ACCOMMODATION AND INTRAOCULAR PRESSURE

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Doctor of Philosophy

ASTON UNIVERSITY

January 2007

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Summary

The relationship between accommodation and intraocular pressure (IOP) has not been addresse question for over 20 years, when measurement of both of these parameters was less advanced tha the central aim of this thesis was to evaluate the effects of accommodation on IOP. The instru throughout this thesis was the *Pulsair EasyEye* non-contact tonometer (NCT) due principally design which allowed the measurement of IOP in one eye and simultaneous stimulation of accom other eye. A second reason for using the *Pulsair EasyEye* NCT was that through collabo manufacturers (Keeler, UK) the instrument's operational technology was made accessible. Hen components underpinning non-contact IOP measures of 0.1 mmHg resolution (an order of ma than other methods) were made available. The relationship between the pressure-output and c has been termed the pressure-response relationship, aspects of which have been shown to be r biometric parameters. Further, analysis of the components of the pressure-response relationshi high-speed photography of the cornea during tonometry has enhanced our understanding of the IOP measure with the *Pulsair EasyEye* NCT.

The NCT samples the corneal response to the pressure pulse over a 19 ms cycle photoele computes the subject's IOP using the data collected in the first 2.34 ms. The relatively instanta the IOP measurement renders the measures susceptible to variations in the steady-state IOP respiratory and cardiac cycles. As such, the variance associated with these cycles was synchronising the IOP measures with the cardiac trace and maintaining a constant pace respirat breathes/minute. It is apparent that synchronising the IOP measures with the peak, middle o cardiac trace significantly reduced the spread of consecutive measures. Of the 3 locatior synchronisation with the middle location demonstrated the least variance (coefficient of variatior strong correlation ($r = 0.90$, $p = <0.001$) with IOP values obtained with Goldmann contact tonome

Accordingly IOP measures synchronised with the middle location of the cardiac cycle were t while the LE fixated low (L; zero D), intermediate (I; 1.50 D) and high (H; 4 D) accommodation continuous measures of accommodation responses were obtained during the IOP measurement p portable infrared Grand Seiko FR-5000 autorefractor. The IOP reduced between L and I accom by approximately 0.61 mmHg ($p <0.001$). No significant reduction in IOP between L and H levels was elicited ($p = 0.65$) ($n = 40$). The relationship between accommodation and IOP was c substantial inter-subject variations. Myopes demonstrated a tendency to show a reductio accommodation which was significant only with I accommodation levels when measured with the $p = 0.01$). However, the relationship between myopia and IOP change with accommodation reach for both I ($r = 0.61$, $p = 0.003$) and H ($r = 0.531$, $p = 0.01$) accommodation levels when me Ocular blood Flow Analyser (OBFA).

Investigation of the effects of accommodation on the parameters measured by the OBFA demons H accommodation levels the pulse amplitude (PA) and pulse rate (PR) responses differed betwe emmetropes (PA: $p = 0.03$; PR: $p = 0.004$). As the axial length increased there was a tendency ocular blood flow (POBF) to reduce with accommodation, which was significant only with H levels ($r = 0.38$, $p = 0.02$). It is proposed that emmetropes are able to regulate the POBF respons ocular perfusion pressure caused by changes in IOP with I ($r = 0.77$, $p <0.001$) and H ($r = 0$ accommodation levels. However, the relationship between IOP and POBF changes in the m correlated for both I ($r = 0.33$, $p = 0.20$) and H ($r = 0.05$, $p = 0.85$) accommodation levels.

The thesis presents new data on the relationships between accommodation, IOP and parameter and provides evidence for possible IOP and choroidal blood flow regulatory mechanisms. F highlight possible deficits in the vascular regulation of the myopic eye during accommodation, w putative role in the aetiology of myopia development.

Key words: Accommodation, intraocular pressure, pulsatile ocular blood flow, refractive regulation.

ACKNOWLEDGEMENTS

I would like to thank my principal supervisor Professor Bernard Gilmartin for his incisive expertise, help, patience and guidance throughout the course of this thesis. Many thanks to associate supervisor: Dr James Wolffsohn for all his technical assistance and knowledge.

I would also like to thank Keeler (UK) for the donation of the *EasyEye Pulsair* non-contact tonometer and Dr Ukai for the loan of the portable FR-5000 (Grand Seiko) autorefractor used in this thesis. Further, many thanks for all the ongoing support and interest provided by Clive Burrows of Keeler (UK) and the late John Fisher; the original designer of the *Pulsair* non-contact tonometer.

The College of Optometrists (UK) is gratefully acknowledged for the award of a 3 year Research Scholarship for my postgraduate studies.

I would like to extend my thanks to all the staff in the Optometry department and particularly Dr Leon Davies for his assistance in the initial stages of this thesis and warmest thanks to all the students for their endurance while participating in the experimental studies. Special thanks to friends and colleagues: Alejandro Cerviño Exposito and Alexandra Benavente-Perez for the endless cups of tea, chocolate and humour. I am fully thankful to them both for sharing all the highs and lows over the last 3 years.

I am forever grateful to my parents for their understanding, love and support. My gratitude goes to brother Gursharan for his words of wisdom and encouragement in the later stages of this project.

Last but not least, I am for ever indebted to my husband Gurpreet without whom none of this would be possible. I could never repay him for all his love, unfailing support, smiles and encouragement and for all his patience when I have had to work long hours.

Finally I would like to dedicate this thesis to my gorgeous nephew and niece; Jay and Jia. Their antics have kept me smiling when I have been at my lowest and have helped keep life in perspective. I hope that one day this work will be an inspiration to them to aim high but keep their feet firmly on the ground.

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CHAPTER 1

THE ACCOMMODATIVE SYSTEM

Accommodation is a flexible mechanism principally driven by an out-of-focus retinal image. The prime role of the mechanism is to minimize retinal blur (Ong and Ciuffreda, 1997; Ciuffreda, 1998). Accurately controlled changes in the crystalline lens modify the refractive power of the lens and bring near objects in to focus in the pre-presbyopic subject.

1.1 Introduction to accommodation

In 1841, Burows introduced the term "Accommodation" to the world of science (cited by Gilmartin, 1986). However, prior to this, the complex nature of the accommodative system had interested many researchers. Immense advances in the field have followed and in turn assisted our understanding of the accommodative mechanism.

In the last 400 years, a number of theories have been proposed to explain how objects of regard are brought into focus at several distances. The first reference to the eye being able to change the distance of its focal point is Kepler (1611). Kepler (1611) suggested that the lens moved backwards and forwards in order to change the refracting power of the eye (cited by Gilmartin, 1986). Descartes (1677) was first to propose that the eye's ability to focus at different distances was due to changes in the shape of the crystalline lens. Descartes stated that zonular fibres were responsible for the changes in crystalline lens shape and thus failed to mention the action of the ciliary muscle (cited by Fincham, 1937).

For years after, several ideas and theories were devised in an attempt to understand the accommodative mechanism. Despite Descartes work in 1677, until 1800 the consensus was that during accommodation the extraocular muscles contracted and these contractions changed the axial length (Sturm, 1697; Hosack, 1794; Home, 1795; Listing, 1853) and corneal curvature (Lobe, 1742; Home, 1795) of the eye which allowed clear vision at different distances (cited by Duke-Elder, 1970). It was not until 1801, when important observations by the eminent Thomas Young established the absence of axial length and corneal radii changes with extraocular muscle contractions during accommodation. Young calculated that the lens would have to move approximately 10 mm to accommodate and clearly since a movement of

this magnitude was impossible, Young disagreed with Kepler's earlier work on the displacement of the lens during accommodation. Young (1801) demonstrated that spherical aberrations of the lens decreased on accommodation and hence proposed that the variations in optical power during accommodation were achieved by changes in shape of the crystalline lens, thereby agreeing with Descartes work in 1677. At the time, the ciliary muscle had not yet been discovered and therefore Young suggested that a muscular mechanism within the lens was responsible for the shape change during accommodation (cited by Duke –Elder, 1970).

Half a century later in 1849, Langenbeck evaluated the changes in the Purkinje images of the lens and observed that accommodation produced a reduction in the size of the anterior lens surface Purkinje image (i.e., the 3rd Purkinje image). Langenbeck therefore confirmed Young's (1801) observation and concluded that the radius of the anterior surface of the lens reduced (i.e. increased in convexity) on accommodation. Langenbeck was first to suggest that the changes in lens curvature during accommodation were due to the action of the ciliary muscle, the presence of which had independently been established by Bowman and Brucke in 1847. In 1851, Cramer followed up on Langenbeck's (1849) work by using electrical stimulation of the ciliary muscle to evoke accommodation and thereby confirm that changes in the lens curvature during accommodation were a result of the muscular activity of the ciliary muscle (cited by Duke-Elder, 1970).

1.2. Anatomical aspects of accommodation

The intricate accommodative system involves many structures of the eye e.g. the crystalline lens, ciliary body and zonular fibres. The association between these structures can be seen in **Figure 1.1**.

1.2.1 The crystalline lens

The biconvex, transparent, elliptical, avascular human lens is a highly organised structure of collagen fibres that refracts the light entering the eye to focus onto the retina. The crystalline lens is comprised of 66% water and 33% protein. The remaining 1% of the lens weight is made up of other constituents including amino acids, lipids and electrolytes. The lens is located immediately behind the iris between the primary refractive surface, the cornea, and the light sensitive retina, and hence acts as a boundary that divides the globe into the aqueous and vitreous chambers. The lens is suspended from the ciliary body by threadlike structures,

known as zonular fibres, which attach to the lens capsule (see **Figure 1.1**). The lens is composed of 4 layers. From the surface to the centre these are the capsule, subcapsular epithelium, cortex and nucleus.

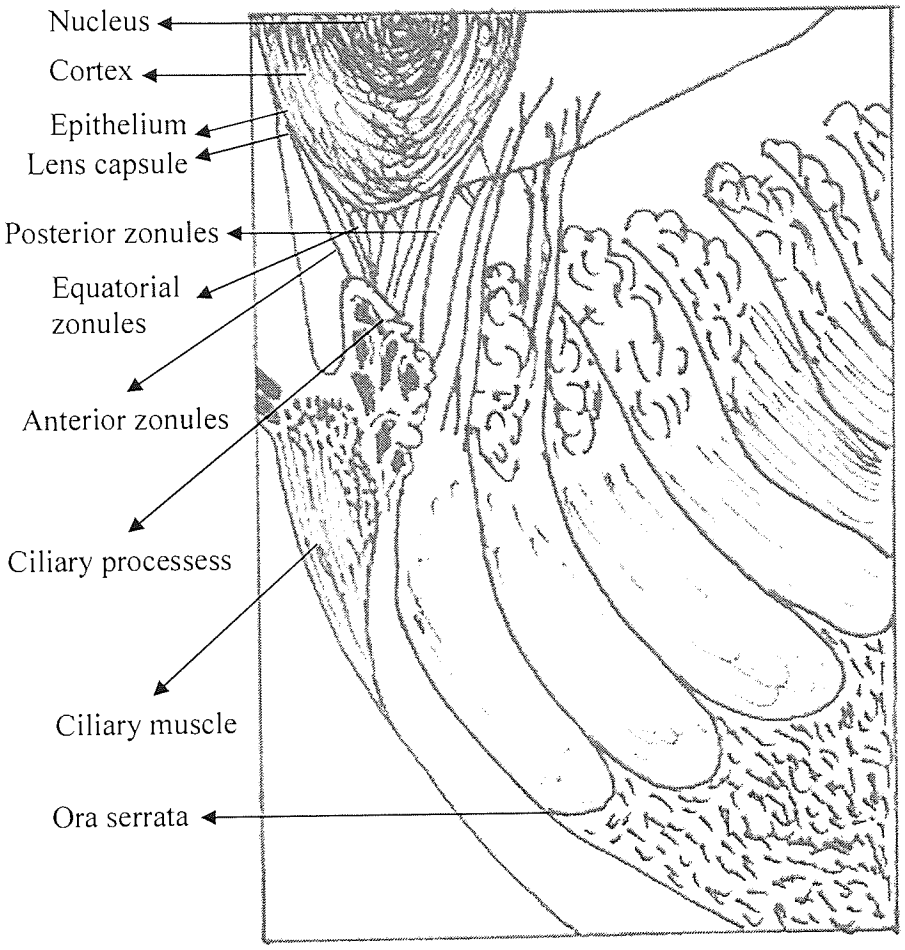


Figure 1.1 Association between the crystalline lens and ciliary body (redrawn from *Histology of the Human Eye.* Hogan, Alvarado and Weddell. Saunders Co: Philadelphia, 1971, p272).

The cornea and the crystalline lens are the two major refractive components of the eye. The power of the entire eye is approximately 60 D and the cornea provides a greater proportion of this power. The corneal power is 43.05 D in Gullstand's (1909) relaxed schematic eye (cited by Bennett and Rabbetts, 1998). The power of the cornea remains relatively stable throughout life although many authors have reported a shift in corneal astigmatism from with-the-rule to against-the-rule with age (Hayashi, Hayashi and Hayashi., 1995; Gudmundsdottir *et al.*, 2000; Goto *et al.*, 2001; Topuz *et al.*, 2004).

The power of the relaxed lens in Gullstrand's (1909) schematic eye is 19.11 D and when fully accommodated it is 33.06 D (cited by Bennett and Rabbetts, 1998). In contrast to the cornea, a distinctive feature of the lens is that the fibres continuously develop throughout life with no concomitant loss of cells. Consequently, both the volume and mass of the lens increase logarithmically with age (Hogan *et al.*, 1971).

The crystalline lens diameter has been evaluated using *post-mortem* eyes (Bluestein *et al.*, 1996; Blum *et al.*, 1997; Lim *et al.*, 1998) and *in-vivo* using magnetic resonance imaging (Fea *et al.*, 2005). The equatorial lens diameter is approximately 9 mm (Rafferty, 1985; Bluestein *et al.*, 1996; Blum *et al.*, 1997; Lim *et al.*, 1998; Fea *et al.*, 2005). The thickness of the lens is dependent on the age of the subject and on the accommodative state of the eye. In Gullstrand's (1909) schematic eye the lens thickness was noted as 3.6 mm (cited by Bennett and Rabbetts, 1998). Evaluation of the lens thickness using advanced techniques for example magnetic resonance imaging (Fea *et al.*, 2005) and partial coherent interferometry (Bolz *et al.*, 2006), demonstrates that the *in-vivo* lens thickness in non-accommodating, 30 year old subjects is approximately 4 mm. The curvature of the anterior and posterior lens surfaces is also dependent on the accommodative state of the eye. In the non-accommodating eye the anterior and posterior curvature of the lens is approximately 11 and 6 mm, respectively measured *in-vivo* using Scheimpflug Purkinje imaging systems (Rosales *et al.*, 2006), magnetic resonance imaging (Koretz *et al.*, 2004) and in *post-mortem* human eyes (Schachar, 2004). The anterior and posterior surfaces of the crystalline lens are hyperbolic with mean conic constants of -4±4.7 and -3±3.6, respectively (Dubbelman and Van der Heijde, 2001).

The main structures of the lens; the nucleus, cortex and capsule are shown in **Figure 1.1**. Although the lens fibres form the bulk of the lens, there is a single layer of epithelial cells immediately under the anterior surface of the crystalline lens (see **Figure 1.1**). Growth of the lens fibres begins with the synthesis of epithelial cells which migrate to the equator, change into lens fibres and stretch over the surface. The new fibres continue to organise themselves, layer upon layer, giving the lens a structure similar to that of a laminated onion.

The nucleus

As the new fibres develop the older fibres are not discarded but instead they are compressed and pushed towards the centre of the lens where they surround the fibres of the foetal nucleus.

Therefore the central part of the crystalline lens, the nucleus, is the oldest and hardest part of the lens. Scheimpflug photography of the lens has demonstrated that there are no zones of discontinuity over a large central region of the lens hence suggesting that the nucleus has a uniform refractive index (Brown, 1974).

The cortex

The cortex consists of younger fibre cells which surround the nucleus. Each fibre is relatively hexagonal in cross section and is approximately 8 to 12 mm long, 10 μ wide and 4.5 μ thick. The young fibres possess nuclei and mitochondria and as the fibres mature they are displaced towards the centre. During maturity the fibres undergo a specialised and unique structural change in which they lose their normal cellular organelles which allows lens transparency. The mature fibres become densely packed with extracellular spaces of only about 20 nm which also aid lens transparency. The fibres are joined by gap junctions which are important in the metabolic function of the lens (Hogan *et al.*, 1971).

In 1801, Young assigned a uniform refractive index of 1.44 to the crystalline lens (cited by Duke - Elder, 1970). Some 80 years later, Matthiessen (1882) described the gradient nature of the refractive index of human lenses, and stated a central index of 1.4106 and an edge index of 1.383 (cited by Smith, 2003). The gradient index structure of the crystalline lens is now well established [Nakao *et al.*, 1968 (rabbits); Palmer and Sivak, 1981 (rabbit, rat and pigeon); Campbell, 1984 (rat); Pierscionek and Chan, 1989 (humans); Jagger, 1990 (cat)].

The literature offers two types of models of lens refractive index, which endeavour to explain the lenticular complexity. In one model, referred to as the Shell Model, the lens has a shell structure, in which each shell has a uniform refractive index (Lotmar, 1971; Mutti *et al.*, 1995). In the alternative model, referred to as the Continuous Gradient Index Model, the refractive index of the lens varies continuously and it is described by a polynomial equation up to the 6^{th} order (Blaker, 1980; Nakao *et al.*, 1968; Jagger, 1990; and Pierscionek and Chan, 1989). Both models agree that the nucleus refractive index remains constant and a gradient index exists only in the cortical regions, which is in accordance with the conclusions drawn from Brown's early Scheimpflug images (Brown, 1974). Both models also state that the refractive index varies between the anterior and posterior surfaces of the lens and that the lens refractive index value is higher than both the aqueous and vitreous humour.

Therefore, due to the complex nature of the refractive index gradient, it is more common to assign an equivalent refractive index to the lens, which is higher than the refractive index value of both the nucleus and cortex. The equivalent refractive index figure depends on age and is typically taken as a value between 1.41 and 1.44 (Dubbelman and Van der Heijde, 2001).

The lens capsule

A collagen capsule encloses the cortex, which is physically distinct from the bulk of the lens. The capsule originates from the basement membrane of the surface ectoderm and is a thin, highly elastic, transparent structure that envelops the lens. A single layer of epithelial cells lies immediately underneath the anterior capsule. The posterior capsule however, is in direct contact with the most superficial lens fibres described above. The capsule is principally composed of collagen and glycosaminoglycans (Sunder-raj and Freeman, 1982). The parallel lamellae structure of the capsule (Hogan *et al.,* 1971; Seland, 1974) is tightly stretched as a result of the force from the internal lens substance (Fincham, 1937).

Many investigators have shown that the anterior capsule is thicker than the posterior capsule (Fisher and Pettet, 1972; Travers, 1990; Krag and Andreassen, 2003 a, b). Krag and Andreassen (2003 a) microscopically measured the capsular thickness of 25 human donor eyes (age range 1-94 years). Their study reports that the posterior capsule thickness ranges between 4 and 9 μm and that the anterior capsule is three to five times thicker than the posterior capsule.

Furthermore, it has been shown that the thickness of the capsule varies across the lens surface (Fisher and Pettet, 1972; Seland 1974). The capsule is thinnest at the posterior pole of the lens at approximately 3.5 microns (Fisher and Pettet, 1972; Travers, 1990; Krag and Andreassen, 2003 a, Barraquer *et al.,* 2006). In the 1970's Fisher and Pettet, (1972) and Seland (1974) suggested that the capsule was thickest at the equatorial region. However, much earlier Fincham (1937) had demonstrated that the thickest region of the anterior and posterior capsule was approximately 2 mm from the lens pole and 3 mm from the equator, respectively. More recent work has also shown that the capsule is thickest at the mid-periphery region of the lens, thus agreeing with Fincham's work (Travers 1990; Barraquer *et al.,* 2006).

Fincham (1937) suggested the concept of 'differential capsular lens shaping in accommodation'. He demonstrated that during accommodation, the thickest part of the lens capsule corresponded with the flattest region of the lens. Fincham (1937) therefore postulated that regional variations in capsular thickness determined the configuration of the lens matrix during accommodation.

There is no doubt that during accommodation the crystalline lens deforms predominantly at the anterior lens surface (Brown, 1973; Koretz, Cook and Kaufman, 2002). Koretz and Handelman (1982) have suggested that the capsule behaves as a 'force distributor' which is able to transmit forces from the ciliary muscle contraction, to the lens substance, so that changes in lens shape can occur. Indeed there is good evidence in the literature which suggests that the lens capsule can act as a force transmitter. For example Krag and Andreassen (2003 a) have demonstrated that the thickness and hence mechanical effectiveness of the anterior lens capsule is three to five times greater than the posterior lens capsule. Furthermore, Krag, Olsen and Andreassen (1996) have reported that the elastic modulus of the lens capsule (0.4 N/mm^2) is one hundred times greater than the elastic modulus of the lens substance [0.7 $N/mm^2 \times 10^{-3}$ (Fisher, 1971)].

However, a paradoxical finding is that during accommodation the cytoplasmatic flow (which occurs during lens fibre deformation) is greater in the nucleus than the cortex (Travers, 1990) which is consistent with the observation that on accommodation the nucleus under goes greater shape change compared to the cortex (Brown, 1973). It is unclear how the lens capsule transmits the force from the zonular fibres and governs the shape change of the nucleus. Travers (1990), in contrast to Fincham, introduced the concept of 'differential lens fibre response to capsular shaping'. Krag and Andreassen (2003 a, b) have more recently suggested that the regional variations in capsular thickness may be a result of stress-induced modelling of the lens capsule and therefore do not influence the shape change during accommodation.

1.2.2 The zonules

The lens is connected to the ciliary processes by a convoluted arrangement of elastic fibres; the Zonulus of Zinn (Streeten *et al,* 1981). The ciliary zonules form a ring around the lens, and in meridonal section are triangular. The base of the triangular shape formed by the

zonules is concave and faces the lens equator and the apex is long and curved and ends at the ora serrata as depicted in **Figure 1.1**.

Each zonular fibre is composed of delicate acellular fibrils, which average approximately 10 nm in cross-section (Bron, Tripathi and Tripathi, 1997). The zonular fibres originate at about 1.5 mm from the bays of the ora serrata, extend forward to the cilary body *pars plana* and attach themselves to the lateral wall of the ciliary processes. The zonular fibres then travel between the ciliary processes and into the valleys of the *pars plicata*. Moving further anteriorly, the zonular plexus splits into the zonular fork and fuses with the zonular lamella of the lens capsule (Coleman, 1970; Hogan *et al.*, 1971; Seland, 1974; Rohen, 1979).

The zonules have three limbs; the anterior, posterior and equatorial limb shown in **Figure 1.1**. The equatorial group of fibres are less dense and attach to the lens equator (Rohen, 1979). The anterior fibres of the zonular fork attach to the anterior lens capsule. However, the posterior zonular fibres are not directly attached to the posterior lens capsule but instead the majority of the posterior zonular fibres are anchored to the anterior hyaloid membrane which attaches to the posterior lens surface (Minsky, 1942; Davanger, 1975; Albrecht and Eisner, 1982; Bernal, Parel and Manns, 2006).

1.2.3 The ciliary muscle

The ciliary muscle is located at the anterior part of the posterior chamber (Coleman, 1970). Posteriorly, the muscle is attached to the elastic network of Bruchs membrane of the choroid (Hogan *et al.*, 1971) and anteriorly, tendons attach the muscle to the scleral spur and trabecular meshwork (Rohen, Futa and Lutjen-Drecoll, 1981). In a meridonal section, the shape of the ciliary muscle is like a right-angled triangle. The right-angle faces the ciliary processes, the posterior acute angle points towards the choroid and the hypotenuse runs parallel to the sclera (see **Figure 1.1**). The muscle fibres originate at the corneo-scleral spur and insert at the span fibrils of the Zonules of Zinn (Rohen, 1979).

Salzmann (1912) organized the fibres into three different groups depending on their orientation (cited by Bron, Tripathi and Tripathi, 1997). Longitudinal fibres (i.e. meridional or Brueck's muscle) are the most external fibres of the ciliary muscle and the innermost fibres are circular fibres (i.e. Sphincter or Mueller's muscle). The intermediate fibres, the radial fibres (oblique) connect the longitudinal and circular fibres. Although three separate bundles

of non-striated fibres constitute the ciliary muscle, the muscle acts as a unitary whole. The simultaneous contraction of the fibres during accommodation causes longitudinal shortening and radial increase in thickness of the muscle (Coleman, 1970).

1.3 Theories of accommodation

Since 1800 it has been known that the principal role of the lens is to accommodate and several theories have been devised in an attempt to explain the accommodative mechanism. However, still, a certain amount of controversy among authors exists. Helmholtz (1909) states:

" There is no other subject in physiological optics about which so many antagonistic opinions have been entertained as concerning the accommodation of the eye."

1.3.1 Helmholtz's (1855) theory

The consensus is that Helmholtz's theory of human accommodation is correct. Helmholtz's theory is supported by results of high resolution anterior segment magnetic resonance imaging studies in which the geometry of the human eye has been observed during accommodation (Strenk, Stenk and Semmlow, 2004) and by finite element simulations of the accommodation process (Martin *et al.,* 2005). Helmholtz hypothesis stated that when the eye was in its normal passive state (i.e. unaccommodated), the lens was under continuous stress from the tension of the zonular fibres, which span the circumferential space between the ciliary body and the lens equator. The pull exerted by the tense zonules resulted in shorter lens axis and flatter lens surfaces. On accommodation, ciliary muscle contraction reduced the ciliary muscle ring diameter. Therefore, the lens equator moved away from the sclera, which released the outward-directed equatorial tension on the lens capsule and hence reduced the resting zonular tension. On release of the tension, the lens takes up its natural state (i.e. more convex). Imaging work on monkeys has shown that the lens equator moves 250 μm away from the sclera on approximately 10D of accommodation which supports Helmholtz's theory (Glasser and Kaufman, 1999). Furthermore, it has been shown that the centre of gravity of the ciliary muscle (a parameter that describes the entire muscle contour) shifts towards the lens equator (Stachs *et al.,* 2002).

Helmholtz did not assign an active role to the vitreous but suggested that the elastic nature of the lens capsule caused the lens to thicken (a forward movement of the anterior surface of 0.4 mm was observed), the equatorial diameter to increase and the curvatures of the anterior and posterior lens surfaces to also increase (cited by Coleman, 1970).

1.3.2 Tscherning's (1895) theory

Tscherning's theory of the mechanism of accommodation was interestingly opposite to the view held by Helmholtz. Tscherning suggested that on contraction of the ciliary muscle the zonular tension would actually increase. The increased zonular tension coupled with the pressure from the anterior vitreous [first proposed by Cramer in 1801 (cited by Duke–Elder, 1970) and later by Coleman (1970)] would result in the plastic cortex of the lens (named the *accommodative layer* by Tscherning) to be pressed against the hard, more steeply curved nucleus. The cortex would therefore attain similar curvatures to that of the nucleus while the peripheral surfaces are flattened. Tscherning's theory did not account for any increase in the lens thickness, which strongly contradicted Helmholtz's (1855) findings (cited by Duke-Elder, 1970).

1.3.3 Tscherning-Pflugk's (1909) theory

Later work by Tscherning showed a forward displacement of the anterior lens surface during accommodation which led Tscherning to accept that the lens thickness increased on accommodation as proposed by Helmholtz (1855). Supported by the work of Pflugk (1909), a modified theory by Tscherning claimed that on contraction of the ciliary muscle, tension was exerted on the choroid, which compressed the vitreous so that pressure was exerted only on the peripheral aspects of the posterior lens surface, and not on the central part of the lens. It was claimed that the pressure on the posterior-peripheral surface of the lens was counteracted by the tension of the zonular fibres at the anterior-peripheral surface. It was proposed that the free pupillary area of the lens hence bulged forward, which was a theory similar to that mentioned by Cramer in 1851 (cited by Duke-Elder, 1970).

1.3.4 Fincham's (1937) theory

Fincham's (1937) explanations favoured the Helmholtz theory on the mechanism of human accommodation. Fincham (1937) suggested that the lens capsule, the zonular fibres and the vitreous were under continual stress in the relaxed state. Helmholtz based his theory of accommodation on the forward displacement of the anterior pole and a stationary posterior

surface. Fincham (1937) considered it important to ascertain whether this forward displacement in the anterior pole was due to an increase in the thickness or to the actual forward movement of the lens. In one subject, Fincham (1937) showed that the lens displaced backwards by 0.24 mm and the overall increase in thickness was 0.58 mm, whereas in another subject the backward movement was only 0.09 mm and an overall increase in thickness was 0.36 mm. His results indicated that on accommodation, the back surface of the lens was displaced backwards. However, the backward displacement of the lens was negligible and only had a small dioptric effect on the overall change in lens power.

Fincham (1937) postulated that the natural state of the lens was unaccommodated and the lens capsule had an important role in deforming the lens substance in accommodation. Fincham thus described the 'capsular theory' of accommodation. He observed that the lens was flatter in the periphery during accommodation where the lens capsule was the thickest. Following these observations, Fincham proposed that during accommodation the lens curvatures changed depending on the regional variations in capsular thickness.

Many studies have shown that the regional variations in capsular thickness increase with age (Fisher and Pettet, 1972; Seland, 1974; Krag, Olsen and Andreassen, 1997; Krag and Andreassen, 2003 b). Therefore, according to Fincham's theory the lens shape change during accommodation should be easier to obtain with increasing age. However, clearly this is not the case as the amplitude of accommodation reduces with age. In respect of these findings Krag and Andreassen (2003 b) disagree with Fincham's 'capsular theory' and state that regional differences in capsular thickness are of no significance in changing the shape of the lens during accommodation.

1.3.5 Coleman's (1970) theory

While both the Helmholtz (1855) and Fincham (1937) theories mentioned the function of the zonules and capsule, they failed to assign an active role to the vitreous body during accommodation. Conversely, the Cramer (1851), Tscherning (1895) and Tscherning-Pflugk (1909) theories referred to the role of the vitreous during accommodation but failed to mention the importance of the zonular fibres and capsular elasticity. Coleman (1970) attempted to unify all five aforementioned theories and hence included the vitreous body, zonular fibres and capsular elasticity in his explanation of the accommodative mechanism. Coleman (1970) observed minimum deformation of the posterior lens surface on

accommodation. He therefore pointed out that if overall the posterior capsule was thinner than the anterior capsule [as proposed by Fincham (1937)], then the lens shape change observed during accommodation should be evident at the posterior rather than the anterior surface. Since little structural change had been observed at the posterior surface during accommodation, Coleman (1970) postulated that the vitreous body in fact supported the posterior lens surface. Coleman's theory also suggested that on contraction of the ciliary muscle, the zonular fibres moved anteriorly and pulled on the ora serrata such that the choroid moved forward. The forward movement of the choroid displaced the vitreous forward causing the anterior vitreous to push up against the posterior surface of the crystalline lens. Simultaneous force from the vitreous and the forward shift of the suspensory ligaments (i.e. relaxation of the zonules) coupled with the elastic properties of the lens and its capsule induced the conoidal conformation of the anterior surface during accommodation (Coleman, 1970).

1.3.6 Schachar's (1995) theory

A more recently proposed theory by Schachar utilizes the idea proposed by Tscherning that on ciliary muscle contraction, anterior and posterior zonular fibre tension is released while increasing the tension in the equatorial zonular fibres (Schachar and Anderson, 1995). Schachar's theory on human accommodation states that the anterior and equatorial zonular fibres insert into the anterior aspect of the ciliary muscle at the iris root, and the posterior zonular fibres insert into the posterior ciliary body. However, experiments on human tissues have noted the absence of zonular bundle insertions at both the iris root and anterior ciliary muscle (Glasser and Campbell, 1998).

In numerous studies Schachar and co-workers have proposed that the simultaneous relaxation of the anterior and posterior zonular fibres and the increase in the traction of the equatorial fibres results in a flattening of the peripheral anterior and posterior lens surfaces, while increasing the anterior and posterior central lens curvatures (Schachar *et al.*, 1995; Schachar *et al.*, 1996; Chien, Huang and Schachar, 2003; Schachar, 2004; Chien *et al.*, 2006; Schachar and Fygenson, 2006).

Furthermore Schachar has observed a posterior-outward movement of the anterior aspect of the ciliary muscle towards the sclera. Schachar *et al.* (1996) have observed that as the accommodation increased between 5.5 and 11 D in humans, the movement of the lens equator

towards the sclera increased between 40-60 μm and have thus concluded that the lens diameter increases on accommodation. However, results obtained from imaging of the accommodative structures in monkeys using ultrasound biomicroscopic imaging, goniovideography and Goldmann lens imaging show the absence of a posterior-outward movement of the anterior aspect of the ciliary muscle on accommodation (Glasser and Kaufman, 1999). Glasser and Kaufman (1999) concluded that Schachar *et al.* had probably observed different regions of the ciliary processes during accommodation and non-accommodation which confounded their results.

1.4 Anatomical changes during accommodation.

Despite Schachar's work, the general view is that Helmholtz theory on human accommodation is correct. On contraction of the ciliary muscle, the pars plana zonules (posterior fibres) stretch, the muscle moves forward and importantly inward, the ciliary muscle collar diameter reduces and consequently releases the tension of the anterior zonular fibres. On release of the zonular tension the equatorial diameter decreases and the lens thickness increases. These changes in lens parameters lead to an increase in dioptric power of the lens (Burd, Judge and Flavell, 1999). Strong evidence supporting Helmholtz's theory has been provided by Strenk and colleagues (Strenk *et al.*, 1999; Strenk, Strenk and Semmlow, 2004) and Ostrin and Glasser (2007). Strenk and co-workers have used magnetic resonance imaging to visualise the anterior segment during non-accommodation and accommodation and their work clearly shows that the lens thickness increases and the equatorial diameter decreases with accommodation as shown in **Figure 1.2** (Strenk *et al.*, 1999; Strenk, Strenk and Semmlow, 2004).

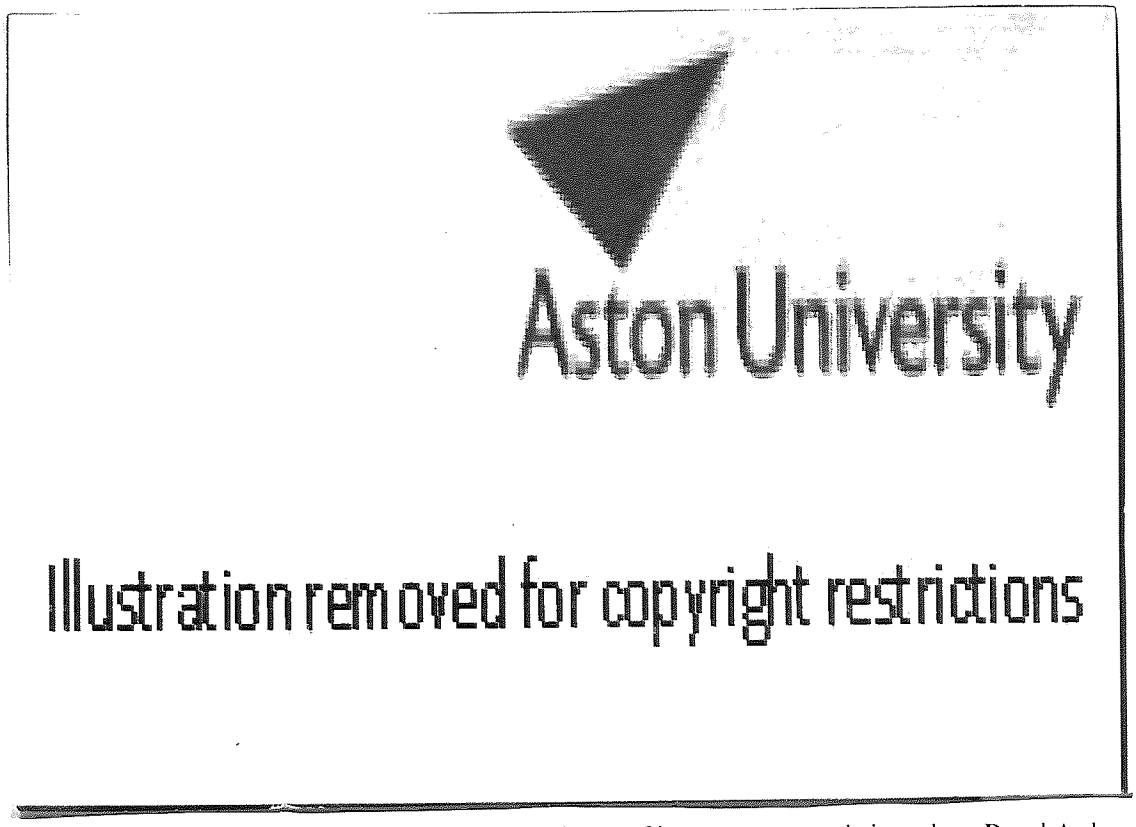


Figure 1.2 1) Helmholtz drawing demonstrating his theory of human accommodation where **R** and **A** show the lens during non-accommodation and accommodation, respectively. 2) Corresponding magnetic resonance image of the human lens where **R** and **A** show the lens during non-accommodation and accommodation (The article from which this figure was reproduced was published in *Progress in Retinal and Eye Research*, **24**, Strenk, Strenk and Koretz, The mechanism of presbyopia, p379-393, Copyright Elsevier, 2005).

Ostrin and Glasser (2007) stimulated accommodation via the Edinger-Westphal and pharmacologically in 3 rhesus monkeys. Accommodative refraction was measured using infrared photorefraction and the movement of the ciliary processes and lens edge was measured using slit-lamp goniovideography. When accommodation was stimulated via the Edinger-Westphal, Ostrin and Glasser (2007) found that the ciliary processes and lens edge moved centripetally by 0.030±0.001 mm/D and 0.027±0.001 mm/D, respectively. Similarly on pharmacologically stimulated accommodation the ciliary processes and lens edge were shown to move centripetally by 0.030±0.005 mm/D and 0.019±0.003 mm/D, respectively. The centripetal movement of the ciliary muscle induces changes in ocular parameters which result in an increase in the refractive power of the eye and are discussed below.

Corneal curvatures

Although early work concluded that corneal curvatures do not change with accommodation (Fairmaid, 1959; Lopping and Weale, 1965; Mandell and Helen, 1968), more recent work has shown quite the opposite. The curvatures of the cornea have been shown to increase with

accommodation i.e. a steepening of the cornea (Brown, 1973; Koretz *et al.,* 1987; Yasuda, Yamaguchi, Ohkoshi, 2003; Saitoh *et al.,* 2004; Yasuda and Yamaguchi, 2005). The steepening has been shown at the anterior and posterior corneal surfaces (Saitoh *et al.,* 2004). Yasuda, Yamaguchi, Ohkoshi (2003) concluded that the ciliary muscle pulled at the limbus of the cornea and sclera and increased the corneal power by between 0.60 and 0.72 D. In contrast He *et al.,* (2003) demonstrated that when convergence was controlled the central cornea flattened whilst the peripheral cornea steepened with accommodation. The authors hypothesised that as the lens moved forward on accommodation, the pressure of the aqueous humour increased and caused the cornea to flatten. However, Lam and Douthwaite (1997) observed that a 6.5 mmHg increase in IOP did not distort the central cornea, although they do not discard the possibility that corneal curvature changes may have occurred in the periphery. Furthermore, it has also been observed that a timolol-induced reduction in IOP did not change the corneal shape (Rosenfield and Gilmartin, 1987) and that the changes in corneal curvature after instillation of pilocarpine preceded the changes in IOP (Yasudu and Yamaguchi, 2005). Therefore, it is concluded that IOP changes are not responsible for the corneal shape changes seen with accommodation.

Furthermore, Pierscionek, Popiolek-Masajada and Kasprzak (2001) used a simple keratometer and concluded that inter-subject variations in corneal shape changes with accommodation existed. Nine of the fourteen subjects in this study showed an increase in corneal curvature in at least one of the meridians with accommodation. Conversely in the remaining five subjects the corneal curvatures were shown to reduce with accommodation.

Cyclotorsion

Buehren *et al.* (2003) have demonstrated that excyclotorsion occurs with accommodation. With 4 and 9 D of accommodative stimuli, excyclotorsion of respectively 1.6±1.1 and 2±1.3° has been noted. Interestingly Buehren *et al.* (2003) noted that when the excyclotorsion which accompanies accommodation is accounted for, the changes in corneal topography with accommodation noted above are diminished.

Lens thickness

The crystalline lens increases in thickness during accommodation (Koretz *et al.,* 1987; Koretz *et al.,* 2004; Ostrin *et al.,* 2006). Ostrin *et al.* (2006) investigated the lenticular changes in 22

subjects (age range of 21 to 30 years). Their study concludes that the lens thickness increased by 0.067±0.008 mm/D of accommodation.

Anterior chamber depth

The anterior chamber depth reduces with accommodation (Fincham, 1937; Duke-Elder, 1970; Coleman, 1970; Brown, 1973; Koretz *et al.,* 1987; Cook *et al.,* 1994; Kortez *et al.,* 2004; Mallen, Kashyap and Hampson, 2006; Ostrin *et al.,* 2006). The anterior chamber depth reduces by approximately 0.05 mm per dioptre of accommodation (Bolz *et al.,* 2006; Ostrin *et al.,* 2006). The reduction in anterior chamber depth with accommodation is attributed to the increase in lens thickness and the forward movement of the anterior pole of the lens (Bolz *et al.,* 2006).

Anterior chamber angle

Interestingly, the changes in anterior chamber angle have not been evaluated widely. It is hypothesised that the forward and inward movement of the ciliary processes on accommodation observed by Glasser and Kaufman (1999) would result in a reduction in the anterior chamber angle. However, to date this has only been demonstrated in a study by Marchini *et al.* (2004) in which ultrasound biomicroscopy was used to investigate the anterior segment changes during accommodation with an intraocular lens implant. Their study concluded that the scleral-ciliary processes angle reduced by approximately 4° with 1D of accommodation which was attributed to the forward and inward movement of the ciliary processes.

Lens curvature

The curvature of the crystalline lens increases during accommodation i.e. the lens surfaces steepen (Koretz *et al.,* 1987; Koretz *et al.,* 2004; Rosales *et al.,* 2006). Rosales *et al.* (2006) have used Scheimpflug and Purkinje imaging systems to investigate the corneal affects of accommodation. Their study reports that with accommodative stimuli levels of 0 and 8 D the radii of curvature of the anterior and posterior lens surface decreases from 11 to 7 mm and 7 to 5 mm, respectively.

Axial length

Changes in axial length with accommodation have also been noted (Shum *et al.,* 1993; Drexler *et al.,* 1998; Mallen *et al.,* 2006). In a recent study Mallen *et al.* (2006) used partial

coherent interferometry to measure the axial length of 30 emmetropes and 30 myopes while fixating accommodative stimuli between 0 and 6 D (the accommodation responses were measured in a separate setup). A positive liner relationship between the transient axial length changes and accommodation was observed in myopes and emmetropes, although this effect was more pronounced in the myopic group. It is thought that ciliary muscle contraction exerts a pulling force on the choroid [as proposed by Coleman (1970), see **section 1.3.5**] and sclera adjacent to the ciliary body, which displaces the posterior portion of the globe posteriorly. Thus an increase in axial length with accommodation occurs (Drexler *et al.,* 1998; Mallen *et al.,* 2006).

It has been noted however that the lenticular refractive index changes occur with accommodation (Garner and Smith, 1997). Since the partial coherent interferometry method assumes a constant refractive index, the accommodation-induced changes in axial length observed may be erroneous (Atchison and Smith, 2004). However, an early study by Shum *et al.* (1993) used A-scan ultrasonography to measure the changes in axial length on 76 subjects. The study also concluded that the axial length increased in the RE by 0.06±0.01 mm while the LE fixated a target at 33 cm.

1.5 Presbyopia

Age influences the human body in an inexorable manner. Atchison (1995) reviews the many theories on the ageing of organisms. The theories include wear-and-tear of non-replaceable body parts, accumulation of toxic metabolites and materials and consumption of irreplaceable substances. Furthermore, variations in certain hormone levels, DNA mutations or damage leading to loss of information, increases in defective proteins as a result of erroneous gene replications, increases in free-radicals and enzymatic and non-enzymatic cross-linking of proteins have also been suggested as ageing mechanisms (Atchison, 1995). Atchison (1995) suggested that like growth and differentiation, ageing may be a pre-programmed event.

The amplitude of accommodation regresses with age and is nearly zero dioptres at the age of 52 years (Hamasaki, Ong and Marg, 1956; Charman, 1989; Koretz *et al.,* 1989). Presbyopia, the Greek term for 'old eye' is therefore a result of the natural and progressive decline in the amplitude of accommodation (Atchison, 1995). A diminution in the clarity of vision results with age which reduces the ability of an individual to perform near work sufficiently (Atchison, 1995; Gilmartin, 1995). Optical (focal length), biometric (mass) and physical

(hardness and surface curvatures) changes in the properties of the lens and changes in the geometry of the zonular attachments often explain the onset and progression of presbyopia which are reviewed by Gilmartin (1995).

A plethora of literature exists surrounding the topic of presbyopia. Controversy exists due to the lack of *in-vivo* information on the changes in the lenticular and extralenticular structures with age. Furthermore, many studies investigate the mechanism of accommodation and presbyopia invasively in primates (Tamm *et al.,* 1991; Tamm *et al.,* 1992 a, b; Croft *et al.,* 1998; Glasser *et al.,* 2001; Croft *et al.,* 2006 a, b) and it is thought that the affects of aging on the eye differ between humans and primates (Strenk, Strenk and Koretz, 2005). Strenk, Strenk and Koretz, (2005) review the classical and current theories of presbyopia and propose a modified geometric theory of presbyopia. The modified geometric theory of presbyopia states that in humans the ciliary muscle contraction does not change with age (Strenk *et al.,* 1999; Strenk, Strenk and Semmlow, 2004; Strenk *et al.*, 2004) although the ciliary muscle apex is displaced inwards and anteriorly (Tamm *et al.,* 1992 a, b; Strenk *et al.,* 1999; Pardue and Sivak, 2000; Strenk, Strenk and Semmlow, 2004; Strenk *et al.*, 2004) due to the increase in lens thickness with age (Cook *et al.,* 1994; Dubbelman and Van der Heijde, 2001; Fea *et al.,* 2005). The displacement of the ciliary muscle with age renders the ciliary muscle contraction ineffective and therefore reduces the amplitude of accommodation (Strenk, Strenk and Koretz, 2005). However, Strenk, Strenk and Koretz (2005) recognise the need for further work to determine the potential significance of the age related structural and mechanical lenticular changes noted by several workers (Fincham, 1937; Fisher and Pettet, 1972; Brown, 1974; Seland, 1974; Travers 1990; Beers and Van der Heijde, 1996; Krag, Olsen and Andreassen, 1997; Glasser and Campbell, 1999; Dubbelman and Van der Heijde, 2001; Moffat, Atchison and Pope, 2002; Krag and Andreassen, 2003 b; Dubbelman *et al.,* 2003; Dubbelman, Van der Heijde and Weeber, 2005; Barraquer *et al.,* 2006) in the onset and development of presbyopia.

1.6 Components of accommodation

The accommodative response comprises of four components: reflex; vergence; proximal and tonic (Heath, 1956a).

Reflex accommodation

The accommodative system responds to spatiotopic and retinotopic cues. The spatiotopic responses are imprecise and are voluntarily controlled, which act in response to body-referenced stimuli e.g. relative size, texture, perspective and position of object in space. Conversely, retinotopic responses are relatively inaccurate and involuntarily controlled (reflex responses), which act in response to eye-referenced stimuli e.g., image blur (Schor *et al.*, 1992).

Under both monocular and binocular conditions, reflex driven accommodation is the principal component of accommodation (Hung, Ciuffreda and Rosenfield, 1996). Blur has been shown to be a sufficient cue to the accommodative system even when other spatiotopic stimuli are minimal or absent (Phillips and Stark, 1977). Blur causes a reduction in both the retinal image contrast and in the retinal image contrast gradient. To attain a sharp in-focus image an automatic change (parasympathetic increase) in the refractive state of the eye is required. The process by which a change in the refractive power of the eye is achieved may be assisted by microsaccades, which produce multiple retinal-image luminance gradients (Fincham, 1951). Due to the microsaccades blur information can more easily be extracted (Stark *et al.*, 1984).

Visual blur is an even-error signal, thus provides information on magnitude but not direction (odd-error signal) (Stark, 1968). It has been demonstrated that reflex accommodation is responsive to blur $\leq$1.50D. However, for blur above this level reflex accommodation requires both spatiotopic and retinotopic information (Stark and Takahashi, 1965; Fincham, 1951; Ciuffreda and Kruger 1988).

Vergence accommodation

Vergence accommodation is the second most important component of the accommodation response (Ciuffreda, 1998). Neural linkage between disparity vergence and accommodative systems allows retinal disparity to stimulate vergence, which in turn evokes accommodation (Fincham and Walton, 1957). In young adults the convergence accommodation/convergence (CA/C) ratio is approximately 0.40 D per meter angle (Rosenfield and Gilmartin, 1988). It is

this CA/C ratio that determines the magnitude of accommodation accompanied with vergence, although proximity is also a factor (Hung, Ciufredda and Rosenfield, 1996).

In addition to the CA/A ratio, reflex accommodation produces accommodative vergence, giving an accommodative convergence/accommodation ratio (AC/A). Accommodative vergence aligns the eyes in response to the blur driven reflex accommodation, which minimizes retinal blur. The AC/A ratio is a separate neurological process to the CA/A ratio; the one is not inversely proportional to the other (Heron *et al.*, 2001). However, both AC/A and CA/A ratios are constants of proportionality (Rosenfield, Ciufredda and Chen, 1995). In addition it has been demonstrated that the CA/A ratio declines with age whereas the AC/A ratio increases with advancing age (Rosenfield, Ciufredda and Chen, 1995; Baker and Gilmartin, 2002; Baker and Gilmartin, 2003).

Thus the purpose of reflex accommodation is to minimize retinal blur, whereas the prime role of vergence accommodation is to eliminate retinal disparity. Although the two components have separate functions, there is a dual interaction between accommodation and convergence (Ciuffreda, 1998). **Figure 1.3** illustrates a model of the interaction.

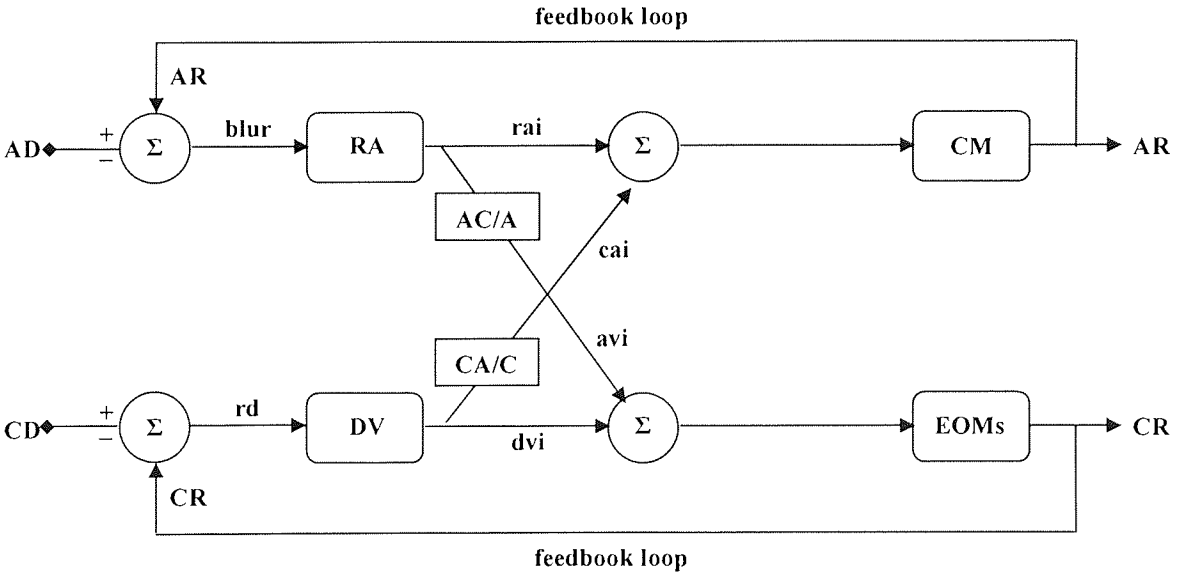


Figure 1.3 Dual interaction between reflex and vergence accommodation (redrawn from McCormack, 1998).

Proximal accommodation

The proximal accommodation component is due to the apparent (or perceived) nearness of objects. Visual cues within 3 metres from the individual can stimulate proximal accommodation (Rosenfield, Ciuffreda and Hung, 1991). Proximal accommodation is also evident when closed-view instruments are used (i.e., instrument myopia), when subjects think near and when the subjects have knowledge of their surroundings (Rosenfield and Ciuffreda, 1991; Cervino *et al.,* 2006). When no visual feedback is available, i.e. accommodation and disparity systems are rendered open-loop, proximal accommodation provides 80 % of the total near response. In closed-loop conditions, proximal accommodation provides only 4-10 % of the near response and the accommodation and disparity vergence systems dominate (Hung, Ciufredda and Rosenfield, 1996).

Tonic accommodation

In a large dark room, where accommodation and disparity vergence are rendered open-loop systems, and no propinquity (i.e., knowledge of nearness) effects exist, the eye adopts its tonic accommodative state (Leibowitz and Owens, 1978; Gilmartin, 1986; Rosenfield *et al.,* 1993; 1994). Fisher, Ciufredda and Hammer, (1987) suggested that the tonic accommodative state represents baseline midbrain neural activity. Work by Leibowitz and Owens (1978) indicates that the tonic accommodation mean is 1.52±0.77 D and ranges between -0.50 and +4.00 D using the He-Ne laser optometer (n=220). Later work has demonstrated a lower tonic accommodation mean of 1.00 D, which ranges between 0 and 2 D (Post, , Johnson and Tsuetaki, 1984). McBrien and Millodot (1987) reported a mean value for tonic accommodation of 0.90±0.53 D, measured using an objective infrared autorefractor (n=62), however, Rosenfield (1989) states a mean value of 1.28±0.15 using an objective, open-field, infrared optometer. The discrepancies present in the literature are due to measurement techniques. A laser optometer presents a moving speckle pattern produced by a low-energy He-Ne laser beam reflecting off a slow moving drum. Higher tonic accommodation values are obtained when using a laser optometer compared to an infrared optometer as more mental effort is required to judge the direction of the speckle motion, which influences tonic accommodation and some proximal accommodative response is also induced (Post, Johnson and Tsuetaki, 1984). The use of infrared in an open-view binocular optometer allows true stimulus-free conditions and therefore induces only minimum proximal accommodation and cognitive effort.

1.7 Static aspects of the accommodative-stimulus response

A characteristic accommodative response profile exists when the optical vergence of a target is altered by varying either the power of the spherical lenses placed in front of the eyes, the target distance in physical space or the target position within a Badal system. The typical accommodative stimulus/response function is divided into six regions; one linear and five non-linear regions, each with definite response features, depicted in **Figure 1.4.**

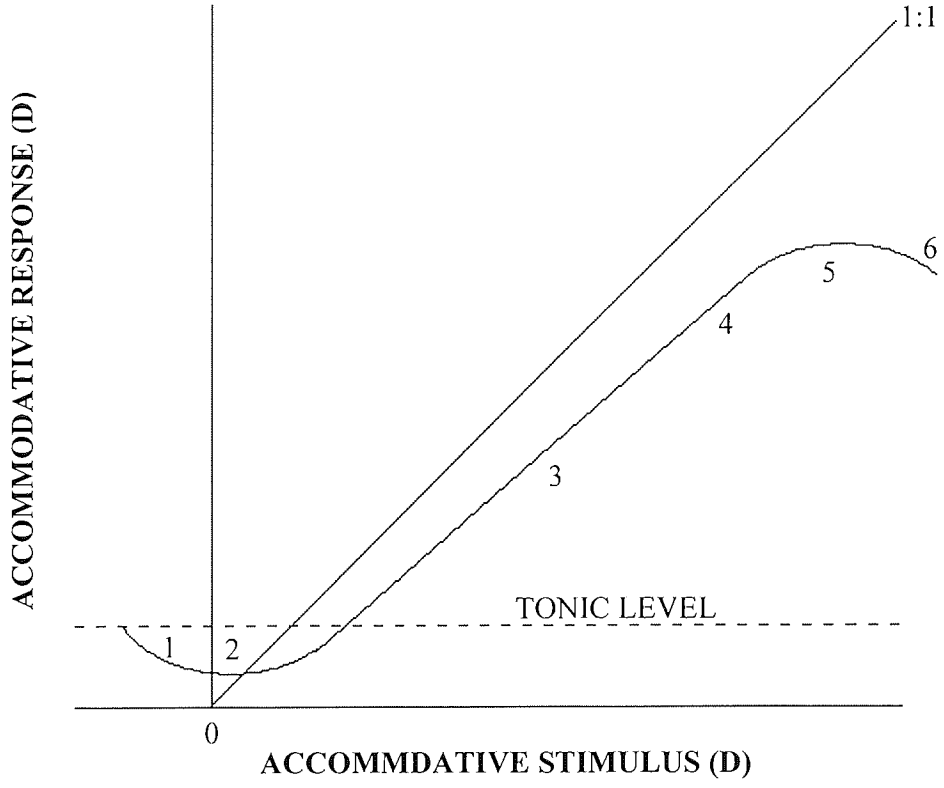


Figure 1.4 Accommodative stimulus–response function (adapted from Ciuffreda, 1998).

1) *Hyperopic non-linear defocus region*: If the stimulus is beyond infinity, an uncompensatible hyperopic retinal defocus is produced. The accommodative response shifts towards the "default" tonic accommodation level.

2) *Initial non-linear zone:* At infinity (i.e., a zero dioptre accommodative stimulus), the accommodative response is between 0.25 and 0.33 D (Rosenfield, Ciuffreda and Rosen, 1992). Therefore, a lead of accommodation is present for targets in the distance.

3) *Linear manifest zone:* A change in accommodative stimulus produces a proportional change in accommodative response over the linear manifest region of the accommodative stimulus/response function. Thus, as the accommodative stimulus increases the accommodative error increases by a proportional amount (Hung and Ciuffreda, 1983). An accommodative lag (i.e., under-accommodation) is evident in this zone (Heath, 1956 b).

4) *"Soft" non-linear saturation zone:* As the accommodative stimulus is further increased, progressively smaller changes in the accommodative response are observed. Thus, the accommodative error or lag of accommodation increases with further increases in accommodative stimulus.

5) *"Hard" non-linear saturation zone:* Further increases in the accommodative stimulus produce a non-linear reduction in the accommodative response. The accommodative system reaches a point where increasing the accommodative stimulus produces no change in the accommodative response.

6) *Myopic non-linear defocus region:* Further increases in the accommodative stimulus continue to exacerbate the under-accommodative response, which eventually leads to an uncompensatible retinal image defocus. A poor contrast retinal image will result when the accommodative stimulus exceeds the amplitude of accommodation by 1 - 2 D. The retinal defocus gradually drives the accommodation system to its tonic accommodative level.

Characteristic features of the accommodative stimulus-response curve have been used to represent the overall accommodative response function which have subsequently been implemented in the aetiology of myopia development (McBrien and Millodot, 1986 a; Bullimore, Gilmartin and Royston, 1992; Rosenfield, 1998; Gwiazda *et al.,* 1993; Seidel, Gray and Heron, 2005). McBrien and Millodot (1986 a) proposed that the linear regression slope (m) of the accommodation stimulus-response function calculated using the least squares method, was an indicator of accommodative stability. However, McBrien and Millodot's method has been questioned by Chauhan and Charman (1995), who have consequently derived another parameter: the accommodative error index.

Early researchers have also used the cross-over point of the accommodation stimulus-response curve and the virtual unity line as an indicator of tonic accommodation (TA)

(Ramsdale and Charman, 1989). However, more recent work has questioned the validity of TA values calculated from the cross-over point of the stimulus-response function. Several workers have demonstrated that TA values obtained using open-loop conditions are typically larger than the TA values calculated from the cross-over point (Ciuffreda *et al.,* 1984; Ong, Ciuffreda and Tannen, 1993; Rosenfield *et al.,* 1993; Davies, 2004).

Aniso-accommodation

Many studies suggest that binocular accommodation is a consensual response in which the accommodative response changes equally in both eyes to a change in accommodative stimuli (Campbell, 1960; Fisher, Ciuffreda and Hammer, 1987; Hokoda and Ciuffreda, 1982). An unequal accommodative response is also possible to unequal accommodative stimuli. Results of several studies investigating the presence of aniso-accommodation are equivocal owing to confounds introduced in the experimental designs (Rosenberg *et al.,* 1953; Spencer and Wilson, 1954). Marran and Schor (1998) used monocular dichoptic blur cues in a binocular stimulus target and demonstrated that aniso-accommodation of >0.50D can occur. The authors also concluded that whereas the shape of the accommodative-stimulus response function (see **Figure 1.4**) is generally sigmoidal in shape, the aniso-accommodative response function is best described as linear (Marran and Schor, 1998). The latency of the consensual accommodative and aniso-accommodative response has been noted as 0.40 and 11.0s, respectively (Marran and Schor, 1998). Marran and Schor (1999) postulated that the aniso-accommodative response is not simply a reflux blur response. Instead it is thought that volitional responses may be involved, thus suggesting that aniso-accommodation involves high level processing (Marran and Schor, 1999).

1.8 The autonomic system

The autonomic nervous system is composed of the parasympathetic and sympathetic divisions, each with unique structural and functional features. Structurally these divisions differ in the location of their preganglionic neuron cell bodies within the central nervous system, the location of their autonomic ganglia, the relative lengths of their preganglionic and postganglionic axons, and the ratio of preganglionic and postganglionic neurons.

Parasympathetic innervation

The first order neurons to the ciliary muscle are contained in the Edinger-Westphal nucleus, which receives excitatory or inhibitory impulses. The neurons travel with the oculomotor

nerve and synapse with the second order neurons at the ciliary ganglion. The axons then travel as short and long posterior ciliary nerves and provide the parasympathetic innervation to the ciliary muscle.

Sympathetic innervation

The axons travel from the hypothalamus to the spinal cord and synapse at the superior-cervical-ganglion. The axons follow the carotid artery reaching the ciliary muscle in the company of the short and long posterior ciliary nerves.

1.8.1 The autonomic innervation of the accommodative system

The predominant parasympathetic supply of the autonomic system to the ciliary body is well established. Over the last 170 years the supplementary innervation of the sympathetic branch has been emphasised, suggesting the dual innervation of the ciliary muscle.

The parasympathetic effect is a result of the action of acetycholine on muscarinic receptors (sub-type M_3). An increase or decrease in receptor stimulation increases or decreases the accommodative response, respectively (Biggs *et al.*, 1959). The sympathetic supply is relatively antagonistic to the parasympathetic supply and is a result of the action of noradrenaline on adrenoceptors (β_2). The supplementary sympathetic innervation is inhibitory in nature and results in inhibition of accommodation (Törnqvist, 1966; Törnqvist, 1967; Gilmartin, 1986; Gilmartin, 1998).

Concurrent baseline parasympathetic innervation is required for any sympathetic innervation to be present and furthermore, an increase in parasympathetic activity directly augments sympathetic activity (Tornqvist, 1967; Davies, Wolffsohn and Gilmartin, 2005). The sympathetic innervation is inhibitory, relatively small i.e. less than 2 D and exhibits slower temporal aspects (20-40 seconds) than the parasympathetic innervation (fast positive accommodation in 1-2 seconds) (Gilmartin, 1998). Hence it is thought that the inhibitory sympathetic facility may be more pertinent during sustained near-work compared to during reflexive accommodation (Gilmartin, 1998). The slow nature of the sympathetic innervation complements the fast reflexive nature of the parasympathetic innervation and together, the two branches of the autonomic nervous system may constitute an adaptive facility (Gilmartin 1986; Gilmartin, 1998).

Gilmartin and Bullimore (1991) concluded that post-task regression of accommodation to pre-task TA levels is attenuated in late-onset myopes (myopic error onset after the age of 15 years) compared to emmetropes. Strang, Winn and Gilmartin (1994) demonstrated that variations in inter-trial regression patterns for an individual were not significant. It was hence speculated that late onset-myopes may have a deficit of inhibitory sympathetic innervation and that this may be a precursor of myopia development (Gilmartin and Bullimore, 1991; Strang, Winn and Gilmartin, 1994). However, more recent work has added that access to sympathetic innervation is characterised by substantial inter-subject variations and that only a small proportion of the population (approximately 30-40 %) have access to a sympathetic facility (Gilmartin and Winfield, 1995; Gilmartin *et al.,* 2002; Mallen, Gilmartin and Wolffsohn, 2005). It has therefore been concluded that myopes and emmetropes cannot be distinguished based on their access to sympathetic facility (Mallen, Gilmartin and Wolffsohn, 2005).

CHAPTER 2

INTRAOCULAR PRESSURE

The intraocular pressure (IOP) reflects the dynamic equilibrium that exists between the production and drainage of aqueous humour. The importance of maintaining a good balance between aqueous production and drainage to attain a steady IOP level is two fold; first to sustain the globe's functional capacity and second to maintain its structural integrity (Sears, 1994). The main factors that determine IOP are: rate of aqueous secretion, resistance encountered in the outflow channels and level of episcleral venous pressure (Pointer, 1999).

2.1 Regulation of intraocular pressure

Aqueous formation

The ciliary body is a multicellular tissue and consists of ciliary smooth muscle cells, (which are innervated by parasympathetic and sympathetic nerve fibres), a stroma containing blood vessels and a ciliary epithelium. The ciliary epithelium is a bilayer of neuroepithelial cells linked by gap junctions (Raviola and Raviola, 1978). The bilayer constitutes a pigment epithelium layer (PE), which is the innermost layer of the ciliary body formed of pigment cells and a non-pigment epithelium layer (NPE) formed of non-pigment cells.

The ciliary epithelium of the ciliary processes secretes aqueous humour via a convoluted, unidirectional active transport process. The main mechanism of aqueous humour formation is the active secretion of solutes (mainly Na^+) from the ciliary stroma, across the ciliary epithelium and into the posterior chamber of the eye (Civan, 2004). Other solutes e.g. Cl^- and HCO^- are also transferred, but to a lesser degree (Cole, 1969). There is higher metabolic activity in the NPE compared with the PE which therefore indicates the dominant role of the NPE in aqueous humour formation (Cameron and Cole, 1963). The PE assists in providing the solutes to the NPE (Civan, 2004). In addition to the mechanism of active secretion, the mechanisms of ultrafiltration and passive diffusion play an important role in aqueous humour formation (Davson, 1990).

Aqueous drainage

Aqueous humour produced in the anterior part of the posterior chamber by the ciliary processes, enters the anterior chamber via the pupillary aperture. Importantly, in addition to the accommodative role of the ciliary muscle, the muscle has a major role in regulating the outflow of aqueous humour via several drainage routes (shown in **Figure 2.1**) which are:

1) *Conventional route*: The conventional route accounts for approximately 90 % of the drainage. The aqueous leaves the anterior chamber via the trabecular meshwork into the canal of Schlemm and seeps into the intra-and episcleral venous systems.

2) *Uveoscleral route*: The uveoscleral route provides egress for the remaining 10 % of aqueous humour. The aqueous humour leaves the anterior chamber via the anterior face of the ciliary body and into the suprachoroidal space. The aqueous diffuses through the sclera or absorbs into the uveal and vascular systems.

Although a small proportion of aqueous humour is exchanged via subsidiary routes, the overall effect of these supplementary routes is insignificant since the net loss and gain are equivalent. The subsidiary routes include:

3) *Vitreous*: Exchange across the anterior vitreous face.

4) *Iris:* Exchange across the iris vessels.

5) *Cornea:* Exchange across the corneal endothelium.

Figure 2.1 Outflow pathways via **1**) Trabecular meshwork; **2**) Suprachoroidal space; **3**) Vitreous; **4**) Iris and **5**) Cornea (The source from which this figure was reproduced was *Wolff's Anatomy of the eye and orbit*. 8th edition. Bron, Tripathi and Tripathi. Chapman and Hall Medical: London, 1997, p302).

2.2 Autonomic regulation of intraocular pressure

The parasympathetic and sympathetic nerve fibres richly innervate the ciliary processes. In addition to the typical neurotransmitters, noradrenaline and acetylcholine, a variety of peptide and non-peptide co-transmitters exist (Davson, 1990). Interpretation of the role of parasympathetic and sympathetic activity in the regulation of aqueous humour dynamics is complex due to the multiplicity of innervated tissues. However, despite this it is thought that aqueous humour is regulated via aqueous formation and resistance to aqueous outflow (Bergmanson, 1982).

A reduction in IOP has been elicited following electrical (Greaves and Perkins, 1952; Belmonte *et al.,* 1987) and pharmacological (Brubaker, 1991; Galbelt *et al.,* 1994) stimulation of the sympathetic nerve fibres. Conversely, stimulation of the parasympathetic nerve fibres in the ocular motor nerve has been shown to increase aqueous humour formation and therefore IOP (Stjernschantz, 1976; Marci and Cevario, 1975; Kaufman, 1984 a). Lanigan, Clark and Hill, (1989) demonstrated that isometric muscle contractions reduced IOP whilst the Valsalva manoeuvre increased IOP. Since isometric muscle contractions are mediated by the sympathetic branch of the nervous system and the Valsalva manoeuvre is interceded by the parasympathetic branch, the authors concluded that sympathetic and parasympathetic

activity reduces and increases IOP, respectively. Further evidence supporting this hypothesis is provided by studies which utilise the features of selective contralateral hemispheric stimulation by forced unilateral nostril breathing and hemispheric lateralisation of the autonomic nervous system (Backon, Matamoros and Ticho, 1989; Backon *et al.,* 1990; Chen, Brown and Smith, 2004). Right forced nostril breathing has been shown to stimulate the left hemisphere of the brain while left forced nostril breathing has been shown to stimulate the right hemisphere of the brain. Several studies have reported that right hemispheric activation via left forced nostril breathing increases IOP, whereas left hemispheric activation via right forced nostril breathing decreases IOP. The hemispheric lateralisation feature of the autonomic nervous system is such that right and left brain hemispheres induce parasympathetic and sympathetic activity, respectively. Therefore, similar to the conclusions drawn by Lanigan *et al.* (1989), more recent work also concludes that stimulation of sympathetic and parasympathetic activity reduces and increases IOP, respectively (Backon, Matamoros and Ticho, 1989; Backon *et al.,* 1990; Chen, Brown and Smith, 2004).

An autonomic ocular reflex system is thought to be involved in the homeostasis of IOP. The presence of clusters of parasympathetic and sympathetic fibres has led to the hypothesis that inter-neural communication occurs in the regulation of IOP (ten Tusscher *et al.,* 1994). Furthermore, in addition to the autonomic pathway, metabolic and hormonal pathways have also been shown to be significant in generating the steady-state IOP (Denis *et al.,* 1994).

2.3. Factors affecting intraocular pressure

The normal range of human IOP is 10-21 mmHg (Bankes *et al.,* 1968). Despite the inherent homeostatic attempts to maintain a steady-state IOP level, inter- and intra-subject variations in IOP exist. Several factors effect the IOP for example age (Medeiros, Sample and Weinreb, 2006); gender (Pointer, 2000); race (Shimmyo *et al.,* 2003); posture (Liu *et al.,* 2003 a); exercise (Karabatakis *et al.,* 2004); smoking (Lee *et al.,* 2003) and caffeine (Chandrasekaran, Rochtchina and Mitchell, 2005). Pertinent to this study are the long- and short-term cyclic variations in IOP and the effects of the ocular motor system on IOP; a discussion of which follows.

2.3.1 Long-term fluctuations

Seasonal influence

Intraocular pressure has a circannual rhythm, which is the slowest cyclic variation change. Intraocular pressures measured in 1621 subjects demonstrated that the IOP is lower in the summer months compared to the winter months (Bengtsson, 1972). More recent studies have also shown that the IOP is higher in the winter months compared to the summer months by 1 to 3 mmHg (Qureshi *et al.*, 1996; Qureshi *et al.*, 1999).

Diurnal variation

A well-established circadian rhythm exists in IOP which is similar in both eyes (Kitazawa and Horie, 1975; Liu, Sit and Weinreb, 2005) and is closely matched to a cosine curve (Kitazawa and Horie, 1975; Liu *et al.*, 2003 b).

The concept that intraocular pressure is subject to diurnal variations was first recognized by Didler-Huguenin over a century ago in 1898 (cited by Pointer, 1997). Using a Maklakov tonometer, Maslenikov (1904) was first to quantify the diurnal variations in IOP (cited by Aihara, Lindsey and Weinreb, 2003) as a drop of ≤ 2 mmHg between 0900 and 1730 hours (cited by Pointer, 1999). A more recent study reports (on 690 diurnal curves) that, for 63 % of normal subjects, the morning peak in IOP is before 1130 hours and for 44 % of normal subjects the lowest IOP is between 1400 and 1730 hours. The mean value of fluctuation in that study was 5.0±2.7 mmHg (David *et al.*, 1992). The exact time of the IOP peak over a 24 hour period and its magnitude remain equivocal, although an early morning peak in IOP tends to be a frequent finding (Bengtsson, 1972; Smith, 1985; David *et al.*, 1992; Wilensky *et al.*, 1993; Pointer, 1997; Liu *et al.*, 1998; Noel *et al.*, 2001; Liu *et al.*, 2005).

A nocturnal elevation in IOP has also been demonstrated (Frampton, Da Rin and Brown, 1987; Liu *et al.*, 1998; Orzalesi *et al.*, 2000; Noel *et al.*, 2001; Liu *et al.*, 2003 a; Liu, Sit and Weinreb, 2005; Kida, Liu and Weinreb, 2006). The physiological basis for the elevation of IOP during the nocturnal period is unclear. Reiss *et al.* (1984) showed a reduction in nocturnal aqueous humour flow. More recently Liu *et al.* (2003) has concluded that the elevation of IOP at night was not due to changes in blood pressure or heart rate but instead

postulated that the changes observed may be due to changes in the resistance to aqueous outflow.

2.3.2 Short-term fluctuations

Respiration

The steady-state IOP fluctuations approximate to a sine wave (Moses and Arnzen, 1983). The ocular pulse wave form is shown in **Figure 2.2**, analysis of which has shown that the principal spectral component is associated with the cardiac cycle which is discussed below (Leydhecker, 1976).

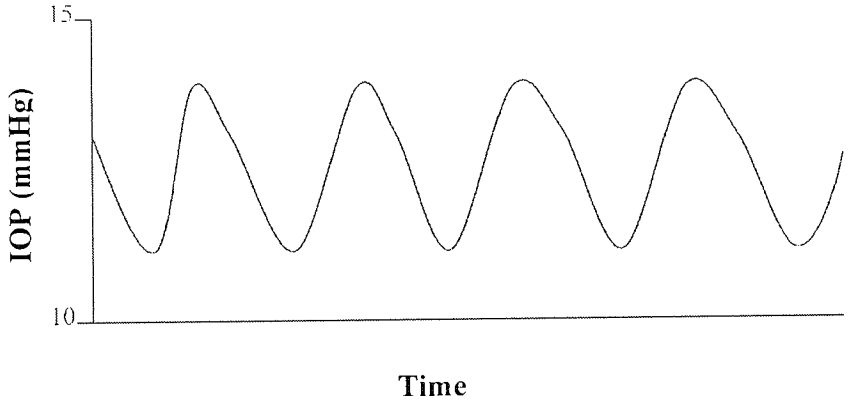


Figure 2.2 Cyclic variations in IOP with time (redrawn from Silver *et al.*, 1989).

A second, lower frequency spectral component of the ocular pulse waveform is related to the respiratory cycle (Moses and Arnzen, 1983; Akselrod *et al.*, 1985) with a slow frequency of 1 every 3 or 4 seconds (Leydhecker, 1976; Perkins, 1981). Hitherto, work on the effects of the respiratory cycle on IOP is limited. This is probably due to the practical and ethical restrictions of manipulating the respiratory cycle. Nevertheless, Leydhecker (1976) concluded that on inspiration and expiration the IOP increased and decreased, respectively. The difference in IOP between inspiration and expiration was recorded as approximately 4mmHg (Leydhecker, 1976). Nanba, Nakayama and Iwata (1989) repeatedly measured the IOP with a non-contact tonometer and demonstrated that measures at either the peak or trough of the cardiac cycle varied by 0.8±0.4 mmHg. The authors concluded that since repeat IOP measurements at each peak or trough should be the equivalent (for the same individual), any variance in the data must be attributable to the influence of the respiratory cycle on IOP measurements (Nanba, Nakayama and Iwata, 1989).

Cardiac influence

The principal spectral component of the ocular pulse wave (shown in **Figure 2.2**) is associated with the cardiac cycle with a frequency of approximately 1 second (Leydhecker, 1976). It is well accepted that with each heart beat, a bolus of arterial blood enters the choroid during the systolic phase. The rhythmic filling of the choroidal vessels set against the constant ocular rigidity results in volumetric changes which give rise to fluctuations in the steady-state IOP, such that the IOP increases with each systole and decreases with each diastole (Bynke and Schéle, 1967). The difference between the maximum IOP (systolic phase) and the minimum IOP (diastolic phase) is known as the ocular pulse amplitude (OPA).

Perkins (1981) reported that the pulse amplitude was 2.84±1.16 mmHg. Nanba *et al.* (1989) also concluded that the mean difference in IOP between the peak and trough of the cardiac pulse waveform (i.e., the OPA) was 1.9±0.8 mmHg. A later study by Trew *et al.* (1991), in which a pneumotonometer was used, concluded that the mean IOP pulse amplitude was between 1.8 and 2.8 mmHg. A more recent study has investigated the effect of the cardiac cycle on IOP measures with the new *Nidek NT-4000* non-contact tonometer. The *NT-4000* detects changes in blood flow with each heart beat in the forehead and allows pulse synchronised IOP measures. As expected the study reported that the mean IOP taken at the peak of the cardiac waveform is higher (16.1±2.6 mmHg) than the mean IOP taken at the middle (15.3±2.6 mmHg) and the trough of the waveform (14.7±2.7 mmHg) and the pulse amplitude was approximately 1.4 mmHg (Lam, Chan and Lam, 2004). Due to differences in methodologies, the exact OPA is equivocal. However, more apposite is that there are substantial inter-subject variations in OPA which need to be considered during the measurement of IOP. Nanba *et al.* (1989) observed that the maximum difference between the peak and trough of the cardiac pulse wave was approximately 4 mmHg. Vernon (1993) has reported that on repeat NCT measures, approximately 60% of his subject group had a range of IOP greater than 4 mmHg and this range increased to more than 10 mmHg in about 8% of the subjects. Analysis of the OPA using the *NT-4000* has reported that the maximum OPA was 6.7 mmHg (Lam, Chan and Lam, 2004).

2.3.3 Oculomotor influences on IOP

Convergence

Armaly and Rubin (1961) noted that during Goldmann contact tonometry (GCT) in clinical practice patients are often instructed to fixate on the fixation-light of the slit lamp. Hence the authors investigated the effect of near fixation on the IOP in 10 subjects using the GCT. The study demonstrated that fixation of a light source at 10 cm reduced the IOP by approximately 5 mmHg. It is thought that the study indirectly investigated the effects of convergence on IOP since the fixation light can be considered as a non-accommodative stimulus.

However, subsequent studies which investigated the effects of gaze position on IOP yielded opposite results to the Armaly and Rubin study. A manometric study on one volunteer (a 26 year old about to undergo enucleation for malignant melanoma) by Coleman and Trokel (1969) observed that on leavoversion of the right eye the IOP increased by approximately 5-10 mmHg which was measured using a radiosonic transducer. In the same study the authors concluded that the IOP increased by 2-4 mmHg with apparent accommodation to a 7.14 D accommodative stimuli. However, of note is that the individual volunteer in the study was cyclopleged with 2% cyclopentolate hydrochloride. Therefore, it is speculated that the increase in IOP observed when viewing the target at 14 cm (i.e. 7.14D accommodative stimuli) was not attributed solely to accommodation but was more likely to be due to the effects of convergence.

A decade later, Helveston, Bick and Ellis (1980) used a pneumotonometer to measure the IOP in 10 subjects in primary gaze, 20° from the primary gaze and in extreme positions of gaze. Their study concluded that a significant increase in IOP of between 4 and 5 mmHg occurred only in extreme positions of gaze but not when fixating a target 20° from the primary position of gaze. Similarly, Saunders (1981) used the Perkins tonometer on 20 subjects and concluded that the IOP increased only in the extremes of gaze. Moses, Lurie and Wette (1982) measured the effects of horizontal gaze on IOP on 6 subjects using the GCT. Although the IOP was not recorded in primary position, the IOP was measured with the eye adducting or abducting by 10, 30 and 50°. The study reported a higher IOP with increasing lateral and nasal gaze. With the eye rotated 50° a 3 and 2 mmHg increase was observed in lateral and medial (mimicking convergence) positions.

More recently, Kumata *et al.* (1994) conducted a study on 20 subjects who were instructed to fixate at 15, 25 and 35° of medial and lateral gaze. The study concluded that compared to the primary gaze, a statistically significant increase in IOP was found in 5 of the 6 positions of gaze evaluated. However, the study does not note which position of gaze did not increase IOP and the technique used to measure the IOP. Nevertheless, the study concluded that the IOP increased in a linear fashion from the primary gaze to increasingly lateral and medial gazes. The difference in IOP from the primary position was approximately 2.68 and 2.12 mmHg for the medial and lateral positions of gaze, respectively.

It is clear that the investigations of the effects of convergence on IOP are limited. It is thought that contraction of the medial recti during convergence may set increased pressure on the globe and hence increase IOP. Of note however is that investigations of convergence in which the eye is rotated from the primary position to extreme gaze positions may not necessary represent the change in IOP on convergence during near work. It is thought that the changes in IOP on contraction of the two medial recti muscles during near work would be somewhat less than the additive effect of the contraction of the ipsilateral medial and contralateral lateral muscle during nasal gaze positions. This may explain the discrepancy in the results of the study conducted by Armaly and Rubin (1961) and the subsequent studies described above. Furthermore, during near work the eyes are fixating in a downwards position and early studies have reported that on down gaze the IOP reduces by 4 to 5 mmHg (Zappia, Winkelman and Gay, 1971; Nardi *et al.*, 1988).

Accommodation

Hitherto, little literature exists on the influence of accommodative effort on IOP, the relationship remaining equivocal. Early workers such as Ware (1813), Donders (1864), Erismann (1871) and von Arlt (1876) believed accommodation raised IOP (cited by Stansbury, 1948). However, Hess and Heine (1898) reported that on maximal ciliary muscle contraction, no increase in IOP was observed (cited by Stansbury, 1948). Several workers also concluded that accommodation had no influence on IOP (summarised by Duke-Elder, 1938). Despite the contradictory results in the literature, Duke-Elder (1938) postulated that accommodation in fact reduced the IOP level. He attributed the reduction in IOP to increases in aqueous outflow following constriction of anterior ciliary arteries, dilation of ciliary veins and widening of the anterior chamber angle. The majority of the studies published subsequently agreed with the tenet that IOP reduces with accommodation.

Armaly and Burian (1958) posed the question as to whether near work should be encouraged or not in glaucoma patients and whether accommodation needs to be minimised during tonometry. The authors used a Mueller electronic tonometer to measure the IOP in one eye while accommodation was simultaneously stimulated in the other eye. No direct measures of accommodation responses were taken. However, it was ensured the subjects were accommodating by instructing them to point at the direction of the opening in the Landolt circle target placed at 25 cm from the eye. The target was rotated to different positions approximately 3 times a minute.

Tonograms showing C values (i.e., facility of aqueous outflow) were obtained from seven young subjects (age 18-25 years) focusing on a target at 25 cm with and without a +4.00 DS lens (presented in a random order). Statistical analysis of the data showed that both the difference in C values obtained during accommodation and relaxation, and the differences in C values attained between subjects were significant at the 1 % level. It was noted that the interpretation of the tonograms was subject to error since fluctuations in IOP caused by the respiratory and cardiac cycles were also recorded. Despite this, the study concluded that for 4D of accommodation, an increase in the C value (and hence decrease in IOP) occurred. Of interest is that the mean calculated reduction in IOP for all 7 subjects was 3.43±1.99 mmHg and although all subjects showed a reduction in IOP, the IOP changes ranged from a decrease of just 1 mmHg to a substantial decrease of 6 mmHg. Mechanisms to explain the increase in C value were suggested by Armaly and Burian and these included an increase in the facility of aqueous outflow, a decrease in formation of aqueous or a reduction in intraocular blood flow (Armaly and Burian, 1958). However, the study did not establish which mechanism(s) was likely to be the principal cause for the change observed.

Work by Armaly and Rubin (1961) used the same apparatus to stimulate accommodation as that used by Armaly and Burian (1958) although the Mueller electronic tonometer was replaced by a Goldmann contact tonometer. The IOP was measured in 10 subjects every minute until three identical and successive measurements were obtained, during accommodation and non-accommodation. The subjects were separated into two age groups; one group aged between 20-25 years and the second group aged between 45-55 years (i.e., reduced amplitude of accommodation). The study quantified the reduction in IOP for the group as a mean value of 3.58±0.16 mmHg with a 4 D accommodative stimulus, which was

significant at the 1 % level. Statistically significant differences between the results of the two groups were observed ($p < 0.01$). In the younger group, a mean reduction in IOP of 4.5±0.167 mmHg was reached after focusing on a 4 D accommodative stimulus for a mean time of 2.7±0.18 minutes. For the older group, the mean reduction in IOP was lower (2.3±0.147 mmHg) and was reached after a longer mean time (4.0±0.27 minutes). However, it was found that since the subjects in the older group could not accommodate to 25 cm, the +4.00 DS was not completely removed but instead it was reduced to a power which stimulated the maximum effortless accommodation. It was concluded that the same amount of accommodative stimulus (i.e., 4 D) had less effect on IOP dynamics in the older group and therefore, the authors postulated that a possible reduction in the response of the trabecular meshwork to ciliary muscle contraction may occur with age. However, it is evident that the two groups were not accommodating exactly 4 D and therefore the conclusions drawn are limited.

Armaly and Rubin (1961) also evaluated the effect of graded accommodation. The +4.00DS lens was reduced in power to stimulate increasing amounts of accommodation. The report concluded that the minimum amount of accommodation that produced a statistically significant change in IOP was as little as 0.50 D. Furthermore, progressive increases in the magnitude of the accommodation stimuli by small increments produced progressively lower IOP measures. However, accommodation stimuli levels in excess of approximately 1.50 D were shown to not produce any further decreases in IOP. Although the authors inferred that the minimum and maximum effects of accommodation on IOP were elicited by a narrow range of accommodation stimuli, no comments were made on the putative regulation of IOP with accommodation.

Pharmacological agents were also used in the same study to confirm the changes in IOP with accommodation. Cycloplegic agents were used which either reduced (with cyclopentolate) or eliminated (with atropine) the alterations in IOP with accommodation. Phenylephrine (a sympathomimetic) a drug that dilates the pupil and increases the sympathetic inhibition of accommodation (Rosenfield *et al.*, 1990; Culhane, Winn and Gilmartin, 1999) was used to conclude that pupil constriction which accompanies accommodation did not contribute to the change observed in IOP (Armaly and Rubin, 1961).

Allen and Burian (1965) constructed a model of the anterior chamber angle, so that the mechanism that modulated IOP change could be investigated. It was demonstrated that contraction of the meridonal fibres of the ciliary muscle resulted in an increase in aqueous outflow. However in contrast, contraction of the circular fibres caused a subsequent decrease in aqueous outflow. The study reported that on contraction of both the aforementioned fibres, the net result observed was an increase in aqueous outflow but was to a lesser extent than that observed when the meridional fibres alone were contracted. Allen and Burian (1965) thus concluded that the outflow tissues behaved like a valve which were controlled by the ciliary muscle which acts as a unitary whole.

A pressure-transducer was used on monkeys (*Macaca nemestrina*) to obtain results on the intraocular pressure changes with accommodation by Young (1981). The study reported that accommodation to an approximately 3 D accommodative stimuli reduced the IOP by 6 mmHg (Young, 1981).

The most recent study which has investigated the effects of accommodation on IOP was conducted by Mauger and co-workers in 1984 (Mauger *et al.*, 1984). The IOP was measured using the Goldmann contact tonometer in 30 subjects aged between 22-35 years. The 30 subjects recruited were split into 3 groups of 10. Group 1 was treated as a control group. In Groups 2 and 3, the effects of accommodation on IOP were investigated. In group 2 the IOP was measured in the RE while the LE fixated a target at 6 m. A -1.50DS lens was added to the LE and the subjects were instructed to attain and maintain a clear image of the 6 m target. Similarly, in Group 3 the IOP measures were taken in the RE while the LE fixated a target at 6 m and through a -4.00DS lens. It was assumed that if the target was clear then the subjects were accommodating to the required level. The addition and removal of the 1.50 and 4D lens in the 2 groups was randomised and the IOP was measured after 30 seconds and 3 minutes of accommodation. The study concluded that with 30 seconds of accommodation to a 1.50 and 4D accommodative stimulus the IOP reduced by 1.15±0.71 and 1.32±0.42 mmHg, respectively. With 3 minutes of accommodation to a 1.50 and 4D accommodative stimulus the IOP was shown to reduce by 2.15±0.78 and 2.38±0.65 mmHg. No statistical tests were performed to establish whether the changes in IOP with 1.50 and 4D of accommodation were statistically different to each other. However, the study has several limitations for e.g., the small sample size used, the effect of repeated applanation tonometry on the steady-state IOP and the omission of the measurement of actual accommodation responses. These limitations

are addressed in subsequent experimental chapters and form the basis for the main aims and objectives of this thesis.

2.4. Intraocular pressure measurement techniques.

Tonometry is a measure of intraocular pressure made by devices known as tonometers. The first attempts to measure IOP were by digital palpation, which observed the differential hardness of the eyeball. Two fingers were placed on the superior globe and a small force applied to get a sense of globe firmness. Classifications to standardise palpation measurements were introduced by Bowman in 1862 and Fick in 1888 (cited by Von Graefe, 1966). The need for objective measurement techniques led workers such as Von Graefe, Donders and Hamer to construct 'impression' tonometers.

The basis for these instruments was that the weight exerted on the eye was proportional to the impression observed. Therefore, if the globe was relatively firm, the depth of the impression would be smaller, indicating a higher IOP. Advances in electronics and signal processing have permitted the invention of more accurate measurement techniques.

Two basic principles are utilised in tonometry: corneal applanation and corneal indentation. In the applanation principal, either the force needed to flatten a given area is measured (e.g. as with the Goldmann or Perkins tonometer) or the area of applanation caused by a given force is measured (e.g. Maklakov tonometer). In the indentation principle the depth of impression caused by a plunger of known weight is measured (e.g. Schiötz tonometer).

2.4.1. Indentation tonometry

The basis of Indentation tonometers is that the depth of impression or deformity caused by a plunger of known weight is measured mechanically or electronically and is proportional to IOP.

The resistance to deformation offered by the cornea is a sum of ocular rigidity and IOP and implies that an ocular rigidity value must be assumed. Comparisons of pressure measurements obtained from indentation tonometers with measures obtained manometrically have been used to specify values for ocular rigidity.

Schiötz tonometer

Hjalmar August Schiötz in 1905 invented the Schiötz tonometer and it has since undergone little change in its design. The instrument is portable, rugged, compact and inexpensive. Different weights (5.5, 7.5, 10 and 15 grams) can be assembled onto the plunger. The movement of the plunger as it indents the cornea is amplified mechanically to a pointer over a scale, which ranges from zero to 20 units. The scale reading is converted to IOP measurements in millimetres of mercury by the use of conversion graphs or tables. More accurate estimates of IOP are obtained by varying the weight on the plunger. If a high IOP is present a heavier weight should be attached and if a low IOP is present then a lighter weight should be assembled on the plunger.

The plunger has a massaging effect on IOP results so repeat measurements should be kept to a minimum although two measurements, using two different weights, are necessary as the estimated IOP results are dependent on ocular rigidity. When taking the second measurement (using a heavier weight), if the IOP is higher compared to that obtained with the first weight, it implies that the eye has a higher than average ocular rigidity. Therefore, in an attempt to minimize this error Friedenwald devised a pressure-rigidity nomogram. The nonogram is used to estimate IOP and approximate the coefficient of rigidity.

The IOP measurement is also influenced by corneal curvature for which Friedenwald devised another conversion chart. A common source of error with the Schiötz tonometer is the build up of tears and mucus, which increase the friction between the plunger and its walls. The use of the instrument as a first choice device is reduced, as it is a contact tonometer and therefore requires thorough sterilisation and corneal anaesthesia. A slight pulsation of the needle is observed and this is due to the cardiac pulse (see **section 2.3.2**). Therefore, the measurement recorded from the scale is subject to inter-observer variations. The instrument requires the subject to be in a semi-supine position, which makes it difficult to use the instrument in accommodation and IOP measures. In addition, only a skilled operator should perform the procedure.

The Schiötz tonometer was widely used to estimate the IOP for years until the introduction of applanation tonometry and thus the Goldmann tonometer.

McKay-Marg/Tono-pen tonometer

The McKay-Marg tonometer is partly an applanation and partly an indentation system. It indicates the movement of a stylus to record the extent of deformity caused by approximately 1g of force on a moving paper, which is electronically amplified. Minimisation of the recording procedures allowed by micro-chip technology (into digital displays) has led to the development of the *Tono-pen*. The *Tono-pen* is essentially a modified McKay-Marg tonometer.

Although the *Tono-pen* tonometer is light, portable and easy to use with the subject in any position it is a contact device which requires corneal anaesthesia. The use of anaesthetics has been shown to influence IOP (see **section 2.4.2**) and therefore the Tono-pen was not used in this study to investigate the relationship between accommodation and IOP.

2.4.2 Applanation tonometers

In 1885 Maklakoff stated that the IOP could be measured from the force needed to displace a constant volume or from the volume displaced by a constant force. In the same year Imbert devised the Imbert law which stated that:

$$\text{pressure against the globe} = \text{IOP} + \text{surface adhesion}$$

Three years later the Imbert-Fick law was developed and subsequently Fick invented a spring loaded tonometer. The Imbert-Fick law states that when a flat surface is pressed against a spherical sphere, equilibrium is attained when the force exerted against the spherical surface is balanced by the internal pressure of the sphere exerted over the area of contact between the sphere and flat surface, i.e.,

$$P = \frac{W\ (g)}{A\ (mm^2)}$$

where **P** is the pressure, **W** is the force required to attain applanation and **A** is the area of flattening.

However, there are assumptions of the Imbert-Fick law which are that the membrane of the sphere is infinitely thin, dry, elastic and flexible, and the only force acting against it is the pressure of the applanating surface. The law also assumes that the volume displaced is insignificant compared to the volume of the eye. The requirements of the Imbert-Fick law are

not truly met by the human eye although they are well approximated under certain conditions. Indeed the cornea is not spherical, is of variable thickness, is wet and is not entirely elastic and flexible. Furthermore, the cornea resists indentation, the applanating surface touches the cornea as well as the pre-corneal tear film which causes capillary attraction and the amount of volume displaced is dependent on ocular rigidity.

2.4.3. Contact tonometers

Goldmann contact tonometer

Hans Goldmann, the Swiss Ophthalmologist invented the Goldmann contact tonometer (GCT) which is regarded globally as the clinical standard for IOP measures and is referred to as the 'Gold standard' of tonometry.

The GCT is based on empirical experimentations which have concluded that when the area applanated is approximately 3 mm the resistance to indentation is balanced by the forces of capillary attraction (which pull the tonometer head towards the cornea), i.e.,

$$P + M = \frac{W - N}{A}$$

where **P** is the pressure, **M** is the elasticity modulus, **W** is the applanation force, **A** is applanation area and **N** is the capillary attraction forces. Goldmann decided that the applanation area has to be precisely 3.06 mm since at this diameter the capillary surface tension and ocular rigidity cancel each other out and the applied force of 0.1 gm corresponds to 1 mmHg, which represents an easy conversion factor (Whitacre and Stein, 1993).

The GCT probe has a flattened tip and consists of an internal doubling prism, which splits a full circle into two semicircles when viewed through slit lamp oculars after instillation of the diagnostic dye, fluorescein. The force applied is varied until the two images of the semicircles, are aligned so that the inner edges of both semicircles are in contact. When this is achieved the area applanated has a diameter of 3.06 mm and translates into an IOP measurement.

Goldmann contact tonometry (or its counterpart Perkins tonometry) is often used in general optometric practice to measure IOP. It is a contact device, which requires corneal

anaesthesia. Owing to the risk of vCJD contamination with re-usable tips disposable tips are now available (*Tonosafe* Grafton Optical) to use with the tonometer, however prior to this, it was necessary to sterilise the probe with sodium hypochlorite and alcohol swabs. The instrument is composed of an applanating head and a spring-loaded level, which needs to be attached to a slit lamp table thus reducing the portability of the tonometer. The final IOP reading taken is practitioner-dependent, as the mires pulsate due to the ocular pulse (see **section 2.3.2**).

The Perkins tonometer is the hand-held derivative of the GCT. The tonometer probe is similar to that used by the GCT and the resistance is adjusted by the rotation of a knurled and calibrated wheel. The illumination system is built into the instrument therefore it is portable and easy to use with the subject in any position. The Perkins tonometer is also a contact device and requires corneal anaesthesia and probe sterilisation and as for the Goldmann tonometer, disposable tips are available (*Tonosafe*).

Limitations of the Goldmann contact tonometer

A review of the factors affecting the IOP measures as taken with the GCT can be found elsewhere (Whitacre and Stein, 1993). Given that the non-contact tonometer and Ocular blood flow analyser used in this thesis is essentially an applanation tonometer, the factors influencing IOP measures pertinent to this study are discussed below.

Inter-subject variations in the physical properties of the cornea result in errors in the measures of IOP. Corneal astigmatism influences the tonometric measures with GCT. With increasing corneal astigmatism the shape of the tonometer-corneal contact changes from a circle to an ellipse which causes errors in the IOP measurements. The GCT underestimates the IOP measures when with-the-rule astigmatism exists. Conversely, the GCT overestimates the IOP when the cornea presents with against-the-rule astigmatism. Hence Goldmann suggested that in eyes with corneal astigmatism of more than 3 D the probe should be aligned to 43°. Empirical experimentations concluded that errors in IOP measurements were minimised when the probe was rotated to 43°. Holladay, Allison and Prager, (1983) however recommended that an IOP measure should be taken in both the vertical and horizontal meridians and the average of these two values would best represent the IOP. Furthermore, irregular astigmatism distorts the fluoroscene rings and hence causes errors in the measurement of IOP (Whitacre and Stein, 1993).

Goldmann stated that inter-subject variations in corneal curvatures could also cause errors in contact tonometry. The steeper the cornea the more force is needed to indent the cornea to the constant applanation area and therefore a higher IOP measure is produced (cited by Mark and Mark, 2003). Furthermore, excessive force applied to the globe displaces more fluid which subsequently increases IOP. Gunvant *et al.* (2004) reported that for every 1 mm increase in corneal curvatures the IOP increases by 1.14 mmHg. However, several recent studies have demonstrated that when the effects of corneal thickness on IOP measurements (discussed below) are accounted for, corneal curvatures do not have significant associations with the IOP (Francis *et al.*, 2007; Kohlhaas *et al.*, 2006; Medeiros *et al.*, 2006; Saleh *et al.*, 2006).

Elhers *et al.* (1975) and later Whitacre *et al.* (1993) stated that the measurements obtained with the Goldmann tonometer are only accurate if it is assumed that the corneal thickness is approximately 520 μm. If the cornea is relatively thicker, then more force is required to deform it and this creates an artificially higher IOP reading. Conversely, if the cornea is thin, an artificially lower IOP is obtained. Indeed, several studies conducted since Goldmann's work have shown similar results (Bhan *et al.*, 2002; Shimmyo *et al.*, 2003; Gunvant *et al.*, 2004; Ko, Liu and Hsu, 2005; Tonnu *et al.*, 2005; Kohlhaas *et al.*, 2006; Medeiros and Weinreb. 2006; Saleh *et al.*, 2006). Several studies have demonstrated that for every 10 μm increase in CCT the IOP increases by between 0.20 and 0.40 mmHg when measured with the GCT (Singh *et al.*, 2001; Bhan *et al.*, 2002; Gunvant *et al.*, 2005; Saleh *et al.*, 2006). Correction models by Ehlers (which uses a nomogram based on manometric experiments) and Orssengo and Pye (a theoretical model) have been used in previous studies to account for the effects of corneal thickness on IOP measures (Argus, 1995; Copt *et al.*, 1999; Herndon *et al.*, 1997; Stodtmeister, 1998; Tamburrelli *et al.*, 2004; Tamburrelli *et al.*, 2005). However, recent studies conclude that the aforementioned correction models overcorrect the IOP such that the corrected IOP is erroneously lower and higher for thicker and thinner corneas, respectively (Gunvant *et al.*, 2005; Kirstein and Targay, 2005).

Schmidt (1960) calculated that for scleral rigidities of 0.0050, 0.0215 and 0.0350 μl^3, an IOP of 26.5 mmHg was overestimated by 0.2, 0.5 and 1 mmHg. He therefore concluded that measures taken with the GCT were influenced by ocular rigidity such that applanation tonometers overestimated IOP as the coefficient of rigidity increased. More recently,

variations in Young's modulus of elasticity between the normal values of 0.1 to 0.9 have been shown to change IOP over a range of approximately 17 mmHg (Liu and Roberts, 2005).

To date the measurement of ocular rigidity has proven to be complex. Friedenwald devised a formula for ocular rigidity which assumed that scleral rigidity was constant. However, scleral rigidity has been shown to decrease with IOP (Gloster and Perkins, 1959) and it is hence thought that Friedenwald's method of calculating ocular rigidity is inaccurate (Foster and Yamamoto, 1978; Brooks, Robertson and Mahonev, 1984). Ocular elasticity has also been modeled in engineering terms but has not yielded simple results (Purslow and Karwatowski, 1996). Orssengo and Pye have more recently modeled the cornea as a shell and have used theoretical equations for the deformation of a shell due to internal and external pressures to calculate the ocular rigidity (Orssengo and Pye, 1999). However, the aforementioned models are largely based on assumptions and can only estimate the *in vivo* ocular rigidity.

As explained earlier when using the GCT the degree of force is varied until the applanated area is 3.06 mm in diameter. Stocker (1956) noted that application of a tonometer probe on one eye reduced the IOP due to aqueous massage caused by the tonometer tip. Goldmann (1959) observed a decrease in IOP of 2 to 3 mmHg after repeated measures (cited by Whitacre and Stein, 1993). Armaly and Rubin (1961) performed GCT once every minute for six minutes and concluded that IOP reduced in the first minute although a progressive decline in IOP after the first minute did not occur. In 140 subjects, Becharakis (1966) found that successive measures over 12 minutes reduced the IOP by approximately 5-6 mmHg (cited by Whitacre and Stein, 1993). Furthermore, Moses and Liu (1968) reported a slightly smaller decrease in IOP on repeat measures of 0.4±1.4 mmHg (n=75) although they noted a decrease of more than 2 mmHg in 35 % of their subject group. The decrease in IOP with the GCT can be partly attributed to the relatively long corneal contact time (approximately 2 seconds) of the probe (Myers and Scott, 1975).

Moreover, Baudouin and Gastaud (1994) have demonstrated a small yet significant reduction in IOP after the instillation of topical anaesthetics. The study reported that the IOP (as measured by a non-contact tonometer) reduced 1 minute after the instillation of 0.4 % oxybuprocaine (aka benoxinate) by approximately 1 mmHg and this reduction remained stable for at least 15 minutes. Of importance is that the study by Baudouin and Gastaud (1994) reports that in some subjects the IOP reduced by 6 and even 8 mmHg after the

instillation of the anaesthetic. The authors hypothesised that, whereas more precise investigations were required, the reduction in IOP may be due to a direct facilitating effect of topical anaesthetics on the aqueous humour outflow. Furthermore an increase in corneal thickness following topical anaesthesia has also been observed as a result of corneal oedema (Herse and Siu, 1992; Asensio *et al.*, 2003; Nam *et al.*, 2006). This increase in corneal thickness may subsequently increase the IOP measures with the GCT. It is thought that the increases in lacrimation and blepharospasm following instillation of anaesthetics observed by Birchall and Kumar, (2001) may disrupt the tear fluid layer and hence lead to erroneous IOP measures, since the fluorescent tear film meniscus is imperative in governing the accuracy of an IOP measure with the GCT (Whitacre and Stein, 1993).

Dynamic Pascal tonometer

The Dynamic Pascal contour tonometer (*Swiss Microtechnology AG, Switzerland)* monitors IOP 100 times per second. The tonometer is similar to the Goldmann tonometer as it is slit-lamp mounted, has a sprung-loaded probe and needs to be in contact with an anaesthetised cornea. The tonometer probe is 7 mm in diameter and is curved to match the curved cornea. A pressure sensor, 1.2 mm in diameter is integrated into the tonometer probe. Therefore, on contact, no bending forces act on the cornea and the pressure exerted on the outside of the cornea is equal to the IOP.

A number of studies have shown that unlike the GCT the Dynamic Pascal contour tonometer is not influenced by variations in central corneal thickness (Kaufmann, Bachmann and Thiel, 2004; Siganos, Papastergiou and Moedas, 2004; Kotecha *et al.*, 2005; Ku *et al.*, 2006; Schneider and Grehn, 2006; Ozbek *et al.*, 2006). For example, Siganos *et al.* (2004) measured the pre-and post-*Lasik* surgery IOP's using the Dynamic Pascal contour tonometer and reported that the former IOP's were significantly lower than the latter and that the IOP measures obtained with the tonometer were not affected by corneal thickness. A recent study in which GCT and Dynamic Pascal contour tonometry was performed in pathological corneas concludes that the Dynamic Pascal contour tonometer measures IOP independent of corneal thickness, curvature and ocular rigidity (Ozbek *et al.*, 2006).

Ocular Blood Flow Tonometers

The Ocular Blood Flow Analyser (OBFA: Paradigm Medical Instruments Inc., Utah, USA) is a pneumotonometer which continuously measures IOP at a high frequency of 200Hz. The pneumotonometer is linked to a computer which allows the changes in IOP with each bolus of blood to be measured. A full account of the measurement principles is given in **Chapter 8**. Briefly the IOP waveform is recorded, digitised and analysed to give a volume profile which is converted to a volume change against time profile, from which pulsatile ocular blood flow (POBF) values are calculated. The OBFA provides measures of IOP, pulse amplitude, pulse volume, pulse rate and pulsatile ocular blood flow (POBF).

An eye of lower ocular rigidity is more distensible and therefore according to the pressure-volume relationship (described in **Chapter 8**) would produce a smaller change in pressure for a given change in volume. The IOP measures obtained with the OBFA are influenced by corneal thickness (Singh *et al.*, 2001; Bhan *et al.*, 2002; Morgan *et al.*, 2002; Ko *et al.*, 2005; Tonnu *et al.*, 2005; Saleh *et al.*, 2006). Morgan *et al.* (2002) concluded that every 10 μm increase in corneal thickness is equivalent to a 0.30 mmHg change in IOP. However, variations in corneal curvatures do not influence IOP measures although a negative correlation between the pulse amplitude measure and corneal curvatures has been reported (Morgan *et al.*, 2002).

2.4.4. Non-contact tonometers

The principle of operation behind non-contact tonometers (NCT) is that the extent of deformity caused by a soft pulse of air directed at the cornea is measured optically by a photoelectric cell and displayed as an IOP measure. The instrument is normally mounted on a height adjustable table and the subject is in the seated position. When taking the IOP measures the air aperture is placed approximately 15 mm from the cornea and does not require contact with the cornea, therefore negating the need for corneal anaesthesia.

In 1972, American Optical produced the first NCT (The Reichert). Here the pressure of the air puff directed towards the cornea increases over time. The time taken from the onset of the puff of air to the required level of applanation of the cornea is measured. The tonometer processes the time signal and digitally displays an IOP value. In a refined version of the Reichert (The Reichert XPERT), the air pressure at the point of applanation is measured rather than the time taken to applanate the cornea. Once the applanation point is detected, the

air pump switches off and limits the force of the air pulse therefore making it more comfortable for the patient. The Topcon tonometer is similar to the Reichert XPERT instrument in that the former also operates by sampling the pressure at the point of corneal applanation and the puff of air is cut-off after applanation.

The Keeler Pulsair tonometer is a hand-held, non-contact device, which is kept in a console that can be desktop or wall mounted. The original *Pulsair* was designed by John Fisher in 1988 (Fisher, Watson and Spaeth, 1988). Revised computer algorithms were applied to improve the instrument and the *Pulsair 2000* and *3000* were launched in 1991 and 1998, respectively. Full operational principles of the Pulsair NCT are discussed in **Chapter 3**.

Since NCT are also based on the Imbert-Fick law described above similar to the GCT, measures taken with the NCT are influenced by physical properties of the cornea and the effects are quantified using a biomechanical model of the cornea (Liu and Roberts, 2005). Several studies have demonstrated that the NCT overestimates and underestimates the IOP in relatively thicker and thinner corneas, respectively (Matsumoto *et al.*, 2000; Eysteinsson *et al.*, 2002; Ko *et al.*, 2005; Tonnu *et al.*, 2005). Tonnu *et al.* (2005) reported that every 10 μm increase in corneal thickness increased the IOP by 0.46 mmHg. Corneal curvatures also influence the IOP measure taken with the NCT, although the effect of corneal curvatures is relatively less than the effect of corneal thickness (Cheng *et al.*, 2006).

Ocular Response Analyser

The Ocular Response Analyser (Reichert ophthalmic instruments, Buffalo, New York) is a relatively recent instrument which primarily measures the IOP by the applanation principle. However, in addition to this the instrument also provides two new measurement parameters that quantify corneal hysteresis and corneal resistance factor. During an IOP measure the air pulse is delivered to the cornea and deforms the cornea to applanation and subsequent concavity. When the air-pulse reaches its maximum pressure output it switches off and the pressure declines in a symmetrical fashion, which means that while the cornea returns from a state of concavity to its normal convex state it passes a second point of applanation (Luce, 2005). The difference between the two IOP measurements at the inward and outward applanation points (see **Figure 2.3**) is considered to represent visco-elastic properties of the cornea i.e., corneal hysteresis (Soergel *et al.*, 1995). The corneal resistance factor is a measurement of the additive effect of viscous and elastic resistance. Reichert claim that the

corneal hysteresis is a measure of corneal dampening caused by viscoelastic properties of the cornea while the corneal resistance factor is dominated by elastic properties of the cornea and may represent the overall resistance of the cornea (Luce, 2005).

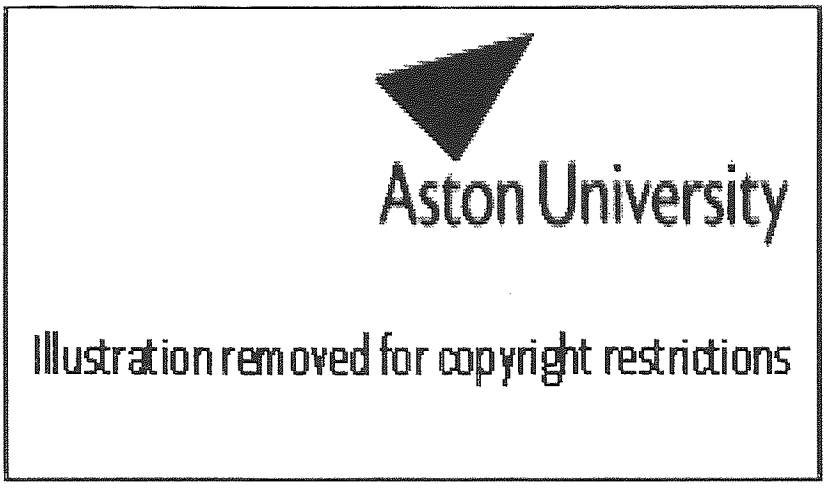


Figure 2.3 Ocular Response Analyser process signals showing the derivation of corneal hysteresis (The article from which this figure was reproduced was published in *Journal of Cataract and Refractive Surgery,* **31**, Luce, D.A. Determining in vivo biomechanical properties of the cornea with an ocular response analyzer, p156-162, Copyright Elsevier, 2005).

In a recent study the relationship between corneal thickness, corneal hysteresis and corneal resistance factor as measured by the Ocular Response Analyzer are investigated. The study concludes that although corneal hysteresis and corneal resistance factor increased with corneal thickness, the association was moderate (corneal hysteresis and corneal thickness: $r=0.426$, $p<0.001$; corneal resistance factor and corneal thickness: $r=0.467$, $p<0.001$). It is therefore postulated that although corneal thickness, hysteresis and resistance factor are related they may actually indicate different biomechanical aspects of ocular rigidity (Shah *et al.,* 2006).

Nidek NT – 4000 non-contact tonometer

The major disadvantage of NCTs is that it samples the IOP within a very short period of time of approximately 1 to 3 ms (Forbes *et al.,* 1974). Although the patient may find the short sampling time comfortable, the IOP reading is affected by the cardiac and respiratory cycles (see **section 2.3.2**). The measure is random with respect to the phase of the cardiac cycle and therefore the spread in successive IOP readings using NCT is typically 4 mmHg (Forbes *et al.,* 1974; Piltz *et al.,* 1985). To account for the variation in IOP measures caused by short-

term fluctuations in IOP, manufacturers of NCTs recommend that an average of 3 or 4 readings are taken to increase the precision of the IOP measure and this has been validated by McCaghrey and Matthews (2001).

The only commercially and currently available NCT which allows pulse synchronous measures is the *Nidek NT-4000* (Nidek Co., Ltd., Japan) which monitors the cardiac cycle from the subjects forehead. Reflected light from a light emitting diode (LED) built into the forehead rest inversely changes with changes in blood flow volume in surface forehead tissue with each heart beat. These fluctuations in reflected light are analysed and when 3 consistent pulse signals are detected the instrument identifies the peak, middle and trough of the cardiac cycle. A pulse synchronous signal is then transmitted and an IOP measure is subsequently taken and recorded (Yaoeda *et al.*, 2005). The manufacturers claim that variance in IOP measures is hence reduced with the *Nidek NT-4000* and this has subsequently been validated by Lam *et al.* (2004), Yaoeda *et al.* (2005) and Queiros *et al.* (2006). The *Nidek NT-4000* is discussed in more detail in **Chapter 5**.

2.5 Accommodation and myopia

The increasing worldwide prevalence of myopia indicates that this is rapidly becoming an epidemic. In the western world (e.g., in Europe and in the United States of America), the prevalence of juvenile onset myopia is approaching 25 %, whereas in the eastern world (i.e., East Asia) the prevalence is much higher at between 60 and 80% (Saw, 2003).

Many factors have been investigated in the development of myopia for example, genetic predisposition, diet, axial length-to-corneal radius ratio, accommodative convergence to accommodation ratio, refractive error at 6 years of age, near work, education or cognitive demand and lighting levels (reviewed by Gilmartin, 2004).

Pertinent to this thesis is the strong emphasis on near work as a precursor to myopia onset and progression. In 1604, Kepler was first to note the association between near work and myopia development (cited by Duke-Elder, 1938). In the 18th century, Dr. M. Tscherning was first to provide data on occupation and myopia prevalence in 7000 Danish subjects. The results of his study are shown in **Table 2.1,** in which the influence of near work on myopia is highlighted.

Occupation	Prevalence of myopia
Students	32
Office and trade workers	16
Artists	13
Tailors/shoe makers	12
Workmen	5
Farmers	2

Table 2.1 Tscherning's data on prevalence of myopia by occupation (n=7000) (compiled from Tscherning, 1924).

Many subsequent studies also demonstrated that the prevalence of myopia was much higher in occupations which required intense near work (Kent, 1963; Goldschmidt, 1968; Diamond, 1957; Provines *et al.*, 1983; Parssinen, 1987; Adams and McBrien, 1992; Midelfart *et al.*, 1992; McBrien and Adams, 1997; Wensor, McCarty and Taylor, 1999; Wu and Edwards, 1999; Shimizu *et al.*, 2003). For example, Adams and McBrien (1992) monitored the refractive status of 251 clinical microscopists. Their study concluded that the prevalence of myopia amongst this group was 71 %. Furthermore, Adams and McBrien (1992) showed that 66 % of the eyes were myopic at the beginning of their longitudinal study. After 2 years, 39 % of the emmetropic eyes became myopic and 48 % of initially myopic eyes progressed further in myopia. A recent study has concluded that the high prevalence of myopia (reported as 73.9%) was positively associated with the amount of reading and writing performed (as a measure of near work) in 946 Singaporean children aged between 5 and 19 years of age (Quek *et al.*, 2004).

The two primary oculomotor responses to near work are accommodation and convergence. Elimination of accommodation using atropine (Shih *et al.*, 2001; Chua *et al.*, 2006) has shown the retardation of myopia progression in children. Shih *et al.* (2001) measured the myopic progression in 188 children aged between 6 and 13 years of age, who were randomly assigned to 3 treatments groups. Their data demonstrated that myopic progression was significantly less (-0.42±0.07D) in Taiwanese children who wore progressive addition lenses and underwent topical instillation of 0.5% atropine, compared to the myopic progression in children who wore single vision lenses only (-1.40±0.09D) and progressive addition lenses only (-1.19±0.07D).

It has however, been noted that the effect of the cycloplegic drug: atropine on myopic progression may be due to a non-accommodative route (McBrien, Moghaddam and Reeder, 1993). McBrien, Moghaddam and Reeder (1993) reported that atropine blocked myopic progression in the chick in which the transmission between the ciliary ganglion and ciliary muscle is via nicotinic receptors. Furthermore, Tan *et al.* (2005) concluded that myopic progression was reduced by approximately 50 % over a 12 month period in 141 Asian children (aged between 6 and 12 years) following topical instillation of 2 % pirenzepine (M1 selective receptor antagonist) twice a day compared to a non-treatment group of 71 children. Similar results were found by Siatkowski *et al.* (2004) in a study based in the United States of America. The myopic progression after 24 months in 53 children aged between 8 and 12 years who were treated to 2 % pirenzepine (twice daily) was 0.58±0.53D. The myopic progression noted was significantly less than that recorded for the non-treatment group of 31 children who progressed by 0.99±0.68 D over the 2 year period. Clarification of the site of action of these muscarinic agonists and their subsequent adverse reactions requires further work.

Animal studies have shown that induced hyperopic blur (using high negative lenses) triggers a compensatory mechanism to achieve focus such that the retina is displaced backwards by a thinning of the choroid (Wallman *et al.*, 1995; Wildsoet and Wallman, 1995). The subsequent remodelling of the sclera at the posterior pole results in myopia (Nickla, Wildsoet and Wallman, 1997; Gentle and McBrien, 1999). Conversely, induced myopic defocus (using high positive lenses) causes the retina to be pushed forward to the image plane by choroidal expansion (Wildsoet and Wallman, 1995; Hung and Ciuffreda, 2000). This mechanism which compensates for both hyperopic and myopic defocus was first hypothesised approximately 60 years ago by Walls (Walls, 1942). It is thought that determining the mechanisms underlying form deprivation myopia in animals may reveal the means for preventing myopia development in humans.

A plethora of research supports (including the results shown in **Appendix 8**) the established view that myopes have less accurate accommodative systems than the emmetropes (McBrien and Millodot, 1986a, b; Ebenholtz and Zander, 1987; Rosenfield and Gilmartin, 1988; Tokoro, 1988; Bullimore, Gilmartin and Royston, 1992; Gwiazda *et al.*, 1993; Jiang 1994 and 1995; Rosenfield, 1998; Nakatsuka *et al.*, 2005). Furthermore, Abbot, Schmid and Strang (1998) concluded that the accommodative responses were reduced in progressing myopes

compared to stable myopes and emmetropes. It is thought that myopes have reduced blur sensitivity, which decreases the error signal (i.e., retinal blur) to the accommodation system and results in impaired accommodative responses (Rosenfield and Abraham-Cohen, 1999; Seidel, Gray and Heron, 2003). Under-accommodation during near work results in the focal plane being behind the retina causing hyperopic blur analogous to that caused by negative lenses in animals. With reference to animal work it was hypothesised by Hung and Ciuffreda (1991) that the sustained hyperopic blur during near work triggers axial elongation in a mechanism similar to that described in animals.

A more accessible method (compared to that possible using pharmacological agents) by which accommodation and hyperopic blur can be eliminated during near work is by using positive powered spectacle lenses. Several studies have investigated the use of this optical method in the retardation of myopia progression (Fulk and Cyert, 1996; Fulk, Cyert and Parker, 2000; Shih *et al.*, 2001; Edwards *et al.*, 2002; Gwiazda *et al.*, 2003; Gwiazda, Thorn and Held, 2005). However, the results of these studies have yielded equivocal results. Gwiazda, Thorn and Held (2005) reported that elimination of the accommodative inaccuracies (i.e. accommodative lag) with progressive addition lenses in 469 children for 3 years impeded myopic progression by only approximately 0.20D (Gwiazda, Thorn and Held, 2005). In addition, under correction of myopia (inducing myopic blur) by 0.75D enhanced rather than inhibited myopia development in 47 children of Malay and Chinese origin (Chung, Mohidin and O'Leary, 2002). Moreover, Mutti *et al.* (2006) more recently has shown that an increase in the accommodative lag does not precede myopic onset and therefore can only be considered as a consequence rather than a cause of myopia development. Given the above evidence, it is thought that the response of the human eye to hyperopic (during accommodation) and myopic (during under-correction) blur is some what different to the response of animal eyes to hyperopic and myopic blur induced by negative and positive lenses.

2.5.1 Accommodation, IOP and myopia development

An alternative and probably less complex hypothesis is that increased intra-ocular forces during accommodation induce axial elongation. Evidence supporting this notion comes from studies which have shown that the axial length increases, albeit only slightly, with accommodation (Shum *et al.*, 1993; Drexler *et al.*, 1998; Mallen, Kashap and Hampson, 2006). This axial elongation has been thought to be a consequence of ciliary muscle

contraction exerting a pulling force on the choroid and sclera adjacent to the ciliary body, which displaces the posterior portion of the globe posteriorly and thus, increases the axial length (Drexler *et al.*, 1998; Mallen, Kashap and Hampson, 2006). Indeed, prolonged periods of axial elongation during sustained accommodation may lead to scleral remodelling (Nickla, Wildsoet and Wallman, 1997; Gentle and McBrien, 1999) and hence myopia. However, the observed increase in axial length during accommodation is questionable since changes in lenticular refractive index during accommodation may effect the measurements of axial length using instruments based on partial coherent interferometry (i.e. Zeiss IOL Master) (Atchison and Smith, 2004).

An interesting hypothesis is that IOP may increase with accommodation which is analogous to an increase in intra-ocular forces. Heine (1899) demonstrated that the posterior globe was particularly expanded in a myopic eye. Since then it has been well-established that the principle structural correlate of myopia is increased axial length (Gilmartin, 2004). In addition, recent magnetic resonance imaging work has shown that the posterior 25 % of the globe is stretched in a myopic eye (personal communication with Professor Bernard Gilmartin). An early proposal by Greene (1980) was that the posterior half of the globe may be either less resistant to forces exerted and therefore mechanically weaker, or the forces exerted are highly concentrated at the posterior globe. Coleman's theory on accommodation (see **section 1.3.5**) which assigned a role to the vitreous body during accommodation is important in this regard. Coleman's theory suggested that on contraction of the ciliary muscle during accommodation, the choroid and hence vitreous moves forward. The consequence is that the vitreous pushes up against the posterior surface of the crystalline lens which combined with the forward shift of the zonules results in a shape change of the crystalline lens. Later work by Fisher (1983) showed normal accommodative ability in a subject who had undergone a vitrectomy. It is clear that the vitreous may not play an important role in the accommodative mechanism although it may still move anteriorly during accommodation as proposed by Coleman.

Based on Coleman's hypotheses that the vitreous moves forward during accommodation, it can be deduced that the pressure in the posterior globe increases during accommodation which may possibly lead to the development of myopia. Kelly (1981) proposed that the anterior movement of the vitreous closed the zonular gaps and therefore interfered with aqueous outflow leading to an increase in IOP and hence myopia. Kelly (1981) therefore

concluded that the mechanism of myopia development was similar to that of glaucoma in that it was a result of increased IOP, and hence referred to myopia as 'juvenile expansile glaucoma'.

Young proposed a biphasic model of myopia development (Young, 1977; 1981). He postulated that sustained near work altered the tonus of the ciliary muscle, which was unable to relax post-task. Prolonged periods of over-accommodation meant that the increased vitreous pressure caused stress-induced changes to the sclera and subsequently led to myopia. In contrast van Alphen (1961) hypothesised that the increased vitreous pressure during accommodation reduced the pressure in the suprachoroidal space, so that the increased vitreous pressure could not be transmitted to the sclera. Van Alphen hence believed that the increased vitreous pressure did not induce stress-related scleral expansion (van Alphen 1961). Furthermore, subsequent work by van Alphen showed that on inflation of the eye (with saline), choroidal expansion occurred only in the regions at which the sclera had been denuded (van Alphen, 1986).

Experimental evidence supporting the above theories was provided by animal studies. Suzuki (1973) conducted a radiographic study and reported an increase in vitreous pressure during accommodation in cats. Simultaneous manometry of the anterior and posterior chambers in animals during electrically stimulated accommodation revealed a reciprocal relationship between the anterior and posterior chambers. The pressure was reportedly lower in the anterior chamber and higher in the posterior chamber during accommodation (Coleman, 1970; Coleman and Young, 1972). In 1975, Young also revealed the presence of this pressure gradient in primate eyes during accommodation. He noted a positive linear relationship between accommodative demand and the pressure of the vitreous chamber, such that the vitreous chamber pressure increased by 1 mmHg per dioptre of accommodation stimuli (cited by Young, 1981).

Furthermore, Greene (1980; 1991) has reported that on extraocular muscle contraction during near work (i.e., convergence), the recti wrap around the globe exerting pressure and hence leading to globe distension. It has also been proposed that the increased pressure exerted by the extraocular muscles is greater in the myopic eye since the distance between the posterior pole and centre of rotation is greater in the myopes (Luedde, 1932). Greene (1980) stated that the contraction of the oblique muscles exerted relatively more force on the posterior globe, as

they insert the globe posteriorly. The effect of oblique muscle contractions coupled with the increase in vitreous pressure during near work was thought sufficient enough to distort the sclera (Greene and McMahon, 1979). Later Greene (1991) reported that the force exerted by medial rectus contraction (150 g) was approximately 400 times greater than the force exerted by ciliary muscle contraction (0.6 g). From Green's work it is concluded that the mechanical effects of convergence on the posterior globe are greater than those of accommodation. More recent work, however, suggests that the extraocular forces are considerably less (40 g) and the force exerted by the posterior attachment is a modest 4 g (Ong and Ciuffreda, 1997).

2.5.2 Accommodation, choroidal blood flow and myopia.

Since the above theory suggests that blur from form-deprivation triggers choroidal changes several workers have hence investigated choroidal blood flow changes during form-deprivation. Reiner and colleagues (Reiner, Shih and Fitzgerald, 1995) have concluded that the reduction in blood flow observed in myopes was a consequence rather than a cause of myopia development. Two mechanisms were proposed: 1) choroidal thinning and hence reduced blood flow accompany axial elongation and 2) the reduction in choroidal blood flow was a result of a neurally-mediated adaptive response due to a reduced need for high choroidal blood flow owing to the thinner choroid. Reductions in choroidal blood flow induced by transecting choroidal nerves, resulted in a reduction in axial elongation. Conversely, pharmacologically increased choroidal blood flow enhanced eye growth. The work by Reiner and co-workers shows that axial elongation only occurs during form-deprivation with an occluder or by corneal scarring. The authors hence concluded that myopia development was associated with form-deprivation rather than reductions in choroidal blood flow (Reiner, Shih and Fitzgerald, 1995).

In addition to the notion that accommodation increased the vitreous pressure, Young also proposed that increased tension on the choroid during accommodation would lead to a reduction in blood supply to the choroid and retina causing these structures to thin and stretch (Young, 1981). Indirect evidence supporting this hypothesis is evident in recent work by Fitzgerald and colleagues, who belong to the same research group as Reiner. Fitzgerald, Wildsoet and Reiner (2002) have shown that when form deprivation was induced by diffusing goggles in the chick eye, a reduction in choroidal blood flow preceded the thinning of the choroid. Indeed, during the recovery phase the increase in choroidal blood flow was more rapid and transient in onset than the increase in choroidal thickness. This may indicate a

vascular aetiology of myopia development. Despite Young's early hypothesis and the dynamic features of choroidal flow and thickness described by Fitzgerald and co-workers, the effects of accommodation on choroidal blood flow are unknown.

2.6 Research aims and objectives

In light of the above literature review, it is thought imperative to establish the relationship between accommodation, IOP and choroidal blood flow. The results obtained from investigating the effects of accommodation and IOP may add weight to the Helmholtz theory of accommodation which suggests that on accommodation the ciliary muscle moves forwards and inwards, the ciliary muscle collar diameter reduces and consequently releases the tension of the anterior fibres. Therefore it is hypothesised that these processes lead to the increased potency of the trabecular meshwork to increase aqueous outflow and hence decrease IOP. Alternatively an increase in IOP during accommodation may add support to Coleman's (1970) proposition that the vitreous body becomes compressed and pushed anteriorly during accommodation. Indeed an increase in IOP during accommodation may be important in our understanding of near work and myopia development, since many early studies have postulated that increased intra-ocular forces during accommodation cause stress-induced scleral expansion and hence myopia (Coleman, 1970; Young, 1977; Greene, 1980; Kelly, 1981; Young, 1981; Greene, 1991).

The nature of the relationship between accommodation and IOP has not been fully addressed as a research question for over 20 years. Mauger's study in 1984 concluded that the IOP reduced by approximately 1.3 and 2.3 mmHg after 3 minutes of sustained fixation of a 1.50 and 4D accommodative stimulus, respectively (Mauger, Likens and Applebaum, 1984). Mauger's work is however subject to several limitations with regard to the study design the most important limitation being the use of the Goldmann contact tonometer (GCT) to measure IOP. Whereas non-contact tonometers have been available since 1972, the large bulky designs of these instruments restricted their use in the investigation of accommodation and IOP. Although the GCT is considered the 'gold-standard' of tonometry, a number of limitations of the technique exist (Whitacre and Stein, 1993). Pertinent to this study are the effects of multiple applanations and the effects of topical anaesthetics on the steady-state IOP. In the present study it is postulated that the changes in IOP with accommodation noted by Mauger and colleagues may have been affected by some degree of 'ocular massage' caused by the contact tonometer probe. Furthermore, the effects of topical anaesthetics on IOP are

equivocal. Whereas Baudouin and Gastaud (1994) reported a reduction in IOP after the instillation of topical anaesthetics, several studies have also concluded that the corneal thickness increases with topical anaesthetics which may lead to a subsequent increase in the IOP measurement (Herse and Siu, 1992; Asensio *et al.*, 2003; Nam *et al.*, 2006). In addition disruption of the tear fluid layer with topical anaesthetics and fluoroscein may lead to erroneous IOP measures, since the fluorescent tear film meniscus is crucial in governing the accuracy of an IOP measure with the GCT (Whitacre and Stein, 1993). Therefore, in this study it is speculated that Mauger's results may be confounded by the putative effects of topical anaesthetics on the steady-state IOP.

A second substantive drawback of Mauger's study is that the measurements of accommodation responses were not incorporated into the study design. Significant within- and between subject variations in the lead and lag of accommodation can occur which approach I D (Ciuffreda, 1998). This study considers that variations in accommodation responses are likely to have subsequently influenced the IOP measures in Mauger's study and hence the conclusions drawn by Mauger and co-workers on the relationship between accommodation and IOP are questionable.

Therefore, this thesis sets out to address the aforementioned limitations of Mauger's study and hence systematically map the relationship between accommodation and IOP. Much of the research uses a non-contact tonometer (NCT) of a high resolution (which is of an order of magnitude greater than other available methods of tonometry) which hence eliminates the need for corneal anaesthesia. The short sampling time of NCTs [approximately 1-3 ms (Forbes, Pico and Grolman, 1974)] means that repeated measures do not progressively reduce the IOP (Grolman, 1972; Forbes *et al.*, 1974; Sorensen, 1975; Myers and Scott, 1975; Chauhan and Henson, 1988; Baudouin and Gastaud, 1994; Lawson-Kopp *et al.*, 2002) in a manner similar to that observed with the GCT. However, the relatively instantaneous IOP measures obtained with NCTs are influenced by inherent short-term variations in the steady-state IOP caused by the respiratory and cardiac cycles (Leydhecker, 1976). The variance in successive non-contact IOP measures has been noted as approximately 4 mmHg (Forbes, Pico and Grolman, 1974; Piltz *et al.*, 1985; Vernon, 1993). Therefore, this thesis will develop a method by which non-contact pulse synchronised IOP measurements can be obtained. A detailed account of the modifications to the apparatus used is given in this thesis. Development of such a method is not only imperative in answering the central research

question in this thesis but will also be of value in other research paradigms and in clinical optometric practice. The research also incorporates the measurement of accommodation responses during the IOP measurement period, which takes advantage of new instrumentation for the quasi-continuous measurements of accommodation.

Furthermore, according to Coleman's (1970) theory on accommodation, Young (1981) speculated that the increased pressure in the vitreous chamber during accommodation would lead to a reduction in blood supply to the choroid. The effects of accommodation on choroidal blood flow are unknown and therefore this study sets out to investigate the effects of accommodation on choroidal blood flow. Reduced choroidal blood flow has been implemented in myopia onset and progression (Fitzgerald *et al.*, 2002). Indeed, if the choroidal blood flow reduces with accommodation, then this may assist in explaining the well documented association between near work and myopia (Rosenfield and Gilmartin, 1998).

It is clear that clarification of the relationship between accommodation, IOP and choroidal blood flow is not only of inherent research interest, but it is more so of importance in understanding the physiology of the human eye during near work, which is a vital part of modern lifestyle.

The onset and development of myopia has over the last three decades attracted substantial interest in clinical vision science. The pursuit of an understanding of the aetiology of myopia is principally driven by a large number of worldwide epidemiological studies from which it has been estimated that approximately 1/6 of the world's population is myopic (Morgan and Rose, 2005). A plethora of research exists on the onset and progression of myopia, and the numerous myopigenic factors identified have been reviewed by Gilmartin (2004). Of particular interest in this thesis is the strong emphasis on near work as a precursor to myopia onset and development. Despite the large amount of work that has associated myopia with near work, the intricacy of the near vision complex coupled with related hereditary and environmental factors hinder our understanding of the causative role of near work (Rosenfield and Gilmartin, 1998). Therefore, a fuller appreciation of the relationship between accommodation and IOP and choroidal blood flow will assist in our understanding of the association between near work and myopia onset and progression.

CHAPTER 3

OPERATIONAL PRINCIPLES OF THE PULSAIR NON-CONTACT TONOMETER

3.1 Introduction

The Goldmann contact tonometer (GCT) is considered 'gold standard' for the measurement of IOP, a fundamental clinical parameter of ocular health. However, there are numerous sources of error that influence the IOP values when measured with the GCT (see **Chapter 2**). In relation to the main aim of this thesis, the use of anaesthetics with the GCT and the affect of repeated applanation tonometry are the two principal factors which influence the IOP and consequently limit the validity of the results of previous studies on accommodation and IOP (Armaly and Burian, 1958; Armaly and Rubin, 1961; Mauger, Likens and Applebaum, 1984).

A study by Baudouin and Gastaud (1994) demonstrated a small yet significant reduction in IOP after the instillation of topical anaesthetics. The study reported that the IOP (as measured by a non-contact tonometer) reduced 1 minute after the instillation of 0.4 % oxybuprocaine (aka benoxinate) by approximately 1 mmHg and this reduction remained stable for at least 15 minutes. Of importance is that the study by Baudouin and Gastaud (1994) reports that in some subjects the IOP reduced by 6 and even 8 mmHg after the instillation of the anaesthetic. The authors hypothesised that, whereas more precise investigations were required, the reduction in IOP may be due to a direct facilitating effect of topical anaesthetics on the aqueous humour outflow.

Some studies suggest that topical anaesthetic agents may cause corneal oedema of the epithelium as a result of alterations to the Na^+/K^+ endothelium pump and subsequent increases in osmotic pressure and hydration of the stroma (Herse and Siu, 1992; Asensio *et al.*, 2003). An increase of approximately 2.9% in central corneal thickness, 1 to 2 minutes after the instillation of 2 drops of 0.5% proparacaine (aka proxymetacaine) has been reported by Herse and Siu (1992). A more recent study has shown that variations in paracentral corneal thicknesses of more than 10 μm can occur in some individuals after anaesthetic eye drops (Asensio *et al.*, 2003). Nam *et al.* (2006) has demonstrated that on instillation of 0.4 % oxybuprocaine and 0.5% proparacaine, central corneal thickness may increase by 7.7 and 8.6

μm, respectively and then returns back to baseline levels after 80 seconds. Furthermore, the authors showed that a second transient increase in central corneal thickness occurred 5 minutes after the instillation of 0.5% proparacaine.

A study by Birchall and Kumar has also observed that use of the topical anaesthetic: 0.4% lidocaine (aka lignocaine) with 0.25% fluorescein, increases the reflex lacrimation and blepharospasm by a greater extent than when 0.5% proxymetacaine with 0.25% fluorescein is used as the anaesthetic agent (Birchall and Kumar, 2001). Since the thickness and reflectance of the resultant fluorescent tear film meniscus is imperative in governing the accuracy of an IOP measure with the GCT (Whitacre and Stein, 1993) it is reasonable to presume that disruption to the tear fluid layer on instillation of anaesthetics can significantly compromise the accuracy of an IOP measurement. To date, although the precise mechanism/s underlying the effect of topical anaesthetics on IOP remains undetermined, the observed changes to the IOP may lead to significant under- or over-estimations of IOP in a clinical and research scenario.

In 1978 Pagenstecher first observed that ocular massage significantly reduced IOP (cited by Stocker, 1956). When acquiring an IOP measure with the GCT, a varying degree of force needs to be applied to the cornea to attain a predetermined amount of corneal applanation (see **Chapter 2**). Repeat corneal applanations with the GCT have been shown to reduce IOP by 3 to 4 mmHg (Moses, 1961; Moses and Liu, 1968). This observation is most likely to be a consequence of aqueous massage from the force of the tonometer which is supported by the finding that GCT does not decrease the corneal thickness readings which may indeed affect the accuracy of the IOP measure (Huang *et al.*, 2005). However, Stocker (1956) noted that application of a tonometer on one eye also reduced the IOP in the contralateral eye during contact tonometry and therefore postulated that aqueous formation reduced bilaterally as a reflex mechanism to the procedure of tonometry. Stocker (1956) also hypothesised that globe deformation during applanation tonometry itself may lead to the obstruction of the aqueous outflow channels resulting in a decrease in aqueous formation as a reflex mechanism.

Therefore, due to the limitations of the GCT discussed above, throughout this thesis a non-contact tonometer (NCT) was used to measure the IOP during accommodation. As the name implies, NCTs do not require corneal contact and therefore negate the need for corneal anaesthesia and hence any errors in the IOP value that may occur as a result of using topical

anaesthetics are eliminated (Herse and Siu, 1992; Moseley *et al.*, 1993; Baudouin and Gastaud, 1994; Birchall and Kumar, 2001; Asensio *et al.*, 2003; Nam *et al.*, 2006). In contrast to the GCT, numerous studies have demonstrated that successive IOP measures with a NCT do not progressively lower the IOP (Grolman, 1972; Forbes *et al.*, 1974; Sorensen, 1975; Myers and Scott, 1975; Chauhan and Henson, 1988; Baudouin and Gastaud, 1994; Lawson-Kopp *et al.*, 2002). Similar to the GCT, NCT's also exploit the applanation principle (based on the Imbert-Fick law, see **section 2.4.2**). The original NCT was designed and patented in 1971 by Bernard Grolman following 10 years of empirical and numerous designs (Grolman, 1972). Briefly, the corneal distortion produced by a controlled air-pulse is monitored and the point of corneal applanation is identified. The time interval required to reach applanation is proportional to the air-pulse force. Calibration factors calculated from clinical trials (comparing IOP measures taken with the GCT and the NCT) are subsequently applied to the IOP measures obtained with NCT to produce measures which are comparable to the gold standard (Grolman, 1972; Forbes *et al.*, 1974).

To investigate the effect of accommodation on IOP more precisely a relatively small NCT was required which would allow unhindered IOP measures in one eye while simultaneously stimulating accommodation in the contralateral eye. The *EasyEye Puslair* NCT (Keeler, UK) was particularly suitable for the study design in mind. The original *Pulsair* was designed by John Fisher in 1988. Revised computer algorithms were applied to improve the instrument and the *Pulsair 2000* and *3000* were launched in 1991 and 1998, respectively. In this chapter the operational principles including the derivation of an IOP measure by the *EasyEye Puslair* NCT are discussed.

3.2 Measurement procedures

The *EasyEye Puslair* NCT which is shown in **Figure 3.1** consists of a base unit comprising the mechanics of the instrument and a handset with which the IOP readings are obtained. The base unit dimensions are 355x305x205 mm (height x width x depth) and weighs 4 kg, whereas the handset is only 265x115x40 mm (height x width x depth) and weighs just 0.9 kg. As shown in the subsequent chapters of this thesis, the relatively small dimensions and light weight of the instrument is particularly advantageous in investigating the relationship between accommodation and IOP.

When the handset is held approximately 25 cm away from the eye the cornea can be viewed through the eyepiece with two green alignment guidance light emitting diodes (LEDs). As the distance to the eye is progressively reduced a central black alignment bar and two red bars are seen. Approximately 15 mm from the corneal plane, a diagonal bow-tie image appears. When this image is in focus and the black alignment bar is at the centre of the bow-tie image the IOP reading is taken automatically. The manufacturers recommend that the mean of 4 measures is used as the actual IOP value to ensure good accuracy compared to measures taken with the GCT. Several studies have confirmed that the acquisition of 4 IOP measures is a reasonable compromise between accuracy and efficiency (Vernon, 1995; McCaghrey and Matthews, 2001; Parker, Herrtage and Sarkies, 2001; Lawson-Kopp *et al.*, 2002).

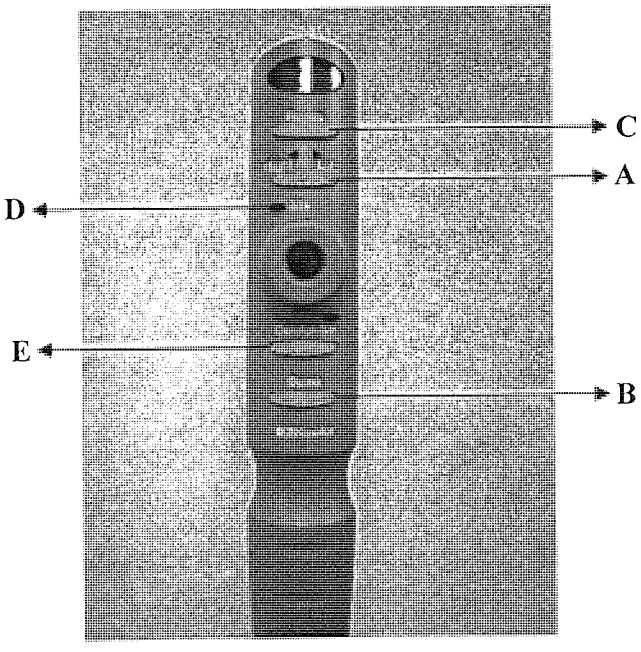


Figure 3.1 The *EasyEye Puslair* NCT where features **A**=RE/LE; **B**=Demo mode; **C**=Review mode; **D**= +30 mode and **E**=Quickpulse mode.

The various features on the handset of the NCT shown in **Figure 3.1** are discussed:

Feature A

Allows the eye under examination to be selected.

Feature B

The calibration of the instrument is confirmed by the 'demo' button on the handset. When pressed once the digital display should read 30 mmHg and when pressed for the second time the display should read 50 mmHg. Deviation from these values indicates a need for recalibration which is done by the manufacturers using the serial connector on the side of the mother unit. There is a tendency for the *Puslair* NCT to be predisposed to a drift in long-term accuracy and therefore requires regular calibration (Atkinson *et al.*, 1992).

Feature C

The digital display shows the running average of the IOP readings. The 'review' button on the handset allows the individual readings to be viewed. When 4 IOP readings are obtained, the average value 'flashes' on the handset and an automatic printout is available showing the average IOP and number of readings taken. The automatic printer facility can be switched off at the mother unit.

Feature D

In the primary mode the instrument takes IOP measures between 7 and 30 mmHg. The '+30' mode is automatically engaged if an IOP above 30 mmHg is detected and the strength of the air pulse is increased commensurately. In the '+30' mode the instrument takes IOP readings between 30 and 50 mmHg.

Feature E

The instrument relies on a clear reflection off the corneal surface. If the cornea is compromised in, for example, dry eye, corneal distortion or scarring, an IOP reading may not be possible. Under these conditions the 'quickpulse' feature of the instrument can be used in which the sensitivity of the instrument to a clear corneal reflection is reduced (see **section 3.4**).

For the purpose of this thesis in collaboration with the manufacturers, the instrument was connected to a personal computer via the serial port on the mother unit. A recording and

analysis program written by Keeler (UK) was used to save the IOP measures on an external medium i.e. a floppy drive. The data was saved in the following format:

XXDDEN.DAT **Format 1**

where *XX* denotes the subjects initials, *DD* refers to the date of data collection, *E* represents the eye tested and finally *N* indicates the number of measurements taken.

A second programme (also written by Keeler, UK) in a Microsoft *Excel* spreadsheet referred to as the 'Puff-Retrieve' programme used the data saved on the floppy drive and displayed the principle components of an IOP measure described in **section 3.4**. However, in order for the Puff-Retrieve program to recognise the data file **Format 1** was changed to **Format 2**:

Puff*N* **Format 2**

where similar to **Format 1**, *N* here represents the number of measures taken. On activation of a macro in the Puff-Retrieve program, the pressure-response relationship was displayed (see **Figure 3.3**) and the IOP calculated to a resolution of 0.01 mmHg which is an order of magnitude greater than all other methods of tonometry. Note however, that on the NCT handset, the IOP is only displayed as an integer as a higher resolution of the IOP measurement is of no relevance in clinical practice.

3.3 Optical design

The pneumatic system consists of a solenoid valve which when energized results in the piston accelerating at a controlled rate, thereby pressurizing a plenum. The positive plenum pressure is transmitted in the direction of the patient's cornea (Grolman, 1972). **Figure 3.2** shows the optical design of the *Pulsair* NCT. Light from a bulb (LB) is collimated by a collimating lens (C). This collimated light is reflected off a beam splitter (BS_1), and then passes through 2 objective lenses (OL_1 and OL_2) and is focused on the patient's cornea (Px). The reflected light from the cornea is split by a second beam splitter (BS_2) and is subsequently received by 3 photodiodes (PD_1, PD_2, and PD_3). The remaining reflected light from the patient's cornea passes through the 2 beam splitters (BS_1 and BS_2) and lenses L_1 and L_2 and is viewed by the operator (Ox).

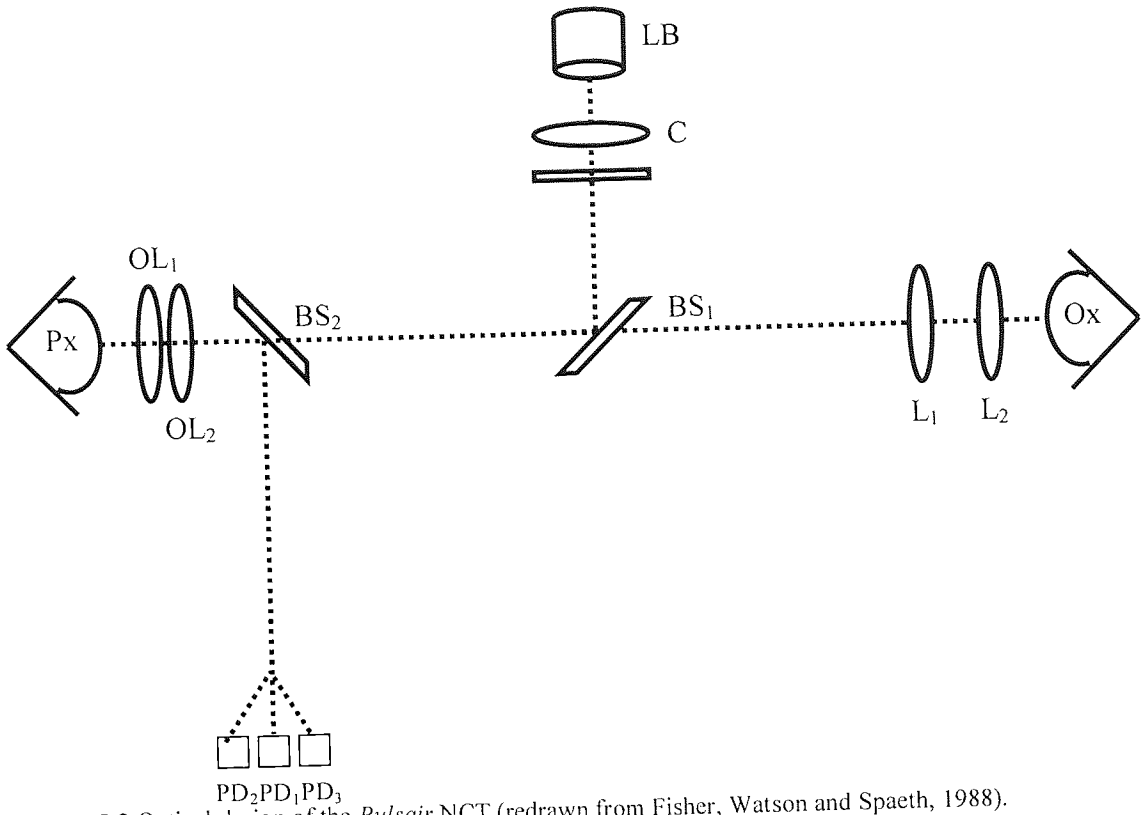


Figure 3.2 Optical design of the *Pulsair* NCT (redrawn from Fisher, Watson and Spaeth, 1988).

3.4 Computation of an IOP measure

Contrast values and K-Factor

A mask in the objective lenses (OL_1 and OL_2) of 2 red bars is reflected off the cornea, the image of which is received by the photodiodes (PD_1, PD_2, and PD_3). A microprocessor monitors the signals received (of the mask image) by each of the photodiodes and computes the contrast values (CV) using **Equation 3.1**. Using the formulae in **Equation 3.2**, the k-factor is computed. **Figure 3.3** shows the relationship between the k-factor and the air pressure output.

$$CV = \frac{PD_1}{\text{Mean} \sum PD_2PD_3} \qquad \textbf{Equation 3.1}$$

$$\text{k-factor} = \frac{CV}{32} \qquad \textbf{Equation 3.2}$$

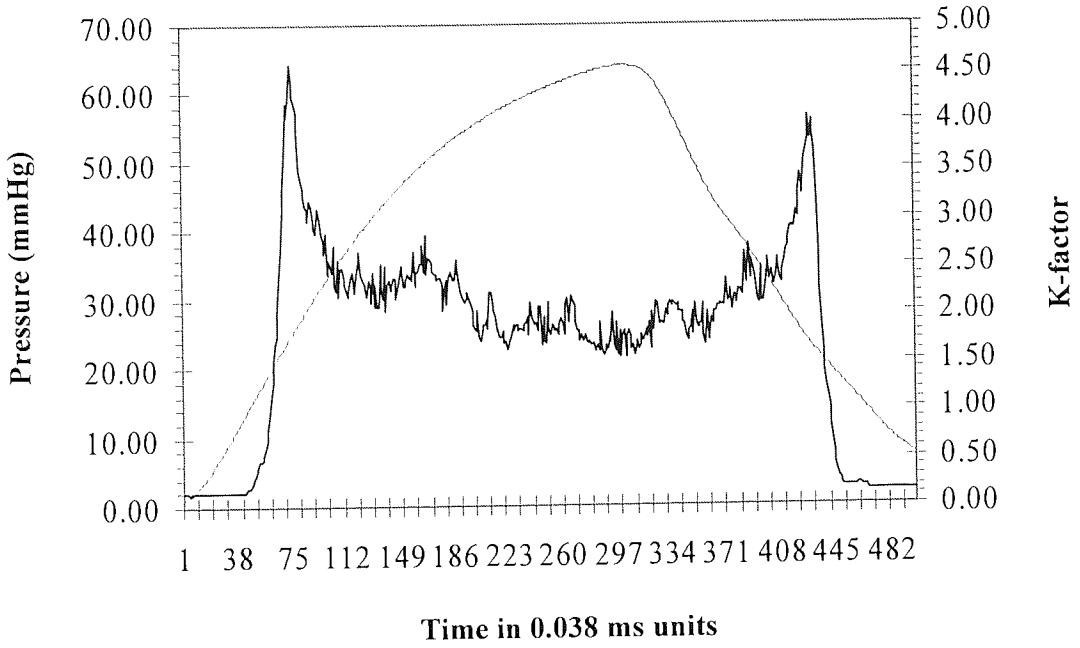


Figure 3.3 Graphical representation of the pressure-response relationship where the grey line represents the pressure output and the black solid line represents the k-factor.

Air pulse criteria

The air pulse is only triggered when the instrument is correctly aligned in the spatial and temporal domains. Correct alignment of the instrument is identified by the microprocessors when the following 4 criteria are satisfied:

1) The two bright areas of the mask image are aligned with the 2 outer photodiodes (PD_2 and PD_3 in **Figure 3.2**) ensuring correct alignment in the horizontal meridian.
2) The light intensity of the mask image on each of the outer photodiodes (PD_2 and PD_3 in **Figure 3.2**) is greater than an arbitrary reference value. This ensures correct alignment in the vertical meridian.
3) The mask image must be in focus on the photodiodes and this is dependent on the distance of the tonometer from the cornea. When the image is in focus on the photodiodes, which is achieved at approximately 15 mm from the corneal plane the observer simultaneously observes a focused image of the mask through the eye piece.
4) All alignment conditions mention in 1-3 above are obtained simultaneously and maintained for 3 to 5 ms.

Criteria 1 to 3 above determines the light intensity (i.e. CV) on the photodiodes and are used to calculate the initial k-factor referred to as k-start. Since there is inevitable human error in judging and maintaining a steady focused image, the criteria is set so that k-start is within a reference range of 0.00 to 0.35 (maximum value is an arbitrary value).

The arbitrary reference values used in the criteria are constant for all Pulsair *EasyEye* NCTs and were determined in clinical trials (by the manufacturers) to permit the measurement of IOPs over a range of eyes. The reference values also ensure that unreliable readings are not possible in a misaligned eye and/or in an eye with corneal shape and/or tear film characteristics outside a pre-determined range. If the above criteria are not met, for example in an excessively dry eye in which the reduced tear quality or quantity effects the amount of light reflected off the cornea, the 'quickpulse' feature can be utilised in which the sensitivity of the instrument to the above criteria is reduced.

Air pulse pressure

Providing the criteria described above are met, an air pulse is triggered and fired via a pneumatic solenoid. The internal pressure of the triggered air pulse is measured by a pressure transducer. The pressure-output increases with time until it reaches a maximum pressure of 30 or 50 mmHg depending on whether the NCT is used in its standard or +30 mode, respectively, after which the output gradually reduces.

Data transmission

The principle components i.e. the pressure-output, CV, and k-factor values of a single IOP measurement are accessed in a Microsoft *Excel* spreadsheet (see **section 3.2**). An example of the 599 *Excel* rows (Column **A**), pressure-output values (Column **B**), CV (Column C), k-factor (Column **D**) and calculated IOP values (Column **E**) transmitted to the *Excel* spreadsheet via the serial port during one air pulse are shown in **Table A1.1** in **Appendix 1**. Note that when the data in **Table A1.1** are plotted the pressure-response relationship shown in **Figure 3.3** is obtained.

IOP Algorithm

An algorithm utilising the pressure-output and contrast values computes the IOP reading at any one moment in time. This globally defined algorithm is:

$$IOP = \left[\frac{aa}{64}\right] + \left[\frac{(PE - P1) \times ba}{262144}\right] \qquad \textbf{Equation 3.3}$$

where:

aa and ba = calibration constants;

PE = pressure at time of event;

P1 = background pressure prior to the event.

Each *Pulsair* NCT instrument is calibrated against GCT since this is considered to be the gold standard for tonometry. There are 3 calibration constants: 'aa', 'ba' and 'skew'. The calibration constants of each instrument vary and for the particular instrument used throughout this thesis the calibration constants are defined as:

1) aa = 77
2) ba = 7667
3) skew = -16

An event occurs every 0.038 ms. The background pressure prior to any event i.e. P1, is defined as:

$$P1 = \frac{CV_1 + CV_2}{2} \qquad \textbf{Equation 3.4}$$

Therefore, the pressure (PE) and thus IOP can be calculated for each of the 599 data points. The calculated IOP for each PE is shown in Column **E** of the example data in **Table 1.1A in Appendix 1.**

Event detection

Although the calculated IOP at each event is available, only one of these values applies to the patient's actual IOP. Therefore, a macro first detects the event at which the patient's IOP is taken. The calibration constant (skew) is then applied to the pressure value at the event and the subsequent IOP measure is displayed on the digital screen of the handset.

The 'actual event' is defined as a point in time at which the CV has risen by 19 counts. The background contrast (BGC) is initially calculated from the formulae in **Equation 3.5** which is essentially the average of the first 5 CV shown in Column **C** of **Table A1.1**. The calculation of the CV at which the actual event occurs i.e. event contrast (EC) is then computed from the formulae shown in **Equation 3.6**.

$$BGC = \frac{CV_1 + CV_2 + CV_3 + CV_4 + CV_5}{5} \qquad \textbf{Equation 3.5}$$

$$EC = BGC + 19 \qquad \textbf{Equation 3.6}$$

The EC value is compared to the 599 CVs received by the instrument (i.e. Column **C** in **Table A1.1**). The *Excel* row (Column **A** in **Table 1.1A**) at which the CV first exceeds the EC value is noted as the event point index (EPI). Since each instrument is calibrated against the GCT the calibrated event index (CEI) is calculated from **Equation 3.7** which incorporates a calibration constant (skew).

$$CEI = EPI + skew \qquad \textbf{Equation 3.7}$$

Each *Excel* row (shown in Column **A** of **Table 1.1A**) represents time intervals of 0.038ms therefore the CEI represents the time at which the calibrated event occurs, the corresponding pressure of the air pulse at CEI represents PE in **Equation 3.3**.

Of note is that a situation can occur in which none of the 599 CV exceeds the EC and hence an IOP is not calculated. This can occur in patients with IOP's greater than 30 mmHg. A stronger air pulse is required to ensure a change in CV which would exceed the EC within the 599 data points. When this condition is detected by the microprocessors, the instrument automatically switches over to the '+30' mode.

Validation

The contrast rise time (CRT) is the time period between the contrast value index 1 (CVI_1) and the contrast value index 2 (CVI_2). The CVI_1 and CVI_2 is the *Excel* row (Column **A** in **Table 1.1A**) at which the corresponding CV (Column C in **Table A1.1**) first exceeds the contrast value x (CV_x shown in **Equation 3.8**) and contrast value y (CV_y shown in **Equation 3.9**), respectively where

$$CV_x = BGC + 12 \qquad \textbf{Equation 3.8}$$

$$CV_y = BGC + 25 \qquad \textbf{Equation 3.9}$$

In terms of *Excel* rows, for the calculated IOP to be valid the number of *Excel* rows between CVI_1 and CVI_2 must be less than 27 which equates to 1.03 ms (as each measure is separated by 0.038 ms). If the *Excel* row or the CRT does not meet the validation criteria an 'error' message appears on the digital display of the handset.

Using the example data in **Table A1.1** and the formulae described the IOP is calculated and validated for the particular Pulsair *EasyEye* NCT used throughout this thesis and is shown in **Appendix 2**.

3.5 The Pressure-response relationship

As explained above the k-factor is calculated from the CVs which are computed from the light signals received by the photodiodes. When the time is plotted against the k-factor the graph in **Figure 3.4** is generated. In **Figure 3.4** it can be seen that the k-factor (i.e. light reflected from the cornea) increases between k_{start} and P_1 and it is postulated that this increase occurs as the corneal convexity reduces in response to the increasing pressure-output. P_1 denotes the point at which the k-factor is at its maximum. Maximum light is reflected from the cornea when it behaves as a flat mirror i.e. when it is applanated (see **Figure 3.5**). Hence it is considered that P_1 corresponds to the point of maximum corneal applanation. The k-factor then reduces between P_1 and PB_1 and since the pressure-output is still on the increase, it is thought that between these points the cornea buckles to form a crater in response to the force of the air pressure. The light received from the crater will subsequently diminish and hence cause a reduction in the k-factor between P_1 and PB_1. The maximum crater is formed at PB_1 and despite some fluctuations, the k-factor and hence the concave corneal shape (crater)

then remains until PB_2. **Figure 3.4** shows that between PB_2 and P_2 the k-factor increases and reaches a second maximum at P_2. It is hypothesised that as the pressure-output begins to reduce the corneal concavity also starts to decrease which is seen as the increase in k-factor between PB_2 and P_2. The cornea then passes a second point of applanation which occurs at P_2 and as the pressure continues to decrease the cornea increases in convexity until the original corneal shape is achieved and the k-factor decreases between P_2 and k_{end}. **Figure 3.4** shows the points on the pressure-response relationship discussed above and the envisaged corneal shape at each point.

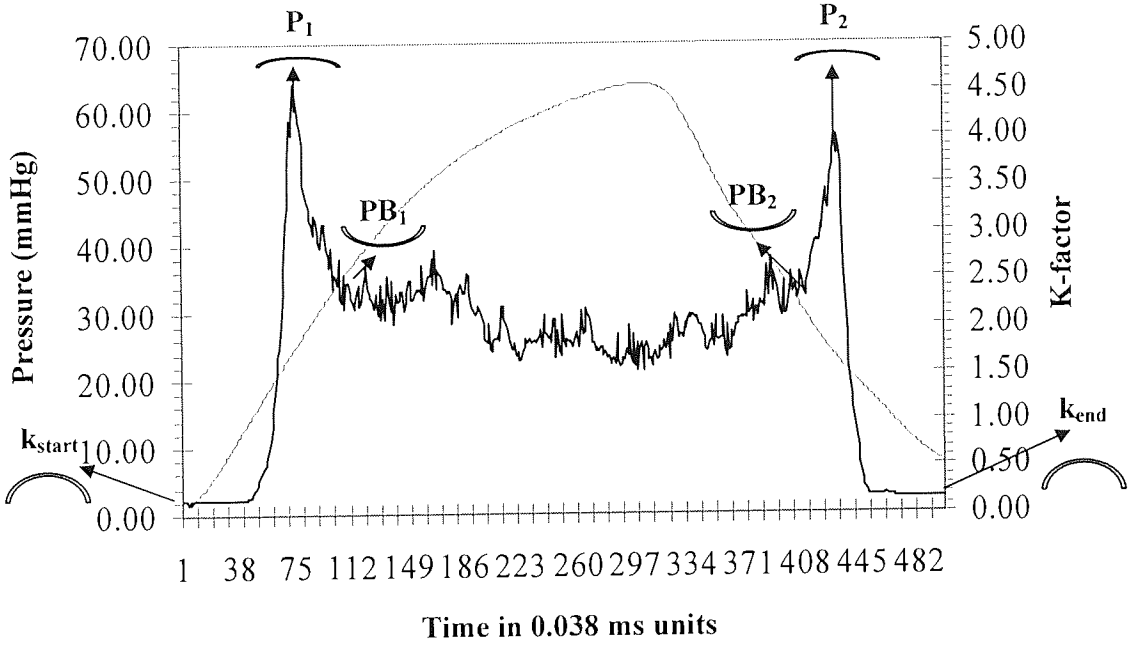


Figure 3.4 Pressure-response relationship showing the envisaged corneal shape at certain parts of the graph.

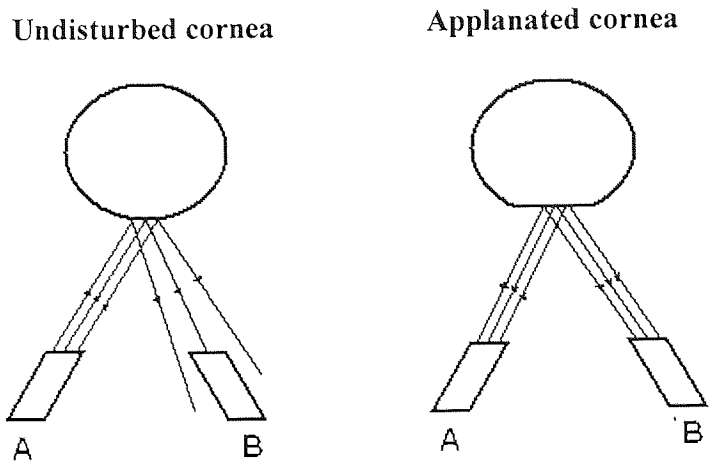


Figure 3.5 Schematic diagram of light transmitted by A and received by B in an undisturbed and applanated cornea (redrawn from Grolman, 1972).

3.6 High Speed Photography

It is of particular interest to determine whether the various components of the pressure-response relationship discussed in **section 3.5** are coincident with the envisaged corneal configurations. From **Figure 3.5** it is assumed that when the cornea is applanated, maximum light is received by the photodiodes. Therefore, since non-contact tonometry is referred to as applanation tonometry (IOP taken when cornea applanated) the IOP at P_1 should represent the patient's IOP. However, it is clear that the computation of a patient's IOP reading precedes the point P_1 (see **section 3.4**). Hence, the exact corneal configuration at the point at which the IOP measure is taken is unknown.

Therefore, high speed motion photography and non-contact tonometry was performed simultaneously in order to investigate the changes in corneal profile. The experimental procedures are discussed.

3.6.1 Methods

3.6.1.1 Subject group

Two postgraduate volunteers took part in this study. The first subject (Subject 1) was a 26 year old male with a myopic prescription of -3.00D (MSE). The other participant (Subject 2) was a 29 year old emmetropic female (MSE=-0.25D).

The research followed the tenets of the Declaration of Helsinki and was approved by the Institution's ethics committee (**Appendix 3**). Written consent was obtained from the subjects willing to participate in the study and copies of the information sheets and consent forms given to the subjects can be found in **Appendix 4**. It was ensured that the visual acuity of both subjects was 0.00 logMAR or better. Both subjects were absent of ocular pathology and none were taking any topical or systemic medications that may affect the measurement of the IOP.

3.6.1.2 Instrumentation

The *Pulsair EasyEye* NCT described earlier was modified (see **section 3.2**) to enable access to the pressure-response relationship of each measure (see **Figure 3.3**). The NCT was mounted on an Ealing optical bench in front of the subject's RE (see **Figure 3.6**).

The Phantom v5.0 (Vision Research Inc., USA) camera was used to film the cornea during tonometry. The camera filmed at a rate of 3800 frames per second and the aperture size used was f5.6. The post-trigger time was set at 253 ms (arbitrary value) i.e. the camera recorded for only 253 ms after it had been activated. This time period was adequate in order to record the full IOP measurement period which occurred over 19 ms. The camera was connected to a personal computer which allowed the camera output to be viewed simultaneously in a *CineView 630* programme (Phantom software, Vision Research Inc., USA).

A battery operated, hand-held Placido disc (Keeler, UK) consisting of white concentric rings was also included in the setup. The Placido disc mires were reflected off the cornea and it was envisaged that the effect on the cornea of the air-pulse could be analysed from the distortion of the concentric rings on tonometry.

3.6.1.3 Experimental procedures

The acquisition of these recordings was technically difficult and time consuming since the ideal setup required the NCT, a high speed camera and the Placido disc be aligned along a common axis. This experimental design was unfeasible mainly due to the bulky designs of the equipment available.

In the initial phases of the lab design, the camera was offset and an image of part of the Placido disc was successfully reflected off the cornea using semi-silvered mirrors. The

opening of the small tube which ejects the air-pulse was measured as 3mm (using digital callipers). A small hole 3 mm in diameter was drilled out of the centre of a 50/50 semi-silvered mirror. This was then attached to the firing end of the NCT and the Placido disc was mounted nasally. An image of part of the Placido disk was reflected off the mirrors and on to the nasal part of the cornea. As the semi-silvered mirrors also affected the light received by the tonometer the alignment criteria described in **section 3.4** was not met and therefore the NCT failed to fire an air-pulse. Even when the 'Quickpulse' feature of the NCT was employed, the NCT still failed to deliver the air-pulse.

Accordingly, the use of the semi-silvered mirrors was discarded and numerous recordings were taken in which the angle and position of the camera and Placido disc were modified. The final experimental setup used is shown in **Figure 3.6**.

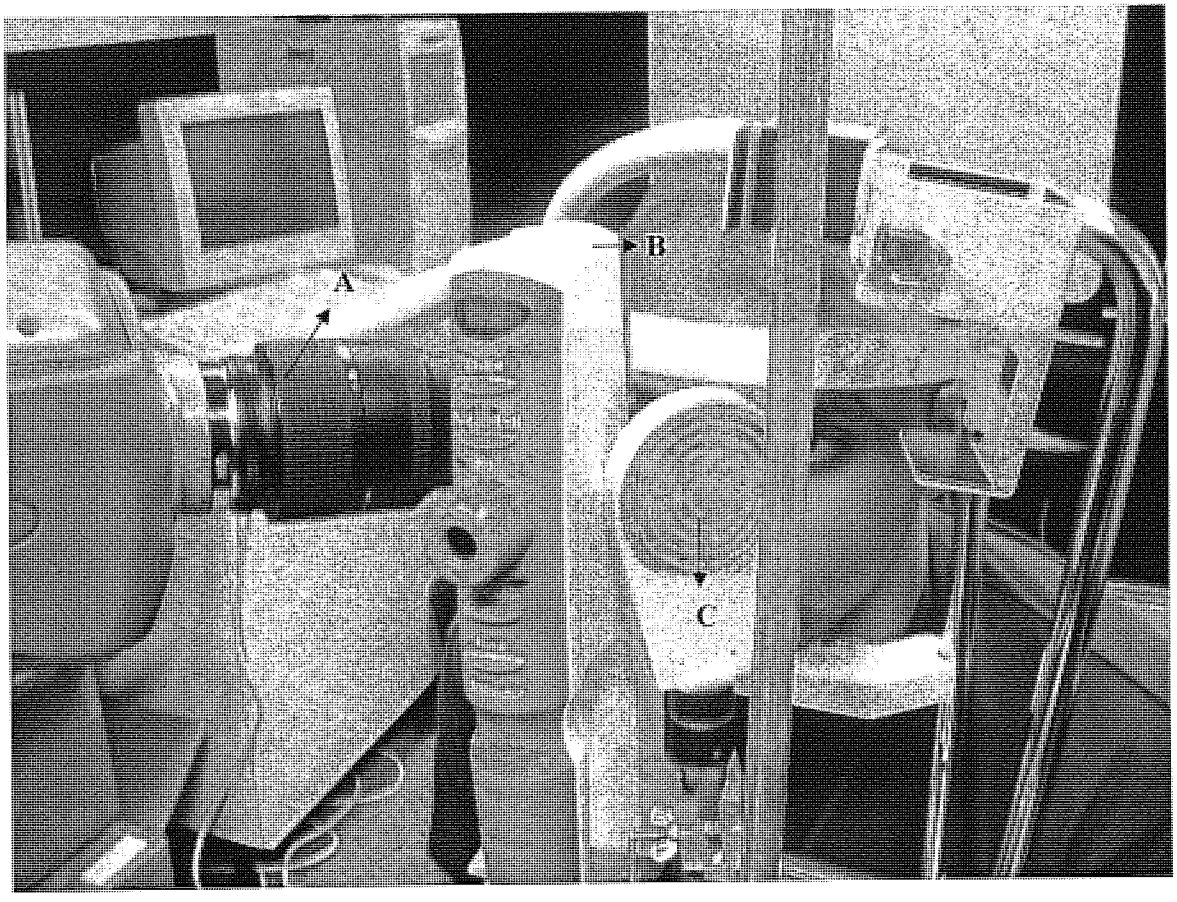
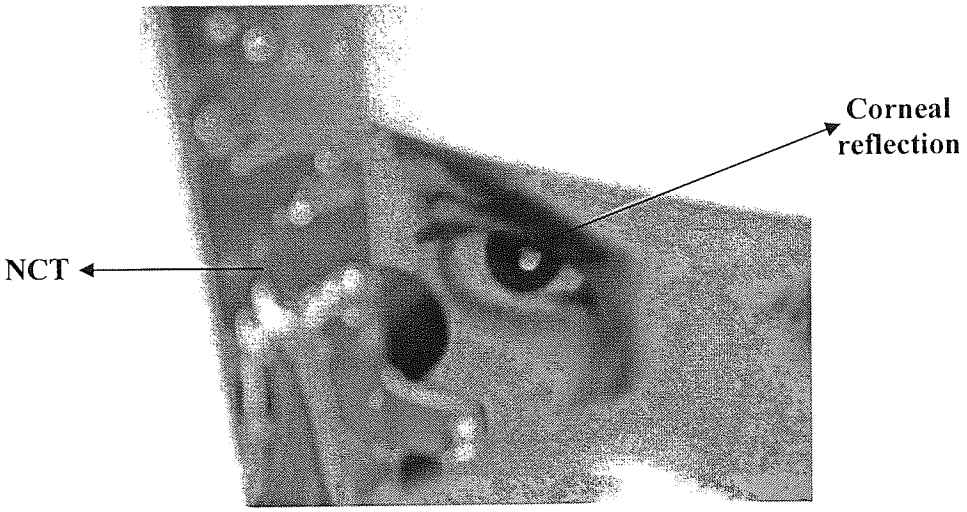


Figure 3.6 Experimental setup where **A**=camera; **B**=NCT and **C**=Placido disc.

On observation of some of the preliminary recordings it was apparent that relatively clear continuous recordings of the cornea were obtained with the camera mounted on a tripod at approximately 80° to the visual axis of the RE (see **Figure 3.6**). The Placido disc needed to

be positioned so that a corneal reflection of the disc could be seen on the subject's RE. Therefore in order to achieve this, with cooperation from the manufacturers, the anterior part of the plastic shroud of the NCT was removed. The upper eyelashes were taped to the upper lid of the RE of Subject 1 so that a clear image of the cornea was captured by the camera. The Placido disc was then mounted in front of the LE and Subject 1 was instructed to fixate on the centre of the Placido disc (see **Figure 3.6**). The position and angle of the disc was then modified until a corneal reflection of the disc rings could be seen on the subject's RE as shown in **Figure 3.7**.

(3.7**a**)



(3.7**b**)

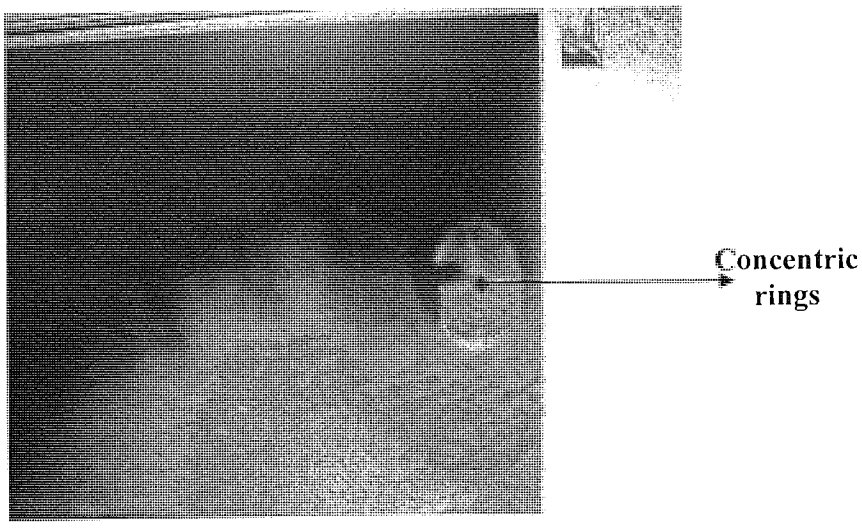


Figure 3.7 Corneal reflection of the Placido disc on the subject's RE (**a**) and (**b**) shows the corresponding image captured by the high speed camera.

The NCT was aligned so that the air-pulse criteria mentioned in **section 3.4** was almost satisfied. The NCT was then only slightly adjusted by operator GR so that the air-pulse was triggered. Another operator (GJ) simultaneously triggered the camera to start recording. Synchronisation of the air-pulse trigger and the camera was crucial to be able to capture the cornea during tonometry as the post-trigger recording time of the camera was only 253 ms.

The image captured is shown in **Video 5.1A** in **Appendix 5.** Unexpectedly, an extended defocused image of the Placido disc rings was seen rather than a distorted clear image. It appears that this phenomenon occurs as the rings are reflected off the tear film which is clearly disrupted and dispersed, albeit momentarily, during non-contact tonometry.

The Placido disc was hence removed from the setup and replaced with a pen torch (Keeler, UK) which provided additional illumination in order to capture a relatively clear image of the cornea with the camera (see **Figure 3.8**). Subject 1 was replaced by Subject 2 who had a larger inter-palpebral aperature, shorter lashes, and was less apprehensive than Subject 1 which meant that the eyelashes did not need to be taped. Subject 2 was instructed to look straight in to the NCT and, as before, the NCT and camera trigger were synchronised.

Figure 3.8 Experimental setup showing the relative position of the pen torch.

3.6.2 Data analyses

The recorded video clip was converted from a CINE file to an AVI file from which 78 individual successive frames were extracted. Given that the recording rate of the camera used was 3800 frames/second, each frame therefore took 0.2632 ms to record [(1/3800)*1000] and hence the full recording was taken in 20.53 ms. A *LabView* Acquistion program (National Instruments) written by Dr. J. Wolffsohn (Aston University, UK) was used to measure the diameter (in pixels) of the deformed area in each extracted frame (see **Figure 3.9**). Due to the angle of the camera and differences in the ease of subjective edge location, there was greater inherent error in the measurement of the deformed area of the cornea in the 180° meridian compared to that in the 90° meridian. Therefore, only the vertical diameter of the deformed area was measured and subsequently used for data analyses.

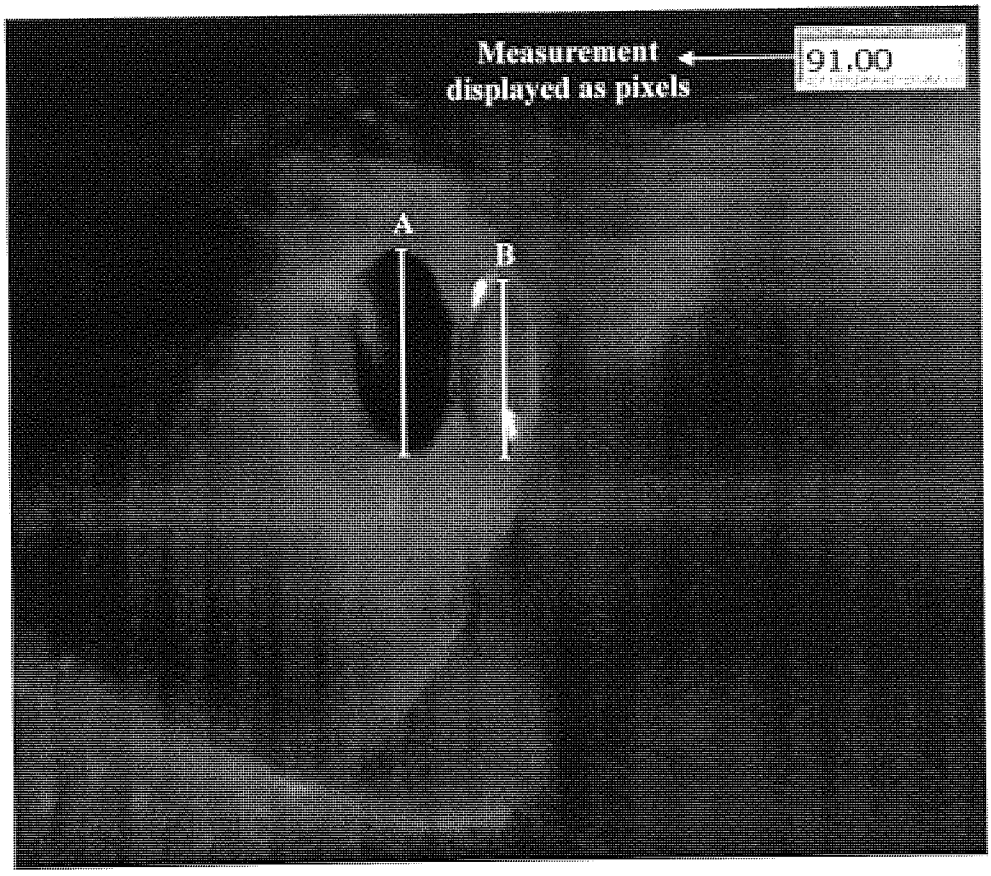


Figure 3.9 Measurement of the vertical diameter of the pupil (**A**) and the deformed corneal area (**B**) in a *LabView* Acquistion window.

A relatively accurate measurement of the vertical height of the distorted corneal area was only possible from frame 15 (f_{15}) to 63 (f_{63}). Although some deformation of the cornea was evident outside this frame range, the edges of the deformed area were too difficult to judge. Although the NCT trigger and the camera trigger were synchronised by two separate operators, the two triggers were not exactly time-locked, and therefore the frames in the video clip could not easily be assigned to the different parts of the pressure-response relationship shown in **Figure 3.4**). Therefore, two different analyses were performed which are discussed in **section 3.6.3.**

Furthermore, in order to calculate the diameter of the deformed corneal area the pupil diameter under the experimental conditions was used as a calibration measure to convert pixels to a physical distance. The *LabView* Acquistion program mentioned above was also used to measure the vertical height of the pupil in each frame (see **Figure 3.9**).

3.6.3 Results

The full recording of the cornea during tonometry is shown in **Video 5.2A** in **Appendix 5.** The corresponding pressure-response relationship for the IOP reading is displayed in **Figure 3.10**.

Since f_{15} to f_{63} were analysed and each frame took 0.2631 ms, the time period over which the data was analysed was 12.63 ms [(63-15)*0.2631]. **Figure 3.11** shows the changes in vertical diameter of the deformed area in the 48 frames analysed.

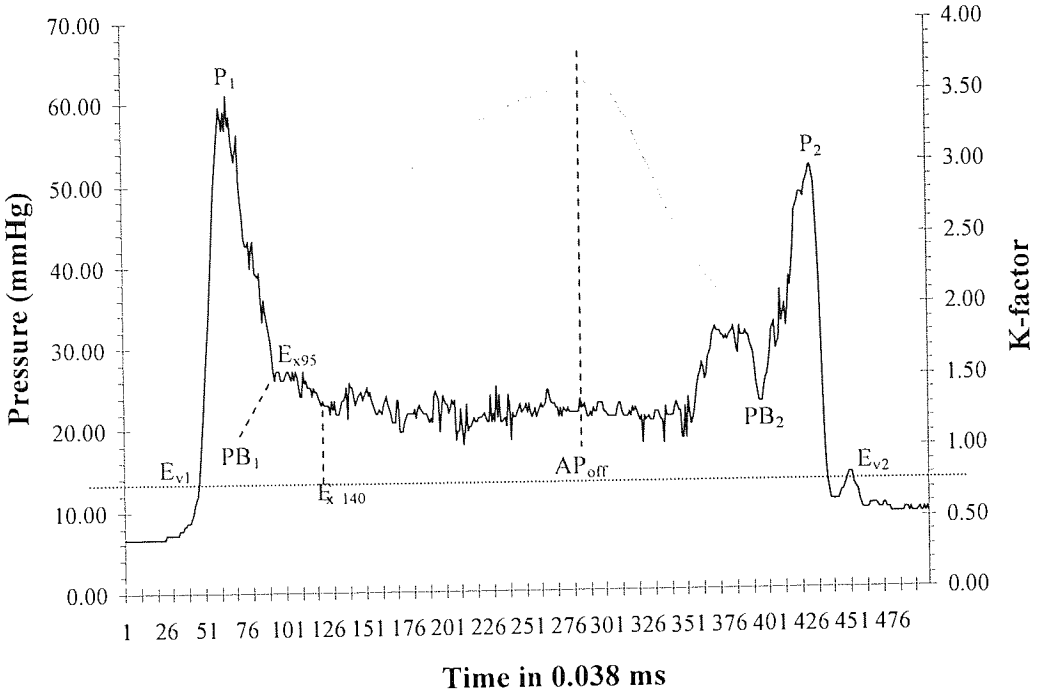


Figure 3.10 Pressure-response relationship corresponding to the video clip in **Video 5.2A** in **Appendix 5** where $\mathbf{E_{v1}}$=event at which IOP taken where the k-factor value is 1.09; $\mathbf{P_1}$= first maximum applanation point; $\mathbf{PB_1}$= bottom of the first peak; $\mathbf{E_{x95}}$ and $\mathbf{E_{x140}}$= *Excel* row 95 and 140 respectively; $\mathbf{AP_{off}}$=time at which the air-pulse pressure starts to decrease; $\mathbf{PB_2}$= bottom of second peak; $\mathbf{P_2}$=second maximum applanation point and $\mathbf{E_{v2}}$= second point at which the k-factor value is 1.09.

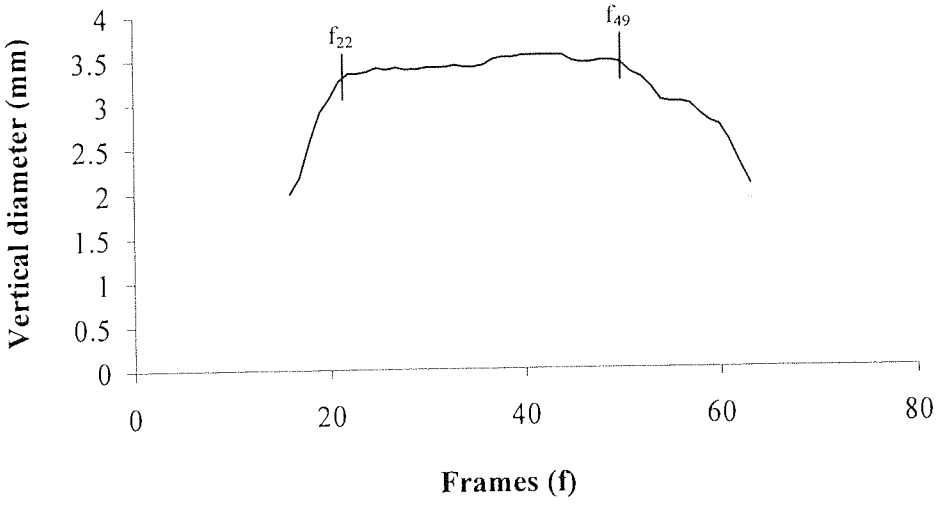


Figure 3.11 Number of frames against vertical diameter of deformed corneal area where f_{22} and f_{49} represent frames 22 and 49 respectively.

Analyses 1

From **Figure 3.11** it is deduced that the deformed area gradually increases in vertical height and reaches maximum in f_{22}. The graph then begins to plateau which indicates that the vertical height of the deformed area remains relatively constant. From f_{49} the vertical size of deformed area starts to gradually reduce. It is postulated that the image in f_{22} therefore represents the point at which the largest deformed area is created which is analogous to P_1 in **Figure 3.10** i.e. the point of maximum applanation which occurs at 2.5 ms (66*0.038) from the start of the measurement period in this particular subject. It is thought that the time between f_{22} and f_{49}, in which the size of the deformed area remains constant, represents the time between PB_1 and PB_2 in **Figure 3.10**. However, the time period between f_{22} and f_{49}, and PB_1 and PB_2 was 7.10 and 13.76 ms, respectively. It is apparent that the two time periods do not match and furthermore the image in f_{49} occurs just before the AP_{off} point in **Figure 3.10**. Moreover, if the image in f_{22} corresponds with P_1, the point P_2 should occur in f_{74} which is clearly inaccurate as the entire measurement cycle occurs in 72 frames. It is therefore evident that what appears to be the point of maximum deformation in **Figure 3.11** does not correspond with the point of maximum deformation in **Figure 3.10**.

Analyses 2

In the second analyses, the y axis scale was reversed and the data points were re-plotted. The resulting graph is shown in **Figure 3.12**.

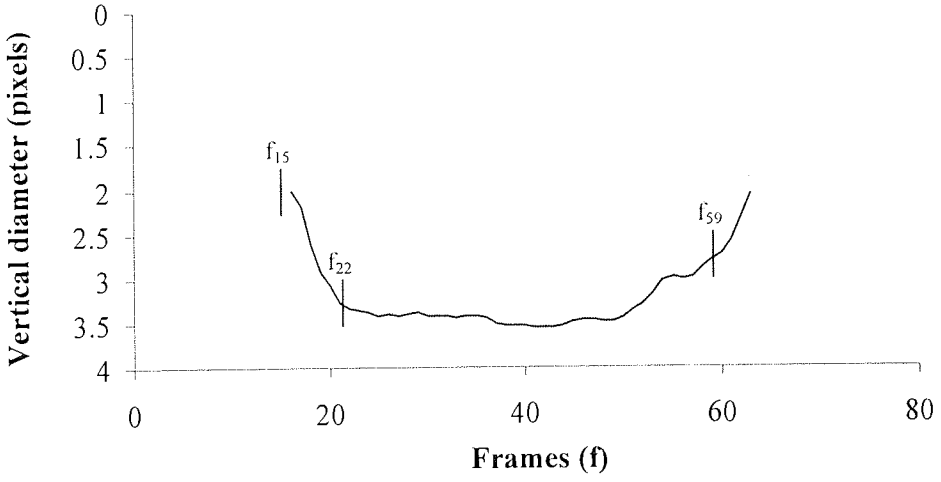


Figure 3.12 Number of frames against vertical diameter of deformed corneal area in which the y-axis is reversed. f_{15}, f_{22} and f_{59} represent frames 15, 22 and 59, respectively.

On analyses of **Figure 3.12**, it was assumed that the image in f_{15} corresponds to PB_1 in **Figure 3.10**. The slight increase in vertical diameter between images f_{15} and f_{22} for 1.84 ms must therefore be analogous to the slight decrease in k-factor between E_{x95} and E_{x140} in **Figure 3.10**, which occurs for 1.71 ms. The time period between PB_1 and PB_2 on the pressure-response relationship is 11.48 ms which should approximately correspond to the time period between f_{22} and f_{59} of the extracted frames (11.58 ms). The first maximum applanation (P_1) occurs at 1.10 ms before PB_1 whilst the second maximum applanation point (P_2) occurs 1.18 ms after PB_2. P_1 and P_2 therefore correspond to f_{11} and f_{63}, respectively. The IOP event occurs 0.61 ms before P_1 and therefore, the image in f_9 must capture the cornea at the time the IOP was taken however, unfortunately this is outside the range of frames analysed.

Figure 3.13 shows that as expected the vertical pupil diameter in each frame remains constant at 105 pixels. Given that the pupil was 4 mm in diameter, 1 mm therefore corresponds to 26.25 pixels (105/4). Hence the average vertical diameter between PB_1 and PB_2 (i.e. between f_{15} and f_{59}) was 3.24±0.38 mm. The diameter of the deformed area at P_2 (i.e. f_{63}) was 1.90 mm. Unfortunately the relatively poor contrast and resolution of the images extracted hinders the analyses of the images between f_0 and f_{14}. Therefore the dimensions of the deformed area at P_1 can not be directly determined. However, intuitively it is thought that the size of the deformed area at P_1 would be greater than that of P_2 (i.e. 1.90 mm) since in **Figure 3.8** the pressure of the air-pulse at the cornea at P_1 (21.71 mmHg) is greater than that at P_2 (18.52 mmHg). Furthermore, the amplitude of the k-factor, which is calculated from the light reflected off the cornea, is much less at P_2 than that at P_1, which also denotes that the area deformed is larger at P_1 than at P_2.

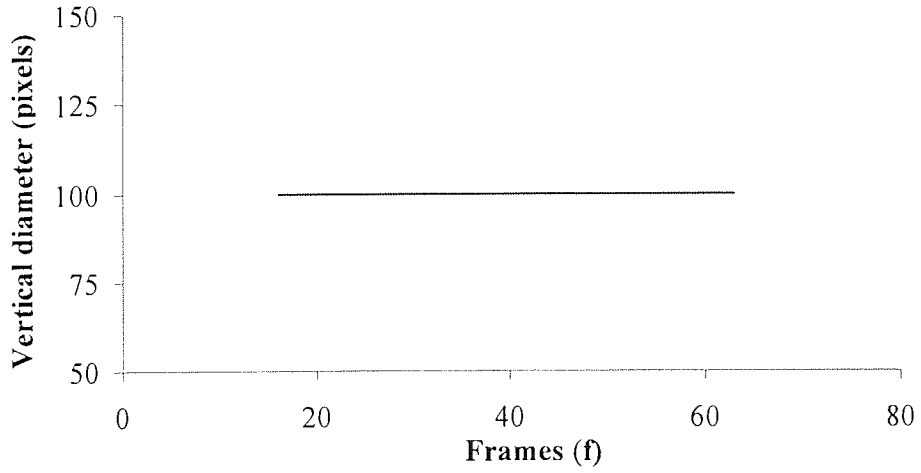
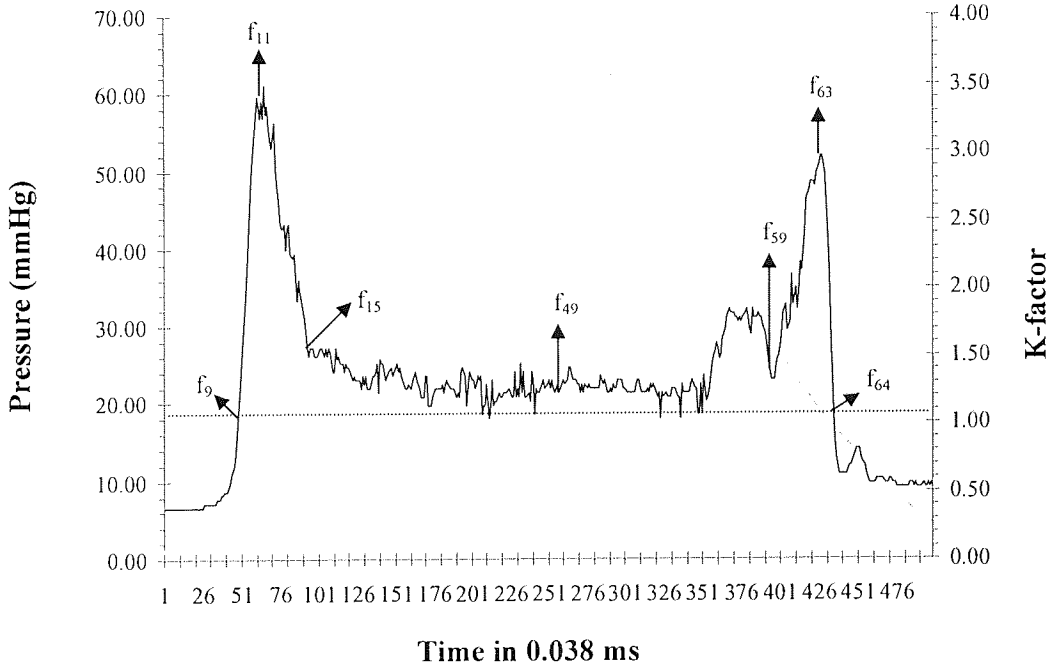
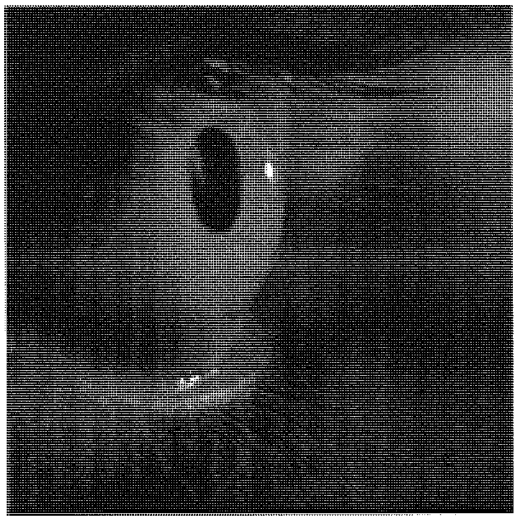


Figure 3.13 Number of frames against the vertical diameter of the pupil.

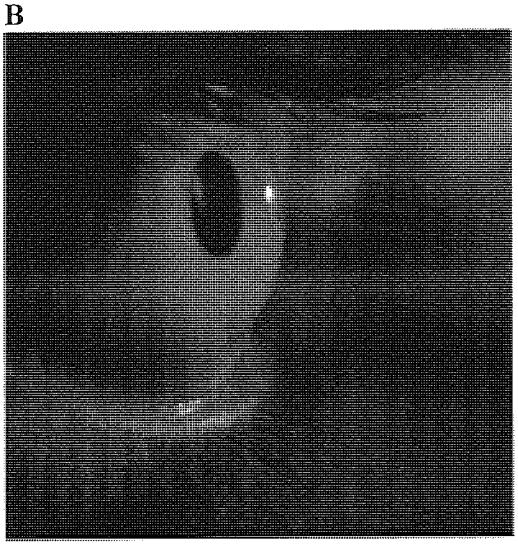
It is thought that the IOP event occurs in f_9 which is also outside the range of frames analysed, therefore the dimensions of the deformed corneal area can not be determined directly. On observation of the principle components of **Figure 3.10**, the EC and EPI were calculated as 31 and 35, respectively (see **section 3.4** for calculations). Using the formula in **Equation 3.2**, the k-factor at the event was calculated as 1.09. A k-factor value of 1.09 also occurs at 16.57 ms from the start of the measurement cycle (E_{v2}) or 0.30 ms after P_2 in **Figure 3.10**. Therefore, it is thought that the deformed area in f_{64} [(0.304/0.2631)+63)] is equivalent to that in f_9 i.e. IOP event point. However, f_{64} is also outside the range of frames analysed. The individual frames which correspond to the various parts of the pressure-response relationship are summarised in **Figure 3.14.**



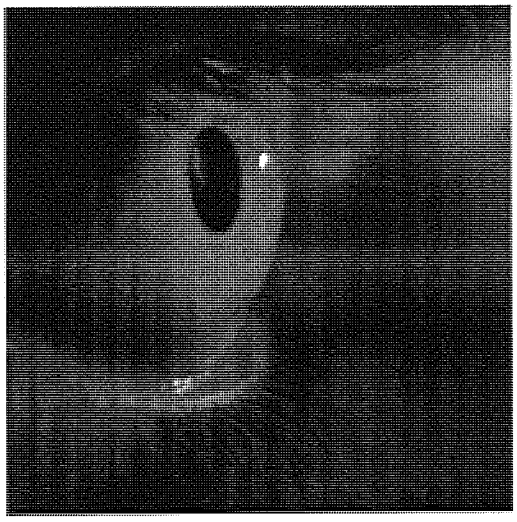
A



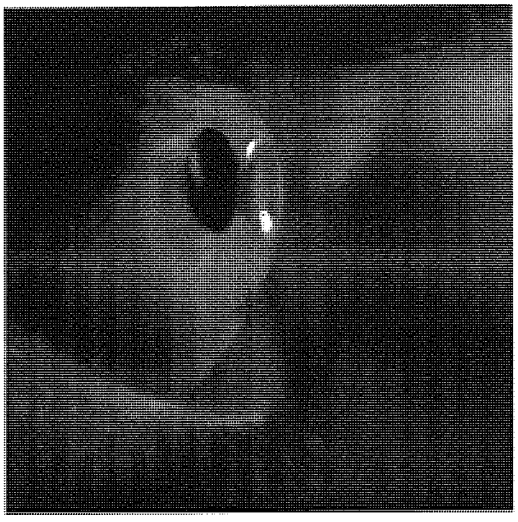
B



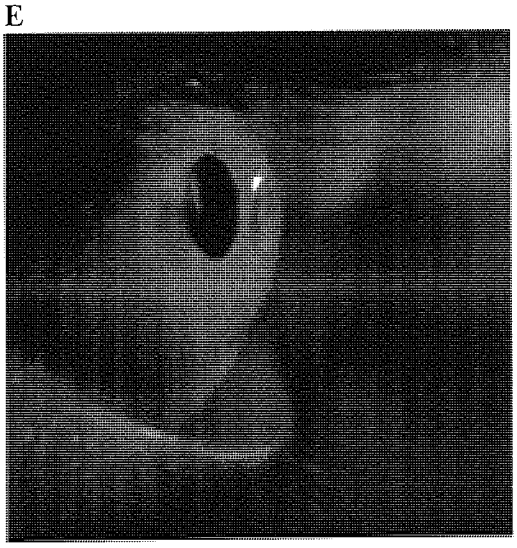
C



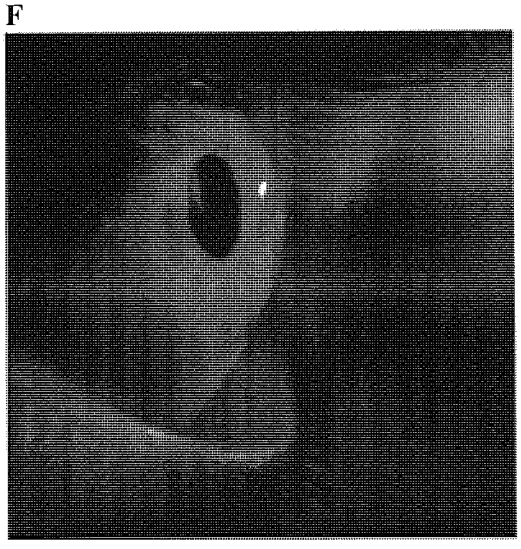
D



E



F



G

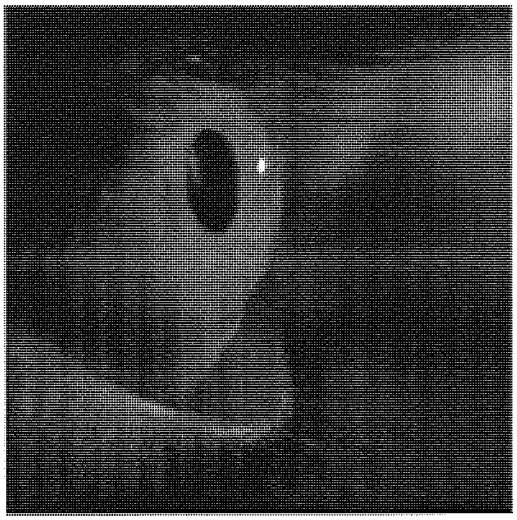


Figure 3.14 The pressure-response relationship where f_9, f_{11}, f_{15}, f_{49}, f_{59}, f_{63}, f_{64}, correspond to image A, B, C, D, E, F and G, respectively.

3.7 Discussion

The *EasyEye Pulsair* NCT is used in the subsequent studies to investigate the effect of accommodation on IOP. The IOP measurement procedures and operational principles are described in the present chapter. The two pertinent advantages of using the *EasyEye Pulsair* NCT are that firstly the relatively small dimensions of the handset allow great flexibility and utility in the experimental setup in which IOP measures can be obtained in one eye and accommodation can be stimulated simultaneously in the other eye. Secondly, high resolution IOP measures are available which enhance the precision of the IOP data. Access to the principle components of an IOP measure have allowed greater understanding of the derivation of the IOP with the *Pulsair* NCT and are shown graphically in **Figure 3.3** and numerically in **Table A1.1** in **Appendix 1**.

The original NCT designed by Grolman in 1971 is referred to as an applanation tonometer as corneal applanation is required in order to obtain an IOP measure. Applanation tonometers exploit the Imbert-Fick law which states that the IOP is equal to the applanation force divided by the area of applanation. The time at which maximum light is recorded by the receiver is analogous to when the cornea behaves as a flat mirror i.e. applanated. Due to the linear correlation between the air-pulse force and time, the patient's IOP is computed. Grolman (1972) used a high speed camera with a frame rate of 5000 to image the cornea during tonometry. He concluded that on direct inspection of the frames in motion with a projector,

the events of corneal applanation and maximum light detection occur simultaneously. Furthermore, Grolman estimated that the diameter of the applanated corneal area was approximately 3.6±0.2 mm (Grolman, 1972: Forbes *et al.*, 1974). However, the corneal profile in terms of size and depth before or after the applanation point was not commented upon.

In the present study, tonometry and video recording were not time-locked and this limitation meant that the individual frames could not be assigned to the components of the pressure-response relationship. Moreover, the relatively poor resolution of the frames limited the analyses to between frames 15 to 63. It was hypothesised that between P_1 and PB_1 the cornea buckles into a crater. From analyses of the diameter of the corneal distortion it is apparent that the time between frames 15 to 59 coincides with the time for which the cornea remains in a state of concavity. The average diameter of the concaved cornea was approximately 3.25 mm and although in the video clip shown in **Video 5.2A** in **Appendix 5** it appears as if the size of the deformed area is increasing between frames 22 and 59, the data show that the vertical size of the crater remains relatively stable.

Unfortunately, it is not clear from the frames whether the cornea is applanated at P_1 although it is clear that the vertical diameter of the deformed area at P_1 must be greater than 1.90 mm. It is evident from the analyses of the algorithm used to calculate an IOP measure with the *Pulsair* NCT, that detection of the point of maximum applanation is not necessary to compute the IOP (i.e. the point at which maximum light is detected is not relevant to the formulae in **Equation 3.3**). In fact, the *Pulsair* NCT detects the point in time at which the cornea has changed shape by a set amount (i.e. 19 CV counts), an event which precedes the event of maximum corneal applanation. Calibration factors are then applied to generate an IOP measure calibrated with the measure obtained with the GCT. It is postulated that frame 9 corresponds to the point at which the IOP reading was taken however, this was outside the range of frames analysed so the exact corneal profile at this point remains unknown. It is possible that as the force of the air-pulse increases the cornea progressively reduces in curvature and a point of complete corneal applanation occurs at only P_1. Therefore, it may be misleading to refer to the *Pulsair* NCT as an applanation tonometer like the GCT, in which corneal applanation is necessary to obtain an IOP measure. However, it is also possible that the cornea applanates much sooner than P_1 and as the force of the air-pulse increases the size of the applanated cornea increases and reaches a state of maximum applanation at P_1.

Another possibility is that a small central portion of the deformed area is constantly applanated during tonometry and the area of flattening gradually increases reaching maximum at PB_1. Hence the corneal concavity seen occurs only in the peripheral portion of the deformed corneal area. Of note is that it has also been suggested that during corneal deformation (in response to the air-pulse) the cornea does not actually reach a point of applanation but instead it reduces in curvature and subsequently crumples to form a crater (personal communication with the opto-electronics designer of the *Pulsair* NCT: John Fisher).

The preliminary work presented in this study describes a method by which the corneal profile during tonometry can be investigated. Indeed, a camera which records at a faster frame rate with better resolution than that used in the current study is needed to judge the depth of the deformed cornea during tonometry. This would then allow the true extent of the deformation to be quantified. Furthermore, the two systems need to be accurately time-locked so that the images in the frames can be accurately assigned to the components of the pressure-response relationship and this will be the aim of future work.

On analysis of the operational principles of the *Pulsair* NCT, it is clear that the IOP reading is taken at the point in time at which the cornea has changed shape by a set amount. Therefore, it is possible that the NCT can be modified so the air-pulse is switched off immediately after this event has been detected. This modification may reduce patient discomfort and reduce any possible changes to the biomechanical structure of the cornea caused by the impact of the increasing air-pulse force on the cornea.

It is well established that NCT is influenced by variations in central corneal thickness, such that higher IOP readings are recorded in eyes with relatively thicker corneas (Eysteinsson *et al.*, 2002; Ko *et al.*, 2005; Tonnu *et al.*, 2005). This phenomenon is explained by the IOP algorithm used to calculate the IOP with the *Pulsair* NCT. If the cornea is relatively thick, the time required to deform the cornea by a set amount is likely to be longer than that of a relatively thin cornea. Hence since the force of the air-pulse increases linearly with time, the pressure of the air-pulse at the time of the event (PE in **Equation 3.3**) would be much higher in a thicker cornea. A larger value of PE in **Equation 3.3** would hence generate a higher IOP.

As stated above the IOP measures obtained with the *Pulsair* NCT are calibrated with the measures taken with the GCT since this is considered 'gold standard' for tonometry. Details

of the subject group used for the calibration process are not disclosed by the manufacturers (Keeler, UK). Previous researchers have found a correlation coefficient of above 0.80 between the IOP measures taken with the *Pulsair* NCT and GCT (Mackie *et al.*, 1996; Parker *et al.*, 2001; Lawson-kopp *et al.*, 2002). However, many factors, for example corneal thickness (Shah *et al.*, 1999; Matsumoto *et al.*, 2000; Recep *et al.*, 2001; Eysteinsson *et al.*, 2002; Ko, Liu and Hsu, 2005; Tonnu *et al.*, 2005) and actual IOP (Moseley, Evans and Fielder, 1989; Parker, Herrtage and Sarkies, 2001) are known to affect the measures obtained with both tonometers. Hence it is possible that differences in the characteristics of the subject group (for example gender, age, corneal thickness and refractive error) used for the calibration process and the subject groups used in other studies may present some variations/error in the IOP measures.

Throughout this thesis, the Pulsair *EasyEye* NCT discussed in this chapter is used and the pressure-response relationship for each IOP measure is attained. As later work will demonstrate the inter-subject variations in IOP responses to accommodation are partly explained by corneal parameters. Therefore, since the pressure-response relationship is essentially derived from the changes in corneal shape, the components of the pressure-response relationship are further evaluated, firstly with respect to biometric parameters and secondly with respect to accommodation and the analyses are discussed in **Chapter 10** of this thesis.

3.8 Conclusions

Evaluation of the principle components of an IOP measure have allowed greater understanding of the derivation of an IOP measure with the *EasyEye Pulsair* NCT. Of relevance to the aims of this thesis was the access to high resolution IOP measures.

An analysis of the pressure-response relationship concludes that:

- Maximum corneal applanation occurs at P_1 and P_2.
- The cornea is forced into a state of concavity, the diameter of which is approximately 3.24 mm.

- It is hypothesised that corneal applanation may not be necessary to obtain an IOP measure with the *Pulsair* NCT and it is hence suggested that referring to the *Pulsair* NCT as an applanation tonometer may be a misnomer.

CHAPTER 4

THE RELATIONSHIP BETWEEN ACCOMMODATION AND INTRAOCULAR PRESSURE; INITIAL CONSIDERATIONS

4.1 Introduction

Many early workers in the 19th century agreed with the tenet that accommodation increased the intraocular pressure (IOP) (reviewed by Stansbury, 1948). In the first half of the 20th century the consensus was that there was no effect of accommodation on IOP (summarised by Duke-Elder, 1938). Despite this, the eminent worker Duke-Elder (1938) postulated that constriction of anterior ciliary arteries, dilation of ciliary veins and widening of the anterior chamber angle on accommodation led to an increase in aqueous outflow and hence concluded that IOP reduced with accommodation. The studies investigating the relationship between accommodation and IOP published thereafter supported the proposal that IOP decreased with accommodation (Armaly and Burian, 1958; Armaly and Rubin, 1961; Allen and Burian, 1965; Mauger *et al.*, 1984).

Pertinent to this thesis was the work done by Mauger and co-workers (Mauger *et al.*, 1984). Thirty subjects, ranging in age from 22 to 35 years were split into 3 groups of 10 (Group 1, 2 and 3). The refractive error of the left eye (LE) was corrected with full aperture trial lenses. Group 1 were instructed to fixate a target at 6m while the IOP was measured in the right eye (RE) with the Goldmann contact tonometer (GCT). Two further IOP measures were taken after 30 seconds and 3 minutes of fixation. In group 2, IOP measures were taken at the same time intervals following the addition of a -4.00D lens to the refractive correction. The same measurement procedure was performed in group 3 following the addition of a -1.50D lens to the refractive correction of the LE. The study demonstrated that owing to the accommodation induced by the -4.00D lens, the IOP reduced by respectively 1.32±0.42 and 2.38±0.65 mmHg after 30 seconds and 3 minutes of accommodation. Accommodation induced by the -1.50D lens reduced the IOP by respectively 1.15±0.71 and 2.15±0.78 mmHg after 30 seconds and 3 minutes of fixation. Therefore the magnitude of IOP reduction increased with an increase in the time of accommodation, although a significant proportion of the change in IOP occurred in the first 30 seconds of accommodation (Mauger *et al.*, 1984).

One major drawback of the Mauger *et al.* (1984) study was the type of tonometer used to take IOP measures. Non-contact tonometers (NCT) have been available since 1971 (Grolman, 1972), however given the bulky designs of these instruments their use has been restricted in this type of clinical research. Inevitably Mauger *et al.* (1984) chose to use the Goldmann contact tonometer (GCT) in their study. Although considered the 'gold standard', there are a number of issues pertaining the IOP measures taken with the GCT (Whitacre and Stein, 1993). Of importance are the effects on IOP of the use of topical anaesthetics with the GCT. Studies have shown that the accuracy of an IOP measure may be reduced by the use of topical anaesthetic agents (Herse and Siu, 1992; Moseley *et al.*, 1993; Baudouin and Gastaud, 1994; Birchall and Kumar, 2001; Asensio *et al.*, 2003; Nam *et al.*, (2006). Although the underlying mechanism(s) remains elusive it has been postulated that topical anaesthetics have a direct facilitating effect on the aqueous humour outflow, subsequently affecting the IOP (Baudouin and Gastaud, 1994). It has also been suggested that topical anaesthetics can cause changes in the tear meniscus (Birchall and Kumar, 2001) and in the thickness of the cornea (Herse and Siu, 1992; Asensio *et al.*, 2003; Nam *et al.*, 2006) which subsequently have indirect effects on the IOP measurement (Whitacre and Stein, 1993). Furthermore, as the GCT probe is in contact with the cornea for approximately 2 seconds (Myers and Scott, 1975) and this time varies substantially between repeat measurements and subjects (Birchall and Kumar, 2001), an inter- and intra-measurement massaging effect may progressively reduce the IOP as found by Moses (1961).

Although Mauger *et al.* (1984) concluded that IOP decreased on accommodation, this result was obtained from a small subject group with a dose effect examined between groups rather than within subjects and furthermore the results may have been contaminated by the effects of topical anaesthetics and repeated and prolonged corneal contact. The aim of this study was therefore to investigate the effects of accommodation on IOP using a modified, high resolution NCT, the Pulsair *EasyEye* (Keeler, UK), on a larger sample size. The data presented in this study were collected in the initial phase of the research programme and were subject to a number of limitations. Revisions to the experimental setup were made subsequently to address these limitations so that a more valid assessment of the relationship between accommodation and IOP could be made (see **Chapter 7**).

4.2 Methods

4.2.1 Subject group

Thirty three subjects (males=14 and females=19) were recruited from the undergraduate population at Aston University for this study. The mean age of the subject group was 21.0±2.1 yrs. The mean spherical equivalent [MSE=sphere + (cyclinder/2)] ranged from +0.50D to -3.69D and the cohort comprised of 21 emmetropes (MSE of ± 0.50D) and 12 myopes (MSE of ≤ -0.50D). The criterion used to divide the subjects in to these refractive groups has been used by many previous studies, such as those conducted by Goh *et al.* (2005), Junghans and Crewther (2005) and Ojaimi *et al.* (2005).

The research followed the tenets of the Declaration of Helsinki and was approved by the Institution's ethics committee (**Appendix 3**). Written consent was obtained from all subjects willing to participate in the study and copies of the information sheets and consent forms given to the subjects can be found in **Appendix 4**. It was ensured that the visual acuity of all the subjects was 0.00 logMAR or better. All subjects were absent of ocular pathology. None of the subjects were taking any topical or systemic medications that may affect the IOP or accommodative function.

4.2.2 Stimulus

Abbot, Schmid and Strang (1998) found a reduced accommodative response to negative-lens induced accommodative demand compared to real target presentations in real dioptric space. Therefore, unlike Mauger *et al.* (1984), who used negative lenses to stimulate accommodation, a high contrast (90%) Maltese cross target was used to stimulate accommodative demand in free-space thus reducing the accommodative response errors. The target was placed at 6m and 25cm to stimulate zero (L; low) and 4D (H; high) of accommodative demand, respectively. Zero and 4D accommodative targets were chosen so that comparisons could be made with previous studies (Armaly and Burian, 1958; Armaly and Rubin, 1961; Mauger *et al.*, 1984) and the IOP measures were taken after 3 minutes of fixation to each target so that comparisons could be made with the work of Mauger *et al.* (1984). Furthermore, there is evidence that suggests that accommodative adaptation occurs after approximately 3 minutes of accommodation (Rosenfield and Gilmartin, 1989). The two accommodative stimuli levels employed were presented in a random order during the IOP measurement period. There is evidence that higher level attentional factors (Francis *et al.*,

2003) and the precise instructions (Ciuffreda and Hokoda, 1985; Stark and Atchison, 1994) given to the subject play an important role in the accuracy of the accommodation responses. All subjects were therefore instructed to "focus on the target carefully", with a number of encouraging reminders as recommended by Stark and Atchison (1994).

4.2.3 Instrumentation

The Shin-Nippon SRW-5000 (Ryusyo Industrial Co., Ltd, Japan) open-view, infrared objective autorefractor was used to measure refractive error while the subjects viewed the Maltese cross target placed at 6m. The autorefractor applies image analyses to an infrared ring which is reflected off the retina (Mallen *et al.*, 2001). The instrument has been shown to provide highly repeatable measures of refractive error in children (Chat and Edwards, 2001) and adults (Mallen *et al.*, 2001). Details of the subject group used for the calibration process are not known but it is presumed that the cohort comprised of subjects mainly from a Japanese background. Therefore differences in the characteristics of the initial subject group (for example gender, age, corneal thickness and curvatures) used for the calibration process and the subject groups used in other studies may present some error in the IOP and POBF measures.

The IOP measures were taken with the portable Pulsair *EasyEye* NCT. Full operational details are given in **Chapter 3**. Keeler (UK) recommends that the average of 4 IOP readings is used to represent the eye's IOP and this has been validated by McCaghrey and Matthews (2001). In collaboration with Keeler the NCT was connected to a PC via an interface port on the instrument's main unit. By means of a macro (a user specified series of actions performed as one new procedure) embedded into a Microsoft *Excel* spreadsheet (written by Keeler, UK), access to high resolution (0.01 mmHg) IOP measures was obtained (see **Chapter 3**). Of great significance were the small dimensions and light weight of the instrument [256 x 115 x 40 mm (height x width x depth) and 0.9 kg weight] which meant that the instrument could be mounted on an optical bench to permit unhindered IOP measures in one eye while simultaneously stimulating accommodation in the other eye (see **Figure 4.1**).

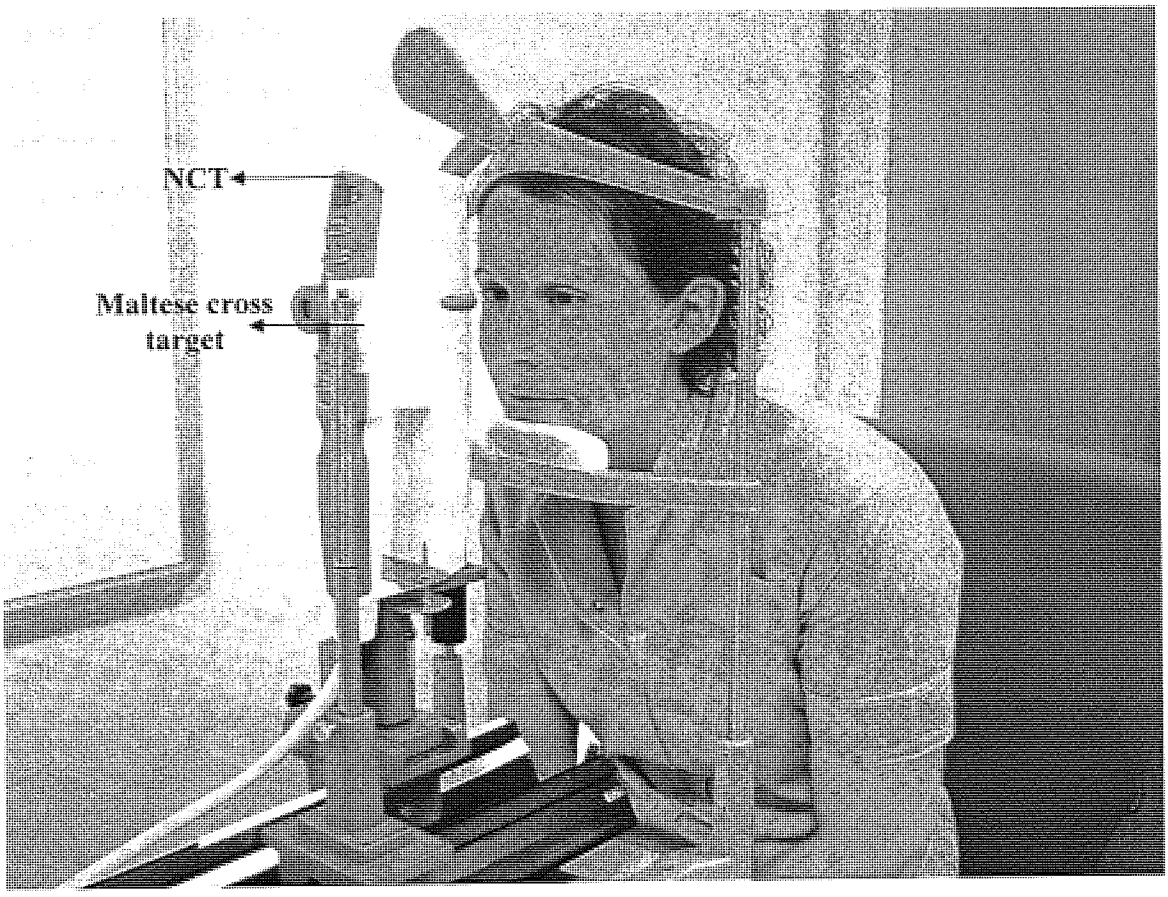


Figure 4.1 The experimental setup allowing IOP measures to be taken in the RE while simultaneously stimulating accommodation in the LE.

4.2.4 Experimental Procedure

On commencement of data collection, each subject had their refractive error measured with the autorefractor and was familiarised with the operation of the NCT. An IOP reading was obtained (not recorded) to demonstrate the sensation experienced when a puff of air was fired at the cornea by the NCT in order to take a measure. This procedure was important as there is evidence that shows that the accuracy of an IOP measure is influenced by increases in apprehension (Forbes *et al.*, 1974; Moses *et al.*, 1984). Following the familiarisation technique each subject was allowed a 10 minute rest period.

Four consecutive IOP measures [as validated by McCaghrey and Matthews (2001)] were obtained in the RE while the LE fixated the two accommodative stimulus targets for 3 minutes. After each set of 4 measures the subjects were allowed a 5 minute rest period. The two accommodative stimulus targets were presented randomly and the results were entered into a Microsoft *Excel* spreadsheet. The experimental setup is shown in **Figure 4.1**.

4.3 Statistical analysis

Data analyses were performed with Microsoft *Excel* for Windows. The accommodative responses were calculated from the distance refractive error and the nominal 4D accommodative response to a target at 25cm (4+MSE). A paired Students t-test was performed on the data to ascertain the effect of accommodation on IOP. A Pearson's product moment correlation coefficient was determined to establish the effect of the variations in the calculated accommodative responses and the associated changes in IOP.

4.4 Results

The mean (±SD) calculated accommodative response for the cohort was 3.16±1.25D which ranged from 0.31 to 4.50D. The mean IOP measured with the eye fixating the L and H accommodative stimulus was 14.13±2.89 and 13.88±1.88 mmHg, respectively. The differences in IOP taken at the two accommodative targets ranged from an increase in IOP of 3.15 mmHg to a decrease of 4.58 mmHg. The mean difference in IOP taken at the two accommodative targets (a mean decrease of 0.27±1.70 mmHg from L to H levels of accommodation) failed to reach statistical significance (t=2.07, p=0.12). **Figure 4.2** illustrates the inter-subject variations in IOP change induced by accommodation for the cohort.

The substantial inter-subject variations in IOP changes on accommodation are considered with respect to the variations in calculated accommodative responses (**Figure 4.3**). A weak, yet significant, positive correlation is exhibited by the data (r=0.52, p=0.002).

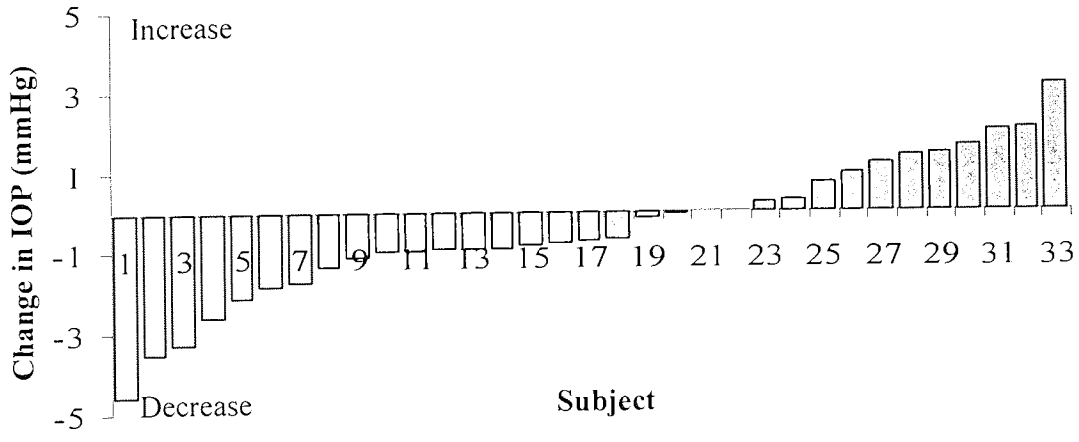


Figure 4.2 Change in IOP between L and H levels of accommodative stimuli ranked in ascending order (n=33).

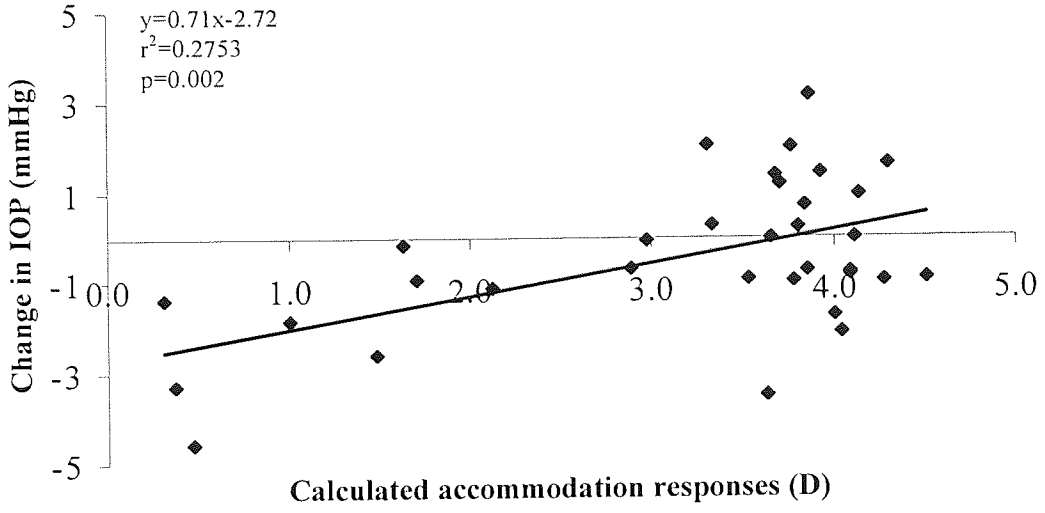


Figure 4.3 Calculated accommodation responses and change in IOP between L and H levels of accommodation (n=33).

4.5 Discussion

A high resolution NCT was used in this study to ascertain the effect of accommodation on IOP. Although a small reduction in IOP on accommodation was evident, in contrast to the previous studies (Armaly and Rubin, 1961; Mauger *et al.*, 1984) the change in IOP was not statistically significant. This discrepancy between studies may be explained by the fact that approximately 40% of the subjects in this study had a calculated accommodative response of 4.00±0.25D. On observation of **Figure 4.2** it is clear that although the difference in IOP between the two accommodative targets did not reach statistical significance there were substantial inter-subject variations in the IOP responses induced by accommodation, such that the differences in IOP between the two accommodative levels spread over a 8 mmHg range. Furthermore, the IOP increased with accommodation in about 1/3 of the subjects and decreased in the other 2/3 of the subjects.

The diverse IOP responses induced by accommodation evident in **Figure 4.2** are of interest. The inter-subject variations in IOP responses to accommodation were considered with respect to the inter-subject variations in the calculated accommodation responses. A significant (albeit weak) relationship between IOP change and the calculated accommodation responses was evident (accounting for 27% of the variance). The reduction in IOP on accommodation is more apparent on relatively lower levels of calculated accommodation responses. For the cohort examined, although it appears that on relatively higher calculated accommodation

response levels the IOP increases slightly, the inter-subject variations in IOP responses is perceptible.

Of note, is that the accommodative responses were calculated from the distance refractive error and that optically, a target at 25cm represents a 4D accommodative stimulus. However, the well documented lead and lag of accommodation to respectively low and high accommodative targets which approaches nearly 1 D (Cuiffreda, 1998), means that there are significant discrepancies between the calculated accommodation responses and the actual accommodation responses in each subject. The spread of calculated accommodation response data exhibited by the cohort coupled with the fact that only less than half of the subjects' had a calculated accommodation response of 4.00±0.25D means that firstly, firm conclusions can not be drawn about the change in IOP on 4D of accommodation and secondly the results can not be compared directly to previous studies mapping the relationship between accommodation and IOP. It is clear that the experimental design needs to be modified in order to control and measure the actual accommodation responses of each subject.

Although the NCT was used to overcome the limitations of the GCT, the NCT instrument is not without disadvantages. As a result of the short measurement time of the NCT which is approximately 1-3 ms (Forbes *et al.,* 1974), the IOP measures are influenced by the inherent short-term fluctuations in IOP. Of particular interest are variations in IOP caused by the respiratory and cardiac cycles (Moses and Arnzen, 1983). Whereas the respiratory cycle affects IOP by approximately 0.8 mmHg (Nanba *et al.,* 1989), the cardiac cycle influences IOP to a much greater extent of between 1.4 and 6.7 mmHg (Lam *et al.,* 2004).

Therefore, the inherent variance in IOP measures associated with the respiratory and cardiac cycles may mask the true effect of accommodation on IOP and furthermore may contribute to the diverse IOP response pattern to accommodation exhibited in this study. Hence, incorporating a stimulus presentation method, which will ascertain more precise accommodation responses and an experimental setup to control the variance in IOP measures associated with biological cycles into a study design, will permit a more accurate investigation of the effect of accommodation on IOP.

Although the results of the present study are of limited value, the study was an opportunity for preliminary work to highlight the experimental design issues which are pertaining to the

measurement of the effects of accommodation on IOP. As later work shows, modifications to the experimental design based on these initial considerations are of relevance in answering the central research question of this thesis.

4.6 Conclusion

The study highlights the need for experimental design modifications, particularly to account for the variance in IOP measurements related to the respiratory and cardiac cycles. Although of limited value, the main findings of the present study are;

- There were substantial inter-subject variations in IOP responses to accommodation.
- The inter-subject variations in IOP responses were weakly related to the calculated accommodation responses.

Chapter 5 discusses the derivation of a method of IOP measurement which takes into account the variance associated with the cardiac and respiratory cycles.

CHAPTER 5

THE EFFECT OF SHORT-TERM FLUCTUATIONS ON THE MEASUREMENT OF INTRAOCULAR PRESSURE.

5.1 Introduction

The full operational details of *Pulsair EasyEye* (Keeler, UK) tonometer are discussed in **Chapter 3**, however briefly, non-contact tonometers (NCT) use a ramped (i.e. force of air increases with time) pulse of air to deform the cornea. The time and extent of corneal deformity is optically measured by a photoelectric cell and displayed as an intraocular pressure (IOP) measure. Although with the *Pulsair EasyEye* NCT the IOP is sampled over a 19 ms cycle, an IOP reading is computed from the data taken in the first 1-3 ms (Forbes *et al.*, 1974). The relatively instantaneous nature of NCTs means that the IOP measures have more variance associated with them and therefore there is less validity in the mean values with reference to measures taken with the Goldmann contact tonometer (GCT).

Moses and Arnzen (1983) reported that the fluctuations in the steady-state IOP closely match a sinewave as illustrated in **Figure 2.2** in **Chapter 2.** Analysis of the ocular pulse waveform shows that the principal spectral component is associated with the cardiac cycle with a frequency of approximately 1 second (Leydhecker, 1976). It is well accepted that with each heart beat, a bolus of arterial blood enters the choroid during the systolic phase. The rhythmic filling of the choroidal vessels set against the constant ocular rigidity results in volumetric changes which give rise to fluctuations in the steady-state IOP, such that the IOP increases with each systole and decreases with each diastole (Bynke and Schéle, 1967). The difference between the maximum IOP (systolic phase) and the minimum IOP (diastolic phase) is known as the ocular pulse amplitude (OPA). Despite differences in methodologies, a number of studies show that the OPA is between 2 to 3 mmHg (Forbes, Pico and Grolman, 1974; Piltz *et al.*, 1985; Nanba *et al.*, 1989; Trew *et al.*, 1991).

Furthermore, a second, lower frequency spectral component of the ocular pulse waveform is related to the respiratory cycle (Moses and Arnzen, 1983; Akselrod *et al.*, 1985) with a slower frequency of 1 every 3 or 4 seconds (Leydhecker, 1976 and Perkins, 1981). Investigating the

effects of respiration on IOP is a complex task as it is practically and ethically difficult to manipulate systematically the respiratory cycle and therefore little relevant literature exists. An early study (using contact tonometry) suggests that inspiring and expiring increases and decreases IOP respectively, the difference being approximately 4mmHg (Leydhecker, 1976). A later study shows that successive IOP measures taken with a NCT at the peak and trough of the cardiac cycle showed a variation of only 0.8±0.4 mmHg which was attributable to the respiratory cycle (Nanba *et al.*, 1989).

During GCT, the OPA can be observed as the rhythmic oscillations in the Goldmann mire images. To date, these oscillations have not been quantified in the spatial and temporal domains. Since the GCT probe is in contact with the cornea for approximately 2 seconds (Myers and Scott, 1975) and therefore over several cardiac cycles, the variability in IOP measures is reduced. In contrast, during NCT the fluctuations in IOP manifest as variability in successive IOP measures (Piltz *et al.*, 1984). As mentioned earlier NCTs take an IOP reading in approximately 1 to 3 ms, which is approximately $1/500^{th}$ of a cardiac cycle (Forbes *et al.*, 1974). Therefore, the measure is random with respect to the phase of the cardiac cycle and the spread in successive IOP readings using NCT is typically 4 mmHg (Forbes *et al.*, 1974, Piltz *et al.*, 1985). However, Vernon (1993) reported that on repeat NCT measures, approximately 60% of his subject group had a range of IOP greater than 4 mmHg and this range increased to more than 10 mmHg in about 8% of the subjects. Consequently, the manufacturers of NCT recommend that an average of 3 or 4 readings are taken to increase the precision of the IOP measure and this has been validated by McCaghrey and Matthews (2001).

It is clear from the above discussion that the short measurement time of the NCT renders the IOP readings susceptible to short-term variations caused by the cardiac and respiratory cycles. The previous chapter concluded that the relationship between IOP and accommodation is characterized by substantial inter-subject variations in IOP responses. However, it is postulated that the fluctuations in IOP associated with the cardiac and respiratory cycles may mask the true effect of accommodation on IOP. Therefore, the aim of this study was to devise a method of IOP measurement in which the spread of IOP readings caused by biological cycles is reduced to permit a more precise investigation of the effects of accommodation on IOP.

5.2 Methods

5.2.1 Subject group

Seventy one subjects (38 males and 33 females) varying in age from 18 to 31 years (mean 21.2±2.7 years) were recruited from the undergraduate population at Aston University. In order to investigate different experimental aspects, the cohort was split into 3 separate groups. Group 1 comprised of 11 subjects (7 males and 4 females) with a mean age of 21.7±2.5 years (range; 20 to 27 years). Group 2 consisted of 50 subjects (25 males and 25 females) with a mean age of 21.2±2.9 years (range; 18 to 31 years) and Group 3 was made up of 10 subjects (6 males and 4 females) with a mean age of 20.7±1.9 years (range; 19 to 24 year).

The research followed the tenets of the Declaration of Helsinki and was approved by the Institution's ethics committee (**Appendix 3**). Written consent was obtained from all subjects willing to participate in the study and copies of the information sheets and consent forms given to the subjects can be found in **Appendix 4.** The visual acuity of all the subjects was 0.00 logMAR or better. All subjects were absent of ocular pathology. None of the subjects were taking any topical or systemic medications that may affect the IOP or cardiovascular function.

5.2.2 Stimulus

Throughout the IOP measurement period, each subject was required to view monocularly [with their left eye (LE)] a stationary high contrast (90%) Maltese cross target placed in open space at 6m. At this distance the target represented a stimulus with minimal accommodative demand and therefore minimised any effects of accommodation on IOP. Furthermore, instructing the subjects to view the Maltese cross target meant that the subject's fixation was kept relatively steady allowing efficient tonometry in the right eye (RE).

5.2.3 Instrumentation

The small dimensions of the *EasyEye Pulsair* allowed the instrument to be mounted on an Ealing optical bench. The setup allowed unhindered IOP measurements in the RE while the LE maintained steady fixation of the Maltese cross target.

A *MLT1010* pulse-transducer (AD Instruments, USA) (**Figure 5.1**) was coupled with a *LabView* Acquisition Programme (National Instruments, USA) to display graphically the cardiac cycle (**Figure 5.2**).

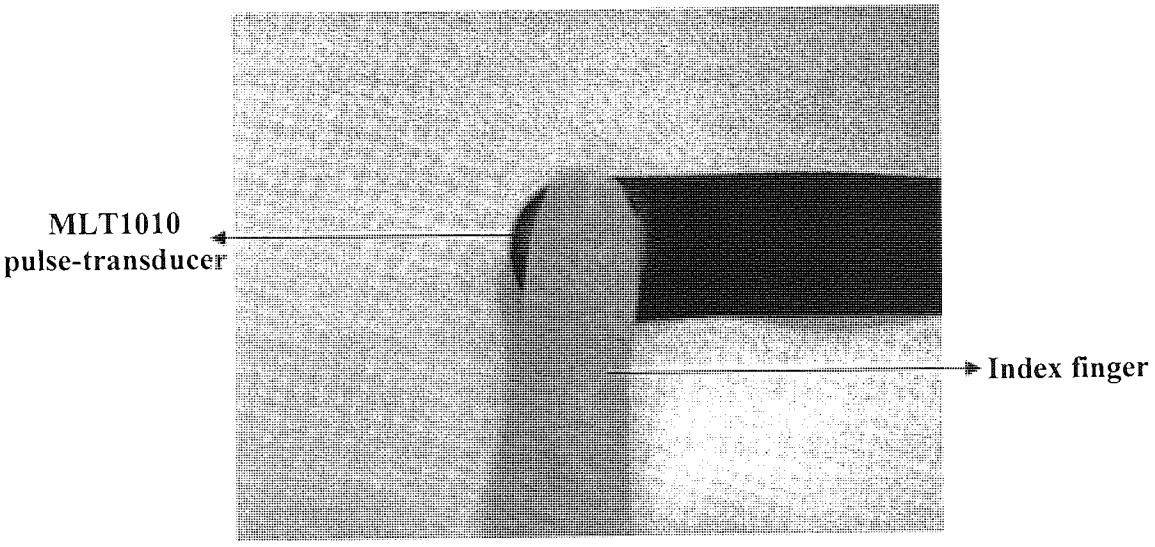


Figure 5.1 The *MLT1010* finger pulse-transducer.

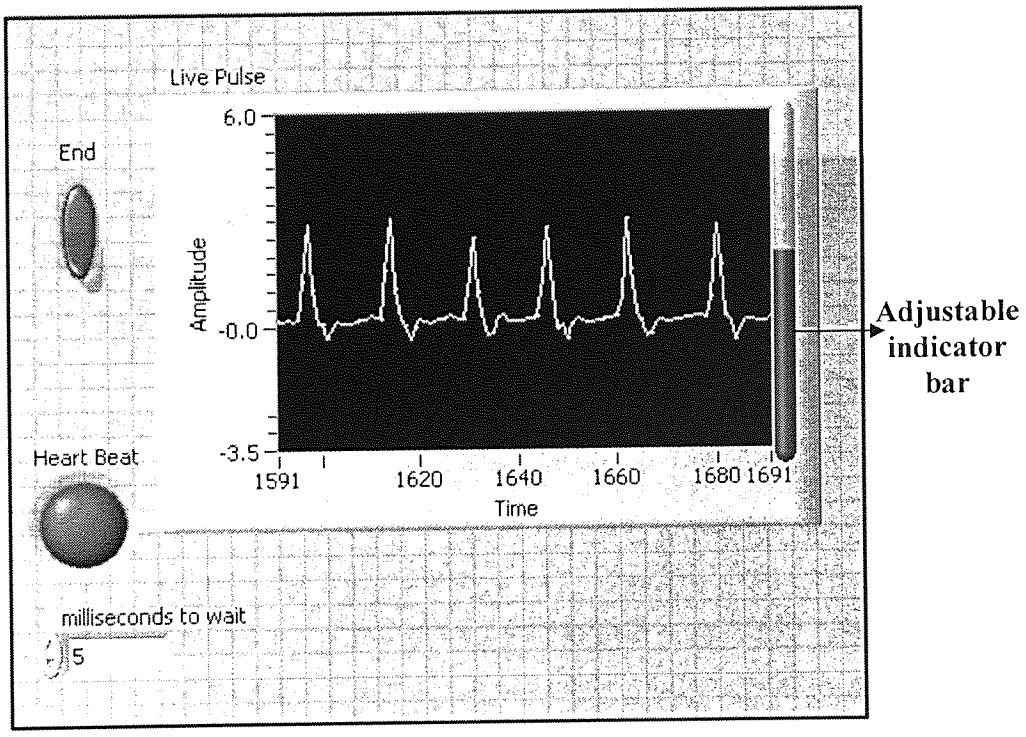


Figure 5.2 Graphical display of the signal obtained from the pulse-transducer (representing the cardiac cycle) in a *LabView* Acquisition programme window.

The pulse-transducer is a device in which the piezo-electric element on the surface of the finger pulse-transducer detects the expansion and contraction of the finger circumference due

to changes in blood volume. The transducer converts the force applied into an electrical signal of between 50 to 200 mV.

Figure 5.3 shows a schematic diagram of the connection between the pulse transducer and the *LabView* Ac quisition programme. The pulse-transducer was connected to a B NC 2090 input/output (I/O) Board via a BIO Amplifier. The BNC 2090 I/O Board is capable of reading signals between ±0.5 and ±10 V. Since the pulse transducer emits a relatively small electrical signal, a BIO Amplifier was required to amplify the signal to approximately 6 V; a magnitude which could be read by the BNC 2090 I/O Board. The I/O board was in turn connected to a personal computer interface card (PCI 6024E) (National Instruments, USA) in a desktop computer. All the connections were made using BNC cables apart from the link between the I/O board and the PCI card which was made with a multi-way cable.

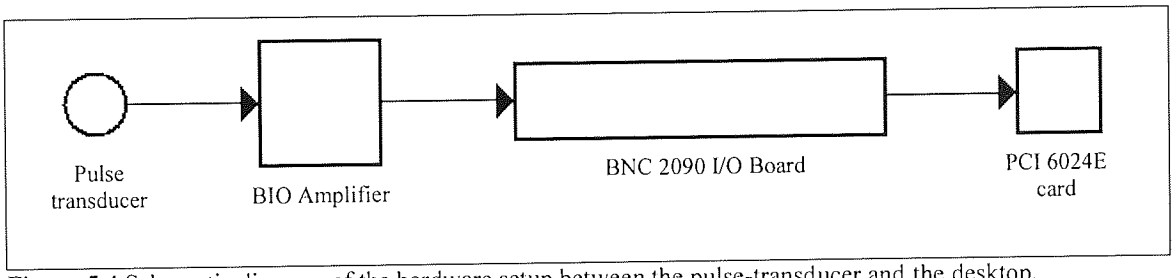


Figure 5.4 Schematic diagram of the hardware setup between the pulse-transducer and the desktop.

The use of a piezo-electric finger pulse-transducer in conjunction with a *LabView Acquisition* programme to display and monitor the cardiac cycle has been used successfully in a previous research study Davies, Wolffsohn and Gilmartin (2005).

In collaboration with the manufacturers (Keeler, UK) of the *EasyEye Pulsair* NCT, the instrument's automatic firing mechanism (on correct alignment with the eye) was disabled. This was achieved by the addition of a small circuit to the main card inside the mother unit of the *EasyEye Pulsair*. A control wire (enable/disable wire) from this circuit was connected to a spare conductor inside the umbilical cord which was extended to the RS232 port on the side of the unit. A RS232 bi-directional cable of which one cable was the control wire (with a red banana type plug) and the other cable was the ground connection (with a black banana type plug) wire were connected to the BNC 2090 I/O Board with BNC cables via an oscilloscope. When the modified *EasyEye Pulsair* unit was connected to the BNC 2090 I/O Board the extra circuit in effect ensured that if the voltage to the unit was below 0.4 V the centre photo-diode had excessive light on it, and therefore the instrument could not fire a puff of air. Conversely,

if the voltage to the unit was above 0.4 V the automatic firing mechanism (on correct alignment with the eye) was enabled. The connections between the PCI 6024E card and the modified *EasyEye Pulsair* unit are summarised in **Figure 5.4**.

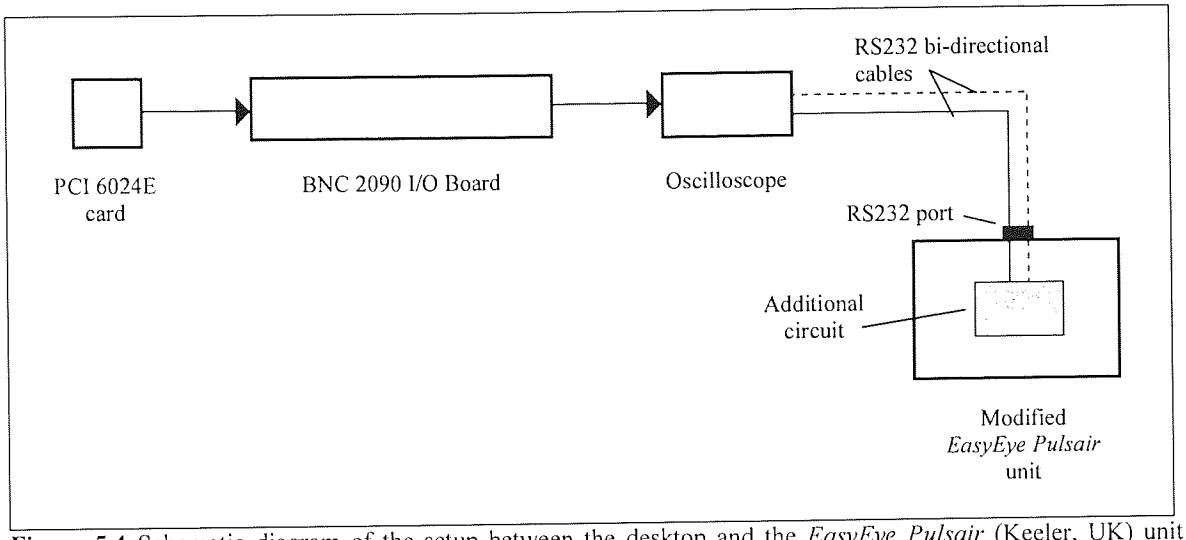


Figure 5.4 Schematic diagram of the setup between the desktop and the *EasyEye Pulsair* (Keeler, UK) unit where --- represents the red wire (enable/disable) and — represents the black wire (ground connection) of the RS232 bi-directional cable.

The *LabView Acquisition* programme permitted the adjustment of the vertical position of the indicator bar in the *LabView* window (shown in **Figure 5.2**) so that it could be aligned with designated positions on the cardiac trace i.e. the peak, middle or trough of the cycle. Two *LabView Acquisition* programs (written by Associate Supervisor Dr. J.S. Wolffsohn, Aston University) ensured that when the point on the cardiac cycle corresponding to the setting of the indicator bar was recognized, a voltage of 10 V (displayed on the oscilloscope) was sent to the NCT. Since this voltage was greater than that required (0.4 V) to enable the *EasyEye Pulsair* NCT, the instrument fired a puff of air on correct alignment of the tonometer with the eye.

The first *LabView Acquisition* programme was written so that an output voltage was fired only when the input signal was greater than the indicator bar setting. With this programme, IOP measures were obtained at the peak (**Figure 5.5a**) of the cardiac cycle. The second *LabView Acquisition* programme was written so that an output voltage was fired only when the input signal was increasing and was within ± 0.2 V of the indicator bar setting. This programme allowed IOP measures to be taken at the middle (**Figure 5.5b**) and trough (**Figure 5.5c**) of the cardiac cycle. Note here that there was some inherent variation in the alignment

of the indicator bar as the relative positions of the peak, middle and trough of the cardiac cycle were estimated by direct inspection of the cardiac trace for each individual. However, peak detection could not be used as the peak would have been missed before the pulse amplitude had changed enough for the computer to have recognised a peak had occurred.

(a)

(b)

(c)

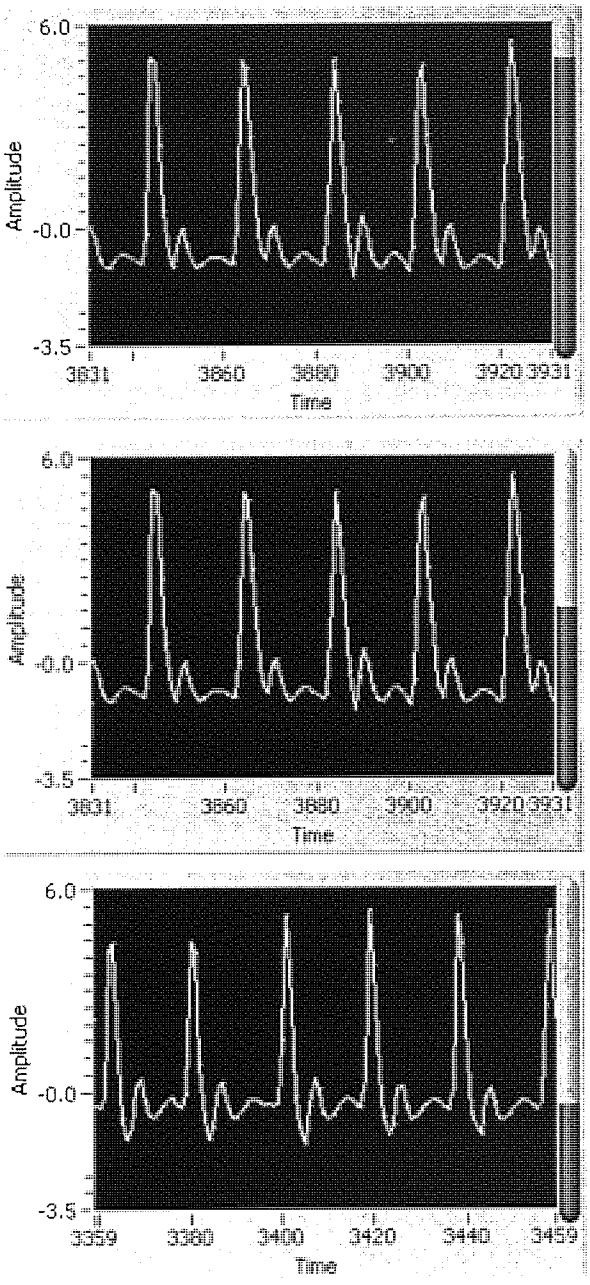


Figure 5.5 The indicator bar corresponding to the peak (**a**), middle (**b**) and trough (**c**) of the cardiac trace.

It was practically difficult to synchronise the IOP readings with definite points of the respiratory cycle (e.g. when the subjects were inhaling or exhaling). Therefore, a digital quartz metronome was set at 60 beats per minute and each subject was instructed to inhale for 2 beats and exhale for 2 beats. This procedure obtained a constant pace respiratory cycle at

15 breaths/minute as the typical frequency of the respiratory cycle is known to be 1 every 4 seconds (Leydhecker, 1976 and Perkins, 1981).

5.2.4 Experimental Procedures

Ametropia was corrected in the LE of each subject using soft daily disposable contact lenses (1-day *Acuvue Dailies*, Etafilcon A, Johnson & Johnson, Vistakon, USA).

Sufficient time was given to each subject to obtain and maintain a constant pace respiratory cycle at 15 breathes per minute which was maintained throughout the IOP measurement period.

A familiarisation procedure was performed in which two NCT measures were taken (but not recorded) to allow the subject to experience the discharge of an air-pulse. This procedure was important as there is evidence that patients express surprise and anxiety during non-contact tonometry which can increase the spread of IOP measures (Forbes *et al.*, 1974). In addition, an increase in muscular tone during tonometry can also increase the IOP (Moses *et al.*, 1984).

After the familiarization procedure the participants were given a 10 minute rest period. The pulse-transducer was wrapped firmly around the index finger of the left hand with the *Velcro*® strap. The transducer was manipulated until the optimum signal was recorded and displayed by the *LabView* Acquisition programme (National Instruments, USA) by repositioning the transducer on the finger, adjusting the tightness of the Velcro® strap or by increasing the temperature of the volunteer's hand. The transducer was highly sensitive to movement and therefore, to avoid any motion artefacts in the signal, the subjects were instructed to keep their left hand extremely still and it was steadied by resting it on a table beside the tonometers.

Stage 1

The within-session repeatability for the pulse-synchronised and pulse-unsynchronised IOP measures was assessed using Group 1 (see **section 5.2.1**). The effect of repeated tonometric measures on IOP was also determined in this group.

The height of the indicator bar was manually positioned to correspond (by direct inspection) with the peak, middle or trough of the cycle as shown in **Figure 5.5a, b** and **c**, respectively.

The corresponding *LabView* Acquisition programme was used to obtain 10 consecutive IOP measures at the peak, middle and trough of the cardiac cycle. Another 10 IOP measures were obtained with a standard unmodified *Pulsair EasyEye* (Keeler, UK). This instrument was not modified, i.e. the tonometer was not mounted on an optical bench nor was it customized to be able to take pulse synchronised measures, which mimics the normal use of the NCT in optometric practice. Furthermore, 10 IOP measures were taken with a GCT after instilling 1 drop of 0.5% Proxymetacaine hydrochloride with 0.25% fluorescein (combined Minims® single dose applicator Chauvin Pharmaceuticals, Ltd.) into the lower fornix of the eye. As the Goldmann mires fluctuated due to the ocular pulse, the measurement was always taken in mid-cycle.

Each set of 10 measurements obtained with the five measurement methods (i.e. GCT, unmodified NCT, and peak, middle and trough synchronised) were randomised although GCT was performed last due the decrease in IOP previously found following repeated measures with the contact device (Moses, 1961; Baudouin and Gastaud, 1994) and due the putative effects of topical anaesthetics previously reported (Herse and Siu, 1992; Moseley *et al.,* 1993; Baudouin and Gastaud, 1994; Birchall and Kumar, 2001; Asensio *et al.,* 2003; Nam *et al.,* 2006). Following each set of 10 measurements, a 5 minute rest period was given to the participant.

Stage 2

In group 2 (see **section 5.2.1**), the IOP measures obtained with an unmodified *EasyEye Pulsair*, and those obtained at the peak, middle and trough of the cardiac cycle were compared to IOP readings taken with the GCT to assess the accuracy of the measurement methods. Furthermore, the magnitude of the OPA was determined from the IOP readings taken at the peak and trough of the cardiac cycle (OPA=peak IOP-trough IOP).

Once a fixed pace respiratory cycle was achieved, 4 consecutive IOP measures [as recommended by Keeler (UK) and validated by McCaghrey and Matthews (2001)] were taken in the RE with an unmodified *Pulsair EasyEye* tonometer and with the GCT (method described above). A further 4 IOP measures were taken with the modified NCT at the peak, middle and trough of the cardiac pulse trace (i.e. 12 measures in total).

The measurement methods were randomised, and since it is known that repeat measures (Moses, 1961; Baudouin and Gastaud, 1994) and topical anaesthetics (Herse and Siu, 1992; Moseley *et al.*, 1993; Baudouin and Gastaud, 1994; Birchall and Kumar, 2001; Asensio *et al.*, 2003; Nam *et al.*, (2006) used with the GCT influence the steady-state IOP, this procedure was performed last. Following each set of 4 measurements, a 5 minute rest period was given to each participant.

Stage 3

From ***Stage 2***, it was shown that synchronising the IOP measures with the middle of the cardiac cycle significantly reduced the variance in IOP readings (see **section 5.4**). Accordingly, the inter-sessional repeatability was assessed using the subjects in Group 3 (see **section 5.2.1**), by taking 10 IOP measurements synchronised with the middle of the cardiac cycle on two different sessions. The 2 sessions for data collection were scheduled on 2 different days at approximately the same time to minimize the effects of diurnal fluctuations on IOP which have previously been reported (Pointer, 1997; Noel *et al.*, 2001; Liu *et al.*, 2003; Kida *et al.*, 2006). The data collected in the first session of ***Stage 3*** of the experimental procedures was used to determine the number of measurements required to obtain a reliable IOP measure.

Although a familiarization procedure was undertaken, invariably the volunteers blinked and 'jumped' when an IOP measure was taken with the NCT. This disturbed the alignment of the NCT, the regularity of the cardiac trace and the fixed pace respiratory cycle. Therefore, in all of the above experiments successive IOP measures were only taken when a steady cardiac cycle signal, correct alignment of the NCT with the eye and a fixed pace respiratory cycle were obtained.

5.3 Statistical Analysis

Software packages *SPSS 12.1* for Windows, *StatView 5* (SAS Institute Inc, USA) and *Microsoft Excel* were used to perform the statistical tests.

From the experimental procedures performed in ***Stage 1*** the coefficient of variation (COV) was calculated for each measurement method to assess the intra-sessional repeatability. The COV is a statistical measure of the relative dispersion of data points in a data series with respect to the mean. The COV is calculated as the standard deviation (SD)/mean. Statistical

comparisons between the COV for each measurement methods were performed by using Wilcoxon signed rank tests as carried out by Yaoeda *et al.* (2005). Wilcoxon signed rank tests are the non-parametric alternative to the paired t-test.

In addition the effect of repeated measures on IOP with each measurement method was determined by performing a repeated measures ANOVA and by calculating the Pearson's product moment correlation coefficient (r) between the IOP reading and the number of measurements (1-10). The Pearson's correlation coefficient is the ratio between the covariance (variance shared by the two variables) and a measure of the separate variances. In the present study and throughout this thesis the significance of r is assessed at the 0.05 level. The r^2 value denotes the percentage of the variance in 1 variable that is explained by the variance in the other variable.

To determine the effect of measurement method on IOP readings, the data in ***Stage 2*** of the experimental procedures were treated to a within-subjects one-way analysis of variance (ANOVA) in randomised blocks followed by Scheffe and Bonferroni *post-hoc* analysis. The significance level taken for the Bonferroni *post-hoc* analyses was 0.005 (since 10 comparisons were performed). An ANOVA is a statistical procedure that uses the F-ratio to test the overall fit of a linear model i.e. tests whether the group means differ. The calculated F-ratio is a ratio of the between-groups variance to the within-groups variance. A number of *post-hoc* analyses are available and vary depending on whether the F-statistic is significant, the number of subjects used in each condition, the normality of the data and the degree of protection required against Type I and II errors (Armstrong *et al.*, 2002).

Agreement between the IOP readings taken with the GCT and those measurements obtained with the unmodified NCT and peak, middle and trough synchronised measures was determined by calculating the Pearson's product moment correlation coefficients and by the method of Bland and Altman (1986). In addition to the COV calculated from data obtained in ***Stage 1*** of the experimental procedures, variance ratio tests (assessed against 2-tailed F-tables, p=0.05) were performed on the data taken in ***Stage 2*** to establish which measurement method exhibited the least variance in IOP data.

Data from ***Stage 3*** was used to assess the inter-session repeatability using the method of Bland and Altman (1986) and by calculating the coefficient of repeatability (COR). The COR

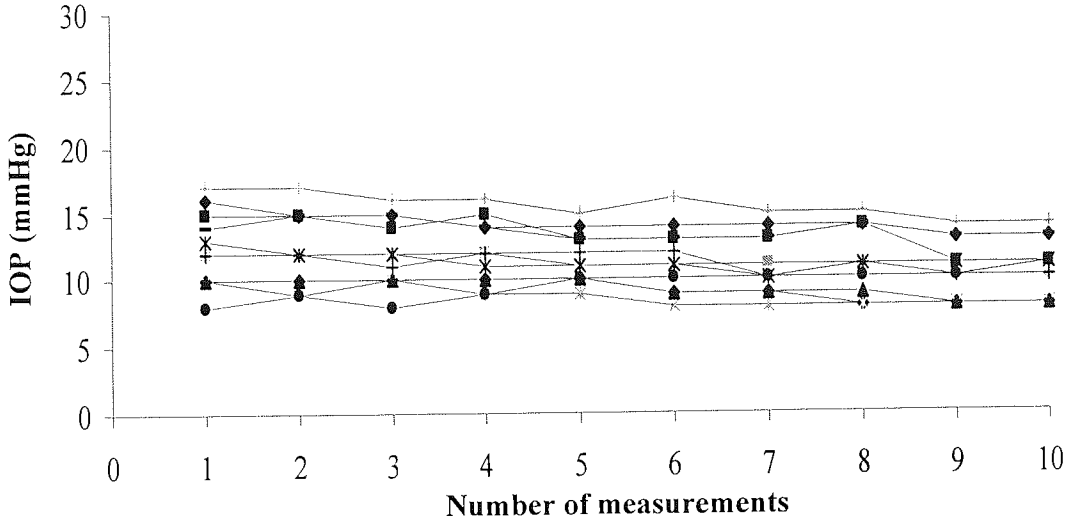
is calculated as 1.96*SD of the differences. In addition the minimum number of pulse synchronised IOP measures required after which any additional measurements had no significant effect on the SD or mean of the IOP measurements was determined by plotting the SD as a function of the number of measurements and identifying the point at which the graph reached a stable plateau. In addition the minimum number of measurements required was also assessed by a repeated measures ANOVA on the running average.

5.4 Results

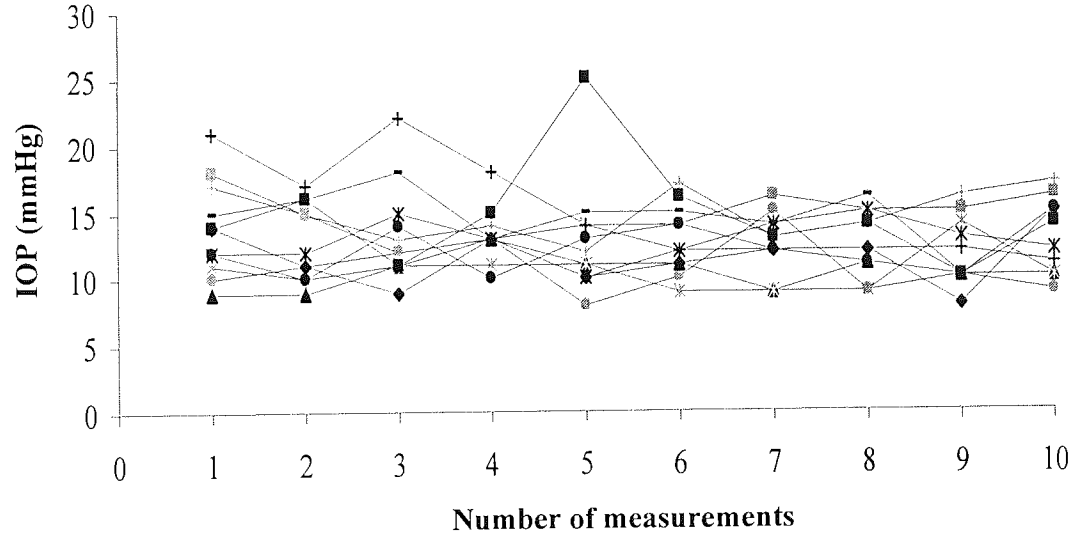
The COV of IOP measures taken with GCT, unmodified NCT, and peak, middle and trough synchronized were 8.2, 17.1, 9.9, 9.1 and 12.0 %, respectively. The Wilcoxon signed rank tests showed that the COV of the NCT was significantly larger than the COV of the GCT and the peak, middle and trough synchronised IOP measures ($p=0.003$, $p=0.008$, $p=0.003$ and $p=0.013$, respectively). The COV of the GCT was significantly smaller than the COV of the trough synchronised measures ($p=0.008$), but was not significantly different to the COV of the peak ($p=0.062$) and middle ($p=0.213$) synchronised IOP measures. The COV of the middle synchronised IOP measures was significantly smaller than that for the trough synchronised IOP measures ($p=0.041$) but was not significantly different to the COV of the peak ($p=0.790$) synchronised IOP measures. Furthermore, the difference between the COVs of the peak and trough synchronised IOP measures approached significance ($p=0.075$).

A highly significant, albeit weak correlation was exhibited between the IOP measures taken with the GCT and the number of measures taken ($r=0.26$, $p=0.006$). Using linear regression the estimated decrease in IOP after 10 consecutive measures with the GCT was 1.94 mmHg. In contrast, the correlation between the IOP measures taken with the unmodified NCT ($r=0.14$, $p=0.14$) and the peak ($r=0.03$, $p=0.74$), middle ($r=0.02$, $p=0.86$) and trough ($r=0.02$, $p=0.84$) synchronised measures and the number of measurements taken did not reach statistical significance. The corresponding graphs are shown in **Figure 5.6**.

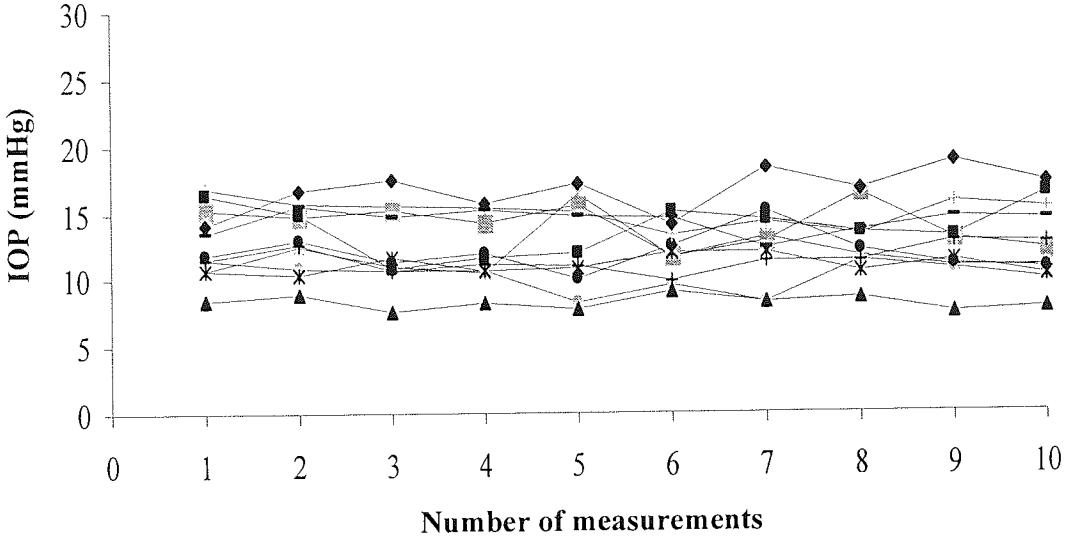
(5.6a)


IOP (mmHg)
30
25
20
15
10
5
0
0 1 2 3 4 5 6 7 8 9 10
Number of measurements

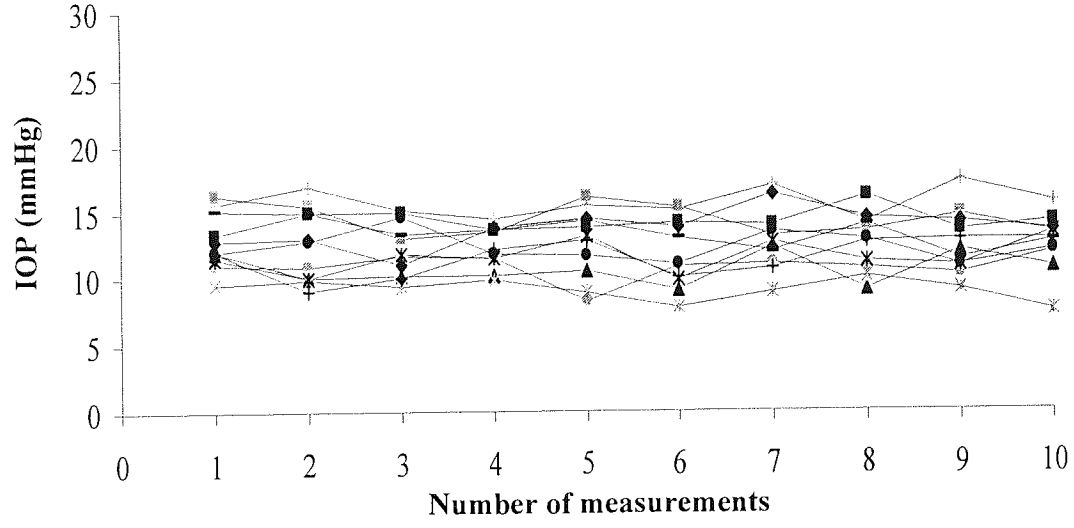
(5.6b)


IOP (mmHg)
30
25
20
15
10
5
0
0 1 2 3 4 5 6 7 8 9 10
Number of measurements

(5.6c)


IOP (mmHg)
30
25
20
15
10
5
0
0 1 2 3 4 5 6 7 8 9 10
Number of measurements

(5.6d)


IOP (mmHg)
30
25
20
15
10
5
0
0 1 2 3 4 5 6 7 8 9 10
Number of measurements

(5.6e)

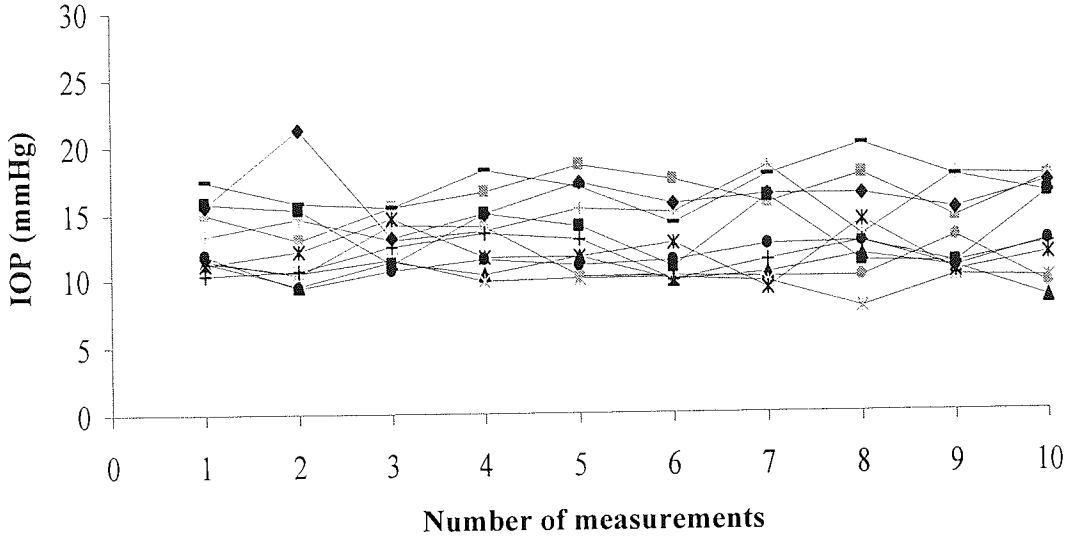


Figure 5.6 The effect of repeated measures on IOP taken with the GCT (**a**), unmodified NCT (**b**) and peak (**c**), middle (**d**) and trough (**e**) synchronised measures where ▲, *, ●, -, ♦, ■ represent subjects 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 and 11, respectively.

Furthermore, the repeated measures ANOVA analyses was significant only for the data taken with the GCT ($F(9, 90)=9.481$, $p=<0.001$) but not for the IOP data obtained with the unmodified NCT ($F(9, 90)=0.678$, $p=0.623$) and with the peak ($F(9, 90)=0.572$, $p=0.711$) middle ($F(9, 90)=0.742$, $p=0.595$) and trough ($F(9, 90)=0.508$, $p=0.752$) synchronised.

Table 5.1 shows the mean IOP findings for the two tonometers (i.e. unmodified NCT and GCT) and the three positions on the cardiac cycle (peak, middle and trough). The results of the one-way ANOVA show that the differences between the IOP measurements taken with the two tonometers and the three positions on the cardiac cycle were unlikely to have arisen by sampling error and were therefore significantly effected by the method of measurement used ($F(2.68, 131.53) = 14.18$, $p=<0.001$). The results from Scheffe and Bonferroni *post-hoc* analyses are shown in **Table 5.2**. The results of Scheffe's *post-hoc* analyses suggest that there were no significant differences in IOP between any of the measurement methods although the difference between IOP measures obtained with the unmodified NCT and the trough synchronised measures approached significance. However, Bonferroni's *post-hoc* analyses depicts that the IOP measures taken with the unmodified NCT differed significantly from all other measurement methods.

Method of IOP measures	Mean IOP (mmHg)	± SD (mmHg)	Range of IOP (mmHg)
Goldmann	14.21	2.96	8.33-23.0
Pulsair	15.61	3.21	10.33-23.0
Peak	14.07	3.08	8.58-23.61
Middle	13.94	3.13	8.19-22.77
Trough	13.75	3.05	8.41-21.44

Table 5.1 Mean IOP measures taken with the 5 different measurement techniques (n=50).

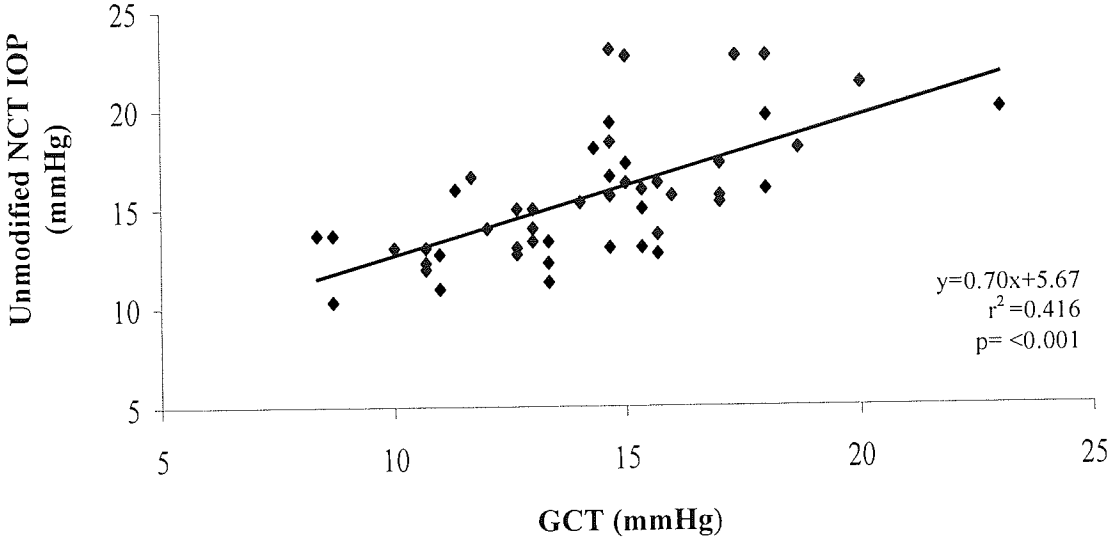
Method of IOP measurement	Mean difference (mmHg)	Scheffe p value	Bonferroni p value
Goldmann-Pulsair	-1.40	0.28	<0.001*
Goldmann-peak	0.14	0.99	0.56
Goldmann-middle	0.27	0.99	0.17
Goldmann- trough	0.47	0.97	0.07
Pulsair-peak	1.54	0.19	<0.001*
Pulsair-middle	1.67	0.12	<0.001*
Pulsair-trough	1.87	0.06 •	<0.001*
Peak-middle	0.13	0.99	0.50
Peak-trough	0.33	0.99	0.14
Middle-trough	0.20	0.99	0.39

Table 5.2 Scheffe and Bonferroni *post-hoc* analyses where • denotes near significant differences and * denotes significant differences (n=50).

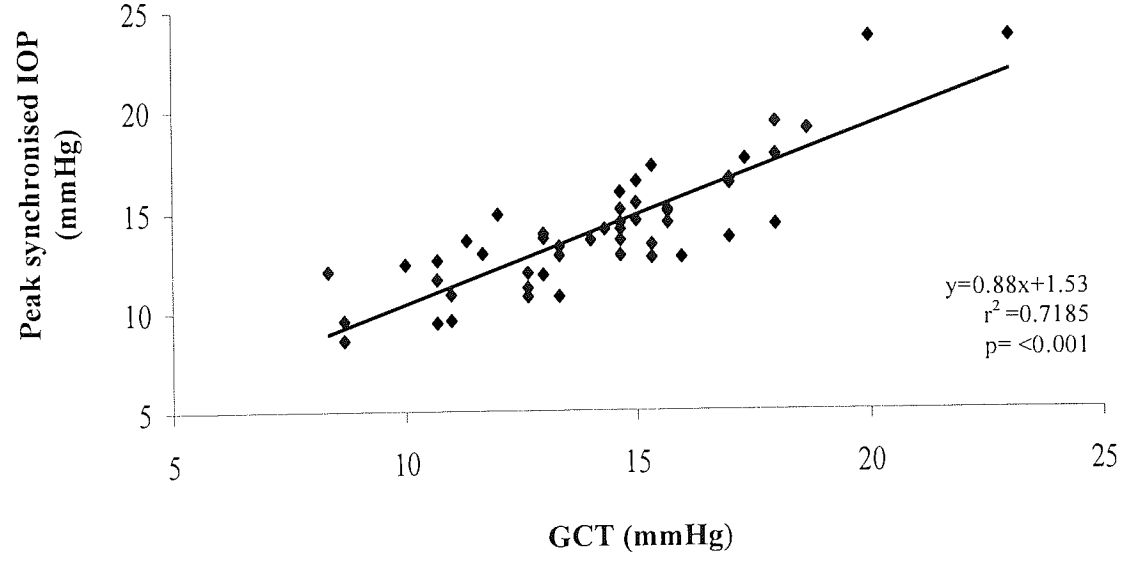
The difference between the peak synchronised and trough synchronised IOP measures (i.e. the OPA) was 0.33±1.54 mmHg and failed to reach statistical significance using both Scheffe's ($p=0.99$) and Bonferroni's ($p=0.14$) *post-hoc* analyses.

As expected the IOP measures taken with the GCT significantly correlated with those taken with the unmodified NCT ($r=0.64$, $p<0.001$) and those taken at the peak ($r=0.85$, $p<0.001$), middle ($r=0.90$, $p<0.001$) and trough ($r=0.83$, $p<0.001$) of the cardiac cycle. The corresponding correlation graphs are shown in **Figure 5.7**.

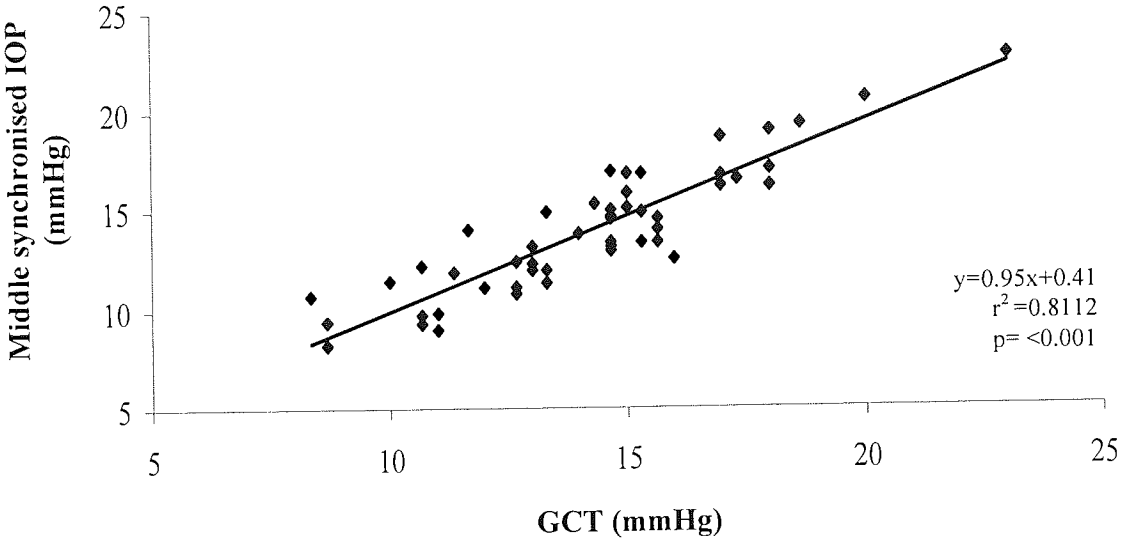
(5.7a)


Unmodified NCT IOP (mmHg)
25
20
15
10
5
5
10
15
20
25
GCT (mmHg)
y=0.70x+5.67
r2 =0.416
p= <0.001

(5.7b)


Peak synchronised IOP (mmHg)
25
20
15
10
5
5
10
15
20
25
GCT (mmHg)
y=0.88x+1.53
r2 =0.7185
p= <0.001

(5.7c)



(5.7d)

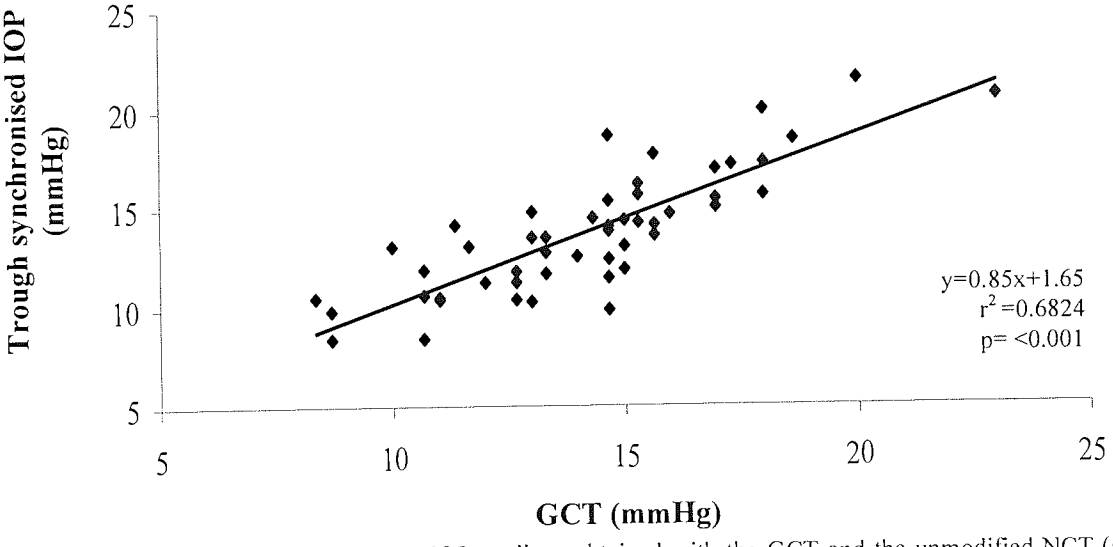


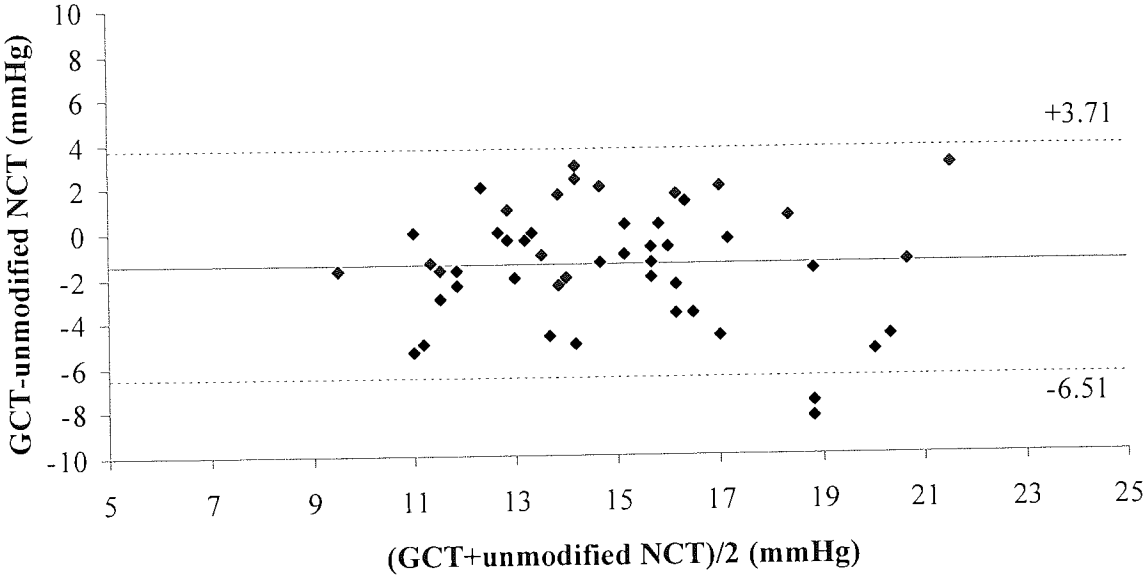
Figure 5.7 Correlation graphs between IOP readings obtained with the GCT and the unmodified NCT (**a**) and peak (**b**), middle (**c**) and trough (**d**) synchronised measures (n=50).

Bland and Altmann dispersion plots of the differences in IOP between the unmodified NCT, the peak, middle and trough synchronised IOP measures and the GCT as a function of their means are plotted in **Figure 5.8** **Table 5.3** shows the mean difference between GCT and the 4 other measurement methods and their 95% confidence intervals (CI). The largest mean difference and 95% CI was exhibited by the unmodified NCT. In contrast, the smallest mean difference and 95% CI was exhibited by the middle synchronised IOP measures where 90 and 98% of the differences in IOP were respectively within 2 and 3 mmHg compared to GCT (**Table 5.3**).

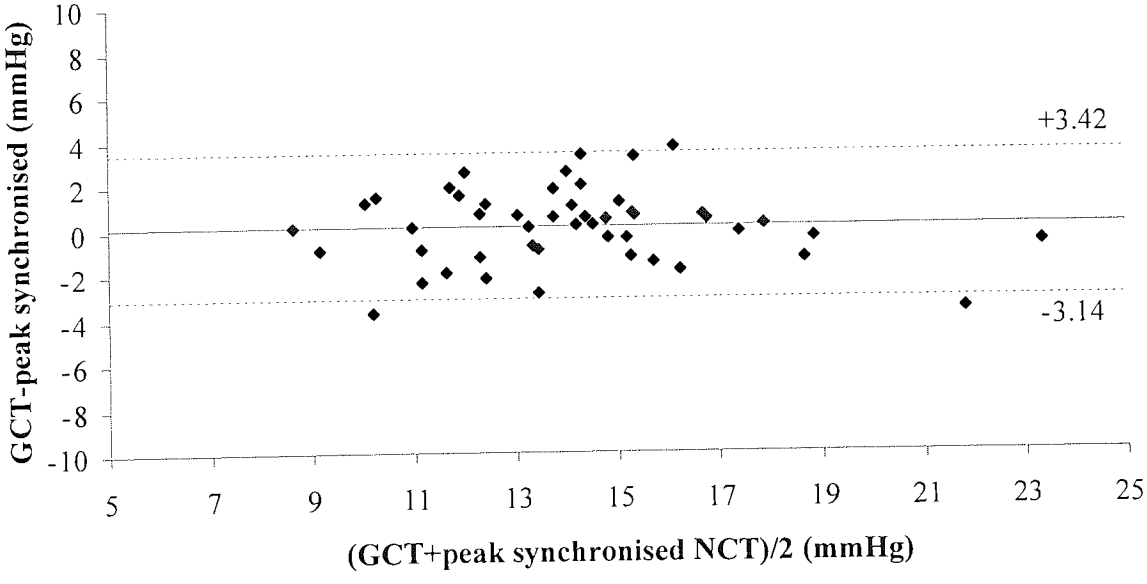
Measurement method	Mean±SD (mmHg)	95% CI (mmHg)	Difference within ±1mmg (%)	Difference within ±2mmHg (%)	Difference within ±3mmHg (%)
Unmodified NCT *vs.* GCT	-1.40±2.61	±5.11	30	64	78
Peak synchronised *vs.* GCT	+0.14±1.67	±3.28	50	78	90
Middle synchronised *vs.* GCT	+0.27±1.37	±2.68	48	90	98
Trough synchronised *vs.* GCT	+0.47±1.77	±3.48	40	72	90

Table 5.3 Mean differences and the 95% CI between GCT and the 4 other measurement methods (n=50).

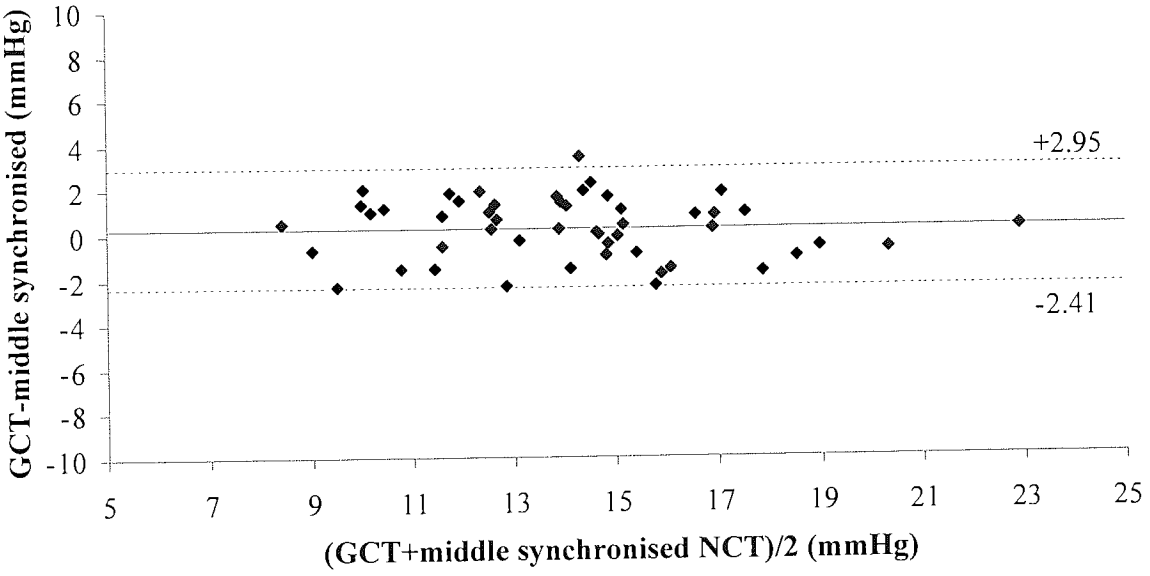
(5.8a)


GCT-unmodified NCT (mmHg)
10
8
6
4
2
0
-2
-4
-6
-8
-10
+3.71
-6.51
5
7
9
11
13
15
17
19
21
23
25
(GCT+unmodified NCT)/2 (mmHg)

(5.8b)


GCT-peak synchronised (mmHg)
10
8
6
4
2
0
-2
-4
-6
-8
-10
+3.42
-3.14
5
7
9
11
13
15
17
19
21
23
25
(GCT+peak synchronised NCT)/2 (mmHg)

(5.8c)



(5.8d)

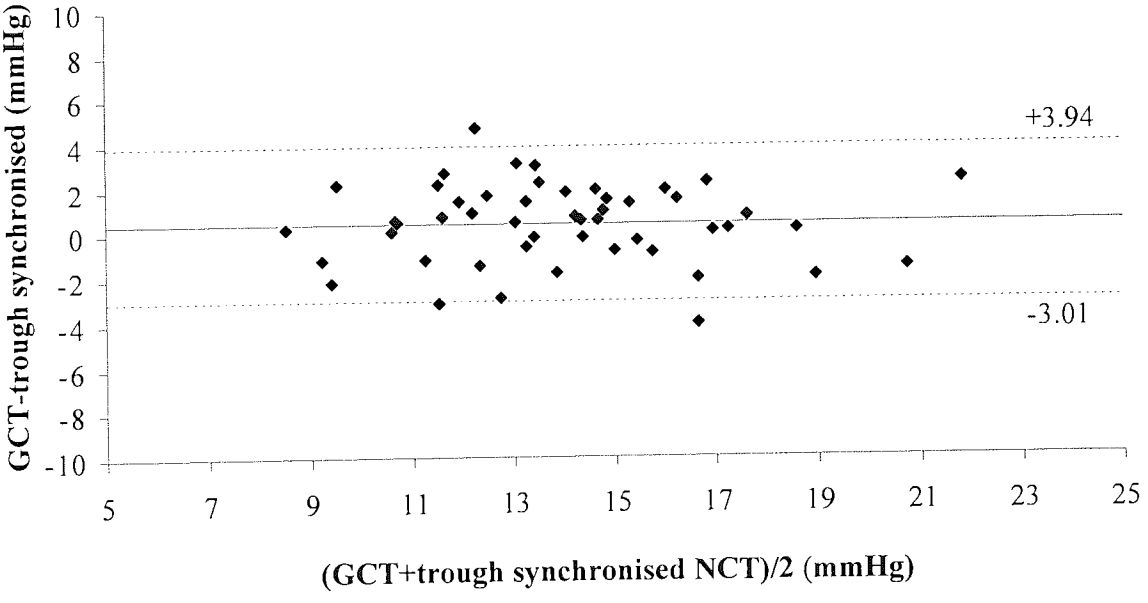


Figure 5.8 Difference between IOP measures from GCT and unmodified NCT (**a**) and peak (**b**), middle (**c**) and trough (**d**) synchronised measures as a function of their mean where — represents the mean bias and the --- represents the 95% limits of agreement.

The variance in IOP measures obtained from the unmodified NCT and those taken at the peak, middle and trough of the cardiac cycle was 14.36, 10.76, 10.69 and 11.03, respectively. The variance in IOP measures was statistically higher in the measures obtained with the unmodified NCT compared to those taken with the peak, middle and trough of the cardiac cycle synchronised with the modified NCT (**Table 5.4**).

Instrument used	Variance ratio	p value
Pulsair-Peak	1.33	0.02*
Pulsair-Middle	1.34	0.02*
Pulsair-Trough	1.30	0.03*
Peak-Middle	1.01	0.47
Peak-Trough	0.98	0.56
Middle-Trough	0.97	0.58

Table 5.4 Results from the variance ratio tests between the IOP measures taken with the unmodified NCT and those taken with the peak, middle and trough synchronised, where *represents a statistically significant difference (n=50).

From the above results it is evident that synchronisation of the NCT with the 3 locations of the cardiac cycle reduces the variance in IOP measures. Of the 3 locations, synchronising the NCT with the middle of the cardiac trace exhibits the least variance and shows a good correlation with GCT. Therefore, the inter-sessional repeatability was assessed by the method of Bland and Altmann (1986) for IOP measures synchronised with the middle of the cardiac cycle. The corresponding Bland and Altmann dispersion plot is shown in **Figure 5.9.** The mean difference between the two sessions was 0.07 mmHg (p=0.72) and the inter-sessional repeatability (i.e., COR) was ± 1.16 mmHg.

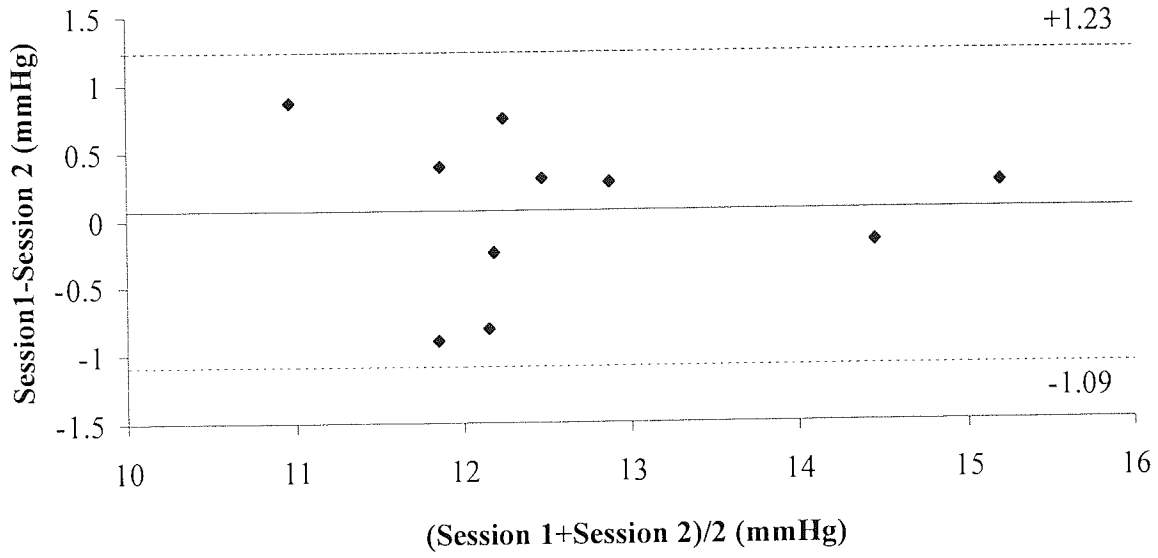


Figure 5.9 Difference between middle synchronised IOP measures taken in session 1 and session 2 as a function of their mean where — represents the mean bias, and --- represents the 95% confidence limits (n=10).

It is valuable to know how many IOP measurements are required, such that after a certain number of readings, any further measures have little effect on the IOP data when the NCT is synchronised with the middle of the cardiac waveform. A repeated measures ANOVA on the running average was insignificant ($F(9, 90) = 1.117$, $p=0.352$). **Figure 5.10** shows the number of IOP measures taken against the SD of the IOP readings. The point at which the graph begins to plateau was identified to provide an approximate guideline to the minimum number of measures required (5 measures) for the practical acquisition of a reliable IOP measure after synchronisation with the middle of the cardiac cycle.

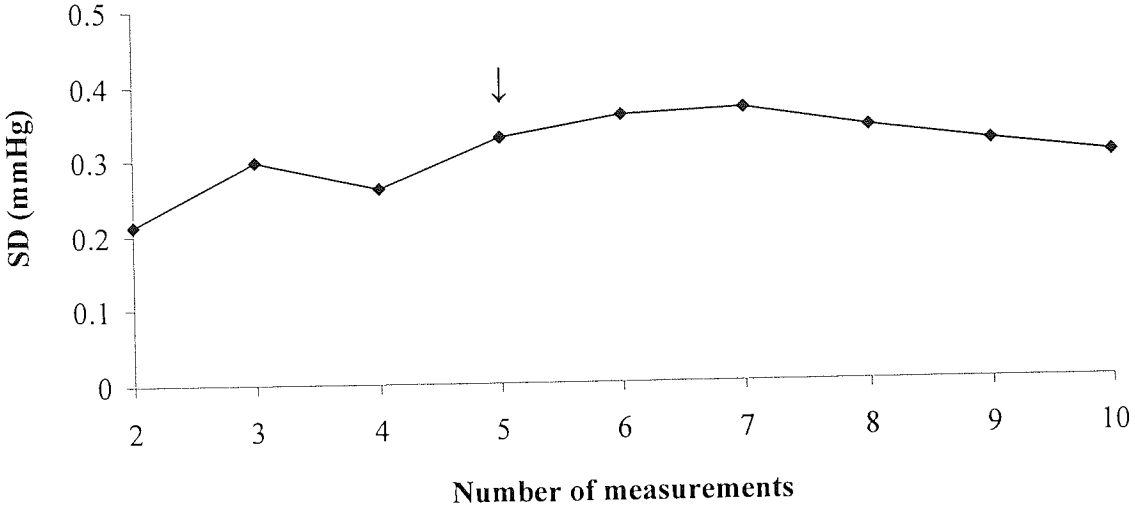


Figure 5.10 Graphical representation of the effects of consecutive measurements on the SD of the IOP measures taken when synchronised with the middle of the cardiac cycle, where ↓ denotes the point at which the graph begins to plateau (n=10).

5.5 Discussion

The aim of the present study was to derive a method of non-contact IOP measurement by which the variance associated with the cardiac and respiratory cycles was reduced. The *Pulsair EasyEye* NCT was coupled with a finger pulse-transducer and a *LabView* Acquisition programme to permit pulse synchronised IOP measurements. The modified instrumentation allowed the investigation of short-term variations in IOP caused by the cardiac cycle and identification of a position on the cardiac cycle which exhibited the least variance.

The only commercially and currently available NCT which allows pulse synchronous measures is the *Nidek NT-4000* (Nidek Co., Ltd., Japan) which monitors the cardiac cycle from the subjects forehead. Reflected light from a light emitting diode (LED) built into the forehead rest inversely changes with changes in blood flow volume in surface forehead tissue with each heart beat. These fluctuations in reflected light are recorded and analysed and on detection of 3 consistent pulse signals, the instrument identifies the peak, middle and trough of the cardiac cycle. A pulse synchronous signal is then transmitted and an IOP measure is taken and recorded (Yaoeda *et al.*, 2005). The manufacturers claim that variance in IOP measures is hence reduced with the *Nidek NT-4000* and this is subsequently validated by Lam *et al.* (2004), Yaoeda *et al.* (2005) and Queiros *et al.* (2006). In any event this NCT could not

be used to investigate the effects of accommodation on IOP due to the bulky design of the instrument. Since the *EasyEye Pulsair* NCT has a slim-line design it was the instrument of choice in the current research program.

In accordance with the recent study by Lam *et al.* (2004), the results from this study show that of the 5 measurement methods, the GCT exhibited the smallest COV. This result is explained by the reduced influence of the ocular pulse on the IOP readings as the GCT is in contact with the cornea over several cardiac cycles and the weight of the contact probe causes a 'damping' effect on the OPA (Perkins, 1981; Piltz *et al.,* 1985). Lam *et al.* (2004) also found that a standard NCT : the *Nidek NT-2000* (Nidek Co., Ltd., Japan) exhibited the highest COV compared to the peak, middle and trough synchronised measures obtained with the *Nidek NT-4000.* Lam *et al.* (2004) also reported that of the three locations on the cardiac cycle investigated in their study, IOP measures synchronised with the middle showed the lowest COV. Similar to the conclusions drawn by Lam *et al.* (2004), Yaoeda *et al.* (2005) concluded that the largest COV was demonstrated with the *Nidek NT-4000* instrument in its standard mode (i.e. no pulse detection feature) compared to the COV of measures taken with the pulse synchronous measurement function switched on. In addition, Yaoeda *et al.* (2005) also found that the middle location on the cardiac cycle exhibited the smallest COV. Queiros *et al.* (2006) concluded that although the standard error of the mean (SEM) was the highest with the *Nidek NT-4000* in its standard mode, this was however only statistically different to the SEM at the trough of the cardiac cycle and not to the SEM at the peak or middle of the cardiac trace. Hence Queiros *et al.* (2006) conclude that synchronising IOP measures with the trough of the cardiac cycle exhibited the least variance.

Similar to the results found by Lam *et al.* (2004), Yaoeda *et al.* (2005) and Queiros *et al.* (2006) the present study concludes that the unmodified NCT shows the highest COV. A greater variation in consecutive measurements was expected with the unmodified NCT as the effects of the ocular pulse on IOP were not accounted for. Of the 3 positions on the cardiac cycle investigated, this study shows that synchronising the tonometric measures with the middle of the cardiac waveform resulted in the smallest COV. In addition, the unmodified NCT shows significantly greater variance in IOP measures compared to the pulse synchronous NCT measurement methods. Although synchronising the middle of the cardiac cycle with the NCT measurements exhibited the least variance (i.e. 10.69), the variance was not significantly different to when the measures were synchronised with the peak (i.e. 10.76)

and trough (i.e. 11.03) of the cardiac cycle. On direct inspection of **Figures 5.7** and **5.8** it is clear that the spread of IOP measures is significantly reduced when the modified NCT is synchronised with the cardiac cycle and that of the three locations on the cardiac cycle investigated, the middle position exhibits the least spread in data.

Furthermore, the acquisition of data at the middle of the cardiac cycle was quicker than that at the peak of the cycle. There is inherent variance in the cardiac cycle trace displayed in the *LabView* window, particularly in the amplitude of the trace which increased or decreased in accordance with motion artefacts. Therefore, when the indicator bar in the *LabView* Acquistion programme was placed in line with the peak of the cardiac cycle (on direct inspection), at times the amplitude of the trace fell short of the indicator bar setting and hence required slight adjustments. In contrast, the middle location of each cardiac cycle was always detected by the *LabView* Acquisition programme as the signal increased from the trough to the peak of the waveform. As a result, the time required for acquisition of data at the peak was relatively longer compared to the time taken to acquire data at the middle of the cardiac trace.

When the IOP measures were synchronised with the middle of the cardiac cycle the measurements were repeatable between sessions. Furthermore, the results of the ANOVA show that 10 consecutive IOP measures were not statically different to each other. On observation of **Figure 5.10**, it is evident that after 5 IOP readings, subsequent measures had no effect on the mean or SD of the reading.

Figure 5.6 indicates that repeat measurements obtained with the unmodified NCT or those taken with the modified NCT did not exhibit a significant 'massaging effect' on the IOP measure. This is consistent with previous studies that conclude that no decrease in IOP is observed after 4 (Lawson-Kopp *et al.*, 2002), 10 (Sorenson, 1975; Forbes *et al.*, 1974) 30 (Forbes *et al.*, 1974) or even 150 (Myers and Scott, 1975) repeat IOP measures with a NCT. In accordance with previous studies a decrease in IOP on repeated applanations was found with the GCT (Stocker, 1958; Moses and Liu, 1968; Whitacre and Stein, 1993). In the present study after 10 repeat measures with the GCT the IOP reduced by approximately 1.94 mmHg. Goldmann (1957) himself observed a decrease in IOP of 2 to 3 mmHg after repeated measures (cited by Whitacre and Stein, 1993). Moses and Liu (1968) reported a slightly smaller decrease in IOP on repeat measures of 0.4±1.4 mmHg (n=75) although they noted a

decrease of more than 2 mmHg in 35 % of their subject group. The decrease in IOP with the GCT is in part associated with the longer corneal contact time of the probe (Myers and Scott, 1975) and is also related to the lowering of IOP on instillation of topical anaesthetics (Baudouin and Gastaud, 1994). Note however that NCTs probably result in more displacement of the ocular volume since the cornea is distorted past applanation into a state of concavity by the air pulse compared to the GCT (see **Chapter 3**). However, the brief impact time of the air puff (Myers and Scott, 1975; Forbes *et al.,* 1974; Grolman, 1972) probably counteracts the effects of the volume displacement during tonometry.

The data were treated to a one-way ANOVA design and this statistical analysis revealed that the measurement method influences the IOP measure. The conservative Scheffe's *post-hoc* analyses failed to show which measurement method significantly affects the IOP reading, although the Bonferroni *post-hoc* analyses suggest that only the unmodified NCT readings are significantly different from the other four measurement methods. In the current study, the unmodified NCT reads approximately 1.40 mmHg higher than the GCT for IOP measures between 8.3 and 23 mmHg. This is in agreement with a previous study that suggests that the IOP readings with a standard *Pulsair* (Keeler, UK) NCT are approximately 1.5 mmHg higher than the GCT for IOPs in the range of 6 to 27 mmHg (Mackie *et al.,* 1996). Although other studies have also shown that the *Pulsair* NCT reads higher than the GCT the difference is much smaller (Parker *et al.,* 2001; Lawson-Kopp *et al.,* 2002). The mean differences between IOP readings obtained with the GCT and the peak, middle and trough synchronised measures in the present study were not statistically significant. On average the IOP measures at the peak, middle and trough of the cardiac cycle were 0.14, 0.27 and 0.47 mmHg lower than the GCT reading, respectively.

A tonometer which yields IOP measures which fall within 3 mmHg from the GCT measures is generally considered clinically acceptable (Mackie *et al.,* 1996; Yang *et al.,* 2000; Rao *et al.,* 2001; Lam *et al.,* 2004). Bland and Altman analyses between the unmodified NCT and GCT showed relatively large limits of agreement and that only 78% of the readings taken with the unmodified NCT were within ±3 mmHg of GCT. The present study yielded a moderate correlation coefficient between the unmodified NCT and the GCT (r=0.64). The *Pulsair EasyEye* NCT is a commercially available instrument and is used in general optometric practice and therefore a better agreement with the GCT was expected as found by previous researchers with a correlation coefficient of above 0.80 (Mackie *et al.,* 1996; Parker

et al., 2001; Lawson-Kopp *et al.*, 2002). In contrast, 90% of the data taken with the peak and trough synchronised measurement methods fell within ±3mmHg of GCT. Furthermore, when the *Pulsair EasyEye* NCT was synchronised with the middle of the cardiac cycle, 90 % of the data fell within ±2mmHg of GCT and 98% fell within ±3mmHg of GCT. Thus, the results demonstrate a satisfactory agreement between the measures taken with the GCT and those taken with the modified NCT synchronised with the peak, middle and trough of the cardiac cycle.

The OPA measured with the new *Nidek NT-4000* NCT equipped with a pulse synchronous measurement function was approximately 1.3mmHg (Lam *et al.*, 2004; Yaoeda *et al.*, 2005; Queiros *et al.*, 2006). However, in the present study the mean IOP measures taken at the peak, middle and trough of the cardiac cycle were not statistically different from each other and thus the mean OPA (0.33±1.54mmHg) did not reach statistical difference. This finding may be partly attributable to limitations of the *LabView* Acquisition program to detect precisely the trough of the cardiac cycle. As described earlier, one of the *LabView* Acquisition program was written to initiate the firing of the puff of air when the signal from the pulse transducer was increasing and within 0.2 V of the indicator bar setting. On inspection of the cardiac waveform, it can be seen that following the initial rise and fall in the signal there are 2 subsequent points on the trace where the signal increases and then decreases (see **Figure 5.5**). Therefore, when the indicator was aligned with the trough of the cardiac cycle, it is possible that the programme may have recognised any of the 3 points on the cycle which meet the criteria and therefore sending a voltage to the NCT enabling it to fire. As a result the IOP measure obtained may not have necessarily corresponded with the trough of the cardiac cycle and in turn may have affected the magnitude of the OPA detected. This limitation of the *LabView* Acquisition program in detecting the trough of the cardiac cycle may explain the reduction in the size of the OPA detected in some subjects, however it does not explain why in approximately 40% the subjects a negative value for the OPA was recorded i.e. the IOP taken at the trough of the cycle was higher than that taken at the peak of the cycle. Although in theory a voltage could have been sent at any 3 points on the cardiac cycle at which an apparent trough was identified, it is possible that 3 consecutive signals with very little time delay between them were sent to the NCT which may have affected the ability of the NCT to take accurate IOP readings. It is evident that the pulse synchronous *Pulsair EasyEye* measurement method is not a good means of studying the range of momentary

fluctuations in the IOP, however since this was not the main aim of the present study this limitation does not present as a serious setback.

The principle aim of this study was to devise a method of IOP measurement in which the variation in IOP measures associated with the cardiac and respiratory cycles are reduced in order to investigate more precisely the effect of accommodation on IOP. It is deduced from this study that coupling a pulse-transducer with a *LabView* Acquisition programme allows pulse synchronised IOP measures which significantly reduce the spread in IOP data compared to the unmodified NCT. Of the 3 positions on the cardiac cycle investigated, the middle location shows a good correlation with GCT and exhibits the least variance in IOP measures.

However, a personal communication with the manufacturers (Keeler, UK) suggested that the reduction in variance observed may be due to the NCT being mounted on an optical bench rather than because of the IOP measures being pulse synchronised. It is hypothesised above that subjects may be more apprehensive when NCT is performed hand-held compared to when the instrument is mounted on a table. Increased apprehension has also been shown to increase the spread of IOP measures (Mackie *et al.*, 1996). Therefore, as suggested by Keeler (UK) the reduction in variance observed may be a result of a decrease in apprehension experienced when the NCT is mounted compared to when hand-held non-contact tonometry is performed. Furthermore, to attain correct alignment of the tonometer with the cornea the instrument is adjusted in the axial, vertical and lateral directions. Indeed, there is a range in which correct alignment can be identified (see **Chapter 3**). Mounting the instrument means that between measures little adjustment is required, particularly in the vertical and lateral directions, to obtain correct alignment and therefore only a small alignment range is required. However, a much larger alignment range is necessary when the IOP measures are obtained with the hand-held instrument and this use of a larger range may in turn increase the variance in IOP measures.

An unpublished BSc project by Mackie *et al.* (1989) on the evaluation of the Keeler *Pulsair* concluded that no difference in inter-sessional repeatability was found when using the *Pulsair* in either the hand-held or the adapted slit-lamp mode (cited by Mackie *et al.*, 1996). In addition to the present study, the IOP was measured in 93 subjects to investigate whether using the NCT as a hand-held or table mounted device had any bearing on the variance of IOP measures. A full description of the experimental procedures can be found in **Appendix 6**.

The results show that the mean IOP was slightly higher when the instrument was used as a hand-held device (15.52±3.34 mmHg) compared to when the instrument was mounted (15.08±3.14 mmHg). This difference in IOP between the 2 measurement methods was small (0.44±2.03 mmHg). The COV of the NCT in its hand-held and mounted modes was approximately 21%. The variance in IOP readings was lower when the NCT was used in the table-mounted mode (9.87) compared to when the instrument was used in the hand-held mode (11.13). However, the difference in variance was not statistically significant ($F=1.13$, $p=0.12$). Therefore, there is evidence to show that the decrease in the variance of IOP data observed on pulse synchronisation of the IOP readings is in fact due to the synchronisation of the measures with the cardiac cycle and not as a result of mounting the NCT, as suggested by (Keeler UK).

In conclusion, analysis of the data suggests that regulating the respiratory cycle and synchronising the modified *Pulsair EasyEye* (Keeler, UK) with the peak, middle or trough of the cardiac cycle significantly increases the accuracy (in terms of GCT) and reduces the variance in IOP measures. Of the 3 locations, synchronising the NCT with the middle of the cardiac cycle demonstrates the least variance in IOP measures, a good correlation with GCT and 98% of the data is within ±2 mmHg of GCT. As an added advantage, less time is required for the acquisition of data at the middle location of the cardiac cycle compared to that needed at the peak and trough of the cardiac trace. Middle synchronised IOP measures are repeatable between sessions and five middle synchronised measures are required to obtain reliable data. This is a significant advance and one that means that the spread of data is more comparable to Goldmann contact tonometry, which is regarded as gold standard.

5.6 Conclusion

This study describes a method of IOP measurement in which the *Pulsair EasyEye* has been coupled with a finger pulse-transducer and a *LabView* Acquisition programme to take IOP measures at 3 chosen locations of the cardiac cycle.

The main findings of this study are:

- In accordance with previous studies IOP reduces on repeat measures with the GCT, however this effect is not evident when using a NCT.

- Measurement method influences IOP, such that unmodified NCT IOP measures are significantly higher than those taken with the GCT and IOP readings synchronised with the peak, middle or trough of the cardiac cycle.

- Attaining a fixed pace respiratory cycle and synchronising the NCT with the peak, middle or trough of the cycle increases the accuracy and reduces the variance in IOP measures.

- Of the 3 locations the middle demonstrates the least variance in IOP measures and a good correlation with GCT.

- The between sessions repeatability of middle synchronised IOP measures was ±1.16 mmHg.

- A minimum of 5 middle synchronised IOP measures are required to attain satisfactory repeatability.

- The pulse synchronous measurement method described does not allow the reliable investigation of the OPA.

CHAPTER 6

THE EFFECT OF ACCOMMODATION ON IOP; CONTROLLING FOR EFFECTS OF SHORT-TERM VARIATIONS.

6.1 Introduction

The principle component of the oculomotor response to near work is accommodation and therefore the change in IOP with accommodation is of intrinsic interest in the context of our understanding of the physiology of sustained near vision with respect to Coleman's and Helmholtz's theory on accommodation (see **Chapter 1**).

Many studies emphasise the role of near work in the development and onset of myopia (Rosenfield and Gilmartin., 1998). The intricacy of the near vision complex means that the exact role of near work in the genesis of myopia remains equivocal. However, a plethora of literature agrees with the tenet that the accommodative system plays an important factor in regulating ocular growth (reviewed by Gilmartin, 2004). The consensus is that physical changes in the structure of the eye which result in myopia occur at the posterior segment, rather than the anterior segment of the globe. This is described very eloquently by recent work using 2-dimensional (Logan *et al.,* 2004) and 3-dimensional MRI imaging (Singh, Logan and Gilmartin, 2006).

A fuller appreciation of the relationship will extend our knowledge on the association between near work and myopia. It is hypothesised that if the IOP changes on nearwork, ocular elongation may occur as a result of mechanical stress on the globe as suggested in early work by Kelly (1981) and Young (1977; 1981). Surprisingly the literature mapping the relationship between accommodation and IOP is however limited; the most recent study being in the early 1980's by Mauger, Likens and Applebaum (1984).

Mauger *et al.* (1984) used a Goldmann contact tonometer (GCT) and hence anaesthetics to measure the IOP during accommodation. The effects of repeated IOP measures with the GCT (Moses, 1961) and the effects of anaesthetics on IOP (Herse and Siu, 1992; Moseley *et al.,* 1993; Baudouin and Gastaud, 1994; Birchall and Kumar, 2001; Asensio *et al.,* 2003; Nam *et*

al., 2006) mean that the results of Mauger's study are of limited value. In a previous study (see **Chapter 4**), the accommodation and IOP experiments, which were analogous to those carried out by Mauger *et al.* (1984), were performed using the *Pulsair EasyEye* non-contact tonometer (NCT) (Keeler, UK). However, it was clear that inherent variations in IOP caused by biological cycles needed to be controlled in order to investigate more precisely the effect of accommodation on IOP. Accordingly in the previous chapter (**Chapter 5**), a measurement method has been described which allows high resolution, pulse-synchronised IOP measures using a modified *Pulsair EasyEye* NCT and a fixed pace respiratory cycle.

A further limitation of the previous research (e.g. Armaly and Burian, 1958; Armaly and Rubin, 1961; Mauger *et al.,* 1984) and of our own preliminary work discussed in **Chapter 4** is that measurements of accommodative response levels were not incorporated in to the study design. It is well documented that accommodative lead and lag exist for distance and near targets respectively, approaching nearly 1D (Ciuffreda, 1998) and there is substantial inter-subject variations in the accommodation stimulus-response profiles (Rosenfield, 1998). Therefore, the changes in IOP on 1.50 (Mauger *et al.,* 1984) and 4D (Armaly and Burian, 1958; Armaly and Rubin, 1961; Mauger *et al.,* 1984) of accommodative stimuli observed in previous studies and reported in our own preliminary work are inconclusive. The variation in accommodative responses within and between subjects may explain the substantial inter-subject variations in IOP changes on accommodation observed.

The aim of this study was therefore to use a high resolution, pulse-synchronised NCT to investigate the effects of accommodation on IOP at a fixed pace respiratory cycle. In addition, the measurements of accommodation responses are incorporated in the study design.

6.2 Method

6.2.1 Subject group

Sixty six subjects were recruited from the undergraduate population at Aston University for this study. The mean±SD age for the group was 21.2± 3.6years of age (range 18-34 years of age). The cohort comprised 26 males and 40 females. The mean spherical equivalent [MSE; sphere+(cyclinder/2)] ranged from +0.50DS to -10.75DS and there were 38 myopes (MSE of ≤-0.50D) and 28 emmetropes (MSE of ±0.50D). Astigmatism was limited to <0.75DC. The criterion used to divide the subjects in to these refractive groups was also used in studies

conducted by Goh *et al.* (2005), Junghans and Crewther (2005) and Ojaimi *et al.* (2005). The accommodative amplitude was measured in each subject using the RAF rule. The mean ±SD of accommodative amplitudes was 12.2±2.1 D.

The research followed the tenets of the Declaration of Helsinki and was approved by the Institution's ethics committee (**Appendix 3**). Written consent was obtained from all subjects willing to participate in the study and copies of the information sheets and consent forms given to the subjects can be found in **Appendix 4**. All subjects had a visual acuity of 0.00 logMAR or better. All subjects were absent of ocular pathology. None of the subjects were taking any topical or systemic medications that may affect the IOP, cardiovascular function or accommodative function.

6.2.2 Stimulus

In **Chapter 4**, accommodation was stimulated by placing a high contrast (90%) Maltese cross target in real dioptric space. However, with such a simple setup in which the target vergence is altered by simply varying target distance 1) the vergence and target size vary inversely with target distance and 2) this setup results in a non-linear vergence scale. Furthermore, to achieve a zero ocular vergence the accommodative target needs to be placed at infinity and due to the impossibility of this setup, the norm is to place the target at 6 meters. However, even with the target placed at 6 meters, the target still presents a 0.17 D accommodative stimulus.

In 1876 Jules Badal (1840-1929), a French ophthalmologist described the principles of a Badal optometer. The simple Badal Optometer consists of a movable target and a fixed single positive lens is placed at its focal distance away from the eye. With this configuration the angular size of the perceived object is independent of the target position and the target vergence perceived by the eye is proportional to the distance of the target from the front focal point of the eye (Bennett and Rabbetts, 1998).

The second principle focus point of the Badal lens can coincide with the eye's anterior focal point, principle point, nodal point or the entrance pupil. Of these four positions, Wittenberg (1988) concluded that the best position would be at the nodal point. However, since on accommodation there is an anterior displacement of the nodal point, a residual small increase in image size results. It is therefore recommended that to attain a constant angular subtense of

the target, the back focal point of the lens must be placed at the entrance pupil of the eye, which is approximately 3mm behind the corneal pole. This ensures a constant angular image size for both unaccommodated and accommodated retinal images (Atchison *et al.,* 1995).

A further advantage of the Badal system is that a linear power scale is achieved, such that there are equal increments between each dioptre reading. In addition, illumination of the target is kept constant. Of note is that to achieve zero ocular vergence the target is only 2 focal lengths of the Badal lens away from the eye. Therefore, although the Badal system has desirable attributes of a linear scale and constant image size, the setup still induces proximal accommodation responses although these are markedly less than those produced with targets in real dioptric space (Atchison *et al.,* 1995).

The changes in IOP to low (L; 0D), intermediate (I; 1.50D) and high (H; 4D) levels of accommodation are investigated. These accommodation stimulus levels were chosen so that the results from this thesis can be compared against the results from previously published studies on the relationship between accommodation and IOP (Armaly and Burian, 1958; Armaly and Rubin, 1961 and Mauger *et al.,* 1984). Therefore, a +5D Badal system was used in the present study. A high contrast Maltese cross target was placed at 200, 140 and 40 mm away from the Badal lens to respectively obtain L, I and H accommodation stimuli. The accommodative targets within the Badal system were fixated for 3 minutes before the IOP measures were taken so that 1) comparisons could be made with previous studies (Mauger *et al.,* 1984) and 2) there is evidence to show that accommodation adaptation occurs after approximately 3 minutes of fixation (Rosenfield and Gilmartin, 1989).

6.2.3 Instrumentation

The Shin-Nippon SWR-5000 open-view, infrared objective autorefractor was used to measure the subjects refractive error (Maltese cross target placed within the Badal system at 200 mm away from the Badal lens) and accommodative responses (Maltese cross target placed within the Badal system at 140 and 40 mm away from the Badal lens). The autorefractor performs analyses of an infrared ring after it has been reflected off the retina (Mallen *et al.,* 2001). The instrument provides highly repeatable measures of refractive error in children (Chat and Edwards, 2001) and adults (Mallen *et al.,* 2001).

The middle location of the cardiac trace was synchronised with the IOP measures taken with the *EasyEye Pulsair* NCT and respiration was kept constant at 15 breathes/minute (see **Chapter 5**).

6.2.4 Experimental Procedures

Before the commencement of data collection, each subject was familiarised with the techniques employed. An IOP measure was obtained (not recorded) to demonstrate the corneal sensation experienced with a NCT since it has been shown that apprehension during non-contact tonometry can increase the IOP (Moses *et al.*, 1984) and increase the spread of consecutive IOP measures (Forbes *et al.*, 1974). Following the familiarisation technique each subject was allowed a 10 minute rest period.

Stage 1

A +5D Badal system was attached to a Shin-Nippon SWR-5000 objective optometer and the distance refractive error of both eyes was measured with a high contrast (90%) Maltese cross target placed at 200 mm away from the Badal lens. The refractive error data obtained in this stage was used to render the subjects LE functionally emmetropic using soft daily disposable contact lenses (1-day Acuvue Dailies, etafilcon A, Johnson & Johnson, Vistakon, USA).

Stage 2

When the target was placed at 200, 140 and 40 mm away from the +5D lens, the Maltese cross target represented an L, I and H accommodative stimulus target, respectively. The RE was occluded with a cream cardboard replication of the *Pulsair EasyEye* plastic shroud with a central red light emitting diode. The subjects were directed to focus on the accommodative target with their LE. The exact phrase used was 'focus on the target carefully', since the precise instructions given have been shown to affect the accuracy of the accommodation responses (Ciuffreda and Hokoda, 1985; Stark and Atchison, 1994). After 3 minutes of fixation of each of the 3 accommodative stimuli levels, the accommodative responses (of the LE) were measured with the autorefractor.

Stage 3

Owing to the relatively small dimensions of the *Pulsair Easyeye* NCT (256 x 115 x 40 mm and weight of 0.9kg), the instrument was mounted on an Ealing optical bench in front of the RE. A +5D Badal system (identical to the one used with the autorefractor in ***Stage 2***) was

mounted on another Ealing optical bench in front of the LE. The set up allowed unhindered IOP measures in one eye and simultaneous stimulation of accommodation in the other eye (see **Figure 6.1**).

A fixed pace respiratory cycle (15 breathes/minute) was achieved by asking subjects to breathe in time to a metronome and a steady cardiac cycle trace was obtained with the finger pulse-transducer. A Maltese cross target was placed within the Badal system at the distances noted above in ***Stage 2*** at which L, I and H accommodative stimuli levels were elicited. The subjects were instructed to fixate on the target for 3 minutes with their LE after which 5 IOP measures synchronised with the middle of the cardiac cycle were taken in the RE (see **Chapter 5**). After each set of 5 measures the subjects were allowed a 5 minute rest period. The 3 distances corresponding to the 3 accommodative levels were presented randomly and the IOP measures were masked from the examiner.

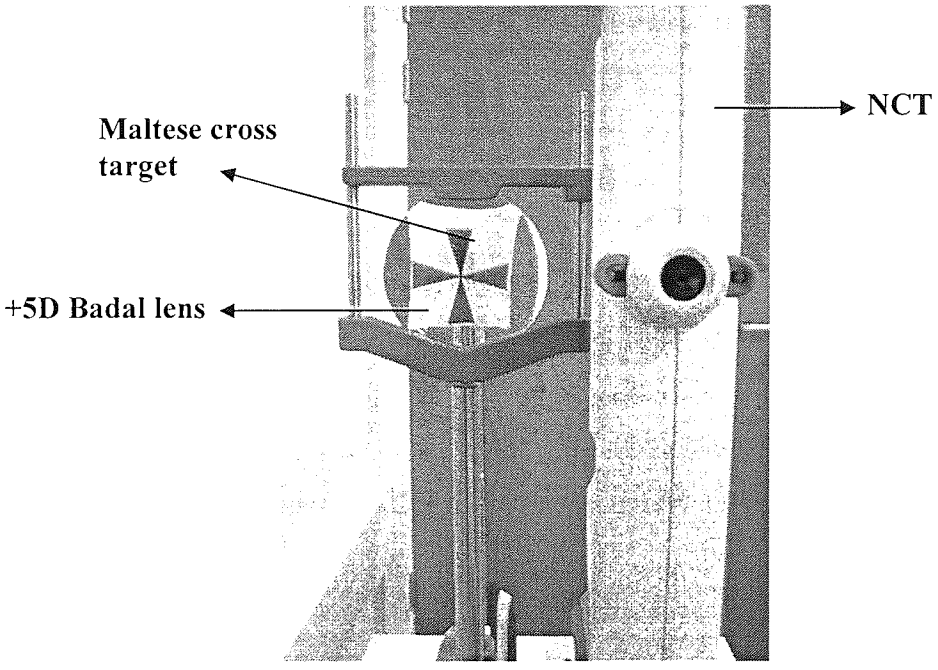


Figure 6.1 Subject view of Pulsair EasyEye NCT in front of RE and Badal system in front of LE.

6.3 Statistical Analysis

The software packages used to perform the statistical analyses were *SPSS 12.1* for Windows, *StatView 5* (SAS Institute Inc, USA) and *Microsoft Excel.*

A one-way ANOVA in randomised blocks was performed followed by both Scheffe and Bonferroni (critical p for multiple comparisons was 0.017) *post-hoc* analyses to determine

whether accommodative response influenced IOP. The IOP measures obtained when the subjects were fixating a L accommodative stimulus were treated as the control for comparison.

The accommodation response errors (*ARE*) were calculated from the difference between the accommodation responses and nominal values for each accommodative stimulus level. The linear regression slopes (m) of the accommodation stimulus-response functions were calculated for each subject as described by McBrien and Millodot (1986 a) and Abbott, Schmid and Strang (1998). The accommodative error indices (I) were also calculated since the accuracy of the response gradient parameter (m) as a single indicator of accommodative stability has been questioned by Chauhan and Charman (1995).

The inter-subject variations in IOP responses to accommodation were investigated with respect to the inter-subject variations in accommodation responses, *ARE*, m and I by computing the correlation coefficients of the relationships. Pearson's product moment correlation coefficients were calculated and a one-way between subjects ANOVA was performed to determine whether refractive error governed the inter-subject variations in IOP responses to accommodation.

6.4 Results

The mean±SD (MN±SD) accommodation responses, accommodation response errors, accommodative stimulus-response gradients (m) and the accommodative error indices (I) for the cohort are shown in **Table 6.1, 6.2,** and **6.3,** respectively.

Accommodation stimulus level	MN±SD Accommodation response (D)
L	+0.16±0.35
I	-1.36±0.31
H	-3.32±0.60

Table 6.1 Summary of mean±SD accommodation responses to the 3 accommodation stimulus levels for the cohort.

Accommodation stimulus level	MN±SD Accommodation response errors (D)
L	0.33±0.20
I	0.27±0.21
H	0.73±0.54

Table 6.2 Summary of mean±SD accommodation response errors to the 3 accommodation stimulus levels for the cohort.

MN±SD stimulus-response gradients (*m*)	MN±SD accommodative error indices (*I*)
0.79±0.17	2.70±3.82

Table 6.3 Summary of accommodative stimulus-response gradients (*m*) and the accommodative error indices (*I*).

The accommodation responses as measured in ***Stage 2*** are shown in **Table 6.1** and the corresponding IOP measures to L, I and H accommodation stimulus levels for the cohort are summarised in **Table 6.4.**

Accommodation stimulus level	MN±SD IOP (mmHg)
L	13.68±1.90
I	13.40±2.01
H	13.29±1.99

Table 6.4 Summary of IOP measures taken at the 3 accommodation stimuli levels.

The within-subjects ANOVA shows that the level of accommodative stimuli influences the IOP [F (2, 98) = 3.919, p=0.022]. The IOP decreases between the L and I, L and H, and I and H accommodation stimulus levels by respectively 0.28±1.19, 0.39±1.12 and 0.11±1.22 mmHg. Scheffe *post-hoc* analyses show no significant differences in IOP taken at the 3 accommodative stimuli levels (L to I p=0.710; L to H p=0.519; I to H p=0.951). However, Bonferroni *post-hoc* analyses revealed that these differences in IOP reached statistical significance only between L and H (p=0.006) accommodative stimulus levels (L and I p=0.056 and I and H p=0.4690).

Of note are the inter-subject variations in the differences in IOP between the 3 levels of accommodative stimuli as illustrated in **Figures 6.2** and **6.3**. The range (RG) and median (MED) of the differences in IOP between L and I, L and H, and I and H are respectively, RG=2.92 to -2.99, MED=-0.18, RG=2.98 to -3.14, MED=-0.37 and RG=2.82 to -2.72, MED=-0.05. The distributions of the differences in IOP between the 3 levels of accommodation stimuli are shown in **Figure 6.4**.

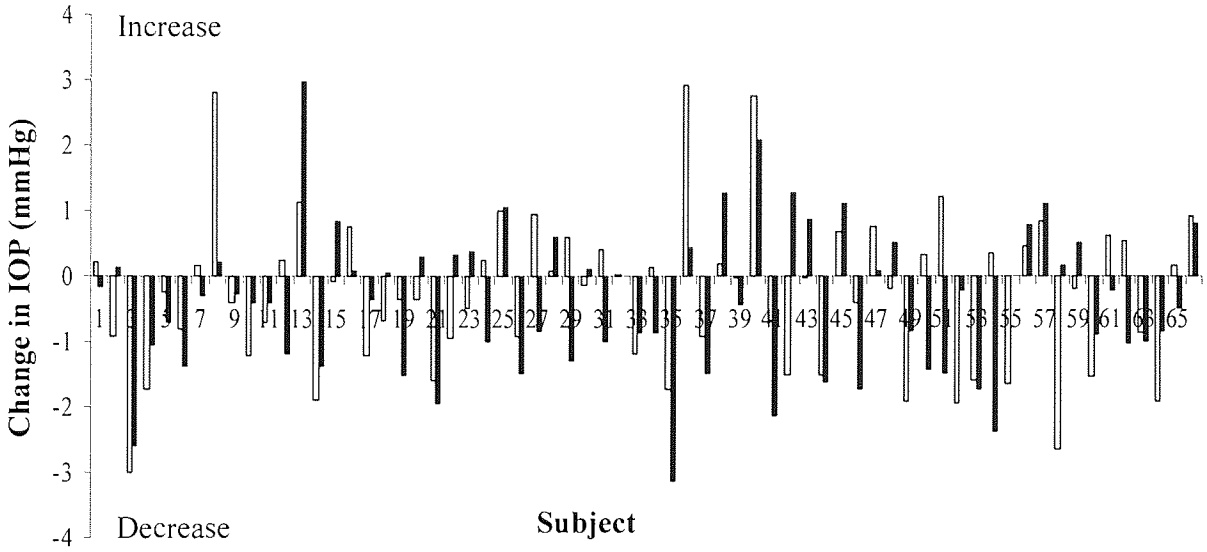


Figure 6.2 Change in IOP (mmHg) between L and I and L and H ■ accommodation stimulus levels (n=66).

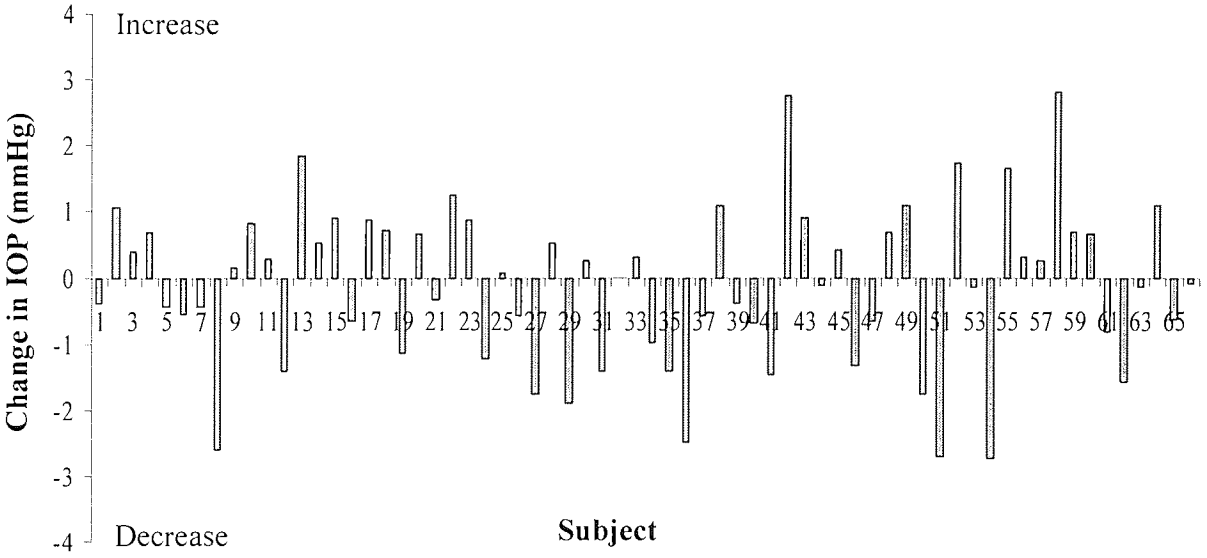
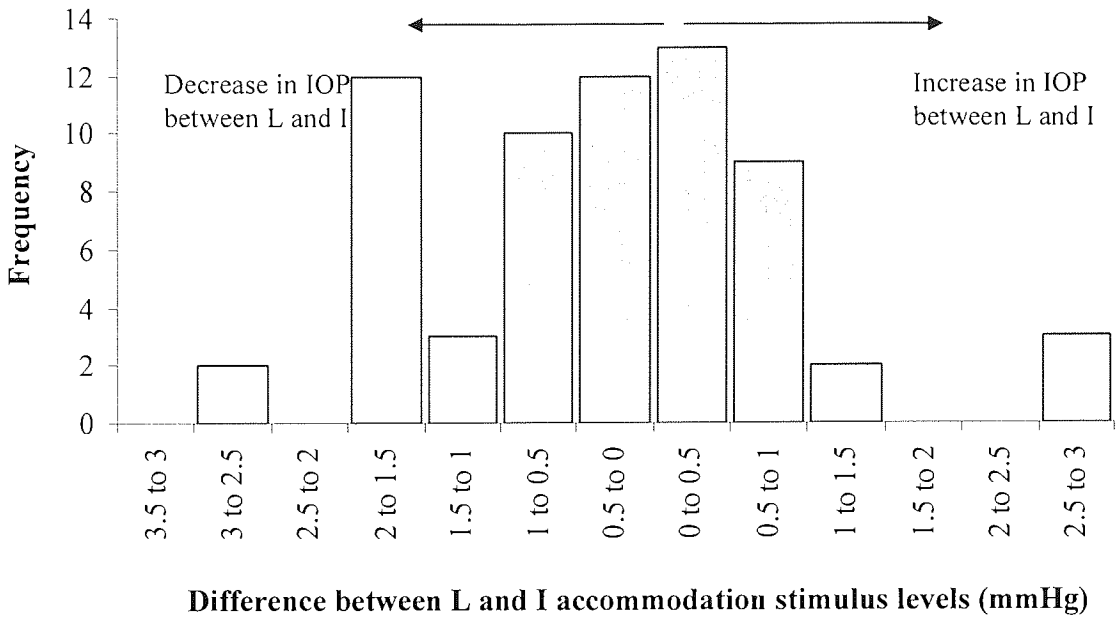
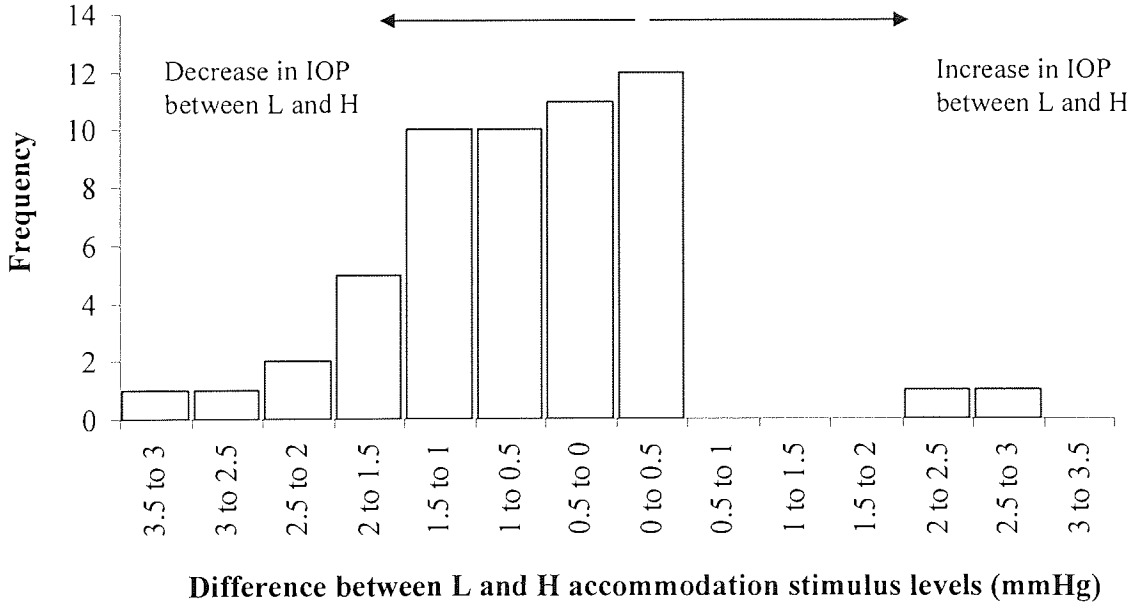


Figure 6.3 Change in IOP (mmHg) between I and H accommodation stimulus levels (n=66).

(6.4 **a**)


Decrease in IOP between L and I
Increase in IOP between L and I
Frequency
0
2
4
6
8
10
12
14
3.5 to 3
3 to 2.5
2.5 to 2
2 to 1.5
1.5 to 1
1 to 0.5
0.5 to 0
0 to 0.5
0.5 to 1
1 to 1.5
1.5 to 2
2 to 2.5
2.5 to 3
Difference between L and I accommodation stimulus levels (mmHg)

(6.4 **b**)


Decrease in IOP between L and H
Increase in IOP between L and H
Frequency
0
2
4
6
8
10
12
14
3.5 to 3
3 to 2.5
2.5 to 2
2 to 1.5
1.5 to 1
1 to 0.5
0.5 to 0
0 to 0.5
0.5 to 1
1 to 1.5
1.5 to 2
2 to 2.5
2.5 to 3
3 to 3.5
Difference between L and H accommodation stimulus levels (mmHg)

(6.4 **c**)

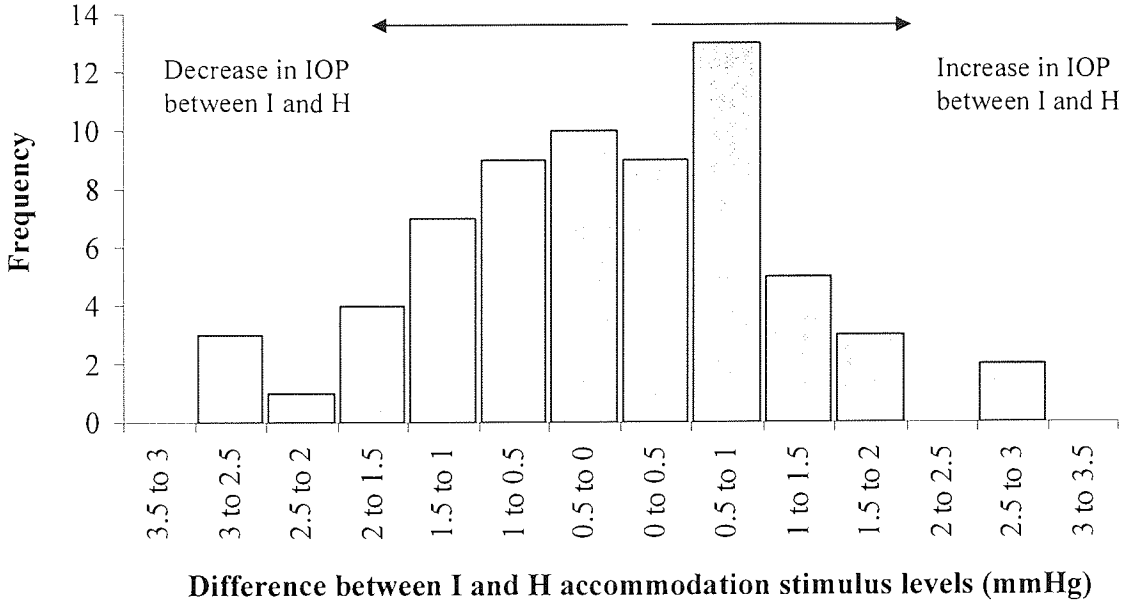
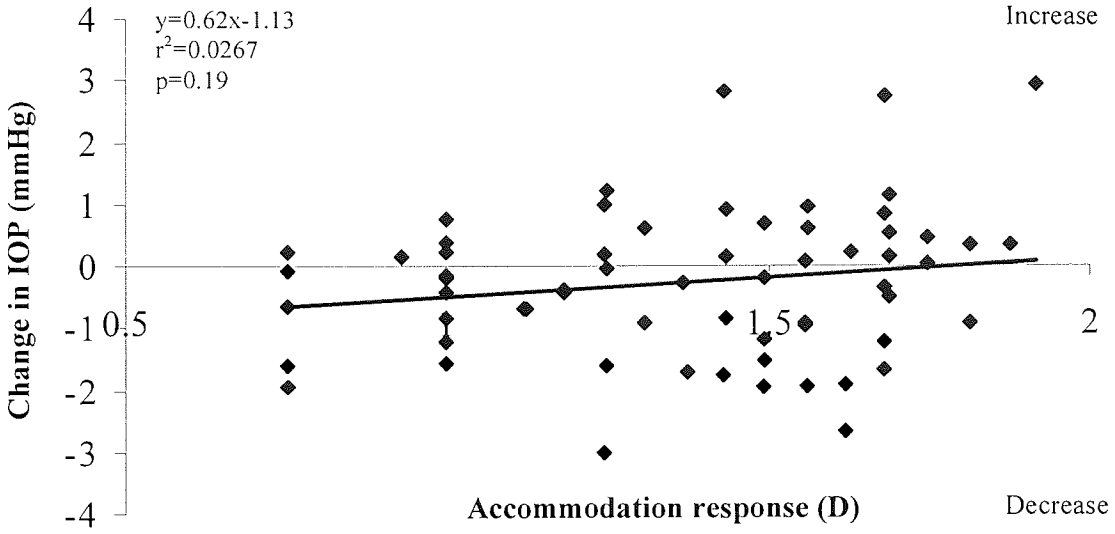


Figure 6.4 Histogram of the differences in IOP between L and I (**a**), L and H (**b**) and I and H (**c**) levels of accommodation stimuli (n=66

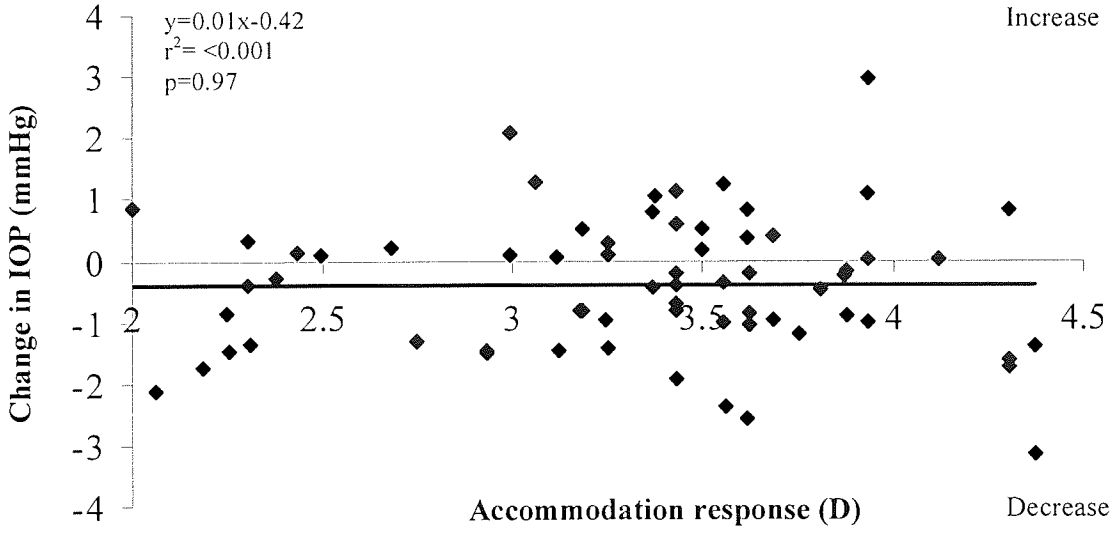
Although a Badal system was utilised to stimulate L, I and H levels of accommodative demand, inevitably a range of accommodation responses were elicited for the 3 accommodation stimuli. **Figure 6.5** shows that the inter-subject variations in the differences in IOP between L and I (r=0.164, p=0.19), L and H (r=0.005, p=0.97) and I and H (r=0.006, p=0.96) accommodation levels are not due to inter-subject variations in accommodation responses. Note that the inter-subject variations in the changes in IOP with accommodation were not considered with respect to the baseline IOPs due to the relatively small inter-subject variations in baseline IOPs.

Figure 6.6 shows that the inter-subject variations in the differences in IOP and accommodation levels are not due to inter-subject variations in *m* (L and I r=0.10, p=0.42; L and H r=0.004, p=0.75; I and H r=0.06, p=0.63). Likewise, **Figure 6.7** shows that the inter-subject variations in the differences in IOP and accommodation levels are not due to inter-subject variations in *I* (L and I r=0.02, p=0.91; L and H r=0.05, p=0.67; I and H r=0.03, p=0.78).

(6.5 a)


y=0.62x-1.13
r²=0.0267
p=0.19
Increase
Change in IOP (mmHg)
4
3
2
1
0
-1
-2
-3
-4
0.5
1.5
2
Accommodation response (D)
Decrease

(6.5 b)


y=0.01x-0.42
r²= <0.001
p=0.97
Increase
Change in IOP (mmHg)
4
3
2
1
0
-1
-2
-3
-4
2
2.5
3
3.5
4
4.5
Accommodation response (D)
Decrease

(6.5c)

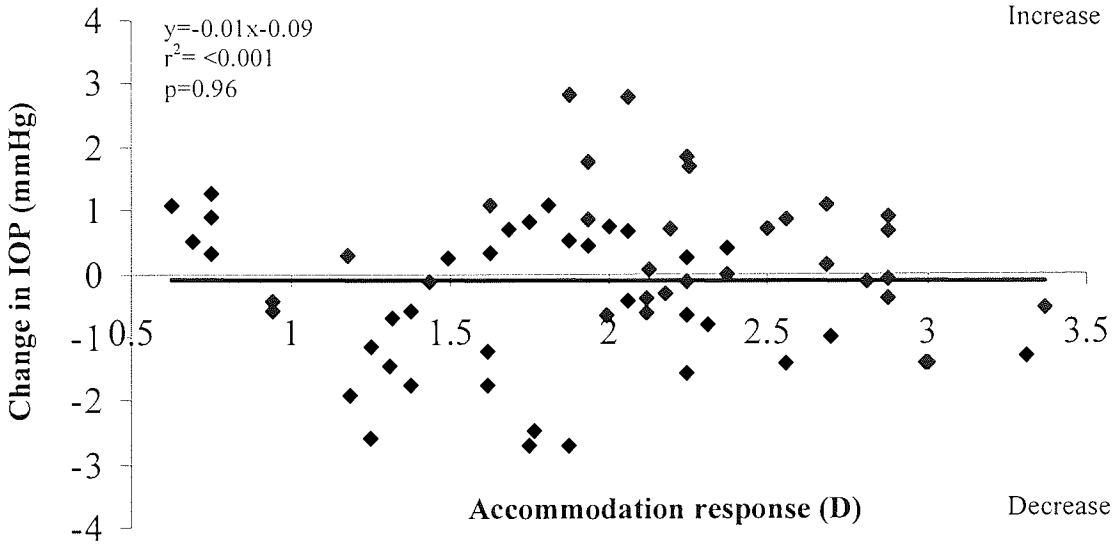


Figure 6.5 The accommodation responses and changes in IOP between (**a**) L and I, (**b**) L and H and (**c**) I and H levels of accommodation stimuli (n=66).

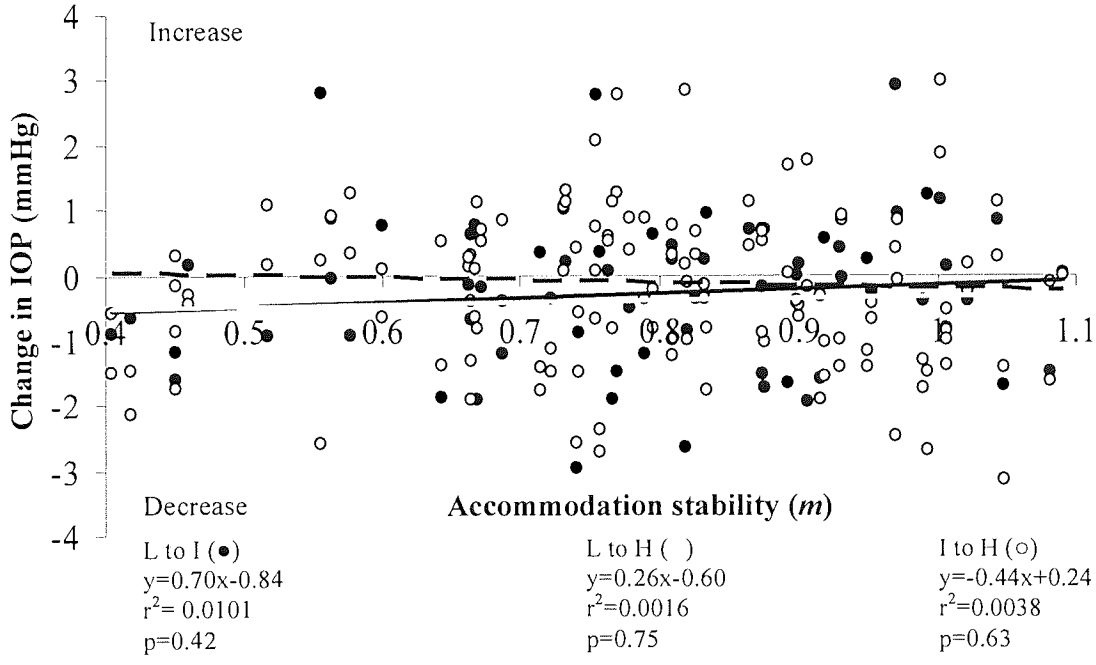


Figure 6.6 Differences in IOP between L and I (—), L and H ( ) and I and H (---) accommodation stimulus levels as a function of accommodative stability (*m*) (n=66).

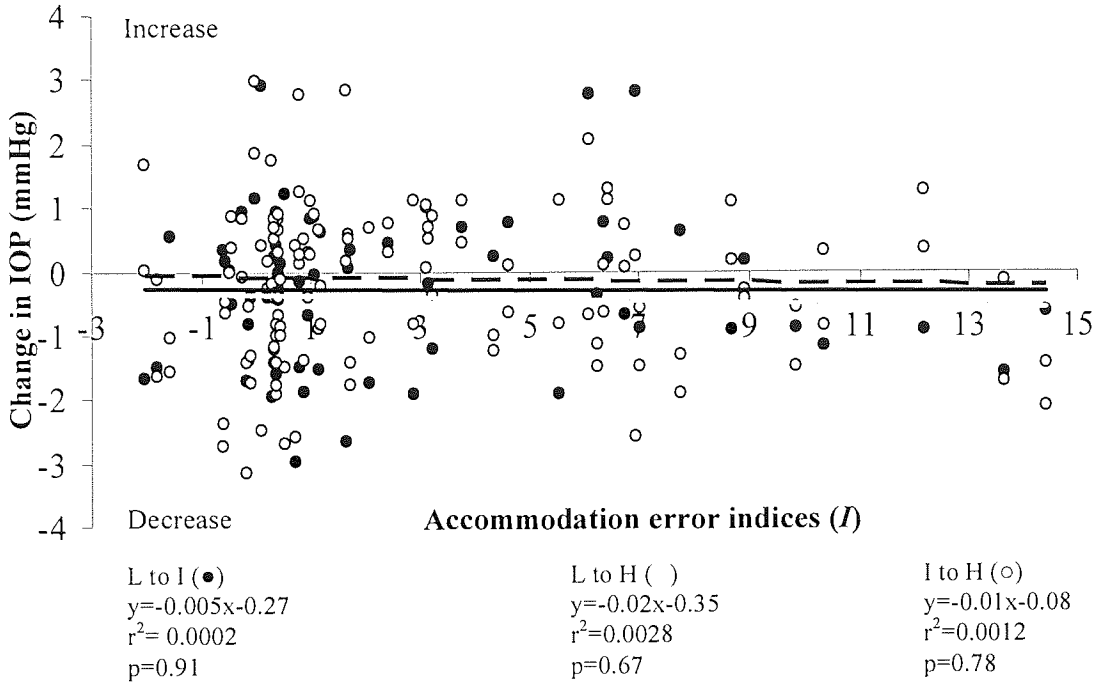


Figure 6.7 Differences in IOP between L and I (—), L and H ( ) and I and H (---) accommodation stimulus levels as a function of accommodative error index (*I*) (n=66).

The MN±SD of the differences in IOP between L and I and L and H accommodation levels for the emmetropes were respectively, -0.36±1.27 and -0.25±1.29 mmHg and for the myopes the MN±SD of the differences in IOP between L and I and L and H accommodation levels were respectively, -0.23±1.13 and -0.50±0.99 mmHg. The between-refractive groups ANOVA results (F=0.061, p=0.805) together with correlation graphs of refractive error and differences in IOP between L and I (r=0.11, p=0.38), L and H (r=0.21, p=0.08) and I and H (r=0.31, p=0.31) levels of accommodation shown provide evidence that the inter-subject variations in refractive error do not account for the inter-subject variations in IOP responses to accommodation (see **Figure 6.8**).

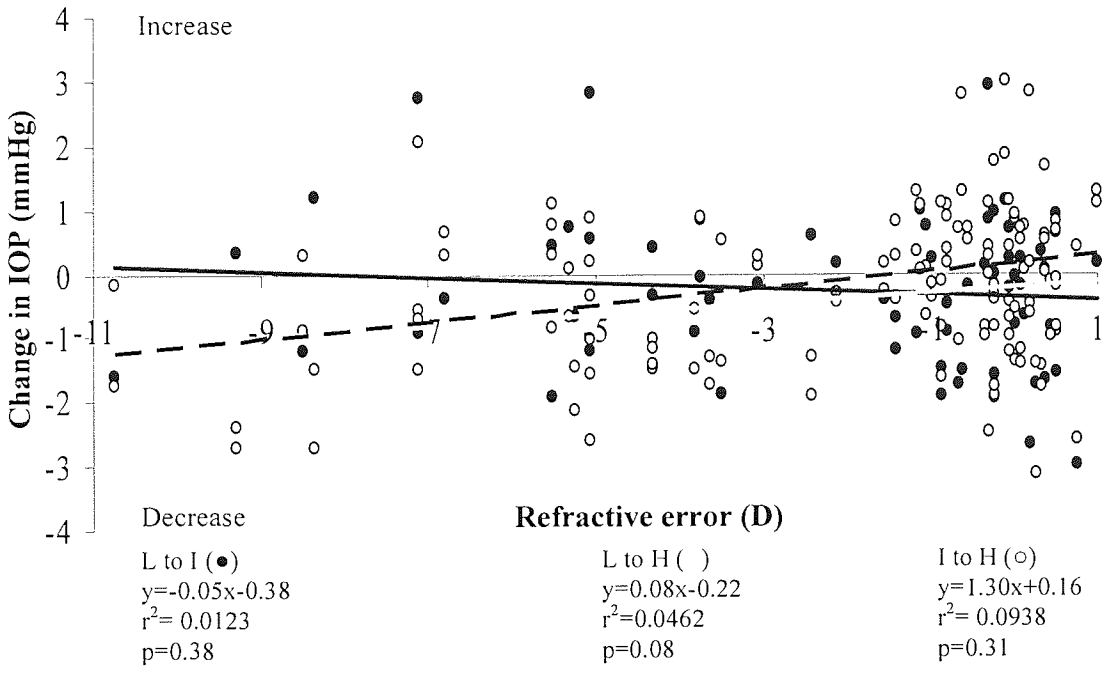


Figure 6.8 Refractive error against the change in IOP between L and I (—), L and H ( ) and I and H (---) accommodation stimulus levels (n=66).

Analyses of the percentage changes in IOP are shown in **Appendix 7**. The results also indicate that accommodation does not influence the percentage changes in IOP and that the inter-subject variations in percentages changes are not explained by refractive error.

The data in the present study was also used to evaluate aspects of the accommodative stimuli-response function, an account of which is given in **Appendix 8.**

6.5 Discussion

IOP measures, synchronised with the middle of the cardiac cycle were taken with a modified *EasyEye Pulsair* NCT after 3 minutes of accommodation to 3 levels of accommodative stimuli presented randomly within a +5D Badal system. Statistical analyses of the IOP change on accommodation showed that the level of accommodation influenced IOP. A small reduction in IOP is present between L and I, L and H, and I and H levels of accommodation. However, only the reduction in IOP between the L and H accommodation levels demonstrated statistical significance on p*ost-hoc* analyses.

The findings of this study may be explained by anatomical changes in the anterior segment of the eye during accommodation. The ciliary muscle is composed of the circumferential, meridional and radial muscle fibres. The muscle fibres are anchored to the scleral spur anteriorly, extend to the ora serrata posteriorly and together with connective tissues they construct the pars plana ciliaris and the pars plicata ciliaris. Based on Helmholtz's theory on accommodation, contraction of the ciliary muscle causes the *pars plana* ciliaris (i.e. posterior fibres) to stretch. The muscle moves forward and inwards, the ciliary muscle collar diameter reduces and consequently releases the tension of the anterior fibres (Hogan *et al.,* 1971; Gilmartin, 1995). These processes lead to the increased potency of the trabecular meshwork and hence an increase in aqueous outflow and thus a decrease in IOP. Evidence supporting this comes from the use of pilocarpine in the treatment of chronic open-angle glaucoma. Pilocarpine acts on muscarinic receptors found in the ciliary muscle (Gupta *et al.,* 1994; Zhang *et al.,* 1995; Gil *et al.,* 1997) causing the muscle to contract (Gabelt and Kaufman, 1992). Induced contraction of the muscle increases tension on the trabecular meshwork and hence increases aqueous humour outflow (Abramson *et al.,* 1974).

Ciliary muscle contraction to accommodate to I levels of accommodation would hence increase outflow (i.e. decrease IOP). However, greater ciliary muscle contraction maybe required to accommodate to higher levels of accommodation and hence result in relatively larger increases in outflow. A dose dependent increase in outflow has also been observed with pilocarpine such that the higher the dose of pilocarpine the higher the aqueous outflow facitily (Pang *et al.,* 1993; Schroeder and Erickson, 1994; 1995; Kiland, Hubbard and Kaufman, 2000). The potential for dose dependent contractibility of the CM may be the basis of the dose effect of accommodation and IOP described in this Chapter of the thesis.

In addition to the contractile characteristics of the ciliary muscle, smooth muscle-like contractile properties have also been found in the trabecular meshwork (Lepple-Wienhues, Stahl and Wiederholt, 1991) and the scleral spur (Tamm *et al.,* 1992 b; Tamm *et al.,* 1995). Hence it is possible that three different contractile elements may be involved in regulating aqueous humour outflow and therefore may explain the increase in IOP between I and H accommodation levels in some subjects. This mechanism is further discussed in **Chapter 7**.

A reduction in IOP on H levels of accommodation has been demonstrated in previous studies (Mauger *et al.,* 1984; Armaly and Burian, 1958; Armaly and Rubin, 1961), although the

magnitude of IOP change is reportedly larger than that found in this study. In the most recent study on accommodation and IOP (Mauger *et al.,* 1984) a significant reduction in IOP was found with I levels of accommodation; a result not observed in this study. It is speculated that the reduction in IOP with accommodation observed in previous studies is emphasised by the use of anaesthetics and repeat applanations with the GCT. Although NCT were introduced in 1972, the bulky design of the instruments, particularly the large plastic shrouds around the firing probe, restricted the utility of these types of tonometers in research, as is the case today with many table-mounted modern tonometers. Mauger *et al.* (1984) therefore chose to investigate the effects of accommodation on IOP using the GCT which allowed simultaneous stimulation of accommodation in one eye and measurement of IOP in the other.

The GCT is in direct contact with the cornea during its measurement procedure and therefore requires topical anaesthetics, the inevitable use of which may lead to significant under- and over-estimations of IOP. A direct facilitating effect of topical anaesthetics on aqueous humour dynamics has been proposed by Baudouin and Gastaud (1994). Increases in CCT values by between 7 to 16 μm have also been demonstrated (Herse and Siu, 1992; Asensio *et al.,* 2003; Nam *et al.,* 2006), which too may result in erroneous IOP measures with the GCT (Whitacre and Stein, 1993). In addition, the chemical composition of the anaesthetic agent disrupts the quality of the tearfilm meniscus (Birchall and Kumar, 2001) which has been shown to influence the IOP reading when taken with the GCT (Whitacre and Stein, 1993). It is postulated that the IOP measures taken with the NCT may also be affected by changes in the tearfilm characteristics and CCT caused by topical anaesthetics.

Mauger *et al.* (1984) used a control group to conclude that repeated measures with the GCT had no significant effect on the IOP. Nevertheless, a progressive reduction in IOP of 3 to 4 mmHg on repeat measurements has also been observed (Moses, 1961; Moses and Liu, 1968). It is thought that a 'massaging effect' of the tonometer probe on the cornea, may increase aqueous outflow and therefore progressively decrease IOP on repeat measures. It has also been suggested that aqueous formation is reduced as a reflex mechanism to the procedure of tonometry (Stocker, 1956). The Goldmann probe is in contact with the cornea for approximately 2 seconds (Myers and Scott, 1975) and this time can vary substantially between measurements and subjects (Birchall and Kumar, 2001). It is possible therefore that an inter- and intra massaging effect which varies between subjects may have varying effects on the aqueous humour. This inter- and intra-measurement massaging effect may explain the

differences in the magnitude of change observed in this study and previous studies (Mauger *et al.*, 1984). It is clear that although Mauger *et al.* (1984) used the GCT which is widely considered as 'gold standard' for tonometric measures, their results on accommodation and IOP may have been contaminated by the effects of anaesthetics on aqueous humour dynamics, tearfilm characteristics and CCT and by the effects of prolonged corneal contact with the tonometer probe.

Although oscillations in IOP measures associated with biological cycles (i.e. cardiac and respiratory cycles) have been controlled, there still remain substantial inter-subject variations in IOP responses to accommodation (magnitude of range of differences was approximately 6 mmHg), as found in our preliminary study (see **Chapter 4**). Somewhat perplexing was the finding that the inter-subject variations in IOP responses were not due to inter-subject variations in accommodation responses, aspects of the stimulus-response function or refractive error.

A failure to measure accommodation responses in previous studies (Armaly and Burian, 1958; Armaly and Rubin, 1961; Mauger *et al.* 1984) and in the study described in **Chapter 4** has been noted as a limitation of the study design. Subsequently, in the current study the measurement of accommodation responses has been incorporated into the study design. The accommodation responses were measured with the autorefractor and Badal system setup (i.e. in ***Stage 2*** of the experimental procedures) and it was assumed that the accommodative system behaved the same during the NCT and Badal system setup (i.e. in ***Stage 3*** of the experimental procedures). However, it is thought that the anticipation of the puff of air from the NCT probably interfered with the subject's concentration to accommodate, hence making it more difficult to obtain and maintain a set amount of accommodation in ***Stage 3*** compared to ***Stage 2*** of the experimental procedures. Furthermore, approximately 70% of subject's reported that the internal red fixation target and the external green alignment LED lights (seen by the subject's RE) of the NCT intermittently superimposed on the Maltese cross target (seen by the LE). Therefore, it is now speculated that the assumption that accommodation responses measured in ***Stage 2*** of the experimental procedures exactly matched those during ***Stage 3*** of the experimental procedures may not have been met.

Therefore, ideally an experimental setup is required in which the exact accommodation responses during the IOP measurement period are known. Consequently, the intra- and inter-

subject variations in accommodation responses may account for the inter-subject variations in IOP responses to accommodation. **Chapter 7** of this thesis goes on to describe an experimental design in which the accommodation responses and IOP measures are measured simultaneously.

6.6 Conclusion

In the present study the effect of IOP on accommodation was investigated using a pulse-synchronised NCT and Badal system, having incorporated the measurement of accommodation responses in to the study design which was conducted in a large cohort. The main findings of this study are:

- IOP decreases progressively on increasing levels of accommodation stimuli.
- The relationship between IOP and accommodation is characterised by substantial inter-subject variations in IOP responses to accommodation.
- The inter-subject variations in IOP changes on accommodation observed are not attributable to inter-subject variations in accommodation responses, aspects of the accommodation stimulus-response functions or refractive error.

CHAPTER 7

THE CHANGE IN IOP WITH ACCOMMODATION WHILE SIMULTANEOUSLY MEASURING ACCOMMODATION RESPONSES.

7.1 Introduction

Hitherto, the literature mapping the relationship between accommodation and IOP is limited. The most recent study was conducted by Mauger *et al.* (1984) who concluded that IOP reduced by 2.15±0.78 and 2.38±0.65 mmHg after 3.5 minutes of fixation to a 1.50 and 4D accommodative stimuli, respectively. However, the results are of limited value due to the drawbacks of the experimental design which are discussed below.

Firstly, although the study by Mauger *et al.* (1984) recruited 30 subjects, a between-subjects design was used where the cohort was divided in to 3 groups of 10. In group 1, the IOP was measured after 30 seconds and after a further 3 minutes of fixation to a low (0D; L) accommodative stimulus. The IOP was measured at the same time intervals while the subjects in groups 2 and 3 fixated a L and intermediate (1.50D; I), and L and high (4D; H) accommodative stimuli levels, respectively. Inherent variations in IOP and accommodation between subjects in each group were therefore not considered. Hence in our preliminary work (see **Chapter 4**) and the study described in **Chapter 6**, a within-subjects design was employed using a larger sample, where each subject fixated all 3 levels of accommodation stimuli which were presented randomly.

Secondly, a Goldmann contact tonometer (GCT) was used to measure the IOP in the Mauger *et al.* (1984) study. The limitations of the GCT are discussed elsewhere (see **Chapter 2**), however pertinent to this study are the effects of topical anaesthetics (Herse and Siu, 1992; Moseley *et al.*, 1993; Baudouin and Gastaud, 1994; Birchall and Kumar, 2001; Asensio *et al.*, 2003; Nam *et al.*, 2006) on IOP. Furthermore, although Mauger *et al.* (1984) demonstrated that no significant reduction in IOP occurred on repeated contact tonometry; reductions in IOP of between 2 and 3 mmHg have been reported previously (Moses, 1961). Hence in this thesis a non-contact tonometer (NCT) the *EasyEye Pulsair* (Keeler, UK) was used in which the need for topical anaesthetics is negated. Furthermore, no reduction in IOP has previously

been reported on repeat measurements using the American Optical NCT (Grolman, 1972; Forbes *et al.,* 1974; Myers and Scott, 1975; Sorensen, 1975; Chauhan and Henson, 1988) and the Pulsair NCT (Baudouin and Gastaud, 1994; Lawson-Kopp *et al.,* 2002). However, the effects of short-term variations in IOP caused by biological cycles, for example the cardiac (Bynke and Schéle, 1967; Forbes *et al.,* 1974; Leydhecker, 1976; Piltz *et al.,* 1985; Nanba *et al.,* 1989; Trew *et al.,* 1991) and respiratory (Leydhecker, 1976; Perkins, 1981; Moses and Arnzen, 1983; Akselrod *et al.,* 1985; Nanba *et al.,* 1989) cycles are emphasised when non-contact tonometry is performed. Consequently, a method of non-contact IOP measures was devised in which these fluctuations were minimised by maintaining a constant pace respiratory cycle and synchronising the IOP measures to the middle of the cardiac cycle (see **Chapter 5** for a full discussion).

A third limitation of Mauger's study was that accommodation responses were not measured and it is well established that an accommodative lead and lag exist for long and short distances, respectively (Ciuffreda, 1998). Furthermore, a series of negative lenses were used to stimulate accommodation in Mauger's study and a reduced accommodative response to negative-lens induced accommodative demand compared to real target presentations in real dioptric space has been demonstrated (Abbott *et al.,* 1998). Hence it is thought that the results of Mauger's study and our preliminary work may have been contaminated by poor control of accommodation responses. Accordingly, in this thesis a Badal system was used to stimulate accommodation in which constant image size and minimum proximal accommodation are elicited. In the study described in **Chapter 6,** the accommodation responses were measured with a Shin-Nippon autorefractor (Ryusyo Industrial Co., Ltd, Japan) and a Badal system and an important assumption was that the accommodative system behaved in a similar way during both the accommodation responses and IOP measurement periods.

However, throughout the IOP measurement period, the ability to maintain a set amount of accommodation may have been affected by increased apprehension (Moses *et al.,* 1984) and anxiety (Forbes *et al.,* 1974) experienced during non-contact tonometry which of course will not have been experienced during the accommodation response measurement period. From the data collection phase of **Chapter 6**, it was noted that subjects reported that the internal target and the alignment lights of the NCT intermittently superimposed on the accommodative stimulus target i.e. the Maltese cross target. This suggests that the stimulus to

accommodation may have occasionally been lost during the IOP measurement period due to the binocular rivalry experienced and hence may have affected the ability to maintain a relatively constant amount of accommodation.

Despite this limitation it was concluded from the results of **Chapter 6** that IOP reduced on higher levels of accommodation. Interestingly, the investigation of the relationship between accommodation and IOP mapped by the experimental protocol described in **Chapter 6,** in which the fluctuations in IOP due to the cardiac and respiratory cycles are reduced, suggests that the relationship is characterised by substantial inter-subject variations in IOP responses to accommodation. These inter-subject variations in IOP responses to accommodation were not explained by inter-subject variations in accommodation responses, aspects of the accommodation stimulus-response function or refractive error. However, as mentioned earlier it was assumed that the accommodative system behaved the same under both experimental designs. It is therefore hypothesised that the inter-subject variations evident in the study described in **Chapter 6** may be due to inter- and intra- subject variations in accommodation responses during the IOP measurement period and this is the basis for the present study.

In the current study, the accommodative target was manipulated so that each subject had similar accommodative responses and furthermore, the simultaneous measurement of accommodation responses during the IOP measurement period was integrated in to the study design.

7.2 Method

7.2.1 Subject group

Forty subjects, from the sixty-six subjects who took part in the study described in **Chapter 6** were randomly recruited from the undergraduate population at Aston University for this study. The MSE [sphere + (cyclinder/2)] ranged from +1.00DS to -8.25DS and comprised of 20 myopes (MSE of $\leq$ -0.50D), 19 emmetropes (MSE of $\pm$ 0.50D) and 1 hypermetrope (MSE of $>$ +0.50D). Astigmatism was limited to <0.75DC. The criterion used to divide the subjects in to these refractive groups was also used in studies conducted by Goh *et al.* (2005), Junghans and Crewther (2005) and Ojaimi *et al.* (2005). The mean±SD (MN±SD) age for the

group was 20.6±3.0 years of age (range 18-31 years of age) and the cohort comprised of 14 males and 26 females.

The research followed the tenets of the Declaration of Helsinki and was approved by the Institution's ethics committee (**Appendix 3**). Written consent was obtained from all subjects willing to participate in the study and copies of the information sheets and consent forms given to the subjects can be found in **Appendix 4**. All subjects had a visual acuity of 0.00 logMAR or better. All subjects were absent of ocular pathology. None of the subjects were taking any topical or systemic medications that may affect the IOP, cardiovascular function or accommodative function.

7.2.2 Stimulus

As in **Chapter 6**, a high contrast Maltese cross target was carefully aligned in a +5D Badal system to stimulate low (0D; L), intermediate (1.50D; I) and high (4D; H) levels of accommodation. This configuration allowed changes in stimulus vergence without changes in the visual angle subtended by the accommodative target (i.e. a linear power scale was obtained) and minimum proximal accommodation was stimulated (Atchison *et al.,* 1995).

Due to the accommodative lead and lag for respectively long and short distances discussed in **Chapter 6** and **Appendix 8** the Maltese cross target was moved within the Badal system until 0.00±0.50 (L), 1.50±0.50 (I), and 4.00±0.50D (H) levels of accommodation were elicited. These accommodation stimulus levels were chosen so that the results from this thesis can be compared to the results from previously published studies on the relationship between accommodation and IOP (Armaly and Burian, 1958; Armaly and Rubin, 1961 and Mauger *et al.,* 1984) and to the results of **Chapter 6**. The accommodative targets within the Badal system were fixated for 3 minutes before the IOP measures were taken as 1) comparisons can be made with previous studies (Mauger *et al.,* 1984) and 2) accommodation adaptation is known to occur after approximately 3 minutes of fixation (Rosenfield and Gilmartin, 1989).

7.2.3 Instrumentation

The Shin-Nippon SWR-5000 open-view, infrared objective autorefractor was used to measure the subject's distance refractive error with the Maltese cross target placed within the Badal system at 200 mm away from the Badal lens. The accommodative responses were measured

with a portable, open-view, infrared, objective autorefractor the Grand Seiko FR-5000 (Grand Seiko Co., Ltd, USA) shown in **Figure 7.1**.

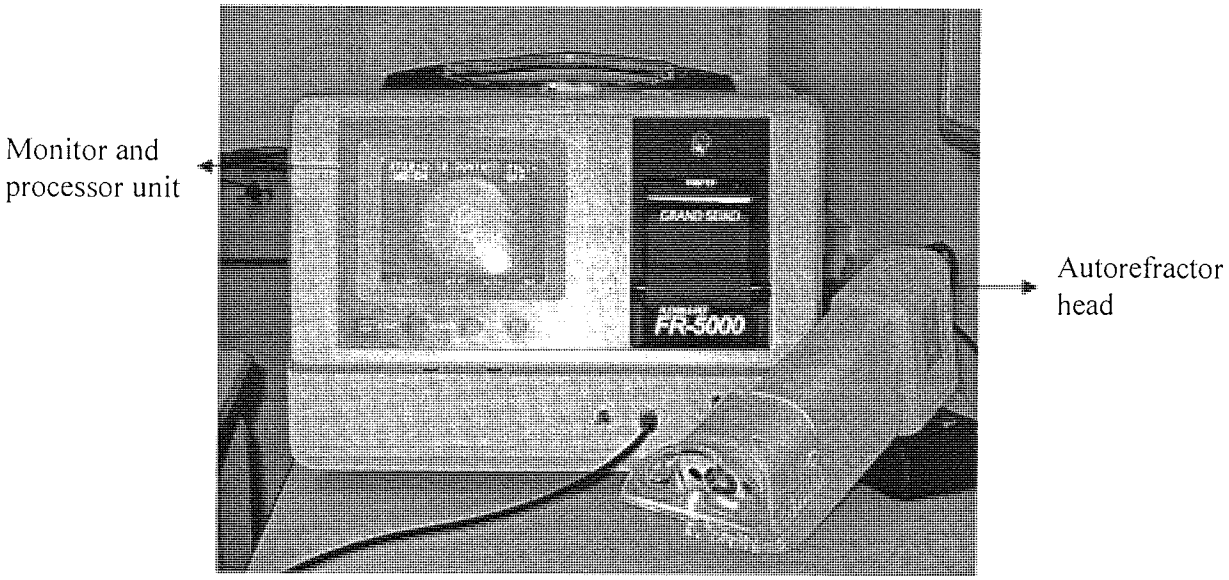


Figure 7.1 Grand Seiko FR-5000 autorefractor

The FR-5000 autorefractor uses the same measurement principal as the Shin-Nippon SWR-5000 and analyses a ring target of infra-red light after reflection off the retina. The optical arrangement of the instrument is however minimised and the autorefractor head is separated from the monitor and processor unit. Due to the small dimensions of the autorefractor head (5x9x21 cm), the head was mounted in front of the +5D Badal lens in alignment with the subjects LE which allowed measurement of the accommodation responses during the IOP measurement period (**Figure 7.2**).

The FR-5000 can measure sphero-cylindrical prescriptions over a range of ±20D spherical and ±10D cylindrical components (power increments of 0.12D or 0.25D; axis increments of 1°). In the instrument's dynamic mode, the system has a 0.01D resolution with pupil sizes >2 mm (Wolffsohn, Ukai and Gilmartin, 2006). To date, a clinical evaluation of the device comparing its performance with subjective refraction has not been performed. However, it is reasonable to assume that the accuracy of the instrument is at least similar to that of the Shin-Nippon SWR-5000.

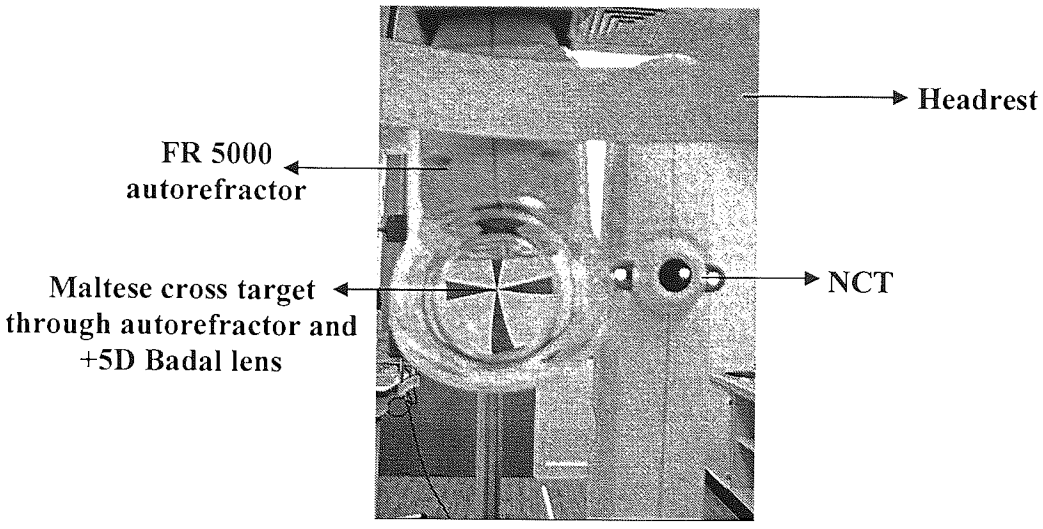


Figure 7.2 Subject view of the NCT in front of the RE and the FR 5000 autorefractor in front of the LE.

7.2.4 Experimental procedures

Forty of the sixty-six subjects who took part in the study described in **Chapter 6** were recruited for this study. Therefore, a familiarisation procedure (described in **Chapter 6**) was not required as the subjects were familiar with the corneal sensation experienced with a NCT.

Stage 1

In *Stage 1* of the experimental procedures a high contrast (90%) Maltese cross target was placed within the +5D Badal system and the distance refractive error was measured using the Shin-Nippon SWR-5000 open-view, infrared, objective optometer. The subjects were rendered functionally emmetropic in the LE using the refractive error data with a soft daily disposable contact lens (1-day *Acuvue Dailies*, etafilcon A, Johnson & Johnson, Vistakon, USA).

Stage 2

A fixed pace respiratory cycle (15 breathes/minute) and a steady cardiac cycle trace was obtained with the finger pulse-transducer (see **Chapter 5**). The experimental setup in this study was identical to that used in **Chapter 6**, with the addition of the FR-5000 autorefractor which was mounted in front of the subjects LE. The Maltese cross target was moved within the Badal system and the accommodation responses of the LE were sampled at 10 second intervals with the FR-5000 autorefractor. The distances at which the accommodation responses of zero±0.50, 1.50±0.50 and 4±0.50D were measured with the autorefractor were noted. The target was placed at the corresponding distances and the subjects were instructed

to "focus on the target carefully" as suggested by Stark and Atchison (1994). After 3 minutes of fixation [as accommodative adaptation occurs after this time period (Rosenfield and Gilmartin, 1989)] the accommodation responses of the LE were measured with the autorefractor by a separate examiner (AC) while 5 IOP measures synchronised with the middle location of the cardiac cycle were taken in the RE with the *Pulsair EasyEye* (Keeler) NCT by examiner GR. Two separate examiners were required as the measurement of the accommodation responses and IOP were required simultaneously. The accommodation responses were measured at 10 second intervals during the 3 minutes of fixation and at 10 second intervals during the IOP measurement period. The experimental set up (shown in **Figure 7.3**) allowed unhindered IOP measures in one eye and simultaneous stimulation and measurement of accommodation in the other eye. Importantly the experimental design assumes that the accommodation responses are consensual, which is a reasonable assumption.

After each set of 5 measures the subjects were allowed a 5 minute rest period. The 3 distances corresponding to the 3 accommodative levels were presented randomly and the results were masked from both examiners.

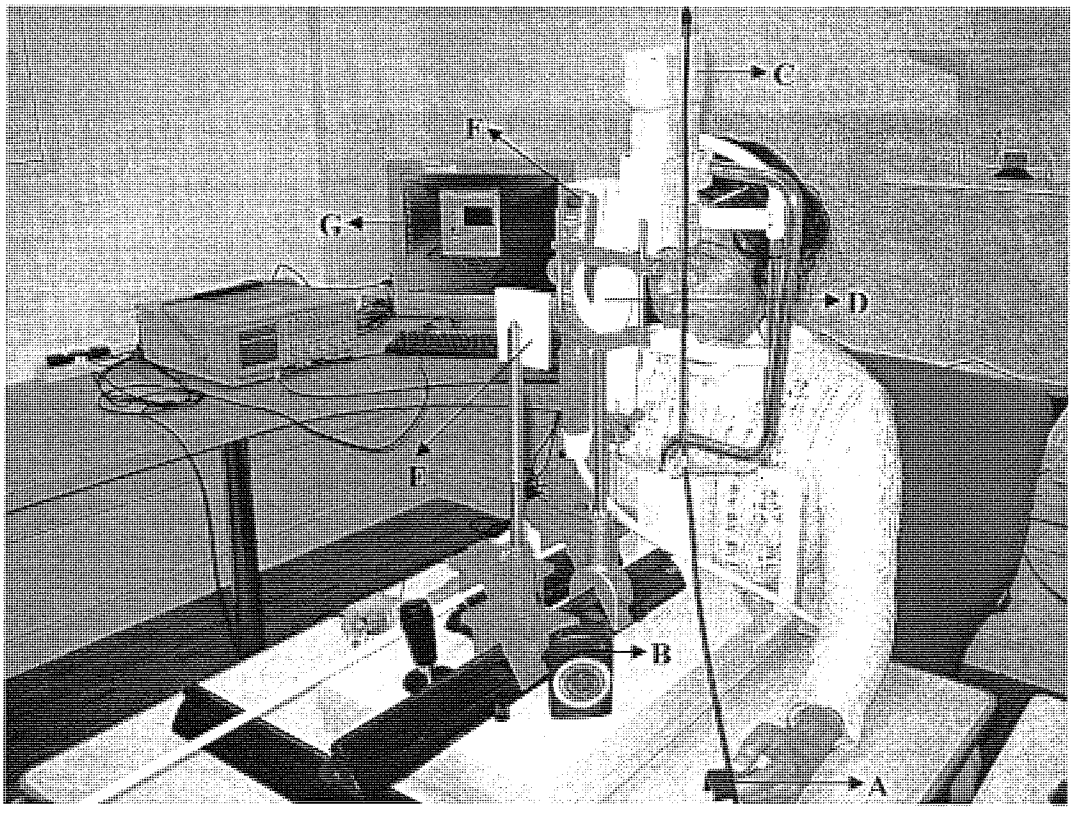


Figure 7.3 The experimental setup allowing IOP measures to be taken in the RE and simultaneous measurement of accommodation responses in the LE. **A**: Pulse-transducer; **B**: Metronome; **C**: FR 5000 autorefractor; **D**: Badal lens; **E**: Maltese cross target; **F**: *Pulsair EasyEye* NCT; **G**: Cardiac trace.

Inter-session repeatability was assessed in 2 volunteers. The 2 data collection sessions were arranged on different days at approximately the same time to minimise the effects of diurnal variations on IOP which have previously been reported (Pointer, 1997; Noel *et al.*, 2001; Liu *et al.*, 2003a, b; Kida *et al.*, 2006).

7.3 Statistical Analysis

Software packages *SPSS 12.1* for Windows and Microsoft *Excel* were used for the statistical analyses of the data.

The average of 5 IOP measures was used for analyses. A within-subjects one-way analysis of variance (ANOVA) in randomised blocks was performed followed by Bonferroni and Scheffe *post-hoc* analyses. For the Bonferroni analyses the critical significance level was taken as 0.017 (0.05/3). The IOP measures obtained when the subjects were fixating a 0D accommodative stimulus were treated as the reference values for comparison. Between sessions repeatability was determined by calculating the coefficient of repeatability (COR) which is defined as the standard deviation of the differences multiplied by 1.96.

To determine whether the myopes and emmetropes differed in their IOP responses to accommodation, a one-way between-subjects analysis of variance (ANOVA) was performed. Furthermore, to establish whether the level of refractive error influenced the change in IOP on accommodation, Pearson's correlation coefficients (r) and the associated significance levels were calculated.

Data collected from the current study was compared against the data for the same 40 subjects collected in **Chapter 6**. The comparison was achieved by the method of Bland and Altman (1986) and by determining Pearson's r and the associated level of significance between the two data sets.

7.4 Results

Accommodation and IOP

The mean±SD (MN±SD) of accommodation responses and IOP for L, I and H accommodation stimuli levels for the cohort are summarised in **Table 7 .1.** The within-subjects ANOVA shows that accommodation significantly influences the IOP ($F(2, 78)=5.678$, $p=0.008$). The IOP decreases between L and I, and L and H accommodative stimuli levels by 0.61±0.99 and 0.13±1.41 mmHg, respectively. The IOP increases between I and H levels of accommodative stimuli by 0.48±1.50 mmHg. On Scheffe *post-hoc* analyses these differences in IOP did not reach statistical significance (L to I: $p=0.39$; L to H: $p=0.98$; I to H: $p=0.51$). However, Bonferroni *post-hoc* analyses revealed that the differences in IOP on accommodation reached statistical significance between L and I ($p<0.001$) and I and H ($p=0.02$) but not between L and H ($p=0.65$) accommodative stimuli levels. The percentage change in IOP between L and I and L and H accommodation levels was a reduction of 4.23±7.05 and 0.55±10.23 respectively and the differences between percentage changes just reached statistical significance ($p=0.04$). The between sessions COR was ±0.17 mmHg.

Accommodation stimulus levels (D)	MN±SD Accommodation response levels (D)	MN±SD IOP (mmHg)
L	-0.14±0.31	13.95±2.09
I	+1.67±0.20	13.34±2.05
H	+4.15±0.36	13.82±2.17

Table 7.1 Summary of accommodation response and corresponding IOP for the 3 accommodation stimuli levels.

The relationship between accommodation and IOP is characterised by substantial inter-subject variations in the differences in IOP between the 3 levels of accommodative stimuli as illustrated in **Figures 7.4** and **7.5.** The range (RG) and median (MED) of the differences in IOP between L and I, L and H, and I and H are respectively, RG=1.01 to -2.57, MED=-0.61; RG=4.47 to -3.27, MED=-0.24 and RG=5.25 to -2.27, MED=+0.59. The distributions of the differences in IOP between the 3 levels of accommodation stimuli are shown in **Figures 7.6.**

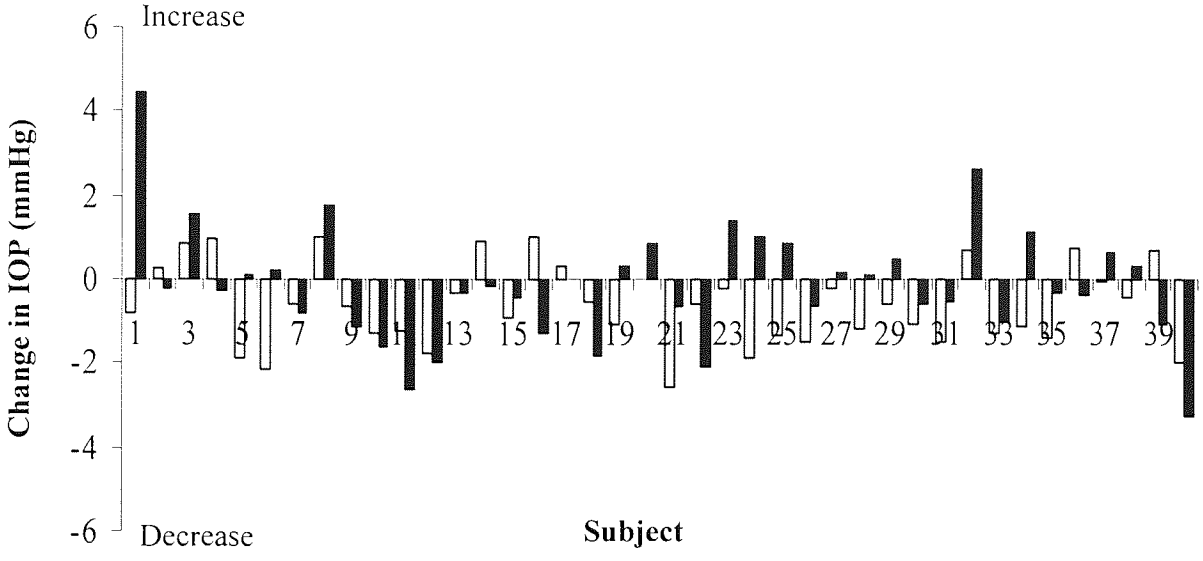


Figure 7.4 Change in IOP (mmHg) between L and I (▒) and L and H (■) accommodation stimulus levels (n=40).

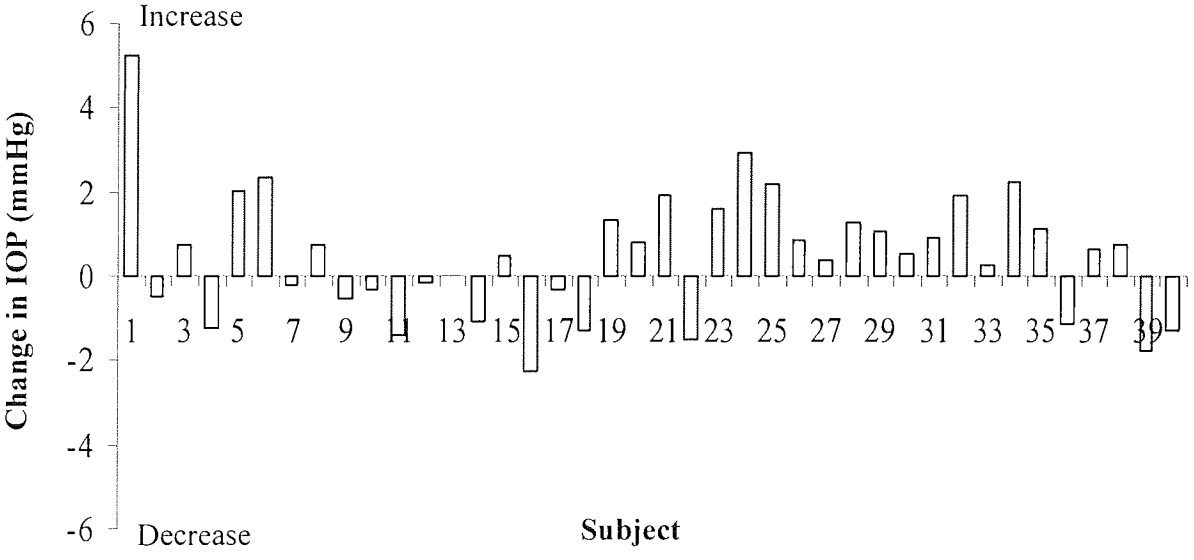
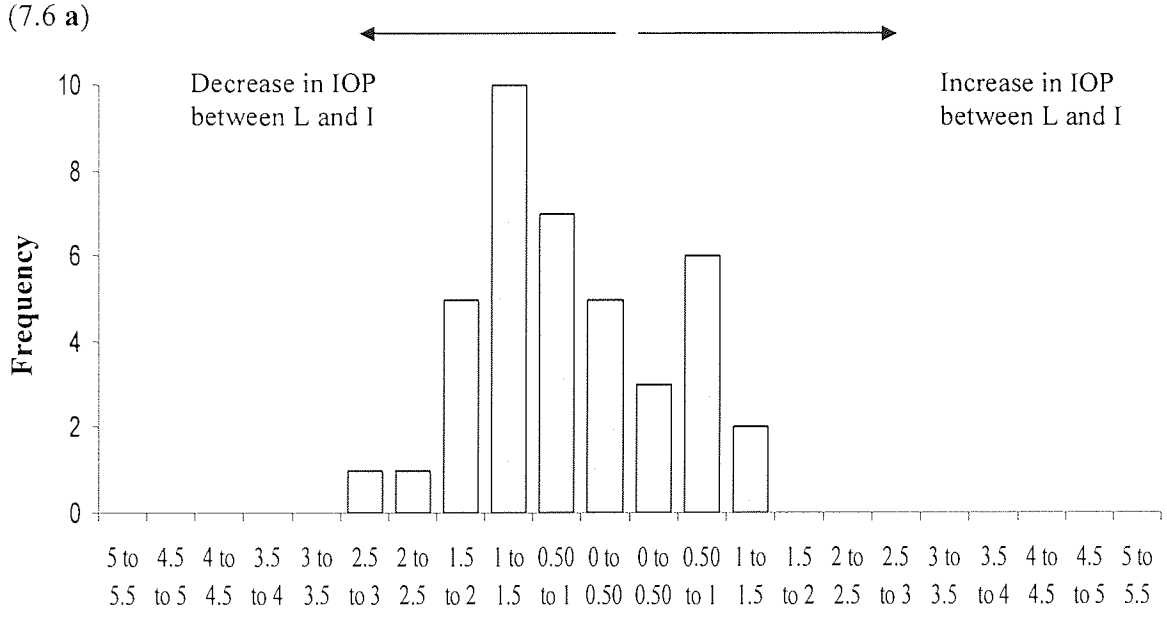


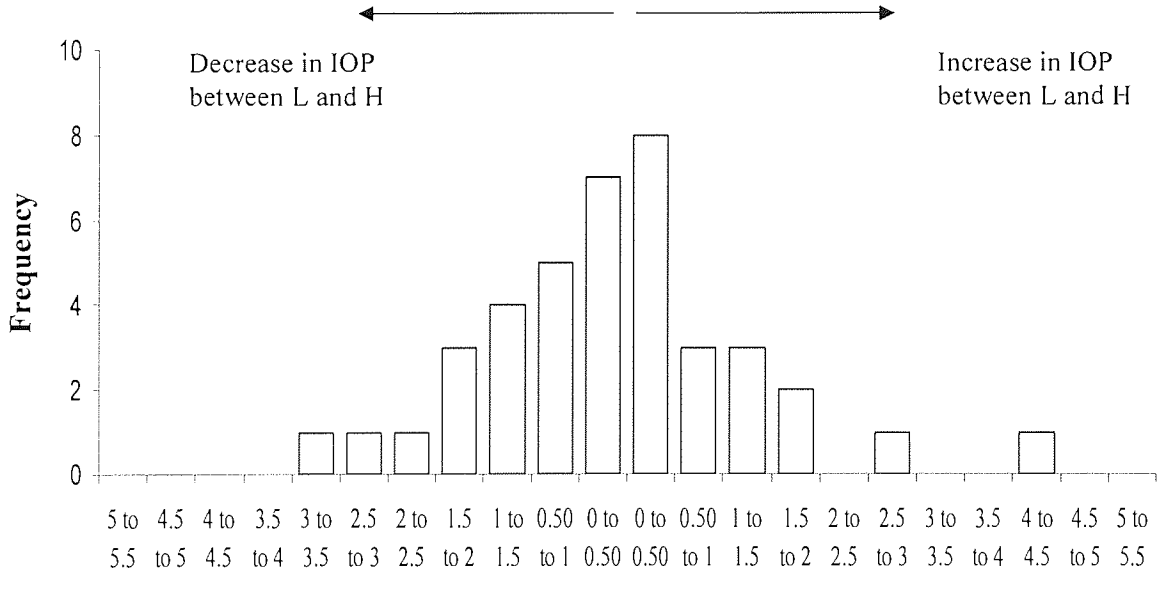
Figure 7.5 Change in IOP (mmHg) between I and H accommodation stimuli levels (n=40).

(7.6 **a**)



Difference between L and I accommodation stimulus levels (mmHg)

(7.6 **b**)



Difference between L and H accommodation stimulus levels (mmHg)

(7.6 c)

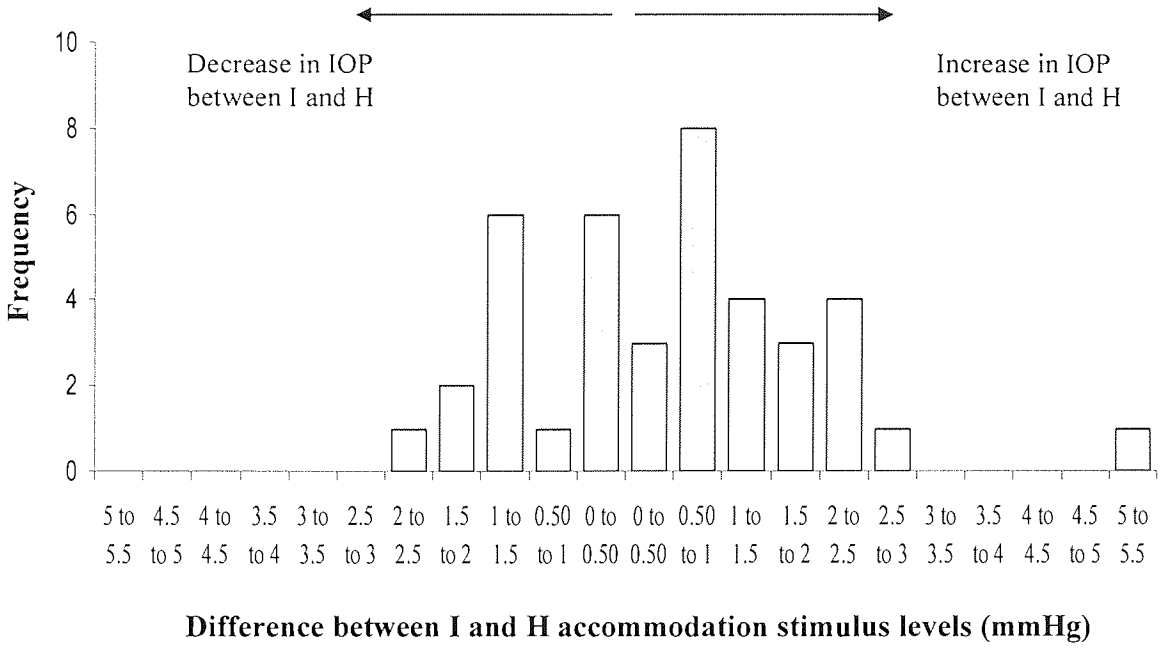


Figure 7.6 Histogram of the differences in IOP between L and I (**a**), L and H (**b**) and I and H (**c**) levels of accommodation stimuli (n=40).

The between-refractive groups ANOVA shows that the differences in IOP responses to accommodation of the emmetropes were not statistically different to the IOP responses to accommodation of the myopes between L and I (F=0.152, p=0.70), L and H (F=0.098, p=0.76), and I and H (F=0.313, p=0.58) levels of accommodation. **Figure 7.7** demonstrates that the variations in IOP responses between L and I (r=0.22, p=0.18), L and H (r=0.19, p=0.24) and I and H (r=0.04, p=0.82) levels of accommodation stimuli are not due to refractive error. Note that the inter-subject variations in the changes in IOP with accommodation were not considered with respect to the baseline IOPs due to the relatively small inter-subject variations in baseline IOPs.

A significant correlation (r=0.41, p=0.008) exists between the change in IOP between L and I levels of accommodation and the change in IOP between I and H levels of accommodation and is shown in **Figure 7.8.**

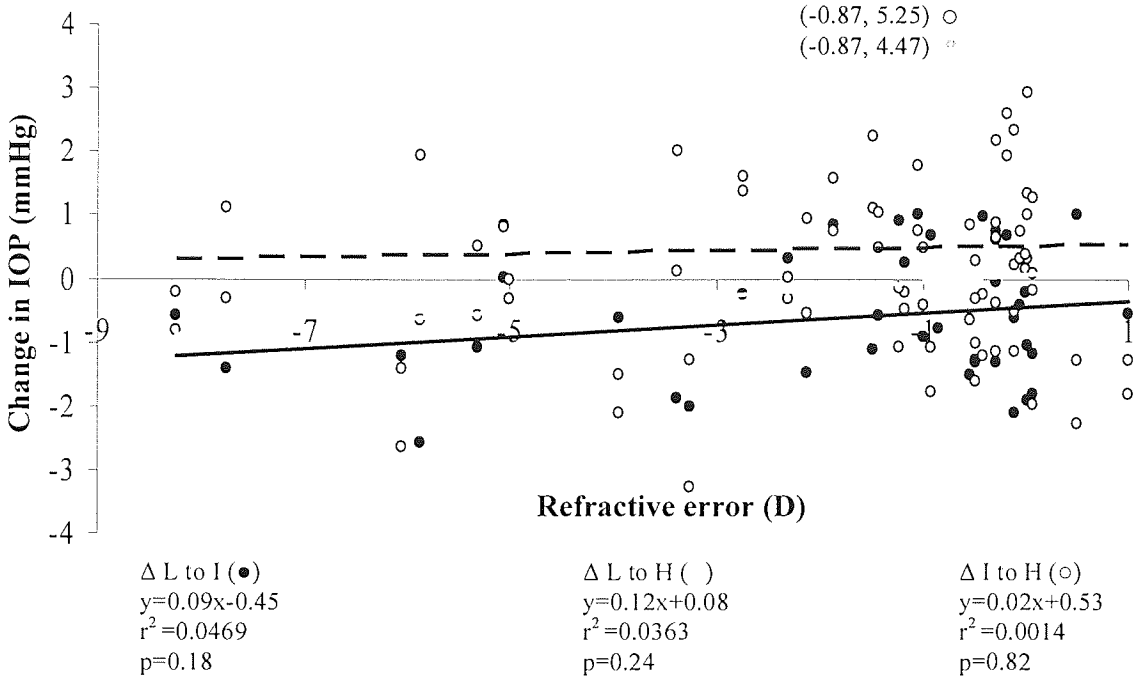


Figure 7.7 Level of refractive error and change in IOP between L and I (—), L and H ( ) and I and H (---) levels of accommodation (n=40).

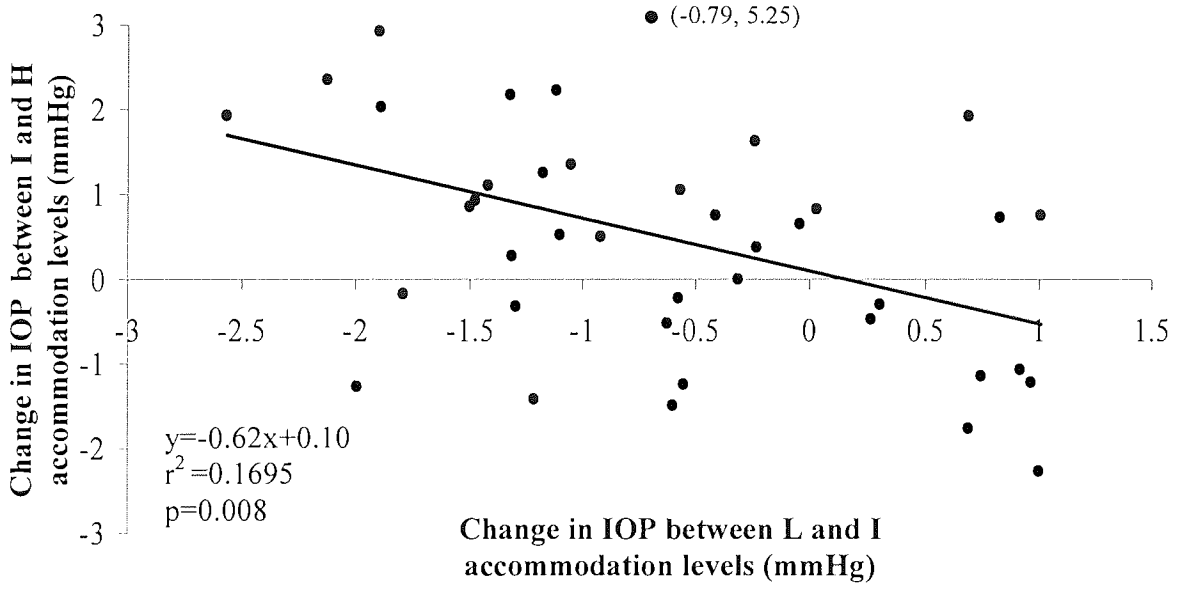


Figure 7.8 Change in IOP between L and I levels of accommodation against the change in IOP between I and H levels of accommodation (n=40).

Analyses of the percentage changes in IOP are shown in **Appendix 9**. The results also indicate that accommodation does influence the percentage changes in IOP. On *post-hoc* analyses the differences in percentages changes in IOP with accommodation reached

statistical significance only between L and I and I and H accommodation levels. Similar to the results shown above, the results in **Appendix 9** also indicate that the inter-subject variations in percentage changes in IOP were not explained by refractive error.

Comparison between the two data sets

The experimental protocol described in **Chapter 6** was performed on sixty-six subjects and 40 of these subjects were recruited for the present study. Therefore, for comparison of the two studies, the results of the same forty participants who were subjected to the experimental protocol described in **Chapter 6** are discussed below.

The accommodation responses and corresponding IOP distributions to L, I and H accommodation stimuli levels are summarised in **Table 7.2.** For the cohort examined the within-subjects ANOVA did not reach statistical significance [F (2, 78) = 1.647, p=0.199]. The IOP decreases between L and I, L and H and I and H accommodative stimuli levels by respectively 0.14±1.04, 0.32±1.10 and 0.18±1.17 mmHg and, as expected, the Scheffe (L and I, p=0.95; L and H, p=0.75; I and H, p=0.91) and Bonferroni (L and I, p=0.41; L and H, p=0.08; I and H, p=0.34) *post-hoc* analyses revealed that none of these differences in IOP were statistically significant.

Accommodation stimulus levels (D)	MN±SD Accommodation response levels (D)	MN±SD IOP (mmHg)
L	-0.18±0.38	13.67±1.90
I	+1.54±0.29	13.53±1.74
H	+4.00±0.35	13.35±1.98

Table 7.2 Summary of accommodation response levels and corresponding IOP to the 3 accommodation stimulus levels for data collected for 40 subjects in **Chapter 6**.

The inter-subject variations in the differences in IOP between the 3 levels of accommodative stimuli are illustrated in **Figures 7.9** and **7.10**. The RG and MED of the differences in IOP between L and I, L and H, and I and H are respectively, RG=2.92 to -1.90, MED=-0.12; RG=2.98 to -3.14, MED=-0.32 and RG=2.77 to -2.60, MED=-0.18. The distributions of the differences in IOP between the 3 levels of accommodation stimuli are shown in **Figures 7.11**.

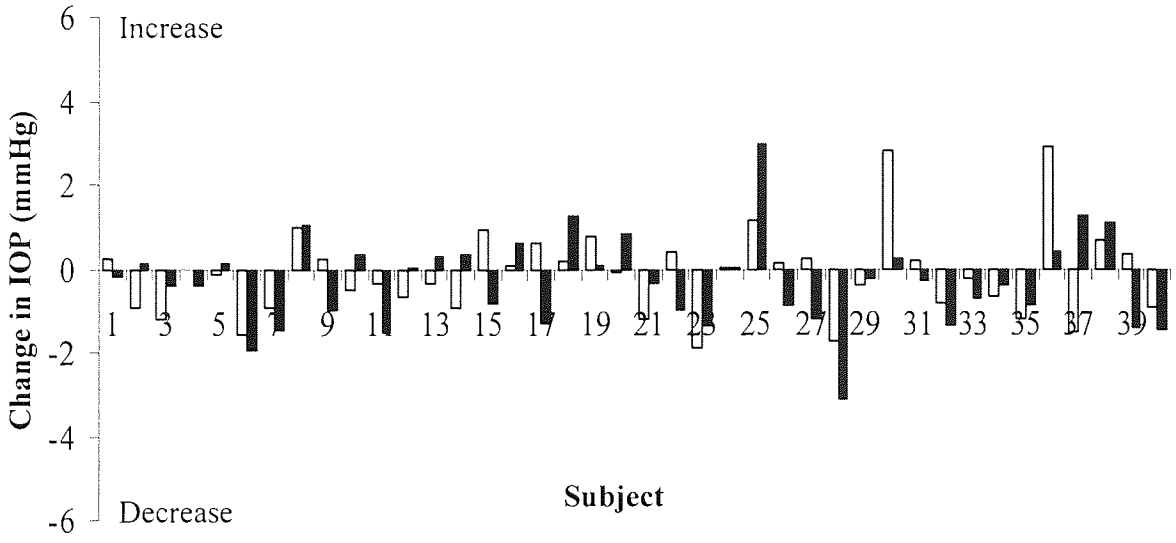


Figure 7.9 Change in IOP between L and I (▩) and L and H (■) accommodation stimulus levels for the data collected for 40 subjects in **Chapter 6**.

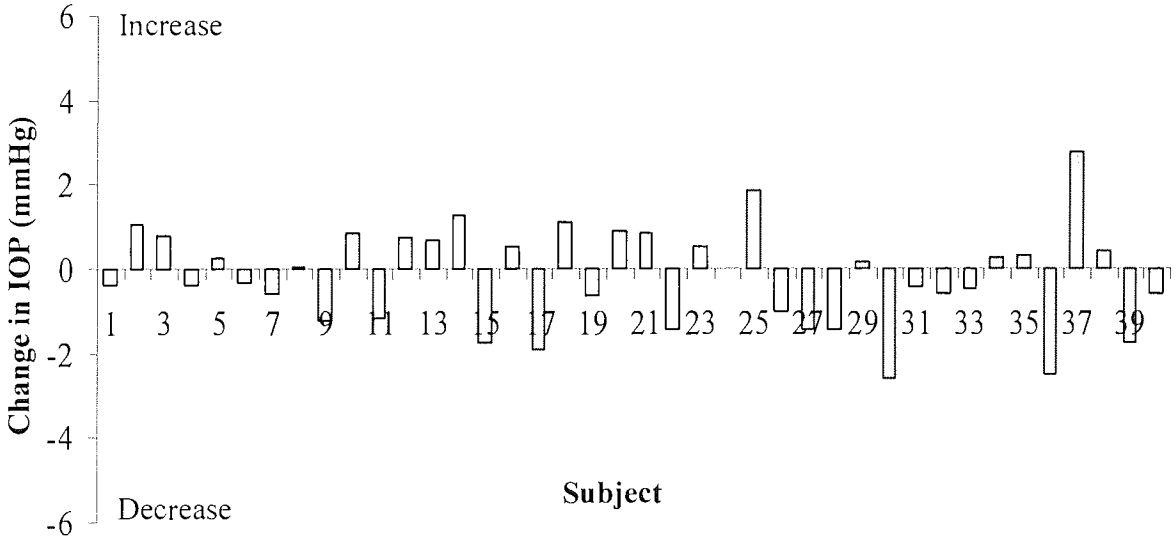
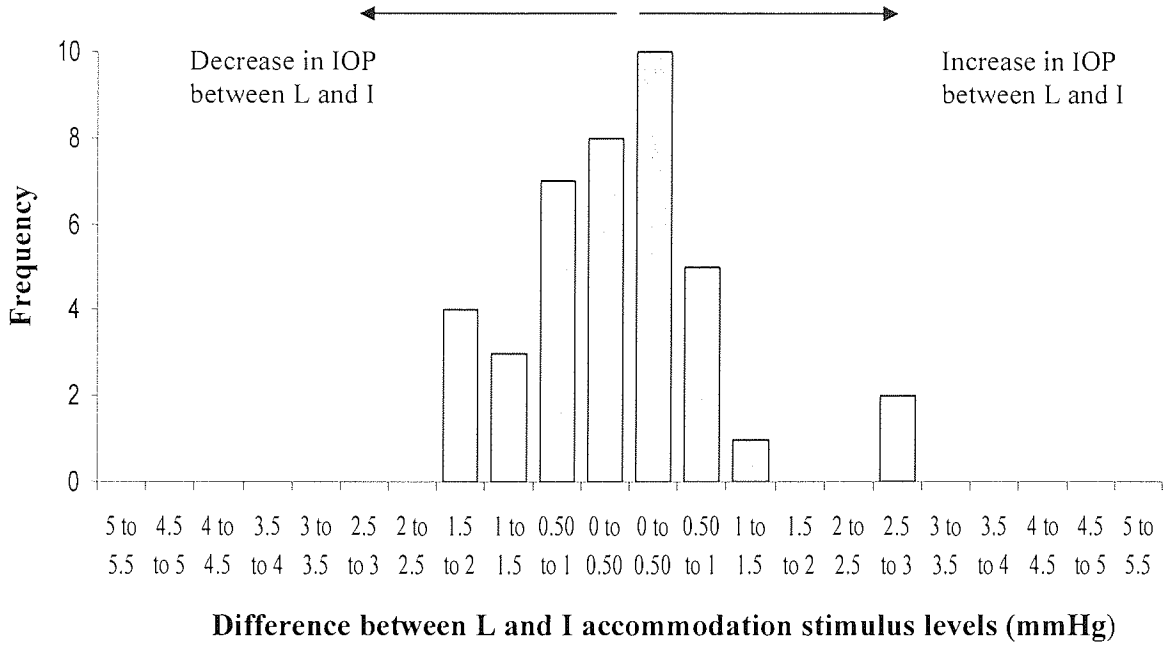


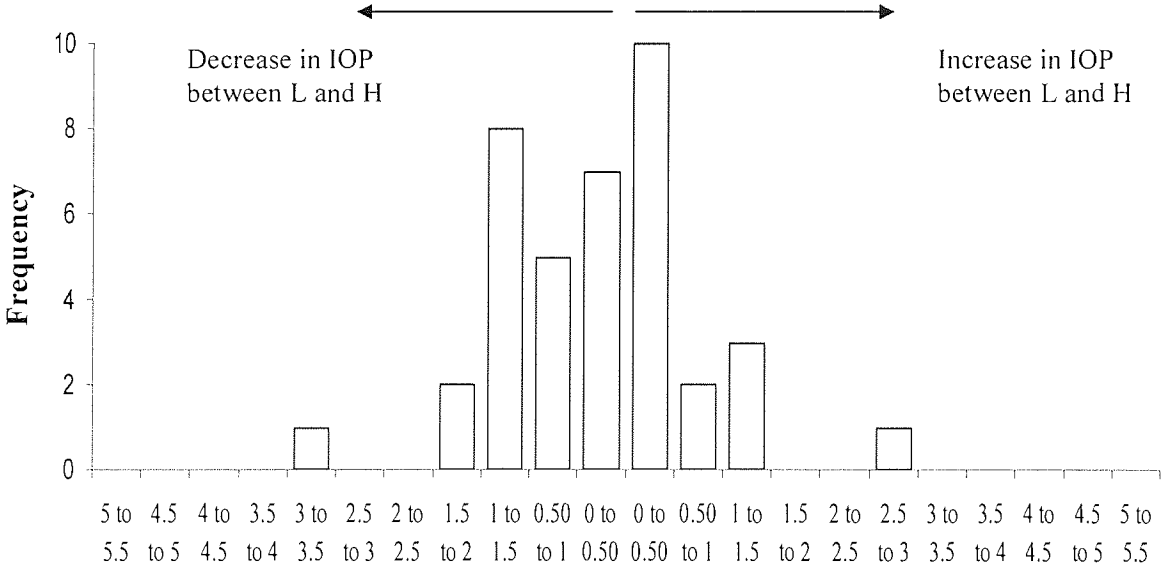
Figure 7.10 Change in IOP between I and H accommodation stimulus levels for the data collected for 40 subjects in **Chapter 6**.

(7.11a)



Difference between L and I accommodation stimulus levels (mmHg)

(7.11b)



Difference between L and H accommodation stimulus levels (mmHg)

(7.11c)

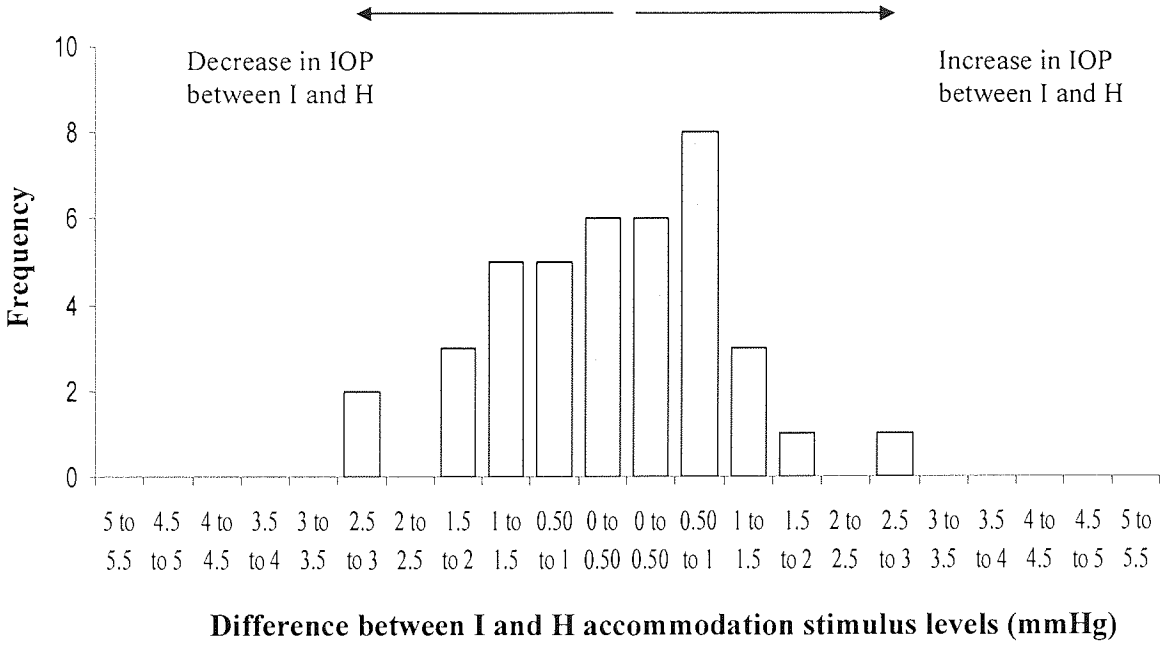
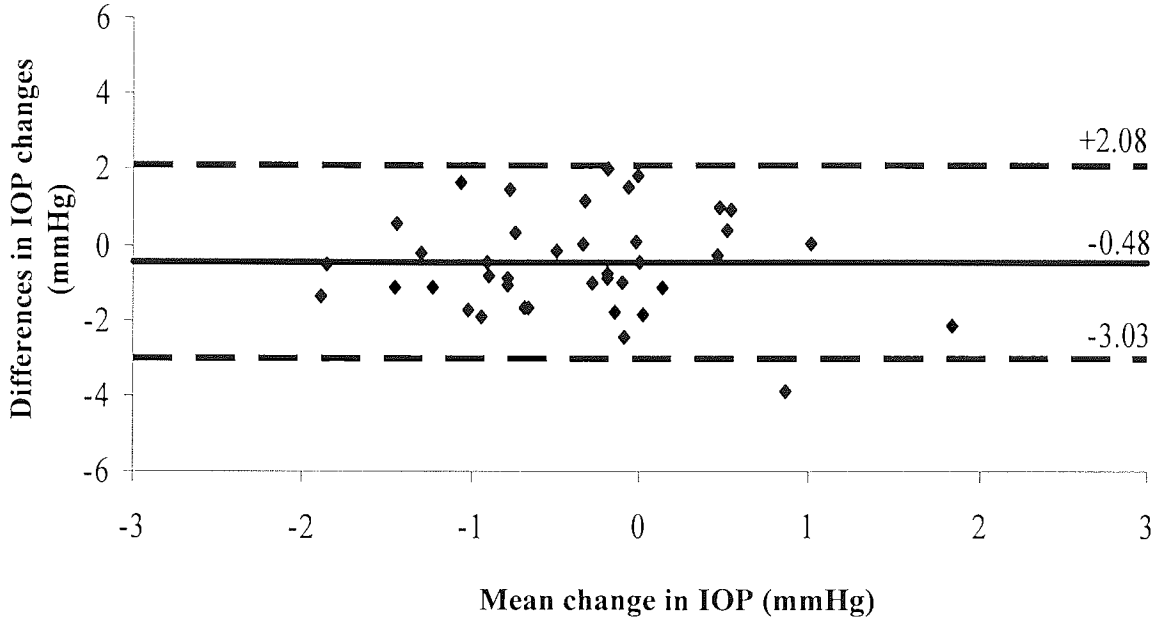


Figure 7.11 Histogram of differences in IOP between L and I (**a**), L and H (**b**) I and H (**c**) levels of accommodation stimuli for the data collected for 40 subjects in **Chapter 6**.

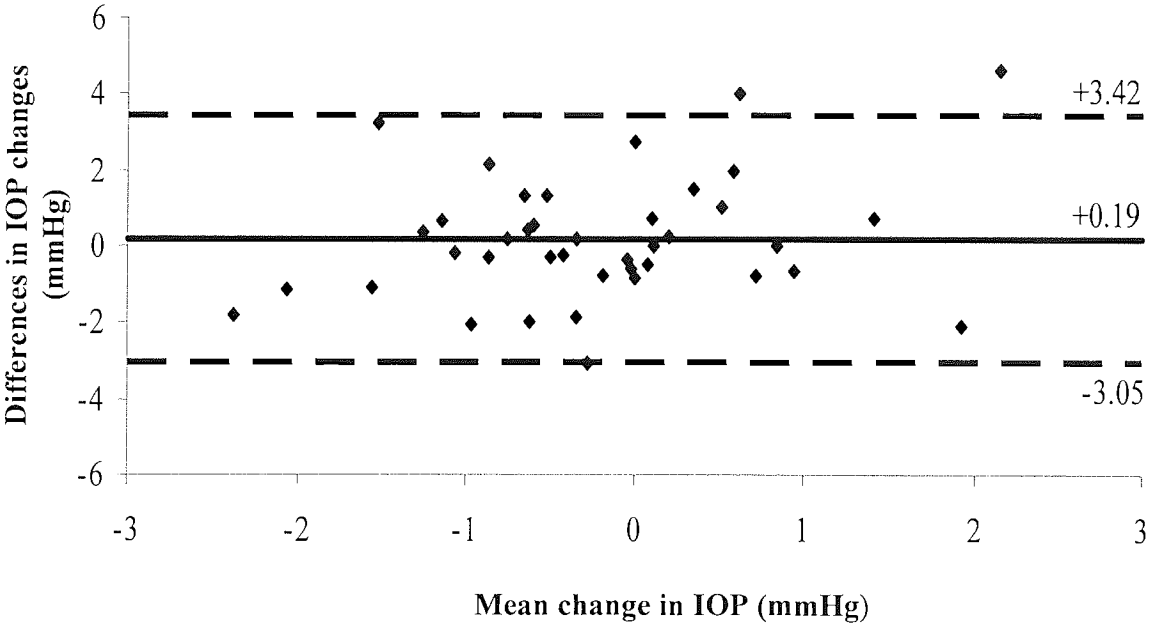
The Bland and Altman plots of the differences in IOP as a function of their means are shown in **Figure 7 .12**. The differences in IOP between L and I (MN±SD: -0.48±1.30 mmHg, p=0.03) and I and H (MN±SD: 0.66±1.73 mmHg, p=0.02) levels of accommodation as measured by the two experimental protocols were statistically significant, whereas the differences in IOP between L and H (MN±SD: 0.19±1.65 mmHg, p=0.48) levels of accommodation were not statistically significant. The confidence intervals for the L and I, L and H, and I and H accommodation levels were respectively ±2.56, ±3.23 and ±3.40 mmHg.

The correlations between the change in IOP between L and I (r=0.17, p=0.28), L and H (r=0.15, p=0.34), and I and H (r=0.17, p=0.28) accommodation levels as measured by the 2 experimental protocols are shown in **Figure 7.13**

(7.12a)


Differences in IOP changes (mmHg)
6
4
2
0
-2
-4
-6
+2.08
-0.48
-3.03
-3
-2
-1
0
1
2
3
Mean change in IOP (mmHg)

(7.12b)


Differences in IOP changes (mmHg)
6
4
2
0
-2
-4
-6
+3.42
+0.19
-3.05
-3
-2
-1
0
1
2
3
Mean change in IOP (mmHg)

(7.12c)

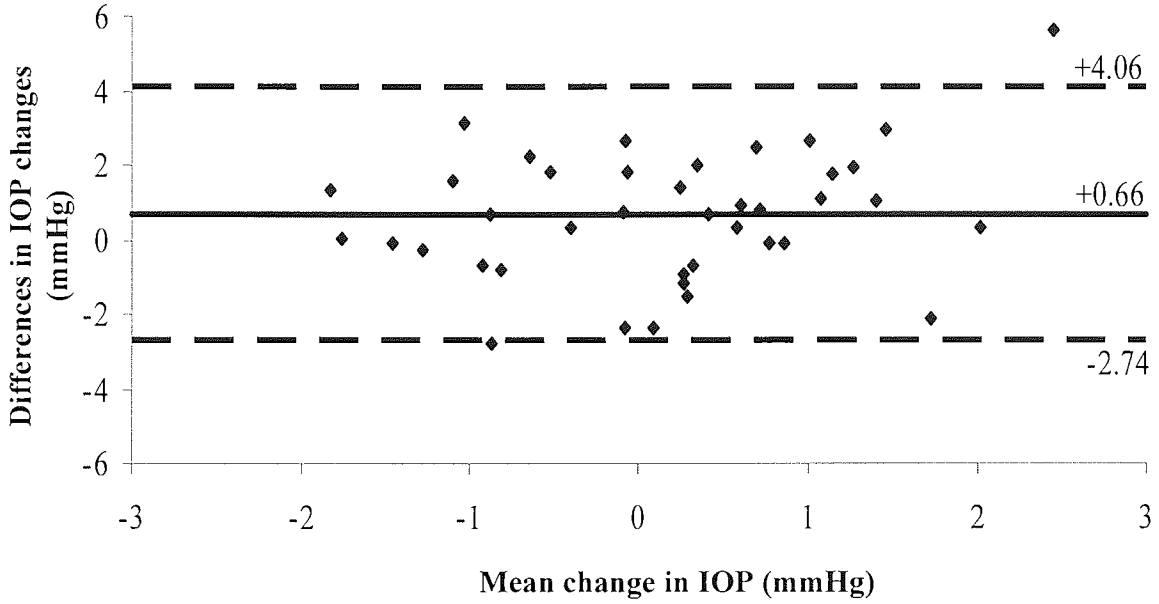
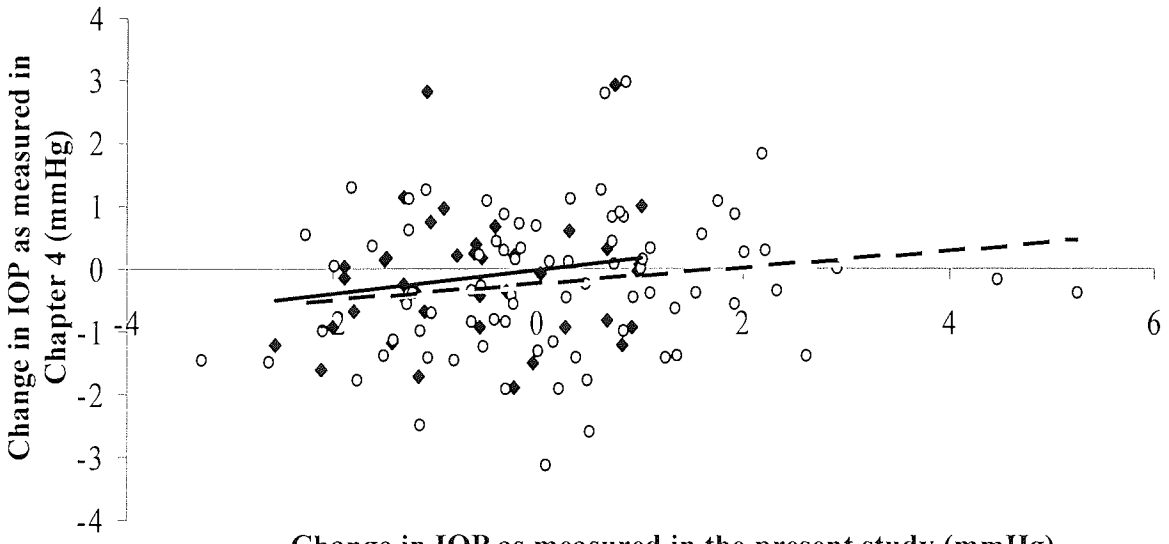


Figure 7.12 Bland and Altman dispersion plots of the differences in the change in IOP between L and I (**a**), L and H (**b**) and I and H (**c**) levels of accommodation as measured in the present study and measured following the experimental protocol described in **Chapter 6** (n=40).



Δ L to I (●)	Δ L to H ( )	Δ I to H (○)
y=0.18x-0.02	y=0.12x-0.30	y=0.14x-0.24
r^2 =0.0303	r^2 =0.024	r^2 =0.0302
p=0.28	p=0.34	p=0.28

Figure 7.13 Correlations between the changes in IOP as measured in the present study and the changes in IOP measured following the experimental protocol in **Chapter 6** for L and I (—), L and H ( ) and I and H (---) accommodation levels (n=40).

7.5 Discussion

IOP measures synchronised with the middle phase of the cardiac cycle (see **Chapter 5**) were taken in the RE with the Pulsair *EasyEye* (Keeler) NCT while the LE fixated L, I and H levels of accommodation stimuli (presented randomly) for 3 minutes. The 3 accommodation levels were stimulated in the LE, within a +5D Badal system and the accommodation responses were measured simultaneously with the FR 5000 (Grand Seiko Co., Ltd, USA) autorefractor. Statistical analyses of the IOP change with accommodation showed that the level of accommodation influenced IOP. *Post-hoc* analyses indicated a significant reduction in IOP for intermediate levels of accommodation but not for higher levels of accommodation. The IOP responses to accommodation were found to be repeatable between sessions. Furthermore, a significant difference in percentage changes in IOP between L and I and L and H levels of accommodation was exhibited suggesting a dose dependency.

A decrease in IOP for intermediate levels of accommodation was also found by Mauger *et al.* (1984). However, there is a difference in the magnitude of the reduction, such that a reduction of <1 mmHg was found in the present study whereas Mauger's study reported a reduction of >2 mmHg for 1.5D of accommodation. In contrast to the present study, the studies described in **Chapter 4**, **Chapter 6** and the study by Mauger *et al.* (1984) also showed that IOP reduced for higher levels of accommodation which was in agreement with the findings of Armaly and Burian (1958) and Armaly and Rubin (1961). It is postulated that since the study conducted by Mauger *et al.* (1984) used a GCT to measure IOP, the results maybe confounded by the use of anaesthetics (Herse and Siu, 1992; Baudouin and Gastaud, 1994; Birchall and Kumar, 2001; Asensio *et al.*, 2003; Nam *et al.*, 2006;) and the effects of repeated contact tonomtery (Stocker, 1956; Moses, 1961; Moses and Liu, 1968).

A simple mechanical explanation of the effects of accommodation on IOP was described in **Chapter 6**. On accommodation, the ciliary muscle contracts, opens the trabecular meshwork, increases aqueous outflow and therefore a decrease in IOP is expected. Hence, on approximately 1.5D of accommodation the ciliary muscle contracts and IOP decreases and indeed, on approximately 4D of accommodation a further decrease in IOP is expected. However, the results of this study show that for the cohort examined, no decrease in IOP is evident for higher levels of accommodation and therefore the existence of a possible regulatory mechanism within the ciliary body or trabecular meshwork is hypothesised.

Stimulation of the muscarinic receptors with pilocarpine has been shown to increase outflow by contraction of the ciliary muscle leading to the widening of the spaces in the trabecular meshwork (Kaufman, 1984 b; Gabelt and Kaufman, 1992; Pang *et al.,* 1992; Kiland *et al.,* 2000). Muscarinic receptors have also been found in the trabecular meshwork (Gupta *et al.,* 1994; Schroeder and Erickson, 1994; 1995; Zhang *et al.,* 1995; Woldesmussie, Feldmann and Chen, 1993) and furthermore the trabecular meshwork has been shown to have smooth muscle-like contractile properties (Lepple-Wienhues, Stahl and Wiederholt, 1991). Therefore similar to the effects on the ciliary muscle, pilocarpine also contracts the trabecular meshwork which has an opposite effect and reduces the aqueous outflow (Wiederholt, Dorschner and Groth, 1997; Wiederholt, 1998) although this effect has been shown to be minimal (Kaufman and Barany, 1976 a, b). The overall increase in outflow and hence decrease in IOP observed with pilocarpine is due to the differential contractibility of the two muscles. Lepple-Weinhues and co-workers have shown that the force of contractibility is 10 times greater in the ciliary muscle compared to that of the trabecular meshwork (Lepple-Weinhues *et al.,* 1991). Nevertheless, the agonism/antagonism between the ciliary muscle and trabecular meshwork has to be considered as it is possible that the trabecular meshwork is involved in the fine-tuning of the regulation of aqueous humour outflow. In addition, contractile cells have also been found in the scleral spur (Tamm *et al.,* 1992 b; Tamm *et al.,* 1995).

Thus it is clear that at least three different contractile elements may be involved in regulating aqueous humour outflow and hence IOP. Furthermore, research undertaken so far indicates a complex signalling cascade which regulates the contractibility of the ciliary muscle, trabecular meshwork and scleral spur and has been reviewed by Wiederholt, Thieme and Stumpff, 2000). In light of the results from the present study it is thought that the IOP reduces on intermediate levels of accommodation, but on higher levels of accommodation it is hypothesised that it may not be ideal for the eye to cope with a further decrease in IOP. A feedback process may lead to changes in the signalling cascade, allowing differential responses of the outflow tissues to reduce the aqueous outflow (i.e. increase IOP). Indeed changes in the autonomic regulation of aqueous formation or a combination of the aqueous inflow/outflow regulating mechanisms may also play a part in regulation of the IOP during high levels of accommodation.

A somewhat perplexing finding was that although the accommodation responses were set and monitored throughout the IOP measurement period, the substantial inter-subject variations in IOP responses to accommodation evident in **Chapter 6** still remained in the present study. Furthermore, similar to the results of **Chapter 6** these inter-subject variations in IOP were not attributable to the refractive status of the eye. **Figure 7.8** shows that in some subjects the IOP increases between L and I accommodation levels and decreases between I and H accommodation levels. Conversely in the majority of subjects the IOP decreases between both L and I accommodation levels and increases between I and H levels of accommodation.

The mean differences in IOP between L and I and L and H levels of accommodation following two separate experimental protocols on the same subjects are not statistically significant. However from observation of the Bland and Altman plots and correlation analyses it is evident that the two data sets are not comparable i.e., the IOP responses to accommodation in the two studies are not similar. This is possibly due to differences in the methods used to control the accommodation responses in the two studies. In the study described in **Chapter 6** an assessment of the accommodation responses were made in a separate experimental setup and it was assumed that the accommodative system behaved the same during the IOP measurement period, whereas in the present study quasi-continuous measures of the accommodation responses were taken which were zero±0.50, 1.5±0.50 and 4±0.50D.

The simultaneous quasi-continuous measurement of accommodation responses during the IOP measurement period increases the validity of the data presented in this study compared to that in **Chapter 6**. The dose dependency evident has instigated a series of investigations to determine whether regulation within the vascular system determines the changes in IOP with accommodation the results of which are discussed in **Chapter 8**. Furthermore, the inter-subject variations in IOP responses may be attributable to inter-subject variations in biometric and oculomotor parameters. Hence the measurement of biometric (e.g. axial length) and oculomotor (e.g. accommodative convergence) parameters were incorporated into the study design and are discussed in **Chapter 9**.

7.6 Conclusion

This study investigates the effects of accommodation on IOP using the *Pulsair Easyeye* NCT to measure the IOP and the FR 5000 autorefractor to simultaneously measure the accommodation responses.

The main findings of this study are:

- The IOP reduces with I levels of accommodation, but not with H levels of accommodation.

- The IOP changes with H accommodation levels may be regulated via aqueous humour outflow or formation.

- The relationship between accommodation and IOP is characterised by substantial inter-subject variations in IOP responses to accommodation.

- These variations are not attributable to the refractive status of the eye.

- The data obtained in the present study and the data obtained following the experimental protocol described in **Chapter 6**, in which the accommodation responses were not measured simultaneously are not comparable.

7.7 Supporting publications

Rai, G. K., Gilmartin, B., Wolffsohn, J. S. and Cervino, A. (2006). The effect of myopia, axial lengths and ocular volumes on IOP response patterns to accommodation. Proceedings of the International Conference on Myopia in Singapore. *Ophthalmic Physiol Opt.* **22;** Suppl I.

CHAPTER 8

ACCOMMODATION AND PULSATILE OCULAR BLOOD FLOW

8.1 Introduction

A plethora of research, including the results shown in **Appendix 8** of this thesis, supports the established view that myopes have less accurate accommodative systems than the emmetropes (McBrien and Millodot, 1986 a; Ebenholtz and Zander, 1987; Rosenfield and Gilmartin, 1988; Tokoro, 1988; Bullimore, Gilmartin and Royston, 1992; Gwiazda *et al.,* 1993; Jiang 1994 and 1995; Rosenfield, 1998; Nakatsuka *et al.,* 2005). It is thought that myopes have reduced blur sensitivity, which decreases the error signal (i.e. retinal blur) to the accommodation system and results in impaired accommodative responses (Rosenfield and Abraham-Cohen, 1999; Seidel, Gray and Heron, 2003) and these accommodation inaccuracies (i.e. increased accommodative lags) have been considered as a predicative factor for the underlying mechanism of myopia onset and/or progression (Goss, 1991; Gwiazda *et al.,* 1993; Drobe and de Saint-Andre, 1995; Gwiazda *et al.,* 1995; Jiang, 1995; Rosenfield and Gilmartin, 1998; Charman 1999; Jiang and Morse, 1999; Gwiazda *et al.,* 2005; Allen and O'Leary, 2006). On elimination of the accommodative inaccuracies with progressive addition lenses, myopic progression is impeded, albeit slightly (Gwiazda *et al.,* 2005). Mutti *et al.* (2006) more recently however, has shown that an increase in the accommodative lag does not precede myopic onset and therefore can only be considered as a consequence rather than a cause of myopia development.

Under-accommodation during nearwork results in the focal plane being behind the retina causing hyperopic blur analogous to that caused by negative lenses. With reference to animal models of myopia it is thought that the blur then triggers a compensatory mechanism to achieve focus such that the retina is displaced backwards by a thinning of the choroid (Hung and Ciuffreda, 1991; Wallman *et al.,* 1995; Wildsoet and Wallman, 1995) and that subsequent remodelling of the sclera at the posterior pole results in myopia (Nickla, Wildsoet and Wallman, 1997; Gentle and McBrien, 1999). Conversely, myopic defocus can be induced by positive lenses such that the focal plane lies in front of the retina. To achieve focus, the retina

is pushed forward to the image plane by choroidal expansion (Wildsoet and Wallman, 1995; Hung *et al.,* 2000). This mechanism which compensates for both hyperopic and myopic defocus was first hypothesised approximately 60 years ago by Walls (Walls, 1942).

Interestingly, when form deprivation is induced and the optic nerve is severed, ocular growth still occurs (Troilo, Gottlieb and Wallman, 1987) which suggests that local changes at the retina influence ocular growth. The underlying mechanism(s) by which the retina may signal to the choroid has been studied extensively and it is thought that a cascade of signals may be involved. The role of dopamine (Ohngemach, Hagel and Schaeffel, 1997; Schaeffel *et al.,* 1995; Bartmann *et al.,* 1994), muscarinic cholinergic receptors (McBrien, Moghaddam and Reeder, 1993; Shih *et al.,* 1999; Kennedy *et al.,* 2000; Shih *et al.,* 2001; Tan *et a*l., 2005; Chua *et al.,* 2006) retinoic acid (Mertz and Wallman, 2000; McFadden, Howlett and Mertz, 2004) and nitric acid (Nickla and Wildsoet, 2004) in the modulation of eye growth have been studied, but the results remain equivocal. It has also been hypothesised that fluid changes across the choroid influence eye growth. It is possible that the thickness of the choroid depends on osmolitical changes within the choroid which may influence the water content of the choroid (Wallmann *et al.,* 1995; Nickla, Wildsoet and Wallman, 1997; Rada *et al.,* 2002). Changes in blood flow have also been implicated in determining choroidal thickness changes. Early work by Shih and co-workers concluded that in the chick eye, the reduction of ocular blood flow was a consequence of myopia and not a cause of axial elongation (Shih, Fitzgerald, Reiner, 1993 a; Shih, Fitzgerald, Reiner, 1993 b; Shih *et al.,* 1993). Fitzgerald, Wildsoet and Reiner (2002) however, showed that when form deprivation was induced by diffusing goggles in the chick eye, a reduction in choroidal blood flow preceded the thinning of the choroid. Indeed, during the recovery phase the observed increase in choroidal blood flow was more rapid and transient in onset than the increase in choroidal thickness.

If form deprivation changes choroidal blood flow and therefore thickness it is intriguing to know whether residual blur from accommodation inaccuracies also influences the blood flow within the choroid and hence the thickness of the choroid. Furthermore, since the magnitude of residual blur during accommodation is less than that caused by form deprivation, it is possible that any accommodation-blur induced changes in choroidal blood flow are autoregulated and that deficits in this autoregulatory mechanism may lead to myopia. The capacity of the choroid to autoregulate is however controversial. The consensus is that the choroid is a passive vascular bed which does not autoregulate (Friedman, 1970; Armaly and

Araki. 1975; Gherezghiher, Okubo and Koss, 1991; Findl *et al.,* 1997; Joos *et al.,* 1999; Delaey and Van de Voorde, 2000). However, many researchers have shown that the choroidal blood flow remains constant despite changes in ocular perfusion, hence suggesting that the choroidal blood flow is infact autoregulated (Kiel, 1994; Kiel and Van Heuven, 1995; Riva *et al.,* 1997; Chou, Lu and Chen, 2002; Lovasik *et al.,* 2003; Reiner, Zagyazdin and Fitzgerald, 2003).

The aim of the current study is to investigate the effects of accommodation on choroidal blood flow. It is hypothesised that the dose dependency evident in **Chapter 7** may be due to regulation within the vascular system. It is thought that the IOP reduction with intermediate accommodation levels observed in the previous study may be accompanied with a reduction in blood flow to the eye during accommodation as proposed by Armaly and Rubin (1961). In addition the inter-subject variations in IOP responses to accommodation may be explained by changes in ocular blood flow during accommodation. Furthermore, Young (1981) postulated that increased vitreous pressure increased the tension on the choroid and resulted in a decrease in choroidal blood flow during accommodation. However, this notion has not been investigated further.

An insight into the vascular status of the eye is provided by blood flow analysers for example the Ocular Blood Flow Analyser (OBFA; *Paradigm Medical Instruments Inc., UK*), Colour Doppler Imaging (CDI; *Acuson Sequoia System, UK*), Heidelberg Retinal Flowmeter (HRF; *Heidelberg Engineering, Germany*) and the Laser-Doppler Flowmeter (Scientific Co., Barrington, Japan). The vascular bed of interest in the present study is the choroid since it supplies 85 % of the total ocular blood flow (Langham *et al.,* 1989). Most of the instruments mentioned have large bulky designs a feature which does not facilitate the investigation of blood flow in one eye while simultaneously stimulating accommodation in the other eye. An exception to this is the OBFA which is a pneumatonometer that measures the pulsatile component of arterial ocular inflow (Langham *et al.,* 1989). Since 75 % of the choroidal blood flow is pulsatile in nature (James, 1998), the OBFA, provides information about the vascular status of the choroid.

A pertinent advantage of the OBFA is that it is a device which principally measures IOP data which is then converted into blood flow data therefore both IOP and blood flow measures are available. The IOP data obtained with the OBFA show good agreement with the IOP measures taken with the Goldmann contact tonometer (GCT) which is considered gold

standard (Spraul *et al.,* 1998). Therefore, the change in IOP on accommodation can be measured with the OBFA and the results can be compared against the results of the study described in **Chapter 7**.

The previous chapter investigated the effects of accommodation on IOP and concluded that IOP reduces for intermediate levels of accommodation, but does not change on higher levels of accommodation. Furthermore, there are substantial inter-subject variations in IOP responses to accommodation. It is hypothesized that some regulatory mechanism within the vascular system may explain why the change in IOP differs for different levels of accommodation. In addition, inter-subject variations in the vascular status of the eye may explain the between-subject differences in IOP responses to accommodation. It is unknown whether vascular autoregulation occurs during accommodation, and if so whether in some subjects this autoregulation is absent or impaired. If there are deficits in the vascular autoregulation, the subsequent changes in choroidal blood flow may influence changes in choroidal thickness and lead to the development of myopia.

8.2 Method

8.2.1 Subject group

The same 40 subjects that were recruited to take part in the study described in **Chapter 7** were recruited for this study from the undergraduate population at Aston University. The cohort comprised of 20 myopes [mean spherical equivalent (MSE) of <-0.50D], 19 emmetropes (MSE of ± 0.50D) and 1 hypermetrope (MSE of >+0.50D) and the MSE ranged from +1.00DS to -8.25DS. Astigmatism was limited to <0.75DC. The criterion used to divide the subjects in to these refractive groups was also used in studies conducted by Goh *et al.* (2005), Junghans and Crewther (2005) and Ojaimi *et al.* (2005). The ages of the group ranged from 18 to 32 years of age (mean age was 20.6±3.0 years of age), and consisted of 14 males and 26 females.

Five of the 40 subjects were unable to comply with the measurement procedures, therefore data was not collected from these subjects. The subjects reported that although the eye was anaesthetised, they were aware of corneal contact as the instrument emitted a whistling sound when the probe was in contact with the cornea. As a result, in these 5 subjects the measurement procedure was hindered due to excessive epiphoria and blephrospasm.

Consequently, the subject group was reduced to 35 subjects (24 females; males 11) with a mean age of 20.9±3.4 years of age (ranging from 18 to 32 years of age). The MSE ranged from +1.00DS to -7.75DS. The group comprised of 17 myopes, 17 emmetropes and 1 hypermetrope.

Written consent was obtained from all subjects willing to participate in the study and copies of the information sheets and consent forms given to the subjects can be found in **Appendix 4**. It was ensured that the visual acuity of all the subjects was 0.00 logMAR or better. All subjects had no ocular pathology. None of the subjects were taking any topical or systemic medications that may affect the IOP, cardiovascular or accommodative function.

8.2.2 Stimulus

The same three accommodative stimulus levels were used as in the previous chapters [i.e. zero (L: low), 1.5 (I: intermediate) and 4D (H: high)]. In **Chapter** 7, a Badal system was used to stimulate accommodation. To investigate the effects of accommodation on ocular blood flow in terms of regulation, ideally the same experimental setup was required. The OBFA was used in its slit lamp mounted mode (as opposed to its handheld mode) and initially attempts were made to mount a Badal system onto a slit lamp. When the Badal system was mounted and aligned with the LE and the subjects were instructed to 'focus on the target', inevitably the RE converged during accommodation. Since the eye (RE) was not in the primary position of gaze, accurate measurements of ocular blood flow readings were difficult to obtain with this experimental setup. Therefore, the accommodative target [a high contrast (90%) Maltese cross target] was presented in real dioptric space.

8.2.3 Instrumentation

The Shin-Nippon SWR-5000 (Ryusyo Industrial Co., Ltd, Japan) open-view, infrared objective autorefractor, which image analyses an infrared ring reflected off the retina, was used to measure the refractive error and associated accommodation responses to the 3 accommodative stimuli. The high repeatability of the refractive error measures obtained with the instrument has been demonstrated previously (Chat and Edwards, 2001; Mallen *et al.*, 2001).

The OBFA was used to measure the vascular status of the eye during accommodation. With each systolic pulse, a bolus of blood enters the choroidal vasculature which expands the globe

and therefore increases the overall volume of the eye (Langham *et al.*, 1989). If the eye is treated as an elastic chamber, a bolus of fluid into the eye would cause the eye to expand, increasing its volume and pressure (Silver and Geyer, 2000). The relationship between ocular volume and IOP is well established such that as the pressure increases the volume increases and vice-versa (Eisenlohr and Langham, 1962; Silver and Geyer, 2000). Hence, these cyclic changes in volume with each heart beat manifest as cyclic changes in IOP which is termed the ocular pulse and is also observed as the pulsations of the mire images during GCT. Perkins, Edwards and Saxena (1977) were the first to devise a tonometer which continuously measured the IOP. Langham and To'mey (1978) argued that if the relationship is inverted, the blood flow could be calculated from these changes in IOP. Essentially, the blood flow is calculated from analyses of the dynamics of the mire pulsations observed during Goldmann tonometry (Silver and Farrell, 1994).

Friedenwald's equation demonstrates a logarithm function between ocular volume and IOP. However, since the cyclic changes in IOP with the cardiac cycle are small, the OBFA assumes that the relationship between ocular volume and IOP is linear. The IOP wave is transferred to the volume wave by multiplying by a constant taken as 1.3 for an IOP in the range of 15 to 25 mmHg. The pulse volume is calculated by the incremental volume change between the peak and trough of the IOP wave over time. The pulsatile ocular blood flow (POBF) value is a product of the pulse volume, the time between the peak and trough of the IOP wave and the pulse rate (Krakau , 1992; Silver and Farrell, 1994).

However, a number of assumptions are made when the POBF is calculated using the OBFA. It is assumed that ocular rigidity is constant, however it has been demonstrated that as axial length and volume increase, ocular rigidity decrease (Perkins, 1981). It is also assumed that that the outflow is constant, no reflux of blood occurs when the pulsatile component of blood flow is zero and that the outflow of blood is non-pulsatile (Krakau, 1992).

The OBFA device can be used in its slit lamp mounted or handheld mode and uses an air piston system to applanate the cornea and continuously record IOP. Gas flows down a central hollow tube of the OBFA at a constant pressure. This pressure pushes against a terminal membrane, deforming the cornea. The gas then escapes into the atmosphere. The resistance offered to the gas is measured as the IOP. In this study, the instrument was used as a slit lamp mounted device, and in this mode the blood flow measures show good reliability and inter-

observer agreement (Yang *et al.*, 1997; Lam *et al.*, 1999). The OBFA gives a POBF reading which essentially represents the pulsing component of the choroidal blood flow (James, 1998), an IOP measure, and measures of the pulse amplitude (PA), volume (PV) and rate (PR). The changes in IOP (i.e. the ocular pulse) are measured 200 times per second. In a single measurement (which takes 7 to 20 seconds) the OBFA records 7 pulses of which 5 consistent pulses are identified and analysed by the instrument's software. A full set of data values are not available when less than 5 reliable pulses are recognised in 20 seconds. The single IOP reading displayed by the instrument is an average of the systolic and diastolic IOP. The PA is the difference between the systolic and diastolic IOP. The pulse rate is the measure of cycles per minute and the PV and POBF are calculated as explained above.

An important feature of the OBFA compared to other instruments that measure ocular blood flow is the relatively small size of the instrument which permits the use of the instrument as a slit lamp mounted device. Therefore, as with the *Pulsair EasyEye* (Keeler, UK) NCT used in this thesis, the small dimensions of the OBFA mean that relatively unhindered ocular blood flow measures can be obtained in one eye while simultaneously stimulating accommodation in the other eye (see **Figure 8.1**). Of note is that as with the *Pulsair* NCT details of the subject group used for the calibration process of the OBFA are not disclosed by the manufacturers. Therefore differences in the characteristics of the subject group (for example gender, age, corneal thickness and curvatures) used for the calibration process and the subject groups used in other studies may present some error in the IOP and POBF measures.

8.2.4 Experimental Procedures

There is a well-established circadian rhythm in IOP characterised initially by an early morning peak (David *et al.*, 1992; Wilensky *et al.*, 1993; Pointer, 1997). Although Claridge and Smith (1994) reported that there was no diurnal variation in blood flow values, more recent work shows that the blood flow varies over a 24 hour cycle, such that it progressively increases during the day and then exhibits a nocturnal dip (Osusky *et al.*, 2000; Lam *et al.*, 2001). Furthermore, the human eye undergoes diurnal fluctuations in axial length. At midday the axial length is at its maximum length (Stone *et al.*, 2004; Wilson *et al.*, 2006). However, these diurnal changes in axial length do not cause the diurnal variations in IOP (Wilson *et al.*, 2006). Since the results from this study were to be compared against the results from the study described in **Chapter 7**, the ocular blood flow data was measured at approximately the same time (±30 minutes) of day as the IOP data in **Chapter 7**.

The experimental procedure was split into 3 separate stages as follows:

Stage 1

A high contrast (90%) Maltese cross target was placed within the +5D Badal system and the distance refractive error was measured using the Shin-Nippon autorefractor. The refractive error data obtained was used to fit a soft daily disposable contact lens (1-day *Acuvue Dailies*, etafilcon A, Johnson & Johnson, Vistakon, USA) to the LE of each subject to render them functionally emmetropic in the one eye.

Stage 2

The accommodative target was moved in open space and the accommodation responses of the LE were measured simultaneously with the autorefractor. The accommodation responses were sampled at 10 second intervals for 3 minutes as it as been shown that accommodative adaptation occurs after 3 minutes of fixation (Rosenfield and Gilmartin, 1989). The distances at which the accommodation responses of zero±0.50, 1.50±0.50 and 4±0.50D were measured with the autorefractor were noted.

Stage 3

Prior to data collection, the measurement technique used to acquire accurate data with the OBFA was mastered as it has been shown that inexperienced operation can affect the accuracy of the results (Morgan and Hosking, 2001). During this practice run, the subjects were familiarised with the measurement technique as it has also been shown that apprehension increases IOP (Myers and Scott, 1975; Moses *et al.,* 1984). Furthermore, from clinical experience, it is common for patients to 'hold their breath' during optometric procedures and there is evidence to show that this can cause errors in routine tonometric measures (Whitacre and Stein, 1993; Rafuse *et al.,* 1994). Inhalation of carbon dioxide, which has analogous effects to 'breath-holding', has been shown to increase POBF values (Kergoat and Faucher, 1999). Conversely, Lam and Lam (2004) found no significant difference in POBF values with or without 'breath-holding', although they do note that the variation in POBF values on consecutive measures is significantly increased when subjects 'hold their breath'. Therefore as recommended by Lam and Lam (2004) the subjects were instructed to not 'hold their breath' during the measurement period.

On the day of data collection, each subject was allowed a 10 minute resting period as it has been shown that following exercise, the POBF returns back to normal levels after a 10 minute resting period (Price *et al.,* 2003). The Maltese cross target was placed in front of the LE at the distances measured in ***Stage 2*** at which zero±0.50 1.50±0.50 and 4±0.50D accommodative responses were measured. The accommodative target was then moved in the horizontal plane such that the RE was in its primary position of gaze. The experimental setup is shown in **Figure 8.1**. As recommended by Stark and Atchison (1994), throughout the experimental procedure the subjects were instructed to 'focus on the target carefully', as higher order attentional factors (Francis *et al.,* 2003) and the precise instructions given to the subjects (Ciuffreda and Hokoda, 1985; Stark and Atchison, 1994) have been shown to influence accommodative accuracy.

One drop of topical anaesthetic, 0.5% proxymetcaine hydrochloride (Chauvin Pharmaceuticals, Ltd.) was instilled only in the subject's RE. This is different from normal clinical practice in which both eyes are anaesthetised before measurements are taken with the OBFA. Normally the unexamined eye is also anaesthetised to help reduce the sensation of drying and therefore the tendency to blink given the relatively long measurement time. However, only the RE (in which the measurement was taken) was anaesthetised to reduce the effects of topical anaesthetics on IOP (Herse and Siu, 1992; Moseley *et al.,* 1993; Baudorin and Gastard, 1994; Birchall and Kumar, 2001; Asensio *et al.,* 2003; Nam *et al.,* 2006).

One reading was obtained in the RE of each subject using the OBFA after fixating the accommodative target at one of the 3 distances, presented randomly for 3 minutes. After 3 minutes of fixation accommodative adaptation occurs (Rosenfield and Gilmartin, 1989) and it is known that autoregulation of ocular blood flow occurs in the first minute of induced change (Ernest, 1968; Riva, Grunwald and Petrig, 1986). Therefore to allow sufficient time for autoregulatory mechanisms to operate, a number of studies measure blood flow after 3 minutes (Ernest, 1968; Riva, Grunwald and Petrig, 1986; Joos *et al.,* 1999).

Correct alignment of the probe was judged by external viewing and the adequacy of applanation was confirmed by listening to the rhythmatic whistling sound emitted by the OBFA. As mentioned earlier one single defined measure is given as a single tonometer application, necessary to capture 5 acceptable pulses. As with the GCT, the probe of the OBFA is in contact with cornea during the measurement period and as expected it has been

reported that IOP readings reduce on repeated measures (Morgan and Hosking, 2001). A reduction in the size of the PA has also been reported (Morgan and Hosking, 2001), but interestingly, the POBF measure is not effected by repeat measures (Morgan and Hosking, 2001; Yang *et al,* 1997) which suggests a robust pressure-volume relationship (Morgan and Hosking, 2001). Recep *et al.* (1998) suggested that repeat measurements should be taken after a 10 minute interval, although more recently no significant difference between baseline and repeat measures was found with time intervals of $\geq$ 2 minutes (Gunvant *et al.,* 2004). Consequently, a 5 minute interval was allowed between measures to eliminate the effect of repeated measurements.

Furthermore, occasionally proprietary artificial teardrops [sodium chloride (NaCl) 0.9% (Chauvin Pharmaceuticals)] were used between measures to ensure good tonometer contact and prevent excessive epithelial drying.

Inter-session repeatability was assessed in 2 volunteers. The 2 data collection sessions were arranged on different days at approximately the same time to minimise the effects of diurnal variations on IOP (Pointer, 1997; Noel *et al.,* 2001; Liu *et al.,* 2003; Kida *et al.,* 2006) and inter-subject variations in diurnal changes in PA, PR and POBF values which have previously been reported (Claridge and Smith, 1994).

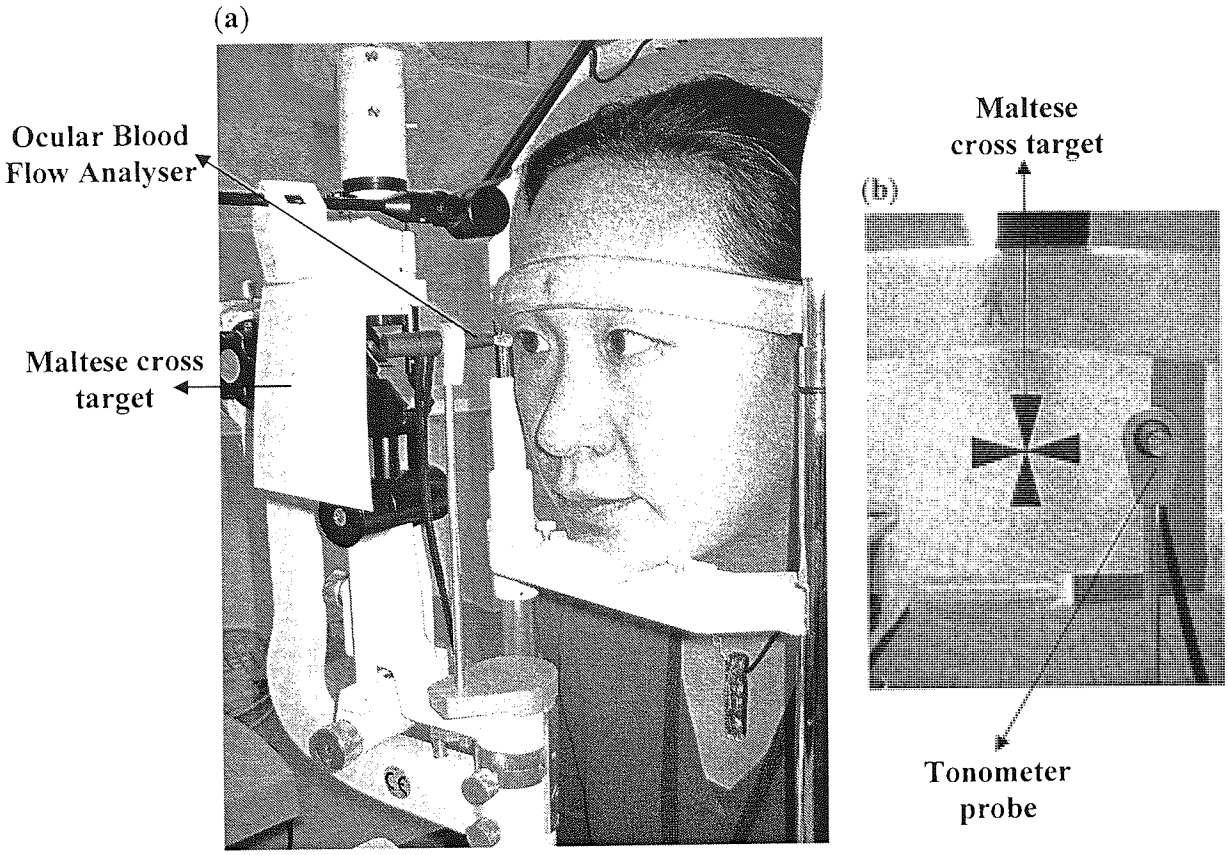


Figure 8.1 (**a**) Experimental setup of accommodative target in front of LE and OBFA in front of RE; (**b**) subject view.

8.3 Statistical analyses

Software packages *SPSS 12.1* for Windows and Microsoft *Excel* were used for the statistical analyses of the data.

The changes in IOP, PA, PV, PR and POBF on accommodation were assessed by within-subjects one-way analysis of variance (ANOVA) tests in randomised blocks followed by Scheffe and Bonferroni *post-hoc* analyses. For the Bonferroni analyses the critical significance level was taken as 0.017 (0.05/3). The IOP measures obtained when the subjects were fixating a L accommodative stimulus were treated as the reference values for comparison. An attempt was made to explain the inter-subject variations in IOP, PA, PV, PR and POBF responses to accommodation by correlating the changes in these parameters with refractive error and axial length. A one-way between subjects ANOVA were performed to determine whether the inter-subject variations in the parameters measured differed between myopes and emmetropes. Furthermore, autoregulation was assessed by correlating the changes in IOP with blood flow measures.

Comparisons between the changes in IOP as measured following the experimental protocol described in **Chapter 7** and those measured in the present study were compared using the method of Bland and Altman (1986). The method involved assessing the differences between the IOP changes on accommodation with paired t-tests. Furthermore, correlation analyses between the 2 measurement techniques were also performed. Finally the changes in IOP as measured in **Chapter 7** were correlated with the changes in POBF measured in the current studies. The relationship between the two parameters was determined by calculating the Pearson's correlation coefficients.

8.4 Results

The results of the within-subjects ANOVAs for each parameter measured with the OBFA are shown in **Table 8.1**.

Acc stimulus level (D)	L	I	H	F value	DF	p
Acc response level (D)	+0.13±0.16	+1.53±0.36	+4.16±0.23			
IOP (mmHg)	10.30±2.41	10.07±2.49	9.38±2.12	3.728	2, 68	0.042*
PA (mmHg)	3.12±1.05	2.97±1.11	3.14±1.34	0.411	2, 68	0.615
PV (μL/min)	8.71±3.17	8.53±3.44	9.69±6.46	0.085	2, 68	0.902
PR (beats/min)	74.03±15.44	73.23±18.59	71.78±15.61	0.240	2, 68	0.770
POBF (μL/min)	1357.60±367.71	1360.69±423.54	1376.37±435.45	0.083	2, 68	0.083

Table 8.1 Mean±SD of accommodation responses and corresponding IOP, PA, PV, PR and POBF and within-subjects ANOVA results where * denotes statistically significant result (n=35).

IOP

The IOP decreases between L and I, L and H and I and H levels of accommodation by respectively 0.23±2.55, 0.92±1.98 and 0.68±1.54 mmHg. Scheffe *post-hoc* analyses indicate the differences in IOP on accommodation were not statistically significant (L and I, p=0.92; L and H, p=0.27; I and H, p=0.48). However, Bonferroni *post-hoc* analyses revealed that these differences in IOP reached statistical significance between L and H (p=0.01) and I and H (p=0.013) accommodative stimulus levels (L and I; p=0.59). The COR of IOP measures was ±1.1 mmHg.

The inter-subject variations in the differences in IOP responses between the 3 levels of accommodative stimuli are illustrated in **Figures 8.2** to **8.3**. The range (RG) and median (MED) of the differences in IOP between L and I, L and H, and I and H are respectively, RG=5.30 to -6.40, MED=-0.50, RG=4.20 to -5.20, MED=-1.00, RG=1.80 to -4.50, MED=-0.60 mmHg.

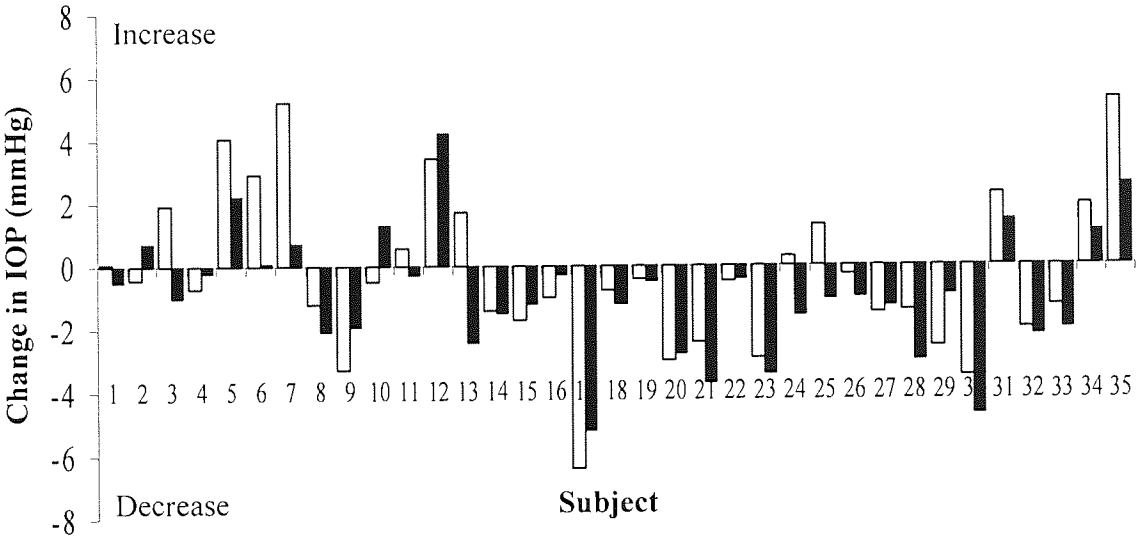


Figure 8.2 Change in IOP (mmHg) between L and I (▩) and L and H (■) accommodation stimulus levels (n=35).

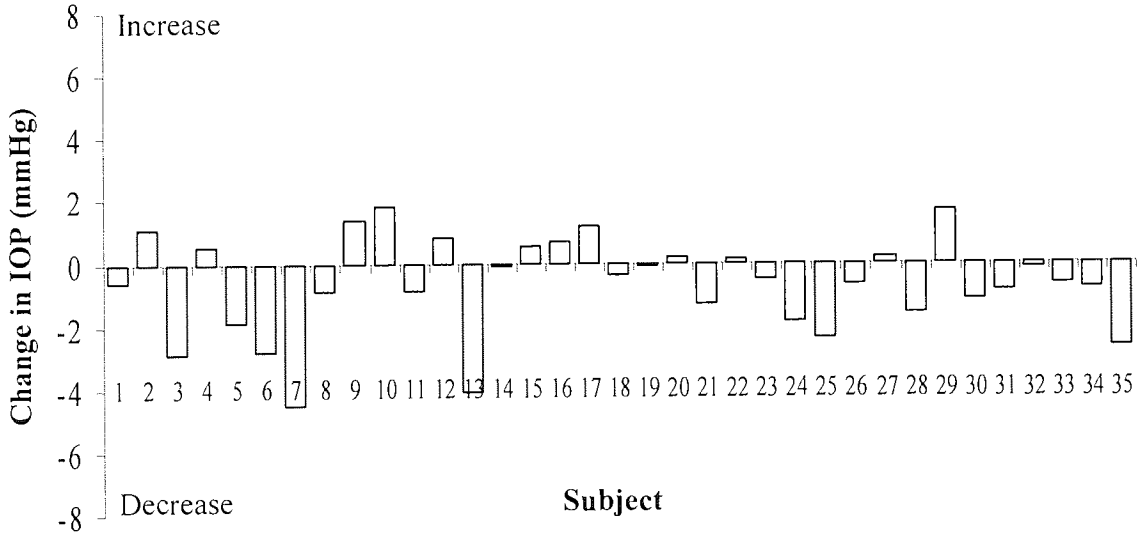


Figure 8.3 Change in IOP (mmHg) between I and H accommodation stimuli levels (n=35).

Figure 8.4 shows that the variations in IOP responses between L and I (r=0.18, p=0.30), L and H (r=0.005, p=0.98) and I and H (r=0.29, p=0.09) levels of accommodation were not due to refractive error. **Figure 8.5** shows that the variations in IOP responses between L and I (r=0.14, p=0.44), L and H (r=0.009, p=0.96) and I and H (r=0.21, p=0.22) were not related to axial length. The between-refractive groups ANOVA shows that the differences in IOP responses to accommodation of the emmetropes were not statistically different to the IOP responses to accommodation of the myopes between L and I (F=0.053, p=0.82), L and H (F=0.359, p=0.55), and I and H (F=1.400, p=0.25) levels of accommodation. Note that the inter-subject variations in the changes in IOP with accommodation were not considered with respect to the baseline IOPs due to the relatively small inter-subject variations in baseline IOPs.

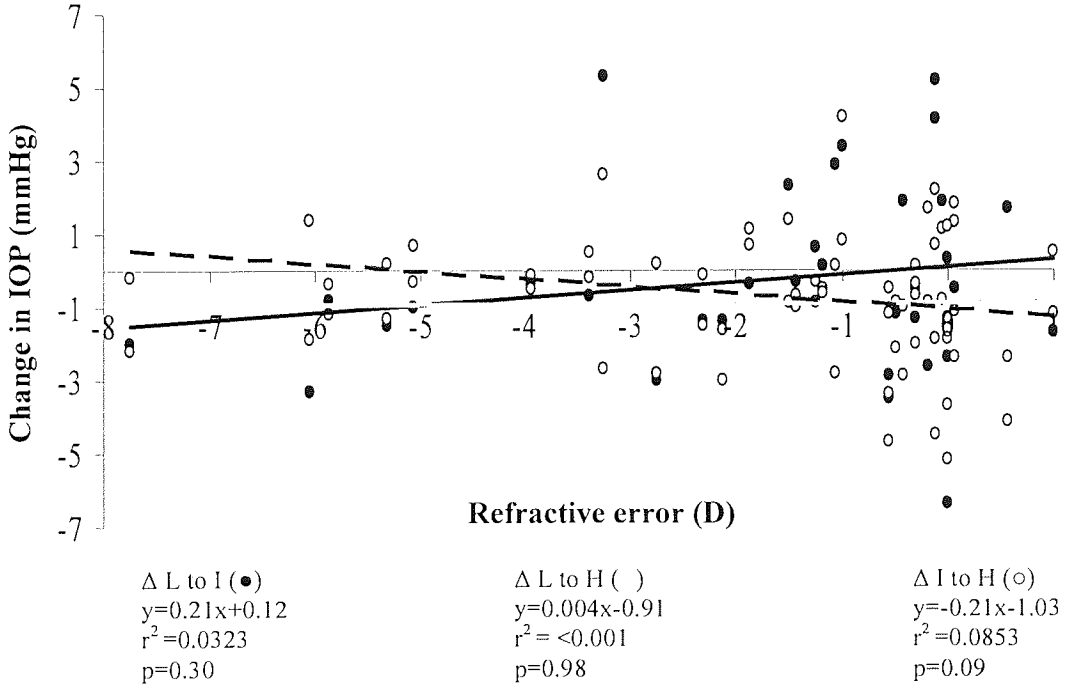


Figure 8.4 Level of refractive error and change in IOP between L and I (—), L and H ( ) and I and H (---) levels of accommodation (n=35).

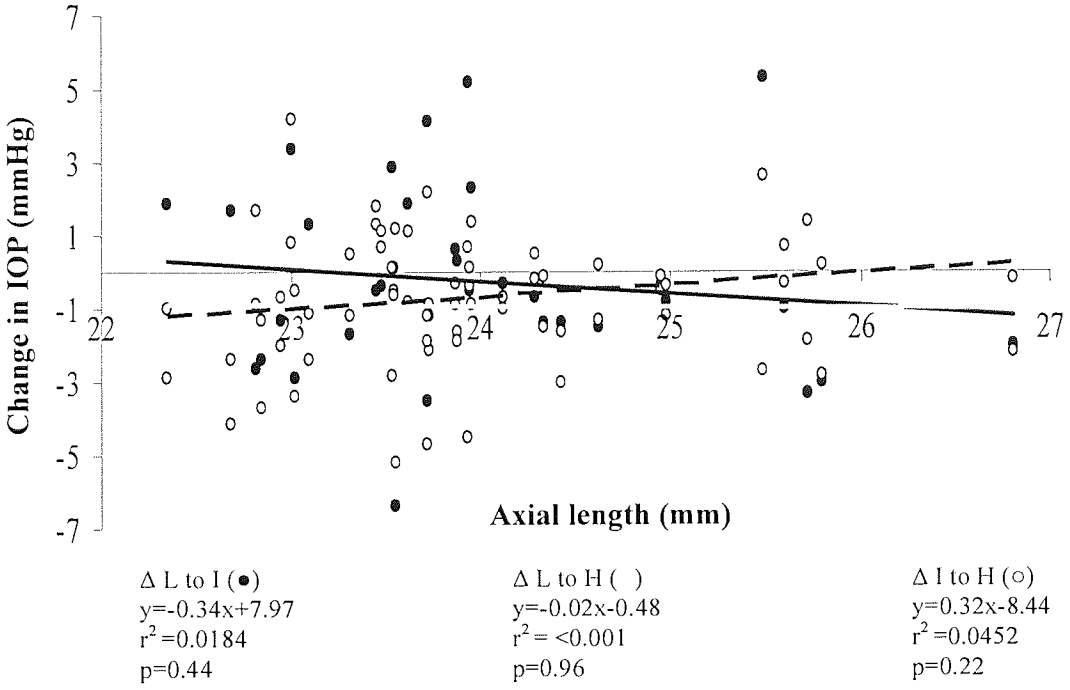


Figure 8.5 Axial length and change in IOP between L and I (—), L and H ( ) and I and H (---) levels of accommodation (n=35).

Analyses of the percentage changes in IOP as measured with the OBFA are shown in **Appendix 10**. The results indicate that accommodation does not influence the percentage changes in IOP. Similar to the results above, the results in **Appendix 10** also suggest that the inter-subject variations in percentage changes in IOP are not explained by variations in refractive error or axial length.

Pulse amplitude

The within subjects ANOVA shows that for the cohort accommodation does not influence the PA (see **Table 8.1**). The COR of PA measures was ±0.3 mmHg. It is evident from **Figures 8.6** and **8.7** that inter-subject variations in the PA changes on accommodation exist.

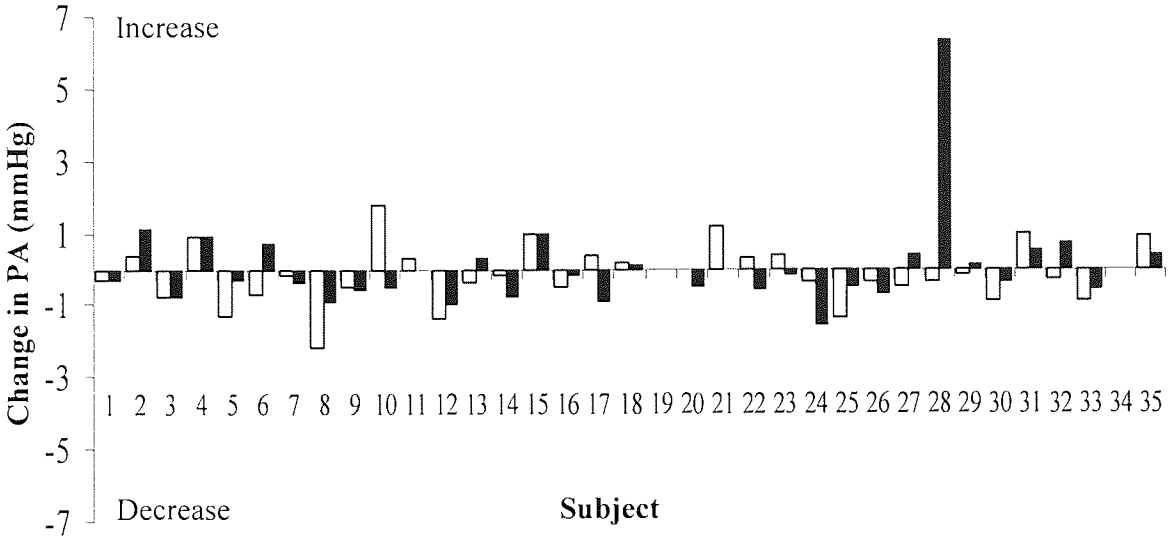


Figure 8.6 Change in PA (mmHg) between L and I ( ) and L and H (■) levels of accommodation stimuli (n=35).

Figure 8.7 Change in PA (mmHg) between I and H accommodation stimuli levels (n=35).

The variations in PA responses between L and I (r=0.007, p=0.97), L and H (r=0.17, p=0.34) and I and H (r=0.15, p=0.40) levels of accommodation were not associated with refractive error as shown in **Figure 8.8.** Likewise, **Figure 8.9** shows the correlation between axial

length and the variations in PA responses between L and I (r=0.07, p=0.68), L and H (r=0.15, p=0.40), and I and H (r=0.09, p=0.61) levels of accommodation.

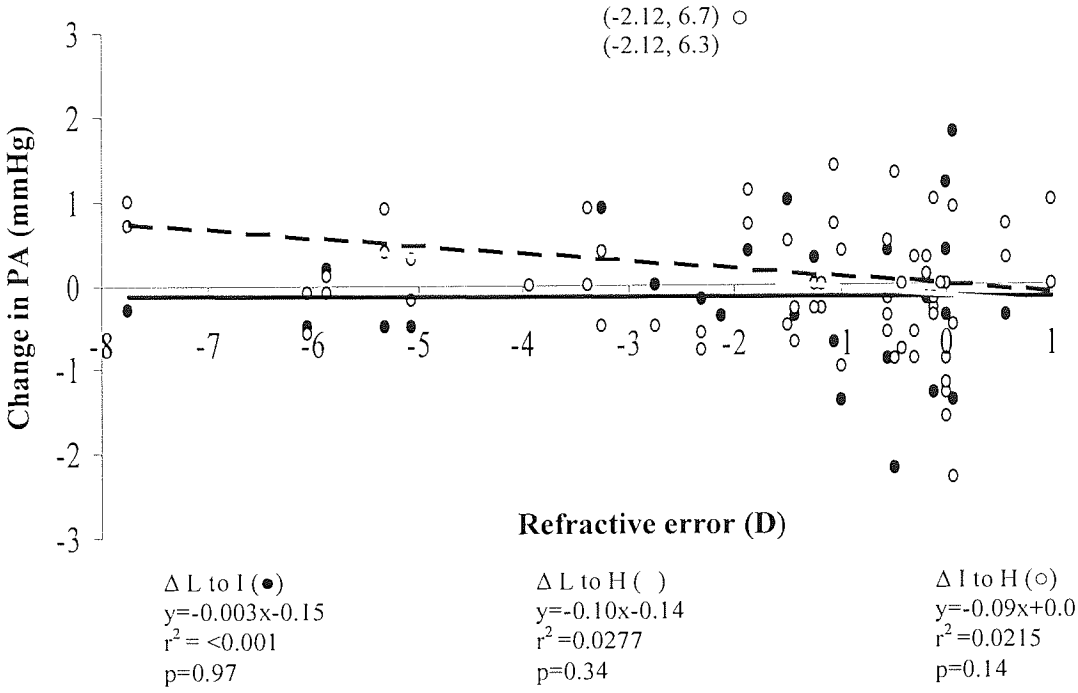


Figure 8.8 Level of refractive error and change in PA between L and I (—), L and H ( ) and I and H (---) levels of accommodation (n=35).

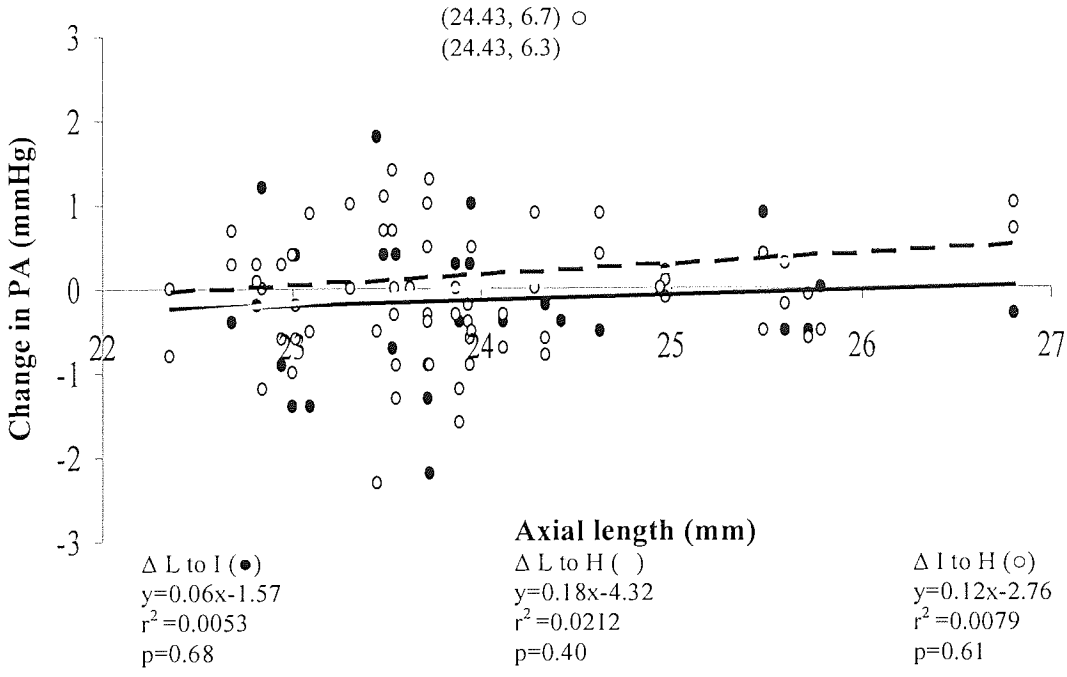


Figure 8.9 Axial length and change in PA between L and I (—), L and H ( ) and I and H (---) levels of accommodation (n=35).

It is clear from **Figure 8.6** and **8.7** that the responses of subject 28 do not match the responses of the other subjects. When the data from subject 28 are excluded the analyses still suggest that the inter-subject variations in PA responses with accommodation are not explained by refractive error or axial length (see **Appendix 11**).

The between-refractive groups ANOVA shows that the differences in PA responses to accommodation of the emmetropes were not statistically different to the PA responses to accommodation of the myopes between L and I ($F=1.577$, $p=0.22$), and between I and H ($F=1.641$, $p=0.21$) accommodation levels. However, the differences in PA responses to accommodation of the emmetropes were statistically different to the IOP responses to accommodation of the myopes between L and H ($F=5.57$, $p=0.03$), such that the PA in the myopes increased (0.47±1.61 mmHg) whilst it decreased in the emmetropes (0.49±0.47 mmHg) between L and H levels of accommodation. When the data of subject 28 are treated as outliers and the results reanalyzed the differences in PA with accommodation between emmetropes and myopes remained statistically significant ($F=10.253$, $p=0.003$).

Pulse volume

For the cohort examined, the within-subjects ANOVA shows that accommodation does not affect PV (see **Table 8.1**). The COR of PV values was 1.2 μL/min. Inter-subject variations in the differences in PV values on accommodation exist and these are shown in **Figure 8.10 and 8.11**. The RG and MED of the differences in PV between L and I, L and H, and I and H were respectively, RG=5.8 to -7.9, MED=-0.2, RG=3.5 to -7.6, MED=0, RG=5.1 to -8.5, MED=+0.2 μL/min.

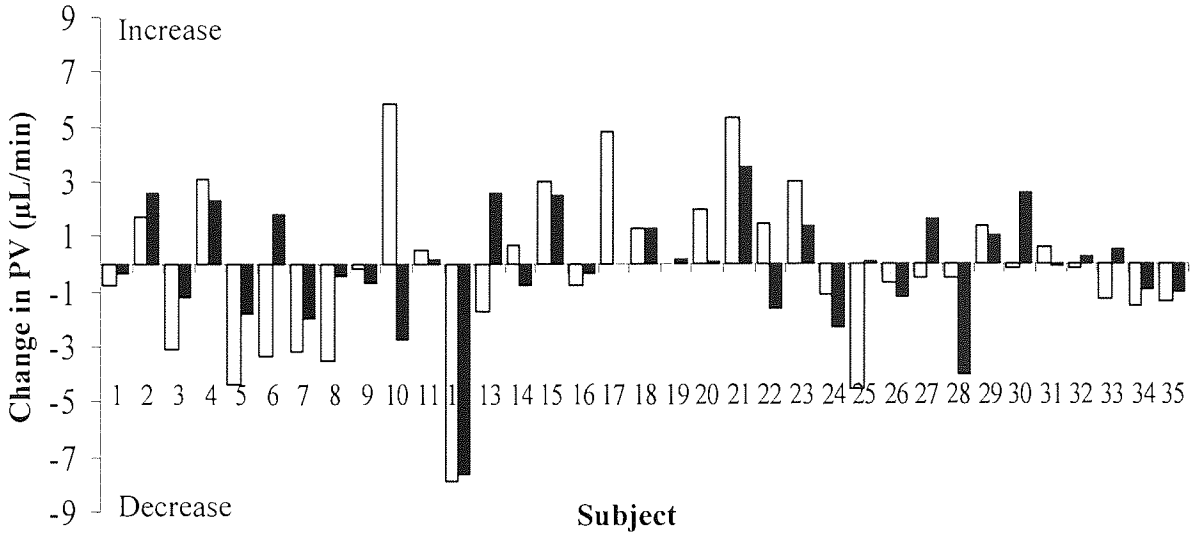


Figure 8.10 Change in PV (µL/min) between L and I (▩) and L and H (■) levels of accommodation stimuli (n=35).

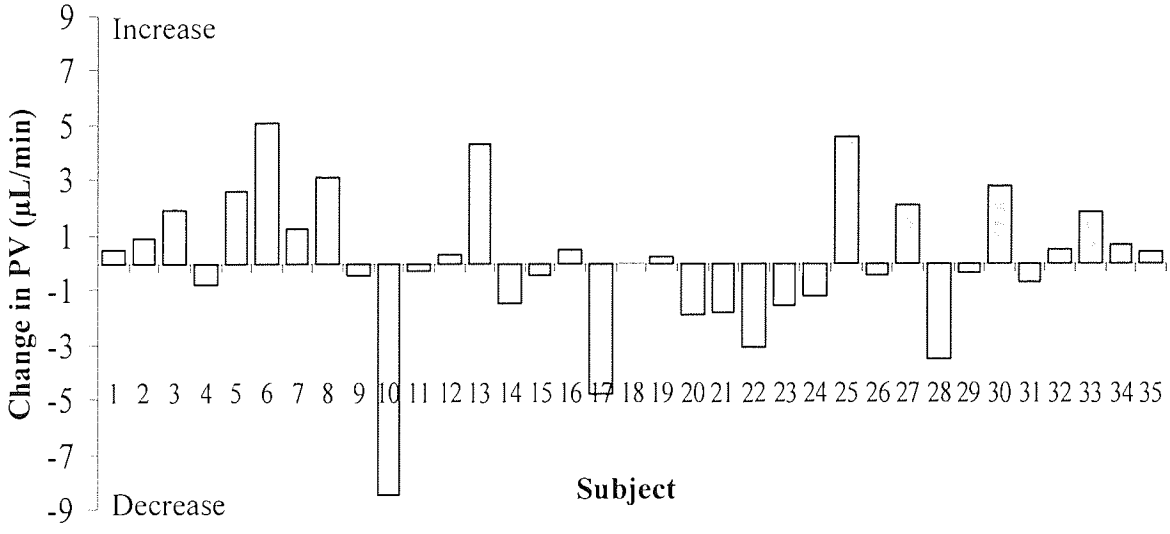


Figure 8.11 Change in PV (µL/min) between I and H levels of accommodation stimuli (n=35).

Figure 8.12 shows that the variations in PV responses between L and I (r=0.03, p=0.84), L and H (r=0.06, p=0.73) and I and H (r=0.01, p=0.95) levels of accommodation were not due to refractive error. **Figure 8.13** shows that the variations in IOP responses between L and I (r=0.08, p=0.66), L and H (r=0.05, p=0.76) and I and H (r=0.13, p=0.47) were not related to axial length.

The between-refractive groups ANOVA shows that the differences in PV responses to accommodation of the emmetropes were not statistically different to the PV responses to accommodation of the myopes between L and I (F=0.303, p=0.59), L and H (F=0.616, p=0.44), and I and H (F=0.001, p=0.97) levels of accommodation.

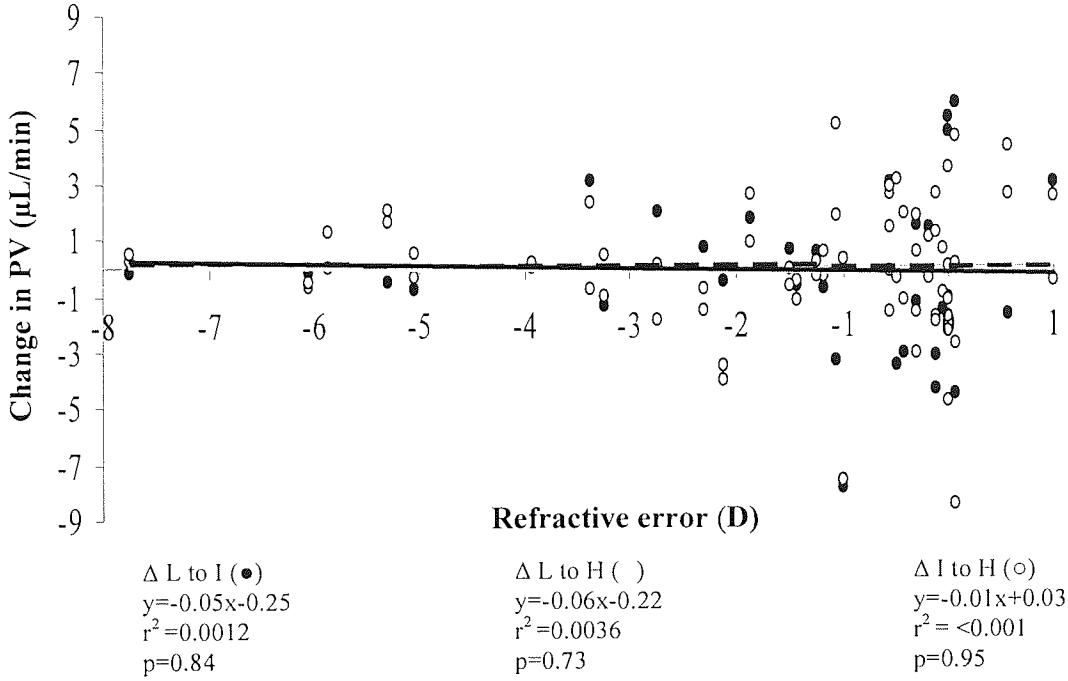
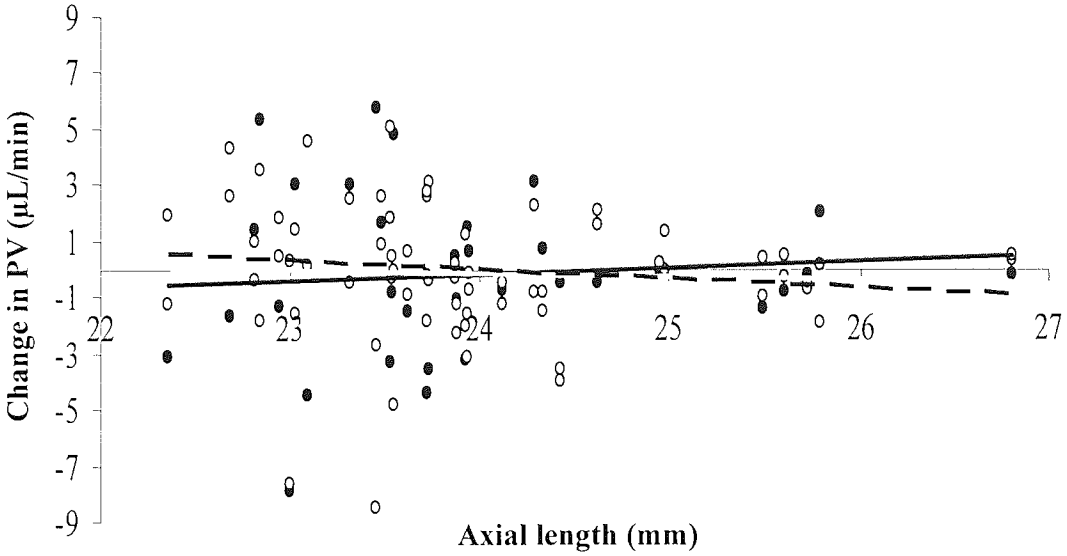


Figure 8.12 Level of refractive error and changes in PV between L and I (—), L and H ( ) and I and H (---) levels of accommodation (n=35).



Δ L to I (●)
y=0.22x-5.37
r^2 =0.0058
p=0.66

Δ L to H ( )
y=-0.11x-2.60
r^2 =0.0029
p=0.76

Δ I to H (○)
y=-0.33x+7.98
r^2 = 0.016
p=0.47

Figure 8.13 Axial length and changes in PV between L and I (—), L and H ( ) and I and H (---) levels of accommodation (n=35).

Pulse rate

The ANOVA results show that accommodation has no significant effect PR (see **Table 8.1**). The COR for PR measures with the OBFA was ±2.6 beats/min. However, the differences in PR values on accommodation are shown in **Figure 8.14 and 8.15**. The RG and MED of the differences in PR between L and I, L and H, and I and H were respectively, RG=33 to -30, MED=-1, RG=23 to -49, MED=2, RG=23 to -53, MED=0 beats/min.

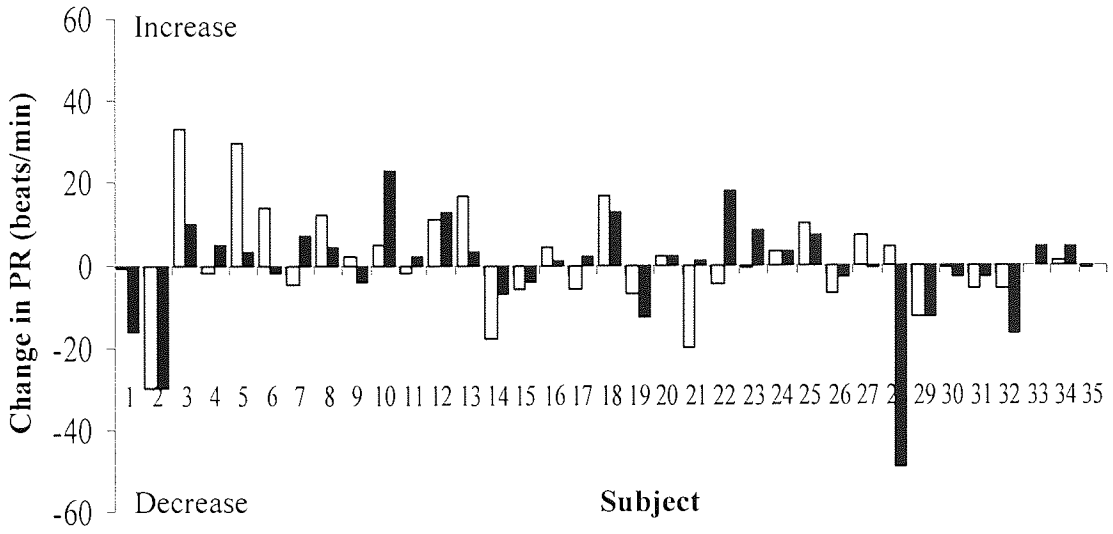


Figure 8.14 Change in PR (beats/min) between L and I (▒) and L and H (■) levels of accommodation stimuli (n=35).

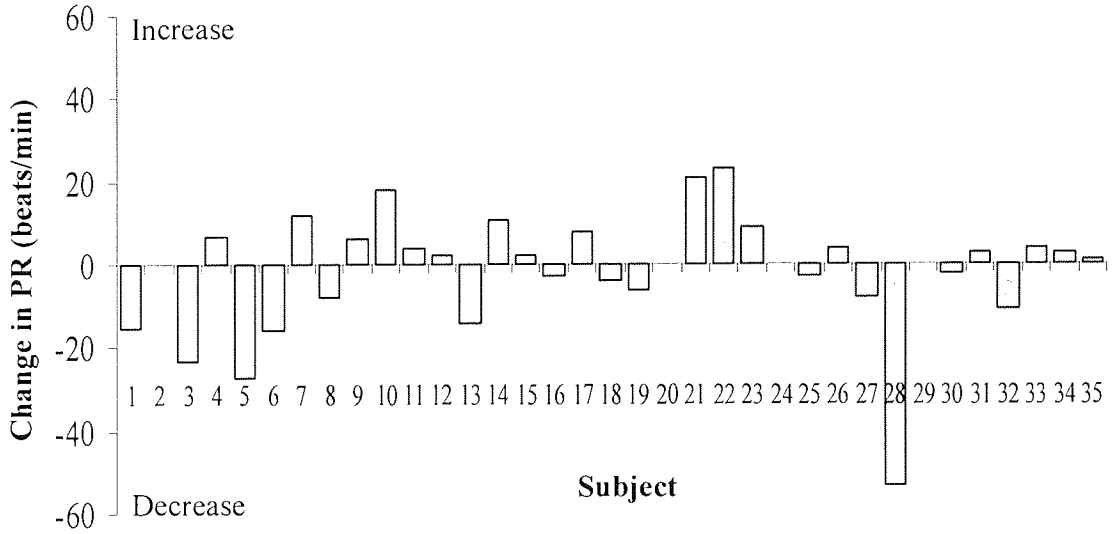
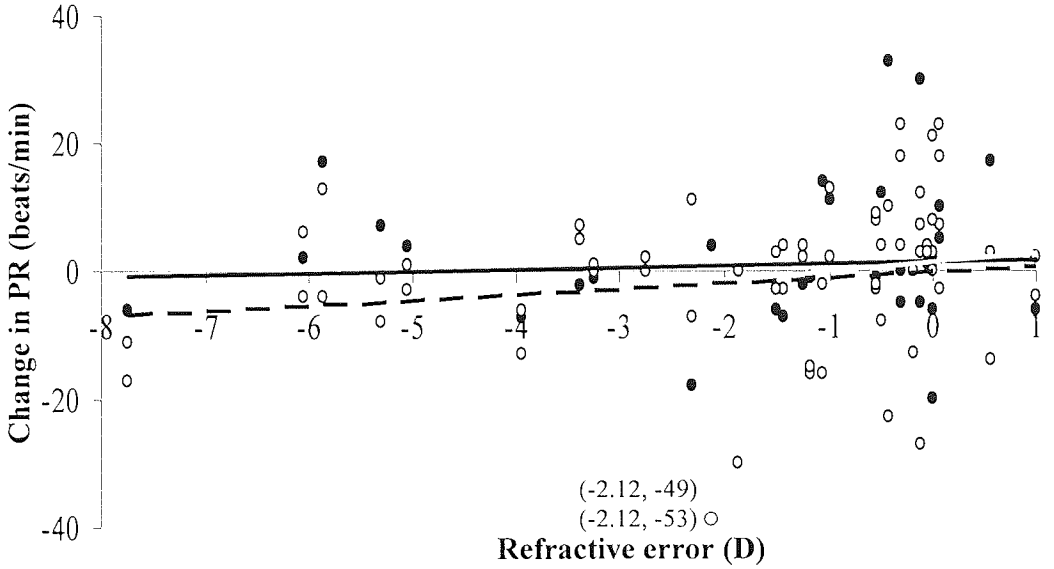


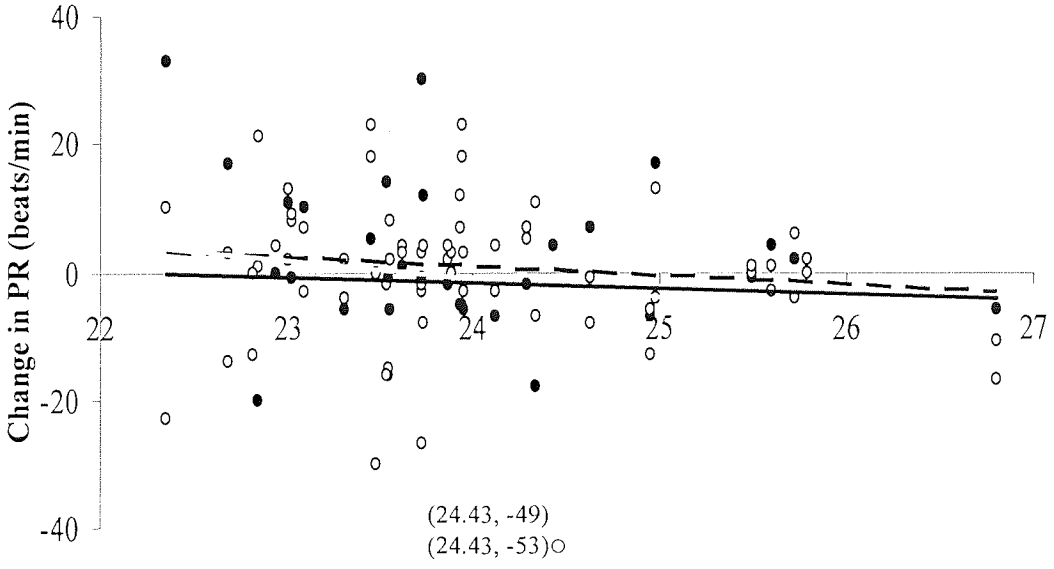
Figure 8.15 Change in PR (beats/min) between I and H levels of accommodation stimuli (n=35).

The variations in PR responses between L and I (r=0.04, p=0.80), L and H (r=0.24, p=0.16) and I and H (r=0.13, p=0.45) levels of accommodation were not due to refractive error as shown in **Figure 8.16**. **Figure 8.17** shows that the variations in IOP responses between L and I (r=0.12, p=0.50), L and H (r=0.23, p=0.19) and I and H (r=0.06, p=0.72) were not related to axial length.



Δ L to I (•)	Δ L to H ( )	Δ I to H (○)
y=0.26x+1.43	y=1.46x+1.53	y=0.87x-0.11
r^2=0.0019	r^2=0.0578	r^2= 0.0176
p=0.80	p=0.16	p=0.45

Figure 8.16 Level of refractive error and changes in PR between L and I (—), L and H ( ) and I and H (---) levels of accommodation (n=35).



Δ L to I (•)	Δ L to H ( )	Δ I to H (○)
y=-14.4x+35.60	y=-2.94x+69.55	y=-0.89x+19.80
r^2=0.0136	r^2=0.0513	r^2= 0.004
p=0.50	p=0.19	p=0.72

Figure 8.17 Axial length and changes in PR between L and I (—), L and H ( ) and I and H (---) levels of accommodation (n=35).

The between-refractive groups ANOVA shows that the changes in PR on accommodation of the emmetropes were not statistically different to the changes in PR of the myopes between L and I (F=1.934, p=0.17), and between I and H (F=1.945, p=0.17). However, the changes in PR to accommodation of the emmetropes were statistically different to the PR responses to accommodation of the myopes between L and H (F=9.862, p=0.004), such that the PR in the myopes decreased (7.18±14.64 mmHg) whilst it increased in the emmetropes (5.53±7.99 mmHg) between L and H levels of accommodation.

Pulsatile Ocular Blood Flow

The differences in POBF values on accommodation are shown in **Figure 8.18 and 8.19.** The COR of POBF values was calculated as ±81.6 μL/min The RG and MED of the differences in POBF between L and I, L and H, and I and H were respectively, RG=699 to -700, MED=-8, RG=679 to -729, MED=-32, RG=627 to -731, MED=+50 μL/min.

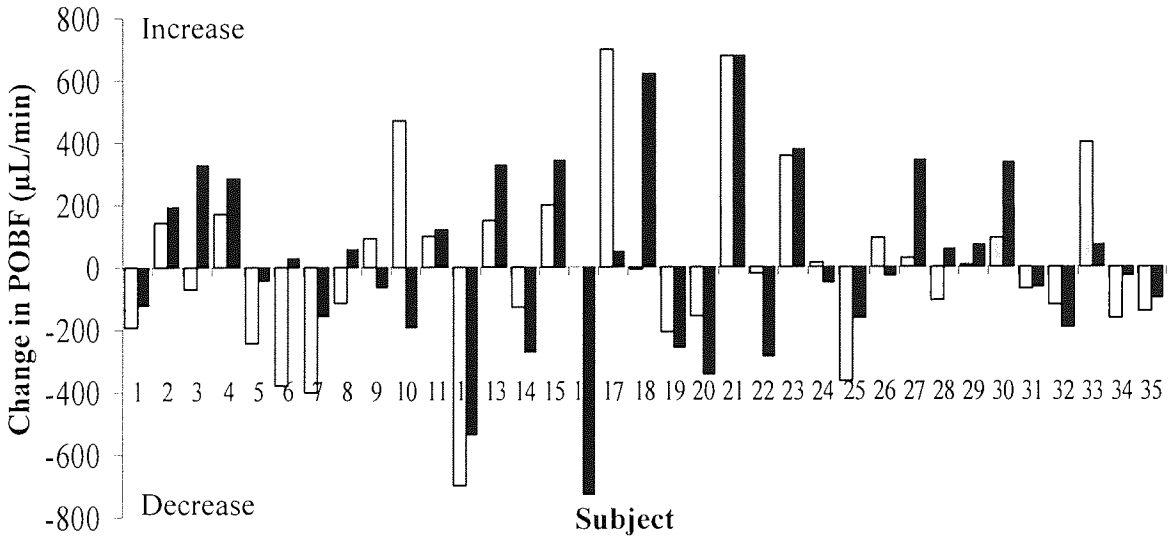


Figure 8.18 Change in POBF (μL/min) between L and I (▩) and L and H (■) levels of accommodation stimuli (n=35).

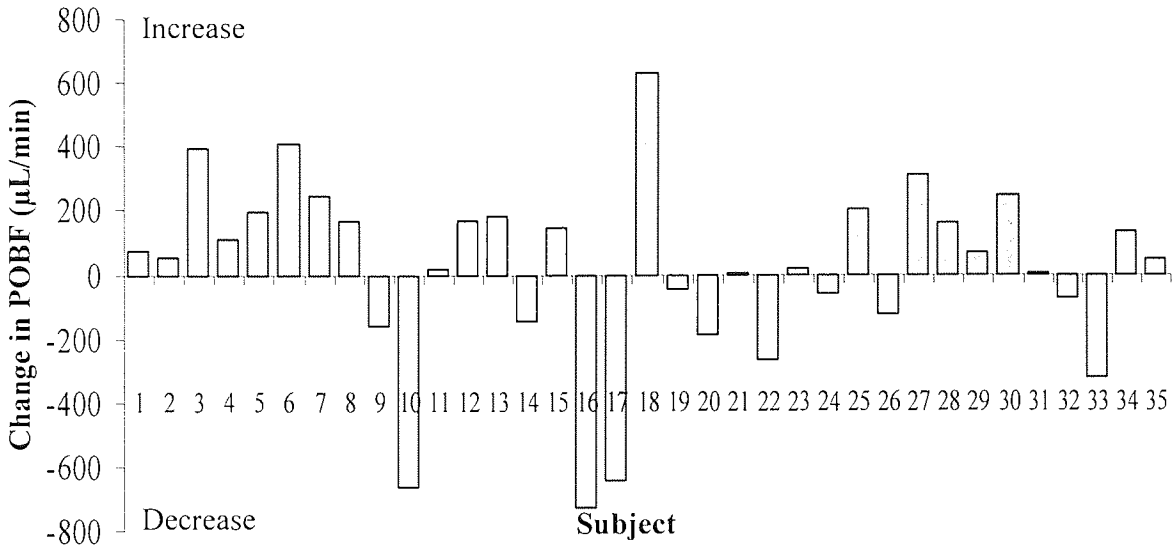


Figure 8.19 Change in POBF (µL/min) between I and H levels of accommodation stimuli (n=35).

The between-refractive groups ANOVA shows that the differences in POBF responses to accommodation of the emmetropes were not statistically different to the POBF responses to accommodation of the myopes between L and I ($F=0.951$, $p=0.34$), L and H ($F=0.616$, $p=0.44$), and I and H ($F=0.026$, $p=0.87$) levels of accommodation. **Figure 8.20** shows that the variations in POBF responses between L and I ($r=0.15$, $p=0.40$), L and H ($r=0.15$, $p=0.38$) and I and H ($r=0.01$, $p=0.94$) levels of accommodation were not due to refractive error. **Figure 8.21** shows the correlation between axial length and the variations in POBF responses between L and I ($r=0.19$, $p=0.28$) and I and H ($r=0.20$, $p=0.24$) levels of accommodation were not statistically significant. However, a significant correlation existed between axial length and the variations in POBF responses between L and H ($r=0.38$, $p=0.02$) levels of accommodation.

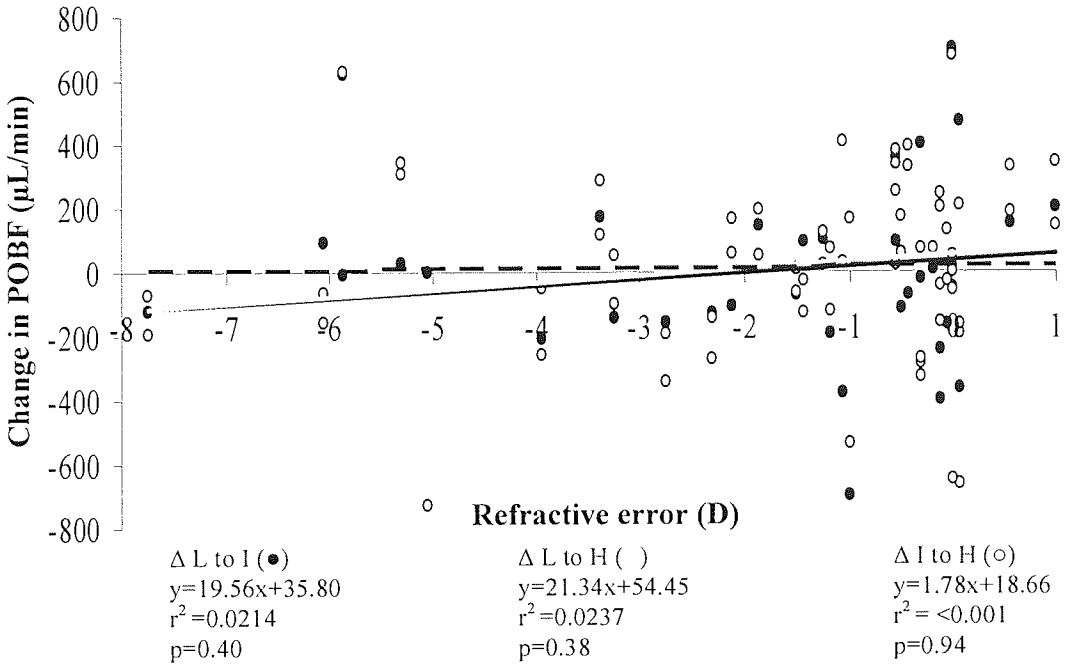


Figure 8.20 Level of refractive error and changes in POBF between L and I (—), L and H ( ) and I and H (---) levels of accommodation (n=35).

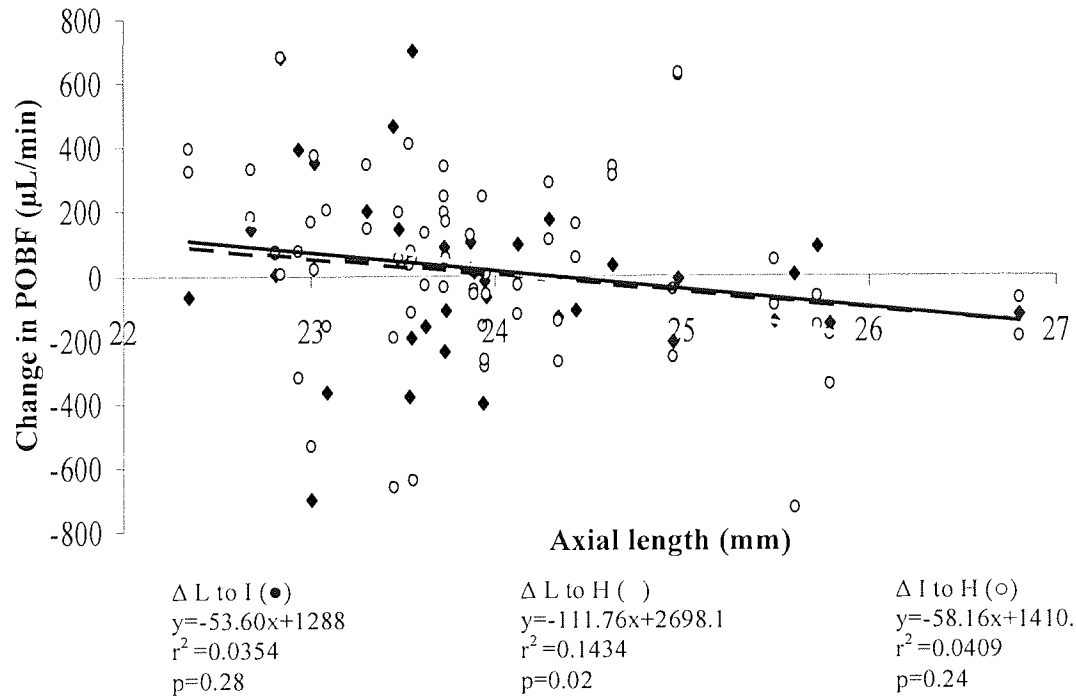


Figure 8.21 Axial length and changes in POBF between L and I (—), L and H ( ) and I and H (---) levels of accommodation (n=35).

Analyses of the percentage changes in POBF are shown in **Appendix 10.** The results indicate that accommodation does not influence the percentage changes in POBF. Similar to the results above, the results shown in **Appendix 10** also suggest that the inter-subject variations in percentage changes in POBF are not explained by differences in refractive error. Furthermore, variations in axial length explained some of the variance in percentage changes in POBF only between L and H accommodation levels.

Modulation of IOP during accommodation

A negative correlation exists between the changes in IOP between L and I, and I and H accommodation levels (**Figure 8.22;** r=0.63, p<0.001), such that if the IOP reduces between L and I levels it increases between I and H accommodation levels. Conversely if the IOP increases between L and I levels, it subsequently decreases between I and H accommodation levels. Likewise, negative correlations are also exhibited between the changes in PA (**Figure 8.23** r=0.47, p=0.007) and PV (**Figure 8.24**; r=0.70, p<0.001) and PR (**Figure 8.25;** r=0.519, p=0.001) between L and I and I and H accommodation levels. In addition, a negative correlation also exists between the changes in POBF between L and I and I and H accommodation levels (**Figure 8.26;** r=0.47, p=0.005), such that if the POBF increases between L and I levels, it decreases between I and H levels. Conversely, if the POBF decreases between L and I levels, the POBF increases between I and H accommodation levels.

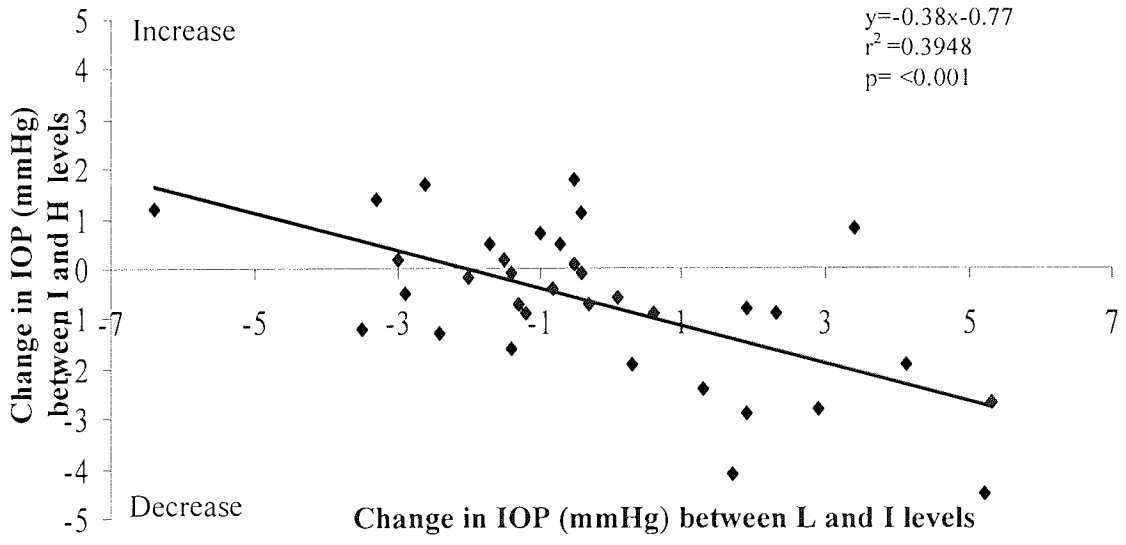


Figure 8.22 Correlation between the change in IOP between L and I and the change in IOP between I and H levels of accommodation stimuli (n=35).

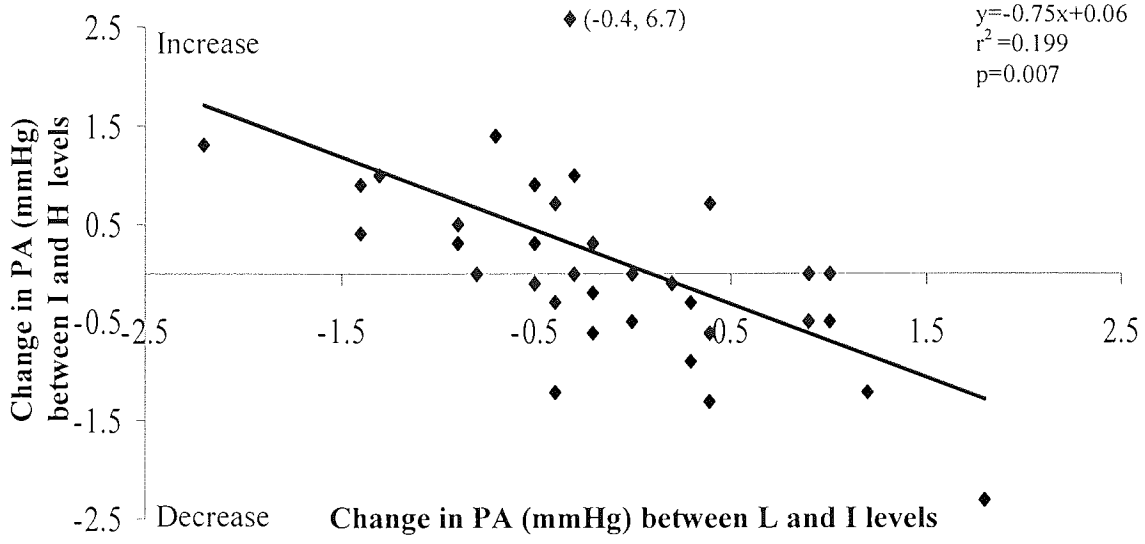


Figure 8.23 Correlation between change in PA (μL/min) between L and I and I and H levels of accommodation stimuli (n=35).

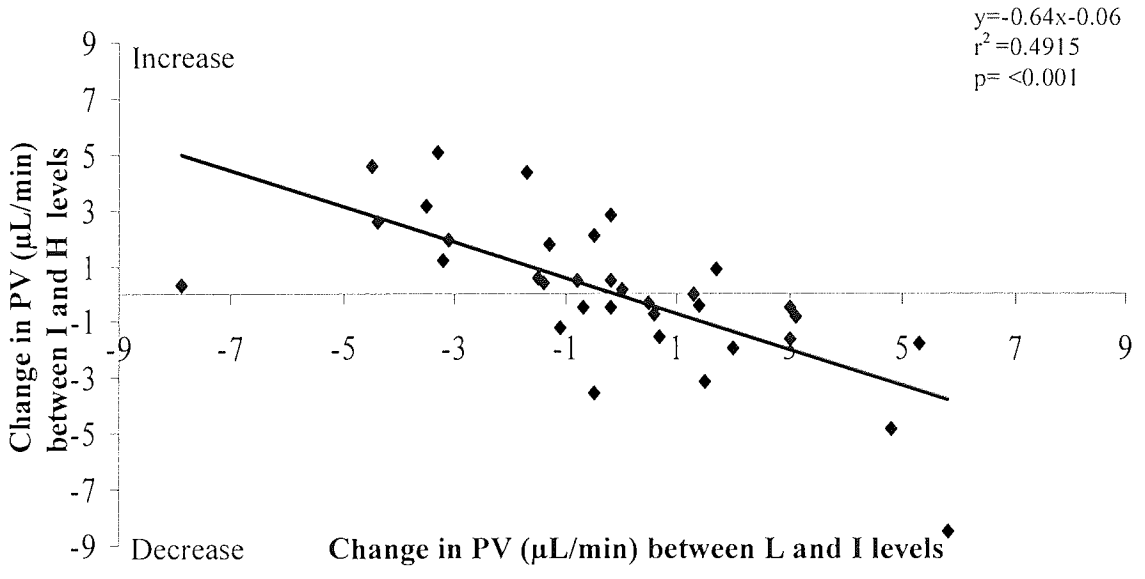


Figure 8.24 Correlation between change in PV (μL/min) between L and I and I and H levels of accommodation stimuli (n=35).

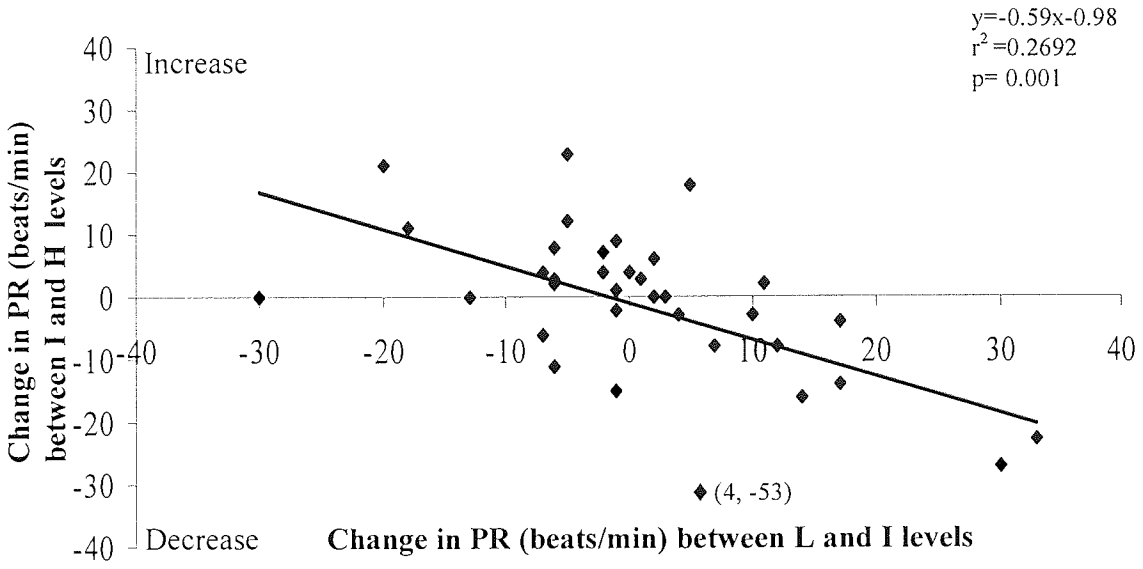


Figure 8.25 Correlation between change in PR (beats/min) between L and I and I and H levels of accommodation stimuli (n=35).

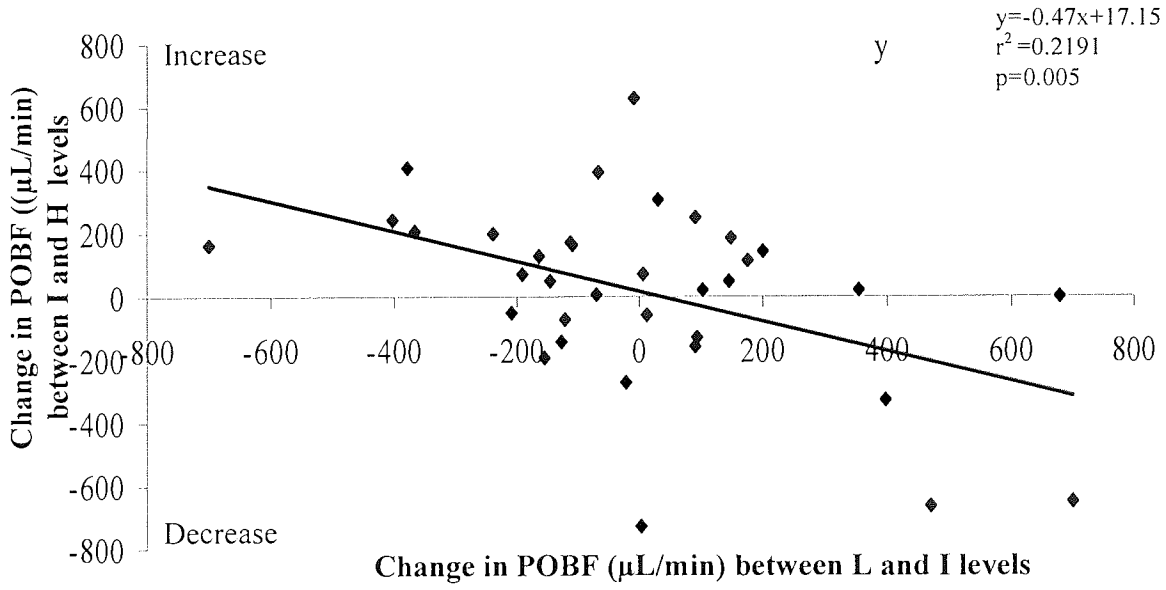


Figure 8.26 Correlation between change in POBF (µL/min) between L and I and I and H levels of accommodation stimuli (n=35).

Autoregulation

A significant relationship between the changes in IOP and changes in PV between L and I (r=0.72, =0.002), and L and H (r=0.81, p=<0.001) accommodation levels has been found in the emmetropes (see **Figure 8.27a**). In contrast **Figure 8.27b** the correlation between the changes in IOP and PV between L and I (r=0.49, p=0.07) and L and H (r=0.32, p=0.20) accommodation levels was not significant. In addition significant correlations were also found between the changes in IOP and POBF between L and I (r=0.77, p=<0.001), and L and H (r=0.73, p=0.001) accommodation levels for the emmetropes (see **Figure 8.28a**). Conversely for the myopes, the changes in IOP and POBF were not correlated for L and I (r=0.33, p=0.20) and L and H (r=0.05, p=0.85) accommodation levels (see **Figure 8.28b**).

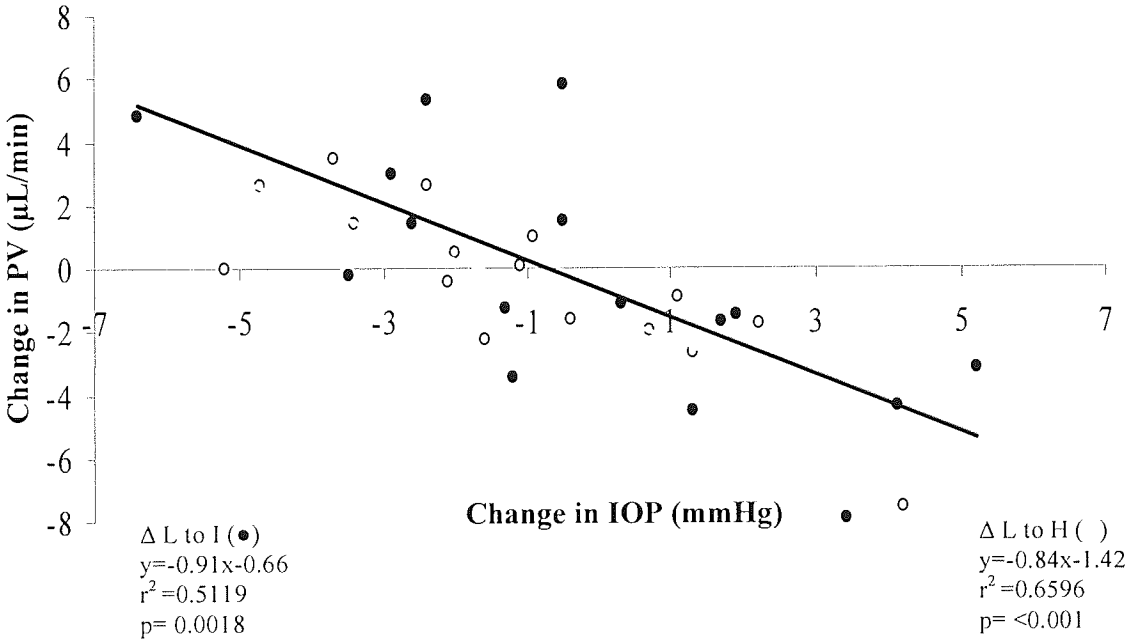


Figure 8.27a Changes in PV as a function of the changes in IOP between L and I (—), and L and H ( ) levels of accommodation for the emmetropes (n=19).

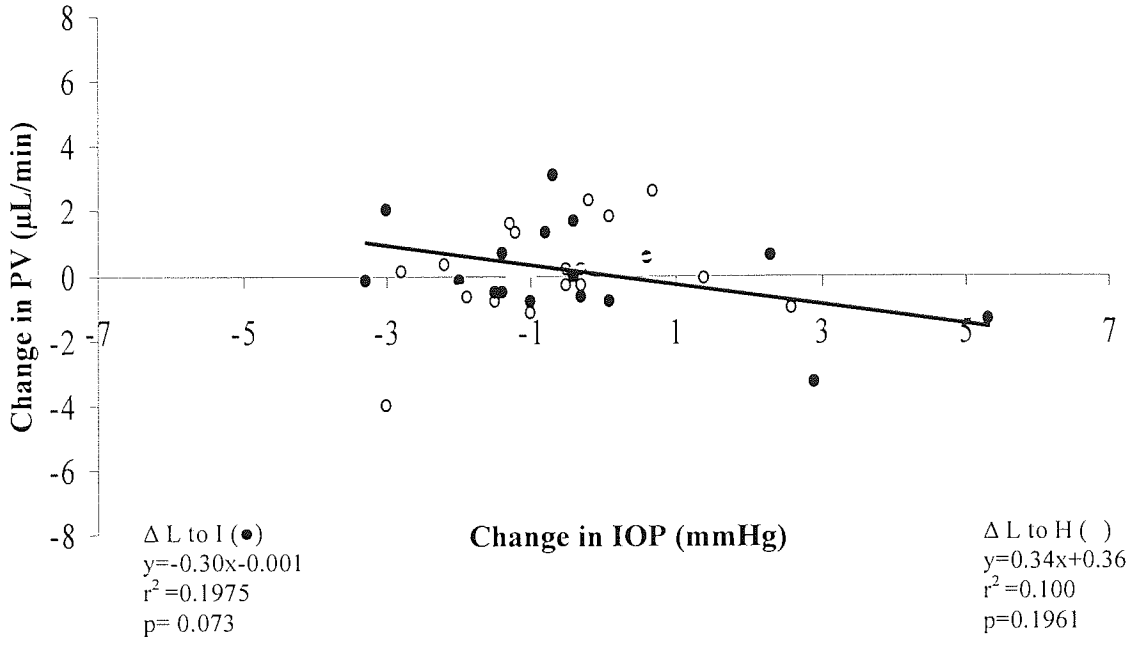


Figure 8.27b Changes in PV as a function of the changes in IOP between L and I (—), and L and H ( ) levels of accommodation for the myopes (n=19).

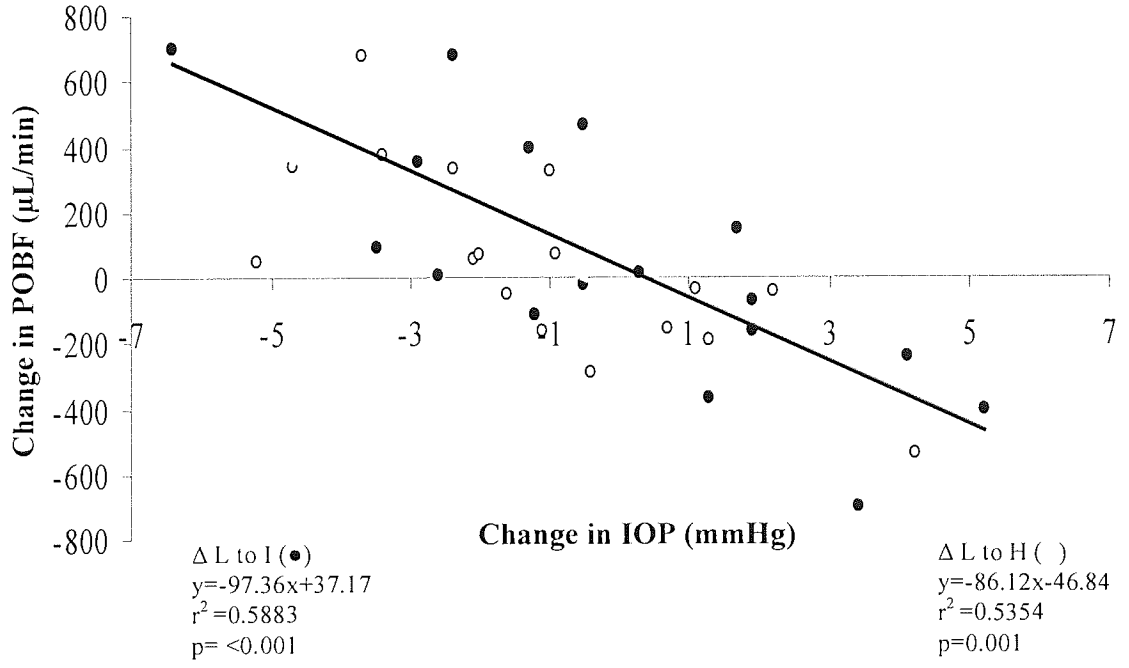


Figure 8.28a Changes in POBF as a function of the changes in IOP between L and I (—), and L and H ( ) levels of accommodation for the emmetropes (n=19).

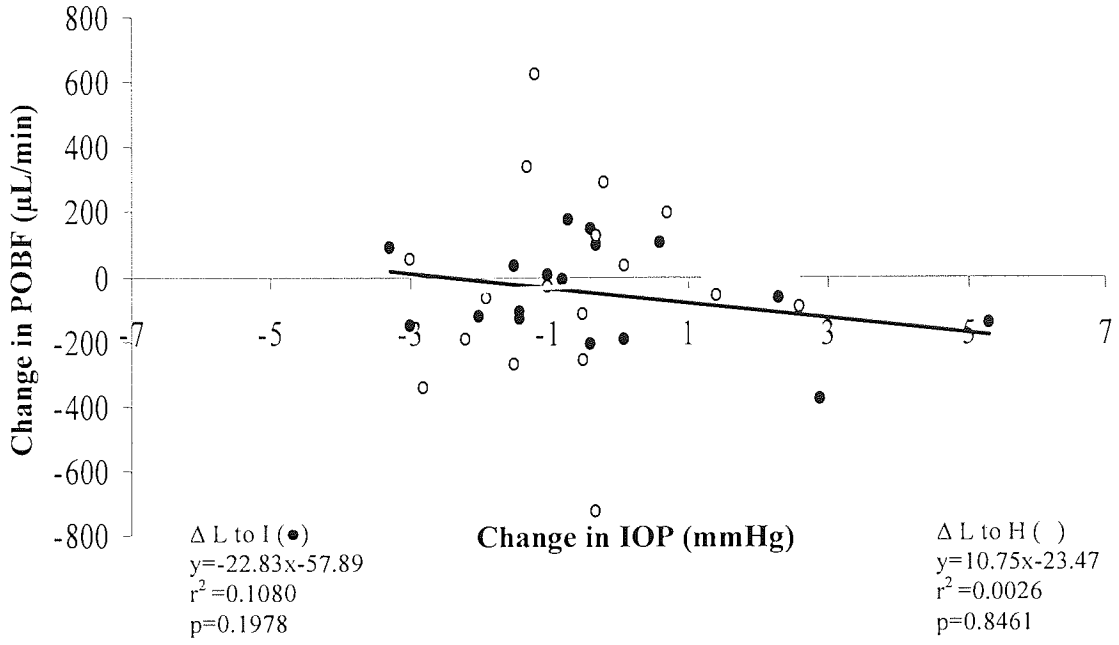


Figure 8.28b Changes in POBF as a function of the changes in IOP between L and I (—), and L and H ( ) levels of accommodation for the myopes (n=19).

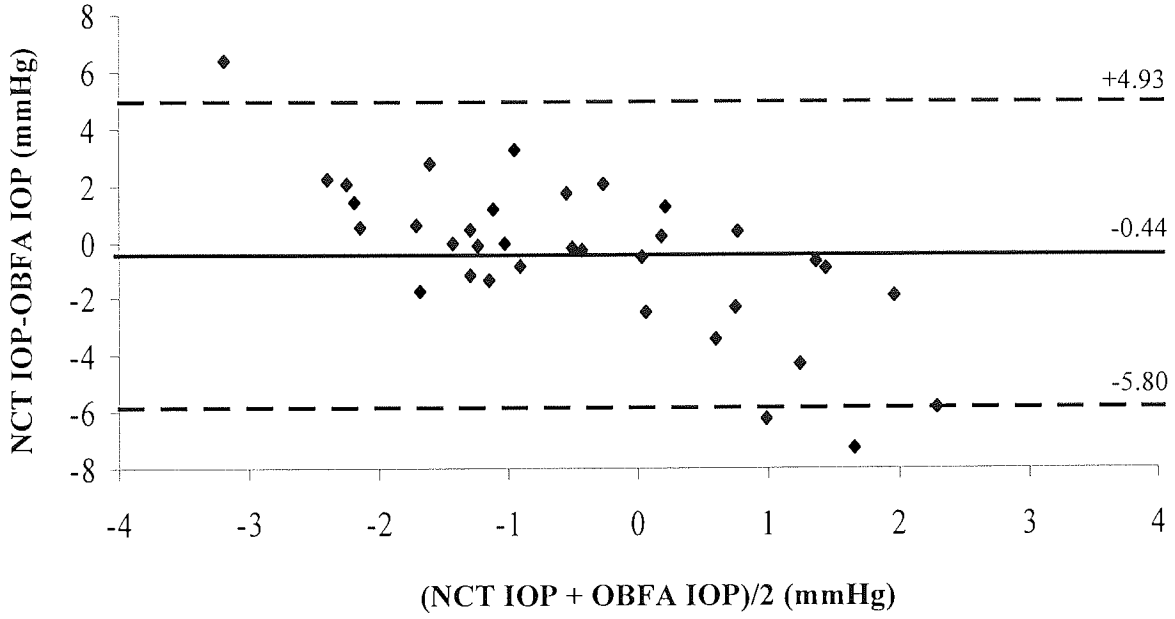
Comparison between NCT and OBFA

The changes in IOP on accommodation, as measured with the OBFA were compared to the changes in IOP on accommodation as measured with the *EasyEye Pulsair* NCT in **Chapter 7** for the same 35 subjects. The Bland and Altman plots of the differences in IOP as a function of their means are shown in **Figure 8.29**.

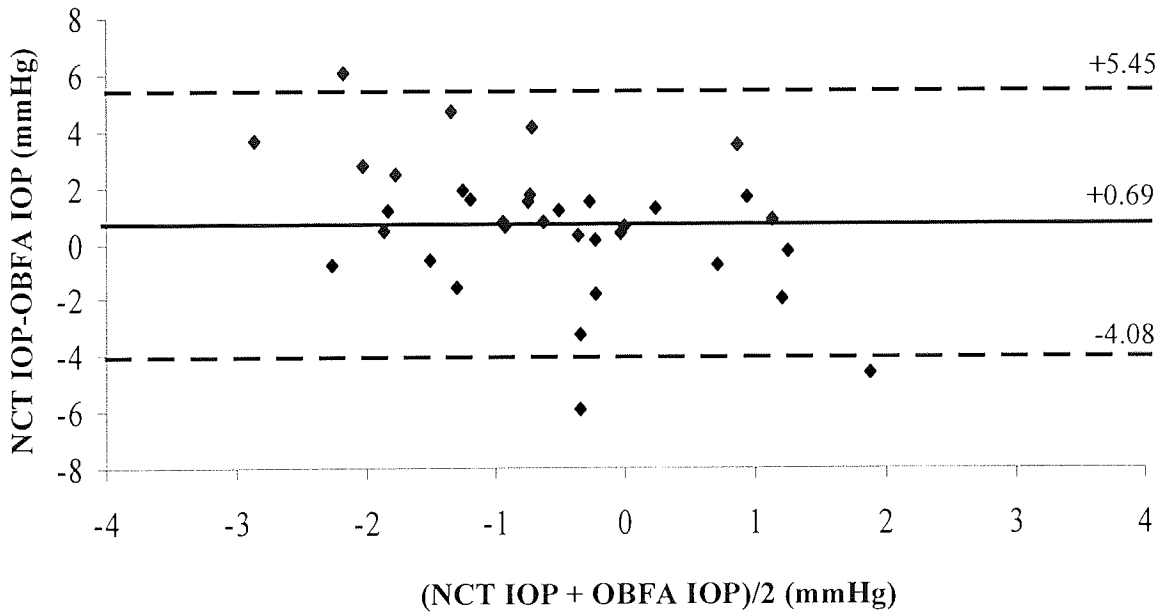
The mean±SD difference between L and I, L and H and I and H accommodation levels were -0.44±2.74 (p=0.35), +0.69±2.43 (p=0.10) and +1.12±1.77 (p=0.001), respectively. The confidence intervals (CI) for the L and I, L and H and I and H accommodation levels were respectively ±5.37, ±4.77 and ±3.47 mmHg.

The correlation graphs in **Figures 8.31** show that the changes in IOP between L and I (r=0.01, p=0.95), L and H (r=0.08, p=0.67) and I and H (r=0.24, p=0.17) accommodation levels, measured with the *EasyEye Pulsair* NCT and with the OBFA were not similar.

(8.29a)


NCT IOP-OBFA IOP (mmHg)
8
6
4
2
0
-2
-4
-6
-8
+4.93
-0.44
-5.80
-4
-3
-2
-1
0
1
2
3
4
(NCT IOP + OBFA IOP)/2 (mmHg)

(8.29b)


NCT IOP-OBFA IOP (mmHg)
8
6
4
2
0
-2
-4
-6
-8
+5.45
+0.69
-4.08
-4
-3
-2
-1
0
1
2
3
4
(NCT IOP + OBFA IOP)/2 (mmHg)

(8.29c)

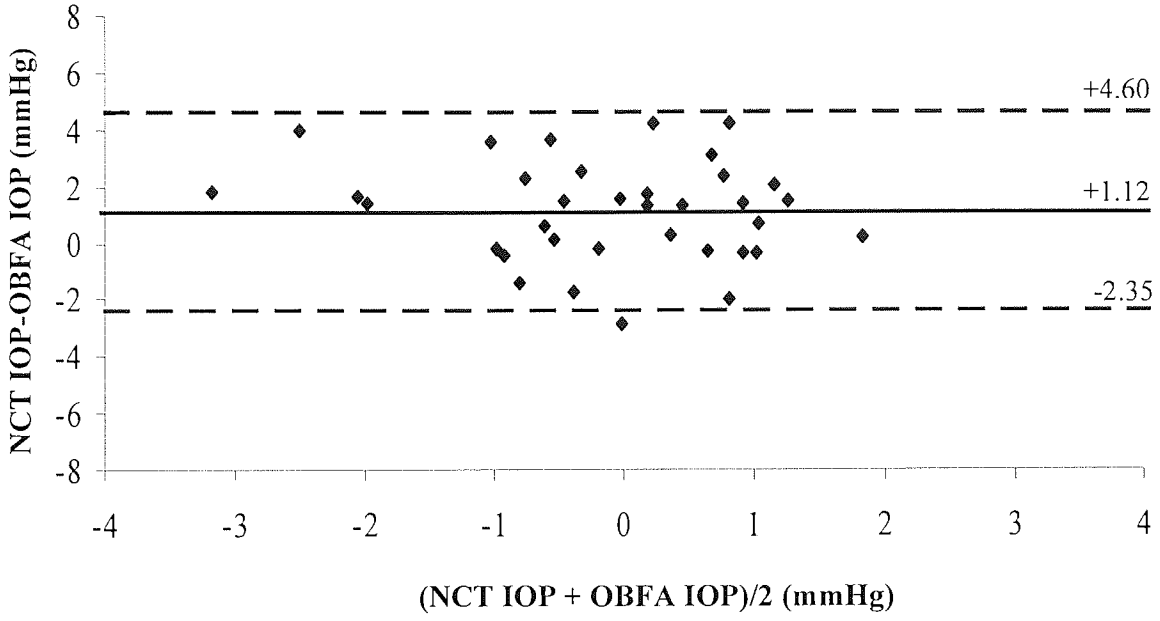


Figure 8.29 Bland and Altman plots for the differences between the changes in IOP between L and I (**a**), L and H (**b**) and I and H (**c**) levels of accommodation as measured with the NCT and OBFA.

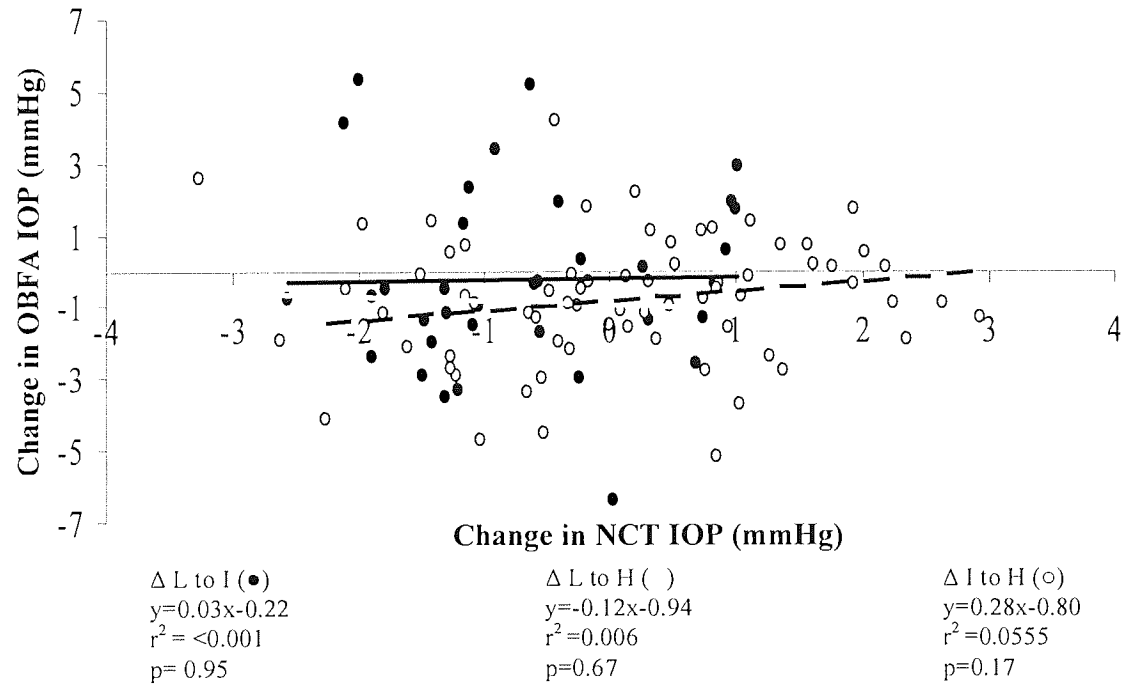


Figure 8.30 Correlations between changes in IOP between L and I (—), L and H ( ) and I and H (---) accommodation levels as measured with the NCT and OBFA (n=35).

The data in the present study was also used to analyses the effects of refractive error and axial length on the blood flow parameters measured with the OBFA. The results are shown in **Appendix 12.**

8.5 Discussion

IOP

The present study found that accommodation influences IOP when measured with the OBFA. A statistically significant reduction in IOP was only observed when relatively high levels of accommodation were stimulated and not when intermediate levels of accommodation were stimulated. These findings were inconsistent with the results from the study described in **Chapter 7** in which the Pulsair *EasyEye* NCT was used to measure IOP and a statistically significant reduction in IOP was reported for I levels of accommodation, but not on H levels of accommodation.

Of note is that in the present study the accommodation responses were measured with the autorefractor in ***Stage 2*** of the experimental procedure and it is assumed that the accommodative system behaved in the same manner during ***Stage 3*** of the experimental procedures when measures with the OBFA were taken. Although a familiarisation procedure was performed, it may have been more difficult to maintain a relatively constant level of accommodation during ***Stage 3*** of the experimental procedure as the whistling sound emitted by the instrument and the relatively long measurement time may have interfered with the subject's concentration to accommodate. Hence, in some subjects the accommodation responses as measured with the autorefractor may not have exactly matched the accommodation responses during the ocular blood flow measurement period and this may have altered the effect of accommodation on IOP.

A similar experimental design problem arose in the study described in **Chapter 6** and consequently a portable, monocular autorefractor was incorporated in the subsequent study design (described in **Chapter 7**). However in the current study, the accommodative target was placed so that the RE remained in the primary position of gaze in order to take blood flow readings and with accommodation, inevitably the LE converged. In order to take simultaneous accommodative response measures with the portable autorefractor, the LE also needed to be in the primary position of gaze. It is clear that with the current instrumentation it

is impractical to measure the ocular blood flow in one eye and simultaneously measure the accommodation responses in the other eye.

A simple mechanical explanation of the effects of accommodation on IOP is described in **Chapter 6** which suggests that with accommodation the ciliary muscle contracts and the aqueous outflow increases hence decreasing IOP. Indeed, a decrease in IOP on I levels of accommodation is expected. Although a reduction in IOP on I accommodation levels was observed in the present study, this did not reach statistical significance when measured with the OBFA. Certainly, on higher levels of accommodation the reduction in IOP is statistically significant when measured with the OBFA.

A reduction in IOP on H levels of accommodation has also been observed by Mauger *et al.* (1984). However, the magnitude of change in IOP on H levels of accommodation as measured with the GCT (i.e. approximately 2.38 mmHg) was approximately twice that found in the present study using the OBFA. The differences in the magnitude of change observed may be due to differences in methodologies. Both the GCT and the OBFA are contact devices and the effects of repeat measures on IOP with the GCT (Moses, 1961) and OBFA (Morgan and Hoskin, 2001) are well documented. However, there still remains a possible intra-measurement massaging effect on the cornea caused by the corneal contact of the probe. As mentioned earlier 7 to 20 seconds are required to take a reading with the OBFA whereas it takes approximately 2 seconds (Myers and Scott, 1975) to take an IOP measure with the GCT. It can be thought that the longer corneal contact time of the OBFA may increase the intra-measurement massaging affect on IOP compared to the GCT. However, although the GCT requires less time to take a reading; more force is necessary to applanate the cornea with the GCT than that required when using the OBFA. This may in turn increase the massaging effect of the GCT compared to the OBFA and explain the discrepancies in the magnitude of change as measured by the two instruments.

Figures 8.2 and **8.3** illustrate the substantial inter-subject variations in IOP responses to accommodation as measured with the OBFA. It is clear that these variations in IOP responses cannot be attributed to inter-subject variations in refractive error or axial length.

Pulse amplitude

The changes in PA with accommodation were considered. The PA changes with accommodation did not reach statistical significance. However, the data demonstrate that PA increases and decreases between L and H accommodation levels in myopes and emmetropes, respectively. The differences in PA between L and I accommodation levels were not statistically different between the myopes and emmetropes. The correlations between refractive error and axial length and the changes in PA did not reach significance.

Pulse volume

The PV changes on accommodation did not reach statistical significance. The PV responses to accommodation of the myopes and emmetropes were not statistically different. No relationship was found between the inter-subject variations in changes in PV with accommodation and refractive error or axial length.

Pulse rate

Similar to the PA and PV data the grouped data demonstrates that the changes in PR with accommodation were not statistically significant. However, with H accommodation levels interestingly the myopes demonstrated a reduction in PR whereas the emmetropes showed an increase in PR. An increase in ocular PR is analogous to an increase in heart rate. A decrease in mean heart rate period (i.e., increase in heart rate) has also been demonstrated with accommodation by Davies (2004). Woldemussie, Feldmann and Chen (1993) proposed that sympathetic and parasympathetic activity increases and decreases the heart rate, respectively. The increased heart rate on accommodation found in the present study could be due to an increase in sympathetic or decrease in parasympathetic activity. Davies (2004) performed Fourier analyses on the cardiac trace during accommodation to conclude that the increase in heart rate was due to a withdrawal of the parasympathetic activity. It is apparent from the present study, that the systemic responses to accommodation of the myopes and emmetropes differ.

Pulsatile ocular blood flow

The effect of accommodation on POBF values approaches significance. It appears that as the level of accommodation increases the POBF values also increase. **Figures 8.18** and **8.19** both show that the relationship between accommodation and POBF was characterised by substantial inter-subject variations. On observation of **Figure 8.20** and **8.21**, the trend

emerging is that on accommodation the POBF progressively reduces as axial length and refractive error increases. However, only the relationship between axial length and POBF changes between L and H accommodation levels reaches significance.

There is some evidence in the literature to show that axial length increases with accommodation due to the contraction of the ciliary muscle pulling the choroid forward and decreasing the circumference of the sclera and thus increasing the axial eye length (Drexler *et al.,* 1998; Mallen, Kashyap and Hampson, 2006). Mallen, Kashyap and Hampson (2006) has demonstrated that the increase in axial length with a 6D accommodative stimuli was greater in myopes (0.058±0.037 mm) than in emmetropes (0.037±0.027 mm) and since a correlation exists between the refractive error and axial length (such that as axial length increases the level of myopia increases) it is inferred that myopes have longer eyes than emmetropes. Mallen, Kashyap and Hampson (2006) also noted that the correlation coefficients between axial elongation with accommodation and baseline axial length were 0.22 and 0.26 for emmetropes and myopes, respectively. Although these correlations were not statistically significant, the trend suggests that the magnitude of axial length increases with accommodation may be greater in more elongated eyes. The data in this study show that the POBF values are dependent on the axial length of the eye (see **Appendix 12**). Therefore, it is possible that the relationship between changes in POBF with H levels of accommodation and axial length observed (see **Figure 8.21**) may be a result of the small changes in axial length with accommodation which subsequently affect the POBF values.

Indeed if axial length changes affect POBF during accommodation then a relationship is expected between PA changes with accommodation and axial length, since data in the present study show that axial length influences both POBF and PA in the same manner (see **Appendix 12**). It is inferred from **Figures 8.8** and **8.9** that no such relationship is present. This may provide evidence against the hypothesis mentioned above which states that changes in axial length on accommodation maybe confounding the POBF.

In summary analyses of the individual parameters shows that PA reduces and increases in emmetropes and myopes, respectively between L and H accommodation levels. Furthermore, the PR increases and decreases in emmetropes and myopes, respectively. The data suggest that on accommodation the bolus of blood entering the eye reduces in emmetropes. It is hypothesised that in order to regulate POBF, an increase in PR is necessary, which is indeed

observed in the present study. In myopes however, it appears that the bolus of blood entering the eye is actually increased which may be attributable to a possible defective feedback process. The reduction in PR observed in the myopic subjects may be necessary to regulate blood flow.

An alternative hypothesis is that emmetropes and myopes differ in their systemic response to accommodation, such that the emmetropes and myopes increase or decrease their PR respectively, during accommodation. This would then lead to subsequent changes in PA in order to regulate the POBF. It is not clear which of the two hypotheses is correct. The data also suggest that subjects with high axial lengths appear to be unable to maintain their blood flow during accommodation resulting in a decrease in POBF. This may possibly be due to an imbalance between PA and PR changes. It is unknown whether these changes in POBF are a cause or effect of myopia development.

Modulation of IOP with accommodation

An interesting observation was that subjects showed either an increase or decrease in IOP, PA, PV and POBF between L and I accommodation levels. This observation may suggest that there may be subsets of subjects: those who increase in the parameters mentioned with I levels of accommodation and those who decrease in the parameters with I levels of accommodation. The direction of change with accommodation may be dependent on some ocular structural component. This observation is supported by the results of **Chapter 7**, which also show that there are subsets of subject who increase or decrease in IOP between L and I accommodation levels.

One may expect that with H levels of accommodation the changes in the parameters continue to change in the same direction as they do with I levels of accommodation. However, the results from this study propose that the eye may not be able to tolerate a larger change in the parameters which may be induced with H levels of accommodation regardless of the direction of change. A feedback process may occur which subsequently induces a compensatory change in the parameters with H levels of accommodation suggesting that the IOP, PA, PV and POBF are modulated. The temporal aspects of these possible compensatory changes in the parameters are unknown as the changes in the parameters were only measured after 3 minutes of accommodation in the present study. It is possible that those who are unable to

modulate the changes on H accommodation levels progress to myopia, due to a defective feed back process.

Autoregulation

Autoregulation is defined as 'the ability of a vascular bed to keep blood flow constant despite changes in perfusion pressure' (Johnson, 1964). The ocular perfusion pressure (OPP) is defined as the difference between ocular arterial and venous pressures with the latter being only slightly higher than the IOP and therefore assumed approximately equal to IOP (Bill, 1962). The mean arterial pressure (MAP) is 1/3 of the systolic pressure plus 2/3 of the diastolic pressure (McKibbin and Verma, 1999). OPP has therefore been proposed to be equal to 2/3 of the MAP minus the IOP. The coefficient of 2/3 is only valid if the subject is in the sitting position during the measurement (Findl *et al.*, 2000).

Therefore, it is apparent that OPP alters with changes in IOP and systemic blood pressure. In the present study, since accommodation alters IOP, OPP must also change with accommodation such that if IOP decreases the OPP increases and if IOP increases then OPP decreases, assuming that systemic blood pressure remains constant. Several workers have suggested that a mechanism in the choroidal circulation adapts vascular resistance to changes in OPP to keep blood flow constant i.e. the choroidal blood flow autoregulates (Kiel, 1994; Kiel and Van Heuven, 1995; Riva *et al.*, 1997; Chou, Lu and Chen, 2002; Lovasik *et al.*, 2003; Reiner, Zagyazdin and Fitzgerald, 2003). **Figures 8.27a** and **8.28a** show that in the emmetrope the choroid does not autoregulate. As IOP changes, the PV and POBF values change in the opposite direction as demonstrated by Findl *et al.* (1997). Several other workers have also found that the choroid does not autoregulate (Alm and Bill, 1972; Friedman, 1970; Armaly and Araki., 1975; Gherezghiher, Okubo and Koss, 1991; Findl *et al.*, 1997; Joos *et al.*, 1999; Delaey and Van de Voorde, 2000). In the myopic eye, it appears that the changes in IOP and PV and POBF between L and I accommodation levels have a similar trend to the responses of the emmetropic eye. However, interestingly with higher accommodation levels it appears that the myopic eye is unable to regulate choroidal pulse volume and POBF blood flow in response to IOP changes. Although not significant, the trend suggests that as IOP increases the blood flow parameters increase and as IOP decreases the blood flow parameters also decrease. It is clear that myopes and emmetropes differ in their vascular regulatory mechanisms during accommodation. This may be due to a defective feedback process which fails to detect the changes in IOP during accommodation or due to an

inability of the choroid to respond to the changes in IOP. Alternatively, it is possible that systemic blood pressure changes during accommodation.

On artificial increases in IOP between 10 to 50 mmHg using a suction cap, the fundus pulsations (Findl *et al.,* 1997; Weigert *et al.,* 2006) and the velocity of the blood flow in the short posterior ciliary arteries (Joos *et al.,* 1999) have been shown to reduce which suggests that the choroid does not autoregulate. It was unknown however, whether the choroidal blood flow autoregulated following small natural changes in IOP. The PV and POBF data suggest that in the emmetropic eye the choroid does not autoregulate even to small changes in IOP. Of note is that the OBFA was performed after 3 minutes of fixation as accommodative adaptation occurs after approximately 3 minutes (Rosenfield and Gilmartin, 1999) and autoregulation is known to occur within the first minute of induced change (Ernest, 1968; Riva, Grunwald and Petrig, 1986; Joos *et al.,* 1999). Sufficient time was therefore allowed for any autoregulatory mechanism to operate although the results from this study cannot rule out a possible delayed autoregulatory response of PV and POBF in both emmetropes and myopes.

Comparison

A comparison was made between the IOP data measured by the NCT in **Chapter 7** and the OBFA for the same 35 subjects. It was concluded that, although the mean differences between the IOP measured with the NCT and the OBFA did not reach statistical significance (except for the differences in IOP between I and H accommodation levels) the large CI (shown in the Bland and Altman plots in **Figure 8.29**) and the corresponding correlation graphs (see **Figure 8.30**) show that the IOP responses to accommodation as measured by the two techniques were not similar. This may be explained by the differences in the methodologies used for the two measurement techniques, that is the type of instrument used to measure IOP (i.e. a NCT in **Chapter 7** and the OBFA in the present study). Furthermore, in the study described in **Chapter 7**, the accommodation responses were measured simultaneously during the IOP measurement period and hence relatively constant levels of accommodation were ensured. However, in the present study, due to limitations of the experimental design, the accommodation responses were measured during a separate stage of the experimental procedure. It was then assumed that the accommodative system behaved the same during the IOP/POBF measurement period. Indeed, the differences in IOP responses

with accommodation observed between these 2 studies may be explained by differences in accommodation responses during the IOP/POBF measurement period.

8.6 Conclusions

This study investigated the effects of accommodation on blood flow parameters using the OBFA. In summary, the results of the current study conclude that:

- Dose effect between IOP and accommodation is present. As the accommodative level increases IOP decreases. However, in the present study only the decrease in IOP with high accommodation levels is statistically significant. The relationship between accommodation and IOP is characterised by substantial inter-subject variations in IOP responses to accommodation which are not attributable to refractive error or axial length.

- No dose effect of accommodation on PA values is apparent. However, in myopes the PA increases whereas in the emmetropes the PA decreases with H levels of accommodation. The correlations between PA changes and refractive error and axial length are not significant.

- The PV data are not significantly influenced by accommodation. The inter-subject variations in PV responses to accommodation are not due to refractive error or axial length.

- The PR decreases in the myopes and increases in the emmetropes with H levels of accommodation. The correlations between PA changes and refractive error and axial length are not significant.

- The relationship between accommodation and POBF values approaches significance. The inter-subject variations in POBF changes between L and I accommodation levels are not attributable to refractive error or axial length. However, the inter-subject variations in POBF changes between L and H accommodation levels are explained by inter-subject variations in axial length.

- The changes in IOP, PA, PR, PV and POBF are modulated. If the parameters increase between L and I accommodation levels, they subsequently decrease between I and H accommodation levels. If however, IOP, PA, PR, PV and POBF values decrease between L and I accommodation levels, they increase between I and H accommodation levels.

- Choroidal vascular responses to IOP changes differ between emmetropes and myopes.

- The changes in IOP with accommodation measured following the experimental protocols described in **Chapter 7** and the present study are not equivalent.

CHAPTER 9

BIOMETRIC AND OCULOMOTOR CORRELATES OF THE EFFECTS OF ACCOMMODATION ON INTRAOCULAR PRESSURE AND BLOOD FLOW

9.1 Introduction

The effects of accommodation on intraocular pressure (IOP) were investigated using a non-contact tonometer (NCT) the *EasyEye Pulsair* (Keeler, UK). The short-term variations in IOP caused by the respiratory and cardiac cycles were minimised by using a metronome and finger pulse-transducer coupled with a *LabView* acquisition programme (National Instruments, USA). Furthermore, the simultaneous measurements of accommodation responses were incorporated into the study design. Despite these modifications to the experimental setup the relationship between accommodation and IOP was consistently characterised by substantial inter-subject variation in IOP responses to accommodation. In addition the effects of accommodation on IOP were analysed using the Ocular Blood Flow Analyser (OBFA; *Paradigm Medical Instruments Inc., UK*). Analyses of the data also demonstrated substantial inter-subject variations in IOP responses (as measured by the OBFA) to accommodation. Furthermore, the effects of accommodation on ocular blood flow were evaluated using the OBFA. Similar to the conclusions drawn from the IOP data, analyses of the ocular blood flow data also demonstrated significant variations in the changes in blood flow with accommodation between-subjects. In the present study, these inter-subject variations in IOP and blood flow responses to accommodation are investigated further with respect to inter-subject variations in biometric and oculomotor parameters.

In recent years, several fundamental optical technologies have been made available which permit non-contact biometric measures of the ocular structures. To date, no single instrument is capable of measuring simultaneously all the biometric parameters of the eye and therefore four instruments are used in the present study namely the Pentacam Comprehensive Eye Scanner (Oculus, Inc.), Orbscan (Bausch and Lomb), Ultrasound Pachymeter (*DGH-550 Pachette 2*, DGH technology, Pennsylvania, US) and the IOL Master (Carl Zeiss). Although

some of these instruments measure the same ocular parameter the results may differ owing to differences in the measuring principles inherent to the system.

9.2 Methods

9.2.1 Subject group

The present study used a sample size of 50 subjects (mean age 20.5±3.1 yrs, range 18-31 years of age) recruited from the undergraduate population at Aston University. The mean spherical equivalent (MSE) of the cohort ranged from +1.00DS to -8.25DS and comprised 28 myopes (MSE of ≤ -0.50D), 21 emmetropes (MSE of ± 0.50D) and 1 hypermetrope (MSE of ≥ +0.50D). The criterion used to divide the subjects into these refractive groups has also been used in several studies for example those conducted by Goh *et al.* (2005), Junghans and Crewther (2005) and Ojaimi *et al.* (2005). In the study group there were 15 males and 35 females. The distribution of refractive error results from supplementing the 40 subjects recruited in the study described in **Chapter 7** with an additional 10 subjects. The additional subjects were recruited principally to increase the number of myopes in the study group to allow analyses of the effects of the degree of myopia on the IOP and blood flow. Biometry and IOP measures (as measured with the NCT) were taken in the 50 subjects.

Three of the additional 10 subjects recruited were unable to comply with the measurement demands of the OBFA and therefore data were not collected from these subjects. The subjects reported that although the eye was anaesthetised, they were aware of corneal contact as the instrument emitted a whistling sound when the probe was in contact with the cornea and this resulted in excessive epiphoria and blephrospasm. Consequently, ocular blood flow data was obtained in 42 subjects (this figure arises from the fact that a full data set was only collected in 35 of the 40 subjects recruited in **Chapter 8** and 7 of the additional 10 subjects recruited in the present study) with a mean age of 20.6±3.2 years of age (ranging from 18 to 31 years of age). The mean spherical error ranged from +1.00DS to -7.75DS. The group comprised of 22 myopes, 19 emmetropes and 1 hypermetrope (30 females; males 12).

The research followed the tenets of the Declaration of Helsinki and was approved by the Institution's ethics committee (**Appendix 3**). Written consent was obtained from all subjects willing to participate in the study and copies of the information sheets and consent forms given to and completed by the subjects can be found in **Appendix 4**. All subjects achieved a

visual acuity of 0.00 logMAR or better. All subjects were absent of ocular pathology and none of the subjects were taking any topical or systemic medications that may affect the IOP, cardiovascular function or accommodative function.

9.2.2 Instrumentation

Pentacam

The Oculus Pentacam is a commercially available, non-contact anterior eye imaging device which uses a rotating Scheimpflug photography principle which captures multiple 2 dimensional slices at different orientations allowing a 3-dimensional image of the anterior segment of the eye to be built up, from the anterior corneal surface to the posterior lens surface.

In a single scan (which takes less than 2 seconds) 12 to 50 single image captures are performed. In total, 25,000 elevation points are measured and evaluated. Any minute eye movements are captured by a second camera and corrected for simultaneously. The Oculus Pentacam provides 3-dimensional chamber analysis, densitometry of the intraocular lens and corneal pachymetry and topography.

Orbscan

In 1999 the Orbscan II became a commercially-available computerised videokeratoscope which allows topographic mapping of the power and shape of the cornea. The instrument is part-based on the Placido disc principle in which the corneal reflection of concentric, alternately light and dark ringed targets allow a quantitative assessment of the cornea to be made. The Placido disc principle combined with the innovative technique of measuring the dimensions of a slit-scanning beam projected on the cornea and complex algorithms facilitates the quantitative assessment of the cornea and provides a virtual reconstruction of the corneal surface.

During data acquisition, 1 scan acquires data from 40 slits which are 12.50 mm high and 0.30 mm wide and projected on the cornea at an angle of 45 degrees to the instrument axis. The software detects and analyses the anterior edge of each slit and creates an elevated topographic representation of the anterior surface of the cornea. The edges of the reflected Placido disc ring mires are also detected and analysed to provide a curvature reconstruction of

the anterior surface of the cornea. Subsequent processing of the sampled data digitally recreates the posterior cornea, anterior iris and lens surfaces.

Pachymeter

Ultrsound pachymetry is considered gold standard for the measurement of central corneal thicknesses. In the present study the *DGH-550 Pachette 2* was used to measure central corneal thickness (CCT). Although the instrument provides quick and accurate measures corneal anaesthesia is required. On contact of the probe with the cornea vibrations in the sound waves are recorded and digital values of the corneal thickness are instantly displayed on a screen. The method relies on the sound waves been echoed by the epithelial and endothelial layers of the cornea. The technique assumes that the velocity of the sound waves through the cornea is constant. The CCT values are calculated from the lag of the echoes from the epithelium and endothelium and the velocity of the sound waves. An important limitation of ultrasound pachymetry is that some degree of operator experience is necessary to place accurately the probe on the cornea and obtain reproducible measures. Furthermore the reflection of ultrasound within the cornea may be adversely influenced by changes in corneal hydration (González-Méijome *et al.,* 2003; Javaloy *et al.,* 2004; Rainer *et al.,* 2004; Wickham, Edmunds and Murdoch, 2005).

IOL Master

The IOL Master provides accurate non-contact measures of axial length (AL), anterior chamber depth (ACD) and corneal curvatures (CC).

The axial length is measured using partial coherent interferometry (PCI) based on the Michelson interferometer (Haigis *et al.,* 2000). Infrared light (780 μm) of short coherence length (160 μm) is split into 2 equal coaxial beams by a beam splitter. The 2 beams are reflected off the cornea and retina and the difference in frequency of the 2 coaxial beams is detected by a photodetector. An optical axial length measure is computed which is converted to a geometric axial length by using a standardised group refractive index (Hitzenberger *et al.,* 1993).

A 0.7 mm wide beam of light is directed through the anterior segment of the eye at an angle of 38° to the visual axis and forms an optical section. The software measures the distance

between the anterior corneal pole and anterior surface of the crystalline lens and the value is displayed as the ACD.

The CCs are measured by analyses of the corneal reflection of 6 points of light arranged in a 2.3 mm diameter hexagonal pattern. The distances between opposite pairs of lights are measured and the toroidal surfaces are calculated by the instrument's software.

Note the details of the initial calibration of the instruments mentioned above are not known. However, as with the Pulsair Non-contact tonometer and the Ocular Blood Flow Analyser used throughout this thesis differences in the characteristics of the subject group (for example gender, age, and ethnicity) used for the calibration process and the subject groups used in subsequent studies may present some error in the measurement of biometric parameters.

Convergence

In the studies described in **Chapter 7** and **8** it was noted that during the IOP and ocular blood flow measurements the RE was occluded with either the *EasyEye Pulsair* NCT or OBFA, respectively and hence retinal disparity was not present during the measurement period. Consequently, convergence was not stimulated via the active feedback mechanism and thus the convergence system was rendered open-loop. Since accommodation was stimulated in the LE, accommodative convergence (AC) still occurred as a result of the well established neural linkage between accommodation and convergence in young eyes and this was evident as the adduction of the LE during the IOP and ocular blood flow measurement periods.

In **Chapters 7** and **8**, the simultaneous measurement of AC was in practical terms complex and furthermore the time and equipment constraints of the study rendered difficult the ability to decouple the accommodation convergence/accommodation(AC/A) system. Therefore AC was measured in a second experimental setup whereby convergence was also rendered open-loop. In the second experimental setup, an accommodative target was used to stimulate set amounts of accommodation response in the LE which thus stimulated AC, which was simultaneously measured. Although in both setups open-loop convergence conditions were present, the two experimental designs did not mirror each other exactly. In one experimental design convergence was rendered open-loop with either the NCT or the OBFA whereas in the second a Maddox rod was used to achieve open-loop conditions in which dissociation was achieved by distortion of the image in one eye and hence eliminated a fusional response.

However, of importance is that the input to, and output from, the accommodation/convergence gain system was equivalent in the two experimental designs.

Ocular volume

Logan (1997) calculated ocular volume from retinal contour shape. The axial length was plotted against the ocular volume of the RE of 28 subjects. The relationship between axial length and ocular volume was defined as that shown in **Equation 9.1** and this was used to calculate the ocular volume in this thesis.

$$y = 109.21x^2 - 4891.57x + 60720.6 \qquad \textbf{Equation 9.1}$$

where y is the ocular volume and x is the axial length of the eye.

Ocular perfusion pressure

The ocular perfusion pressure (OPP) is defined as the difference between ocular arterial and venous pressures with the latter being only slightly higher than the IOP and therefore assumed approximately equal to IOP (Bill, 1962). The mean arterial pressure (MAP) is 1/3 of the systolic pressure plus 2/3 of the diastolic pressure (McKibbin and Verma, 1999). OPP has therefore been proposed to be equal to 2/3 of the MAP minus the IOP (Anderson, 1970). In order to calculate the OPP, the blood pressure was measured in each subject at the start of the experiments using a sphygmomanometer the *UA-767 Fully Automatic Blood Pressure Monitor* (BHS A/A, A & D Co. Ltd, Oxon, UK).

9.2.3 Experimental procedures

The experimental procedures were split into the following stages:

Stage 1

As in the preceding studies, the autorefractor results were used to render the LE functionally emmetropic by fitting a soft daily disposable contact lens (1-day *Acuvue Dailies*, etafilcon A, Johnson & Johnson, Vistakon, USA) to the LE.

Stage 2

The effects of accommodation on IOP were measured in the 10 extra subjects recruited in the present study following the experimental procedures described in **Chapter 7.** The effects of

accommodation on blood flow were measured in the extra 7 of the 10 subjects recruited in this study following the experimental procedures described in **Chapter 8**.

Stage 3

The biometric data from the *Pentacam*, *Orbscan* and *IOL Master* were collected in a random order only in the subject's RE. Once the subject was comfortably positioned at each device with the chin on the chin rest and the forehead against the forehead rest, the subject was instructed to maintain steady fixation on the centre of the fixation target. Once the instrument was aligned correctly, data were collected.

One drop of 0.5% proxymetacaine hydrochloride (Chauvin Pharmaceutical, Ltd.) was instilled into the subject's RE. The ultrasound probe was placed in the centre of the cornea, perpendicular to the corneal plane and one measurement of CCT (consisting of 5 measures) was taken in the RE. Ultrasound pachymetry was performed last owing to the use of anaesthetics and the corneal contact involved.

Stage 4

A measure of the AC was obtained by using the Maddox rod (MR) and wing (MW). The MR is a multiple cylindrical lens which when placed horizontally or vertically in front of one eye, respectively produces a vertical or horizontal streak of light when a light source is viewed by the contralateral eye.

A spot light source was placed at 6 m and a MR was positioned horizontally in front of the subject's RE such that a vertical streak of light was seen. The subjects were instructed to indicate the position of the streak relative to the light source. The horizontal deviation of the streak of light from the light source was measured by placing prisms in front of the same eye as the MR with the base of the prism in the same direction as the displaced streak until the streak passed through the light source. The value in prism dioptres recorded represented the horizontal deviation at L levels of accommodation.

To measure the AC on I and H levels of accommodation a MW scale was replicated and a light emitting diode (LED) was fixed to the centre of the scale. The new tangent scale was attached to the Shin-Nippon autorefractor in line with the visual axis of the LE. The MR was placed horizontally in front of the subject's RE and when the light source (LED) was viewed

a vertical streak of light was seen. The number 2 on the tangent scale was used as an accommodative target. The subjects were instructed to obtain a clear view of the accommodative target and the accommodative responses were sampled at 30 second intervals using the autorefractor. The accommodative target was moved along the visual axis of the LE until accommodation responses of 1.50±0.50 and 4.00±0.50 D were obtained. The subjects were then instructed to indicate the position of the vertical streak on the replicated tangent scale, at each accommodative level, which was presented in a random order. This procedure was repeated 3 times and the average result was used for analyses.

Since the tangent scale was replicated from a standard MW the values obtained were only calibrated for a distance of 33 cm such that each 1^{Δ} was represented by a displacement of the vertical streak by 0.3 cm (measured with digital callipers). Due to the accommodative lag at near the tangent scale was placed at different distances in order to obtain set amounts of accommodative responses. Therefore, the displacement of each 1^{Δ} (x) at a known distance y (see **Figure 9.1**) was calculated from the formulae in **Equation 9.2**.

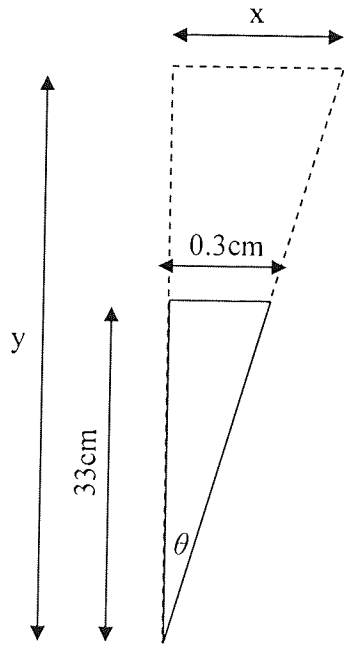


Figure 9.1 Graphical representation for the calculation of the displacement (x) represented by 1^{Δ} at a known distance (y).

From **Figure 9.2**:

$$\text{Tan}\,\theta = \frac{0.3}{33}$$

$$\theta = 0.52$$

therefore:

$$\text{Tan } 0.52 = \frac{x}{y}$$

$$\text{Tan } 0.52\ y = x \qquad \textbf{Equation 9.2}$$

AC was calculated by multiplying the *x* value by the reported displacement of the vertical streak during accommodation.

9.3 Statistical analyses.

Software packages *SPSS 12.1* for Windows and Microsoft *Excel* were used for the statistical analyses of the data.

The inter-subject variations in the response of IOP and blood flow to accommodation were assessed with respect to biometric and oculomotor parameters. Median splits of each parameter were determined and the inter-subject variations in IOP and blood flow responses were assessed with between-subjects one-way ANOVAs.

Pearson's product moment correlation coefficients were determined between each parameter and the changes in IOP and blood flow with accommodation. As several parameters were measured multiple regression analyses were also performed. Although the cases-to-variables ratio was not ideal all the parameters were included in a forward stepwise multiple regression analyses as recommended by Norman and Streiner (2000).

9.4 Results

The comparison of pachymetry, anterior chamber depth and keratometry readings taken with the different modalities are shown in **Appendix 13.**

Analyses of the effects of accommodation on IOP and blood flow parameters are summarised in **Appendix 14.** Consistent with results of **Chapter 7** and **8** the relationship between accommodation and IOP and blood flow is again characterised by substantial inter-subject variations in responses to accommodation.

The influence of the anterior chamber depth (ACDS), anterior chamber angle (ACANS), corneal curvature (CCS), anterior chamber volume (ACVLS), corneal volume (CVLS),

eccentricity (ECCS), central corneal thickness (CCTS), axial length (ALS), ocular volume (OVOLS) and refractive error (Ref Err), accommodative convergence (AC) and accommodative convergence to accommodation ratio (AC/A) values on the inter-subject variations in IOP and blood flow parameters with accommodation were assessed. The mean±SD and the range of each parameter on 50 subjects are shown in **Table 9.1.**

Parameter	Mean±SD	Maximum	Minimum
CCTS (μm)	593.3±38.8	634	463
ACDS (mm)	3.07±0.25	3.60	2.46
ACVLS (ml^3)	197.2±26.9	258	139
ALS (mm)	24.14±1.10	26.80	22.35
ACANS (°)	42.3±4.6	55	32
CVLS (ml^3)	60.03±3.71	71	53.8
CCS (mm)	7.75±0.23	8.35	7.20
ECCS	0.51±0.14	0.87	0.17
Ref Err (MSE; D)	-2.10±2.53	+1.00	-8.25
OVOLS (mm^3)	6399.8±534.7	8055.3	5939.4
Δ AC ($^{\Delta}$) L to I	3.57±2.69	10.77	0.59
Δ AC ($^{\Delta}$) L to H	9.54±5.83	26.53	1.03
AC/A L to I	2.30±1.76	7.17	0.34
AC/A L to H	2.17±1.38	6.63	0.32

Table 9.1 Summary of biometric parameters taken in the RE's of 50 subjects.

9.4.1 Associations between biometric and oculomotor parameters and the changes in IOP as measured with the NCT.

Median split analyses

Median splits of each parameter were determined and the changes in IOP were analysed using between-subject ANOVA tests and the results are shown in **Table 15.1A** in **Appendix 15**. The results suggest that median splits of the corneal volumes (CVLS) demonstrated significant differences in the absolute ($F=5.069$, $p=0.029$) and percentage ($F=4.453$, $p=0.040$) changes in IOP between L and I levels of accommodation. Therefore for CVol values >59.95ml^3 the absolute and percentage reduction in IOP was respectively 0.44±1.32mmHg and 3.11±9.57%. For CVol values <59.95ml^3 the absolute and percentage reduction in IOP was respectively 1.39±1.64mmHg and 9.28±11.07%. None of the other parameters explained

the inter-subject variations in the IOP change between L and H, and I and H levels of accommodation.

Correlation analyses

The inter-subject variations in the absolute and percentage changes in IOP with accommodation as measured with the NCT were correlated with the biometric and oculomotor parameters and the results are shown in **Table 15.2A** in **Appendix 15**. The results show that a negative correlation exists between the changes in IOP between L and I accommodation levels and axial lengths (ALS) (absolute: r=0.297, p=0.04; percentage: r=0.299, p=0.03) and corneal curvatures (CCS) (absolute: r=0.384, p=0.01; percentage: r=0.377, p=0.007). Anterior chamber angles (ACANS) were positively correlated with the absolute (r=0.288, p=0.04) and percentage (r=0.325, p=0.02) changes in IOP between L and H accommodation levels. No significant associations were found between the changes in IOP between I and H accommodation levels and the biometric and oculomotor parameters.

Multiple regression analyses

Parameter	R	R^2	Adjusted R Square	Std. Error of the Estimate	Change Statistics				
					R^2	F	df1	df2	Sig. F
CCS	0.348	0.121	0.103	1.46805	0.121	6.599	1	48	0.013
CCS ACDS	0.445	0.198	0.164	1.41702	0.077	4.519	1	47	0.039

Parameter	Unstandardised Coefficients		Standardised Coefficients	t	Sig.	95% Confidence Interval for B	
	B	Std. Error	Beta			Lower Bound	Upper Bound
Constant	17.275	7.084		2.439	0.018	3.032	31.517
CCS	-2.348	0.914	-0.348	-2.569	0.013	-4.186	-0.510
Constant	30.342	9.194		3.300	0.002	11.845	48.838
CCS	-3.268	0.983	-0.484	-3.326	0.002	-5.245	-1.291
ACDS	-1.936	0.911	-0.309	-2.126	0.039	-3.769	-0.104

Table 9.2 Summary of the results of forward stepwise multiple regression analyses including all independent variables and the dependent variable of absolute changes in IOP (as measured by the NCT) between L and I levels of accommodation (n=50).

Parameter	R	R^2	Adjusted R Square	Std. Error of the Estimate	Change Statistics				
					R^2	F	df1	df2	Sig. F
CCS	0.377	0.142	0.125	10.01580	0.142	7.969	1	48	0.007
CCS ACDS	0.488	0.238	0.205	9.54241	0.095	5.881	1	47	0.019

Parameter	Unstandardised Coefficients		Standardised Coefficients	t	Sig.	95% Confidence Interval for B	
	B	Std. Error	Beta			Lower Bound	Upper Bound
Constant	130.179	48.33		2.694	0.010	33.007	227.351
CCS	-17.606	6.237	-0.377	-2.823	0.007	-30.145	-5.066
Constant	230.555	61.92		3.724	0.001	105.999	355.112
CCS	-24.673	6.618	-0.529	-3.728	0.001	-37.986	-11.359
ACDS	-14.874	6.134	-0.344	-2.425	0.019	-27.214	-2.535

Table 9.3 Summary of the results of forward stepwise multiple regression analyses including all independent variables and the dependent variable of percentage changes in IOP (as measured by the NCT) between L and I levels of accommodation (n=50).

Parameter	R	R^2	Adjusted R Square	Std. Error of the Estimate	Change Statistics				
					R^2	F	df1	df2	Sig. F
ACANS	0.288	0.083	0.064	1.71416	0.083	4.328	1	48	0.043

Parameter	Unstandardised Coefficients		Standardised Coefficients	t	Sig.	95% Confidence Interval for B	
	B	Std. Error	Beta			Lower Bound	Upper Bound
Constant	-4.758	2.285		-2.082	0.043	-9.353	-0.163
ACANS	0.112	0.054	0.288	2.080	0.043	0.004	0.220

Table 9.4 Summary of the results of forward stepwise multiple regression analyses including all independent variables and the dependent variable of absolute changes in IOP (as measured by the NCT) between L and H levels of accommodation (n=50).

Parameter	R	R^2	Adjusted R Square	Std. Error of the Estimate	Change Statistics				
					R^2	F	df1	df2	Sig. F
ACANS	0.325	0.106	0.087	11.22584	0.106	5.664	1	48	0.021

Parameter	Unstandardised Coefficients		Standardised Coefficients	t	Sig.	95% Confidence Interval for B	
	B	Std. Error	Beta			Lower Bound	Upper Bound
Constant	-35.252	14.97		-2.355	0.023	-65.346	-5.159
ACANS	0.838	0.352	0.325	2.380	0.021	0.130	1.545

Table 9.5 Summary of the results of forward stepwise multiple regression analyses including all independent variables and the dependent variable of percentage changes in IOP (as measured by the NCT) between L and H levels of accommodation (n=50).

Parameter	R	R^2	Adjusted R Square	Std. Error of the Estimate	Change Statistics				
					R^2	F	df1	df2	Sig. F
AC	0.283	0.080	0.061	2.07426	0.080	4.166	1	48	0.047
AC ACANS	0.394	0.155	0.119	2.00871	0.075	4.184	1	47	0.046

Parameter	Unstandardised Coefficients		Standardised Coefficients	t	Sig.	95% Confidence Interval for B	
	B	Std. Error	Beta			Lower Bound	Upper Bound
Constant	-0.023	0.533		-0.043	0.966	-1.094	1.048
AC	0.152	0.074	0.283	2.041	0.047	0.002	0.302
Constant	-5.833	2.887		-2.021	0.049	-11.641	-0.026
AC	0.187	0.074	0.348	2.525	0.015	0.038	0.336
ACANS	0.132	0.065	0.282	2.046	0.046	0.002	0.263

Table 9.6 Summary of the results of forward stepwise multiple regression analyses including all independent variables and the dependent variable of absolute changes in IOP (as measured by the NCT) between I and H levels of accommodation (n=50).

Note that none of the parameters evaluated showed a significant association with the percentage changes in IOP between I and H levels of accommodation.

Refractive error

The inter-subject variations in IOP with accommodation were assessed between emmetropes and myopes. A between-subjects ANOVA showed that no significant differences in absolute and percentage changes in IOP were present between L and I (F=0.025, p=0.875; F=0.020, p=0.888), L and H (F=0.002, p=0.962; F=0.004, p=0.951), and I and H (F=0.024, p=0.877; F=0.087, p=0.769) levels of accommodation.

Myopia

Median split

Median split analyses was performed on the myopic group and the results are summarised in **Table 15.3A in Appendix 15**. A median split of their refractive error demonstrated a statistically significant difference in the absolute (F=11.908, p=0.002) and percentage (F=9.156, p=0.006) changes in IOP between L and I levels of accommodation. For a level of myopic refractive error of <-3.29D the absolute and percentage change in IOP was a reduction of respectively 1.61±0.93 mmHg and 10.76±5.69 %. For a level of myopic refractive error of >-3.29D the absolute and percentage change was a decrease of 0.17±1.26 mmHg and 1.38±10.10%, respectively. A median split of the axial lengths (ALS) also demonstrated a statistically significant difference in the absolute and percentage changes in IOP between L and I levels of accommodation. For ALS >24.61 mm the absolute and percentage change in IOP was a reduction of respectively 1.48±0.99 mmHg and 9.79±5.98%, and for ALS <24.61 mm the absolute and percentage change in IOP was a reduction of 0.30±1.36 mmHg and 2.35±10.78%. Furthermore, a median split of the ocular volumes (OVOLS) also demonstrated significant differences in the absolute and percentage changes in IOP between L and I accommodation levels. For OVOLS > 6473.0 mm^3 the absolute and percentage change was a reduction of respectively 1.48±0.99 mmHg and 9.79±5.98%, and OVOL <6473.0 mm^3 the absolute and percentage change in IOP was a reduction of 0.30±1.36 mmHg and 2.35±10.78%, respectively. Median splits of the ocular biometric and oculomotor parameters in the myopic group do not show significant differences in the absolute and percentage changes in IOP between L and H and I and H levels of accommodation.

Correlation analyses

The inter-subject variations in the absolute and percentage changes in IOP with accommodation as measured with the NCT in the myopic group were correlated with the biometric and oculomotor parameters and the results are shown in **Table 15.4A** in **Appendix 15**. Correlation graphs of the level of myopia and the changes in IOP on accommodation are shown in **Figures 9.2** and **9.3**.

The level of myopia was significantly correlated with the absolute (r=0.496, p=0.01) and percentage (r=0.420, p=0.03) changes in IOP between L and I levels of accommodation. The level of myopia was also significantly correlated with the percentage (r=0.390, p=0.04)

changes in IOP between L and H levels of accommodation, but not with the absolute (r=0.321, p=0.10) changes in IOP. No significant correlation was present between the absolute (r=0.008, p=0.97) and percentage (r=0.076, p=0.70) changes in IOP between I and H levels of accommodation.

The results show that a negative correlation exists between the changes in IOP between L and I accommodation levels and ALS (absolute: r=0.484, p=0.009; percentage: r=0.455, p=0.01). OVOLS also show a negative correlation with the absolute changes in IOP between L and I accommodation levels (r=0.394, p=0.04). ECCS were positively correlated with the absolute (r=0.540, p=0.003) and percentage (r=0.513, p=0.005) changes in IOP between L and I levels of accommodation. ACANS were positively correlated with the absolute (r=0.409, p=0.03) and percentage (r=0.445, p=0.02) changes in IOP between L and H accommodation levels. No significant associations were found between the changes in IOP between I and H accommodation levels and the biometric and oculomotor parameters.

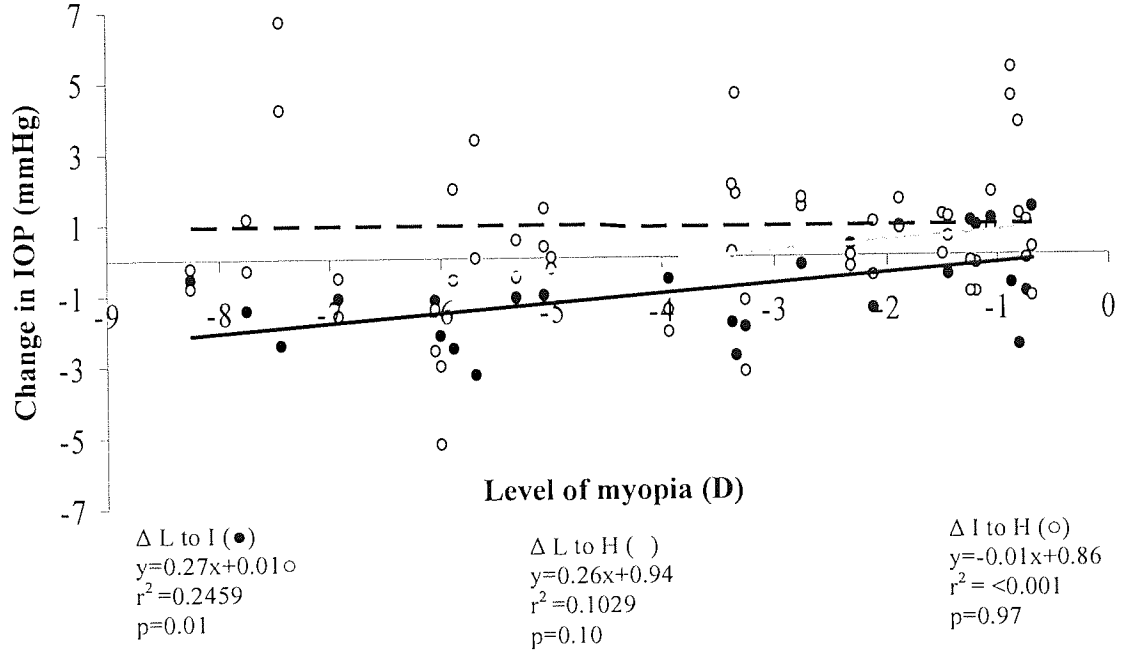


Figure 9.2 Correlation graphs of the level of myopia and the absolute changes in IOP (as measured by the NCT) between L and I (—), L and H ( ) and I and H (---) levels of accommodation (n=28).

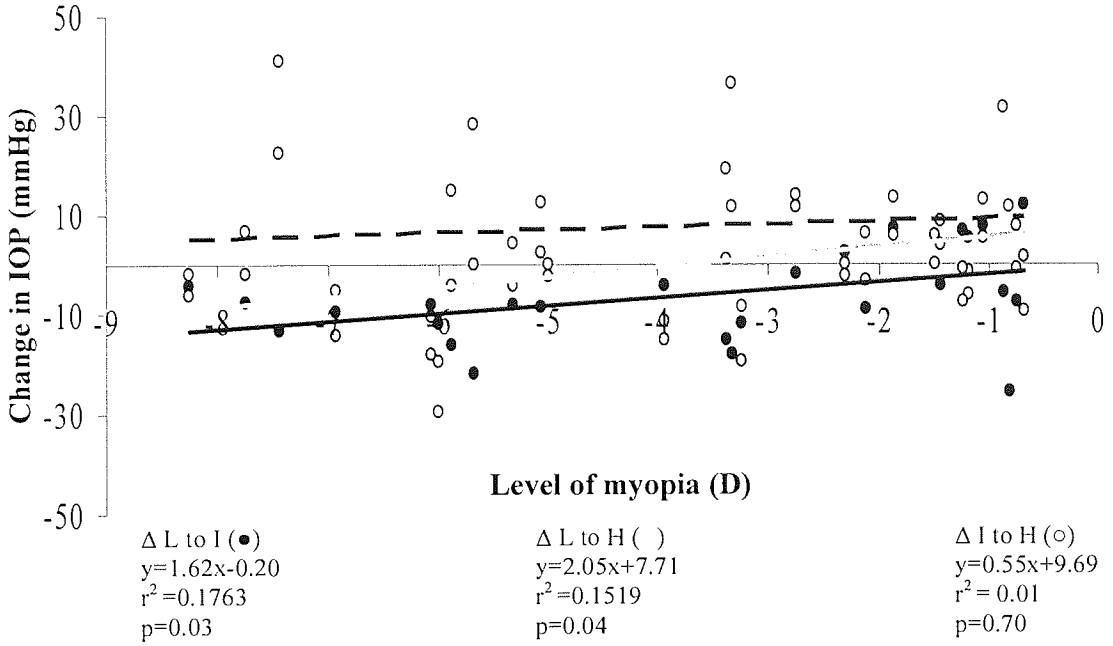


Figure 9.3 Correlation graph of the level of myopia and the percentage changes in IOP (as measured with the NCT) between L and I (—), L and H ( ) and I and H (---) accommodation levels (n=28).

Multiple regression analyses

All the parameters were evaluated using forward stepwise multiple regression analyses with respect to the absolute and percentage changes in IOP with accommodation. The results are shown in **Tables 9.7** to **9.11**.

Parameter	R	R^2	Adjusted R Square	Std. Error of the Estimate	Change Statistics				
					R^2	F	df1	df2	Sig. F
ESSC	0.540	0.292	0.264	1.12800	0.292	10.706	1	26	0.003

Parameter	Unstandardised Coefficients		Standardised Coefficients	t	Sig.	95% Confidence Interval for B	
	B	Std. Error	Beta			Lower Bound	Upper Bound
Constant	-3.692	0.882		-4.185	<0.001	-5.505	-1.878
ESSC	5.273	1.612	0.540	3.272	0.003	1.961	8.586

Table 9.7 Summary of the results of stepwise multiple regression analyses including all the independent variables and the dependent variable of absolute changes in IOP (as measured by the NCT) between L and I levels of accommodation in myopes only (n=28).

Parameter	R	R^2	Adjusted R Square	Std. Error of the Estimate	Change Statistics				
					R^2	F	df1	df2	Sig. F
ECCS	0.513	0.263	0.234	8.18547	0.263	9.2656	1	26	0.005

Parameter	Unstandardised Coefficients		Standardised Coefficients	t	Sig.	95% Confidence Interval for B	
	B	Std. Error	Beta			Lower Bound	Upper Bound
Constant	-24.976	6.401		-3.902	0.001	-38.133	-11.819
ECCS	35.599	11.70	0.513	3.044	0.005	11.559	59.638

Table 9.8 Summary of the results of stepwise multiple regression analyses including all the independent variables and the dependent variable of percentage changes in IOP (as measured by the NCT) between L and I levels of accommodation in myopes only (n=28).

Parameter	R	R^2	Adjusted R Square	Std. Error of the Estimate	Change Statistics				
					R^2	F	df1	df2	Sig. F
ACANS	0.409	0.167	0.135	1.83925	0.167	5.2296	1	26	0.031
ACANS CCS	0.654	0.428	0.382	1.55494	0.260	11.377	1	25	0.002

Parameter	Unstandardised Coefficients		Standardised Coefficients	t	Sig.	95% Confidence Interval for B	
	B	Std. Error	Beta			Lower Bound	Upper Bound
Constant	-7.967	3.499		-2.277	0.031	-15.160	-0.773
ACANS	0.186	0.081	0.409	2.287	0.031	0.019	0.353
Constant	-51.625	13.28		-3.888	0.001	-78.971	-24.28
ACANS	0.258	0.072	0.568	3.583	0.001	0.110	0.406
CCS	5.255	1.558	0.534	3.373	0.002	2.046	8.464

Table 9.9 Summary of the results of forward stepwise multiple regression analyses including all independent variables and the dependent variable of absolute changes in IOP (as measured by the NCT) between L and H levels of accommodation in myopes only (n=28).

Parameter	R	R^2	Adjusted R Square	Std. Error of the Estimate	Change Statistics				
					R^2	F	df1	df2	Sig. F
ACANS	0.445	0.198	0.167	11.64357	0.198	6.423	1	26	0.018
ACANS CCS	0.663	0.439	0.394	9.93200	0.241	10.733	1	25	0.003

Parameter	Unstandardised Coefficients		Standardised Coefficients	t	Sig.	95% Confidence Interval for B	
	B	Std. Error	Beta			Lower Bound	Upper Bound
Constant	-55.597	22.15		-2.510	0.019	-101.14	-10.06
ACANS	1.304	0.514	0.445	2.534	0.018	0.246	2.361
Constant	-326.46	84.81		-3.849	0.001	-501.12	-151.79
ACANS	1.750	0.459	0.597	3.809	0.001	0.804	2.696
CCS	32.602	9.951	0.514	3.276	0.003	12.107	53.097

Table 9.10 Summary of the results of forward stepwise multiple regression analyses including all independent variables and the dependent variable of percentage changes in IOP (as measured by the NCT) between L and H levels of accommodation in the myopes only (n=28).

Parameter	R	R^2	Adjusted R Square	Std. Error of the Estimate	Change Statistics				
					R^2	F	df1	df2	Sig. F
CCS	0.421	0.177	0.145	2.05105	0.177	5.589	1	26	0.026
CCS ACANS	0.620	0.384	0.335	1.80932	0.207	8.412	1	25	0.008

Parameter	Unstandardised Coefficients		Standardised Coefficients	t	Sig.	95% Confidence Interval for B	
	B	Std. Error	Beta			Lower Bound	Upper Bound
Constant	-34.939	15.16		-2.305	0.029	-66.099	-3.778
CCS	4.640	1.963	0.421	2.364	0.026	0.606	8.674
Constant	-55.378	15.45		-3.714	0.001	-89.197	-25.559
CCS	6.199	1.813	0.562	3.419	0.002	2.465	9.932
ACANS	0.243	0.084	0.477	2.900	0.008	0.070	0.415

Table 9.11 Summary of the results of forward stepwise multiple regression analyses including all independent variables and the dependent variable of absolute changes in IOP (as measured by the NCT) between I and H levels of accommodation in the myopes only (n=28).

None of the parameters evaluated showed a significant association with the percentage changes in IOP between I and H levels of accommodation.

9.4.2 Associations between biometric and oculomotor parameters and the changes in IOP as measured with the OBFA.

Median split

Median splits of each parameter were determined and the changes in IOP were analysed using between-subject ANOVA tests and these are shown in **Table 15.5A in Appendix 15**. It was evident that median splits of the centre corneal thickness values (CCTS) partly explained the percentage changes (but not the absolute changes) in IOP between L and I levels of accommodation. For CCTS values >533.5μm and <533.5μm the percentage changes in IOP were respectively an increase of 6.04±31.08% and decrease of 9.97±16.45%. Median splits of the other parameters did not explain the differences in IOP responses between L and H and I and H levels of accommodation.

Correlation analyses

The inter-subject variations in the absolute and percentage changes in IOP with accommodation as measured with the OBFA were correlated with the biometric parameters measured. The results are summarised in **Table 15.6A in Appendix 15.** None of the parameters investigated showed significant associations with the absolute or percentage changes in IOP between L and I, L and H, and I and H accommodation levels.

Multiple regression analyses

Using forward stepwise multiple regression analyses none of the parameters evaluated showed a significant association with the absolute and percentage changes in IOP between L and I, and L and H levels of accommodation. The parameters were also evaluated with respect to the absolute changes in IOP between I and H accommodation levels and the results are shown in **Table 7.12**. None of the parameters evaluated showed a significant association with the percentage changes in IOP between I and H levels of accommodation.

Parameter	R	R^2	Adjusted R Square	Std. Error of the Estimate	Change Statistics				
					R^2	F	df1	df2	Sig. F
Ref Err	0.313	0.098	0.076	1.52011	0.098	4.355	1	40	0.043
Ref Err CCTS	0.452	0.204	0.164	1.44594	0.106	5.209	1	39	0.028

Parameter	Unstandardised Coefficients		Standardised Coefficients	t	Sig.	95% Confidence Interval for B	
	B	Std. Error	Beta			Lower Bound	Upper Bound
Constant	-0.963	0.302		-3.191	0.003	-1.573	-0.353
Ref Err	-0.209	0.100	-0.313	-2.087	0.043	-0.412	-0.007
Constant	6.116	3.115		1.963	0.057	-0.185	12.417
Ref Err	-0.237	0.096	-0.354	-2.462	0.018	-0.431	-0.042
CCTS	-0.013	0.006	-0.329	-2.282	0.028	-0.025	-0.002

Table 9.12 Summary of the results of forward stepwise multiple regression analyses including all independent variables and the dependent variable of absolute changes in IOP (as measured by the OBFA) between I and H levels of accommodation (n=42).

Refractive error

The inter-subject variations in IOP on accommodation were assessed between emmetropes and myopes. A between-subjects ANOVA shows that no significant differences in absolute and percentage changes in IOP were present between L and I (F=0.025, p=0.875; F=0.122, p=0.728), L and H (F=0.852, p=0.362; F=0.356, p=0.554), and I and H (F=2.271, p=0.140; F=1.607, p=0.212) levels of accommodation.

Myopia

Median split

Median split analyses was performed on the myopic group and the results show that median splits of each the parameters do not show significant differences in absolute and percentage changes in IOP between L and I, L and H and I and H accommodation levels (see **Table 15.7A** in **Appendix 15**).

Correlation analyses

The inter-subject variations in the absolute and percentage changes in IOP with accommodation as measured with the OBFA in the myopic group were correlated with the biometric and ocular motor parameters and the results are shown in **Table 15.8A** in **Appendix 15**. The results indicate that a negative correlation exists between ocular volumes

(OVOLS) and the absolute changes in IOP between L and I (r=0.438, p=0.04), and L and H (r=0.484, p=0.02) accommodation levels. The results show that a negative correlation exists between axial lengths (ALS) and the absolute changes in IOP between L and I (r=0.466, p=0.03) and L and H (r=0.511, p=0.02) accommodation levels. However, no significant associations were found between the percentage changes in IOP between L and I and L and H, and the absolute and percentage changes between I and H accommodation levels and the biometric and oculormotor parameters.

The level of myopia was significantly correlated with the absolute and percentage changes in IOP between L and I (r=0.610, p=0.003; r=0.494, p=0.02) and L and H (r=0.531, p=0.01; r=0.434, p=0.04) levels of accommodation. No significant correlation was present between the absolute (r=0.339, p=0.12) and percentage (r=0.239, p=0.28) changes in IOP between I and H levels of accommodation. Correlation graphs of the level of myopia and the changes in IOP on accommodation are shown in **Figures 9.4** and **9.5.**

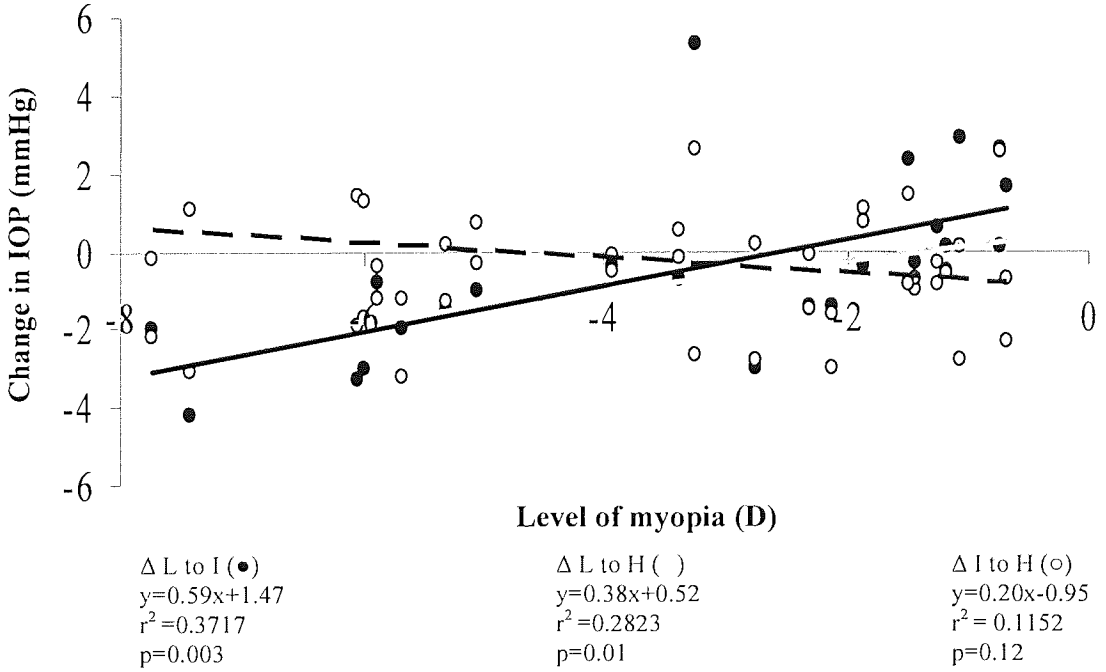
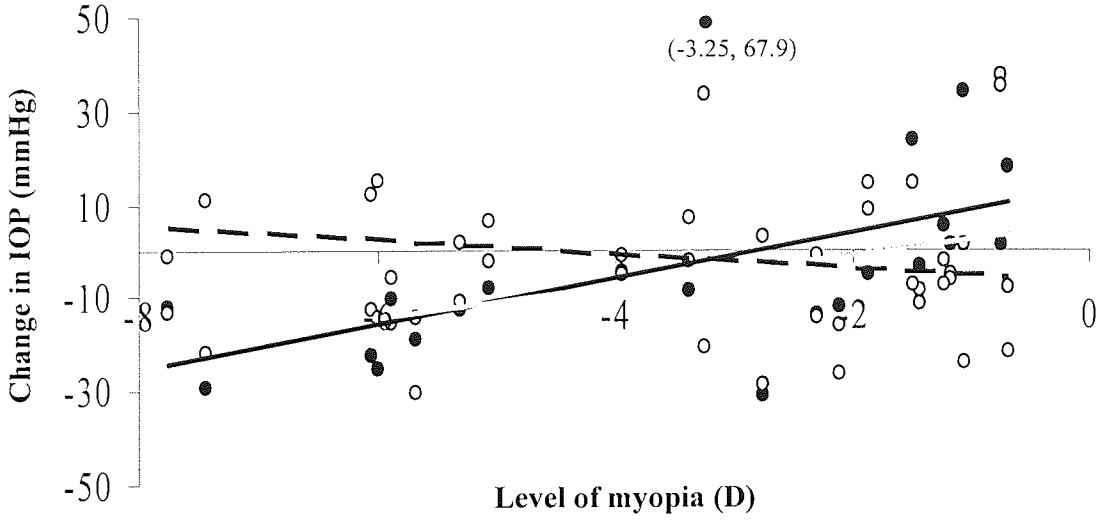


Figure 9.4 Correlation graph of the level of myopia and the absolute changes in IOP (as measured with the OBFA) between L and I (—), L and H ( ) and I and H (---) accommodation levels (n=22).



Δ L to I (•)
y=4.89x+13.98
r^2 =0.2441
p=0.02

Δ L to H ( )
y=3.29x+5.47
r^2 =0.1884
p=0.04

Δ I to H (○)
y=-1.48x-6.52
r^2 = 0.0572
p=0.28

Figure 9.5 Correlation graph of the level of myopia and the percentage changes in IOP (as measured with the OBFA) between L and I (—), L and H ( ) and I and H (---) accommodation levels (n=22).

Multiple regression analyses

The associations between all the parameters and the absolute and percentage changes in IOP with accommodation are shown in **Tables 9.13 to 9.16.**

Parameter	R	R^2	Adjusted R Square	Std. Error of the Estimate	Change Statistics				
					R^2	F	df1	df2	Sig. F
Myopia	0.610	0.372	0.340	1.78007	0.372	11.833	1	20	0.003

Parameter	Unstandardised Coefficients		Standardised Coefficients	t	Sig.	95% Confidence Interval for B	
	B	Std. Error	Beta			Lower Bound	Upper Bound
Constant	1.471	0.704		2.090	0.050	0.003	2.939
Myopia	0.586	0.170	0.610	3.440	0.003	0.230	0.941

Table 9.13 Summary of the results of forward stepwise multiple regression analyses including all the independent variables and the dependent variable of absolute change in IOP (as measured with the OBFA) between L and I levels of accommodation in the myopes only (n=22).

Parameter	R	R^2	Adjusted R Square	Std. Error of the Estimate	Change Statistics				
					R^2	F	df1	df2	Sig. F
Myopia	0.494	0.244	0.206	20.13549	0.244	6.459	1	20	0.019

Parameter	Unstandardised Coefficients		Standardised Coefficients	t	Sig.	95% Confidence Interval for B	
	B	Std. Error	Beta			Lower Bound	Upper Bound
Constant	13.982	7.961		1.756	0.094	-2.625	30.589
Myopia	4.893	1.925	0.494	2.541	0.019	0.877	8.909

Table 9.14 Summary of the results of forward stepwise multiple regression analyses including all the independent variables and the dependent variable of percentage change in IOP (as measured with the OBFA) between L and I levels of accommodation in the myopes only (n=22).

Parameter	R	R^2	Adjusted R Square	Std. Error of the Estimate	Change Statistics				
					R^2	F	df1	df2	Sig. F
Myopia	0.531	0.282	0.286	1.42860	0.282	7.866	1	20	0.011

Parameter	Unstandardised Coefficients		Standardised Coefficients	t	Sig.	95% Confidence Interval for B	
	B	Std. Error	Beta			Lower Bound	Upper Bound
Constant	0.516	0.565		0.913	0.372	-0.662	1.694
Myopia	0.383	0.137	0.531	2.805	0.011	0.098	0.668

Table 9.15 Summary of the results of forward stepwise multiple regression analyses including all the independent variables and the dependent variable of absolute change in IOP (as measured with the OBFA) between L and H levels of accommodation in the myopes only (n=22).

Parameter	R	R^2	Adjusted R Square	Std. Error of the Estimate	Change Statistics				
					R^2	F	df1	df2	Sig. F
Myopia	0.434	0.188	0.148	15.99145	0.188	4.642	1	20	0.044

Parameter	Unstandardised Coefficients		Standardised Coefficients	t	Sig.	95% Confidence Interval for B	
	B	Std. Error	Beta			Lower Bound	Upper Bound
Constant	5.474	6.323		0.866	0.397	-7.714	18.663
Myopia	3.294	1.529	0.434	2.154	0.044	0.105	6.484

Table 9.16 Summary of the results of forward stepwise multiple regression analyses including all the independent variables and the dependent variable of percentage change in IOP (as measured with the OBFA) between L and H levels of accommodation in the myopes only (n=22).

None of the parameters showed a significant association with the absolute or percentage changes in IOP between I and H levels of accommodation.

9.4.3 Associations between biometric and oculomotor parameters and the changes in PA

Median split

Median splits of each parameter were determined and the changes in PA were analysed using between subject ANOVA tests and the results are shown in **Table 15.9A in Appendix 15.** The results suggest that median splits of any of the parameters did not show significant differences in absolute and percentage changes in IOP between L and I, L and H, and I and H accommodation levels.

Correlation analyses

The inter-subject variations in the absolute and percentage changes in PA with accommodation were correlated with the biometric and oculomotor parameters measured (see **Table 15.10A** in **Appendix 15**). None of the parameters investigated showed significant associations with the absolute or percentage changes in PA between L and I, L and H, and I and H accommodation levels.

Multiple regression analyses

The associations between the parameters and the changes in PA with accommodation were investigated using a forward stepwise multiple regression analyses. None of the parameters showed a significant association with the absolute and percentage changes in PA between L and I, L and H, and I and H levels of accommodation.

Refractive error

The inter-subject variations in PA on accommodation were assessed between emmetropes and myopes. A between-subjects ANOVA shows that consistent with the results of **Chapter 8**, no significant differences in absolute and percentage changes in PA were present between L and I ($F=3.826$, $p=0.058$; $F=1.780$, $p=0.190$) and I and H levels of accommodation ($F=1.068$, $p=0.308$; $F=1.037$, $p=0.315$). However, the differences in absolute changes in PA between emmetropes and myopes were statistically significant between L and H levels of accommodation ($F=6.648$, $p=0.014$), such that the PA in the myopes increased (0.34 ± 1.46 mmHg) whilst it decreased in the emmetropes (0.57 ± 0.53 mmHg) on accommodation. The

differences in the percentage changes in PA between L and H levels of accommodation and emmetropes and myopes approached significance (F=3.650, p=0.063).

The differences in the PA responses on H accommodation levels evident between myopes and emmetropes was analysed further with respect to the biometric and oculomotor parameters. Each parameter was treated as a covariate in ANCOVA analyses. The results which are summarised in **Table 9.17** indicate that no single ocular parameter accounts the difference in PA responses to accommodation between emmetropes and myopes.

Covariate	F value	p
Ref Err (D)	7.735	0.008*
Ach Vol (ml^3)	7.690	0.009*
CVol (ml^3)	6.551	0.015*
CC (mm)	6.335	0.016*
CCT (μm)	6.456	0.015*
ACD (mm)	6.791	0.013*
Ach Ang (°)	6.413	0.016*
AL (mm)	7.838	0.008*
OVol (mm^3)	8.025	0.007*
E-values	6.026	0.019*
OPP	6.368	0.016*
AC (Δ)	6.320	0.016*
ACA	6.467	0.015*

Table 9.17 ANCOVA results for each parameter and the PA differences between myopes and emmetropes. * denotes statistically significant result (n=42).

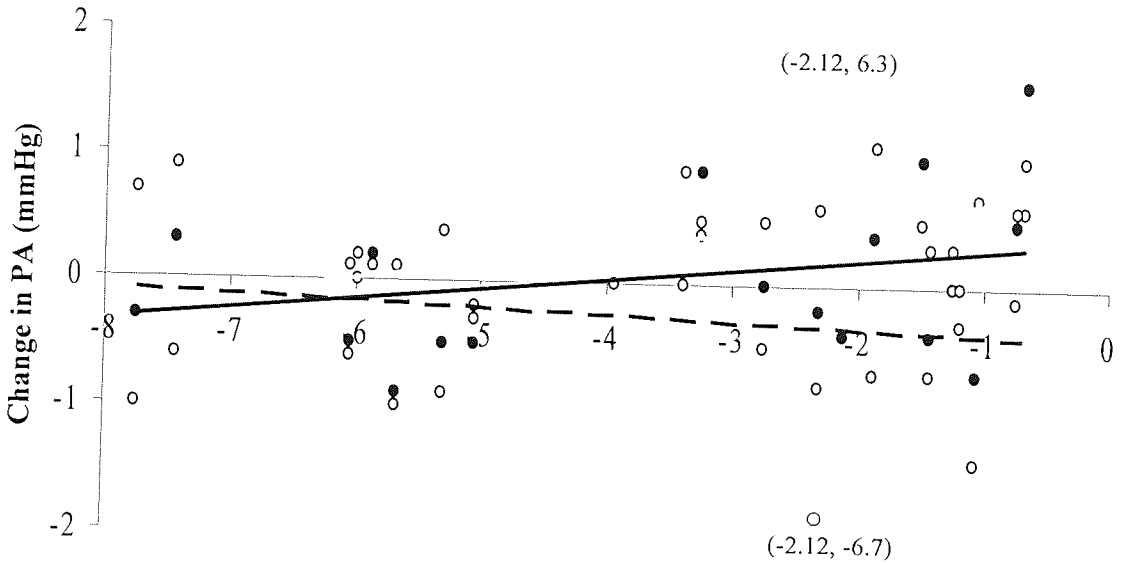
Myopia

Median split

Median split analyses of the parameters were performed on the myopic group and the results are summarised in **Table 15.11A** in **Appendix 15**. The results demonstrate that median splits of each the parameters do not show significant differences in absolute and percentage changes in PA between L and I, L and H, and I and H accommodation levels.

Correlation analyses

The inter-subject variations in the absolute and percentage changes in PA with accommodation in the myopic group were correlated with the biometric and ocular motor parameters and the results are shown in **Table 15.12A** in **Appendix 15**. Correlation analyses of the changes in PA with accommodation and myopia are shown in **Figures 9.6** and **9.7**. No significant correlation was present between the level of myopia and the absolute and percentage changes in PA between L and I (r=0.316, p=0.15; r=0.336, p=0.11), L and H (r=0.204, p=0.36; r=0.206, p=0.36) and I and H (r=0.064, p=0.54; r=0.092, p=0.68) levels of accommodation. Furthermore, none of the parameters investigated showed significant associations with the absolute or percentage changes in PA between L and I, L and H, and I and H accommodation levels.

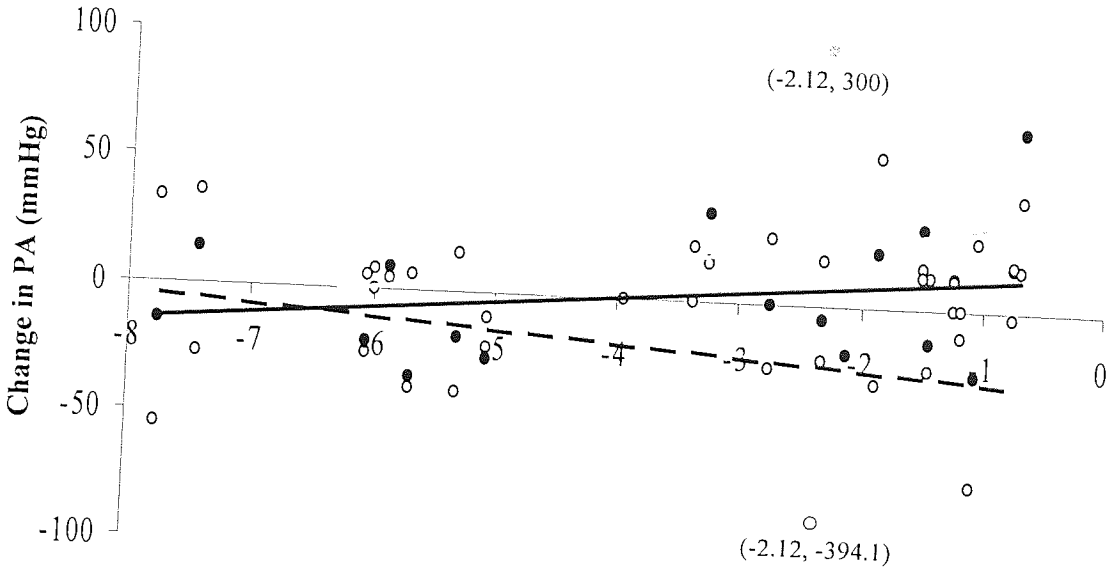


Δ L to I (•)
y=0.09x+0.38
r^2=0.1001
p=0.15

Δ L to H ( )
y=0.13x+0.80
r^2=0.0415
p=0.36

Δ I to H (○)
y=-0.04x-0.42
r^2 = 0.0041
p=0.54

Figure 9.6 Correlation graph of the level of myopia and the absolute changes in PA between L and I (—), L and H ( ) and I and H (---) accommodation levels (n=22).



Δ L to I (●)
y=3.62x+14.66
r^2=0.113
p=0.11

Δ L to H ( )
y=6.14x+37.36
r^2=0.0424
p=0.36

Δ I to H (○)
y=-3.54x-32.18
r^2 = 0.0085
p=0.68

Figure 9.7 Correlation graph of the level of myopia and the percentage changes in PA between L and I (—), L and H ( ) and I and H (---) accommodation levels (n=22).

Multiple regression analysis

The association between all the parameters and the changes in PA with accommodation in the myopic group were investigated using a forward stepwise multiple regression analyses. None of the parameters investigated showed significant associations with the absolute or percentage changes in PA on accommodation.

9.4.4 Associations between biometric and oculomotor parameters and the changes in PR

Median split

Median splits of each parameter were determined and the changes in PR were analysed using between subject ANOVA tests. The results which are summarised in **Table 15.13A** in **Appendix 15** shows that median splits of each of the parameters did not show significant differences in absolute and percentage changes in PR between L and I, L and H, and I and H accommodation levels.

Correlation analyses

The inter-subject variations in the absolute and percentage changes in PR with accommodation were correlated with the biometric and oculomotor parameters measured (see **Table 15.14A** in **Appendix 15**). None of the parameters investigated showed significant associations with the absolute or percentage changes in PR between L and I, L and H, and I and H accommodation levels.

Multiple regression analyses

The associations between the parameters and the changes in PR with accommodation were investigated using a forward stepwise multiple regression analyses. None of the parameters showed a significant association with the absolute and percentage changes in PR between L and I, L and H, and I and H levels of accommodation.

Refractive error

The inter-subject variations in PR with accommodation were assessed between emmetropes and myopes. A between-subjects ANOVA showed that no significant differences in absolute and percentage changes in PR were present between L and I ($F=1.971$, $p=0.168$; $F=2.065$, $p=0.158$) and I and H levels of accommodation ($F=0.900$, $p=0.349$; $F=1.408$, $p=0.242$). However, consistent with the results of **Chapter 8** the differences in absolute changes in PR between emmetropes and myopes were statistically significant between L and H levels of accommodation ($F=6.341$, $p=0.016$), such that the PR in the myopes decreased (4.91±13.96 beats/min) whilst it increased in the emmetropes (4.10±8.18 beats/min) with accommodation. The differences in the percentage changes in PR between L and H levels of accommodation and emmetropes (increased by 7.12±12.70 beats/min) and myopes (decreased by 4.52±15.46 beats/min) was also statistically significant ($F=7.025$, $p=0.011$).

Myopia

Median split

Median split analyses was performed on the myopic group and the results are shown in **Table 15.15A** in **Appendix 15**. The results demonstrate that that median splits of each the parameters do not show significant differences in absolute and percentage changes in PR between L and I, L and H, and I and H accommodation levels.

Correlation analyses

The inter-subject variations in the absolute and percentage changes in PR with accommodation in the myopic group were correlated with the biometric and oculomotor parameters and the results are shown in **Table 15.16A** in **Appendix 15**. Correlation analyses of the changes in PR with accommodation and myopia are shown in **Figures 9.8** and **9.9**. No significant correlation was present between the level of myopia and the absolute and percentage changes in PR between L and I (r=0.022, p=0.46; r=0.036, p=0.44), L and H (r=-0.269, p=0.11; r=-0.331, p=0.07) and I and H (r=-0.242, p=0.14; r=-0.269, p=0.11) levels of accommodation. Furthermore, none of the parameters investigated showed significant associations with the absolute or percentage changes in PR between L and I, L and H, and I and H accommodation levels.

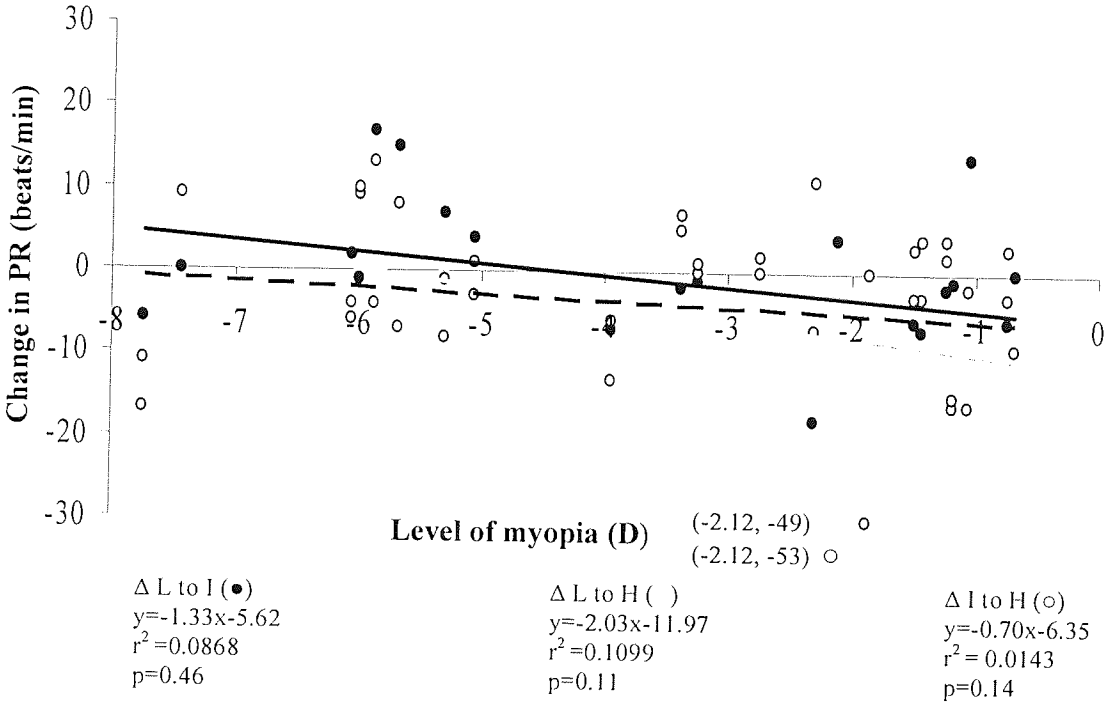


Figure 9.8 Correlation graph of the level of myopia and the absolute changes in PR between L and I (—), L and H ( ) and I and H (---) accommodation levels (n=22).

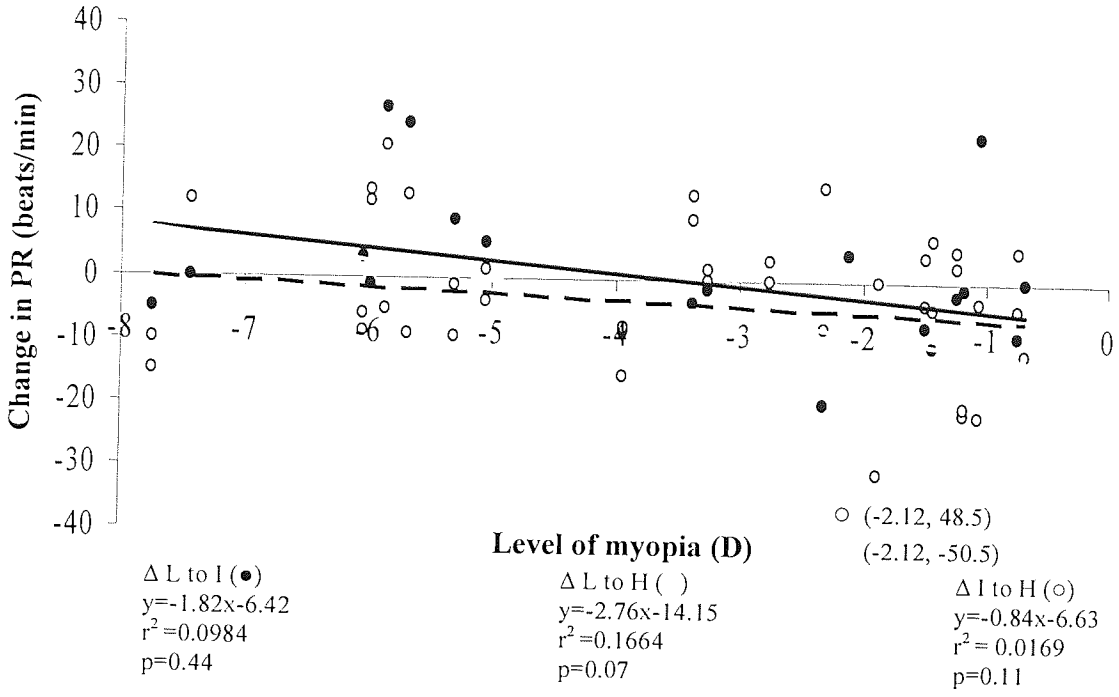


Figure 9.9 Correlation graph of the level of myopia and the percentage changes in PR between L and I (—), L and H ( ) and I and H (---) accommodation levels (n=22).

Multiple regression analyses

The association between all the parameters and the changes in PR with accommodation in the myopic group were investigated using a forward stepwise multiple regression analyses. None of the parameters investigated showed significant associations with the absolute or percentage changes in PR with accommodation.

9.4.5 Associations between biometric and oculomotor parameters and the changes in PV

Biometric and oculomotor parameters

Median split

Median splits of each parameter were determined and the changes in PV were analysed using between-subject ANOVA tests and the results are shown in **Table 15.17A** in **Appendix 15**. The results suggest that median splits of each of the parameters did not show significant differences in absolute and percentage changes in PV between L and I, L and H, and I and H accommodation levels.

Correlation analyses

The inter-subject variations in the absolute and percentage changes in PV with accommodation were correlated with the biometric and oculomotor parameters measured (see **Table 15.18A** in **Appendix 15**). None of the parameters investigated showed significant associations with the absolute or percentage changes in PV between L and I, L and H, and I and H accommodation levels.

Multiple regression analyses

The association between the parameters and the changes in PV with accommodation were investigated using a forward stepwise multiple regression analyses. None of the parameters showed a significant association with the absolute and percentage changes in PV between L and I, L and H, and I and H levels of accommodation.

Refractive error

The inter-subject variations in PV with accommodation were assessed between emmetropes and myopes. A between-subjects ANOVA shows that no significant differences in absolute and percentage changes in PV were present between L and I ($F=0.935$, $p=0.339$; $F=0.215$, $p=0.646$), L and H ($F=0.346$, $p=0.560$; $F=<0.001$, $p=0.995$), and I and H ($F=0.295$, $p=0.590$; $F=1.033$, $p=0.316$) levels of accommodation.

Myopia

Median split

Median split analyses was performed on the myopic group and the results are shown in **Table 15.19A** in **Appendix 15**. The results demonstrate that median splits of each the parameters did not show significant differences in absolute and percentage changes in PV between L and I, L and H, and I and H accommodation levels.

Correlation analyses

The inter-subject variations in the absolute and percentage changes in PV with accommodation in the myopic group were correlated with the biometric and oculomotor parameters (see **Table 15.20A** in **Appendix 15**). Correlation graphs between the level of myopia and the changes in PV with accommodation are shown in **Figures 9.10** and **9.11**. No significant correlation was present between the level of myopia and the absolute and

percentage changes in PV between L and I (r=-0.056, p=0.40; r=-0.094, p=0.34), L and H (r=-0.040, p=0.43; r=-0.009, p=0.49) and I and H (r=0.012, p=0.48; r=0.061, p=0.39) levels of accommodation.

The results show that a negative correlation exists between centre conreal thickness values (CCTS) and the absolute (r=-0.448, p=0.02) and percentage (r=-0.423, p=0.03) changes in PV between L and H accommodation levels. None of the parameters investigated showed significant associations with the absolute or percentage changes in PV between L and I, L and H, and I and H accommodation levels.

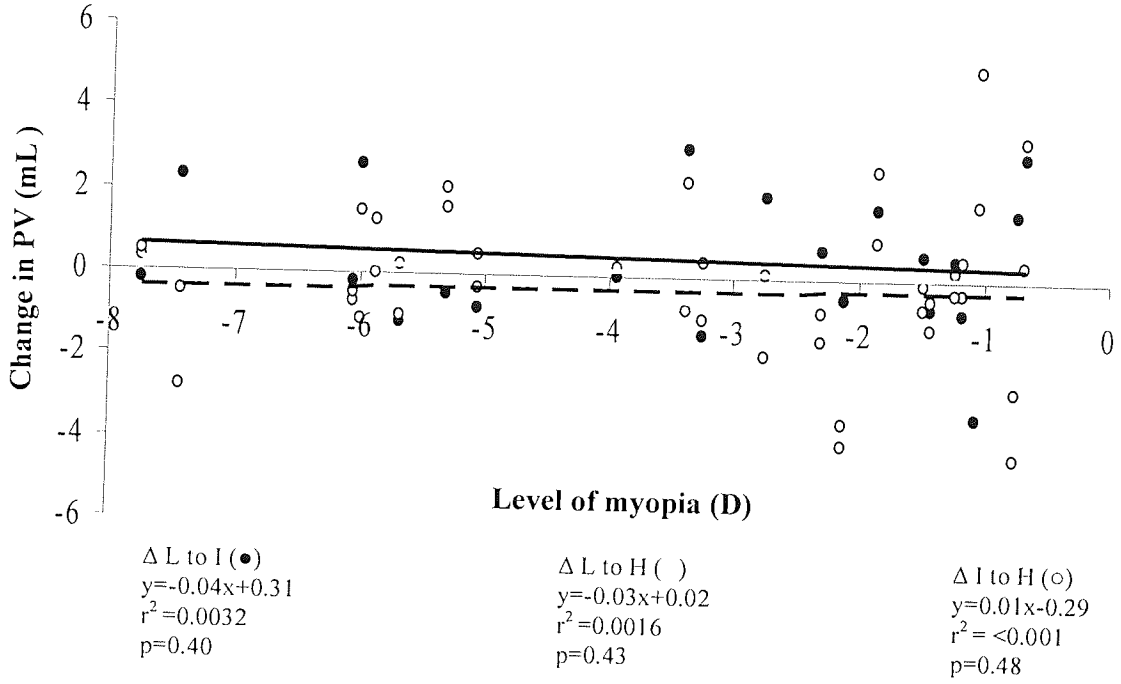


Figure 9.10 Correlation graph of the level of myopia and the absolute changes in PV between L and I (—), L and H ( ) and I and H (---) accommodation levels (n=22).

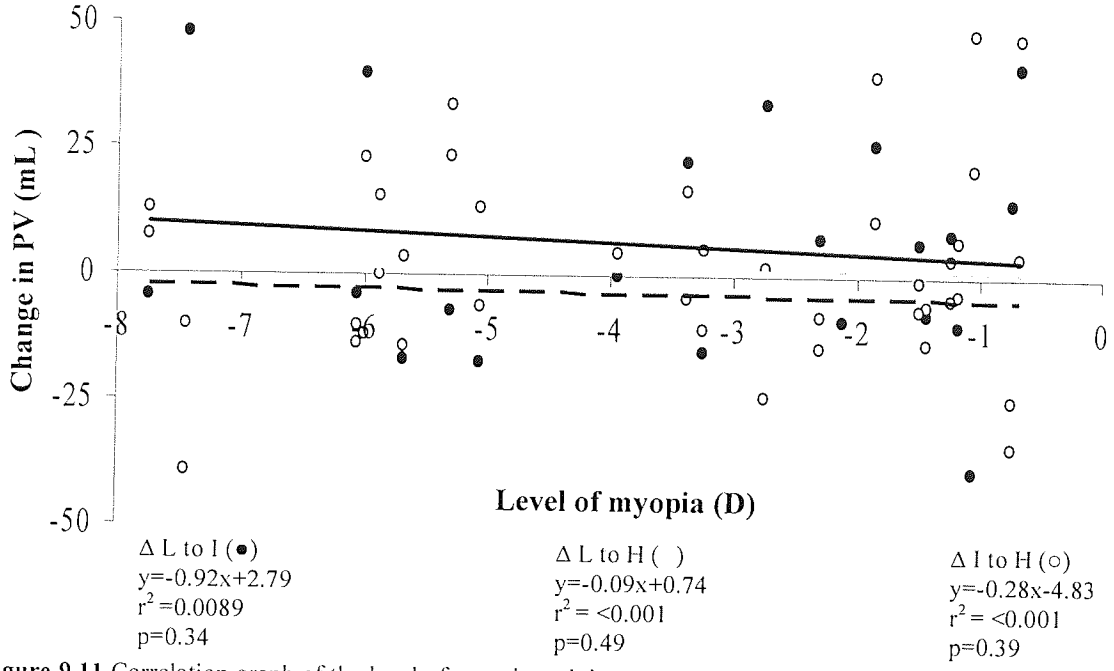


Figure 9.11 Correlation graph of the level of myopia and the percentage changes in PV between L and I (—), L and H ( ) and I and H (---) accommodation levels (n=22).

Multiple regression analyses

The associations between all the parameters and the changes in PR with accommodation in the myopic group were investigated using a forward stepwise multiple regression analyses. Significant associations were evident between CCTS and the absolute **(Table 9.18)** and percentage **(Table 9.19)** changes in PV between L and H accommodation levels. None of the parameters investigated showed significant associations with the absolute or percentage changes in PV between L and I, and I and H accommodation levels.

Parameter	R	R^2	Adjusted R Square	Std. Error of the Estimate	Change Statistics				
					R^2	F	df1	df2	Sig. F
CCTS	0.448	0.200	0.160	1.56801	0.200	5.013	1	20	0.037

Parameter	Unstandardised Coefficients		Standardised Coefficients	t	Sig.	95% Confidence Interval for B	
	B	Std. Error	Beta			Lower Bound	Upper Bound
Constant	10.268	4.543		2.260	0.035	0.790	19.745
CCTS	-0.019	0.008	-0.448	-2.239	0.037	-0.037	-0.001

Table 9.18 Summary of the results of forward stepwise multiple regression analyses including all independent variables and the dependent variable of absolute change in PV between L and H levels of accommodation in myopes only (n=22).

Parameter	R	R^2	Adjusted R Square	Std. Error of the Estimate	Change Statistics				
					R^2	F	df1	df2	Sig. F
CCTS	0.423	0.179	0.138	22.88907	0.179	4.367	1	20	0.050

Parameter	Unstandardised Coefficients		Standardised Coefficients	t	Sig.	95% Confidence Interval for B	
	B	Std. Error	Beta			Lower Bound	Upper Bound
Constant	139.292	66.32		2.100	0.049	0.947	277.638
CCTS	-0.258	0.123	-0.423	-2.090	0.050	-0.516	0.000

Table 9.19 Summary of the results of forward stepwise multiple regression analyses including all independent variables and the dependent variable of percentage changes in PV between L and H levels of accommodation in myopes only (n=22).

9.4.6 Associations between biometric and oculomotor parameters and the changes in POBF

Median split analyses

Median splits of each of the parameters were determined and the changes in POBF with accommodation were analysed using between subject ANOVA tests (see **Table 15.21A** in **Appendix 15**). The results suggest that a median split of the center corneal thickness values (CCTS) at 533.5μm demonstrated a statistically significant difference in the absolute change in POBF between L and I levels of accommodation such that for CCTS values >533.5μm and <533.5μm the absolute change in POBF was respectively a decrease of 46.81±323.94lμl/min and an increase of 149.14±228.47μl/min. A median split of corneal volume values (CVOLS)

showed significant differences in the absolute and percentage changes in POBF between I and H levels of accommodation. For CVOLS values >59.95ml^3 the absolute and percentage changes in POBF values was an increase of respectively 97.43±212.52µl/min and 8.46±18.22%. For CVOLS values <59.95 ml^3 the absolute and percentage changes in POBF values was a reduction of respectively 136.86±298.55µl/min and 9.42±24.74%. None of the other parameters explained the inter-subject variations in the POBF changes between L and H accommodation levels.

Correlation analyses

The inter-subject variations in the absolute and percentage changes in POBF with accommodation were correlated with the biometric and oculomotor parameters measured and the results are summarised in **Table 15.22A** in **Appendix 15**. The results show that a negative correlation exists between the percentage changes in POBF between L and H accommodation levels and axial length (r=0.374, p=0.01) and ocular volumes (r=0.365, p=0.017). No significant associations were found between the changes in POBF between L and I, and I and H accommodation levels and the biometric and oculomotor parameters.

Multiple regression analyses

None of the parameters showed a significant association with the absolute and percentage changes in POBF between L and I levels of accommodation. The parameters (independent variables) were evaluated with respect to the absolute (**Table 9.20**) and percentage (**Table 9.21**) changes in POBF between L and H. **Table 9.22** summarises the results of the analyses between the parameters and the absolute changes in POBF between I and H accommodation levels. None of the parameters evaluated showed a significant association with the percentage changes in POBF between I and H levels of accommodation.

Parameter	R	R^2	Adjusted R Square	Std. Error of the Estimate	Change Statistics				
					R^2	F	df1	df2	Sig. F
ALS	0.307	0.095	0.072	284.44842	0.095	4.177	1	40	0.048
ALS Ref Err	0.508	0.258	0.220	260.78808	0.163	8.587	1	39	0.006

Parameter	Unstandardised Coefficients		Standardised Coefficients	t	Sig.	95% Confidence Interval for B	
	B	Std. Error	Beta			Lower Bound	Upper Bound
Constant	2130.93	1028		2.073	0.045	52.883	4209
ALS	-87.468	42.80	-0.307	-2.044	0.048	-173.96	-0.972
Constant	6691.30	1820		3.678	0.001	3011.10	10371.5
ALS	-285.39	78.11	-1.003	-3.654	0.001	-443.38	-127.39
Ref Err	100.41	34.26	0.805	2.930	0.006	169.71	31.102

Table 9.20 Summary of the results of forward stepwise multiple regression analyses including all independent variables and the dependent variable of absolute changes in POBF between L and H levels of accommodation (n=42).

Parameter	R	R^2	Adjusted R Square	Std. Error of the Estimate	Change Statistics				
					R^2	F	df1	df2	Sig. F
ALS	0.374	0.140	0.118	24.21376	0.140	6.500	1	40	0.015
ALS Ref Err	0.537	0.288	0.252	22.30667	0.148	8.132	1	39	0.007

Parameter	Unstandardised Coefficients		Standardised Coefficients	t	Sig.	95% Confidence Interval for B	
	B	Std. Error	Beta			Lower Bound	Upper Bound
Constant	225.902	87.53		2.581	0.014	49.007	402.796
ALS	-9.288	3.643	-0.374	-2.549	0.015	-16.651	-1.925
Constant	605.490	155.6		3.891	<0.001	290.702	920.279
ALS	-25.762	6.681	-1.037	-3.856	<0.001	-39.276	-12.248
Ref Err	8.357	2.931	0.767	2.852	0.007	14.285	2.429

Table 9.21 Summary of the results of forward stepwise multiple regression analyses including all independent variables and the dependent variable of percentage changes in POBF between L and H levels of accommodation (n=42).

Parameter	R	R^2	Adjusted R Square	Std. Error of the Estimate	Change Statistics				
					R^2	F	df1	df2	Sig. F
CCS	0.329	0.108	0.086	269.69036	0.108	4.854	1	40	0.033

Parameter	Unstandardised Coefficients		Standardised Coefficients	t	Sig.	95% Confidence Interval for B	
	B	Std. Error	Beta			Lower Bound	Upper Bound
Constant	3132.74	1432		2.188	0.035	239.54	6025.95
CCS	-407.33	184.9	-0.329	-2.203	0.033	-781.01	-33.66

Table 9.22 Summary of the results of forward stepwise multiple regression analyses including all independent variables and the dependent variable of absolute changes in POBF between I and H levels of accommodation (n=42).

Refractive error

The inter-subject variations in POBF with accommodation were assessed between emmetropes and myopes. A between-subjects ANOVA showed that no significant differences in absolute and percentage changes in POBF were present between L and I (F=0.104, p=0.749; F=0.047, p=0.830), L and H (F=0.298, p=0.588; F=0.747, p=0.393), and I and H (F=0.052, p=0.821; F=0.876, p=0.355) levels of accommodation.

Myopia

Median split analyses

Median split analyses were performed on the myopic group and the results are summarised in **Table 15.23A** in **Appendix 15**. A median split of the anterior chamber depth values (ACDS) demonstrated a statistically significant difference in the absolute and percentage changes in POBF between L and I levels of accommodation. For ACDS values >3.13 mm the absolute and percentage change in POBF was a reduction of respectively 88.73±159.45 mmHg and 6.31±13.44%, and ACDS values <3.13 mm the absolute and percentage change in POBF was an increase of 150.73±238.48 mmHg and 15.15±23.26%. Median splits of the other parameters in the myopic group do not show significant differences in the absolute and percentage changes in IOP between L and H and I and H levels of accommodation.

Correlation analyses

The inter-subject variations in the absolute and percentage changes in POBF with accommodation in the myopic group were correlated with the biometric and ocular motor parameters (see **Table 15.24A** in **Appendix 15**). Correlation graphs between the level of myopia and the changes in POBF with accommodation are shown in **Figures 9.12** and **9.13**. No significant correlation was present between the level of myopia and the absolute and percentage changes in POBF between L and I (r=0.186, p=0.41; r=0.252, p=0.26), L and H (r=0.088, p=0.70; r=0.011, p=0.96) and I and H (r=0.055, p=0.81; r=0.188, p=0.40) levels of accommodation.

The results show that a positive correlation exists between AC between L and I accommodation levels, and the absolute (r=0.448, p=0.02) and percentage (r=0.423, p=0.03) changes in POBF between L and I accommodation levels. However, when the two data points highlighted in **Figure 9.14** were treated as outliers the associations between absolute (r=-0.132, p=0.290) and percentage (r=-0.153, p=0.260) changes in POBF between L and I accommodation levels and AC was rendered insignificant. Hence none of the parameters investigated showed significant associations with the absolute or percentage changes in POBF between L and I, L and H, and I and H accommodation levels.

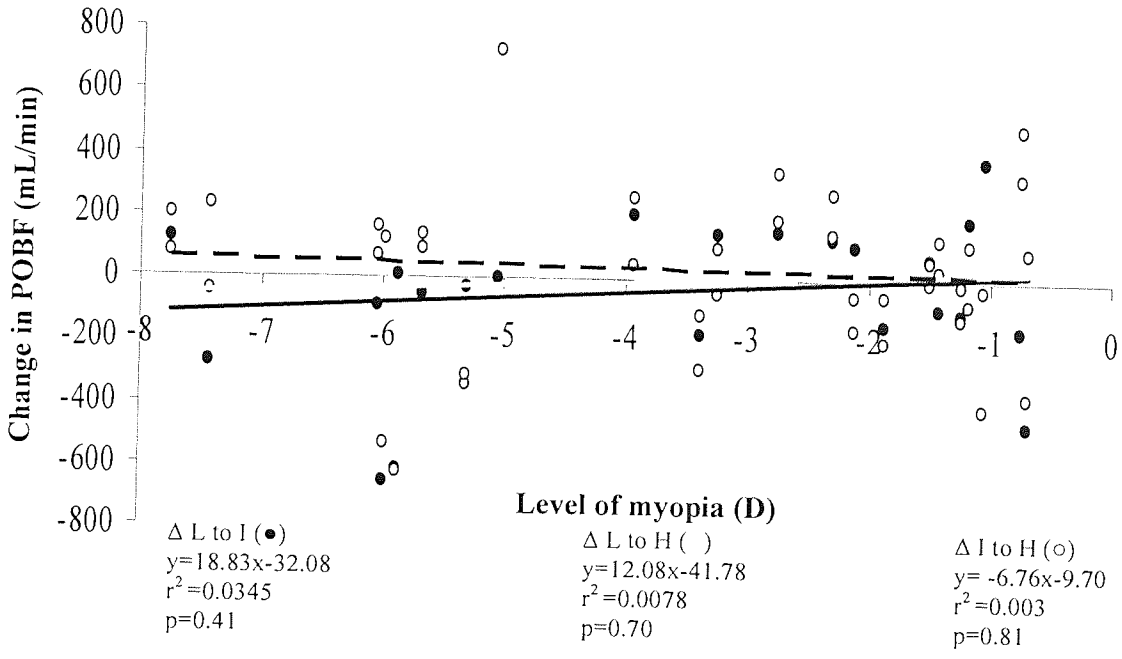


Figure 9.12 Correlation graph of the level of myopia and the absolute changes in POBF between L and I (—), L and H ( ) and I and H (---) accommodation levels (n=22).

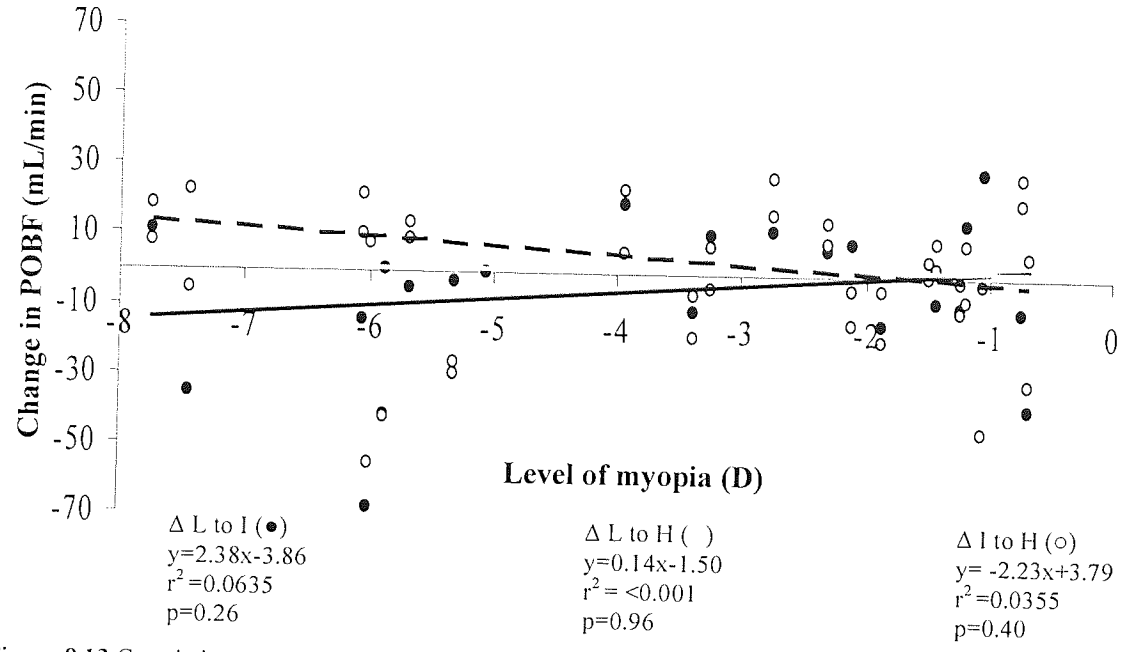
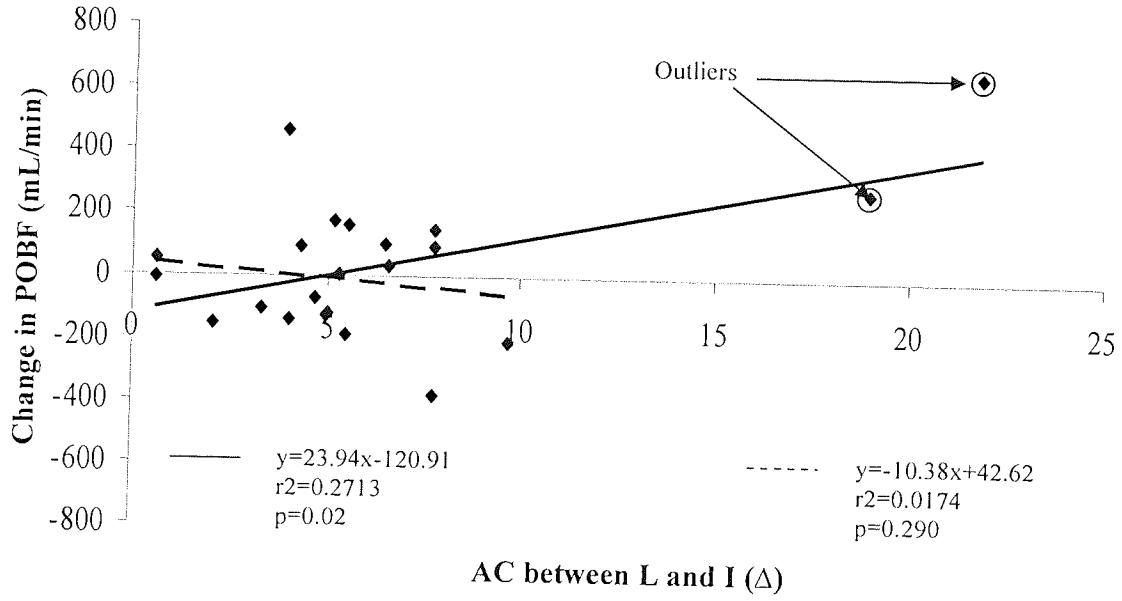


Figure 9.13 Correlation graph of the level of myopia and the percentage changes in POBF between L and I (—), L and H ( ) and I and H (---) accommodation levels (n=22).

(9.14**a**)



(9.14b)

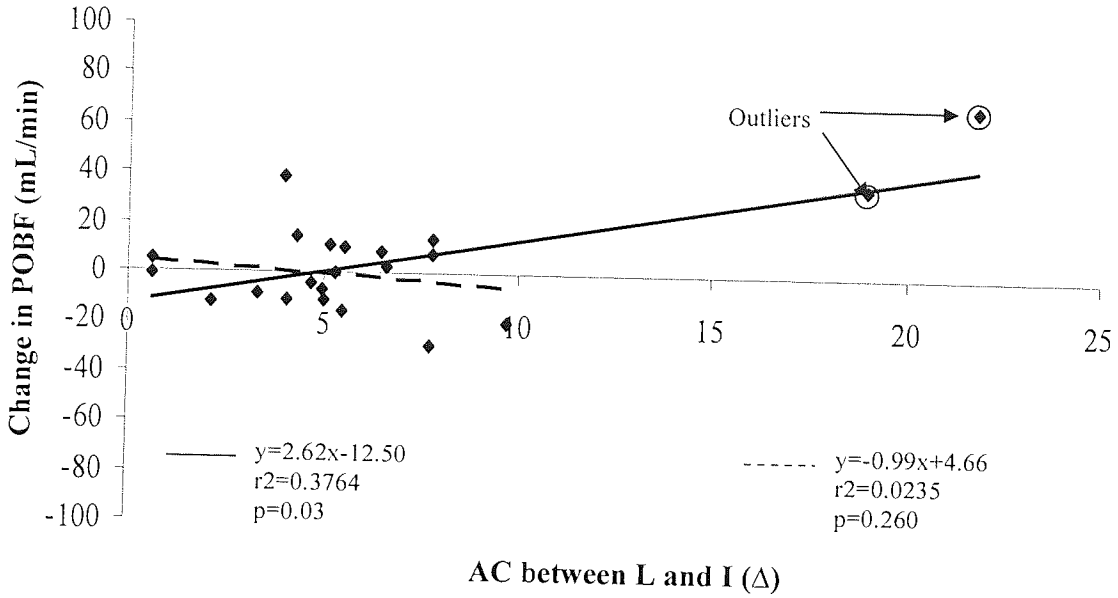


Figure 9.14 Correlation graphs of AC between L and I accommodation levels and the absolute (**a**) and percentage (**b**) changes in POBF between L and I levels of accommodation in the myopes only (n=22).

Multiple regression analyses

The association between all the parameters and the changes in POBF with accommodation were investigated using a forward stepwise multiple regression analyses. The results between the parameters and the absolute and percentage changes in POBF with L and I accommodation levels are shown in **Table 9.23** and **9.24**, respectively. None of the parameters showed significant associations between the POBF changes between L and H, and I and H levels of accommodation.

Parameter	R	R^2	Adjusted R Square	Std. Error of the Estimate	Change Statistics				
					R^2	F	df1	df2	Sig. F
AC	0.521	0.271	0.235	202.47767	0.271	7.448	1	20	0.013
CVOLS	0.688	0.473	0.417	176.69018	0.202	7.264	1	19	0.014

Parameter	Unstandardised Coefficients		Standardised Coefficients	t	Sig.	95% Confidence Interval for B	
	B	Std. Error	Beta			Lower Bound	Upper Bound
Constant	-120.91	71.17		-1.699	0.105	-269.36	27.542
AC	23.937	8.771	0.521	2.729	0.013	5.641	42.233
Constant	2046.7	806.6		2.537	0.020	358.351	3735.0
AC	37.486	9.157	0.816	4.094	0.001	18.319	56.652
CVOLS	-37.740	14.00	-0.537	-2.695	0.014	-67.049	-8.432

Table 9.23 Summary of the results of forward stepwise multiple regression analyses including all the independent variables and the dependent variable of absolute changes in POBF between L and I levels of accommodation in the myopes only (n=22).

Parameter	R	R^2	Adjusted R Square	Std. Error of the Estimate	Change Statistics				
					R^2	F	df1	df2	Sig. F
AC	0.614	0.376	0.345	17.43522	0.376	12.074	1	20	0.002
CVOLS	0.722	0.521	0.471	15.67719	0.145	5.737	1	19	0.027

Parameter	Unstandardised Coefficients		Standardised Coefficients	t	Sig.	95% Confidence Interval for B	
	B	Std. Error	Beta			Lower Bound	Upper Bound
Constant	-12.504	6.128		-2.040	0.055	-25.287	0.279
AC	2.624	0.755	0.614	3.475	0.002	1.049	4.200
Constant	158.416	71.57		2.213	0.039	8.616	308.216
AC	3.693	0.812	0.863	4.545	<0.001	1.992	5.393
CVOLS	-2.976	1.242	-0.455	-2.395	0.027	-5.576	-0.375

Table 9.24 Summary of the results of forward stepwise multiple regression analyses including all the independent variables and the dependent variable of percentage changes in POBF between L and I levels of accommodation in myopes only (n=22).

The two AC data points highlighted in **Figure 9.14** were treated as outliers and the analyses were repeated. The results are shown in **Table 9.25** and **9.26**.

Parameter	R	R^2	Adjusted R Square	Std. Error of the Estimate	Change Statistics				
					R^2	F	df1	df2	Sig. F
CVOLS	0.612	0.375	0.340	148.91278	0.375	10.786	1	18	0.004

Parameter	Unstandardised Coefficients		Standardised Coefficients	T	Sig.	95% Confidence Interval for B	
	B	Std. Error	Beta			Lower Bound	Upper Bound
Constant	2265.59	693.7		3.266	0.004	808.244	3722.94
CVOLS	-38.431	11.70	-0.612	-3.284	0.004	-63.016	-13.846

Table 9.25 Summary of the results of forward stepwise multiple regression analyses including all the independent variables and the dependent variable of absolute changes in POBF between L and I levels of accommodation excluding the outliers in AC values in the myopes only (n=20).

Parameter	R	R^2	Adjusted R Square	Std. Error of the Estimate	Change Statistics				
					R^2	F	df1	df2	Sig. F
CVOLS	0.608	0.370	0.335	12.21166	0.370	10.575	1	18	0.004
CVOLS ACVOLS	0.709	0.502	0.444	11.17079	0.132	4.511	1	17	0.049

Parameter	Unstandardised Coefficients		Standardised Coefficients	t	Sig.	95% Confidence Interval for B	
	B	Std. Error	Beta			Lower Bound	Upper Bound
Constant	184.445	56.89		3.242	0.005	64.935	303.956
CVOLS	-3.121	0.960	-0.608	-3.252	0.004	-5.137	-1.105
Constant	224.408	55.33		4.056	0.001	107.664	341.151
CVOLS	-3.022	0.879	-0.589	-3.437	0.003	-4.876	-1.167
ACVOLS	-0.229	0.108	-0.364	-2.124	0.049	-0.456	-0.002

Table 9.26 Summary of the results of forward stepwise multiple regression analyses including all the independent variables and the dependent variable of percentage changes in POBF between L and I levels of accommodation excluding the outliers in AC values in the myopes only (n=20).

9.5 Discussion

With the addition of the 10 subjects to the main cohort the results in **Appendix 14** show that, consistent with the previous chapters in this thesis, the relationship between accommodation and IOP and blood flow parameters is characterised by substantial inter-subject variations. The present study attempts to explain these inter-subject variations with respect to biometric and oculomotor parameters. The data are treated to median split, independent correlation and multiple regression analyses.

The analyses show that for the grouped data, the inter-subject variations in IOP responses (as measured with the NCT) between L and I accommodation levels are explained by corneal volumes (CVLS), corneal curvatures (CCS), anterior chamber depths (ACDS), and axial lengths (ALS). When the parameters were treated as independent variables median split analyses demonstrates that the magnitude of IOP reduction is lower in subjects with high CVLS compared to those with low CVLS. Independent correlation analyses shows significant negative correlations between ALS (accounting for approximately 9 % of the variance) and CCS (accounting for approximately 15 % of the variance) and the changes in IOP between L and I levels of accommodation. However, following multiple regression analyses in which the relative influence of each parameter is considered, only CCS and ACDS were shown to explain 19.8 % of the variance (CCS: 12.1 %; ACDS: 7.7 %).

Following both independent correlation and multiple regression analyses approximately 10% of the variance in IOP changes between L and H accommodation levels was explained by inter-subject variations in anterior chamber angles (ACANS). 15.5 % of the variance in IOP changes between I and H accommodation levels was explained by accommodative convergence (AC) and ACANS. However, observation of **Table 9.6** shows that the percentage of variance explained by AC ($p=0.047$) and ACANS ($p=0.046$) just reaches significance.

No significant difference in IOP responses to accommodation were elicited between the emmetropes and myopes. However, when the biometric and oculomotor parameters were treated as independent variables in the myopic group significant associations were found between the level of myopia, ALS and ocular volumes (OVOLS) and the inter-subject variations in IOP changes between L and I accommodation levels. The data showed that as these parameters increased the magnitude of IOP reduction was progressively greater.

Therefore the data may indicate that the differences in IOP responses to accommodation between myopes and emmetropes may reach statistical significance, if for example, the mean level of myopia was higher in the cohort tested. The level of myopia, ALS and OVOLS accounted for approximately 25, 23 and 15 % of the variance in the IOP data, respectively. In addition the data demonstrated that 33% of the variance were explained by differences in ECCS. With multiple regression analyses however, only eccentricity (ECCS) was included in the model (accounting for 29.2 % of the variance) and the relative amount of variance in IOP data explained by the level of myopia, ALS and OVOLS did not reach significance.

Significant independent correlations were also found between the level of myopia and ACANS and the inter-subject variations in IOP changes between L and H accommodation levels in the myopic group. However, when all the parameters were entered into a multiple regression analyses the results show that CCS and ACANS together explained 42.8 % of the variance in IOP changes between L and H accommodation levels (CCS: 26.0 %; ACANS: 16.7 %). Multiple regression analyses also shows that 38.4 % of the variance in IOP changes between I and H levels of accommodation is explained by ACANS (20.7 %) and CCS (17.7 %). Although this model just reached significance ($p=0.046$).

The data demonstrate that on multiple regression analyses corneal parameters, for example CCS and ECCS were the strongest predictors of the IOP changes with accommodation. Of interest is that, with accommodation the corneal diameter decreases and the corneal curvature increases (i.e., the cornea steepens) (Brown, 1973; Koretz *et al.,* 1987; Yasuda, Yamaguchi and Ohkoshi, 2003; Saitoh *et al.,* 2004; Yasuda and Yamaguchi, 2005). These changes in corneal parameters have been attributed to the centripetal pull of the cilary muscle on the sclera-corneal limbus (Yasuda, Yamaguchi and Ohkoshi, 2003; Yasuda and Yamaguchi, 2005). However, He *at al.* (2003) demonstrated that when convergence was controlled (as during the NCT-IOP accommodation experiments) the peripheral curvatures increased while the central corneal curvatures decreased. Furthermore, Pierscionek, Popiolek-Masajada and Kasprzak, (2001) used a simple keratometer to measure corneal topography during accommodation and concluded that there were significant inter-subject variations in corneal shape changes with accommodation. In addition, pupil miosis which accompanies accommodation has also been shown to steepen the anterior and posterior cornea (Saitoh *et al.,* 2004).

From these previous studies it is clear that the cornea changes shape with accommodation. However it is not known whether these changes are dependent on baseline corneal measurements. It is well established that non-contact tonometry is influenced by variations in corneal curvatures (Matsumoto *et al.*, 2000; Garzozi *et al.*, 2001; Gunvant *et al.*, 2004; Cheng *et al.*, 2006) such that for every 1 mm increase in corneal curvature the IOP increases by 1.14 mmHg (Gunvant *et al.*, 2004). Therefore, if the changes in corneal curvatures that occur with accommodation are dependent on the baseline values then it is postulated that they may influence the tonometry readings and explain why CCS and ECCS have been shown in the present study to partly account for the variance in IOP data with accommodation.

The tonometric measures may also be influenced by excyclotorsion which also occurs with accommodation (Buehren *et al.*, 2003). The changes in the relative orientation of the cornea with accommodation may indicate that the measurement of IOP during accommodation may have been taken from a different part of the cornea, which varied in corneal curvature and thickness, compared to when the eye was non-accommodating.

Although in the experimental design (described in **Chapter 7**) convergence was rendered open-loop, accommodative convergence still occurred. The adduction of the eye during accommodative convergence meant that the IOP readings were taken in the RE on the temporal region of the cornea. Tonometry was not hindered by the adduction of the eye which indicated that the misalignment of the eye was still within the instrument's tolerance (see **Chapter 3**). It was not possible to precisely quantify the exact position of the temporal cornea at which non-contact tonometry was performed but it is speculated that this varied between subjects depending on the magnitude of accommodative convergence exerted. The misalignment of the tonometer during the measurement period may have subsequently influenced the IOP measures since the corneal curvature progressively reduces from the centre to the periphery of the cornea (Read *et al.*, 2006) and the peripheral cornea changes shape with accommodation (He *et al.*, 2003).

Analyses of the grouped data indicates that the inter-subject variations in IOP responses (as measured with the OBFA) between L and I accommodation levels are explained by CCTS. Median split analyses of CCTS demonstrates that the IOP reduced and increased in subjects with low and high CCTS, respectively. However, on independent correlation and multiple regression analyses none of the parameters showed significant associations with the inter-

subject variations in IOP changes between L and I, and L and H accommodation levels. Multiple regression analyses shows that 20.4 % of the variance in IOP changes between I and H levels of accommodation was explained by CCTS (10.6 %) and refractive error (9.8 %).

No significant difference in IOP responses to accommodation were evident between the emmetropes and myopes. However, in the myopic group, independent correlation analyses shows that significant correlations exist between level of myopia (accounting for 37.2 % of the variance), ALS (accounting for 21.7 % of the variance), and OVOLS (accounting for 19.2 % of the variance) and the changes in IOP between L and I accommodation levels. Similarly, the level of myopia, ALS and OVOLS account for 28.2, 26.1 and 23.4 % of the variance in IOP changes between L and H accommodation levels. The data show that as these parameters increased the magnitude of IOP reduction was progressively greater. Therefore as with the IOP-NCT data the IOP-OBFA data may also be indicating that the differences in IOP responses to accommodation between myopes and emmetropes would have probably reached significance if for example the mean level of myopia was higher in the cohort. On multiple regression analyses, the level of myopia was shown to explain 37.2 and 28.2 % of the variance in IOP data between L and I, and L and H accommodation levels, respectively.

The data suggest that there may be evidence that the changes in IOP with accommodation as measured with the NCT and OBFA are influenced by the level of myopia, ALS and OVOLS. As explained above the results of the multiple regression analyses of the IOP-NCT data show that ECCS and CCS account for the inter-subject variations in IOP responses with accommodation and not the level of myopia, ALS or OVOLS. However, it is thought that since corneal curvatures change on accommodation may subsequently influence non-contact tonometric measures, the relationship between axial length and IOP changes with accommodation may not reach significance due the influence of corneal curvature changes. Interestingly, multiple regression analyses of the IOP data taken with the OBFA, which is not affected by variations in corneal curvatures (Gunvant *et al.*, 2004) shows that as the level of myopia increases the IOP progressively reduces with accommodation. Furthermore, during the OBFA measurements it was ensured that the right eye (on which the measures were taken) was looking in the straight ahead position during accommodation and non-accommodation situations. Hence the OBFA data were always taken from the central part of the cornea (see **Chapter 8**). Therefore, it is speculated that changes in corneal orientation and respective peripheral curvatures that occur with accommodation did not affect the OBFA measurements.

For the grouped data and the data in the myopic group, median split, correlation and multiple regression analyses of the inter-subject variations in PA failed to show any significant associations with the biometric or oculomotor parameters. Consistent with the results of **Chapter 8** significant differences in PA responses to accommodation were found between the emmetropes and myopes. However, none of the parameters investigated accounted for the differences in PA responses between refractive groups. An interesting parameter to note is ocular rigidity. A measure of ocular rigidity was not taken in the present study, since as discussed in **Chapter 2,** the measurement of ocular rigidity is a complex task. Since changes occur in the posterior segment i.e. axial length increases with accommodation (Shum *et al.,* 1993; Drexler et al., 1998; Mallen, Kashyap and Hampson, 2006), it is speculated that changes in ocular rigidity may also occur with accommodation. Moreover, the putative changes in ocular rigidity with accommodation may be dependent on the baseline ocular rigidity. An eye with a lower ocular rigidity is more distensible, and therefore according to the pressure-volume relationship would produce a smaller change in pressure for any given change in volume. The data presented here suggest that with accommodation the ocular rigidity of the myopic eye may increase which would lead to a larger change in pressure for a given change in volume (hence increase PA). Conversely, in the emmetropic eye the ocular rigidity may decrease with accommodation which would result in smaller changes in pressure for any given change in volume and therefore result in a decrease in PA with accommodation. However, this hypothesis requires further work to establish firstly whether the ocular rigidity changes with accommodation and second to determine whether corneal or/and ocular rigidity change with accommodation.

Consistent with the results of **Chapter 8** the changes in PR with accommodation were statistically different between the refractive groups, such that the PR decreased in myopes and increased in emmetropes. Analyses show that no other parameter accounted for the inter-subject variations in PR with accommodation.

The inter-subjects variations in PV with accommodation were not explained by the biometric and oculomotor parameters investigated. However, multiple regression analyses of the changes in PV between L and H accommodation levels in the myopic group shows that CCTS accounted for approximately 20 % of the variance (β= -0.448), although this model only reached significance (p=0.04).

Analyses of the variations in POBF changes with accommodation shows that the POBF values decreased in subjects with high CCTS and increased in subjects with low CCTS between L and I accommodation levels. Independent correlation analyses show significant negative correlations between ALS (accounting for 14.0 % of the variance) and OVOL (accounting for 13.2 % of the variance) and the changes in POBF between L and H accommodation levels. On multiple regression analyses in which the relative influence of each parameter is considered, Ref Err and ALS were shown to explain 25.8 % of the variance (Ref Err: 16.3 %; ALS: 9.5 %). In the myopic group however, median split analyses shows that in subjects with high and low ACDS the POBF reduced and increased between L and I accommodation levels, respectively. On multiple regression analyses CVOLS (37.0 %) and ACVOLS (13.2 %) explained 50.2 % of the variance in POBF changes between L and I accommodation levels.

It is unclear why anterior segment parameters for example CCTS, CVOLS and ACVOLS show significant associations with the variations in PV and POBF changes with accommodation. However, similar to the NCT-IOP and OBFA-IOP data, the POBF changes between L and H accommodation levels are significantly correlated with refractive error.

The results of the present study indicate that no single parameter can be identified which explains the inter-subject variations in IOP and blood flow parameters with accommodation. However, it is clear that changes in several ocular parameters occur during accommodation. It is thought that the magnitude of change in some of the parameters may be dependent on the baseline values which might explain the relationship between these parameters and the variations in IOP and blood flow responses to accommodation.

9.6 Conclusions

The present study investigates the influence of the anterior chamber depth (ACDS), anterior chamber angle (ACANS), corneal curvature (CCS), anterior chamber volume (ACVLS), corneal volume (CVLS), eccentricity (ECCS), central corneal thickness (CCTS), axial length (ALS), ocular volume (OVOLS) and refractive error (Ref Err), accommodative convergence (AC) and accommodative convergence to accommodation ratio (AC/A) values on the inter-subject variations in IOP and blood flow parameters with accommodation. The conclusions are as follows:

For the grouped data:

- The inter-subject variations in IOP changes between L and I accommodation levels (as measured with the NCT) are accounted for by variations in ALS, CCS, ACDS. The variations in IOP changes between L and H accommodation levels are accounted for by variations in ACANS, Ref Err, AL, OVOLS.

- The inter-subject variations in IOP changes between L and I accommodation levels (as measured with the OBFA) are accounted for by variations in CCTS. However, none of the parameters explained variations in IOP changes between L and H accommodation levels.

- None of the parameters investigated accounted for the variations in PA changes with accommodation. The differences in PA responses between myopes and emmetropes on H accommodation levels were not attributed to differences in biometric and oculomotor parameters.

- None of the parameters investigated accounted for the variations in PV changes with accommodation.

- The inter-subject variations in POBF changes between L and I accommodation are accounted for by variations in CCTS. The variations in POBF changes between L and H accommodation levels are accounted for by variations in Ref Err, AL, and OVOLS.

For the myopic group:

- The inter-subject variations in IOP changes between L and I accommodation levels (as measured with the NCT) are accounted for by variations in the level of myopia, ALS, OVOLS and ECCS. The inter-subject variations in IOP changes between L and H accommodation levels are accounted for by variations in level of myopia, CCS and ACANS.

- The inter-subject variations in IOP changes between L and I, and L and H accommodation levels (as measured with the OBFA) are accounted for by variations in level of myopia, ALS and OVOLS.

- None of the parameters investigated accounted for the variations in PA changes with accommodation.

- None of the parameters explained the variations in PV changes between L and I accommodation levels. However, the inter-subject variations in PV changes between L and H accommodation levels are accounted for by variations in CCTS.

- The inter-subject variations in POBF changes between L and I accommodation levels are accounted for by variations in ACDS, CVOLS and ACVOLS. However, none of the parameters explained the variations in POBF changes between L and H accommodation levels.

9.7 Supporting publications.

Rai, G. K., Gilmartin, B., Wolffsohn, J. S. and Cervino, A. (2006). The effect of myopia, axial lengths and ocular volumes on IOP response patterns to accommodation. *11th International Myopia Conference, Singapore.*

CHAPTER 10

THE PRESSURE-RESPONSE RELATIONSHIP IN NON-CONTACT TONOMETRY: AN ANALYSIS OF OCULAR BIOMETRIC CORRELATES.

10.1. Introduction

A feature of the instrumentation used throughout this thesis was that the principle components of the IOP measure were accessed via a dedicated Microsoft *Excel* programme (i.e. 'Puff-Retrieve' (see **section 3.4** in **Chapter 3**). Throughout this thesis the relationship between these principle components is referred to as the pressure-response relationship and is represented graphically in **Figure 10.1**. **Chapter 3** details the operational principles of the *Pulsair EasyEye* non-contact tonometer (NCT) (Keeler, UK). Evaluation of some of the characteristics of the pressure-response relationship has assisted in understanding the derivation of an IOP measure taken with the *Pulsair EasyEye* NCT.

The analysis of the video clip shown in **Video 5.2** in **Appendix 5** was hindered due to the limitations of the instrumentation available (e.g. relatively low frame rate and resolution of the camera and further the inability to image, illuminate and fire an air-pulse along the same axis). Hence the changes in corneal configuration during a tonometric measurement could not be determined and correlated with aspects of the pressure-response relationship. Nevertheless, based on theoretical principles it was postulated in **Chapter 3**, that the initial increase in k-factor (i.e. corneal reflectance) in response to the increasing air-pulse denoted a decrease in corneal convexity. The point at which the k-factor was at its maximum represented the point of maximum 'applanation'. As the pressure-output continued to increase past the point of corneal applanation, the cornea was forced into a state of concavity which was signified by the reduction in k-factor. A decline in the force of the pressure-output resulted in a gradual increase in corneal convexity and therefore, before the original corneal shape was regained, the cornea passed a 2nd point of applanation.

Of interest is that although the pressure-response relationship is sampled over a 19 ms period, only the data collected in the initial stages of the relationship are used to compute the IOP

value. It is speculated that the initial and/or later stages of the pressure-response relationship may provide additional information on the properties of the cornea. Therefore, the aim of the present study was firstly to determine the associations between aspects of the pressure-response relationship and biometric parameters. Secondly, the changes in the components of the pressure-response relationship were assessed with respect to repeated applanations, that is repeated measurements.

A consistent feature of previous results reported in this thesis is that the relationship between accommodation and IOP is characterised by substantial inter-subject variations in IOP responses to accommodation. Although the short-term variations in IOP measures were minimised and the 'quasi-continuous' measurement of accommodation responses was incorporated into the study design, significant inter-subject variations in IOP responses to accommodation remained. It was hypothesised that these variations in IOP responses may partly be due to errors in tonometry caused by alignment of the tonometer, or by changes in corneal properties with accommodation or repeated applanations. Hence, the third aim of this study was to consider the inter-subject variations in aspects of the pressure-response relationship with respect to the inter-subject variations in the IOP responses to accommodation.

10.2. Methods

10.2.1. Subject group

The pressure-response relationship was analysed in the 50 subjects recruited in the study described in **Chapter 9**. The age and refractive error distributions of the sample are detailed in **section 9.2.1**.

Data from 20 of these subjects (selected randomly) were used to assess the effects of tonometry and accommodation on aspects of the pressure-response relationship. Data from this cohort was also used to evaluate further the inter-subject variations in IOP responses as measured with the NCT (see **Chapter 7**). The mean ±SD of the subject group was 20.1±2.5 years of age. The cohort was comprised of 10 myopes (MSE of ≤ - 0.50D) and 10 emmetropes (MSE of ± 0.50D). The criterion used to divide the subjects into these refractive groups has been used by many previous studies, such as those conducted by Goh *et al.* (2005), Junghans and Crewther (2005) and Ojaimi *et al.* (2005).

The research followed the tenets of the Declaration of Helsinki and was approved by the Institution's ethics committee (**Appendix 3**). Written consent was obtained from all subjects willing to participate in the study and copies of the information sheets and consent forms given to the subjects can be found in **Appendix 4**. It was ensured that the visual acuity of all the subjects was 0.00 logMAR or better. All subjects were absent of ocular pathology. None of the subjects were taking any topical or systemic medications that may affect the IOP or accommodative function.

10.2.2. Measurement procedures

The methodologies of data collection are described in **Chapter 7** and **9**. Briefly, 5 pulse synchronised IOP measures were taken in the RE while zero (L; low), 1.50 (I; intermediate) and 4 (H; high) D levels of accommodation were stimulated simultaneously in the LE (see **Chapter 7**). The principle components of each IOP measure were accessed using the 'Puff Retrieve programme' written by Keeler (UK) (see **Chapter 3**). An example of the array of data points for each IOP measure is shown in **Table 1.1A** in **Appendix 1**. The values of the x (i.e. time=*Excel row* x 0.038 ms) and y (i.e. k-factor) coordinates of components A to H (shown in **Figure 10.1**) were determined by placing the curser on the individual components of the pressure-response relationship by direct inspection. The time and k-factor values were confirmed by observation of the array of data points in Columns **A** and **D** of **Table 1.1A,** respectively.

The biometric parameters: central corneal thickness (CCTS), corneal curvatures (CCS), corneal volumes (CVOLS), corneal eccentricity (ECCS), anterior chamber depth (ACDS), anterior chamber volume (ACVOLS), anterior chamber angles (ACANS), axial lengths (ALS) and ocular volumes (OVOLS) were measured using the instrumentation described in **Chapter 9**.

Components of the pressure-response relationship

As explained in **Chapter 3** the k-factor was calculated from the differential contrast values which are computed from the light signals received by the photodiodes and therefore the k-factor essentially represents the magnitude of corneal reflectance. The relationship between time, pressure-output and corneal reflectance can be seen graphically in **Figure 10.1**.

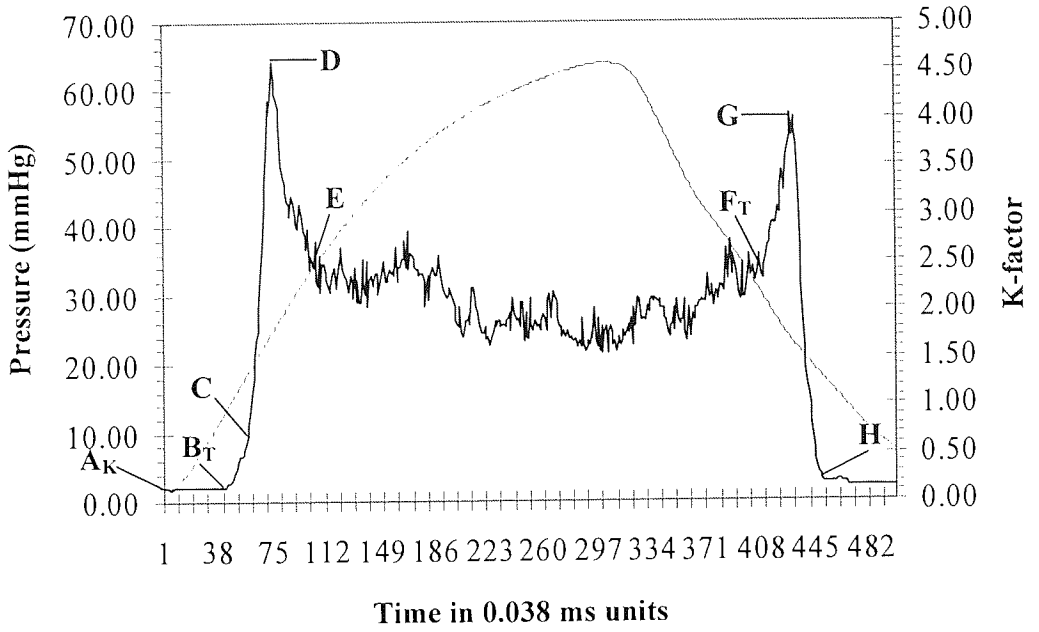


Figure 10.1 Components A to H of the pressure-response relationship where the grey continuous line represents variation in the pressure of the air pulse and the black line denotes the induced perturbations of corneal response (i.e. k-factor). (Pressure-response relationship of an IOP measure taken in the R.E. of a 26 year old, male subject (AC). Rx +0.37 DS).

Components of the relationship labelled in **Figure 10.1** are discussed below:

Component A_K: Represents the corneal reflectance at the start of the measurement i.e. reflectance from an undisturbed in situ convex cornea.

Component B_T: Represents the time at which the cornea starts to change shape in response to the increasing air pulse i.e. the convexity of the cornea begins to reduce.

Component C: Represents the point at which the IOP measure is taken (i.e. when the k-factor has changed by 0.6, see **Chapter 3**). In the following chapter, C_T represents the time of IOP measurement and C_K denotes the corneal reflectance at the point at which the IOP measure is computed.

Component D: Maximum light is reflected from the cornea when it behaves as a flat mirror i.e. when the cornea is applanated (see **Figure 3.5**). It is hence postulated in **Chapter 3** that point D represents maximum corneal applanation. In the following chapter, D_T is the time at maximum applanation and D_K is the corneal reflectance at the point of maximum applanation.

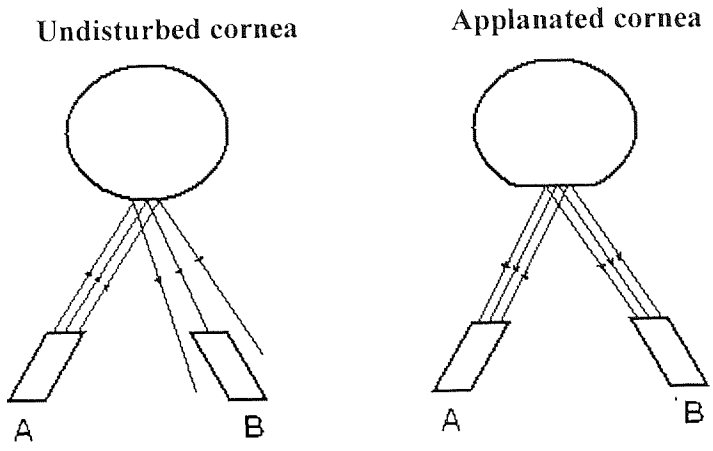


Figure 3.5 Schematic diagram of light transmitted by A and received by B in an undisturbed and applanated cornea (redrawn from Grolman, 1972).

Component E: In **Chapter 3** it is speculated that the reduction in k-factor between D and E represents the cornea 'buckling' into a crater configuration in response to the increasing force of the airpulse. In the following chapter E_T hence represents the time at which the maximum crater is formed and E_K is the corneal reflectance when maximum crater formation is reached.

Component F_T: The force of the air pulse (which is controlled by solenoid) continues to increase until the air pulse is switched off. The pressure-output gradually reduces and the cornea remains in its concave buckled state until F_T. F_T therefore denotes the time at which the crater first begins to recoil i.e. corneal concavity reduces.

Component G: It can be seen that as the pressure-output continues to reduce the corneal concavity also starts to progressively decrease. The cornea hence passes a second point of applanation which is represented by G. In the following chapter, G_T and G_K signify the time and corneal reflectance at the second applanation point, respectively.

Component H: Represents the point at which the corneal convexity is regained. In the following chapter H_T and H_K denote the time and corneal reflectance at which corneal convexity is regained, respectively.

10.3 Statistical analyses

The times and k-factors at each component listed above were inputted in to a Microsoft *Excel* spreadsheet. Software packages *SPSS* 12.1 for Windows was used to perform the statistical tests.

Independent correlation and forward stepwise multiple regression analyses were used to assess the inter-subject variations in the time, k-factor and rate of change in k-factor at each component of the pressure-response relationship and the inter-subject variations in biometric parameters.

The effect of refractive error on components of the pressure-response relationship was assessed with one-way between-subjects analyses of variance (ANOVA) and analyses of covariance (ANCOVA) tests.

The effect of 5 repeated applanations on the times and k-factors at each component of the pressure-response relationship were assessed using repeated measures ANOVA tests followed by Bonferroni corrected t-tests when applicable.

The effect of accommodation on the times and k-factors at each component of the pressure-response relationship were evaluated using one-way ANOVAs in randomised blocks, followed by Scheffé *post-hoc* analyses when necessary.

Furthermore, the inter-subject variations in IOP responses to accommodation demonstrated in **Chapter 7** were investigated with respect to inter-subject variations in times, k-factors and rate of change in k-factor at the components of the pressure-response relationship using independent correlation and forward stepwise multiple regression analyses

10.4. Results

Aspects of the pressure-response relationship

The mean±SD time and k-factor for the components of the pressure-response relationship and the effect of refractive error on these components are summarised in **Table 10.1**.

Table 10.1 demonstrates that component A_K differs between myopes and emmetropes such that the mean±SD for the myopes (0.21±0.03) was larger than that for the emmetropes (0.18±0.03). Furthermore, component C_K was also shown to be influenced by refractive error such that the mean±SD for the myopes and emmetropes was 0.81±0.03 and 0.77±0.05, respectively. However, when component A was treated as a covariate, the differences in component C_K between myopes and emmetropes was rendered insignificant (F=3.085, p=0.086).

Component	Mean±SD for cohort	Mean±SD for Emmetropes	Mean±SD for Myopes	Refractive error ANOVA	
				F-ratio	Sig
A_K	0.20±0.03	0.18±0.03	0.21±0.03	4.704	0.035*
B_T	1.54±0.20	1.57±0.18	1.52±0.21	0.713	0.403
C_T	2.34±0.24	2.29±0.19	2.35±0.25	1.007	0.321
C_K	0.79±0.04	0.77±0.05	0.81±0.03	8.149	0.006*
D_T	3.08±0.36	3.06±0.36	3.10±0.36	0.178	0.675
D_K	4.11±0.53	4.14±0.56	4.09±0.52	0.108	0.744
$F_T - E_T$	10.45±1.73	10.79±0.44	10.19±0.91	1.423	0.239
G_T	15.70±1.10	15.66±0.90	15.74±1.24	0.059	0.809
G_K	3.53±0.32	3.53±0.35	3.53±0.31	0.003	0.957
G_T- D_T	12.62±1.33	12.60±1.16	12.64±1.47	0.008	0.931

Table 10.1 Mean±SD of times (ms) and k-factors at components of the pressure response relationship and the effect of refractive error (N=50). * denotes statistically significant result.

Biometrics and components of the pressure-response relationship

The variance in components A_K, C_K, E_K, and H_K were not explained by inter-subject variations in biometric parameters. However, the inter-subject variations in components D_K and G_K were associated with biometric parameters as shown in **Tables 10.2** and **10.3**, respectively.

(10.2 a)

Parameter	r	r^2	p
CVOL	-0.369	0.136	0.004
CC	-0.353	0.125	0.006
CCT	-0.489	0.239	<0.001

(10.2 b)

Parameter	R	R^2	Adjusted R^2	Std. Error of The Estimate	Change Statistics				
					R^2	F	df1	df2	Sig. F
CCT	0.489	0.240	0.224	0.46237	0.240	15.117	1	48	0.000
CCT CC	0.590	0.348	0.320	0.43271	0.108	7.807	1	47	0.008

	Unstandardised Coefficients		Standardised Coefficients	t	Sig.	95% Confidence Interval for B	
Parameter	B	Std. Error	Beta			Lower Bound	Upper Bound
Constant	7.687	0.920		8.360	<0.000	5.839	9.536
CCT	-0.007	0.002	-0.489	-3.888	<0.000	-0.010	-0.003
Constant	13.406	2.220		6.038	<0.000	8.939	17.872
CCT	-0.006	0.002	-0.473	-4.009	<0.000	-0.010	-0.003
CC	-0.754	0.270	-0.330	-2.794	<0.008	-1.296	-0.211

Table 10.2 Independent correlation (**a**) and multiple regression (**b**) analyses of component D_K and biometric parameters (CCT: centre corneal thickness; CC: corneal curvatures; CVOL: corneal volume).

(10.3a)

Parameter	r	r^2	p
CVOL	-0.242	0.056	0.049
CCT	-0.256	0.066	0.040
ACAN	0.350	0.123	0.007

(10.3b)

Parameter	R	R^2	Adjusted R^2	Std. Error of the Estimate	Change Statistics				
					R^2	F	df1	df2	Sig. F
ACANS	0.350	0.123	0.103	0.29172	0.123	6.4267	1	46	0.015

	Unstandardised Coefficients		Standardised Coefficients	t	Sig.	95% Confidence Interval for B	
Parameter	B	Std. Error	Beta			Lower Bound	Upper Bound
Constant	2.576	0.391		6.596	<0.000	1.790	3.362
ACANS	0.023	0.009	0.350	2.535	0.015	0.005	0.042

Table 10.3 Independent correlation (**a**) and multiple regression (**b**) analyses of component G_K and biometric parameters (CVOL: corneal volume; CCT: centre corneal thickness; ACANS: anterior chamber angles).

Components D_K and G_K were found to be statistically different (F=78.557, p=<0.001). The variations in the differences between these components were associated with biometric parameters as shown in **Table 10.4**.

(10.4a)

Parameter	r	r^2	p
CC	0.410	0.168	0.002
CCT	0.292	0.085	0.022
AL	0.288	0.083	0.024

(10.4b)

Parameter	R	R^2	Adjusted R^2	Std. Error of the Estimate	Change Statistics				
					R^2	F	df1	df2	Sig. F
CC	0.410	0.168	0.150	0.42370	0.168	9.321	1	46	0.004
CC CCT	0.495	0.245	0.211	0.40819	0.077	4.5637	1	45	0.038

	Unstandardised Coefficients		Standardised Coefficients	t	Sig.	95% Confidence Interval for B	
Parameter	B	Std. Error	Beta			Lower Bound	Upper Bound
Constant	-7.031	2.117		-3.322	0.002	-11.292	-2.771
CC	0.835	0.274	0.410	3.053	0.004	0.284	1.386
Constant	-8.707	2.185		-3.985	<0.001	-13.107	-4.306
CC	0.813	0.264	0.400	3.084	0.003	0.282	1.344
CCT	0.003	0.002	0.277	2.136	0.038	0.000	0.007

Table 10.4 Independent correlation (**a**) and multiple regression (**b**) analyses of the differences in components D_K and G_K and biometric parameters (CCT: centre corneal thickness; CC: corneal curvatures; AL: axial length).

The variations in components B_T, C_T, D_T, E_T, F_T, G_T and H_T were not accounted for by variations in biometric parameters. Furthermore, the variations in the differences between components D_T and C_T, E_T and D_T, E_T and B_T, and G_T and F_T were not associated with variations in biometric parameters. However, the variations in the differences between

components C_T and B_T, D_T and B_T, F_T and E_T, and H_T and G_T were accounted for by biometric parameters as shown in **Tables 10.5, 10.6, 10.7** and **10.8**, respectively.

(10.5a)

Parameter	r	r^2	p
CCT	0.308	0.095	0.015
CVOL	0.283	0.080	0.023

(10.5b)

Parameter	R	R^2	Adjusted R^2	Std. Error of the Estimate	Change Statistics				
					R^2	F	df1	df2	Sig. F
CCT	0.308	0.095	0.076	4.62862	0.095	5.048	1	48	0.029

	Unstandardised Coefficients		Standardised Coefficients	t	Sig.	95% Confidence Interval for B	
Parameter	B	Std. Error	Beta			Lower Bound	Upper Bound
Constant	0.051	9.205		0.006	0.996	-18.458	18.559
CCT	0.038	0.017	0.308	2.247	0.029	0.004	0.072

Table 10.5 Independent correlation (**a**) and multiple regression (**b**) analyses of the differences in components C_T and B_T and biometric parameters (CCT: centre corneal thickness; CVOL: corneal volume).

(10.6a)

Parameter	r	r^2	p
CCT	0.311	0.097	0.014

(10.6b)

Parameter	R	R^2	Adjusted R^2	Std. Error of the Estimate	Change Statistics				
					R^2	F	df1	df2	Sig. F
CCT	0.311	0.097	0.078	7.84832	0.097	5.131	1	48	0.028

	Unstandardised Coefficients		Standardised Coefficients	t	Sig.	95% Confidence Interval for B	
Parameter	B	Std. Error	Beta			Lower Bound	Upper Bound
Constant	5.455	15.609		0.349	0.728	-25.929	36.838
CCT	0.065	0.029	0.311	2.265	0.028	0.007	0.123

Table 10.6 Independent correlation (**a**) and multiple regression (**b**) analyses of the differences in components D_T and B_T and biometric parameters (CCT: centre corneal thickness).

(10.7a)

Parameter	r	r^2	P
CC	-0.350	0.123	0.007
AL	0.264	0.070	0.035

(10.7b)

Parameter	R	R^2	Adjusted R^2	Std. Error of the Estimate	Change Statistics				
					R^2	F	df1	df2	Sig. F
CC	0.350	0.122	0.103	43.48556	0.122	6.421	1	46	0.015

	Unstandardised Coefficients		Standardised Coefficients	t	Sig.	95% Confidence Interval for B	
Parameter	B	Std. Error	Beta			Lower Bound	Upper Bound
Constant	824.518	217.232		3.796	<0.001	387.253	1261.783
CC	-71.133	28.071	-0.350	-2.534	0.015	-127.637	-14.629

Table 10.7 Independent correlation (**a**) and multiple regression (**b**) analyses of the differences in components F_T and E_T and biometric parameters (CC: corneal curvatures; AL: axial length).

(10.8a)

Parameter	r	r^2	p
CCT	0.294	0.090	0.021
AL	0.401	0.161	0.002

(10.8b)

Parameter	R	R^2	Adjusted R^2	Std. Error of the Estimate	Change Statistics				
					R^2	F	df1	df2	Sig. F
AL	0.401	0.161	0.143	14.23996	0.161	8.829	1	46	0.005

	Unstandardised Coefficients		Standardised Coefficients	t	Sig.	95% Confidence Interval for B	
Parameter	B	Std. Error	Beta			Lower Bound	Upper Bound
Constant	-104.12	44.657		-2.332	0.024	-194.010	-14.231
AL	5.488	1.847	0.401	2.971	0.005	1.770	9.206

Table 10.8 Independent correlation (**a**) and multiple regression (**b**) analyses of the differences in components H_T and G_T and biometric parameters (CCT: centre corneal thickness; AL: axial length).

The rates of change of k-factor between components of the pressure-response relationship were also considered. Analyses shows that the variations in the rates of change of k-values between the components F and G, and G and H were not related to variations in biometric parameters. However, the variations in rates of change of k-factor between components B and C, C and D, B and D, and D and E were accounted for by variations in biometric parameters as shown in **Tables 10.9**, **10.10**, **10.11** and **10.12**, respectively.

(10.9a)

Parameter	r	r^2	P
CVOL	-0.276	0.076	0.026
CCT	-0.292	0.085	0.020

(10.9b)

Parameter	R	R^2	Adjusted R^2	Std. Error of the Estimate	Change Statistics				
					R^2	F	df1	df2	Sig. F
CCT	0.292	0.086	0.066	0.00636	0.086	4.488	1	48	0.039

	Unstandardised Coefficients		Standardised Coefficients	t	Sig.	95% Confidence Interval for B	
Parameter	B	Std. Error	Beta			Lower Bound	Upper Bound
Constant	0.057	0.013		4.520	<0.001	0.032	0.083
CCT	<0.001	<0.001	-0.292	-2.118	0.039	<0.001	<0.001

Table 10.9 Independent correlation (**a**) and multiple regression (**b**) analyses of the rates of change of k-factor between components B and C and biometric parameters (CVOL: corneal volume; CCT: centre corneal thickness).

(10.10a)

Parameter	r	r^2	p
CVOL	-0.238	0.057	0.048
CC	-0.302	0.091	0.017
CCT	-0.418	0.175	0.001
AL	-0.286	0.082	0.022

(10.10b)

Parameter	R	R^2	Adjusted R^2	Std. Error of the Estimate	Change Statistics				
					R^2	F	df1	df2	Sig. F
CCT	0.418	0.175	0.158	0.05286	0.175	10.191	1	48	0.002
CCT CC	0.504	0.254	0.223	0.05079	0.079	4.989	1	47	0.030

	Unstandardised Coefficients		Standardised Coefficients	t	Sig.	95% Confidence Interval for B	
Parameter	B	Std. Error	Beta			Lower Bound	Upper Bound
Constant	0.513	0.105		4.884	<0.001	0.302	0.725
CCT	-0.001	0.000	-0.418	-3.192	0.002	-0.001	0.000
Constant	1.050	0.261		4.029	0.000	0.526	1.574
CCT	-0.001	0.000	-0.404	-3.206	0.002	-0.001	0.000
CC	-0.071	0.032	-0.282	-2.234	0.030	-0.134	-0.007

Table 10.10 Independent correlation (**a**) and multiple regression (**b**) analyses of the rates of change of k-factor between components C and D and biometric parameters (CVOL: corneal volume; CCT: centre corneal thickness; CC: corneal curvature; AL: axial length).

(10.11a)

Parameter	r	r^2	p
CVOL	-0.276	0.076	0.026
CC	-0.268	0.072	0.030
CCT	-0.424	0.180	0.001
AL	-0.300	0.090	0.017

(10.11b)

Parameter	R	R^2	Adjusted R^2	Std. Error of the Estimate	Change Statistics				
					R^2	F	df1	df2	Sig. F
CCT	0.424	0.180	0.163	0.02607	0.180	10.525	1	48	0.002

	Unstandardised Coefficients		Standardised Coefficients	t	Sig.	95% Confidence Interval for B	
Parameter	B	Std. Error	Beta			Lower Bound	Upper Bound
Constant	0.269	0.052		5.190	0.000	0.165	0.373
CCT	0.000	0.000	-0.424	-3.244	0.002	-0.001	0.000

Table 10.11 Independent correlation (**a**) and multiple regression (**b**) analyses of the rates of change of k-factor between components B and D and biometric parameters (CVOL: corneal volume; CCT: centre corneal thickness; CC: corneal curvature; AL: axial length).

(10.12a)

Parameter	r	r^2	p
CVOL	-0.437	0.191	0.001
CCT	-0.457	0.209	<0.001

(10.12b)

Parameter	R	R^2	Adjusted R^2	Std. Error of the Estimate	Change Statistics				
					R^2	F	df1	df2	Sig. F
CCT	0.457	0.208	0.192	0.09637	0.208	12.644	1	48	0.001

	Unstandardised Coefficients		Standardised Coefficients	t	Sig.	95% Confidence Interval for B	
Parameter	B	Std. Error	Beta			Lower Bound	Upper Bound
Constant	.0758	0.192		3.953	0.000	0.372	1.143
CCT	-0.001	0.000	-0.457	-3.556	0.001	-0.002	-0.001

Table 10.12 Independent correlation (**a**) and multiple regression (**b**) analyses of the rates of change of k-factor between components D and E and biometric parameters (CVOL: corneal volume; CCT: centre corneal thickness).

Effects of tonometry

The effect of repeated applanations on the corneal response was assessed using repeated measures ANOVAs. The overall average corneal response (F=0.784, p=0.480) and the individual components were not effected by repeated measures with the NCT (see **Table 10.13**). The effects of repeated applanations on the rates of change between components were also assessed and the results are also shown in **Table 10.13**.

Component	F-ratio	Sig
A_K	0.327	0.755
B_T	1.258	0.309
C_T	1.491	0.310
C_K	0.279	0.795
D_T	1.190	0.331
D_K	0.661	0.558
E_T	1.295	0.299
E_K	0.661	0.606
F_T	6.027	0.246
F_K	1.519	0.231
G_T	1.657	0.277
G_K	1.573	0.282
H_T	1.213	0.281
H_K	0.958	0.435
Rate of change between B and C	2.866	0.078$^+$
Rate of change between C and D	0.279	0.772
Rate of change between B and D	0.920	0.419
Rate of change between D and E	0.957	0.413

Table 10.13 Effects of repeated tonometry on components of the pressure-response relationship. $^+$ denotes result approaching significance.

The results shown in **Table 10.13** indicate that the effect of repeated measures on the rate of change of k-factor between components B and C approaches significance. The average rate of change between B and C during the 1st, 2nd, 3rd, 4th and 5th applanation was 0.70±0.26, 0.84±0.19, 0.85±0.22, 0.89±0.30 and 1.09±0.46 k-factor/ms, respectively.

Accommodation

The effects of L, I and H accommodation levels on components of the pressure-response relationship were assessed and the results are shown in **Table 10.14**. The results which showed a significant effect were assessed further using Scheffé *post-hoc* analyses.

Component	F-ratio	Sig
A_K	31.206	<0.001*
B_T	10.568	<0.001*
C_T	1.704	0.196
C_K	34.550	<0.001*
D_T	1.301	0.284
D_K	7.052	0.003*
E_T	0.830	0.374
E_K	0.814	0.450
F_T	0.188	0.829
F_K	0.188	0.829
G_T	0.287	0.752
G_K	9.007	0.001*
H_T	0.397	0.676
H_K	13.243	<0.001*
Rate of change between B and C	2.862	0.078
Rate of change between C and D	6.775	0.003*
Rate of change between B and D	8.572	0.001*
Rate of change between D and E	3.058	0.075

Table 10.14 Effects of accommodation on components of the pressure-response relationship

The mean ±SD of component A_K with L, I and H accommodation levels was 0.19±0.03, 0.20±0.03 and 0.26±0.05, respectively. Scheffé *post-hoc* analyses shows that only the differences in component A_K between L and H (0.07±0.06, p= <0.001), and I and H (0.06±0.03, p=<0.001) accommodation levels were significant (L and I: 0.01±0.04, p=0.785).

The mean ±SD of component B_T with L, I and H accommodation levels was 1.54±0.22, 1.48±0.21 and 1.33±0.19 ms, respectively. Scheffé *post-hoc* analyses shows that only the differences in component B_T between L and H (-0.21±0.07, p= 0.009) accommodation levels was significant (I and H: 0.15±0.07, p=0.092; L and I: 0.06±0.06, p=0.636).

The mean ±SD of component C_K with L, I and H accommodation levels was 0.79±0.03, 0.79±0.03 and 0.86±0.05, respectively. Scheffé *post-hoc* analyses shows that only the differences in component C_K between L and H (0.07±0.01, p= <0.001), and I and H (0.06±0.01, p=<0.001) accommodation levels was significant (L and I: 0.01±0.01, p=0.862).

The mean ±SD of component D_K with L, I and H accommodation levels was 4.15±0.61, 4.14±0.55 and 3.76±0.31, respectively. Scheffé *post-hoc* analyses shows that only the differences in component D_K between L and H (-0.39±0.16, p=0.038), and I and H (-0.38±0.16, p=0.045) accommodation levels were significant (L and I: -0.01±0.16, p=0.999).

The mean ±SD of component G_K with L, I and H accommodation levels was 3.62±0.30, 3.63±0.44 and 3.25±0.35, respectively. Scheffé *post-hoc* analyses shows that only the differences in component G_K between L and H (-0.37±0.12, p=0.011), and I and H (-0.38±0.12, p=0.008) accommodation levels were significant (L and I: 0.01±0.12, p=0.995).

The mean ±SD of component H_K with L, I and H accommodation levels was 0.43±0.20, 0.44±0.17 and 0.68±0.36, respectively. Scheffé *post-hoc* analyses shows that only the differences in component H_K between L and H (0.25±0.08, p=0.012), and I and H (0.24±0.08, p=0.018) accommodation levels were significant (L and I: 0.01±0.08, p=0.987).

The mean ±SD of the rate of change between components C and D with L, I and H accommodation levels was 4.62±1.54, 4.55±1.53 and 3.61±1.01 k-factor/ms, respectively. Scheffé *post-hoc* analyses shows that only the differences in the rate of change between L and H accommodation levels approached significance (p=0.08) (L and I: p=0.99; I and H: p=0.11).

The mean ±SD of the rate of change between components B and D with L, I and H accommodation levels was 2.57±0.79, 2.59±.0.86 and 2.00±0.62 k-factor/ms, respectively. Scheffé *post-hoc* analyses shows that the differences in the rate of change between L and H (p=0.07), and I and H (p=0.06) accommodation levels approached significance (L and I: p=0.99).

Inter-subject variations in IOP responses to accommodation

The inter-subject variations in IOP responses to accommodation demonstrated in **Chapter 7** were assessed with respect to inter-subject variations in components of the pressure-response relationship with accommodation. Independent correlation and multiple regression analyses shows that inter-subject variations in the differences in components of the pressure-response relationship did not account for the variations in the differences in IOP measures between L and I accommodation levels. **Tables 10.15** and **10.16** show that inter-subject variations in the differences in components of the pressure-response relationship, partly explained the inter-

subject variations in the differences in IOP responses to L and H, and I and H accommodation levels.

(10.15a)

Component	r	r^2	p
Differences in A_K between L and H accommodation levels	-0.531	0.282	0.01
Differences in C_K between L and H accommodation levels	-0.539	0.291	0.009

Component	R	R^2	Adjusted R^2	Std. Error of the Estimate	Change Statistics				
					R^2	F	df1	df2	Sig. F
C_K L and H	0.539	0.291	0.249	1.77464	0.291	6.974	1	18	0.017

	Unstandardised Coefficients		Standardised Coefficients	t	Sig.	95% Confidence Interval for B	
Parameter	B	Std. Error	Beta			Lower Bound	Upper Bound
Constant	1.025	0.715		1.433	0.170	-0.484	2.534
C_K L and H	-21.074	7.980	-0.539	-2.641	0.017	-37.909	-4.238

(10.15b)

Component	r	r^2	p
Differences in A_K between L and H accommodation levels	-0.579	0.335	0.005
Differences in C_K between L and H accommodation levels	-0.586	0.343	0.004

Parameter	R	R^2	Adjusted R^2	Std. Error of the Estimate	Change Statistics				
					R^2	F	df1	df2	Sig. F
C_K L and H	0.586	0.344	0.305	10.98054	0.344	8.901	1	18	.008

	Unstandardised Coefficients		Standardised Coefficients	t	Sig.	95% Confidence Interval for B	
Parameter	B	Std. Error	Beta			Lower Bound	Upper Bound
Constant	7.844	4.427		1.772	0.094	-1.496	17.184
C_K L and H	-147.31	49.374	-0.586	-2.984	0.008	-251.478	-43.138

Table 10.15 Independent correlation and multiple regression analyses of the absolute (**a**) and percentage (**b**) differences in IOP responses and the differences between changes in components of the pressure-response relationship between L and H accommodation levels.

(10.16a)

Component	r	r^2	p
Differences in A_K between I and H accommodation levels	-0.608	0.370	0.003
Differences in C_K between I and H accommodation levels	-0.573	0.328	0.005

Parameter	R	R^2	Adjusted R^2	Std. Error of the Estimate	Change Statistics				
					R^2	F	df1	df2	Sig. F
A_K I and H	0.608	0.370	0.333	1.74525	0.370	9.995	1	18	0.006

	Unstandardised Coefficients		Standardised Coefficients	t	Sig.	95% Confidence Interval for B	
Parameter	B	Std. Error	Beta			Lower Bound	Upper Bound
Constant	3.296	0.952		3.463	0.003	1.288	5.304
A_K I and H	-42.406	13.413	-0.608	-3.162	0.006	-70.704	-14.107

(10.16b)

Component	r	r^2	p
Differences in A_K between I and H accommodation levels	-0.621	0.386	0.002
Differences in C_K between I and H accommodation levels	-0.589	0.347	0.004
Differences in rate of change between B and D, between I and H accommodation levels	-0.347	0.120	0.073

Parameter	R	R^2	Adjusted R^2	Std. Error of the Estimate	Change Statistics				
					R^2	F	df1	df2	Sig. F
A_K I and H	0.621	0.385	0.349	12.71987	0.385	10.655	1	18	0.005

	Unstandardised Coefficients		Standardised Coefficients	t	Sig.	95% Confidence Interval for B	
Parameter	B	Std. Error	Beta			Lower Bound	Upper Bound
Constant	25.669	6.936		3.701	0.002	11.035	40.303
A_K I and H	-319.11	97.757	-0.621	-3.264	0.005	-525.355	-112.856

Table 10.16 Independent and multiple regression analyses of the absolute (**a**) and percentage (**b**) differences in IOP responses and the differences between changes in components of the pressure-response relationship between I and H accommodation levels.

10.5. Discussion

A facet of the instrumentation used in the present thesis is that, in collaboration with the manufacturers the principle components of the pressure-response relationship were able to be fully accessed. Components of the pressure-response relationship were analysed with respect to biometric parameters, effect of tonometry, accommodation and inter-subject variations in IOP responses to accommodation.

Figure 10.1 shows that the air pulse increases with time and that there is a commensurate change in cornea shape. The corneal response and IOP is sampled over a 19ms cycle. Analysis of the pressure-response relationship demonstrates that the cornea starts to change shape after 1.54±0.20 ms from the start of the measurement cycle. The patient's actual IOP reading is computed from the data taken in the first 2.34±0.24 ms. Maximum applanation occurs at 3.06±0.36 ms after the start of the measurement cycle. Forbes *et al.* (1974) stated that NCTs take IOP measurements within 1 to 3 ms. It appears that this time range encompasses both the NCTs which take IOP measures at the point of maximum applanation such as the Ocular Response Analyser (Reichert Ophthalmic Instruments, Buffalo, New York) (Luce, 2005) and those which take IOP measures that are not dependent on the point of maximum applanation such as the *Pulsair EasyEye* NCT. In response to the increasing pressure-output, after the point of maximum applanation is reached it is seen in **Video 5.2** in **Appendix 5** that the cornea buckles into a state of concavity to form a corneal crater. The present study concludes that this state of concavity remains for 10.45±1.73 ms.

The k-factor at the point at which the IOP measurement is computed (i.e. component C) was shown to be significantly larger in myopes compared to the emmetropes. The k-factor represents the corneal reflectance during deformation. It seems that a reciprocal relationship between k-factor and corneal convexity exists such that as the corneal convexity reduces the k-factor increases. The larger k-factor at C may represent a less convex cornea at C in myopes compared to emmetropes. However, the k-factor at the start of the measurement cycle in the undisturbed eye (i.e. at component A) was also shown to be larger in myopes compared to emmetropes. This suggests that the myopic cornea is less convex (i.e. flatter) than the emmetropic cornea. Davies, Wolffsohn and Gilmartin (2005) also concluded that longer eyes tended to have flatter corneas. However, this relationship seems counterintuitive since myopia is commonly associated with steeper corneas (Carney, Mainstone and Henderson, 1997; Goss *et al.*, 1997; Grosvenor and Goss, 1998). This discrepancy between

studies may be explained by differences in the ability of corneal flattening to maintain pace with axial growth. Van Alphen (1961) suggested that during emmetropization, axial elongation was accompanied by corneal flattening. Therefore it is expected that more elongated globes and hence myopic eyes would have flatter corneas as found in the present study. Van Alphen (1961) also proposed that in some eyes the extent of corneal flattening may be reduced due to restrictions in equatorial growth. This would lead to the expectation that in some elongated and hence myopic eyes, the cornea is more convex than others. Indeed, the differences in k-factor of the undisturbed eye between myopes and emmetropes found in the present study may be confounded by differences in alignment of the tonometer and tear film characteristics which influence the initial k-factor (see **section 3.4**). When differences in component A_K were considered, no differences in component C_K between refractive groups were elicited.

The k-factor at component D of the pressure-response relationship (i.e. point of maximum applanation) was found to be related to biometric parameters. Independent correlation and multiple regression analyses demonstrated that approximately 35% of the variance in component D_K was accounted for by differences in CCTS (24%) and CCS (10.8%). The negative β values for the relationship between D_K and CCTS and CCS denote that in eyes with thicker and flatter corneas the corneal reflectance at maximum applanation was smaller than that in thinner and steeper corneas. This finding may be explained by the proposal that since the pressure-output was constant, a thicker cornea would be applanated to a lesser extent than a thinner cornea. In addition, in a steeper cornea the point of contact of the air-pulse is greater than that of a flatter cornea and therefore the extent of applanation would be greater, which explains the larger D_K values in a relatively steeper cornea.

Independent correlation analyses shows that inter-subject variations in component G_K (i.e. corneal reflectance at 2^{nd} point of maximum applanation) are explained by variations in CVOLS, CCTS and ACANS. However, on multiple regression analyses only ACANS was included in the regression model which was shown to account for approximately 12% of the variance in G_K values. At this stage it is not known why larger ACANS would result in larger values of corneal reflectance at the 2^{nd} maximum applanation point.

The k-factor at component G_K was larger than that at D_K. This is thought to be due to the greater pressure at G_K compared to at D_K, which would result in greater applanation and hence

a larger k-factor value. Following independent correlation analyses, significant associations were demonstrated between the differences in corneal reflectance between components G_K and D_K, and CCS, CCTS and ALS. On multiple regression analyses, approximately 25% of the variance in the differences between components G_K and D_K was accounted for by CCS (16.8%) and CCTS (7.7%). This was probably due the fact that 35% of the variance in component D_K was accounted for by CCTS and CCS as explained above.

The inter-subject variations in the differences between components C_T and B_T (i.e. the differences in time between the point at which the cornea starts to change shape and the point at which the IOP measure is taken) are partly explained by variations in CCTS (accounting for 9.5% of the variance). The positive β value denotes that the thicker the cornea the larger the time difference between components C_T and B_T. This is thought to be due a thicker cornea being more rigid and therefore taking longer to deform by a set amount. On independent correlation analyses, significant associations were found between CCS and ALS and the variations in the differences between components F_T and E_T (i.e. the total time the corneal crater configuration was retained). However, multiple regression analyses demonstrated that the variations in the differences between components F_T and E_T were related to CCS (accounting for 12.2 % of the variance). In eyes with flatter corneas the time for which the state of concavity remained was shorter and *vice versa*. It is not clear at this time why a steeper cornea would remain in a state of concavity for a longer period of time compared to a relatively flatter cornea. Furthermore, the time differences between components H_T and G_T (i.e. the time between the point of 2nd maximum applanation and point at which the original corneal shape is regained) were accounted for by variations in ALS (16.1%). The positive β value suggests that the longer the globe the longer the time taken for the eye to regain its corneal shape from the point of 2nd applanation. Mark, Robbins and Mark (2002) suggested that a longer eye was easier to deform and hence offered less resistance to corneal deformation. Therefore, one would expect that the time taken for the cornea to return back to its original shape would be shorter in longer eyes. However, the present study shows the opposite effect: that a longer eye takes longer to return back to its original shape. This finding may suggest a reduction in elastic properties of the cornea of elongated eyes.

Multiple regression analyses demonstrates that the rate of change of the k-factor between components B and C (i.e. the rate of change between the point at which the cornea begins to change shape and the point at which the IOP measurement is taken), C and D (i.e. the rate of

change between the point at which the IOP measurement is taken and the point of maximum applanation), B and D (i.e. the rate of change between the point at which the cornea begins to change shape and the point of maximum applanation) and D and E (i.e. rate of change between the point of maximum applanation and the point at which the crater is first formed) is associated with CCTS values. The negative β values of the aforementioned relationships signify that the rate of change is slower in a thicker cornea compared to a thinner cornea. It is suggested that a relatively thicker cornea represents a relatively rigid cornea. Hence it is expected that the rate of change in corneal deformation would be slower in a relatively rigid cornea.

Several studies have shown that the IOP is over- or under-estimated in relatively thick and thin corneas, respectively (Shah *et al.*, 1999; Matsumoto *et al.*, 2000; Recep *et al.*, 2001; Eysteinsson *et al.*, 2002; Ko, Liu and Hsu, 2005; Tonnu *et al.*, 2005). This clinical finding is explained by the differences in the times taken to deform corneas of varying thicknesses. It is clear that the rate of change of corneal shape is slower in a thicker cornea compared to that in a thinner cornea. Therefore, the time taken to deform a thick cornea by a set amount is longer than that needed to deform a thin cornea. An increase in the time taken to deform the cornea (due to increased thickness) would result in a high IOP measurement since the pressure-output increases linearly with time.

The data show that 8.6% and 18.0% of the variations in the rate of change between components B and C, and B and D were explained by CCTS, respectively. The result signifies that the rate of change between the point at which the cornea begins to change shape and the point at which the IOP measure is taken is less affected by CCT values than the rate of change between the point at which the cornea starts to change shape and the point of maximum applanation. Thus it is thought that the IOP values taken with NCTs which measure the IOP at the point of maximum corneal applanation for example the Ocular Response Analyser, are more affected by CCT values than the IOP measures taken with the Pulsair NCT.

At present the measurements of IOP and CCT are taken by two separate instruments, a tonometer and pachymeter, respectively. Empirical correction factors are subsequently applied to account for the errors in IOP measures caused by variations in CCTS. An application of the relationships between components of the pressure-response relationship and

CCT described in the present study is that the *Pulsair* NCT may be modified to take an IOP measure and simultaneously analyse the characteristics of the pressure-response relationship. The modified *Pulsair* NCT may subsequently use the data from the pressure-response relationship (for example component D_K which is shown to have the strongest association (r^2=0.24) with CCTS) and accordingly correct for the errors in IOP values caused by CCTS. Therefore, a separate measure of CCT may not be necessary as the data required can be extracted from the pressure-response relationship. Furthermore, the effect of corneal curvatures on IOP measurements can also be simultaneously accounted for in a similar manner by analysing components of the pressure-response relationship. This proposal may present as a great advancement in clinical and research scenarios where the accurate measurement of IOP is imperative e.g. in determining the efficacy of glaucoma drugs.

Repeated measures

It is clear that some of the components of the pressure-response relationship are related to biometric parameters of the cornea. Therefore, the effects of 5 repeated applanations on the components of the pressure-response relationship were evaluated. Repeated measures ANOVAs demonstrate that 5 repeated applanations did not influence the average corneal response, the individual components and the rates of change in k-factor between components of the pressure-response relationship. Of note is that the effects of repeated applanations on the rate of change between components B and C approached significance. The result indicates that the rate of change increases between the point at which the cornea begins to change shape and the point at which the IOP measurement is taken. Although this result does not reach statistical significance, it may signify that the corneal integrity may be reduced (i.e. the eye gets softer) with repeated measures.

The effect of the 'soft' puff of air on the cornea can be seen visually in **Video 5.2** in **Appendix 5.** On observation of the video, the cornea is forced past applanation into a state of concavity and it may be expected that repeated deformations of such magnitude may inevitably affect the integrity of the cornea. In the study described in **Chapter 7**, 5 IOP measures were taken in the RE during accommodation to 3 different levels. Therefore, in total 15 repeated applanations were performed. The effect of 15 applanations on corneal integrity are not known; however based on the results of the present study, there is some evidence to suggest that the corneal integrity may reduce with 15 repeated applanations. It may be considered that the putative changes in corneal integrity may account for some of the

inter-subject variations in IOP responses to accommodation demonstrated in **Chapter 7**. However, of note is that a 5 minute break was given to each subject after every 5 measurements. It may be possible that in the 5 minute break period, the cornea was able to reverse any changes in integrity. Furthermore, the results of **Chapter 5** showed that 10 repeated applanations had a negligible effect on the IOP measurement. Hence the results of **Chapter 5** may in fact provide counter evidence for the proposal that the inter-subject variations in IOP responses to accommodation may be due to the effect of repeated applanations on the corneal integrity.

Of interest, is that the response of the cornea (i.e. rate of change between components B and C) to repeated applanations may be used as an indicator of corneal inflexibility or rigidity. It is proposed that in a thicker/more rigid/stiffer cornea, a greater number of repeated applanations are required to record a change in corneal integrity. Conversely in a thinner/floppy/flexible cornea, a change in corneal integrity may be seen after a fewer number of repeated measures. Although this proposal requires further work, such a measure of corneal plasticity may be useful in identifying patients at risk of corneal pathologies for example kerataconous and in determining the suitability and outcome of corneal refractive surgery.

Several studies have shown that the IOP reduces following repeated measures with the GCT (Stocker, 1956; Moses and Liu, 1968; Myers and Scott, 1975). A reduction in IOP has been thought to be due to the ocular massage caused by the tonometer head. The results of the present study may indicate that the reduction in IOP observed with repeated applanations may also be due to a reduction in corneal integrity following repeated applanations. A softer eye offers less resistance and therefore would produce a lower IOP. It can be thought that the force applied to the cornea with the GCT is greater than that applied with a NCT and therefore fewer applanations are required to change the integrity of the cornea with the GCT.

Accommodation

The effects of accommodation on components of the pressure-response relationship were also analysed. The results show that components A_K (k-factor of the undisturbed eye), C_K (k-factor at the point at which the IOP reading is taken) D_K (k-factor at the 1st point of maximum applanation), G_K (k-factor at the 2nd point of maximum applanation) and H_K (k-factor at the end of the measurement period) were significantly affected by accommodation. In the experimental procedures described in **Chapter 7**, although convergence was rendered open-

loop, accommodative-convergence still occurred. This can be seen as the adduction of the RE while accommodation was stimulated in the LE. Therefore, during the IOP measures taken with I and H accommodation levels the tonometer probe was aligned at least partly with the temporal region of the cornea. It was stated in **Chapter 3**, that the *Pulsair* NCT would fire an air-pulse providing that the initial k-factor was within the range of 0.00 and 0.35 (arbitrary value). The k-factor with L, I and H accommodation levels was approximately 0.19, 0.20 and 0.26, respectively. These values are within the aforementioned initial k-factor range and hence have allowed the measurement of IOP with accommodation. It appears that component A_K with H accommodation levels is significantly higher that with L and I accommodation levels. It is expected that the tonometer is aligned with a more temporal part of the cornea with H accommodation levels than with I accommodation levels due to the greater accommodative convergence which accompanies higher accommodation levels. On alignment of the tonometer on the more peripheral cornea with H accommodation levels, the k-factor appears to increase, indicating that the peripheral cornea is flatter than the central cornea. This finding is in accordance with previous studies which conclude that the corneal curvatures progressively reduce from the centre to the periphery of the cornea (Read *et al.*, 2006). It is speculated that the significant effect of accommodation on the other components i.e. C_K, and H_K may be due to the effect of accommodation on the initial k-factor (A_K).

Component G_K (k-factor at the 2nd point of maximum applanation) and D_K (k-factor at the 1st point of maximum applanation) reduced significantly with H accommodation levels. It is thought that the reduction in corneal reflectance at the 2 applanation points may be related to the size of the applanated area rather than the shape. Since with H accommodation levels the area of the cornea from which the IOP value is measured is relatively flat, further applanation of this area may be limited.

Of interest is that accommodation appeared to influence the time at which the cornea starts to change shape (i.e. component B_T) and the rates of change of k-factor between components C and D, and B and D. The results indicate that the time taken for the cornea to start changing shape and the rate of change between the components decreased with accommodation and was statistically different between L and H accommodation levels. It is postulated above that components of the pressure-response relationship may be indicative of corneal rigidity. Therefore, the decrease in the rate of change of k-factor may suggest that the rigidity of the cornea increases with accommodation. This putative increase in corneal strength may be due

to an increase in corneal rigidity or thickness during accommodation. Although this proposal requires further work it may explain the increase in IOP between I and H accommodation levels demonstrated in **Chapter 7**. As a result of an increase in the patency of the trabecular meshwork during accommodation it was expected that the IOP would progressively reduce with increasing levels of accommodation. However, the data demonstrated that although the IOP reduced with I accommodation levels, with H accommodation levels it appeared that the IOP returned to near baseline measures. It was hypothesised that the dose-dependency may be due to regulatory mechanisms. However, analyses of components of the pressure-response relationship indicate that the cornea may exhibit an increase in thickness/rigidity/stiffness with H accommodation levels which would result in higher IOP measures.

Inter-subject variations in IOP responses to accommodation

The inter-subject variations in IOP responses to accommodation demonstrated in **Chapter 7** were assessed with respect to differences in the components of the pressure-response relationship. Differences in the components of the pressure-response relationship did not explain the variance in the IOP responses to L and I accommodation levels. Independent correlation analyses demonstrated that approximately 30% of the variance in IOP responses to L and H accommodation levels was explained by the variations in the differences in component A_K (i.e. differences in the k-factor of the undisturbed eye) between L and H accommodation levels. Approximately 30% of the variance in IOP responses to L and H accommodation levels was also accounted for by the variations in the differences in component C_K (i.e. differences in the k-factor at the point at which the IOP measure is taken) between L and H accommodation levels. On multiple regression analyses only the differences in component C_K were included in the regression model.

Independent correlation analyses demonstrated that approximately 38% of the variance in IOP responses to I and H accommodation levels was explained by the variations in the differences in component A_K (i.e. differences in the k-factor of the undisturbed eye) between I and H accommodation levels. Approximately 35% of the variance in IOP responses to I and H accommodation levels was also accounted for by the variations in the differences in component C_K (i.e. differences in the k-factor at the point at which the IOP measure is taken) between I and H accommodation levels. A near significant association was also demonstrated between the differences in IOP between I and H accommodation levels and the differences in the rates of change between components B and D (i.e. the rate of change between the point at

which the cornea starts to change shape and the point at which maximum applanation is reached) between I and H accommodation levels. On multiple regression analyses only the differences in component A_K were included in the regression model.

The results suggest that some of the variance in IOP responses to L and H accommodation levels is accounted for by variations in components A_K and C_K. This association between the IOP changes and these components of the pressure-response relationship may be explained by the fact that component A_K in essence represents the corneal shape of the undisturbed cornea. Component A_K varied with accommodation levels due to accommodative-convergence. The IOP measures were calculated from component C_K which were dependent on component A_K since, as stated in **Chapter 3**, the difference between these 2 components is set at a constant of 0.6. The negative association between the differences in the rates of change between B and D and the differences in IOP changes with I and H accommodation levels suggest that the greater the differences in corneal strength/rigidity/thickness between I and H accommodation levels the larger the differences in IOP between I and H accommodation levels. Hence, analyses of the inter-subject variations in IOP responses and the inter-subject variations in components of the pressure-response relationship may provide evidence supporting the above finding that corneal strength/rigidity/thickness increases with accommodation and furthermore that these changes in corneal properties may account for some of the variance in IOP data with accommodation.

In summary the results from the present study show that certain components of the pressure-response relationship are associated with parameters of the cornea for example CCTS and CCS. It is speculated that significant variations in the components of the pressure-response relationship with repeated applanations and accommodation may indicate changes in corneal integrity. Furthermore, some of the inter-subject variations in IOP responses to accommodation evident in **Chapter 7** may be accounted for by variations in the k-factor of the undisturbed cornea. It was proposed in **Chapters 7** and **8** that the increase in IOP between I and H accommodation levels may have been due to some regulatory mechanism. Indeed the findings of the present study suggest that the increase in IOP between I and H accommodation levels may be due to errors in tonometry caused by the alignment of the tonometer with the cornea and possible increases in corneal integrity. Of note however, is that only a third of the variance in IOP changes with accommodation is accounted for by changes in component A_K, and the association with the rates of change between components B

and D and the inter-subject variations approached significance. Therefore the majority of the variance is unexplained and hence may be due to the proposed regulatory mechanism discussed in **Chapter 7**.

The *Ocular Response Analyser* (Reichert Optical Instruments, Buffalo, New York) further utilises the pressure-response relationship of each IOP measure to provide two relatively new biomechanical parameters of the cornea: corneal hysteresis and the corneal resistance factor. The manufacturers of the *Ocular Response Analyser* propose that the corneal hysteresis is a measure of the viscoelastic properties of the cornea while the corneal resistance factor is indicative of the elastic resistance of the cornea (Luce, 2005). As described in **section 2.4.4**, corneal hysteresis is calculated from the difference in IOP of the inward and outward applanation pressures. Such a measure is possible due to the bi-directional, symmetrical pressure-output of the *Ocular Response Analyser*. It is evident from **Figure 10.1** that in the *Pulsair* NCT, the rate of increase in the pressure-output is different to the rate of decease of the pressure-output, i.e. the pressure increase and decrease of the air-pulse is not symmetrical. Therefore, the difference in IOP between the two applanation points does not represent corneal hysteresis. However, it may be possible to mathematically transform the asymmetrical pressure-output into a bi-directional, symmetrical output similar to that of the Ocular Response Analyser. If the same mathematical transformation is also applied to the corneal response (i.e. k-factor) then a measure of corneal hysteresis may be possible and this will be the topic of future work. A measure of biomechanical properties of the cornea with the light-weight slim line *Pulsair* NCT will be an advancement which would allow the measurement of changes in viscoelastic properties of the cornea during accommodation and after the instillation of drugs such as topical anaesthetics. Indeed it would be of interest to investigate the effects of repeated applanations and accommodation on viscoelastic properties of the cornea. Furthermore, it is possible that changes in biomechanical properties of the cornea may further account for the inter-subject variations in IOP responses to accommodation.

10.6. Conclusions

The associations between aspects of the pressure-response relationship and biometric parameters, repeated applanations, accommodation and the inter-subject variations in IOP changes with accommodation were evaluated. The current study concludes that:

- The *Pulsair EasyEye* NCT uses the data collected in the first 2.34±0.24 ms to compute an IOP measure.
- Maximum applanation is reached after 3.08±0.36 ms.
- The cornea remains in a state of concavity for 10.45±1.73 ms.
- A_K (i.e. k-factor of the undisturbed cornea) was higher in myopes than in emmetropes.
- Variations in corneal thicknesses and curvatures accounted for 35% of the inter-subject variations in component D_K (i.e. k-factor at the point of maximum applanation).
- 12% of the variance in component G_K (i.e. k-factor at the 2^{nd} point of maximum applanation) was accounted for by variations in anterior chamber angles.
- Variations in the differences between components D_K and G_K were related to variations in corneal curvatures and thicknesses (accounting for 25% of the variance).
- 10% of the variations in the times between components C_T (i.e. time of IOP measurement) and B_T (i.e. time at which the cornea starts to change), and D_T (i.e. time of 1^{st} maximum applanation) and B_T are explained by corneal thicknesses.
- Variations in corneal curvatures accounted for 12% of the inter-subject variations in the time that the cornea remained in a state of concavity.

- 16% of the variance in the time differences between components H_T (i.e. time at which cornea returns back to its convex state) and G_T (i.e. time of 2nd maximum applanation) is explained by variations in axial lengths.

- 9%, 18% and 21% of the variance in the rates of change between components B (i.e. point at which the cornea starts to change) and C (i.e. point of IOP measurement), B and D (i.e. point of 1st maximum applanation), and D and E (i.e. point of maximum corneal crater formation) is explained by corneal thickness, respectively.

- 25% of the variance in the rates of change between components C and D was accounted for by corneal thicknesses and curvatures.

- The effect of 5 repeated applanations on the rate of change between components B and C approached significance.

- An increase in components A_K, C_K (i.e. k-factor at the point of IOP measurement) and H_K (i.e. k-factor at the point at which the cornea returns back to its convex state) was demonstrated with high accommodation levels.

- A decrease in components B_T, D_K and G_K was demonstrated with high accommodation levels.

- The rate of change between components C and D, and B and D reduced with high accommodation levels.

- Aspects of the pressure-response did not account for the inter-subject variations in the IOP changes between L and I accommodation levels.

- 30% by the inter-subject variations in IOP responses between L and H accommodation levels was explained by differences in C_K between L and H accommodation levels.

- 38% by the inter-subject variations in IOP responses between I and H accommodation levels was explained by differences in A_K between I and H accommodation levels.

CHAPTER 11

DISCUSSIONS, CONCLUSIONS AND FUTURE WORK

11.1 Accommodation and IOP

The central aim of this thesis was to investigate the effects of accommodation on IOP. The effect of accommodative effort on steady-state IOP has not figured in the research literature for over 20 years. A study in the 1980's concluded that the IOP, as measured with the Goldmann contact tonometer (GCT), reduced by 1.3 and 2.3 mmHg respectively on fixation of a 1.50 D (n=10) and 4 D (n=10) accommodative stimuli for 3 minutes (Mauger *et al.*, 1984). However, this study was subject to several limitations. These limitations have been addressed in the present thesis and interpretation of the results has extended significantly our understanding of the effects of accommodation on IOP.

The first and most important limitation of Mauger's study was that the GCT was used to measure the IOP. In accordance with previous studies (Stocker, 1956; Armaly and Rubin, 1961; Moses and Liu, 1968), repeated applanations with the GCT have been shown to reduce the IOP by approximately 2 mmHg due principally to the force of the tonometer head increasing aqueous outflow (i.e. ocular massage). Non-contact tonometers (NCTs) do not require corneal contact and thus several studies have shown that IOP does not reduce with successive applanations using a NCT (Grolman, 1972; Forbes *et al.*, 1974; Sorensen, 1975; Myers and Scott, 1975; Chauhan and Henson, 1988; Baudouin and Gastaud, 1994; Lawson-Kopp *et al.*, 2002). In addition, corneal anaesthesia is necessary to perform contact tonometry with the GCT. Baudouin and Gastaud (1994) suggested that topical anaesthetics reduce IOP by having a direct effect on aqueous humour outflow. Other workers have postulated that topical anaesthetics disrupt tear-film quality (Birchall and Kumar, 2001) or increase corneal thicknesses (Herse and Siu, 1992; Asensio *et al.*, 2003; Nam *et al.*, 2006) which subsequently influence the IOP measurement. The effects of topical anaesthetics on IOP measures are eliminated with the use of NCTs since these instruments do not require corneal contact which therefore precludes the need for corneal anaesthesia.

NCTs have been available since the 1970's and use an air pulse to measure the IOP (Grolman, 1972). However, the large bulky designs of the majority of NCTs have restricted the use of

these instruments in exploring the effects of accommodation on IOP. In the present study the *Pulsair EasyEye* NCT (Keeler, UK) was the instrument of choice due principally to its slim-line design which has allowed great flexibility and utility in the experimental setup such that IOP measures could be obtained in one eye while accommodation was stimulated simultaneously in the other eye. Furthermore, the *Pulsair* NCT was modified to transmit the principle components of an IOP measure into a Microsoft *Excel* programme called 'Puff Retrieve' (written by Keeler, UK). This procedure allowed access to high resolution IOP measures (0.01 mmHg) and the pressure-response relationship, analysis of which has permitted greater understanding of the derivation of an IOP measure.

The pressure-response relationship is the association between the pressure-output of the NCT and the concomitant corneal response as measured by the k-factor (computed from the light reflected off the cornea and received by the NCT). Analyses of the pressure-response relationship demonstrate that although the corneal response to the pressure-output is sampled photoelectronically over a 19 ms cycle, the subject's IOP is computed from the data taken in the initial stages of the cycle; i.e. in approximately the first 2.34 ms. This finding is in accordance with the work by Forbes *et al.* (1974) which states that NCTs obtain an IOP measure within 2 to 3 ms. It is thought that the absence of a progressive reduction in IOP following repeated applanations is due to the brief contact time of NCTs. It was hypothesised that the initial/intermediate/terminal stages of the relationship may be related to corneal properties. **Chapter 10** demonstrates that particular aspects of the pressure-response relationship were related to corneal thickness. Of interest is that the rate of change of k-factor between components B and C (i.e. rate of change between the point at which the cornea starts to change shape and the point at which the IOP measure is taken) was shown to progressively increase over 5 repeated applanations. This increase in rate of change may be indicative of a progressive reduction in corneal rigidity with repeated applanations. It is proposed therefore that the reduction in IOP seen with repeated measures with the GCT may partly be due to a possible reduction in corneal rigidity. Indeed the results in **Chapter 5** show that the IOP values taken with 10 repeated non-contact applanations were not significantly affected by these changes in corneal rigidity.

In a preliminary study described in **Chapter 4**, significant inter-subject variations in IOP responses to accommodation were demonstrated. It was speculated that these variations may have been a result of the effects of short-term variations in IOP caused by the cardiac (Perkins,

1981; Nanba *et al.*, 1989; Lam *et al.*, 2004) and respiratory (Leydhecker, 1976; Moses and Arnzen, 1983; Nanba *et al.*, 1989) cycles on non-contact IOP measurements. Therefore, in order to reduce the spread of IOP measures a pulse transducer was coupled with a *LabView* Acquisition program (National Instruments, USA) to permit pulse synchronised IOP measures. Furthermore, a fixed pace respiratory cycle was achieved using a metronome. The study described in **Chapter 5** shows that synchronising the IOP measurements with the peak, middle or trough of the cardiac cycle significantly reduces the variance in IOP measures. Of the three locations investigated, synchronisation of the IOP measures with the middle location resulted in the least spread of IOP data. Therefore, in subsequent studies each IOP measure was synchronised with the middle location of the cardiac cycle. Furthermore, respiration was kept constant at 15 breathes/minute as it is known that the frequency of the respiratory cycle is 1 every 4 seconds (Leydhecker, 1976; Perkins, 1981).

A second substantive drawback of Mauger's study and the preliminary study described in **Chapter 4** was that it was assumed that accommodation stimulus levels matched exactly the accommodation response levels and hence inter-subject variations in accommodative lag previously described (Ciuffreda, 1998) were not considered. In the study described in **Chapter 6,** having minimised the spread of IOP measures, the measurement of accommodation responses were incorporated into the study design. The significant inter-subject variations in IOP responses to accommodation still remained. It was postulated that the ability to maintain a relatively constant accommodation level may have been hindered during the IOP measurement period. Therefore, the infra-red, monocular, open-view, portable *Grand Seiko FR-5000* (Grand Seiko Co., Ltd, USA) autorefractor was used for the simultaneous quasi-continuous measurements of accommodation responses during the IOP measurement period.

It was hypothesised that, in accordance with Helmholtz's theory of human accommodation, the forward and inward movement of the ciliary muscle would lead to an increase in the patency of the trabecular meshwork to aqueous outflow and this would hence lead to a reduction in IOP. The hypothesised reduction in IOP with accommodation is also supported by the results of studies in which pharmacologically evoked accommodation demonstrated an increase in aqueous outflow and hence a decrease in IOP (Kaufman, 1984b; Gabelt and Kaufman, 1992; Pang *et al.*, 1993; Kiland *et al.*, 2000). Therefore it was envisaged that IOP would reduce with accommodation and this reduction in IOP would be greater with higher

accommodation levels compared to that with lower levels of accommodation. The study described in **Chapter 7** concludes that when the effects of repeated applanations, anaesthetics, variance associated with the cardiac and respiratory cycles are minimised and the exact level of accommodation known, the IOP reduced by approximately 0.61 mmHg with 3 minutes of fixation to an intermediate (I) accommodation target (n=40). However, with high (H) accommodation levels no significant change in IOP was observed hence suggesting that the relationship between accommodation and IOP is dose-dependent. This result is supported by the work of Armaly and Rubin (1961) who found that accommodation stimuli levels in excess of 1.50D produced no further reduction in IOP. It is proposed that the basis of this dose effect is physiological i.e. to maintain ocular integrity some regulatory mechanism within the ciliary body either increases the resistance to outflow or increases aqueous formation for higher accommodation levels. Although for the cohort tested the IOP reduces with I levels of accommodation, this finding cannot be generalised since it is clear that in some subjects the IOP increases between low (L) and I levels.

The effects of accommodation on IOP were also evaluated with the *Ocular Blood Flow Analyser* (OBFA) (Paradigm Medical Instruments Inc., UK) and are reported in **Chapter 8**. The data demonstrate that the IOP reduces between both L and I (approximately 0.23 mmHg), and L and H (approximately 0.92 mmHg) accommodation levels. However, only the change in IOP between L and H accommodation levels reached statistical significance. The data also exhibited substantial inter-subject variations in IOP responses to accommodation. An interesting and repeatable finding in both data sets was that the subjects that showed a decrease in IOP between L and I accommodation levels showed concomitantly an increase in IOP between I and H accommodation levels. Conversely, those subjects that showed an increase in IOP between L and I levels, concomitantly showed a decrease in IOP between I and H accommodation levels. These relationships suggest that there may be subsets of subjects who differ in their IOP responses to accommodation and that the IOP responses are regulated via a feedback mechanism in the ciliary muscle. The mediation may involve the autonomic nervous system and its effect on aqueous humour production and/or outflow via differential contractibility of the outflow muscles (i.e. ciliary muscle, trabecular meshwork and scleral spur).

Bergmanson (1982) proposed that the IOP is regulated via aqueous formation and resistance to aqueous humour outflow. Several studies have established that increases and decreases in

aqueous humour production are mediated by the parasympathetic and sympathetic branches of the autonomic nervous system, respectively (Greaves and Perkins, 1952; Marci and Cevario, 1975; Stjernschantz, 1976; Belmonte *et al.*, 1987; Backon *et al.*, 1989; Backon *et al.*, 1990; Brubaker, 1991; Gabelt *et al.*, 1994; Chen *et al.*, 2004). Furthermore, the presence of clusters of parasympathetic and sympathetic fibres has led to the hypothesis that inter-neural communication occurs in the regulation of IOP (ten Tusscher *et al.*, 2004). Hence it is proposed that between I and H accommodation levels, the putative feedback mechanism within the ciliary muscle initiates an increase in aqueous formation (and hence an increase in IOP), which is augmented by the parasympathetic branch of the autonomic system.

In addition, induced ciliary muscle contraction with the parasympathomimetic pilocarpine via M3 muscarinic receptors (Gupta *et al.*, 1994; Zhang *et al.*, 1995; Gil *et al.*, 1997), increases the tension within the trabecular meshwork which leads to an increase in aqueous humour outflow (Gabelt and Kaufman, 1992; Pang *et al.*, 1993; Kiland *et al.*, 2000). Of relevance is that there is ample evidence to support the theory that the trabecular meshwork also possesses muscarinic receptors (Gupta *et al.*, 1994; Schroeder and Erickson, 1995; Zhang *et al.*, 1995; Woldesmussie *et al.*, 1990) and smooth muscle-like properties (Lepple-Wienhues *et al.*, 1991). Contraction of the trabecular meshwork reduces aqueous outflow and it has hence been suggested that the trabecular meshwork acts as a functional antagonist to the ciliary muscle (Wiederholt *et al.*, 1997; Wiederholt, 1998; Wiederholt *et al.*, 2000). Although the force of contractibility is 10 times greater in the ciliary muscle compared to that of the trabecular meshwork (Lepple-Wienhues *et al.*, 1991), it is proposed that the trabecular meshwork may be actively involved in the regulation of aqueous humour outflow and hence IOP (Lepple-Wienhues *et al.*, 1991; Gupta *et al.*, 1994; Schroeder and Erickson, 1995; Zhang *et al.*, 1995; Woldesmussie *et al.*, 1990; Wiederholt *et al.*, 1997; Wiederholt, 1998; Wiederholt *et al.*, 2000). Furthermore, Selbach and co-workers have raised the possibility that the trabecular meshwork may have some ability to self-regulate aqueous humour outflow (Selbach *et al.*, 2000). Therefore it is suggested that the increase in IOP between I and H accommodation levels may be governed by differential contractibility of the outflow tissues which is augmented by the parasympathetic branch of the autonomic system.

The scleral spur is a protrusion of the sclera into the anterior chamber and is attached to the ciliary body posteriorly and trabecular meshwork anteriorly. One function of the scleral spur is thought to enable the centripetal movement of the ciliary muscle during accommodation. It

is also thought that during ciliary muscle relaxation, the scleral spur works to ensure that the trabecular meshwork and Schlemm's canal are open (Hamanaka, 1989). Therefore, it is possible that during accommodation the action of the sclera spur works to close the outflow of the corneo-scleral meshwork hence reducing aqueous outflow and increasing IOP. Moreover, myofibroblast-like contractile cells have also been found in the scleral spur (Tamm *et al.*, 1995). The effect of stimulation of these contractile cells on the outflow tissues is not known. Indeed, the above proposed mechanisms of regulation of IOP with accommodation may not be mutually exclusive and may work collectively to maintain ocular integrity during accommodation.

Myopia, accommodation and IOP

Near work is thought to be a precursor of myopia onset and development although the exact causation has not been established (Rosenfield and Gilmartin, 1998; Gilmartin, 2004). From the present work it is hypothesised myopia may occur in subjects who are unable to regulate the changes in IOP with accommodation. It is possible that a sustained increase in IOP during near work as a result of a deficit in the regulatory mechanisms may induce stress related expansion of the globe as suggested by several workers (Coleman, 1970; Young, 1971; Greene, 1980; Kelly, 1981; Young, 1981; Greene, 1991). Van Alphen (1986) demonstrated that when the posterior sclera was denuded, axial elongation occurred in eyes which were inflated by up to 14 mmHg.

Van Alphen proposed that the choroid is a smooth, elastic muscle (Van Alphen, 1961) that has contractile properties. Since, Van Alphen's proposal, several studies have experimentally established the presence of contractile cells in the choroid (Flugel-Koch, May and Lutjen-Drecoll, 1996; Poukens, Glasgow and Demer, 1998; May, 2005). Although the network of smooth muscle cells does not continue into the ciliary muscle, the smooth muscle cells have intimate contact with the elastic fibre network of the choroid, which in turn is firmly connected to the posterior tendons of the ciliary muscle (Flugel-Koch, May and Lutjen-Drecoll, 1996). Hence in the course of accommodation ciliary muscle contraction may also lead to the contraction of the choroid. Of note is that these contractile cells are absent in the infant eye and hence may not be involved in the ocular enlargement during emmetropization (Poukens, Glasgow and Demer, 1998). However, it has been suggested that the contractile cells of the choroid may respond to IOP changes and play a role in the regulation of the refractive state (Poukens, Glasgow and Demer, 1998).

Electrically evoked accommodation has been shown to increase the pressure in the posterior chamber while it decreases in the anterior chamber (Suzuki, 1973; Coleman, 1970; Coleman and Young, 1972). Whereas the decrease in the anterior chamber can be explained by the centripetal movement of the ciliary muscle during accommodation, the cause of the increase in the posterior chamber is unclear. One explanation may be due to the simultaneous contraction of the choroid with ciliary muscle contraction. Alternatively, Kelly (1981) proposed that the anterior movement of the vitreous during accommodation closed the zonular gaps and hence interrupted the aqueous outflow. Young (1975) demonstrated the presence of a pressure-gradient in the primate eye and concluded that the posterior chamber pressure increased by 1 mmHg per dioptre of accommodation (cited by Young, 1981). Although the data in the present study suggest that the IOP reduces with I accommodation levels which is in accordance with the Helmholtz theory of accommodation, the IOP measurement obtained with the NCT and the OBFA represents the pressure of the eye as a whole and does not differentiate between the anterior and posterior chambers. Therefore, it remains unknown whether a reciprocal pressure gradient exists between the anterior and posterior chambers during accommodation.

Of interest is that analyses of the pressure-response relationships of IOP measures taken with L, I and H accommodation levels, demonstrates that the time taken for the cornea to start changing shape (i.e. component B see Figure 10.1) and the rate of change between components C (the point at which the IOP measure is taken) and D (the point at which maximum applanation is reached) and B and D decreased with increasing accommodation. However only the changes between L and H accommodation levels reached statistical significance. It is postulated that changes in these components may be indicative of increases in corneal rigidity or thickness with accommodation. A more rigid or thicker cornea would result in higher IOPs when measured with NCTs as found by numerous investigations (Shah *et al.*, 1999; Matsumoto *et al.*, 2000; Recep *et al.*, 2001; Eysteinsson *et al.*, 2002; Ko *et al.*, 2005; Tonnu *et al.*, 2005). It is proposed that with I accommodation levels, ocular rigidity increases but the decrease in IOP is much greater and hence a reduction in IOP is recorded. Conversely, with H accommodation levels the changes in corneal rigidity counteract the reduction in IOP and therefore an increase in IOP between I and H accommodation levels is exhibited.

The present thesis also concludes that although there is no single parameter which accounts for the inter-subject variations in IOP responses to accommodation, there is evidence to suggest that as the level of myopia, axial length and ocular volume increases there is tendency for the IOP to reduce with accommodation. It is not clear however whether this is a cause or effect of myopia. Myopic eyes have been shown to have deeper anterior chamber depths (Rabsilber *et al.*, 2003) and thicker ciliary bodies (Oliveira *et al.*, 2005) than the emmetropic eyes. In **section 2.1** it is noted that although the principal aqueous outflow route is via the trabecular meshwork, the uveoscleral route provides egress for approximately 10% of the aqueous humour, which exits via the anterior face of the ciliary body. It is possible that with accommodation the cellar matrix of the ciliary muscle changes and the thicker ciliary body of the myopic eye may offer a greater area, wider exit, and consequently less resistance to aqueous humour drainage. Thus the reduction in IOP with accommodation may be greater in the myopic eye due to the thicker ciliary body. Furthermore, Touzeau *et al.* (2003) have compared the ocular biometry of emmetropic and myopic eyes and concluded that anterior chamber depth and iridocorneal angle was larger in myopic eyes compared to emmetropic eyes. The larger iridocorneal angle may also indicate that the myopic eye offers less resistance to aqueous humour outflow during accommodation than in the emmetropic eye.

In addition some of the inter-subject variations in IOP responses to accommodation evident in **Chapter 7** may be accounted for by variations in the initial k-factor of the undisturbed cornea (i.e. component A_K in **Figure 10.1**). The differences in the initial k-factor represented the alignment of the tonometer with the cornea which differed between accommodation levels due to the magnitude of accommodative-convergence. The relationship between the inter-subject variations in IOP responses to accommodation and the variations in the rates of change between components B and D of the pressure response relationship approached significance. Hence it is concluded that the inter-subject variations in IOP responses to accommodation may be explained by errors in tonometry caused by the alignment of the tonometer with the cornea and possible increases in corneal rigidity. However, these factors only accounted for approximately 30 % of the variance and thus the majority of the variance in IOP responses to accommodation may be due to proposed regulatory mechanisms.

11.2 Accommodation and Ocular Blood Flow

The effect of accommodation on ocular blood flow was also investigated in the present thesis. The vascular bed of special interest is the choroid since it supplies 85% of the total ocular blood flow (Langham, 1987). Approximately 80% of the choroidal blood flow is pulsatile in nature (Langham *et al.*, 1989). Therefore, the OBFA was used to determine the effects of accommodation on choroidal blood flow. However, this measure is not a direct measure of blood flow since the OBFA essentially measures changes in IOP with each bolus of blood which enters the eye. Although myopia has been associated with accommodation (Rosenfield and Gilmartin, 1998) and choroidal blood flow (Fitzgerald *et al.*, 2002) independently, to date no study has evaluated the effects of accommodation on choroidal flow.

Young (1981) speculated that the increased pressure in the vitreous chamber during accommodation would lead to a reduction in blood supply to the choroid. The results from the present study show that this is not the case since, similar to the IOP data, there is evidence to suggest that there may be subsets of subjects: subjects in which the choroidal blood flow responses decreased then increased between L and I, and I and H accommodation levels, respectively and subjects in which choroidal blood flow increased then decreased between L and I, and I and H accommodation levels, respectively. The data in **Chapter 9** indicates that the choroid does not appear to autoregulate and hence the changes in choroidal blood flow with accommodation are a result of changes in ocular perfusion pressure caused by changes in IOP.

An increase or decrease in IOP causes a decrease or increase in ocular perfusion pressure, respectively. Therefore a decrease or increase in choroidal blood flow is expected. It is possible that to obtain these changes in blood flow, changes in choroidal pulsatility (PA) occur first which then subsequently trigger changes in pulse rate (PR), or alternations in PR changes occur first which then trigger changes in PA. Workers have suggested some time ago that the choroid is involved in the accommodative mechanism (Van Alphen, 1961; Van Alphen, 1986; Coleman, 1970). It is therefore possible that the choroidal plexus changes with contraction of the ciliary muscle and choroid during accommodation which results in changes in PA. To regulate blood flow a subsequent increase in PR is necessary. Indeed the increase in heart rate with accommodation due to a reduction of the parasympathetic innervation has been demonstrated by Davies (2004). It is possible that accommodation induced changes in PR occur first and to regulate blood flow subsequent changes in PA are required. Regardless

of the order of the events, it is speculated that alterations in PA and PR occur to regulate the choroidal blood flow to changes in ocular perfusion pressure.

Myopia, accommodation and Ocular Blood Flow

Indeed it is clear that the response of the myopic eye to ocular perfusion changes is somewhat different to that of the emmetropic eye, particularly with H accommodation levels. In the emmetropic eye, it appears that the PA reduced and the PR increased with accommodation. The balance between these two parameters was such that the choroidal blood flow was regulated with regards to changes in ocular perfusion pressure. In the myopic eye however, the PA increased and the PR decreased. The differences in PA responses to accommodation evident between refractive groups may be a result of structural changes to the myopic choroid which has been shown to be thinner than that of the emmetropic choroid in marmosets (Troilo, Nickla and Wildsoet, 2000). Alternatively, in the myope the changes in PR may be influenced by the autonomic balance or profile, suggesting systemic differences between myopes and emmetropes. Of importance is that the data show that the changes in ocular perfusion pressure with accommodation are not regulated in the myopic eye which may be due the imbalance between the changes in PA and PR. As a consequence of this imbalance it appears that as the level of myopia, axial length and ocular volume increase, there is a tendency for reduced choroidal blood flow with accommodation.

It is not known whether the defective vascular regulation of the choroid during near work is a cause or effect of myopia development. It may be that the thinner choroid of the myopic eye responds differently to accommodation and that the changes observed are a consequence of myopia development. As reduced choroidal blood flow has been implicated in myopia onset and progression (Fitzgerald *et al.*, 2002) an alternative hypothesis is that myopia progression may occur in those subjects who are unable to regulate choroidal blood flow and therefore exhibit a sustained reduction in choroidal blood flow during near work. The reduced blood flow may subsequently lead to the thinning of the choroid and hence axial elongation. Furthermore, myopia progression may also occur in subjects who experience a sustained increase in choroidal blood flow during near work, since Wallman and Winawer (2004) have suggested that an increase in choroidal thickness may act as a signal to induce axial elongation.

11.3 Future work

Derivation of a method by which pulse-synchronised non-contact IOP measures are possible is a significant advance and one that minimises the effects of the cardiac cycle on IOP measures taken with *Pulsair* NCT. Keeler (UK) recommends that the average of 4 IOP measures should be taken with the *Pulsair* NCT (this has been validated by McCaghrey and Matthews, 2001) to represent a valid measure of the steady-state IOP. There is potential for the recommended number of IOP measures to be reduced to fewer readings (e.g. 2) when a modified *Pulsair* NCT incorporates the pulse synchronous feature. It may not be feasible to take just one IOP measure since increased apprehension during tonometry has been shown to increase IOP (Moses *et al.,* 1984) which may affect principally the first IOP measure. Moreover, increased apprehension has also been shown to increase the spread of consecutive IOP measures (Forbes *et al.,* 1974). Therefore it may be necessary that the instrument be modified so that it compares the 1st and 2nd IOP readings. If the 2nd IOP measure is within a predetermined range e.g. within ±1 mmHg then the instrument may indicate that the average of the two measures is a good representation of the steady-state IOP. However, if the 2nd reading is outside the predetermined range then a 3rd measure may be necessary and indicated by the instrument.

Further evaluation of the pressure-response relationship using advanced high-speed photography will extend our understanding of the operational principles of the *Pulsair EasyEye* NCT. It is proposed that the complete trace of the pressure-response relationship may be transformed to provide additional information on biomechanical properties of the cornea similar to that provided by the Ocular Response Analyser (Reichert Ophthalmic Instruments, Buffalo, New York). Indeed the slim-line design of the *Pulsair EasyEye* NCT would provide a measure of possible changes in the corneal biomechanical characteristics with accommodation. Moreover, at present the effects of topical anaesthetics on IOP are equivocal. Utilising the pulse synchronised, non-contact IOP measurement method devised in the present thesis to investigate the effects of anaesthetics on IOP and corneal biomechanical properties could prove fruitful especially as contact tonometry is still considered to be the final arbiter of IOP in clinical ophthalmology.

Future work will involve the investigation of the effects of smaller increments in accommodation responses over a larger range of accommodation levels (e.g. zero to 6D in 0.50D steps) on IOP and choroidal blood flow. Experimentally a larger range of

accommodation responses may be achieved by using a higher powered Badal lens e.g. +8D. The results obtained would provide evidence to support or refute the hypothesis provided in the present thesis which suggests the presence of regulatory mechanisms of IOP and choroidal blood flow during accommodation.

Future work will require a longitudinal study in which the changes in refractive error or axial length (or both) are monitored in a cohort that has been divided into subsets depending on their IOP and choroidal blood flow responses to accommodation. This would allow subjects to be divided into groups which are likely to exhibit sustained increases or decreases in IOP or choroidal blood flow during accommodation. Moreover, the IOP and choroidal blood flow responses during near work could be investigated in stable and progressive myopes. Such studies may determine whether deficits in IOP and choroidal blood flow regulatory mechanisms to accommodation could be treated as a precursor to myopia development.

In the present studies, the changes in IOP and choroidal blood flow with accommodation were only assessed after 3 minutes of accommodation. Future work will involve the evaluation of the changes in IOP and choroidal blood flow following accommodation for longer time periods which will reveal the effect of sustained near work on IOP and blood flow. Furthermore, such a study would provide an insight in to the temporal aspects of IOP and choroidal blood flow changes with accommodation. Data concerning temporal inter-subject variations in IOP and choroidal blood flow responses to accommodation will assist in explaining further the inter-subject variations in responses observed in the present study and vascular aspects of the aetiology of myopia.

In addition, future work will involve the post-task temporal aspects of the decay of IOP and blood flow changes induced by accommodation. Several studies reviewed by Ong and Ciuffreda (1995) have shown a relatively small post-task myopic shift in refraction of approximately 0.40D. This refractive shift has been referred to as near-work induced transient myopia (NITM) and has been implemented in the aetiology of myopia development (Rosenfield and Gilmartin, 1998; Gilmartin, 2004). Previous studies have also demonstrated that both late-onset and early-onset myopes are more susceptible to NITM than emmetropes and hyperopes (Ciuffreda and Wallis, 1998; Ciuffreda and Lee, 2002; Wolffsohn *et al.*, 2003). The time course of NITM is approximately 30 seconds in emmetropes, but in late onset myopes is extended to approximately 1 minute (Ciuffreda and Wallis, 1998). Furthermore,

Wolffsohn *et al* (2003) demonstrated that NITM was evident even 3 minutes after viewing a 5D near task for 5 minutes in young adolescent Hong Kong Chinese. Anomalies in the post-task regression of accommodative responses have been thought to be due to deficits in the sympathetic innervation (Gilmartin and Bullimore, 1991; Strang *et al.,* 1994; Gilmartin, 1998). However, more recent work has shown that access to sympathetic facility is only available in approximately 30 % of the population (Gilmartin, Mallen and Wolffsohn, 2002). Of note is that Armaly and Rubin (1961) concluded that a relatively small amount of accommodation (i.e. 0.50 D), produced a statistically significant change in IOP. Hence, the possibility arises that the sustained post-task accommodative response causes time-integrated cumulative changes in IOP and choroidal blood flow, which may play a role in myopiagenesis.

The experimental setups in the present study were designed to minimise the effects of convergence on IOP and choroidal blood flow so that the effects of accommodation only could be investigated. However, it was not possible to completely and systematically decouple the accommodation-convergence system and therefore accommodative convergence still occurred during the IOP and blood flow measurement period. Despite limitations in the experimental designs of early work, it has been concluded that convergence increases IOP (Coleman and Trokel, 1969; Moses *et al.,* 1982; Kumata *et al.,* 1994). Therefore, medial recti contraction on accommodative convergence during the IOP and blood flow measurement periods may confound the effects of accommodation on IOP and partly account for the inter-subject-variations. Hence future work will investigate the dose and temporal effects of convergence on IOP. Of note however, is that previous research has shown that in extreme positions of gaze, the IOP increased by only 2-3 mmHg (Moses *et al.,* 1982; Kumata *et al.,* 1984). Thus it is predicted that the effect of the relatively modest degrees of convergence evident during near work on IOP would be much less.

Furthermore, pupil miosis also accompanies accommodation. The pupil size decreases linearly with accommodation responses (Alpern, Mason and Jardinico, 1961; Kasthurirangan and Glasser, 2005; Plainis, Ginis and Pallikaris, 2005). Plainis, Ginis and Pallikaris (2005) reported that the pupil decreases by an average of 0.18 mm per dioptre of accommodation response. Inter-subject variations in the pupillary near responses have been demonstrated by Schaeffel, Wilhelm and Zrenner (1993). Moreover, Schaeffel, Wilhelm and Zrenner (1993) have also concluded that myopic subjects have a weaker pupillary near response than the emmetropes and hypermetropes. Pupil constriction and dilatation occur due to the contraction

of the circular sphincter muscle and contraction of the radial dilator muscle, respectively, although the two muscles work synergistically. Contraction of the sphincter muscle during pupil miosis may cause stretching of the anterior face of the ciliary muscle which may lead to a wider area of exit for the aqueous humour and hence offer less resistance to aqueous humour outflow. The effect of pupil miosis on IOP is not well defined and hence will be the topic of further investigations.

It is possible that inter-subject variations in the pupillary near-response and associated changes in IOP may contribute to the inter-subject variations in IOP responses to accommodation demonstrated in the present study. Although the pupil size was not measured in the present study, it is of note that it has been shown that accommodation is not always accompanied by a concomitant pupil response (Stakenberg, 1991). Phillips, Winn and Gilmartin (1992) suggested that blur-driven accommodation alone was not sufficient enough to elicit a pupillary response. The authors proposed that changes in image size and lateral and vertical displacement of a target may be necessary to drive the pupillary response. Furthermore, Kasthurirangan and Glasser (2005) demonstrated that the pupil constricted with accommodation but started to dilate while accommodation was still maintained. Hence it may be that the relatively small changes in pupil size with accommodation may not have a significant effect on IOP. Therefore these studies may provide counter evidence for the possibility that changes in IOP with the pupillary near response may account for the variance in IOP changes with accommodation elicited in the present thesis.

A distinctive facet of the present study is that a method by which high resolution, non-contact, pulse-synchronised IOP measurements are possible has been devised. This method of IOP measurement has potential for great utility in both clinical and research settings. As stated earlier, the present experimental set up could be used to investigate fully the effects of topical anaesthetics on IOP. Clarification of the effects of corneal anaesthesia on IOP may assist in further explaining the discrepancies between the present studies and the work conducted by Mauger and coworkers (Mauger *et al.*, 1984). It is speculated that topical anaesthetics change tear-film and corneal characteristics which result in errors in tonometry. These suggested changes with topical anaesthetics may be detected by changes in the pressure-response relationship.

Furthermore, the effects of topical anaesthetics on IOP could be accounted for in a prospective study in which the effects of accommodation on IOP are evaluated using the Dynamic Pascal tonometer (Swiss Microtechnology AG, Switzerland); an instrument which takes IOP measures that are claimed not to be influenced by variations in corneal thickness, curvature and ocular rigidity (Ozbek *et al.*, 2006). Use of the Dynamic Pascal tonometer in the investigation of accommodation and IOP may assist in understanding the associations between corneal curvatures and thicknesses and the inter-subject variations in IOP changes with accommodation found in the current thesis.

The calculation of POBF with the OBFA is based on several assumptions. The PA and hence POBF values are affected by scleral rigidity and ocular volume and the measurement of these parameters may be contaminated by pulsations generated by other vasculature (Harris *et al.*, 1998). A true indication of the vascular profile during accommodation can be provided by other blood flow measurement techniques. For example the blood flow in the posterior ciliary arteries (which supply the choroid) can be measured with the Colour Doppler Imaging system (*Acuson Sequoia System, UK*) during accommodation. Furthermore, a continuous measure of blood flow is provided by the Laser-Doppler Flowmeter (Scientific Co., Barrington, Japan). Future work will involve the measurement of changes in blood flow during accommodation using the aforementioned techniques. In addition, although approximately 85% of the ocular blood flow is supplied by the choroid, the retinal circulation, which has been shown to adapt to changes in ocular perfusion pressure (Grunwald *et al.*, 1982; Riva *et al.*, 1986; Robinson *et al.*, 1986; Dumskyj *et al.*, 1996), may also play a role in vascular autoregulation during accommodation and should be investigated further.

Finally, in the present thesis it was postulated that the choroidal plexus may change with near work which results in changes in pulse amplitude during accommodation. The Optical Coherence Tomographer (OCT) (Carl Zeiss, Ophthalmic Systems. Inc.) is a relatively new, non-invasive, non-contact imaging system which permits visualisation of the retinal structures *in vivo*. The instrument is based on the principle of low-coherence interferometry where the distance between structures is calculated from the time delay of reflected signals. The high reflectivities of the inner and outer margins of the neurosensory retina permit the measurement of retinal thickness. On observation of the OCT images the choroid is also visible. However, further software development is necessary to determine the thickness of the choroid *in vivo*. A simple approach could be that an edge-detection programme is applied to

the images to attain a measure of choroidal thickness. Thus there is potential to investigate the effects of accommodation on choroidal thickness which may offer an explanation for the changes in choroidal pulsatility found in the current study.

11.4 Concluding statement

The principal aim of this thesis was to investigate the effect of accommodation on IOP and choroidal blood flow. It is apparent that the relationship between accommodation, IOP and choroidal blood flow is characterised by substantial inter-subject variations in responses. It is clear that further work needs to be done in this new and exciting area of research to extend further our understanding of the physiology of the human eye during sustained near work. Of special interest will be the significance of intraocular responses to near work in terms of the onset and progression of myopia.

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APPENDIX 1

A	B	C	D	E
1	316	5	0.16	1.16
2	319	5	0.16	1.25
3	321	5	0.16	1.31
4	326	5	0.16	1.45
5	331	4	0.13	1.60
6	335	5	0.16	1.71
7	341	4	0.13	1.89
8	346	5	0.16	2.04
9	353	5	0.16	2.24
10	360	5	0.16	2.45
11	368	5	0.16	2.68
12	376	5	0.16	2.91
13	384	5	0.16	3.15
14	394	5	0.16	3.44
15	403	5	0.16	3.70
16	412	5	0.16	3.97
17	423	5	0.16	4.29
18	433	5	0.16	4.58
19	444	5	0.16	4.90
20	455	5	0.16	5.22
21	465	5	0.16	5.52
21	465	5	0.16	5.52
22	477	5	0.16	5.87
23	488	5	0.16	6.19
24	500	5	0.16	6.54
25	511	5	0.16	6.86
26	522	5	0.16	7.18
27	533	5	0.16	7.51
28	546	5	0.16	7.89
29	556	5	0.16	8.18
30	570	5	0.16	8.59
31	579	5	0.16	8.85
32	593	5	0.16	9.26
33	605	5	0.16	9.61
34	616	5	0.16	9.93
35	630	5	0.16	10.34
36	642	5	0.16	10.69
37	654	5	0.16	11.04
38	668	5	0.16	11.45
39	678	5	0.16	11.75
40	694	5	0.16	12.21
41	703	5	0.16	12.48
42	719	5	0.16	12.95
43	730	5	0.16	13.27
44	742	6	0.19	13.62
45	757	6	0.19	14.06
46	766	6	0.19	14.32
47	783	7	0.22	14.82

A	B	C	D	E
48	794	8	0.25	15.14
49	806	9	0.28	15.49
50	819	10	0.31	15.87
51	830	12	0.38	16.19
52	844	13	0.41	16.60
53	857	15	0.47	16.98
54	868	15	0.47	17.30
55	883	16	0.50	17.74
56	893	17	0.53	18.03
57	908	19	0.59	18.47
58	920	22	0.69	18.82
59	932	25	0.78	19.18
60	944	30	0.94	19.53
61	956	36	1.13	19.88
62	968	41	1.28	20.23
63	982	48	1.50	20.64
64	993	54	1.69	20.96
65	1005	57	1.78	21.31
66	1019	65	2.03	21.72
67	1029	76	2.38	22.01
68	1043	85	2.66	22.42
69	1055	99	3.09	22.77
70	1067	105	3.28	23.12
71	1079	117	3.66	23.47
72	1091	134	4.19	23.83
73	1103	129	4.03	24.18
74	1117	135	4.22	24.59
75	1126	147	4.59	24.85
76	1140	141	4.41	25.26
77	1151	136	4.25	25.58
78	1162	134	4.19	25.90
79	1174	131	4.09	26.25
80	1186	126	3.94	26.60
81	1198	112	3.50	26.96
82	1210	109	3.41	27.31
83	1222	106	3.31	27.66
84	1234	104	3.25	28.01
85	1246	100	3.13	28.36
86	1258	99	3.09	28.71
87	1271	95	2.97	29.09
88	1281	102	3.19	29.38
89	1294	100	3.13	29.76
90	1305	95	2.97	30.08
91	1316	93	2.91	30.41
92	1328	91	2.84	30.76
93	1339	99	3.09	31.08
94	1351	99	3.09	31.43
95	1363	96	3.00	31.78

96	1373	90	2.81	32.07
97	1384	88	2.75	32.40
98	1394	84	2.63	32.69
99	1406	91	2.84	33.04
100	1416	77	2.41	33.33
101	1426	82	2.56	33.62
102	1436	81	2.53	33.92
103	1446	78	2.44	34.21
104	1457	87	2.72	34.53
105	1466	74	2.31	34.79
106	1478	72	2.25	35.14
107	1487	82	2.56	35.41
108	1498	70	2.19	35.73
109	1509	79	2.47	36.05
110	1518	79	2.47	36.31
111	1530	76	2.38	36.67
112	1539	74	2.31	36.93
113	1549	72	2.25	37.22
114	1559	70	2.19	37.51
115	1569	72	2.25	37.81
116	1579	76	2.38	38.10
117	1588	78	2.44	38.36
118	1597	71	2.22	38.63
119	1607	72	2.25	38.92
120	1615	78	2.44	39.15
121	1625	82	2.56	39.44
122	1635	85	2.66	39.74
123	1644	77	2.41	40.00
124	1653	75	2.34	40.26
125	1662	74	2.31	40.53
126	1671	73	2.28	40.79
127	1680	70	2.19	41.05
128	1689	75	2.34	41.32
129	1697	73	2.28	41.55
130	1706	68	2.13	41.81
131	1715	78	2.44	42.08
132	1724	66	2.06	42.34
133	1733	74	2.31	42.60
134	1741	66	2.06	42.84
135	1750	66	2.06	43.10
136	1758	81	2.53	43.33
137	1767	73	2.28	43.60
138	1776	69	2.16	43.86
139	1783	80	2.50	44.07
140	1792	65	2.03	44.33
141	1800	74	2.31	44.56
142	1807	75	2.34	44.77
143	1816	72	2.25	45.03
144	1823	73	2.28	45.23
145	1831	76	2.38	45.47
146	1839	76	2.38	45.70
147	1846	69	2.16	45.91
148	1854	72	2.25	46.14
149	1860	76	2.38	46.32
150	1868	80	2.50	46.55
151	1875	80	2.50	46.76
152	1882	73	2.28	46.96
153	1890	73	2.28	47.19
154	1898	75	2.34	47.43
155	1904	77	2.41	47.60
156	1911	76	2.38	47.81
157	1918	77	2.41	48.01
158	1926	75	2.34	48.25
159	1933	85	2.66	48.45
160	1940	73	2.28	48.66
161	1947	78	2.44	48.86
162	1954	78	2.44	49.07
163	1961	81	2.53	49.27
164	1967	81	2.53	49.45
165	1973	87	2.72	49.62
166	1981	78	2.44	49.86
167	1986	90	2.81	50.00
168	1993	77	2.41	50.21
169	1999	83	2.59	50.38
170	2005	82	2.56	50.56
171	2011	81	2.53	50.73
172	2018	78	2.44	50.94
173	2024	77	2.41	51.11
174	2030	77	2.41	51.29
175	2037	75	2.34	51.49
176	2042	76	2.38	51.64
177	2048	76	2.38	51.82
178	2054	74	2.31	51.99
179	2060	65	2.03	52.17
180	2066	72	2.25	52.34
181	2071	75	2.34	52.49
182	2076	67	2.09	52.63
183	2083	76	2.38	52.84
184	2088	78	2.44	52.99
185	2094	76	2.38	53.16
186	2098	75	2.34	53.28
187	2104	75	2.34	53.45
188	2109	82	2.56	53.60
189	2114	81	2.53	53.75
190	2119	75	2.34	53.89
191	2124	71	2.22	54.04
192	2128	69	2.16	54.16
193	2133	69	2.16	54.30
194	2139	68	2.13	54.48
195	2142	69	2.16	54.56
196	2147	71	2.22	54.71
197	2152	69	2.16	54.86

198	2156	67	2.09	54.97
199	2161	65	2.03	55.12
200	2166	60	1.88	55.27
201	2170	59	1.84	55.38
202	2175	58	1.81	55.53
203	2180	65	2.03	55.68
204	2184	56	1.75	55.79
205	2189	55	1.72	55.94
206	2193	59	1.84	56.06
207	2197	60	1.88	56.17
208	2203	60	1.88	56.35
209	2207	62	1.94	56.47
210	2212	59	1.84	56.61
211	2216	71	2.22	56.73
212	2221	71	2.22	56.88
213	2226	66	2.06	57.02
214	2230	65	2.03	57.14
215	2235	63	1.97	57.28
216	2239	58	1.81	57.40
217	2243	59	1.84	57.52
218	2248	56	1.75	57.67
219	2251	56	1.75	57.75
220	2255	58	1.81	57.87
221	2258	54	1.69	57.96
222	2261	55	1.72	58.05
223	2265	52	1.63	58.16
224	2268	52	1.63	58.25
225	2272	55	1.72	58.37
226	2275	57	1.78	58.45
227	2280	60	1.88	58.60
228	2283	58	1.81	58.69
229	2287	60	1.88	58.81
230	2289	60	1.88	58.86
231	2293	58	1.81	58.98
232	2296	59	1.84	59.07
233	2299	59	1.84	59.16
234	2303	58	1.81	59.27
235	2306	60	1.88	59.36
236	2310	61	1.91	59.48
237	2312	63	1.97	59.54
238	2315	57	1.78	59.62
239	2320	68	2.13	59.77
240	2322	62	1.94	59.83
241	2326	61	1.91	59.95
242	2329	60	1.88	60.03
243	2331	61	1.91	60.09
244	2335	58	1.81	60.21
245	2338	66	2.06	60.30
246	2341	66	2.06	60.39
247	2345	53	1.66	60.50
248	2348	63	1.97	60.59
249	2350	55	1.72	60.65
250	2354	54	1.69	60.77
251	2357	68	2.13	60.85
252	2360	59	1.84	60.94
253	2363	57	1.78	61.03
254	2366	60	1.88	61.12
255	2369	59	1.84	61.20
256	2372	58	1.81	61.29
257	2375	59	1.84	61.38
258	2378	57	1.78	61.47
259	2379	60	1.88	61.50
260	2382	62	1.94	61.58
261	2384	61	1.91	61.64
262	2387	57	1.78	61.73
263	2390	67	2.09	61.82
264	2392	69	2.16	61.88
265	2394	61	1.91	61.94
266	2398	62	1.94	62.05
267	2401	70	2.19	62.14
268	2404	68	2.13	62.23
269	2406	66	2.06	62.29
270	2408	61	1.91	62.34
271	2411	58	1.81	62.43
272	2414	58	1.81	62.52
273	2416	55	1.72	62.58
274	2418	57	1.78	62.64
275	2421	55	1.72	62.72
276	2423	57	1.78	62.78
277	2425	57	1.78	62.84
278	2428	54	1.69	62.93
279	2429	55	1.72	62.96
280	2432	54	1.69	63.05
281	2434	53	1.66	63.11
282	2436	54	1.69	63.16
283	2438	51	1.59	63.22
284	2440	52	1.63	63.28
285	2442	53	1.66	63.34
286	2444	51	1.59	63.40
287	2445	61	1.91	63.43
288	2446	52	1.63	63.46
289	2449	50	1.56	63.54
290	2450	51	1.59	63.57
291	2452	53	1.66	63.63
292	2453	52	1.63	63.66
293	2454	56	1.75	63.69
294	2456	54	1.69	63.75
295	2458	64	2.00	63.81
296	2458	53	1.66	63.81
297	2461	51	1.59	63.89
298	2461	61	1.91	63.89
299	2462	50	1.56	63.92

300	2464	58	1.81	63.98
301	2463	49	1.53	63.95
302	2464	57	1.78	63.98
303	2463	58	1.81	63.95
304	2463	49	1.53	63.95
305	2463	56	1.75	63.95
306	2462	58	1.81	63.92
307	2462	56	1.75	63.92
308	2460	52	1.63	63.87
309	2459	53	1.66	63.84
310	2458	50	1.56	63.81
311	2455	53	1.66	63.72
312	2453	55	1.72	63.66
313	2451	52	1.63	63.60
314	2446	53	1.66	63.46
315	2443	57	1.78	63.37
316	2440	55	1.72	63.28
317	2434	57	1.78	63.11
318	2429	59	1.84	62.96
319	2423	56	1.75	62.78
320	2416	61	1.91	62.58
321	2409	52	1.63	62.37
322	2401	64	2.00	62.14
323	2392	58	1.81	61.88
324	2381	68	2.13	61.55
325	2370	67	2.09	61.23
326	2359	66	2.06	60.91
327	2347	60	1.88	60.56
328	2334	61	1.91	60.18
329	2321	61	1.91	59.80
330	2308	60	1.88	59.42
331	2294	65	2.03	59.01
332	2279	63	1.97	58.57
333	2264	66	2.06	58.13
334	2250	68	2.13	57.72
335	2234	66	2.06	57.26
336	2219	68	2.13	56.82
337	2204	67	2.09	56.38
338	2188	66	2.06	55.91
339	2172	67	2.09	55.44
340	2158	64	2.00	55.03
341	2141	64	2.00	54.54
342	2127	64	2.00	54.13
343	2110	61	1.91	53.63
344	2093	60	1.88	53.13
345	2078	56	1.75	52.69
346	2061	55	1.72	52.20
347	2045	58	1.81	51.73
348	2029	59	1.84	51.26
349	2013	64	2.00	50.79
350	1998	57	1.78	50.35

351	1981	67	2.09	49.86
352	1966	60	1.88	49.42
353	1950	59	1.84	48.95
354	1935	60	1.88	48.51
355	1921	60	1.88	48.10
356	1905	67	2.09	47.63
357	1890	56	1.75	47.19
358	1876	53	1.66	46.79
359	1862	64	2.00	46.38
360	1848	55	1.72	45.97
361	1835	56	1.75	45.59
362	1822	62	1.94	45.21
363	1810	60	1.88	44.85
364	1798	64	2.00	44.50
365	1784	64	2.00	44.09
366	1773	66	2.06	43.77
367	1761	66	2.06	43.42
368	1750	67	2.09	43.10
369	1740	66	2.06	42.81
370	1728	67	2.09	42.46
371	1717	66	2.06	42.13
372	1707	75	2.34	41.84
373	1696	68	2.13	41.52
374	1686	69	2.16	41.23
375	1675	72	2.25	40.91
376	1666	65	2.03	40.64
377	1656	70	2.19	40.35
378	1645	70	2.19	40.03
379	1635	67	2.09	39.74
380	1625	72	2.25	39.44
381	1615	71	2.22	39.15
382	1604	72	2.25	38.83
383	1595	70	2.19	38.57
384	1584	84	2.63	38.24
385	1574	74	2.31	37.95
386	1564	76	2.38	37.66
387	1552	87	2.72	37.31
388	1542	75	2.34	37.02
389	1531	82	2.56	36.69
390	1519	79	2.47	36.34
391	1508	76	2.38	36.02
392	1498	70	2.19	35.73
393	1486	69	2.16	35.38
394	1475	67	2.09	35.06
395	1464	79	2.47	34.74
396	1451	68	2.13	34.35
397	1440	68	2.13	34.03
398	1428	73	2.28	33.68
399	1416	79	2.47	33.33
400	1405	82	2.56	33.01
401	1392	75	2.34	32.63

402	1380	78	2.44	32.28
403	1367	78	2.44	31.90
404	1354	73	2.28	31.52
405	1343	76	2.38	31.20
406	1329	82	2.56	30.79
407	1318	80	2.50	30.47
408	1305	75	2.34	30.08
409	1292	74	2.31	29.70
410	1280	78	2.44	29.35
411	1267	81	2.53	28.97
412	1255	85	2.66	28.62
413	1243	86	2.69	28.27
414	1230	89	2.78	27.89
415	1218	92	2.88	27.54
416	1207	93	2.91	27.22
417	1195	92	2.88	26.87
418	1182	92	2.88	26.49
419	1172	98	3.06	26.19
420	1160	96	3.00	25.84
421	1149	104	3.25	25.52
422	1139	110	3.44	25.23
423	1128	106	3.31	24.91
424	1118	103	3.22	24.62
425	1107	115	3.59	24.29
426	1097	118	3.69	24.00
427	1087	122	3.81	23.71
428	1077	129	4.03	23.42
429	1068	122	3.81	23.15
430	1058	128	4.00	22.86
431	1048	124	3.88	22.57
432	1039	118	3.69	22.31
433	1029	104	3.25	22.01
434	1019	83	2.59	21.72
435	1010	72	2.25	21.46
436	1000	61	1.91	21.16
437	992	51	1.59	20.93
438	983	45	1.41	20.67
439	974	40	1.25	20.40
440	967	36	1.13	20.20
441	957	32	1.00	19.91
442	950	27	0.84	19.70
443	941	22	0.69	19.44
444	933	17	0.53	19.20
445	925	14	0.44	18.97
446	917	11	0.34	18.74
447	909	9	0.28	18.50
448	901	8	0.25	18.27
449	893	7	0.22	18.03
450	884	6	0.19	17.77
451	877	6	0.19	17.57
452	868	6	0.19	17.30
453	861	6	0.19	17.10
454	852	6	0.19	16.84
455	844	6	0.19	16.60
456	837	6	0.19	16.40
457	827	6	0.19	16.10
458	819	6	0.19	15.87
459	811	6	0.19	15.64
460	802	7	0.22	15.37
461	793	7	0.22	15.11
462	785	6	0.19	14.88
463	776	6	0.19	14.61
464	767	6	0.19	14.35
465	758	6	0.19	14.09
466	750	6	0.19	13.85
467	742	5	0.16	13.62
468	732	5	0.16	13.33
469	724	5	0.16	13.09
470	715	5	0.16	12.83
471	707	5	0.16	12.59
472	699	5	0.16	12.36
473	691	5	0.16	12.13
474	682	5	0.16	11.86
475	675	5	0.16	11.66
476	666	5	0.16	11.40
477	658	5	0.16	11.16
478	651	5	0.16	10.96
479	643	5	0.16	10.72
480	635	5	0.16	10.49
481	628	5	0.16	10.28
482	620	5	0.16	10.05
483	614	5	0.16	9.87
484	607	5	0.16	9.67
485	600	5	0.16	9.47
486	594	5	0.16	9.29
487	587	5	0.16	9.09
488	582	5	0.16	8.94
489	575	5	0.16	8.73
490	569	5	0.16	8.56
491	564	5	0.16	8.41
492	558	5	0.16	8.24
493	553	5	0.16	8.09
494	548	5	0.16	7.94
495	542	5	0.16	7.77
496	537	5	0.16	7.62
497	532	5	0.16	7.48
498	527	5	0.16	7.33
499	522	5	0.16	7.18
500	280	13	0.41	0.11
501	279	13	0.41	0.08
502	278	13	0.41	0.05
503	277	13	0.41	0.02

504	276	13	0.41	-0.01
505	276	13	0.41	-0.01
506	275	13	0.41	-0.04
507	275	13	0.41	-0.04
508	276	13	0.41	-0.01
509	277	13	0.41	0.02
510	278	13	0.41	0.05
511	279	13	0.41	0.08
512	280	13	0.41	0.11
513	282	13	0.41	0.16
514	283	13	0.41	0.19
515	285	13	0.41	0.25
516	288	13	0.41	0.34
517	290	13	0.41	0.40
518	293	13	0.41	0.49
519	295	13	0.41	0.55
520	298	13	0.41	0.63
521	301	13	0.41	0.72
522	303	13	0.41	0.78
523	306	13	0.41	0.87
524	309	13	0.41	0.95
525	313	13	0.41	1.07
526	316	13	0.41	1.16
527	319	13	0.41	1.25
528	322	13	0.41	1.33
529	325	13	0.41	1.42
530	328	13	0.41	1.51
531	330	13	0.41	1.57
532	333	13	0.41	1.66
533	335	13	0.41	1.71
534	337	13	0.41	1.77
535	339	13	0.41	1.83
536	341	13	0.41	1.89
537	342	13	0.41	1.92
538	343	13	0.41	1.95
539	345	13	0.41	2.01
540	346	13	0.41	2.04
541	346	13	0.41	2.04
542	347	13	0.41	2.07
543	348	13	0.41	2.10
544	348	13	0.41	2.10
545	348	13	0.41	2.10
546	348	13	0.41	2.10
547	348	13	0.41	2.10
548	347	13	0.41	2.07
549	347	13	0.41	2.07
550	346	13	0.41	2.04
551	346	13	0.41	2.04
552	345	13	0.41	2.01
553	345	13	0.41	2.01
554	344	13	0.41	1.98
555	343	13	0.41	1.95
556	342	13	0.41	1.92
557	341	13	0.41	1.89
558	341	13	0.41	1.89
559	340	13	0.41	1.86
560	339	13	0.41	1.83
561	338	13	0.41	1.80
562	338	13	0.41	1.80
563	337	13	0.41	1.77
564	337	13	0.41	1.77
565	336	13	0.41	1.74
566	336	13	0.41	1.74
567	336	13	0.41	1.74
568	336	13	0.41	1.74
569	336	13	0.41	1.74
570	337	13	0.41	1.77
571	337	13	0.41	1.77
572	338	13	0.41	1.80
573	339	13	0.41	1.83
574	339	13	0.41	1.83
575	341	13	0.41	1.89
576	341	13	0.41	1.89
577	342	13	0.41	1.92
578	343	13	0.41	1.95
579	344	13	0.41	1.98
580	346	13	0.41	2.04
581	347	13	0.41	2.07
582	348	13	0.41	2.10
583	349	13	0.41	2.12
584	350	13	0.41	2.15
585	351	13	0.41	2.18
586	353	13	0.41	2.24
587	354	13	0.41	2.27
588	354	13	0.41	2.27
589	356	13	0.41	2.33
590	356	13	0.41	2.33
591	357	13	0.41	2.36
592	358	13	0.41	2.39
593	358	13	0.41	2.39
594	359	13	0.41	2.42
595	359	13	0.41	2.42
596	359	13	0.41	2.42
597	359	13	0.41	2.42
598	359	13	0.41	2.42
599	359	13	0.41	2.42

Table 1.1A Principle components of the pressure-response relationship of a single IOP measure, where **A**=Excel row, **B**=pressure output, **C**=contrast values, **D**=k-factor and **E**=IOP values.

APPENDIX 2

IOP CALCULATION AND VALIDATION

The following document details the calculation and validation of an IOP measure taken with the Pulsair EasyEye (Keeler, UK) non-contact tonometer.

Using the example data in **Table 1.1A** an IOP is calculated and validated as follows:

IOP calculation

BGC = Average of C1 to C5 in spreadsheet
= 5

EC = BGC + 19
= 24

EPI = The *Excel* row at which the contrast value first exceeds the EC value
= 59

CEI = EPI + skew
= 43

PE = A43 in *Excel* spreadsheet
= 730

PI = Average of A1 to A2 in spreadsheet
= 317.5

$$\text{IOP} = \left[\frac{aa}{64}\right] + \left[\frac{(PE - PI) \times ba}{262144}\right]$$

$$= \left[\frac{77}{64}\right] + \left[\frac{(730 - 317.5) \times 7667}{262144}\right]$$

= 13.27 mmHg

IOP validation

CV_x = BGC + 12
= 17

CV_y = BGC + 25
= 30

CVI_1 = The *Excel* row at which the contrast value first exceeds the CV_x
= 57

CVI_2 = The *Excel* row at which the contrast value first exceeds the CV_y
= 61

Excel row = 61 – 57
= 4

CRT = 4 x 0.038
= 0.152 ms

Therefore for the IOP calculated since the number of *Excel* rows is less than 27 and accordingly CRT is less than 1.03 ms, the IOP is a valid measure.

APPENDIX 3

HUMAN SCIENCES ETHICAL COMMITTEE SUBMISSION

Application to the Human Sciences Ethical Committee, School of Life and Health Sciences, Aston University for the approval of the experimental procedures performed throughout this study.

ASTON UNIVERSITY **PROJECT NO.....**

THE SENATE **REG/00/174**

HUMAN SCIENCE ETHICAL COMMITTEE

Application for approval of a research project involving human volunteers

Please read the enclosed guidelines before completing this form - in typescript or black ink - and return the form to: The Secretary of the Human Science Ethical Committee, Registry. If you intend to administer any substance or expose the volunteers to a physical procedure other than simple venepuncture **you must also submit an experimental protocol.**

Project title:

The effect of accommodation on intraocular pressure

Outline Scientific Purpose/Objectives for Project and Potential Benefits:
Attached

Investigator(s):	**Department/address:**	**Telephone:**
(First name should be a member of Aston's Academic staff who ill act as main contact)		
Professor Bernard Gilmartin Supervisor	Optometry, School of Life and Health Sciences	X5159
Dr. James Wolffsohn Associate Supervisor	Optometry, School of Life and Health Sciences	X5160
Miss Gurjeet Sammi Postgraduate student	Optometry, School of Life and Health Sciences	X5182

A

Details of sponsoring/collaborating organisation (if any)

1. Name: The College of Optometrists
2. Does the sponsoring/collaborating organisation provide insurance? NO
3. If drugs are used, do any require a clinical trials certificate or clinical trials exemption certificate? NO

*If yes, please provide a copy of the certificate

B

Summary of Project

1 Starting date: October 2003
2 Duration: 3 years
3 Location: Optometry, Life and Health Sciences, Aston University
4 Physical procedures:
Instillation of ophthalmic drugs.
Measurement of:

- Refraction using a standard autorefractor,
- Accommodative response using the *Shinn-Nippon* infrared open-view autorefractor.
- Corneal biometry including corneal thickness and topography using the *Orbscan*
- Axial length and anterior chamber depth using the *IOLMaster.*
- Intraocular pressure (IOP) using the contact *Goldmann* tonometer.
- Intraocular pressure (IOP) using the non-contact *Easy Eye (Keeler)* tonometer.
- Pulse measurements using Goldmann tonometer and pulse transducer.

5. Substances to be administered (a substance is anything other than normal food - chemical constituents

of food stuffs, ethanol and variation of the diet should be included here) and method of delivery should be specified:
A short term topical corneal anaesthetic, proxymetacaine HCl (0.5%) combined with fluorescein (0.25%) will be used. These eye drops are disposable single use applicators (*Minims®*, Chauvin Pharmaceuticals). The duration of corneal anaesthesia with this eye drop is approximately 25 minutes. Topical anaesthetics are used routinely in general optometric practice.
Fluorescein Sodium (1mg) *Fluorets* (Chauvin Pharmaceuticals) are sterile single use applicators that will be used as an indicator dye for detection of corneal abrasions. Diagnostic dyes are used routinely in general optometric practice.

6 Psychological assessment:
N/A

7 Questionnaires: (only to be completed when project contains questionnaire(s) which fall within the types of questionnaire requiring HSEC approval [Guidelines D (3)])
N/A

C

Volunteers

1 Number of volunteers to be used: 30-40
2 Over what time span? 3 year
3 Age of volunteers: 18-26 years
4 Sex of volunteers: Mixed
5 Source: Undergraduate students.
6 Will payments be made to the volunteers and if so, how much will each be paid? No
7 Are the volunteers patients or healthy volunteers? (If patients, give diagnosis, clinic/responsible practitioner).
All volunteers are healthy.
8 Will any volunteers be excluded and if so, on what grounds?
Volunteers will be excluded if they report any previous reactions to topical anaesthetics.
9 Is the activity of the volunteer to be restricted in any way either before or after the procedure? (eg diet, driving)
During the recovery from the topical anaesthetic subjects will be advised to avoid dusty environments, vigorously rubbing their eyes and reinsertion of contact lenses for 20 minutes after instillation of anaesthetic.
10 Consent: Please attach a copy of the consent form you intend to use, detailing how procedures and hazards will be explained.
Attached.

D

Risk Assessment: *a thorough Risk Assessment of the project must be undertaken (including for example welfare issues arising from the procedure, and the possible risk of residual effects in volunteers and the consequences thereof).*

1. Please give full details of any hazards which could affect the health, safety or welfare of any volunteer, or any other person who might be harmed as a result of the experiment.
Potential hazards to subjects result from:
- the instillation of the topical drug proxymetacaine (0.5%) with
- fluorescein (0.25%) during IOP measurements.
- Corneal damage with Goldmann tonometry.
- Transmission of infections between subjects using contact devices e.g., Goldmann tonomtery.

All the instruments and techniques employed are non-invasive and commercially available. They are not considered to hold any risk to health or sight and are routinely used in general optometric practice.

2. What levels of risk are associated with these hazards ?
- A rare adverse reaction has been reported with the use of topical anaesthetics with an incidence of 1 in 1000 patients over 55 years of age. Data are not available for young adults, however there has been only 3 incidences of adverse reactions reported at Aston University over the last 10 years. The reaction is desquamation of the corneal epithelium which results in a significant reduction in visual acuity for up to 2 hours, with no permanent effects.
- There is a small risk of slight corneal abrasions following contact tonometry measurements. Contact tonometry is a technique which is routinely carried out in optometric practice and carries minimal risk when performed by an experienced practitioner.
- There is a small theoretical risk of transmission of vCJD whilst using ocular contact devices, however there is no experimental evidence to quantify the risk.

3. How do you propose to control the risks associated with these hazards?
If any adverse reactions occur with the use of the topical anaesthetic proxymetacaine (0.5%) with fluorescein (0.25%) then the subject's condition will be continuously monitored until visual acuity returns to normal levels.

Subjects will be advised to avoid dusty environments for 20 minutes after corneal anaesthesia as they will not be able to feel foreign bodies entering the eye. They will also be advised not to vigorously rub their eyes after instillation of drugs.
Commercially available disposable tonometer tips (*TonoSafe*®, Haag Streit) will be used when performing Goldmann tonometry to avoid possible contamination.
The corneal integrity of all subjects will be checked at the end of each session using fluorescein (1mg) dye and a slit lamp. This is a standard clinical examination to determine if any corneal damage has being caused by the use of contact devices e.g. Goldmann tonometry.

4. What criteria have you used to determine whether the risks are acceptable?
Acceptability is based on:
   1) The very low level of risk attached to these procedures
   2) The procedures are well established as standard modes of investigation in general optometric practice.

5. Is there any precedent for these experiments? If so, please give details with references if possible.
Aston University, Human Science Ethical Committee,
Ref. 991. The effect of sustained accommodation on pulsatile ocular bloodflow. B. Gilmartin; K.Jones.
Project still continuing-no incidents have occurred.

6. Has this project been considered/is it being considered by any other Ethical Committee? If so, please give details and decision made.
No

E

STATEMENT BY NAMED INVESTIGATORS, HEAD OF SCHOOL AND (if necessary) RESEARCH SUPERVISOR

I consider that the details given constitute a true summary of the project and that the hazards and potential risks to any volunteer are accurately described. The Principal Investigator is the main point of contact for the Human Sciences Ethical Committee, and accordingly should be a member of academic staff of the University (this implies that supervisors of research students will be the main point of contact)

Principal Investigator and Supervisor of Student..... date.....

Investigator..... date.....

Investigator..... date.....

Investigator..... date.....

Investigator..... date.....

Head of School (or nominee)..... date.....

The following should be attached:

- volunteer consent form
- insurance certificate (if available)
- clinical trials certificate or clinical trials exemption certificate (if appropriate)
- experimental protocol

APPENDIX 4

INFORMATION AND CONSENT FORMS FOR PARTICIPANTS

The following documents are copies of the information sheets and consent forms given to each subject who participated in the studies detailed in this thesis.

TITLE: The effect of accommodation on intraocular pressure

RESEARCH WORKERS AND SCHOOL RESPONSIBLE:
Supervisor: Professor. B. Gilmartin, B.Sc., Ph.D., FCOptom, FAAO;
Associate Supervisor. Dr. J. S.W. Wolffsohn, B.Sc., Ph.D., FAAO;
Postgraduate student: Miss G. Sammi, B.Sc., MCOptom;
Neurosciences Research Institute, School of Life and Health Sciences, Aston University, Aston Triangle, Birmingham, B4 7ET.

DESCRIPTION OF PROJECT:
Accommodation is a mechanism that allows the eye to modify its refractive power so that objects of regard can be brought into focus at many distances. The intraocular pressure (IOP) is the pressure within the eyeball and its variations with accommodation will be recorded in this project. The aim is to investigate the effect of different levels of accommodation on IOP. Previous literature suggests a decrease in IOP with sustained accommodation. Knowledge about these changes is important in understanding further the affect on the human eye of sustained nearwork, a common activity in everyday life.

PROCEDURES:
1) Accommodation will be measured using a *Shin-Nippon* autorefractor, a standard clinical instrument. You will be required to look at a target for set time limits, at set distances.
2) Measurements will be made including corneal thickness, shape and eye length using standard clinical non-contact instruments.
3) IOP measurements will be taken using the non-contact *EasyEye* tonometer. You will be required to look at a target at set distances and IOP measurements will be recorded.
4) IOP measurements will be taken using the Goldmann contact tonometer. Before these measurements are taken the front surface of the eye will be numbed for a short time (i.e. 20 minutes) using anaesthetic eye drops, proxymetacaine HCl (0.5%) with fluorescein (0.25%). This is a standard clinical procedure used in general optometric practice.

For at least 20 minutes after the drops (proxymetacaine HCl (0.5%) with fluorescein (0.25%)) have been instilled you are advised to:

1) **avoid dusty environments**
2) **avoid vigorously rubbing your eyes**
3) **avoid wearing contact lenses**

All the instruments and techniques employed are non-invasive and commercially available. They are not considered to hold any significant risk to health or sight. The procedures are well-established modes of investigation and are used routinely in general optometric practice.
You will be required to remove your contact lenses (if worn) for the study.
Contribution to this research project in voluntary and you are free to withdraw from the project at any time.
Personal information and data collected in this experiment will be confidential with respect to any resulting publications.

VOLUNTEER'S STATEMENT
I have read and understood the above explanation. I have had the opportunity to discuss it with the investigators and to ask any questions. I agree to take part in this research project and understand that I am free to withdraw from the project at any time.

Signed..... Print name.....

Date..... Signature of researcher.....

APPENDIX 5

HIGH SPEED PHOTOGRAPHY DURING TONOMETRY

CD-ROM contents:

5.1A High speed photography of the cornea showing dispersion of the Placido disk image during tonometry.

5.2A High speed photography of the cornea showing corneal deformation during tonometry.

APPENDIX 6

THE EASYEYE PULSAIR NCT;
HAND-HELD *versus* TABLE-MOUNTED.

Introduction

Chapter 5 describes a method of IOP measurement in which the variance in IOP data associated with the cardiac and respiratory cycles is significantly reduced using the *EasyEye Pulsair* (Keeler, UK) non-contact tonometer (NCT). A *LabView* acquisition program (National Instruments., USA) was used with a finger pulse-transducer to synchronise the IOP measures with the peak, middle or trough of the cardiac cycle. It is evident from the results that the spread of IOP data significantly reduces when the measures are synchronised with the cardiac cycle. Of the 3 positions investigated, the middle location demonstrated the least variance in IOP measures.

An important feature of the experimental setup used to investigate the effects of accommodation on IOP in this thesis, was that the *EasyEye Pulsair* NCT was mounted on an optical bench in front of the RE which permitted the simultaneous stimulation of accommodation in the LE. To attain correct alignment of the tonometer with the cornea the instrument was adjusted in the axial, vertical and lateral directions with a pillar and saddle attachment mounted on an optical bench. Indeed, there is a range in which correct alignment can be identified (see **Chapter 3**). Mounting the instrument means that between measures little adjustment is required, particularly in the vertical and lateral directions, to obtain correct alignment. However, much larger adjustments are necessary when the IOP measures are obtained with the hand-held instrument. Therefore, although the alignment range is fixed the consistency of alignment varies within this range and is greater when the instrument is used in its hand-held mode which increases the spread of consequent measures. Keeler (UK) engineers suggested that a significant proportion of the reduction in the variance of IOP data demonstrated in **Chapter 5** may in fact be a consequence of mounting the instrument onto an optical bench rather than a direct consequence of pulse synchronised measures.

A study was therefore conducted where the IOP was measured with an unmodified *EasyEye Pulsair* NCT (i.e. without pulse synchronous feature) in both its table-mounted and hand-held modes.

Methods

Subject group.

Ninety three subjects from the undergraduate population at Aston University took part in this study. The mean age of the subject group was 21.1±2.5 years, ranging from 19 to 29 years of age. The cohort comprised of 49 males and 44 females.

The research followed the tenets of the Declarartion of Helsinki and was approved by the institution's ethics committee (**Appendix 3**). Written consent was obtained from all subjects willing to participate in the study and copies of the information sheets and consent forms given to the subjects can be found in **Appendix 4**. All subjects had a visual acuity of 0.00 logMAR or better. All subjects were absent of ocular pathology. None of the subjects were taking any topical or systemic medications that may affect the IOP.

The instrument was used in both modes to take IOP measures at the same time of day to eliminate the effects of diurnal variation on IOP (Pointer, 1997; Noel *et al.*, 2001; Liu *et al.*, 2003; Liu *et al.*, 2005; Kida *et al.*, 2006).

Stimulus presentation

A high contrast (90%) Maltese cross target was placed at 6m. At this distance the target represented a minimal accommodative stimulus to eliminate any effects of accommodation on IOP.

Instrumentation

The full operational details of the *EasyEye Pulsair* NCT are described elsewhere (see **Chapter 3**). The IOP measures were taken with the instrument in its hand-held mode. The NCT was unmodified i.e. not able to take pulse synchronised measures and was not table-mounted. The use of the unmodified NCT in this study mimicked the standard use of the instrument in optometric practice. Throughout this study, the use of the *EasyEye Pulair* NCT in its standard hand-held mode is referred to as 'NCT hand-held'.

A second identical *EasyEye Pulsair* NCT was mounted via an adjustable saddle and pillar device on an Ealing optical bench. Again the NCT was also not modified to take pulse synchronised IOP measures. In the present study, the use of the instrument in its table-mounted mode is referred to as 'NCT table-mounted'.

Experimental procedures
The subjects were instructed to view the fixation target with their LE. A familiarisation procedure described in **Chapter 5** was first performed to reduce any effects of apprehension caused by the method of non-contact tonometry (Forbes *et al.*, 1974; Moses *et al.*, 1984).

Four IOP measures were taken on the RE with the NCT in its hand-held and table-mounted modes. The manufacturers recommend that 4 IOP readings are taken to account for the variance in IOP measures associated with the ocular pulse. This procedure has been validated by McCaghrey and Matthews (2001). NCT hand-held and NCT table-mounted were performed in a random order and the subjects were allowed a 5 minute rest period between each set of 4 readings.

NCT hand-held was performed by 4 experienced practitioners (MD, HB, PS and AB) whereas NCT table-mounted was performed by 1 experienced practitioner (GR).

Statistical analyses
Agreement between the two measurement methods was assessed using the method of Bland and Altman (1986) and by calculating the correlation coefficient of the data set. The variance in IOP measures for both methods was calculated and compared using a variance ratio test. The coefficient of variation (COV) was also calculated for the NCT in its 2 modes.

Results
The mean IOP obtained with the NCT hand-held and NCT table-mounted was 15.52±3.34 and 15.08±3.14 mmHg, respectively. **Figure 6.1A** shows that as expected, the IOP measures obtained with the two instruments demonstrated a strong correlation ($r=0.74$, $p<0.001$).

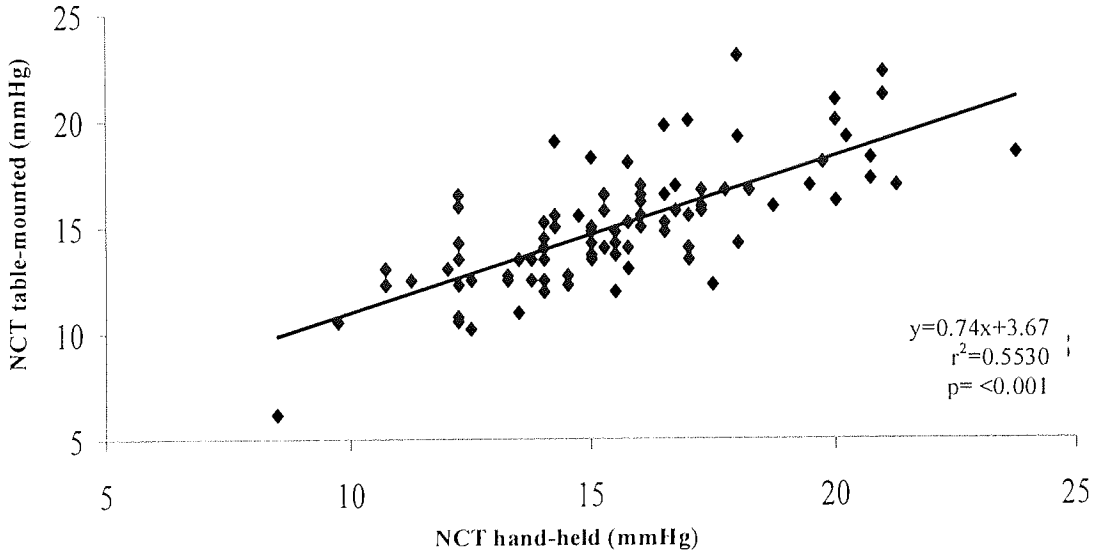


Figure 6.1A Correlation between NCT hand-held and NCT table-mounted (n=93).

The NCT hand-held measured slightly higher IOP measures compared to the NCT table-mounted. This difference between the 2 measurement methods (0.44±2.03 mmHg) just reached statistical significance ($p=0.04$) and the 95% CI was ±3.99 (**Figure 6.2A**).

The COV for the NCT hand-held and the NCT table-mounted was respectively 21.5% and 20.8%. The calculated variance was 11.13 and 9.87 for the NCT hand-held and NCT table-mounted, respectively. This variance calculated for each method was not statistically different ($F=1.13$, $p=0.12$).

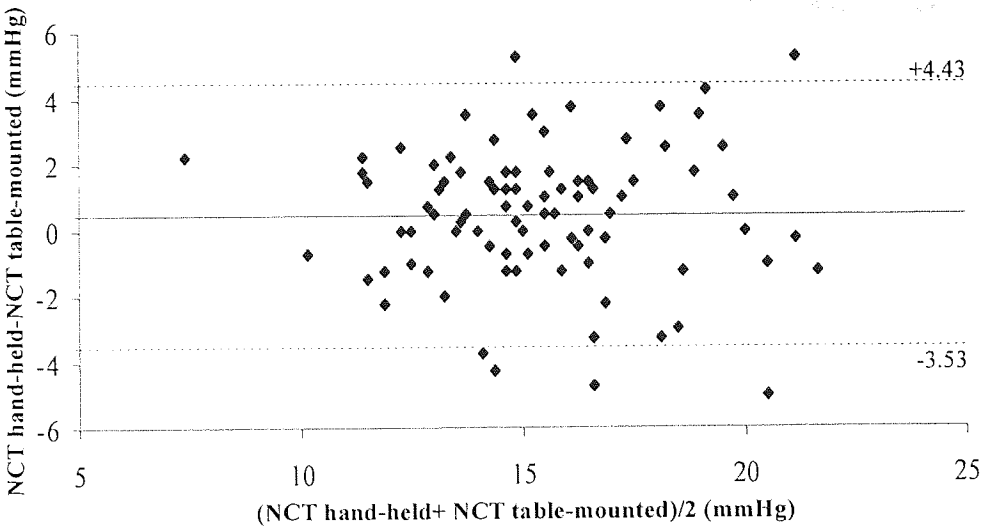


Figure 6.2A Bland and Altman dispersion plot of the IOP measures taken with NCT hand-held and NCT table-mounted, where — represents the mean bias and --- represents the 95% confidence limits (n=93).

Discussion

The main aim of the present study was to evaluate the spread of IOP data taken with the *EasyEye Pulsair* NCT in its standard hand-held mode and in its table-mounted mode. As expected the IOP measures taken with the two instruments were correlated. The measures taken with the NCT hand-held were slightly higher than those taken with the NCT table-mounted although this difference was not significant. A similar result was also found in the study described in **Chapter 5**, where the IOP measures taken with the standard *Pulsair* were higher than those taken at the peak of the cardiac cycle. This maybe explained by possible differences in the degree of apprehension experienced by the subjects when NCT is performed in the 2 different modes. There is evidence in the literature to suggest that increased apprehension (Moses *et al.*, 1984) artificially increases the IOP. It is possible that the subjects are relatively more apprehensive when tonometry is performed hand-held compared to when it is performed in its table-mounted mode which may hence artificially increase the IOP.

Although not statistically different, the variance in the IOP data was slightly higher with NCT hand-held compared to NCT table-mounted. In addition to apprehension artificially increasing IOP, apprehension has also been shown to increase the spread of consecutive IOP measures taken with a NCT (Forbes *et al.*, 1974). Hence given that the subjects may experience more apprehension during hand-held tonometry compared to table-mounted tonometry, the slight differences in the spread of data found in this study may be explained by apprehension. However, Keeler (UK) postulated that the variance in IOP measures is reduced with NCT table-mounted compared to NCT hand-held since less instrument movement is required to obtain correct alignment of the instrument during NCT table-mounted. Therefore, in this mode the IOP measures are taken within a smaller alignment range compared to that required when NCT hand-held is performed.

It can be concluded from the present study that the variance in IOP measures is not significantly influenced by whether the *EasyEye Pulsair* NCT is used in its hand-held or table-mounted mode. This result is in accordance with an unpublished BSc project in 1989 by Mackie *et al.* which concluded no difference in repeatability when using the *Pulsair* NCT in its hand-held or adapted slit-lamp mode (citied by Mackie *et al.*, 1996). Therefore, contrary to the suggestion made by Keeler (UK) the significant reduction in the variance of IOP data achieved in the study described in **Chapter 5**, was attributable to synchronising the IOP measures with the cardiac cycle and maintaining a fixed pace respiratory cycle.

Conclusion

The main results of this study listed below support the results of the study described in **Chapter 5** in which a significant reduction in variance was achieved by synchronising the IOP measures with the cardiac cycle.

- The IOP measures taken with NCT hand-held and NCT table-mounted are well correlated.
- The difference in mean IOP between the two modes is small and just reaches significance.
- The COV of both modes is approximately 21%.
- The variance in IOP measures is not influenced by whether NCT hand-held and NCT table-mounted are used.

APPENDIX 7

PERCENTAGE CHANGES IN IOP WITH ACCOMMODATION

The mean±SD percentage change in IOP between L and I, L and H, and I and H accommodation stimulus levels was a decrease of respectively 1.81±9.17, 2.69±8.12 and 0.38±9.41 mmHg. The within-subjects ANOVA shows that the level of accommodative stimuli does not influence the percentage changes in IOP [F (2, 98) = 1.208, p=0.283].

The MN±SD of the differences in percentage changes in IOP between L and I and L and H accommodation levels for the emmetropes were respectively, -1.93±9.73 and -1.35±9.35 mmHg and for the myopes the MN±SD of the differences in IOP between L and I and L and H accommodation levels were respectively, -1.72±8.87 and -3.68±7.05 mmHg. The between-refractive groups ANOVA results (F=1.334, p=0.252) together with correlation graphs of refractive error and differences in IOP between L and I (r=0.08, p=0.55), L and H (r=0.24, p=0.10) and I and H (r=0.29, p=0.09) levels of accommodation shown provide evidence that the inter-subject variations in refractive error do not account for the inter-subject variations in IOP responses to accommodation (see **Figure 7.1A**).

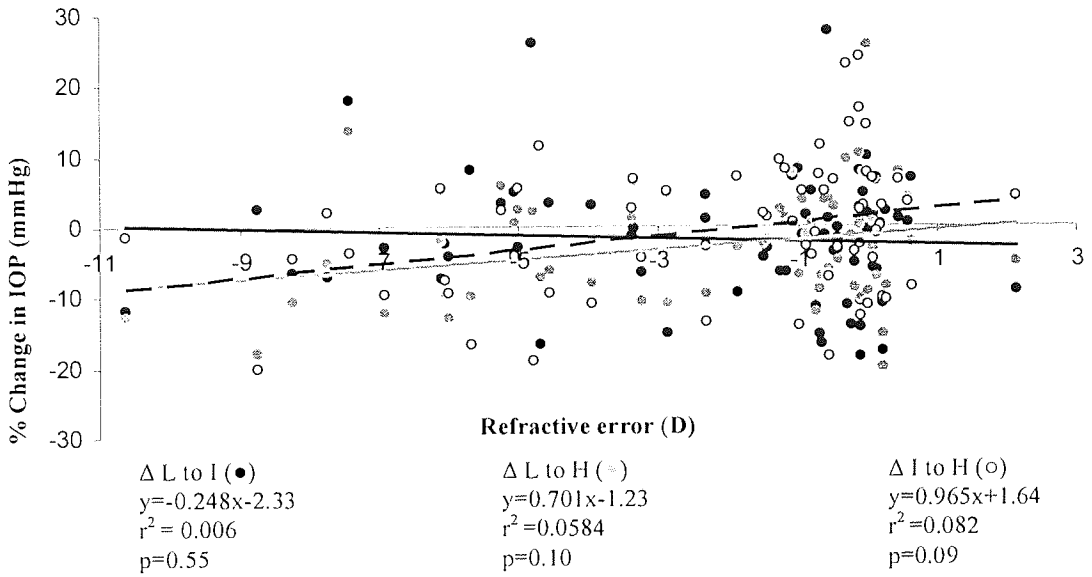


Figure 7.1A Refractive error against the % changes in IOP between L and I (—), L and H ( ) and I and H (---) accommodation stimulus levels (n=66).

APPENDIX 8

ASPECTS OF THE ACCOMMODATION STIMULUS-RESPONSE FUNCTION.

Introduction

In **Chapter 6** the relationship between accommodation and IOP was investigated having incorporated the measurement of accommodation responses in to the study design. The accommodation response data collected was also used to evaluate aspects of the accommodation stimulus-response function, an account of which follows. Descriptions of the subject group and methodologies are given in **Chapter 6**.

Methods

Statistical analyses

The accommodation response errors (*ARE*) were calculated from the difference between the accommodation responses and nominal values for each accommodative stimulus level. A one-way analysis of variance (ANOVA) in randomised blocks was performed followed by Scheffe *post-hoc* analyses to determine whether the accommodation stimulus level influenced the *ARE*. The effect of ammetropia on the *ARE* was determined by a between-subjects ANOVA.

The linear regression slopes (*m*) of the accommodation stimulus-response functions were calculated for each subject using the least squares method (McBrien and Millodot, 1986 a and Abbot, Schmid and Strang, 1998). The accommodative error indices (*I*) were also calculated since the accuracy of the response gradient parameter (*m*) as a single indicator of accommodative stability has been questioned by Chauhan and Charman (1995). Although a full account of the derivation of *I* can be found elsewhere (Chauhan and Charman, 1995), the calculations used are briefly described. The accommodative error indices (*I*) were calculated from **Equation 8.1.A**.

$$I = \frac{E}{r^2}$$ **Equation 8.1A**

where E is the area between the two lines weighted by the interval of x (i.e. between 2 accommodation levels (x_1 and x_2) and r is the correlation coefficient of the line between the intervals of x. For the stimulus-response functions which did not intersect with the unity line, *E* was calculated from the equation shown in **Equation 8.2A**.

$$E = \left[(1-m) \times \left(\frac{x_2 + x_1}{2} \right) - c \right]$$ **Equation 8.2A**

where m is the gradient of the line between point x_1 and x_2 and c is the constant calculated from $y = mx + c$. For the stimulus-response functions which intersected the unity line, *E* was calculated for 2 triangular areas (E_A and E_B) either side of the intersection point using **Equation 8.3A**.

$$E_A = \left[(1-m) \times \left(\frac{x_1 + x_C}{2} \right) - c \right]$$

$$E_B = \left[(1-m) \times \left(\frac{x_2 + x_C}{2} \right) - c \right]$$ **Equation 8.3A**

where x_C is the point of intersection and is calculated from the equation shown in **Equation 8.4A**

$$x_C = \frac{c}{(1-m)}$$ **Equation 8.4A**

The effect of ammetropia on both *m* and *I* was analysed using unpaired Students t-tests.

The cross-over point of the best fit line and the unity line on the accommodation stimulus-response graphs was used to determine the tonic accommodation (TA). To establish whether the TA differed amongst the myopes and the emmetropes an unpaired Students t-test was performed.

Results

The mean±SD (MN±SD) accommodation responses for the cohort and the emmetropes and the myopes are shown in **Table 8.1A**. The associated accommodation stimulus-response curves for the cohort and the two refractive groups are shown in **Figures 8.1A and 8.2A**, respectively.

	Accommodation	
	Stimulus level	**MN±SD Response (D)**
Emmetropes (N=28)	L	+0.09±0.33
	I	-1.39±0.31
	H	-3.66±0.35
Myopes (N=38)	L	+0.21±0.36
	I	-1.35±0.31
	H	-3.07±0.63

Table 8.1A Summary of mean±SD accommodation responses to the 3 accommodation stimulus levels for the two refractive groups.

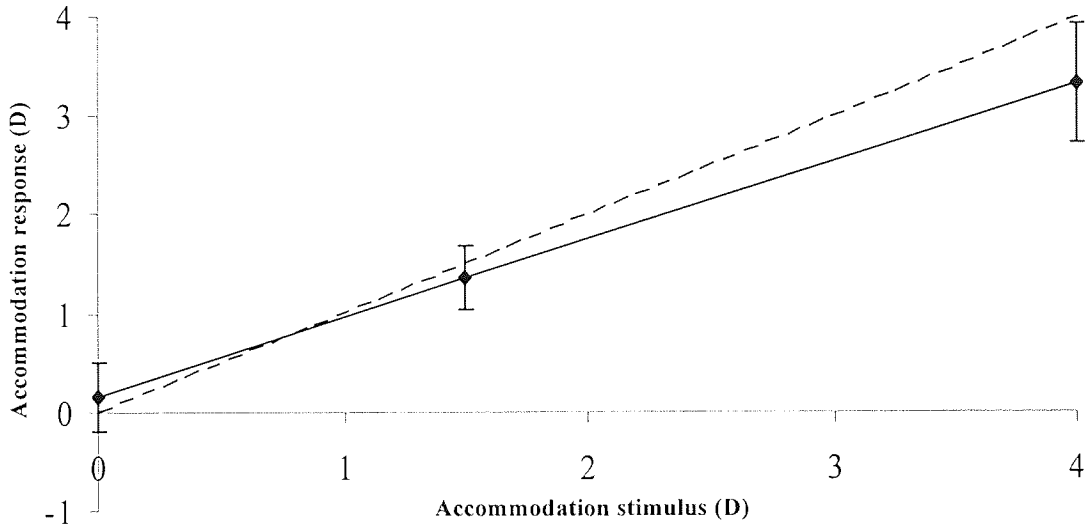


Figure 8.1A Accommodation stimulus-response curve where the error bars represent ±1 SD (n=66).

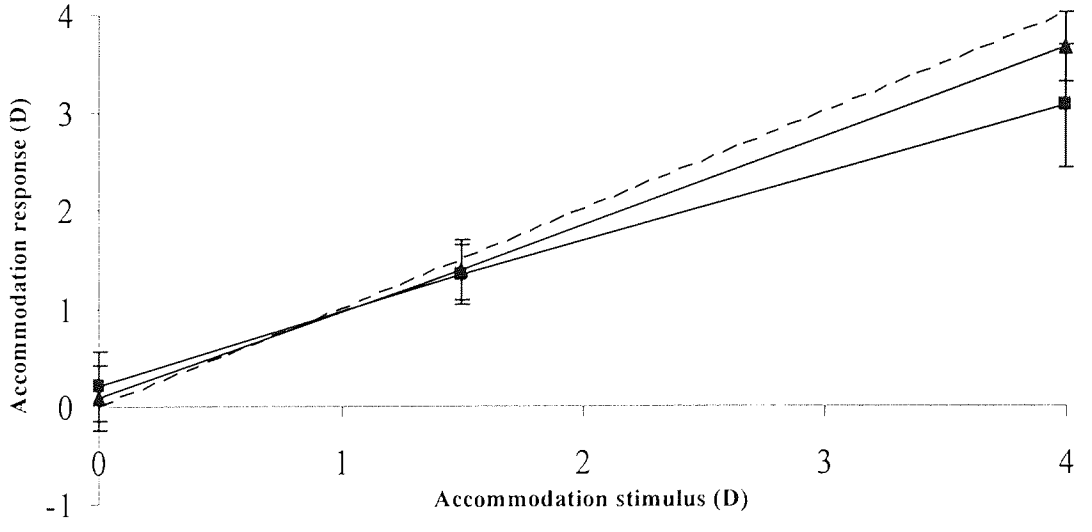


Figure 8.2A Accommodation stimulus-response curves for myopes (■, n=38) and emmetropes (▲, n=28) where the error bars represent ±1 SD.

The MN±SD of the accommodation response errors to the three accommodative stimulus levels are shown in **Table 6.2 (see Chapter 6)**. The ANOVA indicated that the accommodative response error was dependent on

the level of accommodation stimulus [F (1, 65) = 34.764, p=<0.001]. Scheffe *post-hoc* analyses concluded that the accommodation response errors for L and H (p<0.001) and I and H (p<0.001) accommodation stimulus levels varied significantly. However, the accommodation response errors for L and I accommodation stimulus levels were not statistically different (p=0.646). The between-refractive groups ANOVA indicated that the refractive error influenced the accommodative response errors [F (1, 64) = 20.59, p<0.001] such that myopes (MN±SD accommodation response errors; 0.53±0.49D) exhibited higher accommodative response errors than emmetropes (MN±SD accommodation response errors; 0.32±0.22D). Furthermore, a significant interaction effect between refractive groups and level of accommodation stimulus [F (1, 64) = 14.08, p<0.001] was evident.

The accommodative response stability was assessed by calculating the accommodative stimulus-response gradients (*m*) and the accommodative error indices (*I*) for each subject. Overall, the myopes demonstrated lower *m* (MN±SD; 0.72±0.17) compared to the emmetropes (MN±SD; 0.89±0.10) and this difference reached statistical significance (p<0.001). Furthermore, *I* was significantly (p=0.002) higher for the myopes (MN±SD; 4.27±4.63) compared to the emmetropes (MN±SD; 1.20±2.61).

Tonic accommodation was calculated from the cross-over point of the stimulus-response line and unity line on the accommodation stimulus-response graphs. The tonic accommodation was calculated for only 44 subjects, as the others failed to show a point of intersection on the accommodation stimulus-response graphs. The mean±SD tonic accommodation was 1.39±0.80D. Furthermore, the myopes (n=28) showed slightly lower values for tonic accommodation (MN±SD; 1.23±0.69) than the emmetropes (n=16) (MN±SD; 1.66±0.93) and the difference in tonic accommodation between the two refractive groups approached significance (p=0.086).

Discussion

Closed-loop accommodation responses to L, I and H accommodation stimuli within a +5D Badal system were measured with the Shin-Nippon autorefractor. The results show that the magnitude of the *ARE* on L accommodation stimulus levels (i.e. lead of accommodation) were significantly smaller than those on H levels of accommodation stimulus (i.e. lag of accommodation), however the difference between the *ARE* on L and I levels of accommodation stimuli did not reach statistical significance. Furthermore, the *ARE* on I accommodation stimulus levels were smaller than the *ARE* on H levels of accommodation stimuli. The results correspond well with the typical accommodation stimulus-response curve (for both myopes and emmetropes) which shows a lead of accommodation for low stimulus levels and increasingly larger lags at increasingly higher stimulus levels represented by the saturation region of the stimulus-response curve (Rosenfield *et al.*, 1993; Ciuffreda 1998; Gilmartin, 1998).

Overall, the myopes exhibited larger accommodative response errors than the emmetropes, which is in concordance with previous work done using a variety of methodologies (McBrien and Millodot, 1986 a; McBrien and Millodot, 1986 a; Tokoro, 1988; Bullimore, Gilmartin and Royston, 1992; Gwiazda *et al.*, 1993; Rosenfield, 1998; Nakatsuka *et al.*, 2005). The mean *m* for the emmetropes (0.89±0.10) found in this study differs only slightly from the gradient values for the emmetropes (0.79±0.06) found in a recent study by Seidel, Gray and Heron (2005). It has been suggested that the stabilisation of refractive error occurs at circa 15 years of age (Slataper, 1950; Goss and Winkler, 1983). Therefore, many studies (e.g. McBrien and Millodot, 1986 a; McBrien and Millodot, 1986 b; Ciuffreda and Wallis, 1998; Rosenfield and Gilmartin, 1988; Strang, Gray and Heron, 2000) classify myopia in to early-onset myopes (EOM) i.e., onset of myopia before the age of 15 years, and late-onset myopes (LOM) i.e., myopia onset after the age of 15 years. The average *m* for the EOM was 0.72±0.15 in a recent study by Seidel, Gray and Heron (2005), which compares well with the *m* for the myopes (0.72±0.17) in this study. Note that the majority (71%) of subjects in the present study cohort were EOM which may explain why the *m* for the myopes in this study compared well with the *m* for the EOM in the Seidel, Gray and Heron (2005) study. As myopes exhibit larger lags on H levels of accommodation stimuli than the emmetropes (McBrien and Millodot, 1986 a; Bullimore, Gilamrtin and Royston, 1992; Gwiazda *et al.*, 1993; Rosenfield, 1998; Nakatsuka *et al.*, 2005), *m* values for the myopes were significantly lower than the emmetropes. In contrast, Abbot, Schmid and Strang (1998) used a number of methodologies to measure the accommodation stimulus-response curves and concluded that the response gradients of myopes (LOM and EOM) and emmetropes did not differ.

The small number of LOMs in the subject group compared to the number of EOM precluded the useful analyses of the accommodative response function between these two refractive groups. Early research showed that LOMs have lower response slopes compared to the EOMs (McBrien and Millodot, 1986 a). However, a more recent study has concluded that there are no differences in the response gradients between the LOM and EOM when measured monocularly and binocularly in the presence of retinoscopic and/or spatiotopic cues (Seidel, Gray and Heron, 2005).

The accuracy of the linear regression slope *m* as a descriptor of the steady state response has been questioned and an alternative parameter, *I* has been proposed (Chauhan and Charman, 1995). *I* in this study was significantly higher for the myopes compared to the emmetropes. This is in contrast to the Seidel, Gray and Heron (2005) study which showed no significant difference in *I* between the emmetropes, LOM and EOM. Indeed, the validity of *m* and *I* calculations should not be overlooked. In addition to the issues with the use of *m* highlighted

by Chauhan and Charman (1995), thought must be given to the accuracy of the regression slope calculated by the least squares regression method using only 3 data points. Furthermore, Chauhan and Charman (1995) state that calculations of *I* can only be applied to regions on the accommodation stimulus-response curve that are considered to be linear. In this study only 3 data points are used so any 2 points utilised to encompass a linear region (over which *I* can be accurately applied) will result in a best fit line through these 2 data points with a r^2 value of 1. Therefore, *I* is calculated for the whole accommodation stimulus-response function which of course highlights inaccuracies in the results obtained from the analyses of *I* in the present study.

In conclusion, consistent with previous studies (McBrien and Millodot, 1986 a; Rosenfield and Gilmartin, 1988; Bullimore, Gilmartin and Royston, 1992; Gwiazda *et al.*, 1993; Gwiazda *et al.*, 1995; Rosenfield, 1998; Nakatsuka *et al.*, 2005), analyses of all three parameters of the accommodation stimulus-response function (*ARE*, *m* and *I*) indicate that the myopes exhibit greater accommodative inaccuracies than the non-myopes. To date the debate on whether an increased accommodative lag is a cause or consequence of myopia still continues. Recent work by Mutti *et al.* (2006) concludes that no differences in accommodative lag were found in emmetropic subjects and those who became myopic. However, consistent elevations in accommodative lag were present after the onset of myopia. Mutti *et al.* (2006) therefore envisages that increased accommodative lags are not a predicative factor of myopia onset as suggested by others (Gwiazda *et al.*, 2004; Allen and O'Leary, 2005; Gwiazda, Thorn and Held, 2005).

The response of the accommodative system in the absence of any visual stimuli is defined as the tonic accommodation (Rosenfield *et al.*, 1993). A wide range of tonic accommodation values are reported in the literature due to different experimental methodologies (Leibowitz and Owens, 1978; Post, Johnson and Tsuetaki, 1984; McBrien and Millodot, 1987; Rosenfield 1989; Rosenfield *et al.*, 1993; Rosenfield *et al.*, 1994; Jiang 1995; Jiang and Morse, 1999; Zadnik *et al.*, 1999; Allen and O'Leary, 2006). The mean tonic accommodation for the cohort in the present study (1.39±0.38D) falls within this wide range. Although the present study concludes that no differences in tonic accommodation exist between myopes and emmetropes as reported by Woung *et al.* (1993), Strang *et al.* (2000) and Allen and O'Leary, (2006), the difference in TA between myopes and emmetropes approaches significance such that myopes have slightly lower tonic accommodation values than emmetropes. Indeed, a number of studies have reported that myopes have lower tonic accommodation values than emmetropes (Maddock *et al.*, 1981; Bullimore and Gilmartin, 1987; Rosner and Rosener, 1989; Rosenfield and Ciuffreda, 1991; Adams and McBrien, 1993; Jiang 1995; Zadnik *et al.*, 1999, Allen and O'Leary, 2006), although some early work suggests that myopes have higher tonic accommodation values than emmetropes (Gawron, 1981; Simonelli, 1983).

As mentioned earlier, differences in accommodation components between LOM and EOM were not examined in the current study. Previous studies which define myopia based on the age of onset have demonstrated that tonic accommodation is lower in LOM compared to EOM (McBrien and Millodot, 1987; McBrien and Millodot, 1988). However, more recent studies have shown that there are no differences in TA between LOM and EOM (Rosenfield and Gilmartin, 1987; Woung *et al.*, 1993; Strang *et al.*, 2000; Allen and O'Leary, 2006). Furthermore, Allen and O'Leary (2006) have demonstrated that the TA is in fact higher in LOM compared to EOM.

To date the studies mapping the relationship between tonic accommodation and refractive error yield equivocal results. The discrepancies between the results are attributable to differences in methodologies for example, instrumentation used, viewing conditions, cognitive effort, subject group and refractive criterion. Despite this, the inter-subject variations in TA (Rosenfield *et al.*, 1993; Rosenfield *et al.*, 1994) have been implicated in the aetiology of myopia (Strang, Winn and Gilmartin, 1994; Gwiazda *et al.*, 1995; Jiang, 1995; Zadnik *et al.*, 1999; Allen and O'Leary, 2006). For example, it has been suggested that the attenuation of the post-task regression of TA reflects a deficit in the sympathetic innervation of the ciliary muscle which leads to myopia (Gilmartin and Bullimore, 1991; Strang, Winn and Gilmartin, 1994). More recently, Allen and O'Leary, (2006) evaluated different accommodative functions and concluded that tonic accommodation was not a predictive factor of myopic progression.

Of note is that the hypothesis that the cross-over point of the accommodation stimulus-response curve and the virtual unity line is indicative of tonic accommodation (Ramsdale and Charman, 1989) has been challenged (Ciufredda *et al.*, 1989; Ong, Ciuffreda and Tannen, 1993). The value of TA obtained from the stimulus-response curves is typically lower than open-loop accommodation values obtained from other methodologies (e.g. Difference of Gaussian targets) (Ciufredda *et al.*, 1984; Ong, Ciuffreda and Tannen, 1993; Rosenfield *et al.*, 1993; Davies, 2004). Furthermore, in this study, the accuracy of the stimulus-response curve from which the cross-over point is derived is limited as the best-fit line is drawn from only 3 data points.

The relationship between accommodation and myopia onset and development has been extensively studied; however the exact interaction remains elusive (reviewed by Gilmartin, 2004). Despite this, the consensus is that prolonged periods of hyperopic retinal defocus provoke ocular growth. It is therefore postulated that the greater inaccuracies in the accommodative system of the myope, compared to that of the emmetrope is a risk factor or is the underlying mechanism of myopia onset and progression (Goss, 1991; Gwiazda *et al.*, 1993; Drobe and de

Saint-Andre, 1995; Gwiazda *et al.*, 1995; Jiang, 1995; Rosenfield and Gilmartin, 1998; Charman 1999; Gwiazda, Thorn and Held, 2005; Allen and O'Leary, 2006). Many animal studies have induced myopic and hyperopic refractive errors by manipulating hyperopic and myopic defocus, respectively (Wallman *et al.*, 1978; Wiesel and Raviola, 1977; Schaeffel, Glasser and Howland 1988; Irving, Sivak and Callender, 1992; Bartmann *et al.*, 1994; Wildsoet and Wallman, 1995; Diether and Schaeffel, 1997; Wildsoet 1997; Smith and Hung, 1999; McFadden, Howlett and Mertz, 2004).

One proposition is that myopes have reduced blur sensitivity, which decreases the error signal to the blur sensitive (primary retinotopic stimulus) accommodation system and therefore causes impaired accommodative responses and flat regression slopes (Rosenfield and Abraham-Cohen, 1999; Seidel, Gray and Heron, 2003). It has been hypothesised that flat regression slopes decrease the modulation transfer for small details, necessitating a reduced working distance. Objects held closer than normal would result in larger lags than normal and therefore enhanced retinal defocus, which is thought to be a precursor of myopia development (Charman, 1999). This notion is supported by evidence that myopes are inclined to have shorter working distances than emmetropes (Gwiazda *et al.*, 1993; Gwiazda *et al.*, 1995).

An alternative hypothesis is that the slow blur-driven accommodative adaptation serves to decrease accommodative lag during closework (Schor *et al.*, 1986; Rosenfield and Gilmartin, 1998; 1999). A reduction in the slow accommodative adaptation mechanism would result in the accommodative response errors to be maintained throughout the near vision task, resulting in prolonged hyperopic blur and hence myopia (Rosenfield and Gilmartin, 1998; 1999). As fast accommodative adaptation appears to be solely innervated by the parasympathetic nervous system and slow accommodative adaptation by both the sympathetic and parasympathetic nervous systems (Rosenfield and Gilmartin, 1989; Gilmartin and Winfield, 1995; Gilmartin, 1998; Winn *et al.*, 2002), a compromised slow accommodative adaptation system would exhibit compromised sympathetic innervation to the ciliary muscle (Rosenfield and Gilmartin, 1999). Interestingly, reduced sympathetic activity of the ciliary muscle has been suggested in LOM (Rosenfield and Gilmartin, 1998; Gilmartin, 1998; Gilmartin and Bullimore, 1991; Strang, Winn and Gilmartin, 1994) although the proposal is equivocal (Gilmartin and Winfield, 1995).

Interestingly other models mapping the relationship between accommodation and myopia onset and progression have proposed, that the accommodation control system simply refers to the presence of retinal defocus, regardless of the amount or direction of blur present (Yamada and Ukai, 1997; Hung and Ciuffreda, 2000). This notion is supported by the results of the COMET study in which the use of progressive addition lenses, which eliminated retinal defocus at near slowed the progression of myopic development, albeit the magnitude of effect was small (Gwiazda *et al.*, 2003). In addition, the under-correction of human myopia which subsequently induces myopic blur paradoxically increased the rate of myopic progression (Chung, Mohidin and O'Leary, 2002), a result dissimilar to that yielded by animal studies (Irving, Sivak and Callender, 1992; Bartmann *et al.*, 1994; Wildsoet and Wallman, 1995; Diether and Schaeffel, 1997; Wildsoet 1997; Smith and Hung, 1999).

Conclusion

The present study has investigated the accommodative response function in myopes and emmetropes; the results of which are mostly in agreement with previous literature. The main findings of this study are:

- Accommodative response errors increase as the accommodative stimulus levels increase.
- Myopes exhibit larger accommodative response errors, higher accommodative error indices (I) and lower regression slopes (m) compared to the emmetropes.
- Tonic accommodation does not depend on refractive error.

APPENDIX 9

PERCENTAGE CHANGES IN IOP WITH ACCOMMODATION WHILST MEASURING ACCOMMODATION RESPONSES

The mean±SD percentage change in IOP between L and I, L and H, and I and H accommodation stimulus levels was respectively -4.23±7.05, -0.55±10.23 and 4.19±11.50 mmHg. The within-subjects ANOVA shows that the level of accommodative stimuli does influence the percentage changes in IOP [F (2, 78) = 10.106, p=0.002]. Bonferroni *post-hoc* analyses revealed that the differences in percentage changes in IOP on accommodation reached statistical significance between L and I (p<0.001) and I and H (p=0.03) but not between L and H (p=0.78) accommodative stimuli levels.

The MN±SD of the differences in percentage changes in IOP between L and I and L and H accommodation levels for the emmetropes were respectively, -0.95±8.90 and -4.08±7.63 mmHg and for the myopes the MN±SD of the differences in IOP between L and I and L and H accommodation levels were respectively, 0.26±11.60 and -4.44±6.84 mmHg. The between-refractive groups ANOVA results (F=0.106, p=0.747) together with correlation graphs of refractive error and differences in IOP between L and I (r=0.18, p=0.27), L and H (r=0.19, p=0.23) and I and H (r=0.08, p=0.65) levels of accommodation shown provide evidence that the inter-subject variations in refractive error do not account for the inter-subject variations in IOP responses to accommodation (see **Figure 9.1A**).

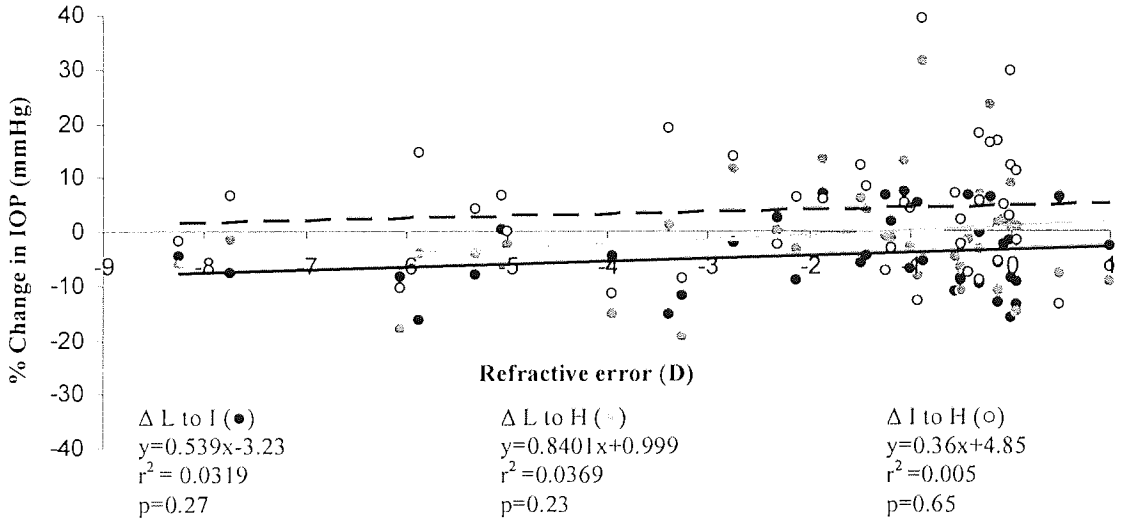


Figure 9.1A Refractive error against the % changes in IOP between L and I (—), L and H ( ) and I and H (---) accommodation stimulus levels (n=40).

APPENDIX 10

PERCENTAGE CHANGES IN IOP AND BLOODFLOW WITH ACCOMMODATION

IOP and accommodation

The mean±SD percentage change in IOP as measured with the Ocular Blood Flow Analyser (OBFA) between L and I, L and H, and I and H accommodation stimulus levels was respectively 0.43±26.76, -6.72±20.51 and -5.10±14.49 mmHg. The within-subjects ANOVA shows that the level of accommodative stimuli does influence the percentage changes in IOP as measured with the OBFA [F (2, 68) = 1.336, p=0.261].

The MN±SD of the differences in percentage changes in IOP between L and I and L and H accommodation levels for the emmetropes were respectively, 1.53±30.28 and -7.81±25.14 mmHg and for the myopes the MN±SD of the differences in IOP between L and I and L and H accommodation levels were respectively, -0.73±23.35 and -5.56±14.82 mmHg. The between-refractive groups ANOVA results (F=0.06, p=0.809) together with correlation graphs of refractive error and differences in IOP between L and I (r=0.15, p=0.38), L and H (r=0.002, p=0.99) and I and H (r=0.23, p=0.18) levels of accommodation shown provide evidence that the inter-subject variations in refractive error do not account for the inter-subject variations in IOP responses to accommodation (see **Figure 10.1A**). Furthermore, **Figure 10.2A** shows that the variations in percentages changes in IOP responses between L and I (r=0.09, p=0.61), L and H (r=0.002, p=0.99) and I and H (r=0.17, p=0.33) were not related to axial length.

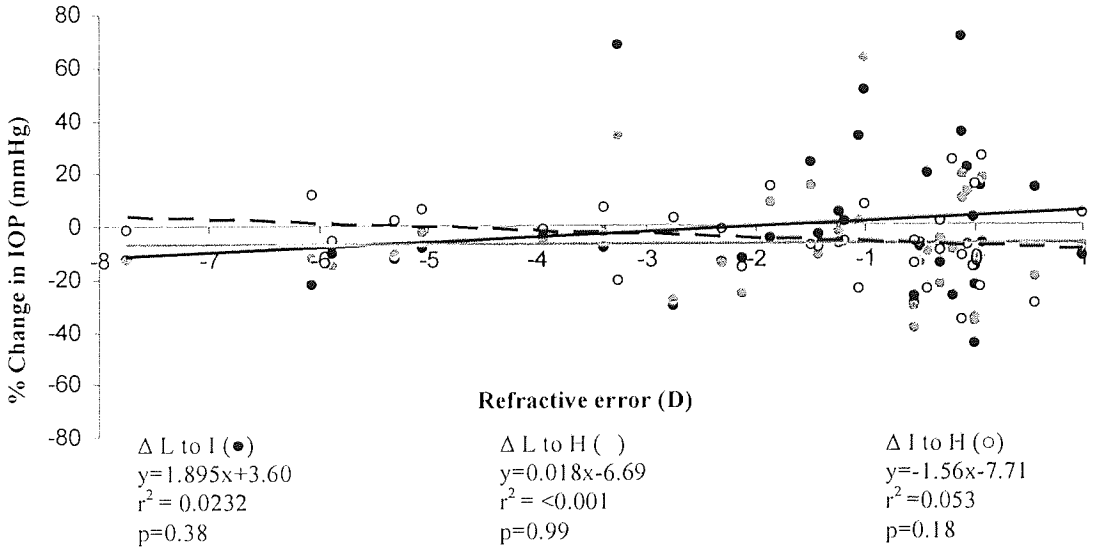


Figure 10.1A Refractive error against the % changes in IOP as measured with the OBFA between L and I (—), L and H ( ) and I and H (---) accommodation stimulus levels (n=35).

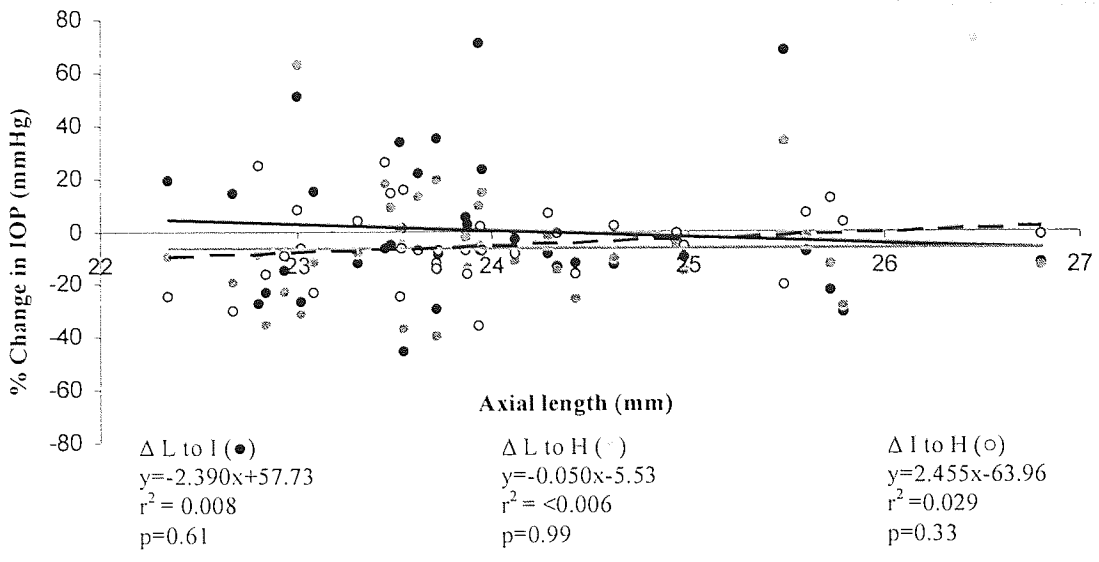
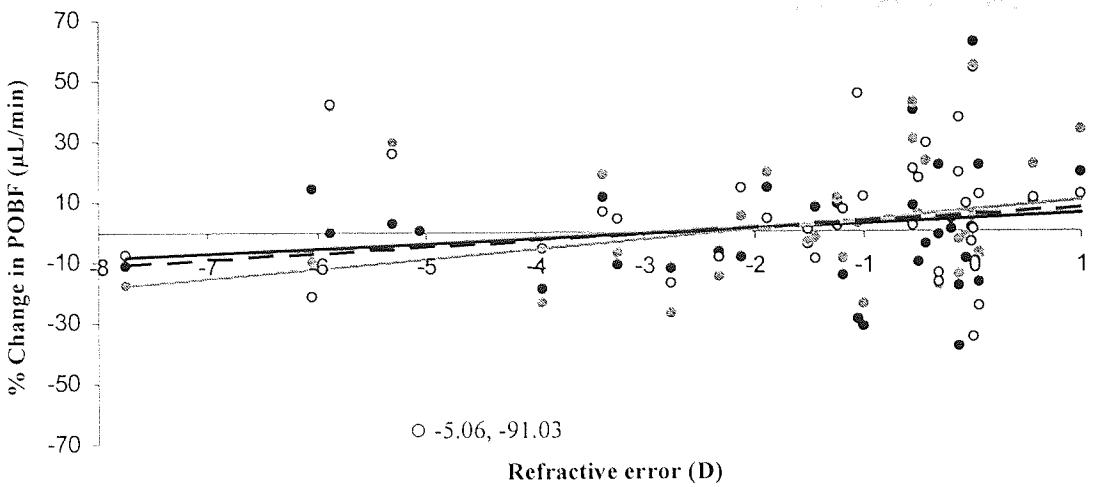


Figure 10.2A Axial length against the % changes in IOP as measured with the OBFA between L and I (—), L and H ( ) and I and H (---) accommodation stimulus levels (n=35).

POBF and accommodation

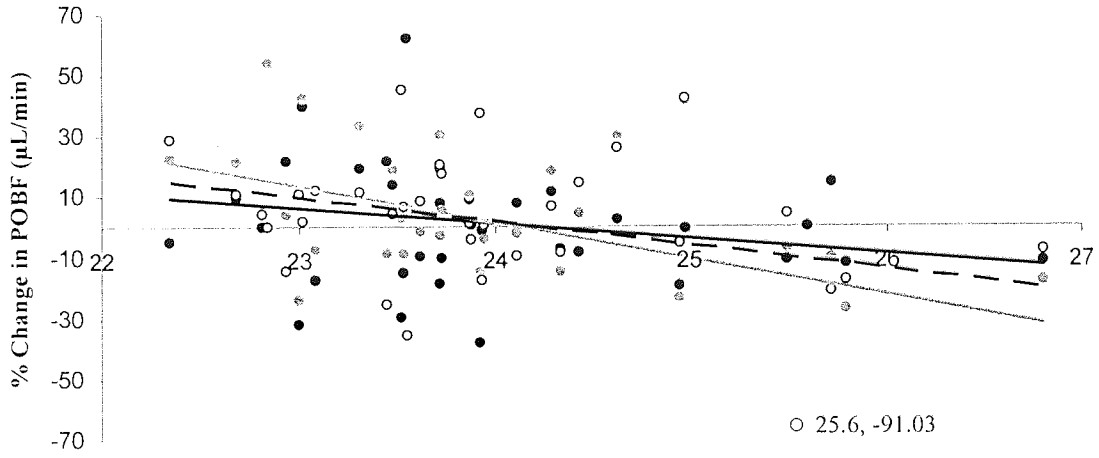
The mean±SD percentage change in POBF between L and I, L and H, and I and H accommodation stimulus levels was respectively 1.29±21.58, 1.57±25.90 and 2.16±24.41 mmHg. The within-subjects ANOVA shows that the level of accommodative stimuli does influence the percentage changes in POBF [F (2, 68) = 0.017, p=0.944].

The MN±SD of the differences in percentage changes in POBF between L and I and L and H accommodation levels for the emmetropes were respectively, 5.83±27.22 and 7.70±21.71 mmHg and for the myopes the MN±SD of the differences in IOP between L and I and L and H accommodation levels were respectively, -3.52±12.43 and -4.92±28.94 mmHg. The between-refractive groups ANOVA results (F=2.581, p=0.118) together with correlation graphs of refractive error and differences in POBF between L and I (r=0.16, p=0.36), L and H (r=0.27, p=0.12) and I and H (r=0.19, p=0.28) levels of accommodation shown provide evidence that the inter-subject variations in refractive error do not account for the inter-subject variations in POBF responses to accommodation (see **Figure 10.3A**). Furthermore, **Figure 10.4A** shows the correlation between axial length and the variations in POBF responses between L and I (r=0.23, p=0.18) and I and H (r=0.32, p=0.08) levels of accommodation were not statistically significant. However, a significant correlation existed between axial length and the variations in POBF responses between L and H (r=0.46, p=0.005) levels of accommodation.



Δ L to I (●)	Δ L to H ( )	Δ I to H (○)
y=1.600x+3.96	y=3.197x+6.91	y=2.138x+5.738
r^2 = 0.0254	r^2 = 0.071	r^2 =0.036
p=0.36	p=0.12	p=0.28

Figure 10.3A Refractive error against the % changes in POBF between L and I (—), L and H ( ) and I and H (---) accommodation stimulus levels (n=35).



Δ L to I (●)	Δ L to H ( )	Δ I to H (○)
y=-5.00x+121.1	y=-11.98x+288.69	y=-7.794x+189.0
r^2 = 0.0547	r^2 = 0.218	r^2 =0.1039
p=0.18	p=0.005	p=0.06

Figure 10.4A Axial length against the % changes in POBF between L and I (—), L and H ( ) and I and H (---) accommodation stimulus levels (n=35).

APPENDIX 11

REANALYSES OF PULSE AMPLITUDE AND ACCOMMODATION DATA

It is clear from **Figures 8.7** and **8.8** that the responses of subject 28 do not match the responses of the other subjects. Reanalyses of the pulse amplitude (PA) data are presented here having excluded the data of subject 28. The within subjects ANOVA shows that for the cohort accommodation does not influence the PA (F=0.302, p=0.611).

The variations in PA responses between L and I (r=0.009, p=0.96), L and H (r=0.27, p=0.12) and I and H (r=0.20, p=0.25) levels of accommodation were not associated with refractive error as shown in **Figure 11.1A.** Likewise, **Figure 11.2A** shows the correlation between axial length and the variations in PA responses between L and I (r=0.08, p=0.66), L and H (r=0.16, p=0.38), and I and H (r=0.04, p=0.81) levels of accommodation.

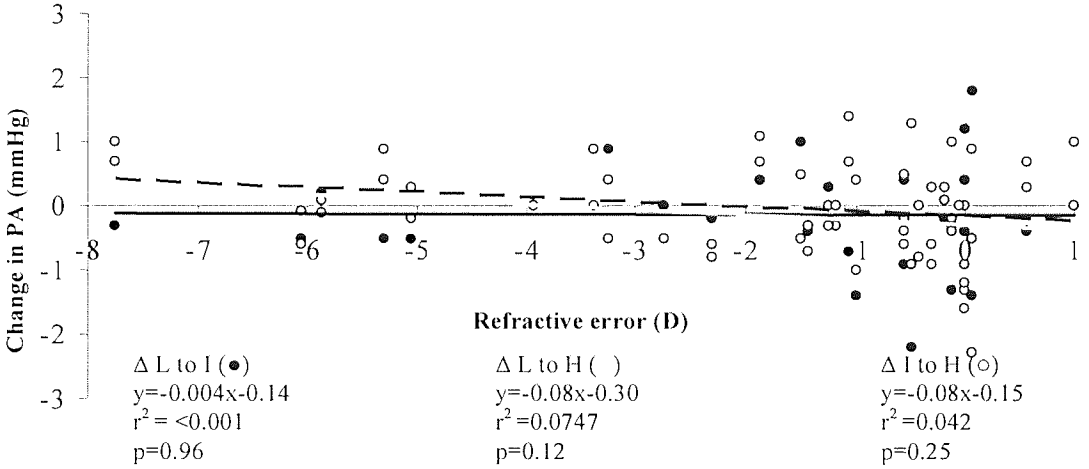


Figure 11.1A Level of refractive error and change in PA between L and I (—), L and H ( ) and I and H (---) levels of accommodation excluding data from subject 28 (n=34).

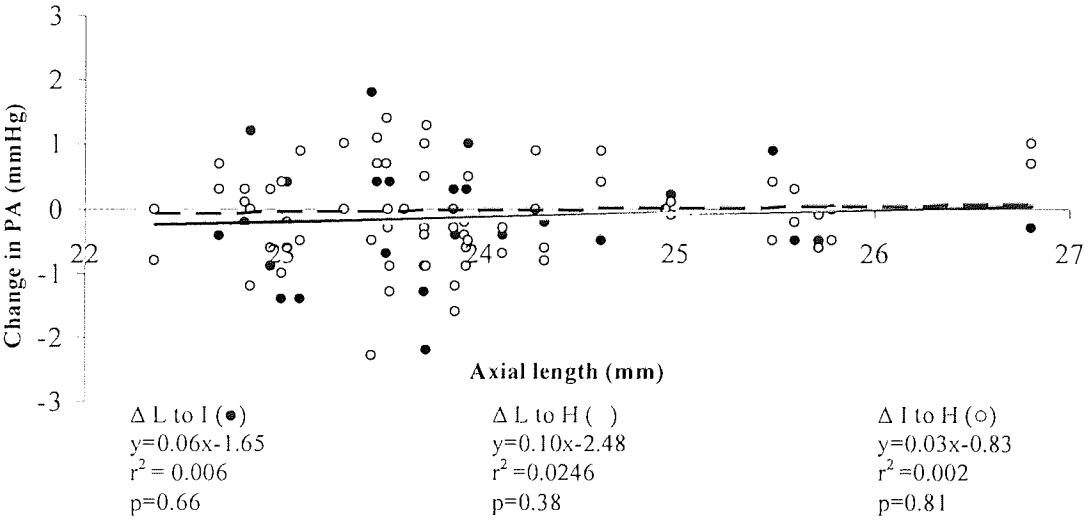


Figure 11.2A Axial length and change in PA between L and I (—), L and H ( ) and I and H (---) levels of accommodation excluding data from subject 28 (n=34).

APPENDIX 12

THE EFFECTS OF REFRACTIVE ERROR AND AXIAL LENGTH ON BLOOD FLOW PARAMETERS

Introduction

The data collected in **Chapter 8**, was also used to determine the effect of refractive error and axial length on ocular blood flow parameters. The effects of refractive error and axial length on pulse amplitude (PA), pulse volume (PV) and pulsatile ocular blood flow (POBF) values were assessed by determining Pearson's correlation coefficients and associated significance levels. Independent t-tests were performed to determine if ocular blood flow parameters differed between refractive groups followed by an analysis of covariance (ANCOVA) to separate the effects of axial length. The subject group and methodologies used are discussed in **Chapter 8**.

Results

Pulse amplitude

The correlation graphs between PA and refractive error and PA and axial length are shown in **Figures 12.1A** and **8.2A**, respectively. The results show that the PA is correlated with refractive error (r=0.44, p=0.009) and axial length (r=0.44, p=0.008), such that as the level of myopia and axial length increases the PA reduces. The difference between the PA values of the myopes (2.59±0.71 mmHg) and emmetropes (3.64±1.12 mmHg) was statistically significant (p=0.003). Several other studies have also found that axial length influences the PA measures (James *et al.*, 1991; Shih *et al.*, 1991; Ravalico *et al.*, 1997; Mori *et al.*, 2001; Lam *et al.*, 2003) therefore when axial length was taken account of, the results of an ANCOVA analyses show that the differences between PA values of the emmetropes and myopes are not statistically different (F (2, 31) = 3.066, p=0.090).

Pulse volume

A significant relationship was evident between PV and refractive error (r=0.41, p=0.01) and axial length (r=0.45, p=0.006). The correlation graphs shown in **Figure 12.3A** and **12.4A** respectively show that as refractive error and axial length increases the PV decreases. The PV values for the myopes and emmetropes were 7.38±2.46 and 10.15±3.30 μL/min, respectively. The difference in PV values between the two refractive groups was significant (p=0.009), although when axial length was accounted for the relationship was subsequently rendered insignificant (F (2, 31) = 1.091, p=0.304).

Pulse rate

No relationship was evident between PR and refractive error (r=0.25, p=0.15) and axial length (r=0.17, p=0.33). The correlation graphs are shown in **Figure 12.5A** and **12.6A.** The PR values for the myopes and emmetropes were 78.6±15.76 and 69.4±14.59 beats/min, respectively. The difference in PR values between the two refractive groups was not significant (F (1, 33) = 3.142, =0.086).

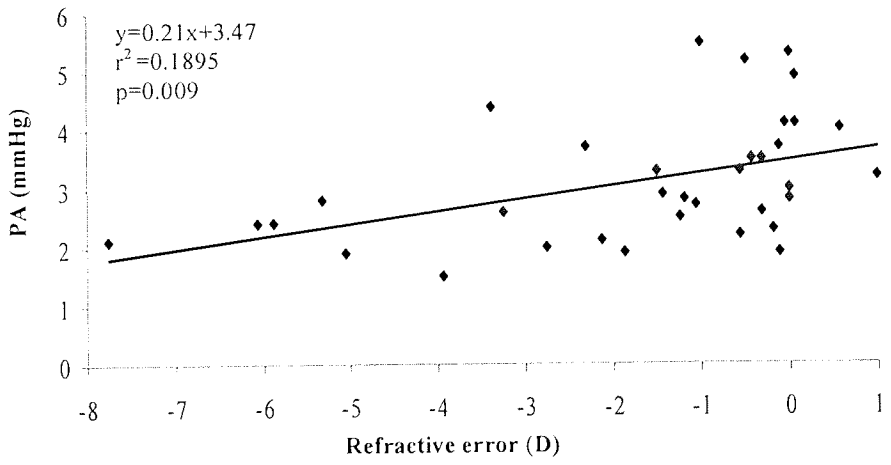


Figure 12.1A Correlation between refractive error and PA (n=35).

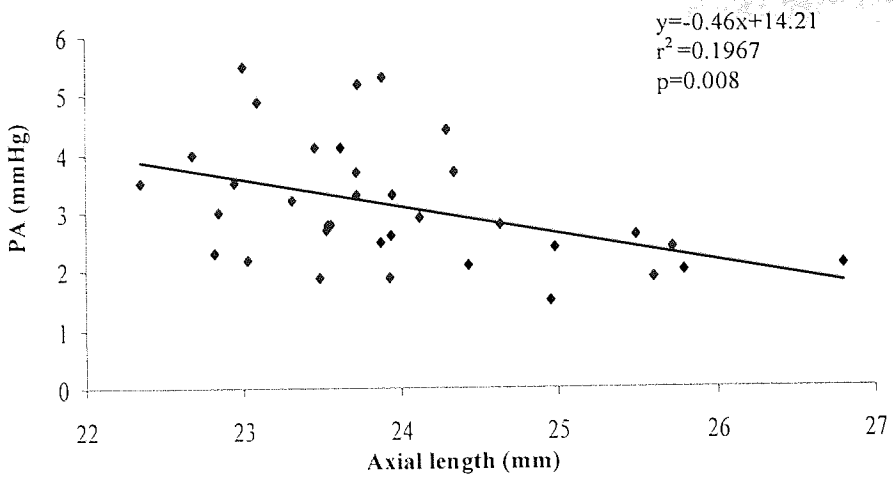


Figure 12.2A Correlation between axial length and PA (n=35).

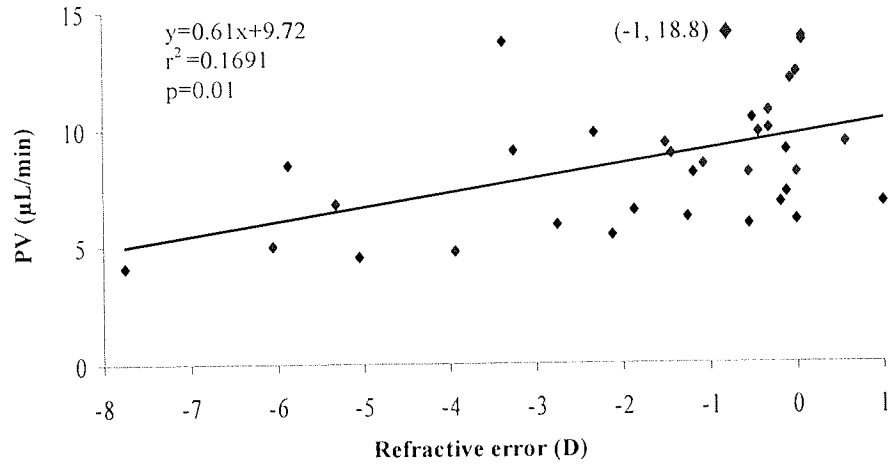


Figure 12.3A Correlation between refractive error and PV (n=35).

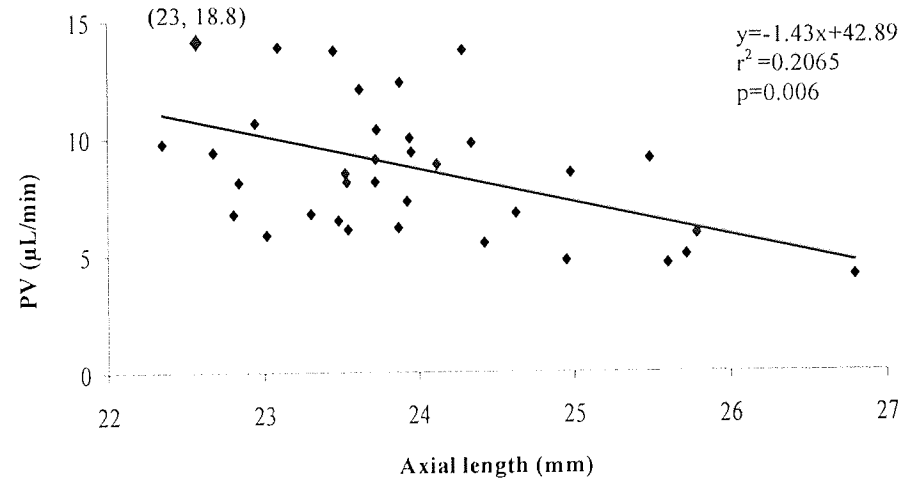


Figure 12.4A Correlation between axial length and PV (n=35).

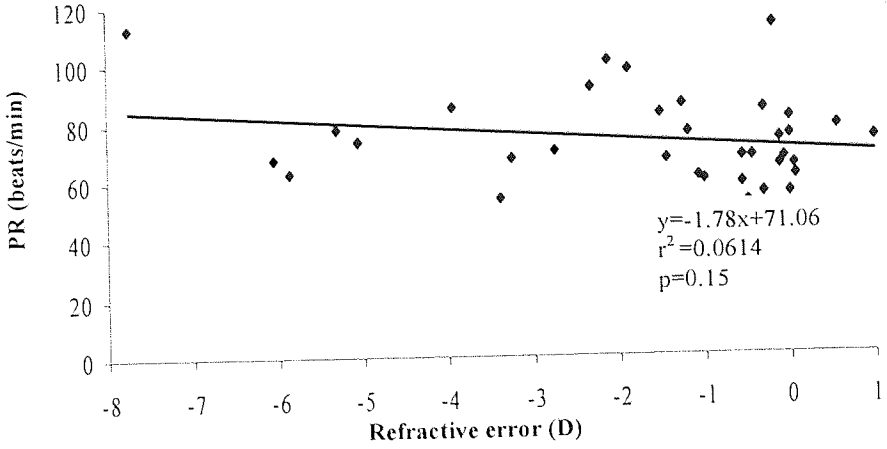


Figure 12.5A Correlation between refractive error and PR (n=35).

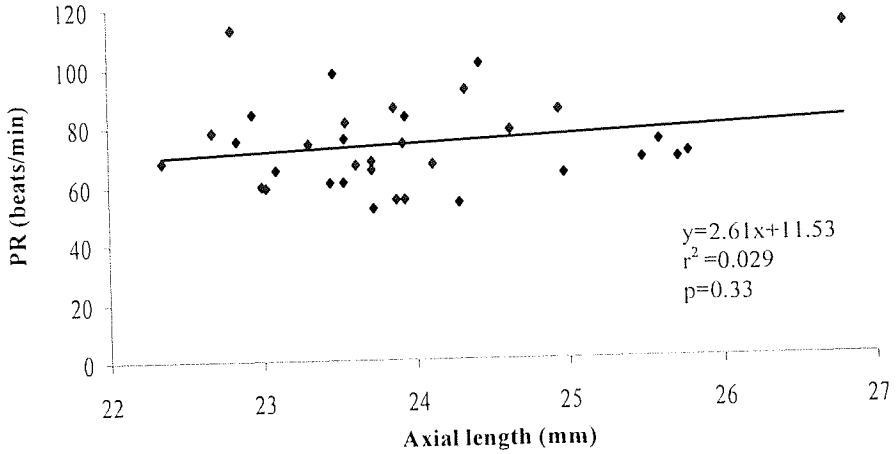


Figure 12.6A Correlation between axial length and PR (n=35).

Pulsatile ocular blood flow

The POBF values were also correlated with refractive error (as shown in **Figure 12.7A**; r=0.36, p=0.03) and axial length (as shown in **Figure 12.8A**; r=0.42, p=0.01), such that as the level of myopia and axial length increases the POBF values reduce. The POBF values for the myopes and emmetropes were respectively 1236.18±281.62 and 1498.06±406.00 μL/min. Since the literature suggests that females have higher POBF values than males (Centofanti *et al.*, 2000; Centofanti *et al.*, 2002; Agarwal *et al.*, 2003), the 2 refractive groups were matched for gender and the difference between the POBF values between the 2 refractive groups just reached statistical significance (p=0.04). Furthermore, it is well documented that axial length influences POBF values (James *et al.*, 1991; Shih *et al.*, 1991; Ravalico *et al.*, 1997; Mori *et al.*, 2001; Lam *et al.*, 2003). Therefore when the effect of axial length was considered, the results of an ANCOVA analyses shows that the POBF values did not differ between myopes and emmetropes ($F(2, 31) = 0.234$, p=0.632).

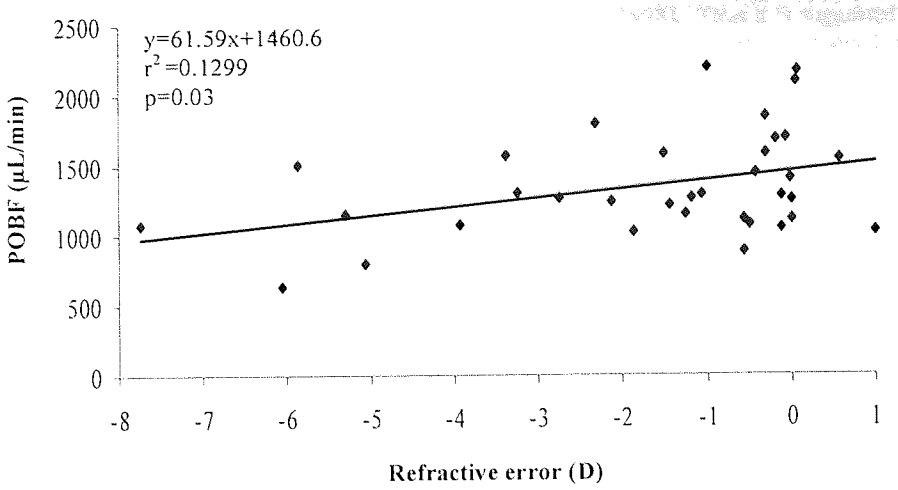


Figure 12.7A Correlation between refractive error and POBF (n=35).

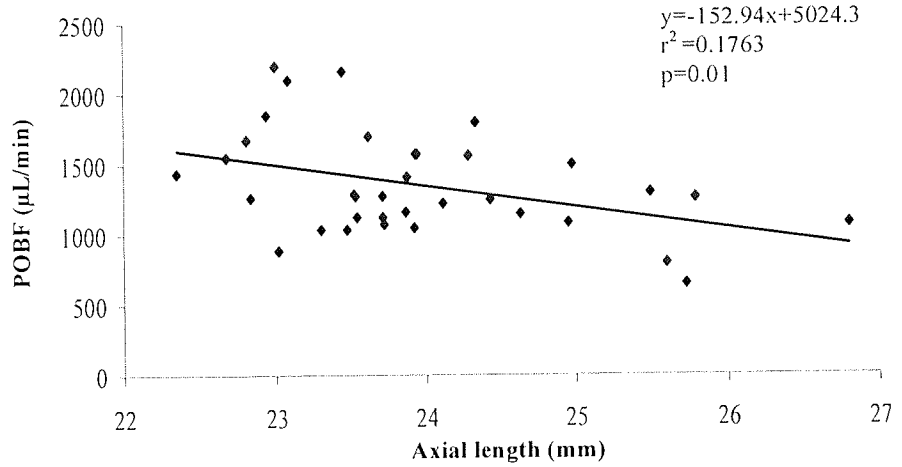


Figure 12.8A Correlation between axial length and POBF (n=35).

Discussion

The results are generally consistent with previous studies in that PA is shown to reduce with increasing axial length (James *et al.*, 1991; Ravalico *et al.*, 1997; Lam *et al.*, 2003; Shih *et al.*, 1991), increasing myopia (James *et al.*, 1991; Ravalico *et al.*, 1997) and that PA is significantly lower in myopes than emmetropes (To'mey *et al.*, 1981; James *et al.*, 1991; Shih *et al.*, 1991; Ravalico *et al.*, 1997). The present study also shows that PV and POBF reduce with increasing axial length (James *et al.*, 1991; Ravalico *et al.*, 1997; Mori *et al.*, 2001; Lam *et al.*, 2002; Lam *et al.*, 2003; Lam and Lam, 2004) and with increasing myopia (James *et al.*, 1991; Ravalico *et al.*, 1997). The correlation coefficients between PA and axial length and refractive error, and POBF values and axial length and refractive error found in the current study are similar to those found by Benevente-Perez (Unpublished PhD data).

There is evidence in the literature which suggests that females have higher POBF values than males (Agarwal *et al.*, 2003; Centofanti *et al.*, 2000; Centofanti *et al.*, 2002). Agrawal *et al.* (2003) suggested and this was due to a higher pulse rate in females compared to males. However, when differences in pulse rate were taken into account, the POBF values remained higher in females compared to males (Gekkieva *et al.*, 2001). It has hence been suggested that choroidal circulation is effected by hormones (Centofanti *et al.*, 2000) and that the gender differences in POBF may be due to differences in the levels of oestrogen (Centofanti *et al.*, 2000; Centofanti *et al.*, 2002). In the present study, the ratio of females to males was approximately 2:1 and therefore this precluded the analyses of the effect of gender on POBF values. Nevertheless, since gender differences in POBF values are well documented, the sample was matched for gender. The results showed that having considered the effects of gender on POBF values, the POBF values were significantly lower in the myopic group compared to the emmetropic group.

Many authors have reported on the relationship between axial length, PA and POBF and it has been suggested that the ocular volume changes proportionally with axial length (James *et al.*, 1991; Shih et al., 1991; Ravalico *et al.*, 1997; Mori *et al.*, 2001; Lam *et al.*, 2003). A limitation of the OBFA is that the transmission of the changes

in ocular volume caused by the cardiac cycle are weakened with increasing axial length and therefore result in a reduction in the PA measures and hence PV and POBF values (James, 1998). Thus it is suggested that the axial length needs to be considered when measuring the PA and POBF (Lam *et al.*, 2003). Indeed, in the present study when differences in axial length are accounted for the differences in PA, PV and POBF between myopes and emmetropes are eliminated.

Hitherto, little literature exists on the vascular status of the myopic eye and it is unclear whether reduced ocular blood flow in the myopic eye is a cause or consequence of myopic onset and progression in humans. In the chick, when form deprivation was induced by goggles a reduction in choroidal blood flow (measured by Laser Doppler Velocimetry) and elongation of the vitreous chamber was observed (Shih *et al.*, 1993). It was unclear whether the changes in chorodial blood flow were a result of vitreous elongation or a consequence of an increase in temperature caused by the goggles as observed by Hodos, Revzin and Kuenzel (1987). In order to induce form deprivation while removing the thermal effects of the goggles, Shih, Fitzgerald and Reiner (1993 a) performed corneal incisions on the chick eye. The results from this study showed that ocular elongation occurred and choroidal blood flow reduced, hence suggesting that the altered blood flow observed was not in response to changes in ocular temperature. Furthermore, when the choroidal blood flow was artificially reduced by transecting the choroidal nerves of the ciliary ganglion, the choroidal blood flow was indeed reduced although an increase in axial length was not observed (Shih, Fitzgerald and Reiner, 1993 b). These studies concluded that in the chick eye, the reduced choroidal blood flow is a consequence of axial elongation rather than a cause of axial growth.

In contrast, Fitzgerald, Wildsoet and Reiner (2002) showed that when form deprivation was induced by diffusing goggles in the chick eye, a reduction in choroidal blood flow preceded the thinning of the choroid. It is unclear why a reduction in choroidal blood flow would result from blur induced from form-deprivation. It is hypothesised that to achieve focus, a compensatory mechanism is triggered in which the reduction in choroidal blood flow would lead to changes in choroidal thickness and hence changes in axial length suggesting a vascular aetiology of myopia. Although the present study demonstrates no significant differences in the blood flow of myopes and emmetropes it is emphasised that the results maybe confounded by the assumptions and limitations of the OBFA. Of interest is that unpublished PhD data (Benevente-Perez) shows the blood velocity is significantly reduced in the central retinal artery in myopes. Since the short posterior ciliary arteries (which supply the choroid) are a branch of the central retinal artery a reasonable assumption would be that the blood flow is also reduced in the former arteries, hence supporting the hypothesis of a vascular aetiology of myopia.

Conclusions

- Myopes have lower PA, PV and POBF values than emmetropes. As the level of myopia and axial length increase the PA, PV and POBF values reduce. However, when the effects of axial length are accounted for, no such differences in PA, PV and POBF between myopes and emmetropes are evident.

APPENDIX 13

COMPARISON OF DIFFERENT TECHNIQUES OF ANTERIOR CHAMBER DEPTH, KERATOMETRIC AND PACHYMETRIC MEASUREMENTS.

Introduction

Anterior chamber depth (ACD) and corneal curvature (CC) measurements were taken with the IOL Master (Carl Zeiss), Pentacam Comprehensive Eye Scanner (Oculus, Inc.) and Orbscan (Bausch and Lomb) instruments in 50 subjects. Furthermore, central corneal thickness (CCT) measurements were also performed on the same 50 subjects with the Ultrasound Pachymeter (*DGH-550 Pachette 2*, DGH technology, US), Pentacam and Orbscan instruments. The methodologies are detailed in **Chapter 9**.

The CCT, ACD and CC data taken with the different instruments were compared. The CCT, ACD and CC measured by the three methods were compared using within-subject one-way ANOVAs in randomised blocks followed by Scheffé *post-hoc* analyses. Furthermore Bland and Altman and correlation analyses were also performed.

Results

Pachymetry

The CCT values for the cohort as measured by the 3 modalities are summarised in **Table 13.1A.** The results of the one-way repeated measures ANOVA was statistically significant ($F(2, 98)=43.761$, $p<0.001$) and the *post-hoc* analyses are shown in **Table 13.2A**. The Bland and Altman plots of the differences in CCT values as a function of their mean and the limits of agreement for the 3 instruments are shown in **Figure 13.1A. Figure 13.2A** illustrates that the CCT measures taken with the Pentacam and Ultrasound Pachymeter ($r=0.45$, $p<0.001$) and Pentacam and Orbscan ($r=0.51$, $p<0.001$) are correlated. The CCT values measured by the Ultrasound Pachymeter and Orbscan were highly correlated ($r=0.92$, $p<0.001$).

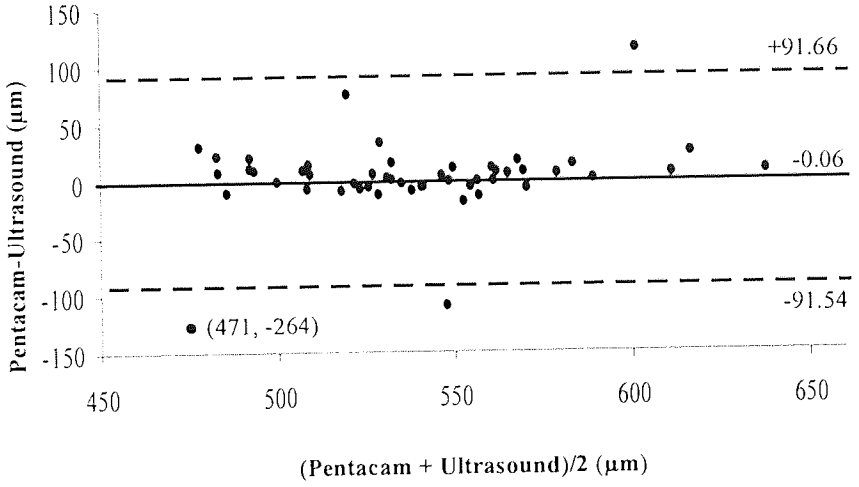
	Instrument		
	Pentacam (μm)	**Orbscan (μm)**	**Ultrasound (μm)**
Mean±SD	539±48.93	583.78±42.09	539.24±38.83
Range	339 to 659	499 to 673	463 to 634

Table 13.1A CCT distribution as measured by the 3 instruments (n=50).

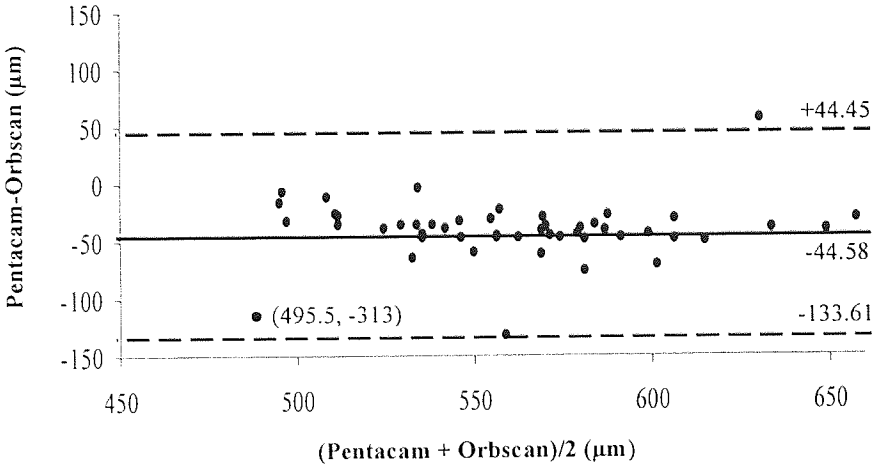
Instruments	**Pentacam-Ultrasound**	**Pentacam-Orbscan**	**Ultrasound-Orbscan**
Mean±SD differences	-0.06±46.74	-44.48±45.42	-44.52±16.97
Scheffé *post-hoc*	1.00	<0.001*	<0.001*

Table 13.2A *Post-hoc* analyses of CCT values as measured by the 3 modalities. * denotes statistically significant results.

(13.1A a)



(13.1A **b**)



(13.1A **c**)

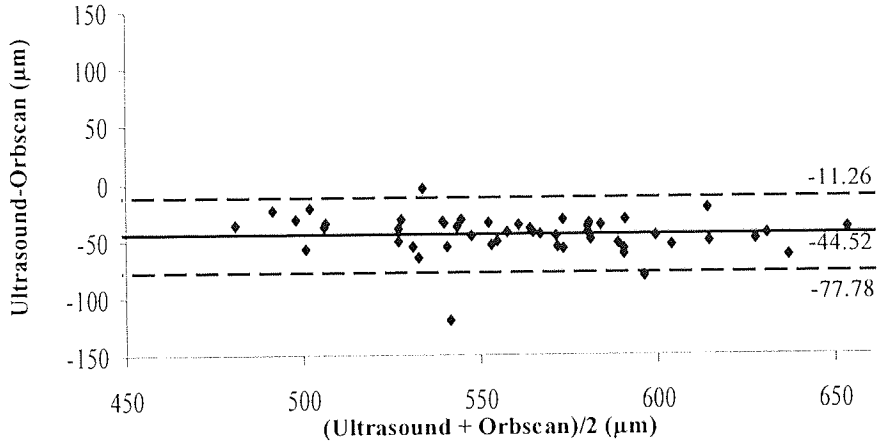
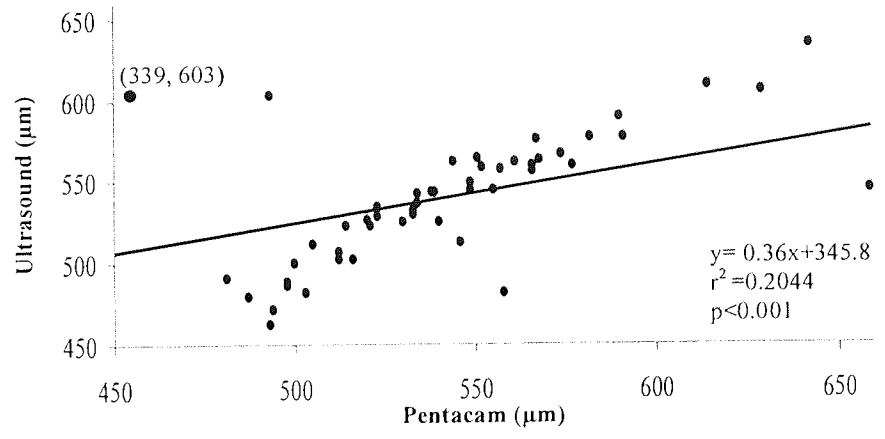
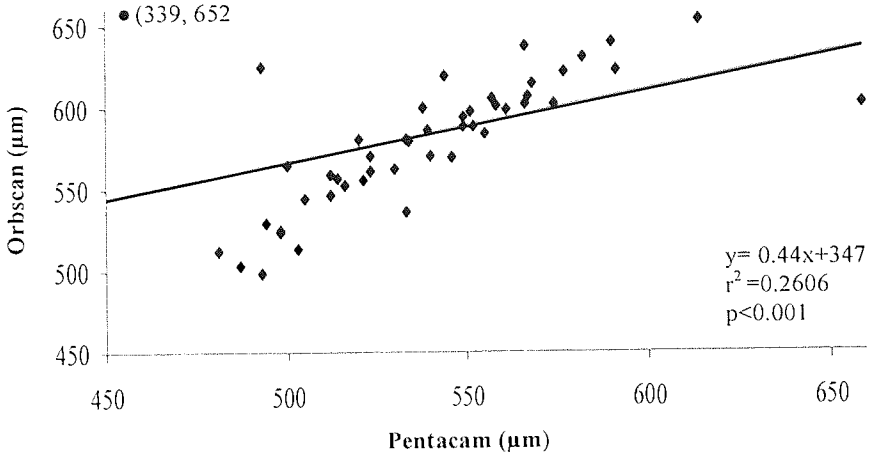


Figure 13.1A Bland and Altman plots for the differences between the CCT values taken with the Pentacam and Ultrasound (**a**), Pentacam and Orbscan (**b**) Ultrasound and Orbscan (**c**) (n=50).

(13.2A **a**)



(13.2A b)



(13.2A c)

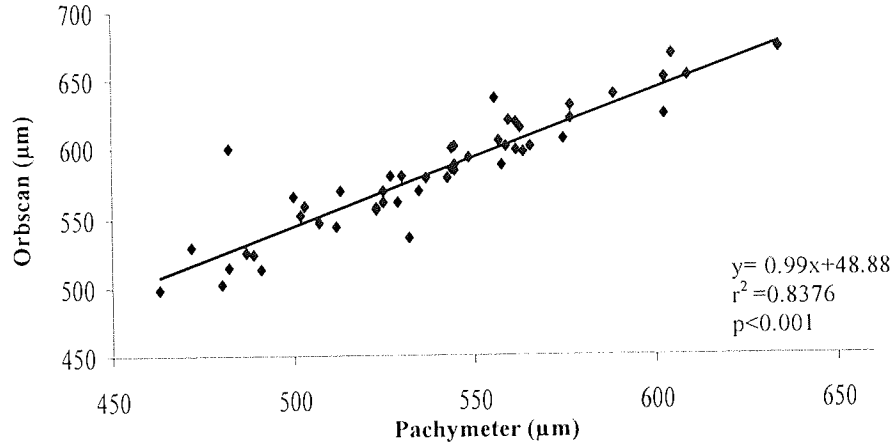


Figure 13.2A Correlation graphs between CCT values taken with the Pentacam and Ultrasound (**a**), Pentacam and Orbscan (**b**) Ultrasound and Orbscan (**c**) (n=50).

Anterior Chamber Depth

The ACD values for the cohort as measured by the 3 modalities are summarised in **Table 13.3A.** The results of the one-way ANOVA reached statistical significance (F (2, 98)=41.084, p<0.001) and the *post-hoc* analyses are shown in **Table 13.4A**. The Bland and Altman plots of the differences in ACD values as a function of their mean and the limits of agreement for the 3 instruments are shown in **Figure 13.3A. Figure 13.4A** illustrates that the correlations in the ACD measures taken with the Pentacam and IOL Master (r=0.19, p=18) and Orbscan and IOL Master (r=0.23, p=0.11) were not statistically significant. The ACD values measured by the Pentacam and Orbscan were highly correlated (r=0.90, p<0.001).

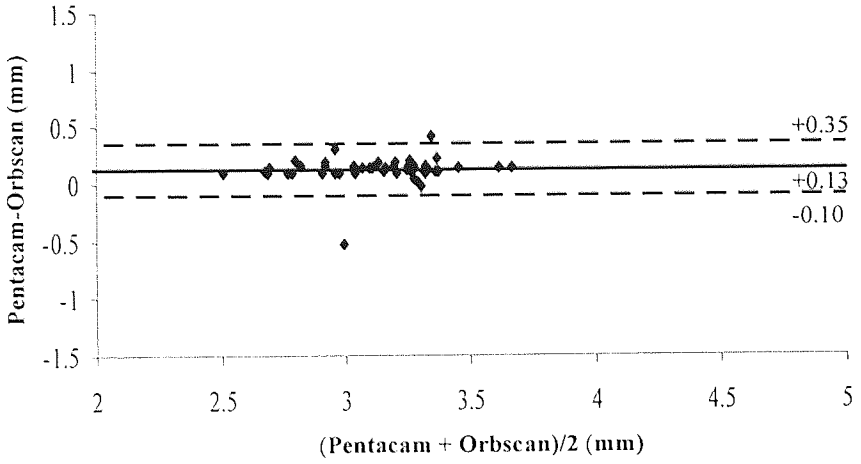
	Instrument		
	Pentacam (mm)	**Orbscan (mm)**	**IOL Master (mm)**
Mean±SD	3.20±0.26	3.07±0.25	3.51±0.40
Range	2.55 to 3.74	2.46 to 3.60	2.16 to 4.13

Table 13.3A ACD distribution as measured by the 3 instruments (n=50).

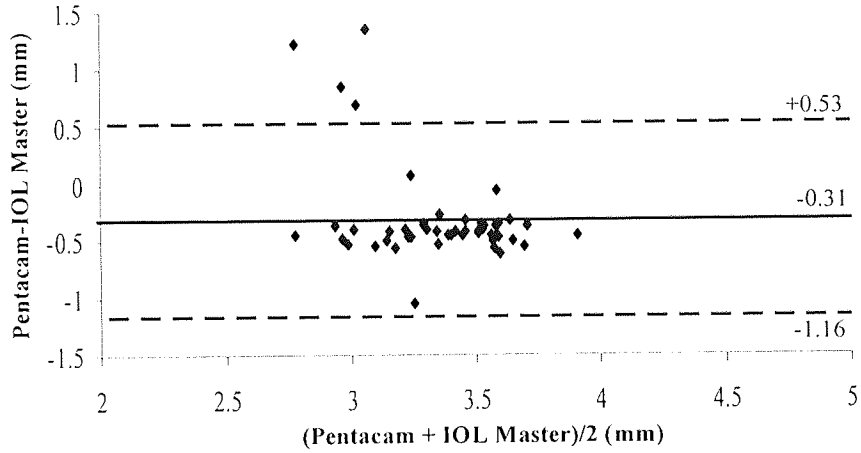
Instruments	**Pentacam-IOL Master**	**Pentacam-Orbscan**	**IOL Master - Orbscan**
Mean±SD differences	-0.31±0.43	0.13±0.12	0.44±0.42
Scheffe	<0.001*	0.120	<0.001*

Table 13.4A *Post hoc* analyses of ACD values as measured by the 3 modalities. * denotes statistically significant results.

(13.3A a)



(13.3A b)



(13.3A c)

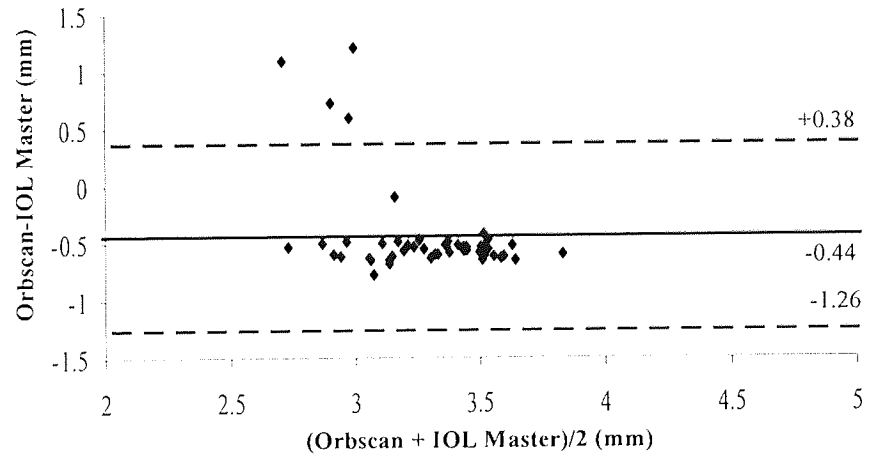
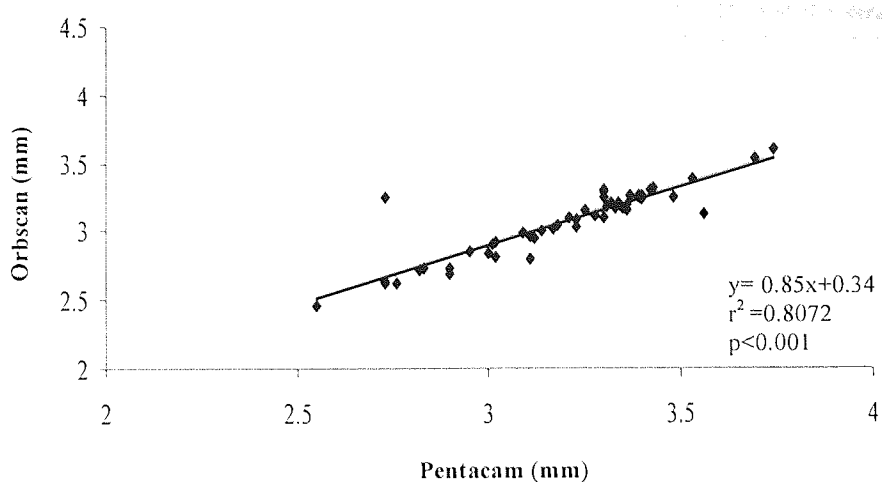
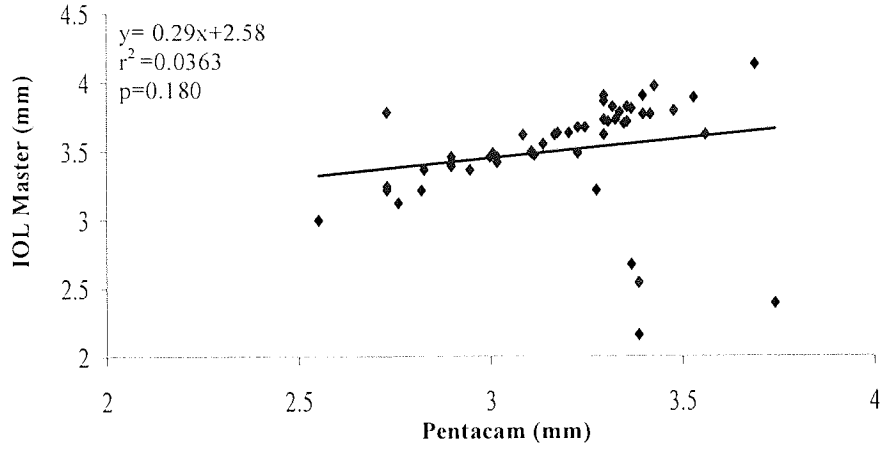


Figure 13.3A Bland and Altman plots for the differences between the ACD values taken with the Pentacam and Orbscan (**a**), Pentacam and IOL Master (**b**) Orbscan and IOL Master (**c**) (n=50).

(13.4A a)



(13.4A b)



(13.4A c)

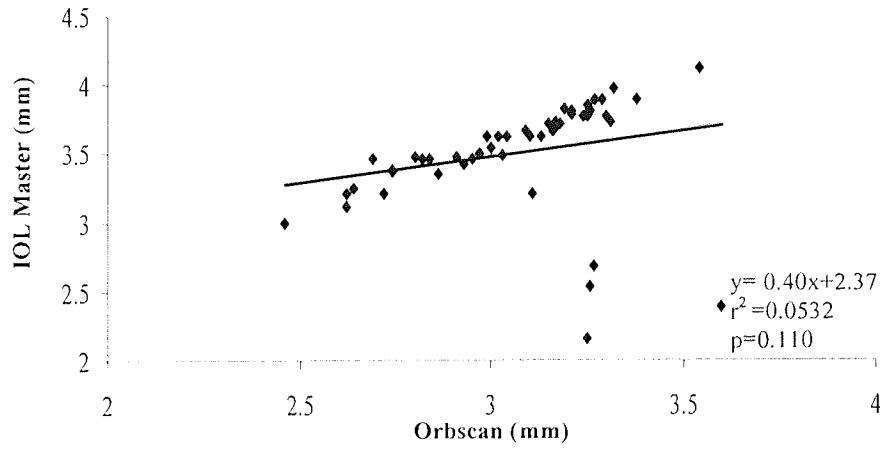


Figure 13.4A Correlation graphs between ACD values taken with the Pentacam and Orbscan (**a**), Pentacam and IOL Master (**b**) and Orbscan and IOL Master (**c**) (n=50).

Keratometry

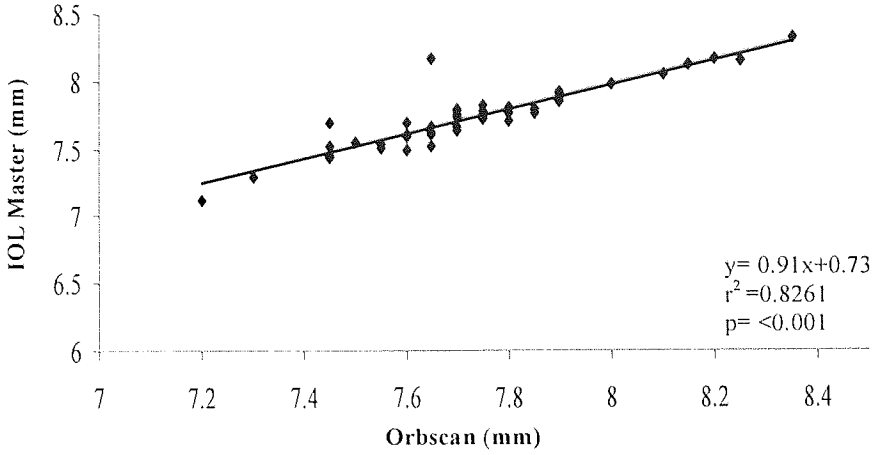
The mean of the keratometry values in the two meridians (CC) for the cohort as measured by the 3 modalities are summarised in **Table 13.5A.** The within-subjects ANOVA did not reach statistical significance (F (2, 98)=0.308, p=0.623).

	Instrument		
	Pentacam (mm)	**Orbscan (mm)**	**Ultrasound (mm)**
Mean±SD	7.77±0.36	7.75±.0.23	7.75±0.23
Range	6.39 to 8.48	7.20 to 8.35	7.13 to 8.33

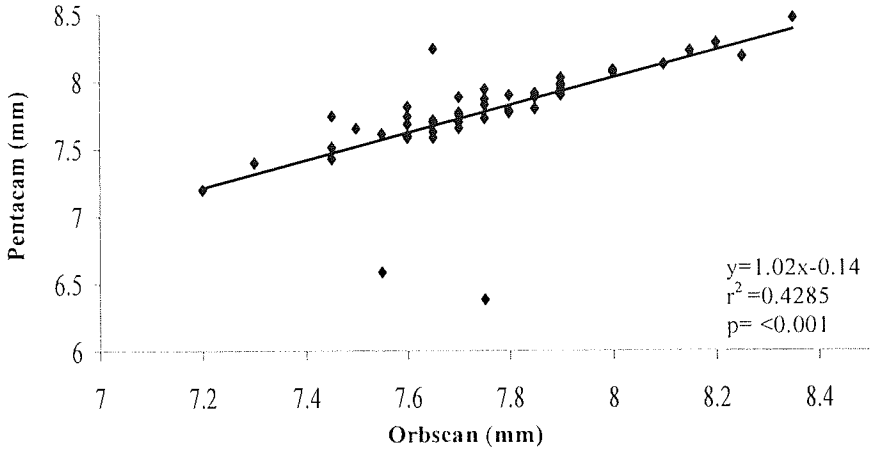
Table 13.5A CC distribution as measured by the 3 instruments (n=50).

Figure 13.5A illustrates that the keratometry values taken with the Pentacam were highly correlated with those measured with the IOL Master ($r=0.70$, $p<0.001$) and Orbscan ($r=0.65$, $p<0.001$) and the keratometry values measured with the Orbscan were highly correlated with those measured with the IOL Master ($r=0.91$, $p<0.001$).

(13.5A **a**)



(13.5A **b**)



(13.5A **c**)

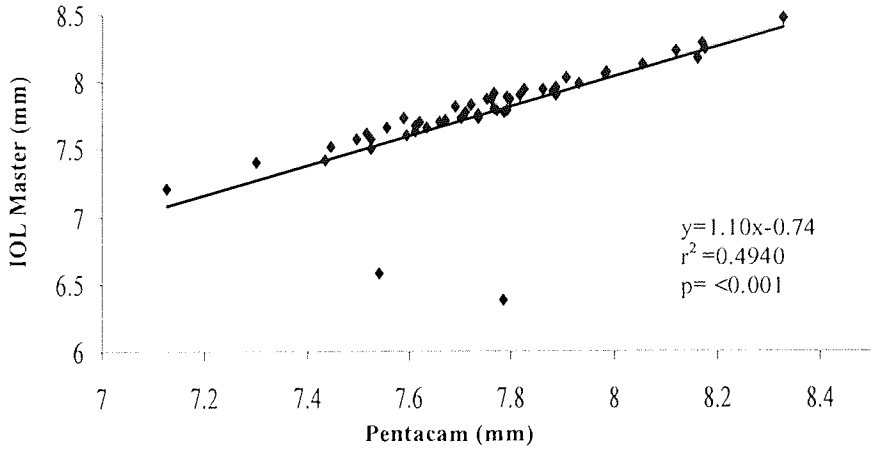


Figure 13.5A Correlation graphs between CC values taken with the Orbscan and IOL Master (**a**), Orbscan and Pentacam (**b**) and Pentacam and IOL Master (**c**) (n=50).

Discussion

CCT

The within-subjects ANOVA demonstrates that the CCT values are significantly influenced by the instrument used. The study concludes that similar to the results of the study conducted by Lackner *et al.* (2005), the CCT values as measured with the Orbscan were on average higher than those measured with the Pentacam. Furthermore, consistent with previous studies (Yaylali, Kaufman and Thompsn, 1997; Modis, langenbucker and Seitz, 2001; Lackner *et al.*, 2005; Basmak, Sahin and Yildirim, 2006) the CCT values measured by the Orbscan

were higher than those measured by the ultrasound method. Lackner *et al.* (2005) and O'Donnell and Maldonado-Codina (2005) found the CCT values measured with the Pentacam were significantly lower than those measured with the ultrasound pachymeter. In contrast the present study concludes that the CCT values measured with the ultrasound pachymeter were only slightly higher than those measured with the Pentacam but this difference did not reach statistical significance.

Previous studies report strong correlations (r >0.90) between the CCT values taken by the Pentacam and Ultrasound (Amano *et al.*, 2006; Ucakhan *et al.*, 2006) and the Pentacam and Orbscan (Amano *et al.*, 2006; Buehl *et al.*, 2006). **Figure 13.5A** shows moderate correlations between these instruments. In addition, consistent with previous literature, the present study demonstrates a strong correlation between the CCT values measured with the ultrasound and Orbscan instrument (Suzuki *et al.*, 2003; Amano *et al.*, 2006; Basmak *et al.*, 2006).

Although the mean difference between the CCT values measured by the Pentacam and the ultrasound pachymeter was not statistically significant the data exhibited the largest 95% confidence intervals (±91.60). The mean difference between the CCT values measured by the Pentacam and Orbscan was significant and the 95% confidence intervals were ±89.03. Finally, a significant mean difference in the CCT values was evident between the Orbscan and ultrasound pachymeter although the data exhibited the smallest confidence intervals (±33.26). It can be concluded from the present data set that of the 3 instruments used, only the Orbscan and Ultrasound pachymeter can be used interchangeably.

ACD

The ANOVA demonstrates that the ACD values are significantly influenced by the instrument used. The present study concludes that the ACD values as measured with the Pentacam were significantly higher and lower than those measured with the Orbscan and IOL Master, respectively. In contrast a recent study by Lackner *et al.* (2005) concluded that the ACD values as measured by the Pentacam were significantly lower than those measured with the Orbscan. A study by Reddy *et al.* (2004) found no significant differences in the ACD values measured y the Orbscan and IOL Master. However, the present study concludes that the ACD values measured with the Orbscan were significantly lower than those measured with the IOL Master, a result consistent with a recent study by Hashemi *et al.* (2005).

A weak, non-significant correlation was evident between the ACD values measured by the IOL Master and the Pentacam and Orbscan. However, consistent with a previous study (Buehl *et al.*, 2006) the ACD data measured with the Orbscan and Pentacam demonstrated a strong correlation.

Although significant, the ACD values measured by the Orbscan and Pentacam showed the least mean difference and the smallest 95% confidence intervals (±0.22). The data taken with the IOL Master and Orbscan and Pentacam demonstrated significant differences in ACD values and relatively large 95% confidence intervals (Pentcam and IOL Master: ±0.84; Orbscan and IOL Master: ±0.82). It is evident from the data that only the Orbscan and Pentacam can be used interchangeably.

Keratometry

The mean of the keratometry readings in the two meridians i.e. k-value as measured by the 3 instruments were assessed with a within-subjects ANOVA. No significant differences in the k-values were evident between the 3 modalities. **Figure 13.5A** demonstrates a strong correlation between the k-value data measured by the IOL Master and that taken with the Pentacam and Orbscan, and between the k-value date measured with the Pentacam and Orbscan.

Conclusions

In the study described in **Chapter 9** several biometric and oculomotor parameters were measured to evaluate the inter-subject variations in IOP and blood flow responses to accommodation. To date, no single instrument is capable of measuring simultaneously all the biometric parameters of the eye and therefore four different instruments were utilised. Some of the instruments mentioned measure the same biometric parameter although the values differ due to different measuring principles inherent to the system. Therefore the present study was conducted to compare the measurements of CCT, ACD and CC values. The results suggest that for the measurement of CCTs values the ultrasound pachymeter and Orbscan can be used interchangeably. For the measurement of ACDs, it is concluded that the Orbscan and Pentacam can be used interchangeably. In addition, for the measurement of CCs it is apparent that all 3 instruments (IOL Master, Orbscan and Pentacam) can be used interchangeably. Therefore in the study discussed in **Chapter 9**, the CCTs values obtained with the ultrasound pachymeter and the ACDs and CCs readings taken with the Orbscan were used to access the inter-subject variations in IOP and blood flow responses to accommodation.

APPENDIX 14

SUMMARY OF EFFECTS OF ACCOMMODATION ON IOP AND BLOOD FLOW PARAMETERS.

A one-way randomised block design ANOVA shows that the level of accommodation influences the IOP as measured by the NCT ($F(2, 98) = 8.004$, $p=0.001$) (n=50). The mean ±SD of accommodation responses to L, I and H accommodation levels were -0.16±0.34, +1.75±0.23 and +4.15±0.21 D, respectively. The corresponding mean ±SD IOP to L, I and H accommodation levels was 14.09±2.36, 13.18±2.68 and 14.06±2.65 mmHg, respectively. The mean ±SD of the differences between L and I, L and H, and I and H accommodation levels was 0.91±1.55, 0.03±1.77 and 0.88±2.14 mmHg, respectively. The results of Scheffe and Bonferroni *post-hoc* analyses are shown in Table **14.1A.**

Accommodation stimulus levels (D)	Scheffe	Bonferroni
L to I	0.187	<0.001
L to H	0.998	0.91
I to H	0.208	0.005

Table 14.1A Summary of *post-hoc* analyses of IOP data measured with the NCT (n=50).

Change	L to I	L to H	p
Absolute	0.91±1.55	0.03±1.77	p=0.005
Percentage	6.19±10.70	0.17±11.75	p=0.003

Table 14.2A Summary of absolute and percentage changes in IOP (as measured with the NCT) between L and I, and L and H accommodation levels.

A one-way randomised block design ANOVA shows that the level of accommodation influences the IOP as measured by the OBFA ($F(2, 82)=5.669$, $p=0.010$). The mean ±SD of accommodation responses to L, I and H accommodation levels were -0.14±0.41, +1.56±0.29, +4.19±0.24 D, respectively. The corresponding mean ±SD IOP to L, I and H accommodation levels was 10.52±2.68, 9.98±2.40 and 9.42±2.13 mmHg, respectively. The mean ±SD of the differences between L and I, L and H, and I and H accommodation levels was 0.54±0.53, 1.10±0.53 and 0.57±0.53 mmHg, respectively. The results of Scheffe and Bonferroni *post-hoc* analyses are shown in Table **14.2A.**

Accommodation stimulus levels (D)	Scheffe	Bonferroni
L to I	0.598	0.187
L to H	0.117	0.001
I to H	0.563	0.025

Table 14.3A Summary of *post-hoc* analyses of IOP data measured with the OBFA.

Change	L to I	L to H	p
Absolute	0.54±0.53	1.10±0.53	0.025
Percentage	1.96±25.86	7.87±20.97	0.037

Table 14.4A Summary of absolute and percentage changes in IOP (as measured with the OBFA) between L and I, and L and H accommodation levels.

A one-way randomised block design ANOVA shows that the level of accommodation does not influence the PA ($F(2, 82)=0.274$, $p=0.720$). The mean ±SD PA to L, I and H accommodation levels was 3.15±1.09, 3.02±1.08 and 3.09±1.28 mmHg, respectively.

A one-way randomised block design ANOVA shows that the level of accommodation does not influence the PR ($F(2, 82) = 0.574$, $p=0.561$). The mean ±SD PR to L, I and H accommodation levels was 73.43±14.39, 74.83±13.49 and 72.81±10.96, beats/min, respectively.

A one-way randomised block design ANOVA shows that the level of accommodation does not influence the PV ($F(2, 82) = 0.574$, $p=0.561$). The mean ±SD PR to L, I and H accommodation levels was 73.43±14.39, 74.83±13.49 and 72.81±10.96, beats/min, respectively.

A one-way randomised block design ANOVA shows that the level of accommodation does not influence the POBF ($F(2, 82)=0.663$, $p=0.518$). The mean ±SD PR to L, I and H accommodation levels was 1332.64±360.36, 1383.81±408.53 and 1364.10±419.65 mL/min, respectively.

APPENDIX 15

SUMMARY OF MEDIAN SPLIT AND CORRELATION ANALYSES OF THE EFFECTS OF BIOMETRIC AND OCULOMOTOR PARAMETERS ON IOP, PA, PR, PV AND POBF.

Parameter	Median Split	L to I				L to H				I to H			
		Absolute		Percentage		Absolute		Percentage		Absolute		Percentage	
		F	p	F	p	F	p	F	p	F	p	F	P
CCTS	543.5µm	0.325	0.571	0.233	0.631	0.003	0.960	0.082	0.776	0.206	0.652	0.433	0.514
ACDS	3.12mm	0.111	0.741	0.118	0.733	0.037	0.848	0.198	0.658	0.160	0.691	0.189	0.666
ACVOLS	199ml^3	0.655	0.422	1.640	0.206	0.435	0.513	0.232	0.632	0.001	0.969	0.144	0.706
ALS	23.83mm	1.378	0.246	1.874	0.177	1.310	0.258	1.955	0.169	0.009	0.923	0.038	0.845
ACANS	41.5°	0.221	0.640	0.549	0.462	0.139	0.711	0.450	0.506	0.001	0.975	0.062	0.804
CVOLS	59.95ml^3	5.069	**0.029***	4.453	**0.040***	0.651	0.424	0.515	0.476	0.800	0.376	1.093	0.301
CCS	7.725mm	0.597	0.443	0.603	0.441	0.447	0.507	0.618	0.436	<0.001	0.996	0.001	0.976
ECCS	0.53	1.040	0.313	1.373	0.247	1.981	0.166	1.185	0.282	3.781	0.058	2.770	0.103
Ref err	-0.94D	<0.001	0.992	0.014	0.907	0.762	0.387	1.110	0.297	0.531	0.470	1.202	0.278
OVOLS	6162mm^3	1.324	0.256	1.828	0.183	0.176	0.677	0.394	0.533	0.227	0.636	0.086	0.771
AC	4.72$^\lambda$	2.172	0.147	2.379	0.130								
	10.32$^\lambda$					0.190	0.467	0.665	0.498				
	5.04$^\lambda$									4.247	0.06	3.504	0.067
AC/A	1.84	0.299	0.587	0.543	0.465								
	1.93					1.497	0.227	2.552	0.117				

Table 15.1A Summary of median split analyses of the biometric and oculomotor parameters and the changes in IOP (as measured with the NCT) with accommodation. * denotes a statistically significant result (n=50).

Biometric parameter	Accommodation level (D)	Absolute change in IOP (mmHg)			% change in IOP (mmHg)		
		r	r^2	p	r	r^2	p
CCT	L to I	0.037	0.001	0.80	0.037	0.001	0.80
	L to H	0.006	<0.001	0.97	0.032	0.001	0.83
	I to H	0.022	0.001	0.88	0.075	0.006	0.61
ACD	L to I	0.096	0.009	0.51	0.111	0.012	0.44
	L to H	0.068	0.005	0.64	0.117	0.014	0.42
	I to H	0.126	0.016	0.38	0.149	0.022	0.30
ACh Vol	L to I	0.149	0.022	0.30	0.185	0.034	0.20
	L to H	0.051	0.003	0.73	0.096	0.009	0.51
	I to H	0.150	0.023	0.30	0.187	0.035	0.19
AL	L to I	0.297	0.088	**0.04***	0.299	0.090	**0.03***
	L to H	0.165	0.027	0.25	0.207	0.043	0.15
	I to H	0.079	0.006	0.59	0.030	0.001	0.84
ACh Ang	L to I	0.051	0.003	0.73	0.067	0.005	0.64
	L to H	0.288	0.083	**0.04***	0.325	0.106	**0.02***
	I to H	0.201	0.041	0.16	0.178	0.032	0.22
CVol	L to I	0.214	0.046	0.14	0.189	0.036	0.19
	L to H	0.146	0.021	0.31	0.120	0.014	0.41
	I to H	0.034	0.001	0.82	0.061	0.004	0.67
CC	L to I	0.348	0.121	**0.01***	0.377	0.142	**0.007***
	L to H	0.010	<0.001	0.95	0.045	0.002	0.76
	I to H	0.260	0.068	0.07	0.252	0.064	0.08
E-values	L to I	0.250	0.063	0.08	0.281	0.079	0.05
	L to H	0.025	0.001	0.86	0.031	0.001	0.83
	I to H	0.202	0.041	0.16	0.192	0.037	0.18
Ref error	L to I	0.218	0.047	0.13	0.195	0.038	0.18
	L to H	0.155	0.024	0.28	0.198	0.039	0.17
	I to H	0.029	0.001	0.84	0.034	0.001	0.82
OVol	L to I	0.216	0.046	0.13	0.210	0.044	0.14
	L to H	0.144	0.021	0.32	0.187	0.035	0.19
	I to H	0.037	0.001	0.80	0.015	<0.000	0.92
AC	L to I	0.112	0.013	0.44	0.083	0.007	0.57
	L to H	0.084	0.007	0.56	0.105	0.011	0.47
	I to H	0.283	0.080	0.05	0.244	0.060	0.09
AC/A	L to I	0.130	0.017	0.37	0.142	0.020	0.33
	L to H	0.167	0.028	0.25	0.184	0.034	0.20

Table 15.2A Summary of the correlations between the changes in IOP with accommodation (as measured with the NCT) and biometric and oculomotor parameters. * denotes a statistically significant result (n=50).

Parameter	Median split	L to I				L to H				I to H			
		Absolute		Percentage		Absolute		Percentage		Absolute		Percentage	
		F	p	F	p	F	p	F	p	F	p	F	p
CCT	545µm	0.011	0.916	0.015	0.902	0.053	0.820	0.166	0.687	0.020	0.888	0.249	0.622
ACD	3.17mm	0.198	0.660	0.890	0.354	0.355	0.556	0.374	0.546	0.640	0.431	1.397	0.248
Ach Vol	201.5ml^3	0.236	0.631	0.631	0.434	0.769	0.389	0.473	0.498	0.236	0.631	0.020	0.890
AL	24.61mm	6.946	**0.014***	5.115	**0.032***	0.773	0.387	1.558	0.223	0.384	0.541	0.057	0.814
Ach Ang	41.5°	2.461	0.129	2.408	0.133	2.456	0.129	2.857	0.103	0.201	0.658	0.075	0.756
CVol	60.4ml^3	0.871	0.359	0.516	0.479	0.244	0.625	0.157	0.696	0.011	0.916	0.013	0.909
CC	7.70mm	0.015	0.904	0.036	0.851	0.269	0.608	0.306	0.585	0.151	0.701	0.151	0.701
E-values	0.555	3.544	0.071	3.019	0.094	0.097	0.758	0.472	0.498	0.607	0.443	0.126	0.726
OVol	6473mm^3	6.946	**0.014***	5.115	**0.032***	0.773	0.384	1.558	0.223	0.384	0.541	0.057	0.814
AC	4.99$^\Delta$	0.112	0.741	0.036	0.852								
	8.94$^\Delta$					1.881	0.182	2.788	0.107				
	4.16$^\Delta$									1.323	0.260	1.605	0.216
AC/A	1.92	0.110	0.743	0.122	0.730								
	1.93					0.002	0.966	0.166	0.687				

Table 15.3A Summary of median split analyses of the biometric and oculomotor parameters and the changes in IOP (as measured with the NCT) with accommodation in the myopes only. * denotes a statistically significant result (n=28).

Biometric parameter	Accommodation level (D)	Absolute change in IOP (mmHg)			% change in IOP (mmHg)		
		r	r^2	p	R	r^2	P
CCT	L to I	0.247	0.061	0.21	0.190	0.036	0.33
	L to H	0.018	<0.001	0.93	0.027	<0.001	0.89
	I to H	0.162	0.026	0.41	0.082	0.007	0.68
ACD	L to I	0.135	0.018	0.49	0.204	0.041	0.30
	L to H	0.034	0.001	0.86	0.001	<0.001	0.99
	I to H	0.049	0.002	0.80	0.113	0.013	0.57
ACh Vol	L to I	0.157	0.025	0.43	0.216	0.047	0.27
	L to H	0.068	0.005	0.73	0.024	<0.001	0.91
	I to H	0.032	0.001	0.87	0.106	0.011	0.59
AL	L to I	0.484	0.234	**0.009***	0.455	0.207	**0.01***
	L to H	0.240	0.058	0.22	0.298	0.089	0.12
	I to H	0.072	0.005	0.71	0.026	<0.001	0.90
ACh Ang	L to I	0.092	0.009	0.64	0.079	0.006	0.69
	L to H	0.409	0.167	**0.03***	0.445	0.198	**0.02***
	I to H	0.310	0.096	0.11	0.301	0.090	0.12
CVol	L to I	0.090	0.008	0.65	0.092	0.008	0.64
	L to H	0.071	0.005	0.72	0.037	0.001	0.85
	I to H	0.117	0.014	0.55	0.069	0.005	0.73
CC	L to I	0.159	0.025	0.42	0.127	0.016	0.52
	L to H	0.366	0.134	0.06	0.337	0.113	0.08
	I to H	0.421	0.177	0.06	0.366	0.134	0.06
OVol	L to I	0.394	0.155	**0.04***	0.361	0.131	0.06
	L to H	0.213	0.045	0.28	0.274	0.075	0.16
	I to H	0.043	0.002	0.83	0.138	<0.001	0.94
Esphercity	L to I	0.540	0.292	**0.003***	0.513	0.263	**0.005***
	L to H	0.007	<0.001	0.97	0.054	0.003	0.79
	I to H	0.327	0.107	0.09	0.275	0.076	0.16
AC	L to I	0.056	0.003	0.78	0.004	<0.001	0.98
	L to H	0.074	0.005	0.71	0.101	0.010	0.61
	I to H	0.314	0.099	0.10	0.283	0.080	0.15
AC/A	L to I	0.096	0.009	0.63	0.130	0.017	0.51
	L to H	0.079	0.006	0.69	0.114	0.013	0.56

Table 15.4A Summary of the correlations between the changes in IOP with accommodation (as measured with the NCT) and biometric and oculomotor parameters in the myopes only. * denotes a statistically significant result (n=28).

Parameter	Median split	L to I				L to H				I to H			
		Absolute		Percentage		Absolute		Percentage		Absolute		Percentage	
		F	p	F	p	F	p	F	p	F	p	F	p
CCT	533.5µm	4.026	0.052	4.348	**0.043***	0.413	0.524	0.318	0.576	6.061	**0.018***	5.451	**0.025***
ACD	3.10mm	0.002	0.961	0.058	0.811	0.515	0.477	0.130	0.720	0.746	0.393	0.514	0.477
Ach Vol	196ml^3	0.556	0.460	0.513	0.478	2.776	0.103	2.224	0.144	0.828	0.368	0.661	0.421
AL	23.73mm	0.042	0.838	0.040	0.842	0.004	0.948	0.140	0.711	0.063	0.803	<0.001	0.995
Ach Ang	41.0°	1.092	0.302	0.856	0.360	2.690	0.109	1.892	0.177	0.156	0.695	0.447	0.507
CVol	59.65ml^3	2.716	0.107	2.376	0.131	0.357	0.553	0.191	0.664	3.662	0.063	3.023	0.090
CC	7.725mm	0.107	0.745	0.059	0.810	0.163	0.688	0.315	0.578	1.164	0.287	0.667	0.419
E-values	0.52	2.548	0.118	1.314	0.258	1.305	0.260	0.665	0.420	1.157	0.289	0.994	0.325
Refer	-0.87D	0.003	0.958	0.004	0.948	1.236	0.273	0.959	0.333	1.908	0.175	0.949	0.336
OVol	6130mm^3	0.009	0.923	0.004	0.950	0.204	0.654	<0.001	0.993	0.188	0.667	0.170	0.682
AC	4.84$^\Delta$	0.038	0.847	0.048	0.828								
	10.89$^\Delta$					0.919	0.343	0.636	0.430				
	6.00$^\Delta$									0.253	0.618	0.017	0.898

AC/A	1.65	0.447	0.508	1.496	0.228								
	1.99					1.041	0.314	0.644	0.427				

Table 15.5A Summary of median split analyses of the biometric and oculomotor parameters and the changes in IOP (as measured with the OBFA) with accommodation. * denotes a statistically significant result (n=42).

Biometric parameter	Accommodation level (D)	Absolute change in IOP (mmHg)			% change in IOP (mmHg)		
		r	r^2	p	r	r^2	p
CCT	L to I	0.182	0.033	0.25	0.203	0.041	0.20
	L to H	0.010	<0.001	0.94	0.008	<0.001	0.96
	I to H	0.284	0.081	0.07	0.280	0.078	0.07
ACD	L to I	0.051	0.003	0.75	0.008	<0.001	0.96
	L to H	0.117	0.014	0.46	0.042	0.002	0.79
	I to H	0.071	0.005	0.65	0.010	<0.001	0.94
ACh Vol	L to I	0.033	0.001	0.83	0.035	0.001	0.83
	L to H	0.073	0.005	0.64	0.026	<0.001	0.87
	I to H	0.151	0.023	0.34	0.112	0.013	0.48
AL	L to I	0.192	0.037	0.22	0.157	0.025	0.32
	L to H	0.085	0.007	0.59	0.087	0.008	0.58
	I to H	0.202	0.041	0.20	0.147	0.022	0.35
ACh Ang	L to I	0.013	<0.001	0.93	0.049	0.002	0.76
	L to H	0.086	0.007	0.59	0.099	0.010	0.53
	I to H	0.091	0.008	0.57	0.110	0.012	0.49
CVol	L to I	0.214	0.046	0.17	0.210	0.044	0.18
	L to H	0.079	0.006	0.62	0.072	0.005	0.65
	I to H	0.246	0.061	0.12	0.224	0.050	0.15
CC	L to I	0.067	0.004	0.67	0.014	<0.001	0.93
	L to H	0.058	0.003	0.71	0.006	<0.001	0.97
	I to H	0.034	0.001	0.83	0.074	0.005	0.64
Esphercity	L to I	0.172	0.030	0.28	0.077	0.006	0.63
	L to H	0.112	0.013	0.48	0.054	0.003	0.74
	I to H	0.135	0.018	0.40	0.125	0.016	0.43
OVol	L to I	0.206	0.042	0.19	0.167	0.028	0.29
	L to H	0.125	0.016	0.43	0.114	0.013	0.47
	I to H	0.172	0.030	0.27	0.123	0.015	0.44
Ref error	L to I	0.268	0.072	0.09	0.240	0.057	0.13
	L to H	0.096	0.009	0.54	0.089	0.008	0.57
	I to H	0.313	0.098	0.10	0.248	0.062	0.11
AC	L to I	0.089	0.008	0.58	0.048	0.002	0.76
	L to H	0.115	0.013	0.47	0.082	0.007	0.61
	I to H	0.044	0.002	0.78	0.005	<0.001	0.98
AC/A	L to I	0.083	0.007	0.60	0.009	<0.001	0.95
	L to H	0.115	0.013	0.47	0.059	0.004	0.71

Table 15.6A Summary of the correlations between the changes in IOP with accommodation (as measured with the OBFA) and biometric and oculomotor parameters. * denotes a statistically significant result (n=42).

Parameter	Median split	L to I				L to H				I to H			
		Absolute		Percentage		Absolute		Percentage		Absolute		Percentage	
		F	p	F	p	F	p	F	p	F	p	F	p
CCT	540µm	0.811	0.379	1.438	0.244	0.003	0.960	0.013	0.912	2.472	0.132	2.104	0.162
ACD	3.13mm	0.636	0.434	0.817	0.377	0.266	0.612	0.227	0.639	0.425	0.522	0.387	0.541
Ach Vol	201ml[1]	0.465	0.503	0.368	0.551	2.056	0.167	1.832	0.191	0.325	0.575	0.488	0.493
AL	24.51mm	4.034	0.058	2.008	0.172	3.236	0.087	2.054	0.167	0.910	0.352	0.399	0.535
Ach Ang	41.8°	0.492	0.491	0.163	0.691	0.815	0.377	0.474	0.499	0.002	0.964	0.021	0.885
CVol	59.7ml[1]	0.076	0.786	0.080	0.781	1.078	0.311	1.186	0.289	0.627	0.438	0.925	0.348
CC	7.70mm	0.001	0.973	0.001	0.971	0.590	0.451	0.567	0.460	0.768	0.391	0.889	0.357
E-values	0.55	0.033	0.859	0.019	0.891	0.259	0.616	0.865	0.363	0.849	0.368	0.910	0.352
OVol	6426mm[1]	4.034	0.058	2.008	0.172	3.236	0.087	2.054	0.167	0.910	0.352	0.399	0.535
AC	5.23[A]	0.026	0.873	0.063	0.804								
	10.04[A]					0.234	0.634	0.364	0.553				
	4.40[A]									0.578	0.456	1.076	0.312
AC/A	2.14	0.087	0.771	0.533	0.474								
	1.99					1.136	0.299	1.570	0.225				

Table 15.7A Summary of median split analyses of the biometric and oculomotor parameters and the changes in IOP (as measured with the OBFA) with accommodation in the myopes only. * denotes a statistically significant result (n=22).

Biometric parameter	Accommodation level (D)	Absolute change in IOP (mmHg)			% change in IOP (mmHg)		
		r	r^2	p	r	r^2	P
CCT	L to I	0.030	<0.001	0.89	0.127	0.016	0.57
	L to H	0.171	0.029	0.45	0.101	0.010	0.65
	I to H	0.255	0.065	0.25	0.249	0.062	0.26
ACD	L to I	0.179	0.032	0.43	0.161	0.026	0.48
	L to H	0.078	0.006	0.73	0.034	0.001	0.88
	I to H	0.194	0.038	0.39	0.175	0.031	0.44
ACh Vol	L to I	0.030	<0.001	0.90	0.043	0.002	0.85
	L to H	0.019	<0.001	0.93	0.044	0.002	0.84

	I to H	0.025	<0.001	0.91	0.016	<0.001	0.94
AL	L to I	0.466	0.217	**0.03***	0.350	0.123	0.11
	L to H	0.511	0.261	**0.02***	0.417	0.174	0.05
	I to H	0.133	0.018	0.56	0.048	0.002	0.83
ACh Ang	L to I	0.284	0.080	0.20	0.259	0.067	0.24
	L to H	0.254	0.064	0.25	0.208	0.043	0.35
	I to H	0.150	0.023	0.50	0.104	0.011	0.65
CVol	L to I	0.095	0.009	0.67	0.025	<0.001	0.91
	L to H	0.192	0.037	0.39	0.132	0.017	0.56
	I to H	0.080	0.006	0.72	0.074	0.006	0.74
CC	L to I	0.134	0.018	0.55	0.067	0.004	0.77
	L to H	0.134	0.018	0.55	0.053	0.003	0.82
	I to H	0.056	0.003	0.80	0.056	0.003	0.80
OVol	L to I	0.438	0.192	**0.04***	0.319	0.101	0.15
	L to H	0.484	0.235	**0.02***	0.375	0.141	0.09
	I to H	0.120	0.014	0.59	0.050	0.002	0.83
Esphercity	L to I	0.319	0.102	0.15	0.269	0.072	0.23
	L to H	0.159	0.025	0.48	0.080	0.006	0.72
	I to H	0.322	0.104	0.14	0.283	0.080	0.20
AC	L to I	0.303	0.092	0.17	0.228	0.052	0.31
	L to H	0.083	0.007	0.71	0.099	0.010	0.66
	I to H	0.097	0.009	0.67	0.045	0.002	0.84
AC/A	L to I	0.131	0.017	0.56	0.078	0.006	0.73
	L to H	0.148	0.022	0.51	0.203	0.041	0.36

Table 15.8A Summary of the correlations between the changes in IOP with accommodation (as measured with the OBFA) and biometric and oculomotor parameters in the myopes only. * denotes a statistically significant result (n=22).

Parameter	Median split	L to I				L to H				I to H			
		Absolute		Percentage		Absolute		Percentage		Absolute		Percentage	
		F	p	F	p	F	p	F	p	F	p	F	p
CCT	533.5µm	0.159	0.692	0.180	0.674	0.544	0.465	0.577	0.452	0.921	0.343	0.965	0.332
ACD	3.10mm	2.544	0.119	1.144	0.291	<0.001	0.991	0.198	0.659	1.034	0.315	0.944	0.337
Ach Vol	196ml^{3}	3.114	0.085	1.341	0.254	0.064	0.802	0.556	0.460	1.915	0.174	1.799	0.187
AL	23.73mm	0.751	0.391	0.004	0.950	0.867	0.357	0.432	0.515	0.091	0.764	0.243	0.625
Ach Ang	41.0°	0.886	0.352	1.000	0.323	2.658	0.111	2.154	0.150	0.775	0.384	0.744	0.393
CVol	59.65ml^{3}	0.382	0.540	0.871	0.356	0.797	0.377	0.870	0.357	1.581	0.216	1.561	0.219
CC	7.725mm	0.106	0.746	0.374	0.544	1.275	0.266	1.017	0.319	1.645	0.207	1.216	0.277
E-values	0.52	1.052	0.311	1.333	0.255	1.158	0.288	1.662	0.205	0.111	0.741	0.613	0.438
Refer	-0.87D	0.084	0.774	0.114	0.738	3.185	0.082	2.055	0.159	2.133	0.152	1.675	0.203
OVol	6130mm^{3}	0.012	0.914	0.188	0.667	0.507	0.481	0.289	0.594	0.355	0.555	0.423	0.519
AC	4.84$^{\Delta}$	2.836	1.000	2.172	0.148								
	10.89$^{\lambda}$					2.437	0.126	1.315	0.258				
	6.00$^{\lambda}$									1.017	0.319	1.002	0.323
AC/A	1.65	1.131	0.294	1.096	0.301								
	1.99					0.311	0.580	0.645	0.427				

Table 15.9A Summary of median split analyses of the biometric and oculomotor parameters and the changes in PA with accommodation. * denotes a statistically significant result (n=42).

Biometric parameter	Accommodation level (D)	Absolute change in IOP (mmHg)			% change in IOP (mmHg)		
		r	r^2	p	r	r^2	p
CCT	L to I	0.065	0.004	0.68	0.064	0.004	0.68
	L to H	0.027	0.001	0.86	0.062	0.004	0.69
	I to H	0.068	0.005	0.67	0.106	0.011	0.50
ACD	L to I	0.182	0.033	0.25	0.131	0.017	0.41
	L to H	0.065	0.004	0.68	0.001	<0.001	0.99
	I to H	0.058	0.003	0.72	0.071	0.005	0.65
ACh Vol	L to I	0.190	0.036	0.23	0.118	0.014	0.46
	L to H	0.029	0.001	0.85	0.108	0.012	0.50
	I to H	0.152	0.023	0.34	0.173	0.030	0.27
AL	L to I	0.019	<0.001	0.91	0.102	0.010	0.52
	L to H	0.087	0.008	0.59	0.039	0.001	0.81
	I to H	0.069	0.005	0.66	0.071	0.005	0.66
ACh Ang	L to I	0.160	0.028	0.31	0.149	0.022	0.35
	L to H	0.066	0.004	0.68	0.042	0.002	0.79
	I to H	0.043	0.002	0.78	0.023	0.001	0.89
CVol	L to I	0.137	0.019	0.39	0.180	0.032	0.25
	L to H	0.067	0.004	0.67	0.040	0.002	0.80
	I to H	0.027	0.001	0.86	0.036	0.001	0.82
CC	L to I	0.028	0.001	0.86	0.061	0.004	0.70
	L to H	0.103	0.011	0.52	0.069	0.005	0.66
	I to H	0.115	0.013	0.47	0.059	0.004	0.71
Esphercity	L to I	0.043	0.002	0.79	0.034	0.001	0.83
	L to H	0.084	0.007	0.59	0.130	0.017	0.41
	I to H	0.107	0.012	0.50	0.139	0.019	0.38
OVol	L to I	0.004	<0.001	0.98	0.117	0.014	0.46
	L to H	0.054	0.003	0.74	0.006	<0.001	0.97

	I to H	0.053	0.003	0.74	0.045	0.002	0.78
Ref error	L to I	0.014	<0.001	0.93	0.085	0.007	0.59
	L to H	0.100	0.010	0.53	0.045	0.002	0.78
	I to H	0.084	0.007	0.59	0.058	0.003	0.72
AC	L to I	0.075	0.006	0.64	0.011	<0.001	0.94
	L to H	0.094	0.009	0.55	0.048	0.002	0.77
	I to H	0.028	0.001	0.86	0.037	0.001	0.82
AC/A	L to I	0.094	0.009	0.55	0.046	0.002	0.77
	L to H	0.043	0.002	0.79	0.043	0.002	0.79

Table 15.10A Summary of the correlations between the changes in PA with accommodation and biometric and oculomotor parameters. * denotes a statistically significant result (n=42).

Parameter	Median split	L to I				L to H				I to H			
		Absolute		Percentage		Absolute		Percentage		Absolute		Percentage	
		F	p	F	p	F	p	F	p	F	p	F	p
CCT	540μm	0.004	0.948	0.003	0.958	0.729	0.403	0.859	0.365	0.693	0.415	0.943	0.343
ACD	3.13mm	0.650	0.430	1.147	0.297	0.540	0.471	0.882	0.359	0.132	0.720	0.306	0.586
Ach Vol	201ml^3	0.110	0.744	0.026	0.872	1.138	0.299	1.199	0.287	1.322	0.264	1.214	0.284
AL	24.51mm	1.712	0.206	1.633	0.216	2.101	0.163	2.138	0.159	0.647	0.431	0.872	0.362
Ach Ang	41.8°	1.155	0.295	1.003	0.329	1.566	0.225	1.314	0.265	0.522	0.478	0.514	0.482
CVol	59.7ml^3	0.284	0.600	0.251	0.622	0.584	0.454	0.729	0.403	0.895	0.355	1.050	0.318
CC	7.70mm	1.875	0.186	2.834	0.108	0.406	0.531	0.309	0.584	1.356	0.258	1.093	0.308
OVol	6426mm^3	1.712	0.206	1.633	0.216	2.101	0.163	2.138	0.159	0.647	0.431	0.872	0.362
E-values	0.55	0.284	0.600	0.055	0.817	0.781	0.387	1.099	0.307	1.127	0.301	1.129	0.301
AC	5.23$^\lambda$	1.034	0.321	0.639	0.433								
	10.04$^\lambda$					0.835	0.372	0.967	0.337				
	4.40$^\lambda$									1.127	0.301	0.926	0.347
AC/A	2.14	0.360	0.555	0.109	0.744								
	1.99					0.346	0.563	0.662	0.426				

Table 15.11A Summary of median split analyses of the biometric and oculomotor parameters and the changes in PA with accommodation in the myopes only. * denotes a statistically significant result (n=22).

Biometric parameter	Accommodation level (D)	Absolute change in IOP (mmHg)			% change in IOP (mmHg)		
		r	r^2	p	r	r^2	p
CCT	L to I	0.115	0.013	0.61	0.089	0.008	0.70
	L to H	0.057	0.003	0.80	0.087	0.008	0.70
	I to H	0.101	0.010	0.66	0.136	0.018	0.55
ACD	L to I	0.064	0.004	0.78	0.128	0.016	0.57
	L to H	0.133	0.018	0.55	0.183	0.033	0.42
	I to H	0.100	0.010	0.66	0.127	0.016	0.57
ACh Vol	L to I	0.067	0.005	0.77	0.115	0.013	0.61
	L to H	0.307	0.094	0.17	0.333	0.111	0.13
	I to H	0.262	0.069	0.24	0.274	0.075	0.22
AL	L to I	0.381	0.145	0.08	0.415	0.172	0.05
	L to H	0.191	0.037	0.39	0.184	0.034	0.41
	I to H	0.026	0.001	0.91	0.039	0.001	0.86
ACh Ang	L to I	0.121	0.015	0.59	0.081	0.006	0.72
	L to H	0.089	0.008	0.69	0.052	0.003	0.82
	I to H	0.034	0.001	0.88	0.031	0.001	0.89
CVol	L to I	0.276	0.076	0.21	0.261	0.068	0.24
	L to H	0.089	0.008	0.69	0.077	0.006	0.73
	I to H	0.028	0.001	0.90	0.034	0.001	0.88
CC	L to I	0.145	0.021	0.52	0.172	0.030	0.44
	L to H	0.044	0.002	0.85	0.022	0.001	0.92
	I to H	0.100	0.010	0.66	0.069	0.005	0.76
OVol	L to I	0.338	0.115	0.12	0.362	0.131	0.10
	L to H	0.195	0.038	0.38	0.187	0.035	0.40
	I to H	0.047	0.002	0.84	0.058	0.003	0.80
Esphercity	L to I	0.243	0.059	0.28	0.163	0.026	0.47
	L to H	0.059	0.003	0.79	0.101	0.010	0.66
	I to H	0.154	0.024	0.49	0.148	0.022	0.51
AC	L to I	0.064	0.004	0.78	0.089	0.008	0.69
	L to H	0.047	0.002	0.84	0.024	0.001	0.92
	I to H	0.164	0.027	0.47	0.137	0.019	0.54
AC/A	L to I	0.058	0.003	0.80	0.004	<0.001	0.98
	L to H	0.092	0.008	0.68	0.142	0.020	0.53

Table 15.12A Summary of the correlations between the changes in PA with accommodation and biometric and oculomotor parameters in the myopes only. * denotes a statistically significant result (n=22).

Parameter	Median split	L to I				L to H				I to H			
		Absolute		Percentage		Absolute		Percentage		Absolute		Percentage	
		F	p	F	p	F	p	F	p	F	p	F	p
CCT	533.5μm	0.079	0.780	0.038	0.847	0.275	0.603	0.183	0.671	0.424	0.519	0.919	0.343
ACD	3.10mm	2.295	0.138	2.511	0.121	0.001	0.974	0.017	0.897	1.251	0.270	1.476	0.232
Ach Vol	196ml^3	0.470	0.497	0.621	0.435	0.418	0.522	0.340	0.563	1.116	0.297	0.467	0.498
AL	23.73mm	1.253	0.270	1.563	0.219	0.207	0.651	0.74	0.787	0.175	0.678	1.038	0.314

Ach Ang	41.0°	0.233	0.632	0.186	0.668	1.613	0.211	1.645	0.207	0.505	0.481	0.627	0.433
CVol	59.65ml^3	0.868	0.357	1.039	0.314	0.565	0.457	0.469	0.497	1.778	0.190	1.265	0.267
CC	7.725mm	0.107	0.745	0.059	0.810	0.163	0.688	0.315	0.578	1.164	0.287	0.667	0.419
E-values	0.52	0.103	0.750	0.159	0.693	1.904	0.175	2.103	0.155	1.992	0.166	1.347	0.253
OVol	6130mm^3	1.253	0.270	1.563	0.219	0.207	0.651	0.074	0.787	0.175	0.678	1.038	0.314
Ref err	-0.87D	3.678	0.062	3.655	0.063	3.803	0.058	4.081	0.050	0.060	0.808	0.876	0.355
AC	4.84$^\Delta$	0.393	0.534	0.560	0.459								
	10.89$^\Delta$					0.046	0.832	0.003	0.959				
	6.00$^\Delta$									2.365	0.132	1.448	0.236
AC/A	1.65	3.455	0.070	2.798	0.102								
	1.99					3.608	0.065	3.715	0.061				

Table 15.13A Summary of median split analyses of the biometric and oculomotor parameters and the changes in PR with accommodation. * denotes a statistically significant result (n=42).

Biometric parameter	Accommodation level (D)	Absolute change in IOP (mmHg)			% change in IOP (mmHg)		
		r	r^2	p	r	r^2	p
CCT	L to I	0.015	<0.001	0.46	0.009	<0.001	0.48
	L to H	-0.036	0.001	0.41	-0.015	<0.001	0.46
	I to H	-0.042	0.002	0.40	-0.042	0.002	0.397
ACD	L to I	-0.074	0.005	0.32	-0.088	0.008	0.29
	L to H	-0.004	<0.001	049	-0.028	0.001	0.43
	I to H	0.051	0.003	0.38	0.051	0.003	0.38
ACh Vol	L to I	-0.107	0.011	0.25	-0.122	0.015	0.22
	L to H	0.065	0.004	0.34	0.034	0.001	0.42
	I to H	0.133	0.018	0.20	0.133	0.018	0.20
AL	L to I	-0.061	0.004	0.70	-0.074	0.005	0.64
	L to H	0.092	0.009	0.56	0.079	0.006	0.62
	I to H	0.031	0.001	0.84	0.052	0.003	0.74
ACh Ang	L to I	-0.076	0.006	0.32	-0.065	0.004	0.34
	L to H	-0.083	0.007	0.30	-0.117	0.014	0.23
	I to H	-0.015	<0.001	0.46	-0.015	<0.001	0.46
CVol	L to I	0.155	0.024	0.16	0.151	0.023	0.17
	L to H	0.079	0.006	0.31	0.082	0.007	0.30
	I to H	-0.146	0.021	0.39	-0.046	0.002	0.39
CC	L to I	-0.016	<0.001	0.46	<0.001	<0.001	0.50
	L to H	0.061	0.004	0.35	0.094	0.009	0.28
	I to H	0.064	0.004	0.34	0.064	0.004	0.34
Esphercity	L to I	0.054	0.003	0.37	0.084	0.007	0.30
	L to H	-0.215	0.046	0.09	-0.243	0.059	0.06
	I to H	-0.222	0.049	0.08	-0.222	0.049	0.08
OVol	L to I	-0.016	<0.001	0.12	-0.194	0.038	0.11
	L to H	-0.066	0.004	0.34	-0.060	0.004	0.35
	I to H	0.077	0.006	0.31	0.077	0.006	0.31
Ref error	L to I	0.190	0.036	0.11	0.197	0.039	0.11
	L to H	0.050	0.003	0.38	0.045	0.002	0.39
	I to H	-0.096	0.009	0.27	-0.096	0.009	0.27
AC	L to I	-0.013	<0.001	0.47	-0.012	<0.001	0.47
	L to H	-0.070	0.005	0.33	-0.082	0.007	0.30
	I to H	-0.118	0.014	0.23	-0.118	0.014	0.23
AC/A	L to I	-0.139	0.019	0.19	-0.139	0.019	0.19
	L to H	-0.224	0.050	0.08	-0.231	0.053	0.07

Table 15.14A Summary of the correlations between the changes in PR with accommodation and biometric and oculomotor parameters. * denotes a statistically significant result (n=42).

Parameter	Median split	L to I				L to H				I to H			
		Absolute		Percentage		Absolute		Percentage		Absolute		Percentage	
		F	p	F	p	F	p	F	p	F	p	F	p
CCT	540µm	0.101	0.753	0.109	0.744	0.687	0.417	0.682	0.419	0.831	0.373	1.062	0.315
ACD	3.13mm	0.008	0.930	0.036	0.851	0.479	0.497	0.350	0.561	0.415	0.527	0.969	0.337
Ach Vol	201ml^3	0.620	0.440	0.488	0.493	0.825	0.374	0.710	0.410	0.073	0.790	0.770	0.391
AL	24.51mm	0.086	0.773	0.031	0.863	2.874	0.160	3.637	0.071	1.455	0.242	1.184	0.289
Ach Ang	41.8°	0.464	0.503	0.457	0.507	1.632	0.216	1.724	0.204	0.394	0.537	0.647	0.431
CVol	59.7ml^3	0.620	0.440	0.732	0.402	0.331	0.571	0.195	0.664	0.994	0.331	1.097	0.307
CC	7.70mm	0.036	0.851	0.119	0.734	1.327	0.263	1.020	0.324	1.212	0.284	1.136	0.299
OVol	6426mm^3	0.086	0.773	0.031	0.863	2.874	0.106	3.637	0.071	1.455	0.242	1.184	0.289
E-values	0.55	0.036	0.851	0.093	0.764	1.976	0.175	2.474	0.131	1.726	0.204	1.255	0.276
AC	5.23$^\Delta$	0.118	0.734	0.152	0.701								
	10.04$^\Delta$					1.475	0.239	1.753	0.200				
	4.40$^\Delta$									3.268	0.086	1.457	0.242
AC/A	2.14	2.583	0.124	2.325	0.143								
	1.99					3.368	0.081	3.672	0.070				

Table 15.15A Summary of median split analyses of the biometric and oculomotor parameters and the changes in PR with accommodation in the myopes only. * denotes a statistically significant result (n=22).

Biometric parameter	Accommodation level (D)	Absolute change in IOP (mmHg)			% change in IOP (mmHg)		
		R	r^2	P	R	r^2	P
CCT	L to I	0.145	0.021	0.260	0.135	0.018	0.275
	L to H	-0.060	0.004	0.395	-0.040	0.002	0.430
	I to H	-0.142	0.020	0.264	-0.137	0.019	0.271
ACD	L to I	0.214	0.046	0.170	0.168	0.028	0.227
	L to H	0.228	0.052	0.153	0.221	0.049	0.162
	I to H	0.060	0.004	0.395	0.062	0.004	0.391
ACh Vol	L to I	0.274	0.075	0.109	0.235	0.055	0.146
	L to H	0.343	0.118	0.059	0.341	0.116	0.060
	I to H	0.120	0.014	0.297	-0.085	0.007	0.353
AL	L to I	-0.026	0.001	0.454	-0.048	0.002	0.417
	L to H	0.163	0.027	0.234	0.214	0.046	0.170
	I to H	0.155	0.024	0.245	0.243	0.059	0.138
ACh Ang	L to I	-0.136	0.018	0.273	-0.140	0.020	0.268
	L to H	-0.009	<0.001	0.484	-0.035	0.001	0.439
	I to H	0.078	0.006	0.366	0.259	0.067	0.122
CVol	L to I	0.238	0.057	0.143	0.234	0.055	0.147
	L to H	0.157	0.025	0..243	0.177	0.031	0.215
	I to H	-0.016	<0.001	0.472	-0.134	0.018	0.277
CC	L to I	-0.040	0.002	0.430	-0.004	<0.001	0.492
	L to H	-0.019	<0.001	0.466	0.010	<0.001	0.483
	I to H	0.009	<0.001	0.484	-0.116	0.013	0.304
OVol	L to I	0.042	0.002	0.427	-0.058	0.003	0.400
	L to H	0.151	0.023	0.252	0.195	0.038	0.193
	I to H	0.154	0.024	0.246	0.233	0.054	0.149
Esphercity	L to I	-0.033	0.001	0.442	0.011	<0.001	0.481
	L to H	-0.232	0.054	0.149	-0.292	0.085	0.094
	I to H	-0.177	0.031	0.216	-0.169	0.029	0.226
AC	L to I	-0.054	0.003	0.406	-0.058	0.003	0.399
	L to H	-0.093	0.009	0.340	-0.116	0.013	0.304
	I to H	-0.223	0.050	0.160	-0.245	0.060	0.136
AC/A	L to I	-0.218	0.048	0.164	-0.204	0.042	0.181
	L to H	-0.362	0.131	0.049	-0.396	0.157	0.034

Table 15.16A Summary of the correlations between the changes in PR with accommodation and biometric and oculomotor parameters in the myopes only. * denotes a statistically significant result (n=22).

Parameter	Median split	L to I				L to H				I to H			
		Absolute		Percentage		Absolute		Percentage		Absolute		Percentage	
		F	p	F	p	F	p	F	p	F	p	F	p
CCT	533.5µm	2.897	0.098	1.774	0.190	1.931	0.172	1.515	0.226	0.383	0.540	0.745	0.393
ACD	3.10mm	1.278	0.265	0.625	0.434	0.122	0.729	0.449	0.507	0.645	0.427	0.865	0.358
Ach Vol	196ml$^{\lambda}$	1.147	0.290	0.518	0.476	0.874	0.356	0.947	0.336	0.199	0.658	0.465	0.499
AL	23.73mm	0.171	0.682	0.031	0.862	0.433	0.514	0.497	0.485	0.207	0.651	0.426	0.518
Ach Ang	41.0°	0.136	0.715	0.491	0.487	1.061	0.309	1.003	0.323	1.309	0.259	1.193	0.281
CVol	59.65ml$^{\lambda}$	2.674	0.110	2.049	0.160	1.576	0.217	1.311	0.259	0.271	0.606	0.660	0.421
CC	7.725mm	0.280	0.599	0.090	0.766	0.241	0.626	0.145	0.705	0.026	0.873	0.079	0.780
E-values	0.52	0.011	0.916	0.035	0.853	0.047	0.830	0.027	0.870	0.085	0.772	0.011	0.919
OVol	6130mm$^{\lambda}$	<0.001	0.987	0.306	0.583	0.354	0.555	0.416	0.522	0.316	0.577	0.470	0.497
Refer	-0.87D	0.184	0.670	0.235	0.631	0.537	0.468	0.710	0.405	0.780	0.382	0.712	0.404
AC	4.84$^{\lambda}$	1.109	0.299	0.870	0.357								
	10.89$^{\lambda}$					0.568	0.455	1.760	0.192				
	6.00$^{\lambda}$									0.085	0.772	0.152	0.699
AC/A	1.65	2.517	0.121	1.498	0.228								
	1.99					0.405	0.528	0.065	0.799				

Table 15.17A Summary of median split analyses of the biometric and oculomotor parameters and the changes in PV with accommodation. * denotes a statistically significant result (n=42).

Biometric parameter	Accommodation level (D)	Absolute change in IOP (mmHg)			% change in IOP (mmHg)		
		R	r^2	P	R	r^2	P
CCT	L to I	-0.201	0.040	0.101	-0.139	0.019	0.190
	L to H	-0.256	0.066	0.051	-0.263	0.069	0.046
	I to H	0.004	<0.001	0.491	-0.048	0.002	0.381
ACD	L to I	0.099	0.001	0.266	0.065	0.004	0.342
	L to H	0.106	0.011	0.252	0.103	0.011	0.258
	I to H	-0.019	<0.001	0.454	-0.063	0.004	0.347
ACh Vol	L to I	0.146	0.021	0.177	0.118	0.014	0.229
	L to H	0.074	0.005	0.321	0.077	0.006	0.313
	I to H	-0.096	0.009	0.273	-0.132	0.017	0.202
AL	L to I	0.058	0.003	0.357	-0.010	<0.001	0.476
	L to H	-0.084	0.007	0.299	-0.196	0.038	0.107
	I to H	-0.132	0.017	0.202	-0.193	0.037	0.111
ACh Ang	L to I	0.109	0.012	0.247	0.154	0.024	0.166
	L to H	-0.006	<0.001	0.486	-0.028	0.001	0.431
	I to H	-0.121	0.015	0.222	-0.073	0.005	0.324
CVol	L to I	-0.277	0.077	0.038	-0.245	0.060	0.059
	L to H	-0.197	0.039	0.106	-0.163	0.027	0.151

	I to H	0.134	0.018	0.199	0.138	0.019	0.192
CC	L to I	0.088	0.008	0.290	0.057	0.003	0.361
	L to H	-0.136	0.018	0.195	-0.165	0.027	0.148
	I to H	-0.207	0.043	0.094	-0.116	0.013	0.233
Esphercity	L to I	-0.094	0.009	0.278	-0.096	0.009	0.273
	L to H	0.042	0.002	0.396	0.089	0.008	0.287
	I to H	0.135	0.018	0.197	0.178	0.032	0.130
OVol	L to I	0.037	0.001	0.408	-0.011	<0.001	0.474
	L to H	-0.057	0.003	0.359	-0.146	0.021	0.179
	I to H	-0.087	0.008	0.291	-0.150	0.023	0.171
Ref error	L to I	-0.082	0.007	0.302	-0.055	0.003	0.365
	L to H	-0.029	0.001	0.427	0.034	0.001	0.414
	I to H	0.064	0.004	0.344	0.145	0.021	0.179
AC	L to I	-0.043	0.002	0.395	0.086	0.007	0.295
	L to H	<0.001	<0.001	0.499	0.086	0.007	0.295
	I to H	0.006	<0.001	0.485	-0.057	0.003	0.360
AC/A	L to I	-0.097	0.009	0.270	0.020	<0.001	0.451
	L to H	-0.130	0.017	0.207	-0.052	0.003	0.372

Table 15.18A Summary of the correlations between the changes in PV with accommodation and biometric and oculomotor parameters. * denotes a statistically significant result (n=42).

Parameter	Median split	L to I				L to H				I to H			
		Absolute		Percentage		Absolute		Percentage		Absolute		Percentage	
		F	p	F	p	F	p	F	p	F	p	F	p
CCT	545µm	1.298	0.268	0.373	0.548	1.562	0.226	1.486	0.237	0.020	0.889	0.045	0.834
ACD	3.17mm	3.595	0.072	4.252	0.052	0.204	0.656	0.102	0.753	4.261	0.052	3.967	0.060
Ach Vol	201.5ml^3	0.032	0.859	0.044	0.836	0.774	0.389	0.121	0.732	0.389	0.540	0.103	0.751
AL	24.61mm	0.066	0.80	0.009	0.926	0.001	0.971	0.014	0.906	0.062	0.805	0.002	0.963
Ach Ang	41.5°	0.892	0.356	0.382	0.544	0.332	0.571	0.059	0.811	0.074	0.789	0.031	0.861
CVol	60.4ml^3	0.409	0.530	0.043	0.837	0.913	0.351	1.001	0.329	0.090	0.768	0.093	0.763
CC	7.70mm	0.945	0.343	1.716	0.205	<0.001	0.990	0.010	0.922	0.683	0.418	1.541	0.229
OVol	6426mm^3	0.066	0.800	0.009	0.926	0.001	0.971	0.014	0.906	0.062	0.805	0.002	0.963
E-values	0.555	0.016	0.899	0.022	0.884	0.162	0.691	0.115	0.738	0.219	0.645	0.068	0.796
AC	4.99$^\Delta$	0.355	0.558	0.039	0.846								
	8.94$^\Delta$					0.004	0.952	0.003	0.954				
	4.16$^\Delta$									0.219	0.645	0.024	0.880
AC/A	1.92	1.363	0.257	0.213	0.649								
	1.93					0.566	0.461	0.396	0.536				

Table 15.19A Summary of median split analyses of the biometric and oculomotor parameters and the changes in PV with accommodation in the myopes only. * denotes a statistically significant result (n=22).

Biometric parameter	Accommodation level (D)	Absolute change in IOP (mmHg)			% change in IOP (mmHg)		
		R	r^2	P	R	r^2	P
CCT	L to I	-0.244	0.059	0.137	-0.072	0.005	0.376
	L to H	-0.448	0.201	**0.018***	-0.423	0.179	**0.025***
	I to H	-0.193	0.037	0.195	-0.225	0.051	0.157
ACD	L to I	-0.196	0.038	0.191	-0.263	0.069	0.118
	L to H	0.064	0.004	0.389	0.055	0.003	0.404
	I to H	0.222	0.049	0.161	0.261	0.068	0.120
ACh Vol	L to I	-0.045	0.002	0.422	-0.055	0.003	0.403
	L to H	-0.021	<0.001	0.464	0.045	0.002	0.421
	I to H	0.019	<0.001	0.466	0.078	0.006	0.366
AL	L to I	-0.143	0.020	0.262	-0.099	0.010	0.330
	L to H	-0.222	0.049	0.160	-0.244	0.060	0.137
	I to H	-0.077	0.006	0.366	-0.135	0.018	0.274
ACh Ang	L to I	-0.072	0.005	0.375	-0.129	0.017	0.283
	L to H	0.187	0.035	0.202	0.129	0.017	0.283
	I to H	0.227	0.052	0.155	0.315	0.099	0.077
CVol	L to I	-0.245	0.060	0.136	-0.101	0.010	0.327
	L to H	-0.216	0.047	0.167	-0.193	0.037	0.195
	I to H	0.014	<0.001	0.475	0.089	0.008	0.347
CC	L to I	0.185	0.034	0.205	0.242	0.059	0.139
	L to H	-0.076	0.006	0.368	-0.070	0.005	0.379
	I to H	-0.224	0.050	0.158	-0.225	0.051	0.157
OVol	L to I	-0.128	0.016	0.284	-0.082	0.007	0.359
	L to H	-0.183	0.033	0.208	-0.186	0.035	0.203
	I to H	-0.054	0.003	0.405	-0.095	0.009	0.336
Esphercity	L to I	-0.382	0.146	0.060	-0.316	0.100	0.076
	L to H	0.014	<0.001	0.476	0.059	0.003	0.397
	I to H	0.335	0.112	0.064	0.359	0.129	0.050
AC	L to I	0.283	0.080	0.101	0.419	0.176	0.070
	L to H	0.034	0.001	0.440	0.071	0.005	0.377
	I to H	0.081	0.007	0.360	-0.002	<0.001	0.496
AC/A	L to I	0.010	<0.001	0.483	0.164	0.027	0.232
	L to H	-0.128	0.016	0.285	-0.103	0.011	0.325

Table 15.20A Summary of the correlations between the changes in PV with accommodation and biometric and oculomotor parameters in the myopes only. * denotes a statistically significant result (n=22).

Parameter	Median split	L to I				L to H				I to H			
		Absolute		Percentage		Absolute		Percentage		Absolute		Percentage	
		F	p	F	p	F	p	F	p	F	p	F	p
CCT	533.5µm	5.132	**0.029***	3.283	0.078	1.155	0.289	0.340	0.563	1.281	0.264	1.304	0.260
ACD	3.10mm	0.316	0.577	0.555	0.461	0.132	0.718	0.065	0.800	0.951	0.335	0.417	0.522
Ach Vol	196ml^3	0.107	0.746	0.155	0.696	0.246	0.623	0.422	0.519	0.031	0.860	0.421	0.520
AL	23.73mm	1.241	0.272	1.271	0.266	1.725	0.197	2.833	0.100	0.041	0.841	0.768	0.386
Ach Ang	41.0°	0.365	0.549	0.157	0.694	0.119	0.732	0.245	0.623	1.001	0.323	1.560	0.219
CVol	59.65ml^3	2.979	0.092	2.014	0.164	0.792	0.379	0.870	0.357	8.583	**0.006***	7.111	**0.011***
CC	7.725mm	0.505	0.482	0.058	0.811	0.584	0.449	1.415	0.241	2.487	0.123	2.003	0.165
E-values	0.52	0.001	0.970	0.014	0.907	0.212	0.648	0.101	0.753	0.272	0.605	0.407	0.527
OVol	6130mm^3	1.359	0.251	1.440	0.237	1.608	0.212	2.776	0.103	0.011	0.916	0.666	0.416
Ref err	-0.87D	3.195	0.081	1.712	0.198	1.687	0.201	2.060	0.159	0.214	0.646	0.184	0.641
AC	4.84$^\lambda$	0.358	0.553	0.078	0.781								
	10.89$^\lambda$					0.436	0.513	0.467	0.498				
	6.00$^\lambda$									0.059	0.809	0.567	0.456
AC/A	1.65	3.490	0.069	1.606	0.212								
	1.99					1.390	0.245	0.791	0.379				

Table 15.21A Summary of median split analyses of the biometric and oculomotor parameters and the changes in POBF with accommodation. * denotes a statistically significant result (n=42).

Biometric parameter	Accommodation level (D)	Absolute change in IOP (mmHg)			% change in IOP (mmHg)		
		r	r^2	p	r	r^2	p
CCT	L to I	0.218	0.047	0.17	0.162	0.026	0.30
	L to H	0.052	0.003	0.74	0.010	<0.001	0.96
	I to H	0.173	0.030	0.27	0.179	0.032	0.26
ACD	L to I	0.156	0.024	0.32	0.149	0.022	0.35
	L to H	0.017	<0.001	0.91	0.010	<0.001	0.94
	I to H	0.182	0.033	0.25	0.091	0.008	0.57
ACh Vol	L to I	0.150	0.023	0.34	0.142	0.020	0.37
	L to H	0.098	0.010	0.53	0.114	0.013	0.47
	I to H	0.054	0.003	0.74	0.032	0.001	0.84
AL	L to I	0.142	0.020	0.37	0.128	0.017	0.42
	L to H	0.308	0.095	0.05	0.374	0.140	**0.01***
	I to H	0.173	0.030	0.27	0.303	0.092	0.05
ACh Ang	L to I	0.061	0.004	0.70	0.085	0.007	0.59
	L to H	0.008	<0.001	0.96	0.027	<0.001	0.86
	I to H	0.072	0.005	0.65	0.018	<0.001	0.91
CVol	L to I	0.242	0.059	0.12	0.183	0.034	0.25
	L to H	0.049	0.002	0.76	0.080	0.006	0.61
	I to H	0.304	0.092	0.05	0.272	0.074	0.08
CC	L to I	0.019	<0.001	0.91	0.048	0.002	0.76
	L to H	0.295	0.087	0.06	0.310	0.096	0.05
	I to H	0.329	0.108	**0.03***	0.248	0.062	0.11
Esphercity	L to I	0.106	0.011	0.50	0.090	0.008	0.57
	L to H	0.084	0.007	0.60	0.058	0.003	0.71
	I to H	0.023	0.001	0.89	0.006	<0.001	0.97
OVol	L to I	0.125	0.017	0.43	0.109	0.012	0.49
	L to H	0.293	0.086	0.06	0.365	0.133	**0.017***
	I to H	0.177	0.031	0.26	0.319	0.102	0.08
Ref error	L to I	0.006	<0.001	0.97	0.057	0.003	0.72
	L to H	0.062	0.004	0.69	0.130	0.019	0.41
	I to H	0.060	0.004	0.71	0.219	0.048	0.16
AC	L to I	0.093	0.009	0.56	0.248	0.062	0.11
	L to H	0.023	<0.001	0.88	0.075	0.006	0.64
	I to H	0.089	0.008	0.57	0.159	0.025	0.32
AC/A	L to I	0.099	0.010	0.53	0.018	<0.001	0.91
	L to H	0.100	0.010	0.53	0.056	0.003	0.72

Table 15.22A Summary of the correlations between the changes in POBF with accommodation and biometric and oculomotor parameters. * denotes a statistically significant result (n=42).

Parameter	Median split	L to I				L to H				I to H			
		Absolute		Percentage		Absolute		Percentage		Absolute		Percentage	
		F	p	F	p	F	p	F	p	F	p	F	p
CCT	540µm	0.519	0.480	0.141	0.711	0.033	0.858	0.066	0.800	0.149	0.704	0.555	0.465
ACD	3.13mm	7.347	**0.013***	7.019	**0.015***	0.606	0.446	0.467	0.502	1.185	0.289	1.128	0.301
Ach Vol	201ml^3	0.407	0.531	0.499	0.488	2.017	0.171	2.045	0.168	1.014	0.326	1.237	0.279
AL	24.51mm	0.036	0.851	0.204	0.656	0.105	0.750	0.392	0.538	0.268	0.610	1.252	0.276
Ach Ang	41.8°	0.197	0.662	0.371	0.549	0.746	0.398	0.624	0.439	1.862	0.188	2.171	0.156
CVol	59.7ml^3	0.156	0.697	0.006	0.940	0.003	0.959	0.122	0.730	0.071	0.792	0.384	0.543
CC	7.70mm	0.033	0.857	0.043	0.837	0.151	0.702	0.358	0.556	0.341	0.566	0.926	0.348
E-values	0.55	0.811	0.379	0.894	0.356	0.994	0.331	0.989	0.332	0.126	0.727	0.101	0.754
OVol	6426mm^3	0.036	0.851	0.204	0.656	0.105	0.750	0.392	0.538	0.268	0.610	1.252	0.276
AC	5.23$^\Delta$	0.340	0.567	0.472	0.500								
	10.04$^\Delta$					0.059	0.810	0.024	0.878				
	4.40$^\Delta$									0.268	0.610	0.515	0.481
AC/A	2.14	0.006	0.938	0.074	0.788								
	1.99					2.160	0.157	1.626	0.217				

Table 15.23A Summary of median split analyses of the biometric and oculomotor parameters and the changes in POBF with accommodation in the myopes only. * denotes a statistically significant result (n=22).

Biometric parameter	Accommodation level (D)	Absolute change in IOP (mmHg)			% change in IOP (mmHg)		
		r	r^2	p	r	r^2	p
CCT	L to I	0.199	0.040	0.37	0.090	0.008	0.69
	L to H	0.045	0.002	0.84	0.058	0.003	0.80
	I to H	0.114	0.013	0.61	0.162	0.026	0.47
ACD	L to I	0.353	0.124	0.11	0.384	0.147	0.08
	L to H	0.109	0.012	0.63	0.104	0.011	0.65
	I to H	0.170	0.029	0.45	0.172	0.030	0.44
ACh Vol	L to I	0.203	0.041	0.36	0.214	0.046	0.34
	L to H	0.233	0.054	0.30	0.234	0.055	0.30
	I to H	0.091	0.008	0.69	0.118	0.014	0.60
AL	L to I	0.158	0.025	0.48	0.099	0.010	0.66
	L to H	0.295	0.087	0.18	0.345	0.119	0.12
	I to H	0.198	0.039	0.38	0.322	0.104	0.14
ACh Ang	L to I	0.145	0.021	0.52	0.144	0.021	0.52
	L to H	0.061	0.004	0.79	0.061	0.004	0.79
	I to H	0.187	0.035	0.40	0.241	0.058	0.28
CVol	L to I	0.089	0.008	0.69	0.019	<0.001	0.93
	L to H	0.066	0.004	0.77	0.152	0.023	0.50
	I to H	0.146	0.001	0.52	0.225	0.050	0.31
CC	L to I	0.065	0.004	0.77	0.059	0.003	0.79
	L to H	0.255	0.065	0.25	0.287	0.082	0.20
	I to H	0.230	0.053	0.30	0.278	0.078	0.21
OVol	L to I	0.145	0.021	0.52	0.097	0.009	0.67
	L to H	0.324	0.105	0.14	0.365	0.133	0.09
	I to H	0.241	0.058	0.28	0.342	0.117	0.119
Esphercity	L to I	0.336	0.113	0.13	0.305	0.093	0.17
	L to H	0.229	0.053	0.30	0.157	0.025	0.49
	I to H	0.023	<0.001	0.92	0.102	0.010	0.65
AC	L to I	0.521	0.271	**0.01***	0.614	0.376	**0.002***
	L to H	0.059	0.004	0.79	0.025	<0.001	0.91
	I to H	0.240	0.058	0.28	0.278	0.077	0.21
AC/A	L to I	0.147	0.022	0.51	0.208	0.043	0.35
	L to H	0.241	0.058	0.28	0.237	0.056	0.29

Table 15.24A Summary of the correlations between the changes in POBF with accommodation and biometric and oculomotor parameters in the myopes only. * denotes a statistically significant result (n=22).

SUPPORTING PUBLICATIONS

Abstracts

Rai, G. K., Gilmartin, B., Wolffsohn, J. S. and Cervino, A. (2006). The effect of accommodation on IOP: evidence for dose dependency. *ARVO*, e-abstract 5859.

Rai, G. K., Gilmartin, B., Wolffsohn, J. S. and Cervino, A. (2006). The effect of myopia, axial lengths and ocular volumes on IOP response patterns to accommodation. *11th International Myopia Conference, Singapore.*

Rai, G. K., Gilmartin, B. and Wolffsohn, J. S. (2007). The Effect of Accommodation on Pulsatile Ocular Blood Flow. . *ARVO*, e-abstract 1040.

THE EFFECT OF ACCOMMODATION ON IOP: EVIDENCE FOR DOSE DEPENDENCY

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Purpose: Literature on the relationship between accommodation and IOP is limited with the most recent study (Mauger *et al.*, 1984 *Am J Optom Physiol Opt*) reporting a reduction of 1.32±0.43 mmHg after 3.5 mins of accommodation to a 4D stimulus using contact tonometry (CT) (N=30, age 22-35 yrs). We investigated the relationship further using a high resolution (0.1 mmHg) non-contact tonometer (NCT) coupled with the cardiac pulse, a fixed pace respiratory cycle (15 breathes/min) and quasi-continuous measures of accommodation responses to 3 levels of accommodation stimuli.

Methods: A modified NCT (*Pulsair* Keeler, UK) was coupled with a finger pulse transducer and a *Labview* Acquisition Program (National Instruments, USA) to trigger the air pulse at 1 of 3 locations on the cardiac cycle; peak, middle, or trough. Of the 3 locations, the middle demonstrated the least variance and a high level of correlation ($r=0.90$, $p<0.001$) with Goldmann CT (N=50, mean age 21.1±3.0 yrs). Accordingly, 5 IOP measures synchronized with the middle location were taken on the RE while the LE fixated zero (low=L), 1.50 (intermediate=I) and 4D (high=H) accommodative stimuli (presented randomly) for 3 minutes (N=40, mean age 20.6±3.0yrs). The LE was rendered functionally emmetropic with soft contact lenses and the accommodative response was measured at 1s intervals during the IOP measurement period with the portable *Flexible-Ref FR-5000* (Grand Seiko Co, Ltd, Japan) monocular IR open-view autorefractor.

Results: Mean (MN) accommodation response levels for L, I, and H stimulus levels were respectively -0.14±0.31, 1.67±0.20 and 4.15±0.36D. The corresponding distributions of IOP (mmHg) were L: MN±SD=13.95±2.09, range (RG)=10.24-19.47, median (MD)=13.51; I: MN±SD=13.34±2.05, RG=9.62-18.92, MD=12.94 and H: MN±SD =13.82±2.17, RG=9.09-20.33, MD=13.34. ANOVA indicated a significant effect of accommodation on IOP ($F=4.82$, $p=0.016$). The MN±SD and RG of the differences in IOP (mmHg) between the L to I and L to H accommodation levels were respectively 0.61±0.99 ($p<0.001$), RG=1.01 to -2.75 and 0.13±1.41 ($p=0.56$), RG=4.47 to -3.27. The % change in IOP between the L to I and L to H accommodation levels were respectively, -4.23±7.05 and -0.55±10.23 ($p=0.04$).

Conclusions: The data demonstrate that the relationship between accommodation and IOP is characterised by substantial inter-subject variation. Despite this, intermediate accommodation response levels are shown to lower IOP significantly. The dose dependency evident has instigated a series of investigations on accommodation and autoregulation within the ocular vascular system.

Support: College of Optometrists,UK

THE EFFECT OF MYOPIA, AXIAL LENGTHS AND OCULAR VOLUMES ON IOP RESPONSE PATTERNS TO ACCOMMODATION.

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Objectives. To examine the effect of myopia and associated ocular biometry on the significant reduction in IOP for intermediate levels of accommodation previously reported using a non-contact tonometer coupled with the cardiac pulse, a fixed pace respiratory cycle (15 breathes/min) and quasi-continuous measures of accommodation responses (see Rai *et al.* ARVO Abstract 2006)

Method. A modified, high resolution (0.1 mmHg) non-contact tonometer (*Pulsair* Keeler, UK) was coupled with a finger pulse-transducer and a *LabView* Acquisition Program (National Instruments) to obtain in the RE of 50 subjects 5 IOP measures synchronized with the middle of the cardiac while the LE (rendered functionally emmetropic with a soft contact lens) fixated a zero (low), 1.50 (intermediate) and 4D (high) accommodative stimuli (presented randomly) for 3 minutes. Accommodation responses were simultaneously obtained at 1 second intervals with the portable monocular *Flexible-Ref FR-5000* (Grand Seiko Co, Ltd, Japan) open-view autorefractor. Ocular biometry measures comprised: non-cycloplegic refractive error (Shin-Nippon open-view autorefractor), anterior chamber depth, axial length (Zeiss IOL Master, Carl Zeiss Meditec) and ocular volume (derived from 3-D MRI data).

Results. Mean±SD accommodation response levels (D) and corresponding IOP (mmHg) measures to zero, 1.50 and 4D accommodation stimuli levels were respectively, -0.16±0.34, 14.09±2.36; 1.75±0.23, 13.50±2.68 and 4.15±0.21, 14.06±2.65. Consistent with our previous finding the change in IOP of 0.59±1.69 mmHg between low and intermediate levels of accommodation was found to be significant ($p<0.016$). No significant differences in IOP changes were elicited by emmetropes (N=21) and myopes (N=28) ($p=0.95$), by differences in axial lengths (median split at 23.87mm, $p=0.10$) and ocular volumes (median split at 6573.31mm^3, $p=0.14$) on accommodation. However, the degree of myopia (D) (median split at -3.29D) demonstrated significant differences in IOP response patterns to accommodation ($p=0.005$).

Conclusion. In concordance to previous results, the dose dependency of IOP responses on accommodation is demonstrated. The inter-subject variations in IOP response patterns can be attributed to differences in the degree of myopia (D) but not to differences in axial lengths or ocular volumes. Other biometric correlates and autoregulation within the vascular system will be investigated.

Support: College of Optometrists UK (GR), Keeler Instruments.

THE EFFECT OF ACCOMMODATION ON PULSATILE OCULAR BLOOD FLOW

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Purpose: The association between sustained accommodative effort and the onset and progression of myopia is well documented, although evidence for causation is equivocal. This study examined the effect of accommodation on pulsatile ocular blood flow (POBF) to evaluate a putative vascular aetiology for myopia.

Methods: The Ocular Blood Flow Analyser (OBFA, Paradigm medical Instruments Inc., UK) was used to measure the changes in the dependent variables intraocular pressure (IOP); POBF and pulse amplitude (PA) in the right eye while the left eye fixated a zero (low); 1.50 (intermediate) and 4D (high) accommodative target (presented randomly) for 3 minutes (myopes=22; emmetropes=19). The fixating eye was rendered functionally emmetropic with soft contact lenses and the accommodative responses were measured in free dioptric space with the Shin-Nippon NVision-K autorefractor. Partial coherent interferometry was used to measure the axial length (AL) and the ocular perfusion pressure (OPP) was calculated as 2/3 mean arterial pressure (MAP) minus IOP, where MAP = 1/3 systolic pressure + 2/3 diastolic pressure.

Results: Mean (+/- sd) accommodation response levels for low, intermediate and high stimulus levels were respectively -0.14 ± 0.41 D, 1.56 ± 0.29 D and 4.19 ± 0.24 D. Only IOP measures were significantly influenced by accommodation ($p=0.01$) with a significant reduction for high accommodation levels by 1.10 ± 2.10 mmHg ($p=0.002$).
Positive correlations were found between the level of myopia and the changes in IOP on intermediate ($r=0.61$, $p=0.003$) and high ($r=0.53$, $p=0.01$) accommodation levels. No differences in the POBF responses to accommodation between refractive groups were evident. However for high accommodation levels PA decreased by 0.57 ± 0.53 mmHg in emmetropes but increased by 0.34 ± 1.46 mmHg in myopes ($p=0.014$), with the difference being independent of inter-subject variations in refractive error, AL or OPP.

Conclusions: Of the dependent variables evaluated, the IOP is shown to reduce significantly only for high accommodation response levels. Consistent with a previous report, the degree of myopia influences IOP on both intermediate and high accommodation levels such that as the error increases the IOP progressively reduces. PA data indicate that choroidal pulsatility increases in myopic subjects whilst it decreases in their emmetropic counterparts, although there is no single parameter which accounts for the relationship. We are currently examining the importance of these findings with reference to associated measures of ocular volume.

Support: College of Optometrists UK (GR), Keeler Instruments.