability	0.98	0.94	0.99	0.89	0.96

Table 5.2 Measurement parameters' coefficients of reliability.

5.4.5. Experimental procedures

Subjects were seated for all measurements. The pneumatonometer probe was mounted on a slit-lamp for this study. All measurements were taken during a single appointment session for each subject. The sequence of measurements is shown in Table 5.3. Completely non-invasive measurements were chosen to be performed first in order to minimise potential secondary influences on corneal topography by pneumatonometer and A-scan probes. Likewise ultrasound measurements were performed last in order to avoid a tonographic effect on IOP and PA measurements.

1. Full eye examination
2. Computerised corneal topography
3. Scheimpflug camera biometry
4. Pneumatonometry
5. A-scan ultrasound biometry

Table 5.3 Order of measurements taken in the corneal topography study.

5.4.6. Statistical analysis

As physiological data from the two eyes of a single subject correlate highly, combining right and left eye measurements can lead to erroneous statistical significance (Ray & O'Day, 1985). To ensure independence of the data, and in convention with other studies (Ravalico *et al.*, 1997), measurements were analysed for the right eye only of each subject. Due to the possible intercorrelations of the independent variables (corneal radius with corneal thickness (Price, Koller & Price, 1999) and corneal radius with axial length (Goss *et al.*, 1997)), a stepwise multiple regression analysis was performed. Multiple regression analysis determines which predictor variables make a significant contribution to the variance in the dependent variable and provides a figure for the unique contribution each variable makes after the other variables have been taken into account (i.e. part correlation coefficient) (Norman & Streiner, 2000b).

5.5. *Results*

Group values for the subjects' measurements are found in Table 5.4. Subjects measurements did not deviate significantly from a normal distribution except for mean spherical refractive error. Subjects' refractive errors had a negative skew: mean value -0.86 dioptres spherical (DS) with a range of +0.75 to -6.50 DS. Table 5.5 shows the results of the stepwise multiple regression analysis for IOP and PA measurements.

	Mean spherical refractive error (DS)	**Central corneal thickness (μm)**	**Axial length (mm)**	**Intraocular pressure (mmHg)**	**Pulse amplitude (mmHg)**
Mean (SD or range) value	-0.86 (range +0.75 to -6.50)	507 (40)	24.05 (1.00)	13.1 (3.3)	3.0 (1.2)

Table 5.4 **Subject characteristics presented as mean (SD or range) values for the group.**

	Central corneal thickness	**Mean corneal radius**	**Axial length**
IOP measurement (significance level)	+ 0.374 ($p = 0.010$)	- 0.262 ($p = 0.078$)	- 0.004 ($p = 0.979$)
PA measurement (significance level)	- 0.022 ($p = 0.888$)	+ 0.459 ($p < 0.001$)	- 0.651 ($p < 0.001$)

Table 5.5 **Part correlation coefficients calculated by stepwise multiple regression analysis for the dependent variables of intraocular pressure (IOP) and pressure pulse amplitude (PA) measurements.**

IOP measurements correlated significantly with CCT measurements ($r = +0.374$, $p = 0.010$) and accounted for 14% of the variance (R^2) in the data. Figure 5.1 shows the plot of IOP measurements versus CCT measurements. The linear regression equation was IOP (mmHg) = 0.030 * CCT (μm) – 2.2: 95% confidence intervals for the slope being 0.007 to 0.053. A 10 μm change in CCT was equivalent to a 0.30 mmHg change in measured IOP. There was no significant correlation between the measurement of IOP and those of corneal radius and

axial length ($p > 0.05$). PA measurements correlated negatively with axial length and positively with mean corneal radius: part correlation coefficients of –0.651 and +0.459 respectively (both $p < 0.001$). The model calculated by stepwise multiple regression attributed 46% of the overall variance in PA measurements to axial length and mean corneal curvature: the individual contributions of variance being 25% for axial length and 21% for mean corneal curvature. There was no significant correlation between PA and CCT measurements ($p > 0.05$).

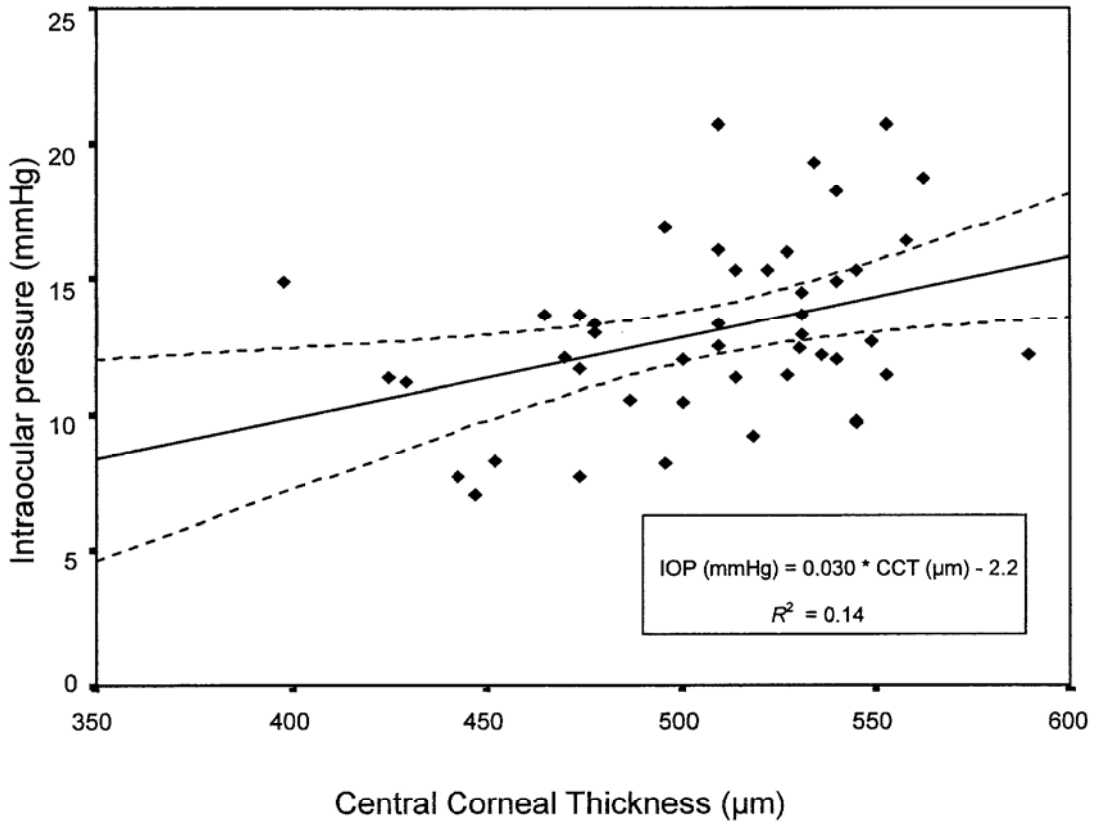


Figure 5.1 **Scatterplot of IOP measurements, taken with the Ocular Blood Flow Analyzer pneumatonometer, and corresponding central corneal thickness measurements. The regression line and its 95% confidence intervals (dashed lines) are shown. Inset box shows the regression equation and variance (R^2) of the association. Confidence intervals for the regression slope are calculated as +/- 1.96 standard errors of the mean regression value.**

5.6. *Discussion*

5.6.1. Intraocular pressure measurements and corneal thickness

This study found that IOP measurements made with the pneumatonometer were significantly influenced by corneal thickness and accounted for 14% of the variance in the measurements. Our results support those of a similar study (Singh *et al.*, 2001) who, using the same pneumatonometer, reported a change in measured IOP of 0.20 mmHg per 10 μm change in CCT. The standard deviation of normal CCT measurements, using a Scheimpflug camera, has recently been reported as 40 μm (Eysteinsson, Jonasson, Sasaki *et al.*, 2002). This would indicate that the influence of CCT on IOP measurements made with the OBFA pneumatonometer is only of clinical significance in those corneas at the extreme limits of their range in thickness.

The influence of corneal thickness on IOP measurements with Goldmann applanation tonometry has long been recognised (Goldman *et al.*, 1957). A number of studies however have concluded that pneumatonometry is less influenced by corneal thickness than Goldmann applanation tonometry (Abbasoglu *et al.*, 1998; Stahl & Vold, 2000; Zadok *et al.*, 1999). Walker & Litovitz (1972) in their theoretical paper on pneumatonometry, also claimed that corneal thickness could be ignored. They stated that it is the bending forces necessary to flatten the cornea that are dependent on corneal thickness and not the tension forces that are used in the balancing of pressures by the pneumatonometer; as the bending forces used in flattening the cornea are taken up by the outer edges of the 5 mm diameter probe, they are independent of the balancing forces occurring under the central (approximately 2.5 mm in diameter) portion of the probe. However, since the introduction of an air-suspension bearing to hold the probe against the cornea for continuous IOP recordings (Tønjum, 1973), a proportion of the pneumatonometer's air flow is required to produce this initial corneal flattening and the influence of corneal thickness may possibly arise here (Figure 1.13). It would be of interest to investigate whether corneal thickness influences the force required to flatten the cornea more than the balancing force created by the gas pressure in the centre of the tube.

A possible explanation for the discrepancy in the conclusions on whether the OBFA pneumatonometer is influenced by CCT, is in the respective probe diameters of the

pneumatic and Goldmann tonometers. The studies that found IOP measurements varied less by pneumatonometry than Goldmann tonometry with a change in corneal thickness were based on measurements taken before and after laser refractive surgery (Abbasoglu *et al.*, 1998; Stahl *et al.*, 2000; Zadok *et al.*, 1999). As the ablation diameters in these studies were 5–6 mm, it would not be unexpected that the force required to applanate a 3.06 mm corneal diameter, as in the case of the Goldmann probe, would be influenced by the reduction in corneal thickness. The larger 5.0 mm diameter pneumatonometer probe however would exert its bending force where the corneal thickness has had no or minimal reduction and therefore may be less influenced by the central corneal thinning. This may also explain why IOP measurements in Goldmann and pneumatic tonometers are both influenced by corneal thickness in normal subjects as the variation in corneal thickness between individuals would occur across the whole cornea. Further investigations to test these hypotheses are required.

A weakness in our study is the lack of a true comparative IOP reading. The gold standard measurement of IOP involves using an invasive manometer and such studies are limited to animal experiments or human eyes undergoing concomitant surgery (Eisenberg *et al.*, 1998). In non-invasive human studies such as ours an assumption is made that the corneal thickness influences the IOP measurement rather than the true IOP: an assumption which is supported by manometric evidence (Whitacre, Stein & Hassanein, 1993b). As subjects were seen between the hours of 10.00 am and 4.00 pm, diurnal variations in IOP and PA may have also introduced additional variance into the measurements taken (Claridge *et al.*, 1994b). Another possible weakness of this study is that the measure of CCT was determined by the image size of an optical section captured by a digital Scheimpflug camera rather than using ultrasound pachometry. Whilst Scheimpflug biometry has the advantage over ultrasound of being completely non-invasive, the corneal section is subject to two types of distortion: one associated with the camera and one ocular (Dubbelman, Van der Heijde & Weeber, 2001).

In optical pachymetry, varying magnification along the image plane arises from the geometry of the camera and the Scheimpflug principle. As the same camera was used under the same conditions for all subjects, this first source of distortion can be ignored. The image of the corneal thickness is refracted by the anterior corneal surface and this ocular source of distortion warrants consideration. The refraction of this corneal section will

depend upon the cornea's refractive index and its anterior corneal radius. A formula for generating the theoretical true corneal thickness from the perceived image has been described by Bennett & Rabbetts (1984) (Equation 5.1).

$$d = \frac{n}{1/d' + (n-1)/r}$$

d = theoretical true corneal thickness
n = refractive index of the cornea
d' = image size of the corneal thickness
r = anterior corneal radius

Equation 5.1 Formula for calculating the theoretical true corneal thickness from an image taken from an optical section.

The potential error in using optical sections to measure corneal thickness may now be determined. As the standard deviation of corneal refractive index (mean 1.376) has been reported (Patel, 1987) to be about 0.0065, the maximum error in corneal thickness measurement (for the limits of a 95% normal distribution) would be no more than 2% and there is no reason to believe that the refractive index values would not be normally spread amongst the subjects. The error in corneal thickness estimation, caused by varying the anterior corneal radius from 6.00 to 8.00 mm, amounts to about 1% (Olsen, Nielsen & Ehlers, 1980). As the subjects' anterior corneal radii were known, the measurement of central corneal thickness was recalculated using Bennett's formula. The results remained the same: corneal thickness measurements correlated with IOP measurements ($r = +0.370$, $p = 0.010$) but not with PA measurements. It therefore appears that the mode of corneal thickness measurement used in this study is robust and that the finding that pneumatonometric IOP measurements are influenced by corneal thickness is reliable.

5.6.2. Intraocular pressure measurements and corneal radius

In this study there was no influence by corneal radius on the IOP measurements made by the pneumatonometer. The effect of corneal curvature on Goldmann tonometry has been shown to be minimal; Mark (1973) found that it accounted for only 3% of the variance in IOP measurements. A positive correlation between corneal curvature and IOP

measurements by applanation-type tonometry is supported in theory as a steeper cornea would need to be indented more, and would displace more intraocular fluid, to produce the same area of applanation than a flatter cornea (Whitacre *et al.*, 1993a).

5.6.3. Intraocular pressure pulse amplitude measurements

PA measurements did not show a significant correlation with corneal thickness. A possible explanation is that as corneal thickness increased, artificially raising the measurement of the lower and higher limits of the pressure pulse, the difference in the limits of the PA remained much the same over the range of IOP in this study. The present study therefore provides no evidence to indicate that the differences found in pulse amplitude between NTG patients and normal subjects (Schmidt *et al.*, 1997), or between POAG patients and ocular hypertensive patients (Kerr *et al.*, 1998), are artifacts due to corneal thickness.

As previously reviewed (1.7) it is common practice to recalculate the PA as an intraocular volume change to determine a measure of the pulsatile blood flow to the eye. As such a calculated volume change is dependent on the IOP level (1.5), these derived measures of pulse volume or POBF may be more influenced by corneal thickness. This can be examined to a first approximation by using the results of this study and a commonly used ocular pressure-volume relationship for POBF calculations (Langham *et al.*, 1976).

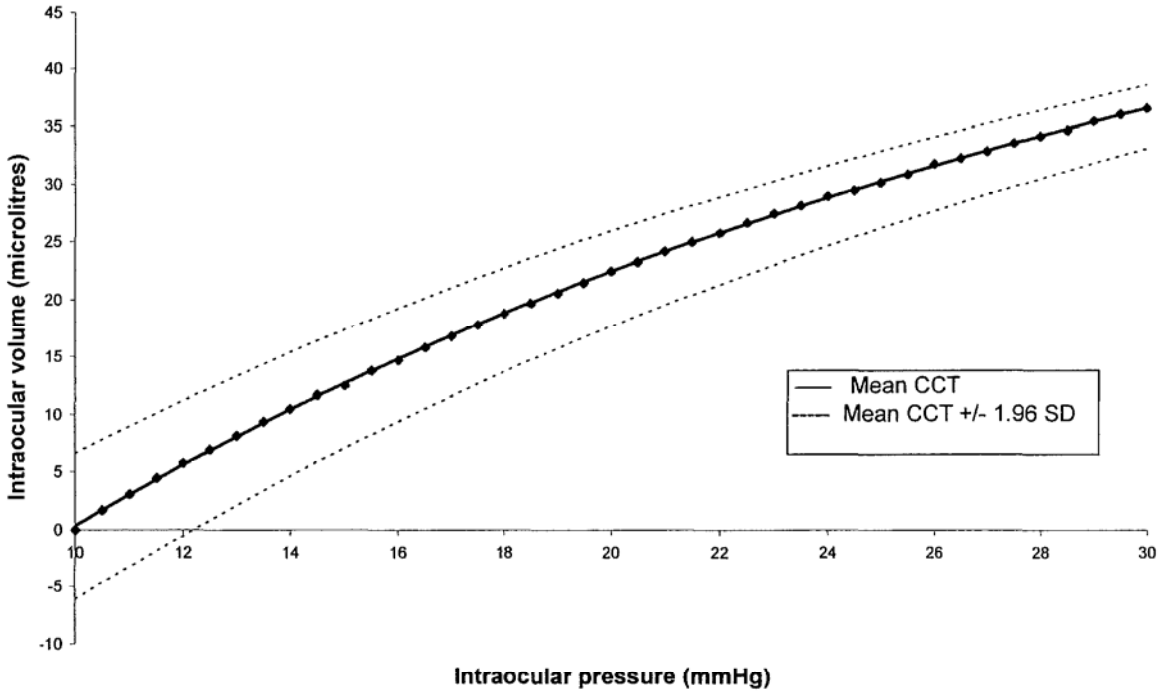


Figure 5.2 **The theoretical effect of central corneal thickness (CCT) variations on the ocular pressure-volume relationship. Adapted from Langham *et al.* (1976) and Eysteinsson *et al.* (2002).**

Figure 5.2 shows the calculated variation in ocular pressure-volume relationship for a normal range in CCT (Eysteinsson *et al.*, 2002). Although the absolute calculated intraocular volume changes markedly as CCT varies, the relative change (slope) of the relationship remains similar. As it is the slope of an ocular pressure-volume relationship that determines the measure of pulse volume or POBF, it appears unlikely that CCT variations are of concern. Taking an extreme example, it would be predicted for a true PA of 6.00 mmHg (a trough of 10.00 mmHg and a peak of 16.00 mmHg) that the calculated pulse volume would only vary by 20% between two subjects having CCT values at the extremes of normality. This is supported in the present study where, on re-examining the data, no significant correlation was found between CCT value and either pulse volume or POBF values.

As this study and others have shown (Ravalico *et al.*, 1997; Shih *et al.*, 1991; To'mey *et al.*, 1981), PA measurements have a strong significant negative correlation with axial length. A number of explanations have been put forward to account for this. First, myopic choroidal atrophy may diminish the cyclic vascular pulsations that produce the variation in IOP

(To'mey *et al.*, 1981). Second, as the posterior sclera in myopia may be associated with anomalous elasticity (Phillips *et al.*, 1995), a relatively smaller pressure variation would occur in an eye with a more distensible shell. Third, the larger ocular volume found in a myopic eye would reduce the relative change in volume, as a bolus of blood enters it, which in turn would reduce the consequent pressure change (Silver *et al.*, 2000).

Using multiple regression to remove the effect of axial length, this study shows for the first time that PA measurements are positively correlated with corneal radius: the flatter the cornea, the higher the pulse amplitude measurement. As the variance in pulse amplitude measurements attributed to corneal curvature was substantial (over 20%), consideration needs to be given to the possible causes and the implications of this finding. As with axial length, the correlation may arise from the effect of ocular volume. For example, taking two eyes of the same axial length, if the one with the steeper cornea has a larger volume the pulse amplitude would be diminished. However, as larger eyes are usually associated with flatter corneas (Goss *et al.*, 1997), this appears to be an unlikely cause. Further, the corneal radius maybe associated with the amount of vascular tissue in an eye; a larger eye possibly having greater vascular tissue. Finally, the positive correlation between PA and corneal radius may reflect an interaction between tonometer probe and cornea, such as a vibration artifact. In their study of the pulsatile response during pneumatonometry, Walker *et al.* (1975a) stated that corneal radius is an important factor in the fundamental vibration frequency (Equation 1.14) of the applanated cornea but that this critical vibration frequency (~150 Hz) is well above that required for clinical studies (~1 Hz).

5.7. *Conclusion*

This study provides evidence that IOP measurements, taken with the pneumatonometer, are correlated with corneal thickness, but not axial length or corneal curvature. Conversely, PA measurements are unaffected by corneal thickness, but correlated with axial length and corneal radius. These parameters should be taken into consideration when interpreting IOP and PA measurements made with the pneumatonometer.

The findings from this study have recently been published and are shown in Appendix A.4.3. (Morgan, Harper, Hosking *et al.*, 2002a).

6. The effect of low-level hypercapnia on Intraocular pressure pulse parameters

6.1. *Abstract*

Purpose: The inhalation of air mixed with CO_2 provides the opportunity to observe the eye under the stimulus of a known vasodilator. The aim of this study was to compare IOP pulse measurements taken during low level hypercapnia to those taken whilst breathing ordinary room air.

Methods: Twenty young healthy subjects with a mean age of 27.7 ± 4.9 yrs, were recruited to a randomised cross-over study. Investigations were undertaken under two breathing conditions: a, using inspired room air (normocapnic condition); and b, using room air mixed with additional CO_2 sufficient to raise their end-tidal CO_2 levels by 15% (hypercapnic condition). Pneumatonometric measurements of POBF and PA were taken under each condition.

Results: In order to achieve an increase in end-tidal CO_2 of 15%, an inhaled CO_2 level of 3.7 ± 0.7 % was required for the group. In comparison to ordinary room air, POBF and PA measures fell by 6.2% ($p = 0.039$) and 8.8% ($p = 0.004$) respectively under hypercapnia. Whereas IOP and systemic parameters of heart rate, arterial pulse pressure, and blood oxygen saturation remained stable, mean arterial blood pressure showed a significant rise of 4.0 mmHg ($p < 0.001$) under the hypercapnic condition.

Conclusions: The findings of this study suggest that in contrast to previous reports using higher degrees of hypercapnia, mild hypercapnia results in a reduction of the IOP pulse parameters, POBF and PA. Explanations to this finding our discussed and the possibility of a shift in pulsatile to non-pulsatile flow in ocular blood flow, under this level of CO_2 inhalation, explored. The study suggests that differences in, or changes to, POBF and PA measures taken in isolation should be treated with caution and ideally interpreted in relation to other indices of ocular blood flow.

6.2. *Introduction*

The IOP pulse parameters present an opportunity to measure the pulsatility of the uveal circulation under different physiological and pathological conditions, or following pharmacological intervention (1.6 to 1.8). Increased arterial carbon dioxide tension (*P*a CO_2) is a potent vasodilator of cerebral arteries and arterioles but has little influence on other vessels of the body (Kety & Schmidt, 1948; Levick, 2000b; Raper, Kontos & Patterson, 1971). Ocular blood vessels share this sensitivity to *P*a CO_2. Animal studies show that ocular blood flow increases in direct proportion to the level of carbon dioxide inhaled (Alm *et al.*, 1972; Friedman *et al.*, 1972; Trokel, 1965; Wilson, Strang & MacKenzie, 1977). In human subjects breathing hypercapnic air, measurements indicative of increased blood flow have been reported for the retinal (Harris, Arend, Wolf *et al.*, 1995; Roff *et al.*, 1999; Sponsel, DePaul & Zetlan, 1992), optic nerve head (Harris *et al.*, 1996) and choroidal circulations (Geiser, Riva, Dorner *et al.*, 2000; Riva *et al.*, 1994). Cerebral vasodilatation from hypercapnia is believed to be mediated through the associated decrease in perivascular pH and involves both nitric oxide-dependent and nitric oxide-independent pathways (Iadecola & Zhang, 1994).

Measurement of POBF and PA during hypercapnia presents an opportunity to observe the effect of a known stimulus to ocular blood flow on the pulsatility of intraocular vascular beds. The effect of hypercapnia on the IOP pulse has had little attention. POBF and PA measurements have been reported to increase during hypercapnic breathing (Kergoat *et al.*, 1999; Schmetterer *et al.*, 2000b). However Roff *et al* (1999) found no change in POBF values during hypercapnia, even though other ocular haemodynamic flow measures increased.

6.3. *Aims*

The purpose of this study therefore was to compare the measurements of PA and POBF taken under hypercapnic and normocapnic conditions.

6.4. *Methods*

6.4.1. Experimental design

This was a single-blinded, randomised cross-over study.

6.4.2. Ethical approval

All experimental procedures of the study conformed to the tenets of the declaration of Helsinki and were approved by the Aston University Human Science Ethical Committee. Written informed consent was obtained from all subjects willing to participate in the study.

6.4.3. Subject sample

Twenty subjects, nine male and eleven female, with an age (mean ± standard deviation) of 27.7 ± 4.9 years (range 18 – 36 years) volunteered for the study. A full eye examination was performed on prospective volunteers. Exclusion criteria used for the study are found in Table 6.1.

- Reported history of present or past eye disease
- Reported history of systemic disease, or current medication, with known cardiovascular properties
- Reported history of respiratory disorders (for example, asthma)
- Reported history of frequent or serious headaches
- Pregnancy
- Corrected visual acuity of less than 6/9
- Corneal or anterior chamber abnormalities as detected by slit-lamp examination
- Posterior segment abnormalities as detected by slit-lamp binocular indirect ophthalmoscopy
- Intraocular pressure greater than 21 mmHg
- Physical or mental incapacity to perform experimental procedures

Table 6.1 Subjects' exclusion criteria for the IOP pulse during hypercapnia study.

A mild increase in inhaled carbon dioxide (up to 5% CO_2) is not usually associated with any side effects (Hosking, Evans, Embleton *et al.*, 2001). However potential hazards, usually associated with levels of 8% or more inhaled CO_2, include nausea, hyperventilation and headaches (Geiser *et al.*, 2000; Schmetterer, Lexer, Findl *et al.*, 1996). For this reason, subjects reporting a history of respiratory disorders, headaches or pregnancy were excluded from the study.

6.4.4. Experimental procedures and instrumentation

Principal of producing a mild rise in Pa CO2

The experimental procedure to produce conditions of isoxic hypercapnia was based on that described by Harris *et al.* (1996) This procedure increases a subject's end-tidal respiratory CO_2 level to approximately 15% above their normal baseline value. It is accepted in pulmonary physiology that end-tidal CO_2 levels are directly proportional to *P*a CO_2 (Sherwood, 1989). The procedure is safe and, by keeping *P*a CO_2 levels to moderate levels, unwanted systemic side effects are usually avoided (Hosking *et al.*, 2001; Niwa, Harris, Kagemann *et al.*, 1999).

Study sequence

Subjects were seated in a well ventilated room wearing a tightly fitting gas mask. The air inhaled by the subject originated, through a non-rebreathing valve, from a mixing chamber that had two inlets: one to room air and one, via a flowmeter, to a cylinder of 100% medical grade CO_2. The expired air was vented to the examination room. The subject sat and adapted to wearing the gas mask, whilst breathing normal room air, for at least 15 minutes prior to testing. This duration of adaptation was chosen to ensure stability cardiac function. Respiratory rate and end-tidal CO_2 measurements were then taken continuously using a rapid response gas analyser (Capnograph Plus V1.06, BCI Inc., Waukesha, Wisconsin, USA) that sampled the inhaled and expired air from a feed line connected to the gas mask. Heart rate and blood oxygen saturation were also monitored continuously using a fingertip pulse oximeter.

Following the adaptation period, measurements of IOP and IOP pulse were taken, along with measurements of blood pressure, under two conditions: one when the subject was

breathing room air mixed with carbon dioxide (hypercapnic air) and one when the subject again breathed solely normal room air. The sequence of the two breathing conditions was randomised (sealed envelope technique) and the subjects were not told which gas they were breathing. During the hypercapnic breathing phase, the end-tidal CO_2 level of each subject was raised by 15% above their individual baseline level. This was achieved through the manual introduction of CO_2 to the mixing chamber from the gas cylinder. For each breathing condition, all measurements were taken 15 minutes after the end-tidal CO_2 level stabilised. A wash out period of 30 minutes was used between the two breathing conditions during which the subject remained seated but could remove the gas mask.

Blood pressure measurements were taken using a validated (Shennan, Rushbrook, Power *et al.*, 1998) automated sphygmomanometer (Omron Rx, Omron Matsusaka Co. Ltd, Japan). Arterial pulse pressure (APP), mean arterial blood pressure (MAP) and mean ocular perfusion pressure (OPP) were calculated as previously described (Equations 1.6, 1.7 and 1.11). The OBFA pneumatonometer (Paradigm Medical Industries, Utah, USA), described above (1.6.4), was used to take measurements of IOP, POBF and PA. Pneumatonometry measurements were taken on one randomly chosen (sealed envelope technique) eye of each subject following topical anaesthesia with one drop of 0.4% benoxinate HCl (*Minims*®, Chauvin, UK). Measurements of IOP, PA and POBF were calculated automatically, by the pneumatonometer's software, from five pulses that it considered acceptable from a maximum recording duration of 20 seconds. The study sequence is summarised in Figure 6.1.

6.4.5. Statistical analysis

Results are presented as mean values for the group, plus and minus the standard deviation of the measurements. Student's two-tailed paired *t*-test was used to compare measurements obtained during the two gas conditions and, where statistically significant, data values are described as percentage change between conditions. Possible correlations between variables were explored using the Pearson Correlation Coefficient. A *p*-value of less than 0.05 was taken as statistically significant.

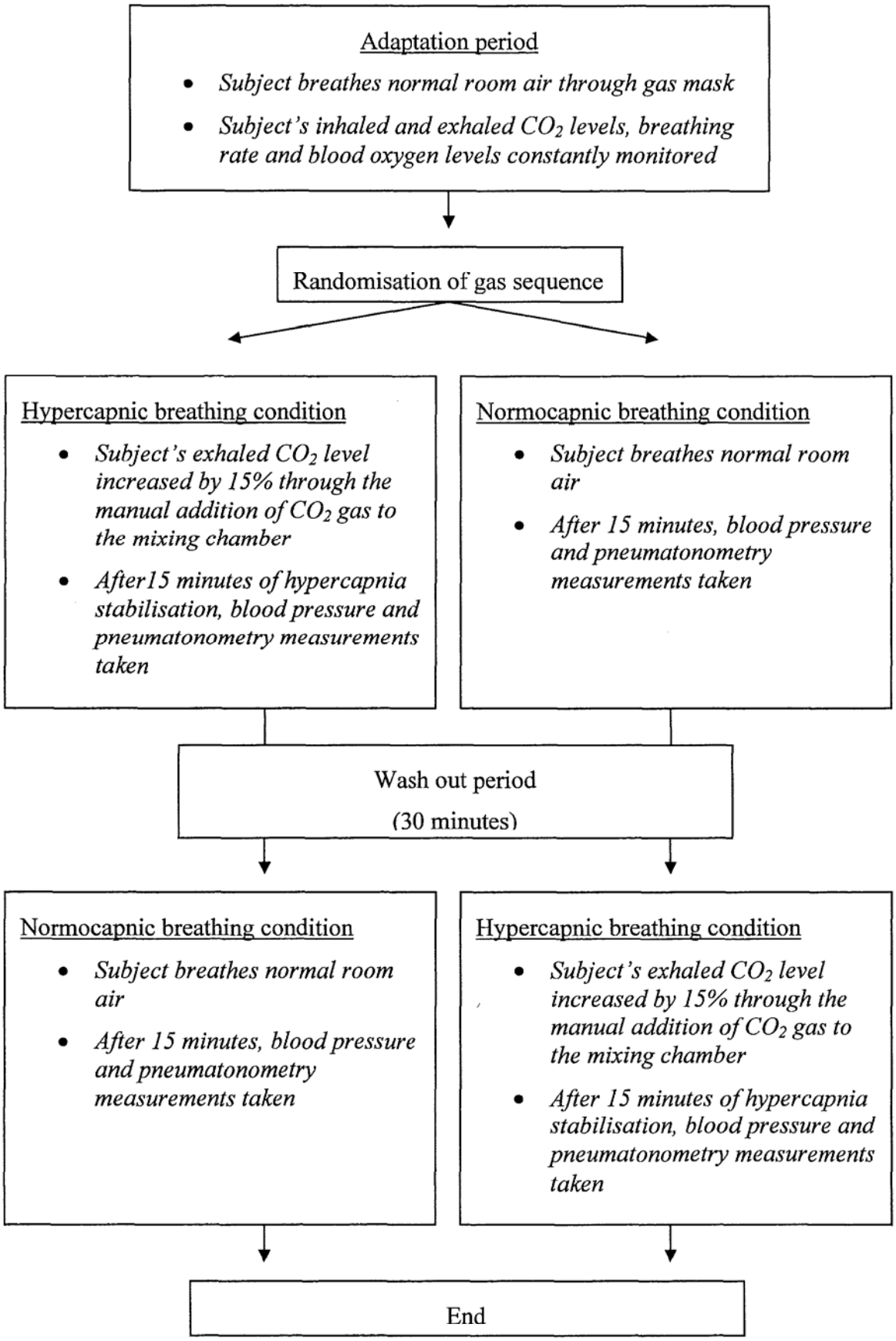


Figure 6.1 Summary of experimental sequence for the hypercapnia study.

6.5. *Results*

Systemic measurements for the two breathing conditions are shown in Table 6.2. During room air breathing, the levels of inhaled CO_2 and end-tidal CO_2 were 0.9 % and 5.2 % respectively. During the hypercapnic phase, the levels of inhaled CO_2 and end-tidal CO_2 increased to 3.7 % and 6.0 % respectively: a successful increase in end-tidal CO_2 of 15.4 % ($p < 0.001$). Whereas heart rate and arterial pulse pressure remained stable, MAP and OPP rose significantly (both $p < 0.001$) during hypercapnia. Respiratory rate and blood oxygen saturation did not significantly alter.

	Room air	**Hypercapnic air**	**Significance**
Mean arterial blood Pressure (mmHg)	85.5 (7.4)	89.5 (9.0)	$p < 0.001$
Mean ocular perfusion pressure (mmHg)	42.9 (4.3)	45.9 (5.9)	$p < 0.001$
Arterial pulse pressure (mmHg)	48.2 (11.7)	47.9 (8.6)	N.S.
Heart rate (beats / min)	66.0 (9.5)	65.6 (8.5)	N.S.
Respiratory rate (breathes / min)	11.7 (2.9)	12.3 (3.0)	N.S.
Blood oxygen saturation (%)	97.2 (0.8)	97.4 (0.9)	N.S.
Inhaled CO_2 level (%)	0.9 (0.2)	3.7 (0.7)	$p < 0.001$
End-tidal CO_2 level (%)	5.2 (0.7)	6.0 (0.7)	$p < 0.001$

Table 6.2 **Systemic parameters, presented as mean (SD) values, during the inhalation of room air and hypercapnic air.**

IOP measurements did not significantly differ between room air and hypercapnic air conditions: 14.1 ± 2.7 mmHg and 13.8 ± 3.2 mmHg respectively. Both the POBF and PA measurements were significantly lower during the hypercapnic phase of the study. The value of the mean POBF value changed from 502 ± 104 μl/min during room air breathing to 463 ± 76 μl/min under hypercapnia: an average decrease of 6.2% ($p = 0.039$). Likewise the mean PA measurement changed from 2.32 ± 0.66 mmHg during room air breathing to 2.08

± 0.56 mmHg under hypercapnia: an average decrease of 8.8% ($p = 0.004$). Figure 6.2 shows the percentage change in IOP, PA and POBF from normocapnia to hypercapnia. No significant correlation was found between the change in IOP pulse parameters and the change in either MAP or OPP.

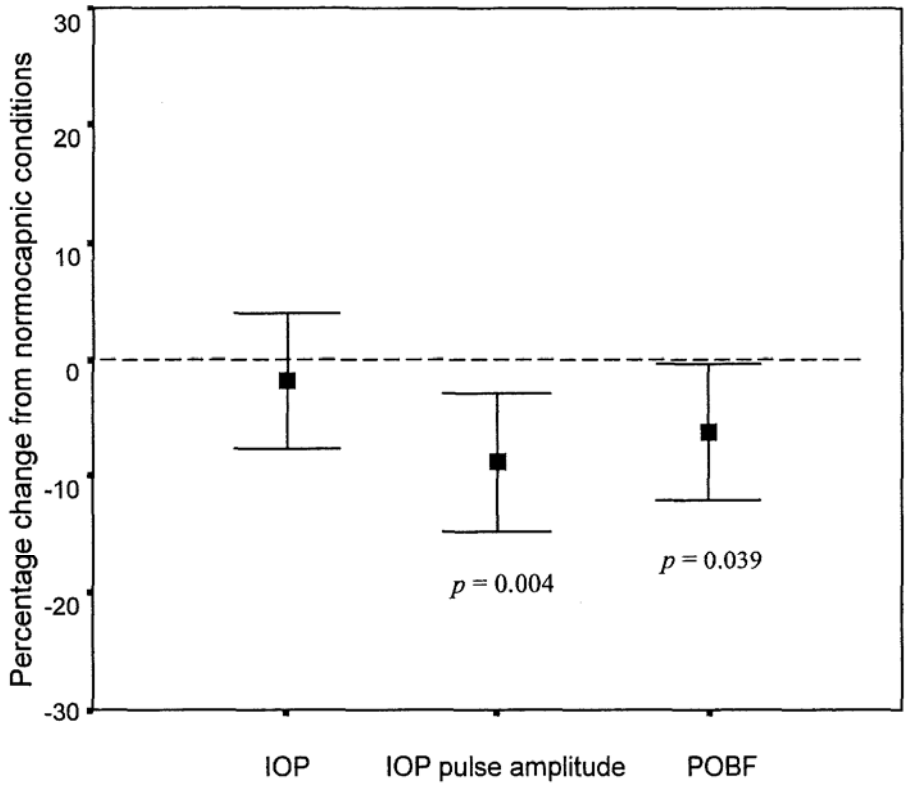


Figure 6.2. Percentage change in intraocular pressure (IOP), IOP pulse amplitude and Pulsatile Ocular Blood Flow (POBF) under hypercapnic compared to normocapnic breathing conditions. Error bars represent the 95% confidence intervals for percentage difference.

6.6. *Discussion*

6.6.1. Previous investigations on the IOP pulse during hypercapnia

The significant increase in end-tidal CO_2 levels and stable pulse oximetry values indicated that the experimental phase of isoxic hypercapnia was successfully achieved in this study. We found a significant reduction in both POBF and PA measures during hypercapnia. This finding appears to contradict two reported studies that investigated the effect of hypercapnia on a similar group of young healthy adults (Kergoat *et al.*, 1999; Schmetterer *et al.*, 2000b). Kergoat *et al.* (1999) found carbogen (95% O_2, 5% CO_2) to increase POBF values by 8%

and concluded it was the 5% CO_2 in carbogen that caused the observed increase, as previous inhalation of 100% O_2 had produced no effect. Schmetterer *et al.* (2000b) reported PA and POBF values to increase by 26% and 15% respectively when subjects breathed 5% CO_2 and 95% air. Schmetterer's group (Bayerle-Eder, Wolzt, Polska *et al.*, 2000; Schmetterer, Findl, Strenn *et al.*, 1997a; Schmetterer *et al.*, 1996; Schmetterer, Wolzt, Lexer *et al.*, 1995) has also repeatedly reported that hypercapnia increases fundus pulsation amplitudes (a parameter, measured interferometrically, as the change in distance between cornea and fundus during the cardiac cycle). These fundus pulsation amplitudes have been shown to correlate highly with the pneumatonometrically derived measures of POBF and PA (Schmetterer, Dallinger, Findl *et al.*, 2000a). Prior reasoning would also perhaps suggest an increase in the pulsatility of the intraocular vascular beds with the rise in inhaled CO_2. Increased levels of *P*a CO_2 relax vascular smooth muscle which, in small arteries and arterioles, leads to an increase in vessel wall compliance or distensibility (Faraci & Heistad, 1998; Nichols *et al.*, 1998f). Unless the arterial pressure pulse dramatically fell, an increased volumetric pulsation of the arterial vessels would be expected. Possible explanations for our observed reductions in IOP pulse parameters therefore need to be determined.

6.6.2. Alternative causes for a change in IOP pulse value

The observed reduction in POBF and PA measures under hypercapnia may have arisen through a concomitant change in a confounding variable. The IOP pulse is highly labile to a number of systemic and ocular factors (1.6). As the study was within-subject and all measurements were taken seated, the influences of refractive error and posture can be dismissed (Ravalico *et al.*, 1997; Trew *et al.*, 1991b). Those variables known to influence the IOP pulse, IOP (Akazawa *et al.*, 1994; Buchanan *et al.*, 1985; Claridge *et al.*, 1994b; Phillips *et al.*, 1992), arterial pulse pressure (Bron *et al.*, 1967; Chopp, Balian & Snitgen, 1983), and heart rate (James *et al.*, 1991c; Ravalico *et al.*, 1996; Trew *et al.*, 1991a) were also stable between the two breathing conditions. During hypercapnia, there was a significant rise in MAP and, as IOP remained unchanged, a similar increase in OPP. As heart rate and arterial pulse pressure remained steady, the mean rise in MAP of 4 mmHg would suggest a small increase in peripheral resistance. A similar change in MAP, without any change in cardiac parameters, was found by Kety *et al.* (1948) in their original studies on cerebral blood flow during hypercapnia. It is now known that hypercapnia acts as a

vasodilator on all vascular beds but many (e.g. cutaneous and skeletal muscle) offset this stimulus with other neurohumoural pathways to maintain a constant blood flow (Faraci *et al.*, 1998). A small rise in MAP under hypercapnic conditions therefore appears to be a normal finding and has been reported elsewhere (Schmetterer *et al.*, 1995). The level of MAP has not been reported to affect IOP pulse parameters (Gekkieva *et al.*, 2001; Mori *et al.*, 2001a; Ravalico *et al.*, 1996) and, in our study, the change in POBF or PA did not correlate with either the change in subject's MAP or OPP.

An additional confounding variable worthy of consideration, is the direct influence of hypercapnia on the ocular pressure-volume relationship. From first principles, the IOP pulse is dependent on this relationship and the eye's ocular, or scleral, rigidity (Friedenwald, 1937). Scleral rigidity is directly influenced by the level of blood oxygen tension (Gallin-Cohen, Podos & Yablonski, 1980), although the effect of hypercapnia has not been documented. Kerty *et al.* (1994) however found no change in scleral rigidity following the systemic injection of acetazolamide, a carbonic anhydrase inhibitor which produces a similar endogenous increase in *P*a CO_2 to that found in hypercapnic breathing studies (Sponsel *et al.*, 1997). The ocular pressure-volume relationship may also be dependent on the level of MAP. Kiel (1995) reported that, in rabbits, increasing levels of MAP produced proportionately steeper ocular pressure-volume relationships (1.5). That is, for the same change in intraocular volume, a higher level of MAP produced a greater change in intraocular pressure. In the present study however, although MAP rose during hypercapnia, IOP pulse parameters decreased. Examination of potential confounding variables, such as the change in MAP, therefore does not reveal an explanation to our observed reductions in IOP pulse parameters.

6.6.3. The effect of carbon dioxide inhalation on intraocular vascular beds

A reduction in ocular blood flow during the hypercapnic phase of our study would be unlikely. Certainly in diseased cerebral arteries, blood flow studies show that the brain can suffer a 'steal' phenomenon to hypercapnia as blood flow to certain cerebral regions increases at the expense of others (Nariai, Senda, Ishii *et al.*, 1998). In the healthy eye, the inhalation of hypercapnic air increases blood flow although the level of response is dependent on the exact vascular bed and level of hypercapnia used. The inhalation of 10% CO_2 mixed with room air has been shown to increase choroidal blood flow by 40% and 60%

in cats and rabbits respectively (Friedman *et al.*, 1972; Trokel, 1965). Similar increases in choroidal blood flow have been demonstrated in primates with the level of increase being directly proportional to the level of arterial CO_2 (Flower *et al.*, 1995; Wilson *et al.*, 1977). Retinal blood flow has a positive linear relationship to arterial CO_2 within the *P*a CO_2 range of 20 to 90 mmHg in monkeys (Tsacopoulis, Baker, Johnson *et al.*, 1973). At the optic nerve head in rabbits, Sugiyama, Utsumi, Azuma *et al.* (1996) have shown 10% inhalation of CO_2 with air increases capillary blood flow by 20%. Studies on young, healthy human subjects, and using the same level of hypercapnia as in our study, also indicate a rise in ocular blood flow. Roff *et al.* (1999) increased end-tidal CO_2 levels of normal subjects by 15% and, using Doppler ultrasound, found flow velocities to increase and the Resistance Index to fall in the short posterior ciliary arteries. The same level of hypercapnia has been shown to increase blood flow values, using scanning laser Doppler flowmetry, at the retina and optic nerve head by 28% and 39% respectively (Harris *et al.*, 1996; Roff *et al.*, 1999). A 10 % increase in end-tidal CO_2 has also been shown to decrease retinal arteriovenous passage times using fluorescein angiography and scanning laser ophthalmoscopy (Harris *et al.*, 1995). By using scanning laser Doppler flowmetry in the avascular foveal region, increased blood flow has been demonstrated in the underlying choriocapillaris during hypercapnia (Riva *et al.*, 1994): the increase in choriocapillaris blood flow being proportional to the level of arterial CO_2 (Geiser *et al.*, 2000).

An increase in *P*a CO_2 reduces the affinity between oxygen and haemoglobin and results in an off-loading of oxygen into the surrounding plasma and a small increase in *P*a O2 (the Bohr effect). Breathing 100% O_2 induces strong vasoconstriction with a concomitant fall in blood flow in the retina (Luksch, Garhöfer, Imhof *et al.*, 2002). In contrast, the effect of 100% O_2 inhalation on choroidal blood flow is negligible (Friedman *et al.*, 1972; Geiser *et al.*, 2000). In the present study, blood oxygen saturation was unaltered during hypercapnia. Whilst a reduction in ocular blood flow during the hypercapnic phase of our study cannot be ruled out (as no other comparative blood flow measures were taken), these preceding studies would suggest it is unlikely.

6.6.4. Pulsatile versus non-pulsatile flow

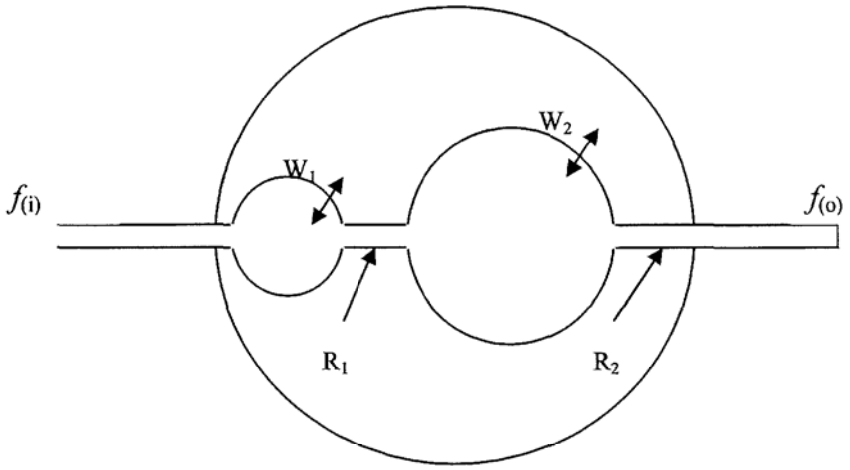
A possible explanation to our results is that, although total ocular blood flow increased during hypercapnia, the proportion of pulsatile ocular blood flow fell. The proportion of

pulsatile ocular blood to the eye has been estimated to be between 33% and 80% (Krakau, 1995; Langham *et al.*, 1989a); the exact proportion being determined by a number of local and systemic factors (Krakau, 1995). In cerebral blood flow, it is the proportion of non-pulsatile flow that increases during low level hypercapnia and the compliance behaviour of pre-capillary arteries is not simple (Curran-Everett, Zhang, Jones *et al.*, 1995).

Examples of shifts in the pulsatile fraction of ocular blood flow exist. Riva, Titze, Hero *et al.* (1997b) using scanning laser Doppler flowmetry at the macula, reported that the pulsatile fraction of choroidal blood flow altered after static exercise. A shift in pulsatile to non-pulsatile ocular blood flow has been proposed to occur when a subject moves from an upright to a supine position (James *et al.*, 1991b). Studies have demonstrated that POBF measurements made on subjects in the supine position are consistently lower than when they stand or sit (James *et al.*, 1991a; Ravalico *et al.*, 1996; Trew *et al.*, 1991b). Rather than a fall in total ocular blood flow being responsible for this change, Doppler ultrasonography indicates that, as end-diastolic velocity of the ophthalmic artery increases in proportion to peak systolic velocity when a subject lies down (Canning *et al.*, 1988), non-pulsatile ocular blood flow increases. Kerty *et al.* (1994) has speculated that reports of reductions in POBF whilst other ocular blood flow measures increase (Yoshida *et al.*, 1991), may be explained by a shift from pulsatile to non-pulsatile flow.

6.6.5. An analogue model to describe pulsatile and non-pulsatile ocular blood flow

The possible biomechanics of a shift in pulsatile to non-pulsatile ocular blood flow can be illustrated using the electrical analogue circuit developed by Krakau (1995). The modelling of pulsatile and non-pulsatile blood flow using the comparable equivalents of direct and alternating current is common practice in circulation research. Each vascular component in an end-arterial bed can be represented by a lumped electrical equivalent in a two-terminal circuit (Figure 6.3).



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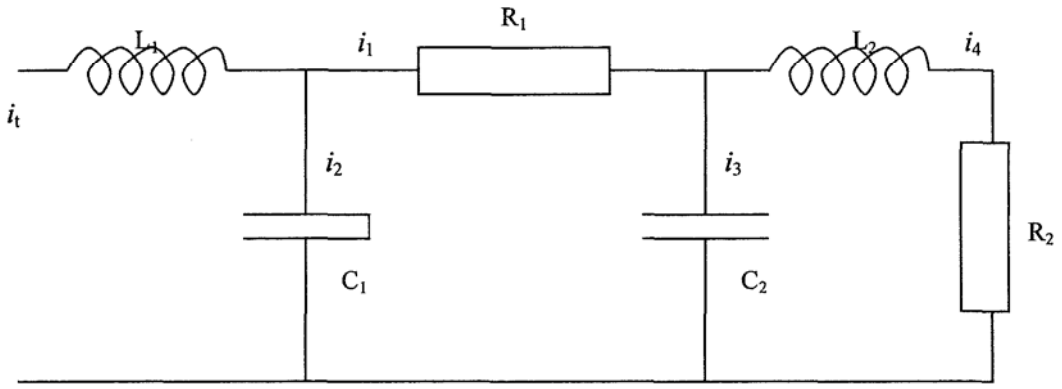

b

Figure 6.3 **a) A schematic of the end-arterial flow components of the eye: W_1 and W_2 are windkessels representing the distensibility of the arterial and capillary beds respectively; R_1 and R_2 represent the resistance to blood flow at the respective arterial and intrascleral venous levels; $f_{(i)}$ and $f_{(o)}$ represent arterial inflow and venous outflow respectively.**

b) The corresponding electrical analogue circuit: C_1 and C_2 are capacitors representing the distensibility of the arterial and capillary beds respectively; R_1 and R_2 are resistors representing resistance to flow at the respective arterial and intrascleral venous level; L_1 and L_2 are inductors necessary to represent blood flow mass at the respective arterial and intrascleral venous level; i_t and i_n represent total ocular blood inflow and subsequent pulsatile currents; i_4 is constant as venous outflow is assumed to be steady. Adapted from Krakau (1995).

The effect of breathing hypercapnic air is taken to reduce resistance at R1, and increase capacitance at C1, due to the relaxation of smooth muscle in the arterial bed. The capacitance of the capillary bed (C2) is assumed not to change because the major intraocular capillary bed (the choriocapillaris) is not surrounded by a muscular layer. A simple sinusoid is used to represent the arterial pressure pulse that drives pulsatile ocular blood flow. A constant pressure head (DBP minus IOP) is used to drive the non-pulsatile component of ocular blood flow. Using values for the analogue circuit described by Krakau, (1995) the model predicts, for a fall in resistance at R1 and a corresponding increase in capacitance at C1, an initial shallow decline in the pulsatile ocular blood flow before an exponential rise (Figure 6.4).

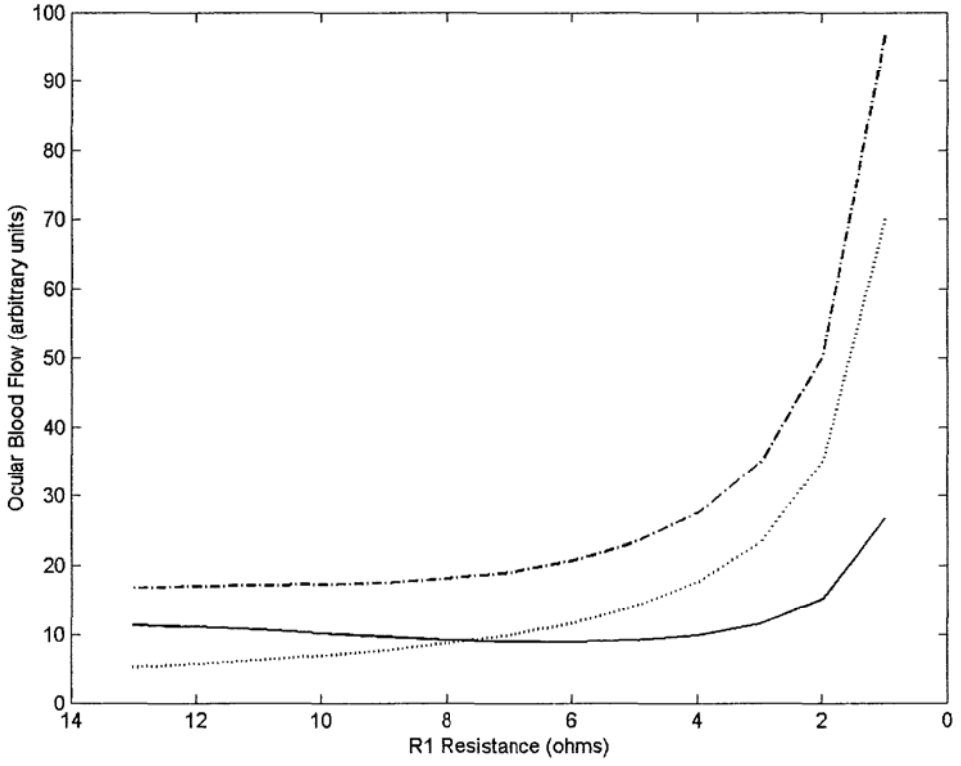

Figure 6.4 **The change in ocular blood flow components, predicted from an electrical analogue model (see Figure 2), as the resistance at R1 falls: — pulsatile flow; ···· steady flow; - - - total flow (pulsatile + steady).**

This bimodal change in pulsatile flow can be explained as follows. As resistance at R1 falls, although capacitance at C1 increases, the increase in voltage across the larger capacitor C2 (whose capacitance remains stable) causes an initial decrease in pulsatile current. The later increase in pulsatile current occurs as capacitance at C1 continues to increase as R1 continues to fall. Returning to the vascular components of the eye, this sequence of events

would represent an initial drop in pulsatile flow at the level of the capillary beds as total flow increases followed by a subsequent rise as compliance at the arterial level continues to increase.

Although speculative, our results may represent an initial reduction in pulsatile flow as resistance in the arterial bed falls and total flow increases. The level of inhaled CO_2 in the hypercapnic phase of the present study was modest. The technique of increasing end-tidal CO_2 by 15% (Harris *et al.*, 1996), requires an individual adjustment to the level of inhaled CO_2 required by each subject. The mean inhaled CO_2 level for subjects in the present study was 3.7%. The studies by Kergoat *et al.* (1999) and Schmetterer *et al.* (2000a) used a predefined level of inhaled CO_2: 5% CO_2 in 95% O_2 and 5% CO_2 in 95% air respectively. We suggest that at the low levels of hypercapnia used in our study, the observed reductions in IOP pulse measures represent a shift in pulsatile to non-pulsatile flow. Greater levels of hypercapnia may be required to elicit an augmentation in the pulsatile component of ocular blood flow. Some support for this conclusion comes from a study by Geiser *et al.* (2000). Using scanning laser Doppler flowmetry at the macula, Geiser *et al.* measured choroidal blood flow in young healthy subjects as they inhaled increasing levels of CO_2 mixed with O_2. Although choroidal flow measures increased in a steep dose-dependent fashion with the level of CO_2, an increase in pulsatility only occurred at the highest levels of hypercapnia (8% CO_2, 92% O_2).

6.7. *Conclusions*

The possible implications of our study are far reaching. We have shown that both the measurements of POBF and PA decrease during mild hypercapnia, a known vasodilator of ocular blood vessels. We suggest that, at the levels of hypercapnia used in this study, a shift in pulsatile to non-pulsatile ocular blood flow may occur. Such a shift is consistent with a previously described electrical analogue model used to predict the components of pulsatile and steady ocular blood flow. A reduction in IOP pulse measures, despite a presumed increase in total ocular blood flow, suggests that ocular pulsatility data should be used with caution in the interpretation of haemodynamic status, and should ideally be confirmed in the context of other vascular measures and known physiological responses. Further investigations to look at the possible change in pulsatility quotient under increasing levels of hypercapnia would be of interest.

7. The intraocular pressure pulse: a comparison of spectral analysis techniques

7.1. *Abstract*

Purpose: Previous authors have indicated that measurement of the IOP pulse in the frequency domain provides greater differentiation between diseased and non-diseased eyes. In addition our group has previously speculated that the higher spectral components of the IOP pulse waveform are characteristic of the eye's internal vascular compliance. As part of the hypothesis validation, this study investigated the test-retest variability of the original technique in obtaining the harmonic components of the IOP pulse and compared it to two other alternative methods of spectral analysis.

Methods: Recordings of continuous IOP were made using a high-fidelity pneumatonometer on 30 volunteers (63.8 ± 7.5 yrs). The IOP pulse waves were analysed using three different techniques to obtain the spectral components: the originally described technique; a Power Spectral Density (PSD) technique; and an averaged single pulse (ASP) technique. The repeatability (within-visit variation) and reproducibility (between-visit variation) of the three techniques were calculated as coefficients of reliability (CoR) for the harmonic moduli of the IOP pulse waveforms.

Results: The original spectral analysis technique produced acceptable repeatability for the third and fourth harmonics. Correlation of within-visit measurements diminished at lower and higher harmonics. Correlation of between-visit measurements with the original technique was poor and only the third and fourth harmonics attained significant reproducibility. In comparison the repeatability and reproducibility of the PSD technique was good up to the fourth harmonic. The ASP technique was the only technique capable of measuring beyond the 4^{th} harmonic with any significant correlation within- and between-visits.

Conclusions: Our results indicate that the originally described technique for analysing the spectral components of the IOP pulse is inadequate for measuring the lowest and highest harmonics. Spectral leakage from the discontinuous start and end of the waveform signal, as well as that arising from normal heart-rate variation, probably account for its poor correlation of measurements. Further investigations on IOP pulse moduli would benefit from using these alternative spectral analysis techniques.

7.2. *Introduction*

Any repeating waveform can be represented as a series of component sinusoids (Bloomfield, 2000). The breaking down of an isolated waveform into its harmonic components uses a process known as Fourier transformation (Appendix 2). Commonly the results of such a transformation are displayed as the amplitudes (or moduli) and phase values of the components plotted against their respective frequencies. Representing a waveform in this way is known as spectral analysis, or analysis in the frequency domain rather than the original recording (usually time) domain. Spectral analysis has provided a valuable investigative technique in the field of cardiovascular research. Particularly, research has focussed on the harmonics of the arterial blood pressure pulse (Nichols *et al.*, 1998e). The first harmonic, commonly referred to as the fundamental, falls at a frequency that is the reciprocal of the waveform's time period: which in the case of the arterial blood pressure pulse is approximately 1 Hz. Further harmonics then fall at successive multiples of this fundamental frequency: 2 Hz, 3 Hz, 4 Hz, etc. It has been shown that the higher harmonics of an arterial blood pressure pulse are dependent on the compliance of that artery's vascular bed and the wave-reflections that those end-arterial vessels produce (O'Rourke, 1999b). Furthermore, combined with similar analysis of the artery's flow wave, spectral analysis of the blood pressure pulse can be used to calculate the impedance of that arterial system (1.3.3) (Mills *et al.*, 1970).

IOP demonstrates a pulsation arising from the volumetric distension and decompression of the eye's internal vascular beds during systole and diastole (Weber, 1850). Evans *et al.* (2002) has speculated that the higher harmonics of this IOP pulse represent similar spectral characteristics of end-arterial beds found in the eye. Part of the validation of such a hypothesis requires an assessment of the precision of the measurements made and the calculations used in the spectral analysis of the IOP pulse (Hulley *et al.*, 1988).

7.2.1. Aims

The purpose of this study was to investigate the test-retest variability of Evans' spectral analysis technique and compare it to two alternative methods of analysing the IOP pulse in the frequency domain.

7.3. *Method*

7.3.1. Experimental design

This was a validation study investigating the repeatability (within-visit variation) and reproducibility (between-visit variation) of measurements.

7.3.2. Ethical approval

All experimental procedures of the study conformed to the tenets of the declaration of Helsinki and were approved by the Oxfordshire Research & Ethics Committee and the Aston University Human Science Ethical Committee. Written informed consent was obtained from all subjects willing to participate in the study.

7.3.3. Subject sample

30 volunteers (mean 63.8 ± 7.5 yrs; 13 male and 17 female) were recruited from optometric practice. Prospective subjects were given a full eye examination and the exclusion criteria for the study are shown in Table 7.1.

- Reported history of present or past eye disease
- Reported history of systemic disease or current medication with known cardiovascular properties
- Previous ocular surgery
- Corrected visual acuity of less than 6/9
- Visual field defect as detected by automated perimetry
- Corneal or anterior chamber abnormalities as detected by slit-lamp examination
- Posterior segment abnormalities as detected by slit-lamp binocular indirect ophthalmoscopy
- Intraocular pressure greater than 21 mmHg
- Physical or mental incapacity to perform experimental procedures

Table 7.1 Exclusion criteria used for the comparison of spectral analysis techniques' study.

7.3.4. Instrumentation and experimental procedures

The pneumatonometer (Ocular Blood Flow Analyzer pneumatonometer, Paradigm Medical Industries, Utah, USA) used in this study has been previously described (1.6.4). The pneumatonometer was set to its 'data-only' function which provides the operator with the option of recording IOP continuously for 10 seconds duration. Recordings were made on one randomly chosen eye (sealed envelope technique) of each subject following topical anaesthesia with a drop of 0.4% benoxinate HCl (*Minims,*® Chauvin, UK). All measurements were taken in the seated position with the pneumatonometer mounted on a slit-lamp. All subjects were allowed to rest for 20 minutes whilst seated before recordings were made in order to stabilise cardiac function. Two recordings, one followed directly by the second, were made at the first appointment. A third recording, made on the same chosen eye, was made approximately five weeks later (mean interval 38.4 ± 6.5 days). Again measurements were taken seated after the subject had been allowed to rest for 20 minutes. An example of a 10-second continuous IOP recording is shown in Figure 7.1 and subsequent examples to illustrate the results of the three spectral analysis techniques use this same 10-second recording.

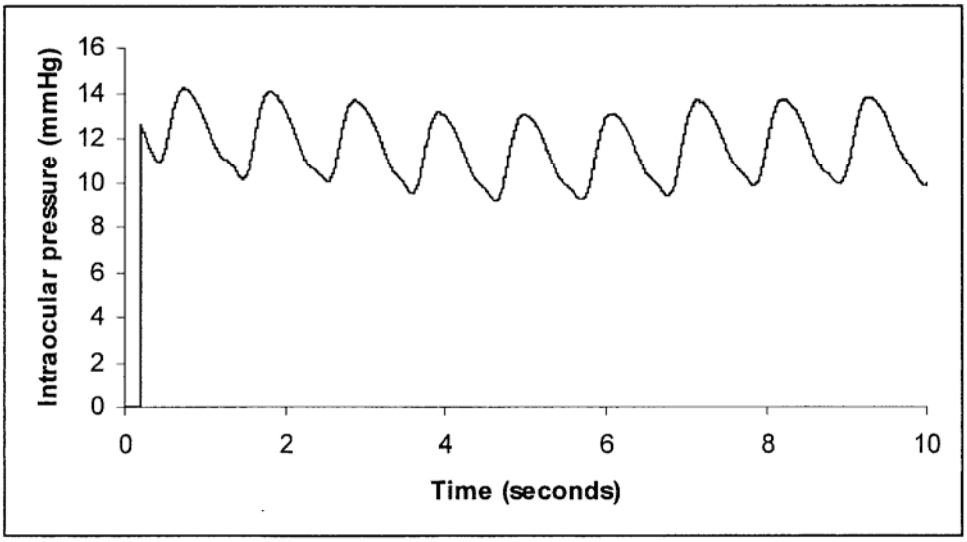

Figure 7.1 Example of a 10-second continuous IOP recording made with the Ocular Blood Flow Analyzer pneumatonometer.

The individual 10-second recordings were later downloaded from the pneumatonometer's base unit to a computer for the necessary spectral analysis.

7.4. *Spectral Analysis Techniques*

The sampling frequency of the pneumatonometer was 200 Hz, which, combined with a 10 s capture time, provided 2000 IOP data points available for analysis per recording. The frequency response of a pneumatonometer has been previously studied (Walker *et al.*, 1975a) and, for the model used in this study, the level of dynamic amplification is believed to be within less than 1% for driving frequencies up to 10 Hz (Silver *et al.*, 1994). Taking resting heart rates to have a maximum frequency of 90 beats per minute (1.5 Hz)(Levick, 2000d), spectral analysis of the IOP pulse up to the 7th harmonic can therefore be justified (7 x 1.5 = 10.5). Harmonic moduli of the IOP pulse were calculated using three spectral analysis techniques.

7.4.1. Technique 1: Evans' Spectral Analysis Technique

Evans' technique of analysing the IOP pulse has been described elsewhere (Evans *et al.*, 2002). In brief, three overlapping sections, each consisting of 1024 data points were analysed using a Fast Fourier Transform (FFT) (Cooley & Tukey, 1965). Resultant moduli were averaged and expressed as a percentage of the total. An example of the resultant spectral distribution is shown in Figure 7.2. The harmonic frequencies of the IOP pulse were found by visual inspection and the corresponding peak moduli recorded.

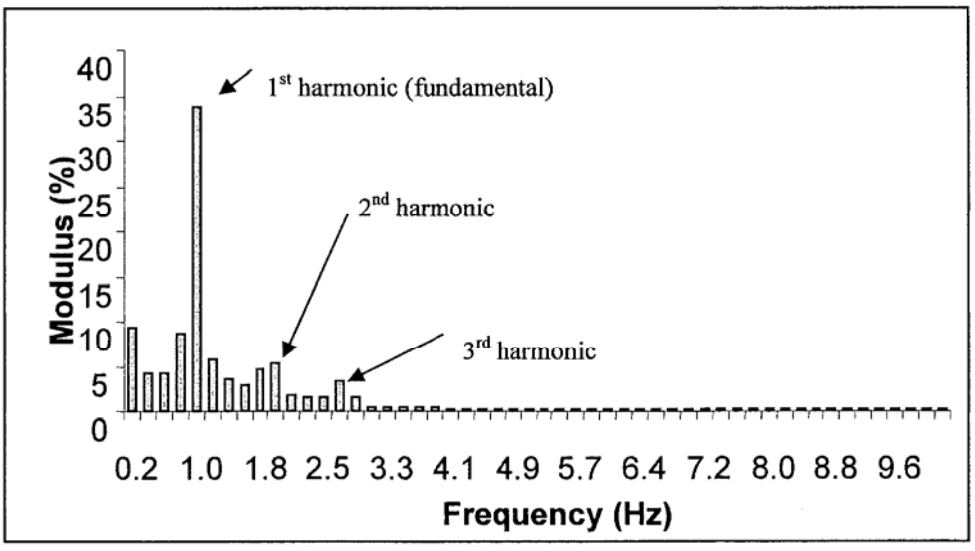

Figure 7.2 Example of the harmonic distribution, using the spectral analysis technique described by Evans *et al.* (2002) of the IOP waveform in Figure 7.1.

7.4.2. Technique 2: Power Spectral Density Analysis

The energy of a waveform signal can be expressed using Parseval's theorem (Bloch, 2000) Equation 7.1:

$$E = \int_{-\infty}^{+\infty} | f(t) |^2 \, dt = \frac{1}{2\pi} \int_{-\infty}^{+\infty} | F(\omega) |^2 \, d\omega$$

E = energy of the waveform
$f(t)$ = the function of the waveform in the original time domain
$F(\omega)$ = the function of the waveform in the frequency domain

Equation 7.1 Parseval's theorem used to express the energy of a waveform signal.

The advantage of plotting the square of each component function ($[F_n(\omega)]^2$) against its frequency, is that each component can then be integrated to find the power of the signal. This is known as a Power Spectral Density (PSD) function and allows quantification of the resultant components. In addition, it is recommended in PSD analyses to pre-process the data. Pre-processing of waveform data is recommended because, as the FFT assumes the end of a waveform is continuous with its beginning, an abrupt difference in data values at the beginning and end of a signal appear as a square wave. Unless the ends of an input signal are reduced to zero, the abrupt difference in IOP data will produce unnecessary spectral components. The pre-processing of a waveform signal, so that the start and finish reduce to zero, is known as windowing. An example of a windowed IOP waveform is shown in Figure 7.3.

Following transformation, the harmonic frequencies corresponding to the IOP pulse were again determined from visual inspection of the PSD. The three frequency bins centered on each harmonic were integrated to give a measure of signal power and recorded. An example of a PSD function derived from a 10-second IOP recording is shown in Figure 7.4.

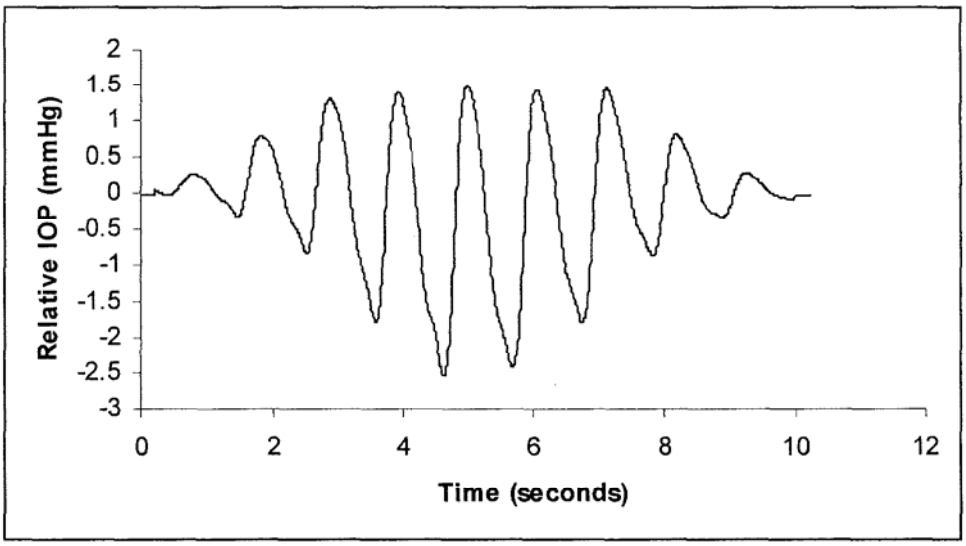

Figure 7.3 **Example of IOP data taken from Figure 7.1 having been pre-processed by a windowing function.**

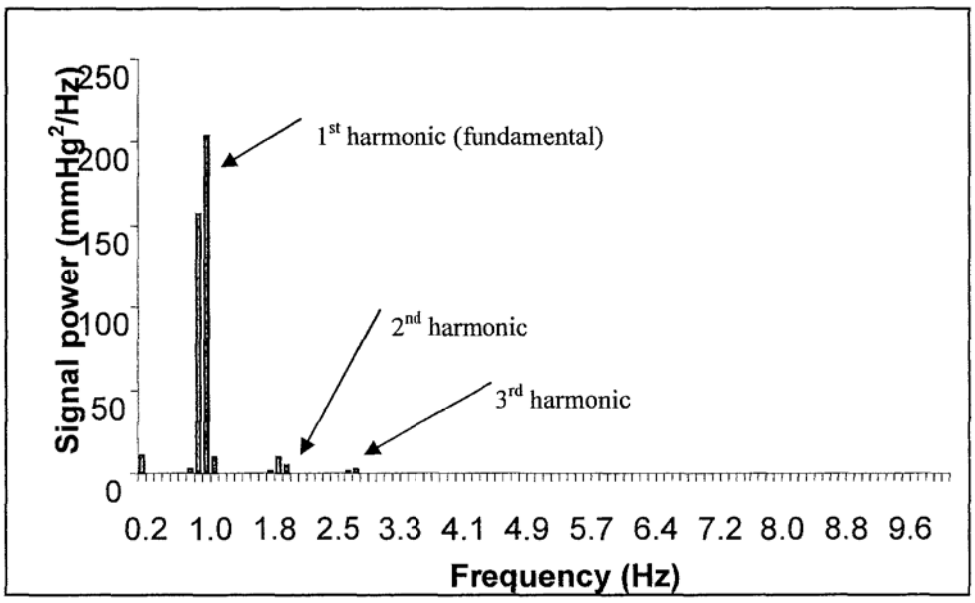

Figure 7.4 **An example of the Power Spectral Density function originating from the input signal in Figure 7.1.**

7.4.3. Technique 3: Averaged Single Pulse (ASP) technique

In contrast to the above two techniques, the ASP technique performed an FFT on a section of IOP data corresponding solely to one IOP pulse rather than a non-specific section. The

mean of six IOP pulses from an original IOP recording was first taken to produce an 'averaged single pulse'. This resultant pulse was then sampled at 32 points (Figure 7.5) before processing with the FFT.

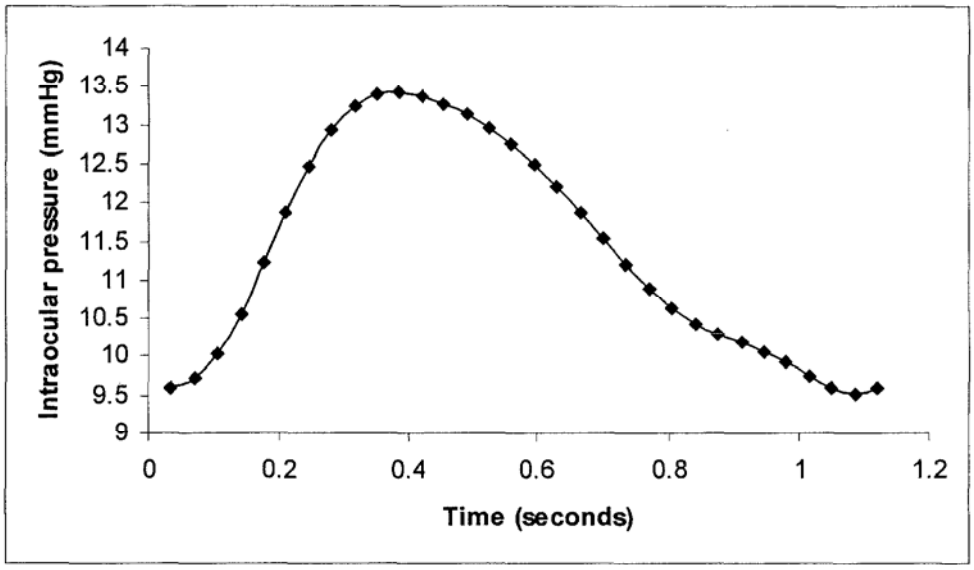

Figure 7.5 **An average IOP pulse created from six IOP pulses of the original 10-second IOP recording shown in Figure 7.1: ♦ - sampled data points (32 in total) used to calculate the Fourier components**

The 32 sample data points allowed the creation of 16 harmonic frequencies. This is more than twice the sampling frequency necessary to calculate the harmonics of interest (justified by the frequency response of the pneumatonometer) and avoids potential aliasing errors (Pugh, Eadie, Winn *et al.*, 1987). An example of the resultant harmonics calculated from an averaged IOP pulse is shown in Figure 7.6.

As the harmonics created by the ASP technique are solely those arising from IOP pulse, it is not necessary to isolate them from the background noise of other moduli created in the transformation of longer lengths of IOP data (as in the first two spectral analysis techniques).

7.4.4. Statistical Analysis

In order to assess within-visit variability of measurements (repeatability) of each spectral

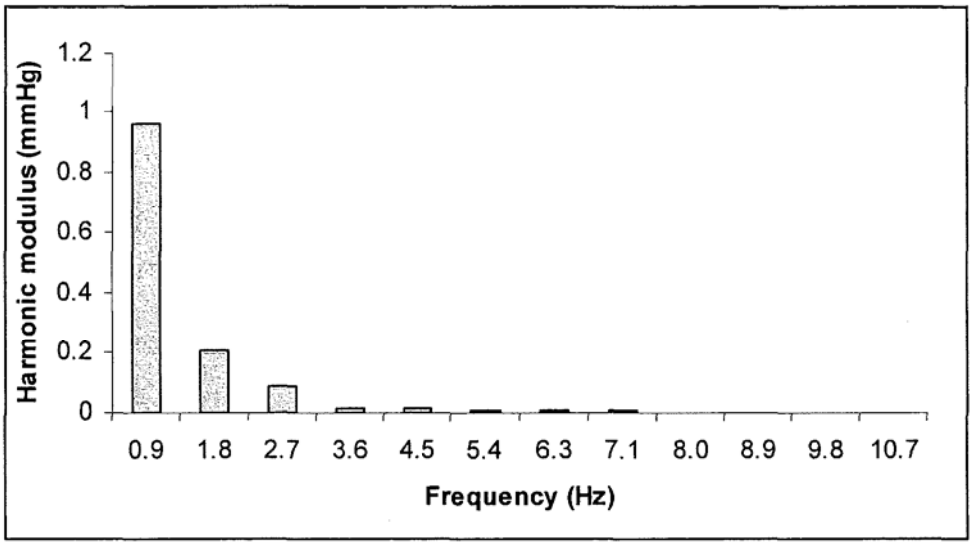

Figure 7.6 **Example of harmonic moduli calculated using the averaged single pulse technique for the IOP wave shown in Figure 7.1.**

analysis technique, coefficients of reliability (CoR) were calculated from the pairs of harmonics produced from the first two IOP recordings. Between-visit variability of measurements (reproducibility) was likewise assessed by comparing the first IOP recording with one repeated on the same eye of the same patient approximately five weeks later (mean interval 38.4 ± 6.5 days). CoR has been previously described (3.3.6) and are preferred (for assessing the degree of agreement between measurements) over the use of Pearson correlation coefficients as they estimate the average correlation among all possible orderings of pairs (Bland *et al.*, 1996).

7.5. *Results*

The CoR for pairs of harmonic moduli, for each method of analysis, were calculated from IOP recordings taken on the same day (Figure 7.7) and approximately five weeks apart (Figure 7.8).

7.5.1. Original spectral analysis technique

The original spectral analysis technique produced acceptable repeatability for the third and fourth harmonics: CoR values, 0.83 ($p < 0.001$) and 0.68 ($p < 0.001$) respectively. Correlation of within-visit measurements dropped off at lower and higher harmonics.

Correlation of between-visit measurements with the original technique was poor and only the third and fourth harmonics attained significant reproducibility: CoR values, 0.45 (p = 0.004) and 0.35 (p = 0.022) respectively.

7.5.2. Power spectral analysis technique

In comparison, the repeatability and reproducibility of the PSD technique was good up to the fourth harmonic. The within-test CoR values of the PSD technique were: 1st harmonic, 0.91; 2nd harmonic, 0.80; 3rd harmonic, 0.75; and 4th harmonic, 0.63 (all $p < 0.001$). The between-test CoR values of the PSD technique were: 1st harmonic, 0.84; 2nd harmonic, 0.76; 3rd harmonic, 0.83; and 4th harmonic, 0.63 (all $p < 0.001$). Further harmonic moduli of the IOP pulse could not be distinguished from the background noise of other spectral components.

7.5.3. Averaged single pulse technique

The ASP technique was the only technique capable of measuring beyond the 4th harmonic with any significance. The within-test CoR values of the ASP technique were: 1st harmonic, 0.93; 2nd harmonic, 0.85; 3rd harmonic, 0.84; 4th harmonic, 0.53; 5th harmonic, 0.59 (all $p <$ 0.001); 6th harmonic, 0.51 (p = 0.001); and 7th harmonic, 0.46 (p = 0.004). The between-test CoR values of the ASP technique were: 1st harmonic, 0.88; 2nd harmonic, 0.82; 3rd harmonic, 0.57 (all $p < 0.001$); 4th harmonic, 0.29 (p = 0.048); 5th harmonic, 0.27 (p = 0.059); 6th harmonic, 0.36 (p = 0.017); and 7th harmonic, 0.37 (p = 0.015).

7.6. *Discussion*

7.6.1. Evans' spectral analysis technique

Evans' spectral analysis technique performed poorly, in terms of repeatability and reproducibility, for measuring the lowest and highest harmonics of the IOP pulse. The technique's poor precision at measuring the lowest harmonics (for example the fundamental) was probably due to the spread of the signal into the surrounding frequency bins. There are two reasons for this. First, normal heart rate variability (such as sinus arrhythmia) slightly shifts the moduli relating to the IOP pulse into neighbouring frequencies.

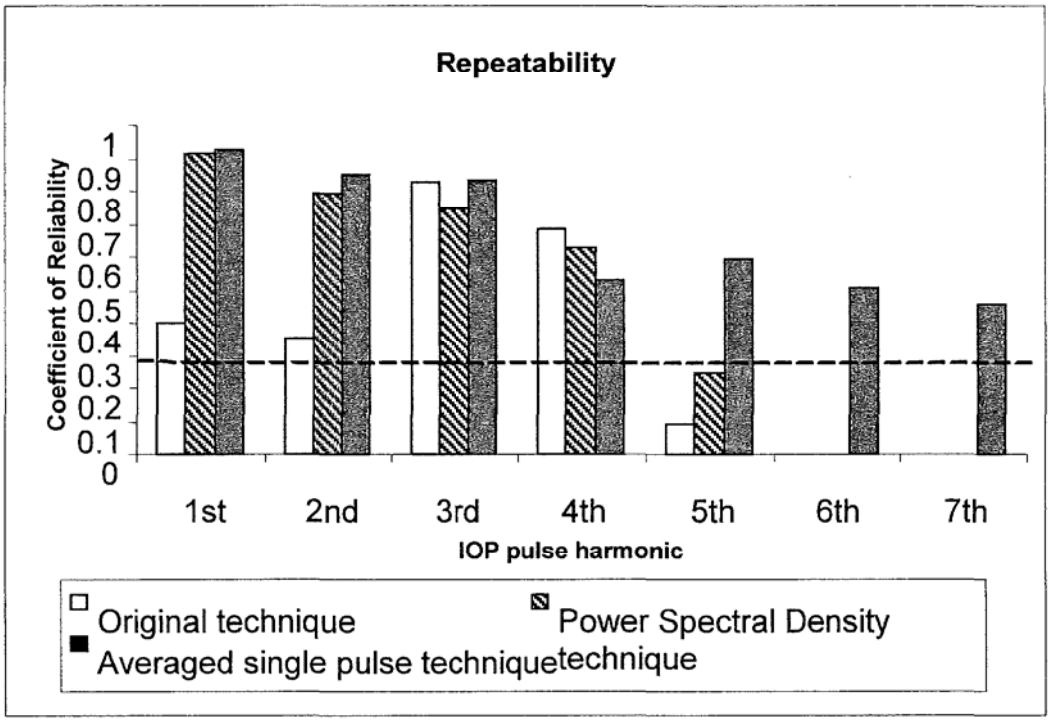

Figure 7.7 **Repeatability (within-visit agreement) of the three spectral analysis techniques at measuring the IOP pulse in the frequency domain: dashed line indicates the $p = 0.05$ level of significance.**

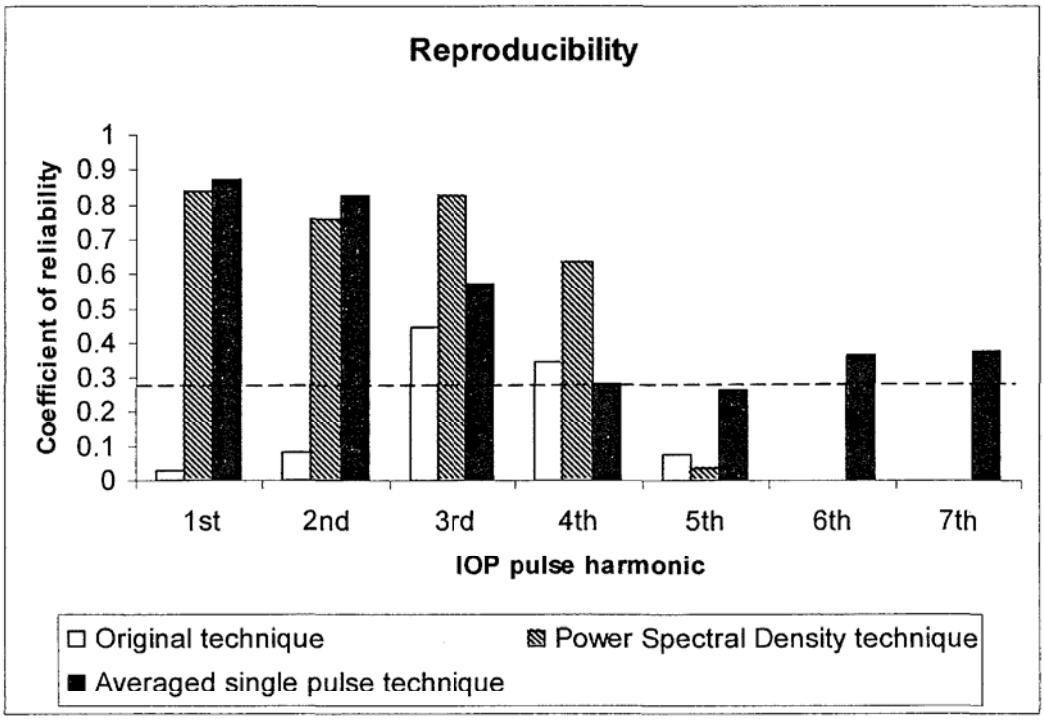

Figure 7.8 **Reproducibility (between-visit agreement) of the three spectral analysis techniques at measuring the IOP pulse in the frequency domain: dashed line indicates the $p = 0.05$ level of significance.**

Second, in the majority of IOP sections, the IOP value at the start of the 1024 data point stream would be different to the end. Such harsh transitions in the signal would produce additional unwanted spectral components: this is known as spectral leakage (Bloch, 2000). As the IOP pulse moduli had the greatest value in the lowest two harmonics, such spreading into surrounding frequency bins would cause greater variability in pairs of measurements.

7.6.2. Power spectral analysis technique

By windowing the input signal, the PSD technique greatly improved the resolution of the IOP pulse harmonics: cf. Figure 7.2 and Figure 7.4. The quantification of signal power also allowed the principle IOP pulse harmonic to be integrated with frequency bins either side of it. This reduced the variability in repeated measurements caused by spectral power shifting slightly with small changes in heart rate. Both these factors would have helped the resultant improvement seen in repeatability and reproducibility of the PSD technique over the original.

7.6.3. Averaged single pulse technique

Neither the PSD, nor the original spectral analysis technique, were capable of distinguishing the harmonics produced by the IOP pulse at the highest frequencies: beyond the 4^{th} harmonic (approximately beyond 5 Hz). This is undoubtedly due to the small amount of power produced by the IOP pulse at these frequencies compared to other spectral components arising from a continuous section of IOP waveform. By isolating the IOP pulse, as in the ASP technique, the Fourier transform produces a series of harmonics specific to the IOP pulse and no manual extraction is required from the resultant spectral components. That is, the first component is the first harmonic (or fundamental) of the IOP pulse, the second component is the second IOP pulse harmonic, etc. The ASP technique therefore automatically produced values for the higher IOP pulse harmonics. No pre-processing of the signal was required as the averaged single pulse began and ended at the same IOP value. An advantage of the ASP technique is that the resultant IOP pulse moduli are measured in the original time domain units (mmHg) which perhaps provides greater clinical relevance.

The repeatability and reproducibility at measuring the higher harmonics (5^{th} to 7^{th}) were modest. However, considering that the modulus values at these frequencies were equivalent

to approximately $1/100^{th}$ mmHg in pressure, it is a tribute to the FFT effectiveness and the high-fidelity of the pneumatonometer that correlations were found at all.

7.6.4. Comparison to spectral analysis of the arterial pressure pulse

In cardiovascular research, the higher harmonics of the arterial blood pressure pulse are commonly measured up to 12 Hz (approximately the 8^{th} harmonic). Such precision is possible because the blood pressure pulse is a whole order of magnitude larger than that of an IOP pulse and, in cardiovascular research, pressure measurements are often taken directly using an invasive manometer (Nichols & O'Rourke, 1998d). As previously reviewed (1.3.3), in arterial systems the higher harmonics of the pressure and flow waves are associated with vascular impedance and the degree of wave reflection produced at the end-arterial bed. A less compliant vascular bed, such as that found in a patient with arteriosclerosis, produces a higher degree of wave reflection. Shifts also occur in the spectral profile as the level of tonus in these end-arterioles changes with vasoconstriction and vasodilatation (Westerhof *et al.*, 1972).

In the case of the IOP pulse, it must be remembered that the waveform arises from the volumetric distension of the internal ocular vascular beds rather than the change in blood pressure. Whether similar spectral characteristics of an end-arterial bed exist in the IOP pulse, as in the arterial blood pressure pulse, remain to be confirmed. This study has shown that, given the instrumentation and the modified spectral analysis techniques used, further investigation of such a hypothesis is reasonable.

7.7. *Conclusion*

In conclusion, the results of this study suggest that Evans' technique for analysing the spectral components of the IOP pulse is inadequate for measuring its lowest and highest harmonic moduli. Spectral leakage from the discontinuous start and end of the waveform signal, as well as that arising from normal heart-rate variation, probably account for its poor correlation of measurements. Further investigations in this work will use the alternative spectral analysis techniques described in this study.

8. Spectral analysis of the intraocular pressure pulse: validatory investigations

8.1. *Abstract*

Purpose: It has been proposed that spectral analysis presents a more precise method of differentiating physiological and pathological differences in the IOP pulse. Furthermore the harmonic components of the IOP pulse wave may be characteristic of the status of the intraocular vascular beds in an analogous way that spectral analysis of blood pressure and flow waves reveals impedance characteristics of an artery. The purpose of this study was to attempt to validate such hypotheses.

Method: Three studies were performed in which theoretical differences in ocular vascular impedance may be expected. *Study A* compared IOP pulse moduli in two differently aged groups of subjects. *Study B* investigated any change in a subject's IOP pulse moduli during the inhalation of hypercapnic air (a known ocular vasodilator) compared to breathing normal room air. *Study C* compared IOP pulse moduli in groups of subjects with normal and raised blood pressure.

Results: *Study A*. The spectral profile of IOP pulse moduli, for both young and old subject groups, exhibited a steep decay with increased frequency that best approximated an inverse cubed function: for the young subject group, $y = 0.55\ x^{-2.80}$ ($r = 0.95$); for the old subject group, $y = 0.51\ x^{-2.74}$ ($r = 0.94$). No significant difference existed in IOP pulse moduli between the two age groups. *Study B*. A significant fall in IOP pulse modulus occurred in the first two frequency bins during hypercapnia: IOP pulse moduli in the first frequency bin (0.5 to 1.5 Hz) fell from 0.56 ± 0.15 mmHg to 0.49 ± 0.13 mmHg (a mean decrease of 9.7%, $p = 0.010$); IOP pulse moduli in the second frequency bin (1.5 to 2.5 Hz) fell from 0.11 ± 0.05 mmHg to 0.09 ± 0.05 mmHg (a mean decrease of 22.4%, $p = 0.001$). No other IOP pulse moduli altered significantly between the two breathing conditions. *Study C*. No significant difference existed in IOP pulse moduli between the group with high blood pressure and the group with normal blood pressure.

Conclusion: Spectral analysis provided greater statistical confidence of a hypercapnic-induced change over the traditional IOP pulse parameters of PA and POBF (cf. Chapter 5). However the present studies do not provide any evidence that spectral analysis of the IOP pulse offers any further information on the eye's vascular characteristics.

8.2. *Introduction*

Spectral analysis of the arterial pressure pulse has proved a valuable tool in cardiovascular research (Nichols *et al.*, 1998e). Deconstruction of continuous blood pressure recordings into the component wave cycles has provided a method of detecting low frequency rhythms such as those associated with the respiratory and vasomotor cycle (Akselrod, Gordon, Madwed *et al.*, 1985). Womersley (1955) showed that examination of the arterial pressure pulse gradient in the frequency domain can be used to predict the oscillatory blood flow pattern (Equation 1.4). It is in the calculation of vascular impedance however, that spectral analysis of arterial blood pressure and flow measurements has found greatest value (Nichols *et al.*, 1998g).

8.2.1. Vascular impedance revisited

The concept of impedance has previously been introduced (1.3.3) and will be further explained. Impedance describes the opposition to flow in an oscillatory system. McDonald ((1955) first suggested that the term impedance, rather than resistance, should be used when considering arterial 'pulsatile' flow and pressure and that the term resistance should be confined only to the state of 'static', or mean flow. Like resistance, vascular impedance describes the relationship between blood flow and blood pressure but is frequency specific (Equation 1.10). In order to characterise this relationship, the individual harmonic components of the pressure and flow waves are calculated, through the use of Fourier transformation. Linearity is assumed between the components of pressure and flow at each harmonic frequency. The impedance characteristics of a particular artery and its distal end-arterial bed can therefore be described by the ratios of pressure to flow moduli, and the phase angle differences at each frequency. An example of an impedance spectrum is shown in Figure 8.1.

Vascular impedance spectra have a number of common characteristics:

- Data points from a single recording are found solely at the harmonic frequencies of the arterial pulse wave; the first data point lying at the frequency of the heart rate and subsequent data points lying at multiples of this frequency. It is common practice in

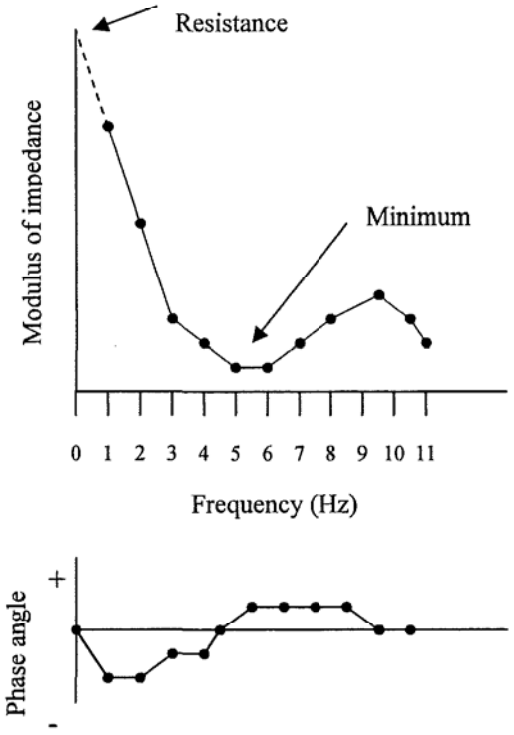

Figure 8.1 **An example of a typical vascular impedance spectrum, represented by the harmonic components of moduli (above) and phase (below), recorded and calculated from a large peripheral (e.g. femoral) artery. Adapted from a figure by Mills *et al.* (1970).**

circulation research to join these points graphically to extrapolate impedance values at intervening frequencies. This practice is supported by the fact that when the heart rate is experimentally driven at different frequencies, the impedance profile remains the same: evidence that the arterial tree can be treated as a linear system (O'Rourke *et al.*, 1966).

- Vascular resistance (opposition to flow in a steady, non-pulsatile, state) is equivalent to vascular impedance at zero frequency: i.e. at the non-oscillatory point (Mills *et al.*, 1970). The modulus value of vascular resistance can therefore be extrapolated from the impedance graph by calculating where the impedance line intersects the ordinate axis (Figure 8.1).

- The majority of vascular impedance spectra exhibit a frequency where the moduli are at a minimum and the phase lag is zero (Figure 8.1). Such minima are caused by wave reflections, in pressure and flow, from the high resistance arterioles distal to

the measured site producing constructive and destructive interference with the initial waveforms (Westerhof, Sipkema, Van den Bos, *et al.*, 1972). The frequency at which a minimum occurs is dependent on the distance between measurement and reflection site and the velocity of wave travel along the artery (

- Equation 1.5). The degree of the minimum is mostly dependent on the level of wave reflection from the distal small arteries and arterioles. Vascular beds whose end-arteries have a high muscle tone, such as the cutaneous beds of the hands and face, have a high reflection factor. Other vascular beds, such as the renal, produce minimal arterial wave reflections.
- The initial slope of the impedance spectra (represented by the moduli at the lower frequencies) is dependent on the capacitance, or distensibility, of the artery and end-arteriole bed distal to the recording site (Westerhof & Elzinga, 1991). A low, flat spectrum represents a compliant peripheral vascular network that produces little opposition to the component flow waves; a high, steep spectrum represents the opposite.
- At zero frequency, there is no phase difference between pressure and flow. At low frequencies, pressure phase usually lags behind flow and returns to zero close to the frequency where moduli are at a minimum (Figure 8.1).

In addition vascular impedance spectra exhibit a number of features consistent with certain physiological, pathological and pharmacological states. As it is pertinent to the current study, the effects on vascular impedance of aging, high blood pressure and vasodilatation will be covered.

8.2.2. Effects of aging on vascular impedance

With increasing age, arterial walls show characteristic and progressive changes in their structure and function (Benetos, Waeber, Izzo *et al.*, 2002). Of these, the increasing thinning, splitting and fragmentation of the load-bearing elastin fibres in the tunica media of the central elastic arteries (such as the carotid) is of greatest influence on vascular impedance. The degeneration of elastin is accompanied by an increase in collagenous material and ground substance. The functional result of such structural change is a reduction in arterial distensibility (the 'stiffened' arteries of arteriosclerosis) and an increase in arterial

waveform velocity. This functional change impacts on an artery's impedance spectrum (Nichols & O'Rourke, 1998a). Reduced capacitance heightens the values of the impedance moduli and increases the difference in phase lag between flow and pressure. In addition, the increased velocity that a waveform travels down an artery and reflects back, shifts the frequency of the modulus minimum to the right of the impedance spectrum. The typical features of a vascular impedance spectrum from an aged artery are consistent with profile 'C' in Figure 1.7.

8.2.3. The effects of vasodilatation on vascular impedance

In contrast to aging and high blood pressure, peripheral vasodilatation increases arterial compliance both at the central elastic arteries (by reducing blood pressure and the level of distension in the elastin fibres) and at the periphery (through relaxation of the vascular smooth muscle). The position of the minimum modulus value on the impedance spectrum becomes less pronounced as wave reflection reduce, and shifts to lower frequencies as wave travel slows (O'Rourke, 1999b). As capacitance in the arterial system increases, the slope of the initial impedance moduli flattens. A vascular impedance spectrum representative of a dilated end-arterial bed is shown in 'B' of Figure 1.7.

8.2.4. The effects of high blood pressure on vascular impedance

The effect of high blood pressure on vascular impedance is similar to that seen with aging (O'Rourke, 1999a). The immediate effect of high blood pressure is to distend the central elastic arteries. This shifts the pulsatile load onto the collagenous elements of the arterial wall as the elastin fibres reach their limit of elasticity (O'Rourke, 1989). Stretched central arteries are less distensible and exhibit faster wave velocities. At the periphery, the increased perfusion pressure stimulates the muscular arteries and end-arterioles to vasoconstrict in order, through autoregulation, to maintain the distal organ with the same flow of blood. Vasoconstriction of these arteries and arterioles reduces pulse wave dampening and increases wave reflection from the 'hard' end-arterial terminus (Nichols & O'Rourke, 1998b). As with aging, these changes seen in high blood pressure produce increased impedance moduli, increased phase lag and a shift of the modulus minimum to higher frequencies: a 'C' type impedance spectrum in Figure 1.7. With time, as seen in

chronic high blood pressure, the sustained load on the arterial system produces structural damage to the vascular walls as a form of premature aging.

8.2.5. Spectral analysis of the IOP pulse

Two previous studies have indicated that spectral analysis may provide greater confidence in detecting clinical differences in the IOP pulse. Best *et al.* (1974) compared a number of analysis methods at detecting different levels of experimentally induced carotid stenosis in anaesthetised rabbits. Whilst PA and crest time analysis (1.7) detected carotid stenoses of 50% or greater, only spectral analysis of the IOP pulse detected lesser degrees of carotid stenosis. The first IOP pulse harmonic modulus was significantly lower in rabbits with 20% or more carotid stenoses and the authors concluded that spectral analysis of the IOP pulse may increase diagnostic ability. More recently, Evans *et al.* (2002) reported that spectral analysis differentiated a group of eyes with POAG from a normal age-matched sample. Although the parameters of PA, pulse volume and POBF showed no significant difference, the mean percentage IOP pulse moduli for the 3rd, 4th and 5th harmonics were significantly lower for the glaucoma group compared to the control group. In addition to concluding that spectral analysis may provide greater precision in detecting differences in IOP pulse, the authors speculated that the higher IOP moduli may represent vascular characteristics analogous to those found in the arterial pressure pulse.

8.3. *Aims*

The purpose of this study was two-fold: first, to investigate whether spectral analysis provides greater precision in detecting a change in IOP pulse character; second, to elucidate whether the moduli of the IOP pulse exhibit any of the above characteristics noted in vascular impedance spectra. Three studies were performed. Two studies, the effect of age and high blood pressure on IOP pulse moduli, examined the potential increase in vascular impedance upon the spectral components of the IOP pulse. The third study investigated the effect of a known ocular vasodilator (the inhalation of hypercapnic air) using data derived from the study previously reported in Chapter 6.

8.4. *Methods*

8.4.1. Experimental design

Studies A and C were group control comparisons. Study B was a randomised single-blinded cross over study.

8.4.2. Ethical approval

All experimental procedures of the study conformed to the tenets of the declaration of Helsinki and were approved by the Aston University Human Science Ethical Committee. In addition Study C had ethical approval from the East Birmingham Local Research Ethics Committee, Birmingham Heartlands Hospital, Birmingham, UK. Written informed consent was obtained from all subjects willing to participate in the study.

8.4.3. Subject samples

Study A: The effect of age on IOP pulse moduli

In order to create two distinct age groups, subjects were recruited between the ages of either 30 - 40 years or 60 - 70 years. Subjects were recruited from the general eye examination clinic at Aston University, Birmingham, UK and from staff of the university. Subjects were given a full eye examination prior to being accepted for the study. The exclusion criteria used for the study are shown in Table 8.1.

- Reported history of present or past eye disease
- Reported history of systemic disease or current medication with known cardiovascular properties
- Previous ocular surgery
- Corrected visual acuity of less than 6/6
- Corneal or anterior chamber abnormalities as detected by slit-lamp examination
- Posterior segment abnormalities as detected by slit-lamp binocular indirect ophthalmoscopy
- Intraocular pressure greater than 21 mmHg
- Physical or mental incapacity to perform experimental procedures

Table 8.1 Subjects' exclusion criteria for validatory study A – investigation of age and IOP pulse moduli.

Study B: The effect of hypercapnia on IOP pulse moduli

Details of subject recruitment and methodology for this study can be found in 6.4.4.

Study C: The effect of high blood pressure on IOP pulse moduli

Patients, either newly diagnosed with high blood pressure or whose blood pressure was uncontrolled on their present medication, were recruited to the study. Volunteer patients were recruited from local GP surgeries and a specialist hypertension clinic at Birmingham Heartlands Hospital, Birmingham, UK. Diagnosis and classification of high blood pressure was made according to the recommendations of the sixth report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure (1997):

Table 8.2.

Category	Ranked classification	Systolic (mmHg)		Diastolic (mmHg)
Optimal	0	<120	and	<80
Normal	1	<130	and	<85
High-normal	2	130-139	or	85-89
Hypertension				
Stage 1	3	140-159	or	90-99
Stage 2	4	160-179	or	100-109
Stage 3	5	≥ 180	or	≥ 110

Table 8.2 Classification of blood pressure for adults (age 18 and older). Adapted from the Sixth Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure. (VI, 1997)

Patients with high blood pressure were given a full eye examination before the study and were subject to the exclusion criteria found in Table 8.3 before recruitment.

- Reported history of present or past eye disease other than that associated with high blood pressure
- Previoius ocular surgery
- Corrected visual acuity of less than 6/9
- Corneal or anterior chamber abnormalities as detected by slit-lamp examination
- Posterior segment abnormalities as detected by slit-lamp binocular indirect ophthalmoscopy other than attributed to high blood pressure
- Intraocular pressure greater than 21 mmHg
- Physical or mental incapacity to perform experimental procedures

Table 8.3 Exclusion criteria for the subjects with high blood pressure used in Study C of the validatory investigations on IOP pulse moduli.

A control group of subjects, matched for age and gender, were recruited from patients attending for eye examinations in optometric practice. Details of the recruitment criteria used for these subjects can be found in Section 7.3.3.

8.4.4. Experimental procedures and instrumentation

All measurements were taken on seated subjects who had rested for 20 minutes. Blood pressure measurements were taken using a validated (Shennan *et al.*, 1998)automated sphygmomanometer (Omron Rx, Omron Matsusaka Co. Ltd, Japan). In addition to diastolic and systolic blood pressure, calculations of APP and MAP were made as previously described (Equations 1.6 and 1.7). The OBFA pneumatonometer (Paradigm Medical Industries, Utah, USA) was used to record measurements of IOP and continuous IOP waveforms. This instrument has previously been described (1.6.4). Pneumatonometry measurements were taken on one randomly chosen eye of each subject following topical anaesthesia with one drop of 0.4% benoxinate HCl (*Minims*®, Chauvin, UK). The pneumatonometer was mounted on a slit-lamp. Measurements of IOP were calculated automatically, by the pneumatonometer's software, from five or more IOP pulses that it considered acceptable from a ten second recording. Recording and calculation of IOP pulse moduli was performed as described in 7.4. The two spectral analysis techniques, Power Spectral Density (PSD) and Average Single Pulse (ASP), found to give greater precision in

Chapter 6 were used to analyse IOP pulse moduli. Phase lags of the harmonic components were not examined.

8.4.5. Statistical analysis

Appropriate paired (Study B) and non-paired (Studies A and C) Student's *t*-tests were used with parametric data. The Mann-Whitney test was used for ordinal data where necessary. Possible correlations were explored using the Pearson correlation coefficient. Results are presented as mean values for the group, plus and minus the standard deviation of the measurements. A probability value of $p < 0.05$ was taken as statistically significant.

8.5. *Results*

8.5.1. Study A: The effect of age on IOP pulse moduli

The group characteristics for the young and old subjects are shown in Table 8.4. IOP values for the young and old subject groups were not significantly different ($p > 0.05$): 12.9 ± 2.3 mmHg and 12.3 ± 3.1 mmHg respectively. IOP pulse moduli results were similar using either the PSD or ASP analysis technique and so results are presented for only one technique (ASP method). The spectral profile of IOP pulse moduli, for both age groups, exhibited a steep decay with increased frequency. The individual moduli for the younger subject group are shown in Figure 8.2. The decay curves that best fitted the data were approximately inverse cubed functions: for the young subject group, $y = 0.55\ x^{-2.80}$ ($r = 0.95$); for the old subject group, $y = 0.51\ x^{-2.74}$ ($r = 0.94$).

The IOP pulse moduli, grouped by 1 Hz frequency bins, are shown for the young and old subjects in Figure 8.3 and Figure 8.4; note that the ordinate axis in Figure 8.4 is magnified to differentiate the harmonic moduli at higher frequencies. No significant difference in IOP pulse moduli existed between the two subject age groups.

	Age Band (yrs)		Significance level
	30 to 40	**60 to 70**	
Mean age (yrs)	34.0 ± 2.7	66.5 ± 2.5	$p < 0.001$
Gender (male/female)	10/10	10/10	N.S.
Mean spherical spectacle refraction (DS)	-0.80 (2.50)	+1.50 (1.34)	N.S.
Systolic blood pressure (mmHg)	124.79 (12.33)	119.70 (14.73)	N.S.
Diastolic blood pressure (mmHg)	79.16 (9.60)	78.25 (7.96)	N.S.
Arterial pulse pressure (mmHg)	45.21 (10.96)	42.80 (20.12)	N.S.
Mean arterial blood pressure	88.28 (5.91)	100.55 (13.98)	N.S.
Heart rate (beats/min)	72.00 (11.61)	70.85 (12.17)	N.S.

Table 8.4 Group characteristics for Study A: mean (SD) values.

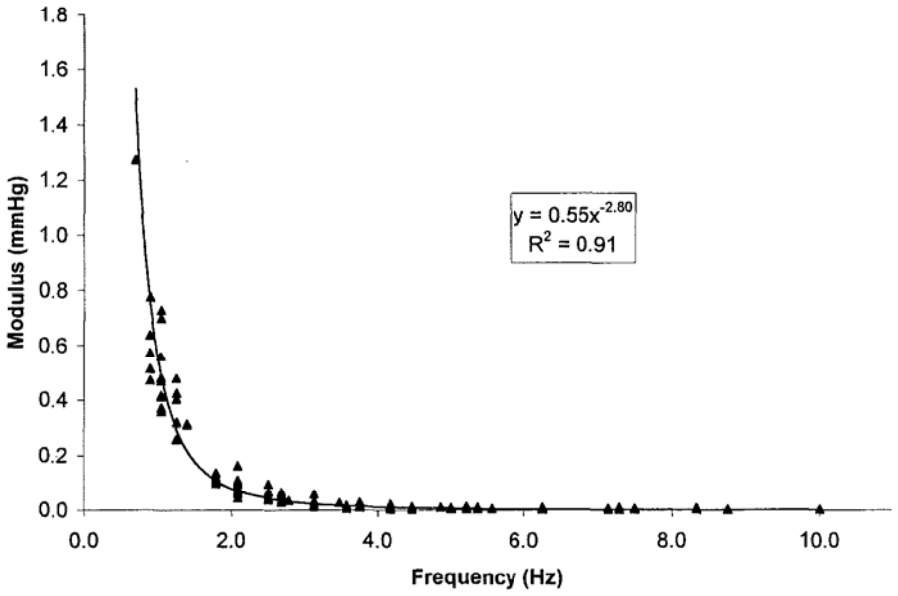

Figure 8.2 Individual harmonic moduli of the intraocular pulse for subjects in their third decade.

No statistical difference was found between age group for the traditional IOP pulse parameters of either POBF or PA (Table 8.5).

Age Group	Pulsatile Ocular Blood Flow (µl/min)	IOP pulse amplitude (mmHg)	Significance
Young (30-40 years)	1155 (272)	3.8 (1.5)	N.S.
Old (60-70 years)	1237 (416)	3.8 (1.2)	N.S.

Table 8.5 **Mean (SD) values for the traditional IOP pulse parameters in the two age groups of Study A.**

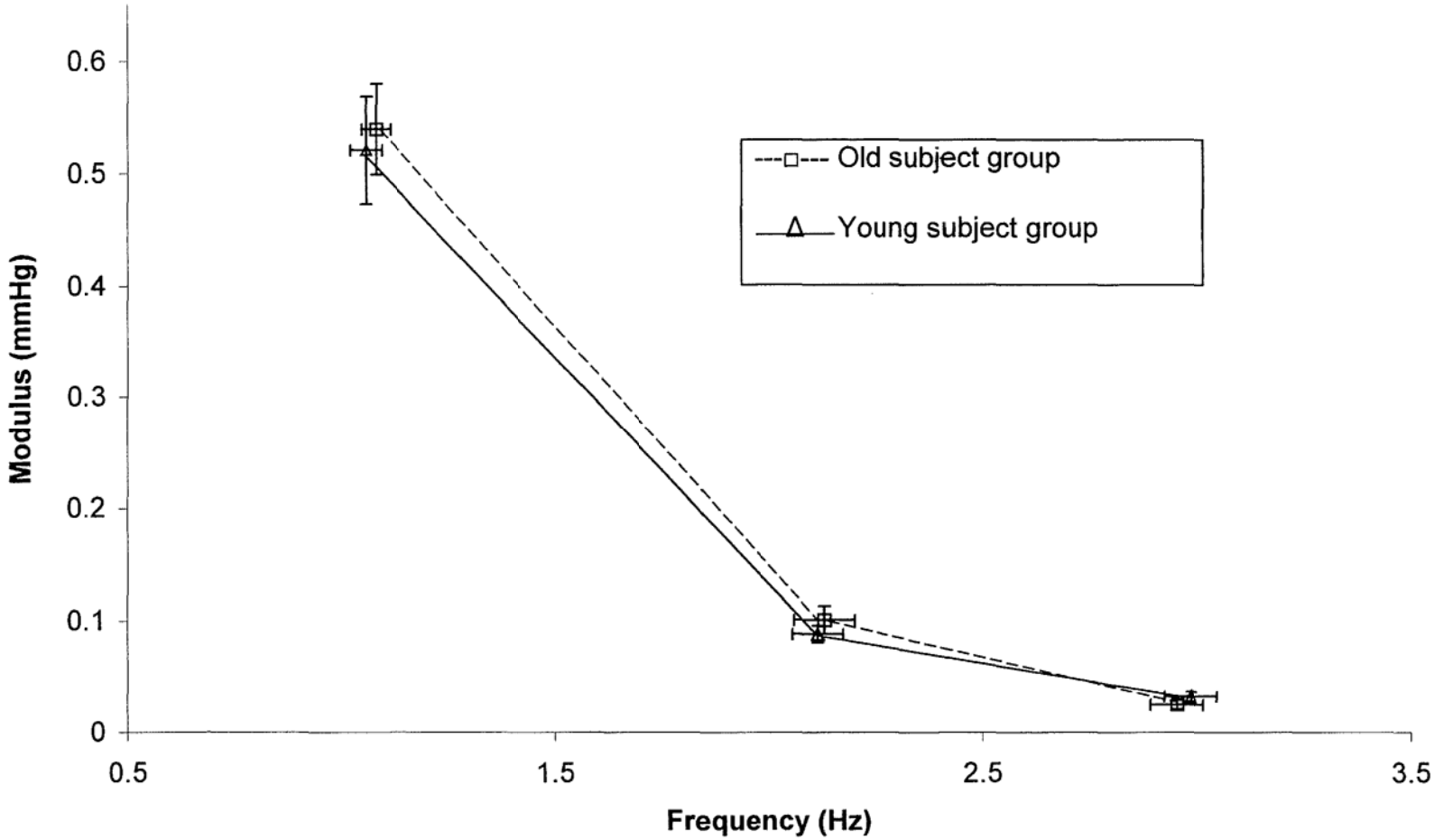

Figure 8.3 **The mean harmonic moduli, binned by frequency up to 3.5 Hz, of the intraocular pressure pulse for two age groups: error bars represent one standard error of the mean. No significant difference, at any of the 1 Hz frequency bins, existed between age groups.**

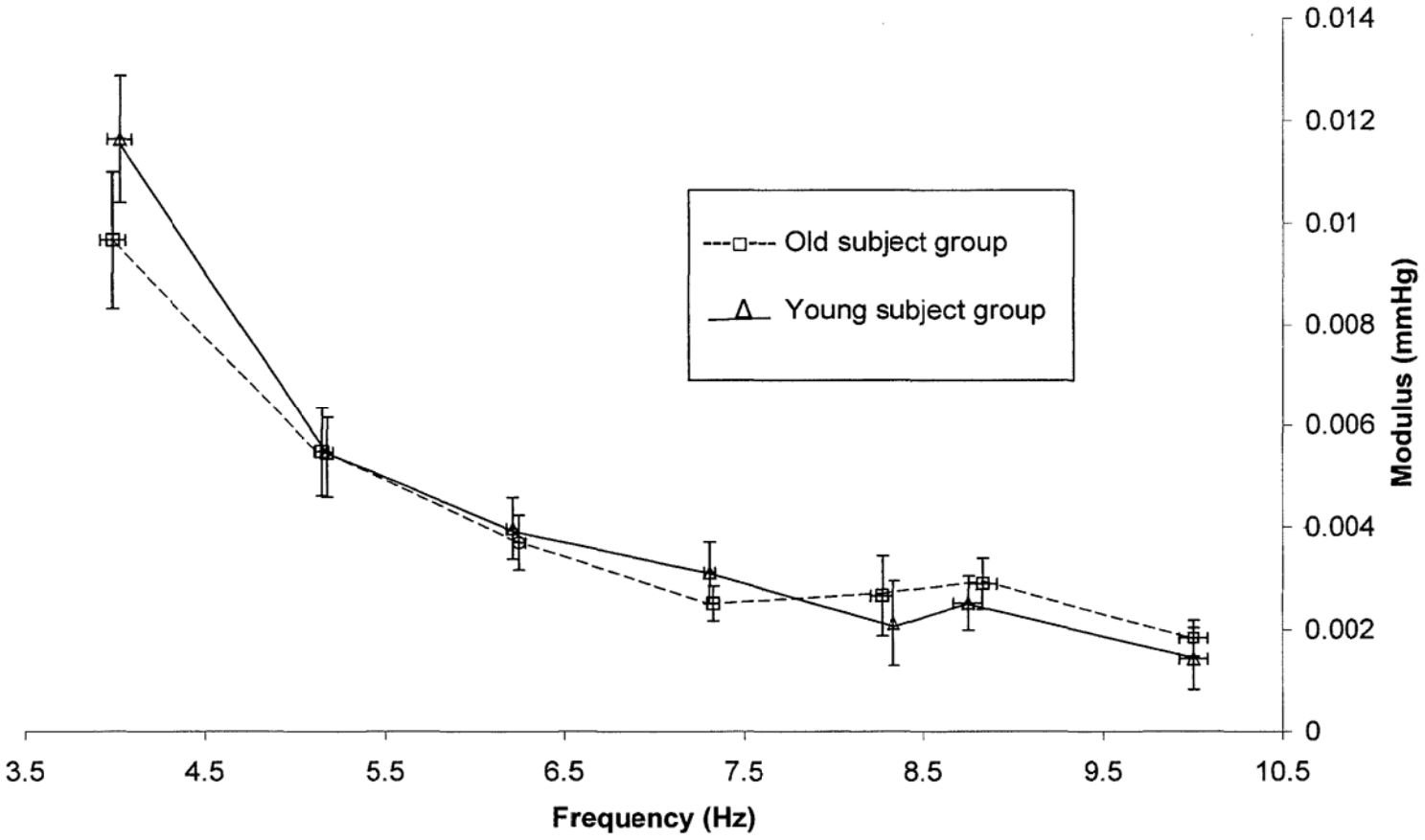

Figure 8.4 **The harmonic moduli, binned by frequency from 3.5 to 10.5 Hz, of the intraocular pressure pulse for two different aged subject groups: error bars represent one standard error of the mean. No significant difference, at any of the 1 Hz frequency bins, existed between age groups.**

8.5.2. Study B: The effect of hypercapnia on IOP pulse moduli

Full details of the systemic and ocular parameters measured in this study can be found in Chapter 6. As reported, no significant change in heart rate occurred between normocapnic and hypercapnic breathing conditions. This allowed within-subject changes in IOP pulse modulus to be compared at each 1 Hz frequency bin. A significant fall in IOP pulse modulus occurred in the first two frequency bins during hypercapnia (Figure 8.5). IOP pulse moduli in the first frequency bin (0.5 to 1.5 Hz) fell from 0.56 ± 0.15 mmHg to 0.49 ± 0.13 mmHg (a mean decrease of 9.7%, $p = 0.010$); IOP pulse moduli in the second frequency bin (1.5 to 2.5 Hz) fell from 0.11 ± 0.05 mmHg to 0.09 ± 0.05 mmHg (a mean decrease of 20.2%, $p = 0.001$). No other IOP pulse moduli altered significantly between the two breathing conditions.

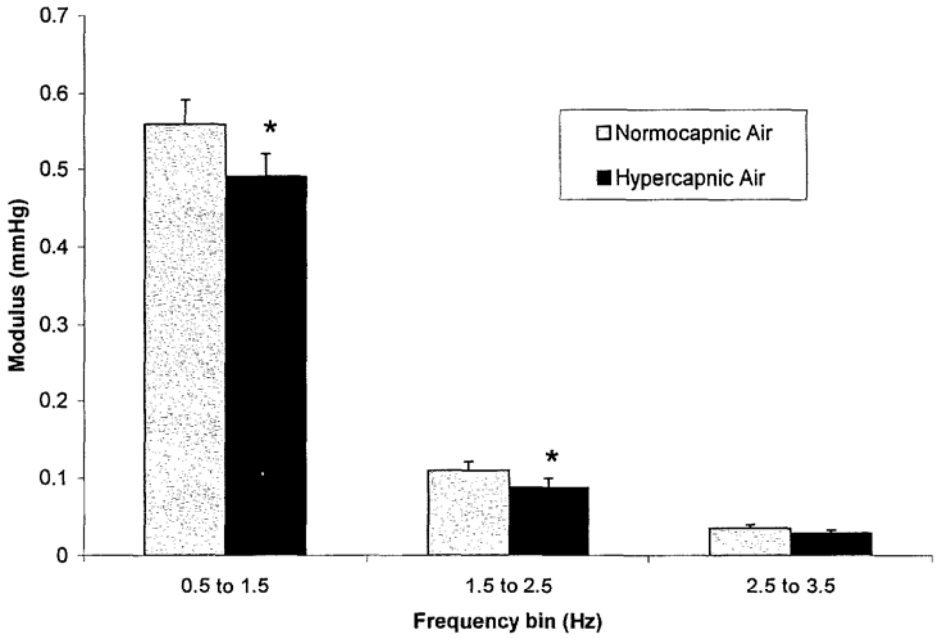

Figure 8.5 **Harmonic moduli of the intraocular pressure pulse, binned by frequency up to 3.5 Hz, during the inhalation of ordinary room air (normocapnia) and during the inhalation of increased carbon dioxide (hypercapnia): error bars indicate one standard error of the mean; * indicates a significant change in modulus between breathing conditions ($p < 0.05$).**

8.5.3. Study C: The effect of high blood pressure on IOP pulse moduli

The systemic characteristics of the subjects with high blood pressure and those of the control group are shown in Table 8.6. Whilst the normal blood pressure group reported taking no medication, the high blood pressure group reported the following systemic medications: 1 patient on ACE-inhibitor; 2 patients, beta-blockers; 1 patient, thiazide diuretic; and 1 patient on a statin.

	Normal blood pressure group	**High blood pressure group**	**Significance level**
Age (yrs)	54.1 (13.0)	55.4 (17.7)	N.S.
Gender (male/female)	5/5	5/5	N.S.
Mean spherical spectacle refraction (DS)	-0.05 (1.6)	-1.25 (3.2)	N.S.
Systolic blood pressure (mmHg)	117.0 (8.8)	145.5 (14.2)	$p < 0.001$
Diastolic blood pressure (mmHg)	67.8 (7.2)	84.8 (14.0)	$p = 0.003$
Arterial pulse pressure (mmHg)	49.2 (6.2)	60.7 (16.4)	$p = 0.05$
Mean arterial blood pressure	83.4 (6.2)	104.6 (11.1)	$p < 0.001$
Heart rate (beats/min)	64.5 (10.5)	64.9 (15.8)	N.S.
JNC blood pressure classification (median, range)	0, 0 to I	III, III to IV	$p = 0.001$

Table 8.6 **Group characteristics of those subjects with diagnosed high blood pressure and a matched control group: presented as mean (SD) values.**

The IOP pulse moduli, grouped by frequency bin, are shown in Figures 8.6 and 8.7 for the group with high blood pressure and the control group. No significant difference in IOP pulse modulus existed between the two groups at any of the frequency bins.

No statistical difference was found between normal and high blood pressure groups for the traditional IOP pulse parameters of either POBF or PA (Table 8.7).

Group	Pulsatile Ocular Blood Flow (µl/min)	IOP pulse amplitude (mmHg)	Significance
Normal blood pressure	1038 (266)	2.8 (1.6)	N.S.
High blood pressure	1095 (381)	3.0 (1.7)	N.S.

Table 8.7 **Mean (SD) values for the traditional IOP pulse parameters in the groups of subjects with normal and high blood pressure of Study C.**

8.6. *Discussion*

8.6.1. Frequency dependence and IOP moduli

Our results showed that the IOP pulse moduli were highly frequency dependent. This is a characteristic found in arterial flow and pressure waves and represents the response of a vascular system to various driving frequencies (O'Rourke *et al.*, 1966). It is therefore important to compare IOP pulse moduli at the same frequency rather than at the same harmonic number. This is a weakness of the previous two studies that have looked at IOP pulse moduli (Best *et al.*, 1974; Evans *et al.*, 2002). An example may help clarify this point. Taking two subjects with very different heart rates (1.5 Hz and 0.5 Hz), it is important to compare the first harmonic of subject one (1 x 1.5 Hz) with the third harmonic of the subject two (3 x 0.5 Hz) as these are the components that fall at the same frequency.

8.6.2. Measurement precision and IOP moduli

No differences in either IOP pulse moduli, or the traditional IOP pulse parameters of POBF and PA, were found between the groups of subjects that differed by age or blood pressure. The lower frequency IOP pulse moduli decreased significantly during the inhalation of hypercapnic air. This is consistent with the falls in traditional IOP pulse parameters (POBF

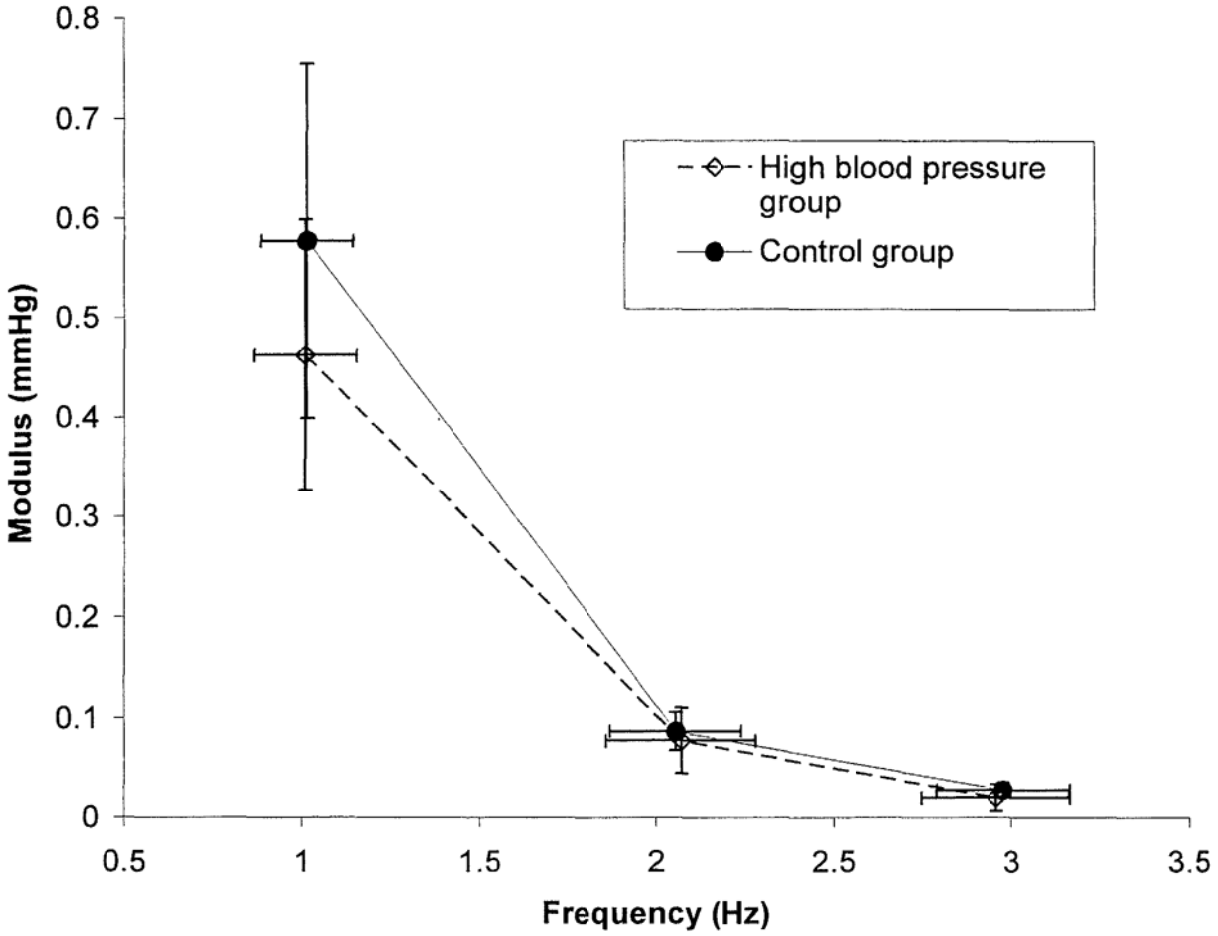

Figure 8.6 **The harmonic moduli, binned by frequency up to 3.5 Hz, of the intraocular pressure pulse for a group of patients with high blood pressure and a control group: error bars represent two standard errors of the mean. No significant difference, at any of the 1 Hz frequency bins, existed between age groups.**

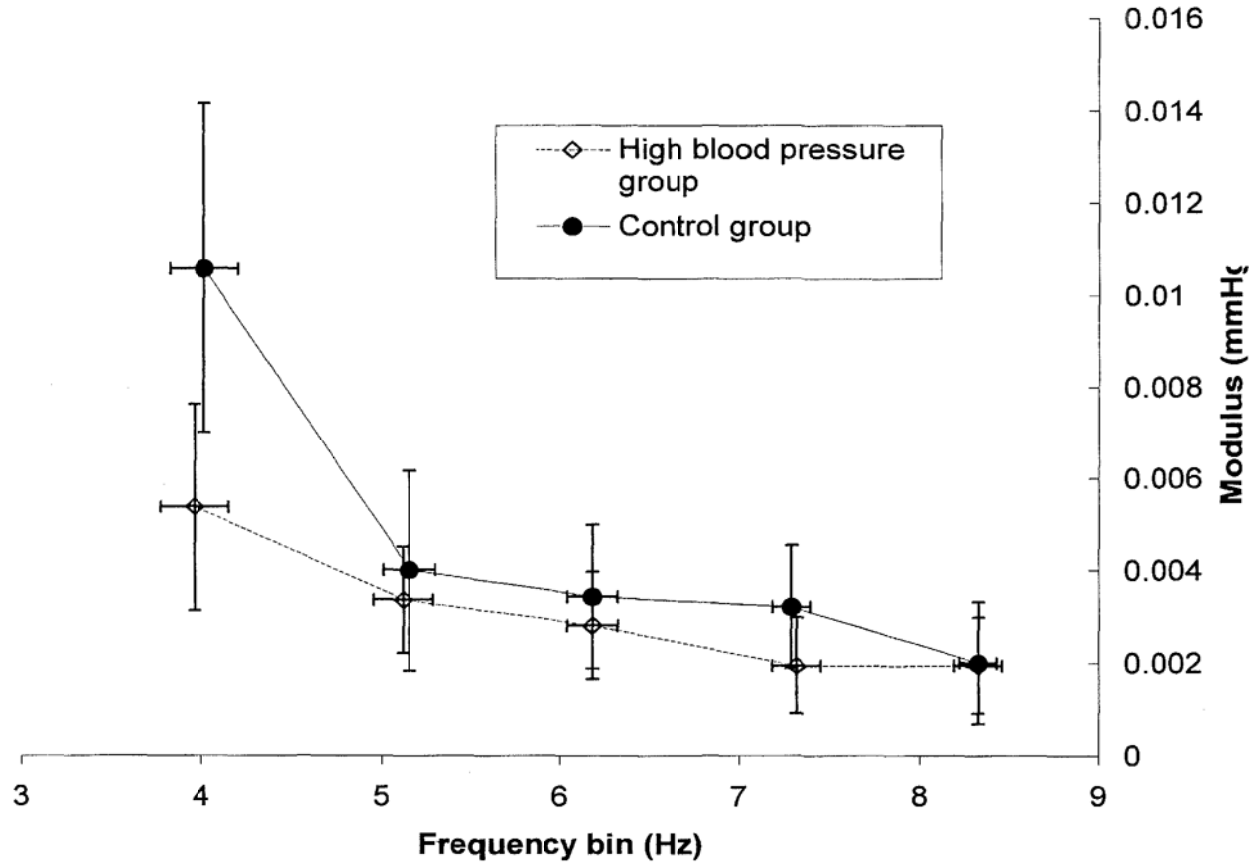

Figure 8.7 **The harmonic moduli, binned by frequency from 3.5 to 9.0 Hz, of the intraocular pressure pulse for a group of patients with high blood pressure and a control group: error bars represent two standard errors of the mean.No significant difference, at any of the 1 Hz frequency bins, existed between age groups.**

and PA) reported for the same study in Chapter 6. Such falls were surprising given the expected change in vascular smooth muscle, and arterial wall distensibility, with this known vasodilator. As discussed in Chapter 6 however, such changes may reflect the drop in compliance of the relatively larger capillary beds, over the arterial vessels, as blood flow increases. This is consistent with falls in cerebral capillary compliance demonstrated at low levels of hypercapnia (Curran-Everett *et al.*, 1995). The measured change in IOP pulse moduli at low frequency during hypercapnia was of greater significance than that found with the traditional parameters of POBF and PA reported in Chapter 6. The mean change in the second IOP pulse modulus was 20.2% (95% confidence intervals, -9.2% to -31.1%) in comparison to a mean change in PA of 8.8% (95% confidence intervals, -3.5% to -16.5%). This indicates that calculation of IOP pulse moduli may detect changes in the IOP pulse with greater confidence than conventional parameters of PA and POBF. A similar conclusion has been made by the two previous studies that used spectral analysis on the IOP pulse (Best *et al.*, 1974; Evans *et al.*, 2002).

8.6.3. Higher IOP pulse moduli

The studies found in these validatory investigations did not provide any evidence that the IOP pulse moduli contained vascular characteristics analogous to the moduli of arterial pressure pulses. Before discussing further, it is important to differentiate impedance moduli from the moduli of an IOP pulse. Vascular impedance moduli describe the frequency specific relationship between blood flow and pressure in an artery. IOP pulse moduli represent the component waveforms of the oscillation in IOP produced from the change in internal ocular volume as the vascular beds expand and contract with each heart beat. As a first approximation, the IOP pulse moduli are most closely related to the component flow waves: the denominator of the impedance equation. One can therefore speculate that, as long as the driving pressure moduli remain fixed or are similar between groups, IOP pulse moduli should behave inversely to vascular impedance moduli. For example, if ocular vascular impedance was to increase, IOP pulse moduli would be expected to decrease. In order to support the hypothesis that IOP pulse moduli relate to vascular impedance, evidence of reduced IOP moduli in the more aged or hypertensive group would be required. Neither groups differentiated for age, or those differentiated for blood pressure, showed any significant difference in IOP pulse moduli at any of the frequencies calculated.

If ocular vascular characteristics do exist in the higher IOP pulse moduli then a number of possible reasons may exist for the lack of significant findings in the present studies. First, differences in group IOP pulse moduli may have been too small to detect with the statistical power of the studies. Taking the mean and standard deviation values of the population to be 2.0 mmHg and 0.6 mmHg respectively (Gekkieva *et al.*, 2001), and the estimated difference between groups as 30%, the statistical power of a study using 20 subjects per group is approximately 50%. The sample sizes of the study groups were small, which would necessitate a large difference between groups to attain significance. Clinical studies that used arterial pulse waves to show arterial stiffening with age required over 400 subjects (Avolio, Chen, Wang *et al.*, 1983). In addition, as the reproducibility of IOP pulse moduli appreciably falls above 5 Hz (Chapter 6), the difference necessary between groups to achieve statistical significance becomes all the greater for higher frequencies. The IOP pulse is a whole order of magnitude smaller than that of the arterial pressure pulse and differences in wave components, if they exist, between groups would likewise reflect this.

Second, although the frequency response of the OBFA pneumatonometer has been theoretically assessed as being acceptable upto 10 Hz this has not been verified in a laboratory. Indeed one of the original developers of the POBF measure, David Silver, has speculated that the IOP waveform signal recorded by the pneumatonometer may be pre-processed by a smoothing function (personal communication). Any such high-pass filters may have removed any potential higher harmonics of interest in these studies. If future investigations on the higher spectral components of the IOP pulse are to be pursued, a frequency response study of the dynamic tonometer used is essential.

Third, the IOP pulse represents a composite of volume changes from the numerous vessels within the eye rather than a single artery. The multiplicity of vessels, individually dilating and contracting with each heart beat, is likely to smudge the component wave signals particularly at higher frequencies. The finding that low frequency IOP pulse parameters fall during low level hypercapnia also indicate that the volume change produced by the combined intraocular vascular beds is more complex than that predicted in a simple single artery.

Fourth, by ignoring the contribution to ocular haemodynamics that the APP makes (equivalent to numerator of the vascular impedance relationship), sole examination of the IOP pulse moduli may be of limited value. Although one can match groups for APP and attempt to keep systemic blood pressure parameters stable for within-subject studies, disregarding the contribution the arterial pressure pulse makes to ocular haemodynamics is unwise and at the least will contribute greater variance to the data (Bron *et al.*, 1967; Chopp *et al.*, 1983).

8.7. *Conclusion*

In summary, the present studies found that IOP pulse moduli were highly frequency dependent. It is recommended that comparisons of IOP moduli between subjects or groups are made by frequency rather than harmonic number. During the inhalation of hypercapnic air, IOP pulse moduli at low frequency changed with greater statistical confidence than the comparative measures of PA and POBF. The present studies do not provide any evidence that calculation of IOP pulse moduli by themselves contain additional vascular characteristics analogous to those of the arterial pulse waves used in the calculation of vascular impedance.

9. Intraocular Pressure Pulse Parameters and Systemic Vascular Characteristics in Glaucoma

9.1. *Abstract*

Purpose: To investigate the IOP pulse and vascular status of patients with glaucomatous optic neuropathy.

Methods: Two groups of newly diagnosed, untreated patients, consisting of 20 patients with normal-tension glaucoma (NTG group) and 20 patients with high-tension glaucoma (POAG group), were group-matched for age and IOP with respective groups of 20 normal subjects (Normal group) and 20 ocular hypertensive subjects (OHT group). Traditional measurements of IOP pulse (PA and POBF) were compared to the moduli of the groups' IOP pulses calculated from a previously described spectral analysis technique. Measurements of the groups' blood pressure parameters were taken using acknowledged formulae. Vasoreactivity was determined from finger blood flow values at discrete time points during a cold-provocation test using cutaneous laser Doppler flowmetry.

Results: Both glaucoma groups were successfully matched for age and IOP with their respective control groups. A significant difference in POBF value existed between the NTG group (840 (249) μl/min) and its control group of normal subjects (1068 (344) μl/min): $p = 0.034$. Moduli determined from spectral analysis of the IOP pulse failed to show any significant difference between glaucoma groups and their respective control groups. Between all four groups, mean systolic blood pressure and mean arterial pulse pressure was significantly greater in POAG patients in comparison to those with NTG ($p = 0.011$ and $p = 0.014$ respectively). The influence of gender was found to be a significant factor in baseline cutaneous finger blood flow values.

Conclusion: This study found evidence of a reduction in the pulsatile fraction of ocular blood flow of NTG patients and a difference in systolic blood pressure between high- and normal-tension glaucoma patients. Spectral analysis of the IOP pulse did not give greater confidence in separating glaucomatous from non-glaucomatous eyes over traditional IOP pulse parameters. It is recommended that the influence of gender in future investigations of vasoreactivity and glaucoma be recognised and that this factor in possible subcategories of glaucoma warrants further investigation.

9.2. *Introduction*

Anomalous IOP pulse parameter values in patients suffering with POAG and NTG have been repeatedly described (1.9.2). In brief, both POAG and NTG patients exhibit reduced PA, pulse volume and POBF values in comparison to groups of IOP-matched normal subjects. The predictive value of these traditional IOP pulse parameters in differentiating glaucomatous and healthy eyes however is poor (Lambrou *et al.*, 1989). Recently Evans *et al.* (2002) reported that the spectral analysis of the IOP pulse showed greater potential as a clinical predictor of glaucoma. Previous work has shown that calculation of the initial harmonics of the IOP pulse provides a parameter that is highly repeatable (Chapter 7) and may detect change in the IOP pulse with greater confidence compared to traditional pulse values (Chapter 8). These studies support the concept that spectral analysis of the IOP pulse may provide an improved technique in detecting glaucomatous disease.

It has been proposed that an aetiological factor in POAG, and particularly its NTG variant, is a vascular dysregulation of the optic nerve head circulation (Flammer *et al.*, 2002). Possible mechanisms that could cause optic nerve head ischaemia are reduced ocular perfusion pressure and a systemic tendency to arterial vasospasm (1.9.2). It would be of interest to investigate whether these cardiovascular properties differ in a glaucomatous group and whether any associations with IOP pulse characteristics exist.

9.3. *Aims*

The purpose of this study was to investigate the IOP pulse, using both traditional and newly developed parameters, in glaucomatous and healthy eyes. In addition, due to the suspected role of ocular perfusion pressure and/or a systemic vasospastic phenotype in the aetiology of POAG, associations between blood pressure measures, and the response of finger blood flow to cold-provocation, were investigated.

9.4. *Methods*

9.4.1. Experimental design

This was a group control study comparing two groups of newly-diagnosed glaucoma patients with two respective groups of age- and IOP-matched control subjects.

9.4.2. Ethical Approval

All experimental procedures of the study conformed to the tenets of the declaration of Helsinki and were approved by the Oxfordshire Research Ethics Committee and the Aston University Human Science Ethical Committee. Written informed consent was obtained from all volunteers.

9.4.3. Subject sample

Patients with newly-diagnosed POAG were prospectively recruited from the Primary Assessment Clinic at the Oxford Eye Hospital. Diagnosis of POAG was made by an experienced ophthalmologist based on visual field results (SITA 24-2 program, Zeiss-Humphrey Visual Field Analyser) and ophthalmoscopic examination of the optic nerve. Patients were placed into one of two glaucoma groups; those patients with a Goldmann tonometry value of 21 mmHg or less at diagnosis were allocated to a group referred to as the normal-tension glaucoma (NTG) group, and those with a presenting IOP of 22 mmHg or more were allocated to the POAG group. Patients were excluded from the study for any of the reasons found in Table 9.1.

- Narrow anterior chamber angles (grade 2 or less, Shaffer gonioscopy grading system)
- Concurrent or previous eye disease
- Amblyopia
- Previous ocular surgery or treatment
- Significant lens opacification (grade 2 or more, Oxford Clinical Cataract Classification and Grading System) (Brown, Bron, Ayliffe *et al.*, 1987)
- Physical or mental incapacity to perform experimental procedures

Table 9.1 Glaucoma study exclusion criteria

9.4.4. Control Sample

In order to match the glaucoma groups for age and IOP, subjects were recruited from optometric practice and the Primary Assessment Clinic of the Oxford Eye Hospital. The two control groups therefore consisted of ocularly healthy subjects and subjects with raised IOP but having no signs of glaucomatous optic neuropathy. These latter subjects are described as having ocular hypertension (OHT).

Control subjects were given a full eye examination in order to rule out eye disease. In addition to being subject to the necessary exclusion criteria in Table 9.1, control subjects were required to have a visual acuity of 6/9 or better and no evidence of glaucoma in either eye based on the criteria in Table 9.2.

1. An ophthalmoscopically normal optic nerve head as defined by:

   - The proportion of neuroretinal rim thickness obeying Jonas' ISNT rule
   - A vertical cup to disc ratio of less than 0.7
   - Absence of focal narrowing of the neuroretinal rim
   - A healthy coloured neuroretinal rim
   - Absence of slit-like cribrosal pores in the optic cup
   - Absence of disc haemorrhages
   - Absence of focal or diffuse optic disc arteriole narrowing
   - Absence of slit defects in the retinal nerve fibre layer on red-free ophthalmoscopy
   - Absence of extensive beta-zone parapapillary chorioretinal atrophy
   - Absence of any other sign indicative of optic disc pathology

2. Normal visual fields as defined by two consecutive visual field examinations (SITA 24-2 program, Zeiss-Humphrey Visual Field Analyser).

Table 9.2 **Criteria used to define absence of glaucoma in an eye (Alexander, 1994; Hodapp *et al.*, 1993; Jonas *et al.*, 1999).**

9.4.5. Experimental Procedures and instrumentation

Ocular measurements were taken on one eye only of each subject. For the glaucoma groups, ocular measurements were taken on the eye diagnosed as having glaucoma or, in the case of bilateral glaucoma, on the eye with the more advanced disease. For the control subjects, measurements were taken on a randomly chosen (sealed-envelope method) eye. All measurements were taken at one appointment which, in the case of the glaucoma patients, took place before they began topical ocular anti-hypertensive medication. All measurements were taken with the patient in the seated position, after 20 minutes of rest in order to stabilise cardiovascular and cutaneous blood flow measurements. The sequence of the study procedures is shown in Table 9.3. The order of study procedures was chosen for the following reasons. The more ocularly invasive tests were performed last in order that tests of visual function were not affected. Pneumatonometry was performed prior to biometry so as to avoid tonographic reduction in IOP with the ultrasound probe. Two measurements of blood pressure and heart rate, before and after the pneumatonometry measurements, were taken in order to confirm cardiac stability.

1. Patient history
2. Subjective refraction
3. Visual parameters
    - Visual acuity
    - Contrast sensitivity
    - Visual fields
4. 1st blood pressure and heart rate measurement
5. Pneumatonometry measurements
6. 2nd blood pressure and heart rate measurement
7. Cold-provocation test
8. Ocular dimension measurements
    - Keratometry
    - A-scan ultrasound biometry

Table 9.3 Sequence of glaucoma study procedures

Subject history

A clinical grading of a subject's history of digital vasospasm was made using the modified Taylor-Pelmear scale (Table 9.4) and recorded as an ordinal score (0 – 4) (Allen, Devlin, McGrann *et al.*, 1992).

Grade	Symptoms
0	No tingling, numbness or blanching of digits
1	Blanching of one or more fingertips with or without tingling and numbness
2	Blanching of one or more fingers beyond tips, usually confined to winter
3	Extensive blanching of digits. Frequent episodes summer and winter
4	Extensive blanching of most fingers with frequent episodes summer and winter. Trophic skin changes in fingers

Table 9.4 Clinical grading of vasospasm (Allen *et al.*, 1992).

Migraine was recorded as present in a subject on the basis of a reported history of unilateral headaches associated with one or more of the following: nausea; photophobia; sensitivity to sound; and scintillating scotoma (Lance, 1994).

Visual performance

A subject's visual acuity was measured using a logMAR chart and recorded following the recommended procedure described by Bailey, Bullimore, Raasch *et al.* (1991). Contrast sensitivity was measured using a Pelli-Robson chart following the original scoring procedure (Pelli, Robson & Wilkins, 1988). Visual field analysis was performed using automated perimetry (SITA 24-2 operating system, Humphrey Field Analyzer, Carl Zeiss Ophthalmic Systems, Inc., Humphrey division, Dublin, California). All subjects had performed at least one visual field examination using the same instrumentation on a previous occasion in order to minimise potential learning effects.

Blood pressure parameters

Measures of systemic blood pressure and heart rate were made using a validated (Shennan *et al.*, 1998) automated sphygmomanometer (Omron Rx, Omron Matsusaka Co. Ltd, Japan). Blood pressure and heart rate measurements were taken twice (before and after pneumatonometry measurements) and averaged. In addition the parameters of APP, MAP and OPP were also calculated as previously described (Equations 1.6, 1.7 and 1.11).

Pneumatonometry

Pneumatonometry measurements were taken using the OBFA pneumatonometer (Paradigm Medical Ind., Utah, USA) mounted on a slit-lamp, following topical anaesthesia of the subject's eye with one drop of 0.4% benoxinate HCl (*Minims*®, Chauvin, UK). Measurements of IOP, PA and POBF were calculated automatically, by the pneumatonometer's software, from five or more IOP pulses that it considered acceptable from a maximum recording duration of 20 seconds. Calculation of the IOP pulse's moduli was performed by analysing a 10 second continuous IOP waveform as previously described (7.4). Both the Power Spectral Density technique and the averaged single pulse technique were used to analyse the IOP pulse moduli.

Cutaneous finger blood flow

Measurements of finger blood flow were taken using a cutaneous laser Doppler flowmeter (PF 5010: Perimed, Stockholm, Sweden). The principle of cutaneous laser Doppler flowmetry (LDF) will be described in brief (Bonner & Nossal, 1981).

Incident laser light, produced from a fibre optic probe, is absorbed, transmitted or scattered by underlying skin tissue. Light reflected from a moving corpuscle is subject to a Doppler shift. The total scattered light therefore consists of the original frequency (reflected from non-moving tissue) and slightly shifted frequencies above and below the original (reflected from corpuscles moving towards and away from the source respectively). The light scattered back effectively has its frequencies spread around the original incident frequency. Measurement of this frequency broadening is the principle of LDF. The amount of frequency shift (Δf) can be calculated from Equation 9.1.

$$\Delta f = (2\pi / \lambda)(K_s - K_i) * V$$

Δf	shift in frequency		
K_s	wave vector of scattered light	V	the velocity of the particle (the corpuscle)
K_i	wave vector incident light	λ	the wavelength of the incident light.

Equation 9.1 Optical Doppler shift calculation.

The reflected light is therefore composed of a spectrum of frequencies each associated with a particular intensity: the so-called Doppler shift power spectrum (Bonner & Nossal, 1990). If this spectrum is integrated across the relevant frequencies, a quantity that describes the distance travelled by all the moving particles inside the sample volume per unit time is calculated. Similarly the Doppler shift power spectrum can be further integrated to find the mean number of photon collisions with moving particles and this is proportional to the number of moving corpuscles. These two quantities, termed 'flow' and 'volume' form the basis of laser-Doppler flowmetry.

For this study, the flowmeter's fibre optic probe was attached to the skin overlying the terminal phalanx of the right middle finger using purpose-made double-sided adhesive tape. The fibre optic probe radiates a monochromatic beam of light produced from a laser diode of 780 nm wavelength. The light penetrates the skin to a depth of about 1 mm and so blood flow measurement below that of the superficial arteriole and venular plexus is not possible. Some light is absorbed by the tissue and some is reflected back to the probe that contains a photodiode receiver. The source laser and receiving detector are slightly separated, by 300 μm, and blood flow information is gathered from a shallow, up to 1 mm, hemisphere of tissue.

LDF flow values, recorded in arbitrary units (AU), were recorded continuously during the experimental procedure. The method for cold-provocation was based on a number of previous studies (Drance, Douglas, Wijsman *et al.*, 1988; O'Brien *et al.*, 1999; Usui *et al.*, 1992). The same standardised procedure was followed for each subject. An initial baseline period of cutaneous finger blood flow was followed by placing the hand in ice-cold (4.0 ±

0.5 °C) water for 30 seconds. The hand was then removed, dried carefully with a towel (in order to avoid evaporative effects), and then monitored for a following 10 minutes. An example of a section of LDF recording taken from a subject during cold-provocation is shown in Figure 9.1.

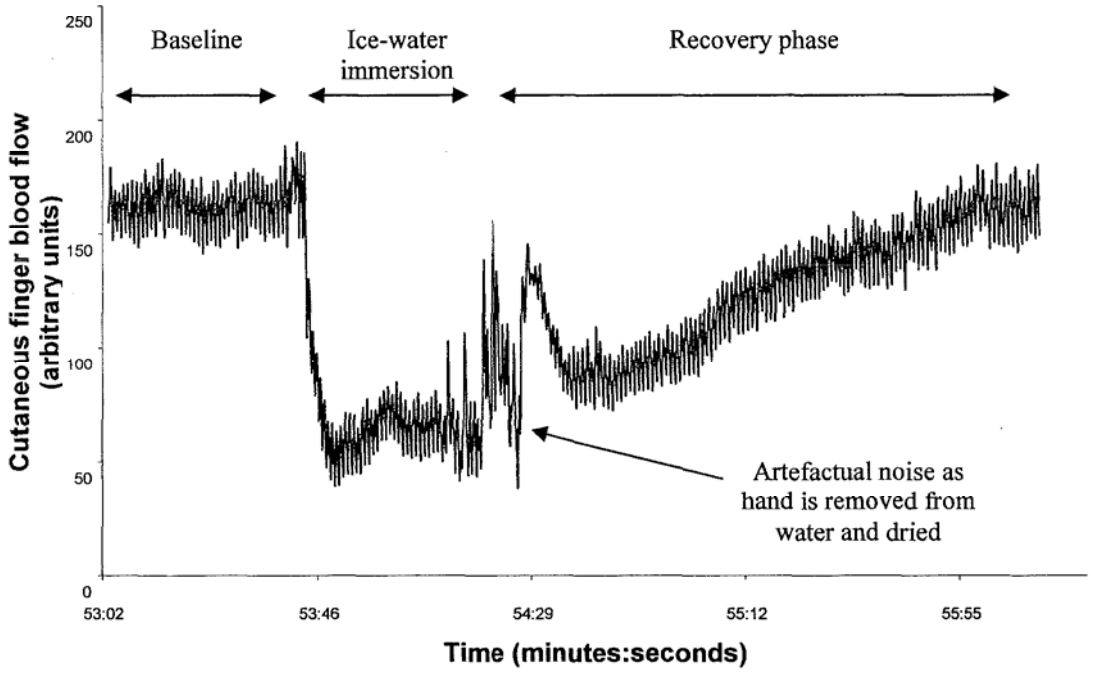

Figure 9.1 Example of a section of a subjects cutaneous laser Doppler flowmetry recording during cold water provocation.

Mean flow values were calculated from sections of, artefact-free, 30-second LDF recordings at the following points in the cold-provocation test:

- Baseline (recorded as the maximum flow plateau found after at least 20 minutes of rest in a seated postion).
- Cold water immersion
- Immediately after cold-water immersion
- 10 minutes after removing hand from the ice-cold water

In addition to these mean flow values, the ratio of baseline to minimum flow and the time to recover to baseline value were calculated. These latter two measures have been found to be anomalous in patients with NTG (Drance *et al.*, 1988; O'Brien *et al.*, 1999).

Given the influence of ocular volume (James *et al.*, 1991c), and possibly corneal curvature (Chapter 5) on IOP pulse parameters, mean corneal radius and axial length were measured using a Javal-Schiötz keratometer and A-scan ultrasound, respectively.

9.4.6. Statistics

Data were first examined in order to verify normality of distribution. Data showing significant skew or kurtosis were transformed (logarithmically) in order to better approximate a normal distribution. Data were presented in the format of their mean (standard deviation) values. Comparisons between the two glaucoma groups and their respective control groups were made using independent sample t-tests. Where comparison between all four groups was appropriate, univariate analysis was used to investigate significant factors in a dependent variable. Relationships between continuous variables were investigated using multiple regression. Ordinal and categorical data (vasospasm and migraine history) were analysed using the appropriate non-parametric statistical tests.

9.5. *Results*

9.5.1. Group characteristics

The background characteristics of the NTG group and the matched control group of normal subjects are shown in Table 9.5. Likewise Table 9.6 displays the background characteristics for the POAG group and their matched control group of OHT patients. Both glaucoma groups (NTG and POAG) exhibited significantly worse visual parameters in visual acuity, contrast sensitivity and visual field score compared to their respective control groups (all ($p = \text{or} < 0.001$). The number of prescribed cardiovascular medications that were reported by subjects and patients in each group are tallied in Table 9.7.

Parameter	Normal subject group	Normal-tension glaucoma group	Significance
Gender (m/f)	8/12	8/12	N.S.
Race (Caucasian/Afro-Caribbean/Oriental/Asian)	20/0/0/0	18/1/1/0	N.S.
Number of subjects with diabetes	1	3	N.S.
Age (yrs)	62.3 (7.7)	65.5 (10.7)	N.S.
Goldmann IOP (mmHg)	14.8 (3.8)	17.4 (2.4)	N.S.
Mean spectacle Rx (D.S.)	-0.44 (2.49)	0.12 (1.81)	N.S.
Visual acuity (logMAR)	-0.09 (0.07)	0.03 (0.07)	$p < 0.001$
Contrast sensitivity (Pelli-Robson)	1.60 (0.11)	1.39 (0.15)	$p = 0.001$
Mean Deviation in visual field score	-0.46 (1.17)	-9.31 (6.8)	$p < 0.001$
Mean corneal radius (mm)	7.68 (0.18)	7.71 (0.21)	N.S.
Axial length (mm)	23.58 (1.33)	23.48 (0.64)	N.S.

Table 9.5 Background characteristics, presented as mean (SD) values, of the normal-tension glaucoma and normal subject groups.

9.5.2. Blood pressure values

The respective blood pressure and blood pressure-derived values for the two group comparisons are found in Table 9.8 and Table 9.9. The mean APP of the POAG group was significantly greater than the OHT group ($p = 0.046$). No other significant differences existed between the glaucoma groups and their respective control groups for the blood pressure parameters and heart rate. As mean ages were not significantly different, the IOP-independent blood pressure parameters of SBP, DBP, APP and MAP were compared between all four groups. A significant difference between groups was found for the SBP and APP value ($p = 0.011$ and $p = 0.014$ respectively, one-way ANOVA). Tukey's LSD post-hoc comparison test found the differences to arise from the higher SBP and APP mean

Parameter	OHT subjects	POAG patients	Significance
Gender (m/f)	13/7	13/6	N.S.
Race (Caucasian/Afro-Caribbean/Oriental/Asian)	19/1/0/0	20/0	N.S.
Diabetes	2	1	N.S.
Age	65.5 (5.3)	67.2 (12.0)	N.S.
Goldmann IOP (mmHg)	25.5 (2.7)	26.4 (3.5)	N.S.
Mean spectacle Rx (D.S.)	1.29 (1.96)	1.86 (5.17)	N.S.
Visual acuity (logMAR)	-0.07 (0.08)	0.05 (0.11)	$p < 0.001$
Contrast sensitivity (Pelli-Robson)	1.53 (0.13)	1.35 (0.21)	$p = 0.007$
Mean Deviation in visual field score	-0.38 (1.61)	-8.20 (7.44)	$p < 0.001$
Mean corneal radius (mm)	7.67 (0.28)	7.64 (0.24)	N.S.
Axial length (mm)	22.87 (0.96)	23.12 (0.85)	N.S.

Table 9.6 **Background characteristics, presented as mean (SD) values, of the Ocular Hypertensive (OHT) subject group and the Primary Open Angle Glaucoma (POAG) group.**

values in the POAG patients (Figure 9.2 and Figure 9.3). Post-hoc comparison found: 1, the SBP of the POAG group significantly higher than that of the NTG group (($p = 0.001$); and 2, the APP of the POAG group significantly higher than both the NTG group and the OHT group ($p = 0.003$ and $p = 0.010$) respectively.

Prescribed systemic cardiovascular medication	Normal control group	Normal-tension glaucoma group	Ocular hypertensive control group	Primary open angle glaucoma group
Beta-blocker	4	2	3	3
statin	2	2	2	3
aspirin	1	3	2	5
diuretic	2	3	5	5
ACE inhibitors	1	2	3	2
Calcium-channel blocker		2	1	4
Nitrate		1		1
Antiplatelet drugs				2
digoxin			1	
Angiotensin II receptor antagonist				1

Table 9.7 **Reported systemic cardiovascular medications taken by subjects and patients for each group**

Parameter	Normal subject group	Normal-tension glaucoma group	Significance
Systolic blood pressure (mmHg)	134.9 (20.7)	130.0 (12.2)	N.S.
Diastolic blood pressure (mmHg)	77.8 (11.7)	77.0 (11.6)	N.S.
Arterial pulse pressure (mmHg)	57.0 (15.5)	53.0 (10.5)	N.S.
Mean arterial blood pressure (mmHg)	96.8 (13.4)	94.6 (10.7)	N.S.
Mean ocular perfusion pressure (mmHg)	49.8 (8.6)	46.3 (7.5)	N.S.
Heart rate (beats/minute)	68.9 (11.1)	70.8 (12.8)	N.S.

Table 9.8 **Blood pressure and heart rate mean (SD) values for normal-tension glaucoma and normal subject groups.**

Parameter	OHT subjects	POAG patients	Significance
Systolic blood pressure (mmHg)	135.1 (13.3)	146.5 (15.7)	N.S.
Diastolic blood pressure (mmHg)	80.1 (8.3)	79.9 (11.2)	N.S.
Arterial pulse pressure (mmHg)	55.0 (8.5)	66.6 (18.7)	$p = 0.046$
Mean arterial blood pressure (mmHg)	98.5 (9.4)	102.1 (9.4)	N.S.
Mean ocular perfusion pressure (mmHg)	40.2 (6.9)	41.7 (6.6)	N.S.
Heart rate (beats/minute)	76.8 (8.0)	73.5 (14.3)	N.S.

Table 9.9 **Blood pressure and heart rate mean (SD) values for the Ocular Hypertensive (OHT) subject group and the Primary Open Angle Glaucoma (POAG) group.**

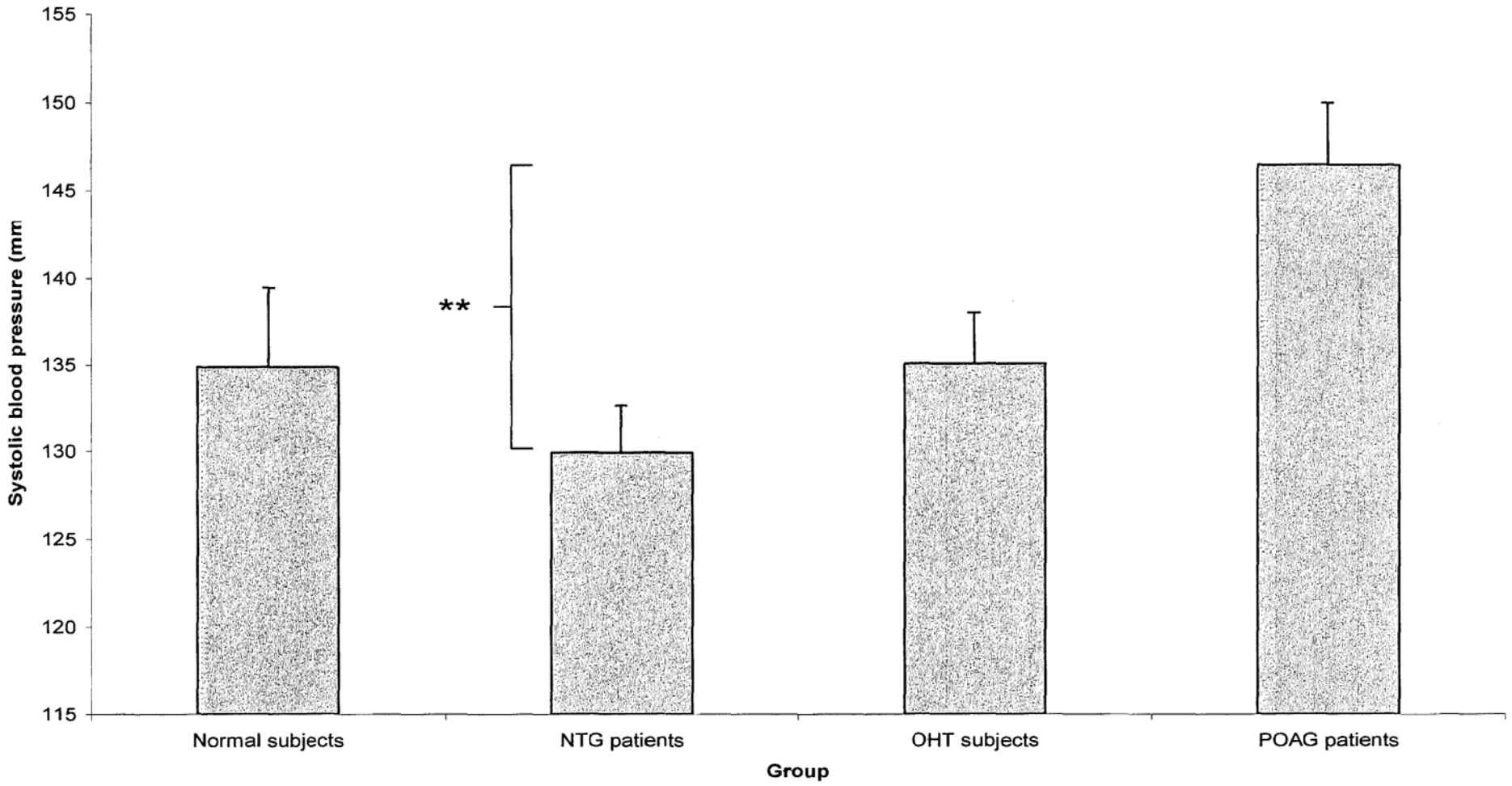

Figure 9.2 **Mean systolic blood pressure values for all four subject and patient groups: error bars indicate one standard error of the mean; ** significant difference between mean values at the $p < 0.01$ level (Tukey's LSD post-hoc comparison test).**

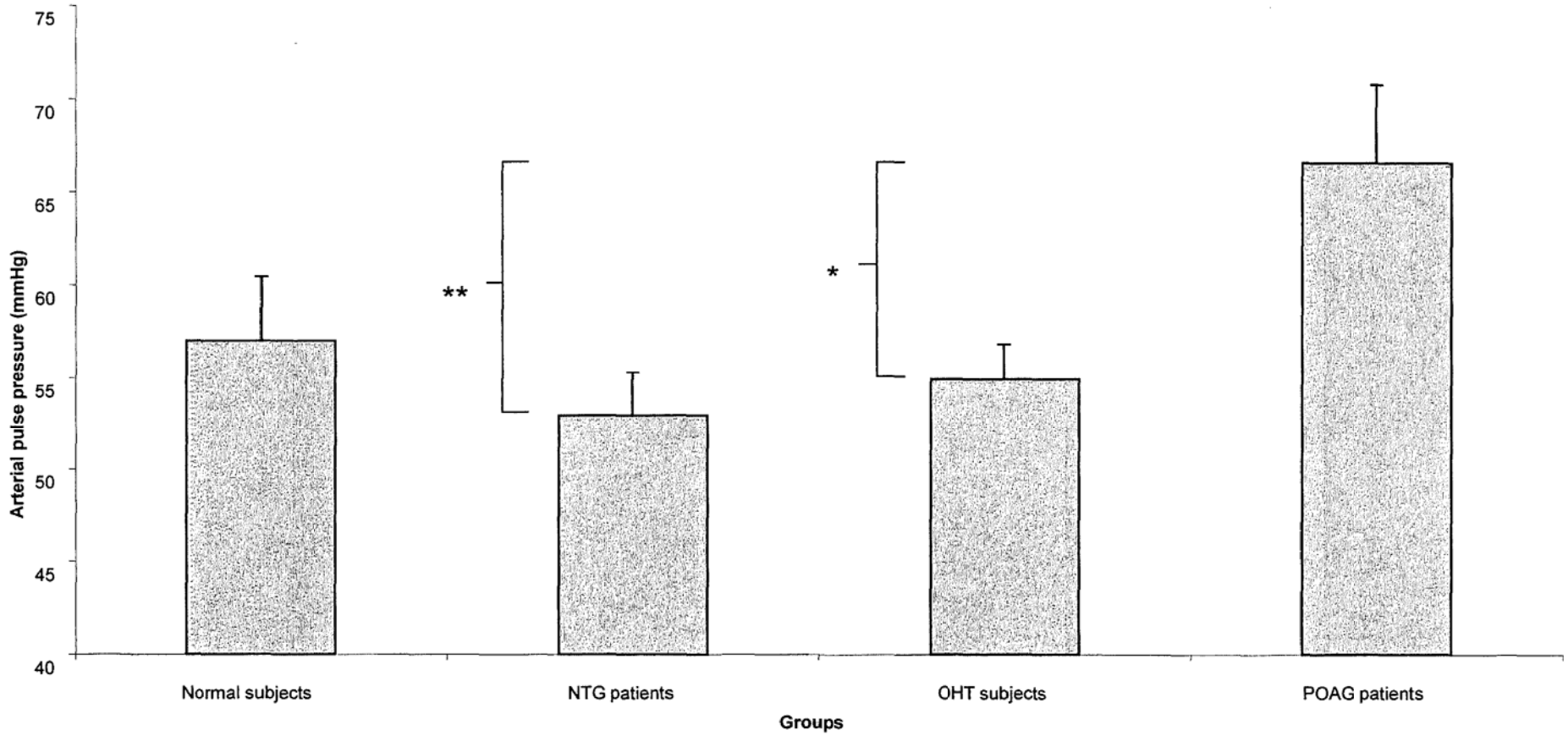

Figure 9.3 **Mean arterial pressure pulse values for all four subject and patient groups: error bars indicate one standard error of the mean; * significant difference between mean values at the $p < 0.05$ level; ** significant difference between mean values at the $p < 0.01$ level (Tukey's LSD post-hoc comparison test).**

9.5.3. Clinical history of migraine and vasospasm

The prevalence of reported migraine was higher in the glaucoma groups compared to their controls (Table 9.10) but was not of statistical significance (Kruskal-Wallis test). Likewise although the total group score on the clinical grading of vasospasm was highest in the glaucoma groups, it was not statistically significant (Table 9.11).

Group	Number of subjects reporting a history of migraine
Normal subjects	1/20
Normal-tension glaucoma patients	4/20
Ocular hypertension subjects	2/20
Primary open-angle glaucoma patients	4/20

Table 9.10 **Prevalence of migraine in subject groups. No significant difference between group proportions ($p > 0.05$).**

Group	Total Taylor-Pelmear vasospasm score for group
Normal	7
Normal Tension Glaucoma	10
Ocular Hypertension	9
Primary Open Angle Glaucoma	10

Table 9.11 **Total vasospasm score by group. No significant difference between group grades ($p > 0.05$).**

9.5.4. Traditional IOP pulse parameters

No significant difference in mean PA existed between either glaucoma group and its respective control group (Figure 9.4). The mean POBF value was significantly lower ($p = 0.034$) in the NTG group than the normal subject group: 840 (249) μl/min and 1068 (344) μl respectively (Figure 9.5). No significant difference existed between the mean values of POBF in the POAG group and its matched group of OHT subjects.

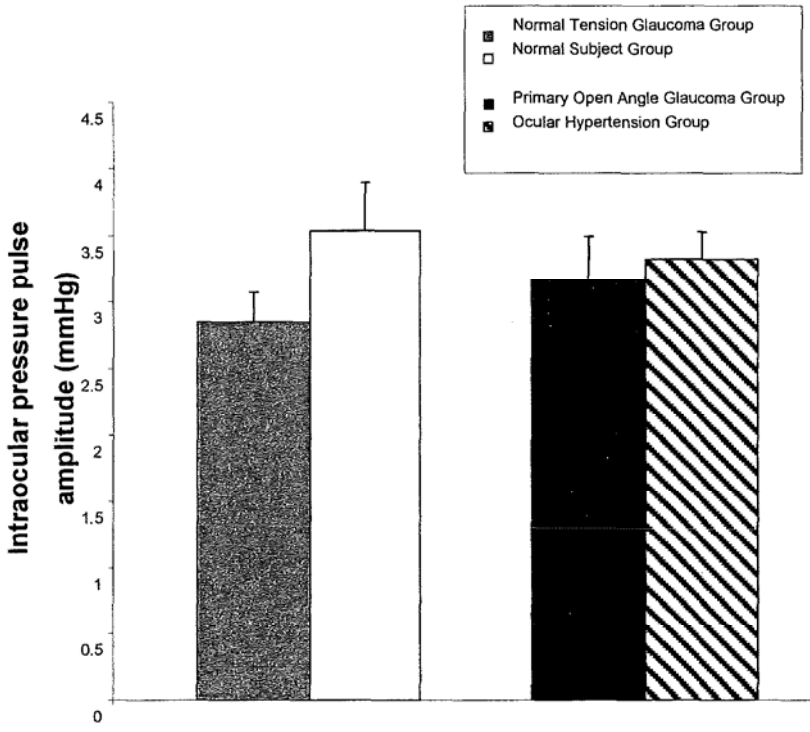

Figure 9.4 **Intraocular pressure pulse amplitude in the two glaucoma groups and their respective matched control groups. Error bars represent one standard error of the mean. No significant difference between glaucoma groups and their control groups ($p > 0.05$).**

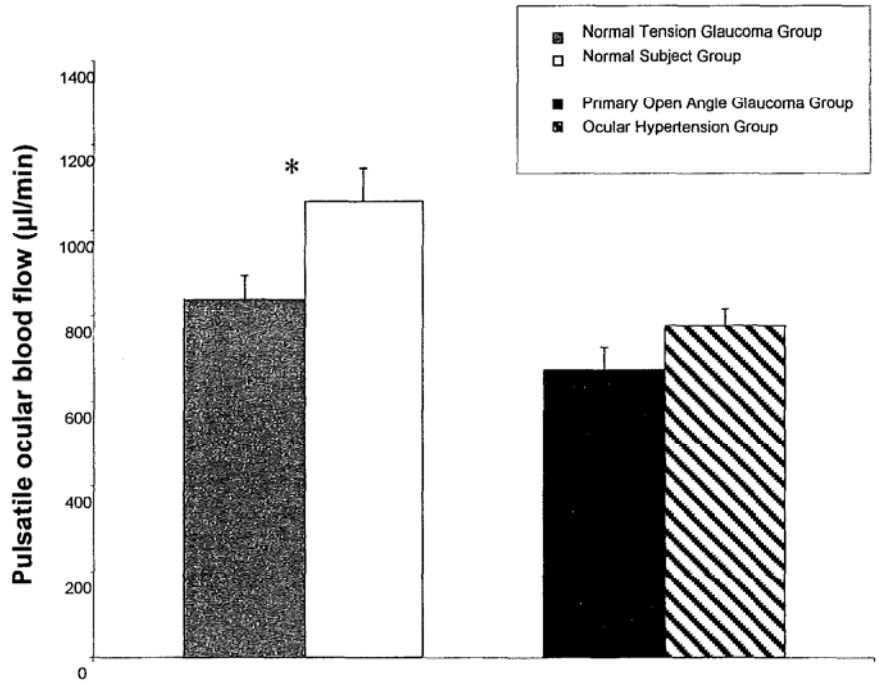

Figure 9.5 **Pulsatile ocular blood flow in the two glaucoma groups and their respective matched control groups: error bars represent one standard error of the mean (SEM); * indicates a significant difference in value ($p < 0.05$) between the glaucoma group and its matched control group.**

9.5.5. IOP pulse moduli

Figure 9.6 and Figure 9.7 show the IOP pulse moduli, calculated using the ASP technique, of the NTG and normal control group for the frequency ranges 0 to 3.5 Hz and 3.5 to 10 Hz respectively. Similarly Figure 9.8 and Figure 9.9 show the IOP pulse moduli of the POAG group and OHT control group for the frequency ranges 0 to 3.5 Hz and 3.5 to 10 Hz respectively. No significant difference in the mean values of the IOP moduli between glaucoma groups and their respective control groups was demonstrated in any of the one Hertz frequency bins. Calculation of the IOP pulse moduli using the alternative PSD technique again showed no statistically significant difference.

9.5.6. Cutaneous laser Doppler flowmetry

The cutaneous blood flow values, as measured using laser Doppler flowmetry, at each stage in the cold provocation test for each group are shown in Figure 9.10. Cutaneous blood flow values demonstrated significant positive skew and were log transformed in order to produce normal distributions. No significant difference was demonstrated between any of the patient or subject groups in finger blood flow at any of the four stages of the cold-provocation test, nor in the ratios of baseline flow to flow after cold water immersion. In addition, the groups showed no significant difference in the time taken for the blood flow value to recover to its baseline value after cold-provocation. The median times for recovery were: normal control group, 5.5 minutes; NTG group, 5.5 minutes; OHT control group, 5.0 minutes; and the POAG group, 4.5 minutes. Factorial ANOVA revealed gender to be a significant factor in cutaneous blood flow values. The mean (SD) baseline value for all 38 female subjects was 185 (77) AU in comparison to 147 (61) AU for all 42 male subjects ($p = 0.013$). Factorial ANOVA revealed no interaction between gender and subject group or migraine to be significant. No significant correlation was found between any of the IOP pulse parameters and cutaneous finger blood flow values.

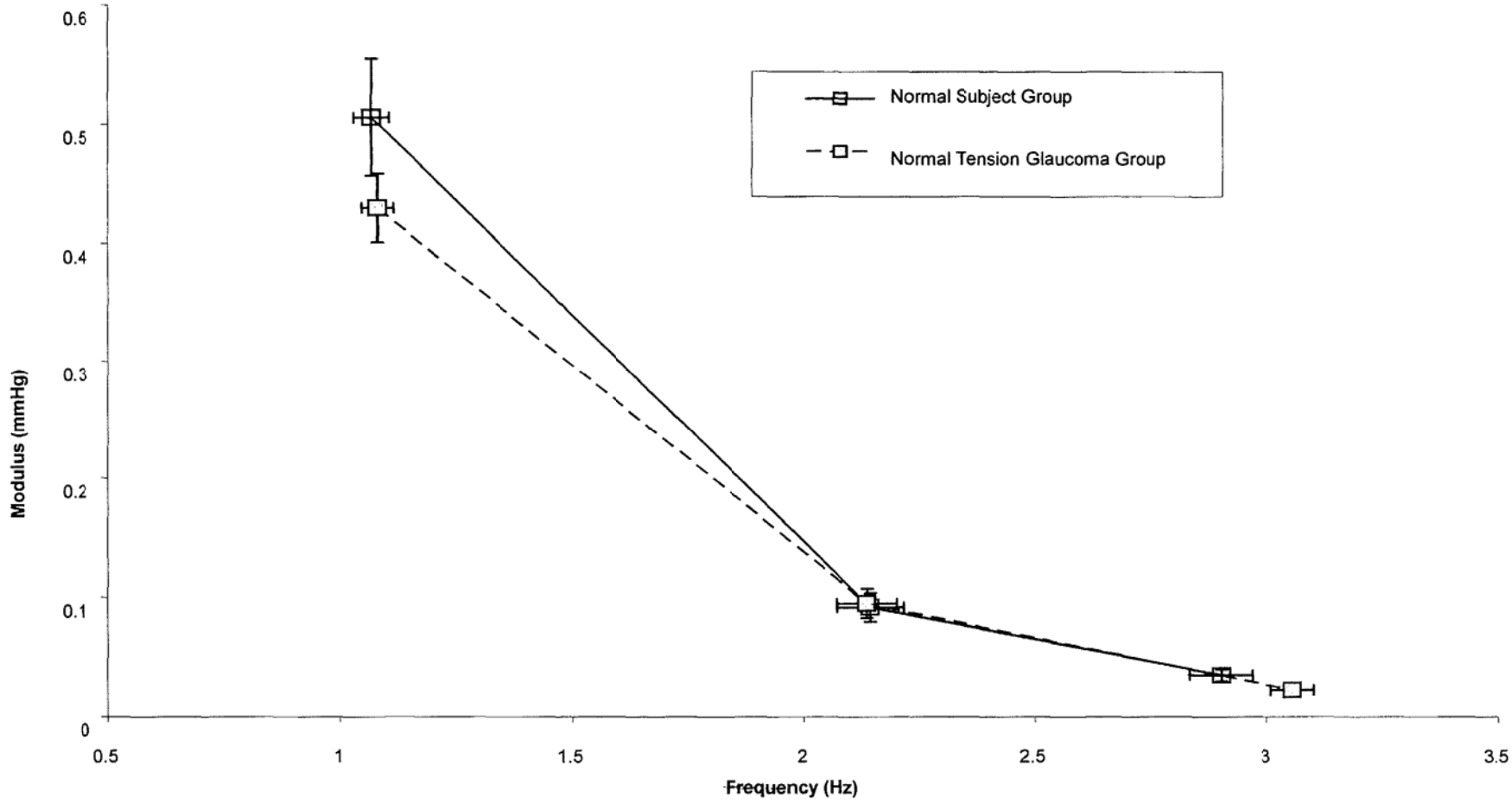

Figure 9.6 **The mean harmonic moduli, in one Hertz frequency bins up to 3.5 Hz, of the intraocular pressure pulse for the normal-tension glaucoma group and normal subject group: error bars indicate one standard error of the mean. No significant difference existed between groups at any of the 1 Hz frequency bins ($p > 0.05$).**

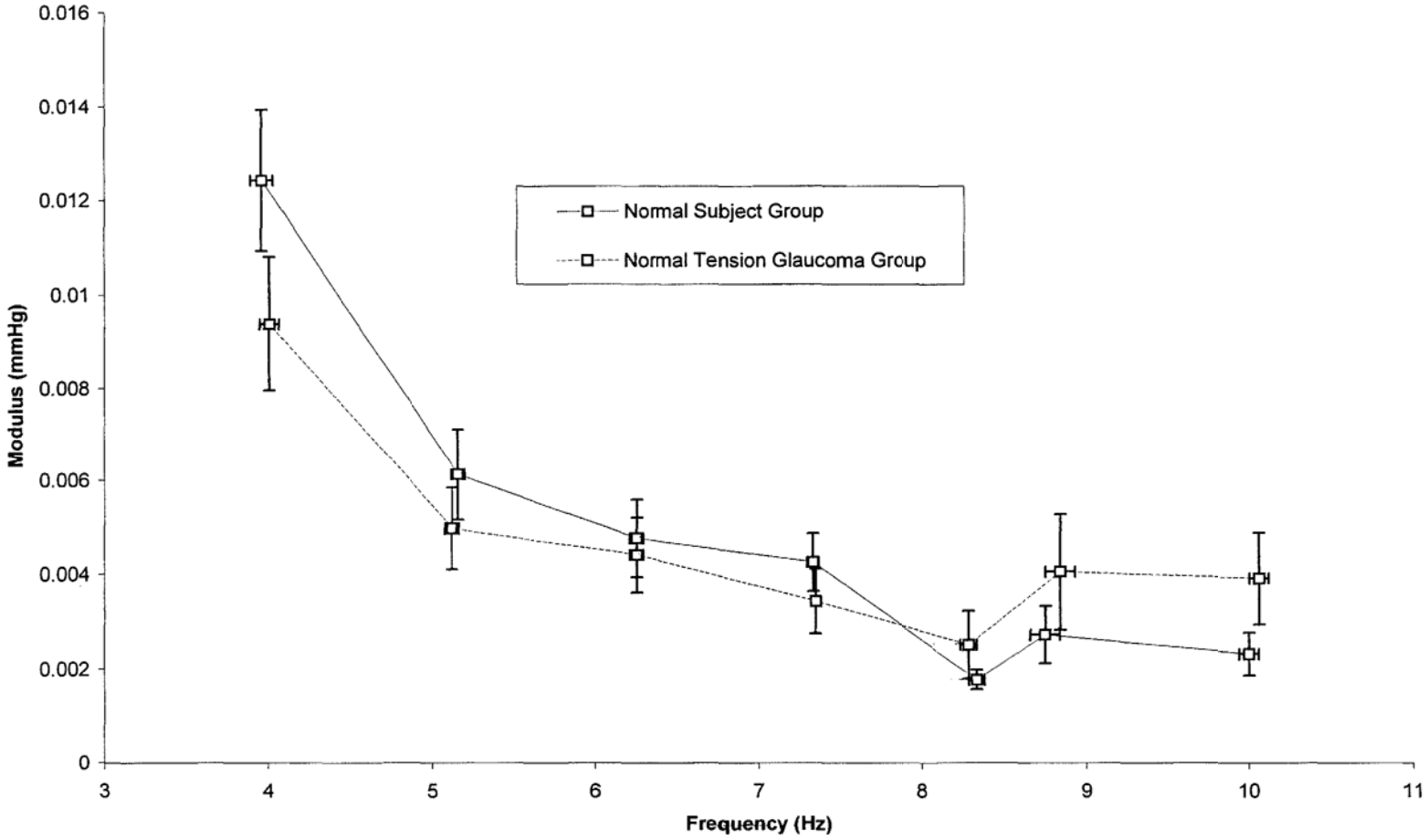

Figure 9.7 **The mean harmonic moduli, in one Hertz frequency bins from 3.5 Hz to 10 Hz, of the intraocular pressure pulse for the normal-tension glaucoma group and normal subject group: error bars indicate one standard error of the mean. No significant difference existed between groups at any of the 1 Hz frequency bins ($p > 0.05$).**

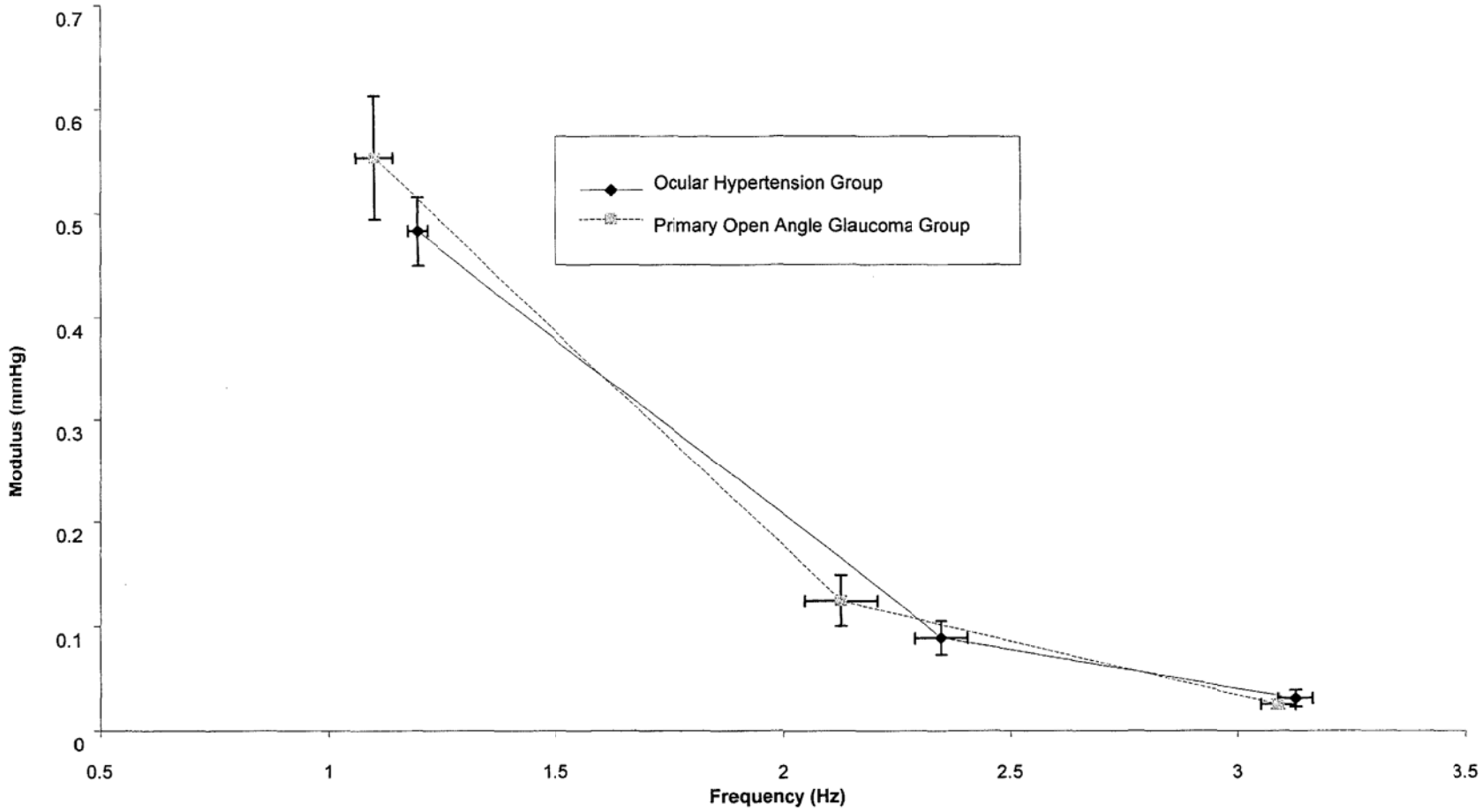

Figure 9.8 **The mean harmonic moduli, in one Hertz frequency bins up to 3.5 Hz, of the intraocular pressure pulse for the normal-tension glaucoma group and normal subject group: error bars indicate one standard error of the mean. No significant difference existed between groups at any of the 1 Hz frequency bins ($p > 0.05$).**

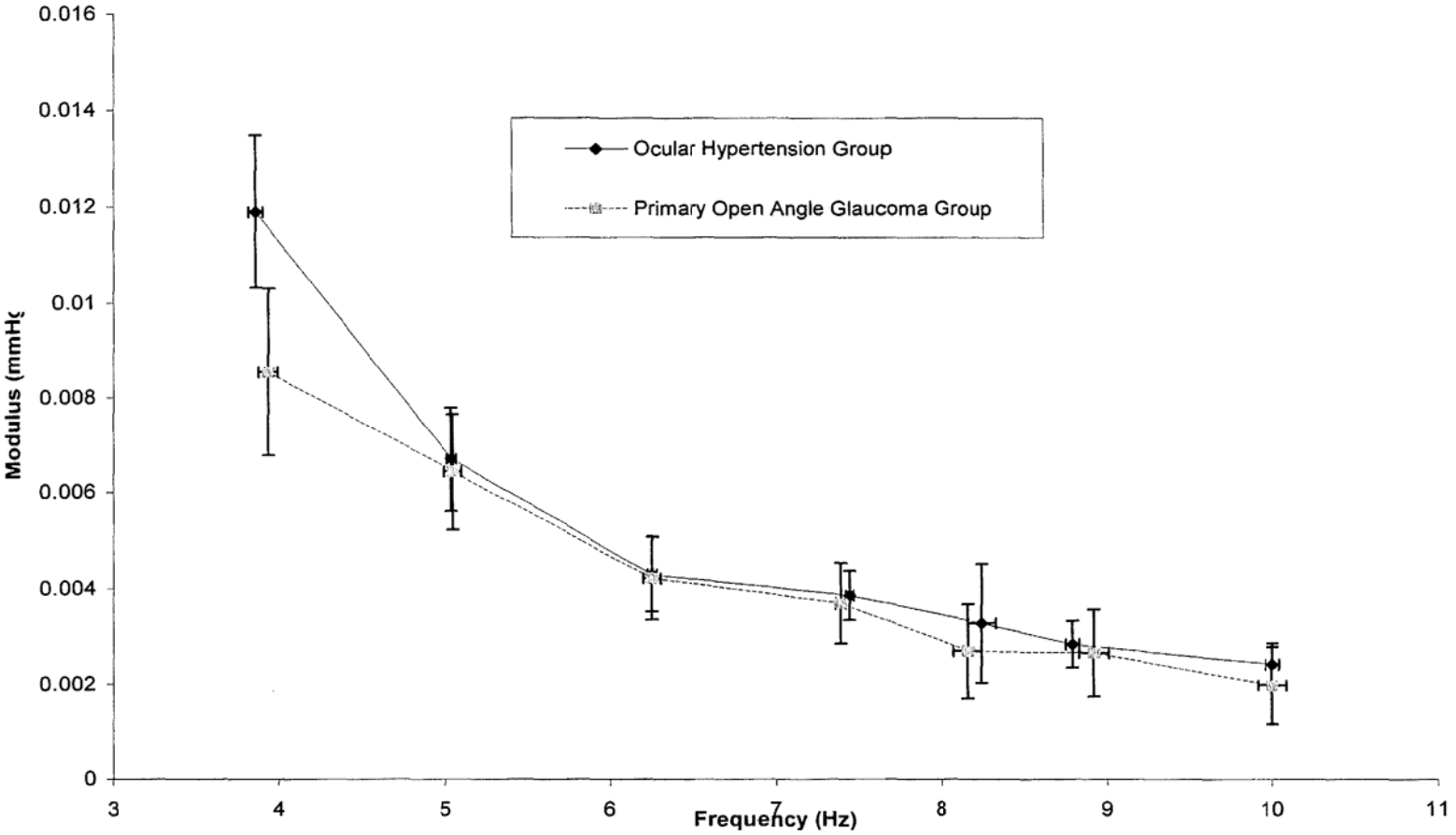

Figure 9.9 **The mean harmonic moduli, in one Hertz frequency bins from 3.5 Hz to 10 Hz, of the intraocular pressure pulse for the normal-tension glaucoma group and normal subject group: error bars indicate one standard error of the mean. No significant difference existed between groups at any of the 1 Hz frequency bins ($p > 0.05$).**

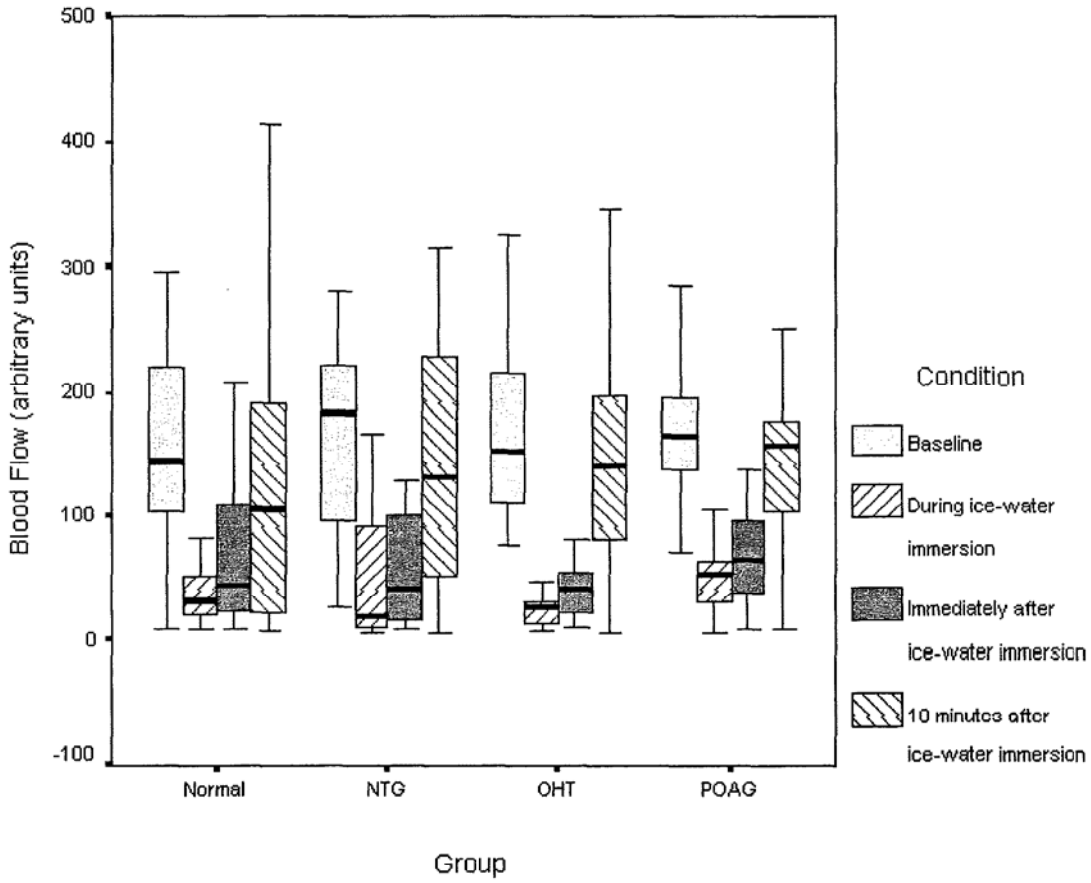

Figure 9.10 **Boxplot display of cutaneous laser Doppler flowmetry measurements taken from the index finger fn the four subject groups at baseline, during and after cold-provocation: normal, normal subject group; NTG, normal-tension glaucoma group; OHT, ocular hypertension group; POAG, primary open angle glaucoma group.**

9.6. *Discussion*

9.6.1. Group characteristics

The strict control of age and IOP between glaucoma groups and their respective control groups allowed comparisons to be faithfully made between the variables of interest. In addition, those physiological factors known to influence the IOP pulse were comparable between groups: gender; heart rate; mean refractive error and axial length; and mean corneal radius (1.6). There were differences between group mixes in terms of diabetes status and numbers of systemic medications. These differences may have introduced confounding variables.

Unsurprisingly the visual parameters of contrast sensitivity and mean deviation in visual field score were significantly worse in the glaucoma groups. The significant reduction in visual acuity of the glaucoma groups was unexpected as these were newly diagnosed patients and reduced VA is not recognised as a sign of early glaucoma. As concurrent eye disease and clinically significant cataract were excluded, it is likely that the reduction in VA was due to glaucoma. This is supported in that the mean difference in log MAR acuity between diseased and normal groups was approximately only 5 letters on the chart and that a minimum level of acuity was not an exclusion criterion for glaucoma patients.

A possible weakness of the present study was the inclusion criteria for the NTG group. Patients were allocated to this group solely on a single presenting IOP measurement by Goldmann tonometry. Accepted guidelines to define a patient as having NTG recommend diurnal IOP phasing and additional procedures, such as MRI scans, to rule out non-glaucomatous causes of visual field loss (Hodapp *et al.*, 1993; Levene, 1980; Stürmer & Meier-Gibbons, 1994). In addition, central corneal thickness has been shown to be an increasingly important factor in the classification of NTG (Copt *et al.*, 1999). Unfortunately, a pachymeter was unavailable for this study. The consequence of these study weaknesses was that the NTG group may not have been as pure a population as ideally required.

9.6.2. Blood pressure values

The systemic arterial pressure pulse of the POAG group was significantly greater than its comparison OHT group. In addition, on comparing between all four groups, systolic and APP was significantly greater in patients with high-tension glaucoma than those with normal IOP values. There was no evidence that NTG patients had abnormally low blood pressure as their values were similar to the two control groups.

There is conflicting data on the role of blood pressure in glaucoma. Large epidemiological studies found that the risk of glaucoma rose with increasing (particularly systolic) blood pressure (Bonomi *et al.*, 2000; Dielemans *et al.*, 1995; Tielsch *et al.*, 1995b). Other, predominately group-control studies, have found systemic *hypo*tension associated with

progressive POAG and NTG (Demailly *et al.*, 1984; Hayreh *et al.*, 1994; Kaiser *et al.*, 1993).Sommer (1996) has squared this circle by suggesting the following. In early systemic hypertension (prior to secondary vascular sclerosis) high perfusion pressure may offer a protective vascular influence to the optic nerve head. In contrast, late stage hypertension may be haemodynamically disabling to the optic nerve through the associated increase in arterial resistance. The significant difference in systolic and arterial pulse pressure between the two glaucoma groups in this study is suggestive of a systemic cardiovascular distinction between these two notional glaucoma groups.

In this study, no difference in the eye's perfusion pressure was found between glaucoma and control groups. Some investigators have found an association between the level of OPP and prevalence of glaucoma but as the OPP calculation is dependent on the level of IOP, the association may arise solely through the latter (Bonomi *et al.*, 2000; Leske *et al.*, 1995; Tielsch *et al.*, 1995b). The present study, which controlled for the level of IOP, does not support the concept of a systemic difference in perfusion pressure as a causative factor in glaucoma. It may be that the proposed fall in OPP to cause optic nerve head ischaemia either occurs transiently, such as in an excessive drop in overnight blood pressure (Graham *et al.*, 1995), or at a more local site in the ocular vasculature (Pillunat *et al.*, 1989).

9.6.3. Traditional IOP pulse parameters

The mean POBF value was significantly lower in the NTG group compared to the control group of normal subjects. This is in agreement with other studies (Fontana *et al.*, 1998; James *et al.*, 1991b; Quaranta *et al.*, 1994; Ravalico *et al.*, 1994) and, as important physiological variables were controlled for, indicates that the pulsatile component of ocular blood flow appears to be, on average, lower in patients with NTG. The diminished POBF value in NTG could occur for a number of reasons.

First, it may represent a causative factor in NTG. Previous authors have speculated that the IOP pulse values in NTG represent diminished ciliary perfusion to the optic nerve head, either through faulty autoregulation (Fontana *et al.*, 1998) or a vasculature under vasospasm (Schmidt *et al.*, 1997). Given the known association between reduced IOP pulse values and carotid artery stenosis (1.7.1), it is natural to ask whether this disease plays a part in a

possible ischaemic induced NTG. Levene (1980)in a major review of NTG, concluded that there was little evidence for carotid artery disease as a cause of NTG. As in the present study, the majority of IOP pulse studies in glaucoma have not investigated or ruled out the presence of carotid artery disease (Pillunat *et al.*, 1989). Future IOP pulse studies would possibly benefit from investigating this known cause of reduced ocular perfusion pressure.

Second, the diminished POBF value may be a consequence of glaucomatous nerve damage: for example, reflecting a reduced demand for nutrients as retinal metabolism falls. Third, a confounding factor may be responsible for the association. James *et al.* (1991b) speculated that an anomalous scleral elasticity could cause glaucomatous damage (through a structurally weaker lamina cribrosa) and lower IOP pulse values (through the effect on the ocular pressure-volume relationship). It is important to reiterate that a diminished POBF value does not necessarily indicate a reduced total ocular blood flow as the proportion of non-pulsatile flow is unknown.

A similar reduction in POBF values for POAG patients in comparison to OHT patients has been reported by previous investigators (Kerr *et al.*, 1998; Trew *et al.*, 1991c), but was not found in this study. A possible cause may have been the significantly greater arterial pressure pulse of the POAG patients inflating the IOP pulse values. A retrospective univariate analysis of POBF values between POAG and OHT groups, with APP as a covariant, supported this possibility: significance level changed from $p = 0.588$ to $p = 0.097$.

9.6.4. IOP pulse amplitude and IOP pulse moduli

Neither simple IOP pulse amplitude values, nor IOP pulse moduli, exhibited mean differences in value between glaucoma groups and their respective control groups. The lack of significant difference found in PA values, in comparison to the POBF values, may reflect a greater variance in this parameter that a volume transfer function removes. Some investigators (Schmidt *et al.*, 1997) who have reported lower mean IOP pulse amplitudes in glaucomatous groups used much larger group sample sizes ($n = 32$). The similar lack of significant difference in IOP pulse moduli indicates that use of a spectral analysis technique offers no improvement over traditional IOP pulse values (e.g. POBF) in differentiating

glaucomatous from healthy eyes. This finding is contrary to an initial report (Evans *et al.*, 2002) that showed comparison of the third to fifth IOP pulse moduli gave greater confidence (over PA, pulse volume and POBF) in separating a glaucoma group from a normal group. As the present study used a more repeatable spectral analysis technique and larger subject samples than the initial report (n =10), it is likely the current finding carries greater evidential weight. A possible explanation to the previous differences in IOP pulse harmonics is the different IOP level in the glaucoma and control group in the initial report; that is, differences in IOP pulse may have arisen solely due to the glaucoma group not being matched in IOP with its control group.

9.6.5. Cutaneous blood flow

The present study did not find any significant prevalence of a vasospastic tendency or anomalous finger blood flow values in the glaucoma groups. This contradicts the findings of a number of earlier authors (Drance *et al.*, 1988; Gasser *et al.*, 1991; O'Brien *et al.*, 1999). Drance *et al.* (1988) using a similar LDF measurement and cold-provocation test as in the present study, found NTG patients (without migraine) exhibited both lower baseline flow values, and lower flow values in response to ice-cold water, than normal controls without migraine. Obscurely the results for NTG patients with migraine were not presented. Gasser *et al.* (1991) used nailfold video capillaroscopy and a blast of cold air over their subjects' fingers to provoke a vasospastic reaction. Vasospasm, defined as a blood-flow standstill in the nailfold capillaries of greater than 12 seconds, was more common in the NTG group than the POAG or normal control group. Using a similar cold-provocation and LDF system as the present study O'Brien *et al.* (1999) found their NTG group to have lower minimum flow values and longer recovery times than both POAG and normal control subjects.

Possible reasons why the present study did not repeat the observations of the above are that the NTG group was not a pure population or that the exact methodology differed between groups. Certainly the acknowledged high level of variance in cutaneous LDF studies makes differentiation between possible groups difficult and a validated technique for cold-provocation would help minimise inter-study differences (Allen *et al.*, 1992; Braverman, 1997). The present study is not alone in being unable to find anamolous finger blood flow values in NTG patients (Rojanapongpun & Drance, 1993b; Usui *et al.*, 1992). In recent years, the Vancouver group has modified their position on the association of vasospasm and

glaucoma (Broadway *et al.*, 1998); rather than being associated with NTG patients, vasospasm is apparently more prevalent in a subgroup of POAG known as 'focal ischaemic' glaucoma (Spaeth, 1994). However as this association was based on a selected 38 pure 'focal ischaemic glaucoma' patients (out of a total of 1870 glaucoma patients), this finding may be difficult to corroborate.

Other investigators have proposed that the simple questioning of a patient as to whether they suffer from 'cold hands', or through the clinical impression of shaking a patient's hand, is a better determinant of a patient's vasospastic tendency than a formal finger blood flow test (Broadway *et al.*, 1999; Guthauser *et al.*, 1988; Hasler, Orgül, Gugleta *et al.*, 2002). Such clinical techniques however may be criticised for subjective bias. The present study used a recognised grading scale for vasospasm and although scores were higher in the glaucoma groups, the difference was not significant.

Baseline finger blood flow values in the present study were significantly dependent on gender, with female subjects demonstrating greater blood flow. Gender differences in cutaneous blood flow have been found using LDF on the forearm (Bircher, de Boer, Agner *et al.*, 1994; Rodrigues, Pinto & Leal, 2001). There is evidence that such flow differences are due to gender specific levels of circulating ET-1 and the proportion of endothelin system receptors in the cutaneous vasculature (Kellogg, Liu & Pergola, 2001). It is possible that the effect of gender on finger blood flow is responsible for some of the findings in the above studies. Raynaud's disease is more prevalent in females and the female to male ratio in focal ischaemic glaucoma is approximately 3:1 (Flammer *et al.*, 2001; Spaeth, 1994). In two of the above studies that found NTG patients to have anomalous finger blood flow values, the gender mix of the groups was not reported (Drance *et al.*, 1988; O'Brien *et al.*, 1999). In the third study, the gender mix in each group was not equal (Gasser *et al.*, 1991). The role of gender in determining a glaucoma patient's finger blood flow and his or her response to cold provocation is a possible area for further investigation.

The proportion of subjects reporting a history of migraine was not significantly different between groups in the present study. Phelps *et al.* (1985) first found an association between migraine and NTG and speculated that, as migraine is a vasospastic disorder, there may be an underlying common aetiology. A reported history of migraine has been found to be an

increased risk factor for the rate of progressive visual field loss in untreated NTG patients (Drance *et al.*, 2001). The small numbers of subjects in the present study may have been responsible for the lack of significant difference in migraine prevalence. Alternatively the present study agrees with others that have failed to find an increased prevalence of migraine in patients with NTG or POAG (Klein *et al.*, 1993; Usui, Iwata, Shirakashi *et al.*, 1991).

9.6.6. Conclusion

This study found evidence of a reduction in the pulsatile fraction of ocular blood flow in NTG patients. In addition differences in systolic and arterial pulse pressure suggest there is a cardiovascular distinction between normal- and high-tension glaucoma. Spectral analysis of the IOP pulse did not provide greater sensitivity in differentiating glaucomatous eyes from normal eyes over current pulse parameters such as the POBF measure. Finger blood flow measurements were found to be gender specific. It is recommended that any future studies in the relationship between glaucoma and acral vasospasm recognise this factor in cutaneous blood flow differences. In addition, as possible subcategories of POAG (e.g. focal ischaemic glaucoma) are gender specific, this finding may offer future investigative opportunities in the role of vasoreactivity in POAG.

10. Intraocular Pressure Pulse Parameters and Systemic Vascular Characteristics in Diabetes Mellitus

10.1. *Abstract*

Purpose: To investigate the IOP pulse and its relation to haemodynamic and haemoglycaemic values in diabetic patients with and without retinopathy.

Methods: Measurements were taken on four age-matched groups (each $n = 20$): normal control subjects (age 63.0 ± 8.1 yrs); diabetic patients with no retinopathy (age 60.6 ± 9.5 yrs); diabetic patients with background retinopathy (age 60.1 ± 10.5 yrs); and diabetic patients with preproliferative retinopathy (age 60.9 ± 8.5 yrs). A pneumatonometer was used to take IOP pulse measurements on one eye only of each subject. Traditional measurements of IOP pulse (PA and POBF) were compared to the moduli of the groups' IOP pulses which were calculated from a previously described spectral analysis technique. Blood pressure parameters were measured using an automated sphygomomanometer and percentage glycosylated haemoglobin levels were taken from a venous blood sample. Cutaneous blood flow was measured over the patient's temple ipsilateral to, and at the same level to, the eye under investigation using cutaneous laser Doppler flowmetry.

Results: One-way ANOVA found the mean POBF values significantly greater ($p = 0.037$) in diabetic patients with background retinopathy (1370 (415) μl/min) compared to normal control subjects (1047 (316) μl/min) and diabetic patients without retinopathy (1050 (313) μl/min). Neither PA nor IOP pulse moduli showed any significant difference amongst groups. Systemic arterial pressure pulse was found to contribute greatest variance to the POBF measurement (part-correlate +0.61, $p < 0.001$). Neither cutaneous LDF measurement nor glycosylated haemoglobin level was found to have any association with the IOP pulse parameters.

Conclusion: This study found that the pulsatile fraction of ocular blood flow to be greater in diabetic patients with background retinopathy than either normal subjects or non-retinopathic diabetic patients. Raised POBF values may reflect the greater prevalence of augmented arterial pulse pressures found in diabetic patients with retinopathy. It is recommended that future investigations study this possible confounding or causative factor in diabetic retinopathy further.

10.2. *Introduction*

As reviewed in Chapter 1, the diabetic eye has been shown to demonstrate anomalous blood flow in the retinal and choroidal circulations. As with other methods of measuring ocular blood flow, published results have been inconsistent in describing the association between IOP pulse value and the degree of diabetic retinopathy (Esgin *et al.*, 2001; Geyer *et al.*, 1999; Langham *et al.*, 1991b; MacKinnon *et al.*, 1997). As spectral analysis provides a technique for measuring change in the IOP pulse with greater confidence (Chapter 7) and high repeatability (Chapter 6) in comparison to traditional IOP pulse parameters, it would be of interest to apply this technique to the IOP pulse waves of diabetic subjects. It has been hypothesised that the higher harmonics of the IOP pulse contain characteristics of the intraocular vascular beds such as arterial compliance (Evans *et al.*, 2002). Waveform analysis of the arterial pressure pulse has found diabetic patients to exhibit anomalous values that are attributed to the reduced compliance of the arterial vessel wall in this diseased group (McVeigh, Brennan, Hayes *et al.*, 1993). Spectral analysis of the IOP pulse provides the opportunity to investigate whether any such similar waveform abnormalities exist in diabetic patients.

High blood pressure is now a recognised risk factor in the development of diabetic complications, including diabetic retinopathy (Aiello, Cahill & Wong, 2001). Whether retinopathy is advanced through the direct damaging effects of high perfusion pressure on the diabetic vascular endothelium, or indirectly (through, for example, anomalous autoregulation of diabetic retinal vessels) is not completely known (Quigley & Cohen, 1999; Rassam *et al.*, 1995). Esgin *et al.* (2001) found blood pressure to be an important factor in the POBF values in their group of untreated diabetic patients. The possible influential role of blood pressure in IOP pulse studies and diabetic retinopathy requires further investigation.

The blood glucose level of a diabetic patient has been shown to affect ocular blood flow, including IOP pulse values (Findl *et al.*, 2000a; Perrott *et al.*, 2001). This additional source of variance is worthy of investigation as it may further explain the contradictory results in previous IOP pulse studies in diabetes.

Diabetic patients exhibit anomalous skin blood flow characteristics (Benbow, Pryce, Noblett *et al.*, 1995). The anomalous flow values are believed to be due to the degree of diabetic neuropathy in a patient. No previous comparisons of cutaneous and ocular blood flow in diabetic patients appear to have been reported. As retinal and epidermal vessels both represent microvascular beds any association between skin blood flow and the degree of retinopathy would be of interest.

10.3. *Aims*

The purpose of this study was to investigate the IOP pulse, using traditional and newly developed analyses, in diabetic eyes with varying severity of retinopathy. A further aim was to investigate any association between IOP pulse value and the values of blood pressure, glycaemia and cutaneous blood flow.

10.4. *Methods*

10.4.1. Experimental design

This was a group control study that compared a group of normal control subjects with groups of diabetic subjects with differing severity of retinopathy. The groups were matched in terms of age and IOP.

10.4.2. Ethical Approval

All experimental procedures of the study conformed to the tenets of the declaration of Helsinki and were approved by the East Birmingham Local Research & Ethics Committee and the Aston University Human Science Ethical Committee. Written informed consent was obtained from all subjects willing to participate in the study.

10.4.3. Subject Sample

Diabetic subjects were recruited from patients attending the ophthalmology out-patient department at Birmingham Heartlands Hospital. Both insulin and non-insulin dependent diabetic patients were recruited. Patients were allocated to one of the four following

groups: no retinopathy; background retinopathy; pre-proliferative retinopathy; and proliferative retinopathy. Allocation to a group was made by an experienced ophthalmologist using dilated ophthalmoscopy and, when necessary, fluorescein angiography. The clinical signs used in differentiating the four diabetic retinopathy grades are found in Table 10.1.

Diabetic Retinopathy Grade	**Clinical Signs**
No retinopathy	No retinal diabetic signs seen on dilated ophthalmoscopy or retinal photography
Background retinopathy	No retinal diabetic signs other than the following: • retinal haemorrhages • retinal microaneurysms • retinal exudates • one or two cotton wool spots isolated to one quadrant
Pre-proliferative retinopathy	Background retinopathy lesions plus one or more of the following: • venous beading • venous loops • intra-retinal microvascular abnormalities (IRMA) • large, deep intra-retinal haemorrhages • multiple cotton wool spots in two or more quadrants
Proliferative retinopathy	New vessels at disc or elsewhere on retina

Table 10.1 Clinical signs used in the allocation of a diabetic patients eye to a retinopathy grade group.

Patient exclusion criteria are shown in Table 10.2.

- Past or present ocular disease other than diabetic related
- Previous ocular surgery including laser photocoagulation
- Significant lens opacification (grade 2 or more, Oxford Clinical Cataract Classification and Grading System) (Brown *et al.*, 1987)
- Intraocular pressure greater than 21 mmHg
- Physical or mental incapacity to perform experimental procedures

Table 10.2 Diabetic study subjects' exclusion criteria

10.4.4. Control Sample

Non-diabetic control subjects were recruited from optometric practice. All prospective control subjects underwent a full eye examination and were excluded for any of the reasons found in Table 10.3.

- Reported history of present or past eye disease
- Diabetes mellitus
- Previous ocular surgery
- Corrected visual acuity of less than 6/9
- Visual field defect as detected by automated perimetry
- Corneal or anterior chamber abnormalities as detected by slit-lamp examination
- Significant lens opacification (grade 2 or more, Oxford Clinical Cataract Classification and Grading System)(Brown, 1987)
- Posterior segment abnormalities as detected by slit-lamp binocular indirect ophthalmoscopy
- Intraocular pressure greater than 21 mmHg
- Physical or mental incapacity to perform experimental procedures

Table 10.3 Exclusion criteria used for the normal control subjects in the diabetic study.

10.4.5. Experimental Procedures and instrumentation

Ocular measurements were taken from one eye only of each subject. For suitable diabetic patients the choice of eye measured was made using the following criteria:

- the eye that qualified by inclusion criteria (for example, the fellow having had laser photocoagulation)
- if both eyes qualified, the eye with the most advanced retinopathy grade
- if both eyes qualified and were equal in retinopathy grade, a randomly chosen eye

For control subjects, measurements were taken on a randomly chosen eye (sealed envelope method). All measurements were taken in a single appointment with the patient in the seated position. Measurements were taken after 20 minutes of rest in order to stabilise cardiovascular and cutaneous blood flow measurements. The sequence of the study procedures is shown in Table 10.4.

1. Patient history
2. Subjective refraction
3. Visual parameters
Visual acuity
Contrast sensitivity
Visual fields
4. 1st blood pressure and heart rate measurement
5. Pneumatonometry measurements
6. 2nd blood pressure and heart rate measurement

Table 10.4 Sequence of diabetic study procedures

The order of study procedures was chosen for the following reasons. Pneumatonometry was performed towards the end in order that tests of visual function were not affected. Two measurements of blood pressure and heart rate, before and after the pneumatonometry measurements, were taken in order to confirm cardiac stability. The venous blood sample was taken last as it involved the patient walking to a nearby venepuncture clinic.

Visual function

A subject's visual acuity was measured using a logMAR chart and recorded following the recommended procedure described by Bailey *et al.* (1991). Contrast sensitivity was measured using a Pelli-Robson chart following the original scoring procedure (Pelli *et al.*,

1988). Visual field analysis was performed using the SITA 24-2 program (Humphrey Field Analyzer, Carl Zeiss Ophthalmic Systems, Inc., Humphrey division, Dublin, California). Due to the possibility of corneal curvature influencing IOP pulse measurements, mean corneal radius was measured using a Javal-Schiötz keratometer. The measurement of axial length in this study was not possible.

Blood pressure parameters

Measures of systemic blood pressure and heart rate were made using a validated (Shennan *et al.*, 1998) automated sphygmomanometer (Omron Rx, Omron Matsusaka Co. Ltd, Japan). The two blood pressure measurements were averaged. In addition the parameters of APP, MAP and OPP were also calculated as previously described (Equations 1.6, 1.7 and 1.11).

Pneumatonometry

Pneumatonometry measurements were taken using the OBFA pneumatonometer (Paradigm Medical Ind., Utah, USA) mounted on a slit-lamp, following topical anaesthesia of the subject's eye with one drop of 0.4% benoxinate HCl (*Minims*®, Chauvin, UK). Measurements of IOP, PA and POBF were calculated automatically, by the pneumatonometer's software, from five or more IOP pulses that it considered acceptable from a maximum recording duration of 20 seconds. Calculation of the IOP pulse's moduli was performed by analysing a 10 second continuous IOP waveform as previously described (7.4). Both the Power Spectral Density technique and the averaged single pulse technique were used to analyse the IOP pulse moduli.

Cutaneous blood flow

Measurements of cutaneous blood flow were taken using a laser Doppler flowmeter (PF 5010: Perimed, Stockholm, Sweden). The principle of cutaneous LDF has been previously described (9.4.5). The flowmeter's fibre optic probe was attached to the skin of the patient's temple ipsilateral to, and at the same level of, the eye under study. Cutaneous temple flow measurements were taken as the mean of a 30 second, artefact free, recording of a maximum flow plateau after at least 20 minutes rest in a seated position.

Glycaemic control

In order to assess the possible influence of glycaemic control upon IOP pulse parameters, the percentage of glycosylated haemoglobin (HbA_{1c}) of each diabetic subject was measured at the end of the appointment from a venous blood sample. In brief, HbA_{1c} provides an index of the average blood glucose concentration over the life of the haemoglobin molecule (approximately 6 weeks). All blood samples were measured in the same clinical blood chemistry department of Birmingham Heartlands Hospital.

10.4.6. Statistical analysis

Data were first examined in order to verify normality of distribution (Norman *et al.*, 2000a). Data showing significant skew or kurtosis were transformed (natural log) in order to better approximate a normal distribution. Data were presented in the format of their mean (standard deviation) values. Comparisons of interval data between groups were initially made using one-way ANOVA, with post-hoc comparisons of paired data made using Tukey's (LSD) test. Variables that contributed significant variance to the dependent parameter were isolated using stepwise multiple regression analysis. Any such isolated variables were then used in a factorial ANCOVA using subject group and gender as factors. Comparisons of ordinal and categorical data between groups were made using the Kruskal-Wallis test, with post-hoc comparisons of paired data made using either the chi-squared or Mann-Whitney U test.

10.5. *Results*

10.5.1. Background characteristics

20 subjects were recruited to each group except that of the proliferative retinopathy group. Only four patients were recruited to this latter group. Diabetic patients with untreated proliferative retinopathy were uncommon in the hospital recruitment clinic due to the practice of applying pre-emptive laser photocoagulation and the short window of time between diagnosis and treatment. The proliferative retinopathy group was therefore discarded and only the remaining four groups were analysed. The clinical characteristics of these four groups are shown in Table 10.5. There was no significant difference between groups in clinical characteristic, except as follows. There was a significantly greater

proportion of non-caucasian subjects in the pre-proliferative group compared to the normal subject group (chi-squared comparison, $p = 0.032$). Compared to the normal subject group, visual acuity was significantly reduced in the subject groups with background retinopathy ($p = 0.001$) and pre-proliferative retinopathy ($p = 0.035$). Similarly, compared to the normal subject group, contrast sensitivity values were significantly worse in the subject groups with background retinopathy and pre-proliferative retinopathy (both $p < 0.001$). The mean deviation in visual field score was significantly lower in the pre-proliferative retinopathy group in comparison to the group of normal subjects ($p = 0.024$).

Parameter	**Normal subjects**	**Diabetic subjects**			**Significance**
		No retinopathy	**Background retinopathy**	**Pre-proliferative retinopathy**	
Gender (m/f)	11/9	12/8	13/7	16/4	N.S.
Race (Caucasian/Afro-Caribbean/Oriental/ Asian)	20/0/0/0	16/3/0/1	16/1/3/0	14/3/3/0	$p = 0.032$
Age (yrs)	63.0 (8.1)	60.6 (9.5)	60.1 (10.5)	60.9 (8.5)	N.S.
Intraocular pressure (mmHg)	14.2 (3.9)	14.0 (3.2)	15.9 (3.4)	15.1 (4.2)	N.S.
Mean spectacle Rx (D.S.)	-0.13 (2.57)	0.31 (1.55)	0.66 (1.17)	0.56 (1.40)	N.S.
Visual acuity (logMAR)	-0.08 (0.07)	-0.01 (0.11)	0.09 (0.17)	0.04 (0.16)	$p = 0.001$
Contrast sensitivity (Pelli-Robson)	1.60 (0.11)	1.47 (0.19)	1.26 (0.24)	1.31 (0.24)	$p < 0.001$
Mean Deviation in visual field score	-0.46 (1.17)	-1.78 (1.88)	-3.73 (5.58)	-4.29 (5.22)	$p = 0.011$
Mean corneal radius (mm)	7.69 (0.19)	7.65 (0.28)	7.68 (0.26)	7.76 (0.31)	N.S.

Table 10.5. Characteristics of each subject group. Values presented as mean (SD) values.

In the diabetic groups, the proportion of insulin-dependent to non-insulin patients was 7:13, 11:9, 10:10 for the no retinopathy, background retinopathy and pre-proliferative retinopathy

groups respectively. Blood pressure, heart rate and other blood pressure-derived values for the groups are found in Table 10.6. No significant differences between groups' blood pressure parameters were indicated with one-way ANOVA.

Parameter	Normal subjects	Diabetic subjects			Significance
		No retinopathy	Background retinopathy	Pre-proliferative retinopathy	
Systolic blood pressure (mmHg)	130.9 (16.9)	136.6 (16.2)	144.8 (18.3)	144.1 (28.5)	N.S.
Diastolic blood pressure (mmHg)	75.6 (11.5)	79.8 (9.3)	81.2 (12.1)	83.2 (11.1)	N.S.
Arterial pulse pressure (mmHg)	55.3 (12.3)	56.9 (12.9)	63.7 (15.3)	60.9 (26.0)	N.S.
Mean arterial blood pressure (mmHg)	93.8 (12.3)	98.73 (10.43)	102.37 (12.51)	103.48 (14.2)	N.S.
Mean ocular perfusion pressure (mmHg)	48.6 (8.5)	51.8 (7.0)	52.4 (7.7)	53.9 (10.2)	N.S.
Heart rate (beats/minute)	69.2 (11.2)	72.7 (13.4)	75.3 (13.3)	77.1 (14.7)	N.S.

Table 10.6 **Blood pressure, heart rate and other blood pressure-derived values for the normal subject and diabetic groups. Values presented as mean (SD) values.**

10.5.2. Traditional IOP pulse parameters

No significant difference existed in PA value amongst normal or diabetic groups (Figure 10.1). A significant difference in POBF value did exist amongst groups (one-way ANOVA, $p = 0.037$): Figure 10.2. Tukey's LSD post-hoc comparison test found mean POBF values significantly greater in diabetic patients with background retinopathy (1370 ± 415 μl/min) compared to normal control subjects (1047 ± 316) μl/min) and diabetic patients without retinopathy (1050 ± 313 μl/min): $p = 0.012$ and $p = 0.013$ respectively.

10.5.3. IOP pulse moduli

Figure 10.3 and Figure 10.4 show the IOP pulse moduli for the normal control and diabetic groups as calculated using the ASP technique. No significant difference between groups existed at any of the one Herz frequency bins. Similarly no difference in group moduli was found using the alternative PSD technique.

10.5.4. Further analysis of the POBF values

For all subjects, stepwise multiple regression analysis found APP, heart rate and IOP to be significant correlates of the POBF value: part correlates were +0.61 ($p < 0.001$), +0.20 ($p = 0.025$), and -0.23 ($p = 0.011$) respectively. APP, heart rate and IOP accounted for 38% of the variance in total of the POBF value: APP alone accounted for 32% of the variance in the POBF value (Figure 10.5).

Factorial ANCOVA was then performed on the POBF values using the above important covariates (APP, heart rate and IOP). The resulting analysis found gender to be a significant factor ($p = 0.003$) but not subject category. The mean POBF value for the total 52 male subjects was 1117 (427) μl/min and 1264 (378) μl/min for the total 28 female subjects.

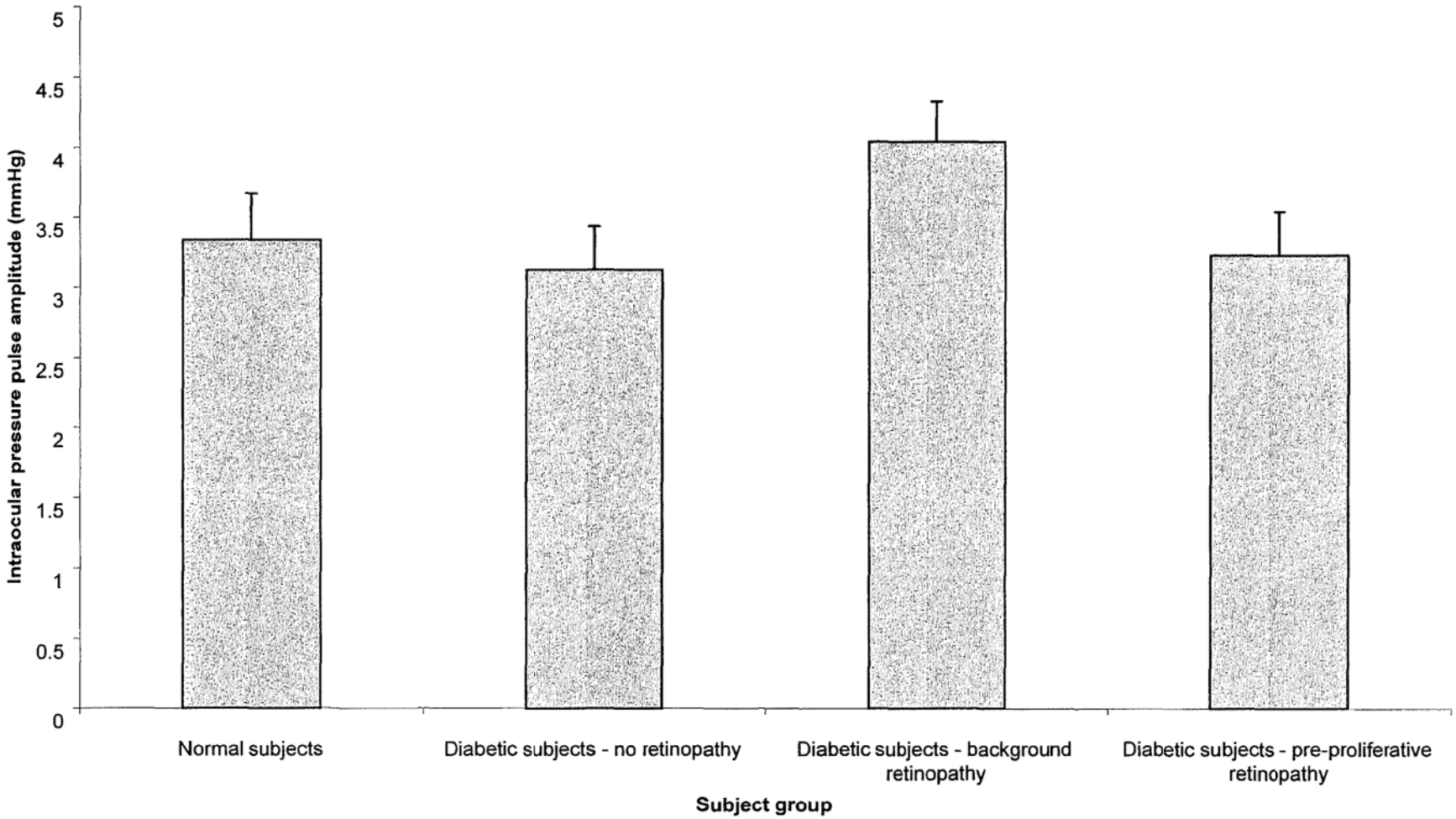

Figure 10.1 **Intraocular pressure pulse amplitude mean values for the normal control and diabetic groups. Error bars represent one standard error of the mean. No significant difference between group values ($p > 0.05$).**

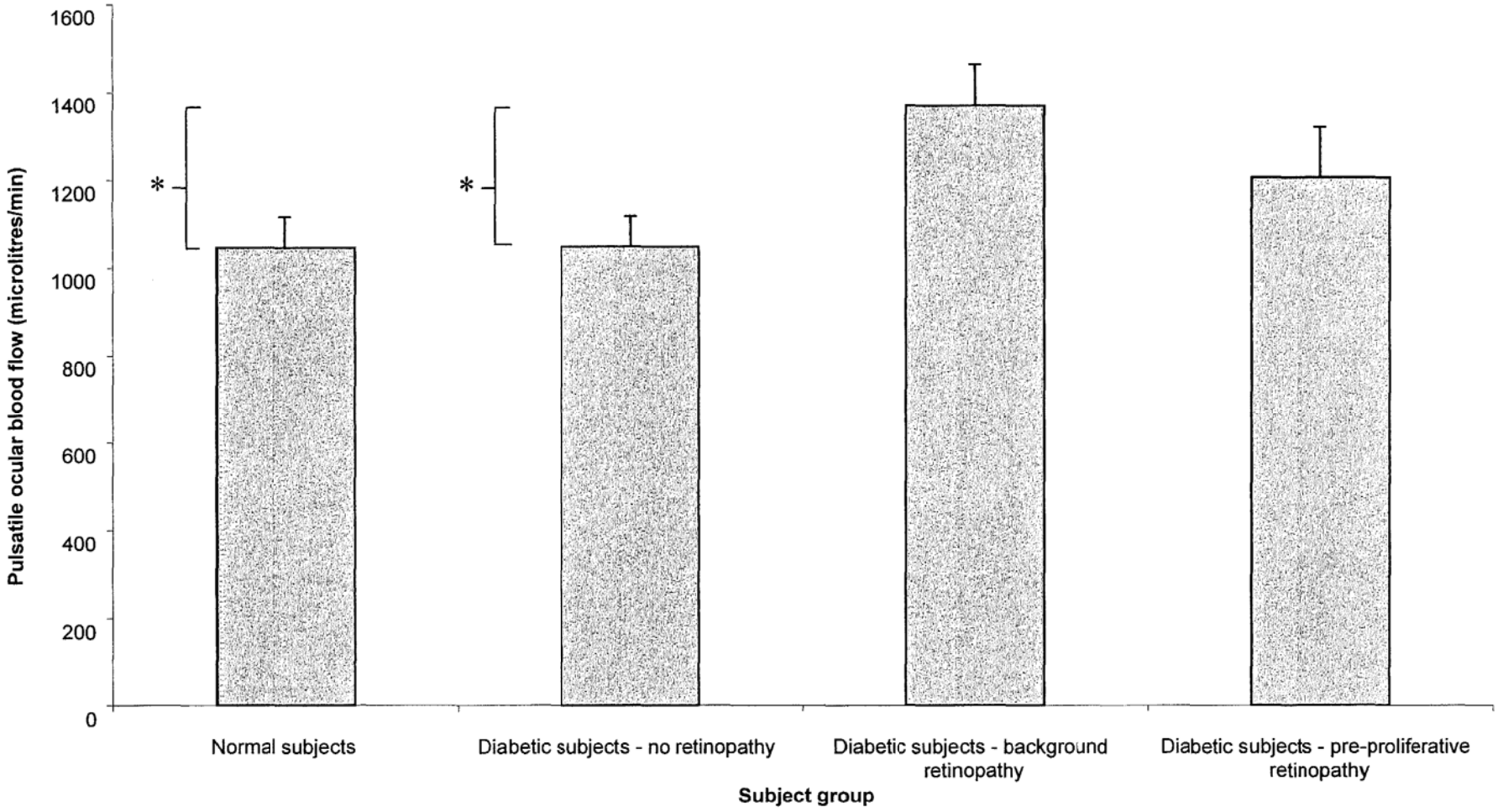

Figure 10.2 Pulsatile ocular blood flow mean values for the normal control and diabetic groups. Error bars represent one standard error of the mean. * indicates a significant difference between mean values at the $p < 0.05$ level (Tukey's LSD post-hoc comparison test).

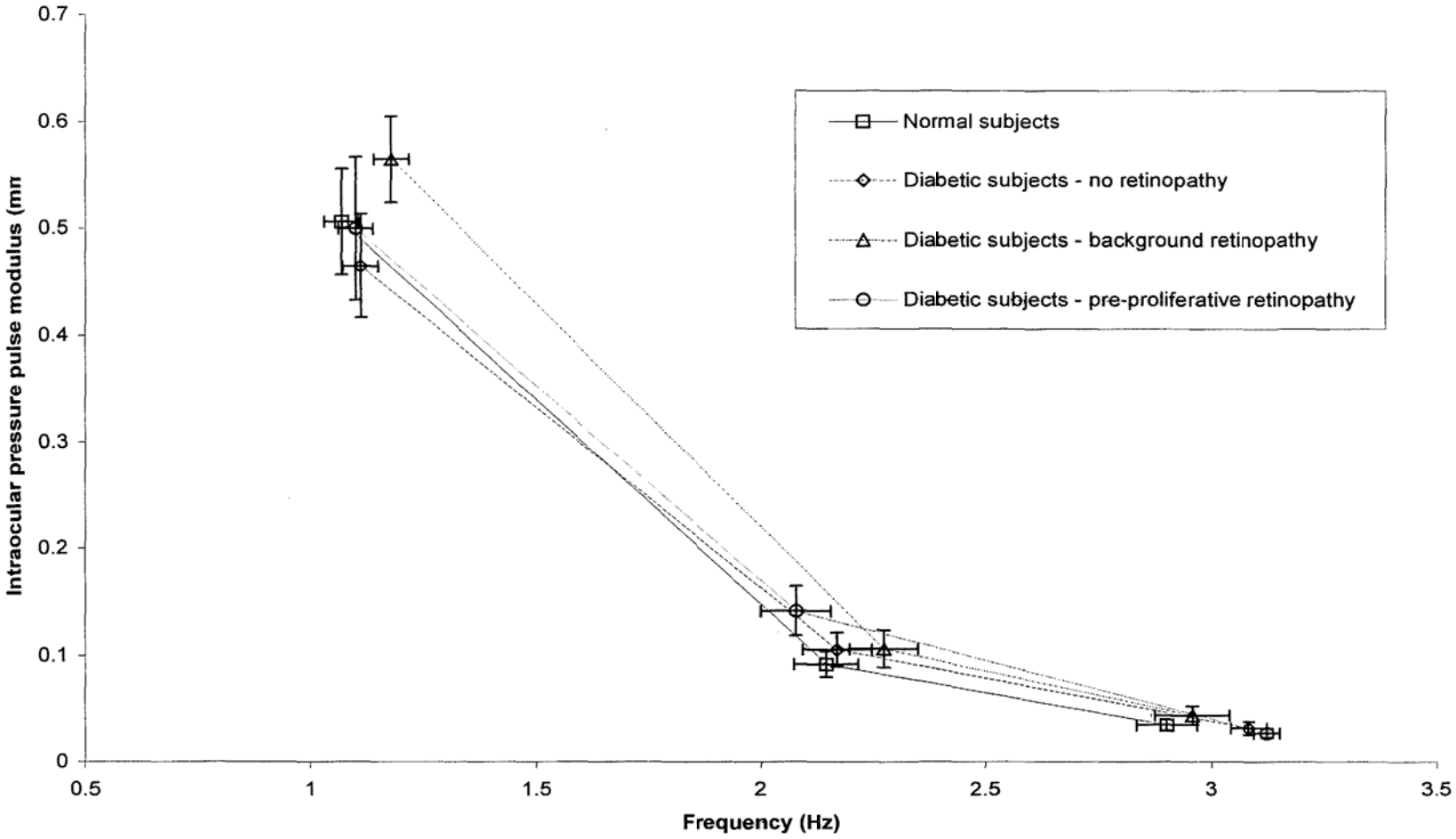

Figure 10.3. The mean harmonic moduli, in one Hertz frequency bins up to 3.5 Hz, of the intraocular pressure pulse for the normal control and diabetic groups: error bars indicate one standard error of the mean. No significant difference existed between groups at any of the 1 Hz frequency bins ($p > 0.05$).

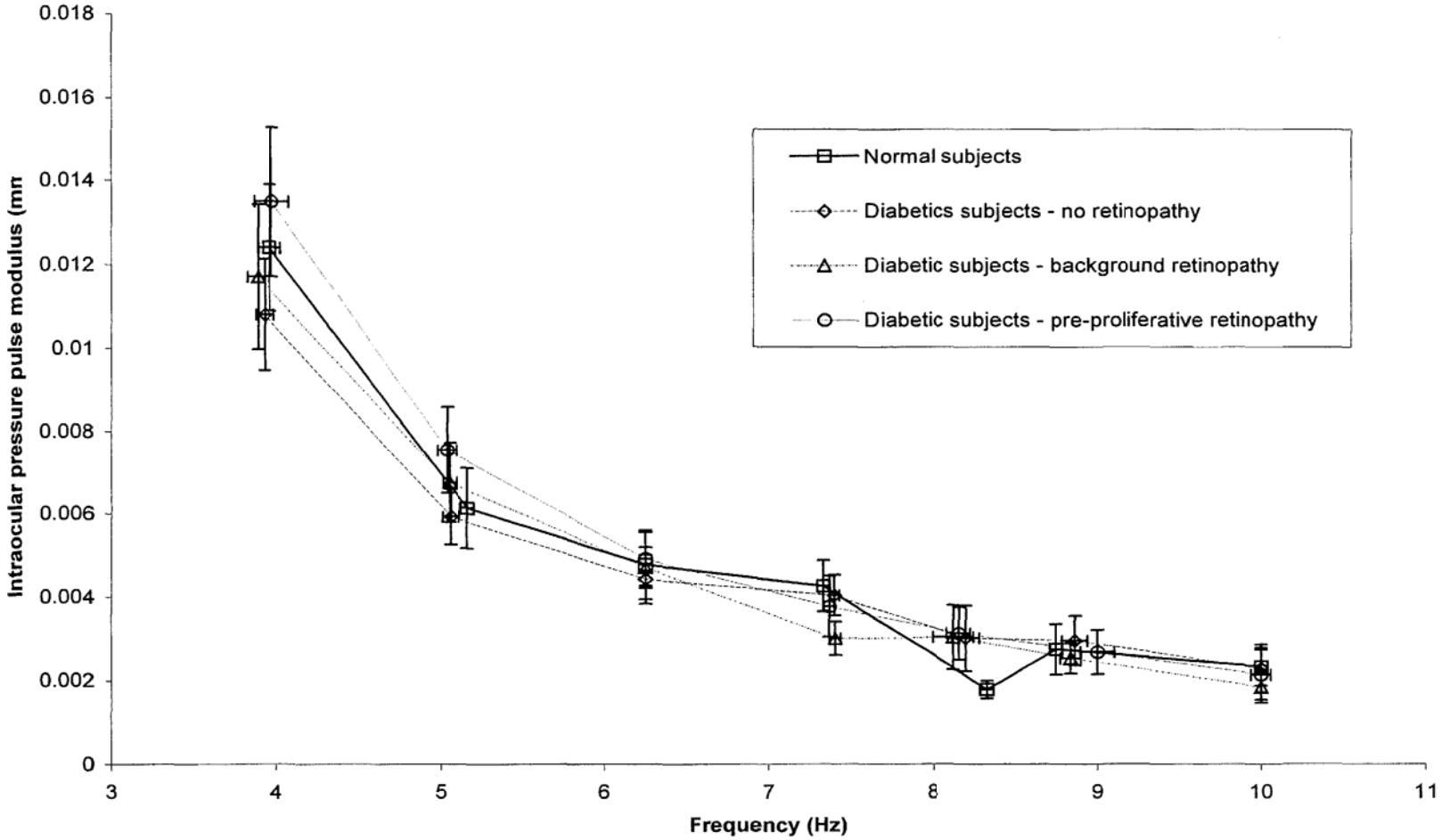

Figure 10.4. The mean harmonic moduli, in one Hertz frequency bins from 3.5 to 10 Hz, of the intraocular pressure pulse for the normal control and diabetic groups: error bars indicate one standard error of the mean. No significant difference existed between groups at any of the 1 Hz frequency bins ($p > 0.05$).

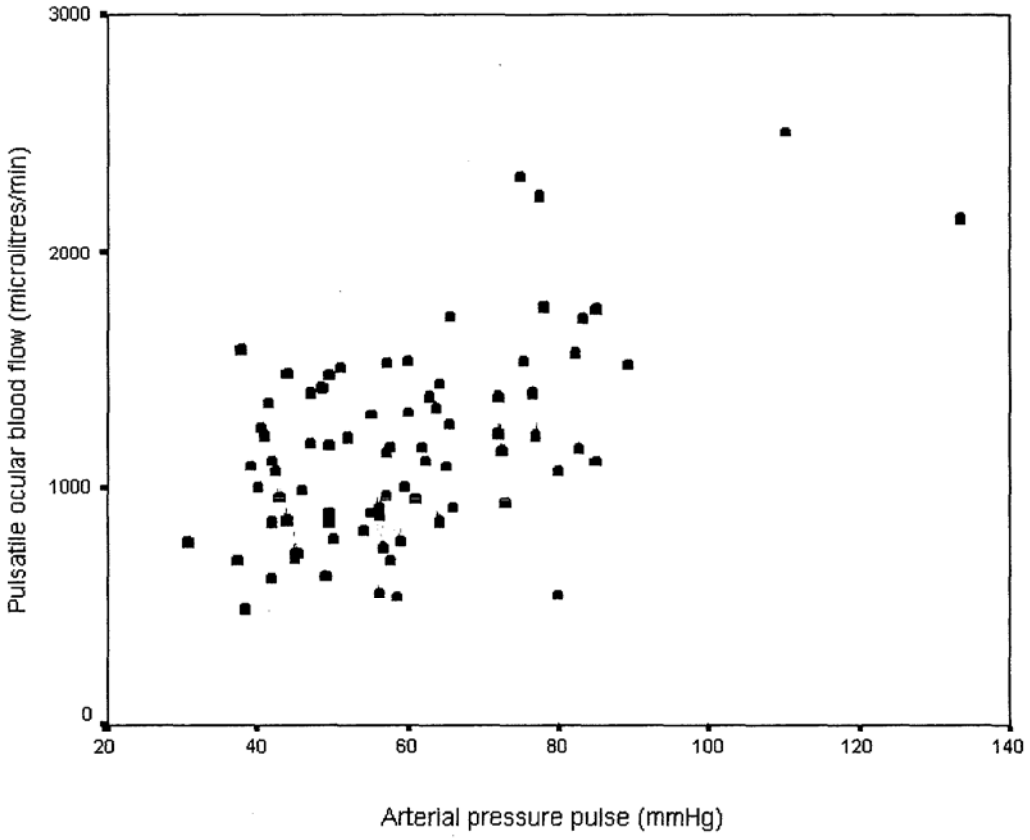

Figure 10.5. Scatterplot of all subjects pulsatile ocular blood flow values against their arterial pressure pulse value

10.5.5. Cutaneous blood flow

The cutaneous LDF values exhibited significant positive skew and were log transformed before analysis. There was no significant difference in mean cutaneous LDF value between subject groups. Stepwise multiple regression analysis found only heart rate to be a significant correlate to cutaneous LDF value (part-correlate +0.22, $p = 0.047$) of the considered variables of SBP, DBP, MAP, heart rate and POBF value. Factorial ANCOVA, with heart rate as a covariant, identified gender ($p = 0.001$) but not subject group to be a significant factor in cutaneous temple LDF value: the 52 male subjects had, on average, an LDF flow value 15.5% higher than the 28 female subjects (Figure 10.6).

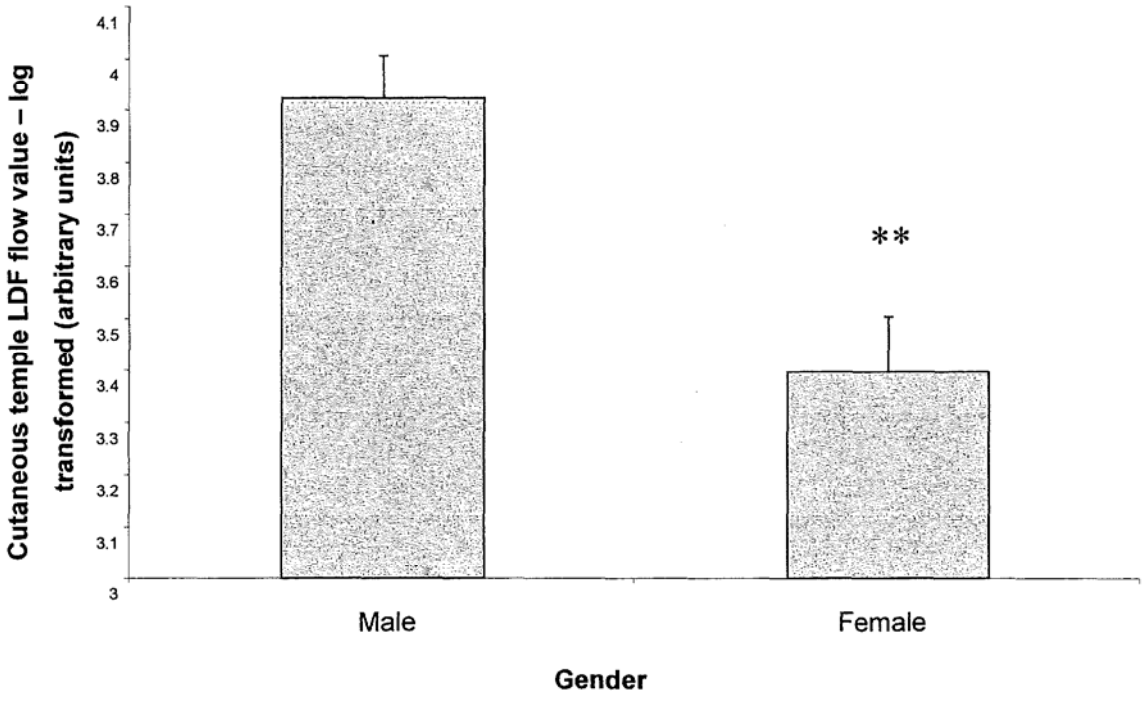

Figure 10.6 **Cutaneous temple LDF flow values (log transformed) for the total male and female subjects of all normal control and diabetic groups. Error bars represent one standard error of the mean. ** indicates a significant difference between groups at the $p < 0.01$ level.**

10.5.6. Glycosylated haemoglobin values

For the 60 diabetic subjects, glycosylated haemoglobin results were successfully taken in 55 patients (blood test results were missing from one subject of the no diabetic retinopathy group and two subjects of both the background and pre-proliferative retinopathy groups). No significant difference in HbA_{1c} existed between diabetic group: Table 10.7. Multiple regression analysis found no association between HbA_{1c} and either IOP pulse amplitude value, POBF value, or cutaneous LDF flow value.

	Diabetic group		
	No retinopathy	Background retinopathy	Pre-proliferative retinopathy
Percentage glycosylated haemoglobin level	6.99 (1.78)	7.58 (1.32)	7.85 (1.79)

Table 10.7 **Glycated haemoglobin values for the three diabetic subject groups. Values are presented as mean (SD). No significant difference between groups ($p > 0.05$).**

10.6. *Discussion*

This study found diabetic patients with background retinopathy to have significantly greater levels of POBF than either normal controls or diabetic patients without retinal complications.

10.6.1. Background characteristics

The poor recruitment of patients having untreated proliferative retinopathy was disappointing but similar to the experiences found by other investigators (Geyer *et al.*, 1999; MacKinnon *et al.*, 1997). The remaining groups of control subjects and diabetic patients were successfully matched in terms of age and, fortuitously, in IOP. In addition other physiological variables (gender, heart rate, mean spectacle refraction, keratometry) that may affect IOP pulse values were similar between groups. The racial mix in the groups was significantly different. Although no associations between race and IOP pulse value have been reported (Chapter 1) this difference may have been a confounding variable. NIDDM is twice as prevalent in people of Afro-Caribbean ancestry, and three to five times more prevalent in people from South Asia, than in white Europeans (Kumar & Clark, 1994). This group control study may have been improved by sampling a similar racial mix for the normal control subjects.

10.6.2. Visual function and diabetic retinopathy

Unsurprisingly the visual parameters of visual acuity and contrast sensitivity were reduced in the diabetic groups with retinopathy compared to the normal controls. In addition, at the level of pre-proliferative retinopathy, visual field performance was significantly reduced in comparison to the normal control group. The reduction in visual field performance as diabetic retinopathy advances has been previously reported and correlates with the degree of ischaemia present in the retina (Henricsson & Heijl, 1994; Pahor, 1998).

10.6.3. Blood pressure parameters

Mean arterial blood pressure increased with severity of retinopathy but only approached significance ($p = 0.067$). The additional risk of high blood pressure in the development of diabetic retinopathy is well established and greater subject numbers in the study may have

produced significance. The result that MAP was closer to attaining significance over OPP suggests that it is not simple perfusion pressure that underlies the advancement of retinopathy and the concept of IOP management being of therapeutic benefit in controlling diabetic retinopathy is not supported (Rassam *et al.*, 1995).

10.6.4. IOP pulse parameters

No significant difference existed either in IOP pulse amplitude or in the IOP pulse moduli between any of the groups under study. This finding agrees with Schmidt *et al.* (2000) who found no difference in PA value amongst young type 1 diabetic patients who were split into similar retinopathy-dependent groups. The present study found that the spectral analysis technique neither provided an improved method of differentiating between normal controls and diabetic eyes over traditional IOP pulse parameters, nor gave any evidence that the higher IOP pulse harmonic moduli contained information analogous to that found in the arterial pressure profiles of diabetic patients (McVeigh *et al.*, 1993).

In contrast to the PA results, the POBF values were significantly higher in the diabetic groups with background retinopathy in comparison to both normal subjects and diabetic patients without retinopathy. This agrees with the findings of two previous studies that, similar to the present study, looked at the POBF values in mixed groups of insulin and non-insulin dependent diabetics in this age category. Mackinnon *et al.* (1997) found POBF values to be higher in diabetic patients with background and preproliferative / proliferative changes by 58% and 70% respectively compared to normal controls. Geyer *et al.* (1999) reported a mean POBF value of 33% above that of normal controls for her group of diabetics with preproliferative / proliferative retinopathy. In both the above studies, as in ours, no significant difference in PA measurement existed between diabetic subjects with retinopathy and normal controls. This suggests that the POBF measure is of greater value in differentiating diseased from healthy eyes. In contrast to the above studies, Langham *et al.* (1991b) reported decreasing POBF values with increasing severity of retinopathy. Mackinnon *et al.* 1997) suggested that the results by Langham *et al.* differed because of the earlier model of POBF pneumatonometer used and measurements were taken in the supine position. A number of possible reasons may exist for the rise in POBF value with the onset of diabetic retinopathy.

Increased POBF values in patients with diabetic retinopathy may reflect a rise in total ocular blood flow. A number of reports on retinal blood flow, using either video fluorescein angiography or laser Doppler velocimetry, have found that bulk retinal blood flow is above normal in diabetic eyes prior to vasoproliferation (Cunha-Vaz *et al.*, 1978; Kohner, 1976; Patel, Rassam, Newsom *et al.*, 1992; Yoshida *et al.*, 1983). The increase in retinal perfusion has been presumed to occur in response to both metabolic demand and faulty autoregulation (Ciulla, Harris, Latkany *et al.*, 2002). Thickening of capillary basement membranes lead to occlusive angiopathy and tissue hypoxia which increases retinal metabolic demand. Retinal pericytes, the contractile cells responsible for autoregulation of flow, have been shown to be particularly susceptible to hyperglycaemia (Cai & Boulton, 2002). If total ocular blood flow mirrored these reported retinal blood flow changes, increases in POBF value may be seen.

Alternatively a rise in POBF value may indicate a shift in the proportion of pulsatile to non-pulsatile ocular blood flow. As review in Section 1.9.5, later studies indicated that total retinal blood flow in non-proliferative retinopathy is similar or even diminished in comparison to normal control subjects (Bursell *et al.*, 1996; Feke *et al.*, 1994; Grunwald, Riva, Sinclair *et al.*, 1986). Investigators who have examined other diabetic ocular circulations, which are perhaps of more comparative value to the POBF measure, do not find results indicative of a rise in blood flow. Weinberger *et al.* (1998) in one of the few studies to look directly at the filling properties of the choriocapillaris using indocyanine green angiography, found approximately half the eyes studied with non-proliferative retinopathy exhibited late-phase filling. Dimitrova *et al.* (2001)studied the choroidal circulation of a similar mixed type I / type II age group of diabetic patients as the present study, by measuring blood velocity of the posterior ciliary arteries with Doppler ultrasound. Background diabetic retinopathy was associated with a stable peak-systolic velocity (PSV) and a fall in end-diastolic velocity (EDV) of the PCA. The increased proportion of the pulsatile velocity change (PSV-EDV) to non-pulsatile velocity (EDV) indicated increased downstream resistance (resistance index = (PSV-EDV)/EDV). Similar increases in resistance index (due to a fall in EDV in comparison to PSV) have been reported for the ophthalmic artery in non-proliferative diabetic patients (Ino-ue, Azumi & Yamamoto, 2000; Mackinnon, McKillop, O'Brien *et al.*, 2000). It is not known how close the ratio of pulsatile to non-pulsatile ocular blood flow agrees with the ratio of pulsatility as determined by Doppler ultrasound, but previous authors suggest it is likely to be very near (Kiss, Dallinger, Polak *et al.*, 2001). If this is the case, the increased POBF values found in this

study may represent such a shift in ocular blood pulsatility with no change, or even a decrease, in total ocular blood flow.

Further analysis of our results that allowed for influencing covariates (for example, APP) found gender to be a factor in POBF with female subjects demonstrating higher values. Higher POBF values in females have been previously reported (Fontana *et al.*, 1998; Gekkieva *et al.*, 2001) and Centofanti *et al.* (2000a) has proposed this difference is due to the vasoactive effect of oestrogens on the choroidal vasculature.

APP was the strongest part-correlate with POBF value and, as its incorporation into the factorial ANOVA diminished group category to borderline significance ($p = 0.052$), it deserves further discussion. APP appears to be the forgotten variable in IOP pulse studies. As previously reviewed (1.3.1), pulsatile arterial pressure change drives pulsatile arterial blood flow. Early clinical IOP pulse studies highlighted the finding that APP represented a close approximation to this driving pulsatile change in ocular arterial pressure (Bron *et al.*, 1967; Knox, 1973). Although frequently measuring systolic, diastolic, and mean blood pressure (and usually OPP), recent investigators have almost uniformly failed to recognise the importance of APP in IOP pulse studies. An example is the study by Esgin *et al.* (2001). This group reported lower PA and POBF values in a group of diabetic patients with normal MAP in comparison to a group with high MAP (retinopathy levels were not reported). Measurement of the APP may have explained some of this reported difference. High blood pressure is commonly associated with an augmented APP value (Black *et al.*, 1999). Although not found to be significantly different amongst groups in the present study, some very high APP values were found in the study's subjects (Figure 10.5). MacKinnon *et al.* (1997) similarly suspected that high APP values in her diabetic groups may have been responsible for the anomalous POBF values. Recently Knudsen, Poulsen, Hansen *et al.* (2002) reported that increased APP in itself was associated with the development of diabetic microangiopathies including retinopathy. The authors proposed that the increased shear stress experienced by the vascular endothelium from the greater pulsatile flow could produce renal and retinal microvascular complications. Further research into whether increased POBF values in diabetic retinopathy reflect just a parallel increase in APP, or possibly represent a causative sign, is needed.

10.6.5. Cutaneous blood flow values

Subject group was not found to be a significant factor in the cutaneous blood flow measurements at the temple. As found in Chapter 9, gender was again found to be a factor in skin blood flow. Although POBF values were also gender specific, the lack of correlation between LDF flow and POBF values gave no evidence of a common physiological basis. Of peripheral interest in this study was that cutaneous blood flow measurements made at the temple were higher in males, whereas the cutaneous flow measurements made in Chapter 9, on the distal phalanx of the middle finger, were higher in females. This indicates that gender differences in cutaneous blood flow are location specific. This finding does not appear to have been previously reported.

10.6.6. Glycosylated haemoglobin levels

In the present study, glycosylated haemoglobin levels were not associated with stage of diabetic retinopathy, or with any other measured variable. Acute increases in blood glucose increase both retinal blood flow, as determined by video fluorescein angiography, and POBF values (Bursell *et al.*, 1996; Perrott *et al.*, 2001). The lack of any correlation in the present study probably reflects how glycosylated haemoglobin represents a mean value of blood glucose over approximately 6 weeks. Measurement of the exact plasma glucose concentration at the time of POBF measurement may have provided the necessary temporal resolution to better investigate the effect of glycaemia on IOP pulse value. Unfortunately in the present study it was not possible to measure plasma glucose within sufficient time of the pneumatonometric recording. Findl *et al.* (2000a) reported a positive correlation between HbA_{1c} and the interferometric measure, fundus pulsation amplitude. As it has been previously shown that both POBF and PA correlate strongly with the fundus pulsation amplitude measure it is perhaps surprising no association was found in the current study. The study by Findl *et al.* (2000a) was conducted on young (maximum age 32 years) type I diabetics, which may explain the difference in the studies' findings.

10.7. *Conclusion*

The present study demonstrated that the pulsatile fraction of ocular blood flow greater in diabetic patients with background retinopathy than either normal controls or diabetics without retinal complications. The use of spectral analysis to investigate the IOP pulse in

diabetic patients provided no statistical differentiation of retinopathy grade. Whether the raised POBF values reflect an increase in total ocular blood flow or, alternatively, a shift in the quotient of pulsatile flow remains to be determined. In addition, the present study found that the systemic differences in APP may be responsible for a large part of the variation seen in diabetic POBF values. It is recommended that future investigations study the possible confounding or causative factor of APP in diabetic retinopathy further.

11. Summary and Conclusions

11.1. *Summary*

Scientists have investigated the nature of the IOP pulse for over 150 years and the relevant scientific literature has been reviewed in Chapter 1 of this thesis. The IOP pulse represents a unique interaction between cardiovascular physiology and local ocular structure. As the IOP pulse is a relatively non-invasive measure, investigators have hoped to prove the pulse's clinical utility in determining an aspect of the eye's haemodynamic function. In addition to providing insights into the eye's basic physiology, such a measure would find employment in the detection and monitoring of a number of sight-threatening diseases where haemodynamic malfunction is known or suspected. The IOP pulse has yet to fulfil this ideal role. There are a number of reasons for this: the large number of influencing variables; the wide range of normal values which overlap considerably with diseased states; and the uncertainty in knowing exactly what the measurements define. This thesis has been concerned with investigating how to realise the IOP pulse's potential. In summary, the findings of this work were:

11.1.1. A comparison of two dynamic tonometers

At present, human studies on the IOP pulse are, in the main, limited to measuring the fluctuation in IOP as interpreted by a dynamic tonometer (Krakau *et al.*, 1995). The purpose of this part of the work was to compare a dynamic tonometer against an accepted pneumatonometer (OBFA) in order to determine which instrument to use in further studies. The Dynamic Observing Tonometer (DOT) has a number of novel features including a long data collection window (up to four minutes) and visual inspection of the retina during recording (Entenmann *et al.*, 1997). Validation of the instrument was performed by assessing its accuracy against accepted clinical gold standards and its precision by comparing agreement of two repeated measurements. The clinical gold standards chosen were Goldmann applanation tonometry for measuring IOP and pneumatonometry for PA measurement. Both these instruments have been validated in manometric studies and have pedigrees for measuring these parameters (Goldman *et al.*, 1957; Tønjum, 1972).

In the study, the DOT was found to over-estimate IOP to a clinically unacceptable level with a wide range of disagreement in comparison to Goldmann tonometry. This is in accord with another study that found an even greater disparity of IOP measurement (Troost *et al.*, 2001). In addition, the precision of IOP and PA measurements with the DOT was inferior to that of the pneumatonometer. The level of measurement repeatability found with the DOT agreed with that of a recent paper by Vogel *et al.* (2001). The clinical impression of the DOT's poor performance was that it arose from the inconsistent contact between lens and eye during hand-held application.

In order to pursue further studies on the IOP pulse, the OBFA pneumatonometer was chosen as the measurement instrument due to its greater agreement with Goldmann tonometry in IOP measurement and its superior repeatability of PA recording. Publication of this work is shown in Appendix 3.1.

11.1.2. The effect of operator experience and mode of application on pneumatonometer repeatability

The OBFA pneumatonometer is marketed primarily for measuring the derived parameter of the IOP pulse, POBF. The instrument is fully automated and has been described as being as reliable in the hands of a novice as in those of an experienced operator (Crowhurst *et al.*, 1996). In addition, the pneumatonometer probe may be mounted in a hand-held instrument for greater clinical flexibility and research opportunities. The purpose of this study was to investigate operator experience and mode of pneumatonometer application on POBF measurement precision. The benefit of taking repeated measurements was also determined by inspecting the change in measurement standard deviation as recommended by Weinreb *et al.* (1993).

The study found that operator experience did influence POBF measurement precision as evidenced by the fall in standard deviation values. Hand-held measurements showed the greater improvement in precision with operator experience. It is therefore recommended that new operators ensure they have acquired sufficient experience in taking recordings with the OBFA pneumatonometer, particularly if using the hand-held probe, before embarking on a study.

There was evidence that successive applanations with the OBFA pneumatonometer produced a tonographic effect upon the IOP and PA measurement. This did not occur with the POBF measurement suggesting perhaps the ocular pressure-volume relationship, used in the calculation of POBF, helps counter this source of variation. For further studies in the work, a maximum of two recordings were taken on an eye. Publication of this work is shown in Appendix 3.2.

11.1.3. The effect of corneal thickness and corneal curvature on pneumatonometer measurements

The influence of corneal thickness and curvature on IOP pulse measurements has not previously been reported. The effect of corneal thickness on the IOP measurements of Goldmann applanation tonometry is of clinical concern (Brubaker, 1999). Previous investigators have found contradictory data as to whether pneumatonometric IOP measurements are prone to the same effects of corneal topography (Abbasoglu *et al.*, 1998; Singh *et al.*, 2001). The purpose of this work was to investigate these possible sources of variation on the pneumatonometric measurements of IOP and PA.

Although IOP measurements taken with a pneumatonometer have been shown theoretically not to rely on corneal thickness (Walker *et al.*, 1972), the study found a significant positive correlation between the two. This is in agreement with two recent papers (Bhan, Browning, Shah *et al.*, 2002; Singh *et al.*, 2001). It is speculated that the re-routing of a proportion of the pneumatonometer's gas flow, to provide the initial applanation of the probe, is responsible for this effect. Moses *et al.* (1979) also criticised the effect of this method of pneumatonometric probe applanation.

Over the range investigated in the study, PA measurements did not show any correlation with corneal thickness. In contrast, mean corneal radius was isolated as a part-correlate with PA but not IOP measurements. It is recommended that future investigations using these pneumatonometric measurements consider these influences of corneal topography. Publication of this work is shown in Appendix 3.3.

11.1.4. IOP pulse parameters during low level hypercapnia

The inhalation of increased levels of CO_2 provides the opportunity to study the IOP pulse during exposure to a known ocular vasodilator with minimal systemic side-effects (Hosking *et al.*, 2001). As in the cerebral circulation, blood flow parameters in both animal and man have been shown to increase in all ocular circulations in direct proportion to the level of *P*a CO_2 (Geiser *et al.*, 2000; Wilson *et al.*, 1977). Increases in ocular and cerebral blood flow are believed to occur through arterial vasodilatation from the decrease in perivascular pH (Iadecola *et al.*, 1994). As the IOP pulse has been questioned as to its exact haemodynamic property (Hitchings, 1991), the purpose of this study was to elucidate any change in IOP pulse characterised by mild hypercapnia.

In contrast to the findings of two previous papers (Kergoat *et al.*, 1999; Schmetterer *et al.*, 2000b), the study found a reduction in both PA and POBF during inhalation of low-level CO_2. Possible explanations to this have been discussed and it is speculated that a shift in the proportion of pulsatile to non-pulsatile blood flow may occur under levels of mild hypercapnia. Shifts in ocular pulsatility, under other physiological provocations, have been suspected by previous investigators and are supported by an analogue model of ocular blood flow (James *et al.*, 1991b; Kerty *et al.*, 1994; Krakau, 1995).

The possible implications of this study are far reaching. A reduction in IOP pulse measures, despite a presumed increase in total ocular blood flow, suggests that ocular pulsatility data should be used with caution in the interpretation of haemodynamic status and should ideally be confirmed in the context of other vascular measures and known physiological responses.

11.1.5. A comparison of spectral analysis techniques

Evans *et al.* (2002) previously reported that analysis of the IOP pulse in the frequency domain gave superior differentiation between normal and glaucomatous eyes over traditional IOP pulse parameters. The purpose of this study was to investigate the precision of this original spectral analysis technique. In addition, two alternate spectral analysis

techniques were devised and compared in terms of within- and between-visit measurement variation.

Although attaining reasonable repeatability for the 3rd and 4th IOP pulse harmonics, the originally described spectral analysis technique performed poorly at lower and higher frequencies. The technique's poor precision at measuring the lowest harmonics (for example the fundamental) was probably due to the spread of signal into the surrounding frequency bins caused by normal heart rate variation and spectral leakage from the hard junction at the start of the pulse wave.

The two alternate spectral analysis techniques described, showed greater repeatability and reproducibility and were recommended for further studies on investigating the IOP pulse in the frequency domain.

11.1.6. Validatory investigations on the IOP pulse in the frequency domain

Two previous studies have indicated that spectral analysis of the IOP pulse provided superior differentiation of diseased and healthy eyes compared to traditional IOP pulse parameters (Best *et al.*, 1974; Evans *et al.*, 2002). In addition, Evans *et al.* (2002) speculated that the higher spectral components of the pulse may contain characteristics of the intraocular vascular beds analogous to the impedance spectra obtained from arterial pulse waves. The purpose of this part of the work was to attempt to validate these hypotheses. Three studies were performed in which theoretical differences in ocular vascular impedance may be expected.

The results showed that the IOP pulse moduli were highly frequency dependent. This is a characteristic found in arterial flow and pressure waves and represents the response of a vascular system to various driving frequencies (O'Rourke *et al.*, 1966). It is therefore important to compare IOP pulse moduli at the same frequency rather than at the same harmonic number. In addition, the results indicated that calculation of the IOP pulse moduli detected changes with greater confidence than the conventional parameters of PA and POBF.

The studies found in this section of work did not provide any evidence that the IOP pulse moduli contained vascular characteristics analogous to those found from the moduli of arterial pressure pulses. Possible reasons for this are discussed. In particular, the limitation of only measuring part of the impedance relationship is highlighted. A future technique for calculating a measure of ocular vascular impedance is described and its advantages discussed.

11.1.7. IOP pulse parameters and systemic vascular characteristics in glaucoma

This study investigated the IOP pulse, using both traditional and newly developed parameters, in glaucomatous and healthy eyes. In addition, due to the suspected role of ocular perfusion pressure and/or a systemic vasospastic phenotype in the aetiology of glaucoma, associations between blood pressure measures, and the response of finger blood flow to cold-provocation, with glaucoma group were investigated.

The study found evidence of a reduction in the pulsatile fraction of ocular blood flow of NTG patients. This is in agreement with a number of previous studies (Fontana *et al.*, 1998; James *et al.*, 1991b; Quaranta *et al.*, 1994; Ravalico *et al.*, 1994). In addition, the POAG patients had significantly higher systolic and arterial pulse pressure than the group of NTG patients and OHT subjects respectively. These findings are suggestive of cardiovascular differences between these groups. Spectral analysis of the IOP pulse did not give greater confidence in separating glaucomatous from non-glaucomatous eyes over traditional IOP pulse parameters.

No difference in vasoreactivity was found between the groups of glaucoma patients and control subjects. This contradicts some studies (Drance *et al.*, 1988; Gasser *et al.*, 1991; O'Brien *et al.*, 1999) but supports the findings of others (Rojanapongpun *et al.*, 1993b; Usui *et al.*, 1992). The study did find gender to be an influencing factor in cutaneous finger blood flow and the possible implications for vasoreactivity studies in glaucoma are discussed. It is recommended that the influence of gender in future investigations of vasoreactivity and

glaucoma be recognised and that this factor in possible subcategories of glaucoma (e.g. focal ischaemic glaucoma) may warrant further investigation.

11.1.8. IOP pulse parameters and systemic vascular characteristics in diabetes mellitus

This study investigated the IOP pulse, using traditional and newly developed analyses, in diabetic eyes with varying severity of retinopathy. In addition, measurements of blood pressure, glycaemic level and cutaneous blood flow were taken and any association with IOP pulse value investigated.

The study found diabetic patients with background retinopathy to have, on average, significantly higher POBF values than either normal control subjects or diabetic patients without retinopathy. This is in general agreement with two previous studies (Geyer *et al.*, 1999; MacKinnon *et al.*, 1997). Possible explanations to this finding are explored including the possibility that the value represents a shift in the proportion of pulsatile to non-pulsatile blood flow. There was evidence that raised arterial pulse pressures in the diabetic patients contributed to the anomalous POBF values. The confounding or causative factor of raised arterial pulse pressures in diabetic retinopathy is a possible avenue of further research (Knudsen *et al.*, 2002).

No differentiation in study group was found using spectral analysis of the IOP pulse or cutaneous blood flow values. Likewise, glycosylated haemoglobin levels did not correlate with any IOP pulse value in contrast to the findings of a previous published study (Findl *et al.*, 2000a).

11.2. *Conclusions*

The aims of this work were three-fold:

1. To optimise the measurement and analysis of the IOP pulse.

The findings of the work were:

- The Dynamic Observing Tonometer was inferior to the OBFA pneumatonometer both in terms of its accuracy of IOP measurement and its precision of both IOP and PA measurement
- There is a period of learning in which a new operator masters measurements taken with an OBFA pneumatonometer, particularly if the probe is used hand-held
- Repeated applications of a pneumatonometer have a tonographic effect both on the IOP and PA measurement.
- A previously described spectral analysis technique for calculating the IOP pulse moduli was improved upon by two alternative methods in terms of both within- and between-visit reliability
- No evidence has been found to suggest the higher spectral components of the IOP pulse contain characteristics analogous to the impedance spectra of arterial pulse waves.

2. To investigate any ocular and systemic variables which influence IOP pulse measurements

The findings of the work were:

- Unlike IOP, corneal thickness does not affect the measurement of PA
- Axial length and mean corneal radius both affect PA measurements
- IOP pulse moduli are highly frequency specific and can be best described by an inverse-cubed function
- The driving pressure wave of pulsatile blood flow, the arterial pressure pulse, correlates positively with the POBF measure

3. To explore the nature of the pulse in health and disease and its relation to systemic cardiovascular variables.

The findings of the work were:

- Inferring total ocular blood flow changes from the behaviour of the IOP pulse may be erroneous due to shifts in the quotient of pulsatile to non-pulsatile flow
- The POBF measure, indicative of the pulsatile fraction of total ocular blood flow, is reduced in patients with normal-tension glaucoma
- The POBF measure is abnormally high in diabetic patients with background retinopathy and may be related to their arterial pressure pulse values

11.3. *Future areas of research arising from this work*

Of the number of new questions that this thesis uncovered, two particular avenues of future research are perhaps worth highlighting.

11.3.1. The relationship between pulsatile and non-pulsatile ocular blood flow

In order for the IOP pulse to be of use as an ocular haemodynamic function, it is imperative that shifts in the ratio of pulsatile to steady ocular blood flow be determined. Comparative blood flow measurements may highlight situations where shifts in this ratio occur. A possible example study could explore the effect of different levels of hypercapnia on both IOP pulse and retrobulbar blood velocities as determined by colour Doppler ultrasound. The proportion of pulsatile to steady flow as determined by ultrasound could then be related to IOP pulse values.

11.3.2. A relative measure of ocular vascular impedance

The limitation of simply measuring a blood flow value is the unknown contributory effects of driving pressure gradient and local opposition to flow; for example, is a low flow value due to poor perfusion pressure or narrowed non-compliant arterioles? As it is local vascular dysfunction that is of interest in ophthalmic studies, it would make sense to measure the driving pressure gradient in addition to blood flow values.

The estimation of mean ocular perfusion pressure may be of some use in situations of steady blood flow; although there is no guarantee that brachial blood pressure values are representative of those found at the head (Nicholls *et al.*, 1998a). For pulsatile flow studies,

the use of mean ocular perfusion pressure makes less sense. Instead it is necessary to relate pulsatile pressure to pulsatile flow. Future investigations may therefore benefit from attempting to combine ocular flow and pressure data to calculate a measure of 'ocular vascular impedance'. In theory this would provide a stronger parameter of ocular haemodynamics. A proposed technique for calculating a measure of 'ocular vascular impedance' is shown in Figure 11.1. Ideally the calculation of ocular vascular impedance would involve the measurement of a pulse pressure profile from the retrobulbar arteries feeding the eye and a similarly located pulse flow profile. Acknowledging all the assumptions and limitations that it carries with it, the derived measure of POBF provides a pulse flow profile. For a suitable arterial pressure pulse, the ipsilateral carotid artery presents probably the best site for a non-invasive measurement. Specialised arterial tonometers, that are usually used to calculate aortic impedance (Wilkinson, Fuchs, Jansen *et al.*, 1998), would be suitable to measure the carotid pressure pulse profile. Knowing the profiles of blood pressure and blood flow would allow the calculation of impedance spectra using known methods (Mills *et al.*, 1970). The different measuring sites for pressure and flow would necessitate that the location of any significant opposition to flow, indicated by high impedance values, would not be precise: it would lie anywhere between carotid artery and the intraocular vascular beds. However a measure of ocular vascular impedance would still retain the advantage over a measure of simple flow as it would be characteristic of local vascular properties; a more relevant parameter to investigators studying the eye in health and disease.

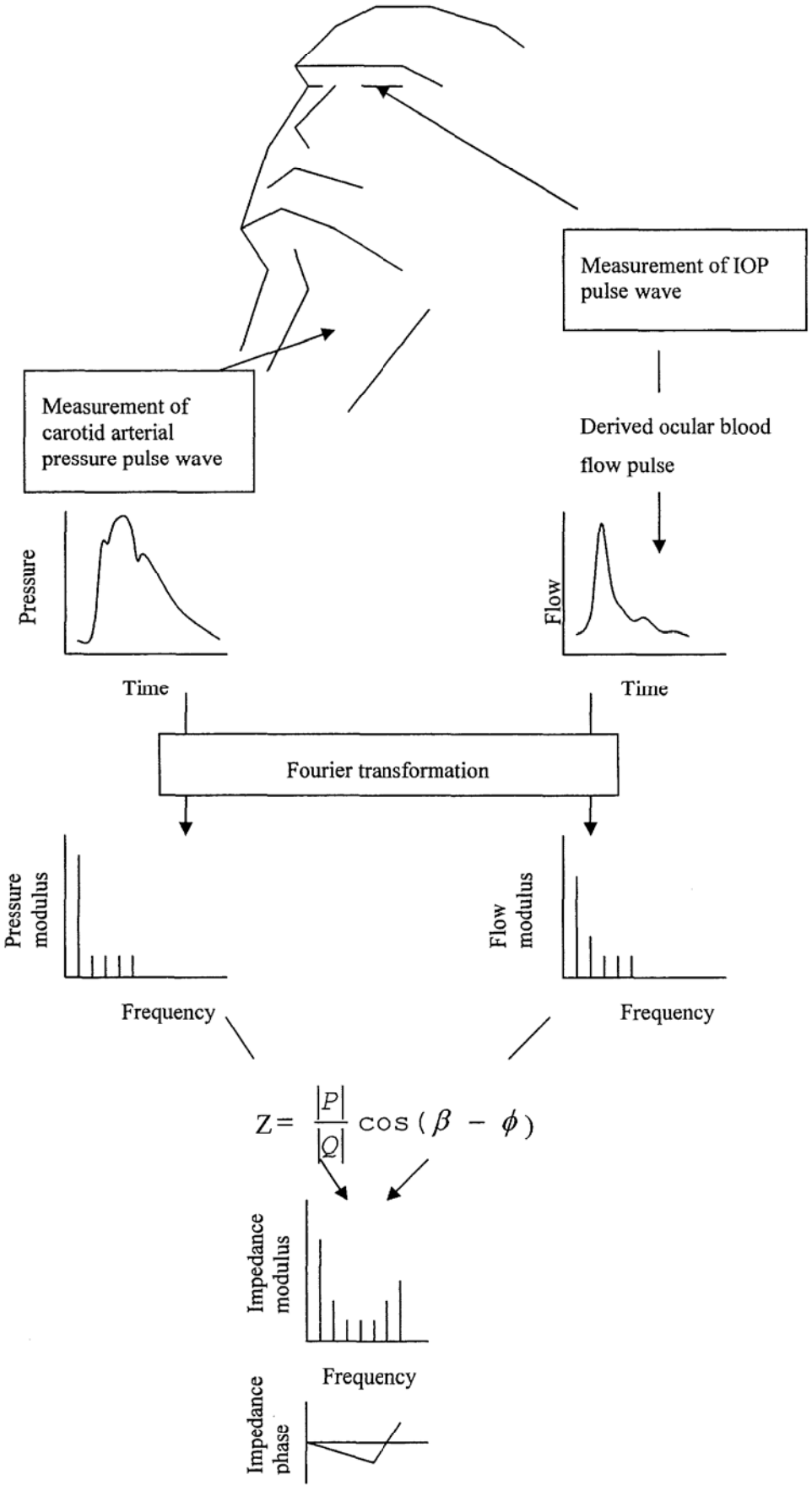

Figure 11.1 A proposed method of calculating a measure of ocular vascular impedance.

Appendices

Appendix 1: Figures for Chapter 4

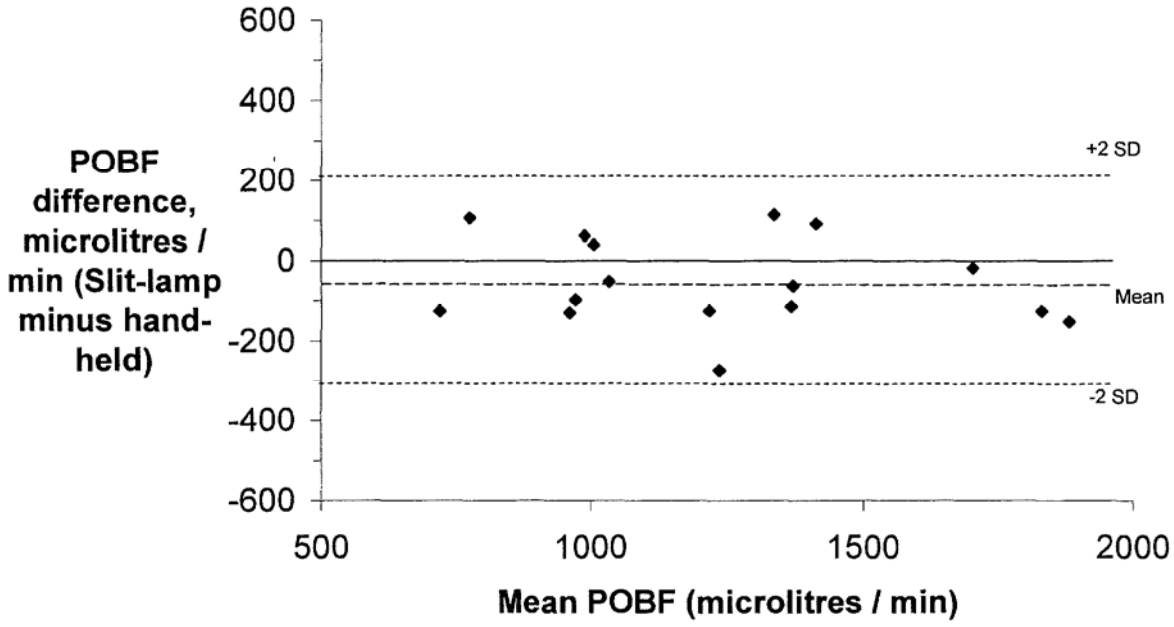

Figure A.1.1 The between-method differences in POBF as a function of their mean: ♦ indicates the mean value difference between each method; dashed lines indicate the overall mean difference between methods and the corrected two standard deviation limits of agreement.

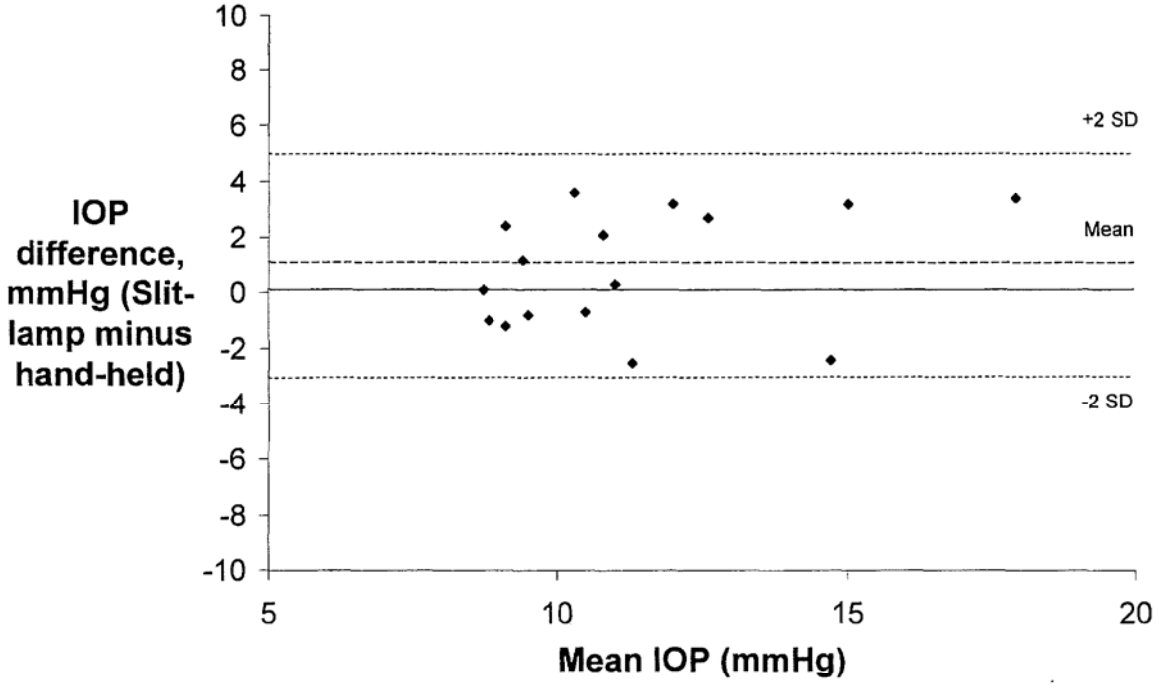

Figure A.1.2 The between-method differences in IOP as a function of their mean: ♦ indicates the mean value difference between each method; dashed lines indicate the overall mean difference between methods and the corrected two standard deviation limits of agreement.

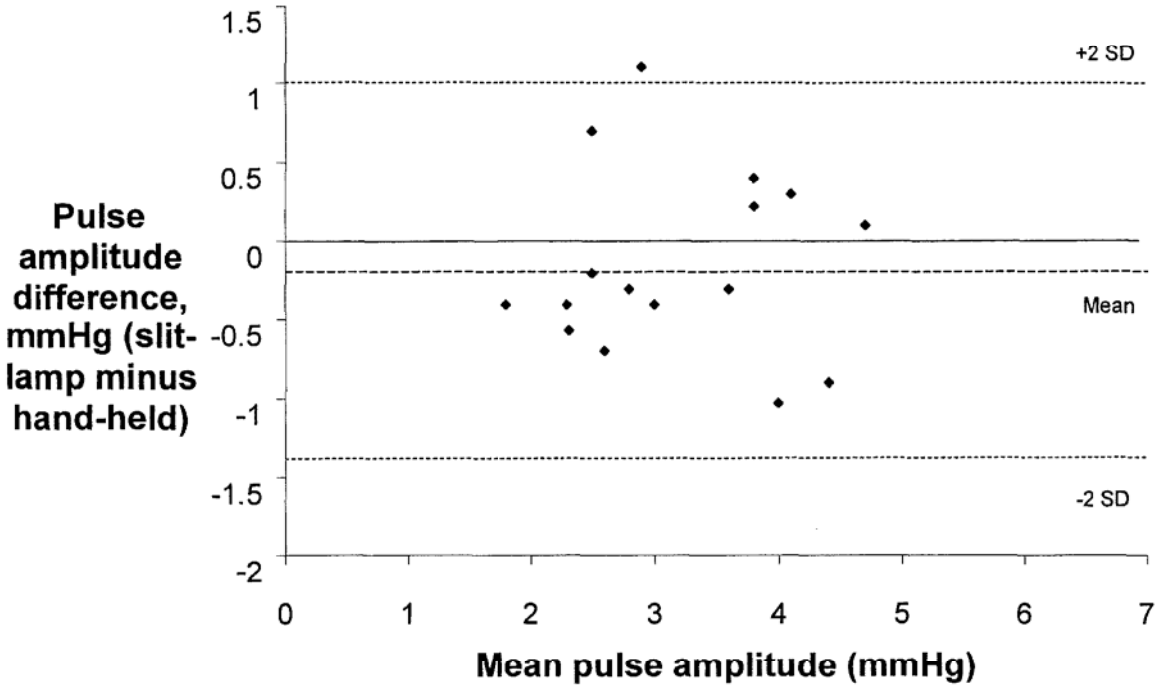

Figure A.1.3 The between-method differences in pulse amplitude as a function of their mean: ♦ indicates the mean value difference between each method; dashed lines indicate the overall mean difference between methods and the corrected two standard deviation limits of agreement.

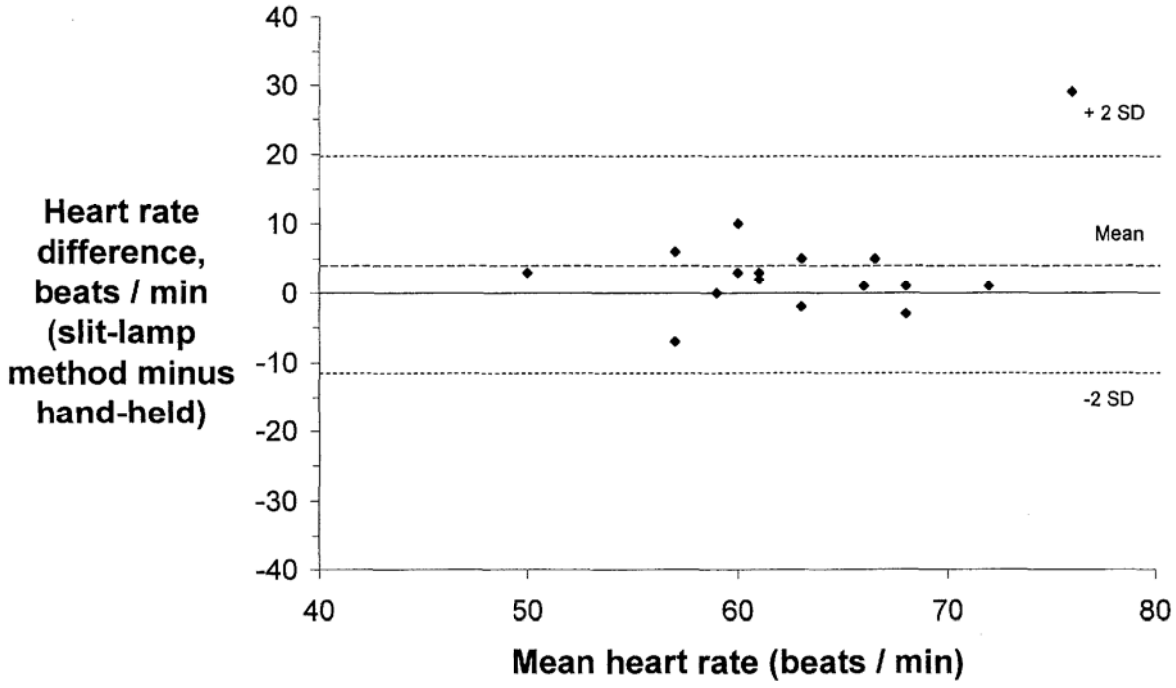

Figure A.1.4 The between-method differences in heart rate as a function of their mean: ♦ indicates the mean value difference between each method; dashed lines indicate the overall mean difference between methods and the corrected two standard deviation limits of agreement.

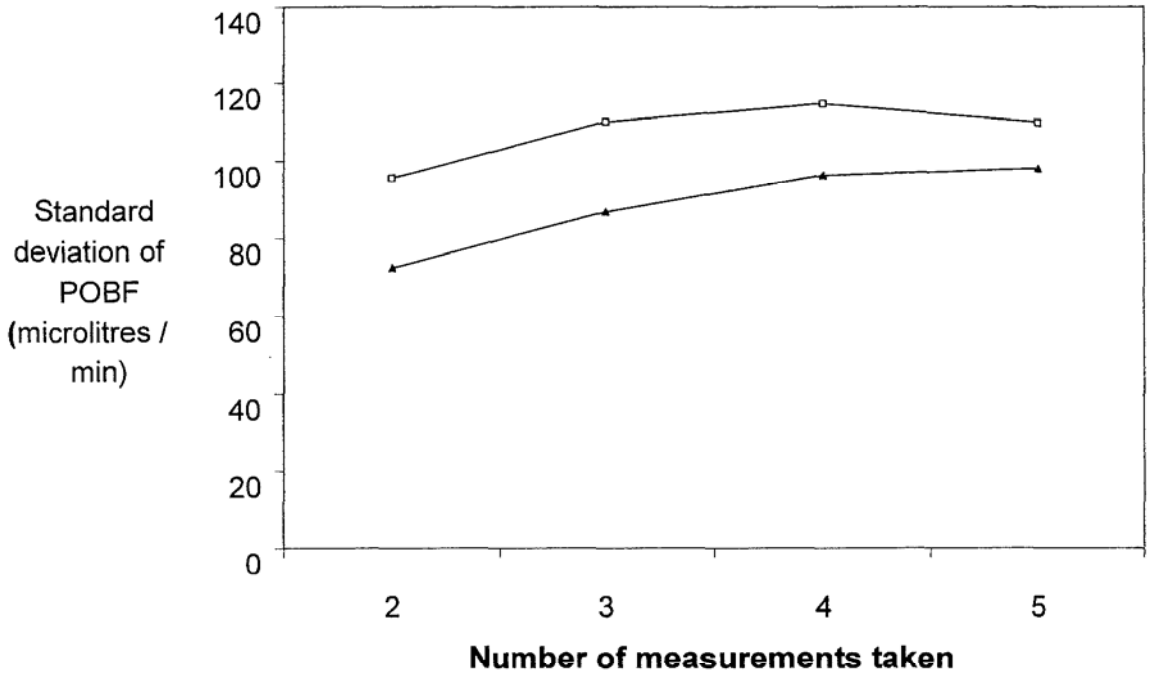

Figure A.1.5 Mean standard deviation of POBF as a function of the number of measurements taken: ▲slit-lamp method; □ hand-held method.

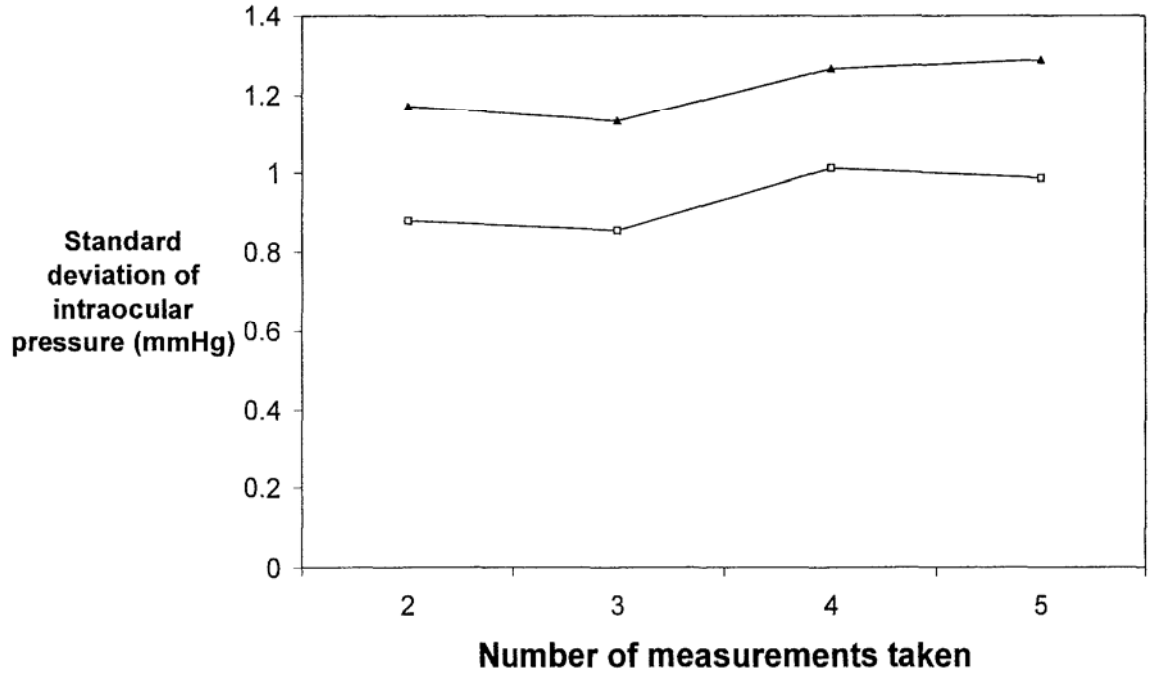

Figure A.1.6 Mean standard deviation of intraocular pressure as a function of the number of measurements taken: ▲slit-lamp method; □ hand-held method.

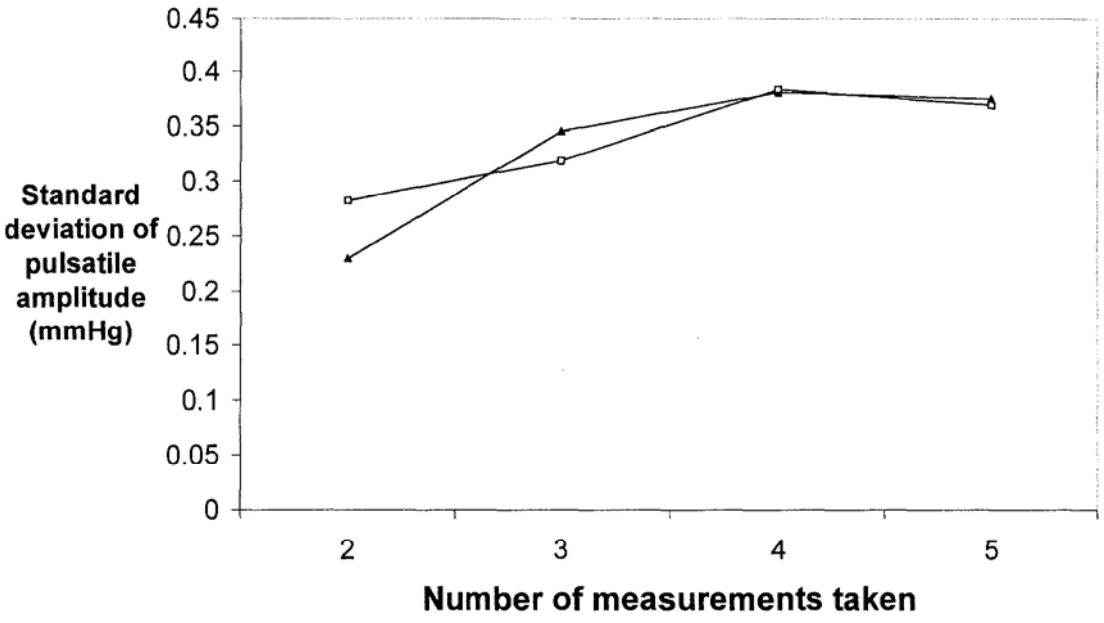

Figure A.1.7 Mean standard deviation of pulse amplitude as a function of the number of measurements taken: ▲slit-lamp method; □ hand-held method.

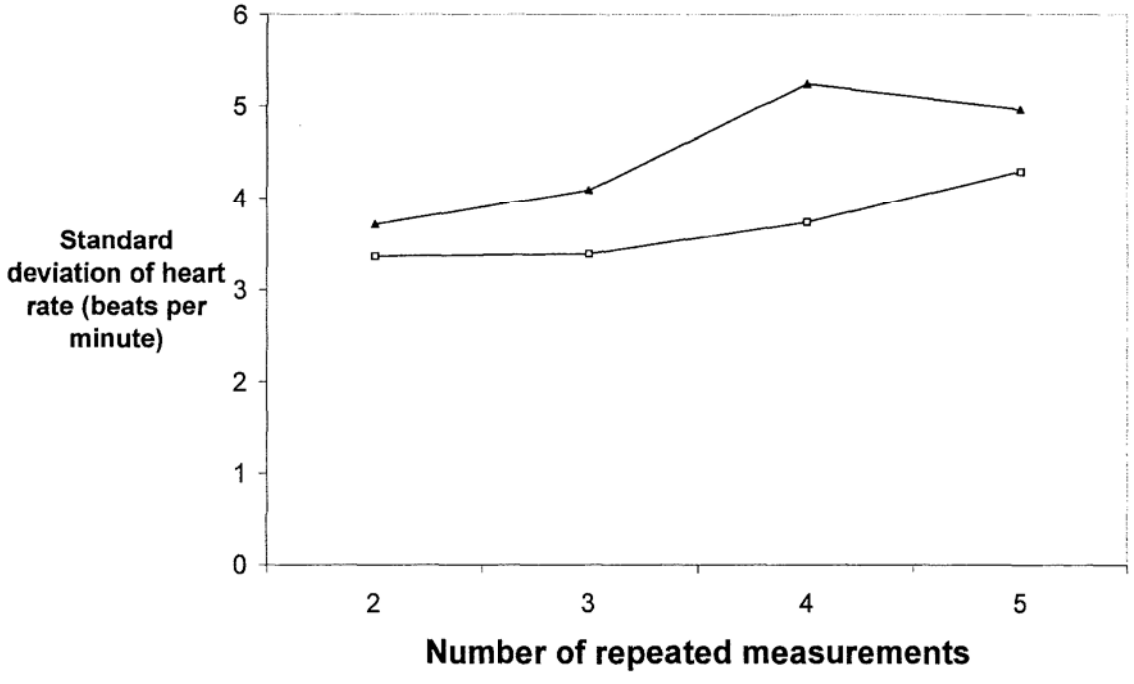

Figure A.1.8 Mean standard deviation of heart rate as a function of the number of measurements taken: ▲slit-lamp method; □ hand-held method.

Appendix 2: Fourier Analysis

Count Jean Baptiste Joseph Fourier, while stranded in Africa with Napoleon's army, created a number of mathematical laws to describe the properties of waveforms. He discovered that any steady state oscillation could be described as the sum of a set of sinusoidal waveforms whose frequencies are all integral multiples of the original oscillation. This led to two main applications: the adding of Fourier components to make a periodic function (*Fourier series*) and the dissecting of a function into its components (*Fourier analysis*).

The Fourier series allows any periodic waveform to be created from a number of component sinusoidal functions, or harmonic components, and a convenient form of the series is shown in Equation A.1 (Bloch, 2000).

$$f(t) = \frac{A_0}{2} + \sum_{n=1}^{\infty}\left[A_n \cos\left(\frac{2\pi nt}{T}\right) + B_n \sin\left(\frac{2\pi nt}{T}\right)\right]$$

f(t)	the function, or waveform, in the time domain (t)
$A_0/2$	the mean value of the function
A_n	the Fourier coefficients of the even parts of the series
B_n	the Fourier coefficients of the odd parts of the series
T	the period of the periodic function

Equation A.1 A general Fourier series

In contrast, by deconstructing a function into its component sinusoids, Fourier analysis (or transformation) allows any waveform to be described as a series of harmonic components each associated with a particular frequency. The ability to analyse a waveform in the frequency domain rather than its original data domain (usually time) is extremely useful in scientific fields as diverse as molecular biology and oceanography (Bracewell, 1989). One advantage is that small amounts of energy associated with certain component frequencies can be detected even when extremely large amounts of energy are associated at other

frequencies. There are several ways to define the transform, but a general form is shown in Equation A.2.

$$F(\omega) = \int_{-\infty}^{+\infty} f(t) e^{-i\omega t} dt$$

$F(\omega)$	the function (or waveform) in the frequency domain
$f(t)$	the waveform in the original domain (which in this case is time t)
ω	frequency (measured in radians/s)

Equation A.2 The forward Fourier transform

Similarly the function in the frequency domain can be reversed (Equation A.3) which is the same as Equation A.1 but written in the exponent form. Equation A.2 is known as the forward Fourier transform and equation A.3 as the inverse Fourier transform.

$$f(t) = \frac{1}{2\pi} \int_{-\infty}^{+\infty} F(\omega) e^{+i\omega t} d\omega$$

$F(\omega)$	the function (or waveform) in the frequency domain
$f(t)$	the waveform in the original domain (which in this case is time t)
ω	frequency (measured in radians/s)

Equation A.3 The inverse Fourier transform

It is worth noting that a Fourier series represents an infinite waveform with a finite number of sinusoidal functions and an analytic Fourier transform represents a finite waveform (i.e. the section under analysis) as a continuous spectra of frequencies. Although this latter fact appears unsatisfactory in describing the waveform, the outstanding usefulness of the transform is that the curve described by a certain number of terms is the best approximation

for that number of terms. Computing further terms will only improve the description of the original curve and does not alter the lower harmonics.

Commonly when a particular waveform is subjected to Fourier transformation the resultant harmonics are complex numbers (they contain the imaginary number *i*, the square root of -1). As complex numbers are not readily analysed, it is usual in the biological sciences to express them in their polar notation: magnitude (or modulus) and phase. The modulus and phase are related to the original sinusoidal components in Equation A.4.

$$M \cos(\omega t - \phi) = A \cos \omega t + B \sin \omega t$$

M	modulus and has the same units as the waveform (e.g. mmHg)
ω	angular frequency (e.g. measured in radians per second)
t	time
ø	phase, in radians and equal to $\tan^{-1}$ (B/A).

Equation A.4 The relationship between polar and trigonometric expressions of a Fourier component

The modulus and phase of each harmonic can then be plotted against frequency. A problem with Fourier transformation is that, unless the original waveform is a relatively simple integrand, the computation is extremely laborious. For this reason the use of Fourier analysis was restricted until the advent of computers and faster analysis algorithms such as the Fast Fourier Transform (FFT) (Cooley *et al.*, 1965). The Fast Fourier Transform calculations in this thesis were performed using the supplied program in Microsoft Excel Toolpak.

Appendix 3: Method of Literature Search used in Chapter 1

A search of the MEDLINE database was conducted with the following search words: intraocular pressure pulse, ocular pressure pulse, ocular pulse amplitude, pulsatile ocular blood flow. The search covered articles published in English during the years 1960 to 2002. Additional sources included publications in peer-reviewed journals and published books cited in other articles or relevant to the review. Criteria for inclusion or exclusion of articles were originality, importance, and new findings regarding the IOP pulse. Only descriptions and findings of techniques that measure pulsations in intraocular pressure were considered for the review.

Appendix 4: Publications arising from this Work

4.1

Morgan, A.J., Hosking, S.L., Salmon, J.F. Clinical evaluation of the dynamic observing tonometer. *Journal of Glaucoma* **11**: 334-339

4.2

Morgan, A.J., Hosking, S.L. Ocular blood flow tonometer reproducibility: the effect of operator experience and mode of application. *Ophthalmic and Physiological Optics* **21**:401-406

4.3

Morgan, A.J., Harper, J., Hosking, S.L., Gilmartin, B. The effect of corneal thickness and corneal curvature on pneumatonometer measurements. *Current Eye Research* **25**: 107-112

References

Abbasoglu, O.E., Bowman, R.W., Cavanagh, H.D., & McCulley, J.P. (1998) Reliability of Intraocular Pressure Measurements after Myopic Excimer Photorefractive Keratectomy. *Ophthalmology* **105:** 2193-2196.

Aiello, L.P., Cahill, M.T., & Wong, J.S. (2001) Systemic considerations in the management of diabetic retinopathy. *American Journal of Ophthalmology* **132:** 760-76.

Akazawa, Y., Tokoro, T., & Funata, M. (1994) Ocular pulse and pulsatile change of scleral strain in living in situ rabbit eyes. *Optometry and Vision Science* **71:** 207-211.

Akselrod, S., Gordon, D., Madwed, J.B., Snidman, N.C., Shannon, D.C., & Cohen, R.J. (1985) Hemodynamic regulation: investigation by spectral analysis. *American Journal of Physiology* **249:** H867-H875.

Alexander, L.J. (1994) Congenital and acquired anomalies of the optic nerve head. In L. J. Alexander (Ed.), *Primary Care of the Posterior Segment* (pp. 89-170). Norwalk, Connecticut: Appleton & Lange.

Alimgil, M.L., Benian, Ö., Esgin, H., & Erda, S. (1999) Ocular pulse amplitude in patients with Graves' disease: A preliminary study. *Acta Ophthalmologica Scandinavica* **77:** 694-696.

Allen, D.G., & Kentish, J.C. (1985) The cellular basis of the length-tension relation in cardiac muscle. *Journal of Molecular and Cellular Cardiology* **17:** 821-840.

Allen, J.A., Devlin, M.A., McGrann, S., & Doherty, C.C. (1992) An objecive test for the diagnosis and grading of vasospasm in patients with Raynaud's syndrome. *Clinical Science* **82:** 529-534.

Alm, A. (1989) Microspheres in optic nerve blood flow measurements. In G. N. Lambrou & E. L. Greve (Eds.), *Ocular Blood Flow in Glaucoma* (pp. 65-68). Amsterdam: Kugler & Ghedini.

Alm, A. (1992) Ocular Circulation. In W. M. Hart (Ed.), *Adler's Physiology of the Eye* (pp. 198-227).

Alm, A., & Bill, A. (1972) The oxygen supply to the retina II. Effects of high intraocular pressure and of increased arterial carbon dioxide tension on uveal and retinal blood flow in cats. A study with radioactively labelled microspheres including flow determinations in brain and some other tissues. *Acta Physiologica Scandinavica* **84:** 306-319.

Alward, W.L.M. (2000) The genetics of open-angle glaucoma: the story of GLC1A and myocilin. *Eye* **14:** 429-436.

Anderson, D.R. (1989) Anatomy and physiology of ocular blood flow. In G. N. Lambrou & E. L. Greve (Eds.), *Ocular Blood Flow in Glaucoma* (pp. 55-59). Amsterdam: Kugler & Ghedini.

Ariffin, A., Hill, R.D., & Leigh, O. (1992). *Diabetes and Primary Eye Care*. Oxford: Blackwell Scientific Publications.

Ates, H., Uretmen, O., Killi, R., Akkin, C., & Andac, K. (2000) Color Doppler imaging in glaucoma patients with asymmetric visual field loss. *Annals of Ophthalmology* **32:** 123-126.

Avolio, A.P., Chen, S.-G., Wang, R.-P., Zhang, C.-L., Li, M.-F., & O'Rourke, M.F. (1983) Effects of aging on changing arterial compliance and left ventricular load in a northern Chinese urban community. *Circulation* **68:** 50-58.

Bafa, M., Lambrinakis, I., Dayan, M., & Birch, M. (2001) Clinical comparison of the measurement of the IOP with the ocular blood flow tonometer, the Tonopen XL and the Goldmann applanation tonometer. *Acta Ophthalmologica Scandinavia* **79:** 15-18.

Bailey, I.L., Bullimore, M.A., Raasch, T.W., & Taylor, H.R. (1991) Clinical Grading and the Effects fo Scaling. *Investigative Ophthalmology & Visual Science* **32:** 422-432.

Bakalash, S., Kipnis, J., Yoles, E., & Schwartz, M. (2002) Resistance of retinal ganglion cells to an increase in intraocular pressure is immune-dependent. *Investigative Ophthalmology & Visual Science* **43:** 2648-53.

Baxter, G.M., Williamson, T.H., McKillop, G., & Dutton, G.N. (1992) Color Doppler ultrasound of orbital and optic nerve blood flow: effects of posture and Timolol 0.5%. *Investigative Ophthalmology & Visual Science* **33:** 604-610.

Bayerle-Eder, M., Wolzt, M., Polska, E., Langenberger, H., Pleiner, J., Teherani, D., Rainer, G., Polak, K., Eichler, H.G., & Schmetterer, L. (2000) Hypercapnia-induced cerebral and ocular vasodilation is not altered by glibenclamide in humans. *American Journal of Physiology* **278:** R1667-R1673.

Becker, B. (1971) Diabetes mellitus and primary open-angle glaucoma. *American Journal of Ophthalmology* **71:** 1-16.

Becker, B., & Friedenwald, J.S. (1953) Clinical Aqueous Outflow. *Archives of Ophthalmology* **50:** 557-571.

Bellarminoff, L. (1886) Anwendung der graphischen Methode bei Untersuchung des intraocularen Druckes. *Pflugers Arch Ges Physiol* **39:** 449-472.

Benbow, S.J., Pryce, D.W., Noblett, K., MacFarlane, I.A., Friedmann, P.S., & Williams, G. (1995) Flow motion in peripheral diabetic neuropathy. *Clinical Science* **88:** 191-196.

Benetos, A., Waeber, B., Izzo, J., Mitchell, G., Resnick, L., Asmar, R., & Safar, M. (2002) Influence of age, risk factors, and cardiovascular and renal disease on arterial stiffness: clinical applications. *American Journal of Hypertension* **15:** 1101-8.

Bennett, A.G., & Rabbetts, R.B. (1984) Examination of the Eye and Ophthalmoscopy. In A. G. Bennett & R. B. Rabbetts (Eds.), *Clinical visual optics* (pp. 323-350). London: Butterworths.

Berne, R.M., & Levy, M.N. (1997a) The arterial system. In R. M. Berne & M. N. Levy (Eds.), *Cardiovascular physiology* (7th ed., pp. 135-152). St Louis: Mosby.

Berne, R.M., & Levy, M.N. (1997b) The cardiac pump. In R. M. Berne & M. N. Levy (Eds.), *Cardiovascular Physiology* (7th ed., pp. 55-82). St Louis: Mosby.

Berne, R.M., & Levy, M.N. (1997c) The peripheral circulation and its control. In R. M. Berne & M. N. Levy (Eds.), *Cardiovascular Physiology* (7th ed., pp. 171-194). St Louis: Mosby.

Bertram, B., Wolf, S., Fiehöfer, S., Schulte, K., Arend, O., & Reim, M. (1991) Retinal circulation times in diabetes mellitus type 1. *British Journal of Ophthalmology* **75:** 462-465.

Best, M., Kelly, T.A., & Galin, M.A. (1970) The Ocular Pulse -Technical Features. *Acta Ophthalmologica* **48:** 357-368.

Best, M., Keyes, M., Plechaty, G., & Galin, M. (1971a) Graphic analysis of the ocular pulse in carotid occlusion. *Archives of Ophthalmology* **85:** 315-319.

Best, M., Plechaty, G., Harris, L., & Galin, M.A. (1971b) Ophthalmodynamometry and ocular pulse studies in carotid occlusion. *Archives of Ophthalmology* **85:** 334-338.

Best, M., Pola, R., Plechaty, G., Masket, S., & Galin, M.A. (1971c) Ocular Pulse Studies in Carotid Stenosis. *Archives of Ophthalmology* **85:** 730-737.

Best, M., & Rogers, R. (1974) Techniques of Ocular Pulse Analysis in Carotid Stenosis. *Archives of Ophthalmology* **92:** 54-58.

Bhan, A., Browning, A.C., Shah, S., Hamilton, R., Dave, D., & Dua, H.S. (2002) Effect of corneal thickness on intraocular pressure measurements with the pneumotonometer, Goldmann applanation tonometer, and Tono-Pen. *Investigative Ophthalmology & Visual Science* **43:** 1389-92.

Bienfang, D.C. (1989) Loss of the ocular pulse in the acute phase of temporal arteritis. *Acta Ophthalmologica Supplementum* **191:** 35-37.

Bill, A. (1985) Some aspects of the ocular circulation. *Investigative Ophthalmology & Visual Science* **26:** 410-424.

Bill, A. (1993) Vascular Physiology of the Optic Nerve, *The Optic Disc in Glaucoma* (pp. 37-50).

Bill, A., & Sperber, G.O. (1990) Control of Retinal and Choroidal Blood Flow. *Eye* **4:** 319-325.

Bircher, A., de Boer, E.M., Agner, T., Wahlberg, J.E., & Serup, J. (1994) Guidelines for measurement of cutaneous blood flow by laser Doppler flowmetry. *Contact Dermatitis* **30:** 65-72.

Black, H.R., Kuller, L.H., O'Rourke, M.F., Weber, M.A., Alderman, M.H., Benetos, A., John, B., Cohn, J.N., Franklin, S.S., Mancia, G., Safar, M., & Zanchetti, A. (1999) The first report of the Systolic and Pulse Pressure (SYPP) Working Group. *Journal of Hypertension* **17:** S3-S14.

Blair, N.P., Feke, G.T., Morales-Stoppello, J., Riva, C.E., Goger, D.G., Collas, G., & McMeel, W.J. (1982) Prolongation of the retinal mean circulation time in diabetes. *Archives of Ophthalmology* **100:** 764-768.

Bland, J.M., & Altman, D.G. (1986) Statistical Methods For Assessing Agreement Between Two Methods of Clinical Measurement. *The Lancet* **8:** 307-310.

Bland, J.M., & Altman, D.G. (1996) Statistics Notes: Measurement error and correlation coefficients. *British Medical Journal* **313:** 41-42.

Bloch, S.C. (2000) Analysis Toolpak: Fourier Analysis. In S. C. Bloch (Ed.), *Excel for Engineers and Scientists* (First Edn ed., pp. 11-1 to 11-24). New York: John Wiley & Sons, Inc.

Bloomfield, P. (2000). *Fourier analysis of time series: an introduction* (2nd ed.). New York: Wiley.

Boles Carenini, A., Sibour, G., & Boles Carenini, B. (1994) Differences in the Longterm Effect of Timolol on the Pulsatile Ocular Blood Flow. *Survey of Ophthalmology* **38:** S118-S124.

Bonner, R., & Nossal, R. (1981) Model for laser Doppler measurements of blood flow in tissue. *Applied Optics* **20:** 2097-2107.

Bonner, R.F., & Nossal, R. (1990) Principles of laser-Doppler flowmetry. In A. P. Shepherd & P. Å. Öberg (Eds.), *Laser-Doppler blood flowmetry* (pp. 17-46). Boston: Kluwer Academic.

Bonomi, L., Marchini, G., Marraffa, M., Bernardi, P., Morbio, R., & Varotto, A. (2000) Vascular Risk Factors for Primary Open Angle Glaucoma. *Ophthalmology* **107:** 1287-1293.

Bosem, M.E., Lusky, M., & Weinreb, R.N. (1992) Short-term Effects of Levobunolol on Ocular Pulsatile Flow. *American Journal of Ophthalmology* **114:** 280-286.

Bracewell, R.N. (1989) The Fourier Transform. *Scientific American*: 62-69.

Braunwald, E., & Ross, J. (1979) Control of cardiac performance. In R. M. Berne (Ed.), *Handbook of Physiology, Cardiovascular System, Vol 1, The Heart* (Vol. 1, pp. 533-579). Bethesda: American Physiological Society.

Braverman, I.M. (1997) The Cutaneous Microcirculation: Ultrastructure and Microanatomical Organization. *Microcirculation* **4:** 329-340.

Broadway, D.C., & Drance, S.M. (1998) Glaucoma and vasospasm. *British Journal of Ophthalmology* **82:** 862-870.

Broadway, D.C., Nicolela, M.T., & Drance, S.M. (1999) Optic Disk Appearances in Primary Open-Angle Glaucoma. *Survey of Ophthalmology* **43:** S223-S243.

Bron, A.J., Knox, D.L., & Gaasterland, D. (1967) Tonographic studies in carotid occlusive disease. *British Journal of Ophthalmology* **51:** 577-595.

Bron, A.J., Tripathi, R.C., & Tripathi, B.J. (1997a) The choroid and uveal vessels, *Wolff's Anatomy of the Eye and Orbit* (8th ed., pp. 371-410). London: Chapman & Hall.

Bron, A.J., Tripathi, R.C., & Tripathi, B.J. (1997b) The eyeball and its dimensions, *Wolff's Anatomy of the Eye and Orbit* (8th ed., pp. 211-232). London: Chapman & Hall.

Bron, A.J., Tripathi, R.C., & Tripathi, B.J. (1997c) The orbital and cerebral vessels, *Wolff's Anatomy of the Eye and Orbit* (pp. 85-106). London: Chapman & Hall.

Brooks, A.M.V., Robertson, I.F., & Mahoney, A.-M. (1984) Ocular rigidity and intraocular pressure in keratoconus. *Australian Journal of Ophthalmology* **12:** 317-324.

Brown, N., Bron, A., Ayliffe, W., Sparrow, J., & Hill, A. (1987) The objective assessment of cataract. *Eye* **1:** 234-246.

Brown, S., & Jampol, L.M. (1996) New concepts of regulation of retinal vessel tone. *Archives of Ophthalmology* **114:** 199-204.

Brubaker, R.F. (1999) Tonometry and corneal thickness. *Archives of Ophthalmology* **117:** 104-5.

Buchanan, R.A., & Williams, D. (1985) Intraocular pressure, ocular pulse pressure and body position. *American Journal of Optometry & Physiological Optics* **62:** 59-62.

Buckley, C., Hadoke, P.W.F., Henry, E., & O'Brien, C. (2002) Systemic vascular endothelial cell dysfunction in normal pressure glaucoma. *British Journal of Ophthalmology* **86:** 227-232.

Bursell, S.-E., Clermont, A.C., Kinsley, B.T., Simonson, D.C., Aiello, L.M., & Wolpert, H.A. (1996) Retinal blood flow changes in patients with insulin-dependent diabetes mellitus and no diabetic retinopathy. *Investigative Ophthalmology & Visual Science* **37:** 886-897.

Butt, Z., & O'Brien, C. (1995) Reproducibiity of pulsatile ocular blood flow measurements. *Journal of Glaucoma* **4:** 214-218.

Butt, Z., O'Brien, C., McKillop, G., Aspinall, P., & Allan, P. (1997) Color Doppler Imaging in Untreated High- and Normal-Pressure Open-Angle Glaucoma. *Investigative Ophthalmology & Visual Science* **38:** 690-696.

Bynke, H.G. (1968) Influence of intraocular pressure on the amplitude of the corneal pulse. *Acta Ophthalmologica* **46:** 1135-1145.

Bynke, H.G. (1969) Further studies on the ocular pulse in rabbits at experimental carotid occlusion and at loading of the bulb. *Acta Ophthalmologica* **47:** 514-522.

Bynke, H.G., & Krakau, C.E.T. (1964) A handy Instrument for oculo-sphygmography with some clinical results. *Ophthalmologica* **148:** 367-373.

Bynke, H.G., & Schele, B. (1967) On the origin of the ocular pressure pulse. *Ophthalmologica* **153:** 29-36.

Cai, J., & Boulton, M. (2002) The pathogenesis of diabetic retinopathy: old concepts and new questions. *Eye* **16:** 242-60.

Campbell, M.J., & Machin, D. (1999). *Medical statistics: a commonsense approach* (3rd ed.). Chichester: John Wiley & Sons, Ltd.

Canning, C.R., & Restori, M. (1988) Doppler ultrasound studies of the ophthalmic artery. *Eye* **2:** 92-95.

Carter, C.J., Brooks, D.E., Doyle, D.L., & Drance, S.M. (1990) Investigations into a Vascular Etiology for Low-tension Glaucoma. *Ophthalmology* **97:** 49-55.

Cartwright, M.J., Grajewski, A.L., Friedberg, M.L., Anderson, D.R., & Richards, D.W. (1992) Immune-related disease and normal-tension glaucoma. *Archives of Ophthalmology* **110:** 500-502.

Castren, J.A., & Lavikainen, P. (1963) New method of diagnosing carotid diseases. *Acta Ophthalmologica* **41:** 219-223.

Cellini, M., Possati, G.L., Profazio, V., Sbrocca, M., Caramazza, N., & Caramazza, R. (1997) Color Doppler imaging and plasma levels of endothelin-1 in low-tension glaucoma. *Acta Ophthalmologica Scandinavica* **?:** 11-13.

Centofanti, M., Bonini, S., Manni, G., Guinetti-Neuschuler, C., Bucci, M.G., & Harris, A. (2000a) Do sex and hormonal status influence choroidal circulation? *British Journal of Ophthalmology* **84:** 786-787.

Centofanti, M., Migliardi, R., Bonini, S., Manni, G., Bucci, M.G., Pesavento, C.B., Amin, C.S., & Harris, A. (2002) Pulsatile ocular blood flow during pregnancy. *European Journal of Ophthalmology* **12:** 276-80.

Centofanti, M., Zarfati, D., Manni, G.L., Bonini, S., Migliardi, R., Oddone, F., Harris, A., & Bucci, M.G. (2000b) The influence of oestrogen on the pulsatile ocular blood flow. *Acta Ophthalmologica Scandinavica* **132:** 38-39.

Chandra, S.R., & Friedman, E. (1972) Choroidal blood flow. II. The effects of autonomic agents. *Archives of Ophthalmology* **87:** 67-69.

Chang, B.Y.P., Hee, W.C.L., Ling, R., Broadway, D.C., & Beigi, B. (2000) Local anaesthetic techniques and pulsatile ocular blood flow. *British Journal of Ophthalmology* **84:** 1260-1263.

Chao, P., & Bettman, J.W. (1957) The relative volume of blood in the choroid and retina. *American Journal of Ophthalmology* **162:** 294.

Chapleau, M.W. (1999) Arterial baroreflexes. In J. L. Izzo & H. R. Black (Eds.), *Hypertension Primer* (pp. 83-86). Baltimore: Lippincott Williams & Wilkins.

Chen, S.-J., Cheng, C.-Y., Lee, A.-F., Lee, F.-L., Chou, J.C.K., Hsu, W.-M., & Liu, J.-H. (2001) Pulsatile ocular blood flow in asymmetric exudative age related macular degeneration. *British Journal of Ophthalmology* **85:** 1411-1415.

Cheng, H.-M., Singh, O.S., Kwong, K.K., Xiong, J., Woods, B.T., & Brady, T.J. (1992) Shape of the myopic eye as seen with high-resolution magnetic resonance imaging. *Optometry and Vision Science* **69:** 698-701.

Chidlow, G., Nash, M.S., Crowhurst, C., Bron, A.J., & Osborne, N.N. (1996) The Ocular Blood Flow Tonograph: A New Instrument for the Measurement of Intraocular Pressure in Rabbits. *Experimental Eye Research* **63:** 463-469.

Chopp, M., Balian, J.V., & Snitgen, C.R. (1983) Systems analysis of intraocular pressure pulse waves. *Glaucoma* **5:** 168-172.

Chumbley, L.C., & Brubaker, R.F. (1976) Low-tension glaucoma. *American Journal of Ophthalmology* **81:** 761-767.

Cioffi, G.A., & Buskirk, E.M.V. (1996) Vasculature of the Anterior Optic Nerve and Peripapillary Choroid. In R. Ritch, M. B. Shields, & T. Krupin (Eds.), *The Glaucomas* (2nd ed., Vol. I, pp. 177-188). St Louis: Mosby.

Ciulla, T.A., Harris, A., Latkany, P., Piper, H.C., Arend, O., Garzozi, H., & Martin, B. (2002) Ocular perfusion abnormalities in diabetes. *Acta Ophthalmologica Scandinavica* **80:** 468-77.

Claridge, K.G. (1993) The effect of topical pilocarpine on pulsatile ocular blood flow. *Eye* 7: 507-510.

Claridge, K.G., & James, C.B. (1994a) Ocular pulse measurements to assess pulsatile blood flow in carotid artery disease. *British Journal of Ophthalmology* **78:** 321--323.

Claridge, K.G., & Smith, S. (1994b) Diurnal variation in pulsatile ocular blood flow in normal and glaucomatous eyes. *Survey of Ophthalmology* **38:** S198-S205.

Collaborative Normal Tension Glaucoma Study Group. (1998a) Comparison of glaucomatous progression between untreated patients with normal-tension glaucoma and patients with therapeutically reduced intraocular pressures. *American Journal of Ophthalmology* **126:** 487-497.

Collaborative Normal Tension Glaucoma Study Group. (1998b) The effectiveness of intraocular pressure reduction in the treatment of normal-tension glaucoma. *American Journal of Ophthalmology* **126:** 498-505.

Coleman, D.J., & Lizzi, F.L. (1979) In vivo choroidal thickness measurement. *American Journal of Ophthalmology* **88:** 369-375.

Cooley, J.S., & Tukey, J.W. (1965) An algorithm for the machine calculation of complex Fourier series. *Mathematics of Computation* **19:** 297-301.

Copt, R.P., Thomas, R., & Mermoud, A. (1999) Corneal thickness in ocular hypertension, primary open-angle glaucoma, and normal tension glaucoma. *Archives of Ophthalmology* **117:** 14-16.

Coupland, S.G., Deschenes, M.C., & Hamilton, R.C. (2001) Impairment of ocular blood flow during regional orbital anesthesia. *Canadian Journal of Ophthalmology* **36:** 140-144.

Crowhurst, C., & Massey, A.D. (1996) Reliability of Pulsatile Ocular Blood Flow Values in normal eyes. *Investigative Ophthalmology & Visual Science* **37:** S434

Cunha-Vaz, J.G., Fonseca, J.R., de Abreu, J.R.F., & Lima, J.J.P. (1978) Studies on retinal blood flow II. diabetic retinopathy. *Archives of Ophthalmology* **96:** 809-811.

Curran-Everett, D., Zhang, Y., Jones, R.H., & Jones, D. (1995) Hypoxia, hypercapnia and hypertension: their effects on pulsatile cerebral flow. *Journal of Applied Physiology* **79:** 870-878.

Curtin, B.J., Iwamoto, T., & Renaldo, D.P. (1979) Normal and staphylomatous sclera of high myopia. *Archives of Ophthalmology* **97:** 912-915.

Dallinger, S., Dorner, G.T., Wenzel, R., Graselli, U., Findl, O., Eichler, H.G., Wolzt, M., & Schmetterer, L. (2000) Endothelin-1 contributes to hyperoxia-induced vasoconstriction in the human retina. *Investigative Ophthalmology & Visual Science* **41:** 864-869.

Dandona, L., Hendrickson, A., & Quigley, H.A. (1991) Selective effects of experimental glaucoma on axonal transport by retinal ganglion cells to the dorsal lateral geniculate nucleus. *Investigative Ophthalmology* **32:** 1593-1599.

Dekker, P.W., Kanngiesser, H., & Robert, Y.C. (1996) [Contact glass tonometer]. *Klin Monatsbl Augenheilkd* **208:** 370-2.

Dekker, P.W., Robert, Y.C.A., Kanngiesser, H., Pirani, P., & Entenmann, B. (1999) Principles of Contact Lens Tonometry. *International Ophthalmology* **22:** 105-111.

Delaey, C., & Van de Voorde, J. (2000) Regulatory mechanisms in the retinal and choroidal circulation. *Ophthalmic Research* **32:** 249-256.

Demailly, P., Cambien, F., Plouin, P.F., Baron, P., & Chevalier, B. (1984) Do patients with low tension glaucoma have particular cardiovascular characteristics? *Ophthalmologica* **188:** 65-75.

Dielemans, I., Vingerling, J.R., Algra, D., Hofman, A., Grobbee, D.E., & de Jong, P.T.V.M. (1995) Primary open-angle glaucoma, intraocular pressure, and systemic blood pressure in the general elderly population - The Rotterdam Study. *Ophthalmology* **102:** 54-60.

Dielemans, I., Vingerling, J.R., Wolfs, R.C.W., Hofman, A., & Grobbee, D.E. (1994) The prevalence of primary open-angle glaucoma in a population-based study in the Netherlands - The Rotterdam Study. *Ophthalmology* **101:** 1851-1855.

Dimitrova, G., Kato, S., Tamaki, Y., Yamashita, H., Nagahara, M., Sakurai, M., Kitano, S., & Fukushima, H. (2001) Choroidal circulation in diabetic patients. *Eye* **15:** 602-607.

Doughty, M.J., & Zaman, M.L. (2000) Human Corneal Thickness and Its Impact on Intraocular Pressure Measures: A Review and Meta-analysis Approach. *Survey of Ophthalmology* **44:** 367-408.

Draeger, J., Michelson, G., & Rumberger, E. (2000) Continuous Assessment of Intraocular Pressure - Telematic Transmission, even under Flight- or Space Mission Conditions. *European Journal of Medical Research* **5:** 2-4.

Drance, S., Anderson, D., & Schulzer, M. (2001) Risk Factors for Progression of Visual Field Abnormalities in Normal-tension Glaucoma. *American Journal of Ophthalmology* **131:** 699-708.

Drance, S.M., Douglas, G.R., Wijsman, K., Schulzer, M., & Britton, R.J. (1988) Response of Blood Flow to Warm and Cold in Normal and Low-Tension Glaucoma. *American Journal of Ophthalmology* **105:** 35-39.

Dubbelman, M., Van der Heijde, G.L., & Weeber, H.A. (2001) The thickness of the aging human lens obtained from corrected Scheimpflug images. *Optometry and Vision Science* **78:** 411-416.

Duijm, H.F., van den Berg, T.J.T.P., & Greve, E.L. (1997) A Comparison of Retinal and Choroidal Hemodynamics in Patients With Primary Open-Angle Glaucoma and Normal-pressure Glaucoma. *American Journal of Ophthalmology* **123:** 644-656.

Dumskyj, M.J., & Kohner, E.M. (1999) Retinal Blood Flow Regulation in Diabetes Mellitus: Impaired Autoregulation and No Detectable Effect of Autonomic Neuropathy Using Laser Doppler Velocimetry, Computer Assisted Image Analysis, and Isometric Exercise. *Microvascular Research* **57:** 353-356.

Durham, D.G., Bigliano, R.P., & Masino, J.A. (1965) Pneumatic Applanation Tonometer. *Transactions of the American Academy of Ophthalmology & Otolaryngology* **69:** 1029-1047.

Edmund, C. (1988) Corneal elasticity and ocular rigidity in normal and keratoconic eyes. *Acta Ophthalmologica* **66:** 134-140.

Ehlers, N., Hansen, F.K., & Aasved, H. (1975) Biometric correlations of corneal thickness. *Acta Ophthalmologica* **53:** 652-659.

Eisenberg, D.L., Sherman, B.G., McKeown, C.A., & Schuman, J.S. (1998) Tonometry in adults and children: a manometric evaluation of pneumatonometry, applanation, and TonoPen in vitro and in vivo. *Ophthalmology* **105:** 1173-1181.

Eisenlohr, J.E., & Langham, M.E. (1962a) The relationship between pressure and volume changes in living and dead rabbit eyes. *Investigative Ophthalmology & Visual Science* **1:** 63-77.

Eisenlohr, J.E., Langham, M.E., & Maumenee, A.E. (1962b) Manometric studies of the pressure-volume relationship in living and enucleated eyes of individual human subjects. *British Journal of Ophthalmology* **46:** 536-548.

Entenmann, B., Robert, Y.C.A., Pirani, P., Kanngiesser, H., & Dekker, P.W. (1997) Contact Lens Tonometry - Application in Humans. *Investigative Ophthalmology & Visual Science* **38:** 2447-2451.

Esgin, H., Alimgil, M.L., & Erda, S. (2001) The effect of systemic hypertension on pulsatile ocular blood flow in diabetic patients. *Acta Ophthalmologica Scandinavia* **79:** 160-162.

Evans, D.W., Harris, A., Danis, R.P., Arend, O., & Martin, B.J. (1997) Altered retrobulbar vascular reactivity in early diabetic retinopathy. *British Journal of Ophthalmology* **81:** 279-282.

Evans, D.W., Harris, A., Garrett, M., Chung, H.S., & Kagemann, L. (1999) Glaucoma patients demonstrate faulty autoregulation of ocular blood flow during posture change. *British Journal of Ophthalmology* **83:** 809-813.

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. *Graefe's Archive for Clinical and Experimental Ophthalmology* **240:** 475-480.

Evans, J. (1995) Causes of blindness and partial sight in England and Wales 1990/91, *Studies on Medical and Population Subjects No. 57* . London: HMSO.

Eysteinsson, T., Jonasson, F., Sasaki, H., Arnarsson, A., Sverrisson, T., Sasaki, K., & Stefansson, E. (2002) Central corneal thickness, radius of the corneal curvature and intraocular pressure in normal subjects using non-contact techniques: Reykjavik Eye Study. *Acta Ophthalmologica Scandinavica* **80:** 11-15.

Fallon, T.J., Chowincyzk, P., & Kohner, E.M. (1986) Measurement of retinal blood flow in diabetes by the blue-light entoptic phenomenon. *British Journal of Ophthalmology* **70:** 43-46.

Faraci, F.M., & Heistad, D.D. (1998) Regulation of the cerebral circulation: role of endothelium and potassium channels. *Physiology Review* **78:** 53-97.

Feke, G.T., Buzney, S.M., Ogasawara, H., Fujio, N., Goger, D.G., Spack, N.P., & Gabbay, K.H. (1994) Retinal circulatory abnormalities in type 1 diabetes. *Investigative Ophthalmology & Visual Science* **35:** 2968-2975.

Feke, G.T., Tagawa, H., Deupree, D.M., Goger, D.G., Sebag, J., & Weiter, J.J. (1989) Blood Flow in the Normal Human Retina. *Investigative Ophthalmology & Visual Science* **30:** 58-65.

Feucht, M., Pillunat, L.E., Mueller, M.F., Bernd, A.S., Koeller, A.U., Boehm, A.G., & Mollenhauer, K.A. (2001) Comparison of measurements by the SmartLens®-System and the OBF-System U.K.®. *Investigative Ophthalmology & Vision Science* **42:** s21.

Findl, O., Dallinger, S., Rami, B., Polak, K., Schober, E., Wedrich, A., Ries, E., Eichler, H.-G., Wolzt, M., & Schmetterer, L. (2000a) Ocular haemodynamics and colour contrast sensitivity in patients with type 1 diabetes. *British Journal of Ophthalmology* **84:** 493-498.

Findl, O., Rainer, G., Dallinger, S., Dorner, G., Polak, K., Kiss, B., Georgopoulos, M., Vass, C., & Schmetterer, L. (2000b) Assessment of Optic Disk Blood Flow in Patients with Open-angle Glaucoma. *American Journal of Ophthalmology* **130:** 589-596.

Flammer, J. (1994) The vascular concept of glaucoma. *Survey of Ophthalmology* **38 Suppl:** S3-6.

Flammer, J. (1996) To What Extent Are Vascular Factors Involved in the Pathogenesis of Glaucoma? In H. J. Kaiser, J. Flammer, & P. Hendrickson (Eds.), *Ocular Blood Flow* (pp. 12-39). Basel: Karger.

Flammer, J., Guthauser, U., & Mahler, F. (1987) Do ocular vasospasms help cause low tension glaucoma? In E. L. Greve & A. Heijl (Eds.), *Seventh International Visual Field Symposium* (pp. 397-399). Dordrecht: Martinus Nijhoff / Dr W. Junk Publishers.

Flammer, J., Haefliger, I.O., Orgul, S., & Resink, T. (1999) Vascular Dysregulation: A Principal Risk Factor for Glaucomatous Damage? *Journal of Glaucoma* **8:** 212-219.

Flammer, J., Orgul, S., Costa, V.P., Orzalesi, N., Krieglstein, G.K., Serra, L.M., Renard, J.P., & Stefansson, E. (2002) The impact of ocular blood flow in glaucoma. *Progress in Retinal and Eye Research* **21:** 359-93.

Flammer, J., Pache, M., & Resink, T. (2001) Vasospasm, its role in the pathogenesis of diseases with particular reference to the eye. *Progress in Retinal and Eye Research* **20:** 319-49.

Flower, R.W., Fryczkowski, A.W., & McLeod, D.S. (1995) Variability in choriocapillaris blood flow distribution. *Investigative Ophthalmology &Visual Science* **36:** 1247-1258.

Flower, R.W., & Klein, G.J. (1990) Pulsatile flow in the choroidal circulation: a preliminary investigation. *Eye* **4:** 310-318.

Flügel-Koch, C., May, C.A., & Lütjen-Drecoll, E. (1996) Presence of a contractile cell network in the human choroid. *Ophthalmologica* **210:** 296-302.

Fontana, L., Poinoosawmy, D., Bunce, C.V., O'Brien, C., & Hitchings, R. (1998) Pulsatile ocular blood flow investigation in asymmetric normal tension glaucoma and normal subjects. *British Journal of Ophthalmology* **82:** 731-736.

Foulds, W.S. (1990) The choroidal circulation and retinal metabolism - an overview. *Eye* **4:** 243-248.

Frenkel, R.E.P., Hong, Y.J., & Shin, D.H. (1988) Comparison of the Tono-Pen to the Goldmann Applanation Tonometer. *Archives of Ophthalmology* **106:** 750-753.

Friedenwald, J.S. (1937) Contribution to the theory and practice of tonometry. *American Journal of Ophthalmology* **20:** 985-1024.

Friedland, A.B. (1983) Relationship Between Arterial Pulsations and Intraocular Pressure. *Experimental Eye Research* **37:** 421-428.

Friedman, E., & Chandra, S.R. (1972) Choroidal blood flow III. Effects of oxygen and carbon dioxide. *Archives of Ophthalmology* **87:** 70-71.

Fryczkowski, A.W., Sherman, M.D., & Walker, J. (1991) Observations on the lobular organization of the human choriocapillaris. *International Ophthalmology* **15:** 109-120.

Funk, R.H.W. (1997) Blood supply of the retina. *Ophthalmic Research* **29:** 320-325.

Galassi, F., Nuzzaci, G., Sodi, A., Casi, A., Cappelli, S., & Vielmo, A. (1994) Possible correlations of ocular blood-flow parameters with intraocular-pressure and visual-field alterations in glaucoma - A study by means of color Doppler imaging. *Ophthalmologica* **208:** 304-308.

Gale, E.A.M., & Anderson, J.V. (1994) Diabetes mellitus and other disorders of metabolism. In P. J. Kumar & M. L. Clark (Eds.), *Clinical Medicine* (Third ed., pp. 829-870). London: W B Saunders.

Galin, M.A., & Best, M. (1971) Studies in intra-ocular vascular dynamics. *Transactions of the Ophthalmological Society of the UK* **91:** 279-290.

Galin, M.A., Best, M., Plechaty, G., & Nussbaum, P. (1972) The ocular pulse. *Transactions of the American Academy of Ophthalmology and Otolaryngology* **76:** 1535-1541.

Galin, M.A., & Harris, L. (1966) The ocular pulse in carotid-cavernous sinus fistula. *American Journal of Ophthalmology* **61:** 1472-1479.

Gallin-Cohen, P.F., Podos, S.M., & Yablonski, M.E. (1980) Oxygen lowers intraocular pressure. *Investigative Ophthalmology & Visual Science* **19:** 43-8.

Gasser, P., & Flammer, J. (1987) Influence of vasospasm on visual function. *Documenta Ophthalmologica* **66:** 3-18.

Gasser, P., & Flammer, J. (1991) Blood-Cell Velocity in the Nailfold Capillaries of Patients with Normal-Tension and High-Tension Glaucoma. *American Journal of Ophthalmology* **111:** 585-588.

Gee, W. (1985) Ocular Pneumoplethysmography. *Survey of Ophthalmology* **29:** 276-292.

Gee, W., & Reed, J.F. (1999) Ocular Volume Change per minute with Ocular Pneumoplethysmography. *Ophthalmic Research* **31:** 351-357.

Geijer, C., & Bill, A. (1979) Effects of raised intraocular pressure on retinal, prelaminar, laminar, and retrolaminar optic nerve flow in monkeys. *Investigative Ophthalmology & Visual Science* **18:** 1030-1042.

Geiser, M.H., Riva, C.E., Dorner, G.T., Diermann, U., Luksch, A., & Schmetterer, L. (2000) Response of choroidal blood flow in the foveal region to hyperoxia and hyperoxia-hypercapnia. *Current Eye Research* **21:** 669-676.

Gekkieva, M., Orgül, S., Gherghel, D., Gugleta, K., Prünte, C., & Flammer, J. (2001) The influence of sex difference in measurements with the langham ocular blood flow system. *Japanese Journal of Ophthalmology* **45:** 528-532.

Geyer, O., Man, O., Weintraub, M., & Silver, D.M. (2001) Acute effect of Latanoprost on pulsatile ocular blood flow in normal eyes. *American Journal of Ophthalmology* **131:** 198-202.

Geyer, O., Neudorfer, M., Snir, T., Goldstein, M., Rock, T., Silver, D.M., & Bartov, E. (1999) Pulsatile ocular blood flow in diabetic retinopathy. *Acta Ophthalmologica Scandinavia* **77:** 522-525.

Gherghel, D., Orgul, S., Dubler, B., Lubeck, P., Gugleta, K., & Flammer, J. (1999) Is Vascular Regulation in the Central Retinal Artery Altered in Persons With Vasospasm. *Archives of Ophthalmology* **117:** 1359-1362.

Gherghel, D., Orgul, S., Gugleta, K., & Flammer, J. (2001) Retrobulbar blood flow in glaucoma patients with nocturnal over-dipping in systemic blood pressure. *American Journal of Ophthalmology* **132:** 641-7.

Gilchrist, J.M. (1996) On the precision and reliability of IOP measurements. *British Journal of Ophthalmology* **80:** 586-587.

Glucksberg, M.R., & Dunn, R. (1993) Direct measurement of retinal microvascular pressures in the live, anesthetized cat. *Microvascular Research* **45:** 158-165.

Goh, W.S.H., & Lam, C.S.Y. (1994) Changes in refractive trends and optical components of Hong Kong Chinese aged 19-39 years. *Ophthalmic and Physiological Optics* **14:** 378-382.

Goldman, V.H., & Schmidt, T. (1957) Über Applanationstonometrie. *Ophthalmologica* **134:** 221-242.

Golnik, K.C., & Miller, N.R. (1992) Diagnosis of cavernous sinus arteriovenous fistula by measurement of the ocular pulse amplitude. *Ophthalmology* **99:** 1146-1152.

Gordon, M.O., Beiser, J.A., Brandt, J.D., Heuer, D.K., Higginbotham, E.J., Johnson, C.A., Keltner, J.L., Miller, J.P., Parrish II, R.K., Wilson, M.R., Kass, M.A., & Ocular Hypertension Treatment Study Group. (2002) The Ocular Hypertension Treatment Study - Baseline factors that predict the onset of primary open-angle glaucoma. *Archives of Ophthalmology* **120:** 714-720.

Goss, D.A., Van Veen, H.G., Rainey, B.B., & Feng, B. (1997) Ocular Components Measured by Keratometry, Phakometry, and Ultrasonography in Emmetropic and Myopic Optometry Students. *Optometry and Vision Science* **74:** 489-495.

Graham, S.L., & Drance, S.M. (1999) Nocturnal Hypotension: Role in Glaucoma Progression. *Survey of Ophthalmology* **43:** S10-S16.

Graham, S.L., Drance, S.M., Wijsman, K., Douglas, G.R., & Mikelberg, F.S. (1995) Ambulatory Blood Pressure Monitoring in Glaucoma. *Ophthalmology* **102:** 61-69.

Grajewski, A.L., Ferrari-Dileo, G., Feuer, W.J., & Anderson, D.R. (1991) Beta-adrenergic responsiveness of choroidal vasculature. *Ophthalmology* **98:** 989-995.

Grant, W.M. (1950) Tonographic method for measuring the facility and rate of aqueous flow in human eyes. *Archives of Ophthalmology* **44:** 204-214.

Grebe, R., Hopkins, S., & Langham, M. (1990) Comparison of an indirect and direct method for the measurement of ocular blood flow in the conscious and anaesthetized rabbit. *Investigative Ophthalmology & Visual Science* **31:** 381.

Greene, P.R. (1985) Closed-form ametropic pressure-volume and ocular rigidity solutions. *American Journal of Optometry & Physiological Optics* **62:** 870-878.

Grødum, K., Heijl, A., & Bengtsson, B. (2001) Refractive error and glaucoma. *Acta Ophthalmologica Scandinavica* **79:** 560-566.

Grunwald, J.E., Hariprasad, S.M., & DuPont, J. (1998a) Effect of aging on foveolar choroidal circulation. *Archives of Ophthalmology* **116:** 150-154.

Grunwald, J.E., Piltz, J., Hariprasad, S.M., & DuPont, J. (1998b) Optic nerve and choroidal circulation in glaucoma. *Investigative Ophthalmology & Visual Science* **39:** 2329-36.

Grunwald, J.E., Riva, C.E., Sinclair, S.H., Brucker, A.J., & Petrig, B.L. (1986) Laser Doppler velocimetry study of retinal circulation in diabetes mellitus. *Archives of Ophthalmology* **104:** 991-996.

Grunwald, J.E., Riva, C.E., Stone, R.A., Keates, E.U., & Petrig, B.L. (1984) Retinal autoregulation in open-angle glaucoma. *Ophthalmology* **91:** 1690-4.

Grunwald, J.E., Sinclair, S.H., & Riva, C.E. (1982) Autoregulation of the retinal circulation in response to decrease of intraocular pressure below normal. *Investigative Ophthalmology & Visual Science* **23:** 124-127.

Guthauser, U., Flammer, J., & Mahler, F. (1988) The relationship between digital and ocular vasospasm. *Graefe's Archive for Clinical and Experimental Ophthalmology* **226:** 224-226.

Guttridge, N.M. (1994) Changes in ocular and visual variables during the menstrual cycle. *Ophthalmic and Physiological Optics* **14:** 38-48.

Haefliger, I.O., & Anderson, D.R. (1996a) Blood Flow Regulation in the Optic Nerve Head. In R. Ritch, M. B. Shields, & T. Krupin (Eds.), *The Glaucomas* (Vol. I, pp. 189-197). St Louis: Mosby.

Haefliger, I.O., & Anderson, D.R. (1996b) Pericytes and Capillary Blood Flow Modulation. In H. J. Kaiser, J. Flammer, & P. Hendrickson (Eds.), *Ocular Blood Flow* (pp. 74-78). Basel: Karger.

Haefliger, I.O., Dettman, E., Liu, R., Meyer, P., Prünte, C., Messerli, J., & Flammer, J. (1999) Potential role of nitric oxide and endothelin in the pathogenesis of glaucoma. *Survey of Ophthalmology* **43:** S51-S58.

Haefliger, I.O., Flammer, J., & Lüscher, T.F. (1992) Nitric oxide and endothelin-1 are important regulators of human ophthalmic artery. *Investigative Ophthalmology & Visual Science* **33:** 2340-2343.

Haefliger, I.O., Fleischhauer, J.C., & Flammer, J. (2000) In glaucoma, should enthusiasm about neuroprotection be tempered by the experience obtained in other neurodegenerative disorders? *Eye* **14:** 464-472.

Harris, A., Anderson, D.R., Pillunat, L., Joos, K., Knighton, R.W., Kagemann, L., & Martin, B.J. (1996) Laser Doppler flowmetry measurement of changes in human optic nerve head blood flow in response to blood gas perturbations. *Journal of Glaucoma* **5:** 258-265.

Harris, A., Arend, O., Wolf, S., Cantor, L.B., & Martin, B.J. (1995) CO_2 dependence of retinal arterial and capillary blood velocity. *Acta Ophthalmologica Scandinavica* **73:** 421-424.

Harris, A., Ciulla, T.A., Chung, H.S., & Martin, B. (1998a) Regulation of Retinal and Optic Nerve Blood Flow. *Archives of Ophthalmology* **116:** 1491-1495.

Harris, A., & Jonescu-Cuypers, C.P. (2001) The impact of glaucoma medication on parameters of ocular perfusion. *Current Opinion in Ophthalmology* **12:** 131-137.

Harris, A., Kagemann, L., & Cioffi, G.A. (1998b) Assessment of Human Ocular Hemodynamics. *Survey of Ophthalmology* **42:** 509-533.

Harris, A., Sergott, R.C., Spaeth, G.L., Katz, J.L., Shoemaker, J.A., & Martin, B.J. (1994) Color Doppler Analysis of Ocular Vessel Blood Velocity in Normal-tension Glaucoma. *American Journal of Ophthalmology* **118:** 642-649.

Hart, W.M. (1992) Intraocular Pressure. In W. M. Hart (Ed.), *Adler's Physiology of the Eye* (pp. 248-267).

Harwerth, R.S., Crawford, M.L., Frishman, L.J., Viswanathan, S., Smith, E.L., 3rd, & Carter-Dawson, L. (2002) Visual field defects and neural losses from experimental glaucoma. *Progress in Retinal and Eye Research* **21:** 91-125.

Hasler, P.W., Orgül, S., Gugleta, K., Vogten, H., Zhao, X., Gherghel, D., & Flammer, J. (2002) Vascular dysregulation in the choroid of subjects with acral vasospasm. *Archives of Ophthalmology* **120:** 302-307.

Hayreh, S.S. (1989) Blood supply of the optic nerve head in health and disease. In G. N. Lambrou & E. L. Greve (Eds.), *Ocular Blood Flow in Glaucoma* (pp. 3-48). Amsterdam: Kugler & Ghedini.

Hayreh, S.S. (1990) *In Vivo* choroidal circulation and its watershed zones. *Eye* **4:** 273-289.

Hayreh, S.S. (1995) The Optic Nerve Head Circulation in Health and Disease. *Experimental Eye Research* **61:** 259-272.

Hayreh, S.S., Zimmerman, M.B., Podhajsky, P., & Alward, W.L. (1994) Nocturnal arterial hypotension and its role in optic nerve head and ocular ischemic disorders. *American Journal of Ophthalmology* **117:** 603-24.

Hedges, T.R., Baron, E.M., Hedges, T.R.I., & Sinclair, S.H. (1994) The retinal venous pulse: Its relation to optic disc characteristics and choroidal pulse. *Ophthalmology* **101:** 542-547.

Hegyalijai, T., Meienberg, O., Dubler, B., & Gasser, P. (1997) Cold-induced acral vasospasm in migraine as assessed by nailfold video-microscopy: prevalence and response of migraine prophylaxis. *Angiology* **48:** 345-349.

Hendrickson, P. (1999) Mechanical ocular blood-flow model to challenge the 'Ocular Blood Flow' (O.B.F.) - Device with known pulsatile-flow values. *Klinische Monatsblatter fur Augenheilkunde und Augenarztliche Fortbilding* **214:** 272-274.

Henricsson, M., & Heijl, A. (1994) Visual fields at different stages of diabetic retinopathy. *Acta Ophthalmologica* **72:** 560-569.

Hidayat, A.A., & Fine, B.S. (1985) Diabetic Choroidopathy. *Ophthalmology* **92:** 512-522.

Hitchings, R. (1991) The ocular pulse. *British Journal of Ophthalmology* **75:** 65.

Hitchings, R.A. (1998) Can Pulsatile Ocular Blood Flow be a Predictor of Future Visual Field Loss in Normal-Tension Glaucoma? In Krieglstein (Ed.), *Glaucoma Update VI* (pp. 153-155). Berlin Heidelberg: Springer Verlag.

Hodapp, E., Parrish II, R.K., & Anderson, D.R. (1993). *Clinical Decisions in Glaucoma.* St Louis: Mosby - Year Book, Inc.

Holland, M.G., Madison, J., & Bean, W. (1960) The ocular rigidity function. *American Journal of Ophthalmology* **50:** 958-974.

Holló, G., Lakatos, P., & Farkas, K. (1998) Cold Pressor Test and Plasma Endothelin-1 Concentration in Primary Open-Angle and Capsular Glaucoma. *Journal of Glaucoma* **7:** 105-110.

Hopkins, S.D., Grebe, R., & Langham, M.E. (1989) Pulsatile blood flow in cataractous eyes. *Investigative Ophthalmology & Vision Science* **30:** 499.

Hørven, I. (1968) Dynamic tonometry. *Acta Ophthalmologica* **46:** 1213-1221.

Hørven, I. (1969) Dynamic tonometry and choroidal melanoma. *Archives of Ophthalmology* **82:** 440-445.

Hørven, I. (1970a) Dynamic tonometry III. The corneal indentation pulse in normal and glaucomatous eyes. *Acta Ophthalmologica* **48:** 39-58.

Hørven, I. (1970b) Dynamic tonometry IV. The corneal indentation pulse in giant cell arteritis. *Acta Ophthalmologica* **48:** 710-718.

Hørven, I., & Nornes, H. (1971) Crest Time Evaluation of Corneal Indentation Pulse. *Archives of Ophthalmology* **86:** 5-11.

Hørven, I., & Sjaastad, O. (1977) Cluster headache syndrome and migraine: Ophthalmological support for a two-entity theory. *Acta Ophthalmologica* **55:** 35-51.

Hørven, I., & Syrdalen, P. (1969) Corneal indentation pulse and general anesthesia. *Acta Ophthalmologica* **48:** 59-66.

Hosking, S.L., Evans, D.W., Embleton, S., Good, D., Houde, B., & Bartlett, J. (2000) Spectral Content of IOP Pulse Wave Reveals Differences Between Glaucoma Patients and Normal Subjects. *Investigative Ophthalmology & Visual Science* **41:** S556.

Hosking, S.L., Evans, D.W., Embleton, S.J., Houde, B., Amos, J.F., & Bartlett, J.D. (2001) Hypercapnia invokes an acute loss of contrast sensitivity in untreated glaucoma patients. *British Journal of Ophthalmology* **85:** 1352-6.

Hulbert, M.F.G. (1998) Pulsatile ocular blood flow and intraocular pressure during retrobulbar injection of lignocaine: influence of additives. *Journal of Glaucoma* **7:** 413-416.

Hulley, S., & Cummings, S. (1988) Planning the Measurements: Precision and Accuracy. In S. B. Hulley & S. R. Cummings (Eds.), *Designing Clinical Research* (1st Edn ed., pp. 31-42). Baltimore: Williams & Wilkins.

Iadecola, C., & Zhang, F. (1994) Nitric oxide-dependent and independent components of cerebrovasodilation elicited by hypercapnia. *American Journal of Physiology* **266:** R546-R552.

Ino-ue, M., Azumi, A., & Yamamoto, M. (2000) Ophthalmic artery blood flow velocity changes in diabetic patients as a manifestation of macroangiopathy. *Acta Ophthalmologica Scandinavica* **78:** 173-176.

Isambert, M.G. (1971) Le pouls choroïdien et ses variations. *Bull Soc Ophthal Fr* **71:** 341-348.

Iwata, K., Shirakashi, T., Fukuchi, T., & Nanba, K. (1991) Vascular Reduction Following Axonal Damage in Glaucoma. *Glaucoma Update* **IV:** 99-102.

Jain, M.R., & Marmion, V.J. (1976) A clinical evaluation of the applanation pneumatonograph. *British Journal of Ophthalmology* **60:** 107-110.

James, C.B. (1993). *Pulsatile Ocular Bloodflow in Glaucoma.* Unpublished Doctarate of Medicine, Oxford, Oxford.

James, C.B. (1994) Effect of trabeculectomy on pulsatile ocular blood flow. *British Journal of Ophthalmology* **78:** 818-822.

James, C.B. (1998) Pulsatile Ocular Blood Flow - Editorial. *British Journal of Ophthalmology* **82:** 720-721.

James, C.B., & Smith, S.E. (1991a) The effect of posture on the intraocular pressure and pulsatile ocular blood flow in patients with non-arteritic anterior ischaemic optic neuropathy. *Eye* **5:** 309-314.

James, C.B., & Smith, S.E. (1991b) Pulsatile ocular blood flow in patients with low tension glaucoma. *British Journal of Ophthalmology* **75:** 466-470.

James, C.B., Trew, D.R., Clark, K., & Smith, S.E. (1991c) Factors influencing the ocular pulse - axial length. *Graefe's Archive for Clinical and Experimental Ophthalmology* **229:** 341-344.

Jänig, W. (1988) Pre- and postganglionic vasoconstrictor neurons. *Annual Reviews of Physiology* **50:** 525-539.

Jay, J.L. (1992) The vascular factor in low tension glaucoma: alchemist's gold? *British Journal of Ophthalmology* **76:** 1.

Jennings, B.J. (1991) Ophthalmodynamometry. In J. B. Eskridge, J. F. Amos, & J. D. Bartlett (Eds.), *Clinical Procedures in Optometry* (pp. 550-565). Philadelphia: J B Lippincott Company.

Johnson, P.C. (1964) Review of Previous Studies and Current Theories of Autoregulation. *Circulation Research* **14/15:** 2-9.

Jonas, J.B., Budde, W.M., & Panda-Jonas, S. (1999) Ophthalmoscopic Evaluation of the Optic Nerve Head. *Survey of Ophthalmology* **43:** 293-320.

Kahn, H.A., & Milton, R.C. (1980) Alternative definitions of open-angle glaucoma. *Archives of Ophthalmology* **98:** 2172-2177.

Kaiser, H.J., Flammer, J., Graf, T., & Stumpfig, D. (1993) Systemic blood pressure in glaucoma patients. *Graefe's Archive for Clinical and Experimental Ophthalmology* **231:** 677-680.

Kashiwagi, K., Hosaka, O., Kashiwagi, F., Taguchi, K., Mochizuki, J., Ishii, H., Ijiri, H., Tamura, K., & Tsukahara, S. (2001) Systemic circulatory parameters. comparison between patients with normal tension glaucoma and normal subjects using ambulatory monitoring. *Japanese Journal of Ophthalmology* **45:** 388-96.

Kass, M.A., Heuer, D.K., Higginbotham, E.J., Johnson, C.A., Keltner, J.L., Miller, J.P., Parrish II, R.K., Wilson, M.R., Gordon, M.O., & Ocular Hypertension Treatment Study Group. (2002) The Ocular Hypertension Treatment Study - A randomized trial determines that topical ocular hypotensive medication delays or prevents the onset of primary open-angle glaucoma. *Archives of Ophthalmology* **120:** 701-713.

Kaufmann, C., Fierz, A., Kollias, S., & Robert, Y.C.A. (2002) Ocular pulse amplitude in a case of innominate steal sydrome. *American Journal of Ophthalmology* **133:** 155-156.

Kellogg, D.L., Jr., Liu, Y., & Pergola, P.E. (2001) Selected contribution: Gender differences in the endothelin-B receptor contribution to basal cutaneous vascular tone in humans. *Journal of Applied Physiology* **91:** 2407-11; discussion 2389-90.

Kergoat, H. (1997) Using the POBF as an index of interocular blood flow effects during unilateral vascular stress. *Vision Research* **37:** 1085-9.

Kergoat, H., & Faucher, C. (1999) Effects of oxygen and carbogen breathing on choroidal hemodynamics in humans. *Investigative Ophthalmology & Visual Science* **40:** 2906-2911.

Kerr, J., Nelson, P., & O'Brien, C. (1998) A comparison of ocular blood flow in untreated primary open-angle glaucoma and ocular hypertension. *American Journal of Ophthalmology* **126:** 42-51.

Kerty, E., Hørven, I., Dahl, A., & Nyberg-Hansen, R. (1994) Ocular and cerebral blood flow measurements in healthy subjects: A comparison of blood flow velocity and dynamic tonometry measurements before and after acetazolamide. *Acta Ophthalmologica* **72:** 401-408.

Kety, S.S., & Schmidt, C.F. (1948) The effects of altered arterial tensions of carbon dioxide and oxygen on cerebral blood flow and cerebral oxygen consumption of normal young men. *Journal of Clinical Investigation* **12:** 484-492.

Khan, J.C., Hughes, E.H., Tom, B.D., & Diamond, J.P. (2002) Pulsatile ocular blood flow: the effect of the Valsalva manoeuvre in open angle and normal tension glaucoma: a case report and prospective study. *British Journal of Ophthalmology* **86:** 1089-1092.

Kiel, J.W. (1992) Autoregulation of Choroidal Blood Flow in the Rabbit. *Investigative Ophthalmology & Visual Science* **33:** 2399-2410.

Kiel, J.W. (1995) The effect of arterial pressure on the ocular pressure-volume relationship in the rabbit. *Experimental Eye Research* **59:** 267-278.

Kiel, J.W. (1999) Modulation of choroidal autoregulation in the rabbit. *Exp Eye Research* **69:** 413-29.

Kiel, J.W. (2000) Endothelin modulation of choroidal blood flow in the rabbit. *Exp Eye Research* **71:** 543-50.

Kiss, B., Dallinger, S., Polak, K., Findl, O., Eichler, H.G., & Schmetterer, L. (2001) Ocular hemodynamics during isometric exercise. *Microvascular Research* **61:** 1-13.

Kitaya, N., Yoshida, A., Ishiko, S., Mori, F., Abiko, T., Ogasawara, H., Kato, Y., & Nagaoka, T. (1997) Effect of timolol and UF-021 (a prostaglandin-related compound) on pulsatile ocular blood flow in normal volunteers. *Ophthalmic Research* **29:** 139-144.

Kitazawa, Y., Shirai, H., & Go, F.J. (1989) The effect of Ca2+ -antagonist on visual field in low-tension glaucoma. *Graefe's Archive for Clinical and Experimental Ophthalmology* **227:** 408-412.

Klein, B.E., Klein, R., Meuer, S.M., & Goetz, L.A. (1993) Migraine headache and its association with open-angle glaucoma: the Beaver Dam Eye Study. *Investigative Ophthalmology & Visual Science* **34:** 3024-7.

Klein, B.E.K., Klein, R., & Jensen, S.C. (1994) Open-angle glaucoma and older-onset diabetes - The Beaver Dam Eye Study. *Ophthalmology* **101:** 1173-1177.

Klein, M.L., Jampol, L.M., Condon, P.I., Rice, T.A., & Serjeant, G.R. (1982) Central retinal artery occlusion without retrobulbar hemorrhage after retrobulbar anesthesia. *American Journal of Ophthalmology* **93:** 573-577.

Knox, D.L. (1973) Ocular pressure studies in the diagnosis of carotid artery disease. *Transactions of the American Ophthalmological Society* **71:** 459-487.

Knudsen, S.T., Poulsen, P.L., Hansen, K.W., Ebbehoj, E., Bek, T., & Mogensen, C.E. (2002) Pulse pressure and diurnal blood pressure variation: association with micro- and macrovascular complications in type 2 diabetes. *American Journal of Hypertension* **15:** 244-50.

Kohner, E.M. (1976) The problems of retinal blood flow in diabetes. *Diabetes* **25:** S839-S844.

Kondo, M., Wang, L., & Bill, A. (1997) The role of nitric oxide in hyperaemic response to flicker in the retina and optic nerve in cats. *Acta Ophthalmologica Scandinavica* **75:** 232-235.

Konno, S., Feke, G.T., Yoshida, A., Fujio, N., Goger, D.G., & Buzney, S.M. (1996) Retinal Blood Flow Changes in Type I Diabetes: A long-term, Follow-Up study. *Investigative Ophthalmology & Visual Science* **37:** 1140-1148.

Kothe, A.C. (1994) The Effect of Posture on Intraocular Pressure and Pulsatile Ocular Blood Flow in Normal and Glaucomatous Eyes. *Survey of Ophthalmology* **38:** S191-S197.

Kothe, A.C., & Vachon, N. (1992) Factors affecting the pulsatile ocular blood flow: axial length and ocular rigidity. *Optometry and Vision Science* **69:** S74-S75.

Krakau, C., Lindberg, S., & Havelius, U. (1995) An instrument for recording the ocular pulse wave. *Acta Ophthalmologica Scandinavica* **73:** 472-474.

Krakau, C.E. (1992) Calculation of the pulsatile ocular blood flow. *Investigative Ophthalmology & Visual Science* **33:** 2754-6.

Krakau, C.E.T. (1995) A model for pulsatile and steady ocular blood flow. *Graefe's Archive for Clinical and Experimental Ophthalmology* **233:** 112-118.

Krishna, N., & Botelho, S.Y. (1963) Rhythmic Changes in Intraocular Pressure Due to Periodic Changes in Vasomotor Tone. *Archives of Ophthalmology* **69:** 125-129.

Kubota, T., Jonas, J.B., & Naumann, G. (1993) Decreased choroidal thickness in eyes with secondary angle closure glaucoma. *British Journal of Ophthalmology* **77:** 430-432.

Kumar, P., & Clark, M. (1994) Diabetes Mellitus. In P. Kumar & M. Clark (Eds.), *Clinical Medicine* . London: Saunders.

Lam, A.K., Chan, H., Fan, W., & To, C.H. (1999) A preliminary study on the ocular blood flow (OBF) of Hong Kong Chinese. *Ophthalmic and Physiological Optics* **19:** 512-517.

Lam, A.K.C., Wong, S., Lam, C.S.Y., & To, C.-H. (2002) The effect of myopic axial elongation and posture on the pulsatile ocular blood flow in young normal subjects. *Optometry and Vision Science* **79:** 300-305.

Lambrou, G.N., Sindhunata, P., van den Berg, T.J.T.P., Geijssen, C.H., Vyborny, P., & Greve, E.L. (1989) Ocular pulse measurements in low-tension glaucoma. In G. N. Lambrou & E. L. Greve (Eds.), *Ocular Blood Flow in Glaucoma* (pp. 115-120). Amsterdam: Kugler & Ghedini.

Lance, J.W. (1994) The classification of headache. In J. W. Lance (Ed.), *Mechanism and Management of Headache* (pp. 23-24). Oxford: Butterworth-Heinemann Ltd.

Langham, M.E. (1987) Ocular Blood Flow and Visual Loss in Glaucomatous Eyes. In G. K. Krieglstein (Ed.), *Glaucoma Update III* (pp. 58-66). Berlin: Springer-Verlag.

Langham, M.E., Farrell, R., Krakau, T., & Silver, D. (1991a) Ocular Pulsatile Blood Flow, Hypotensive Drugs, and Differential Light Sensitivity in Glaucoma. In G. K. Krieglstein (Ed.), *Glaucoma Update IV* (pp. 162-172). Berlin Heidelberg: Springer-Verlag.

Langham, M.E., Farrell, R.A., O'Brien, V., Silver, D.M., & Schilder, P. (1989a) Blood flow in the human eye. *Acta Ophthalmologica Supplementum* **191:** S9-S13.

Langham, M.E., Farrell, R.A., O'Brien, V., Silver, D.M., & Schilder, P. (1989b) Non-invasive measurement of pulsatile blood flow in the human eye. In G. N. Lambrou & E. L. Greve (Eds.), *Ocular Blood Flow in Glaucoma* (pp. 93-99). Amsterdam: Kugler & Ghedini.

Langham, M.E., Grebe, R., Hopkins, S., Marcus, S., & Sebag, M. (1991b) Choroidal Blood Flow in Diabetic Retinopathy. *Experimental Eye Research* **52:** 167-173.

Langham, M.E., & Kramer, T. (1990) Decreased Choroidal Blood Flow Associated with Retinitis Pigmentosa. *Eye* **4:** 374-381.

Langham, M.E., Leydhecker, W., Krieglstein, G., & Waller, W. (1976) Pneumatonographic studies on normal and glaucomatous eyes. *Advances in Ophthalmology* **32:** 108-133.

Langham, M.E., & McCarthy, E. (1968) A rapid pneumatic applanation tonometer. Comparative findings and evaluation. *Archives of Ophthalmology* **79:** 389-99.

Langham, M.E., & Preziosi, T.J. (1984) Non-invasive diagnosis of mild to severe stenosis of the internal carotid artery. *Stroke* **15:** 614-621.

Langham, M.E., & To'mey, K.F. (1978) A Clinical Procedure for the Measurements of the Ocular Pulse-Pressure Relationship and the Ophthalmic Arterial Pressure. *Experimental Eye Research* **27:** 17-25.

Lassen, N.A. (1964) Autoregulation of cerebral blood flow. *Circulation Research* **14/15:** 201-204.

Lawrence, C., & Schlegel, W.A. (1966) Ophthalmic pulse studies I. Influence of intraocular pressure. *Investigative Ophthalmology* **5:** 515-525.

Lawrenson, J.G. (2000) Histopathology and pathogenesis of diabetic retinopathy. In A. R. Rudnicka (Ed.), *Diabetic Eye Disease - identification and co-management* (pp. 32-43). Oxford: Butterworth Heinemann.

Leske, M.C., Connell, A.M.S., Wu, S.-Y., Hyman, L.G., Schachat, A.P., & Barbados Eye Study Group. (1995) Risk factors for open-angle glaucoma. *Archives of Ophthalmology* **113:** 918-924.

Lester, H.A. (1966) Ocular Oscillometry in Cerebrovascular Disease. *Archives of Ophthalmology* **76:** 391-398.

Levene, R.Z. (1980) Low Tension Glaucoma: A Critical Review and New Material. *Survey of Ophthalmology* **24:** 621-664.

Levick, J. (2000a) Control of stroke volume and cardiac output. In J. Levick (Ed.), *An Introduction to Cardiovascular Physiology* (3rd ed., pp. 86-117). London: Arnold.

Levick, J.R. (1995) Coordinated cardiovascular responses. In J. R. Levick (Ed.), *An Introduction to Cardiovascular Physiology* (1st ed., pp. 276-291). Oxford: Butterworth-Heinemann.

Levick, J.R. (2000b) Control of blood vessels I: Local control. In J. R. Levick (Ed.), *An introduction to cardiovascular physiology* (3rd ed., pp. 261-278). London: Arnold.

Levick, J.R. (2000c) Haemodynamics: pressure, flow and resistance. In J. R. Levick (Ed.), *An Introduction to Cardiovascular Physiology* (3rd ed., pp. 118-147). London: Arnold.

Levick, J.R. (2000d) Overview of the cardiovascular system. In J. R. Levick (Ed.), *An Introduction to Cardiovascular Physiology* (3rd ed., pp. 1-14). London: Arnold.

Levkovitch-Verbin, H., Martin, K.R., Quigley, H.A., Baumrind, L.A., Pease, M.E., & Valenta, D. (2002) Measurement of amino Acid levels in the vitreous humor of rats after chronic intraocular pressure elevation or optic nerve transection. *Journal of Glaucoma* **11:** 396-405.

Leydhecker, W. (1976) The Intraocular Pressure: Clinical Aspects. *Annals of Ophthalmology* **8:** 389-399.

Lieberman, M.F., Maumanee, A.E., & Green, R.W. (1976) Histologic studies of the vasculature of the anterior optic nerve. *American Journal of Ophthalmology* **82:** 405-423.

Liu, B., & Neufeld, A.H. (2000) Expression of nitric oxide synthase-2 (NOS-2) in reactive astrocytes of the human glaucomatous optic nerve head. *Glia* **30:** 178-186.

Liu, C.J., Chiou, H.J., Chiang, S.C., Chou, J.C., Chou, Y.H., & Liu, J.H. (1999a) Variations in ocular hemodynamics in patients with early and late glaucoma. *Acta Ophthalmologica Scandinavica* **77:** 658-62.

Liu, C.J., Ko, Y.C., Cheng, C.Y., Chou, J.C., Hsu, W.M., & Liu, J.H. (2002) Effect of latanoprost 0.005% and brimonidine tartrate 0.2% on pulsatile ocular blood flow in normal tension glaucoma. *British Journal of Ophthalmology* **86:** 1236-9.

Liu, J.H.K., Kripke, D.F., Twa, M.D., Hoffman, R.E., Mansberger, S.L., Rex, K.M., Girkin, C.A., & Weinreb, R.N. (1999b) Twenty-Four–Hour Pattern of Intraocular Pressure in the Aging Population. *Investigative Ophthalmology & Visual Science* **40:** 2912-2917.

Luksch, A., Garhöfer, G., Imhof, A., Polak, K., Polska, E., Dorner, G.T., Anzenhofer, S., Wolzt, M., & Schmetterer, L. (2002) Effect of inhalation of different mixtures of O2 and CO2 on retinal blood flow. *British Journal of Ophthalmology* **86:** 1143-1147.

Lumme, P., Tuulonen, A., Airaksinen, P.J., & Alanko, H.I. (1991) Neuroretinal rim area in low tension glaucoma: Effect of nifedipine and acetazolamide compared to no treatment. *Acta Ophthalmologica* **69:** 293-298.

Lynn, B., & Cotsell, B. (1991) The delay in onset of vasodilator flare in human skin at increasing distance from a localized noxious stimulus. *Microvascular Research* **41:** 197-202.

Mackie, S.W., Jay, J.L., Ackerley, R., & Walsh, G. (1996) Clinical comparison of the Keeler Pulsair 2000, American Optical MkII and Goldmann applanation tonometers. *Ophthalmic and Physiological Optics* **16:** 171-177.

Mackinnon, J.R., McKillop, G., O'Brien, C., Swa, K., Butt, Z., & Nelson, P. (2000) Colour Doppler imaging of the ocular circulation in diabetic retinopathy. *Acta Ophthalmologica Scandinavica* **78:** 386-389.

MacKinnon, J.R., O'Brien, C., Swa, K., Aspinall, P., Butt, Z., & Cameron, D. (1997) Pulsatile ocular blood flow in untreated diabetic retinopathy. *Acta Ophthalmologica Scandinavia* **75:** 661-664.

Mäepea, O. (1992) Pressures in the anterior ciliary arteries, choroidal veins and choriocapillaris. *Experimental Eye Research* **54:** 731-736.

Mann, R.M., Riva, C.E., Stone, R.A., Barnes, G.E., & Cranstoun, S.D. (1995) Nitric oxide and choroidal blood flow regulation. *Investigative Ophthalmology & Visual Science* **36:** 925-930.

Mark, H.H. (1973) Corneal curvature in applanation tonometry. *American Journal of Ophthalmology* **76:** 223-224.

Maurice, D.M. (1958) A recording tonometer. *British Journal of Ophthalmology* **42:** 321-335.

McBain, E.H. (1958) Tonometer calibration II. Ocular Rigidity. *Archives of Ophthalmology* **60:** 1080-1091.

McDonald, D.A. (1955) The relation of pulsatile pressure to flow in arteries. *Journal of Physiology* **127:** 533-552.

McKibbin, M., Cassidy, L., Dabbs, T.R., & Verma, D. (1999a) Letter to the editor. *Eye* **?:** 266-267.

McKibbin, M., & Menage, M.J. (1999b) The effect of once-daily latanoprost on intraocular pressure and pulsatile ocular blood flow in normal tension glaucoma. *Eye* **13:** 31-34.

McKibbin, M., & Verma, D. (1999c) Recurrent amaurosis fugax without haemodynamically significant ipsilateral carotid stenosis. *Acta Ophthalmologica Scandinavica* **77:** 224-226.

McLeod, D.S., & Lutty, G.A. (1994) High-Resolution Histologic Analysis of the Human Choroidal Vasculature. *Investigative Ophthalmology & Visual Science* **35:** 3799-3811.

McVeigh, G., Brennan, G., Hayes, R., Cohn, J., Finkelstein, S., & Johnston, D. (1993) Vascular abnormalities in non-insulin-dependent diabetes mellitus identified by arterial waveform analysis. *American Journal of Medicine* **95:** 424 - 430.

Meininger, G.A., & Davis, M.J. (1992) Cellular mechanisms involved in the vascular myogenic response. *American Journal of Physiology* **263:** H647-659.

Melkumyants, A.M., Balashov, S.A., & Khayutin, V.M. (1995) Control of arterial lumen by shear stress on endothelium. *News in Physiological Sciences* **10:** 204-210.

Meyer, P., Flammer, J., & Lüscher, T.F. (1993) Local anesthetic drugs reduce endothelium-dependent relaxations of porcine ciliary arteries. *Investigative Ophthalmology & Visual Science* **34:** 2730-2736.

Michelson, G., Groh, M., & Gründler, A. (1994) Regulation of ocular blood flow during increases of arterial blood pressure. *British Journal of Ophthalmology* **78:** 461-465.

Michelson, G., & Harazny, J. (1997) Relationship between ocular pulse pressures and retinal vessel velocities. *Ophthalmology* **104:** 664-671.

Michelson, G., Langhans, M.J., & Groh, M.J.M. (1996) Perfusion of the Juxtapapillary Retina and the Neuroretinal Rim Area in Primary Open Angle Glaucoma. *Journal of Glaucoma* **5:** 91-98.

Miller, K., & Quigley, H.A. (1988) The clinical appearance of the lamina cribrosa as a function of the extent of glaucomatous optic nerve damage. *Ophthalmology* **95:** 135-138.

Mills, C.J., Gabe, I.T., Gault, J.H., Mason, D.T., Ross Jr., J., Braunwald, E., & Shillingford, J.P. (1970) Pressure-flow relationships and vascular impedance in man. *Cardiovascular Research* **4:** 405-417.

Mitchell, P., Hourihan, F., Sandbach, J., & Wang, J.J. (1999) The relationship between glaucoma and myopia: the Blue Mountains Eye Study. *Ophthalmology* **106:** 2010-5.

Mitchell, P., Smith, W., Chey, T., & Healey, P.R. (1997) Open-angle glaucoma and diabetes. *Ophthalmology* **104:** 712-718.

Mittag, T.W., Serle, J., Schumer, R., Brodie, S., & al, e. (1994) Studies of the ocular pulse in primates. *Survey of Ophthalmology* **38:** S183-190.

Moalem, G., Leibowitz-Amit, R., Yoles, E., Mor, F., Cohen, I.R., & Schwartz, M. (1999) Autoimmune T cells protect neurons from secondary degeneration after central nervous system axotomy. *Nature Medicine* **5:** 49-55.

Morgan, A., Harper, J., Hosking, S., & Gilmartin, B. (2002a) The effect of corneal thickness and corneal curvature on pneumatonometer measurements. *Current Eye Research* **25:** 107-112.

Morgan, A., & Hosking, S. (2001) Ocular blood flow tonometer reproducibility: the effect of operator experience and mode of application. *Ophthalmic and Physiological Optics* **21:** 401-406.

Morgan, A.J., & Hosking, S.L. (2003) Measurement of ocular blood flow in optometric practice. In S. Doshi & W. Harvey (Eds.), *Investigative Techniques and Ocular Examination* (pp. 69-74). Edinburgh: Butterworth-Heinemann.

Morgan, A.J., Hosking, S.L., & Salmon, J.F. (2002b) Clinical evaluation of the dynamic observing tonometer. *Journal of Glaucoma* **11:** 334-9.

Morgan, W.H., Yu, D.-Y., Cooper, R.L., Alder, V.A., Cringle, S.J., & Constable, I.J. (1995) The Influence of Cerebrospinal Fluid Pressure on the Lamina Cribrosa Tissue Pressure Gradient. *Investigative Ophthalmology & Visual Science* **36:** 1163-1172.

Mori, F., Konno, S., Hikichi, T., Yamaguchi, Y., Ishiko, S., & Yoshida, A. (2001a) Factors affecting pulsatile ocular blood flow in normal subjects. *British Journal of Ophthalmology* **85:** 529-530.

Mori, F., Konno, S., Hikichu, T., Yamaguchi, Y., Ishiko, S., & Yoshida, A. (2001b) Pulsatile ocular blood flow study: decreases in exudative age related macular degeneration. *British Journal of Ophthalmology* **85:** 531-533.

Morsman, C.D., Bosem, M.E., Lusky, M., & Weinreb, R.N. (1995) The Effect of Topical Beta-Adrenoceptor Blocking Agents on Pulsatile Ocular Blood Flow. *Eye* **9:** 344-347.

Moses, R.A. (1958) The Goldmann applanation tonometer. *American Journal of Ophthalmology* **46:** 865-869.

Moses, R.A., & Grodzki, W.J. (1979) The pneumatonograph - a laboratory study. *Archives of Ophthalmology* **97:** 547-552.

Nagaoka, T., Mori, F., & Yoshida, A. (2002) Retinal Artery Response to Acute Systemic Blood Pressure Increase during Cold Pressor Test in Humans. *Investigative Ophthalmology & Visual Science* **43:** 1941-1945.

Nariai, T., Senda, M., Ishii, K., Wakabayashi, S., Yokota, T., Toyama, H., Matsushima, Y., & Hirakawa, K. (1998) Posthyperventilatory steal response in chronic cerebral hemodynamic stress: a positron emission tomography study. *Stroke* **29:** 1281-92.

Neal, M.J. (1992) Autonomic Nervous System. In M. J. Neal (Ed.), *Medical Pharmacology at a Glance* (pp. 20-21). Oxford: Blackwell Science Ltd.

Németh, J., Michelson, G., & Harazny, J. (2001) Retinal microcirculation correlates with ocular wall thickness, axial eye length, and refraction in glaucoma patients. *Journal of Glaucoma* **10:** 390-395.

Nicastro, N.J., Requa, R.K., Campo, R.V., & Barnet, R.W. (1987) Decreased Ocular Pulse Amplitudes in Glaucoma. *Glaucoma* **9:** 45-52.

Nicholls, W.W., & O'Rourke, M.F. (1998a) Classification of arterial pressure waves. In W. W. Nicholls & M. F. O'Rourke (Eds.), *McDonald's Blood Flow in Arteries: Theoretical, experimental and clinical principles* (4th ed., pp. 215-218). London: Arnold.

Nicholls, W.W., & O'Rourke, M.F. (1998b) Properties of the arterial wall: practice. In W. W. Nicholls & M. F. O'Rourke (Eds.), *McDonald's Blood Flow in Arteries: Theoretical, experimental and clinical principles* (4th ed., pp. 73-97). London: Arnold.

Nicholls, W.W., & O'Rourke, M.F. (1998c) Properties of the arterial wall: theory. In W. W. Nicholls & M. F. O'Rourke (Eds.), *McDonald's Blood Flow in Arteries: Theoretical, experimental and clinical principles* (4th ed., pp. 54-72). London: Arnold.

Nichols, W.W., Conti, C.R., Walker, W.E., & Milnor, W.R. (1977) Input Impedance of the Systemic Circulation in Man. *Circulation Research* **40:** 451-458.

Nichols, W.W., & O'Rourke, M.F. (1998a) Aging. In W. W. Nichols & M. F. O'Rourke (Eds.), *McDonald's Blood Flow in Arteries - Theoretical, experimental and clinical principles* (4th ed., pp. 347-376). London: Arnold.

Nichols, W.W., & O'Rourke, M.F. (1998b) Hypertension. In W. W. Nichols & M. F. O'Rourke (Eds.), *McDonald's Blood Flow in Arteries - Theoretical, experimental and clinical principles* (4th ed., pp. 377-395). London: Arnold.

Nichols, W.W., & O'Rourke, M.F. (1998c) Measurement of blood flow. In W. W. Nichols & M. F. O'Rourke (Eds.), *McDonald's Blood Flow in Arteries - Theoretical, experimental and clinical principles* (4th ed., pp. 135-136). London: Arnold.

Nichols, W.W., & O'Rourke, M.F. (1998d) Measuring principles of arterial waves. In W. W. Nichols & M. F. O'Rourke (Eds.), *McDonald's Blood Flow in Arteries: Theoretical, experimental and clinical principles* (4th ed., pp. 114-153). London: Arnold.

Nichols, W.W., & O'Rourke, M.F. (1998e) Principles of recording and analysis of arterial waveforms. In W. W. Nichols & M. F. O'Rourke (Eds.), *McDonald's Blood Flow in Arteries: Theoretical, experimental and clinical principles* (Fourth ed., pp. 223 - 242). London: Arnold.

Nichols, W.W., & O'Rourke, M.F. (1998f) The role of smooth muscle in the arterial wall. In W. W. Nichols & M. F. O'Rourke (Eds.), *McDonald's Blood Flow in Arteries* (4th ed., pp. 85-87). London: Arnold.

Nichols, W.W., & O'Rourke, M.F. (1998g) Vascular impedance. In W. W. Nichols & M. F. O'Rourke (Eds.), *McDonald's Blood Flow in Arteries - Theoretical, experimental and clinical principles* (4th ed., pp. 243-283). London: Arnold.

Nichols, W.W., & O'Rourke, M.F. (1998h) Wave reflections. In W. W. Nichols & M. F. O'Rourke (Eds.), *McDonald's Blood Flow in Arteries - Theoretical, experimental and clinical principles* (4th ed., pp. 201-222). London: Arnold.

Nickells, R.W. (1996) Retinal ganglion cell death in glaucoma: the how, the why, and the maybe. *Journal of Glaucoma* **5:** 345-356.

Nicolela, M.T., Hnik, P., & Drance, S.M. (1996) Scanning Laser Doppler Flowmeter Study of Retinal and Optic Disk Blood Flow in Glaucomatous Patients. *American Journal of Ophthalmology* **122:** 775-783.

Niwa, Y., Harris, A., Kagemann, L., Yamamoto, T., Matsubara, M., Takahashi, & Kitazawa, Y. (1999) A new system to supply carbon dioxide safely to glaucoma patients. *Japanese Journal of Ophthalmology* **43:** 16-9.

Noble, M.I.M. (1968) The contribution of blood momentum to left ventricular ejection in the dog. *Circulation Research* **23**.

Norman, G.R., & Streiner, D.L. (1993) Factorial ANOVA, *Biostatistics - The Bare Essentials* (pp. 73-82). St Louis: Mosby Year Book, Inc.

Norman, G.R., & Streiner, D.L. (2000a) Elements of statistical inference. In G. R. Norman & D. L. Streiner (Eds.), *Biostatistics - The Bare Essentials* (pp. 42-55). Hamilton, Ontario: B.C. Decker Inc.

Norman, G.R., & Streiner, D.L. (2000b) Multiple Regression. In G. R. Norman & D. L. Streiner (Eds.), *Biostatistics: The Bare Essentials* (pp. 127-137). Hamilton, Ontario, Canada: B.C. Decker Inc.

O'Brien, C., & Butt, Z. (1999) Blood Flow Velocity in the Peripheral Circulation of Glaucoma Patients. *Ophthalmologica* **213:** 150-153.

Oku, H., Sugiyama, T., Kojima, S., Watanabe, T., & Azuma, I. (1999) Experimental optic cup enlargement caused by endothelin-1-induced chronic optic nerve head ischemia. *Survey of Ophthalmology* **44 Suppl 1:** S74-84.

Olsen, T., Nielsen, C.B., & Ehlers, N. (1980) On the optical measurement of corneal thickness I. Optical principle and sources of error. *Acta Ophthalmologica* **58:** 760-766.

Olver, J.M. (1990) Functional anatomy of the choroidal circulation: methyl methacrylate casting of human choroid. *Eye* **4:** 262-272.

Olver, J.M., Spalton, D.J., & McCartney, A.C.E. (1990) Microvascular study of the retrolaminar optic nerve in man: the possible significance in anterior ischaemic optic neuropathy. *Eye* **4:** 7-24.

Orgül, S., Gugleta, K., & Flammer, J. (1999) Physiology of Perfusion as It Relates to the Optic Nerve Head. *Survey of Ophthalmology* **43:** S17-S26.

O'Rourke, M. (1989) Systolic blood pressure: arterial compliance and early wave reflection, and their modification by antihypertensive therapy. *Journal of Human Hypertension* **3:** 47-52.

O'Rourke, M.F. (1981) Letter to the editor. *American Journal of Physiology* **240:** R393-R395.

O'Rourke, M.F. (1999a) Arterial stiffness and hypertension. In J. L. Izzo & H. R. Black (Eds.), *Hypertension Primer* (2nd ed., pp. 160-162). Baltimore: Lippincott Williams & Wilkins.

O'Rourke, M.F. (1999b) Wave travel and reflection in the arterial system. *Journal of Hypertension* **17:** S45-S47.

O'Rourke, M.F., & Taylor, M.G. (1966) Vascular impedance of the femoral bed. *Circulation Research* **18:** 126-139.

Osborne, N.N., Ugarte, M., Chao, M., Chidlow, G., Bae, J.H., Wood, J.P., & Nash, M.S. (1999) Neuroprotection in relation to retinal ischemia and relevance to glaucoma. *Survey of Ophthalmology* **43 Suppl 1:** S102-28.

Osei, K., Fields, P.G., Cataland, S., Craig, E.L., George, J.M., & O'Dorisio, T.M. (1985) Abnormal retinal artery responses to stress in patients with type I diabetes. *American Journal of Medicine* **78:** 595-601.

Pache, M., Kräuchi, K., Cajochen, C., Wirz-Justice, A., Dubler, B., Flammer, J., & Kaiser, H.J. (2001) Cold feet and prolonged sleep-onset latency in vasospastic syndrome. *Lancet* **358:** 125-126.

Pahor, D. (1998) Automated static perimetry as a screening method for evaluation of retinal perfusion in diabetic retinopathy. *International Ophthalmology* **21:** 305-309.

Palmer, R.M.J., Ashton, D.S., & Moncada, S. (1988) Vascular endothelial cells synthesize nitric oxide from L-arginine. *Nature* **333:** 664-666.

Pappenheimer, J.R. (1984) Contributions to microvascular research of Jean Leonard Marie Poiseuille. In E. M. Renkin & C. C. Michel (Eds.), *Handbook of Physiology, Cardiovascular System, Vol 4, The Microcirculation* (pp. 1-10). Bethesda: American Physiological Society.

Parver, L.M., Auker, C., & Carpenter, D.O. (1980) Choroidal blood flow as a heat dissipating mechanism in the macula. *American Journal of Ophthalmology* **89:** 641-646.

Parver, L.M., Auker, C.R., Carpenter, D.O., & Doyle, T. (1982) Choroidal Blood Flow II. Reflexive Control in the Monkey. *Archives of Ophthalmology* **100:** 1327-1330.

Patel, S. (1987) Refractive index of the mammalian cornea and its influence during pachometry. *Ophthalmic and Physiological Optics* **7:** 503-506.

Patel, V., Rassam, S., Newsom, R., Jutta, W., & Kohner, E. (1992) Retinal blood flow in diabetic retinopathy. *British Medical Journal* **305:** 678-683.

Pelli, D.G., Robson, J.G., & Wilkins, A.J. (1988) The design of a new letter chart for measuring contrast sensitivity. *Clinical Vision Science* **2:** 187-199.

Perkins, E.S. (1981a) The ocular pulse. *Current Eye Research* **1:** 19-23.

Perkins, E.S. (1981b) Ocular volume and ocular rigidity. *Experimental Eye Research* **33:** 141-145.

Perkins, E.S. (1985) The ocular pulse and intraocular pressure as a screening test for carotid artery stenosis. *British Journal of Ophthalmology* **69:** 676-680.

Perkins, E.S., Edwards, J., & Saxena, R.C. (1977) A new recording tonometer. *Transactions of the ophthalmological Society UK* **97:** 679-682.

Perkins, E.S., & Gloster, J. (1957) Distensibility of the eye. *British Journal of Ophthalmology* **41:** 93-102.

Perrott, R.L., North, R.V., Drasdo, N., Ahmed, K.A., & Owens, D.R. (2001) The influence of plasma glucose upon pulsatile ocular blood flow in subjects with Type II diabetes mellitus. *Diabetologia* **44:** 700-705.

Peterson, L.H., Jensen, R.E., & Parnell, J. (1960) Mechanical properties of arteries in vivo. *Circulation Research* **8:** 622-639.

Phelps, C.D., & Corbett, J.J. (1985) Migraine and Low-Tension Glaucoma. *Investigative Ophthalmology & Visual Science* **26:** 1105-1108.

Phelps, C.D., & Phelps, G.K. (1976) Measurement of intraocular pressure: a study of its reproducibility. *Albrecht Von Graefes Arch Klin Exp Ophthalmol* **198:** 39-43.

Phillips, C.I., Tsukahara, S., Hosaka, O., & Adams, W. (1992) Ocular pulsation correlates with ocular tension: the choroid as piston for an acqueous pump? *Ophthalmic Research* **24:** 338-343.

Phillips, J.R., & McBrien, N.A. (1995) Form deprivation myopia: elastic properties of sclera. *Ophthalmic and Physiological Optics* **15:** 357-362.

Pianka, P., Weintraub-Padova, H., Lazar, M., & Geyer, O. (2001) Effect of sub-Tenon's and peribulbar anesthesia on intraocular pressure and ocular pulse amplitude. *Journal of Cataract & Refractive Surgery* **27:** 1221-1226.

Pillunat, L.E., Anderson, D.R., Knighton, R.W., Joos, K.M., & Feuer, W.J. (1997) Autoregulation of human optic nerve head circulation in response to increased intraocular pressure. *Experimental Eye Research* **64:** 737-44.

Pillunat, L.E., Stodtmeister, R., Marquardt, R., & Mattern, A. (1989) Ocular perfusion pressures in different types of glaucoma. *International Ophthalmology* **13:** 37-42.

Pillunat, L.E., Stodtmeister, R., & Wilmanns, I. (1987) Pressure compliance of the optic nerve head in low tension glaucoma. *British Journal of Ophthalmology* **71:** 181-7.

Poinoosawmy, D., Indar, A., Bunce, C., Garway-Heath, D.F., & Hitchings, R.A. (2002) Effect of treatment by medicine or surgery on intraocular pressure and pulsatile ocular blood flow in normal-pressure glaucoma. *Graefe's Archive for Clinical and Experimental Ophthalmology* **240:** 721-726.

Polak, K., Dallinger, S., Polska, E., Findl, O., Eichler, H.-G., Wolzt, M., & Schmetterer, L. (2000) Effects of Insulin on Retinal and Pulsatile Choroidal Blood Flow in Humans. *Archives of Ophthalmology* **118:** 55-59.

Price, F.W., Koller, D.L., & Price, M.O. (1999) Central Corneal Pachymetry in Patients Undergoing Laser In Situ Keratomileusis. *Ophthalmology* **106:** 2216-2220.

Prünte, C., Orgül, S., & Flammer, J. (1998) Abnormalities of microcirculation in glaucoma: facts and hints. *Current Opinion in Ophthalmology* **9:** 50-55.

Pugh, J., Eadie, A., Winn, B., & Heron, G. (1987) Power Spectrum Analysis in the Study of Ocular Mechanisms. *Ophthalmic and Physiological Optics* **7:** 321-324.

Purslow, P.P., & Karwatowski, W.S.S. (1996) Ocular Elasticity: Is Engineering Stiffness a More Useful Characterization Parameter than Ocular Rigidity. *Ophthalmology* **103:** 1686-1692.

Quaranta, L., Manni, G., Donato, F., & Bucci, M.G. (1994) The effect of increased intraocular pressure on pulsatile ocular blood flow in low tension glaucoma. *Survey of Ophthalmology* **38 Suppl:** S177-81; discussion S182.

Quigley, H.A. (1989) Histological and clinical features of the optic nerve head in early glaucoma diagnosis. In G. N. Lambrou & E. L. Greve (Eds.), *Ocular Blood Flow in Glaucoma* (pp. 83-84). Milano: Kugler & Ghedini.

Quigley, H.A. (1993) Open-angle glaucoma. *New England Journal of Medicine* **328:** 1097-1106.

Quigley, H.A. (1996) Number of people with glaucoma worldwide. *British Journal of Ophthalmology* **80:** 389-393.

Quigley, H.A., & Addicks, E.M. (1980) Chronic experimental glaucoma in primates II. Effect of extended intraocular pressure elevation on optic nerve head and axonal transport. *Investigative Ophthalmology & Visual Science* **19:** 137-152.

Quigley, H.A., & Anderson, D.R. (1976) The dynamics and location of axonal transport blockade by acute intraocular pressure elevation in primate optic nerve. *Investigative Ophthalmology* **15:** 606-616.

Quigley, H.A., Hohman, R.M., Addicks, E.M., Massof, R.W., & Green, R.W. (1983) Morphologic changes in the lamina cribrosa correlated with neural loss in open-angle glaucoma. *American Journal of Ophthalmology* **95:** 673-691.

Quigley, M., & Cohen, S. (1999) A new pressure attenuation index to evaluate retinal circulation. *Archives of Ophthalmology* **117:** 84-89.

Qureshi, I.A. (1997) Intraocular pressure: a comparative analysis in two sexes. *Clinical Physiology* **17:** 247-255.

Ramrattan, R.S., van der Schaft, T.L., Mooy, C.M., de Bruijn, W.C., Mulder, P.G.H., & de Jong, P.T.V.M. (1994) Morphometric analysis of Bruch's membrane, the choriocapillaris, and the choroid in aging. *Investigative Ophthalmology & Visual Science* **35:** 2857-2864.

Rankin, S.J.A. (1999) Color Doppler Imaging of the Retrobulbar Circulation in Glaucoma. *Survey of Ophthalmology* **43:** S176-S182.

Raper, A.J., Kontos, H.A., & Patterson, J.L. (1971) Response of pial precapillary vessels to changes in arterial carbon dioxide tension. *Circulation Research* **28:** 518-523.

Rassam, S.M.B., Patel, V., & Kohner, E.M. (1995) The Effect of Experimental Hypertension on Retinal Vascular Autoregulation in Humans: a Mechanism for the Progression of Diabetic Retinopathy. *Experimental Physiology* **80:** 53-68.

Ravalico, G., Pastori, G., Crocè, M., & Toffoli, G. (1997) Pulsatile ocular blood flow variations with axial length and refractive error. *Ophthalmologica* **211:** 271-273.

Ravalico, G., Pastori, G., Toffoli, G., & Croce, M. (1994) Visual and Blood Flow Responses in Low-Tension Glaucoma. *Survey of Ophthalmology* **38:** Suppl S173-S176.

Ravalico, G., Toffoli, G., Pastori, G., Croce, M., & Calderini, S. (1996) Age-related ocular blood flow changes. *Investigative Ophthalmology & Visual Science* **37:** 2645-2650.

Ray, W.A., & O'Day, D.M. (1985) Statistical Analysis of Multi-Eye Data in Ophthalmic Research. *Investigative Ophthalmology & Vision Science* **26:** 1186-1188.

Recep, O.F., Hasiripi, H., Vayisoglu, E., Kalayci, D., & Sarikatipoglu, H. (1998) Accurate time interval in repeated tonometry. *Acta Ophthalmologica Scandinavia* **76:** 603-605.

Renkin, E.M. (1984) The control of the microcirculation. In E. M. Renkin & C. C. Michel (Eds.), *Handbook of Physiology, Cardiovascular System, Vol 4, The Microcirculation* (pp. 627-688). Bethesda: American Physiological Society.

Rich, W.J., & James, M.L. (1989) Cataract surgery and vascular changes in the eye. *Acta Ophthalmologica Supplementum* **191:** 39-42.

Ridley, F. (1930) The intraocular pressure and drainage of aqueous humour. *The British Journal of Experimental Pathology* **11:** 217-240.

Riva, C.E., Cranstoun, S.D., Grunwald, J.E., & Petrig, B.L. (1994) Choroidal blood flow in the foveal region of the human ocular fundus. *Investigative Ophthalmology & Visual Science* **35:** 4273-4281.

Riva, C.E., Grunwald, J.E., Sinclair, S.H., & Petrig, B.L. (1985) Blood velocity and volumetric flow rate in human retinal vessels. *Investigative Ophthalmology & Visual Science* **26:** 1124-1132.

Riva, C.E., Hero, M., Titze, P., & Petrig, B. (1997a) Autoregulation of human optic nerve head blood flow in response to acute changes in ocular perfusion pressure. *Graefe's Archive for Clinical and Experimental Ophthalmology* **235:** 618-26.

Riva, C.E., Sinclair, S.H., & Grunwald, J.E. (1981) Autoregulation of retinal circulation in response to decrease of perfusion pressure. *Investigative Ophthalmology & Visual Science* **21:** 34-8.

Riva, C.E., Titze, P., Hero, M., Movaffaghy, A., & Petrig, B.L. (1997b) Choroidal blood flow during isometric exercises. *Investigative Ophthalmology & Visual Science* **38:** 2338-2343.

Riva, C.E., Titze, P., Hero, M., & Petrig, B.L. (1997c) Effect of Acute Decreases of Perfusion Pressure on Choroidal Blood Flow in Humans. *Investigative Ophthalmology & Visual Science* **38:** 1752-1760.

Rizq, R.N., Choi, W.-H., Eilers, D., Wright, M.M., & Ziaie, B. (2001) Intraocular pressure measurement at the choroid surface: a feasibility study with implications for implantable microsystems. *British Journal of Ophthalmology* **85:** 868-871.

Robinson, R., White, M., McCann, P., Magner, J., & Eustace, P. (1991) Effect of anaesthesia on intraocular blood flow. *British Journal of Ophthalmology* **75:** 92-93.

Rockwood, E.J., Fantes, F., Davis, E.B., & Anderson, D.R. (1987) The response of retinal vasculature to angiotensin. *Investigative Ophthalmology & Visual Science* **28:** 676-682.

Rodrigues, L.M., Pinto, P.C., & Leal, A. (2001) Transcutaneous flow related variables measured in vivo: the effects of gender. *BMC Dermatology* **1:** 4.

Roff, E.J., Harris, A., Chung, H.S., Hosking, S.L., Morrison, A.M., Halter, P.J., & Kagemann, L. (1999) Comprehensive assessment of retinal, choroidal and retrobulbar haemodynamics during blood gas perturbation. *Graefe's Archive for Clinical and Experimental Ophthalmology* **237:** 984-990.

Rojanapongpun, P., Drance, S.J., & Morrison, B.J. (1993a) Ophthalmic artery flow velocity in glaucomatous and normal subjects. *British Journal of Ophthalmology* **77:** 25-29.

Rojanapongpun, P., & Drance, S.M. (1993b) The response of blood flow velocity in the ophthalmic artery and blood flow of the finger to warm and cold stimuli in glaucomatous patients. *Graefe's Achive for Clinical and Experimental Ophthalmology* **231:** 375-377.

Ruiz Barranco, F. (1960) Modificaciones de la tension y volumen oculares por la presion arterial. *Arch Soc Oftal Hisp-Amer* **20:** 484-492.

Ruskell, G.L. (1971) Facial parasympathetic Innervation of the choroidal blood vessels in monkeys. *Experimental Eye Research* **12:** 166-172.

Schilder, P. (1989) Ocular blood flow changes with increased resistance external and internal to the eye. *Acta Ophthalmologica Supplementum* **191:** 19-23.

Schilder, P. (1994) Ocular Blood Flow Responses to Pathology of the Carotid and Cerebral Circulations. *Survey of Ophthalmology* **38:** S52-S58.

Schiotz, H.J. (1920) Tonometry. *British Journal of Ophthalmology* **4:** 201-210.

Schlegel, W.A., & Lawrence, C. (1969) Doppler measurement of vortex vein blood flow in animals. *Investigative Ophthalmology* **8:** 201-205.

Schmetterer, L., Dallinger, S., Findl, O., Eichler, H.G., & Wolzt, M. (2000a) A comparison between laser interferometric measurement of fundus pulsation and pneumotonometric measurement of pulsatile ocular blood flow. 1. Baseline considerations. *Eye* **14:** 39-45.

Schmetterer, L., Dallinger, S., Findl, O., Graselli, U., Eichler, H.G., & Wolzt, M. (2000b) A comparison between laser interferometric measurement of fundus pulsation and pneumotonometric measurement of pulsatile ocular blood flow. 2. Effects of changes in pCO2 and pO2 and of isoproterenol. *Eye* **14:** 46-52.

Schmetterer, L., Dallinger, S., Findl, O., Strenn, K., Graselli, U., Eichler, H.-G., & Wolzt, M. (1998) Noninvasive Investigations of the Normal Ocular Circulation in Humans. *Investigative Ophthalmology & Visual Science* **39:** 1210-1220.

Schmetterer, L., Findl, O., Strenn, K., Graselli, U., Kastner, J., Eichler, H.-G., & Wolzt, M. (1997a) Role of NO in O2 and CO2 responsiveness of cerebral and ocular circulations in humans. *American Journal of Physiology* **273:** R2005-R2012.

Schmetterer, L., Findl, O., Strenn, K., Jilma, B., Graselli, U., Eichler, H.G., & Wolzt, M. (1997b) Effects of endothelin-1 (ET-1) on ocular hemodynamics. *Current Eye Research* **16:** 687-92.

Schmetterer, L., Lexer, F., Findl, O., Graselli, U., Eichler, H.-G., & Wolzt, M. (1996) The effect of inhalation of different mixtures of O2 and CO2 on ocular fundus pulsations. *Experimental Eye Research* **63:** 351-355.

Schmetterer, L., & Wolzt, M. (1999) Ocular blood flow and associated functional deviations in diabetic retinopathy. *Diabetologia* **42:** 387-405.

Schmetterer, L., Wolzt, M., Lexer, F., Alschinger, C., Gouya, G., Zanaschka, G., Fassolt, A., Eichler, H.-G., & Fercher, A.F. (1995) The effect of hyperoxia and hypercapnia on fundus pulsations in macular and optic disc region in healthy young men. *Experimental Eye Research* **61:** 685-690.

Schmidt, K.G., Pillunat, L.E., Kohler, K., & Flammer, J. (2001) Ocular pulse amplitude is reduced in patients with advanced retinitis pigmentosa. *British Journal of Ophthalmology* **85:** 678-82.

Schmidt, K.G., von Ruckmann, A., & Mittag, T.W. (1998a) Okuläre Pulsamplitude bei okulärer Hypertension und verschiedenen Glaukomformen. *Ophthalmologica* **212:** 5-10.

Schmidt, K.G., von Ruckmann, A., & Pillunat, L.E. (1998b) Topical carbonic anhydrase inhibition increases ocular pulse amplitude in high tension primary open angle glaucoma. *British Journal of Ophthalmology* **82:** 758-62.

Schmidt, K.-G., Mittag, T.W., Pavlovic, S., & Hessemer, W. (1996) Influence of physical exercise and nifedipine on ocular pulse amplitude. *Graefe's Archive for Clinical and Experimental Ophthalmology* **234:** 527-532.

Schmidt, K.-G., Ruckmann, A.V., Mittag, T.W., Hessemer, V., & Pillunat, L.E. (1997) Reduced ocular pulse amplitude in low tension glaucoma is independent of vasospasm. *Eye* **11:** 485-488.

Schmidt, K.-G., von Ruckmann, A., Kemkes-Matthes, B., & Hans-Peter Hammes, H.-P. (2000) Ocular pulse amplitude in diabetes mellitus. *British Journal of Ophthalmology* **84:** 1282-1284.

Schottenstein, E.M. (1996) Intraocular Pressure and Tonometry. In R. Ritch, M. B. Shields, & T. Krupin (Eds.), *The Glaucomas* (Vol. 1, pp. 407-428). St Louis: Mosby.

Schulzer, M., Drance, S.M., Carter, C.J., Brooks, D.E., Douglas, G.R., & Lau, W. (1990) Biostatistical evidence for two distinct chronic open angle glaucoma populations. *British Journal of Ophthalmology* **74:** 196-200.

Schwartz, B. (1994) Circulatory defects of the optic disk and retina in ocular hypertension and high pressure open-angle glaucoma. *Survey of Ophthalmology* **38 Suppl:** S23-34.

Schwartz, M. (2001) Physiological Approaches to Neuroprotection: Boosting of Protective Autoimmunity. *Survey of Ophthalmology* **45 (Suppl 3):** S256-S260.

Schwenn, O., Troost, R., Vogel, A., Grus, F., Beck, S., & Pfeiffer, N. (2002) Ocular pulse amplitude in patients with open angle glaucoma, normal tension glaucoma, and ocular hypertension. *British Journal of Ophthalmology* **86:** 981-984.

Shaikh, M.H., & Mars, J.S. (2001) The acute effect of pilocarpine on pulsatile ocular blood flow in ocular hypertension. *Eye* **15:** 63-66.

Shennan, A.H., Rushbrook, J., Power, J., Wright, J., & Poston, L. (1998) An accurate oscillometric wrist blood pressure monitor: validation of the Omron Rx (HEM-608). *Journal of Human Hypertension* **12:** 794.

Sherwood, L. (1989) Respiratory System: Gas Exchange. In L. Sherwood (Ed.), *Human Physiology: From Cells to Systems* (pp. 434-435). St Paul, MN: West Publishing Company.

Shih, Y.-F., Horng, I.-H., Yang, C.-H., Lin, L.L.-K., Peng, Y., & Hung, P.-T. (1991) Ocular Pulse Amplitude in Myopia. *Journal of Ocular Pharmacology* **7:** 83-87.

Shiose, Y. (1990) Intraocular Pressure: New Perspectives. *Survey of Ophthalmology* **34:** 413-435.

Siliprandi, R., Canella, R., Carmignoto, G., Schiavo, N., Zanellato, A., Zanoni, R., & Vantini, G. (1992) N-methyl-D-aspartate-induced neurotoxicity in the adult rat retina. *Visual Neuroscience* **8:** 567-573.

Silver, D.M., & Farrell, R.A. (1994) Validity of pulsatile ocular blood flow measurements. *Survey of Ophthalmology* **38:** S72-S80.

Silver, D.M., Farrell, R.A., Langham, M.E., O'Brien, V., & Schilder, P. (1989) Estimation of pulsatile ocular blood flow from intraocular pressure. *Acta Ophthalmologica Supplementum* **191:** 25-29.

Silver, D.M., & Geyer, O. (2000) Pressure-volume relation for the living human eye. *Current Eye Research* **20:** 115-120.

Singh, R.P., Goldberg, I., Graham, S.L., Sharma, A., & Mohsin, M. (2001) Central Corneal Thickness, Tonometry, and Ocular Dimensions in Glaucoma and Ocular Hypertension. *Journal of Glaucoma* **10:** 206-210.

Smith, P. (1891a) Amount of the intraocular pressure, *The Pathology and Treatment of Glaucoma* (pp. 13). London: J & A Churchill.

Smith, P. (1891b). *The Pathology and Treatment of Glaucoma*. London: J & A Churchill.

Sommer, A. (1996) Glaucoma risk factors observed in the Baltimore Eye Survey. *Current Opinion in Ophthalmology* **7:** 93-98.

Sommer, A., Tielsch, J.M., Katz, J., Quigley, H.A., Gottsch, J.D., Javitt, J., Singh, K., & Baltimore Eye Survey Research Group. (1991) Relationship between intraocular pressure and primary open angle glaucoma among white and black Americans. *Archives of Ophthalmology* **109:** 1090-1095.

Spaeth, G.L. (1994) A new classification of glaucoma including focal glaucoma. *Survey of Ophthalmology* **38 Suppl:** S9-17.

Sponsel, W., Paris, G., Trigo, Y., & Pena, M. (2002a) Comparative effects of latanoprost (Xalatan) and unoprostone (Rescula) in patients with open-angle glaucoma and suspected glaucoma. *American Journal of Ophthalmology* **134:** 552.

Sponsel, W.E., DePaul, K.L., & Zetlan, S.R. (1992) Retinal hemodynamic effects of carbon dioxide, hyperoxia, and mild hypoxia. *Investigative Ophthalmology & Visual Science* **33:** 1864-1869.

Sponsel, W.E., Mensah, J., Kiel, J.W., Remky, A., Trigo, Y., & Friberg, T. (2000a) Effects of Latanoprost and Timolol-XE on Hydrodynamics in the Normal Eye. *American Journal of Ophthalmology* **130:** 151-159.

Sponsel, W.E., Paris, G., Sandoval, S.S., Sanford, D.K., Harrison, J.M., Elliott, W.R., & Trigo, Y. (2000b) Sildenafil and Ocular Perfusion. *The New England Journal of Medicine* **342:** 1680.

Sponsel, W.E., Paris, G., Trigo, Y., Pena, M., Weber, A., Sanford, K., & Mckinnon, S. (2002b) Lantanoprost and brimonidine: therapeutic and physiologic assessment before and after oral nonsteroidal anti-inflammatory therapy. *American Journal of Ophthalmology* **133:** 11-18.

Sponsel, W.E., & Shipman, D. (1997) Effects of blood gases on ocular perfusion. In S. M. Drance (Ed.), *Vascular Risk Factors and Neuroprotection in Glaucoma - Update 1996* (pp. 65-71). Amsterdam: Kugler.

Spraul, C., Lang, G.E., Ronzani, M., Hogel, J., & Lang, G.K. (1998) Reproducibility of measurements with a new slit lamp-mounted ocular blood flow tonograph. *Graefe's Archive for Clinical and Experimental Ophthalmology* **236:** 274-279.

Stahl, J., & Vold, S. (2000) Effect of corneal thickness on the accuracy of intraocular pressure measurement in rabbits after excimer laser photoablation. *Journal of Cataract and Refractive Surgery* **26:** 736-743.

Stürmer, J., & Meier-Gibbons, F. (1994) The diagnosis of normal-tension glaucoma. *Current Opinion in Ophthalmology* **5:** 64-68.

Sucher, N.J., Lipton, S.A., & Dreyer, E.B. (1997) Molecular basis of glutamate toxicity in retinal ganglion cells. *Vision Research* **37:** 3483-93.

Sugiyama, T., Utsumi, T., Azuma, I., & Fujii, H. (1996) Measurement of optic nerve head circulation: comparison of laser speckle and hydrogen clearance methods. *Japanese Journal of Ophthalmology* **40:** 339-43.

Sullivan, P., Cioffi, G.A., Wang, L., Johnson, C.A., Van Buskirk, E.M., Sherman, K.R., & Bacon, D.R. (1999) The Influence of Ocular Pulsatility on Scanning Laser Doppler Flowmetry. *American Journal of Ophthalmology* **128:** 81-87.

Suzuki, I. (1962) Corneal pulsation and corneal pulse waves. *Japanese Journal of Ophthalmology* **6:** 190-194.

Syrdalen, P., & Hørven, I. (1970) Corneal indentation pulse and retrobulbar anesthesia. *Acta ophthalmologica* **48:** 719-730.

Tezel, G., Kass, M.A., Kolker, A.E., Becker, B., & Wax, M.B. (1997) Plasma and aqueous humor endothelin levels in primary open-angle glaucoma. *Journal of Glaucoma* **6:** 83-89.

Thorburn, W. (1972) Recordings of applanating force at constant intraocular pressure I. Basic principle and apparatus description. *Acta Ophthalmologica* **50:** 720-736.

Thorburn, W. (1978) The Accuracy of Clinical Applanation Tonometry. *Acta Ophthalmologica* **56:** 1-5.

Tiedeman, J.S., Kirk, S.E., Srinivas, S., & Beach, J.M. (1998) Retinal oxygen consumption during hyperglycaemia in patients with diabetes without retinopathy. *Ophthalmology* **105:** 31-36.

Tielsch, J.M., Katz, J., Quigley, H.A., Javitt, J.C., & Sommer, A. (1995a) Diabetes, intraocular pressure, and primary open-angle glaucoma in the Baltimore Eye Survey. *Ophthalmology* **102:** 48-53.

Tielsch, J.M., Katz, J., Sommer, A., Quigley, H.A., & Javitt, J.C. (1995b) Hypertension, perfusion pressure, and primary open-angle glaucoma. *Archives of Ophthalmology* **113:** 216-221.

To'mey, K.F., Faris, B.M., Jalkh, A.E., & Nasr, A.M. (1981) Ocular Pulse in High Myopia: A Study of 40 Eyes. *Annals of Ophthalmology* **13:** 569-571.

Tomlinson, A., & Leighton, D.A. (1972) Ocular dimensions in low tension glaucoma. *British Journal of Ophthalmology* **56:** 97-105.

Tønjum, A.M. (1972) Studies on the ocular pulse pressure wave in human, dog, and rabbit eyes with a pneumatic tonometer. *Acta Ophthalmologica* **50:** 677-687.

Tønjum, A.M. (1973) A new sensor for pneumatic tonometry. Construction and working principle. *Acta Ophthalmol Suppl* **120:** 12-9.

Trew, D.R., James, C.B., Thomas, H.L.S., Sutton, R., & Smith, S. (1991a) Factors influencing the ocular pulse - the heart rate. *Graefe's Archive for Clinical and Experimental Ophthalmology* **229:** 553-556.

Trew, D.R., & Smith, S.E. (1991b) Postural studies in pulsatile ocular blood flow: I. Ocular hypertension and normotension. *British Journal of Ophthalmology* **75:** 66-70.

Trew, D.R., & Smith, S.E. (1991c) Postural studies in pulsatile ocular blood flow: II. Chronic open angle glaucoma. *British Journal of Ophthalmology* **75:** 71-75.

Trew, D.R., Wright, L.A., & Smith, S.E. (1991d) Ocular responses in healthy subjects to bunazosin 0.3% - an alpha1 adrenoceptor antagonist. *British Journal of Ophthalmology* **75:** 411-413.

Trokel, S. (1965) Effect of respiratory gases upon choroidal hemodynamics. *Archives of Ophthalmology* **73:** 838-842.

Troost, R., Vogel, A., Beck, S., Schwenn, O., Grus, F., & Pfeiffer, N. (2001) Clinical comparison of two intraocular pressure measurement methods: SmartLens dynamic observing tonography versus Goldmann. *Graefe's Archive for Clinical and Experimental Ophthalmology* **239:** 889-892.

Tsacopoulis, M., Baker, R., Johnson, M., Strauss, J., & David, N.J. (1973) The effect of arterial PCO2 on inner-retinal oxygen availability in monkeys. *Investigative Ophthalmology* **12:** 449-455.

Tuck, M.W., & Crick, R.P. (1998) The age distribution of primary open angle glaucoma. *Ophthalmic Epidemiology* **5:** 173-83.

Tuulonen, A., Nagin, P., Schwartz, B., & Wu, D.-C. (1987) Increase of Pallor and Fluorescein-filling Defects of the Optic Disc in the Follow-up of Ocular Hypertensives Measured by Compterized Image Analysis. *Ophthalmology* **94:** 558-563.

Ulrich, A. (1996) Choroidal Angiography with Perfusion Pressure Videoangiography. In H. J. Kaiser, J. Flammer, & P. Hendrickson (Eds.), *Ocular Blood Flow* (pp. 195-206). Basel: Karger.

Ulrich, W.D., Ulrich, C., & Bohne, B.D. (1986) Deficient autoregulation and lengthening of the diffusion distance in the anterior optic nerve circulation in glaucoma: an electro-encephalo- dynamographic investigation. *Ophthalmic Research* **18:** 253-9.

Ulrich, W.-D., & Ulrich, C. (1985) Oculo-Oscillo-Dynamography: A Diagnostic Procedure for Recording Ocular Pulses and Measuring Retinal and Ciliary Arterial Blood Pressures. *Ophthalmic Research* **17:** 308-317.

Ulrich, W.-D., Ulrich, C., & Walther, G. (1989) Ocular perfusion pressure and oculo-oscillodynamography. In G. N. Lambrou & E. L. Greve (Eds.), *Ocular Blood Flow in Glaucoma* (pp. 101-114). Amsterdam: Kugler & Ghedini.

Usui, T., & Iwata, K. (1992) Finger blood flow in patients with low tension glaucoma and primary open-angle glaucoma. *British Journal of Ophthalmology* **76:** 2-4.

Usui, T., Iwata, K., Shirakashi, M., & Abe, H. (1991) Prevalence of migraine in low-tension glaucoma and primary open-angle glaucoma in Japanese. *British Journal of Ophthalmology* **75:** 224-6.

Vafidis, G.C. (2000) Features of diabetic eye disease. In A. R. Rudnicka & J. Birch (Eds.), *Diabetic Eye Disease* (pp. 52-67). Oxford: Butterworth-Heinemann.

van der Werff, T.J. (1981) A new single-parameter ocular rigidity function. *American Journal of Ophthalmology* **92:** 391-395.

Varma, R., Hilton, S.C., Tielsch, J.M., Katz, J., Quigley, H.A., & Sommer, A. (1995) Neural rim area declines with increased intraocular pressure in urban Americans. *Archives of Ophthalmology* **113:** 1001-1005.

Vernon, S. (1995) Reproducibility with the Keeler Pulsair 2000 non-contact tonometer. *British Journal of Ophthalmology* **79:** 554.

Vetrugno, M., Maino, A., Cantatore, F., Ruggeri, G., & Cardia, L. (2001) Acute and chronic effects of brimonidine 0.2% on intraocular pressure and pulsatile ocular blood flow in patients with primary open-angle glaucoma: an open-label, uncontrolled, prospective study. *Clinical Therapeutics* **23:** 1519-1528.

VI, J. (1997). *The Sixth Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure* (98-4080): National Institutes of Health.

Vogel, A., Beck, S., Schwenn, O., Grus, F., Krummenauer, F., & Pfeiffer, N. (2001) [Reproducibility of measurement of ocular pulse amplitude and intraocular pressure using Smartlens]. *Ophthalmologe* **98:** 944-9.

Waldo, A.L., & Wit, A.L. (1993) Mechanisms of cardiac arrhythmias. *Lancet* **341:** 1189-1193.

Walker, R.E., Compton, G.A., & Langham, M. (1975a) Pneumatic Applanation Tonometer Studies. IV. Analysis of Pulsatile Response. *Experimental Eye Research* **20:** 245-253.

Walker, R.E., & Langham, M.E. (1975b) Pneumatic applanation tonometer studies. III. Analysis of the floating tip sensor. *Exp Eye Res* **20:** 167-72.

Walker, R.E., & Litovitz, T.L. (1972) An Experimental and Theoretical Study of the Pneumatic Tonometer. *Experimental Eye Research* **13:** 14-23.

Walter, P., Schnakenberg, U., vom Bogel, G., Ruokonen, P., Kruger, C., Dinslage, S., Ludtke Handjery, H.C., Richter, H., Mokwa, W., Diestelhorst, M., & Krieglstein, G.K. (2000) Development of a completely encapsulated intraocular pressure sensor. *Ophthalmic Research* **32:** 278-84.

Wang, L., & Bill, A. (1997) Effects of constant and flickering light on retinal metabolism in rabbits. *Acta Ophthalmologica Scandinavica* **75:** 227-231.

Watkins, R., Beigi, B., Yates, M., Chang, B., & Linardos, E. (2001) Intraocular pressure and pulsatile ocular blood flow after retrobulbar and peribulbar anaesthesia. *British Journal of Ophthalmology* **85:** 796-798.

Wax, M.B. (2000) Is there a role for the immune system in glaucomatous optic neuropathy? *Current Opinions in Ophthalmology* **11:** 145-150.

Weber, C. (1850). *Nonnullae disquisitiones, quae ad facultatem oculum rebus longinquis et propinquis accomodandi spectant.* Unpublished Inaugural dissertation, Marburg University, Marburg.

Weinberger, D., Kramer, M., Priel, E., Gaton, D.D., Axer-Siegel, R., & Yassur, Y. (1998) Indocyanine Green Angiographic Findings in Nonproliferative Diabetic Retinopathy. *American Journal of Ophthalmology* **126:** 238-247.

Weinreb, R.N. (1992) Editorial: Why Study the Ocular Microcirculation in Glaucoma? *Journal of Glaucoma* **1:** 145-147.

Weinreb, R.N., Lusky, M., Bartsch, D.U., & Morsman, D. (1993) Effect of repetitive imaging on topographic measurements of the optic nerve head. *Archives of Ophthalmology* **111:** 636-638.

Wessely, K. (1908) Experimentelle Untersuchungen über den Augendruck, sowie über qualitative und quantitative Beeinflussing des intraokularen Flüssigkeitswechsels. *Arch Augenheilk* **60:** 1-48.

Westerhof, N., & Elzinga, G. (1991) Normalized input impedance and arterial decay time over heart period are independent of animal size. *American Journal of Physiology* **261:** R126-R133.

Westerhof, N., Sipkema, P., Van den Bos, G.C., & Elzinga, G. (1972) Forward and backward waves in the arterial system. *Cardiovascular Research* **6:** 648-656.

Whitacre, M.M., & Stein, R. (1993a) Sources of error with use of Goldmann-type tonometers. *Survey of Ophthalmology* **38:** 1-30.

Whitacre, M.M., Stein, R.A., & Hassanein, K. (1993b) The effect of corneal thickness on applanation tonometry. *American Journal of Ophthalmology* **115:** 592-6.

Wilkinson, I.B., Fuchs, S.A., Jansen, I.M., Spratt, J.C., Murray, G.D., Cockcroft, J.R., & Webb, D.J. (1998) Reproducibility of pulse wave velocity and augmentation index measured by pulse wave analysis. *Journal of Hypertension* **16:** 2079-84.

Wilson, T.M., Strang, R., & MacKenzie, E.T. (1977) The response of the choroidal and cerebral circulations to changing arterial P CO2 and acetazolamide in the baboon. *Investigative Ophthalmology & Visual Science* **16:** 576-580.

Wolf, S., Arend, O., Sponsel, W.E., Schulte, K., Cantor, L.B., & Reim, M. (1993) Retinal hemodynamics Using Scanning Laser Ophthalmoscopy and Hemorheology in Chronic Open-angle glaucoma. *Ophthalmology* **100:** 1561-1566.

Wolfs, R.C.W., Borger, P.H., Ramrattan, R.S., Klaver, C.C.W., Hulsman, C.A.A., Hofman, A., Vingerling, J.R., Hitchings, R.A., & de Jong, P.T.V.M. (2000) Changing Views on Open-Angle Glaucoma: Definitions and Prevalences - The Rotterdam Study. *Investigative Ophthalmology & Visual Science* **41:** 3309-3321.

Wolfs, R.C.W., Klaver, C.C., Ramrattan, R.S., van Duijn, C.M., Hofman, A., & de Jong, P.T.V.M. (1998) Genetic risk of primary open-angle glaucoma - population-based familial aggregation study. *Archives of Ophthalmology* **116:** 1640-1645.

Womersley, J.R. (1955) Method for the calculation of velocity, rate of flow and viscous drag in arteries when the pressure gradient is known. *Journal of Physiology* **127:** 553-563.

WuDunn, D. (2002) Genetic basis of glaucoma. *Current Opinion in Ophthalmology* **13:** 55-60.

Yamazaki, S., & Baba, H. (1993) Acute effect of topical carteolol on ocular pulsatile volume change. *Acta Ophthalmologica* **71:** 760-764.

Yan, D.B., Coloma, F.M., Metheetrairut, A., Trope, G.E., Heathcote, J.G., & Ethier, C.R. (1994) Deformation of the lamina cribrosa by elevated intraocular pressure. *British Journal of Ophthalmology* **78:** 643-648.

Yang, Y.C., & Hulbert, M.F. (1995) Effect of trabeculectomy on pulsatile ocular blood flow. *British Journal of Ophthalmology* **79:** 507-508.

Yang, Y.C., Hulbert, M.F.G., Batterbury, M., & Clearkin, L.G. (1997a) Pulsatile Ocular Blood Flow Measurements in Healthy Eyes: Reproducibility and Reference Values. *Journal of Glaucoma* **6:** 175-179.

Yang, Y.C., Illango, B., Cook, A., & Batterbury, M. (2000) Intraocular pressure and pulse rate measurements by the OBF tonograph - comparison to reference instruments. *Ophthalmic and physiological optics* **20:** 401-407.

Yang, Y.C., Kent, D., Fenerty, C.H., Kosmin, A.S., & Damato, B.E. (1997b) Pulsatile ocular blood flow in eyes with untreated choroidal melanoma. *Eye* **11:** 331-334.

Yoshida, A., Feke, G.T., Morales-Stoppello, J., Collas, G.D., Goger, D.G., & McMeel, J.W. (1983) Retinal blood flow alterations during progression of diabetic retinopathy. *Archives of Ophthalmology* **101:** 225-227.

Yoshida, A., Feke, G.T., Ogasawara, H., Goger, D.G., Murray, D.L., & McMeel, J.W. (1991) Effect of Timolol on Human Retinal, Choroidal and Optic Nerve Head Circulation. *Ophthalmic Research* **23:** 162-170.

Yoshida, A., Hirokawa, H., Ishiko, S., & Ogasawara, H. (1992) Ocular circulatory changes following scleral buckling procedures. *British Journal of Ophthalmology* **76:** 529-531.

Ytteborg, J. (1960) The role of intraocular blood volume in rigidity measurements on human eyes. *Acta Ophthalmologica* **38:** 410-436.

Zadok, D., Tran, D.B., Twa, M., Carpenter, M., & Schanzlin, D.J. (1999) Pneumotonometry versus Goldmann tonometry after laser in situ keratomileusis for myopia. *Journal of Cataract and Refractive Surgery* **25:** 1344-8.

Zeimer, R.C. (1996) Circadian variations in intraocular pressure. In R. Ritch, M. B. Shields, & T. Krupin (Eds.), *The Glaucomas* (Vol. 1, pp. 429-445). St Louis: Mosby.