BIOMETRIC AND PHYSIOLOGICAL FACTORS IN HUMAN OCULAR PERFUSION

Alexandra Benavente-Perez

Doctor of Philosophy

Aston University, Birmingham
March 2007

Aston University

BIOMETRIC AND PHYSIOLOGICAL FACTORS IN HUMAN OCULAR PERFUSION

ALEXANDRA BENAVENTE-PEREZ

Doctor of Philosophy
2007

SUMMARY

The structural characteristics of a myopic eye describe an elongated vitreous chamber, which in high myopia is related to a stretched sclera, increased risk of choroidal atrophy and additionally macular degeneration, choroiditis and glaucoma. Despite extensive research in the field of myopia, the ocular haemodynamics of the healthy human myopic eye remain unexplored, with limited data available from animal and degenerative human myopia studies. By addressing the vascular features that characterise myopia, this thesis aims to provide an understanding of the early structural changes associated with human myopia and the progression to co-morbidity with age.

This thesis addresses three main areas of study:

1. Ocular perfusion features and autoregulatory mechanisms in human myopia.
The various vascular beds in the eye – retrobulbar, choroidal and microretinal- are assessed to provide a detailed analysis of the myopic ocular haemodynamics.

- The resting state vascular profile of the myopic eye shows a lower pulsatile ocular blood flow (POBF), central retinal artery (CRA) and choroidal blood velocity than the emmetropic eye.
- The pulsatile and microretinal blood flow reduce significantly with age.
- The ocular vascular reactivity is highly sensitive to changes in oxygenation, and it increases with increased axial length; the basis of which may lie in autonomic dysregulation in myopic patients.
- The inter-eye differences in axial length and refractive error in anisomyopes correlate significantly with the inter-eye differences in retrobulbar and microretinal blood velocity.

2. Choroidal thickness at the macular area of myopic eyes.
The blood flow and thickness of the choroid have been related in animal models of myopia suggesting an active mechanism to regulate myopic eye growth; however, it remains unexplored in human eyes to date. A new optical coherence tomography (OCT) instrument, the SD OCT' Copernicus' (SOCT), whose repeatability and reproducibility of the choroidal calculations are also investigated, is used to measure the choroidal thickness.

- SOCT measurements of choroidal thickness are repeatable and reproducible in normal eyes.
- Choroidal thickness at the macular area correlates negatively with axial length.

3. Effect of chronic smoking on the ocular haemodynamics and autoregulation.
The increased risk of smoking-related cardiovascular diseases and the relationship between smoking and age related macular degeneration (ARMD) highlight the importance of the evaluation of the effects of smoking on the ocular haemodynamics. This study showed:

- The resting state ocular haemodynamics of young smokers with a smoking history ranging from 3 to 10 years does not differ significantly from that of non-smokers.
- The mechanism of the systemic and ocular vascular regulation is exacerbated in young smokers and it correlates with the consumption of nicotine.

This thesis demonstrated a reduced resting ocular pulse amplitude and retrobulbar blood flow in human myopia, associated with an apparent oversensitivity to the vasodilatory effects of hypercapnia, which may be due to anatomical differences in the volume of the vessel beds. In young smokers, normal resting state vascular characteristics were present; however there also appeared to be increased reactivity to hypercapnia, possibly due to relative chronic hypoxia. The systemic circulation in myopes and smokers over-reacted similarly to hypercapnia suggesting that physiologic differences are not confined to the eye. Age also showed a negative effect on autoregulatory capacity in otherwise normal eyes. Collectively, these findings suggest that myopes and smokers require greater autoregulatory capacity to maintain appropriate oxygenation of retinal tissue, and since the capacity for such regulation reduces with age, these groups are at greater risk of insufficient autoregulation and relative hypoxia with age.

Key words: ocular haemodynamics, myopia, autoregulation, choroidal thickness, smoking

Para papa y mama

(To dad and mum)

ACKNOWLEDGEMENTS

I am very grateful to my supervisors, Dr. Sarah L Hosking and Dr. Nicola S Logan, without whom I would definitely not be here today. Thank you for believing in me in the first instance and for your support, expertise, guidance, patience during my time at Aston and, above all, thanks for your phone calls during the past week! I would also like to thank Prof. Bernard Gilmartin for having his door always opened and for his endless enthusiasm for research.

A very big thank you to Dr. Dheeraj Bansal (halla) for his IT and engineering support that allowed the completion of the data collection when this thesis seemed impossible to finish, for his SOCT loan, for being the first one daring to breathe my lethal CO_2 and not killing me when we found out that I made him inhale what he was supposed to exhale...! A special thanks for his constant and unconditional support, and for making me smile every day.

Thanks to Dr. Gurjeet Rai (wow!) for being much more than a friend throughout these 3 and a half years. Thanks for your wise emotional support, for controlling my sugar intake and for making me explain you 'only once' the meaning of OPP (please, read OPP with Spanish accent). My gratitude is also extended to the great third inhabitant of the MB712 office Alejandro Cerviño for his friendship, professional advice and supply of 'el jueves'.

A very special thank you to my amazing family; to mum, dad, my sister, my brother, Ana and the two most beautiful and fun nieces in the world Alma and Violeta. I hope this thick book will help you reach that top shelf where all the cool games are! Thank you mum and dad for giving me everything...

Muchas gracias a Kirstin for being there for me when I needed most, I cannot explain how much you have helped me...!

Many thanks to Dr. Miriam Conway and Rebekka Heitmar for those great vascular conversations in our lab that made me learn in 1 month more than what I had learnt in 2 years! Thanks to Dr. Helena Workman for helping me decipher the mystery of the repeatability studies and for being the master of OCT. Thanks are also due to Dr. Andrew Morgan for his hypercapnia knowledge and to Dr. Richard Amstrong and Dr. Darmesh Maniyar for their statistical advice.

I would also like to thank all those students, members of staff and friends who dedicated some of their time to sit for my research, my most sincere thank you.

And big thanks to 'El Bicho', for their inspiring music that brought to my office that little piece of Spain during my dark days.....

Contents

LIST OF FIGURES

Figure

LIST OF TABLES

Table

LIST OF ABBREVIATIONS

The abbreviations more frequently used in this thesis are listed below in alphabetical order:

ACD: Anterior chamber depth
AL: Axial length
BP: Blood pressure
CDI: Colour Doppler Imaging
CO_2: Carbon dioxide
CRA: Central retinal artery
CRV: Central retinal vein
CT: Corneal thickness
DBP: Diastolic blood pressure
EDv: End diastolic blood velocity
FTQ: Fagerstrom tolerance questionnaire
HRF: Heidelberg Retinal Flowmeter
HRFmaxflow: Maximum blood flow in the retinal microvessels
HRFmaxvel: Maximum blood velocity in the retinal microvessels
HRFmaxvol: Maximum blood volume in the retinal microvessels
HRFminflow: Minimum blood flow in the retinal microvessels
HRFminvel: Minimum blood velocity in the retinal microvessels
HRFminvol: Minimum blood velocity in the retinal microvessels
IH: Isoxic hypercapnia
IOP: Intraocular pressure
MAP: Mean arterial pressure
MSE: Mean spherical equivalent
OA: Ophthalmic artery
OBFa: Amplitude of the ocular pulse
OBFA: Ocular Blood Flow Analyser
OBFiop: Intraocular pressure measured with OBFA
OBFr: Rate of the ocular pulse
OBFv: Volume of the ocular pulse
ONH: Optic nerve head
OPP: Ocular perfusion pressure
pCO_2: Carbon dioxide partial pressure
POBF: Pulsatile ocular blood flow
PSv: Peak systolic blood velocity
RI: Resistance index
SBP: Systolic blood pressure
SPCA/SCPAs: Short posterior ciliary arteries

List of equations

LIST OF APPENDICES

CHAPTER ONE
INTRODUCTION

> "After reviewing the literature on myopia, one is impressed by the many divergent points of view and the many contradictions encountered, but as a result of this interest and theorizing, the subject is kept alive and fresh, and eventually a clear understanding will ensure"
>
> (Edwin W. Burton, 1942)

Animal studies (Fitzgerald, *et al.* 2005; Fitzgerald, *et al.* 2001; Jin and Stjernschantz 2000; Wildsoet and Wallman 1995) and research performed on human pathological myopia (Dimitrova, *et al.* 2002; Akyol, *et al.* 1996) represent the only available data to date on the perfusion features of the human myopic eye. A reduction in the blood velocities of the retrobulbar vessels and ocular blood pulse have been found to correlate with increasing levels of myopia (Dimitrova, *et al.* 2002). Additionally, myopes are at higher risk of defective ocular conditions, such as glaucoma (Mitchell, *et al.* 1999) and chorioretinal atrophy (Brasil, *et al.* 2006) which have previously shown some degree of ocular blood flow reduction. In view of the findings described by the literature, the investigation of the ocular haemodynamics and vascular autoregulation in healthy human myopia is of intrinsic interest, as autoregulatory dysfunction in myopes has not yet been investigated and the aetiology of myopia may be associated with vascular autonomic dysfunction.

This thesis provides a detailed analysis of the ocular perfusion characteristics of the healthy eye by assessing the retinal, retrobulbar and choroidal circulation and the vascular autoregulatory features of healthy myopic eyes. Additionally, due to the known effect of age (Fleg 1986; Kannel, *et al.* 1961) and smoking (Campisi, *et al.* 1998) on the cardiovascular performance, the study of the ocular blood flow in the ageing eye and in the eye of smokers was also performed.

Section one: MYOPIA

1.1. Myopia definition and prevalence

Myopia has become a research topic of growing interest during the past 30 years. Research groups from various countries have directed their investigations towards the study of the myopic refractive error, whose world prevalence continues to increase with time. An increase in the percentage of refereed research publications on myopia (table 1.1) -many of which between 2000

and 2007 are references on laser refractive surgery- coincides with the dramatic increase in the prevalence of myopia, which reached 80 per cent among school children of Taiwan in 1995 (Lin, *et al.* 1999) and 89.8 per cent among medical students in Singapore in 2004 (Woo, *et al.* 2004).

Myopia is a condition in which the refractive power of the eye is excessive with respect to the axial length such that the eye's focal point is anterior to the retina, which may arise as a result of a relatively long eye (axial myopia), a steep cornea or crystalline lens (refractive myopia) or a combination of all three (figure 1.1). Additionally, variations in refractive index of the ocular refractive media can induce changes towards myopic refractions (index myopia). As a result of this augmented power, in the unaccommodated eye, the image from a distant object does not fall on the retina, as it does in an emmetropic eye. A myopic eye has its focal point in front of the retina, which results in a blurred retinal image.

Axial length is the biometric feature mostly correlated with myopia, axial myopia being the most common type of myopia, whereas it is mainly among elderly patients that refractive index myopia is found due to age-related changes occurring in the crystalline lens.

Decade	1890 - 1900	1900 - 1910	1910 - 1920	1920 - 1930	1930 - 1940	1940 - 1950	1950 - 1960	1960 - 1970	1970 - 1980	1980 - 1990	1990 - 2000	2000 - 2007
Number of myopia papers *	2	2	3	1	1	27	229	732	1277	1863	3348	3831
Total number of papers *	1200	1383	3058	6399	10250	104022	147188	1821238	2686689	3645560	4830194	4425669
Percentage of myopia papers (%)	0.16	0.14	0.10	0.01	0.01	0.001	0.02	0.04	0.04	0.05	0.06	0.09

Table 1.1 *Number of publications on animal and human myopia per decade since 1890 (source: National Library of Medicine and the National Institutes of Health).*

Despite reports describing variations in the prevalence of myopia across countries and ethnicities, myopia is becoming a world epidemic. The differences in prevalence rates are primarily due to the differences in sample size, age of the sample, environmental exposure and genetic factors.

The prevalence of myopia has increased in the past 20 years, especially in rural areas of East Asian countries (Gilmartin 2004). A report from 1989, which reviewed approximately 500 papers from U.S and European journals since 1812 on myopia prevalence, suggested that the distribution of refractive errors in Caucasian school-children and young adults had not changed for the past 100

years (Adams, *et al.* 1989). The prevalence of myopia was correlated with level of family income, education of parents, refractive status of parents, reading ability, school success and intelligence.

The report also described the problems of standardising myopia study protocols, since different refractive techniques were used to determine prevalence statistics, and the levels of myopia chosen to delimit group samples also differed among studies.

During the past 100 years the main refractive techniques have been ophthalmoscopy (the refraction being the lens that brought into focus the retinal image), subjective refraction, retinoscopy and more recently automated refraction (Lin, *et al.* 1999).

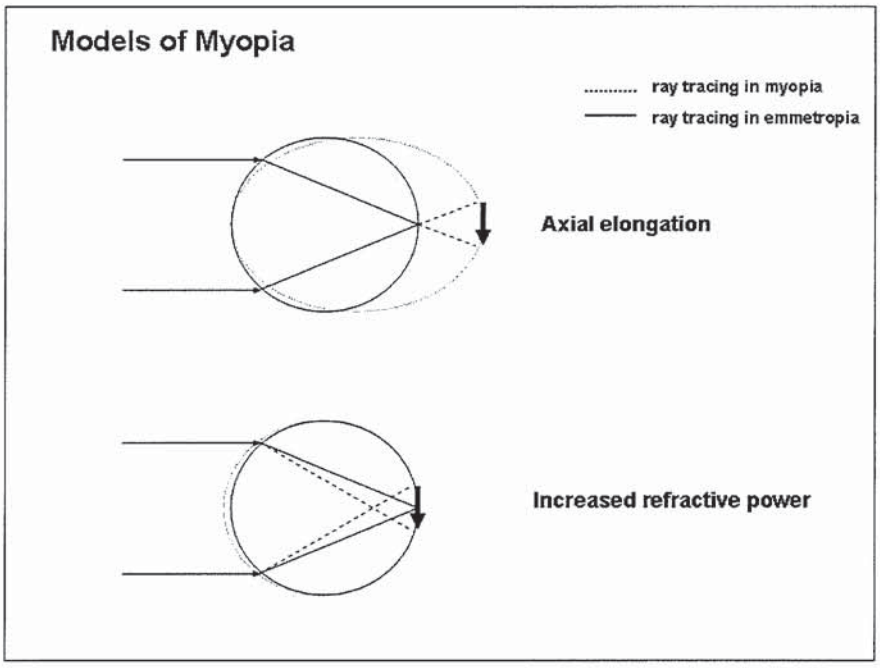


Figure 1.1 *Schematic models of myopia: eye representing the features of ray tracing in emmetropia and axial and refractive myopia.*

The prevalence of myopia in children varies across countries, and these variations may also be due to differences in age, recruitment criteria and technique used. Despite these potential differences, results show a trend towards an increase in the prevalence of myopia in children once they enter school. Early reports examined German school children with ages ranging from 5 to 16 years. The percentages of myopia prevalence as measured by ophthalmoscopy varied from 1.4 per cent in 10,060 children ages 5- 7, to 35 per cent for 2,172 children aged 14 - 16. (Cohn, 1867 in (Adams, *et al.* 1989)). In England, two studies performed at the beginning of the 20th century provided

similar prevalence rates in different decades. In 1955, a total of 1,066 children aged 5 to 15 years were evaluated and 21.1 per cent were found to be myopic (Mc Neil 1955), which did not differ significantly from the data of 1932 in which 21.6 per cent of 1,702 children with ages ranging 6 to 14 were myopic (McIllroy and Hamilton, 1932 in (Adams, *et al.* 1989)). Studies on the prevalence of myopia in US children performed from 1928 to 1952 were mostly based on data from cycloplegic subjective refraction. In 1928, Kempf found an overall myopia prevalence of 7.1 per cent after assessing 1,828 participants with ages ranging from 6 to 18 years. Evaluation by subgroups for age showed only 2.1 per cent of myopia among the youngest children (aged 6 to 8); whereas, the percentage of children with myopia also increased to 5.5 per cent in children aged 9 to 11. The myopic rate reported in children between 12 and 18 years of age was the highest of all at 9.5 per cent (Kempf, 1928 in (Adams, *et al.* 1989)). Eighty years later an analogous increase in the rate of myopia before and after children were first admitted to school was observed and data showed higher prevalence rates for children above the age of 6 years (6 per cent) than pre-school children (3 per cent) (Junghans and Crewther 2003; Zadnik, *et al.* 2003; Robinson 1999).

The reported increase in the rate of myopia in children appears to be supported by recent UK data from the Avon Longitudinal Study of Parents and Children (ALSPAC) in which the prevalence of 0.5D myopia or greater was 13.6 per cent in children at the age of 7 years. However, this percentage may include some false positive numbers, leading to a higher prevalence, since the readings were not taken under cycloplegia (Williams 2006).

It is necessary to highlight prematurity as a factor to consider before assessing the prevalence of myopia in children. Premature children are those born at less than 37 weeks postconceptional age, or more than 37 weeks but weighing < 2 500 g at birth. The world prevalence of prematurity in 2003 was approximately 13%, which means that one child in eight is born prematurely (Martin et al. 2005). Babies born prematurely and/or with low birth weight, have been reported to be at a higher risk of developing myopia. A study by Castren in 1955 showed a two-fold increase in myopia prevalence for children who were between 1,000 and 2,000 grams when they were born (Castren 1955). Later studies have shown similar results (Saunders, *et al.* 2002; Gerhard 1983).

The myopia prevalence in the young adult population is higher than that of children. The prevalence of myopia in college students has been analysed on numerous occasions from the end of the 19th century due to their accessibility for study purposes. Early reports studying college students of white origin in the US, UK, and Germany show similar prevalence rates that ranged from 28 per cent to 40 per cent (Agnew and Williams 1877; Derby 1877; Ware 1813). The prevalence rate of myopia in college students around 1950 varied across countries, as shown by

the differences between the 32 per cent reported in England (Parnell 1951) and 44 per cent for the US (Dunphy, *et al.* 1968).
The percentage of students with myopia increases in some East Asian populations, with rates of 70 per cent in China (Goh and Lam 1994), 65 per cent in Japan (Matsumura and Hirai 1999) and 89 per cent in Singapore (Woo, *et al.* 2004).

The pattern of refractive error among adults also differs among countries, although the age profiles show similarities; for example, the prevalence of myopia above the age of 80 seems to be higher, with a decrease in the myopic rate for those whose ages range from 40 to 70 (Tarczy-Hornoch, *et al.* 2006; Wickremasinghe, *et al.* 2004; Wong, *et al.* 2000). This myopic shift has been related to nuclear opacities, while the recruitment criteria have been suggested by some to account for the discrepancy. Wong (2006) evaluated the effect of age and cohort on the distinct pattern of myopia observed in adults (Wong 2006). After analysing data from adults in Singapore, a decrease in axial length with age was found, since it is unlikely that the eye shrinks with age, this suggested a cohort effect. However, studies in Mongolian and Latin populations corrected for a potential cohort effect also showed a similar refractive profile in adults. Wong therefore concluded that the pattern of the refractive status of the adult eye is affected by both, a cohort and an age effect.

1.2 Biochemical markers of myopia

Early mechanical theories of myopia include that by Levinsohn in 1929 among others (Levinsohn 1929). Levinsohn suggested in 1929 that the traction of the optic nerve over the eyeball in downward gaze position may the reason for myopic elongation, and the results of his study showed how myopia could be induced in primates after they were raised in cages and forced to look down.

Current animal myopia research is mostly orientated towards the evaluation of animal models known to result in induced myopia. Rose and colleagues reported having induced myopia in cats that had been deprived from distance vision (Rose, *et al.* 1974). However, it was not until the study by Wiesel and Raviola that an animal model for myopia was validated. Wiesel and Raviola reported axial myopia after lid suture of juvenile macaque monkeys (Wiesel and Raviola 1977). No changes in corneal thickness, corneal curvature, and anterior chamber depth or lens thickness were reported. However, all changes that accompanied the induced axial myopia occurred in the posterior segment of the eye; a deeper vitreous chamber and a thinner sclera was observed (Wiesel and Raviola 1977). Deprivation myopia was then explored in chick eyes. Wallman and co-authors

reported local changes in axial length and vitreous chamber depth in those retinal areas that were deprived from light using translucent occluders (Wallman, *et al.* 1987). Several studies followed that by Wiesel and Raviola, and confirmed that lid suture (Fitzgerald, *et al.* 2005), patterned goggles (Howlett and McFadden 2006), opaque contact lenses and negative refractive lenses also trigger axial length elongation (Wildsoet and Wallman 1995 ; Schaeffel, *et al.* 1990; Schaeffel, *et al.* 1988).

A study performed in 1980 reported induced anisometropia in kittens after placing a negative lens in front of one of the eyes of the animals (Smith, *et al.* 1980). A correlation between degree of refraction and axial length of the eyes stimulated with negative lenses was found. All the previous studies on animal models suggest that, despite the uncertainty regarding the mechanisms that result in axial elongation, there is evidence to indicate that it may be a vision-dependent feature.

1.2.1 Vasoactive intestinal polypeptide (VIP)

Other animal studies were directed towards the evaluation of the biochemistry and neurochemistry of deprivation myopia (Howlett and McFadden 2006; Brand, *et al.* 2005; Fitzgerald, *et al.* 2005; Wildsoet 1997). The evaluation of possible molecular and neural pathways involved in animal induced axial myopia may provide a better understanding of the mechanisms implicated in deprivation myopia. Immunohistochemistry has demonstrated a higher reactivity of retinal vasoactive intestinal polypeptide (VIP) in the closed eyes of macaques with myopia induced through lid suture than in those eyes that remained opened (Stone, *et al.* 1988). However, it remains unknown whether the increase in VIP activity was due to an increase in VIP synthesis, or a decrease in VIP release.

VIP is a neuropeptide that belongs to the glucagon hormone family and it is localised in a type of amacrine cells whose dendrites reach the central sub-lamina of the inner plexiform layer, the bistratified cone amacrine cell. VIP had previously been shown to stimulate the formation of cyclic AMP in the rabbit retina *in-vitro* (Schorderet, *et al.* 1981). Cyclic adenosine monophosphate (cAMP) is a molecule derived from adenosine triphosphate (ATP) that takes part in intracellular signal transduction, which in turn is involved in transferring the effects of hormones like glucagon and adrenaline. An increase in cyclic AMP may therefore suggest involvement of VIP in eye growth, as it is known to be affected by growth hormones.
This is further supported by findings in chick eyes, in which VIP appeared to be involved in both normal and deprivation eye growth (Seltner and Stell 1995).

1.2.2 The glucagon family

The expression at the ribonucleic acid (RNA) level of several other members of the glucagon hormone family in the mouse retina has recently been investigated together with the effect of visual experience (Mathis and Schaeffel 2006). Early growth response protein 1 (Egr-1), glucagon, glucose-dependent insulinotropic polypeptide (GIP), peptide histidine isoleucine (PHI), growth hormone-releasing hormone (GHRH), pituitary adenylate cyclase-activating polypeptide (PACAP) and VIP were the proteins and hormones evaluated. From these, only VIP, PHI, PACAP and GIP were localized in the mouse retina. VIP was co-localized with PHI and Egr-1, which in turn was strongly regulated by retinal illumination.

Therefore, despite the fact that various members of the glucagon family have been localised in the mouse retina, there is not enough evidence to demonstrate their involvement in visually related mechanisms (Mathis and Schaeffel 2006).

1.2.2.1 Egr-1

Egr-1, also known as EGR1, Krox-24 protein and nerve growth factor-induced protein among others belongs to the EGR family of C2H2-type zinc-finger proteins. It is a nuclear protein and functions as a transcriptional regulator. Egr-1 activates the products of target genes required for differentiation and mitogenesis. The possibility of a relationship between Egr-1 and myopia was first suggested by Fisher and colleagues in 1999. The expression of Egr-1 in chicks with form deprivation myopia using positive lenses correlated with the sign of defocus in the glucagon subset of amacrine cells (Fischer, *et al.* 1999). Also, in the mouse Egr-1 appeared to be strongly regulated by light and by the transition between light and darkness (Brand, *et al.* 2005). Similar results in the retina of macaques mention the immunoreactivity of Egr-1 in amacrine cells to be sensitive to focus (Zhong, *et al.* 2004). Since these results were obtained in the retina of high primates, Egr-1 therefore appears to be an interesting protein whose expression is affected by visual experience, potentially being part of the signalling pathway that leads to eye growth.

1.2.2.2 Dopamine

One of the first observations on the biochemistry of visual deprivation myopia was a decrease in the levels of dopamine parallel to ocular enlargement in chicks and rhesus monkeys (Stone, *et al.* 1989). Dopamine is a neurotransmitter that acts over specific dopamine and adrenergic receptors. Dopamine has been found in approximately 0.5% of amacrine and interplexiform retinal cells. Despite the small percentage, dopaminergic cells cover a large part of the retina due to their long processes (Teakle, *et al.* 1993). The decrease in the dopamine levels that accompanied axial myopia was tested by local supply of a dopamine agonist, apomorphine, in neonatal chicks.

Apomorphine appeared to delay ocular growth only in the axial direction. Apomorphine did not delay equatorial growth, which raised the possibility of a selective effect on the chick eye (Stone, *et al.* 1989). The significance of dopamine on eye growth is highlighted by these results, in which a delay in axial growth occurs after the supply of a dopamine agonist.

A recent study by Megaw and colleagues (Megaw et al. 2006) reconsidered the role of dopamine, and investigated the balance between circadian- and light control over dopamine release in chickens. The results suggested that the activity of the dopaminergic amacrine cell in the chicken retina was dominated by light-activated input.

1.3 Eye growth and myopia

Advancement in the myopia research field has been possible mainly due to the experimental work performed in animals, and to the application of ideas and theories to the human eye. One of the most interesting features of the myopic eye is, without question, the elongation of the vitreous chamber. Thus, the study of the eye growth mechanisms would provide a wider understanding of the aetiology of axial myopia. Many of the studies on myopia based their findings and conclusions in animal models manipulated to induce eye growth. To date, two main experimental techniques have been employed to trigger myopia in animals: form-deprivation and lens-induced defocus. Both methods resulted in obscured or blurred retinal images that stimulated eye growth. However, the mechanisms responsible for eye growth have not been identified yet. Biochemical involvement has been suggested to play an important role in the pathogenesis of eye growth (section 1.2), and it is the author's opinion that understanding the molecular biology and biochemistry of eye growth will widen our insight and will aid in designing a valid way of controlling ocular growth.

Form deprivation myopia was the first validated animal model for myopia, followed by the use of translucent occluders, lid suture, patterned goggles and opaque contact lenses (Howlett and McFadden 2006; Fitzgerald, *et al.* 2005; Schaeffel and Burkhardt 2005; Wildsoet 1997; Schaeffel, *et al.* 1990; Wiesel and Raviola 1977). In all cases, depriving the eye from a sharp retinal image resulted in eye growth or change in shape of the posterior components of the eye as long as vision was obscured. Once the image was restored, with the resultant induced myopic defocus, there was a thickening of the choroidal layer followed by a decrease in the ocular growing rate (Fitzgerald, *et al.* 2001; Shih, *et al.* 1993).

Animal myopia has also been induced using lenses to trigger retinal defocus. Negative lenses diverge light entering the eye, which results in an apparent increase in focal length. The eye has shown its ability to adjust axial length to the new focal length, diminishing the hyperopic retinal defocus imposed by the negative lens. Once clarity is achieved, the rate of ocular elongation diminishes. The increase in axial length was preceded by a thinning of the choroid, which appeared to lead the sclera towards the new focal length (Wildsoet 1997; Wildsoet and Wallman 1995). The effect of myopic-defocus on ocular growth has also been assessed using positive lenses and findings have revealed the opposite effect. Myopic-induced defocus triggered an expansion and thickening of the choroid, which seemed to drive the slowing of ocular elongation. This mechanism eventually resulted in the reallocation of the image plane back to the retina (Hung, *et al.* 2000; Wildsoet and Wallman 1995).

Ocular growth therefore appeared to be visually guided, but the limits to this growth do not seem to agree among species. Temporal evaluation of ocular growth has shown that after normal animal development, there is a trend towards a decreased regulatory mechanism in tree-shrews and chicks (Wallman, *et al.* 1987). However, adolescent primates and marmosets also show a decrease in susceptibility towards form-deprivation myopia (Troilo, *et al.* 2000b; Troilo, *et al.* 2000a). Wallman therefore suggested the possibility of a double emmetropisation pathway, one through which short-term adjustments are performed to ensure the clarity of the retinal image, similar to the accommodative mechanism, and another of slower temporal resolution, whose aim is to ensure final emmetropia (Wallman and Winawer 2004).

A local control of eye growth was suggested by studies in which deprivation of a retinal section with diffusers or negative lenses lead to induced myopia exclusively in that retinal area (Wallman, *et al.* 1987). Also, positive lenses covering half of the retina resulted in the same half of the retina becoming elongated and myopic (Diether and Schaeffel 1997). Additionally, if the optic nerve was sectioned, deprivation myopia still occurred (Troilo and Wallman 1991).

Experimentally induced eye growth could therefore be interpreted as an autoregulatory mechanism that occurs at a local level. This would be in agreement with the homeostatic theory, as homeostasis aims to maintain stability within an internal media, the eye. In such a case, induced-myopia would be an example of homeostatic ocular autoregulation, guided by visual clues. The eye may be simply trying to constantly correct defocus, with the final aim of reaching a sharp image, also called emmetropisation.

Furthermore, ocular growth is subject to discussion due to the unanswered question as to whether ocular growth results from scleral growth or scleral stretching. The scleral behaviour of birds and

mammals after deprivation myopia differs significantly. The dopaminergic amacrine cells of deprived chicks had larger dendritic trees than those of control eyes. The total dendritic area in deprived eyes was approximately 40% larger than in the control eye. This suggested production of new tissue, therefore growth, in myopic eyes as a response to visual deprivation (Teakle, *et al.* 1993). However, deprivation experiments by Raviola and Weisel in monkeys showed stretching of the pigment epithelium and thinning of the sclera, which are in accordance with findings in human myopia (Raviola and Wiesel 1985) which may indicate a stretching mechanism in deprived eyes of mammals versus scleral growth in deprived chick-eyes. An interesting point was raised by Beebe in the Ciba Foundation Symposium, in which he clarified how thinning of the sclera does not necessary mean stretching. Thinning, he said "could result from a change in the metabolic rate of the tissue, leading to more degradation than synthesis". In this way, myopia may well be associated with both scleral growth, part of the physiological maturation of the body, and stretching (Beebe in (Widdows 1990)).

1.4 Emmetropisation and myopia

Emmetropisation can be described as the mechanism that maintains the formation of the optical image on the retina by matching eye length to the optical length of the refractive components of the eye. In essence, it can be referred to as the relationship between axial length and ocular refractive state to ensure a constant sharp retinal image. At present, emmetropisation is interpreted as both a passive and an active process occurring as a result of physiological eye growth, without any visual stimulation, as well as a mechanism resulting from constant visual input and feedback.

The eye is programmed to achieve emmetropisation with advancing years through growth and maturation. The refractive error most common in children is hyperopia, with recent numbers showing 13% of hyperopia compared to 9% of myopia in a study with 2523 children of African American, Asian, Hispanic and white ethnicities with ages ranging from 5 to 17 (Kleinstein, *et al.* 2003). The growth of a child's body affects every organ; therefore an initially shorter hyperopic eye would be expected to grow as part of the general physiological growth. The passive process of emmetropisation occurs due to normal child's eye growth. The hyperopic refractive state in newborn infants progresses towards emmetropia in the first months of life followed by a slower period of growth (Sorsby, *et al.* 1961). The consequent eye enlargement reduces the dioptric power of the ocular refractive system ensuring a balance with the increasing axial length. The power of the cornea and the lens reduces by increasing the radii of curvature; whereas the effect of the lens

also decreases by deepening of the anterior chamber. The lens may also change in refractive index, and the resultant effect is to render the eye emmetropic (Brown, *et al.* 1999).

If an emmetropic animal eye that is initially of normal size and shape can compensate for the defocus induced using positive and negative lenses, vision must play an important role in emmetropisation. The possibility of an active visual-feedback mechanism has been supported by animal studies and human eye observations. Monkeys (Smith, *et al.* 2002; Raviola and Wiesel 1985), marmosets (Graham and Judge 1999; Troilo and Judgeb 1993), ostriches (Ofri, *et al.* 2001), cats (Smith, *et al.* 1980), chicks (Mathis and Schaeffel 2006; Smith, *et al.* 2002; Silver and Geyer 2000; Wallman, *et al.* 1987) and mice (Mathis and Schaeffel 2006), deprived from vision by lid suture, goggles or patterned contact lenses have been reported to become myopic. Additionally, chronic degradation of the retinal image before the age of 3 in humans appears to lead to axial myopia as shown by ocular elongation associated with corneal scars or ptosis (Curtin 1988). The significance of retinal blur is therefore supported from both animal and human observations. Using animal eyes in order to investigate the mechanisms that trigger ocular growth has allowed the clarification of many points that were unanswered. However, it is important to highlight the uncertainty regarding the similarity between form deprivation myopia and human myopia. Some scientists have questioned the number of discretely different myopic forms that exist, the extent of overlap between the physiology of clinically detected human myopia and deprivation-induced myopia, and the possibility that different species have varying mechanisms or causative factors for myopia development (Wallman in (Widdows 1990)). To date, these questions remain unanswered. Research has moved forward, and the knowledge acquired after years of investigation has helped to unveil some of the mechanisms of deprivation myopia. Until 1988 the active theory of emmetropisation lacked evidence to show whether axial growth could occur as a result of positive or negative defocus. It was known that negative lenses induced axial growth. However, the effect of positive lenses on eye growth had not yet been evaluated. It was hypothesised that emmetropisation may be bidirectional, thereby counterbalancing positive and negative defocus; the process of emmetropisation may be analogous to the accommodative control of refractive state, in which accommodative fluctuations ensured a sharp retinal image over time.

It has subsequently been shown in animal models that positive and negative lenses induce both myopic and hyperopic growth (Schaeffel, *et al.* 1990; Schaeffel, *et al.* 1988). Interestingly, studies supporting an active and bidirectional emmetropisation mechanism have shown that changes occur in other ocular structures such as corneal thickness and anterior chamber depth, as well as axial length. In view of these results, emmetropisation may also be explained as a mechanism in which reshaping of other ocular features is involved. However, corneal and anterior chamber depth changes have been reported to occur after emmetropia was achieved, which suggests a

faster visually-mediated rather than shape-related mechanism in emmetropia (Troilo and Wallman 1991).

Chick eyes can detect defocus (Wallman and Winawer 2004). However, the optical signals that allow defocus detection remain unknown. It remains unproven whether the eye worked as a trial-error system to detect the direction of growth, or whether it was able to detect the signal of blur and work accordingly. Surprisingly, recent findings by Zhu and co-workers reported that chick eyes only required approximately 10 minutes to experience growth towards the new direction imposed by lens defocus. Consequently it was suggested that chick eyes may not be dependent on trial and error techniques to determine the sign of the blur, but they may actually recognise the sign of the blur, and grow accordingly (Zhu, *et al.* 2005).

Several other questions remain unanswered, such as the temporal features of animal induced myopia. Chick eyes were the first to be subjected to periods of unrestricted vision in a study in which myopia was induced by -10.00D lenses. Brief periods of clear vision, starting from 3 hours, showed a reduction in the development rate of myopia. Tree shrews with form deprivation myopia showed similar results after 1 hour of unrestricted vision (Shaikh, *et al.* 1999). Likewise, results from studies of deprivation myopia in monkeys suggested that only a few weeks of continuous form deprivation could promote axial growth, with brief daily periods of unrestricted vision appearing to prevent axial myopia (Smith, *et al.* 2002).

If brief periods of unrestricted vision reduce the rate of ocular growth, this may have direct implications in human myopia. The progression of infant myopia induced by ptosis could therefore be reduced by short periods of exposure of the eye to an unrestricted corrected vision, by, for example, holding the eyelid. However, it may not work in all cases, such as in cataracts, as the lack of light resulted in degenerative myopia alone in a group of patients who were probably genetically predisposed (Balacco-Gabrieli, *et al.* 1986).

In 2004, Wallman and Winawer proposed that the eye is under homeostatic control (Wallman and Winawer 2004). They suggested that the mechanisms that regulate eye growth resemble those of a homeostatic balance. Homeostasis mechanisms in a living organism regulate the environment in order to maintain a stable and constant condition. This is achieved by multiple adjustments, controlled by interrelated regulation mechanisms. The term homeostasis was first used in 1959 to refer to the changes in both physical and chemical parameters that allow an organism (cells, tissues and organs) to function properly. The main parameters whose variations actively regulate homeostasis are: concentration of oxygen and carbon dioxide, internal pH, concentration of salt and other electrolytes (osmoregulation), as well as concentration of nutrients and waste products

(secretion), and volume and pressure of extra-cellular fluid. In higher animals, such as human beings and primates, the homeostasis control system can be extrinsic or intrinsic. Extrinsic systems are the nervous system and the endocrine system. The nervous system operates directing sensorial signals and stimuli towards the spinal cord and brain, where the signal is processed and sent back in the form of stimulus response. The endocrine system, on the other hand, requires chemical components to convey the message. Intrinsic systems, on the other hand, refer to those within the organ or tissue. They are local autoregulatory mechanisms, which mean that any signal is processed and dealt with within the same tissue or organ. A homeostatic response, therefore, occurs after a change in variable and the information provided is received by the homeostatic system in charge in the form of specific feedback. There are two main types of feedback to which the system reacts: negative feedbacks, in which the system responds to reverse the direction of the change; and positive feedbacks, whose responses aim to amplify the change in the variable.
The homeostatic growth control suggested by Wallman and Winawer comprised two components, and it was based on both the intrinsic and nervous systems. The active non-visual component would regulate physiological maturation and growth, whereas the visually guided component of homeostatic growth would appear to have a primary role due to its ability to transform the emmetropic eye in which physiological growth is taking place into a larger ametropic eye (Wallman and Winawer 2004).

The earlier theory of emmetropisation by Van Alphen (1961) proposed that the refractive state of the eye may be determined by a stretch factor that is controlled by the tonus of the ciliary muscle-choroid layer which in turn appeared able to resist partly the IOP. This theory was confirmed by the results obtained after stimulating the parasympathetic system, which resulted in an increased tension in the whole choroid leading to a decrease in pressure on the sclera. Stimulation of the sympathetic system showed the opposite effect but to a lesser extent. From these results, Van Alphen predicted that individuals with high levels of ciliary muscle tonus would tend to be hyperopic; whereas subjects with low levels of tonic accommodation would be myopic (Van Alphen and Graebel 1991; Van Alphen 1986).

1.5 Myopia and accommodation

Accommodation is the increase in refractive power caused by the reshaping of the crystalline lens to allow reallocation of the retinal image. Helmholtz first suggested the theory of accommodation in the 19th century. His theory described a flattening of the curvature of the lens induced by a relaxation mechanism of the ciliary muscle to focus distant objects. To bring near objects in to focus a steepening mechanism of the crystalline lens is triggered by contraction of the ciliary

muscle with no pulling action on the lens via the zonular fibres (Garner 1983). This theory represents the traditional view of the ophthalmic science and it had not been questioned until the end of the 20th century. Schachar subsequently suggested that the ciliary muscle may in fact contract on accommodation, exerting tension on the equator of the lens that steepens the central curvature of the lens and flattens the periphery. Therefore, accommodation would be associated with an increase in zonular tension leading to a rise in central lens volume, which contradicts the theory of accommodative relaxation of the lens zonulae proposed by Helmholtz (Schachar, *et al.* 1994; Schachar, *et al.* 1993a; Schachar, *et al.* 1993b).

Several accommodative functions such as amplitude of accommodation (AA), accommodative convergence/accommodation ratio (AC/A ratio) and lag of accommodation are said to play a key role in myopia onset and progression in young adults. The amplitude of accommodation describes the range of the accommodative response, and is the difference between the accommodation at the far-point and near-point (Rosenfield and Cohen 1996). Various studies have reported differences in AA with refractive error, myopes having larger amplitudes than emmetropes and hyperopes (McBrien and Millodot 1986 ; Maddock, *et al.* 1981).

1.5.1 The role of AC/A ratio in myopia

The AC/A ratio refers to the amount of accommodative convergence (AC) per unit of accommodative (A) response and is an important parameter of the convergence system. Myopic children and young adults with late onset myopia have been found to have high AC/A ratios (Gwiazda, *et al.* 1999), although previous research was not able to identify significant differences in the AC/A ratio between myopes and emmetropes (Jiang 1995).

The accommodative response triggered by a near target tends to be smaller than the response required. There is therefore an under-accommodative response which is referred to as lag of accommodation, and is quantified by the difference between the accommodative stimulus and the measured accommodative response. The importance of the lag of accommodation in human myopia resides in the hyperopic retinal defocus that results from an increased lag of accommodation during near-vision tasks, which is supported by studies in which myopes were found to exhibit reduced accommodative responses than emmetropes (Bullimore, *et al.* 1992; Rosenfield and Gilmartin 1988; McBrien and Millodot 1986). Additionally, accommodative lag has been found to increase after the first year of myopia onset, varying with ethnicity. Asian children showed the highest accommodative lag followed by African-American and white children (Mutti, *et al.* 2006; Gwiazda, *et al.* 1995). Recently 605 children 6 to 14 years of age were assessed as part of the CLEERE study and those children who became myopic were found to have more

hyperopic relative peripheral refractive errors than emmetropes, thus suggesting the role of the peripheral retinal on the onset and progression of myopia (see section 1.7) (Mutti, *et al.* 2007).
The importance of the lag of accommodation in the aetiology of myopia is highlighted by findings in animal models of myopia in which small hyperopic defocus induced with negative lenses appeared to drive ocular growth (Wildsoet and Wallman 1995; Schaeffel, *et al.* 1990; Schaeffel, *et al.* 1988) (section 1.3).

1.5.2 Cognition and accommodation in myopia

Additionally, the interaction between cognition and ocular accommodation is a topic of interest. On accommodation, the addition of a mental task has shown to induce a positive increase in tonic accommodation that is significantly larger in myopes than in emmetropes (Bullimore and Gilmartin 1987) and the sympathetic nervous system (SNS) has been suggested to influence the negative changes in accommodation to a near target (Bullimore and Gilmartin 1987). The SNS is responsible for the regulation of homeostatic mechanisms such as blood flow regulation (i.e. vasoconstriction), pupil diameter control (midriasis), blood pressure response (increase in blood pressure) and accommodation (relaxation of lens). It may be hypothesized that for distant targets, mental effort has little effect (as sympathetic influences are minimal), whereas for near targets, cognitive demand leads to a decrease in the accommodative response (Davies, et al. 2005; Winn, et al. 1991). Davies and co-workers assessed the effect of cognition on accommodation from the cardiovascular (SNS) and ocular perspective in emmetropes and late-onset myopes, and reported that an increase in the cognitive demand reduced the accommodative responses in all subjects. The average heart rate decreased with increased workload, and cardiovascular and ocular variations with increasing cognition significantly correlated with parasympathetic activity (Davies, *et al.* 2005).

The relationship between muscarinic receptors, accommodation and myopia

Muscarinic receptors are present in the main structures involved in accommodation, the ciliary muscle and lens. Atropine is a non-selective anti-muscarinic agent that appears to block the accommodative response. Several studies have assessed the effect of atropine on myopia progression, the majority of which have found a decrease in the progression rate of myopia after atropine supply. A recent retrospective study in which records of Chinese school-aged children were analysed described a myopia progression of 0.28D in those children treated with 0.05% atropine solution; whereas the control group formed by children without treatment had an increase of 0.75D (Lee, *et al.* 2006). Prospective randomised studies overcome the potential disadvantages which appear after studying old records in retrospective studies. Kennedy and colleagues assessed 214 children who were treated with atropine for 7 years. Their myopic

progression rate was evaluated and compared to a matched sample of 194 subjects. After the atropine treatment period, the mean myopic progression adjusted for age and refractive error was significantly smaller in those subjects who underwent treatment (0.05 D per year) than that of controls (0.36 D per year) ($p<0.001$). Follow-up over 20 years estimated the remaining effects of atropine treatment. The myopia progression of those under atropine treatment was 2.79 D, whereas the control group without atropine had a progression of 3.78 D. These results suggest a potential beneficial effect of atropine therapy over the control of myopia progression as the effects stayed the same once treatment was discontinued (Kennedy, *et al.* 2000). Atropine has therefore been considered to reduce the progression of myopia through accommodation-blocking mechanisms. However, this has later been questioned in light of results on an animal study showing decreased progression of myopia even after the removal of accommodative-related receptors and cells (Luft, *et al.* 2003). A recent publication by Chua and colleagues (2006) describes the significantly different myopia progression in a randomised placebo-controlled study in which atropine was supplied to 346 children with ages ranging 6 to 12 years (Chua, *et al.* 2006). The eyes receiving atropine drops had a myopia progression of 0.28±0.92 D and the axial length remained unchanged from baseline (0.02±0.35 mm); whereas those children in the placebo-treated control eyes progressed by -1.20±0.69 D, with an axial length increase of 0.38±0.38 mm.

Therefore, the mechanisms through which atropine leads to a reduction in the myopia progression remain undetermined and additionally, secondary effects have been reported after using atropine, such as mydriasis or accommodative and myotic blockage.

In addition, pirenzepine, a relatively selective muscarinic-1 antagonist, was evaluated to control the progression of myopia in 353 children with ages 6 to 12 years (Tan, *et al.* 2005). Pirenzepine was supplied as a gel, and its effect was compared to placebo. After one year, the pirenzepine group had a progression of 0.47 D, which differed significantly from the progression of the placebo group (0.84 D). Thus, pirenzepine appears as the muscarinic receptor antagonist of choice due to the reduction in secondary effects (mydriasis and cycloplegia) attributed to atropine, as pirenzepine is a selective M1 receptor.

1.6 Risk factors for myopia

Risk factors are characteristics that increase the probability of suffering from a disease or developing a condition. Risk factors are variables related to an increased risk, but they do not necessarily trigger the condition; therefore, they are not causal. The term risk factor was first used by Dr. Thomas R. Dawber, a heart researcher, in a scientific paper from 1961 (Dawber and Kannel

1961). He described blood pressure, cholesterol and smoking as risk factors for a heart disease, offering the use of a valuable new terminology (Sullivan 2005; Kannel, *et al.* 1967; Dawber and Kannel 1961; Kannel, *et al.* 1961).
The mathematical interpretation of a risk factor describes the ratio between patients experiencing a condition (N) and those exposed to the risk factor (E) (Equation 1.1)

$$Risk = N \div E \tag{1.1}$$

However, recent epidemiological research uses a wide range of statistical methods to describe risk factors, such as multiple correlations, exponential functions, survival rates and odd ratios. Thus, applying the definition of risk factor to the myopia field, it can be said that risk factors play an important role in the aetiology of myopia; however they are not the direct cause of myopia. A family history of myopia, intensive close work and being East Asian in ethnicity are among the main risk factors for myopia development.

1.6.1 Inheritance as risk factor for myopia

A genetic input to the development and progression of myopia was suggested due to reported increased ratios of myopia inheritance in twins, or longitudinal studies in which children with parental history of myopia were more likely to have greater increases in axial length (Saw, *et al.* 2005; Teikari, *et al.* 1991). However, it is necessary to be aware of the limitations of separating genetic from environmental factors when assessing myopia risk factors, as human eyes are influenced by both environmental and genetic experience. Nonetheless, the similarity of monozygotic twin's refraction, brought up in similar environment as digozygotic twins has been reported to be higher than that of binovular twins, which may represent the genetic value of myopia (Chen, *et al.* 1985). Additionally, the question of twins reared apart was answered in a study by Knobloch and colleagues (1985) in which the ophthalmologic features of 26 pairs of twins (18 monozygotic, 8 dizygotic) were evaluated. The refraction data showed a significantly higher refractive error correlation between the eyes of monozygotic twins than between those of dizygotic twins (Knobloch, *et al.* 1985).

Epidemiological studies have also supported the influence of parental myopia on the incidence of myopia. The analysis of parental myopia to date has been principally performed in two different ways: incidence of myopia as a function of the number of parents being myopic (Mutti, *et al.* 2002), and percentage of myopic children with myopic parents (Pacella, *et al.* 1999). The outcomes of the Orinda study, which evaluated a mixed ethnic population of Carolina, showed

inheritance factors to account for approximately 80 per cent of juvenile myopia. Statistics evaluating the incidence of myopia in children with one or two myopic parents reported 33 to 57 per cent incidence when both parents were myopic and 18 to 25 per cent when only one parent was myopic in two different studies throughout (Mutti, *et al.* 2002; Wu and Edwards 1999). Similar findings were reported by Saw and colleagues after parental history of myopia was found to correlate significantly to the refractive error group ($p < 0.001$). For Chinese children, the proportion of children with myopia in one or two myopic parents was higher than in those with no myopic parents; whereas for non-Chinese children, the proportion with myopia in two myopic parents was higher than in those with one myopic parent or none (Saw, *et al.* 2002). Additionally, the differences in the pattern observed among ethnicities suggested an inheritance profile that may run in families and countries (Grice 1997).

Ethnicity, therefore, also appears to be one of the main risk factors for the onset of myopia, with East Asians having the highest prevalence of myopia at present. Population studies described an increase of approximately 40 per cent in the prevalence of myopia in Taiwanese and Chinese children with ages 7 to 15 (Lin, *et al.* 1996; Lam and Goh 1991). The increase in the prevalence of myopia of East Asian countries contrasts with the decrease experienced by Nordic countries such as Sweden(Gronlund, *et al.* 2006).

However, as highlighted by Schaeffel and colleagues in 2003, if genetic factors were to be the main trigger for myopia, visual experience would not be able to activate the onset of myopia on emmetropic eyes (Schaeffel, *et al.* 2003). The visual information received throughout life is of vital importance for the correct development of visual function, as shown by the development of amblyopia in some cases after deprivation of form vision (i.e. reduction of visual input due to congenital cataract, ptosis) or abnormal binocular interaction (i.e. strabismus). In eyes with poor accommodative amplitude, comparable anomalous visual stimulation may occur after performing long near tasks in an attempt to focus near objects.

1.6.2 Near work as a risk factor for myopia

Near work has been associated with myopia; however, as suggested by Rosenfield and Gilmartin, an association between myopia and close work should not be interpreted as a causative relationship (Rosenfield and Gilmartin 1998). A comprehensive evaluation of the near and distance work habits of 366 fourteen-year-old school children revealed that children with myopia were significantly more prone to spend more time studying and reading than playing sports (Mutti, *et al.* 2002). The Tanjong Pagar Survey described the educational and socio-economical characteristics of a sample of 951 adults with ages ranging from 40 to 81 years and evaluated their

correlation with their ocular dimensions. Higher education was associated with longer axial lengths longer vitreous chambers and more myopic refractions after correcting for age, sex, occupation, income and housing. Interestingly, adjustment for axial length attenuated the refractive association of education, which may represent the strength of highly demanding cognitive near work and myopia (Wong, *et al.* 2002).

Despite the noticeable relationship between near work and myopia, it is interesting to highlight the link between these results and those previously reported by Goldschmidt in which the prevalence of myopia was found to be higher in university students than in a population with lower level of education (Goldschmidt 1968). Outcomes of this nature might suggest a role for cognitive demand in the association between near work and myopia, a role that has been investigated by several research groups, with contradicting results. Bullimore and Gilmartin (1987) reported a significantly higher increase in tonic accommodation for late onset myopes than for emmetropes (Bullimore and Gilmartin 1987), which has been recently supported by the differences described by Davies and co-workers in the accommodative response of late onset myopes after increasing cognitive demand (Davies, *et al.* 2005). These disparities in accommodative responses between late onset myopes and emmetropes seemed to be affected by the sympathetic nervous system (Bullimore and Gilmartin 1987). However, the effect of cognitive demand was not associated with the degree of refractive error in a study by Rosenfield and Ciuffreda, in which the transient myopic shift induced by sustained near-work tasks appeared related to the within-task accommodative response rather than to variations in cognitive demand during the course of the near-visual activity (Rosenfield and Ciuffreda 1994).

1.6.3 Diet and myopia

Another risk factor thought to be involved in the onset and progression of myopia was diet (Werbach 2003; Edwards 1996; Rosner, *et al.* 1995 ; Gardiner 1956). Changes in the Eskimo diet and cultural habits occurred parallel to an increase in the myopia incidence. Eskimos moved from hunter-gatherer diets with high consumption of proteins, medium levels of fat and low levels of carbohydrates to diets with high levels of sugar and carbohydrates (Schaefer 1977; Schaefer 1971; Scott 1956). The nature of the carbohydrates consumed by the hunter societies allowed the absorption of carbohydrates gradually with low rise of the plasma glucose. However, the glucose levels produced by the refined sugars increase insulin levels automatically (Cordain, *et al.* 2005), which may be related to the rise in the incidence of diabetes type II experienced by the Eskimo and Australian aborigine societies after their contact with the western society (Thorburn, *et al.* 1987).

Additionally, hyperinsulinaemia has shown activation of the pathway that triggers scleral growth, by inactivating genes that normally restrict scleral proliferation (Ferry, *et al.* 1999). This, together with the results observed in an animal study with rats, which showed increased incidence in the development of relative myopia after their diet was altered with high sucrose levels, supports the theory that diet may be associated to the aetiology of myopia (Bardiger and Stock 1972).

Additionally, a retrospective study by Lane revealed a significant difference in the consumption of refined carbohydrates in the diet of myopes compared to that of emmetropes. As these results were drawn from an analysis performed once the child had already developed myopia, the results are not conclusive (Lane 1982). Another study assessed in a prospective way the diet of children who became myopic, and compared it to that of children who did not develop myopia. Lower energy intake resulted in lower mean percentages of protein, fat, vitamins B1, B2 and C, phosphorus, iron, and cholesterol in myopic children (Lam, *et al.* 2002). However, interestingly, myopes have not been reported to have decreased weight or height (Edwards 1996).

It is also interesting to note that the levels of chromium in red blood cells, which have been indicated to reduce the blood glucose levels, appeared to be decreased in myopes compared to emmetropes. This supports the potential effect of diet on myopia (Lane 1982).

1.7 Peripheral defocus in myopia

Typically, the refractive state of an eye is defined by the refractive error measured along the visual axis. However, the refraction measured off-axis differs from that centrally. The evaluation of the peripheral refractive state of myopes, emmetropes and hyperopes has revealed different patterns in these peripheral areas, myopic eyes having elongated vitreous chamber and a relatively more prolate profile than emmetropes (Logan, *et al.* 2004; Singh, Logan and Gilmartin 2006; Logan, Gilmartin and Dunne 1995).

The traditional management of myopia with negative lenses places the defocused myopic image back on the retinal plane. This approach aims to correct the central vision, with no correction for the peripheral retina. However, owing to the elongated myopic vitreous chamber and its relatively prolate shape, the management of the central vision alone may lead to a hyperopic state of the peripheral retina. Therefore, the correction of the central vision only in myopia results in relative peripheral hyperopia, which may stimulate an active emmetropisation mechanism. Such emmetropisation may in turn trigger ocular growth, leading to myopia progression (Schmid 2003).

Seidemann and colleagues used an infrared autorefractor to measure the spherical and cylindrical refractive components in the central visual field, and at 45 deg eccentricity. The central refraction showed no differences between emmetropes, myopes and hyperopes; however, peripheral readings revealed significantly more hyperopic spherical equivalents in myopic subjects than in emmetropic subjects and more myopic spherical equivalents in hyperopic subjects (Seidemann, *et al.* 2002).

Recent studies have supported this hypothesis. The study by Schmid and co-workers (2003) recorded axial length and spherical equivalent at central location and 15 degrees temporally, nasally, inferiorly and superiorly in 63 children with ages ranging from 7 to 15 years of age. Additionally, an Optical Low Coherence Reflectometer (OLCR) was developed to determine retinal steepness. The results showed correlation between relative peripheral axial length and mean spherical equivalent between refractive groups (Schmid 2003). Charman also claimed the need of human longitudinal studies to corroborate whether the retinal defocus associated with peripheral hyperopia may result in the activation of mechanisms that lead to axial myopia (Charman 2005).
A possible correlation between retinal steepness and peripheral refraction has also been reported, which suggests the possibility of detecting children at risk of myopic progression by the evaluation of their retinal steepness (Schmid 2003).

The effect of age was analysed recently in two studies in which no significant differences in peripheral refraction were attributed to age. Atchison and co-workers compared the outcomes from different subjects in 2 age groups; 55 young subjects (24 ± 4 years of age) and 41 older subjects (59 ± 3 years) (Atchison, *et al.* 2005), whereas Charman and Jennings reported a 26-year follow up of 2 emmetropic subjects (Charman and Jennings 2006). Despite the differences between both study designs, the conclusions reached by both studies agreed on the invariability of the peripheral refraction with age.

1.8 Genetics

The role played by genetic factors in the development of myopia is supported by the fact that myopia is one of the clinical features of several genetic syndromes such as Stickler syndrome, Marfan syndrome or Knobloch syndrome. However, a very low percentage of myopes suffer from any of these syndromes (Van Camp, *et al.* 2006; Paluru, *et al.* 2005; Olivier Menzel 2004; Passos-Bueno, *et al.* 1994). Twin and family studies have supported a genetic input to the development and progression of myopia. As mentioned above (section 1.6) , several studies have shown

increased ratios of myopia inheritance in twins, greater increases in axial length in children with parental myopia and similar refraction of monozygotic twins born and raised in analogous environment compared to that of binovular twins (Saw, *et al.* 2005; Teikari, *et al.* 1991; Chen, *et al.* 1985). Therefore, despite the known influence of visual input on the development of myopia in animal models (section 1.3), it is also evident that human myopia is influenced by genetic factors, more specifically, high myopia, as suggested by analysis of selected pedigrees in high myopia (Francois 1961). The genetic approach aims to identify potential genes involved in the development and progression of myopia, and it has two main purposes: to help understanding the molecular physiology of myopia, and to develop potential targets for pharmacological intervention in the future (Young 2004; Schaeffel, *et al.* 2003).

The concept of genetic involvement must not be interpreted simply as the unit of genetic inheritance. A gene comprises a linear group of nucleotides able to undergo replication, expression and mutation. Genes control the synthesis of proteins, which is a key function in understanding the physiology of immunology, as genes regulate the production and synthesis of proteins which will become immunological elements.

1.8.1 Mapping genes

The number of human genes is not known exactly, but an approximation of 30,000 to 35,000 has been suggested. This large number imposes several limitations on genetic mapping, since an average chromosome would comprise 1,000 genes, which approximately equals from 45 to 280 million base pairs (Mb) of DNA. By linkage analysis a region of 1 to 5 million base pairs is delimited, which contains approximately 10 to 50 genes. These numbers give an indication of how complicated it is to map a gene through a family, as in the case of high myopia.

Linkage analysis has allowed the genetic assessment of several families with nonsyndromic high myopia. Three autosomal dominant loci have been determined using this mapping technique: MYP1, MYP2 and MYP3. In 1990, the first locus for an X-linked form of high myopia (MYP1) was described on chromosome Xq28, which represents the long arm of chromosome X, location 28. Two autosomal dominant high myopia loci, MYP2 and MYP3, were located at chromosome 18p11.31 (small arm of chromosome 18, location 11.31) and at chromosome 12q23.1-24 (long arm of chromosome 12, location 23). Most recently, chromosome 17q21-22 (long arm of chromosome 17, location 21) has also been related to autosomal dominant high myopia, and a suggestive fourth locus for autosomal dominant high myopia was reported at chromosome 7q36.

MYP1 was named Bornholm eye disease locus, as the family studied was originally from Bornholm in Denmark (Young, *et al.* 2004). Another family from Minnesota with similar X-linked phenotype was also assessed, and all males showed protanopia, abnormal photonic electroretinographic responses along with high myopia. Both families were then suggested to have a novel cone dystrophy associated to myopia, and not just simply myopia. Therefore, MYP1 high myopia locus was concluded to be linked to a novel cone dystrophy.

MYP2 is also called myosin II heavy chain. It has been related to autosomal dominant high myopia, and it is involved in cytokinesis and contractile ring formation. MYP2 is considered one of the most interesting loci at the moment as MYP2 has been linked to autosomal dominant myopia in Italian and Hong Kong Chinese families (Lam, *et al.* 2003 ; Heath, *et al.* 2001).

MYP3, or myopia high grade autosomal dominant locus, was first discovered in 1998 after the genetic assessment of a German/Italian family whose average mean spherical error from all the individuals was approximately -9.47 D. Lumican (LUM) and fibromodulin (FMOD) had been suggested by an animal study as potential functional candidate genes for high myopia (Chakravarti, *et al.* 2003). In view of these outcomes, a later study screened individuals with myopia for LUM and FMOD. However, the results did not show LUM and FMOD as a candidate genes for autosomal dominant high myopia at MYP3 (Paluru, *et al.* 2005). A study by Farbrother and co-workers evaluated the representation of the myopia loci to date in the UK population and found MYP3 to be the most representative locus, with approximately 25% of UK families having autosomal dominant transmission of high myopia. No significant linkage was found for the MYP2 locus (Farbrother, *et al.* 2004).

Transforming growth factor (TGF)-β-induced factor (TGIF) is one of the identified candidate genes within locus MYP2. TGIF was investigated for mutations in Chinese subjects with high myopia, and it was suggested as a candidate gene for high myopia after being found to have sequence alterations (Lam, *et al.* 2003b). However, a focused positional candidate gene analysis at the MYP2 locus on TGIF, EMLIN-2, MLCB and CLUL1 by Young showed that none of the genes assessed as candidate genes were actually associated with high myopia at the MYP2 locus (Young 2004).

1.9 Methods to assess refractive and biometric status of the eye

A large quantity of the work described in this thesis concentrates on myopia; therefore adequate methods were required to assess the refractive and biometric status of the eyes of the participants.

1.9.1 Measurements of refractive error

The measurements of the participants refractive error were performed objectively in order to reduce the bias that a subjective refraction may have induced (i.e. tiredness) using an objective automated autorefractor, as automated refraction is more repeatable than subjective refraction and therefore more appropriate for myopia studies (Bullimore, *et al.* 1998). In this study an automated refractor was used, the Shin-Nippon NVision-K 5001 Autorefractor (Japan).

The Shin-Nippon NVision-K 5001 is an open view auto-refractor that uses an infra-red source to calculate the refractive condition of the human eye. The instrument can take measurements ranging from ±22.00D sphere and ±10.00D cylinder, in 0.125 steps for spherical and cylindrical power and 1° for the axis of the cylinder. It calculates the refractive error of the eye in two main steps: an infra-red ring is moved backwards and forward until a sharp image of this ring is obtained. The refractive error of the eye is given by a digital analysis of the ring resulting from the target reflected onto the retina.

Prior to the data collection, the test eye included in the accessories of the Shin-Nippon NVision-K 5001 was used to ensure the adequate calibration of the instrument. To take refractive measurements, the patient was asked to rest forehead and chin on the rests of the Shin-Nippon SRW 5000 Autorefractor and to look straight onto a high contrast Maltese cross situated 6m in front. The projection of the infra-red ring was brought into focus on the display screen by a joystick where the location and size of the eye's pupils can be visualised. By pressing the joy-stick, the image of the ring reflected on the retina was analysed digitally in different meridians that allow the calculation of the toroidal prescription. Five readings were taken for each patient, and the instrument provided an average of the refractive error.

1.9.2 Biometric readings

In this thesis, the biometric features of the participants' eyes assessed were the thickness of the cornea (pachymetry), axial length, anterior chamber depth and vitreous chamber volume.

1.9.2.1 Pachymetry

The thickness of the cornea can be measured using topographic pachymetry and ultrasound. Ultrasonic pachymetry is an efficient way to measure corneal thickness; however, the probe must touch the corneal surface, for which topical anaesthesia is required, and the measurement relies on the high reflectance of the corneal endothelium and epithelium layers. A high-frequency sound wave is sent towards the cornea, and echoed by the endothelium and the epithelium due to the differences in tissue density. Knowing the time lag of the reflections from these two layers and the

velocity of the sound wave, the thickness of the cornea can be calculated, assuming that the velocity of the ultrasonic wave in the cornea is constant. Its accuracy is dependent on the perpendicularity of the probe's application to the cornea and reproducibility relies on precise probe placement on the corneal centre. Thereby, repeated measurements are recommended to reduce the variability of the thickness recording (Ehlers and Hjortdal 2004).

The pachymeter used in this thesis was the DGH-550 Pachette 2 (DGH technology, Pennsylvania, US). The DGH 500 ultrasonic pachymeter is composed of a base unit, where the measurements are displayed, linked by a cable to the ultrasonic probe. It is a portable device that can be tilted to facilitate the viewing of the readings. The DGH 500 pachymeter can store up to 33 readings, each of which is displayed with its standard deviation. It allows the analysis of the cornea in any location, and is not affected by the gaze position of the patient. As the DGH 500 pachymeter touches the cornea, a drop of anaesthetic was instilled in the patient's eye (proxymetacaine HCl 0.5% Minims®, Chauvin Pharmaceuticals). The patient was asked to look straight ahead and the probe was directed towards the centre of the cornea. An acoustic signal was emitted by the pachymeter as soon as a successful reading had been taken. In the current study, the average of five readings was taken per patient's eye.

1.9.2.2 Axial length and anterior chamber depth measurements

The IOLMaster from Zeiss (Carl Zeiss Jena, Germany) calculates axial length, corneal radii, anterior chamber depth and white to white readings needed mostly in cataract surgery (Eleftheriadis 2003). The IOLMaster is a non-invasive device, which ensures the comfort of the patient as the four measurements are taken with the same machine, without anaesthetic, minimising the risk of infection from patient to patient reported with conventional ultrasonography. The instrument comprises a unit from where the laser light is directed towards the patient's eye. This unit is linked to a keyboard where the patient's data is typed.

The IOLMaster calculates axial length and anterior chamber depth based on partial coherence interferometry (PCI). PCI is based on Michelson's interferometer, by which precise distance measurements can be taken. The Michelson interferometer produces interference fringes by splitting a beam of monochromatic light. One of the split beams strikes a fixed mirror, while the other hits a movable mirror. An interference pattern results when the reflected beams are brought back together. Accurate distance measurements can be taken by moving the mirror and counting the interference fringes which move by a reference point (Equation 1.2). The distance "d" associated with m fringes is

$$d=m\lambda/2 \qquad (1.2)$$

, where d is the distance to be measured, m is the number of fringes, and λ is the wavelength of the monochromatic light.

Michelson´s interferometry has been applied to different scientific fields, such as molecular biology, ophthalmology and dermatology (Jaffe and Caprioli 2004; Welzel 2001; Haigis, *et al.* 2000).

To calculate axial length, the IOLMaster measures the distance between the anterior surface of the tear film and the retinal pigment epithelium (RPE). A graphical representation is obtained with the different surfaces the laser crossed. The cornea and RPE appear as the main peaks, from which axial length is calculated. The IOLMaster is programmed to provide an automatic axial length calculation, however reference points can be manually selected to calculate a customised measurement of the axial length if wanted.

The pattern projected onto the eye and principles used vary with the measurement taken. To assess the anterior chamber depth, the IOLMaster directs a 0.7 mm width slit beam of light through the anterior segment of the eye at an angle of 38 degrees to the visual axis. The instrument forms an optical section and the internal software measures the distance between the anterior corneal pole and the anterior crystalline lens surface to calculate the anterior chamber depth (Sheng, *et al.* 2004).

The IOLMaster is one of the most recent techniques used in ophthalmology for the calculation of intraocular lens (IOL) power for cataract surgery. Besides the cataract application (Basu 2005; Rose and Moshegov 2003) , PCI has been found to be highly reproducible and repeatable when measuring axial length (Eleftheriadis 2003; Santodomingo-Rubido, *et al.* 2002), anterior chamber depth (Hashemi, *et al.* 2005) and keratometry. The IOLMaster is currently used in research due to the accuracy of its readings. The calculation of the axial length has not shown significant differences with ultrasound readings although the anterior chamber depth has been reported to be significantly shorter than the values obtained with ultrasound, since the IOLMaster measures more directly along the visual axis (as the instrument's internal target is fixated) and, being a non-contact device, does not indent the patient's cornea.Additionally, the corneal radius measurements taken with IOLMaster matched more closely those of the keratometer than those of the videokeratoscope. The coefficient of repeatability for the axial length calculation has been reported to be from 0.00 to 0.04 mm (Basu 2005; Hashemi, *et al.* 2005; Sheng, *et al.* 2004; Eleftheriadis 2003; Rose and Moshegov 2003; Santodomingo-Rubido, *et al.* 2002; Vogel, *et al.* 2001).

These characteristics, together with a better resolution than ultrasound, and the fact that it is a non-invasive technique, made IOLMaster the instrument of choice for the calculation of axial length and anterior chamber depth in this thesis.

Prior to data collection, the calibration of the IOLMaster was verified using the test eye provided. For calibration purposes, the test eye needed to be clean, especially for the keratometry and ACD measurements. To take the readings, the patient was asked to rest both chin and forehead against the rests of the IOLMaster once their data had been entered. The patient only needed to stare at the different spots of light provided to measure the biometric parameters with continuous blinking to spread an optically smooth tear film over the cornea that ensured the quality of the readings taken. The readings were then saved on disk or printed out on a pre-defined form provided with the machine.

1.9.2.3 Vitreous chamber volume calculation

Colour Doppler Imaging (CDI) was used to calculate the volume of the vitreous chamber. CDI provides with a 2-D image of the orbit after placing the probe on the closed eyelid of the patient.

The volume of the vitreous chamber was calculated by delimiting with calipers the three main diameters of the vitreous chamber. Since the image given by the instrument only covers 2 dimensions simultaneously, the three main diameters were obtained by rotating the probe 90 degrees. The image of the lens obtained on the grey 2D ultrasound image was used as a reference for this calculation (figure 1.2).

Appendix 1.1 shows the repeatability of the vitreous chamber volume calculation, which showed a coefficient of variation (CoV) of 0.48 and a coefficient of repeatability (CoR) of 1.92.

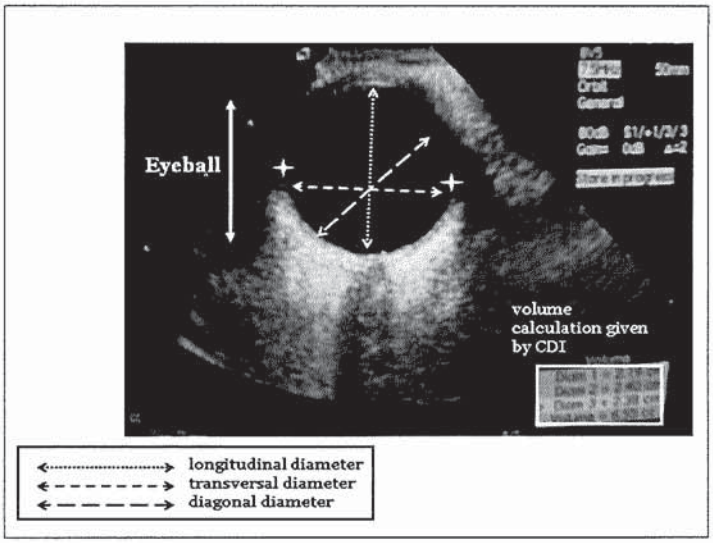


Figure 1.2 *Diagram showing the procedure followed for the calculation of vitreous chamber volume*

Section two: Vascular concepts

An overview of the ocular vascular system, together with an explanation of the terminology and vascular instruments used throughout the thesis is given in this section. Blood composition and the physiology of blood flow are depicted to show some of the essential ocular vascular concepts.

1.10 Ocular vascular supply

In order to understand the perfusion characteristics of the human myopic eye, knowledge of the ocular vascular supply is required. The ocular circulation is complex, due to the number of different structures that require blood supply and drainage. The blood vessels that nourish the eye can be divided into two main groups comprising retinal and choroidal vasculature.

1.10.1 Retinal blood supply

The retina is the innermost layer of the eye. Its function is to receive and process the visual information that first passes through other optic surfaces located anterior to it (tear film, cornea, aqueous humour, crystalline lens and vitreous humour). The retina is characterized by its high metabolic rate. It is considered as one of the tissues with the largest oxygen consumption per unit weight (Forrester, *et al.* 2001). The retinal blood supply in humans is provided by the central retinal artery (CRA). The CRA supplies blood directly to the inner two thirds of the retina and the choroid supplies the outer third of the retina. Via a process of diffusion, the CRA supplies rods,

cones and outer layers. The retinal circulation has a low flow rate (25mm/s) and a high oxygen exchange (Forrester, *et al.* 2001).

The choroidal circulation has a high flow rate (150 mm/s), low oxygen exchange and a fenestrated capillary bed. The choroidal circulation is thought to have the highest flow rate of the entire body due to its large vessels and low vascular resistance (Forrester, *et al.* 2001). The choroidal circulation is thought to lack local autoregulatory properties, although as will be explained later, this is a topic of controversy. The regulation of the choroid is innervated by the sympathetic system; whereas the retinal circulation is ruled by autoregulatory mechanisms that are locally controlled, some of which are mediators released by the endothelial cells (Delaey and Van de Voorde 2000). The percentage of ocular vascular transportation attributed to the uveal tract is 96% (85% choroid, 10% ciliary body, 1% iris), this high percentage reflecting the importance of the choroidal circulation, whereas the retinal percentage is only 4% (Alm 1992).

The retinal layers posterior to the outer plexiform layer (OPL) are avascular and are considered to receive nutrients from retinal and choroidal vessels while the middle limiting membrane is considered to be the border between choroidal and retinal supply (Remington 1997). There is also a capillary network that spreads like a vast cobweb throughout the retina providing a supply of extra-nourishment (Flammer, *et al.* 2002).

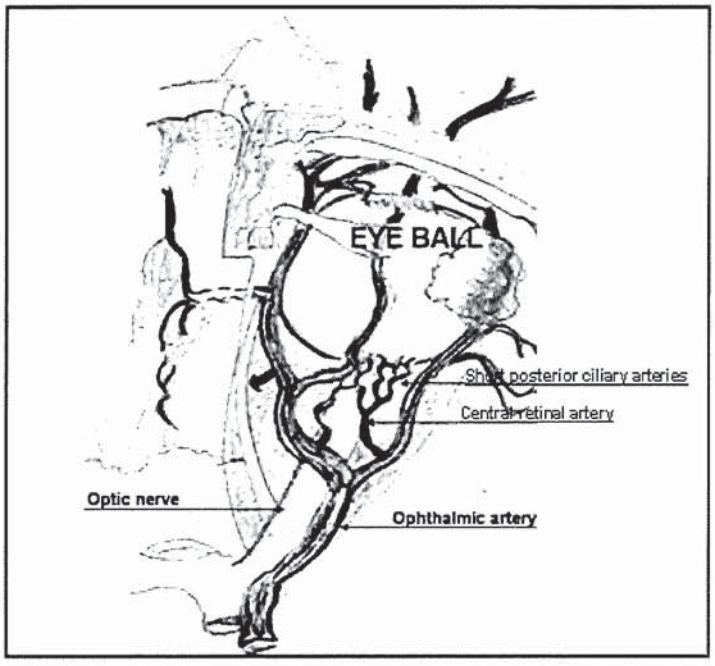


Figure 1.3 *Distribution of the retrobulbar vessels: ophthalmic artery (OA), central retinal artery (CRA), short posterior ciliary arteries (SPCAs) (right eye).*

1.10.1.1 Central retinal artery

The CRA nourishes the inner retinal layers. It is one of the branches of the ophthalmic artery (OA), which in turn is a subdivision of the internal carotid artery. The OA arteries can be subdivided into an orbital group and an ocular group. The orbital group includes the lacrimal artery, the supraorbital, the anterior and posterior ethmoidal, the internal palpebral artery, the supratrochlear artery and the dorsal nasal artery as the orbital branches. The ocular group comprises the long and short posterior ciliary arteries, the anterior ciliary artery, the muscular artery and the central retinal artery. The OA provides nourishment to the orbit and scalp and is supplemented by the external carotid (Bron, *et al.* 1997).

The CRA pierces the inferomedial area of the optic nerve (figure 1.3), and is accompanied by the central retinal vein (CRV) and some sympathetic fibres as it runs forward within the optic nerve. It then passes through the lamina cribosa and enters the optic disc branching superiorly and inferiorly. These branches in turn are subdivided into nasal and temporal branches and continue to branch dichotomously within the retinal nerve fiber layer (Remington 1997). Each of the four major branches of the CRA supplies a sector of the retina without overlap (the retinal vessels are functional end-arteries) (Warwick 1976). The large retinal arterial branches travel in the nerve fibre layer beneath the inner limiting membrane. The central retinal artery has been reported to be a very tortuous vessel (Rouviere 1976).

1.10.1.2 Choroidal circulation

The choroid is a thin brown highly vascular layer that extends from the optic nerve towards the ciliary body. The choroid is part of the uveal tract (comprising iris, ciliary body and choroid) and it is located between the sclera and the retina providing nutrients to the outer retinal layers (figure 1.4). Three differentiated layers make up the choroid: vessel layer, capillary layer and Bruch's membrane (Remington 1997).

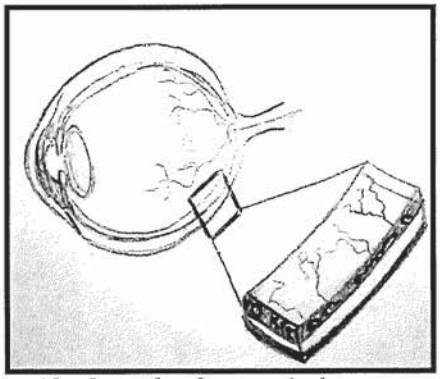


Figure 1.4 *Drawing of the choroid where the three main layers can be differentiated: Bruch's membrane (innermost), capillary layer and vessel layer (outermost).*

The choroidal vascular supply is derived from the ciliary arteries. There are three groups of ciliary arteries: long posterior ciliary arteries (LPCAs), short posterior ciliary arteries (SPCAs) and anterior ciliary arteries. The arteries that supply the choroid are the SPCAs, whereas the LPCAs are mainly in charge of nourishing the iris and the anterior arteries supply the sclera and the conjunctiva (Araki 1975). The SPCAs are a branch of the OA, which pierce the optic nerve through the temporal region (figure 1.3) (Olver JM 1990).

Choroidal blood flow represents 85% of the total ocular blood flow (Kaufman and Alm 2003) and the main role of this layer is to nourish the external third of the retinal structure, allowing good vision without obstructing the fovea. The choroidal circulation is mainly controlled by sympathetic innervation and it is thought not to be autoregulated (Delaey and Van de Voorde 2000). Additionally, Van Alphen (1986) suggested that the ciliary muscle choroid layer also behaves like a solid sheet of smooth muscle able to resist part of the IOP.

1.10.1.3 Retinal capillaries

The capillaries are the smallest vessels of the whole vascular system with diameters ranging from 5 to 10 μm. Capillaries are the microvessels responsible for connecting arteries and veins, and additionally they are the vascular entities closest to body tissues, therefore transporting oxygen, water and lipids by diffusion directly to the tissues (figure 1.5). Capillaries are nearly exclusively composed of an endothelial layer that allows this diffusion transport, together with a basal lamina and pericytes that ensure the physical support of the microvessel. The flow of blood in the capillaries is controlled by the so called precapillary sphincters. These structures are made up of muscle fibers, and they regulate the perfusion of blood by their opening and closing. Precapillary sphincters are located between the arterioles and the capillaries (Forrester, *et al.* 2001).

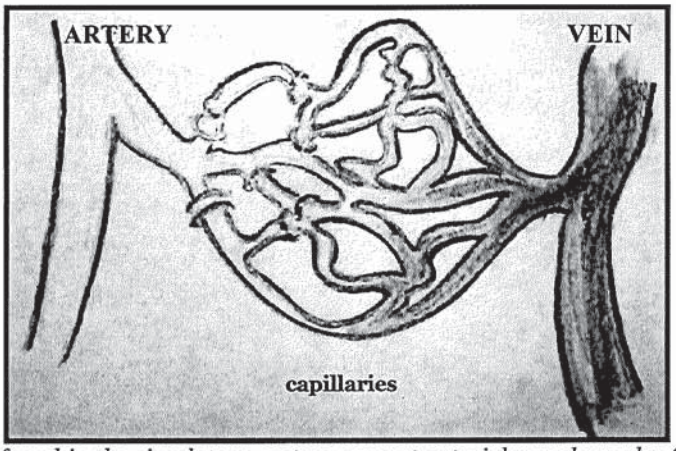


Figure 1.5 *The capillaries found in the circulatory system connect arterioles and venules (as shown in figure), and they measure approximately 5-10μm*

The retinal capillary network spreads throughout the retina. The diameter of retinal capillaries is yet smaller than those of conventional capillaries. These retinal capillaries contribute to the blood-retinal barrier, due to the non-leaky tight junctions of the endothelial cells (zonulae occludents) (Bron, *et al.* 1997).

All vascular beds are highly permeable to lipid-soluble substances, such as oxygen (O_2) and carbon dioxide (CO_2), which pass readily through the endothelial cells that make up the capillaries. For water-soluble substances, the permeability of the vessel wall is determined by the structure of the capillary endothelium. The thick basal lamina and astrocyte processes also protect this blood-retinal barrier, compensating in turn for the more permeable nature of the retinal vascular endothelium (Stewart PA 1994).

There are two main levels of capillary networks in the retina. A deep network lies on the inner nuclear layer (INL) near the outer plexiform layer, whereas the superficial network is located around the nerve fibre or ganglion cell layer (Warwick 1976). The retinal capillary network reaches only the scleral margin of the inner nuclear layer. The outer retina is normally avascular. Capillaries are most abundant in the macula, but are absent from the fovea itself (capillary-free zone). The fovea, therefore, gets its nutrients from the choriocapillaries (Bron, *et al.* 1997).
Another capillary network spreads over the nerve fibre layer in the peripapillary region. Interest in these capillaries in glaucoma arose due to their increased vulnerability to raised intraocular pressure (IOP) in glaucoma due the infrequent arterial input, lack of anastomoses and the long course of the capillaries (over 1000μm) which as shown in equation 1.6 increases the resistance to flow. Flame-shape haemorrhages or cotton-wool spots occur predominantly in this capillary network. This is due to the high permeability to low molecular weight substances of this capillary network (Bill, *et al.* 1983).

The opening and closing of capillaries in response to tissue need controlled by the precapillary sphincters is termed vasomotion, thus the number of open capillaries depends on the metabolic needs of the target tissue. Therefore, some of the capillaries are open in skeletal muscles during rest, while maximal exercise may increase blood flow tenfold to twenty fold (Johnson and Henrich 1975).

1.10.1.4 Pericytes

Pericytes are non differentiated cells that have been implicated in many vascular-related functions such as regulation of the microcirculation, permeability of the capillary wall, and modulation of endothelial cell growth in angiogenesis and in the immune system (Chakravarthy and Gardiner 1999).When pericytes are found as support of small blood vessels, they take the shape of small

cells with long processes that run parallel to the long axis of microvessels that give off shorter processes that encircle the capillary walls (figure 1.6).

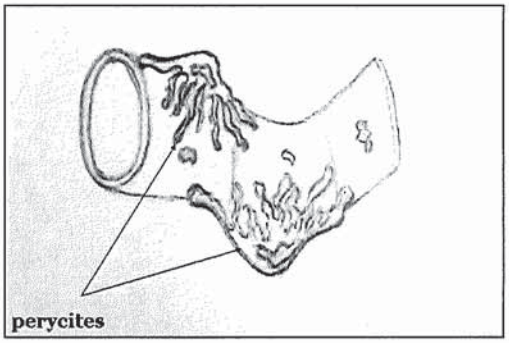


Figure 1.6 *Drawing of the shape and location in which pericytes are found in the vascular system, as support of small blood vessels, taking the shape of small cells with long processes that run parallel to the long axis of microvessels that give off shorter processes that encircle the capillary walls*

In the retina, pericyte processes cover a large part of the circumference of the capillary endothelial tubes (Kaufman and Alm 2003). Pericytes are located within basal lamina and are in intimate contact with the endothelial cells by peg and socket contacts, adhesion plaques and gap junctions. They express protein typical of contractile cells (actin & myosin) and therefore, they contain the morphologic basis for contraction and deformation of the walls of the micro vessels (Haefliger, *et al.* 1999). Pericytes contract in the presence of peptides such as angiotensin II, endothelin-1 (the most potent natural vasoconstrictor) and bradykinin, and relax in the presence of adrenergic β2-receptor agonists, nitric oxide, carbon dioxide, forskolin or adenosine. Based on these findings retinal pericytes have been suggested to have an important role in the regulation of the microcirculation (Kaufman and Alm 2003).

1.10.2 Optic nerve head supply

The optic nerve (also known as cranial nerve II) is a continuation of the axons of the ganglion cells in the retina. There are approximately 1.1 million nerve cells in each optic nerve. The optic nerve, which acts as a cable connecting the eye with the brain, shares more similarities with brain tissue features than with those of nerve tissue. The area considered the anatomical beginning of the optic nerve inside the retina is referred to as optic nerve head (ONH).

The tissue that forms the optic nerve resembles brain tissue and similarly the vascular supply of the optic nerve is also analogous to that of the brain in the sense that in both optic nerve and brain the blood supply comprises two networks (Bron, *et al.* 1997). In a simplified manner (since this thesis did not evaluated the haemodynamics of the ONH), the vascular supply of the ONH can be resumed as provided by:

-CRA, which supplies the superficial zone of the optic nerve head and
-SPCA, whose vessels supply with nutrients the small area within the ONH close to the lamina cribosa (Flammer, *et al.* 2002; Hayreh 2001).

The central retinal vein (CRV) facilitates the venous drainage of this structure in almost all the ONH areas. The prelaminar region may also drain into the peripapillary retinal veins (Hayreh 1996) (figure 1.7). The blood

A later study from 1997 reported an efficient mechanism of autoregulation in the ONH after IOP elevation with a suction cup. A compensatory decrease in ONH vascular resistance during IOP elevation correlated with the increase in blood flow (Riva, *et al.* 1997) .

An animal study by Riva and co-workers in 1990 described the ONH variations in the ocular blood flow velocity and volume of minipigs as measured with Laser Doppler Flowmetry (LDF). The frequency of the variations found ranged from 2.5 to 5.5 cycles/min. Additionally, volume fluctuations were studied in ONH, retina and choroid. The results showed that the volume fluctuations in the choroidal and retinal vessels were higher than those in the ONH, suggesting an autoregulatory mechanism in the ONH (Riva 1990).

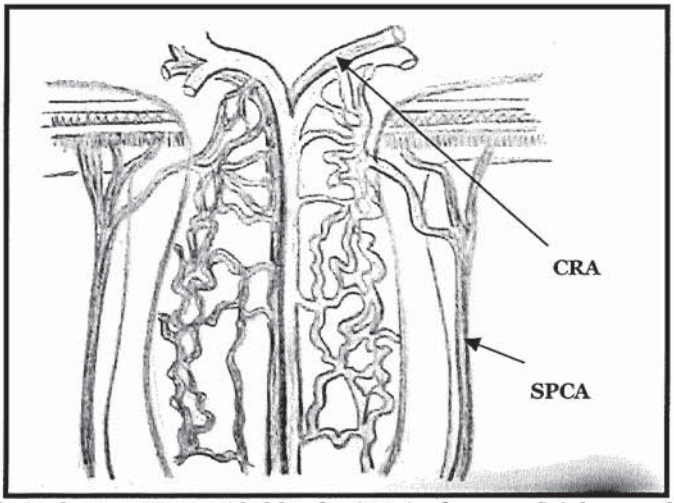


Figure 1.7 *The blood supply in the ONH is provided by the CRA in the superficial zone of the optic nerve head and by the SPCA in the area close to the lamina cribosa*

1.10.3 Ocular haemodynamics

Haemodynamics is the term used to refer to the wide range of events that occur within our cardiovascular system. In order to further understand the haemodynamics of blood flow, it is

necessary to characterize the features that describe the movement of the particles that form the flow of blood.

1.10.3.1 Blood composition

Blood is a suspension of deformable cells in plasma (45% and 55% of the total concentration respectively). Red blood cells constitute the largest percentage of cells in normal blood (99%), and they are the primary cell species influencing the rheological properties of blood. The major function of blood is to transport oxygen necessary to life throughout the body. It also supplies the tissues with nutrients, removes waste products, and contains various components of the immune system defending the body against infection, such as white cells and immunoglobulins. There are about 5.6 litres of blood in an average human body, accounting for approximately 8% of body mass (Renkin and Michel 1984). The normal values for the pH of the blood, that is, the activity of the hydrogen molecules, range from 7.35 to 7.45. Values below 7.35 are acidic and reflect those blood profiles with a tendency to donate hydrogen ions. Values over 7.45, however, are suggestive of an alkaline blood formulation. Blood pH along with arterial carbon dioxide tension ($PaCO_2$) and acid carbonate (HCO_3) readings are helpful in determining the acid-base balance of the body. On average, 86-90% of CO_2 in the body is converted into H_2CO_3.

The vascular circulatory system consists of a network of blood vessels with complicated geometry, including branching, curvature and a wide range of diameters. To analyse the haemodynamic functions in the complex *in vivo* vascular system, one must first understand *in vitro* perfusion features of flow in a tube with simple geometry.

It is first important to indicate that large vessels such as arteries and veins are anatomical entities, whereas microvessels (arterioles, venules and capillaries) are structurally and functionally part of the tissue they supply (Bron, *et al.* 1997), and the two physical conditions necessary before blood enters tissues are a relatively high upstream pressure to ensure sufficient quantities of blood reach the tissues and a low pressure to protect the capillary walls from downstream flow.

1.10.3.2 Blood flow definitions

Pressure (P) in a fluid is the mean compressive force (F) per unit area (A) acting on fluid surfaces in a direction normal to the surface at any point (equation 1.3)

$$P = F/A \tag{1.3}$$

Shear stress (σ) on a surface is the force acting on a unit area in a direction tangential to the surface. In a fluid at rest, shear stresses are zero.

Shear rate (γ) is the velocity gradient in a moving fluid. For motions in parallel layers, the shear rate is the difference in velocity per unit distance of separation between adjacent layers.

Viscosity (η) is a measure of the resistance of the fluid to friction when the fluid is in motion. For simple shear flows, it is defined as the ratio of the shear stress to the shear rate (equation 1.4):

$$\eta=\sigma/\gamma \tag{1.4}$$

For steady laminar flow of a Newtonian fluid in a cylindrical tube with uniform radius r, Poiseuille's law gives the pressure drop (ΔP) between two points separated by a length, l (Poiseuille 1797) (equation 1.5):

$$\Delta P=8l\eta/\pi r^4 \tag{1.5}$$

Examples of Newtonian fluid are simple solutions like water and aqueous solutions. In these fluids the viscosity depends on the temperature, and typically decreases when temperature rises. Non-Newtonian fluids are those whose viscosity changes when they are stirred or shaken.

The ratio between pressure drop and volumetric flow gives the resistance (R). Therefore, in laminar flow through a tube with uniform radius, the resistance is (equation 1.6):

$$R= 8l\eta / \pi r^4 \tag{1.6}$$

The radius of the vessel is thus a critical factor in determining viscous resistance. The shear rate in Poiseuille flow through a cylindrical tube varies from zero in the centre to a maximum value at the wall.

1.11 Clinical blood flow measures

The perfusion characteristics of blood comprise a variety of terms that refer to an extensive range of vascular parameters and features. The following terms describe the main parameters that will be used throughout this thesis.

1.11.1 Rate of blood flow

Blood flow describes the flow of the blood components in the cardiovascular system. Flow through a blood vessel is determined entirely by two factors: the pressure difference between the two ends of the vessel, and the vascular resistance. It can be described from the approximation of Poiseuille´s law as follows (equation 1.7):

$$F= \Delta P / R \tag{1.7}$$

where F is the flow, ΔP is the difference in pressure between two ends, and R the vascular resistance.

Differences in the rate of blood flow among the distinct retinal areas have been previously reported: blood flow near the fovea and around the optic nerve is much higher than blood flow in the peripheral retina, whereas blood flow through the choroid is extremely high in comparison with the retina. These disparities may be attributed in part to the difference in photoreceptor density between central and peripheral retina. The number of ganglion cells in the foveal area has been quantified as 32,000 to 38,000 cells/mm^2 (Curcio and Allan 1990), whereas the nasal portion of the peripheral retina exceeds by 300% the number of cells in the temporal retina, and the superior portion exceeds the inferior by 60%. The fact that the macular area comprises a larger amount of retinal cells results in a higher supply need. On the other hand, the flow in the choroid is higher due to the larger size of the choroidal vessels, and its low vascular resistance (Forrester, *et al.* 2001; Harris, *et al.* 1998).

1.11.2 Pulsatile ocular blood flow

In *in-vivo* circulation, flow velocity fluctuates with changes in vessel diameter and with the cardiac cycle. The cardiac cycle is partly controlled by the autonomous nervous system, which is the part of the nervous system not requiring conscious involvement of the brain in order to work. The fluctuations induced by both variations in vessel alterations and cardiac cycle are predominantly changes in flow rate, without changes in direction. These alterations in blood velocity, added to heart-rate variations, result in the blood pulse.

At the ocular level, the pressure in the eye is also affected by the cardiac cycle. The intraocular pressure (IOP) has been reported to increase during the systolic phase, decreasing with the diastole. The difference between the maximum and minimum IOP variations due to cardiac cycle has been reported to be approximately 2 to 3 mmHg.

Similarly, the heart rate affects the flow of blood in the eye. With every heart beat, a bolus of blood enters the eye, provoking a subtle change in eye volume. An intraocular pressure waveform is generated every time the blood bolus passes through the choroidal layer, which can be analysed to obtain the value of the pulsatile ocular blood flow (Silver and Geyer 2000). The maximum IOP change during the cardiac cycle is called the pulse amplitude (PA). The use of a pneumotonometric probe (related to the respiratory rhythm) linked to a Langham ocular blood flow system enables readings of intraocular pressure and its variation with heart rate (ocular pulse) (Langham, *et al.* 1989). From these values, the pulsatile ocular blood flow (POBF) is calculated as a ratio of pressure/volume as follows (equation 1.8):

$$POBF=cf(dV(tb)/dtb)- (dV(ta)/dta) \quad (1.8)$$

where "c" is a constant that related to the systolic duration of the pulse, "f" is the pulsatile rate and dV/dt is the incremental change in volume at either point "b" or "a" on the pulse profile.

The Ocular Blood Flow Analyser (OBFA, Paradigm Medical Instruments Inc., Utah, USA) enables the calculation of the pulsatile component of blood flow. OBFA estimates the pulsatile component of ocular blood flow based on the pneumotonometry principle, which is based on the principle by Langham described above. The ocular pressure rises and falls with each heartbeat creating a pressure waveform every time the bolus of blood from each heartbeat passes through the ocular choroid. The OBFA measures intraocular pressure 200 times per second and the pulse amplitude (PA) is calculated from the maximum IOP changes during the cardiac cycle. Based on a hydrodynamic eye model, the total pulsatile ocular blood flow (POBF) is given from the IOP variation with time as a ratio of pressure and volume values. The ocular pulsatile component is believed to represent 75% to 85% of the total ocular blood flow, and it is believed to be mainly determined by choroidal circulation (Mori, *et al.* 2001; Ravalico, *et al.* 1997; Ravalico, *et al.* 1996).

The instrument consists of a probe connected to the on board computer by a cable in which a current of air flows towards the end of the probe. The calculation of POBF is automatically derived from the five pulses that are closest to each other in the IOP beat to beat variation. These data are analysed by the on board computer in real time and intraocular pressure, pulse amplitude, pulse volume and POBF are calculated. This model is based on the assumption that venous outflow from the eye is non-pulsatile. Moreover, the ocular rigidity, which is used to derive ocular volume changes from changes in IOP, is assumed to be standard for all subjects. These assumptions are very important in order to understand the results obtained with this instrument.

The OBFA was used in this thesis, and an explanation of the protocol followed is given below.

OBFA protocol

The OBFA probe uses a disposable plastic tip held onto a flexible end that allows the sensor to move backwards and forwards with the pulsations of the cardiac cycle. The calibration of the OBFA is done by introducing the flexible metal end that holds the plastic tip onto an elastic tube attached to the instrument. A pulse of air is sent through the tube and the calibration test is passed when the metal end detects properly the air pulse.

In order to take accurate readings, the plastic tip has to be placed perfectly perpendicular to the corneal surface ensuring perfect contact between the tip and the eye surface (figure 1.8). The OBFA tip touches the surface of the eye; therefore the patient's cornea must first be anaesthetised. In this study, one drop of anaesthetic (proxymetacaine HCl 0.5%) was instilled in the patient's eye to prevent any uncomfortable sensation. The patient was then asked to stare, with eyes wide open, at a target located 6m in front. At least 5 quality pulse waves were needed for the instrument to provide data. The instrument automatically stopped recording when quality data was obtained.

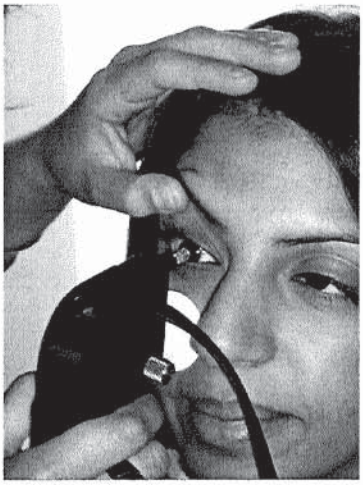


Figure 1.8 *Hand-held recording of ocular blood flow using OBFA on an anaesthetised cornea. Note the perpendicularity of the instrument with respect to the cornea.*

The reliability of the readings obtained with OBFA has been assessed in a study by Yang and colleagues (Yang, *et al.* 1997). The reliability coefficient was reported to be 0.92 with no significance in the variation bias and in the first exposure effect. POBF appears as being normally distributed with mean values that vary with gender (due to the effect of the female menstrual cycle on blood flow), age and refractive error. The average has been found to be 669.90 ± 233 µl/min in men, and 841 ± 254.6 µl/min in women, decreasing with age and larger ocular axial lengths. This high inter-individual variation may lead to false results when using POBF as screening tool, therefore, gender and axial length are important variables to consider when interpreting OBFA results (Yang, *et al.* 1997). Moreover, the ratio pulsatile/non-pulsatile ocular blood flow cannot be assumed to be constant, especially in the event of changes in systemic blood pressure; therefore, the pulsatile blood flow should not be understood as an estimate of total ocular blood flow (Schmetterer 1998). IOP has been recently reported to be affected by repeated measures, decreasing with exposure time. When repeated measures were performed within 15 minutes, a significant decrease in IOP was observed. However, there was no significant decrease in OBF. It is therefore recommended that measures are obtained within the first two attempts. Otherwise, a

minimum of 2 minute-break between attempts should be given to ensure good quality recordings (Gunvant, *et al.* 2004).

The OBFA has also been used to evaluate the pulsatile component of blood flow in diseased eyes, such as in patients with retinitis pigmentosa, where the amplitude of the ocular blood pulse in 13 patients showed a significant decrease compared to that of 10 healthy matched controls (Langham and Kramer 1990). The pulsatile choroidal blood flow also appears reduced in patients with diabetic retinopathy as shown by another study of the same group where 11 diabetic patients with background retinopathy were compared to 9 diabetics with no apparent retinopathy and 19 healthy volunteers. The ocular pulsatile blood flow values were 210 ± 37 µl/min, 471 ± 70 µl/min and 648 ± 42 µl/min respectively ($p<0.001$), which suggests choroidal blood flow decreases with the severity of the retinopathy in diabetes (Langham, *et al.* 1991). Additionally, the POBF has also been used to assess the pulsatile blood flow in glaucoma and hypertension (Kerr, *et al.* 2003). The POBF of 24 glaucoma subjects and 50 ocular hypertensives (OHT) (IOP>25 mmHg) was found to decrease significantly compared to a matched control sample ($p<0.001$). Interestingly, a significant increase in POBF was observed in 24 patients (combination of POAG and OHT patients) after the commencement of IOP lowering medication (Betaxolol 0.5% bd). POBF increased from 589.9 µl/min to 670.8 µl/min ($p=0.02$) (Kerr, *et al.* 2003).

1.11.3 Ocular perfusion pressure (OPP)

The ocular perfusion pressure is the difference between the pressure in the arteries entering the tissue (Pa) and in the veins leaving it (Pv). A relationship between blood flow (BF), perfusion pressure (OPP) and vascular resistance (R) exists, and it has been described as follows (equation 1.9, equation 1.10):

$$OPP = Pa - Pv \tag{1.9}$$

$$BF = OPP/R \tag{1.10}$$

Blood flow in any tissue is generated by the perfusion pressure, which is also defined as the difference between mean arterial pressure and venous pressure. Mean arterial pressure (MAP) equals the diastolic blood pressure (Dbp) added to a third of the pulse pressure (Pp), as follows (equation 1.11):

$$MAP = Dbp + 1/3Pp \tag{1.11}$$

, where Pp equals the difference between systolic blood pressure (Sbp) and Dbp.

Therefore, Pa can be approximated to MAP and additionally a good estimate of the pressure in the veins leaving the eye (Pv) can be made by measuring the IOP. Such approximation can be used as the venous pressure should be marginally higher than the IOP for an adequate blood circulation, therefore Pv ≈ IOP, in healthy eyes. These two pressures are almost equal at both normal and elevated IOP (Maepea 1992). Spontaneous pulsations, which can be visualized in the central retinal vein at the optic disc, are a consequence of the pressure within the vein being nearly equal to the IOP.

The transmural pressure (pressure difference between that inside and outside the vessel) in the intraocular veins and the venous side of the capillaries is small. A small increase in intravascular pressure may raise the transmural pressure to several times its normal value. The development of microaneurysms in diabetic retinopathy may be caused in part by venous stasis (Kaufman and Alm 2003). Patients with impaired blood flow in one eye have been found to have a lower tendency to diabetic retinopathy than in the other eye with normal blood flow (Gay and Rosenbaum 1996). In this situation it appears that a reduction of the transmural pressure in the veins and capillaries contributes to protection of the walls of the vessels. The potential damage to vessels characteristic of diabetic retinopathy (e.g. haemorrhages, exudates) is lower if the blood flow is reduced (Kaufman and Alm 2003).

From all the above it is observed that OPP can be reduced by either a reduction in the mean arterial pressure or an increase in IOP. However, a reduction in perfusion pressure (Pa-Pv) does not imply reduction in blood flow (BF) unless the vascular resistance (R) is reduced to the same extent as the perfusion pressure. In tissues such as brain and kidney a reduction in vascular resistance occurs when the perfusion pressure is reduced.

1.11.4 Resistance indices

Resistance (R) refers to the impedance or opposition to blood flow created by the friction the blood encounters as it passes through the vessels. It is related to blood viscosity, the length of blood vessels, and vessel radius by Poiseuille's law. Since R cannot be measured by any direct means, it is of interest to establish other parameters reflecting vascular resistance.

The downstream vascular resistance can be calculated by Pourcelot's resistive index. This resistive index (RI) is considered to reflect vascular resistance peripheral to the measuring location. The RI is derived from the characteristics of the spectral wave form as described by Pourcelot (Pourcelot 1975). It is calculated using velocity data as assessed by colour Doppler imaging (CDI) and

represents the ratio that relates the peak systolic and end diastolic velocity (PSV and EDV, respectively) according to the formula 1.12

$$RI = PSV\text{-}EDV/PSV \quad (1.12)$$

Pourcelot's ratio is independent of the Doppler angle, so that the resistance index is not affected by the angle at which the blood velocities are calculated. This index varies from zero to one, with higher values indicating higher distal vascular resistance. However, Polska and colleagues have demonstrated that retinal vascular resistance and RI are not truly associated. A parallel evaluation of the CRA vascular resistance was performed using CDI and laser Doppler velocimetry (LDV). LDV values were combined with vessel size measurements and calculation of OPP. An increase in vascular resistance was induced, which did not appear as such when using CDI. These data indicated that RI as assessed with color Doppler imaging (CDI) does not reflect accurate measures of retinal vascular resistance (Polska, *et al.* 2001).

1.11.5 Assessment of retrobulbar vessels

CDI was the instrument used in this thesis to assess the ocular circulation in the retrobulbar vessels and it is based on the Doppler Effect, by which the speed of blood (or any moving object) can be calculated (Wells 1990). Christian Andreas Doppler (1803-1853) first described the principle of using a change in the frequency of waves to measure the velocity of an object (Doppler 1842). This principle was applied to ultrasound in the investigation of human diseases in 1957 and subsequently became established in routine clinical practice for the examination of larger vessels in the body, such as the carotid artery and the heart (Yoshida, *et al.* 1961; Satomura 1957). The Doppler shift has been widely used for estimation of ocular blood flow. It was first introduced by Riva and colleagues as a technique to measure red blood cell velocity in the eye in 1972 (Riva, *et al.* 1972). The signal used can be either optical (Laser Doppler Velocimetry or Laser Doppler Flowmetry) or auditory (Ultrasound Colour Doppler Imaging).

1.11.5.1 Colour Doppler Imaging (CDI)

CDI detects the Doppler shift induced by moving red blood cells through the use of an ultrasonic signal. CDI combines conventional b-scan (grey-scale imaging of anatomical structures) together with the Doppler shift frequency measurement of blood flow velocities and the colour representation of blood flow based on these frequency shifts.

The Doppler equation relates the frequency of the ultrasound beam (f), the velocity of the blood (Vblood), the velocity of the ultrasound through the blood (Vsound), the Doppler shift, and the

angle of incidence between the direction of the blood flow and the approaching sound beam (θ) as follows (equation 1.13):

$$\text{Shift: } 2fV\text{bloodCos}\theta/V\text{sound} \qquad (1.13)$$

Within soft tissues such as the eyelids, a small proportion of the ultrasound is scattered or reflected and arrives back to the probe as an echo. The speed of ultrasound in the body is constant (1540 m/s), so the depth of any scatterer or reflector can be found from the time delay from emitting the pulse to receiving the echo. The main pulse continues deeper into the body to be scattered or reflected from deeper structures. When the echoes from one pulse have died down, the next pulse is emitted from a slightly different position along the probe. In this way, it is possible to create an image of a plane in the orbit, with depth into the orbit as the vertical axis and position along the probe as the horizontal axis.

Acuson Sequoia® Ultrasound system (Siemens Medical Solutions, Malvern, US) was the CDI used in this thesis. The Sequoia system by Siemens consists of a screen unit linked to a keyboard where the data and the optimization of the image can be adjusted. Different probe sizes are available with this instrument: 3V2, 8V and 8L5. The size of the probe to use depends on the area of the body assessed. The probe selected for the orbit was the 8V5, as it was the smallest probe available. A surgical bed was used in conjunction with this instrument, to allow the patients to rest in a supine position with their eyes closed.

The parameters measured with the CDI were the peak systolic velocity (PSv), which represents the velocity of the blood during the cardiac systolic phase, the end diastolic velocity (EDv), which represents the velocity during the diastolic phase, the ratio between the systolic and the diastolic velocity, and the resistance index (RI). A speed-time graph was provided, on which the velocity parameters were plotted against time, and from this, the peak systolic and end diastolic velocities were calculated. Additionally, as indicated previously, the resistance index (RI) was calculated from the values obtained from the PSV and EDV (equation 1.12)

Optimization of parameters

The Sequoia system allows the manipulation of certain parameters with the purpose of obtaining an adequate quality of the 2D image representation. The parameters that can be adjusted to improve the quality of the image are Space Time® resolution control, edge, DELTA (differential echo amplification) and persistence:

-The Space Time® resolution control adjusts the balance between spatial (detail) resolution and temporal (frame rate) resolution by offering a choice between four possibilities: T1, T2, S1 and S2. T1 and T2 are useful in imaging moving structures, and therefore useful in vascular analysis. S1 and S2, however, are excellent for imaging detailed small structures.
-EDGE control is a 2D spatial filter that allows the user to modify between pixel smoothness and sharpness with a range from -2 for the smoothest to +2 for the sharpest.
-DELTA™ enhances the contrast among tissues, which helps to distinguish between normal anatomy and subtle pathologies. DELTA values range from 1 to 5, the later being the value with the highest contrast analysis.
-PERSISTENCE represents temporal averaging for noise reduction and image smoothing. There are 6 levels, from which low values such as 0 or 1 are recommended for vascular features.

The set up of these parameters was the same for all the subjects in this study, and it was as follows:
Usage of 8V5 probe, which works at 7.5MHz
GAIN: 33dB
Space Time resolution control: 1
EDGE: +1
DELTA (differential echo amplification): 4
PERSISTENCE: 1

CDI protocol
In order to perform CDI measurements, the author of this thesis was trained by the instrument manufacturer in a 2-day course, which allowed the author to carry out the CDI measurements by herself. To ensure that the vascular readings were correctly performed, the initial CDI data was analysed for intra-observer repeatability (see appendix 1.2)

The ultrasound gel was applied on the selected probe, after which the patient was asked to close their eyelids to allow the gently placement of the probe over the closed eyelid of the patient. Patients were advised to imagine looking directly up to facilitate an adequate and fast imaging of the orbit. The hand of the examiner was rested on the patient's forehead to minimize the pressure on the globe. Initially, a cross-sectional image of the eye and optic nerve was obtained using the 2D ultrasound mode only, as reference for the location of the ocular vessels.

The vessels studied were the OA, CRA and SPCA. Knowledge of the ocular vessel anatomy and the profile of the speed-time graph were used to differentiate between the ocular vessels. Each vessel had a distinctive speed-time profile due to the size of the vessel and its relationship with the

cardiovascular system. It was important to ensure that the CDI readings were taken approximately at the same vessel area for all patients to avoid the influence of inter-patient variations in physical features of the vessels on the CDI readings.

Every vessel was assessed at a specific location to ensure the consistency of the readings. The anatomy and large size of the OA facilitated its location, and its speed-time profile was of significant help as it resembled the speed-time profile of the heart (figure 1.9). The OA crosses the optic nerve in approximately 85% of cases to reach the medial wall of the orbit. It then proceeds forward horizontally and divides into 2 terminal branches, frontal and dorsal nasal. Throughout the course of the study the OA was assessed at this later dorsal nasal side (figure 1.9).

The CRA is a branch of the OA, therefore being of smaller calibre. This explains the smaller size of the CRA speed-time graph (figure 1.10). CRA velocities at both systolic and diastolic phases are significantly reduced compared to those of the OA. Additionally, the positive pulsatile graphical representation of the velocity in CRA is always accompanied by a negative non-pulsatile plot that corresponds to the Central Retinal Vein (CRV), which runs parallel to the artery.

The blood flow in the CRV results in a mirror plot of the CRA plot, being the main difference the lack of pulsatility of the vein, and the quality of the blood transported (non-oxygenated). The CRA runs within the optic nerve to finally pierce the eyeball wall and supply the retina with blood. The haematic features of the CRA were examined using CDI in the last 3mm of its course within the optic nerve, before penetrating the eyeball.

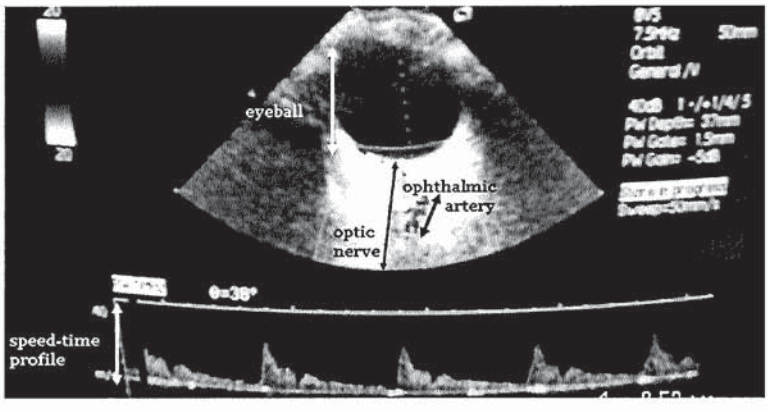


Figure 1.9 *CDI image displaying the location at which the ophthalmic artery (OA) vascular readings were performed and its characteristic speed-time profile*

The speed-time profile of the SPCA is similar to that of the CRA; however the velocities at both the peak and trough are higher in SPCA than in the CRA. The explanation for that is not due to the

calibre of the SPCA, which is smaller than the calibre of the CRA; but to larger number of vessels over which the reading is taken when evaluating the short arteries. The SPCAs arise from the OA and they pass forward around the optic nerve to the posterior part of the eyeball. These numerous arteries then pierce the sclera around the entrance of the nerve nasally and temporally to supply the choroid and ciliary processes. The location chosen to assess the SPCA was their temporal bifurcation before perforating the sclera (figure 1.11).

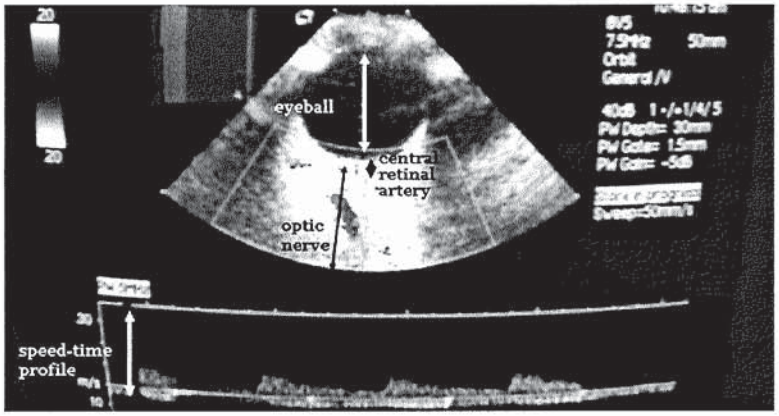


Figure 1.10 *CDI image displaying the location at which the central retinal artery (CRA) vascular readings were performed and its characteristic speed-time profile*

Studies have reported the reproducibility of the CDI readings taken on OA, CRA and PCAs, being the most reliable and reproducible vessels the OA and CRA. Greater variation was noted in the posterior ciliary vessels (Vilchez, *et al.* 2004; Baxter and Williamson 1995). The vascular readings have recently been found to be significantly dependent on age and sex (Ustymowicz, *et al.* 1999), therefore, it is important that these variables are considered to ensure the right interpretation of the values obtained.

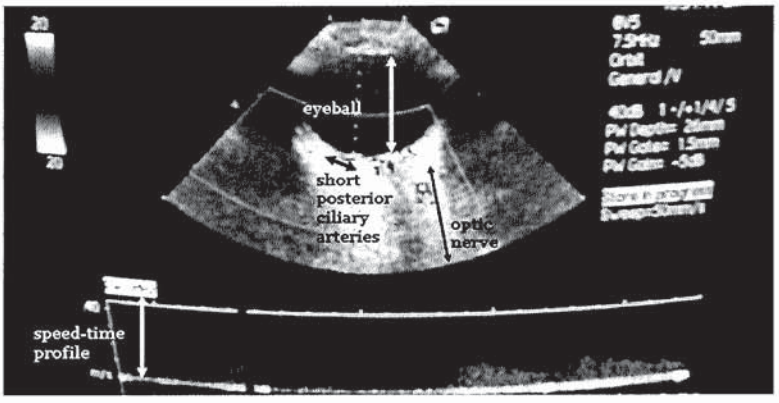


Figure 1.11 *CDI image displaying the location at which the vascular readings in the short posterior ciliary arteries (SPCAs) were recorded*

CDI has also been used to assess the ocular perfusion features of the OA, CRA and SPCAs in diseased eyes, such as those with age-related macular degeneration (Ciulla, *et al.* 1999). Twenty-five subjects with nonexudative age-related macular degeneration were compared with 25 age-matched control subjects, and the results described a significant decrease in SPCA diastolic blood velocity ($p = .0012$). In the CRA, the diastolic blood velocity was significantly lower, and it was accompanied by a significantly higher RI ($p<0.001$, $p<0.001$).
The retrobulbar circulation in primary angle glaucoma (POAG) (Nicolela, *et al.* 1996) had been previously assessed in one eye of 20 POAG patients and it was compared to the results from 20 ocular hypertensive patients, and the results showed that glaucoma patients had lower blood flow velocity in the CRA compared with that of ocular hypertension patients of similar age and level of untreated intraocular pressure. Another study also assessed POAG patients, normal tension glaucoma (NTG) and ocular hypertension (OHT) in both eyes of 25 healthy normal volunteers, 13 NTG and 19 POAG. The systolic and diastolic blood velocities of the CRA and SPCAs appeared significantly decreased in POAG patients compared to the normal control group ($p<0.01$), and the RI was also significantly higher in the CRA and SPCAs ($p<0.01$) in acute glaucoma patients than in the control group. However, no significant differences were reported for NTG or OHT (Liu, *et al.* 1999).

In view of these outcomes, it appeared interesting to evaluate the effect of dorzolamide on the ocular blood flow of glaucomatous eyes compared to that in healthy eyes. The OA, CRA and central retinal vein (CRV) in 26 POAG eyes and 13 normal control eyes were evaluated before and after the use of topical dorzolamide. In all eyes, there was a significant increase in OA and CRA diastolic blood velocities; however, it was only in POAG eyes that the CRA systolic velocity appeared significantly higher after application of dorzolamide (Martinez, *et al.* 1999).

Additionally, a study by Nicolela and colleagues (Nicolela. *et al*, 1996) evaluated the haemodynamics of the retrobulbar vessels in patients with different optic nerve appearances (Broadway. *et al*, 1998) and reported that after performing CDI in 24 patients with focal ischemic discs, 26 patients with myopic glaucomatous discs, 16 patients with senile sclerotic discs and 16 patients with generalized enlargements of the cup, the patients with senile sclerotic discs had statistically significantly lower diastolic velocity and a higher resistance index in their OA, CRA and SPCA.

CDI also has the option of calculating blood flow within a delimited area so that the volume can be assessed by measuring the three dimensions of the vessel. However, this method to calculate the blood flow has been found to be unreliable due to the small size of the vessels assessed (Zeitz, *et al.* 2005), reason for which blood volume was not calculated in this study.

1.11.6 Blood flow in the retinal microvessels

The vascular perfusion of the retinal capillary network can be evaluated using a non-invasive technique that provides quantitative measurements of blood flow, volume and velocity. The instrument consists of a confocal scanning device that can investigate various depths of tissue using a low intensity infrared laser beam that scans the fundus through the undilated pupil. The instrument is the scanning laser Doppler flowmeter, and the one used in this thesis was the Heidelberg Retinal Flowmeter (HRF, Heidelberg Engineering, Heidelberg, Germany).

1.11.6.1 Heidelberg Retinal Flowmeter, HRF

The HRF offers the possibility of either a retinal scan or a superficial optic nerve head scan by means of a 780nm infrared diode that provides an image of the back of the eye. HRF measures the retinal capillary perfusion at approximately 16,000 different locations on the ocular fundus. The HRF is limited to the detection of temporal frequency variations (Doppler shifts) below 2000Hz, which is adequate for determining the velocity of the red blood cells in the retinal capillaries (Michelson, *et al.* 1996). The image obtained by HRF resembles the image generated by a retinal tomograph. However, the HRF scanning system creates a two-dimensional picture whereas a retinal tomograph provides a three-dimensional image.

Optimisation of parameters

HRF field of view can be adjusted manually from 10° × 2.5° to 20° × 5° with a focusing range from -12 to +12 dioptres. In this study, the field assessed was 10° × 2.5° to allow comparison with results from previous papers. The focus was adjusted to the prescription of each patient to achieve the best visualization of the retinal area. For example, the focus of the scanning device was adjusted to a value close to -2.00D in a -2.00D myopic patient. The resolution of the instrument is limited transversally to 10 μm and longitudinally to 300 μm.

Standard analysis can be performed using a 10x10 pixel frame, however, a local search strategy has been suggested by Hosking and co-workers in order to optimize differentiation of ocular blood flow within subjects (Hosking, *et al.* 2001a). In order to do this, a transparency with a delimited 15x15 pixel square was placed on the monitor screen over the area to be evaluated. The 10x10 pixel frame used for the standard analysis was manually relocated within the 15x15 pixel to identify the highest and lowest values of blood flow.

HRF protocol

All the HRF measurements were carried out by the author of this thesis, who was trained by an expert user (Morgan AJ).

In order to take readings, the patient was asked to rest with his/her chin and forehead on the respective rests attached to the instrument. A fixating light was used to direct the patient's gaze position to the desired area to allow the viewing of the retinal region of interest.
The patient was asked to remain still on the chinrest while at least three quality pictures of the fundus were taken to prevent errors from movement or focus variations.

HRF has been reported to be highly reliable for blood flow, volume and velocity calculations at the choroidal level (82%, 81% and 83% respectively) (Michelson, *et al.* 1996). HRF for the assessment of the peripapillary retina has also shown reproducible measurements (Jonescu-Cuypersa, *et al.* 2001). The results are highly dependent on the area selected for the measurement as both the brightness of the illuminated fundus and the ocular pulsatility related to the cardiac pulse influence the measurements (Harris, *et al.* 2003). The effect of photodiode sensitivity on the DC (brightness) value and the resultant blood flow measurements of retina and rim tissue have been found to alter the readings. Blood flow reached the optimum value when the DC value was between 70 and 110. It is also not recommended to keep the DC value above 190, as readings taken with DC higher than 190 have been shown to be significantly lower than readings taken with lower DC values (Hosking, *et al.* 2001b). For this study, therefore, any reading with DC above 190 was rejected and was not considered for data analysis.

HRF has also been used to monitor the blood flow changes in retinal capillaries with age and after assessing 15 healthy volunteers with mean age 65 years, there was a significant decrease in retinal blood volume compared with the young subject group whose mean age was 28 years. The older subjects also exhibited reduced blood flow and velocity at the neuroretinal rim and lamina cribosa (Embleton, *et al.* 2002).

Also anatomical differences in the perfusion features of the optic nerve head (ONH) have shown a reduced ONH perfusion to OHN thickness ratio in the inferior retinal sector compared to the superior, which suggests that the inferior sector of retinal nerve fibre layer and optic nerve head may have lower blood flow per unit nerve tissue volume compared to the superior sector (Harris, *et al.* 2003)

Moreover, the HRF has been able to detect changes in disease, such as in diabetes. The nasal, papillo-macular and foveal retinal areas were assessed using HRF in 80 diabetic patients and 49 controls. A significant negative correlation with the blood glucose level was found in the perifoveal area of patients with proliferative diabetic retinopathy ($R=-0.585$, $p<0.001$) (Cuypers, *et al.* 2000).

1.11.7 Measurement of choroidal blood flow

The features of the choroidal blood flow are of inherent interest in the field of myopia, especially after animal studies in which chick eyes were induced with visual deprivation myopia described changes in choroidal blood flow and choroidal thickness (Wildsoet and Wallman 1995) (Fitzgerald, *et al.* 2001).

1.11.7.1 Laser Doppler Flowmetry (LDF)

In this thesis, *in-vivo* assessment of the choroidal blood flow was attained using the Laser Doppler Flowmeter (LDF) (Riva method, Institut de Recherche en Ophtalmologie, Switzerland). The LDF, also based on the Doppler Effect, provides semi quantitative measurements of blood flow derived from the analysis of frequency changes induced by moving red blood cells. LDF was first used by Riva and colleagues, to gauge the velocity in individual retinal vessels of the rabbit eye (Riva et al., 1972). In 1975 Stern applied this technique to a microcirculatory bed and later obtained measurements of blood flow in various tissues (Stern et al, 1977). The change in the laser frequency when the laser falls on moving red blood cells is calculated by an exponential function from which blood volume, velocity and flow are given. These parameters are referred to as LDF parameters.

The LDF, designed by Prof. Riva and colleagues (Feke and Riva 1978), contains two laser lights, one of which has the purpose of being merely a fixation target while the other laser light (λ=785 nm, 90 μW at the cornea) is directed to the vessels to provide the velocity reading. Both lasers are built on a retinal fundus camera, which enables the user to image the back of the eye while directing the laser to the vascular bed of interest. It has a theoretic depth of tissue penetration of 1000μm, which makes it a sensitive tool to gauge the blood flow changes in the superficial layers of the anterior optic nerve and retina. The scatter induced by the movement of red blood cells and surrounding tissue is collected by an optical fibre, and it is analysed by the LDF computer. The parameters provided are blood velocity, volume and flux. Velocity is expressed in kilohertz and volume and flux are expressed in arbitrary units (AU). LDF also provides continuous and sensitive measurements of relative choroidal blood flow (Riva, *et al.* 1994).

LDF protocol

The LDF measurements were carried out by the author of this thesis after receiving training from the designer (Prof. Riva) and developer (Petrig B) of the instrument.

In order to take the readings, a minimum pupil size of 5mm is required to obtain accurate readings, thus one drop of tropicamide (Tropicamide 0.5% Minims®, Chauvin Pharmaceuticals) was instilled in the eye of the subjects. Once the pupil was dilated, the subject was asked to place

their chin and forehead on the rests of the LDF. Optic nerve rim readings, as well as choroidal readings can be taken with this instrument. For this study, only macular readings (choroid) were taken. To direct the laser to the macula, where choroidal measurements were performed, we asked the patient to fixate on the measuring laser. The heart rate of the patient was recorded using an ear infrared plethysmometer and the average time course of each LDF parameter during the cardiac cycle was obtained by averaging over a period T, taking into account the phase of the heart cycle. Detailed descriptions of the choroidal blood flow measurements are given in section 7.4.4.

The measurements of choroidal blood velocity, volume and flow have been shown to repeatable using the LDF, and the percentages of repeatability found for the choroidal velocity, volume and flow were 93%, 74.6% and 77.5% respectively (Gugleta, *et al.* 2002). The reliability of the parameters measured by the LDF significantly improve when the noise is reduced by correcting the influence of yield (yield = direct current/gain). The reliability improves from 74.6% to 94% when assessing volume, whereas the reliability of the flux parameter reaches 86% after correcting for the noise (Gugleta, *et al.* 2002). The influence of yield was therefore corrected through the collection of data for this project and the power of the measuring laser was kept within 0.5mW and 1mW.

Similarly to the study by Martinez and co-workers in which the effect of dorzolamide was assessed on glaucoma patients using the HRF, the effect of topical dorzolamide in glaucoma patients has also been evaluated using LDF (Pillunat, *et al.* 1994). Dorzolamide eye drops were applied to both eyes of 15 healthy subjects, and readings were taken on the papillary area prior to application of drops, 90 minutes after and 3 days after. Interestingly, the results showed the expected drop in IOP ($p<0.001$); however, the flow of blood in the optic nerve head capillaries did not change during therapy, which was suggested to be to the effective autoregulation in human optic nerve head circulation, which was not affected by dorzolamide (Pillunat, *et al.* 1994).

Of particular interest was the study by Movaffaghy and colleagues , in which the viability of using LDF to help with an early diagnosis of malaria was tested (Movaffaghy, *et al.* 2002). The diagnosis of malaria in Malawi is currently performed by taking measurements of cerebral blood flow, which is not a task easy to perform in the tropics. Therefore, the aim was to validate the measurement of optic nerve head blood flow instead of cerebral blood flow to help outcome prediction. A portable LDF was used to measure one eye of 13 children (2.7 +/- 1.1 years) during a period of about 8 min. The average coefficients of variation (CoV) of the flow parameters ranged from 13% to 27% and 23% for the optic nerve head velocity, volume and flow respectively. Thus, LDF was found to be feasible for the measurement of cerebral malaria in children. However, the large range of these

coefficients of variations due to the presence of fluctuations of ONH blood flow limits its use as a diagnostic tool (Movaffaghy, *et al.* 2002).

LDF studies by Riva and colleagues (Riva, *et al.* 1997) on human eyes suggested for the first time a possible autoregulatory mechanism in the choroid that had not been described in previous studies. Riva and colleagues first described some level of choroidal autoregulation after experimentally inducing a rapid increase in IOP in 6 subjects and a slower increase in 14 subjects using a suction cup, and measuring choroidal blood flow with Laser Doppler Flowmetry (LDF). Increased IOP results in reduced ocular perfusion pressure, as explained by equation 1.9. However, small changes in OPP did not result in choroidal blood flow changes, which pointed towards some kind of local regulation. Additionally, the time response to the experimentally induced variations in IOP was reported to appear as a neural regulatory response (Riva, *et al.* 1997). The theoretical background needed to understand the mechanisms that rule autoregulation are presented in the following section.

1.12 Mechanisms of blood regulation

Blood circulation in the peripheral system is affected by the relative distance from the heart, and it is regulated by two main mechanisms, one centrally based through the nervous system (extrinsic regulation), and other locally located within the tissues (intrinsic regulation).

1.12.1 Extrinsic regulation

1.12.1.1 Neural regulation

The central nervous system coordinates the two major nervous pathways, the somatic and the autonomic pathways. The somatic system normally contains one sensor and one motor neuron that group resulting in nerve pairs. The reflexes that initiate the somatic cascade are mostly consciously controlled by the organism. On the contrary, the stimulation of the autonomic system is not controlled by the organism in most of the cases. The autonomic system consists of two subsystems, the sympathetic and the parasympathetic systems.

Blood circulation in specific tissues such as the skin or muscle is controlled by two neural sympathetic subsystems: sympathetic adrenergic active vasoconstrictor system, and sympathetic non-adrenergic active vasodilator system. Adrenergic-mediated neural reactions are those in which adrenaline works as a neurotransmitter. During heat stress, the adrenergic active vasodilator system accounts for 85 to 95% of the overall cutaneous vasodilator response (Kellogg,

et al. 1995). Moreover, blood flow in exercising skeletal muscle is a balance between metabolic vasodilation and sympathetic non-adrenergic vasoconstriction (Clifford, *et al.* 2002).

1.12.2 Intrinsic or local regulation

1.12.2.1 Local myogenic regulation

Vascular smooth muscle is the main tissue responsible for the control of peripheral blood flow. In many situations, vascular smooth muscle cells are in contact with endothelial cells, promoting the interaction between both tissues. A myogenic vascular regulation describes the changes that occur in blood flow related to the smooth muscle, which are typically in response to variations in transmural pressure. Thus, an initial increase in blood flow due to an increase in the perfusion pressure will be followed by a decrease in blood flow as a response to myogenic needs. An example of myogenic regulation is every change occurring in the capillaries of the lower extremities when moving from a vertical to a horizontal position, caused by the variations in perfusion pressure.

1.12.2.2 Local metabolic regulation

The metabolic requirements of the tissues can also regulate blood flow. Vasodilation describes the widening of the blood vessels that follows the relaxation of the smooth muscle, and it can occur to satisfy the metabolic needs of a tissue. Actively metabolizing cells surrounding arterioles release vasoactive substances that cause vasodilation. This is termed the metabolic theory of blood flow regulation. Increases or decreases in metabolism lead to increases or decreases in the release of these vasodilator substances (Klabunde 2004). Several different mechanisms involved in the metabolic regulation of blood flow are summarized below:

1.12.2.3 Adenosine

Adenosine is the combination of a nucleoside and the sugar ribose. Adenosine is derived from hydrolysis of intracellular ATP and ADP, and its formation increases during oxygen consumption. Adenosine-induced vasodilation is thought to occur via endothelial dependent relaxation of smooth muscle as adenosine is found inside the artery walls (Leuenberger, *et al.* 1999).

1.12.2.4 Potassium ion (K^+)

Potassium ions are found in cardiac and skeletal muscle, working as part of the Na^+ / K^+ pump, which ensures the maintenance of the ionic concentration gradients across the membrane. Energy in the form of ATP hydrolysis is needed to maintain the balance of the pump. During muscle contractions, K^+ accumulates in the extracellular space, which leads to

relaxation of the vascular smooth cells, which may lead to vasodilation (Haddy, *et al.* 2006; Klabunde 2004).

1.12.2.5 Hypoxia

A decrease in pO_2 has been found to result in vasodilation, either via a direct mechanism due to insufficient O_2 to maintain smooth muscle contraction; or via an indirect mechanism through the production of vasodilator metabolites (Gow 2005).

1.12.2.6 Carbon dioxide (CO_2)

CO_2 is produced in the parenchymal cells and it is later diffused to the vascular smooth muscle, on which it exerts its effect. An increase in PCO_2 (hypercapnia) results in an increase in ocular blood flow, as well as in the cerebral supply (Hosking, *et al.* 2004; Chung, *et al.* 1999b; Roff, *et al.* 1999; Mansberger, *et al.* 1993; Ringelstein, *et al.* 1992).

The vasodilatory effect induced by CO2 on optic nerve head vessels of pigs appears to be mediated by prostaglandins, as indomethacin inhibited vasodilation induced by hypercapnia (Petropoulos and Pournaras 2005). Additionally, hypercapnia appears to be associated with decreased extracellular pH, activation of potassium channels, and membrane hyperpolarization (Tian, *et al.* 1995; Kontos 1989; Nesterov, *et al.* 1978).

1.12.2.7 pH

Variations in pH are one of the main metabolic regulatory mechanisms of blood flow. The principal changes in pH occur at extracellular and arterial levels. An extracellular decrease in pH results in vasodilation. Similarly, an arterial decrease of pH accompanied by an increase in pCO_2 leads to respiratory acidosis, which finally result in vasodilation. However, a decrease in arterial pH with constant pCO_2 levels does not give any vasodilation effect.

Vasodilation is accompanied by a decrease in blood pressure, as the vessel area is enlarged, decreasing the resistance of the blood to flow. The nerves and muscles that take part and control vasodilation are referred to as vasomotors. Some of the main natural vasodilators are:

- Adenosine
- Adrenaline
- Bradikinin
- Histamine
- Tetrahydrocannabinnol
- Endothelium-mediated local regulation

The endothelium is the thin layer of squamous epithelial cells located in the inner part of the blood vessels. The endothelium defines the line between blood and vessel wall, therefore housing many of the regulatory mechanisms of blood flow. For years the endothelium was thought to be an inert single layer of cells passively allowing the passage of water and other small molecules across the vessel wall. However, this dynamic tissue performs many other active functions, such as the secretion and modification of vasoactive substances and the contraction and relaxation of vascular smooth muscle (Berne and Levy 1997). Prostacyclin is formed in the endothelium from arachidonic acid and its main function appears to be preventing intravascular clot formation through the inhibition of platelet adhesion. The endothelial-derived relaxing factor, identified as nitric oxide, is also formed and released from the endothelium.

Endothelin-1 (ET-1) is one of the most potent natural vasoconstrictors involved in vascular homeostasis. From the two ET receptor subtypes, ET_AR and ET_BR, the former is found in the smooth muscle of blood vessels and it promotes vasoconstriction and retention of sodium, which results in increased blood pressure; whereas ET_BR is found on the vascular endothelial cells. The binding to ET_BR activates vasoconstriction, while it also appears to mediate vasorelaxation through the release of nitric oxide (the precise effects of endothelin B receptor activation depends on the type of cells involved.) (Ergul 2002).

1.12.2.8 Nitric oxide

Nitric oxide (NO) has been found to be involved in several vascular mechanisms, although initially it was once just considered as a simple gas. NO is a primary relaxing factor released from endothelial cells that was first observed in 1980 by Furchgott and Zawadzki after several studies of arterial smooth muscle (Furchgott and Zawadzki 1980). Endogenous relaxing factor (EDRF), as NO was called at the beginning, is now known to be a biological messenger in numerous physiological responses throughout the body, usually related to increased intracellular cyclic GMP concentrations (Ignarro, *et al.* 1987). Living organisms are constantly responding to stimuli from environmental factors as well as from substances produced by cells within the same organism. These responses are clustered under the term of "cell signalling systems". One of these mechanisms of interaction between cells is the cyclic GMP signalling system, in which NO is involved. Cyclic GMP is produced by GTP (Guanine nucleotide TriphosPhate), a compound used in metabolic reactions for the exchange of energy; sugar in exchange for energy. Cyclic GMP is converted into a non-cyclic form (GMP) by a group of enzymes known as phosphodiesterases (PDE). After degradation to GMP, ATP (supplier of metabolic energy) is required again to obtain the initial compound: GTP. NO is known to activate guanylyl cyclase (sGC), one of the molecules that take part in the production of cyclic GMP out of GTP (Denninger and Marletta 1999).

NO is synthesized from oxygen and L-arginine by the enzyme nitric oxide synthase (NOS). It was initially difficult for biochemists to accept that NO, which is an extremely simple diatomic gas, was able to transport specific information from one cell to another. To date, three different forms of the molecule nitric oxide synthase (NOS) that produces NO have been identified: inducible NOS, neuronal NOS and endothelial NOS. Inducible NOS is only present under pathologic conditions in response to inflammatory or allergic reactions whereas both the neuronal and endothelial NOS are expressed as constitutive elements (Luksch, *et al.* 2000).

NOS are found throughout the body and in all compartments of the eye. Available data suggest that hypertension decreases the corporal production of nitric oxide (Forte, *et al.* 1997) and ischemia has shown to be related to a decrease in the production of NO (Watanabe, *et al.* 1990). NOS have been found in peripheral ocular nerve fibres, related cranial ganglia, and the retina of the rat. Indeed, ocular tissues were among the first in which NO producing enzymes were identified (Yamamoto, *et al.* 1993).
Nitric oxide synthase-like immunoreactive peripheral nerve fibers were visualized mainly in the choroid and around limbal blood vessels. There was, however, no indication of nitric oxide in the cornea. Nitric oxide synthase thus localizes to peripheral ocular nerve fibers, chiefly parasympathetic in nature, and to several cell types in the retina (Yamamoto, *et al.* 1993). Of special interest is the fact that nitric oxide synthase has been observed within the vascular endothelium of the retina and choroid.

Moreover, the human retina can express mRNA for both two of the three NO isoforms mentioned above: inducible and constitutive NO isoforms (Chen, *et al.* 1998; Park, *et al.* 1994). Nitric oxide probably acts as a choroidal vasodilator of parasympathetic origin in the eye (Yamamoto, *et al.* 1993). These findings were supported by those describing the presence of neural NO synthase in retinal amacrine cells and photoreceptor cells from a variety of species (Yamamoto, *et al.* 1993). Using immunochemistry, heavy staining assessing the choroidal vasculature of humans has indicated the presence of nitric oxide synthase molecules (Flugel, *et al.* 1994).

The importance of NO in the regulation of blood flow has been shown in many studies, either *in vitro* or *in vivo*. NO-mediated control of basal ocular blood flow is demonstrated by the vasoconstriction seen in experiments where vascular endothelial cells were removed, or when NO synthase was inhibited (Koss 1999).

Laser Doppler flowmetry has demonstrated a reduced choroidal and retinal blood flow in response to inhibition of nitric oxide synthesis in in the cat optic nerve head (Buerk, *et al.* 1996) and in the pigeon choroid (Zagvazdin, *et al.* 1996). NO has also been shown to regulate basal ocular blood

flow in rats. In humans, systemic administration of a non-selective inhibitor of NO synthase (L-NAME) decreased basal choroidal blood flow as measured by pulsatile flow analysis (Schmetterer, *et al.* 1997).

Arterial vasodilation on ocular blood vessels has also been shown after studying the effect of drugs which `donate' nitric oxide in different animal species (Benedito, *et al.* 1991).

Studying the role of NO on cerebral blood flow under stress conditions such as hypercapnia or hypoxia suggested that NO is involved in the responses of cerebral vasculature to elevated levels of CO_2 in the arterial blood whereas it does not appear to play a major role in hypoxia-evoked vasodilation (Buchanan and Phillis 1993).

Nitric oxide has also been shown to modulate the tone of pericytes, having the potential therefore to influence blood flow in capillaries, which can be explained by the capillary role pericytes have in blood flow autoregulation (Haefliger, *et al.* 1999).

1.12.3 Autoregulation

Autoregulation is the mechanism that ensures constant levels of blood flow and blood supply at a local level despite moderate variations in perfusion pressure or metabolic demand. Perfusion pressure may change throughout the day; however, tissue flow should remain stable to maintain the metabolic activity of each organ. This autoregulation occurs independently of neural influence.

If autoregulation is explained from the myogenic perspective, an increase in the arterial blood pressure would affect OPP (equation 1.9), resulting in a higher OPP value, which in turn will increase the ocular blood flow (OBF) (equation 1.10).
An autoregulatory mechanism in this case will be a proportional resistive index raise that will guarantee a constant blood flow. Similarly, when blood flow falls, an autoregulatory response will trigger a decrease in the vascular resistance index to keep the perfusion pressure within the previous values (figure 1.12).

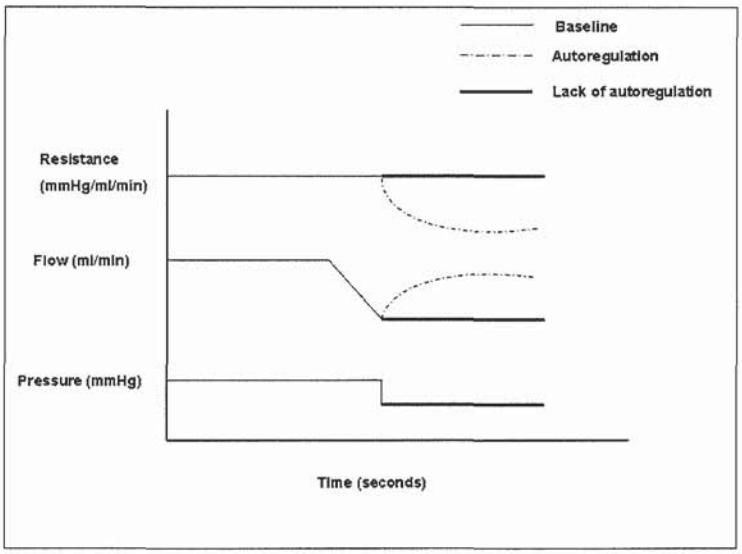


Figure 1.12. *Graph showing the changes involved in a vascular autoregulatory response at baseline and after variation in blood flow*

Autoregulation may be achieved by either:

- Metabolic mechanisms that involve all the chemical changes that take place in the organism. These metabolic mechanisms are characterised by a local control and the effect of oxygen partial pressure (pO_2), pH and adenosine, which is a molecule that provides energy to the organism (ATP).
- Myogenic mechanisms that refer to those processes originating in the muscles which do not depend upon neural influences.

Each of these components, as a rule, operates in concert. The stimuli for the myogenic mechanism (related to the muscles) are variations in the transmural pressure difference. When this pressure difference is decreased, as with increased IOP, the activity of pacemaker cells in the arteriolar wall is reduced, resulting in reduced arteriolar tone and consequently lowered vascular resistance (Flammer and Orgul 1998).

Presence or absence of autoregulation varies among species and even among tissue beds of a single organ of a specific species. The renal, cerebral and coronary tissues show exceptional autoregulation, for example in cases of hypotension where there is a change in systemic blood pressure. Hypotensive patients do not suffer from acute lack of cerebral supply, unless extreme cases, as the brain ensures a constant supply of oxygen and nutrients (Aaslid, *et al.* 1989).

Autoregulation has been demonstrated in the vascular beds of the anterior uvea in cats and monkeys and in the retina in rabbits, cats, pigs and monkeys (Alm and Bill 1972). In most studies examining autoregulation, the ocular perfusion pressure is reduced by elevating the IOP; however, reductions in blood pressure have the same effect on retinal oxygen tension as elevations in IOP. Several studies have been performed in order to further understand the haemodynamics within the eye, and to detect a possible impairment of vascular autoregulation in ocular pathologies such as in glaucoma and diabetes (Tutaj, *et al.* 2004; Wangsa-Wirawan and Linsenmeier 2003; Flammer, *et al.* 2002; Nagel, *et al.* 2002; Chakravarthy and Gardiner 1999; Chung, *et al.* 1999a; Joos, *et al.* 1999; Koss 1999; Michael 1999; Roff, *et al.* 1999; Findl, *et al.* 1997; Wolf, *et al.* 1995; Buchanan and Phillis 1993; Paulson 1990; Riva, *et al.* 1986; Johnson and Henrich 1975).

Therefore, since measuring the ocular blood flow under normal conditions alone might not be enough to detect damaged autoregulation; its assessment can be carried out using stress testing. As IOP appears linked to variations in ocular perfusion pressure, inducing changes in IOP has been used as a reliable stress test. In addition, the assessment of the ocular haemodynamics under pO_2 and carbon dioxide partial pressure (pCO_2) variations, as well as the use of the suction cup leading to an increase in IOP, are the main stress testing methods currently used.

1.13 Stress testing

The assessment of ocular blood flow under normal conditions solely may not show deficiencies in blood autoregulation. Therefore, to study a possible impairment of the autoregulatory function, it is necessary to create extreme conditions under which the autoregulatory mechanisms can be evaluated (Roff, *et al.* 1999). These extreme conditions are also called stress testing.

Stress methods currently in use are changes in IOP through the use of a suction cup (Azuara-Blanco, *et al.* 1998; Ustymowicz, *et al.* 1997), changes in oxygen partial pressure (pO_2) (Luksch, *et al.* 2002; Roff, *et al.* 1999) and changes in carbon dioxide partial pressure (pCO_2) (Chung, *et al.* 1999b; Niwa, *et al.* 1999). Additionally, acetazolamide, a carbonic anhydrase inhibitor, has also been used to study blood flow (Dallinger, *et al.* 1998).

1.13.1.Changes in pO2

It is well known that changes in carbon dioxide partial pressure (pCO_2), and oxygen partial pressure (pO_2) strongly influence cerebral and ocular blood flow (Chung, *et al.* 1999a; Chung HS 1999; Wilson, *et al.* 1977; Wilson TM 1977; Alm and Bill 1972; Alm A 1972). Partial pressure of any gas is the pressure the gas would have if it was the sole occupier of a container. In a mixture of

gases, therefore, each gas will have a partial pressure equal to the pressure each gas would have if just that gas occupied the volume.

Inhalation of oxygen, which leads to an increase in inhaled pO2, significantly decreases retinal blood velocity and flow. Increasing the concentration of pO2 has also been found to be accompanied by a reduction of retinal venous vessel diameters.
This decrease has been found to be significantly higher when subjects were administrated pure oxygen or a gas mixture with lower partial pressure of CO_2 (Luksch, *et al.* 2002). Increased levels of oxygen also initiate metabolic autoregulatory activity at the cerebral level by decreasing cerebral blood-flow through vasoconstriction of cerebral vasculature (Roff, *et al.* 1999).

These observations suggest that elevated arterial blood oxygen tension (pO_2) results in vasoconstriction; however, the mechanisms underlying this vasoconstriction are not clear. Some of the proposed theories are:

- The vasoconstriction may be mediated by a receptor called endothelin, as it has been reported to play a part in hyperoxia induced vasoconstriction in healthy subjects (Findl, *et al.* 2000). Endothelin is a peptide hormone that is released by endothelial cells. It is considered to be the most potent vasoconstriction hormone known.
- Intracellular and extracellular alkalosis, which is the condition in which the alkalinity of body fluids is abnormally high. This arises because of a failure of the mechanisms that usually maintain a balance between alkalosis and acids within the neutral values, which are around pH: 7.4.

1.13.2 Changes in pCO_2

Increasing pCO_2 has been reported to increase blood flow through arterial and arteriolar vasodilation due to a potential decrease in brain extracellular fluid pH, which also occurs in the optic nerve head and the retina (Wilson, *et al.* 1977). Under hypercapnic conditions (increased pCO_2), the cerebral blood flow has also been found to increase (Bayerle-Eder, *et al.* 2000; Schmetterer, *et al.* 1997; Goadsby 1994; Buchanan and Phillis 1993; Ringelstein, *et al.* 1992).

In addition, in normal-tension glaucoma, vasodilation caused by CO_2 inhalation normalises retrobulbar arterial flow velocities, suggesting that some vascular deficits in glaucoma may be reversible (Chung, *et al.* 1999a).

The haemodynamic response to vasodilatory agents (pCO_2) varies across the different vascular beds (Roff, *et al.* 1999). Therefore, any study which assesses exclusively only one ocular vascular

bed will most certainly not be able to describe adequately the vascular perfusion characteristics of the eye (Gupta, *et al.* 2002).

1.13.3 Suction cup

The suction cup oculopressor is an ophthalmodynamometer which consists of a suction pump that is connected by plastic tubing to a rigid, plastic suction cup with an inner diameter of 11 mm. The cup is placed on the temporal conjunctiva and with an increase in the negative pressure in the suction cup the intraocular pressure rises.

Riva and colleagues (Riva, *et al.* 1997) studied the effects of changes in retinal perfusion pressure caused by a suction cup. By increasing IOP, therefore decreasing OPP (see equation 1.10) retinal blood flow was observed to decrease in the first 30 seconds. However, after 30 seconds, volumetric blood flow recovered its normal value, suggesting an autoregulatory response. These results support the autoregulatory capacity of the retina (Riva, *et al.* 1986).

Findl and co-workers (Findl, *et al.* 1997) evaluated the perfusion characteristics of the macula, optic disc and retrobulbar vessels by means of a suction cup that induced changes in IOP on 10 healthy volunteers. The results from fundus pulsation measurements indicated that choroidal blood flow decreased when IOP is increased, whereas the findings giving by Doppler ultrasound in the CRA indicated reduced blood flow velocity in this artery during raised IOP.

Several other studies have also made use of the suction cup (Gugleta, *et al.* 2002; Nagel, *et al.* 2002; Joos, *et al.* 1999). Azuara-Blanco and co-workers (Azuara-Blanco, *et al.* 1998) used the suction cup to provoke acute increases in IOP in the healthy eyes of 10 myopes and 10 emmetropes and reported a significant increase in disc cup area and cup volume on both refractive groups that pointed towards a mechanism by which the optic nerve head appears to have a dynamic topography dependent on IOP.

1.13.4 Carbonic anhydrase stimulation

Carbonic anhydrase, an enzyme that was first observed 70 years ago, has two chief functions: the conversion of CO_2 into a dissolved form (carbonic acid, HCO_3^-), and the acid-base balance in the body (Chegwidden and Carter 2000). Carbonic acid (or dissolved form of CO_2) facilitates its transport to the lungs, where it is transformed back into CO_2 to be expired. A correct acid-balance in the body will also facilitate the secretion of fluids such as saliva, bile, cerebral spinal fluid and aqueous humour. The carbonic anhydrase molecule has been found in the ciliary body, cornea,

lens and retina. It has also been located in the choroidal capillary endothelium (Wistrand, *et al.* 1986).

Acetazolamide is a carbonic anhydrase inhibitor that acts by blocking the action of the enzyme carbonic anhydrase, which accelerates the reaction between CO_2 and H_2O to form carbonic acid. It was first created in 1950 and it was used first as diuretic. The oral administration of acetazolamide was found to lower IOP, which lead its use towards the treatment of glaucoma (Becker 1954).

However, acetazolamide has also been found to increase blood flow (Kiss, *et al.* 1999; Dallinger, *et al.* 1998; Rassam, *et al.* 1993), and interestingly both acetazolamide and CO_2 have been found to be valid techniques to induce the stimulation of the cerebral vasomotors, which lead to its evaluation as a potential ocular stress test (Dallinger, *et al.* 1998; Ringelstein, *et al.* 1992). The administration of acetazolaminde has the advantage of reduced side effects compared to those caused by CO_2, reported to range from mild dizziness, light headedness, or headache in 1/50 of the population, and since acetazolamide is also commercially available and commonly used in the treatment of glaucoma to reduce the IOP, the adverse reactions to its administration are controlled (Ringelstein, *et al.* 1992). The results obtained in the OA and choroidal vasculature, in which a decrease in blood flow was found, suggest that acetazolamide may be used as provocation test to evaluate the autoregulatory capacity of the different ocular vascular beds (Dallinger, *et al.* 1998).

Acetazolamide, however, was not used for this study due to its invasive nature, as the patients would have had to be injected with the substance. Additionally, it would have not been possible to control the concentration of acetazolamide in blood, which would have not permitted us to correlate changes in concentration with potential changes in blood flow.

The stress testing used in our study needed to be supplied in a non-invasive, controlled manner, allowing the subsequence analysis of data. For these reasons, the stress testing of choice in our study was to raise the partial pressure of carbon dioxide (pCO_2) by increasing the blood levels of carbon dioxide (hypercapnia), which allowed a detailed analysis of the ocular metabolic autoregulation of blood flow.

1.14 Ocular blood flow in myopia

Structural features of myopic eyes describe an eye with an elongated vitreous chamber, axial length being the ocular biometric component that correlates best with refractive error (Sorsby, *et*

al. 1961; Sorsby, *et al.* 1957). Myopia greater than -6.00D has been shown to be mainly axial (Jones, et al. 2005; Sorsby, *et al.* 1957) and due to the increase in axial length with increasing myopia, the fundus of a myopic eye is frequently characterised by a series of changes, a summary of which is provided in table 1.2.

One of the complications described in the summary table are lacquer crack lesions (LCL), which consist of breaks in the structure formed by Bruch's membrane, retinal pigment epithelium and choriocapillaris. The paper by Ohno-Matsui and co-workers (Ohno-Matsui, *et al.* 2003) described lacquer cracks in 51 of 325 myopic eyes examined (15.7%) whose average axial length was above 26mm. This finding supports myopic elongation of the posterior segment as the potential underlying trigger of LCL.

Additionally, chorioretinal atrophy, which appears as small punched-out yellow/white lesions relatively close to LCL that eventually expose the sclera, may also be the result of a stretched sclera and thinned choroid as a consequence of axial elongation.

Thus, the thinning of the choroid in human axial myopia places myopes at high risk of vascular-related ocular disorders, such as choroidal atrophy and consequent macular degeneration, choroiditis and glaucoma (Saw, et al. 2005).

There is also evidence of myopic choroidal thinning in animal studies. Thinning of the choroidal layer was observed after lens induced myopia in chick eyes, which was followed by a reduction in the choroidal blood flow (Wildsoet and Wallman 1995).
A later study by Fitzgerald and co-workers described a large increase in choroidal blood flow preceding the thickening of the choroidal layer in the recovery phase of induced deprivation myopia also in chick eyes (Fitzgerald, *et al.* 2001). Therefore, the choroid seems to thicken and thin to alter the position of the retina thereby compensating the defocus.
Furthermore, changes in choroidal blood flow seem to occur in the same direction as changes in choroidal thickness, and outcomes such as those reported by Fitzgerald and co-workers (Fitzgerald, *et al.* 2001) in which choroidal blood flow variations preceded those in choroidal thickness, raise the possibility that changes in choroidal blood flow may actually trigger changes in choroidal thickness.

Table 1.2 *Summary of fundus changes in myopia (adapted from Swann P, 2002)*

Thus, the literature provides evidence to support a potential link between myopic structural changes and changes in ocular haemodynamics. These vascular findings on animal models of myopia suggest a potential contribution of ocular perfusion towards the onset and progression of myopia. However, little research has been performed to evaluate the vascular features of the healthy human myopic eye. Degenerative myopia in humans appears to be due to changes at the choroidal and retinal level, as reported by Akyol and co-workers, and Dimitrova and colleagues (Dimitrova, *et al.* 2002; Akyol, *et al.* 1996). The retrobulbar blood velocities measured with colour Doppler imaging (CDI) in the central retinal artery (CRA) and posterior ciliary arteries (PCA) were found to be reduced in 21 patients with degenerative myopia (Akyol, *et al.* 1996). Similar outcomes were reported using the same ultrasound instrument in patients with myopic choroidal neovascularisation. The blood velocity in the central retinal vein (CRV), CRA and PCAs decreased with increasing myopia. Additionally, the resistance index (RI) in the PCA was also reported to be higher in these patients (Dimitrova, *et al.* 2002). These results, however, only describe the perfusion features of pathological myopia.

Logan and colleagues evaluated the pulsatile component of the ocular blood flow on a sample of 14 anisomyopes, and reported a significant relationship of inter-eye differences in refractive error and ocular blood flow (Logan, *et al.* 2002). Additionally, Lam evaluated the pulsatile ocular blood flow (POBF) and CDI vascular parameters in the ophthalmic artery (OA) of 31 anisometropes. Lower POBF amplitudes were found in longer eyes; however, no difference was found in the OA blood velocity between the more and less myopic eyes (Lam, *et al.* 2003a). The importance of supporting OBFA results with additional vascular techniques resides in the assumptions related to eye volume and scleral rigidity, which may be particularly pertinent to the myopic eye.

Anisomyopia has been referred to as a good model for the investigation of myopia; however, the results of Lam and colleagues using CDI do not appear to back up POBF outcomes.

These studies represent our current knowledge on the vascular features of human myopia, and yet they lack an extensive evaluation of the different ocular vascular beds on healthy myopic eyes. The use of different techniques to evaluate the wide spectrum of vascular parameters that affect the physical characteristics of ocular haemodynamics is necessary to further our understanding on human myopia. Furthermore, the regulatory features of the healthy human myopic eye remain unexplored, and their assessment is crucial to fully understand the vascular aspects of ocular perfusion in myopia.

CHAPTER TWO
RESEARCH RATIONALE

Evidence from animal studies, mainly performed in chick eyes, describe a decrease in choroidal blood flow after form deprivation and lens-induced myopia (Fitzgerald, *et al.* 2001; Jin and Stjernschantz 2000) and research performed on human pathological myopia show a reduction in the blood velocity of the retrobulbar vessels in patients with degenerative myopia (Dimitrova, *et al.* 2002; Akyol, *et al.* 1996) and a decrease in the amplitude of the ocular blood pulse with increasing myopia (Lam, *et al.* 2003; Logan, *et al.* 2002; Logan, *et al.* 1997; To`mey, *et al.* 1981). In view of the data provided by the literature to date, the investigation of the ocular haemodynamics and autoregulation in human myopia appears of intrinsic interest and it is hypothesised that the healthy human myopic eye shows some degree of decrease in blood flow that predisposes it to complications of the posterior pole. Additionally, autoregulatory dysfunction in myopes has not yet been investigated and myopia may be associated with vascular autonomic dysfunction and exacerbated by ageing and smoking (as both are known to affect blood flow). By furthering our understanding of the vascular features of myopia, this will widen our current knowledge on the aetiology of a refractive error of growing prevalence.

2.1 Research aims

The principal aims of this thesis were:

1. To describe the ocular perfusion features and autoregulatory mechanisms in the retrobulbar, choroidal and microretinal circulation of the human myopic eye.
2. To investigate the residual effect of chronic smoking on the ocular haemodynamics and its potential interaction with refractive error and axial length.
3. To assess the relationship between axial length and choroidal thickness on choroidal blood flow.

Thus, smoking and age were used as challenges to the vascular system in human myopia to show deficits in vascular function rather than imply that smoking and age had implicit aetiological significance in myopia onset and development

2.2 Perfusion features of the human myopic eye

High myopes are at higher risk of ocular complications, such as glaucoma (Mitchell, *et al.* 1999), chorioretinal atrophy and macular degeneration (Swann and Schmid 2002), all of which have

been previously found to exhibit some degree of reduction in ocular blood flow (Pournaras, *et al.* 2006; Aydin, *et al.* 2003; Findl, *et al.* 2000; Chung, *et al.* 1999a; Chung, *et al.* 1999b; Joos, *et al.* 1999; Roff, *et al.* 1999; Duijm, *et al.* 1997; Findl, *et al.* 1997; Ustymowicza, *et al.* 1997). Animal studies in which myopia was induced in avian (chicks and pigeons) and primate (marmosets) by means of form deprivation or imposed defocus have also reported decreased choroidal blood flow (Fitzgerald, *et al.* 2001; Wildsoet and Wallman 1995). Therefore, there is evidence to highlight the importance of further investigation of the perfusion characteristics of the healthy human myopic eye.

In order to establish the resting state vascular profile and responses to stress testing, a series of investigations were conducted. In each case it was necessary to identify and control for possible factors affecting the ocular circulation, such as age, systemic blood pressure, smoking and alcohol intake.

The first experimental chapter provided an evaluation of the resting ocular characteristics of human myopia by assessing a young sample of high myopes (MSE ≥ -5.00D), low myopes (MSE -1.00D to -4.50) and emmetropes (± 0.50 D). Since age and blood pressure are variables inherent to the participants, their effect was assessed by performing a second study within the same chapter in which two age groups were recruited and their vascular data compared. The mechanism of ocular blood flow regulation in myopia was assessed in Chapter 4 by assessing the difference in vascular response given by myopes and emmetropes to a mild gas provocation (hypercapnia). Chapter 5 evaluated the chronic effect of smoking on the ocular haemodynamics and vascular regulation; whereas Chapter 6 furthered the investigation of the ocular vascular features of myopia by assessing a group of anisomyopes. The blood flow in the choroidal layer, which has been reported to be actively involved in induced-myopia animal studies, was measured in Chapter 7, and its relationship with choroidal thickness and axial length and refractive error was assessed in Chapter 8.

2.2.1 The human myopic eye: vascular profile and effect of ageing on ocular perfusion characteristics.

Identifying the vascular physiological characteristics of the healthy myopic eye and the changes undergone by the ageing eye is essential to understand the perfusion features of the degenerative myopic eye. This study comprised two parts to provide an independent evaluation of the effect of age and ocular biometrics on the ocular haemodynamics, both of which remained unexplored. In the first study the hypothesis tested was that the ocular blood flow may be reduced in young healthy myopes with MSE greater than -5.00D. Thus, the resting state retrobulbar, pulsatile and

microretinal blood flow was determined in 3 refractive groups: 22 emmetropes (MSE ±0.50D), 22 low myopes (MSE -1.00 to -4.50D) and 22 high myopes (MSE ≤-5.00D). The consecutive refractive groups (emmetropes, low myopes and low myopes to high myopes) were arranged to differ by 0.50D to minimise the confounding effect that overlapping refractive groups would exert on the vascular data. In order to identify whether a specific myopic category could be differentiated from other categories in terms of ocular haemodynamics the degree of myopia was evaluated as a discrete variable rather than a continuous. The separation into discrete categories of myopia has been used widely in previous literature that has compared, for example, biometric parameters of the myopic eye (Wildsoet 1998, In Myopia and Nearwork Rosenfield and Gilmartin pp30-32) and has been adopted here principally to ascertain whether ocular haemodynamics are significantly compromised in myopia that exceeds 5 D, a level known to be associated with ocular pathology (Curtin 1985).

The three refractive groups were matched for age, gender and ethnicity to provide results that reflected the true resting vascular state of human myopia, without the influence of the confounding effects of age and the age-related changes in BP. In all the studies the subjects were matched one-to-one for age, gender, refractive error, axial length and ethnicity. When the different age groups were compared, the subjects were subsequently matched for gender, refractive error, axial length and ethnicity.

The second study in this chapter focused on the effect of age on ocular blood flow and to avoid the confounding effect of ocular biometrics on the assessment of the ocular circulation, the participants were matched for mean spherical equivalent and axial length. Ninety-eight volunteers were subdivided into 2 age groups; a young group with 48 participants with ages ranging from 19 to 30 years (mean age 24.83 ± 4.16 years), and an older group with 48 subjects whose age was above 40 years and ranged from 42 to 78 years (mean age age 55.27 ± 10.99 years). This approach was chosen due to the initial design of the study in which the data was going to be compared with those from patients suffering from glaucoma, whose age tends to be above the age of 40 years old.

2.2.2 Vascular autoregulation in human myopia

The body is constantly exposed to variations in perfusion pressure such as changes in arterial pressure and changes in body position, and it is also exposed to variations in the metabolic demands of the tissues, for which the ability to ensure constant blood flow is essential. To assess the correct functioning of any vascular system, the evaluation of its response to autoregulatory stimuli is as fundamental as the assessment of its haemodynamic features at rest. The ocular and cerebral circulation have previously shown autoregulatory capacity (Riva, *et al.* 2005; Longo, *et al.*

2004; Hafez, *et al.* 2003; Riva, *et al.* 1997; Aaslid, *et al.* 1989; Riva, *et al.* 1986) however the autoregulatory response of the healthy myopic eye has not been assessed to date. This is of particular importance since abnormal vascular regulation may be demonstrated even when resting state perfusion is within normal limits, and may be the underlying mechanism for future damage. Thereby, the second experimental chapter of this thesis studied the ocular vascular autoregulation in human myopes using hypercapnia as a provocation test.

Briefly, hypercapnia consists of an increase in the carbon dioxide (CO_2) end tidal partial pressure (pCO_2) (for a detailed explanation see section 1.13.2). To be able to assess the effect of breathing enriched CO_2 air, it is first necessary to record the ocular blood flow while breathing normal room air (baseline) and compare it to the measurements obtained while breathing CO_2 increased approximately 15% above baseline. The study consisted of 33 myopes (mean age 36.78± 15.00 years; mean MSE -4.51 ± 2.56 D) and 33 emmetropes (mean age 40.27 ± 16.13 years; mean MSE 0.08 ± 0.41), whose ocular blood flow responses to hypercapnia were compared. Both groups were matched for age, gender and ethnicity.

2.2.3 The effect of smoking on human haemodynamics

The evaluation of the effects of chronic smoking on the ocular perfusion features was considered necessary due to the known acute effect that smoking has on the ocular haemodynamics of healthy eyes (Lietz-Partzsch, *et al.* 2001; Tamaki, *et al.* 2000 ; Monfrecola, *et al.* 1998; Morgado, *et al.* 1994; Robinson, Petrig and Riva 1985). Of further importance is the link between smoking and age related macular degeneration (AMD) (Smith, *et al.* 2001; Klein, *et al.* 1993). Since myopes are known to be at higher risk of macular degeneration due to their fundus physiology, smoking may increase the risk of AMD on the predisposed myopic fundus. Several papers have been published assessing the acute effect of smoking on ocular perfusion; however, there remain two important omissions in the understanding of the impact of smoking on ocular perfusion and ultimately ocular disease: 1. The chronic effects of smoking following smoking cessation are unexplored, and 2. The impact of smoking on vascular autoregulation in a young and otherwise healthy population.

The purpose of the study was firstly to evaluate the chronic effect of long-term smoking on the ocular haemodymanic profile and secondly to assess the autoregulatory characteristics of the ocular vascular system in young smokers. Additionally, the interaction effect between human myopia and smoking on ocular haemodynamics was also addressed. The Fagerström Tolerance Questionnaire (FTQ) provided an objective tool to recruit the smoking sample group. Twenty-one non-smokers (mean age of 26.52 ± 5.17 years, average MSE -1.55 ± 2.06D) and 21 smokers (mean age 26.79 ± 4.09 years, average MSE -1.41 ± 2.09D), who had been smoking for 8 years on average

were recruited for the first part of the study; whereas a subgroup of 11 non-smokers (mean age 25.00 ± 2.93years; average MSE -1.42 ± 2.11D and 11 smokers (mean age 25.81 ± 3.70 years; average MSE -1.21 ± 2.04D) comprised the subject sample for the second part. Both groups were matched for age, refractive error and ethnicity.

2.2.4 Choroidal blood flow in the myopic eye

The choroidal circulation is a vascular bed of remarkable value to investigate in human myopia, as it has been suggested to be involved in the active process of myopic growth in animal studies. Changes in choroidal thickness appear to precede alterations in choroidal blood flow in the recovery phase of form deprivation myopia (Fitzgerald, *et al.* 2001; Wildsoet and Wallman 1995) and additionally, they are noticeable in the fundus view of highly myopic eyes (Saw, *et al.* 2005; Ohno-Matsui, *et al.* 2003). Thereby, there is evidence that suggests that the circulation of the choroidal layer may be affected in human myopia.

The purpose of this investigation was to evaluate the resting choroidal circulation in human myopia and additionally to assess the vascular autoregulatory properties of the choroid in myopia. For the first part of the investigation 23 emmetropes (mean MSE -0.006 ±0.47; mean age 35.60 ± 14.94 years), and 28 myopes (average MSE-3.80 ± 2.12D; mean age 35.60 ± 14.94 years) were assessed and the choroidal readings obtained were compared. The evaluation of the choroidal circulation with respect to autoregulation was performed on a subgroup of 10 emmetropes (mean MSE -0.21 ± 0.30; mean age 33.10± 10.37 years) and 10 myopes (mean MSE -3.90 ± 2.06; mean age 30.90 ± 12.16 years). The two sample groups assessed in both studies were matched for age, refractive error and ethnicity.

2.2.5 The relationship between axial length, refractive error, choroidal thickness and choroidal blood flow

There is evidence of choroidal thinning in myopia studies in animals. Thinning of the choroidal layer was observed after lens induced myopia in chick eyes, which was followed by a reduction in the choroidal blood flow (Wildsoet and Wallman 1995). Additionally, myopic eyes appear to exhibit significantly lower capillary density and capillary diameter in the choroid and significantly greater distance between adjacent intercapillary meshes than the control chick eyes (Hirata and Negi 1998). Thereby, the relationship between choroidal thickness and choroidal blood flow in human myopic eyes was considered of inherent interest to further the understanding of human myopia.

The high resolution of a new optical coherence tomography (OCT) instrument (SD OCT 'Copernicus'(SOCT) Optopol, Poland) allows the visualisation of the choroid, thereby allowing the measurement of choroidal thickness in this study. Since the SOCT has not been previously used for the assessment of the choroidal layer, the choroidal thickness was measured manually by the use of callipers and the repeatability and reproducibility of the choroidal thickness measurement were also assessed by measuring choroidal thickness at the macular region of 12 healthy volunteers (mean age 26.66±6.44 years) at each of 2 visits. The scan used was a radial macular scan that comprised 15 radial line scans of 7mm in length arranged in a star-like pattern with the centre on the fovea. Each radial scan spaced 12 degrees from each other. Since the SOCT was found to be repeatable, this study evaluated the relationship between axial length, choroidal thickness and choroidal blood flow. Ten healthy volunteers (different from those taking part in the repeatability study) participated in the second part of the study. Their mean age was 35.72 ± 13.19 years and mean MSE -1.10 ± 1.79 D.

2.3. Summary

These studies intended to add to the current knowledge base on the vascular profile of myopes, paying particular attention to the ocular and systemic relationship of the vascular parameters, the vascular regulatory capacity of ocular circulation and the impact of other confounding factors, such as smoking and age, on ocular blood flow.

CHAPTER THREE
HUMAN MYOPIA AND OCULAR BLOOD FLOW: PERFUSION FEATURES OF THE HUMAN MYOPIC EYE AND THE EFFECT OF AGE ON OCULAR BLOOD FLOW

Chapter three is divided into two studies: Study one assessed the eyes of myopic volunteers below the age of 30 and Study two evaluated those from myopes over the age of 40.

3.1 Study one
Perfusion features of the young human myopic eye.

3.1.1 Abstract

Purpose: To provide a detailed analysis of the systemic and ocular perfusion characteristics of the healthy myopic eye by comparing the retinal and choroidal vasculature of emmetropes and myopes. A secondary aim was to evaluate the correlation between ocular biometric parameters and ocular blood flow.

Methods: Retrobulbar vessels, retinal capillaries and ocular vascular pulsatility were evaluated using CDI, HRF, and OBFA respectively. 66 healthy volunteers were grouped into 3 refractive groups: 22 high myopes with spherical equivalent (MSE) ≥ -5.00D (average MSE -6.87 ± 1.52 D; mean age 22.63 ± 4.33 years, range: 18-37 years) were compared to 22 low myopes whose MES ranged between -1.00D and -4.50D (mean MES -2.31 ± 1.12 D; mean age 28.36 ± 3.62 years, range: 18-32 years). The control group consisted of 22 emmetropes (mean MSE of -0.11 ±0.33D; mean age 23.22 ± 4.59 years, range: 18-37 years). The three groups were matched for age, gender and ethnicity.

Systemic blood pressure, heart rate, intraocular pressure and patient height and weight were recorded. Additionally, axial length (AL), vitreous chamber volume (VCV) and corneal thickness were measured to evaluate potential correlations with ocular blood flow parameters.

Results: The ocular pulse amplitude ($p=0.016$) and CRA ratio between the systolic and diastolic velocities ($p=0.014$) were significantly lower in high myopes, whereas CRA RI was significantly higher in high myopes ($p=0.004$). MSE correlated with AL ($p<0.001$), VCV ($p<0.001$), OBFa ($p=0.03$), OBFv ($p=0.02$), POBF ($p=0.014$), CRA RI ($p=0.007$) and CRA ratio ($p=0.05$).

Conclusions: High myopia was characterised by a significantly lower CRA blood velocity at both systole and diastole, which was accompanied by a significantly higher resistance index. The pulsatile component of blood flow also appeared significantly decreased in high myopia. These findings suggested a lower pulsatile and CRA blood flow in myopia, which appeared unaffected at the retinal capillary level.

3.1.2 Introduction

High myopia is related to a stretched sclera, increased risk of choroidal atrophy and consequent macular degeneration, choroiditis and glaucoma (Saw 2006; Tano 2002). Another complication of human myopia is lacquer crack lesions (LCL), which are characterized by breaks in the complex formed by Bruch's membrane, retinal pigment epithelium and choriocapillaris. LCL are attributed to the myopic progressive posterior segment elongation, which is supported by a recent paper from Ohno-Matsui and co-workers in which lacquer cracks were found in 51 of 325 myopic eyes examined (15.7%) whose axial length was above 26mm (Ohno-Matsui, *et al.* 2003). There is also evidence of myopic choroidal thinning in animal studies. Thinning of the choroidal layer was observed after lens induced myopia in chick eyes, which was followed by a reduction in the choroidal blood flow (Wildsoet and Wallman 1995). A later study by Fitzgerald and co-workers described a large increase in choroidal blood flow preceding the thickening of the choroidal layer in the recovery phase of induced deprivation myopia also in chick eyes (Fitzgerald, *et al.* 2001).

These vascular findings in animal myopia associate the ocular circulation with the myopic eye growth. Research performed on myopic eyes with degenerative myopia described a reduction in the blood velocities of retrobulbar vessels. Peak systolic (PSv) and end diastolic velocities (EDv) at the CRA and SPCA were measured using CDI. A decrease in PSv, EDv and RI in CRA and SPCA in was found in 21 patients with degenerative myopia compared to 20 aged-matched controls (Akyol, *et al.* 1996). Dimitrova and colleagues also used CDI to compute CRA, Central retinal vein (CRV) and SPCA blood velocities. The degree of myopia was found to correlate with blood velocity in CRA and SPCA after assessing 52 participants which included hyperopes, healthy myopes, patients with cataract surgery and myopes with choroidal neovascularisation (CNV). Refractive error was found to correlate linearly with CRA, CRV and SPCA velocities, such that blood velocity appeared diminished with increasing myopia. Additionally, the affected eye of the 8 patients with CNV was compared to the fellow eye and a significant increase in SPCA resistance index (RI) was reported (Dimitrova, *et al.* 2002). The retinal microcirculation of 25 pathological myopes with glaucoma was assessed with a scanning laser Doppler flowmeter in a study by Németh and co-workers in which mean blood flow in the retinal microvessels was found to correlate with refractive error and axial length (Németh, *et al.* 2001). These results, however, only had direct implications on the perfusion features of pathological myopia, and it remains unconfirmed whether a) pathological myopia may be the cause or the consequence of altered ocular blood flow, and b) whether healthy human myopes share this perfusion deficit.

Logan and colleagues evaluated the pulsatile component of ocular blood flow in one of the few studies performed to date on healthy human myopic eyes. They evaluated a sample of 14

anisomyopes, and reported a significant relationship of inter-eye differences in refractive error, ocular pulse volume and ocular pulse amplitude (Logan, *et al.* 2002; Logan 1997). Lam and co-workers also measured pulsatile ocular blood flow using POBF in 31 anisometropes, to which the evaluation of OA blood velocity using CDI was added. Lower POBF amplitudes were found in longer eyes; however, no difference was found for the OA (Lam, *et al.* 2003).

These studies represent our current knowledge on the vascular profile of human myopia, and yet they lack the evaluation of the different ocular vascular beds. The use of different techniques to evaluate the wide spectrum of vascular parameters that affect the physical characteristics of ocular haemodynamics is necessary to further our understanding on human myopia.

3.1.3 Aims and objectives

The aim of this study was to provide a detailed analysis of the systemic and ocular perfusion characteristics of the healthy myopic eye by assessing both the retinal and choroidal vasculature using OBFA and HRF on emmetropes and myopes. An evaluation of the correlation between ocular biometric parameters and ocular blood flow was also provided.

3.1.4 Methods

3.1.4.1 Study Sample and Recruitment Criteria

Power statistics revealed that a sample size with a statistical power of 80%, allocation ratio 1:1 and a significant value of 5% (α=0.05) required a sample ranging from 11 to 17 participants (11 for OA, 17 for CRA and 15 for SPCA) to show statistical significance between the retrobulbar blood velocity of emmetropes, low mopes and high myopes. Sixteen was the sample size calculated to evaluate POBF and 62 to analyse the microretinal circulation (sample size calculations performed following Simple Interactive Statistical Analysis, SISA). Therefore, for this study 66 healthy volunteers were evaluated for the study of subjects below the age of 40. All volunteers were recruited from the student and staff population of Aston University

Since a physiological correlation is known to exist between the two eyes of the same subject, only one eye randomly chosen from each subject was evaluated (Kimura, *et al.* 2003).

The participants were recruited and grouped according to their refractive error measured objectively without cycloplegia. Emmetropes 22 high myopes with spherical equivalent (MSE) ≤ -5.00D (average MSE-6.87 ± 1.52 D; range: -5.00D to -9.40D; mean age 22.63 years ± 4.33, range: 18-37 years) were compared to 22 low myopes whose MSE ranged between -1.00D and -4.50D (mean MSE-2.31 ± 1.12 D; range: -1.00D to -4.50; mean age 28.36 years ± 3.62, range: 18-

32years). The control group consisted of 22 emmetropes with MSE ±0.5 D (mean MSE of -0.11D ±0.33; mean age 23.22 years± 4.59, range: 18-37). All the three groups were matched for age, gender and ethnicity (i.e. each subject was individually matched for age, gender and ethnicity with a subject from each refractive group).

All the participants were healthy volunteers, which was confirmed by the author following investigation of the fundus using Volk lens (90D), recording of blood pressure and a detailed recording of systemic and ocular history and symptoms. Those subjects exhibiting any sign of myopic fundus changes (e.g. lacquer cracks, staphylomas, chorioretinal atrophy, lattice degeneration or pavingstone degeneration) were not included in the study. Exclusion factors included any ocular disorder, diabetes, hypotension, hypertension or any other systemic disorder or medication likely to affect the systemic or ocular vasculature. Since no blood test was performed, all of the previous conditions were self-reported by the participants. A corrected visual acuity of 6/9 or better was required together with astigmatism of less than 1.5 diopters cylinder, which reduced the percentage of astigmatic patients being taken as myopic subjects after MSE calculation.

In total 9 subjects were found to be smokers, 3 of which were emmetropes (MSE ±0.50 D), 3 were low myopes (MSE -1.00D and -4.50D) and 3 were high myopes (MSE ≤ -5.00D).

3.1.4.2. Ethical Approval and Informed Consent

Written and verbal information about the exact procedures to be performed during the visit was given to all the subjects prior to data collection. The participants were encouraged to ask any questions and to clarify any doubts they might have before signing the written consent. The volunteers were requested to sign two copies of the written consent, one of which was given to them for their own records. All investigations were approved by the Ethical Review Committee and conformed to the declaration of Helsinki (appendix 2.1).

3.1.4.3 Study Sample: Dietary Restrictions

Alcohol, nicotine and caffeine containing products have been reported to affect the flow of blood in the eye (Domino, *et al.* 2004; Gdovinova 2001). To ensure the reliability of the data, the participants recruited for this study were asked to refrain from smoking and consuming alcohol and caffeine containing products 12 hours prior to the study.

Exercise is known to increase the mean arterial pressure (MAP); however the maximal BP falls rapidly on stopping exercise (Tzemos, Lim and MacDonald 2002). Therefore, since the total duration of the experiment was approximately 1 hour and the vascular readings were performed approximately in the last 30 minutes, the potential effect of exercise on the systemic circulation

was considered to be minimal. Thus, the subjects were not asked to refrain from exercising before the appointment.

3.1.4.4. Experimental Protocol and Investigations

The features, calibration and protocol of the instruments used to measure ocular blood flow are explained in detail in chapter 1.

The experimental protocol of the study was design to give priority to subjective versus objective tests and to have those tests that involved posture changes performed at the end. The protocol was as follows:

- Autorefraction (Shin-Nippon NVision-K 5001 Autorefractor, Japan) (section 1.9.1) and biometry data (IOLMaster, Carl Zeiss Jena, Germany Zeiss) (section 1.9.2) were taken once written consent was obtained from the volunteer.
- Proxymetacaine HCl 0.5% (Minims®, Chauvin Pharmaceuticals) was instilled as anaesthetic before corneal thickness was measured (DGH-550 Pachette 2, DGH technology, Pennsylvania, US) (section 1.9.2) and intraocular pressure (IOP) were recorded (Tono-Pen®XL, Medtronic Xomed, Inc., Minnesota, USA).
- Pulsatile ocular blood flow was then assessed with the Ocular Blood Flow Analyser (OBFA, Paradigm Medical Industries, Utah, USA) (section 1.11.2) followed by the evaluation of the retinal microvasculature using the Heidelbergh Retinal Flowmeter (HRF, Heidelberg Engineering, Heidelberg, Germany) (section 1.11.6). Ultrasonography (Colour Doppler Imaging, CDI. Sequoia CDI System, Siemens Medical Solutions, USA) (1.11.5) was performed next on the OA, CRA and short posterior ciliary arteries (SPCA). The integrity of the cornea was confirmed using a slitlamp after the instillation of fluorescein (Fluorets strips, Chauvin Pharmaceutical), once all the tests mentioned above had been performed.
- Systemic blood pressure was recorded with an automated sphygmomanometer placing the cuff on the upper part of the left arm while the patient rested sitting with the arm on the chair arm-rest (UA-767 Fully Automatic Blood Pressure Monitor BHS A/A, A & D Co. Ltd, Oxon, UK).
- Weight and height were then measured on all the participants.

3.1.4.5 Statistical analysis

All data are given as mean ± SD. The data collected exhibited was normally distributed (see appendix 1.2 for explanation and histograms), which allowed the use of parametric tests; one-way ANOVA was used to assess the differences between high myopes, low myopes and emmetropes.

and a Tukey- Kramer HSD post-hoc test was used to follow up statistically significant results to determine the groups that differed significantly.
There are several ways of performing *post-hoc* analysis between group means; Fisher's Protected Least Significance Difference (Fisher's PLSD), Tukey-Kramer Honestly Significant Difference (Tukey-Kramer HSD), Student-Newman-Keuls (SNK) and Tukey Compromise among others. In this study, Tukey-Kramer was the *post-hoc* analysis of choice due to its lower risk of type I errors and the fact that it can be used for experiments which have equal or unequal number of observations in each group(Amstrong, Slade and Eperjesi 2000).

Bonferroni correction was performed when associated parameters calculated from the same piece of equipment were compared. Bonferroni correction aimed to avoid creating type I errors (α), by which the null hypothesis would be rejected when, in fact, it was true. Since the calculation of pulsatile ocular blood flow (POBF) is based on the pulsatile rate and the incremental changes in volume, the initial significance level adopted for POBF ($p=0.05$) was divided by 3 resulting in a critical level of $p=0.016$. In the retrobulbar blood flow analysis, the resistance index and ratio of each vessel evaluated are based on the direct measurement of the blood velocity; thus the significance level changed from $p=0.05$ to $p=0.025$ for both RI and ratio parameters.
Since maximum and minimum values were measured for each microretinal parameter, the significance level changed from $p=0.05$ to $p=0.025$ for the microretinal blood velocity and to $p=0.012$ for the blood volume and blood flow as their calculation was based on ocular blood velocity. When Bonferroni correction was performed, it was indicated in the tables in the following manner:

* value statistically significant prior to Bonferroni correction (therefore not significant)

** value statistically significant after Bonferroni correction (therefore significant)

Pearson's linear correlation analysis was used to determine the relationship between vascular parameters, MSE, axial length and vitreous chamber volume by bivariate analysis. Multiple regression was used to assess the correlation between MAP and OPP with the microretinal circulation. Stepwise multiple regression analysis was used to explore the relationship between ocular biometry and ocular perfusion features and to define the biometric parameter that contributed the most to the perfusion profile of the human myopic eye.

The comparative results between refractive groups are represented in the form of box-plots, as they are known to provide an accurate visual summary of many important aspects of a distribution. The box stretches from the lower hinge (defined as the 25th percentile) to the upper hinge (the 75th percentile) and therefore contains the middle half of the scores in the distribution. The median is shown as a line across the box. Therefore 1/4 of the distribution is between this line

and the top of the box and 1/4 of the distribution is between this line and the bottom of the box. Thus, box-plots were able to depict the large intra-group variability that a bar chart would not be able to provide. Statistical significance was defined as $p<0.05$.

3.1.5 Results

3.1.5.1. General Characteristics

There were no significant differences in age, gender, height, weight, body mass index and IOP between the three groups assessed. Moreover, systemic haemodynamics, MAP and OPP did not differ among groups (table 3.1). Two sets of data analysis were performed in this study, the first of which evaluated differences between the refractive groups (ANOVA), whereas the second analysis assessed the profile of ocular blood flow as a function of refractive error and axial length (Pearson's correlation).

	Emmetropes	Low myopes	High myopes	p
N	22	22	22	n/a
Gender	10m; 12f	10m; 12f	12m; 10f	n/a
Ethnicity	9w/12a	9w/12a	9w/12a	n/a
MSE (Dioptres)	-0.11 ± 0.33	-2.31 ± 1.12	-6.87 ± 1.52	**<0.001
Age (years)	23.22 ± 4.59	23.86 ± 3.62	22.63 ± 4.33	ns
Systolic BP (mmHg)	111.85 ± 12.69	107.90± 11.65	106.95 ± 16.05	ns
Diastolic BP (mmHg)	66.00 ± 10.94	63.95 ± 9.07	60.00 ± 9.24	ns
Pulse rate (bpm)	72.65 ± 7.14	74.22 ± 7.74	74.90 ± 11.83	ns
MAP(mmHg)	97.25 ± 14.79	89.68 ± 16.44	88.90 ± 13.48	ns
OPP (mmHg)	51.87 ± 10.93	46.16 ± 11.83	46.13 ± 7.90	ns
Height (m)	1.67 ± 0.09	1.69 ± 0.11	1.70 ± 0.10	ns
Weight (kg)	65.05 ± 12.68	63.88 ± 13.39	69.32 ± 15.50	ns
BMI	22.92 ± 3.05	22.09 ± 3.06	23.46 ± 3.20	ns
Axial length (mm)	23.53 ± 0.73	24.2 ±0.79	25.98 ± 0.92	**<0.001
ACD (mm)	3.55 ± 0.3	3.66 ±0.23	3.67 ± 0.47	** 0.048
VCV (cm^3)	5.04 ± 0.64	5.19 ± 0.70	6.00 ±0.55	**<0.001
CT (μm)	546.81 ± 42.84	542. 62 ± 40.50	545.04 ± 40.77	ns
IOP (mmHg)	13.05 ± 3.58	13.61 ± 3.01	13.71 ± 2.40	ns

Table 3.1. *Summary of physical, ocular and vascular systemic features by refractive group (explanation of abbreviations:f, female; m, male; w, white ethnicity; a, east asian; MAP, mean arterial pressure; OPP, ocular perfusion pressure; BMI, body mass index; ACD, anterior chamber depth; CT, corneal thickness; IOP, intraocular pressure) * statistically significant*

3.1.5.2. Ocular biometry and perfusion characteristics

Ocular biometry

Of all the biometric parameters assessed, only axial length and vitreous chamber volume differed significantly between the three groups. High myopes had larger ACD (p=0.048), axial length (p<0.001) and vitreous chamber volume (p=0.001) compared to emmetropes and low myopes. IOP and corneal thickness did not show significant differences across the three refractive groups (table 3.1).

Pulsatile ocular blood flow

The analysis of OBFA parameters exhibited a significantly lower ocular pulse amplitude (OBFa) (p=0.016) with increasing myopia. OBFA readings obtained for each group are summarized in table 3.2 and figure 3.1.

	Emmetropes	Low myopes	High myopes	p
OBFiop (mmHg)	10.75 ± 3.53	11.45 ± 2.84	10.64 ± 2.55	ns
OBFa (mmHg)	3.68 ± 1.45	3.17 ± 0.71	2.46 ± 1.20	**0.016
OBFv (µl)	9.84 ± 3.19	8.13 ± 1.89	6.98 ± 3.09	*0.017
OBFr	58.71 ± 16.76	65.81 ± 6.64	70.46 ± 14.54	0.047
POBF (µl/min)	1299.14 ± 318.6	1149.62 ± 306.18	987.20 ± 386.24	*0.032

Table 3.2. *Pulsatile ocular blood flow outcomes for the different group (explanation of abbreviations: OBFiop, intraocular pressure; OBFa, amplitude of ocular pulse; OBFv, volume of ocular pulse; OBFr, rate of ocular pulse; POBF, pulsatile ocular blood flow) ** significant value after Bonferroni correction; ns: not significant difference*

VESSEL	PARAMETER	Emmetropes	Low myopes	High myopes	p
OA	**PSv (m/s)**	0.464 ± 0.13	0.395 ± 0.15	0.449 ± 0.18	ns
	EDv (m/s)	0.132 ± 0.15	0.116 ± 0.14	0.091 ± 0.04	ns
	RI	0.783 ± 0.07	0.787 ± 0.05	0.796 ± 0.05	ns
	Ratio	5.100 ± 1.75	5.009 ± 1.22	5.344 ± 1.84	ns
CRA	**PSv (m/s)**	0.127 ± 0.03	0.122 ± 0.03	0.119 ± 0.02	ns
	EDv (m/s)	0.044 ± 0.02	0.044 ± 0.01	0.033 ± 0.01	** 0.037
	RI	0.655 ± 0.08	0.635 ± 0.07	0.714 ± 0.06	** 0.004
	Ratio	3.125 ± 0.98	2.857 ± 0.60	3.76 ± 1.33	**0.014
SPCA	**PSv (m/s)**	0.149 ± 0.04	0.149 ± 0.03	0.143 ± 0.03	ns
	EDv (m/s)	0.064 ± 0.02	0.061 ± 0.02	0.057 ± 0.02	ns
	RI	0.574 ± 0.11	0.602 ± 0.10	0.612 ± 0.02	ns
	Ratio	2.659 ± 1.25	2.708 ± 0.84	2.752 ± 0.73	ns

Table 3.3. *Results obtained at the retrobulbar vessels by refractive group as measured with CDI (explanation of abbreviations: PSv, peak systolic velocity; EDv, end diastolic velocity; RI, resistance index) ** statistically sig*

Retrobulbar data

Of all the retrobulbar vessels evaluated (OA, CRA and SPCA), only the CRA velocity appeared significantly higher in high myopes when compared with low myopes and controls. After analysing the profile of high myopes, CRA was found to have a lower velocity at both systole and diastole (p=0.014) accompanied by a higher resistance index (p=0.004) when compared to low myopes. The resistance index was also found to be significantly higher in high myopes compared to emmetropes (p=0.036). All the results obtained in the retrobulbar vessels are shown in table 3.3, figure 3.1 and figure 3.2.

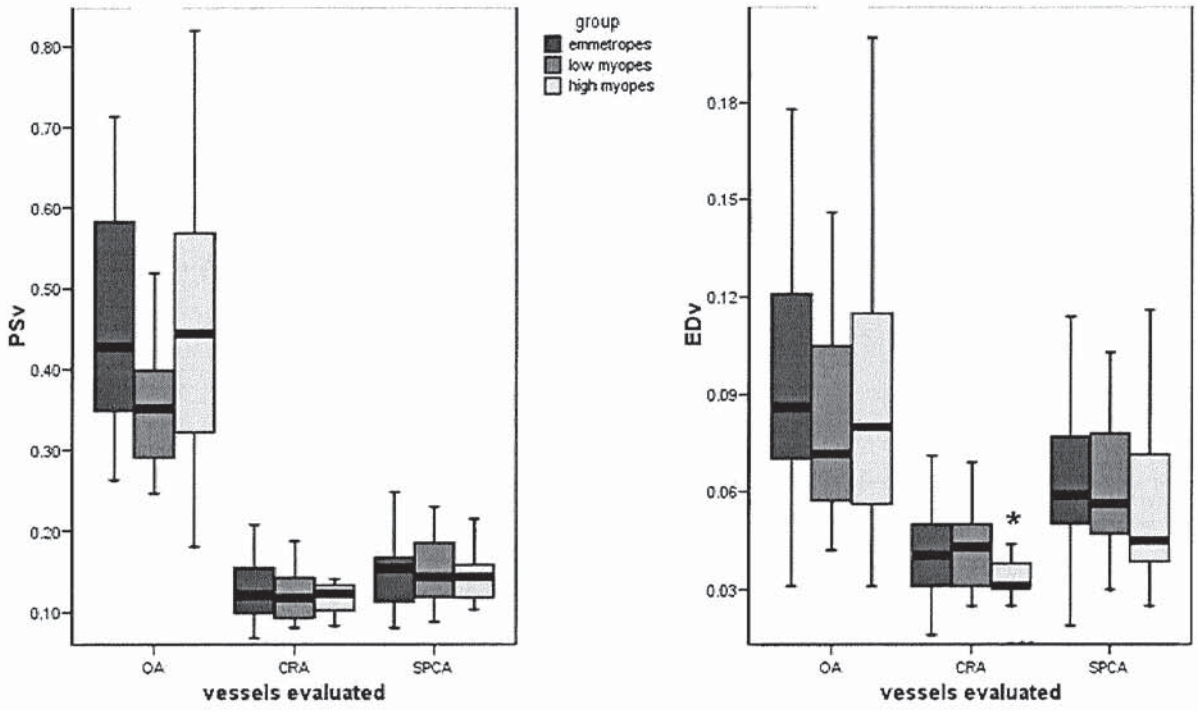


Figure 3.1. *Peak systolic (PSv) and end diastolic velocities in the OA, CRA and SPCA measured using CDI for each refractive group given in the form of box plot * statistically significant*

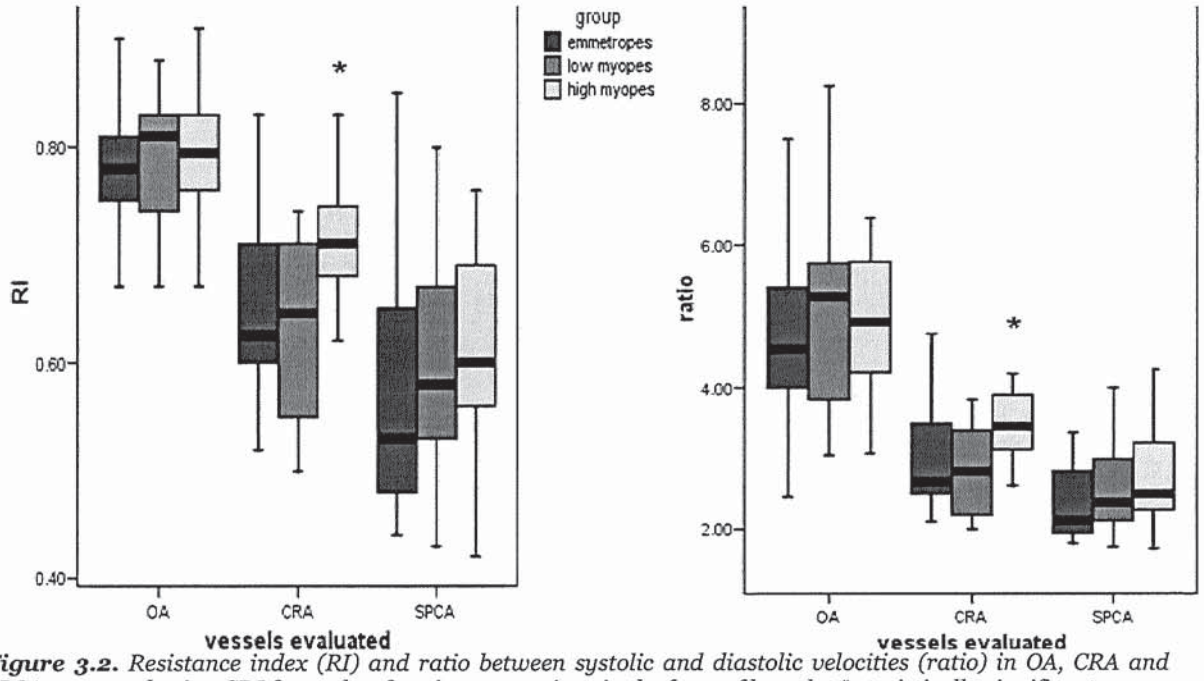


Figure 3.2. *Resistance index (RI) and ratio between systolic and diastolic velocities (ratio) in OA, CRA and SPCA measured using CDI for each refractive group given in the form of box plot * statistically significant*

Retinal capillaries

Maximum and minimum values of retinal blood flow, volume and velocity were obtained from the retinal capillary bed around the foveal area using HRF. There was no significant difference between the maximum and minimum values for low and high myopic patients compared to data obtained from emmetropes (table 3.4 and figure 3.3).

	PARAMETER	Emmetropes	Low myopes	High myopes	p
MAX	**Volume (AU)**	28.64 ± 8.75	25.87 ± 8.80	26.68 ± 5.55	ns
	Flow (AU)	499.87 ± 164.42	439.53 ± 177.82	467.44 ± 138.88	ns
	Velocity (AU)	1.64 ± 0.46	1.52 ± 0.53	1.57 ± 0.44	ns
MIN	**Volume (AU)**	19.15 ± 7.86	16.99 ± 5.86	16.94 ± 3.72	ns
	Flow (AU)	308.97 ± 133.61	252.80 ± 97.57	267.62 ± 104.22	ns
	Velocity (AU)	1.07 ± 0.40	0.89 ± 0.32	0.94 ± 0.35	ns

Table 3. 4. *Maximum and minimum values of volume, flow and velocity measured in the retinal capillaries using HRF (AU: arbitrary units)*

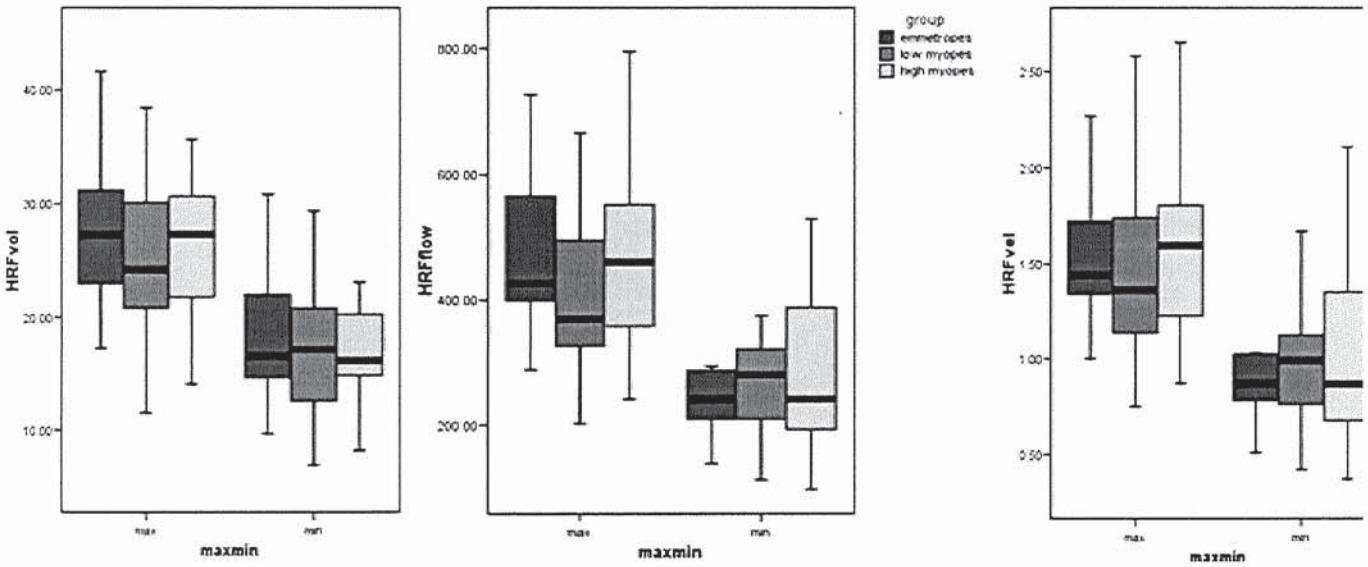


Figure 3.3 *Maximum and minimum blood volume (HRFvol), flow (HRFflow) and velocity (HRFvel) in the foveal capillaries measured using HRF.*

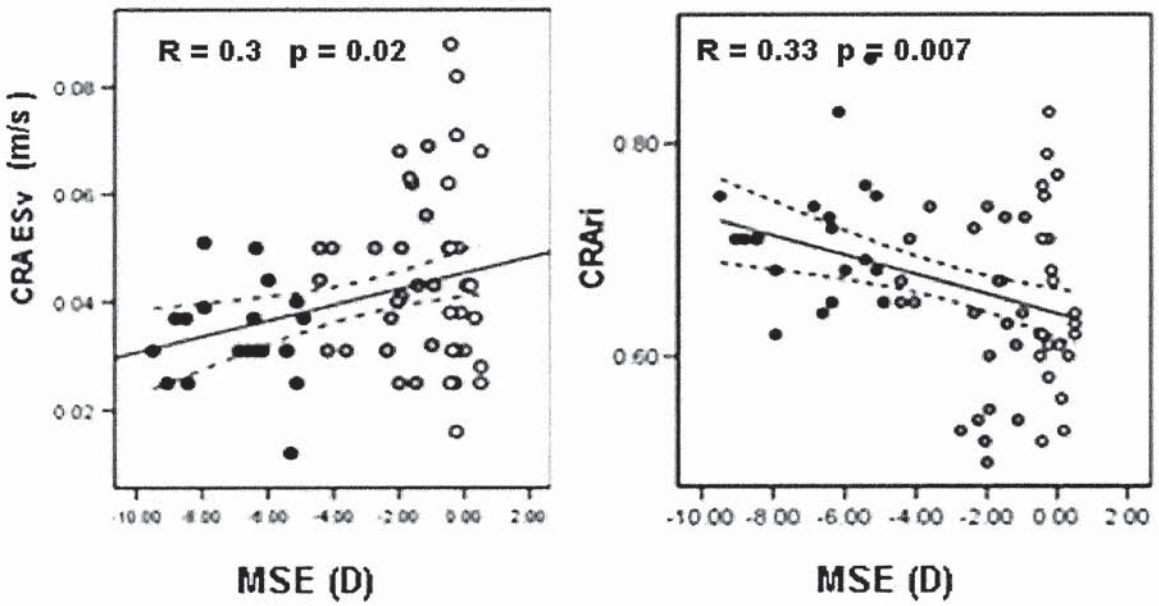


Figure 3.4a *Significant correlations between MSE and Central Retinal Artery end diastolic velocity (CRA EDv) and resistance index (CRA RI) (Light coloured data points: emmetropes (MSE±0.50D)and moderate myopes (MSE -1.00D to -4.50); dark coloured data points: high myopes(≤-5.00D)).*

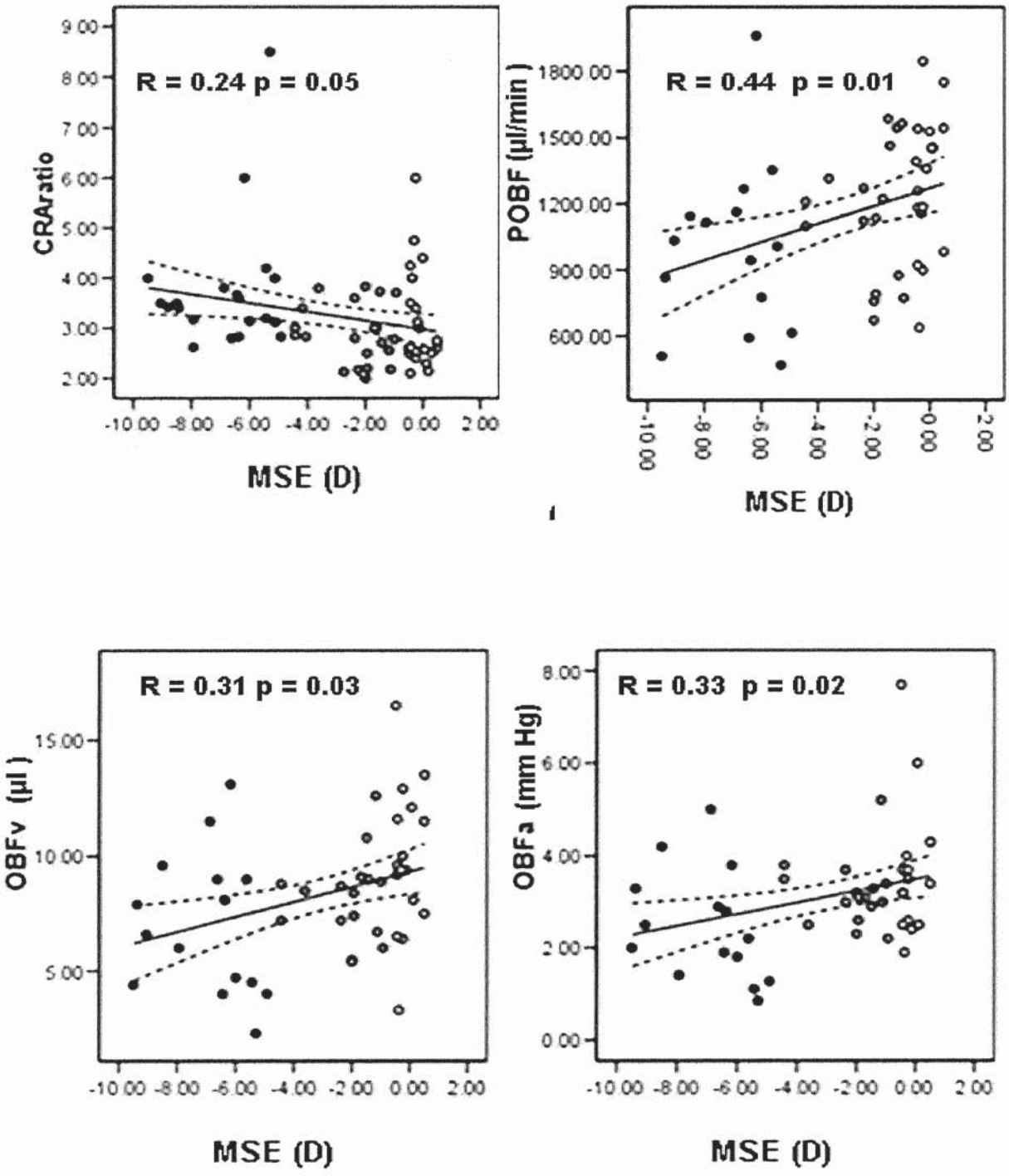


Figure 3.4b *Significant correlations between MSE, the volume of the ocular pulse (OBFv) and the amplitude of the ocular pulse (OBFa).(Light coloured data points: emmetropes (MSE±0.50D)and moderate myopes (MSE -1.00D to -4.50); dark coloured data points: high myopes(≤-5.00D)).*

3.1.5.3 Relationship of OBF to ocular biometry

The relationship between refractive error, axial length, vitreous chamber and ocular vascular parameters was performed using the Bivariate Pearson's product-moment correlation test. The bivariate correlation between systemic circulation and ocular circulation was also evaluated.

The vascular beds that differed significantly between the three groups, the pulsatile and retrobulbar circulation, correlated significantly with refractive error and axial length, but not with vitreous chamber volume. Refractive error (MSE) correlated with OBFv (R =0.31; p=0.03), OBFa (R=0.33; p=0.02), POBF (R=0.44; p=0.01), CRA EDv (R=0.30; p=0.02), CRA RI (R=-0.33; p=0.007) and CRA ratio (R=0.24; p=0.05) (figure 3.4). AL correlated with OBFa (R=0.42; p=0.003), OBFv (R=0.49; p<0.001), POBF (R=0.52; p<0.001) and CRA RI (R=0.24; p=0.05) (figure 3.5). Vitreous chamber volume correlated with OBFv (R=-0.36; p=0.025) and POBF (R=0.44; p=0.005).

The correlation between the three main components of arterial blood pressure (systolic blood pressure, SBP, diastolic blood pressure, DBP and pulse) and the ocular vascular outcomes showed a positive correlation of OA PSV and CRA EDv with SBP (R=0.13 p=0.002, R=0.29 p=0.02). The systemic pulse correlated significantly with the rate the ocular pulse (OBFr) (R=0.5 p<0.001) and with the ratio and resistance index between systolic and diastolic velocities in the CRA (R=-0.33 p=0.007; R=0.31 p=0.01) (table 3.5).

		SBP R value	p	Pulse	p	MAP R value	p	OPP R value	p
POBF	**OBFr**	0.04	ns	0.5	* <0.001	0.02	ns	-0.03	ns
CDI	**OA PSv (m/s)**	0.13	*0.002	0.06	ns	0.15	ns	0.25	ns
	CRA EDv (m/s)	0.29	* 0.02	-0.14	ns	0.15	ns	0.25	ns
	CRA RI	0.002	ns	-0.33	* 0.007	0.14	ns	0.18	ns
	CRA Ratio	-0.17	ns	0.31	* 0.01	0.02	ns	-0.02	ns
HRF	**Max Volume (AU)**	-0.004	ns	-0.13	ns	0.21	ns	0.31	* 0.02
	Min Volume (AU)	0.09	ns	-0.08	ns	0.31	* 0.01	0.44	* 0.001
	Max Flow (AU)	0.15	ns	-0.1	ns	0.17	ns	0.27	0.04
	Min Flow (AU)	0.6	ns	-0.05	ns	0.31	* 0.02	0.45	* <0.001
	Max Velocity (AU)	-0.30	ns	-0.08	ns	0.14	ns	0.22	ns
	Min Velocity (AU)	0.03	ns	-0.05	ns	0.27	* 0.04	0.43	* 0.001

Table 3.5 *Vascular parameters that showed significant Pearson´s product-moment correlation with systolic BP (SBP), pulse, MAP and OPP *statistically significant*

The calculated average blood pressure (mean arterial pressure, MAP) correlated significantly with the minimum blood volume, flow and velocity in the retinal microvessels, which also showed correlation with the ocular perfusion pressure (OPP) (table 3.5). Multiple regression of MAP, OPP and IOP with HRF readings showed significant correlation in all the parameters evaluated (table 3.6). No systemic pressure reading correlated with OBFA parameters.

The OPP correlated significantly with CRA PSv (R= 0.37, p= 0.005), CRA EDv (R= -0.32, p=0.012), SPCAPSv (R=-0.27, p=0.039), HRF maxvolume (R=-0.36, p=0.005), HRFminvolume (R= -0.45, p< 0.001), HRFminflow (R= -0.38, p= 0.003), HRFminvel (R= -0.38, p= 0.002).

Stepwise multiple regression performed on OBFA readings showed AL as the biometric parameter with the largest contribution to pulsatile blood flow variation.

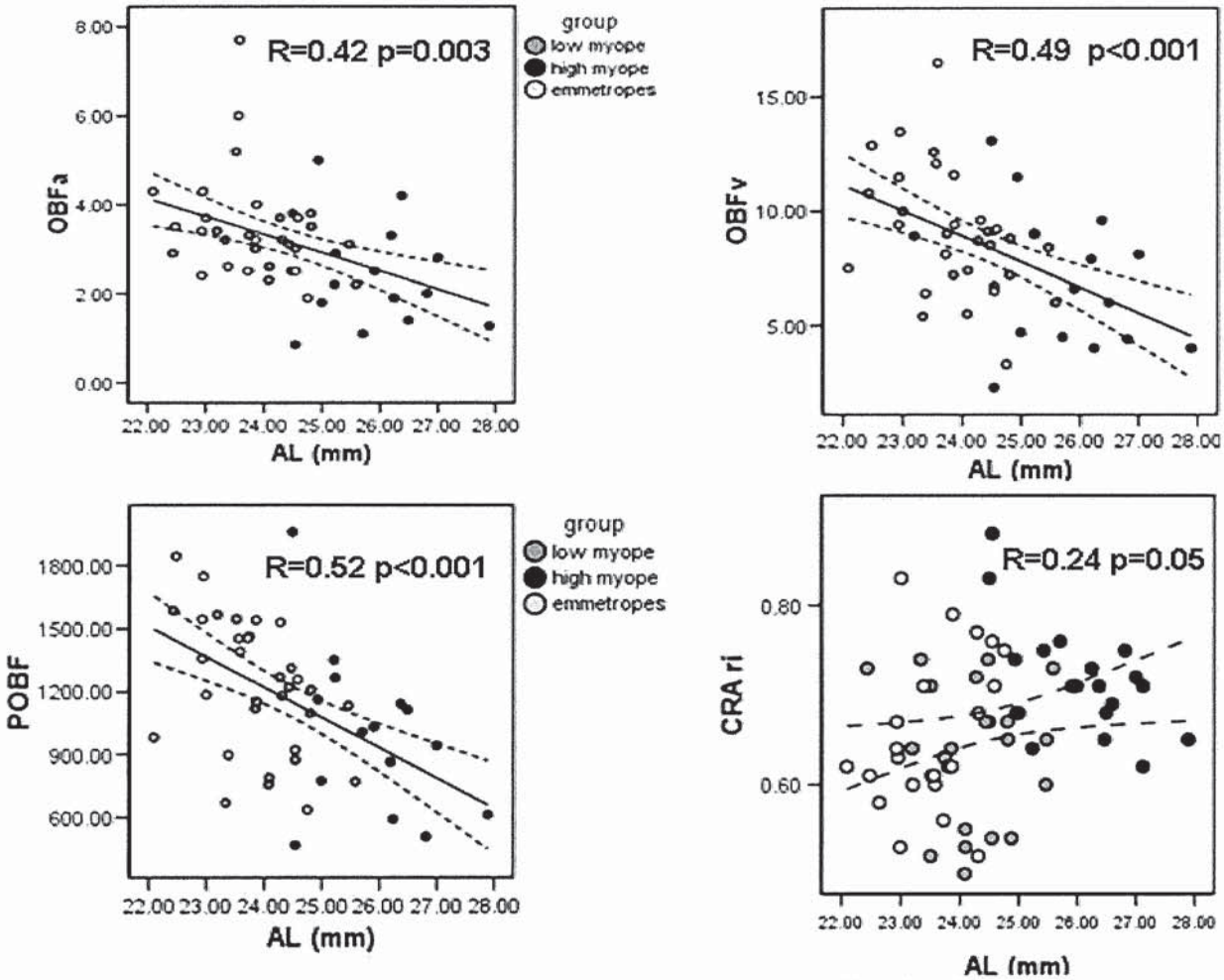


Figure 3.5 *Correlation analysis showing significant relationship between axial length and vascular parameters.*

Parameter	Multiple correlation MAP, OPP, IOP and HRF	p
MaxVolume (AU)	0.41	* 0.017
MaxFlow (AU)	0.43	* 0.009
MaxVelocity (AU)	0.41	* 0.018
MinVolume (AU)	0.55	* < 0.001
MinFlow (AU)	0.67	* < 0.001
MinVelocity (AU)	0.68	* < 0.001

Table 3.6 *Multiple correlation between MAP, OPP, IOP and HRF parameters *statistically significant*

3.1.5.4. Vascular systemic correlates by refractive group

The relationship between pulse, MAP, OPP, systolic and diastolic blood pressure and the vascular outcomes obtained with the different ocular techniques was analysed separately for each refractive group. The results are shown in table 3.7 and 3.8.

	SBP R value	p	DBP R value	p	Pulse R value	p
EMMETROPES						
OBFiop (mmHg)	0.58	ns	0.07	ns	0.32	ns
CRA PSv (m/s)	0.4	ns	-0.07	ns	-0.07	ns
HRFmaxvol (AU)	0.23	ns	-0.04	ns	-0.14	ns
HRFmaxflow (AU)	0.55	*0.06	0.27	ns	-0.02	ns
HRFmaxvel (AU)	0.59	*0.006	0.29	ns	0.11	ns
HRFminvol (AU)	0.40	ns	0.12	ns	-0.13	ns
HRFminflow (AU)	0.48	*0.03	0.16	ns	-0.71	ns
HRFminvel (AU)	0.44	ns	0.08	ns	-0.07	ns
LOW MYOPES						
OBFiop (mmHg)	0.47	ns	0.57	0.02	-0.03	ns
HIGH MYOPES						
OBFa (mmHg)	-0.33	ns	-0.5	ns	-0.57	ns
OBFr	0.2	ns	0.52	*0.04	0.77	*0.001

Table 3.7 *Systemic blood pressure correlation with ocular vascular readings by refractive group *stat sig*

In the emmetropic group, OBFiop correlated with OPP, as well as CRA PSv and maximum retinal capillary volume, flow and velocity, and minimum retinal capillary volume, flow and velocity. MAP was found to correlate with HRF maximum flow and velocity, minimum volume, flow and velocity. The SBP correlated with maximum flow and velocity, and minimum flow. In the

moderate myopic group, only OBFiop showed a correlation with diastolic blood pressure, MAP and OPP. For the high myopes, significant correlation was only found between OBFa and OBFr and heart rate, MAP and OPP.

	MAP	p	OPP	p
EMMETROPES				
OBFiop (mmHg)	-0.30	ns	-0.55	* 0.03
CRA PSv (m/s)	0.38	ns	0.44	* 0.05
HRFmaxvol (AU)	0.42	ns	0.57	* 0.01
HRFmaxflow (AU)	0.61	* 0.04	0.6	* 0.001
HRFmaxvel (AU)	0.60	* 0.005	0.64	* 0.002
HRFminvol (AU)	0.60	* 0.005	0.72	* <0.001
HRFminflow (AU)	0.63	* 0.003	0.75	* <0.001
HRFminvel (AU)	0.57	* 0.008	0.72	* <0.001
LOW MYOPES				
OBFiop (mmHg)	0.7	* 0.003	0.7	* 0.003
HIGH MYOPES				
OBFa (mmHg)	-0.51	* 0.05	-0.55	* 0.03
OBFr	0.45	ns	0.48	ns

Table 3.8 *MAP and OPP correlations with ocular vascular readings by refractive group *statistically significant.*

3.1.5.5 Ocular blood flow as a function of refractive error and axial length

Since axial length correlated significantly with some of the ocular vascular parameters assessed and there appeared to be a trend towards a decrease in pulsatile and retrobulbar blood flow taking place for a specific level of myopia was hypothesised and address as followed.

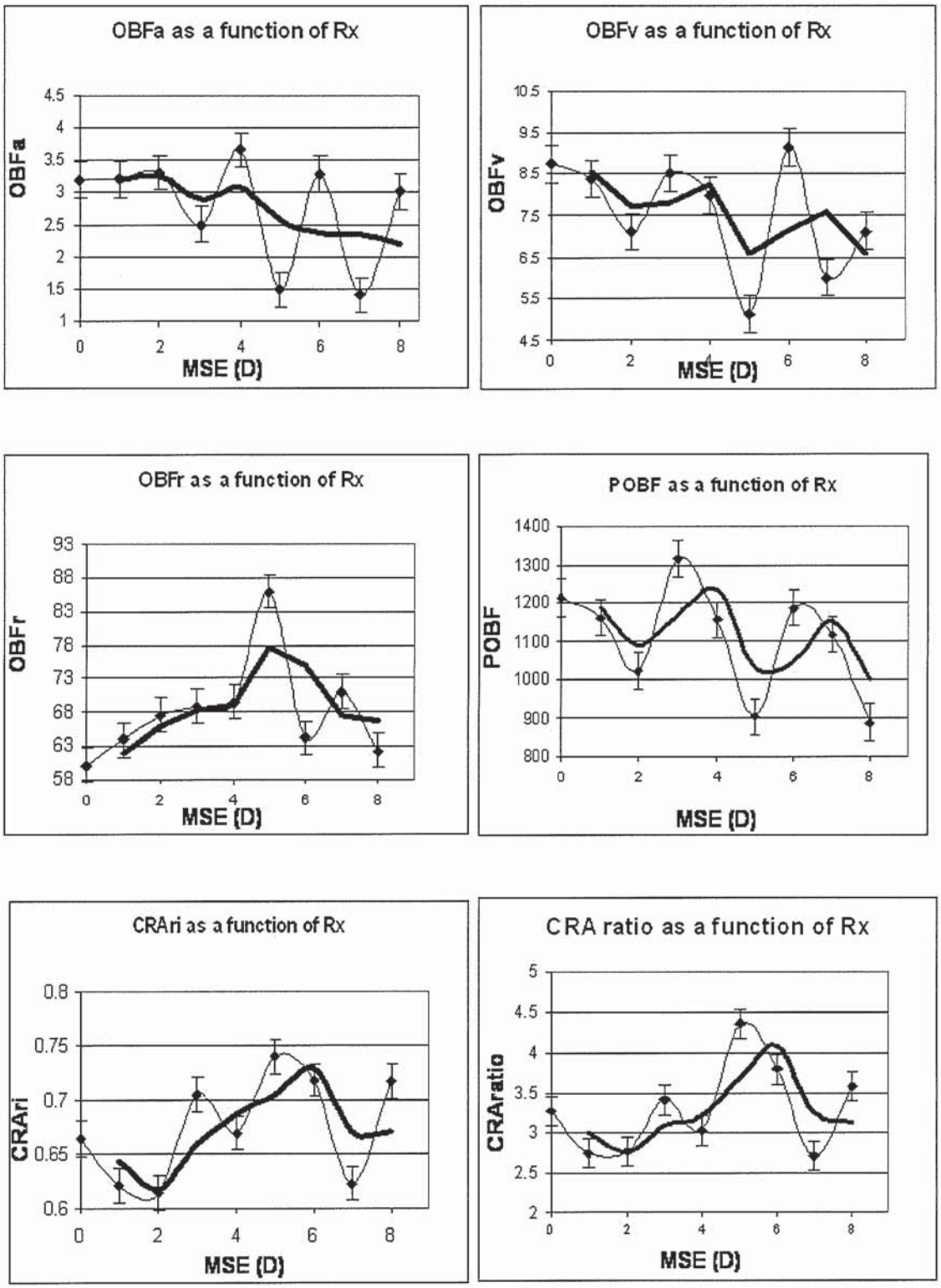


Figure 3.6 *Results from the parameters that differed between refractive groups plotted as a function of refractive error. The thin line represents the connection line between data points; whereas the dark line represents the moving average (MA).*

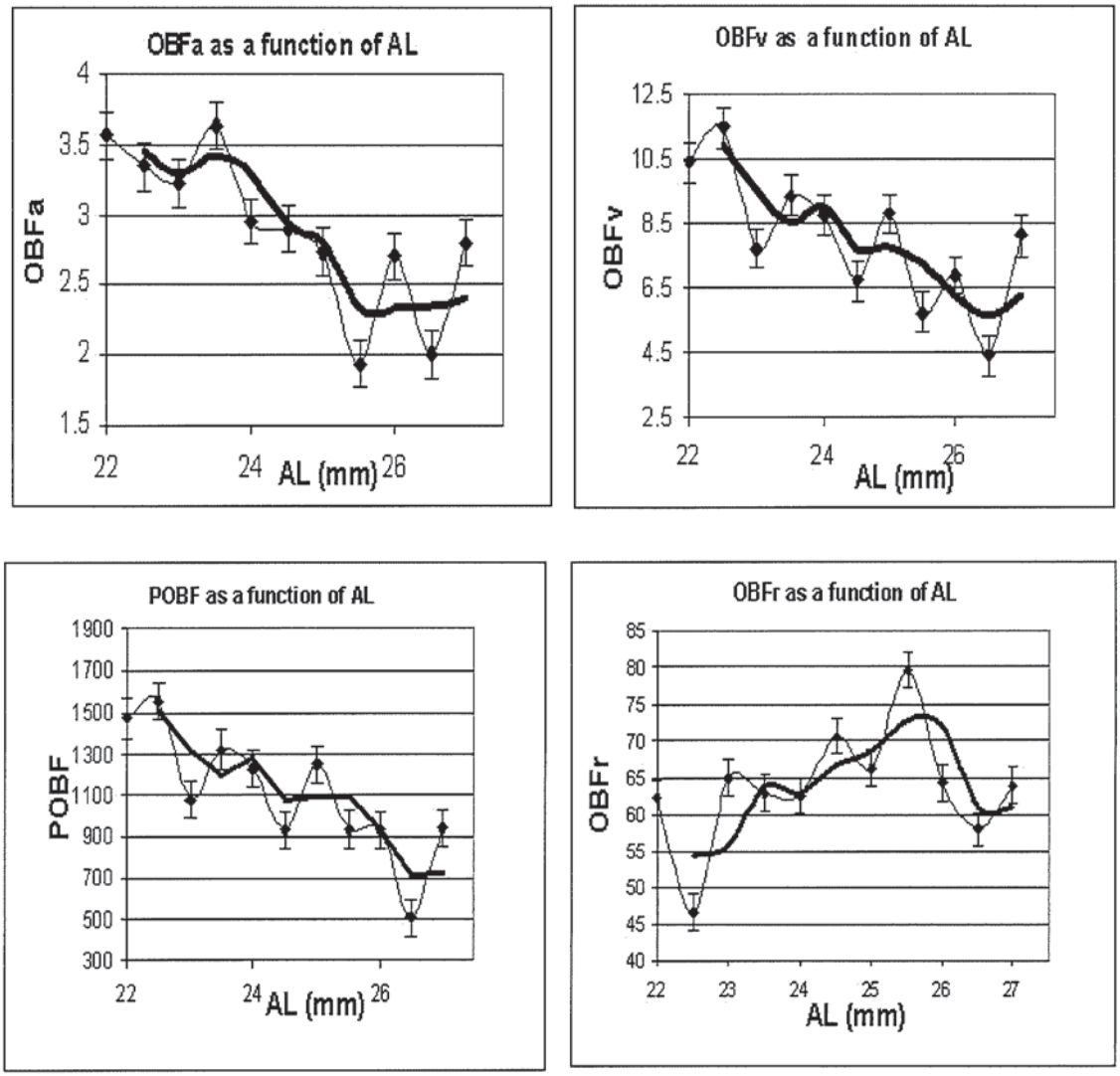


Figure 3.7 *Results of the parameters that differed between refractive groups plotted as a function of axial length (AL). The thin line represents the connecting line between data points; whereas the dark line represents the moving average (MA).*

To address this trend, a graphical evaluation of blood flow as a function of refractive error and axial length was performed. Being aware that not all of the OBFA readings reached statistical significance after Bonferroni correction, those vascular readings that showed significance or approached levels of significance appeared to decrease with increasing MSE and axial length. Thereby, graphs were designed to represent an average vascular reading (y axis) for every dioptre (or mm of AL) (x axis) (figure 3.6, figure 3.7). The data points in the graph were connected by moving average (MA). MA is a trendline normally used to smooth short-term fluctuations, thus highlighting longer-term trends or cycles. The MA between both myopic refractive groups (MSE -1.00D and -4.50D and from -5.00D onwards) was based on 22 data points. Averaging technique's

inherent disadvantage of inferring higher influence depending upon the number of subjects is overcome by the use of MA.

3.1.6 Discussion

The present study assessed the vascular differences in systemic and ocular blood flow between three refractive groups: emmetropes, low myopes and high myopes. Three instruments were used to investigate different ocular vascular beds and perfusion features: OBFA, CDI and HRF. The outcomes revealed no difference in systemic blood pressure, MAP, OPP and IOP, or in weight, height or body mass index between the three refractive groups. However, the ocular pulsatile component of blood flow and the CRA systolic and diastolic blood velocities were significantly lower in high myopia, which was accompanied by a significantly higher CRA resistance index, which suggests a decrease in CRA blood flow (see equation 1.10). There were no significant differences in the retinal capillaries blood flow parameters between the three refractive groups. In the emmetropic group all the ocular vascular beds correlated significantly with systemic blood supply, whereas in low and high myopes only the pulsatile blood flow correlated with the systemic blood pressure. Moreover, the pulsatile ocular blood flow and CRA blood velocity correlated with axial length and refractive error.

3.1.6.1 Differences between refractive groups

No differences were found in arterial blood pressure readings between refractive groups, which suggests a similar cardiovascular profile among the refractive groups. The three refractive groups differed significantly in axial length, and myopes had greater axial lengths than emmetropes. The level of myopia was found to correlate significantly with AL, a finding that is supported by many previous studies (Logan, *et al.* 2005; Wickremasinghe, *et al.* 2004; Zadnik, *et al.* 2003; Gwiazda, *et al.* 2002; Lam, *et al.* 2002; Grosvenor and Scott 1991; Goss, *et al.* 1990; McBrien and Millodot 1987; Fledelius 1982; Larsen 1971).

The ACD of high myopes was significantly higher than those of low myopes and emmetropes, which has been linked to the increased axial length of the myopic eye (Saw, *et al.* 2005; Lin, *et al.* 1999).

Our results did not show significant differences between the IOP of myopes and emmetropes. However, one of the hypotheses associated with the pathogenesis of myopia has been elevated IOP. Increased IOP has been suggested to impose scleral stress and, therefore, axial elongation (Quinn, *et al.* 1995; Jensen 1992), and several previous papers have reported an increased IOP in myopic subjects (Saw, *et al.* 1996; Pruett 1988). A study by Wong and collaborators (Wong, *et al.*

2003) showed a significant correlation between myopic prescriptions and increasing IOP ($p<0.001$) in a Caucasian population aged 43 to 86 years of age. However, when the groups were controlled for baseline IOP, age and gender, IOP did not show evidence of significance. The study by Wong and that by Bonomi and colleagues (Bonomi, *et al.* 1982) support the lack of IOP difference between myopes and emmetropes found in the present study. Bonomi and co-workers showed no difference between the IOP of 137 pairs of anisomyopic eyes suggesting that high myopia is neither the cause or the effect of high intraocular pressure.

The use of three different vascular instruments in this study helped to depict a wider picture of the haemodynamic profile of myopia. Analysis of OBFA parameters revealed a significantly lower ocular pulse amplitude and rate that were accompanied by a trend towards a decreased ocular pulse volume and total pulsatile ocular blood flow in the myopic group. The significant differences were found between emmetropes and high myopes and no differences were found between emmetropes and low myopes, or low and high myopes; which suggests that healthy myopic eyes below -4.50D do not exhibit a lower pulsatile ocular blood flow. The differences, however, appear significant for those healthy myopes whose MSE is above -5.00. Various studies have described a decrease in the ocular pulse amplitude of eyes with myopic prescriptions or greater axial lengths (Lam, *et al.* 2002; Logan, *et al.* 2002; Shih, *et al.* 1991; To`mey, *et al.* 1981). Lam *et al* (2003) assessed the pulsatile and OA profile of 31 anisometropic eyes using OBFA and CDI. Anisometropia represents a reliable technique for the assessment of myopia, as the same individual exhibits different prescription in each eye despite both eyes being exposed to the same genetic and environmental conditions. The paper by Lam described lower pulse amplitude in longer eyes, and a significant correlation between axial length and pulsatile ocular blood flow. However, no difference was reported between paired OA readings, which they attributed to the volume and pressure relationship assumed by POBF calculation (Lam, *et al.* 2003).

It is however misleading to draw conclusions like those suggested by Lam and colleagues, in whose paper, due to the lack of agreement between the results obtained using POBF and CDI, the lower POBF found in the more myopic eye of anisomyopes was reported to be due to the scleral rigidity and eye volume assumptions (Lam, *et al.* 2003). The location of the retrobulbar vessels and the technique used for their assessment differ significantly from those of the OBFA, which analyses ocular pulsatile blood flow. Thus, interpretation of the outcomes obtained with different vascular instruments should be made from both a technical, anatomical and clinical perspective. The present study highlighted the need to assess the ocular perfusion features of the myopic eye using various vascular instruments that assess different ocular vascular beds to gain an overall picture of the resting state blood flow.

The evaluation of the retrobulbar vessels showed a significantly lower systolic and diastolic velocity in the CRA in high myopes (MSE ≥ -5.00 D) compared to low myopes (-1.00D to -4.50D) and emmetropes (± 0.50). The outcomes of the present study on the retrobulbar vessels were consistent with previous studies on ocular blood flow and myopia in which a reduction in CRA peak systolic (PSv) and end diastolic velocity (EDv) was found (Dimitrova, *et al.* 2002; Akyol, *et al.* 1996). However, it is necessary to emphasize that these previous studies evaluated patients with myopic retinopathy and myopic choroidal neovascularisation respectively and additionally, both eyes of each patient were evaluated in the study of Akyol *et al.*, which might have induced statistical errors due to physiological correlation between the two eyes of a single subject (Ray and O'Day 1985).

The CRA vascular resistance index was significantly higher in high myopes. Blood flow resistance (R) through a vessel is dependent on vessel length (l) and radius (r), as described by Poiseuille's law on laminar flow analysis of Newtonian fluid through a cylindrical tube. The resistance is calculated using: $R= 8l\eta / \pi r^4$, where η is the viscosity of the fluid. Despite blood being a non-Newtonian fluid, most blood flow analyses are currently performed assuming blood as a Newtonian fluid, which seems to be more accurate when considering large arteries with simple geometries. The increased RI described in the present study might, therefore, suggest a reduction in vessel radii and, consequently, diameter. Narrowing of the retinal arterial diameter will lead to higher RI.

Our results are in agreement with those from a study by Shimada and co-workers in which high myopes (< -8.00 D) and mild myopes (-3.00 D to -8.00 D) exhibited narrowing of retinal artery diameters, alongside decreased velocities in the temporal branch of the CRA (Shimada, *et al.* 2004). Patton *et al.* likewise reported the association between increased axial length and narrowing of both arteriolar and venular retinal diameters (Patton, *et al.* 2005). An increase in CRA resistance index parallel to a decrease in systolic and diastolic velocities may, thus, represent different aspects of the same feature.

The blood velocities at the OA and SPCA did not differ significantly between the three refractive groups; results that may be accounted for the larger calibre of the vessels in the case of the OA, which may result in increased noise, and the significant correlation with the systemic vasculature, which did not exhibit significant differences across the refractive groups. The SPCAs, in contrast, are relatively small vessels compared to the CRA. However, it does not appear to be a matter of the sample size, as power statistics revealed that a sample size with a statistical power of 80%, allocation ratio 1:1 and a significant value of 5% (α=0.05) requires a sample of 11 participants to show statistical significance between the OA blood velocity of myopes and emmetropes and a

sample size of 15 to show significance between the SPCAs blood velocity (sample size calculations performed following Simple Interactive Statistical Analysis, SISA).

The HRF evaluated the blood flow, velocity and volume for the macular retinal capillaries; emmetropes and myopes did not differ significantly in any of the readings. Capillaries are the smallest vessels of the vascular system, responsible for interconnecting arteries and veins and with diameters that range from 5 to 10 μm. There are two main levels of capillary networks in the retina; a deep network laying on the inner nuclear layer (INL) and a superficial network located around the nerve fibre or ganglion cell layer. The superficial capillary network has been previously reported to be vulnerable to raised IOP, such as in hypertensive glaucoma, due to the long course of the vessels (over 1000μm), infrequent arterial input, and lack of anastomosis (Bill, *et al.* 1983). The elevated susceptibility of the retinal capillaries to raised IOP may, therefore, account for the lack of difference between the perfusion of the retinal microvessels in the three refractive groups. IOP plays an important role in the perfusion pressure of the eye (see equation 1.9), which suggests that similar IOPs most likely may lead to similar haemodynamic features of the retinal capillaries. Reduced CRA blood velocity accompanied by stable retinal microvascular circulation suggests a supply mechanism in myopes with MSE greater than 5.00 D by which the retinal microvessels ensure constant supply of blood to the photoreceptors despite a decrease in retrobulbar velocities.

3.1.6.2 Relationship between systemic circulation and ocular blood flow

When the vascular outcomes from all the three groups were analysed together, systemic blood pressure correlated with pulsatile ocular blood flow, blood velocity in the CRA and retinal microcirculation. However, the correlation between systemic blood pressure and vascular outcomes in emmetropic eyes differed from the correlation found in low and high myopes. In emmetropes, the arterial pressure correlated with retinal microcirculation blood flow and CRA systolic velocity, whereas the retinal microcirculation correlated with OPP. However, in low and high myopes only pulsatile ocular blood flow correlated with arterial blood pressure.

Emmetropes were the only refractive group exhibiting a significant correlation between retinal microcirculation and systemic blood pressure. The correlation coefficient reached higher levels of significance when HRF readings were correlated with both systemic and ocular pressure parameters (MAP, OPP and IOP). It is, therefore, important to highlight these differences in correlation between emmetropes, low myopes and high myopes. The analysis described an emmetropic eye whose pulsatile, retrobulbar and retinal microvascular blood supply significantly correlated with the arterial blood pressure; whereas only pulsatile ocular blood flow correlated

with the arterial blood pressure in the myopic eye. These results may reflect a differentiated vascular pattern of ocular blood supply with refractive error or eye size.

The results of this study could be interpreted from the perspective of choroidal homeostasis and eye growth would place our results in the context of current myopia research. The choroid has been suggested to thin and thicken as a compensatory mechanism aiming to relocate the blurred retinal image in the myopic eye (Wallman and Winawer 2004). Several papers have reported a thinning of the choroidal layer in animal deprivation myopia (Fitzgerald, *et al.* 2001; Wildsoet and Wallman 1995). The choroid has also been reported to thicken after the recovery phase of animal deprivation myopia, which supports the existence of active mechanisms of visual growth control in which the choroid may be involved. Walls hypothesised in 1942 a push-pull mechanism by which the choroid would pull or push the retina backwards or forwards in response to retinal defocus (Walls 1942). Supporting Walls theory, the dynamic explanation by Wallman and Winawer suggested an active choroid that would change thickness to accelerate of decelerate the growing mechanism of the eye (Wallman and Winawer 2004).

The choroid has previously been suggested to be part of the signal cascade of ocular growth between the retina and the sclera. Visual stimulation in the form of positive or negative defocus over the retinal layer might result in a cascade of events in which the amplitude of the choroidal blood pulse might be involved. A decrease or increase in the amplitude of the pulse (as a result of positive or negative defocus) might potentially be translated into a decreased or increased pulsatile ocular blood flow, which may lead to changes in choroidal thickness due to variations in metabolic needs. Directing the hypothesis towards our research interest, myopic defocus, known to stimulate eye growth, might be linked to a reduction in pulse amplitude that could well act as a trigger for changes in choroidal thickness to relocate the retinal image. If the thickness of the choroid decreases, it might be accompanied by a retinal thinning, as suggested by a recent paper in which decreased macular thickness and volume had been reported in Singaporean myopic children (Luo, *et al.* 2006).

Workman also very recently described a relationship between axial length and retinal thickness influenced by retinal location, such that as axial length increases, the retinal thickness decreases in regions at greater distances from the fovea (Workman 2006). A thinning of the retinal layer would consequently be recognised by the vascular supply as a need to decrease in metabolic needs, which might be translated into decreased velocities of those arteries supplying the retina. The CRA is the main retinal blood supply, and in this study it has been shown to exhibit both a decrease in velocity and increase in resistance index with myopia. The retinal microcirculation would ensure

that the minimum supply is accomplished, and it would remain stable, a hypothesis that would agree with our results.

3.1.6.3. Relationship between ocular blood flow, axial length and refractive error

In addition to the vascular differences found between myopes and emmetropes, a correlation between refractive error, axial length and ocular blood flow was also found. Pulse amplitude, pulse volume and POBF correlated with axial length, which agrees with several previous papers reporting a significant negative correlation between axial length and ocular pulse amplitude (Lam, *et al.* 2003; Logan, *et al.* 2002; Morgan, *et al.* 2002; Ravalico, *et al.* 1997; Shih, *et al.* 1991; To`mey, *et al.* 1981). Various reasons have been given to explain this marked correlation. An increased ocular volume in larger myopic eyes has been suggested to mask pulse-induced volume changes, therefore resulting in decreased pulse amplitudes (Silver and Geyer 2000). However, the results reported in this study showed larger correlation between axial length and pulsatile parameters than that between vitreous chamber volume and OBFa values. Thus, it could be argued that changes in vitreous chamber volume due to ocular blood pulsations may be significantly more affected by axial length than by vitreous chamber volume. Larger axial lengths may be linked to true reduced pulse amplitudes, which may represent a potential reduction in total pulsatile blood flow. The relationship described between increased axial length and scleral rigidity has also been suggested to play a role in masking ocular pulse amplitude readings (Honmura 1968). However, the significant correlation obtained in this study between axial length and perfusion features of the CRA could be considered an additional factor to support the effect of axial length on ocular blood flow, at both choroidal level (pulsatile blood flow) and retrobulbar level (CRA).

Figure 3.6 shows the vascular parameters that differed between the refractive groups as a function of MSE. The six graphs presented describe a trend until MSE 4 to 6 dioptres, beyond where the linear profile becomes irregular, with peaks and troughs that illustrate an increase or decrease in the vascular values. Therefore, the fluctuations in linearity found beyond 4 dioptres may be linked to the differences in ocular perfusion found between emmetropes and high myopes. The dioptric power chosen to delimit the boundary between low and high myopia in this study was -5.00D. Therefore, part of the participants whose results induced fluctuations in the MA series (those with MSE ranging -4.00D to -6.00D) were in the group defined as low myopes. Therefore, significant differences may not have been found in this study between low and high levels of myopia due to the criteria followed to determine the refractive subgroups. These results may also suggest a

declining vascular supply in healthy human myopic eyes beyond refractions of 4.00 to 6.00 D of myopia or an axial length of 25mm.

Additionally, it is of special interest to observe the complementary pattern given by OBFa and CRA RI in figure 3.6. It is noticeable that peaks in the pulsatile ocular blood flow amplitude graph correspond with troughs in the CRA RI graph. This relationship was further analysed by Pearson's correlation between OBFa and CRA RI. No significant correlation was found, which may partly be due to the use of different instruments to assess different vascular beds. However, both choroid and CRA are pulsatile structures, which would explain that an increase in pulse amplitude looked complementary to a decrease in resistance index.

Figure 3.7 shows the profile of pulsatile flow and CRA blood flow as a function of AL. The graphs describe a linear trend, which suggest a linear effect of axial length on the ocular perfusion features of human myopia. In an attempt to comprehend the effect of axial length on the ocular perfusion features of the myopic eye, further analysis was performed.

The data obtained from the 66 participants were subdivided into 3 axial length-based groups based on reports suggesting high myopes to have an AL above 25 mm (Swann and Schmid 2002; Tano 2002). The first group (AL1) comprised 22 subjects whose axial length ranged from 22m to 23.85 mm. The second group (AL2) had 22 subjects with axial lengths ranging between 23.86 mm and 24.93 mm. The third group (AL3) consisted of 22 subjects with axial lengths above 24.95 mm. The three groups were matched for age and gender. AL1, AL2 and AL3 only showed differences in OBFA readings, with no significant changes in retrobulbar or retinal microvascular circulation. OBFa ($p=0.003$), OBFv ($p=0.003$) and POBF ($P=0.003$) were decreased in the group with longest axial length. These results support the lack of significant differences in microretinal vessels found between emmetropes, low myopes and high myopes.

These results confirm the linear correlation between AL and pulsatile ocular blood flow, as no significant decrease is observed from a specific axial length value, contrary to the profile described by the refractive error.

Our results appear to support the push-pull theory by Walls, being, however, aware of the limitations of this study, such as the assumptions of the OBFA system calculation. Lack of longitudinal data does not allow this study to evaluate whether the decrease in pulsatile and retrobulbar blood flow occurs prior to the progression of myopia in human eyes, or whether the eye becomes myopic, thereby resulting in decreased ocular circulation. Chapter seven of this thesis uses Laser Doppler Flowmetry data (Riva et al), which measures choroidal blood flow, and it will

help unveil the choroidal perfusion features of the myopic eye by overcoming eye volume and scleral rigidity assumptions known to affect POBF readings. Additionally, *in-vivo* evaluation of retinal and choroidal thickness in chapter eight will help understand the hypothesised relationship between retinal and choroidal blood flow and thickness.

Another limitation of the study may be a lack of subjects with MSE greater than -10.00 D, which would have offered a wide vascular perspective of myopia. However, the inclusion criterion was such that myopes greater than 10D, who are more likely to have associated pathologies, would not bias the results obtained. This study, therefore aimed to evaluate healthy myopic eyes, free of retinal pathologies. To our knowledge, there are no studies evaluating the vascular features of myopia by combining three different techniques (i.e. assessing the different ocular vascular beds), which limits the comparison of our results with studies by other research groups.

In summary, high myopes showed a significantly lower ocular pulse amplitude and CRA blood velocity, which was accompanied by a significantly higher CRA RI. The retinal microcirculation did not differ between refractive groups and only in emmetropes was the retinal microcirculation correlated with systemic and ocular perfusion pressure, which indicates the influence of the systemic blood pressure on the microvascular circulation of myopes. The decrease in pulsatile parameters and retrobulbar velocities correlated with the degree of myopia and increased axial length.

3.2 Study two

The effect of age on the perfusion features of the human myopic eye

3.2.1. Abstract

Purpose: To assess the effect of age on the perfusion features of the human eye.

Methods: The retrobulbar vessels (Colour Doppler Ultrasound, CDI), retinal microvessels (Heidelberg Retina Flowmeter, HRF), and ocular pulsatility (Ocular Blood Flow Analyser, POBF) were assessed in one eye (randomly selected) of each of 96 healthy volunteers subdivided into two age groups: a) A younger group comprising 48 subjects whose age ranged from 19 to 30 years (average age 24.83 ± 4.16 years; mean MSE -2.18 ± 2.81D), b) A mature group (over 40years) of 48 participants with ages ranging from 42 to 78 years (average age 55.27 ± 10.99 y; mean MSE -1.90 ± 3.03D).

The two groups were matched for gender and ethnicity, and they did not differ significantly in the mean distribution of refractive error or axial length. Blood pressure, intraocular pressure, and body mass index were recorded to evaluate potential correlations with ocular blood flow parameters.

Results: There were no significant differences in height, weight, body mass index or MSE between the two groups. The older group exhibited higher systolic (SBP) and diastolic blood pressures (DBP) ($p=0.002$; $p<0.001$ respectively) as well as higher intraocular pressure (IOP); there was no difference in ocular perfusion pressure (OPP) between the groups. Ophthalmic artery end diastolic velocity (OA EDv) was significantly higher in the older group ($p=0.007$). The ratio between the peak systolic and end diastolic velocities in the OA (SDratio) and the pulsatile ocular blood flow (POBF) were significantly reduced in subjects >40 years ($p<0.001$ and $p=0.008$ respectively).

Increased age exhibited a significant trend towards higher IOP values ($R=0.26$; $p= 0.03$), decreasing POBF ($R=0.27$; $p=0.03$), maximum retinal microvessels flow and velocity ($R=0.26$; $p= 0.03$) and additionally with an increase in systolic and diastolic systemic blood pressure ($R=0.2$; $p<0.001$).

Conclusions: Pulsatile ocular blood flow and retinal microvasculature flow significantly reduced with age. The decrease in the ratio between the peak systolic and end diastolic blood velocity (SDratio) may also infer a decreasing vessel area with age, which would explain the increased susceptibility of the senescent eye to ocular vascular pathologies.

3.2.2 Introduction

The previous study provided a detailed analysis of the ocular and systemic vascular characteristics of the young human myopic eye. However, in order to understand the haemodynamics of human myopia, it is necessary to provide an insight into the vascular features of the ageing human eye. Ageing is associated with several well-recognized changes in the cardiovascular system such as an increase in systolic and diastolic blood pressures and a reduction in cerebral blood flow and velocity among others (Fleg 1986b; Kannel and Gordan 1978). Therefore, an exhaustive and complete evaluation of the haemodynamics of human myopia should include the effect of age on ocular blood flow and the relationship between ocular blood flow and systemic blood changes with age.

Ageing is associated with numerous changes in the heart at the cellular and biochemical level (Pugh and Wei 2001). Many of the changes observed in the cardiovascular systme of elderly people, such as increased blood pressure, are cardiac adjustments to compensate for arterial stiffening with age. Those cardiac and vascular changes can be grouped into morphological and functional changes (Cheitlin 2003).

With age, the cardiac morphology reveals a decrease in the myocyte number, as well as an increase in size and stiffening of myocyte cells (Olivetti, *et al.* 1991). Myocytes are muscle cells located in the heart, also called cardiac myocytes, whose size is approximately 25 μ in diameter and 100 μ in length. The myocyte is composed of bundles of myofibrils, composed in turn by distinct repeating microanatomical units, called sarcomeres, each of which is composed of thick and thin filaments (myosin and actin, respectively). Chemical and physical interactions between actin and myosin cause the sarcomere length to shorten, and therefore the myocyte to contract during the process of excitation, also called contraction coupling. The interactions between actin and myosin serve as the basis for the sliding filament theory of muscle contraction. Impaired excitation-contraction mechanisms, calcium homeostasis, myocyte apoptosis and growth controlling factors have been reported with advancing age (Priebe 2000). The cardiac function is therefore affected by age and shows a decrease in myocardial contraction velocity and coronary blood flow reserve, accompanied by an increase in myocardial contraction time, myocardial stiffness and ventricular filling pressures (Gardin, *et al.* 1998; Lakatta 1994).

The principal variations of the vascular morphology with age are an increase in diameter and stiffness of large arteries, an increase in medial and intimal thickness, and changes in vascular cell proliferation and vascular wall matrix (Lakatta, *et al.* 1997; Lakatta 1994; Wei 1992). Reported changes in vascular function include a decrease in β-adrenoceptor mediated vasodilation, as well

as a decrease in nitric oxide production and an increase in the pulse wave velocity (Groenink, *et al.* 1999; Lakatta, *et al.* 1997; Lakatta 1994; Wei 1992).

The influence of age on the retinal and ONH circulation has been previously evaluated with different vascular techniques such as the scanning laser Doppler flowmeter, pulsed Doppler sonography, laser Doppler flowmetry and colour Doppler imaging. The results reported a significant decrease in retinal and ONH blood flow that appears to be accompanied by a decrease in choroidal and foveolar blood flow (Embleton, *et al.* 2002; Mori, *et al.* 2001; Harris, *et al.* 2000; Gillies, *et al.* 1999; Dallinger, *et al.* 1998; Grunwald, *et al.* 1998; Groh, *et al.* 1996; Ravalico, *et al.* 1996). On average, only one instrument was used in each study, which restricts gaining a wider understanding of the perfusion features of the aging eye. Additionally, the data from most of those studies were matched for gender; whereas only Mori and colleagues matched the sample for axial length. Evaluating the relationship between the systemic circulation and the blood supply in the aging eye would also provide a wider approach on the haemodynamics of the aging eye.

3.2.3 Aims and objectives

The aim of this study was to further the understanding of the effect of age on ocular blood flow by assessing different vascular beds on two age groups matched for axial length and MSE. The retrobulbar supply was evaluated using CDI, whereas ocular pulsatile blood flow and foveal capillaries was assessed with OBFA and HRF respectively. Additionally, the potential relationship between ocular blood flow and systemic blood supply was examined.

3.2.4 Methods

3.2.4.1 Study Sample and Recruitment Criteria

96 healthy volunteers were evaluated for the study. All volunteers were recruited from the student and staff population of Aston University.

Since a physiological correlation is known to exist between the two eyes of the same subject, only one eye randomly chosen from each subject was evaluated (Kimura, *et al.* 2003).

Our hypothesis was to test whether patients above the age of 40 exhibited a reduced ocular blood flow compared to subjects below the age of 30. Therefore, the subject sample consisted of one eye from 96 healthy volunteers divided into two groups according to their age. The younger group consisted of 48 subjects whose age ranged from 19 to 30 years (average age 24.83 ± 4.16 years; mean MSE -2.18 ± 2.81D), whereas 48 participants with ages ranging from 42 to 78 years (average age 55.27 ± 10.99 years; mean MSE -1.90 ± 3.03D) formed the older group. Forty was the age

chosen to delimit the older group, as it is in this age that systemic and ocular-related physiological changes such as increase in blood pressure, change in levels of natural hormones and decrease in ocular accommodation amplitude occur (Gavrilov and NS 2006), and the age beyond which diseases with a potential vascular basis, such as glaucoma, are more likely to onset. The two groups were matched coincidently for MSE, axial length, gender and ethnicity (i.e. each subject was individually matched for MSE, axial length, gender and ethnicity with a subject from the other age group).

All the participants were healthy volunteers, which was confirmed by the author by investigation the fundus using Volk lens (90D), recording blood pressure and a detailed recording of systemic and ocular history and symptoms. Exclusion factors included any ocular disorder, diabetes, hypotension, hypertension or any other systemic disorder or medication likely to affect the systemic or ocular vasculature. Those subjects exhibiting any sign of myopic fundus changes (lacquer cracks, staphylomas, chorioretinal atrophy, lattice degeneration or pavingstone degeneration) were not included in the study. Since no blood test was performed, all of the previous were self-reported by the participants. A corrected visual acuity of 6/9 or better was required together with astigmatism of less than 1.5 diopters cylinder, which reduced the percentage of astigmatic patients being taken as myopic subjects after MSE calculation.

In total 16 subjects were found to be smokers, 8 of which were below the age of 30 (mean age 24.32 ± 3.66 years) and 8 were above the age of 40 (mean age 45.81 ± 4.67 years).

3.2.4.2. Ethical Approval and Informed Consent

Written and verbal information about the exact procedures to be performed during the visit was given to all the subjects prior to data collection. The participants were encouraged to ask any questions and to clarify any doubts they might have before signing the written consent. The volunteers were requested to sign two copies of the written consent, one of which was given to them for their own records. All investigations were approved by the Ethical Review Committee and conformed to the declaration of Helsinki (appendix 2.1).

3.2.4.3 Study Sample: Dietary Restrictions

Alcohol, nicotine and caffeine containing products have been reported to affect the flow of blood in the eye (Domino, *et al.* 2004; Gdovinova 2001). To ensure the reliability of the data, the participants recruited for this study were asked to refrain from smoking or consuming alcohol and caffeine containing products 12 hours prior to the study.

Exercising is to increase the mean arterial pressure (MAP); however the maximal BP falls rapidly on stopping exercise (Tzemos, Lim and MacDonald 2002). Therefore, since the total duration of

the experiment was approximately 1hour and the vascular readings were performed approximately in the last 30 minutes, the potential effect of exercising on the systemic circulation was minimal. Thus, the subjects were not asked to refrain from exercising before the appointment.

3.2.4.4 Experimental Protocol and Investigations

The instruments and experimental procedure followed in this second study of chapter one were the same as those followed in study one (section 3.1.4.4).

3.2.4.5 Statistical analysis

A one-way ANOVA was used to test for significance between the two age groups for each blood flow parameter. All data are given as mean ± SD. Bonferroni correction was performed to correct for multiple comparisons when associated parameters calculated from the same piece of equipment were compared. Bonferroni correction aimed to avoid creating type I errors.

Pearson's bivariate linear correlation analysis was used to determine the relationship between ocular vascular parameters and systemic circulation, MSE, axial length and vitreous chamber volume.

ANCOVA explored the differences between age groups while controlling for those vascular systemic variables that showed significant correlation with each of the age groups independently.
A two-way ANOVA was performed to assess the interaction between age and refractive error on the ocular vascular parameters.

Statistical significance was defined as $p<0.05$.

3.2.5 Results

There were no significant differences in gender, height, weight, body mass index or MSE between the young and the adult group. The systemic circulation differed significantly between the two age groups, with the older subjects exhibiting higher systolic and diastolic blood pressures ($p=0.002$; $p<0.001$). Pulse, MAP and OPP were not significantly different (table 3.9).

Two major statistical approaches were performed to analyse these data. Firstly, the vascular parameters obtained in the older group were compared with those from the younger matched sample to evaluate the effect of age and its relationship with systemic circulation. Subsequently, the interaction between age and refractive error, and age and axial length on ocular blood flow was evaluated.

	Subjects < 40	Subject > 40	p
N	48	48	n/a
Ethnicity	33w; 15a	36;12a	n/a
Gender	20m; 28f	20m; 28f	ns
Age (years)	24.83 ± 4.16	55.27 ± 10.99	*<0.001
MSE	-2.18 ± 2.81	-1.90 ± 3.03	ns
Systolic BP (mmHg)	109.06 ± 15.19	119.87± 17.47	* 0.002
Diastolic BP (mmHg)	64.93 ± 11.01	77.00 ± 9.90	*<0.001
Pulse rate	71.77 ± 9.94	68.25 ± 9.94	ns
MAP (mmHg)	90.45 ± 16.38	94.95 ± 15.14	ns
OPP	47.12 ± 11.38	47.76 ± 10.63	ns
Height (m)	1.70 ± 0.10	1.68 ± 0. 09	ns
Weight (kg)	69.47 ± 15.71	74.68 ± 12.61	ns
BMI	23.84 ± 4.00	25.55 ± 4.95	ns
Axial length (mm)	24.22 ± 1.17	24.55 ± 1.38	ns
ACD (mm)	3.59 ± 0.34	3.42 ± 0.37	*0.021
VCD (mm)	5.42 ± 0.87	5.89 ± 0.84	ns
CT (μm)	553.76 ± 40.52	561. 54 ± 43.06	ns
IOP (mmHg)	13.47 ± 3.34	15.49 ± 3.77	*0.007

Table 3.9 *Summary of physical, ocular and vascular systemic features in the two age groups (ns: not significant * statistically significant).*

3.2.5.1 Effect of age on ocular haemodynamics, ocular biometry and systemic blood supply

Ocular biometry

The two age groups showed no differences in axial length, corneal thickness and vitreous chamber volume; however, there was a significant decrease in ACD in those patients over the age of 40 (p= 0.021). Additionally, IOP showed a significant increase with age (p= 0.007) (table 3.9).

Pulsatile ocular blood flow

After comparing OBFA results from the two age groups, a significant lower pulsatile ocular blood flow (POBF) (p=0.008) was observed in the older group.

IOP was assessed as a potential covariate to be included in the analysis but it did not show significant correlation with OBFA parameters, therefore, as it did not satisfy the ANCOVA assumptions, IOP was not included in the analysis. Table 3.10 and figure 3.8 summarise OBFA findings by age group.

	Subjects < 40y	Subject > 40y	p
OBFiop (mmHg)	10.54 ± 2.59	13.08 ± 4.60	**0.007
OBFa (mmHg)	3.05 ± 1.15	3.10 ± 1.14	ns
OBFv (µl)	8.40 ± 2.60	7.36 ± 3.21	ns
OBFr	65.46 ± 12.86	64.58 ± 9.27	ns
POBF (µl/min)	1184.38 ± 287.63	971.67 ± 369.67	**0.008

Table 3.10 *Pulsatile ocular blood flow outcomes for the different age groups (ns: not significant difference ** statistically significant)*

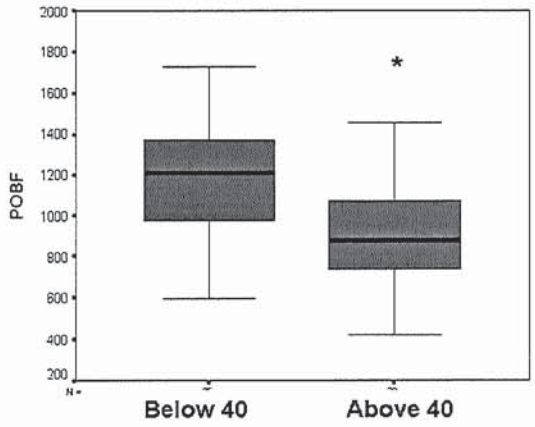


Figure 3.8 *OBFA results for each age group in the form of box plots (OBFiop, OBFv: volume of pulsatile blood flow; OBFa: amplitude ocular pulse; OBFr: rate of ocular pulse) * statistically significant*

VESSEL	PARAMETER	Subjects < 40	Subject > 40	p
OA	**PSv (m/s)**	0.434 ± 0.14	0.492 ± 0.23	ns
	EDv (m/s)	0.096 ± 0.04	0.140 ± 0.07	*0.001
	RI	0.776 ± 0.06	0.783 ± 0.45	ns
	Ratio	4.969 ± 1.56	3.72 ± 1.39	*<0.001
CRA	**PSv (m/s)**	0.120 ± 0.02	0.117 ± 0.03	ns
	EDv (m/s)	0.044 ± 0.01	0.054 ± 0.07	ns
	RI	0.636 ± 0.08	0.631 ± 0.07	ns
	Ratio	2.947 ± 0.75	2.820 ± 0.59	ns
SPCA	**PSv (m/s)**	0.143 ± 0.04	0.147 ± 0.04	ns
	EDv (m/s)	0.064 ± 0.02	0.061 ± 0.03	ns
	RI	0.584 ± 0.11	0.583 ± 0.09	ns
	Ratio	2.657 ± 1.04	2.550 ± 0.72	ns

Table 3.11 *Results obtained at the retrobulbar vessels using CDI by age group (ns: not significant difference ** statistically significant after Bonferroni correction).*

Retrobulbar data

OA EDv was significantly higher in the older group (p=0.001), which was accompanied by a significantly lower ratio between systolic and diastolic velocity (p<0.001). All the results obtained in the retrobulbar vessels are shown in table 3.11, figure 3.9a and figure 3.9b.

Retinal capillaries

There was no significant difference in microretinal circulation between the young and the older group (table 3.12 and figure 3.10).

	PARAMETER	Subjects < 40	Subject > 40	p
MAX	**Volume (AU)**	24.49 ± 9.40	22.29 ± 6.91	ns
	Flow (AU)	436.94 ± 190.72	381.87 ± 122.59	ns
	Velocity (AU)	1.51 ± 0.58	1.31 ± 0.38	ns
MIN	**Volume (AU)**	20.22 ± 23.24	19.76 ± 24.63	ns
	Flow (AU)	256.36 ± 126.42	222.77 ± 104.50	ns
	Velocity (AU)	1.07 ± 0.40	0.89 ± 0.32	ns

Table 3. 12 *Maximum and minimum blood volume, flow and velocity measured in the retinal capillaries in each age group using HRF (ns: not significant)*

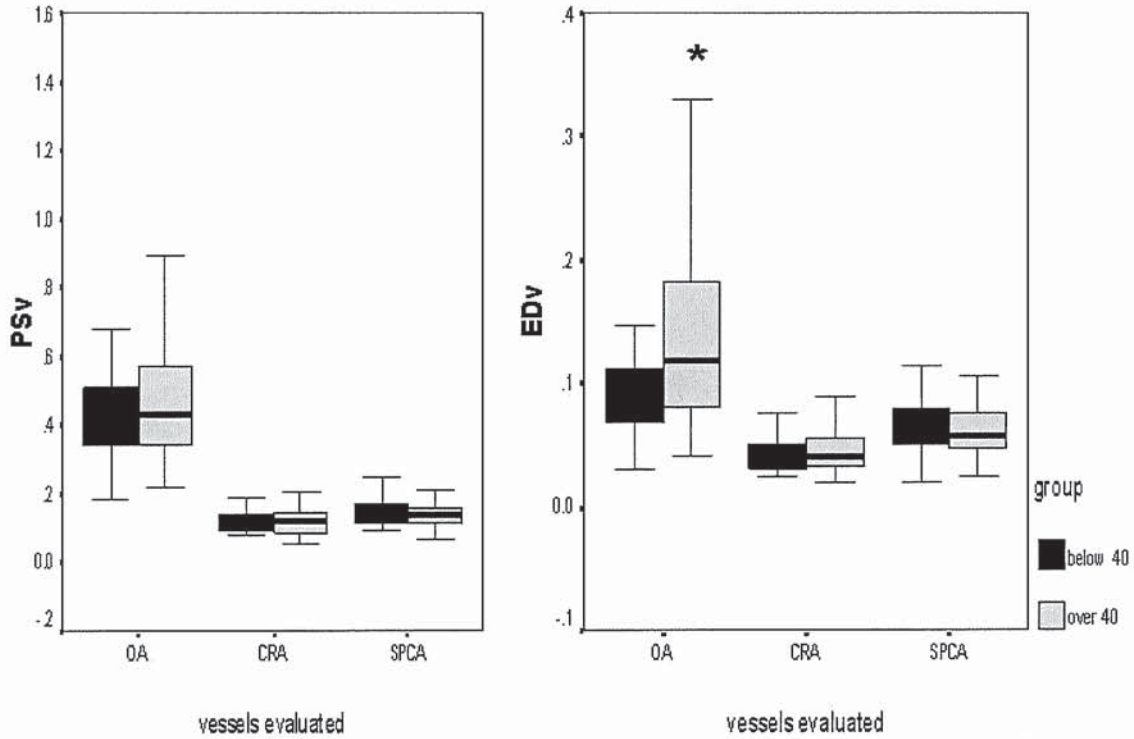


Figure 3.9a. *Peak systolic (PSv) and end diastolic velocities in the OA, CRA and SPCA measured using CDI for each refractive group given in the form of box plot * statistically significant*

3.2.5.2 Correlations between systemic blood pressure, ocular perfusion pressure and ocular blood flow

Evaluation of the correlations between the pulsatile components of ocular blood flow and systemic blood pressure demonstrated a significant correlation between systolic and diastolic blood pressure (SBP, DBP) and OBFiop (for SBP, R=0.42 p<0.001; for DBP, R=0.50 p<0.001), OBFv (for SBP, R=-0.24 p=0.04; for DBP, R=-0.30 p=0.01) and POBF (for SBP, R=-0.30 p=0.01; for DBP, R=-0.38 p=0.001). Additionally, the pulse rate correlated with ocular pulse amplitude (R=-0.34 p=0.004), volume (R=-0.30 p=0.01) and rate (R=-0.38 p=0.001).

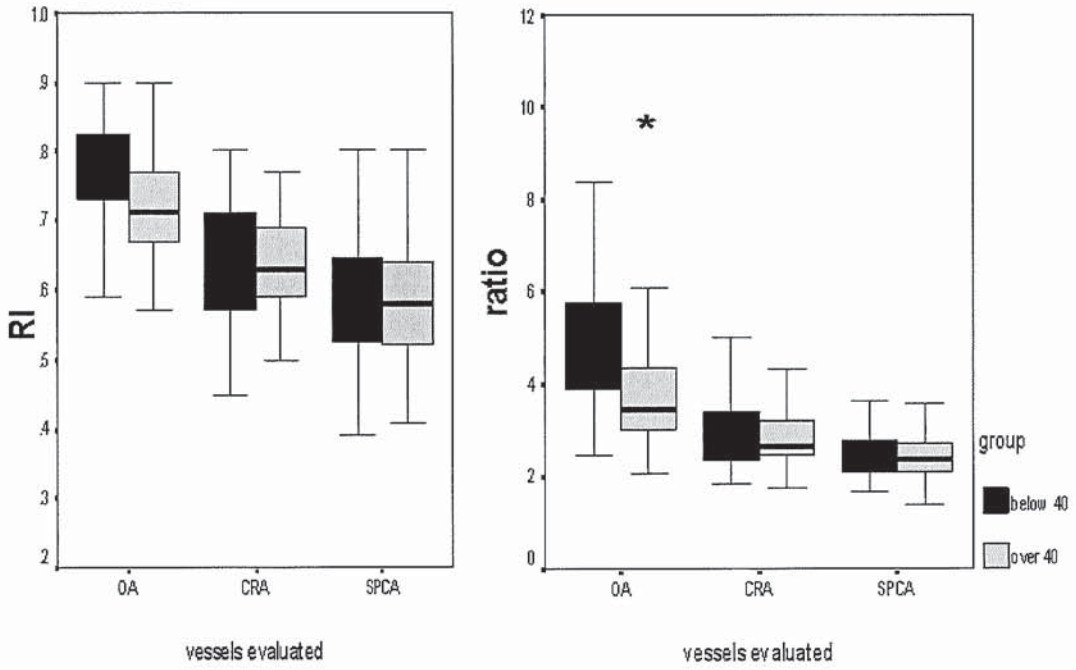


Figure 3.9b. *Peak systolic (PSv) and end diastolic velocities in the OA, CRA and SPCA measured using CDI for each age group given in the form of box plot * statistically significant*

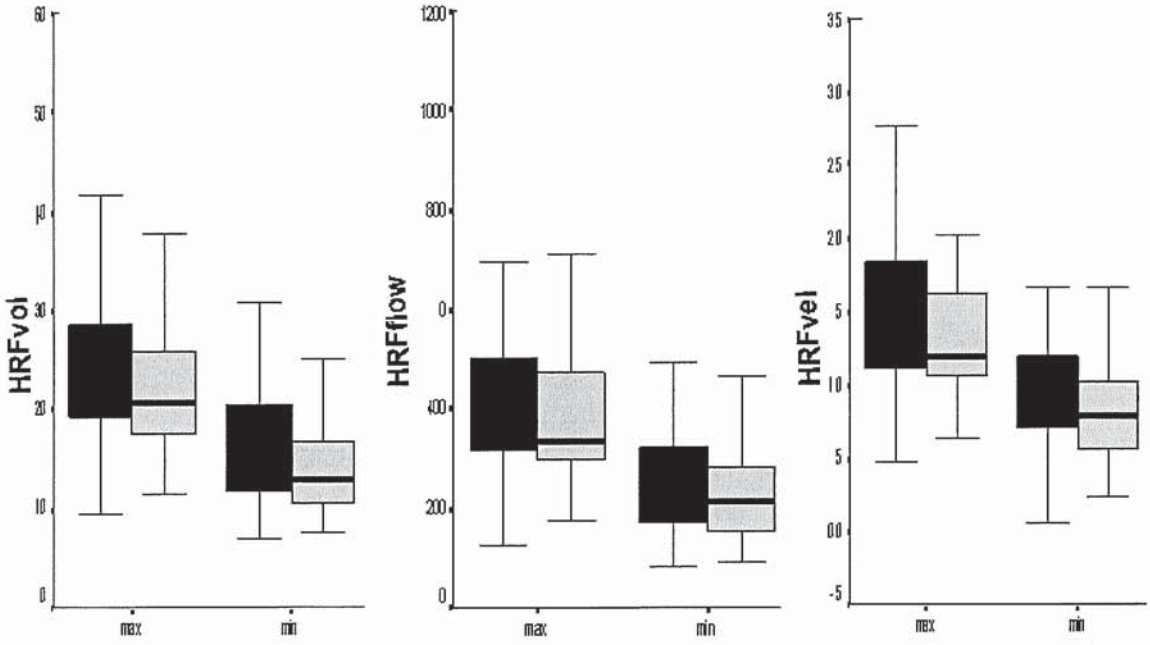


Figure 3.10 *Maximum and minimum blood volume (HRFvol), flow (HRFflow) and velocity (HRFvel) in the foveal capillaries measured using HRF by age group.*

The systolic phase of the systemic blood pressure correlated significantly with OA systolic velocity (R=0.24 p=0.02); whereas the diastolic phase correlated significantly with OA diastolic velocity (R=0.31 p=0.003), with the systolic to diastolic velocities of the OA (R=-0.45 p<0.001), CRA (R=-0.37 p<0.001) and SPCA (R=-0.25 p=0.017) and with the resistance index at the SPCA (R=-0.23 p=0.017).

MAP and OPP were not found to correlate with ocular pulsatile or retrobulbar blood flow components. However, MAP and OPP significantly correlated with the maximum blood volume, flow and velocity at the retinal microvessels (R=0.25 p=0.025; R=0.25 p=0.024; R=0.24 p=0.029 respectively for MAP), (R=0.32 p=0.004; R=0.34 p=0.002; R=0.32 p=0.003 respectively for OPP). Additionally, OPP also correlated with retinal capillaries minimum values of blood volume, flow and velocity (R=0.34 p=0.002; R=0.27 p=0.012; R=0.28 p=0.011 respectively).
The correlations between systemic circulation and ocular blood flow parameters are summarised in table 3.13.

3.2.5.3 Differences in ocular blood flow analysis between age groups correcting for systemic circulation

ANCOVA was used to investigate the differences between age groups while controlling for systemic circulation. There are several assumptions that must be checked before performing ANCOVA, all of which were checked for this analysis. Assumptions:

1. The covariate should be measured prior to main data collection
2. The covariate should correlate with each group independently
3. The correlation should be linear
4. The slopes of the independent correlations should be different

Each ocular vascular variable evaluated was tested against systolic and diastolic blood pressure, heart rate, MAP and OPP. ANCOVA was performed only on those systemic vascular parameters that agreed with the assumptions mentioned above, which were as follows:

a. Diastolic blood pressure for OBFiop and OA ratio
b. Pulse for OBFr
c. OPP for HRFmaxflow

The results obtained after ANCOVA analysis showed no significant difference between in OBFiop, OA ratio, OBFrate and HRFmaxflow the two age groups after correcting for diastolic blood pressure, pulse and OPP respectively (p=0.66; p=0.75; p=0.055; p=0.064).

Parameter	SBP Pearson's R	P *	DBP Pearson's R	P *	Pulse Pearson's R	P *	MAP Pearson's R	P *	OPP Pearson's R	P *
OBFiop	0.42	<0.001	0.50	<0.001	0.02	ns	0.16	ns	-0.05	ns
OBFa	-0.02	ns	-0.06	ns	-0.38	0.004	-0.14	ns	-0.20	ns
OBFv	-0.24	0.04	-0.30	0.013	-0.33	0.007	-0.19	ns	-0.16	ns
OBFr	-0.10	ns	0.005	ns	0.50	<0.001	0.03	ns	-0.01	ns
POBF	-0.30	ns	-0.39	0.001	-0.01	ns	-0.10	ns	-0.04	ns
OA PSv	0.24	0.02	0.05	ns	-0.01	ns	-0.02	ns	-0.07	ns
OA EDv	0.18	ns	0.31	0.003	-0.004	ns	0.08	ns	0.02	ns
OA RI	0.02	ns	-0.06	ns	0.05	ns	-0.06	ns	-0.05	ns
OA Ratio	-0.07	ns	-0.45	<0.001	0.004	ns	-0.12	ns	-0.10	ns
CRA PSv	0.19	ns	0.04	ns	-0.05	ns	0.10	ns	0.09	ns
CRA EDv	0.09	ns	0.12	ns	0.02	ns	0.03	ns	0.03	ns
CRA RI	-0.15	ns	-0.14	ns	0.12	ns	-0.18	ns	-0.11	ns
CRA Ratio	-0.12	ns	-0.37	<0.001	-0.03	ns	-0.07	ns	-0.09	ns
SPCA PSv	0.18	ns	0.001	ns	0.07	ns	0.10	ns	0.18	ns
SPCA EDv	0.09	ns	0.012	ns	0.06	ns	0.10	ns	0.15	ns
SPCA RI	0.02	ns	-0.27	0.013	0.02	ns	-0.08	ns	0.10	ns
SPCA Ratio	-0.04	ns	-0.25	0.017	0.04	ns	-0.03	ns	-0.04	ns
HRF MaxVolume	-0.09	ns	-0.17	ns	0.05	ns	0.25	0.02	0.32	0.004
HRF MaxFlow	-0.03	ns	-0.10	ns	0.06	ns	0.25	0.024	0.34	0.002
HRF MaxVelocity	-0.06	ns	-0.11	ns	0.08	ns	0.24	0.03	0.32	0.003
HRF MinVolume	0.08	ns	-0.02	ns	-0.10	ns	0.26	0.02	0.34	0.002
HRF MinFlow	0.04	ns	0.06	ns	0.07	ns	0.16	ns	0.27	0.012
HRF MinVelocity	-0.18	ns	-0.20	ns	-0.14	ns	0.24	0.03	0.28	0.01

Table 3.13 *Correlations between ocular blood flow parameters and systemic circulation.*

3.2.5.4 Effect of age, refractive error and axial length on ocular haemodynamics.

To evaluate the relationship between age and ocular blood flow, the ocular vascular readings obtained from patients aged 30 to 40 that were not considered for the previous analysis were added to this correlation analsysis (mean age 37.02 ± 16.84 years, age range 18 to 78 years).

The results obtained from bivariate correlation analysis showed how age correlated significantly with POBF, OA ratio, CRA ratio and HRF maximum flow (figure 3.11 to figure 3.15).

MSE and axial length significantly correlated with IOP and ocular pulse amplitude, volume and flow. MSE also correlated with CRA systolic velocity (table 3.14).

3.2.5.5 Analysis of the interaction effect between age and refractive error on ocular blood flow

Two-way ANOVA allows the evaluation of two independent variables (e.g age and refractive error) on one dependent variable (e.g each of the ocular vascular parameters assessed). None of the ocular vascular variables showed significant interaction with age and refractive error (appendix 1.3).

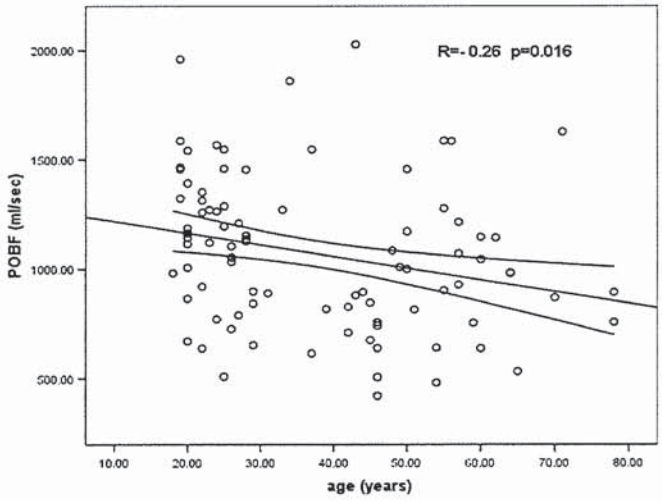


Figure 3.11 *Scatter plot showing the significant correlation between age and POBF.*

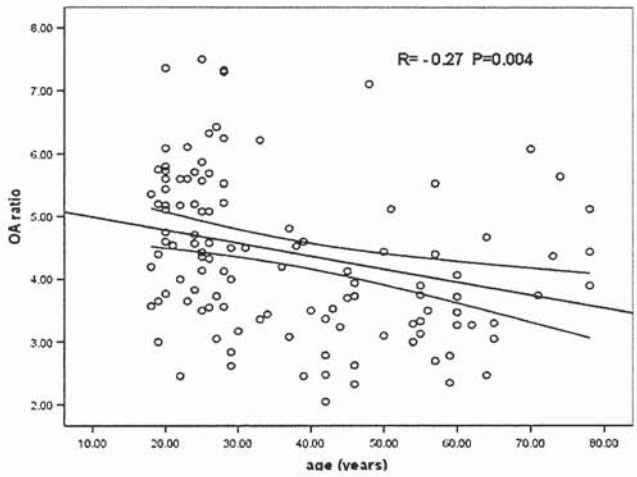


Figure 3.13 *Scatter plot showing the significant correlation between age and OA ratio*

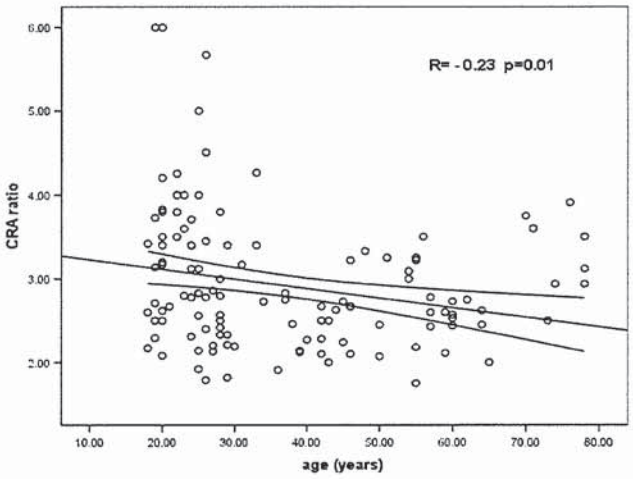


Figure 3.14 *Scatter plot showing the significant correlation between age and CRA ratio*

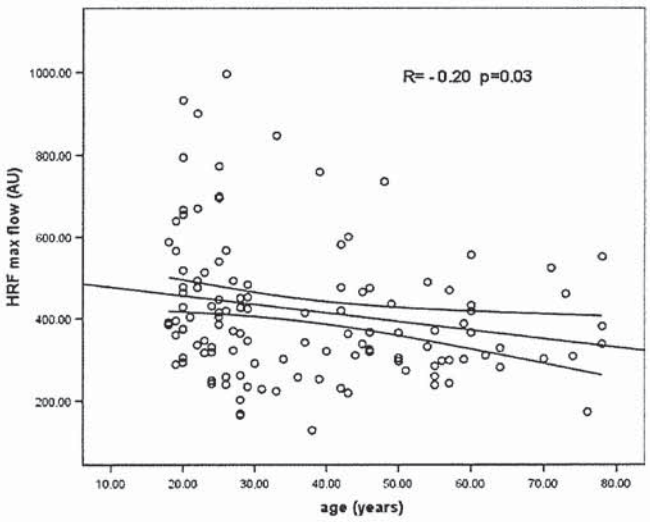


Figure 3.15 *Scatter plot showing the significant correlation between age and the maximum flow in the retinal microvessels (AU: arbitrary units)*

Parameter	MSE Pearson's R	p	AL Pearson´s R	p
OVERALL ANALYSIS				
OBFiop (mmHg)	-0.13	ns	0.30	*0.012
OBFa (mmHg)	0.30	*0.012	-0.29	*0.017
OBFv	0.29	ns	-0.37	*0.002
POBF (µl/min)	0.30	*0.01	-0.44	*< 0.001
CRA PSv (m/s)	0.24	*0.02	-0.17	ns
Subjects < 40				
POBF (µl/min)	0.34	*0.046	-0.49	*0.003
Subjects > 40				
OBFa (mmHg)	0.40	*0.018	-0.47	*0.004
OBFv (µl)	0.30	ns	-0.41	*0.013
POBF (µl/min)	0.30	ns	-0.36	*0.029
OA ratio (m/s)	0.27	ns	-0.30	*0.05
CRA PSv (m/s)	0.30	*0.045	-0.32	*0.031

Table 3.14 *MSE and AL correlations with ocular vascular readings performing a) overall analysis; b) young group analysis; c) adult group analysis (ns: not significant *statistically significant)*

3.2.6 Discussion

The objective of this second study of chapter three was to evaluate the effect of age on the ocular haemodynamics to further understand the systemic and ocular vascular features of human myopia. After comparing two samples matched for axial length, MSE and ethnicity, no differences were observed in height, weight, BMI or corneal thickness between the participants below and above the age of 40 years. Additionally, MAP and OPP were not found to differ between the two age groups either. IOP and systolic and diastolic blood pressures, however, were significantly higher in the older group. No differences were described at the retinal capillary level, but a significantly lower ocular pulse amplitude and pulsatile blood flow were found. The OA PSv and EDv in the older group were higher than in the young group. The level of correlation between the systemic blood pressure and ocular blood flow varied between age groups. In the young group,

MAP and OPP correlated with the blood velocity in the CRA, SPCA and retinal capillaries; whereas only the retinal microvessels appeared correlated with MAP and OPP in the older group.
Age correlated with IOP, pulsatile blood flow, the OA systolic to diastolic velocities ratio and the velocity, flow and volume of blood in the retinal capillaries.

3.2.6.1 Differences in ocular haemodynamics, ocular biometry and systemic blood supply between the two age groups

Ocular and body biometry

The two age groups assessed were matched for gender and ethnicity, and no statistical differences were found in MSE and axial length. Corneal thickness did not differ between groups, however the adult group exhibited a significantly decreased anterior chamber depth. The ACD was measured using IOLMaster, which has been proved to be a valid technique for ACD assessment (Hashemi, *et al.* 2005). The reduction in ACD with age found in this study is in agreement with previous papers in which a decrease in ACD with age was also reported (Bourne and Alsbirk 2006; Klatt, *et al.* 2006; Rabsilber, *et al.* 2006). It has been hypothesised that the age-related decrease in ACD, which was also found to correlate with an age-related decrease in anterior chamber volume, may be due to the increasing maturation of the crystalline lens with age and the decrease in the accommodation ability known to occur from the 3rd to the 5th decade (Klatt, *et al.* 2006).

IOP in those participants over the age of 40 has been found to be significantly higher than in the subjects below the age of 40, a result that has previously been reported in several papers (Hashemi, *et al.* 2005; Rochtchina, *et al.* 2002; Carel, *et al.* 1984). However, lack of association between age and IOP has also suggested after controlling for blood pressure, which questions the nature of the relationship (Weih, *et al.* 2001). Results from investigations like the Blue Mountain Study, in which the size of the sample could be representative of the relationship between age and IOP (n=3260), recorded IOPs in subjects only above the age of 49 years, which limits interpretation of results (Weih, *et al.* 2001). Nevertheless, a study undertaken in Japan with a sample size of 200,000 healthy subjects disclosed that obesity, systolic BP, and age were the most influential factors for IOP. Subgroup analysis suggested that young, obese and hypertensive subjects had higher IOP; whereas older, thin and hypotensive had lower IOPs. Therefore, it appears that IOP is physiologically maintained by a counterbalance between the IOP-lowering effect of age and the IOP-raising effects of obesity and systolic hypertension, which are largely age dependent (Shiose 1984).

The young and old group evaluated in the present study did not differ in MSE, axial length or corneal thickness. Past papers that evaluated the effect of age on corneal thickness and found a

significant decrease in corneal thickness assessed principally participants of East Asian origin (Hong-Kong Chinese, Thai and Mongolian) (Lekskul, *et al.* 2005; Liu, *et al.* 1999; Flammer and Orgul 1998; Foster, *et al.* 1998). However, as mentioned above, no significant relationship was found between corneal thickness and age in this study, a finding that agrees with two studies the first of which assessed corneal thickness in subjects of white origin, and the second a mixed sample of Caucasian, Asian, Hispanic and African American (Stefansson, *et al.* 2005; Shimmyo, *et al.* 2003). Thus, decreased corneal thickness with age appears to be a cohort effect.

Systemic and ocular blood flow

Previous literature addressing the effect of age on the cardiovascular system highlighted those changes occurring in cardiac morphology such as the thickening of the arterial walls and decrease in collagen activity, which ultimately lead to increased vascular stiffness. In agreement with many other research publications, a significant increase in systolic and diastolic blood pressure with age was found in this study (Nagy, *et al.* 2006; Hillen, *et al.* 2000; Fleg 1986a; Kannel and Gordan 1978). Higher systolic and diastolic blood pressures are an adaptation to the biological changes occurring with age. When the walls of the vessels start thickening, the pressure in the vessels rises to ensure the minimum supply of blood. Additionally, due to arterial stiffening there is an increase in pulse wave velocity that results in an augmentation of the tension in the left ventricle and cardiac mechanical load (Priebe 2000; Lakatta 1994).

It was interesting, however, to observe no difference between mean arterial pressure and ocular perfusion pressure of the younger and older group, which may represent adjustments undergone by the arterial pressure and ocular perfusion pressure to ensure sufficient blood supply in the healthy adult eye, acting as protective mechanisms. Dallinger and colleagues reported a significant correlation between age and MAP and OPP, which we did not observed in this study (Dallinger, *et al.* 1998), which may in turn be related to the lack of significantly different MAP and OPP found in the present study.

The analysis of the pulsatile ocular blood flow demonstrated a significantly lower pulsatile blood flow for the adult group that remained significant after Bonferroni correction, which therefore reflects a reduction of POBF with age. A decrease in POBF with age has previously been reported by Ravalico and co-workers, after they assessed 105 subjects with ages ranging from 10 to 80 years (Ravalico, *et al.* 1997). Geyer and colleagues also found a significant reduction in POBF with age after evaluating 259 eyes of patients whose ages ranged from 40 to 60 years (Silver and Geyer 2000). A later study by Mori et al on Japanese subjects did not find any significant differences in POBF (Mori, *et al.* 2001). Mori and coworkers argued that the study by Lam et al did not take axial length into consideration, thus masking the actual effect of age on POBF. In our study both groups

were matched for axial length, therefore, the potential effect of axial length on POBF was controlled. The thickness of the central cornea has been reported to affect OBFiop (Gunvant, *et al.* 2004). In this study, there were no significant differences between the corneal thickness of the young and adult groups, therefore, pulsatile ocular blood flow should not have been influenced by this variable. Additionally, Lam and colleagues measured POBF in 118 eyes and measured scleral rigidity using a Schiotz tonometer. Age was found to correlate significantly with scleral rigidity, which may suggest that the reduction in POBF found with age may be due to increase in scleral rigidity. However, multiple regression analysis indicated that the most influential factor affecting POBF was ageing, which is in agreement with the POBF reduction found in the older group (Lam, *et al.* 2003).

The haemodynamic profile of the retrobulbar vessels revealed significantly higher OA EDv, as well as a higher OA systolic to diastolic velocities ratio with increasing age. Additionally, the correlation between age and OA ratio was statistically significant. Harris and co-workers and Lam and colleagues previously reported reduced OA and SPCA blood flow velocities with age using the CDI (Harris, *et al.* 2003; Lam, *et al.* 2003). They assessed the vascular profile of the retrobulbar vessels in 128 and 118 healthy subjects respectively. Both studies reported a significant negative correlation between age and OA and SPCA PSv and EDv. However, none of these studies assessed the systemic circulation or correlations between ocular and systemic blood flow. Additionally, it is well known that increased vascular stiffness with age leads to elevated systolic arterial pressure and increased pulse-wave velocity. CDI evaluates velocities at the systolic and diastolic phases of the cardiac pulse; therefore, they are waves reflecting the velocity of the pulse in the retrobulbar vessels. It could even be said that CDI analyses the pulsatile retrobulbar profile, since the OA, CRA and SPCA are known to pulsate (Priebe 2000; Lakatta 1994). If we take into consideration the significant correlation found between OA, CRA and SPCA blood velocities and systemic blood flow, higher OA velocities could be hypothesised, even if they did not exhibit statistical significance. A decrease in OA velocity, therefore, does not fit the systemic profile of the ageing circulatory system. Additionally, only the OA correlated significantly with the systemic blood pressure in the adult group, which may explain why only the OA appeared significantly increased. The OA was, therefore, the retrobulbar vessel mostly affected by the systemic circulation in those subjects over the age of 40.

The significant increase in OA velocities with age can also been explained from the theoretical point of view. The velocity of a fluid inside a tube equals the volume occupied by the fluid divided by the area travelled by the fluid as follows (equation 3.1):

$$\text{Velocity} = \text{volume}/(\text{area} \times \text{time}) \qquad (3.1)$$

If one moves the variable "area" to the other side of the equation, one would have

Velocity x area=volume/time

From which flow would equal (equation 3.2)

Flow=volume/time (3.2)

, in which flow describes velocity (distance per unit of time) travelled per unit of area. Therefore, an increase in the OA blood velocity may just represent a decrease in vessel area, which would in turn be associated with a decrease in vessels radii, known to occur with age.

There were no significant differences in CRA or SPCA vascular parameters between the two age groups. A study by Gillies and colleagues (Gillies, *et al.* 1999) assessed the CRA and SPCA in 20 participants below the age of 30, and 30 above the age of 60, and reported a significant increase in the systolic velocities of the CRA and SPCA. The OA was not evaluated. One of the reasons why our study did not find any significant differences in CRA or SPCA may have been the refractive and axial length match performed between groups. Gillies and Harris did not match for AL and MSE, and as shown in the first study of chapter four, there is a significant correlation between axial length, refractive error and CRA blood velocity. Lack of difference between CRA and SPCA velocities may also be due to the age taken to delimit the adult group. The vascular profile of a subject's eye over 40 may still not differ significantly from that of an eye below the age of 30. For this reason, the adult group was further subdivided into 2 subgroups, below and above the age of 60 (see appendix 1.4 for data). No differences were found, but it was noticeable that the three age subgroups (below 30, below 60 and above 60) differed significantly in AL and MSE. It is important to make sure that the groups evaluated do not differ significantly in AL or MSE, as CRA blood velocity correlates with these parameters. An ANCOVA was then performed, to correct for the effect of AL and MSE on the velocity of the CRA. ANCOVA analysis did not result in significant differences either. It was therefore concluded that blood velocity at the OA was the only parameter significantly affected by age, and this may possibly be due to the correlation found between the systemic circulation and OA.

The increase in blood velocity in the CRA may also be related to the adaptation mechanism resulting from the increase in BP with age reported previously, by which the pressure in the vessels rises to ensure the minimum supply of blood resulting in increased CRA blood velocity (Priebe 2000; Lakatta 1994)

The blood flow in the retinal microvessels did not show a significant difference between the age groups. A paper by Embleton and colleagues investigated the effect of age on the capillary blood flow in the retinal, neuroretinal rim and lamina cribosa. A total of 30 patients were evaluated,

which were subdivided into two groups, young (20 to 38 years) and mature (48-82). Decreased capillary blood flow at the retina, rim and lamina cribosa was reported with increasing age (Embleton, *et al.* 2002). Our study did not find a reduction in blood supply at the foveal retinal capillaries, which differs from the retinal area evaluated in the study by Embleton and co-workers. We hypothesise that the eyes of healthy subjects over the age of 40 attempt to ensure a constant blood supply to the central fovea, as the diameter of the vessel in the choriocapillaris decreases with age, accompanied by a decrease in vessels density (Ramrattan, *et al.* 1994). Therefore, if the blood supply from the choroidal layer is reduced, the blood supply at the retinal microvessels may remain unchanged with age to ensure constant blood supply to the fovea.

3.2.6.2 Relationship between the systemic and ocular vascular parameters

The systemic circulation appears to affect the vascular behaviour of the ocular blood perfusion, as observed by the correlation between pulsatile ocular blood flow and arterial systolic, diastolic and pulse pressure. These correlations suggest that the pulsatile blood flow in the eye is highly affected by variations in systemic blood pressure. Whereas the pulsatile component of blood flow has been suggested to represent 80 per cent of the total ocular blood flow, POBF does not provide information regarding the steady component of ocular blood flow (Langham, *et al.* 1989). Therefore, the pulsatile component of ocular blood flow appears largely affected by the systemic circulation. No previous study assessed the correlation between arterial blood pressure and POBF; therefore, these outcomes cannot be compared to previous research.

The systolic and diastolic velocities of the OA, CRA and SPCA showed significant correlation with arterial systolic and diastolic blood pressure (DBP). However, more parameters correlated with diastolic blood pressure (SBP) than with systolic blood pressure, which indicates a larger impact of diastolic blood pressure on retrobulbar vessels blood velocity than systolic pressure.

The vascular parameters in the retinal capillaries correlated significantly with MAP and OPP. The retrobulbar vessels and the pulsatile circulation did not show significant correlation with MAP or OPP. Thus, the behaviour of the retinal capillaries at the fovea appeared significantly more affected by the ocular perfusion pressure than by the systemic arterial perfusion pressure. This may reflect a local vascular mechanism, by which the retinal microvessels around the fovea respond actively to changes in IOP and OPP.

Additionally, there were evident differences between the correlations with arterial blood pressure between the age groups. The arterial pressure of the younger group correlated significantly with pulsatile ocular blood flow and retrobulbar velocities; whereas the arterial blood pressure of the older group was found to correlate significantly only with POBF and OA. The difference in the

correlation pattern between young and older subjects may account for local variations in the perfusion features of the aging eye which may be affected by the changes in systemic blood pressure experienced with age.

However, the assessment of the correlation between ocular vascular parameters and systemic blood pressure may not be sufficient to evaluate autoregulatory properties. For this reason, a provocation test was used to test the vascular autoregulatory features of emmetropes and myopes (see chapter four).

3.2.6.3 Vascular correlations with axial length and refractive error

The data obtained from the 96 subjects was assessed for potential correlations with AL or MSE. Negative significant correlations were found between axial length and all the pulsatile components of ocular blood flow, which described a relationship by which an increase in axial length would result in decreased ocular pulse amplitude, volume, rate and blood flow. MSE was also found to correlate negatively with the amplitude, rate and blood flow. Many papers have reported similar correlations, and it has been a topic of discussion whether decreases in POBF actually mean a decrease in flow, or whether they are just due to larger axial lengths (Lam, *et al.* 2003; Lam, *et al.* 2002; Logan, *et al.* 2002; Shih, *et al.* 1991; To`mey, *et al.* 1981). The correlation between AL and MSE with POBF variables did not vary when it was analysed by age group, suggesting a correlation across the whole age spectrum.

The CRA peak systolic velocity also correlated with MSE, which has been previously reported in a paper in which myopes with choroidal neovascularisation were assessed (Dimitrova et al. 2002). In our study, however, the participants were healthy emmetropes and healthy myopes, which supports the correlation between AL and blood velocity in healthy individuals.

When the two age groups were analysed independently, the correlations varied significantly. In the adult group, axial length correlated with POBF parameters and velocities in the OA, and in the CRA; whereas only POBF correlated with AL in the young group. These results suggest a relationship between AL and ocular haemodynamics that becomes more apparent with increasing age.

All the vascular beds assessed correlated significantly with age. A negative relationship was obtained for all the correlations, which exhibits the tendency towards a decreased ocular blood flow with age, which has been reported previously. Interestingly, however, not all the parameters that correlated significantly with age differed between the two age groups, which may represent the large variability in the percentage of change across vascular variables.

3.3 Conclusion

The perfusion characteristics of the human myopic eye differ significantly from those of the emmetropic eye. High myopia is characterised by lower CRA blood velocities at both systole and diastole accompanied by a significantly higher CRA resistance index. The pulsatile component of blood flow also appears to be significantly decreased in high myopia. These findings suggest a lower pulsatile and CRA blood flow but unaffected microretinal circulation in healthy myopes whose MSE was greater than -5.00D. Additionally, the pulsatile component of ocular blood flow and retinal microvasculature flow were significantly reduced with age, which suggests that the perfusion in the choroid and retrobulbar vessels is reduced. The decrease in SDratio may also infer a decreasing vessel area with age, which would explain the increased susceptibility of the senescent eye to ocular vascular pathologies. However, there was no interaction effect between the decrease with age and increasing myopia, possibly due to the increase in OA velocity with age, which countered the reduction found in myopia.

CHAPTER FOUR
VASCULAR AUTOREGULATORY FEATURES OF THE HUMAN MYOPIC EYE AND THE EFFECT OF AGE ON THE REGULATION OF OCULAR HAEMODYNAMICS

4.1 Abstract

Purpose: To evaluate the ocular blood flow autoregulatory properties of human myopia using a mild provocation test by which the percentage of carbon dioxide concentration (pCO_2) was significantly increased.

Methods: Retinal and choroidal circulations were analysed with Colour Doppler Imaging (CDI), Ocular Blood Flow Analyser (OBFA) and Heidelberg Retinal Flowmeter (HRF) in myopes and emmetropes at 2 sessions: baseline (B1, breathing room air) and isoxic hypercapnia (end tidal $pCPO_2$ increased approximately 15% above B1 with constant O_2 supply). CDI measured the ophthalmic artery (OA), central retinal artery (CRA) and short posterior ciliary arteries (SPCA). HRF evaluated the retinal microcirculation and OBFA measured pulsatile ocular blood flow (POBF). The results from each of thirty-three myopic subjects with ages ranging 20 to 73 years (mean age 36.78± 15.00 years; mean MSE -4.51 ± 2.56D) were compared to those from 33 emmetropes with ages ranging 18 to 78 years (mean age 40.27 ± 16.13 years; mean MSE 0.08 ± 0.41D). Both groups were matched for age, gender and ethnicity.

Blood pressure (BP), intraocular pressure (IOP), and body mass index (BMI) were also recorded.

Results: At baseline no differences in systemic or ocular blood pressure, mean arterial pressure (MAP) or ocular perfusion pressure (OPP) were found between myopes and emmetropes. Under hypercapnia, emmetropes experienced an increase in OA PSv and OA RI (p=0.008; p=0.019). Myopes exhibited an increase in POBF and microretinal blood velocity (p=0.011, p=0.020 respectively) during hypercapnia. Age correlated with the percentage change induced by hypercapnia in OA PSv (R=0.44 p=0.011), CRA PSv (R=0.47 p=0.008) and CRA EDv (R=0.57 p=0.001) only in emmetropes.

Conclusions: Evaluation of the autoregulatory features of the human eye demonstrated differences in the ocular vascular response between emmetropes and myopes. The potential regulatory response in the retrobulbar vessels to hypercapnia in emmetropes contrasted with the lack of retrobulbar reactivity in myopes. The POBF and microvascular responsiveness in myopes suggests a regulatory mechanism highly sensitive to changes in oxygenation, as shown by the lack of responsiveness described in the emmetropic group. The significant correlation between the increased retrobulbar blood velocities as a response to hypercapnia with age may be indicative of a protective mechanism occurring parallel to the decrease in vessel diameter occurring with age.

4.2 Introduction

As already mentioned in section 1.13 of this thesis, autoregulation comprises those metabolic and myogenic mechanisms that maintain constant blood supply to the tissues, despite potential variations in perfusion pressure. The body is constantly exposed to variations in perfusion pressure such as changes in arterial pressure and changes in body position among others, and it is the ability to ensure constant blood flow that preserves the function of the surrounding tissues. Ocular circulation and cerebral circulation have been demonstrated to have this autoregulatory capacity (Riva, Logean and Falsini 2005; Longo, Geiser and Riva 2004; Hafez, *et al.* 2003; Riva, *et al.* 1997; Aaslid, *et al.* 1989; Riva, Grunwald and Petrig 1986). However, autoregulation seems to be a complex phenomenon due to the wide spectrum of mechanisms involved in it, such as metabolic, myogenic, neurogenic or endothelium-mediated mechanisms.

Evaluation of the perfusion features of any vascular bed should include the assessment of the resting haemodynamic profile and, additionally, the study of its vascular properties. As mentioned in previous papers, the assessment of ocular blood flow under normal conditions may not be sufficient to detect vascular dysfunction; thereby provocation tests have become of extended use to assess the vascular autoregulatory properties of the human eye (Hosking, *et al.* 2004; Hafez, *et al.* 2003; Embleton, *et al.* 2002; Logan, Gilmartin and Cox 2002; Schmetterer 1998; Schemetterer, *et al.* 1996; Riva 1990). To provoke the excitation and regulatory response of the ocular haemodynamics, acute changes in perfusion pressure or in the metabolic conditions are needed. At present the main provocation tests use are changes in IOP, changes in oxygen partial pressure (pO_2) and changes in carbon dioxide partial pressure (pCO_2).

Artificially raised IOP has been reported to stimulate autoregulation at the optic disc, retrobulbar vessels, macula and retina (Joos, *et al.* 1999; Findl, *et al.* 1997; Riva, *et al.* 1997; Riva, Grunwald and Petrig 1986), whereas increased oxygen partial pressure concentration has been found to induce vasoconstriction and a decrease in blood flow at both the retinal and cerebral level (Chung, *et al.* 1999b; Langhans, Michelson and Groh 1997; Riva 1990; Alm and Bill 1972). Hypercapnia, or increased carbon dioxide partial pressure concentration, appears to provoke vasodilation and an increase in blood flow accompanied by reduced vascular resistance (Hosking, *et al.* 2004; Roff Hilton, *et al.* 2003; Luksch, *et al.* 2002; Chung, *et al.* 1999a; Roff, *et al.* 1999; Schmetterer, *et al.* 1997; Schemetterer, *et al.* 1996; Riva, *et al.* 1994).

There are two main approaches to evaluating vascular regulation: static and dynamic testing. Static autoregulation testing was described by Tiecks and colleagues as the comparison between baseline blood flow and blood flow post-perfusion pressure manipulation. This technique was said

to be time consuming and usually invasive, as it normally involves inducing metabolic changes (e.g using vasoactive medications or inhalation of gases). However, it has been proved to deliver reliable results, therefore becoming a repeatable valid technique (Tiecks, *et al.* 1995).

The dynamic approach first described by Aaslid and co-workers used changes in arterial blood pressure induced by the release of a tight blood pressure cuff as an autoregulatory stimulus to cerebral blood flow (Aaslid, *et al.* 1989). This technique was questioned by Kontos, who argued that the results given by Aaslid and colleagues after the provocation were derived blood flow calculations from measurements of blood flow velocity at the middle cerebral artery without the recording of vessel calibers (Kontos 1989). However, the technique was subsequently validated in several papers in which the velocity and the blood flow were recorded simultaneously and velocity and flow correlated significantly (Newell, *et al.* 1994; Aaslid, *et al.* 1989). Thus, vascular autoregulation can be studied either statically or dynamically.

It is also important to highlight the two main autoregulatory mechanisms that can occur in the pressence of changes in perfusion pressure; metabolic and myogenic. Schmetterer and co-workers (Schmetterer, et al. 1995) evaluated choroidal blood flow pulsations with a laser interferometer, and found that under hypercapnic conditions there was no variation in fundus pulse amplitude (FPA), whereas hyperoxia resulted in decreased FPA in the macular and papillae region. These results may mistakenly appear to show autoregulation only under hypercapnic conditions, as FPA remained stable after increasing the carbon dioxide partial pressure. However, Schmetterer stressed the importance of analyzing ocular changes exerted by increased pO_2 and pCO_2 in relation to systemic changes. In the study by Schmetterer and colleagues, hyperoxia did not affect the systemic blood pressure; however, it drove a decrease in FPA in the macula and papilla, which suggested local metabolic autoregulation. However, raised carbon dioxide partial pressure induced an increase in mean arterial pressure which was accompanied by unchanged FPA, which mainly described a metabolic regulatory mechanism (Schmetterer, *et al.* 1995) (see section 1.12.2 of this thesis for a detailed explanation of vascular autoregulation).

Despite several studies suggesting a reduced resting pulsatile blood flow in myopia, and decreased retrobulbar velocities in pathological myopia (Dimitrova, *et al.* 2002; Logan, Gilmartin and Cox 2002; Németh, Michelson and Harazny 2001; Akyol, *et al.* 1996), to date the vascular regulatory features of the human myopic eye remain to be explored.

4.3 Aims and objectives

The aim of this study was to evaluate the ocular blood flow autoregulatory properties of human myopia. A minimally invasive method of stress testing involving gas perturbations to induce hypercapnia was adopted in order to investigate and reflect as much as possible the normal physiological vascular autoregulatory responses.

4.4 Methods

4.4.1 Study Sample and Recruitment Criteria

Sixty-six healthy participants were recruited from the students and staff at Aston University, and assigned to one of two groups a) emmetropes (MSE ±0.50D) and b) myopes (MSE ≤ -1.00D). The emmetropic group consisted of 33 participants whose age ranged from 18 to 78 years (average age 40.27 ± 16.13 years; mean MSE 0.08 ± 0.41), whereas the myopic group comprised 33 participants with ages ranging from 20 to 73 years (average age 36.78± 15.00 years; mean MSE -4.51 ± 2.56). The two groups were matched at baseline for age, gender and ethnicity.

Since a physiological correlation is known to exist between the two eyes of the same subject, only one eye randomly chosen from each subject was evaluated (Kimura, *et al.* 2003).

All the participants were healthy volunteers, which was confirmed by the author following investigation of the fundus using Volk lens (90D), recording of blood pressure and a detailed recording of systemic and ocular history and symptoms. Those subjects exhibiting any sign of myopic fundus changes (e.g. lacquer cracks, staphylomas, chorioretinal atrophy, lattice degeneration or pavingstone degeneration) were not included in the study. Exclusion factors included any ocular disorder, diabetes, hypotension, hypertension or any other systemic disorder or medication likely to affect the systemic or ocular vasculature. Since no blood test was performed, all of the previous conditions were self-reported by the participants. A corrected visual acuity of 6/9 or better was required together with astigmatism of less than 1.5 diopters cylinder, which reduced the percentage of astigmatic patients being taken as myopic subjects after MSE calculation.

4.4.2 Ethical Approval and Informed Consent

Written and verbal information about the exact procedures to be performed during the visit was given to all the subjects prior to data collection. The participants were encouraged to ask any questions and to clarify any doubts they might have before signing the written consent. The volunteers were requested to sign two copies of the written consent, one of which was given to them for their own records.

All investigations were approved by the Aston University Ethical Review Committee and conformed to the declaration of Helsinki (appendix 2.1).

4.4.3 Study Sample: Dietary Restrictions

Alcohol, nicotine and caffeine containing products have been reported to affect the flow of blood in the eye (Domino, *et al.* 2004; Gdovinova 2001). To ensure the reliability of the data, the participants recruited for this study were asked to refrain from smoking or consuming alcohol and caffeine containing products 12 hours prior to the study.

Exercise is known to increase the mean arterial pressure (MAP); however the maximal BP falls rapidly on stopping exercise (Tzemos, Lim and MacDonald 2002). Therefore, since the total duration of the experiment was approximately 1 hour and the vascular readings were performed approximately in the last 30 minutes, the potential effect of exercise on the systemic circulation was considered to be minimal. Thus, the subjects were not asked to refrain from exercising before the appointment.

4.4.4. Experimental Protocol and Investigations

The experimental procedure followed in the current study comprised two main sets of data collection, which were performed in the same session in order to avoid daily fluctuations in systemic blood pressure. The sessions consisted of a baseline measurement of ocular blood flow using OBFA, HRF and CDI performed under normal breathing conditions followed by repeated measures during induced hypercapnia. The instruments used in this study were the following (for more detailed explanation of instruments, see section 1.9.2 and section 1.11):

a. Ocular measurements

- Autorefraction (Shin-Nippon NVision-K 5001 Autorefractor, Japan)
- Biometric analysis (IOLMaster, Carl Zeiss Jena, Germany Zeiss)
- Corneal thickness (DGH-550 Pachette 2, DGH technology, Pennsylvania, US).
- Intraocular pressure (Solan Tono-Pen®XL Tonometer (Medtronic Solan, Florida)

b. Vascular instruments

- Ocular Blood Flow Analyser (OBFA, Paradigm Medical Industries, Utah, USA)
- Heidelbergh Retinal Flowmeter (HRF, Heidelberg Engineering, Heidelberg, Germany)
- Colour Doppler Imaging (Colour Doppler Imaging, CDI. Sequoia CDI System, Siemens Medical Solutions, USA).

c. Systemic measurements

- Blood pressure (UA-767 Fully Automatic Blood Pressure Monitor BHS A/A, A & D Co. Ltd,

Oxon, UK).

- Measurements of height and weight.

d. Gas monitoring and perturbation methods

- End tidal carbon dioxide and oxygen levels were continuously recorded using a gas analyser (pulse oximeter and capnograph) (Capnocheck Plus Capnograph, Smiths Medical, Waukesha, Wisconsin, USA).

4.4.4.1 Pulse oximeter and capnograph

Pulse oximetry and capnography are techniques that monitor the concentration of oxygen (O2) and carbon dioxide (CO2) in patient's blood. These instruments were used to ensure the wellbeing of the participants throughout the duration of the study.

Pulse oximetry. Principle

A pulse oximeter is a non-invasive medical device that assesses the concentration of oxygen in the blood by measuring the amount of oxygenated haemoglobin (Hb). It consists of two small light-emitting diodes (LED) and a photodetector (photodiode) normally attached to a clip. A LED emits light that becomes electrically altered when it is crossed by a semiconducting material. In this case, two infrared lights of different wavelengths (650nm and 805nm) are directed towards the bloodstream. The blood behaves differently depending on the percentage of oxygen contained and the amount of infrared light absorbed by both the oxygenated and non-oxygenated blood is also different. By calculating the variations in the absorption profiles of the two wavelengths, a processor computes the proportion of oxygenated haemoglobin and provides a number that is interpreted as the concentration of oxygen in the blood (Fearnley 1995).

The pulse oximeter consists of a probe that can be attached to the patient's finger, which in turn is linked to a computerized unit. The unit displays the percentage of Hb saturated with oxygen together with an audible signal for each pulse beat, a calculated heart rate and a graphical display of the blood flow. Audible alarms can be programmed to alert to high or low levels of O_2 and CO_2. This feature was used to prevent hypoxia being allowed to occur during the course of the study.

Capnography. Principle

A capnograph measures the amount of CO_2 in an air sample. In the case of monitoring expired gases, capnography provides direct readings of the inhaled and exhaled concentration of CO_2 (FCO_2), and it can also describe indirectly the CO_2 concentration in patient's blood. It monitors the concentration of breathing CO_2 as a time-concentration curve, giving a graphic display of instantaneous FCO_2 versus time or expired volume during a respiratory cycle. Capnography provides information about CO_2 production, pulmonary perfusion, alveolar ventilation, respiratory patterns, and elimination of CO_2. A capnograph may incorporate one of two types of

analyzer: mainstream or sidestream. Mainstream analysers insert a sampling window into the ventilator circuit for measurement of CO_2, (i.e respiratory system) whereas a sidestream analyser aspirates gas from the ventilator circuit (i.e air in a mask) (Branson, Hess and Chatburn 1994; Block and McDonald 1992).

Capnography has been shown to be effective in the early detection of adverse respiratory events, and at present it is the technique most currently used along with pulse oximetry to monitor the administration of general anaesthesia. Capnography and capnometry may be used sometimes as synonyms; however, capnometry only monitors the dynamic pattern of inflow CO_2 concentration (F CO_2) without a graphical representation of the F CO_2 profile.

The capnograph used for this study had a sidestream analyser based on infrared light. The infrared transducer was connected to the end of a monitoring tube placed at the breathing circuit to record. The tube that conducts the exhaled air is taken onto the transducer where the CO_2 concentration is monitored and shown on the display unit (figure 4.1).

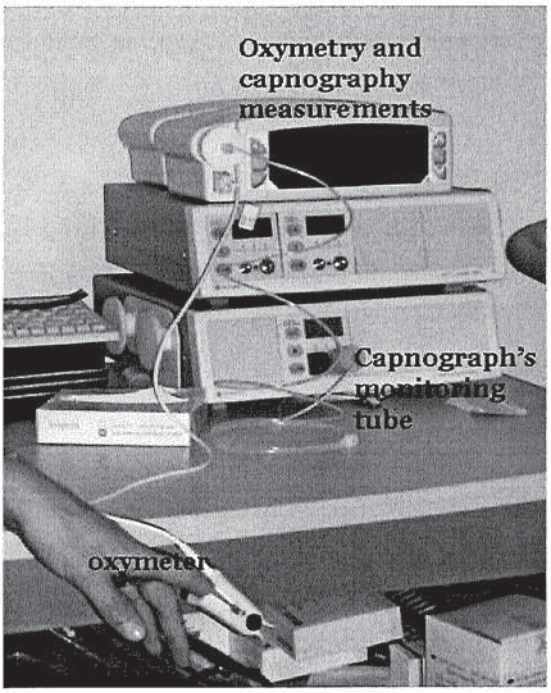


Figure 4.1 *Picture of the capnograph and oxymeter appearance used to monitor the supply of oxygen and carbon dioxide to the participants*

4.4.4.2 Protocol

Baseline condition

Prior to the collection of baseline data, ocular biometric readings (axial length, anterior chamber

depth and corneal thickness) and refractive error were recorded in all the subjects.

The participants were asked to breathe normal room air through a face mask with the non-rebreathing inlet valve of a gas mask connected to a mixing chamber (1014 series non-rebreathing mask, Hans Rudolph Inc., Missouri, USA) (figure 4.2).

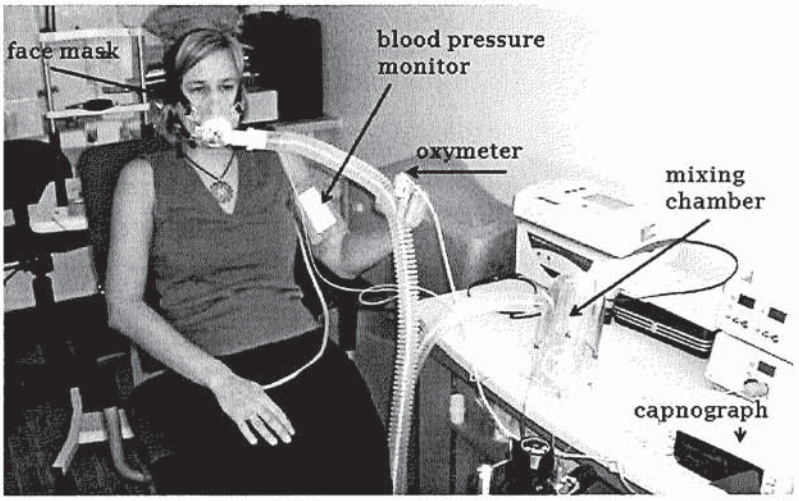


Figure 4.2 *Picture of the setup followed during the baseline condition prior to hypercapnia*

Baseline conditions were achieved by breathing normal room air for approximately 5 minutes until the patient's respiration rate stabilized. The oxygen and carbon dioxide saturation was constantly recorded. However, since the capnograph, oximeter and blood pressure monitor did not allow the storage of data, the readings were manually recorded on the patient's record sheet as indicated below.

The order of the tests performed during the baseline condition was as follows:

- Systemic blood pressure, oxygen saturation and carbon dioxide end-tidal concentration were recorded before and after placing the face mask on the patient.
- The pulsatile component of ocular blood flow was recorded using the Ocular Blood Flow Analyser (OBFA), after which systemic blood pressure, oxygen saturation and carbon dioxide end-tidal concentration were recorded.
- The blood flow in the retinal capillaries and the retrobulbar vessels was subsequently measured using the HRF and CDI, after which systemic blood pressure, oxygen saturation and carbon dioxide end-tidal concentration were again collected

As indicated above, despite the constant monitoring of systemic blood pressure, oxygen saturation and carbon dioxide end-tidal concentration, these values were manually recorded on the patient's

record sheet prior to each ocular blood flow measurement.

Hypercapnic condition

After taking readings at baseline, the patients were asked to rest with the mask on for 3 minutes, during which blood pressure and the saturation of CO_2 and O_2 were constantly recorded.

The average respiratory end-tidal CO_2 level was then increased by approximately 15% above baseline by adding CO_2 to room air in a mixing chamber; throughout this procedure the capnograph's oximeter constantly monitored and displayed the pCO_2 values of the patient. The pulse oximeter alarm was set at a high pitch for pCO_2 readings above 15% increase for each patient, which allowed the experiment to be carried out safely. CO_2 was supplied ensuring unaffected O_2 blood levels (isoxic hypercapnia) and by limiting the end tidal CO_2 levels to approximatelly 15% above normal baseline, the possibility of undesirable side effects was minimised.

The sequence of the tests was the same as for the baseline condition to make sure that postural changes did not induce bias in the results obtained, and it was as follows:

- Systemic blood pressure was recorded once the average respiratory end-tidal CO_2 level was increased by approximately 15% above baseline and stabilised for 5 minutes.
- Proxymetacaine HCl 0.5% was instilled to assess pulsatile ocular blood flow using the Ocular Blood Flow Analyser (OBFA).
- The systemic blood pressure was measured again before recording the blood flow of the retinal microvasculature around the macular area using the Heidelberg Retinal Flowmeter (HRF); after which blood pressure was again recorded and Colour Doppler Imaging (CDI) was used to assess OA, CRA and SPCA.

Once the tests were finalised, the mask was removed and water was provided to the patient to drink while the volunteers rested for 5 minutes since having the mask caused slightly dry mouth. The blood pressure was then taken to ensure recovery from hypercapnia to a normal breathing condition.

Throughout the session, a fan was used to ensure adequate room air ventilation for baseline measurement and for hypercapnic conditions. The integrity of the cornea was checked using slitlamp after the instillation of fluorescein (Fluorets strips, Chauvin Pharmaceutical) once all the tests mentioned above had been performed. Weight and height were then measured on all the participants at the end of the session.

A flowchart of the protocol is presented in figure 4.3.

4.4.5 Statistical analysis

All data are given as mean ± SD. Two-tailed paired Student's *t*-test was used to test for significance between baseline and hypercapnic conditions in myopes and emmetropes. Multiple comparisons were corrected using Bonferroni analysis. One-way repeated measures ANOVA allowed the assessment of changes in variable measured on the same scale under three or more different occasions. In addition, one-way repeated measures ANOVA was used to evaluate potential differences in systemic blood pressure prior to each ocular blood flow measurement.

This study also assessed the effect of age on the response to hypercapnia by subdividing the total sample in those participants below the age of 40 and those above the age of 40. The OPP increased significantly from baseline to hypercapnia in both the young group and in the older group. Thereby, to account for the confounding effects of OPP, the ocular response to hypercapnia was evaluated with a repeated measured ANCOVA in which OPP was included as a covariate. To assess the vascular response to hypercapnia it was necessary to ensure that the results provided were the actual response to pCO_2 changes and not the resultant of a higher pressure of perfusion.

Additionally, two-way between-groups ANOVA was used to assess the interaction effect between age and refractive error on the vascular responses to hypercapnia. Pearson's product moment correlation was used to assess the effect of age on the vascular response to hypercapnia. Statistical significance was defined as $p<0.05$.

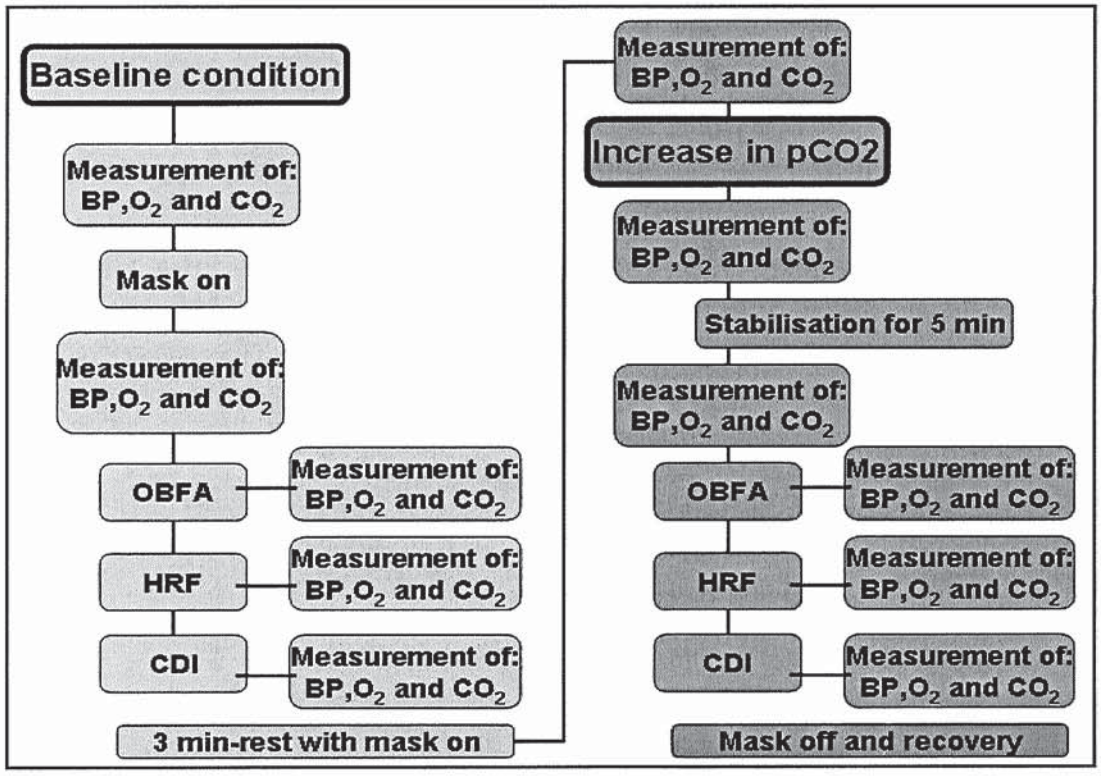


Figure 4.3 *Flowchart summarising the protocol followed for baseline measurements (light shading) and for hypercapnia measurements (darker shading).*

4.5 Results

4.5.1 Baseline Condition

There were no significant differences in age, gender, height, weight or body mass index between the two groups at baseline. Moreover, resting systemic blood pressure, MAP and OPP did not differ significantly between the two refractive groups (table 4.1, figure 4.4).

From the ocular biometric parameters assessed (axial length (AL), corneal thickness (CT), anterior chamber depth (ACD) and IOP), only AL and ACD differed significantly between the two refractive groups ($p<0.001$) (table 4.1).

The ocular blood flow parameters in emmetropes and myopes did not differ significantly (appendix 1.5).

	Emmetropes	Myopes	p
N	36	36	n/a
MSE (D)	0.09 ± 0.43	-4.51 ± 2.56	*<0.001
Gender	14m; 19f	16m; 17f	n/a
Age (years)	40.54 ± 15.82	36.78 ± 15.00	ns
Ethnicity	27w; 9a	27w; 9a	n/a
Systolic BP (mmHg)	113.63 ± 12.26	115.84± 16.24	ns
Diastolic BP (mmHg)	72.09 ± 8.24	73.15 ± 9.76	ns
Pulse rate (bpm)	67.03 ± 11.95	68.81 ± 9.63	ns
MAP (mmHg)	85.81 ± 9.04	86.21 ± 13.50	ns
OPP (mmHg)	44.39 ± 6.47	42.35 ± 8.75	ns
Height (m)	1.71 ± 0.08	1.69 ± 0.08	ns
Weight (kg)	75.92 ± 15.74	71.05 ± 12.10	ns
BMI	25.86 ± 4.41	24.63 ± 3.68	ns
Axial length (mm)	23.68 ± 0.72	25.31 ± 1.14	*<0.001
ACD (mm)	3.44 ± 0.27	3.60 ±0.33	*0.031
CT (μm)	555.21 ± 42.89	569. 45 ± 33.60	ns
IOP (mmHg)	14.31 ± 3.82	14.67 ± 4.04	ns

Table 4.1. *Summary of physical, ocular and vascular systemic features by refractive group (explanation of abbreviations: MAP, mean arterial pressure; OPP, ocular perfusion pressure; BMI, body mass index; ACD, anterior chamber depth; CT, corneal thickness; IOP, intraocular pressure) ns: not significant *significant value*

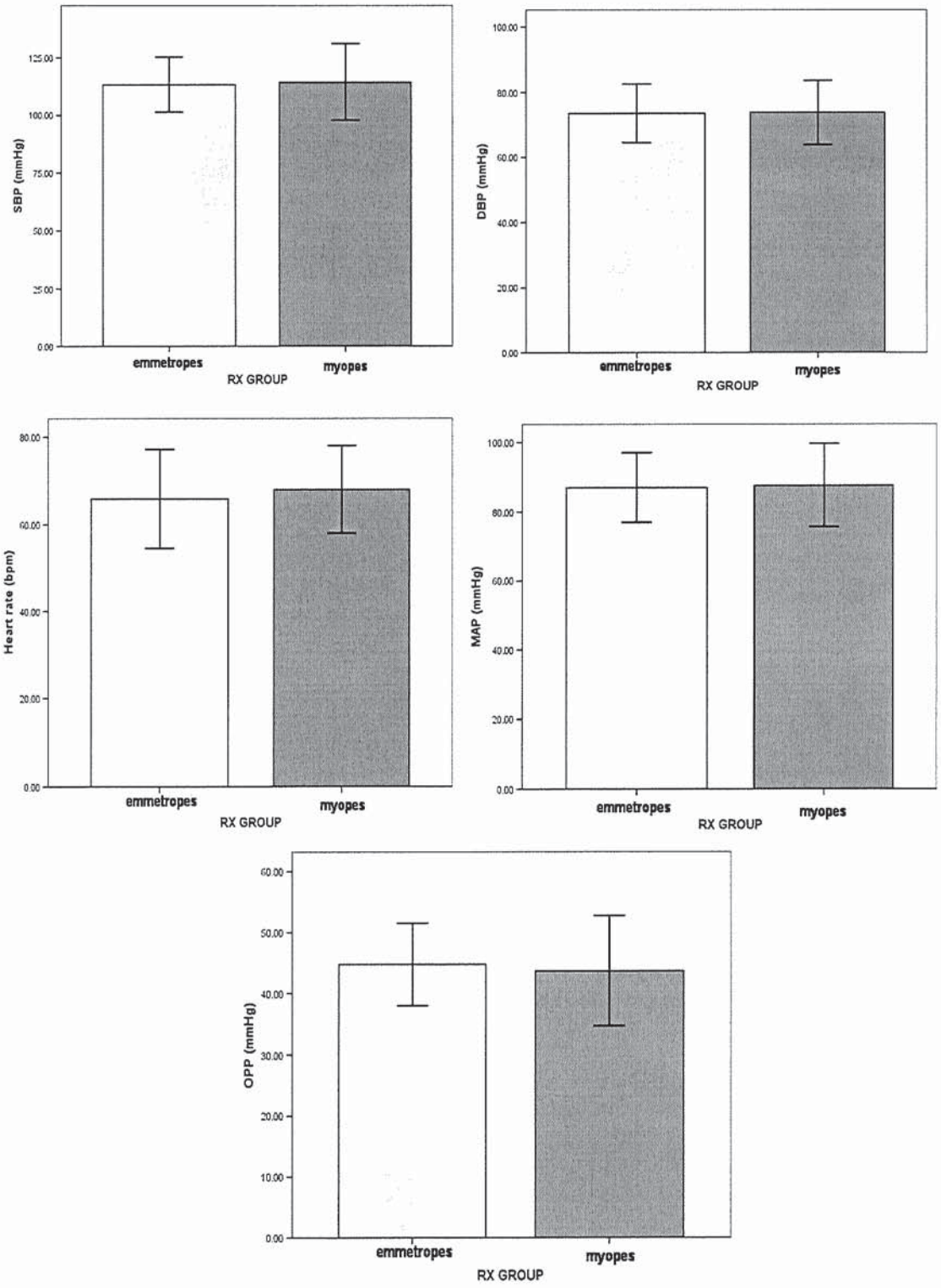


Figure 4.4 *Bar plots showing lack of statistical difference between systolic blood pressure (SBP), diastolic blood pressure (DBP), heart rate, mean arterial pressure (MAP) and ocular perfusion pressure (OPP) between myopes and emmetropes during baseline condition*

BP did not vary throughout the baseline session from one instrument to another in either of the refractive groups (table 4.2).

Recording	SBP (mmHg)	DBP (mmHg)	Heart rate (bpm)	MAP (mmHg)	OPP (mmHg)
			overall		
1	113.09 ± 13.67	69.78 ± 9.15	61.68 ± 11.97	84.09 ± 9.33	42.05 ± 6.24
2	111.92 ± 13.28	72.68 ± 10.20	65.51 ± 11.54	85.76 ± 9.99	43.16 ± 6.20
3	114.42 ± 14.64	72.72 ± 8.42	64.34 ± 9.31	86.62 ± 9.69	43.74 ± 6.32
			emmetropes		
1	118.90 ± 13.05	76.71 ± 9.94	67.84 ± 13.04	90.78 ± 10.10	46.94 ± 7.12
2	116.40 ± 12.19	75.75 ± 9.88	68.25 ± 13.85	89.30 ± 9.66	45.95 ± 5.98
3	118.25 ± 10.99	74.71 ± 12.07	63.93 ± 10.86	89.22 ± 10.84	45.90 ± 7.49
			myopes		
1	114.21 ± 24.86	74.09 ± 10.36	66.96 ± 8.79	87.46 ± 13.15	43.90 ±10.15
2	117.39 ± 14.17	76.09 ± 16.70	68.78 ±11.52	89.85 ±13.93	45.50 ± 10.69
3	118.12 ± 13.70	72.84 ± 9.91	64.90 ± 11.53	87.93 ± 9.98	44.22 ± 8.12

Table 4.2. *Summary of systemic blood pressure readings at baseline taken prior to 1. CDI, 2. OBFA, 3. HRF (SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial pressure; OPP, ocular perfusion pressure). There were no significant differences between the three sets of BP measurements for any of the refractive groups.*

	Baseline	Hypercapnia	p
	Emmetropes		
End tidal CO_2 (mmHg)	38.82	44.11	*<0.001
O_2 saturation levels (%)	96.69	97.73	*<0.001
	Myopes		
End tidal CO_2 (mmHg)	39.02	45.85	*<0.001
O_2 saturation levels (%)	96.81	97.61	*<0.001

Table 4.3 *Changes in CO_2 and O_2 from baseline to hypercapnic conditions for each refractive group (*statistically significant)*

4.5.2 Hypercapnia condition

4.5.2.1 Hypercapnic characteristics

The end tidal carbon dioxide concentration in emmetropes increased significantly from 38.82 mmHg at baseline to 44.11 mmHg during hypercapnia (17.08% increase; $p<0.001$). End tidal

concentration in the myopic group changed significantly from 39.02 mmHg at baseline to 45.85 mmHg (17.5% increase; p<0.001) (table 4.3).

4.5.2.2 Systemic response to hypercapnia

The saturation of oxygen increased parallel to CO_2 rise, which ensured that the increase in O_2 during gas perturbation was similar for each group. Indeed, blood oxygen saturation increased significantly from 96.69% at baseline to 97.73% during hypercapnia in emmetropes (p<0.001) and from 96.81% to 97.61% in myopes (p<0.001). A summary of the data describing changes in end tidal carbon dioxide and oxygen saturation is given in table 4.3.

The repeated measures analysis of BP did not show significant changes throughout the hypercapnic session. Analysis of BP for each refractive group individually reported a significant increase in diastolic BP and MAP (p=0.02; p=0.028) only in myopes (table 4.4).

	Baseline	Hypercapnia	p
Emmetropes			
SBP (mmHg)	113.06 ± 11.88	115.09 ± 22.46	ns
DBP (mmHg)	72.87 ± 9.06	75.60 ± 9.61	ns
Heart rate (bpm)	65.12 ± 11.63	65.42 ± 10.67	ns
MAP (mmHg)	86.27 ± 9.06	88.76 ± 11.79	ns
OPP	44.13 ± 5.69	45.79 ± 7.94	ns
Myopes			
SBP (mmHg)	113.00 ± 17.26	119.84 ± 16.72	ns
DBP mmHg)	71.36 ± 8.45	77.06 ± 10.81	*0.02
Heart rate (bpm)	66.93 ± 10.85	68.24 ± 9.83	ns
MAP (mmHg)	85.24 ± 9.89	91.32 ± 11.97	*0.028
OPP	42.30 ± 8.48	46.35 ± 9.44	ns

Table 4.4. *Systemic BP changes from baseline to hypercapnia: overall analysis and by refractive group.*

4.5.2.3 Ocular vascular response to hypercapnia

Retrobulbar response to hypercapnia

During hypercapnia none of the refractive groups experienced significant changes (table 4.5 and table 4.6).

Pulsatile ocular blood flow response to hypercapnia

Emmetropes did not exhibit any significant changes in OBFA parameters between baseline and hypercapnia. Myopes showed an increase in POBF during hypercapnia (p=0.011) (figure 4.5, table 4.5 and table 4.6). After Bonferroni correction, the level of significance was reduced from <0.05 to p<0.016, thus, only POBF reached significance.

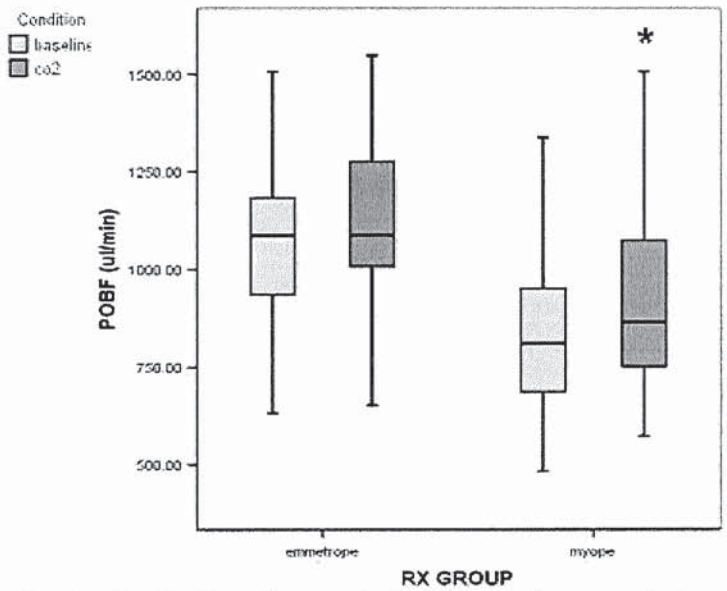


Figure 4.5 *Box plots showing the significant increase in POBF during hypercapnia in myopes (POBF, pulsatile ocular blood flow) * statistically significant after Bonferroni correction*

Retinal capillaries response to hypercapnia

Hypercapnia in emmetropes did not induce any significant changes in the blood flow, volume or velocity in the retinal capillaries using HRF around the macular area. In myopes, however, the minimum blood velocity appeared significantly increased after hypercapnia (p=0.020) (table 4.5, table 4.6 and figure 4.6).

The ocular vascular results obtained using CDI, OBFA and HRF during hypercapnia are summarised in table 4.5 and table 4.6

Instrument	Parameter	Mask Baseline	Hypercapnia	P
		Emmetropes		
OBFA	**OBFa (mmHg)**	3.50 ± 1.34	3.65 ± 1.34	ns
	OBFv (µl)	8.58 ± 3.06	8.91 ± 3.00	ns
	OBFr	62.36 ± 11.72	57.36 ± 14.86	ns
	POBF (µl/min)	1060 ± 212.42	1098.25 ± 255.10	ns
CDI	**OA PSv (m/s)**	0.469 ± 0.18	0.498 ± 0.19	ns
	OA EDv (m/s)	0.128 ± 0.07	0.129 ± 0.07	ns
	OA RI	0.705 ± 0.08	0.745 ± 0.07	*0.019
	OA ratio	3.83 ± 1.29	4.33 ± 1.41	ns
	CRA PSv (m/s)	0.120 ± 0.03	0.123 ± 0.04	ns
	CRA EDv (m/s)	0.063 ± 0.01	0.046 ± 0.01	ns
	CRA RI	0.600 ± 0.08	0.625 ± 0.08	ns
	CRA ratio	2.60 ± 0.54	2.75 ± 0.61	ns
	SPCA PSv (m/s)	0.158± 0.07	0.156 ± 0.05	ns
	SPCA EDv (m/s)	0.066 ± 0.03	0.064 ± 0.02	ns
	SPCA RI	0.575± 0.10	0.575 ± 0.09	ns
	SPCA ratio	2.43 ± 0.58	2.50 ± 0.74	ns
HRF	**MaxVolume (AU)**	21.86 ± 9.74	21.92 ± 6.12	ns
	MaxFlow (AU)	401.48 ± 338.19	393.35 ± 200.05	ns
	MaxVelocity (AU)	1.34 ± 0.91	1.37 ± 0.57	ns
	MinVolume (AU)	14.45 ± 6.99	14.55 ± 4.48	ns
	MinFlow (AU)	337.45 ± 181.33	258.15 ± 129.73	ns
	MinVelocity (AU)	0.91 ± 0.35	0.87 ± 0.39	ns

Table 4.5. *Results of OBFA, CDI and HRF vascular parameters during hypercapnia condition for the emmetropic group (ns: not significant ** not significant after Bonferroni correction)*

Instrument	Parameter	Mask baseline	Hypercapnia	p
		Myopes		
OBFA	**OBFa (mmHg)**	2.95 ± 0.99	3.07 ± 0.82	ns
	OBFv (µl)	8.46 ± 10.17	9.09 ± 9.87	* 0.038
	OBFr	64.40 ± 7.27	63.44 ± 11.00	ns
	POBF (µl/min)	837.80 ± 235.31	930.08 ± 273.78	**0.011
CDI	**OA PSv (m/s)**	0.468 ± 0.20	0.493 ± 0.20	ns
	OA EDv (m/s)	0.144 ± 0.05	0.118 ± 0.05	ns
	OA RI	0.732 ± 0.08	0.747 ± 0.09	ns
	OA ratio	5.30 ± 1.06	4.47 ± 1.57	ns
	CRA PSv (m/s)	0.110 ± 0.03	0.113 ± 0.03	ns
	CRA EDv (m/s)	0.042 ± 0.01	0.045 ± 0.01	ns
	CRA RI	0.601 ± 0.10	0.597 ± 0.08	ns
	CRA ratio	2.69 ± 0.58	2.61 ± 0.59	ns
	SPCA PSv (m/s)	0.141± 0.04	0.143 ± 0.04	ns
	SPCA EDv (m/s)	0.061 ± 0.02	0.065 ± 0.02	ns
	SPCA RI	0.525 ± 0.04	0.543 ± 0.07	ns
	SPCA ratio	2.34 ± 0.36	2.24 ± 0.34	ns
HRF	**MaxVolume (AU)**	20.34 ± 6.06	20.40 ± 4.97	ns
	MaxFlow (AU)	350.09 ± 108.40	374.17 ± 95.69	ns
	MaxVelocity (AU)	1.30 ± 0.59	1.30 ± 0.31	ns
	MinVolume (AU)	13.52 ± 5.06	16.45 ± 8.48	ns
	MinFlow (AU)	214.30 ± 74.85	245.92 ± 92.58	0.032
	MinVelocity (AU)	0.75 ± 0.27	0.88 ± 0.30	**0.020

Table 4.6. *Results of OBFA, CDI and HRF vascular parameters during hypercapnia condition for the myopic group (ns: not significant ** statistically significant * not significant after Bonferroni correction)*

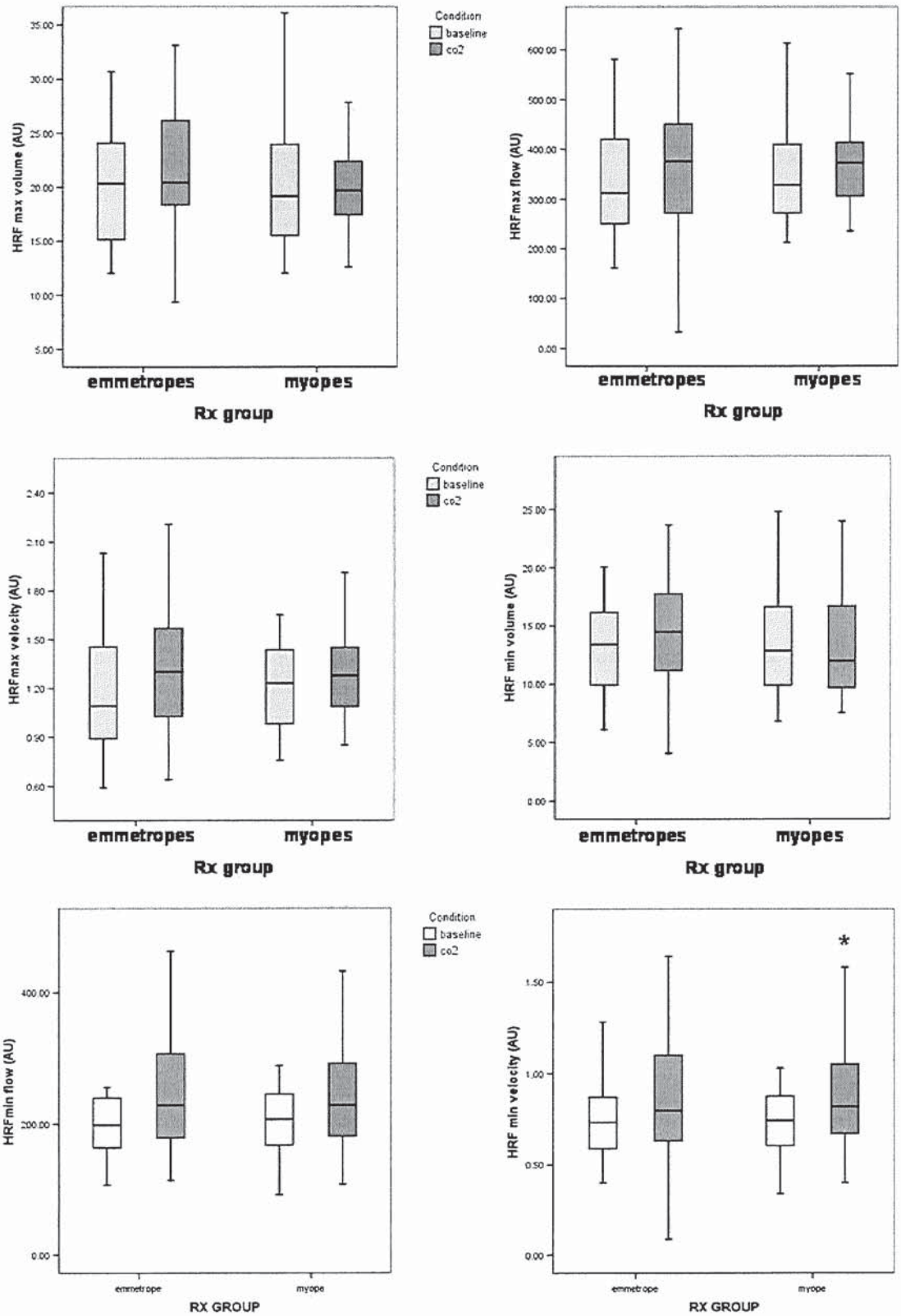


Figure 4.6 *Hypercapnia responses from the retinal microvessels assessed with HRF in emmetropes and myopes (* statistically significant).*

4.5.3. Effect of age on haemodynamic autoregulation

The evaluation of the differences in autoregulatory response to hypercapnia with age was performed in a similar way to that in the second study of chapter three (section 3.2) and the total sample size of the current study was subdivided into two age groups; under 40 (N=37) and over 40 (N=29). The two age groups differed significantly in systolic BP and diastolic BP at baseline ($p=0.02$; $p<0.001$), but not in MAP or OPP. No significant differences were found between the ocular biometric data of the two age groups (table 4.7).

	Subjects <40	Subjects >40	p
Age (years)	26.43 ± 5.06	53.96 ± 9.43	* <0.001
Gender	19m; 18f	11m; 18f	n/a
MSE (D)	-2.40 ± 2.81	-1.96 ± 3.20	ns
Systolic BP (mmHg)	109.18 ± 13.12	115.41 ± 12.86	* 0.02
Diastolic BP (mmHg)	68.81 ± 8.85	77.62 ± 9.80	*<0.001
Pulse rate (bpm)	67.75 ± 12.76	62.65 ± 9.21	ns
MAP (mmHg)	82.27 ± 9.10	90.21 ± 9.41	ns
OPP (mmHg)	42.90 ± 6.23	43.49 ± 6.24	ns
IOP (mm Hg)	13.06 ± 3.42	13.55 ± 4.16	ns
Height (m)	1.69 ± 0.08	1.70 ± 0.09	ns
Weight (kg)	71.64 ± 14.63	74.89 ± 16.16	ns
BMI	24.29 ± 4.52	25.63 ± 3.84	ns
Axial length (mm)	24.42 ± 1.13	24.65 ± 1.37	ns

Table 4.7. *Summary of physical, ocular and vascular systemic features by age group (explanation of abbreviations: MAP, mean arterial pressure; OPP, ocular perfusion pressure; BMI, body mass index; ACD, anterior chamber depth; CT, corneal thickness) ns: not significant *significantly different*

4.5.3.1 Systemic response to hypercapnia by age group

The MAP and OPP increased significantly from baseline to hypercapnia in both the young group and in the older group ($p<0.001$ for all parameters). Thereby, to account for the confounding effects of OPP on the ocular response to hypercapnia, this variable was included as a covariate in the ANOVA repeated-measures analysis performed in the following sections.

4.5.3.2 Ocular vascular response to hypercapnia by age group

Pulsatile ocular blood flow

None of the age groups exhibited any significant variations in OBFA parameters (table 4.8).

	Baseline	hypercapnia	p
	<40 years		
OBFa (mmHg)	3.04 ± 0.88	3.09 ±0.84	ns
OBFv (µl)	7.66 ± 2.25	7.95 ± 2.51	ns
OBFr	64.90 ± 10.10	62.36 ± 9.95	ns
POBF (µl/min)	1022.40 ± 190.38	1059.88 ± 251.77	ns
	>40 years		
OBFa (mmHg)	3.35 ± 1.27	3.56 ± 1.30	ns
OBFv (µl)	9.30 ± 10.30	9.97 ± 9.89	** 0.03
OBFr	62.16 ± 9.05	59.04 ± 15.49	ns
POBF (µl/min)	871.28 ± 276.00	963.84 ± 292.78	ns

Table 4.8 *OBFA measurements during baseline and hypercapnia for the two age groups (ns: not significant **not significant after Bonferroni correction)*

Retrobulbar data

The ophthalmic artery peak systolic blood velocity (OA PSv) (p=0.003) in the patients above the age of 40 increased significantly as response to hypercapnia (table 4.9).

Retinal capillaries

Maximum and minimum values of retinal blood flow, volume and velocity were obtained from the retinal capillary bed around the foveal area using HRF. The younger group showed a trend towards increased minimum blood volume with hypercapnia that did not reach statistical significance (p=0.056). No changes were observed in the older group.

VESSEL	PARAMETER	Baseline	Hypercapnia	p
		<40 years		
OA	**PSv (m/s)**	0.440 ± 0.20	0.460 ± 0.20	ns
	EDv (m/s)	0.129 ± 0.14	0.101 ± 0.04	ns
	RI	0.737 ± 0.08	0.767 ± 0.08	ns
	Ratio	4.18 ± 1.27	4.73 ± 1.44	* 0.035
CRA	**PSv (m/s)**	0.110 ± 0.03	0.104 ± 0.02	ns
	EDv (m/s)	0.057 ± 0.07	0.042 ± 0.11	ns
	RI	0.589 ± 0.10	0.590 ± 0.07	ns
	Ratio	2.60 ± 0.57	2.54 ± 0.53	ns
SPCA	**PSv (m/s)**	0.144 ± 0.07	0.142 ± 0.05	ns
	EDv (m/s)	0.063 ± 0.03	0.064 ± 0.02	ns
	RI	0.537 ± 0.11	0.538 ± 0.08	ns
	Ratio	2.287 ± 0.40	2.262 ± 0.60	ns
		>40 years		
OA	**PSv (m/s)**	0.489 ± 0.15	0.540 ± 0.18	ns
	EDv (m/s)	0.145 ± 0.06	0.152 ± 0.07	ns
	RI	0.697 ± 0.07	0.721 ± 0.08	ns
	Ratio	5.06 ± 7.59	3.99 ± 1.45	ns
CRA	**PSv (m/s)**	0.122 ± 0.03	0.135 ± 0.04	**0.003
	EDv (m/s)	0.047 ± 0.16	0.050 ± 0.18	ns
	RI	0.614 ± 0.08	0.636 ± 0.08	ns
	Ratio	2.70 ± 0.56	2.84 ± 0.64	ns
SPCA	**PSv (m/s)**	0.156 ± 0.04	0.157 ± 0.05	ns
	EDv (m/s)	0.064 ± 0.02	0.065 ± 0.02	ns
	RI	0.773 ± 1.00	0.584 ± 0.081	ns
	Ratio	2.50 ± 0.54	2.49 ± 0.54	ns

Table 4.9 *Retrobulbar vascular parameters during baseline and hypercapnia for each age group (ns: not significant ** statistically significant * not significant after Bonferroni correction)*

PARAMETER	Baseline	hypercapnia	p
	<40 years		
MaxVolume (AU)	20. 23 ± 6.07	19.99 ± 5.94	ns
MaxFlow (AU)	331.82 ± 119.31	343.20 ± 116. 50	ns
MaxVelocity (AU)	1.13 ± 0.35	1.21 ± 0.34	ns
MinVolume (AU)	13.46 ± 4.58	13.75 ± 5.32	0.056
MinFlow (AU)	203.57 ± 73.05	235.40 ± 91.42	ns
MinVelocity (AU)	0.71 ± 0.27	0.81 ± 0.32	ns
	>40 years		
MaxVolume (AU)	22.16 ± 10.02	22.60 ± 4.80	ns
MaxFlow (AU)	429.81 ± 344.46	434.10 ± 182.60	ns
MaxVelocity (AU)	1.44 ± 0.91	1.48 ± 0.54	ns
MinVolume (AU)	14.75 ± 7.55	14.16 ± 4.09	ns
MinFlow (AU)	362.86 ± 501.43	265.46 ± 110.44	ns
MinVelocity (AU)	0.97 ± .77	0.94 ± 0.36	ns

Table 4.10 *Vascular measurements in the retinal microvessels during baseline and hypercapnia for each age group (ns: not significant * statistically significant after Bonferroni correction)*

4.5.3.3 Relationship between age and the vascular response to hypercapnia

Age correlated with the percentage change in OA EDv (R=0.38 p=0.016) (figure 4.7), and the ratio between the peak systolic and end diastolic blood velocities in the SPCAs (SPCA ratio) (R=0.34 p=0.032) (figure 4.8).

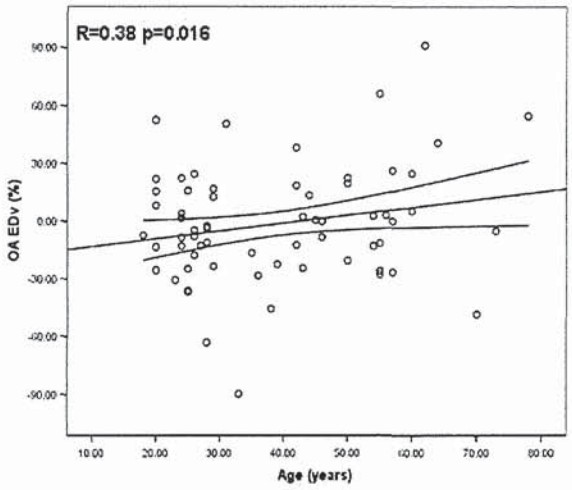


Figure 4.7 *Scatter plot showing the correlation between age and % change in OA EDv during hypercapnia.*

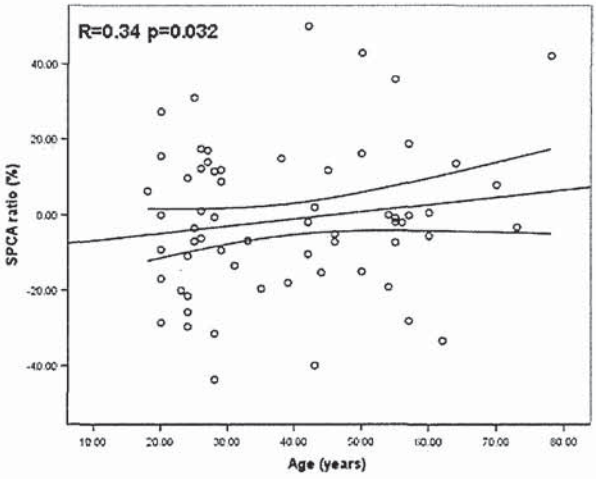


Figure 4.8 *Scatter plot showing the correlation between age and % change in SPCA ratio during hypercapnia*

Age correlated significantly with the percentage change in DBP (R=0.30 p=0.01) and MAP (R=0.26 p=0.033), whereas MSE did not correlate significantly with any of the percentage changes in BP, MAP or OPP (table 4.11).

	SBP R value	p	DBP R value	p	MAP R value	p	OPP Rvalue	p
Age	0.15	ns	0.30	* 0.01	0.26	* 0.033	0.09	ns
MSE	0.05	ns	0.22	ns	0.16	ns	0.14	ns

Table 4.11 *Pearson's correlation test between percentage change in age, MSE, BP, MAP and OPP *statistically sig*

4.5.4 Relationship between axial length and the ocular response to hypercapnia

Axial length correlated significantly with the percentage change in ocular pulse amplitude, volume and flow (R=0.31 p=0.03; R=0.35 p=0.016; R=0.31 p=0.033). Additionally, AL correlated with the percentage change in maximum retinal microvessel velocity (R=-0.29 p=0.024) (figure 4.9).

4.5.3.3 Interaction effect between age and refractive error on ocular vascular responses to hypercapnia

Assessment of the differences between the vascular responses to hypercapnia with age in myopes and emmetropes revealed a significant interaction effect for OA diastolic velocity (p=0.019), CRA systolic velocity (p=0.025) and CRA EDv (p=0.001).

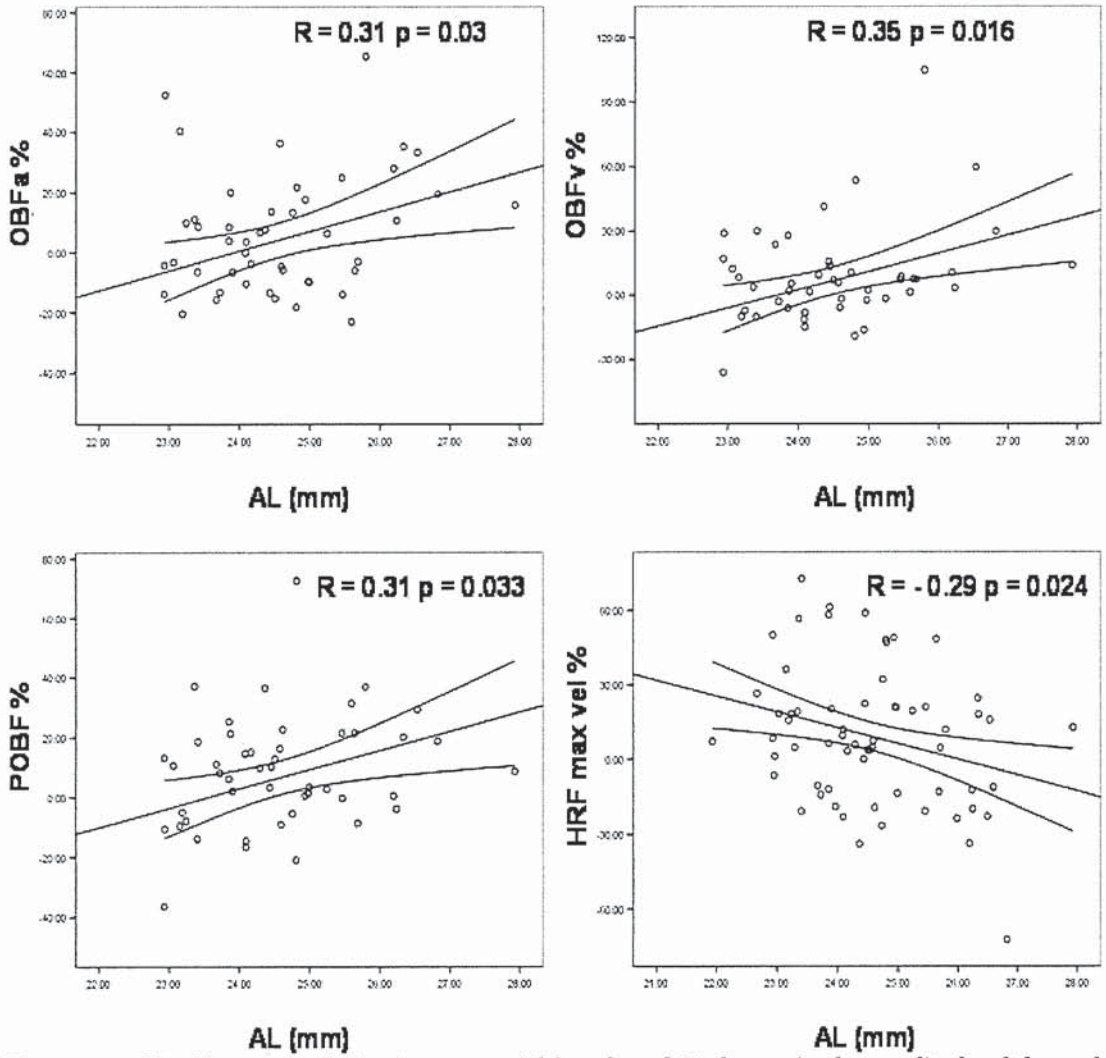


Figure 4.9 *Significant correlation between axial length and % change in the amplitude of the ocular pulse (OBFa), volume of the ocular pulse (OBFv), pulsatile ocular blood flow (POBF) and maximum velocity in the retinal microvessels during hypercapnia (p<0.05)*

4.6 Discussion

The differences in vascular response given by emmetropes and myopes to isoxic hypercapnia were evaluated in the present study. Three instruments were used to investigate different ocular vascular beds and perfusion features: OBFA, CDI and HRF. The outcomes revealed no difference in systemic blood pressure, MAP, OPP and IOP between the two refractive groups at baseline. Hypercapnia induced an increase in diastolic BP and MAP in myopes that did not reach significance for the emmetropic group. Hypercapnia in myopes revealed a significant increase in POBF and microretinal blood velocity.

The response to hypercapnia did not show differences between the two age groups evaluated (below and above 40 years); however, age was found to correlate significantly with the increases in POBF, OA EDv and the ratio between the PSv and EDv in the SPCA (SPCA ratio). Axial length correlated with the changes induced by hypercapnia in ocular pulse amplitude, volume and pulsatile ocular blood flow, as well as with those in microretinal blood velocity.

4.6.1 Systemic response to hypercapnia

The two refractive groups evaluated differed significantly in AL and ACD, the latter being possibly due to the larger number of patients over the age of 60 years in the emmetropic group (5) than in the myopic group (2) and the effect the maturation of the lens may have on the ACD (see section 3.1.6).

During this study, hypercapnia was induced by supplying significantly increased end-tidal carbon dioxide concentration. Blood oxygen saturation also increased significantly under hypercapnic conditions, which has been reported to be a consequence of mild hypercapnia and it is known as the Bohr Effect. Haemoglobin (Hb), a protein that contains iron and is responsible for the oxygen transport in blood red cells, distributes oxygen from the lungs to the rest of the body, where it releases the oxygen load. The Bohr Effect describes a decrease in haemoglobin's oxygen binding affinity after a decrease in pH or an increase in CO_2 or temperature (Antonini et al., 1962). Reduction in binding affinity increases the release of oxygen by Hb in tissues. Thereby, during exercise, for example, the body increases the end-tidal concentration of CO_2 to satisfy the oxygen need, as more oxygen will be released. Therefore, it can be summarised that the production of CO_2 increases with elevated metabolic activity of a tissue and the Bohr effect facilitates the increase in O_2 release to satisfy the increased demand.

In our study, a significant increase in oxygen saturation was observed after increasing CO_2 levels, which demonstrates that hypercapnia was achieved without inducing hypoxia, therefore ensuring

a constant supply of oxygen to the tissues. Several previous studies reported similar increases in oxygen saturation after induced hypercapnia (Huber, *et al.* 2006; Hosking, *et al.* 2001).

A static technique was the choice to assess the vascular autoregulatory properties of human myopia in this study, as it has been proved to deliver reliable results, therefore becoming a repeatable and valid technique (Tiecks, *et al.* 1995). Additionally, previous studies from this research group also used hypercapnia as a provocation test, which allows the comparison of results. The vascular outcomes were recorded once the hypercapnic status was achieved for 5 minutes. The results showed a significant increase in diastolic BP and MAP; whereas emmetropes did not experience any significant changes in systemic or ocular blood pressure. Huber and co-workers evaluated changes in contrast sensitivity and blood velocity at the OA and CRA on 31 participants under hypercapnic conditions. They reported a significant increase in systolic blood pressure, MAP and OPP in response to hypercapnia ($p=0.02$; $p=0.009$; $p=0.001$) (Huber, *et al.* 2006). There are, however, other studies in which hypercapnia was induced using the same technique, and did not exert any influence on systemic or ocular circulation. Roff et al evaluated one eye of 14 healthy subjects and reported no significant variations between BP at baseline or during hypercapnic conditions (Roff, *et al.* 1999). From the same research group, a study on 10 untreated glaucoma patients and 10 controls did not find significant changes in BP or heart rate when baseline was compared to hypercapnia (Hosking, *et al.* 2001). Therefore, the characteristics and size of the sample assessed appear to play a role on the systemic response given to hypercapnia. In this study, only the myopic group exhibited an increase in DBP and MAP during hypercapnia. Hence, the ocular and systemic responses to hypercapnia differed significantly between the two refractive groups suggesting differences in regulatory abilities at both systemic and ocular levels between emmetropes and myopes, as no differences were found between both refractive groups at baseline.

Hypercapnia did not affect IOP readings in any of the refractive groups, which agrees with previous studies (Gugleta, *et al.* 2005; Hosking, *et al.* 2004; Hosking, *et al.* 2001; Roff, *et al.* 1999).

4.6.2 Ocular blood flow changes with hypercapnia

4.6.2.1 Retrobulbar response to hypercapnia

There were no significant changes in retrobulbar circulation during hypercapnia in either myopes or emmetropes.

Huber *et al* reported a significant increase in OA and CRA PSv and EDv using CDI during

hypercapnia and concluded that retrobulbar perfusion behaves in an analogous way to cerebral perfusion during hypercapnia, which is known to increase. Huber and colleagues mentioned how increased OA and SPCA velocities during hypercapnia suggest an overall increase in blood flow. However, for a correct interpretation, it is important to remember that CDI only measures blood velocity and ratios, therefore, blood flow analysis from CDI ocular measurements should only be made if vessel diameter is accounted for. Blood flow is proportional to two main components, pressure difference between the two ends of the vessel, and vascular resistance, which is in turn related to the diameter of the vessel evaluated. Thus, for an objective appraisal of increased blood flow, blood velocity should appear increased accompanied by reduced resistance index (see section 1.11.5). A concomitant increase in both peak diastolic (PSv) and end diastolic velocity (EDv) has been described in *in-vitro* models as an indicator for an increase in volumetric flow (Spencer, *et al.* 1991), however in the present study only the PSv appeared significantly increased. The increase in OA PSv may be occurring as a metabolic regulatory mechanism to facilitate the flow of blood due to the significant decrease in oxygenation. Despite a certain degree of disparity in vascular reactivity to changes in perfusion pressure between the cerebral and ophthalmic artery (Kolodjaschna, *et al.* 2005), increased pCO_2 has been shown to stimulate blood flow in the cerebral and OA (Bayerle-Eder, *et al.* 2000; Schmetterer, *et al.* 1997; Harris, *et al.* 1996; Faraci, Breese and Heistad 1994).

However, in the present study no variations were observed in the OA, CRA or SPCA during hypercapnia. Differences in the blood vessels that supply the orbit have been previously described in a paper by Harris and colleagues (Harris, *et al.* 1996) in which the OA and the CRA did not provide the same degree of response as the internal carotid or cerebral artery, thus suggesting regional differences in the regulatory response within the same subject. Differences in the OA and CRA autoregulatory mechanisms using CDI have also been reported by Nemeth and colleagues on 12 healthy young volunteers using short dynamic exercise as stress testing to induce changes in OPP (Nemeth, *et al.* 2002). Exercising resulted in an increase in BP and significant decrease in IOP ($p<0.004$; $p<0.006$). The velocities in the OA decreased significantly, accompanied by a significant increase in resistance index; whereas the vascular parameters in the CRA remained stable. The decrease in OA blood velocity was suggested to occur as a potential over-compensatory mechanism due to the increase in OPP induced by exercising. Michelson and co-workers reported similar outcomes in OA and iris microcirculation of 22 healthy subjects after physical exercise using pulsed Doppler sonography and LDF. The resulting elevated perfusion pressure after exercising was associated with an increased systolic and decreased diastolic velocity (constant mean blood velocity) in the ophthalmic artery and iridal vessels, and an increased vascular resistance. It was suggested that the parallel elevation of vascular resistance and blood pressure

during exercise indicated constant blood flow in the ophthalmic artery and the iris, which in turn may account for by a sympathetic mechanism for protecting the eye from over-perfusion (Michelson, Groh and Grundler 1994). These papers support the autoregulatory response described in the present study in the OA of emmetropes. However, the mechanisms that control changes in perfusion pressure are mainly of a myogenic origin; whereas the ones described in the present study point to an active metabolic regulatory mechanism in the OA of emmetropes due to the lack of changes in OPP.

The paper by Huber et al (2006) additionally suggested an increase in volumetric flow in both retrobulbar vessels (Huber, *et al.* 2006). However, the statistical approach followed to compare baseline and hypercapnia readings was ANOVA and additionally, no correction was performed for the significant increase in OPP reported during hypercapnia. This analysis may result in a false positive increase in blood velocities thought to occur due to a decrease in oxygenation, when it is occurring due to the increase in perfusion pressure observed during hypercapnia.

4.6.2.2 Pulsatile ocular blood flow response to hypercapnia

The OBFA response to hypercapnia in emmetropes did not show significant changes; whereas myopes exhibited an increase in POBF during hypercapnia. Pulsatile ocular blood flow has not been shown to respond to variations in pCO_2, as reported by Roff and colleagues (Roff, *et al.* 1999). Fourteen healthy volunteers were assessed at baseline and during hypercapnia, and the values obtained at baseline did not differ from those acquired during hypercapnia. The proposed mechanism to explain the lack of OBFA response to hypercapnia mentioned the limitations of the POBF readings, known to be affected by scleral rigidity and ocular volume assumptions, and the lack of IOP response to hypercapnia, on which the POBF calculations are based.

In the present study, myopes showed a significantly increased POBF during hypercapnia, which may suggest that the pulsatile component of the ocular blood flow is extremely sensitive to changes in carbon dioxide concentration, thus resulting in an amplified response to compensate for the decrease in O2 concentration.

4.6.2.3 Retinal microvasculature response to hypercapnia

The response to hypercapnia given by the retinal microcirculation in the macular area differed between emmetropes and myopes. HRF values obtained during hypercapnia in emmetropes did not reveal any significant changes in vascular parameters; whereas in myopes there was an increase in microvascular blood velocity during hypercapnia. These results suggest an active regulatory function in the microvascular circulation of myopes, a finding that is supported by the hypercapnia-induce increase in retinal blood flow and velocity reported in several previous papers

(Hosking, *et al.* 2001; Chung, *et al.* 1999b; Roff, *et al.* 1999; Schmetterer, *et al.* 1997; Schmetterer, *et al.* 1995). Chung and co-workers (Chung, *et al.* 1999b) reported a regional variation in the response to vasodilatory and vasoconstrictive stimuli after assessing the ocular vascular response from 14 healthy volunteers to hypoxia and isoxic hypercapnia. The analysis compared the responsiveness from the superior and inferior peripapillary retinal quadrants, and found that the temporal quadrant of the inferior peripapillary retina was less responsive to vasodilation and more responsive to vasoconstriction. Despite the marked differences between the retinal areas assessed in the present study (macular area) and that by Chung *et al.*, the lack of significant response to hypercapnia in emmetropes may be related to the anatomical differences between the emmetropic and a myopic retina. The elongation of the posterior pole in myopia is accepted (Logan, *et al.* 2004; Fredrick 2001) and myopic eyes appear to have a prolate shape in which the axial and transverse dimensions regulate independently (Singh, Logan and Gilmartin 2006; Logan, *et al.* 2004). Since the vascular regulatory capacity of the myopic eye has not been explored previously, the independent regulation of the ocular dimension could be related to differences in ocular vascular regulation.

Additionally, it is also necessary to highlight the lack of information regarding the refractive state of the participants evaluated in the previous papers mentioned above. The refractive status of the participants assessed in each study that evaluated ocular reactivity to hypercapnia to date most likely included hyperopes, myopes and emmetropes); thus reducing the possibility of unveiling differences in vascular reactivity between refractive groups. To support this hypothesis, the haemodynamic response to hypercapnia from the 66 participants recruited in the present study was calculated. The overall analysis of CDI data showed a significant increase in the OA systolic and diastolic velocities ($p=0.006$), accompanied by a significant increase in resistance index ($p=0.007$), whereas OBF analysis showed a significant increase in pulsatile ocular blood volume and flow (OBFv and POBF) during hypercapnia ($p=0.016$; $p=0.013$). However, no significant changes during hypercapnia were reported in the microretinal vasculature. These results suggest a vasodilatory effect of hypercapnia on the retrobulbar and pulsatile but not microretinal blood flow, which would be supported by the literature reviewed above.

The results from this study could be related to those obtained by Rai (2007), who has recently suggested that myopes and emmetropes respond differently to high and relatively low levels of accommodation (Rai 2007; Rai, Gilmartin and Wolffsohn 2007.). An overall significant decrease in IOP at high levels of accommodation (4D) was reported, and independent evaluation of the ocular pulse amplitude by refractive group showed myopes to have significantly increased ocular pulse amplitude at high levels of accommodation; whereas emmetropes exhibited a decrease in ocular pulse amplitude at high levels of accommodation. These results point towards differences

in the autoregulatory mechanism induced by the decrease in IOP at high levels of accommodation, which in turn, may suggest a role in accommodation and vascular autoregulation in the onset and progression of myopia.

4.6.3 Effect of age on the regulation of ocular haemodynamics

In order to study the effect of age on ocular haemodynamic regulation, the sample evaluated was grouped by age (below and above 40 years). This approach was the one followed, as it allowed the comparison of results between the age analysis performed in the second part of chapter three, which evaluated the resting ocular vascular profile of the ageing eye, and the present study assessing the effect of age on the ocular regulatory mechanisms of the human eye.

There was no significant difference in ocular haemodynamics at baseline (which was performed with the breathing mask on to minimise the effect of the mask on the hypercapnic response); however, the systolic and diastolic blood pressure readings were higher in the older group. These differences in BP would not have confounded the statistical analysis of the response to hypercapnia had the ocular perfusion pressure not changed during hypercapnia. However, OPP increased significantly during hypercapnia, and an elevated ocular perfusion pressure may induce changes in ocular blood flow that may be understood as local metabolic regulation if OPP is not controlled. Therefore, after controlling for the changes in OPP during hypercapnia, no significant changes in retrobulbar, pulsatile or microretinal circulation were observed. Thus, the ocular vascular reactivity to increased pCO_2 did not differ between age groups. The lack of difference in vascular response may be due to the exclusion of a gap age that would have differentiated the two age groups to a greater extend. Despite not finding differences in haemodynamic regulation between the two age groups, a positive correlation was found between age and the percentage change in OA EDv and SPCA ratio induced by hypercapnia. Increase in wall rigidity, accompanied by a significant increase in blood pressure, may also be related to a greater increase in retrobulbar blood velocity, as the vessel diameter reduces with age. Normal human aging is associated with a gradual decrease in cerebral blood flow in regions of the brain thought to be important for higher order cognitive functions. This reduction has been related to changes in vascular architecture, vessel walls and decrease in blood flow regulation, which result in a reduced capacity of blood vessels to respond to metabolic changes. Changes in vascular smooth muscle cells might contribute to such functional impairment (Hughes, *et al.* 2006). Pericytes, non differentiated cells that have been implicated in many vascular-related functions (see section 1.10.1.4) have also been implicated in the age-dependent decrease in autoregulation of blood flow; pericyte coverage of human cortical capillaries decreases with age (Farrell, *et al.* 1987) and evidence of pericyte degradation in the central nervous system of ageing rats has also been reported (Hellstrom, *et al.*

1999). Therefore, the increase in retrobulbar blood velocity that occurred with increasing age may be due to a protective mechanism by which the retrobulbar blood velocity in the OA and SPCA over-respond to changes in oxygenation due to their age-related sensitivity reduction to metabolic changes.

4.6.4 The effect of AL on the vascular autoregulation of the eye

A significant correlation between AL and the percentage change induced by hypercapnia on the ocular pulse amplitude, volume, flow, and retinal microcirculation was found. The results suggested that the difference in hypercapnic response between myopes and emmetropes was possibly related to the differences in AL. Thus, AL appears as an important factor, as it correlated with the resting POBF and CRA blood velocity (section 3.1.5.3), and it also appeared to be related to the response to hypercapnia. The slope of the correlation described a mechanism by which the increase in ocular pulse amplitude and blood flow was larger in eyes with increased axial length. However, the slopes and significant values obtained (p ranging from p=0.016 to p=0.03) need to be considered and suggest a cautious approach. Additionally, the lower resting POBF in the myopic group reported in chapter 3 (section 3.1.5.2) together with the greater POBF response to hypercapnia with increasing axial length may be indicative of a relative vasoconstriction in myopic subjects that was partially reversed during increased pCO_2. The differences in the slopes may be due to the fact that POBF is a calculation of blood flow; whereas the parameter that changed significantly in the microretinal circulation was the blood velocity. A decrease in blood velocity is representative of an increase in blood flow, if the resistance index decreases. Since the resistance index could not be calculated for the retinal microvasculature, no assumptions could be made. However, an increased POBF response to hypercapnia with increased axial length and a decreased microretinal blood velocity response to hypercapnia may represent different aspects of an increase in reactivity to hypercapnia with increasing axial length.

4.6.5 Interaction between the effect of age and refractive error on ocular autoregulation

Evaluation of the combined effect of age and refractive error on the ocular vascular responses during hypercapnia revealed a significant interaction between OA PSv, CRA PSv and CRA EDv. These results suggested that while the ocular regulation in myopes did not appear to vary significantly with age, in emmetropes the raised retrobulbar blood velocity response to hypercapnia seemed to increase with age. Myopic retinal blood vessels have been reported to be significantly decreased in diameter compared to emmetropes, which in turn has been suggested to be related to the elongated myopic vitreous chamber. Thus, the shape of the myopic eye may induce these changes in vessel geometry, and it could be for this reason that myopic ocular vessels

do not exhibit a progressive increase in retrobulbar blood velocity. The results from the present study are in agreement with those from a study by Shimada and co-workers in which mild myopes (> -3.00 D) and high myopes (-3.00 D to -8.00 D) exhibited narrowing of retinal artery diameters, alongside decreased velocities in the temporal branch of the CRA (Shimada, *et al.* 2004). Patton and colleagues likewise reported the association between increased axial length and narrowing of both arteriolar and venular retinal diameters (Patton, *et al.* 2005).

4.7 CONCLUSION

The evaluation of the autoregulatory features of the human eye demonstrated differences in the ocular vascular response between emmetropes and myopes. The POBF and microvascular responsiveness in myopes suggests a regulatory mechanism highly sensitive to changes in carbon dioxide concentration, as shown by the lack of responsiveness described in the emmetropic group..

The significant correlation found between the responsiveness to hypercapnia and axial length, high myopes may be coincidentally related to an increased reactivity due to their greater axial length.

Additionally, the significant correlation between the increased retrobulbar blood velocities as a response to hypercapnia with age may be indicative of a protective mechanism occurring parallel to the decrease in vessel diameter occurring with age

CHAPTER FIVE
THE EFFECT OF SMOKING ON HUMAN OCULAR HAEMODYNAMICS

5.1 Abstract

Purpose: To evaluate the chronic effect of smoking on the vascular perfusion characteristics of the human eye.

Methods: The sample comprised one eye of 42 healthy volunteers, 21 non-smokers and 21 smokers. The smoking group (n=21, mean age 26.79 ± 4.09 years, range 19 to 36 years) had an average MSE of -1.41 ± 2.09D (range +0.56D to -6.18D), had been smoking for 7.92 years (range 3 to 15 years) and from the Fagerström Tolerance Questionnaire (FTQ) exhibited a mean score of 7.38 (range 6 to 10). The non-smoking group (mean age of 26.52 ± 5.17 years, range 19 to 38 years) had an average MSE of -1.55 ± 2.06D (range +0.38D to -5.12D) and scored O points in the FTQ. For all participants a baseline vascular profile was determined from a) CDI measurements of blood velocity at the ophthalmic artery (OA), central retinal artery (CRA) and short posterior ciliary arteries (SPCA), b) HRF measures of flow, volume and velocity in the retinal microcirculation, and c) OBFA measures of pulsatile ocular blood flow (POBF) with all volunteers breathing normal room air. Autoregulation was assessed in a subgroup of volunteers by repeating all measurements, initially at a second baseline breathing room air through a mask, and then during isoxic hypercapnia (breathing CO_2 enriched air through a mask until end tidal pCO_2 increased approximately 15% above baseline). The subgroup comprised one eye of each of 11 non-smokers (mean age 25.00 ± 2.93 years, range 18 to 29 years; average MSE -1.42 ± 2.11D, range +0.50 to -6.44D) who scored O points in the FTQ, and 11 smokers (mean age 25.81 ± 3.70 years, range 19 to 31 years; average MSE -1.21 ± 2.04D, range +0.06D to -6.18D) who scored more than 6 in the FTQ (mean score 6.63, range 6 to 8) and had smoked on average 8.77 years (range 3 to 8 years) In each case the groups were matched for age, gender, ethnicity and MSE. Blood pressure (BP), intraocular pressure (IOP), and body mass index (BMI) were also recorded.

Results: Baseline Perfusion: no differences in BP, mean arterial pressure (MAP), ocular perfusion pressure (OPP) or ocular vascular perfusion was found between smokers and non-smokers. Hypercapnia: hypercapnia induced a significant increase in systolic blood pressure (SBP), diastolic blood pressure (DBP), mean arterial pressure (MAP) and ocular perfusion pressure (OPP) only in smokers. After controlling for the effects of OPP in smokers there were no significant response to hypercapnia in the pulsatile, retrobulbar or microretinal circulation of either smokers or non-smokers. FTQ score correlated significantly with HIPC OA ratio (R=-0.63 p=0.05) and CRA PSv (R=-0.65 p=0.04).

Conclusions: In normal resting conditions there was no significant reduction in baseline flow for smokers compared to non-smokers. During stress testing, only smokers demonstrated significant changes to systemic blood pressure which may be indicative of systemic hypoxia and autonomic dysregulation. Additionally, the lack of metabolic vascular reactivity to carbogen after controlling for OPP may be related to an endothelial dysfunction widely suggested in smoking.

5.2 Introduction

Smoking constitutes a risk factor for several conditions related to tissue destruction contributing to lung disease, cellular changes increasing the risk of cancer, and cellular and molecular reinforcing effects leading to smoking dependence. Our knowledge on the pathophysiology of smoking has improved greatly in the past two decades and it is reflected by the increasing number of research publications on the mechanisms, consequences and prevention of smoking (Henningfield, Schuh and Jarvik 1995).

Recent smoking prevalence surveys in the UK describe approximately 25 per cent smokers among the adult population. After analysing 15,000 participants from two administrative boroughs in the North West of England (55 per cent women and 45 per cent men) 27.2 per cent of the women were found to smoke (mean age 45.2 years), which differed significantly from 25.6 per cent of smokers among men (mean age 45.6) (Edwards, *et al.* 2006; Henningfield, Schuh and Jarvik 1995). Greece has currently the highest smoking prevalence among the members of the European Union, as shown by recent epidemiological studies that estimated that 40% of the adult population are daily smokers (Vardavas and Kafatos 2006).

Studies from other European countries, such as Sweden, show a trend towards a decrease in smoking prevalence. School-based community intervention combined with repeated cross-sectional surveys over 7 years on approximately 1600 students (ages 14 and 15 years) showed a significant decrease of nearly 50% in smoking prevalence in the intervention area. At the start of the intervention, smoking prevalence in grade nine was 16.1% in the intervention area compared to 23% in the national reference group. Although the prevalence in the national sample remained stable, there was a decrease to 9.0% in the intervention area at the end of the study period (Nilsson, *et al.* 2006).

Cigarette smoking is one of the most important aetiologic factors of diseases in the respiratory and cardiovascular system. The mechanism by which smoking affects these systems involves several mediators and pathways. Cigarette smoke is composed of many chemicals, including tar with

several carcinogens, nicotine, free radicals and gaseous compounds, such as carbon monoxide (CO). The gas component of cigarette smoke contains 4.5% CO, which is considered a toxic chemical at high concentrations, leading to a severe hypoxic condition by displacing oxygen from haemoglobin (Hb). Reports indicate that even low levels of chronic CO exposure may have important effects on health. Epidemiological studies have shown that ambient CO levels correlate with onset of heart diseases, increased mortality rates, and hospital admission for cardiovascular diseases (Burnett, *et al.* 1997; Kleinman, *et al.* 1989; Stern, *et al.* 1977). In addition, recent animal studies have shown that inhalation of CO at doses corresponding to tobacco smoking worsens cardiac failure in rats with induced myocardial infarction (Melin, *et al.* 2005; Mirza, *et al.* 2005). Furthermore, CO exposure has been suggested as an important aetiological factor for atherosclerosis (Kleinman, *et al.* 1989; Astrup, Kjeldsen and Wanstrup 1970). However, these findings have been questioned by other experimental studies, which did not show any association between CO exposure and atherosclerotic diseases (Penn, Currie and Snyder 1992; Weir and Fabiano 1982). Effects of acute high dose CO exposure on the respiratory system are well known, including pulmonary cell damage, endothelial and alveolar swelling and oedema (Niden and Schulz 1965).

Smoking has also been found to be a risk factor for systemic and peripheral vascular conditions. Smoking is associated with a two to four-fold increased risk of coronary heart disease and it has also been related to a higher risk of stroke and stroke related mortality (Lakier 1992).

Evaluation of the association between smoking and several ophthalmologic disorders reveals a continuous expansion of the negative effect induced by smoking on the ocular tissues. Cataract development and age-related macular degeneration are accelerated by smoking. Additionally, retinal ischemia, anterior ischemic optic neuropathy and Graves ophthalmopathy have also been linked to smoking, and the offspring of smoking mothers appear to be at higher risk of suffering from strabismus (Solberg, Rosner and Belkin 1998).

To date, mainly the acute effect of smoking on the vascular features of the eye has been analysed and it has been performed using several techniques such as blue field entoptic simulation, Doppler velocimetry and pulse wave velocity. The findings from different studies agree in increased blood velocities (pulse wave velocity and macular velocity), and decreased blood flow (retinal blood flow, microretinal blood flow) (Lietz-Partzsch, *et al.* 2001; Tamaki, *et al.* 2000 ; Monfrecola, *et al.* 1998; Morgado, *et al.* 1994; Robinson, Petrig and Riva 1985). The acute effect of smoking on the ocular perfusion of 14 healthy smokers using the blue field stimulation technique, which allows quantifying the velocity of leucocytes flowing in one's own macular capillaries, described an increase in macular velocity. The increase in velocity was hypothesised to be caused by the acute

increase that smoking induces on MAP (Robinson, Petrig and Riva 1985). This increase in systemic blood pressure after smoking has also been reported by several other studies, suggesting that increased ocular blood velocity may, therefore, be the result of the increase in systemic blood pressure induced by smoking, to which the ocular local regulatory mechanisms respond with an increase in resistance to ensure constant blood flow (Mahmud and Feely 2003; Ahlborg and Lundberg 2001; Tamaki, *et al.* 2000 ; Morgado, *et al.* 1994). Supporting this hypothesis, Tamaki and co-workers reported an increase in ocular vascular resistance on 10 healthy smokers after smoking (Tamaki, *et al.* 2000).

Williamson and colleagues (Williamson, Lowe and Baxter 1995) assessed the influence of age, BP and smoking on the retrobulbar blood flow of 95 healthy participants. The results showed a weak association between smoking and reduced blood velocity in the OA ($p=0.05$), but no correction analysis was performed to prevent the effect of age and BP on the results obtained in smokers. A later study by Steigerwalt and co-workers assessed the retrobulbar blood velocity in the OA, CRA and SPCAs of 10 smokers who smoked on average 20 cigarettes a day using CDI (Steigerwalt, *et al.* 2000). After comparing the results to those obtained from 11 non-smokers, a significant decrease in the CRA and SPCAs systolic and diastolic blood velocities of smokers was reported ($p<0.05$). However, this study did not assess the systemic profile of the participants, thus lacking essential information to understand the chronic effect of smoking on the ocular haemodynamics.

The autoregulatory properties of ocular blood flow in chronic smokers have also been reported to be affected, as shown by abnormally sustained choroidal blood flow in smokers compared to non-smokers after isometric exercise (Kiowski, *et al.* 1994). The vascular reactivity of the retinal bed in smokers was assessed in a study by Wimpissinger and colleagues (Wimpissinger, *et al.* 2005) during inhalation of 100% oxygen for 10 min. The response from 12 smokers and 12 nonsmokers to hyperoxia was assessed using the Retinal Vessel Analyzer (RVA) and laser Doppler velocimetry (LDV). The results showed a significant decrease in arterial and venous diameters in smokers and nonsmokers ($p<0.001$ both); however, the decrease was significantly more pronounced in smokers ($p<0.001$).

Thus, smoking appears to have an effect on the autoregulatory and physiological mechanisms of vascular supply to the healthy eye. However, at present, the literature available on the chronic effects of smoking on ocular haemodynamics lacks a detailed evaluation of the different vascular beds and systemic circulation with a correction analysis to prevent the confounding effects of age and BP. Additionally, the washout periods (periods without smoking) prior to data collection from previous studies ranged from 30 minutes to 1 hour, which may not be sufficient to describe the residual chronic effect of smoking. This motivated a study to evaluate the human ocular

haemodynamics of smokers by assessing the various ocular vascular beds, considering systemic circulation and age as potential variables, and including a larger washout period to provide data on the actual chronic effect of smoking.

5.3 Aims and objectives

The aim of this study was to evaluate the chronic effect of long-term smoking on the haemodymanic profile and autoregulatory characteristics of the ocular vascular system in humans.

5.4 Methods

The current chapter was subdivided in two sections, the first of which described the effect of chronic smoking on ocular blood flow by evaluating the differences between a group of smokers (n=21) and non-smokers (n=21), both groups being matched for age, gender, ethnicity, MSE and axial length. In the second section, the ocular vascular regulatory profile of smokers was investigated using a stress test on a subgroup of smokers (n=11) and non-smokers (n=11), also matched for age, gender, ethnicity, MSE and axial length.

In addition, the interaction between AL and the FTQ score on the percentage change of ocular haemodynamics was evaluated by subdividing the smokers' subgroup that underwent hypercapnia into two groups based on FTQ score and AL. The FTQ subdivision included those smokers that scored less than 7 in FTQ in one subgroup and those smokers whose FTQ score was greater than 7 in another subgroup. The AL subdivision was performed with 24mm as the subdivision criterion.

5.4.1 Study Sample and Recruitment Criteria

The participants were recruited from the students and staff at Aston University, Birmingham, UK. In order to provide a quantitative indicator of smoking category, the subjects were asked to complete the Fagerström Tolerance Questionnaire (FTQ). Only those who reported smoking for at least three years were included in the study, since nicotine has shown to exert an effect on systemic circulation after smoking for one year.

The sample for the first study consisted of 42 healthy volunteers, 21 smokers and 21 non-smokers. Smokers had been smoking for 7.92 years on average (range 3 to 15 years). All the 21 smokers that participated in the study scored more than 6 points in the FTQ (mean score 7.38, ranging from 6 to 10). Smokers age ranged from 19 to 36 years (average age 26.79 ± 4.09 years) and their average MSE was -1.41 D ± 2.09 (range +0.56D to -6.18D). The control group consisted of 21 non-

smokers, all of whom scored O points in the FTQ. The mean age of the control group was 26.52 ± 5.17 years (age range 19 to 38 years), and their average MSE was -1.55 ± 2.06D (range +0.38D to -5.12D). Each smoker was individually matched for age, gender, ethnicity and refractive error to a non-smoker (table 5.1).

The sample group for the second study comprised one eye of each of 11 non-smokers (mean age 25.00 ± 2.93 years, range 18 to 29 years; average MSE -1.42 ± 2.11D, range +0.50 to -6.44D) who scored O points in the FTQ, and 11 smokers (mean age 25.81 ± 3.70 years, range 19 to 31 years; average MSE -1.21 ± 2.04D, range +0.06D to -6.18D) who scored more than 6 in the FTQ (mean score 6.63, range 6 to 8) and had smoked on average for 8.77 years (range 3 to 8 years). This study also had individual matching for age, gender, ethnicity and refractive error between smokers and non-smokers.

To evaluate the relationship between AL and FTQ score on the ocular vascular response to hypercapnia, the smokers that underwent the second study were subdivided into: AL <24mm or ≥24mm and FTQ score (≤7 score or >7 score) as indicated in table 5.2. The reason for this AL subdivision was to segregate further the smokers from into average smokers and heavy smokers. Additionally, since chapter 4 of this thesis has shown AL to correlate significantly with ocular blood flow, smokers were further subdivided according to their AL. Thereby, the potential interaction between FTQ score and AL was evaluated.

Since a physiological correlation is known to exist between the two eyes of the same subject, only one eye randomly chosen from each subject was evaluated (Kimura, *et al.* 2003). All the participants were healthy volunteers, which was confirmed by ophthalmologic investigation of the fundus, blood pressure measurements and detailed recording of systemic and ocular history and symptoms. Exclusion factors included any ocular disorder, diabetes, hypotension, hypertension or any other systemic disorder or medication likely to affect the systemic or ocular vasculature. A corrected visual acuity of 6/9 or better was required together with astigmatism of less than 1.5 diopters cylinder, which reduced the percentage of astigmatic patients being taken as myopic subjects after MSE calculation.

Since the aim of this study was to describe the long-term effect of smoking on the ocular haemodynamics, only those smokers able to refrain from smoking for 12 hours prior to the study were recruited. Refraining from smoking for 12h was a requirement of marked importance in this study as nicotine has a half-life of 2 hours (Benowitz. et al, 1982), and 6 hours after smoking only 0.031 mg of nicotine remains in the body (average 1mg of nicotine per cigarette). Thus, asking the subjects to refrain from smoking for 12 hours ensured that the chronic effect was evaluated.

Due to the addictive nature of smoking, smokers were asked to be truthful prior to participating in the study. Only those smokers who satisfied the requirement were included in the analysis.

5.4.2 Ethical Approval and Informed Consent

Written and verbal information about the exact procedures to be performed during the visit was given to all the subjects prior to data collection. The participants were encouraged to ask any questions and to clarify any doubts they might have before signing the written consent. The volunteers were requested to sign two copies of the written consent, one of which was given to them for their own records. All investigations were approved by the Ethical Review Committee and conformed to the declaration of Helsinki (appendix 2.1)

	Smokers	Non-smokers	p
FTQ score	7.38 ± 1.07	0	* <0.001
MSE (D)	-1.41 ± 2.09	-1.55 ± 2.06	ns
Gender	11m; 10f	11m; 10f	n/a
Age (years)	26.76 ± 4.09	26.52 ± 5.17	ns
Systolic BP (mmHg)	107.45 ± 13.26	113.85 ± 16.39	ns
Diastolic BP (mmHg)	72.09 ± 8.24	73.15 ± 9.76	ns
Pulse rate (bpm)	66.80 ± 11.04	67.85 ± 10.29	ns
MAP (mmHg)	91.01 ± 15.11	93.98 ± 18.19	ns
OPP	48.61 ± 10.33	49.80 ± 12.86	ns
Height (m)	1.69 ± 0.08	1.72 ± 0.08	ns
Weight (kg)	72.11 ± 16.72	70.51 ± 14.94	ns
BMI	25.02 ± 4.67	23.55 ± 3.82	ns
Axial length (mm)	23.83 ± 0.89	24.22 ± 1.12	ns
ACD (mm)	3.54 ± 0.29	3.64 ±0.25	ns
CT (μm)	538.28 ± 41.68	558.71 ± 37.82	ns
IOP (mmHg)	12.58 ± 3.29	12.85 ± 3.20	ns

Table 5.1. *Summary of physical, ocular and vascular systemic features in smokers and non-smokers (FQT score, Fagerström Tolerance Questionnaire score; MAP, mean arterial pressure; OPP, ocular perfusion pressure; BMI, body mass index; ACD, anterior chamber depth; CT, corneal thickness; IOP, intraocular pressure)*

5.4.3 Study Sample: Smoking and dietary restrictions

As indicated in section 5.4.1, to ensure the reliability of the data, the smokers recruited in this study were asked to refrain from smoking. Additionally, due to the reported effect of alcohol and caffeine on ocular blood flow (Domino, *et al.* 2004; Gdovinova 2001), all the participants, smokers and nonsmokers, were asked to refrain from alcohol and consuming caffeine containing products 12 hours prior to the study.

	FTQ score		
AL	**score <7**	**score>7**	
<24mm	**2**	**3**	**5**
≥24mm	**3**	**3**	**6**
	5	**6**	**TOTAL=11**

Table 5.2 *Sample size for the analysis of the effect of AL and FTQ score on the ocular vascular response to hypercapnia*

5.4.4 Fagerström Tolerance Questionnaire, FTQ

All the participants were asked to answer eight questions regarding their smoking habits that provided an objective tool for the recruitment of smokers. The questionnaire was designed in 1978 by Fagerström to measure the degree of physical dependence on smoking (Fagerstrom 1978) and has been validated showing that its scores correlate significantly with nicotine blood levels and chronic nicotine intake (Pomerleau, *et al.* 1990).

The questionnaire consists of eight items related to physiological dependence to nicotine. It can score from zero (0) to eleven (11) points, and scores equal to or higher than 6 usually indicate a high degree of dependence (Fagerström 1978;) (table 5.3).

5.4.5. Experimental Protocol and Investigations

The experimental procedure followed in the current study comprised two main sets of data collection, which were performed in the same session in order to avoid daily fluctuations in systemic blood pressure.

The sessions consisted of a baseline measurement of ocular blood flow using OBFA, HRF and CDI performed under normal breathing conditions followed by repeated measures during induced hypercapnia for a subgroup of participants.

The instruments and protocol followed in this study were the same as those in chapter 4 (section 4.4.4).

Table 5.3 *Fagerström Tolerance Questionnaire, FTQ (from Fagerström, 1978).*

5.4.6 Statistical analysis

One-way ANOVA was used to assess the differences in ocular haemodynamics between smokers and non-smokers. Bonferroni correction was performed when associated parameters calculated from the same piece of equipment were compared. Pearson's correlation test evaluated the relationship between the scores given by FQT, the ocular vascular readings and the response to hypercapnia. The response to hypercapnia for the correlation analysis was given as percentage change between baseline and hypercapnia vascular measurements calculated for each individual and assigned positive or negative depending on the direction of the change (positive for an increase in absolute value, negative for a decrease).

A two-tailed paired Student's t-test was used to test for significance between baseline and hypercapnic conditions in non-smokers. The OPP increased significantly from baseline to hypercapnia in the smokers group. Thereby, to account for the confounding effects of OPP, the ocular response to hypercapnia was evaluated with a repeated measured ANCOVA in which OPP was included as a covariate. To assess the vascular response to hypercapnia it was necessary to ensure that the results provided were the actual response to pCO_2 changes and not the resultant of a higher pressure of perfusion. One-way repeated measures ANOVA was used to evaluate potential differences of each patient's systemic blood pressure prior to each ocular blood flow measurement.
Two-way between-groups ANOVA was used to assess the interaction effect between axial length, smoking, age and refraction on the vascular responses to hypercapnia
Two-way between-groups ANOVA was used to assess the interaction effect between refractive error and smoking group on the vascular responses to hypercapnia.
Statistical significance was defined as $p<0.05$. All data are given as mean ± SD.

5.5 Results

5.5.1 The effect of smoking on ocular haemodymanics

There were no significant differences in age, gender, height, weight and BMI between smokers and non-smokers. Moreover, systemic blood pressure, MAP and OPP did not differ significantly between the two groups (table 5.1).

None of the ocular biometric parameters assessed (AL, corneal thickness, ACD and IOP) differed significantly between the smokers and non-smokers (table 5.1).

5.5.1.1 Pulsatile Ocular Blood Flow

The analysis of OBFA parameters did not exhibit significant differences between smokers and non-smokers (table 5.4).

5.5.1.2 Retrobulbar haemodynamics

No significant differences were found between the retrobulbar blood velocities in smokers and non-smokers (table 5.5).

5.5.1.3 Retinal microvessels

Maximum and minimum values of retinal blood flow, volume and velocity were obtained from the retinal capillary bed around the foveal area using HRF. There was no significant difference between smokers and non-smokers (table 5.6).

	Non-smokers	Smokers	p
OBFa (mmHg)	3.01 ± 1.11	3.09 ± 1.46	ns
OBFv (µl)	7.87 ± 2.83	8.51 ± 3.52	ns
OBFr	66.75 ± 10.07	64.31 ± 18.79	ns
POBF (µl/min)	1072.79 ± 304.64	1186.86 ± 393.17	ns

Table 5.4. *Pulsatile ocular blood flow outcomes in smokers and non-smokers (explanation of abbreviations: OBFiop, intraocular pressure; OBFa, amplitude of ocular pulse; OBFv, volume of ocular pulse; OBFr, rate of ocular pulse; POBF, pulsatile ocular blood flow) (ns: no significant difference).*

VESSEL	Parameter	Non-smokers	Smokers	p
OA	**PSv (m/s)**	0.445 ± 0.12	0.404 ± 0.07	ns
	EDv (m/s)	0.103 ± 0.04	0.101 ± 0.04	ns
	RI	0.767 ± 0.06	0.756 ± 0.06	ns
	Ratio	2.85 ± 0.66	3.603 ± 1.90	ns
CRA	**PSv (m/s)**	0.121 ± 0.02	0.113 ± 0.03	ns
	EDv (m/s)	0.045 ± 0.01	0.037 ± 0.02	ns
	RI	0.632 ± 0.08	0.664 ± 0.12	ns
	Ratio	2.857 ± 0.60	3.60 ± 1.90	ns
SPCA	**PSv (m/s)**	0.157 ± 0.04	0.147 ± 0.04	ns
	EDv (m/s)	0.069 ± 0.02	0.064 ± 0.02	ns
	RI	0.560 ± 0.08	0.566 ± 0.10	ns
	Ratio	2.37 ± 0.56	2.46 ± 0.71	ns

Table 5.5 *Results obtained in the retrobulbar vessels of smokers and non-smokers measured with CDI (explanation of abbreviations: PSv, peak systolic velocity; EDv, end diastolic velocity; RI, resistance index). (ns: no significant difference).*

	Parameter (arbitrary units)	Smokers	Non-smokers	p
MAX	**Volume**	58.47 ± 4.93	25.78 ± 9.73	ns
	Flow	356.34 ± 155.61	450.39 ± 202.27	ns
	Velocity	2.18 ± 0.48	1.52 ± 0.59	ns
MIN	**Volume**	17.88 ± 6.53	17.55 ± 7.88	ns
	Flow	265.83 ± 109.01	270.06 ± 135.93	ns
	Velocity	0.98 ± 0.46	0.94 ± 0.41	ns

Table 5.6 *Maximum and minimum values of volume, flow and velocity measured in the retinal capillaries using HRF in smokers and non-smokers (ns: no significant difference)*

5.5.1.4 Correlation between FTQ score and ocular blood flow

The correlation between FTQ score and ocular blood flow readings was assessed in the group of 21 smokers by Pearson' product correlation. No significant correlation was found between any of the ocular parameters and FTQ scores (appendix 1.6).

5.5.2 Differences in vascular response to hypercapnia between smokers and non-smokers

There were no significant differences in age, gender, height, weight and BMI, MAP or OPP between the smokers and non-smokers that underwent the second study during baseline (table 5.7).

Baseline condition

BP did not change significantly throughout the baseline session, which lasted for approximately 45 minutes, in either of the groups (table 5.8). The maximum blood flow at the microretinal vessels was significantly lower in smokers than in nonsmokers ($p=0.02$). Bonferroni correction was used to correct for microretinal blood flow and volume, as they are both calculated from blood velocity. Additionally, all the HRF parameters were corrected with Bonferroni, since maximum and minimum values were given. For HRF volume and velocity the corrected p value was $p=0.012$, whereas the HRF velocity corrected p value was $p=0.025$ (see section 3.1.4.5). Thus, after Bonferroni correction, there was no significant difference between the ocular perfusion parameters of smokers and non-smokers at baseline (table 5.9).

Hypercapnic condition

Hypercapnic characteristics

The end tidal carbon dioxide concentration was successfully increased in both smokers and non-smokers. Smokers showed a significant increase in pCO_2 from 38.19 mmHg at baseline to 44.47 mmHg during hypercapnia (16.44 % increase) ($p<0.001$). Non-smokers end tidal concentration changed significantly from 39.88 mmHg at baseline to 46.62 mmHg (16.90 % increase) ($p<0.001$).

The oxygen concentration increased proportionally to CO_2 rise and varied significantly from 97.18 % at baseline to 98.20 % during hypercapnia in smokers ($p<0.001$) and from 97.27 to 98.31 in non-smokers ($p<0.001$). A summary of the data describing changes in end tidal carbon dioxide and oxygen saturation is given in table 5.10.

	Non-smokers	Smokers	p
MSE (D)	-1.42 ± 2.11	-1.21 ± 2.04	ns
Gender	4m; 7f	4m; 7f	n/a
Age (years)	25.00 ± 2.93	25.81 ± 3.70	ns
Systolic BP (mmHg)	112.27± 12.96	109.18 ± 10.85	ns
DiastolicBP (mmHg)	70.45 ± 11.68	68.45 ± 8.84	ns
Pulse rate (bpm)	70.18 ± 16.16	71.81 ± 13.70	ns
MAP (mmHg)	84.39 ± 11.34	81.84 ± 9.36	ns
OPP (mmHg)	43.14 ± 5.71	42.76 ± 7.24	ns
Height (m)	1.68 ± 0.09	1.66 ± 0.06	ns
Weight (kg)	63.74 ± 9.70	73.79 ± 19.35	ns
BMI	22.34 ± 2.82	26.30 ± 5.70	ns
Axial length (mm)	24.14 ± 1.04	23.76 ± 0.86	ns
ACD (mm)	3.61 ± 0.27	3.49 ± 0.23	ns
CT (μm)	552.43 ± 39.72	528.20 ± 33.72	ns
IOP (mmHg)	13.12 ± 3.73	11.80 ± 3.20	ns

Table 5.7. *Summary of physical, ocular and vascular systemic in smokers and non-smokers (explanation of abbreviations: MAP, mean arterial pressure; OPP, ocular perfusion pressure; BMI, body mass index; ACD, anterior chamber depth; CT, corneal thickness; IOP, intraocular pressure) ns: not significant*

recording	SBP (mmHg)	DBP (mmHg)	Heart rate (ppm)	MAP (mmHg)	OPP (mmHg)
1	108.04 ± 14.62	68.59 ± 10.53	71.86 ± 16.40	81.74 ± 11.10	42.03 ± 5.77
2	111.18 ± 12.16	68.63 ± 8.19	69.22 ± 12.68	82.81 ± 8.94	42.75 ± 5.13
3	114.09 ± 10.15	67.04 ± 10.23	62.50 ± 9.71	81.33 ± 8.70	41.76 ± 4.81

Table 5.8. *Summary of systemic blood pressure readings at baseline taken prior to 1. CDI, 2. OBFA, 3. HRF (explanation of abbreviations: SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial pressure; OPP, ocular perfusion).There were no significant differences between the three sets of BP measurements.*

5.5.2.1 Systemic response to hypercapnia

BP did not show significant changes throughout the hypercapnic session (table 5.11).

When the BP response induced by hypercapnia was analysed in smokers and non-smokers, only smokers showed a significant increase in SBP, DBP, MAP and OPP (p=0.007; p=0.040; p=0.024; p=0.024) (table 5.12). Thereby, to account for the confounding effects of OPP on the ocular response to hypercapnia, this variable was included as a covariate in the ANOVA repeated-measures analysis performed in the following sections.

Instrument	Parameter	Non-smokers	Smokers	p
OBFA	**OBFiop (mmHg)**	11.53 ± 1.74	10.07 ± 1.94	ns
	OBFa (mmHg)	3.38 ± 0.83	2.99 ± 1.08	ns
	OBFv (µl)	8.58 ± 2.34	8.13 ± 2.37	ns
	OBFr	61.80 ± 14.13	68.45 ± 12.25	ns
	POBF (µl/min)	1061.00 ± 89.58	1146.36±228.86	ns
CDI	**OA PSv (m/s)**	0.408 ± 0.273	0.466 ± 0.16	ns
	OA EDv (m/s)	0.098 ± 0.04	0.122 ± 0.06	ns
	OA RI	0.711 ± 0.120	0.742 ± 0.05	ns
	OA ratio	3.98 ± 1.50	4.00 ± 0.78	ns
	CRA PSv (m/s)	0.113 ± 0.03	0.121 ± 0.03	ns
	CRA EDv (m/s)	0.087 ± 0.13	0.052 ± 0.02	ns
	CRA RI	0.596 ± 0.07	0.584 ± 0.11	ns
	CRA ratio	2.54 ± 0.51	2.57 ± 0.66	ns
	SPCA PSv (m/s)	0.135 ± 0.04	0.181 ± 0.10	ns
	SPCA EDv (m/s)	0.061 ± 0.02	0.076 ± 0.05	ns
	SPCA RI	0.548 ± 0.06	0.559 ± 0.10	ns
	SPCA ratio	2.24 ± 0.34	2.33 ± 0.44	ns
HRF	**Max Volume (au)**	21.40 ± 4.62	17.26 ± 4.03	ns
	Max Flow (au)	353.60 ± 97.35	257.84 ± 61.89	* 0.02
	Max Velocity (au)	1.22 ± 0.32	0.91 ± 0.22	ns
	Min Volume (au)	13.48 ± 4.11	13.16 ± 3.51	ns
	Min Flow (au)	207.74 ± 81.25	187.43 ± 43.84	ns
	Min Velocity (au)	0.74 ± 0.28	0.67 ± 0.16	ns

Table 5.9. *Results of OBFA, CDI and HRF vascular parameters during baseline condition for smokers and non-smokers (ns:not significant; * not significant after Bonferroni correction)*

	Baseline	Hypercapnia	
	Smokers		
End tidal CO_2 (mmHg)	38.19 ± 5.99	44.47 ± 7.03	<0.001
O_2 saturation levels (%)	97.18 ± 1.16	98.20 ± 1.13	<0.001
	Non-smokers		
End tidal CO_2 (mmHg)	39.88 ± 9.12	46.62 ± 7.59	<0.001
O_2 saturation levels (%)	97.27 ± 1.19	98.31 ± 116	<0.001

Table 5.10. *Changes in CO_2 and O_2 from baseline to hypercapnic conditions for smokers and non-smokers.*

recording	SBP (mmHg)	DBP (mmHg)	Heart rate (ppm)	MAP (mmHg)	OPP (mmHg)
			overall		
1	113.77 ± 13.50	71.81 ± 11.11	70.13 ± 13.78	85.80 ± 11.28	43.47 ± 7.84
2	113.86 ± 11.93	71.63 ± 10.58	69.63 ± 11.02	85.71 ± 10.20	43.41 ± 7.09
3	114.33 ± 12.39	70.19 ± 13.79	66.38 ± 12.46	85.06 ± 12.69	43.04 ± 8.60
			smokers		
1	113.70 ± 8.70	71.00 ± 7.61	71.20 ± 12.45	85.85 ± 7.42	44.67 ± 4.42
2	111.80 ± 9.36	70.10 ± 8.31	70.20 ± 7.56	84.07 ± 8.62	43.49 ± 4.68
3	112.30 ± 9.49	68.60 ± 4.27	70.30 ± 9.88	84.33 ± 5.60	43.89 ± 4.29
			non-smokers		
1	114.45 ± 17.59	73.45 ± 13.92	70.18 ± 15.68	87.12 ± 14.49	42.80 ± 10.52
2	115.45 ± 15.04	73.18 ± 12.95	68.69 ± 14.43	87.27 ± 12.55	42.90 ± 9.70
3	117.36 ± 13.55	72.54 ± 18.57	69.27 ± 13.72	87.48 ± 16.00	43.05 ± 11.28

Table 5.11. *Summary of systemic blood pressure readings at baseline taken prior to 1. CDI, 2. OBFA, 3. HRF (explanation of abbreviations: SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial pressure; OPP, ocular perfusion).There were no significant differences between the three sets of BP measurements in either of the groups.*

	Baseline	Hypercapnia	p
		smokers	
SBP (mmHg)	108.45 ± 9.42	113.18 ± 8.96	*0.007
DBP (mmHg)	67.81 ± 8.17	74.81 ± 14.25	*0.04
Heart rate (bpm)	69.09 ± 12.66	70.27 ± 10.92	ns
MAP (mmHg)	81.36 ± 8.08	87.60 ± 11.81	*0.024
OPP (mmHg)	42.06 ± 4.52	46.22 ± 7.48	*0.024
		non-smokers	
SBP (mmHg)	111.63 ± 14.59	115.00 ± 15.68	ns
DBP (mmHg)	68.63 ± 11.31	75.45 ± 15.46	ns
Heart rate (bpm)	69.18 ± 13.94	69.54 ± 15.46	ns
MAP (mmHg)	82.96 ± 11.60	88.63 ± 14.73	ns
OPP	40.04 ± 8.60	43.81 ± 10.47	ns

Table 5.12. *Systemic BP changes from baseline to hypercapnia: overall analysis and independent response from smokers and non-smokers (ns: not significant; * statistically significant)*

5.5.2.2 Ocular vascular response to hypercapnia

Neither of the groups exhibited a significant retrobulbar change during hypercapnia (table 5.13).

VESSEL	PARAMETER	Baseline	Hypercapnia	p
		NON-SMOKERS		
OA	**PSv (m/s)**	0.408 ± 0.27	0.431 ± 0.27	ns
	EDv (m/s)	0.098 ± 0.04	0.091 ± 0.04	ns
	RI	0.710 ± 0.11	0.762 ± 0.11	ns
	Ratio	3.981 ± 1.50	4.878 ± 1.81	ns
CRA	**PSv (m/s)**	0.113 ± 0.03	0.113 ± 0.02	ns
	EDv (m/s)	0.087 ± 0.13	0.046 ± 0.01	ns
	RI	0.060 ± 0.07	0.059 ± 0.09	ns
	Ratio	2.547 ± 0.51	2.580 ± 0.71	ns
SPCA	**PSv (m/s)**	0.134 ± 0.03	0.149 ± 0.04	ns
	EDv (m/s)	0.061 ± 0.02	0.068 ± 0.02	ns
	RI	0.548 ± 0.06	0.539 ± 0.07	ns
	Ratio	2.246 ± 0.34	2.218± 0.33	ns
		SMOKERS		
OA	**PSv (m/s)**	0.432 ± 0.13	0.474 ± 0.15	ns
	EDv (m/s)	0.116 ± 0.05	0.114 ± 0.05	ns
	RI	0.741 ± 0.05	0.760 ± 0.06	ns
	Ratio	3.986 ± 0.82	4.555 ± 1.33	ns
CRA	**PSv (m/s)**	0.121 ± 0.03	0.099 ± 0.02	ns
	EDv (m/s)	0.052 ± 0.02	0.037 ± 0.01	ns
	RI	0.581 ± 0.11	0.620 ± 0.10	ns
	Ratio	2.567 ± 0.70	2.862 ± 0.97	ns
SPCA	**PSv (m/s)**	0.191 ± 0.10	0.170 ± 0.06	ns
	EDv (m/s)	0.081 ± 0.05	0.067 ± 0.02	ns
	RI	0.550 ± 0.09	0.582 ± 0.09	ns
	Ratio	2.284 ± 0.43	2.578 ± 0.93	ns

Table 5.13 *Vascular results obtained during baseline and hypercapnia in smokers and non-smokers using CDI (ns: no significant difference)*

The pulsatile and microretinal blood flow response to hypercapnia did not differ significantly between smokers and non-smokers (table 5.14, table 5.15).

	Baseline	Hypercapnia	p
	NON-SMOKERS		
OBFiop (mmHg)	11.64 ± 1.92	11.78 ± 2.52	ns
OBFa (mmHg)	3.38 ± 0.83	3.34 ± 1.05	ns
OBFv (µl)	8.58 ± 2.34	8.56 ± 3.07	ns
OBFr	61.80 ± 14.13	59.40 ± 15.14	ns
POBF (µl/min)	1061.00 ± 189.58	1062.30 ± 236.05	ns
	SMOKERS		
OBFiop (mmHg)	10.07 ± 1.94	10.00 ± 3.23	ns
OBFa (mmHg)	3.14 ± 1.01	3.11 ± 0.90	ns
OBFv (µl)	8.45 ± 2.24	8.82 ± 2.45	ns
OBFr	68.50 ± 12.92	64.00 ± 7.91	ns
POBF (µl/min)	1172.00 ± 223.97	1204.50 ± 299.09	ns

Table 5.14 *Vascular results obtained during baseline and hypercapnia in smokers and non-smokers using OBFA (ns: no significant difference)*

	PARAMETER	Baseline	Hypercapnia	p
		NON-SMOKERS		
Max	**Volume (au)**	21.40 ± 4.62	22.50 ± 7.08	ns
	Flow (au)	353.60 ± 97.35	383.84 ± 106.78	ns
	Velocity (au)	1.22 ± 0.32	1.32 ± 0.35	ns
Min	**Volume (au)**	13.48 ± 4.11	14.23 ± 5.98	ns
	Flow (au)	207.74± 81.25	231.23 ± 103.73	ns
	Velocity (au)	0.748 ± 0.28	0.758 ± 0.423	ns
		SMOKERS		
Max	**Volume (au)**	17.26 ± 4.03	17.32 ± 2.69	ns
	Flow (au)	257.84 ± 61.89	282.13 ± 74.38	ns
	Velocity (au)	8.69 ± 23.42	0.97 ± 0.29	ns
Min	**Volume (au)**	13.16 ± 3.51	13.61 ± 3.16	ns
	Flow (au)	187.43 ± 43.84	221.74 ± 56.73	*0.035
	Velocity (au)	0.67 ± 0.15	0.80 ± 0.20	*0.027

Table 5.15 *Vascular results obtained during baseline and hypercapnia in smokers and non-smokers using HRF (ns: no significant difference) * not significant with Bonferroni correction.*

5.5.3 Relationship between smoking score and hypercapnia response

In smokers, the FTQ score correlated significantly with the hypercapnia-induced increase in blood velocity in the ophthalmic artery, central retinal artery and retinal microvessels. Additionally, the number of years smoking correlated significantly with the hypercapnia-induced percentage change in OA and CRA (table 5.16, figure 5.1).

		Score FTQ		Years smoking	
Vascular parameter changes	**Change (mean± sd)**	**Pearson's R**	**p**	**Pearson's R**	**p**
OBFiop (%)	**0.507 ± 29.44**	0.02	ns	0.20	ns
OBFa (%)	**1.354 ± 21.39**	0.32	ns	-0.50	ns
OBFv (%)	**5.499 ± 21.68**	0.14	ns	-0.30	ns
OBFr (%)	**-5.071 ± 11.51**	-0.14	ns	0.38	ns
POBF (%)	**3.453 ± 20.23**	-0.27	ns	0.07	ns
OA PSv (%)	**12.677 ± 33.79**	-0.70	*0.02	-0.30	ns
OA EDv (%)	**-0.397 ± 22.69**	0.03	ns	0.05	ns
OA RI (%)	**3.048 ± 8.862**	-0.61	0.08	-0.65	0.06
OA ratio (%)	**15.474 ± 30.50**	-0.63	*0.05	-0.53	ns
CRA PSv (%)	**-15.204 ± 21.43**	-0.65	*0.04	0.09	ns
CRA EDv (%)	**-22.542 ± 19.63**	-0.58	0.08	-0.04	ns
CRA RI (%)	**7.991 ± 13.76**	0.36	ns	0.09	ns
CRA ratio (%)	**12.030 ± 22.46**	0.05	ns	0.13	ns
SPCA PSv (%)	**6.872 ± 45.30**	-0.14	ns	-0.40	ns
SPCA EDv (%)	**7.162 ± 54.11**	-0.07	ns	-0.05	ns
SPCA RI (%)	**9.888 ± 35.41**	0.29	ns	-0.34	ns
SPCA ratio (%)	**20.426 ± 68.71**	0.33	ns	-0.37	ns
HRF max vol (%)	**3.158 ± 18.70**	0.15	ns	0.63	ns
HRF max flow (%)	**11.843 ± 26.91**	0.22	ns	0.63	ns
HRF max vel (%)	**-5.566 ± 40.19**	0.02	ns	-0.07	ns
HRF min vol (%)	**14.977 ± 52.73**	-0.50	ns	-0.13	ns
HRF min flow (%)	**5.247 ± 15.80**	0.51	ns	0.66	ns
HRF min vel (%)	**19.850 ± 21.57**	0.70	*0.03	0.78	**0.01

Table 5.16 *Correlations in smokers between the score obtained in FTQ, years smoking and percentage change in vascular parameters. ** significant with Bonferroni correction*

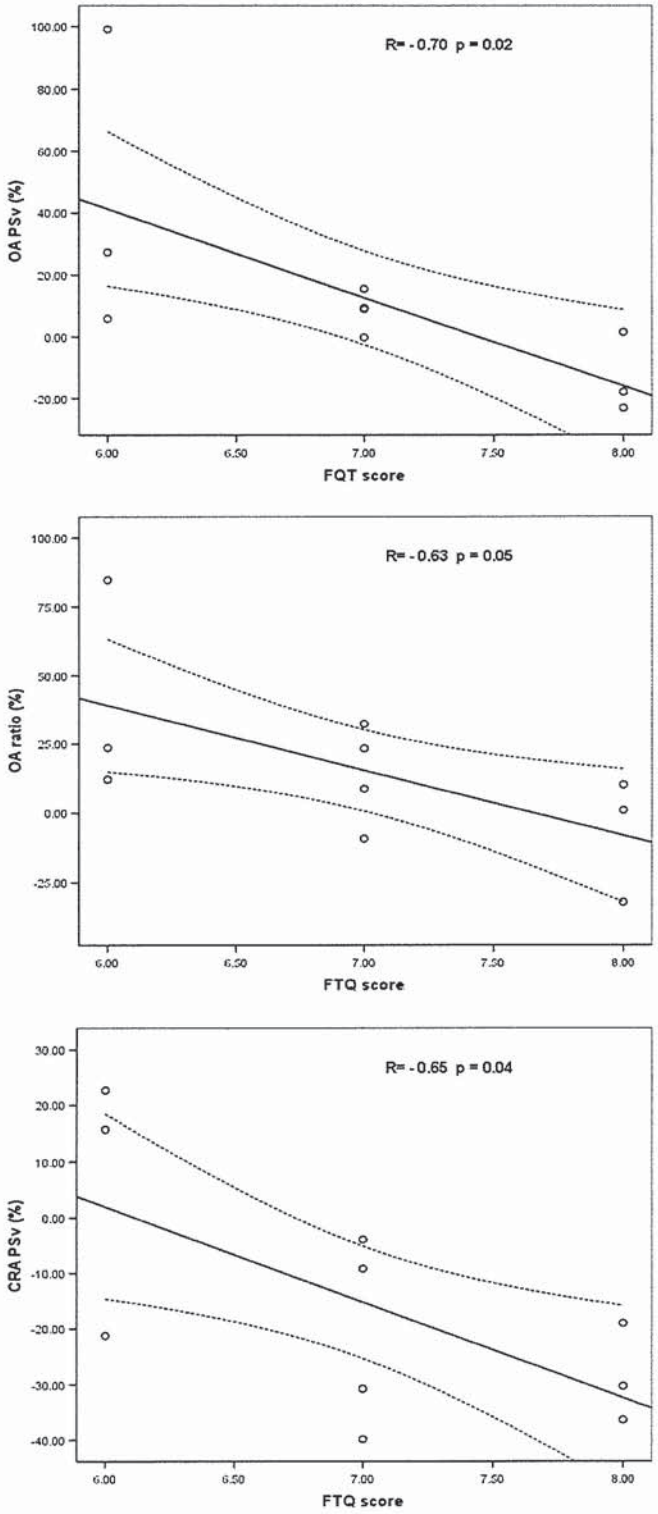


Figure 5.1 *Scatter plots showing the significant correlation between the score obtained in the FTQ (FTQ score) and the ocular vascular response to hypercapnia calculated as percentage change (OA PSv:ophthalmic artery peak systolic velocity, OA ratio: ophthalmic artery systolic to diastolic blood velocity ratio, CRA PSv: central retinal artery peak systolic velocity).*

5.5.4 Relationship between hypercapnia induced changes in BP and ocular blood flow.

The maximum velocity percentage change at the retinal microvessels correlated significantly with percentage change in diastolic blood pressure, mean arterial pressure and ocular perfusion pressure (p=0.014; p=0.008; p=0.014) (table 5.17, figure 5.2) in smokers only.

	SBP R value	p	DBP R value	p	MAP R value	p	OPP R value	p
OBFiop (%)	-0.45	ns	-0.42	ns	-0.34	ns	-0.34	ns
OBFv (%)	0.35	ns	0.51	ns	0.42	ns	0.38	ns
POBF (%)	0.29	ns	0.31	ns	0.27	ns	0.24	ns
CRA PSv (%)	0.13	ns	0.36	ns	0.27	ns	0.23	ns
HRF max flow (%)	-0.07	ns	0.009	ns	-0.17	ns	-0.16	ns
HRF max vel (%)	-0.61	ns	-0.82	*0.014	-0.81	*0.008	-0.77	*0.014
HRF min vel (%)	0.062	ns	-0.18	ns	-0.17	ns	-0.15	ns

Table 5.17 *Correlations in smokers between percentage change in ocular haemodynamics and systolic blood pressure (SBP), diastolic blood pressure (DBP), pulse rate, mean arterial pressure (MAP), and ocular perfusion pressure (OPP) (ns: no significant difference * significantly different)*

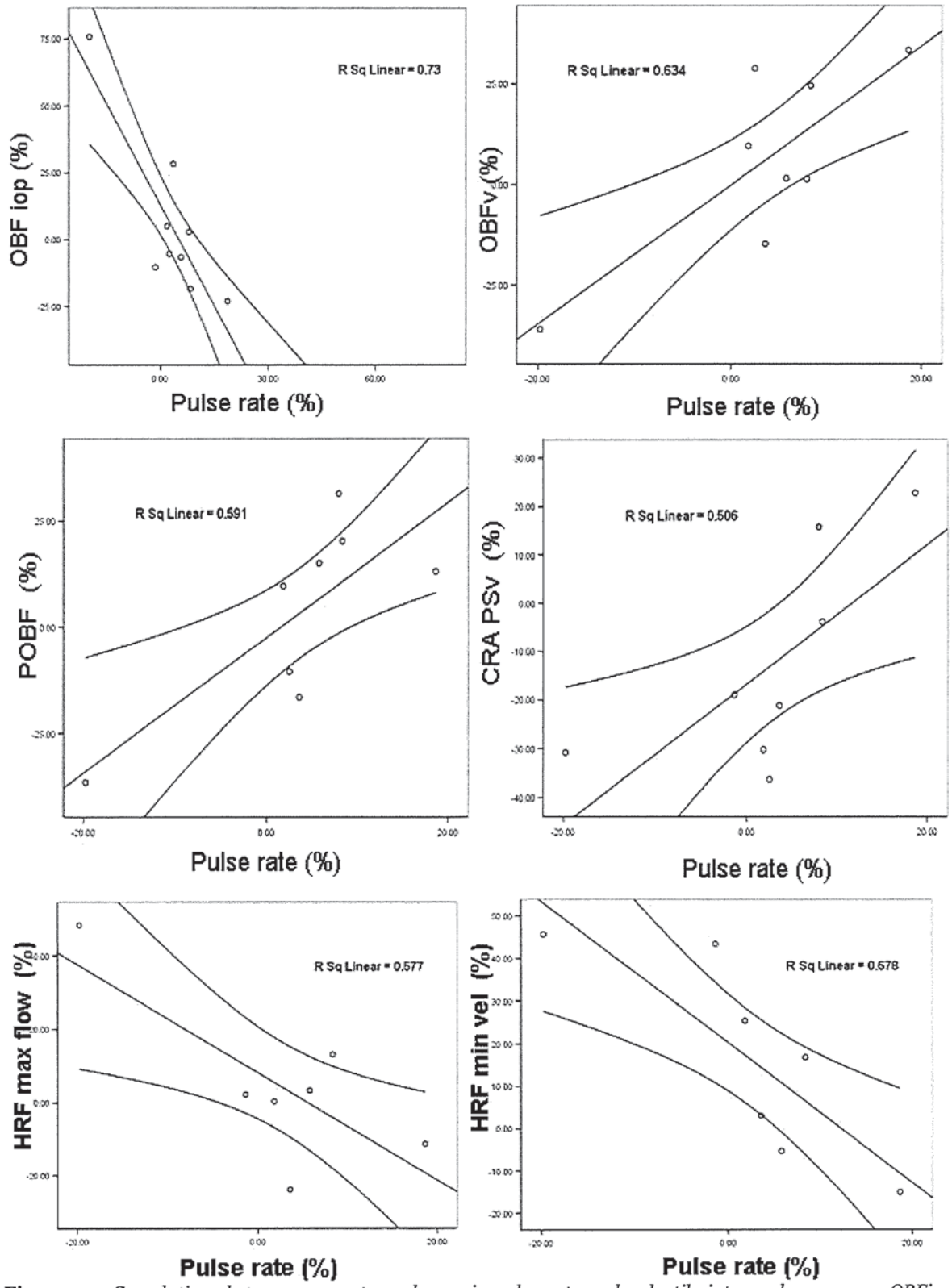


Figure 5.2 *Correlations between percentage change in pulse rate and pulsatile intraocular pressure OBFiop, volume of the ocular pulse (OBFv), pulsatile ocular blood flow (POBF), central retinal artery systolic velocity (CRA PSv) and retinal microvascular flow and velocity (HRF max flow, HRF min vel).*

5.5.5 The effect of AL and FTQ score on the ocular vascular response to hypercapnia

The hypercapnia-induced percentage change in the minimum retinal capillaries blood velocity in smokers showed statistical interaction between AL and the FTQ score (p=0.03)(figure 5.3).

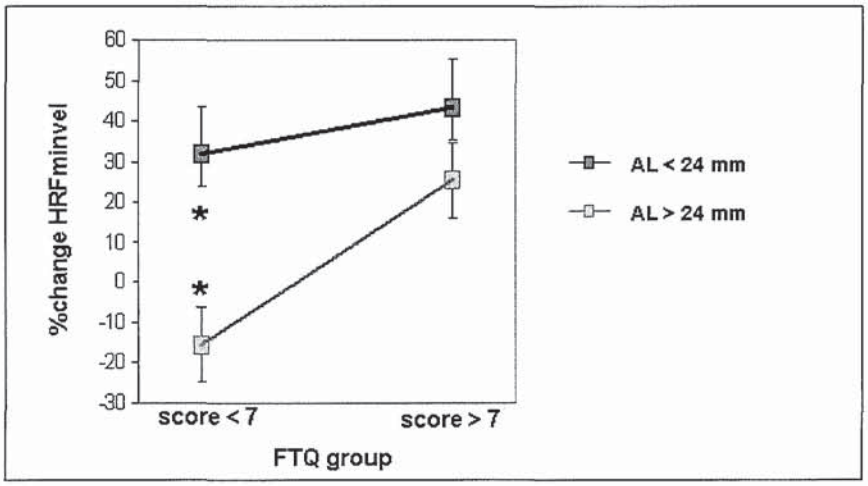


Figure 5.3 *Interaction effect for the smoking group between FTQ score and AL (FTQ: Fagerström Tolerance Questionnaire; AL: axial length) *the HRFminvel percentage change for those smokers with AL greater than 24mm differed significantly from the percentage change of the smokers with AL less than 24mm*

5.6 Discussion

The chronic effect of smoking on the retrobulbar, pulsatile and microretinal blood flow of young healthy smokers was evaluated using Colour Doppler Imaging (CDI), Ocular Blood Flow Analyser (OBFA) and Heidelberg Retinal Flowmetry (HRF). No significant differences in resting systemic blood pressure, MAP, OPP or ocular biometric parameters were found between smokers and non-smokers. Evaluation of the resting ocular vascular features between smokers and non-smokers did not reveal any significant differences. The systemic response to hypercapnia exhibited a significant increase in systolic blood pressure (SBP), diastolic blood pressure (DBP), mean arterial pressure (MAP) and ocular perfusion pressure (OPP) in the smokers. The analysis of the vascular regulatory properties after the inclusion of OPP as a covariate did not show significant changes during hypercapnia in smokers or non-smokers.

There was a significant relationship between the FTQ score of smokers and the hypercapnia-induced increase in blood velocity in the ophthalmic artery, central retinal artery and retinal

microvessels. Also in smokers, a significant interaction between axial length and the FTQ score on the microretinal response to hypercapnia was found.

5.6.1 Chronic effect of smoking on ocular haemodynamics

5.6.1.1Chronic effect of smoking on BP

The chronic effect of smoking on systemic blood pressure appears controversial, as smoking has been shown to induce an acute increase in blood pressure and heart rate, which in turn has been associated with malignant hypertension (Cryer, *et al.* 1976). Several studies have reported significantly higher BP in smokers than in non-smokers, which differs from findings describing lower BP (Berglund and Wilhelmsen 1975) or similar BP in smokers compared to non-smokers (Berglund and Wilhelmsen 1975; Seltzer 1974). The issue raised by Mann and colleagues was whether the real BP of smokers is higher on average due to the habit of smoking, which maintains an elevated daytime blood pressure; whereas at the time of measuring BP in the clinic, as the patient refrains from smoking, the BP appears reduced (Mann, *et al.* 1991). A study by Primatesta and co-workers (Primatesta, *et al.* 2001) aimed to overcome the influences of BMI, alcohol intake, age and gender on the effect of smoking on BP. The sample consisted of 33,860 volunteers with ages ranging from 16 to 97 years, recruited throughout 3 years by the annual Health Survey for England. After adjusting for age, BMI, social class and alcohol intake, older male smokers (> 45 years of age) were found to have higher systolic BP. No differences were reported for those below the age of 44. Therefore, it was concluded that the differences in BP reported by previous studies between smokers and non-smokers were likely to be due to the interaction between smoking, alcohol intake, and BMI (Primatesta, *et al.* 2001). Our results agree with those of Primatesta and colleagues and support the finding that BP does not differ between smokers and non-smokers below the age of 44 years when they are matched for BMI, age and gender.

5.6.1.2 Chronic effect of smoking on ocular blood flow

In the present study, the ocular perfusion features of smokers and non-smokers were not found to differ significantly. These results agree with those obtained by Hara in an animal study in which the chronic effect of smoking on the choroidal circulation of Wistar rats was assessed after inhalation of cigarette smoke for 30 minutes daily (Hara 1991). Choroidal blood flow and the index of choroidal vascular resistance were measured in one subgroup after 16 weeks and in another subgroup after 25 weeks of cigarette smoke inhalation. There were no significant differences in choroidal blood flow between those rats which underwent cigarette smoke inhalation and the animals in the control group, and additionally, no significant differences were reported between the two smoking groups (16 weeks and 25 weeks). However, the index of choroidal vascular resistance increased significantly after 25 weeks of inhalation. Thereby, these

results showed no significant effect of smoking on the choroidal blood flow of rats after 25 weeks smoking, but they reflect the results of chronic smoking on the choroidal circulation secondary to an alteration on the vascular resistance.

The results of the present study do not agree with those presented in a paper by Steigerwalt and co-workers in which the retrobulbar velocities in the OA, CRA and PCA were measured in 10 smokers and compared to those from 11 non-smokers using Colour Doppler Ultrasonography. They found a trend towards decreased blood velocities that reached significance for CRA and PCA (Steigerwalt, *et al.* 2000). The lack of agreement between the study by Steigerwalt and co-workers and the present study was probably due to differences in the recruitment criteria, as sample group for the present study was younger and the smoking history shorter than that assessed by Steigerwalt. Additionally, the participants involved in the study by Steigerwalt and colleagues smoked on average more cigarettes per day than the participants included in the present study. Therefore, the differences in the design of both studies suggest that greater nicotine intake for a longer period of time may result in decreased retrobulbar velocities. Our results, thus, agree with those from Hara in animals, with the limitation that our study did not record choroidal resistance, and it may be one possible reason why the current results study was unable to detect a significant effect of chronic smoking on the ocular blood flow parameters evaluated.

Power statistics revealed that a sample size with a statistical power of 80%, allocation ratio 1:1 and a significant value of 5% (α=0.05) required a sample of 20 participants to show statistical significance between the retrobulbar blood velocity of smokers and non-smokers, 13 to exhibit differences in POBF and 29 to show a difference in microretinal circulation. The sample size of this study is smaller than the values from the calculated sample size, which limited the study from showing a significant change during hypercapnia (sample size calculations performed following Simple Interactive Statistical Analysis, SISA).

Williamson and colleagues (Williamson, Lowe and Baxter 1995) assessed the influence of age, BP and smoking on the retrobulbar blood flow of 95 healthy participants. The results showed a weak association between smoking and reduced blood velocity in the OA of 24 healthy smokers (p=0.05). This study, however, did not correct for age or BP on the results obtained in smokers, and the relationship between smoking and reduced blood velocity was withdrawn from the correlation between the number of cigarettes smoked, without considering other factors such as those included in the FTQ used in the present study.

The acute effect of cigarette smoking on retinal blood flow has been investigated previously using Laser Doppler velocimetry (Morgado, et al. 1994), CDI (Kaiser, Schoetzau and Flammer 1997) and

Laser Speckle method (Tamaki, *et al.* 2000) among others. Morgado and colleagues reported a reduction in retinal blood flow on 21 volunteers, 11 of which had diabetes mellitus (Morgado, *et al.* 1994). Additionally, Tamaki evaluated the acute effect of smoking on the optic nerve head tissue circulation of 10 healthy smokers using the Laser Speckle method, by which normalised blur gives a quantitative index of tissue velocity. Sham smoking did not result in significant changes in blood velocity; however, after smoking there was a significant reduction in the tissue blood velocity in the posterior fundus, which may suggest an increase in resistance index (Tamaki, *et al.* 2000). Lietz-Partzsch evaluated the acute effect of smoking a cigarette on the retinal capillaries of 14 smokers and compared it to 14 non-smokers. The microretinal blood flow was assessed at baseline, after which the smoking group was asked to have a cigarette, and the blood flow was then assessed in all the participants ten minutes later. A significant decrease in microretinal blood flow was found in smokers ($p<0.01$) (Lietz-Partzsch, *et al.* 2001).

Interestingly, Kaiser and colleagues reported a significantly higher blood velocity in the OA, CRA and long posterior ciliary arteries in 46 healthy chronic smokers compared to 189 non-smokers using the same instrument as the one used for this study, the Siemens CDI. However, in their study the interval between the last cigarette and the recording of retrobulbar velocities was 30 to 60 minutes. Additionally, the sample size differed largely from that in the present study and smokers were reported to have significantly lower BP than non-smokers, which may explain the different outcomes described in the current study (Kaiser, Schoetzau and Flammer 1997).

Cigarette smoking acutely increases plasma catecholamines and results in higher blood pressure, heart rate, and sympathetic outflow (Narkiewicz, *et al.* 1998). Cigarette smoking has been reported to have a powerful sympathetic excitatory effect, influencing sympathetic drive to muscle blood vessels, to skin, and to the heart. A study by Kim and co-workers monitored changes in BP, pulse wave velocity (PWV) and pulse pressure at baseline and 5, 15, 30, 45 and 60 minutes after smoking (Kim, *et al.* 2005). PWV is a generally accepted parameter to estimate arterial stiffness based on the absorption of the entire pulse wave caused by ventricular contraction by a perfectly elastic artery compared to the partial reflection of the wave in the case of a rigid artery (Mahmud and Feely 2003). At baseline only the systolic BP differed significantly between smokers and non-smokers, showing no differences in DBP, pulse pressure, heart rate or PWV. These results agree with the vascular parameters described in the current study, and could be applied to the ocular haemodynamics profile found in the participants that took part in this study. After 5 minutes of cigarette smoking a significant increase in SBP, DBP, heart rate and PWV was observed and all the parameters returned to baseline values after 15 minutes, excluding PWV which returned to baseline readings 45 minutes after the onset of smoking. These results would support the outcomes of this study, since the readings were recorded 12 hours after the last cigarette. By the

time the ocular vascular profile of smokers was assessed, the perfusion parameters had recovered their normal values, thus, not showing significant differences, as occurred with the baseline readings in the study by Kim et al.

The results of the present study, therefore, suggested that young volunteers who had been smoking for more than 3 years did not show reduced retrobulbar, pulsatile or microretinal circulation. These findings may be attributed to the vascular capacity of the young healthy eye, reported in chapter four of this thesis, which appeared to decrease with age. Since the age of the smokers that participated in this study ranged from 19 to 31 years, the acute effects of smoking reported by several papers may be compensated by the vascular capacity to regulate ocular blood flow

5.6.1.3 Vascular response to hypercapnia in smokers and non-smokers

During hypercapnia, the systemic blood pressure changed significantly in smokers only, showing an increase in SBP, DBP, mean arterial pressure and ocular perfusion pressure. No changes were observed in ocular blood flow during hypercapnia in either smokers or non-smokers.

A reduced ocular vascular reactivity in smokers has been reported previously by several papers using adaptation to darkness, exercise and changes in breathing gas concentration as main provocation tests. Wimpissinger and colleagues reported a significantly decreased retinal arterial diameter in smokers during inhalation of 100% oxygen (Wimpissinger, *et al.* 2005). These findings were further supported by the reduced capacity of smokers to increase blood flow velocity in darkness in a study by Havelius and Hansen, in which the vascular reactivity to darkness was assessed in 20 smokers compared to 20 matched non-smokers (Havelius and Hansen 2005). Subfoveal choroidal blood flow has also been evaluated after six minutes of isometric exercise on 12 smokers and 12 non-smokers, and the increase in choroidal blood flow observed in smokers and non-smokers was significantly higher in the smoking group (Wimpissinger, *et al.* 2003).

To our knowledge, the ocular vascular response to increased pCO_2 has only been described in two papers to date. Wimpissinger and colleagues evaluated the choroidal blood flow, amplitudes of the ocular pulsations and retinal vessel diameter response to increased pCO_2 breathing on 13 smokers and 12 non-smokers using LDF, laser interferometry and Retinal Vessel Analyser (RVA) respectively. The smokers had smoked for at least two years, similarly to our study, and they smoked on average 15 to 25 cigarretes a day, which is more than the smokers in the present study. A choroidal and retinal response to carbogen was observed in non-smokers, which differed from the lack of changes in smokers, thereby leading to suggest an abnormal choroidal vascular reactivity in smokers. However, it is necessary to highlight the increase in SBP, DBP and MAP

during carbogen breathing found in smokers only. These changes in systemic blood pressure suggest a potential change in ocular perfusion pressure, which was not measured in the study by Wimpissinger and colleagues. If OPP changed significantly, as was the case in the present study, the effect of carbogen breathing should be evaluated controlling for the confounding effects that an increase in OPP may have on the ocular blood flow. Since OPP was not accounted for, the decrease in optic nerve head red blood cell volume reported during increased pCO2 may be the result of variations in OPP and the lack of significant changes during carbogen breathing may be related to myogenic mechanisms, and not due to the actual metabolic regulation. This hypothesis is supported by the findings observed in the present study in smokers when OPP was not included in the statistical analysis during hypercapnia (appendix 5.1). Smokers exhibited a significant decrease in central retinal artery systolic and diastolic blood velocity ($p=0.04$; $p=0.011$) and an increase in minimum flow and velocity during hypercapnia ($p=0.035$, $p=0.027$), that did not, however, reach the level of significance after Bonferroni correction.

The significant decrease in CRA PSv and EDv suggested a decrease in CRA blood flow during hypercapnia, as indicated in section 4.6.2.1, which might have been of myogenic origin, since the decrease in CRA blood velocity was not significant after controlling for the effects of OPP.

No significant differences in vasoactive reactivity were reported by Huber and colleagues (Huber, *et al.* 2006) in a subgroup of 11 smokers from a sample of 31 volunteers after CO_2 breathing, which was explained by the differences in instruments used; Huber *et al* used CDI, whereas Wimpissinger *et al* mainly used LDF techniques.

Despite the differences in statistical approach followed by Wimpissinger *et al* and those followed in the present study, the results described above suggest that smoking alters the mechanisms involved in blood flow regulation, which agree with the trend described by our results. Smoking is a risk factor for systemic and peripheral vascular conditions, and it has been associated with a two to four-fold increased risk of coronary heart disease and a higher risk of stroke and stroke related mortality (Lakier 1992). Retinal ischemia, anterior ischemic optic neuropathy and Graves ophthalmopathy have also been linked to smoking (Solberg, Rosner and Belkin 1998). Thus, the lack of response to hypercapnia in the ocular vascular bed of smokers may be related to a compromised autoregulatory mechanism.

Smokers have shown lower basal endothelin (ET)-1 plasma levels (Ahlborg and Lundberg 2001) and short-term smoking has been shown to increase vasoconstriction induced by ET-1 in the forearm (Kiowski, *et al.* 1994), all of which suggest a close relationship between the endothelin system and smoking habits. Endothelin is a peptide known to play a key role in vascular

homeostasis and it is one of the strongest vasoconstrictors currently investigated. It is located in the endothelium (see section 1.12.2.7) and its activation has shown to initiate vasoconstriction as well as vasodilation by nitric oxide release due to the binding of the ET-1 receptor (ET_BR (the precise effects of endothelin B receptor activation depends on the type of cells involved.) (Ergul 2002).The results of the present study could therefore be related to a decrease in the vasodilatory capacity of the ocular bed due to the lower basal ET-1 described in smokers.

It was interesting, however, to observe no significant response to hypercapnia in the ocular vascular perfusion of the non-smoking group. The reason for this may lie in the sample size, not being large enough. In order to observe a significant response to hypercapnia in the non-smoking group, power statistics revealed that a sample with a statistical power of 80%, allocation ratio 1:1 and a significant value of 5% (α=00.05) required 80 participants to show statistical significance in the retrobulbar vessels, 15 to exhibit differences in POBF and 32 to show a difference in microretinal circulation. The sample size of this study is smaller than the calculated sample size, which may limit the study from showing a significant change during hypercapnia (sample size calculations performed following Simple Interactive Statistical Analysis, SISA). Additionally, the refractive error of the participants included in the smoking and non-smoking groups ranged from +1.06 to -6.18 D and each group consisted of 8 emmetropes and 3 myopes. The lack of significant response to hypercapnia on the pulsatile and microretinal circulation in emmetropes would agree with the results described in chapter four, where no significant reactivity to increased pCO_2 was observed in emmetropes.

5.6.1.4 Correlation between hypercapnia-induced percentage change in ocular perfusion and FTQ score, years smoking and BP parameters

The significant correlation found between the FTQ score and the percentage change in blood velocity and resistance index induced by hypercapnia in the OA and CRA suggested a relationship between the degree of nicotine-dependence in smokers (given by FTQ) and the regulatory activity in the ocular beds in smokers. The FTQ was designed to provide an objective measurement of the degree of physical dependence to smoking (Fagerstrom 1978) and its scores correlate significantly with nicotine blood levels and chronic nicotine intake (Pomerleau, *et al.* 1990). Thus, it can be suggested that the vascular response to hypercapnia from the vascular beds in the healthy eyes of smokers is affected by the nicotine intake. The significant negative correlation between the hypercapnia induced percentage change in OA, CRA and the FTQ score represents a trend towards decreased blood velocities with increasing FTQ scores. The decrease in retrobulbar blood velocity can be understood as an increase in blood flow, if the resistance index also decreases. Since the resistance index could not be calculated for the retinal microvasculature, no assumptions can be

made. However, the systemic response given by the smokers, which was similar to that given by the myopes in the chapter 4, was probably related to their oxygen uptake ability, that is, the poor O2 exchange resulted from smoking made them more sensitive to changes in oxygenation. This hypothesis would explain their systemic and ocular changes. If the lack of response given to hypercapnia was related to the dysfunction of the endothelial tissue, as suggested above, this would increase the risk of vessel damage in smokers, thereby, putting them at greater risk with age. This might have also been the case for the myopic group, who reacted systemically to changes in pCO2, whereas emmetropes did not. Therefore, the reactivity to hypercapnia in the retrobulbar vessels appears to decrease with increasing nicotine intake, which may be related to an endothelial dysfunction in the circulatory system of smokers (Karatzi, *et al.* 2007; Winkelmann, *et al.* 2001; Zeiher, Schachinger and Minners 1995). Smoking appeared to cause endothelial dysfunction through impairment of nitric oxide (NO) production, or increased oxidative stress, but the exact mechanism still needs to be elucidated (Karatzi, *et al.* 2007).

5.7 CONCLUSION

Evaluation of the resting state vascular profile of a group of young smokers did not differ significantly from that of a group of non-smokers matched for age, gender and MSE. Hypercapnia induced a significant increase in SBP, DBP, MAP and OPP in smokers only. After controlling for the confounding effects of increased OPP on the ocular response to hypercapnia, neither of the groups exhibited significant ocular blood flow variations during increased pCO_2, which may be related to the endothelial dysfunction known to occur in smokers.
It is, however, important to highlight that the studies were under-power statistically due to the sample sizes; thereby further recruitment and data collection may be needed.

It was interesting to find that the systemic response to hypercapnia from the smokers was similar to that of myopes, which suggests that both groups needed to make systemic changes to cope with relatively small changes in pCO_2.

Future work could include the assessment of older smokers with a longer smoking history, as the perfect vascular autoregulatory response found in the young smokers of the present study is probably compromised with age, which would provide further evidence of the chronic effect of smoking on the ocular blood flow and its interaction with the effect of age.

CHAPTER SIX
OCULAR BLOOD FLOW AND VASCULAR AUTOREGULATION IN HUMAN ANISOMYOPIA

6.1 Abstract

Purpose: To determine the resting perfusion and autoregulatory characteristics of the human eye in anisomyopia.

Methods: The sample comprised both eyes of 14 anisomyopes whose ages ranged from 20 to 78 years (mean age 36.92 ± 17.73 years), and whose axial anisometropia expressed as mean spherical equivalent (MSE) was 2.39 ± 0.57D. Resting state ocular perfusion was determined at an initial baseline investigation (B1: breathing room air) using CDI to measure blood velocities at the ophthalmic artery (OA), central retinal artery (CRA) and short posterior ciliary arteries (SPCA), HRF to evaluate retinal microcirculation, and OBFA to measure pulsatile ocular blood flow (POBF). In order to investigate autoregulatory characteristics, investigations were repeated on a subgroup of volunteers comprising both eyes of 7 healthy anisomyopes whose ages ranged from 20 to 54 years (mean age 33.42 ± 13.74 years) with axial anisometropia expressed as mean spherical equivalent (MSE) 2.25 ± 0.33D at a second baseline (B2: breathing normal room air through a face mask) and then during and isoxic hypercapnia (IH: breathing CO2 enriched air through a mask until end tidal $pCPO_2$ increased approximately 15% above B2). Blood pressure (BP), intraocular pressure (IOP), and body mass index (BMI) were also recorded.

Results: B1: there was a significant difference in MSE between the more and less myopic eye ($p=0.041$). The differences in axial length (AL) and MSE between each pair of anisomyopic eyes correlated significantly with the differences in blood velocity in the short posterior ciliary arteries (SPCA) ($R=0.60$, $p=0.02$; $R=0.56$, $p=0.037$) and with the minimum blood volume, flow and velocity of the retinal microcirculation ($R=-0.85$, $p<0.001$; $R=0.54$, $p=0.047$; $R=-0.93$, $p<0.001$). IH: there was a significant increase in pulse rate ($p=0.002$) in the less myopic eyes only. The response to hypercapnia from the more myopic eyes showed an increased SPCAs blood velocity ($p=0.029$) and decreased SPCAs resistance index (RI) that did not reach the level of significance after Bonferroni correction. The differences in microretinal blood velocity and flow percentage change during hypercapnia between each pair of anisomyopic eyes correlated significantly with the in AL and MSE difference between anisomyopic eyes ($R=0.91$, $p=0.004$; $R=-0.82$, $p=0.023$; $R=0.92$, $p=0.004$; $R=-0.86$, $p=0.01$; $R=0.76$, $p=0.04$ respectively).

Conclusions: These results highlighted the relationship between ocular haemodynamics and axial length, as larger eyes had shown significantly lower ocular blood flow. The role that axial length appears to play in the ocular haemodynamics was emphasised by the relationship between

the vascular reactivity in the retinal capillaries and axial length and refractive error, which suggests a greater vascular reactivity in larger eyes.

6.2 Introduction

The previous experimental chapters of this thesis explored the vascular features of the human myopic eye using several instruments that evaluated the retrobulbar, choroidal pulsatile and microretinal circulation, and they also assessed the vascular regulatory features of the myopic eye using a provocation test by which the percentage of carbon dioxide concentration ($pCPO_2$) was significantly increased.

The findings obtained showed a lower OA blood velocity, which was accompanied by a significantly higher resistance index. The pulsatile component of blood flow was also significantly lower in high myopes; however, there were no significant differences at the retinal capillary level. The assessment of the autoregulatory features of myopia demonstrated differences in the ocular vascular response between emmetropes and myopes. The regulatory response in the retrobulbar vessels to hypercapnia in emmetropes contrasted with the lack of retrobulbar reactivity in myopes. The POBF and microvascular responsiveness in myopes suggested a regulatory mechanism highly sensitive to changes in oxygenation, as shown by the lack of responsiveness described in the emmetropic group. The significant correlation between the increased retrobulbar blood velocities as a response to hypercapnia with age may be indicative of a protective mechanism occurring parallel to the decrease in vessel diameter occurring with age.

Thereby, the ocular blood flow in myopes appeared to be decreased compared to emmetropes but it seemed highly reactive to changes in oxygenation interacting with age in later stages of life. The participants included in the previous studies described in this thesis were emmetropes, low myopes and high myopes perfectly matched for age, gender and ethnicity. Matching ensured an increase in the power of the statistical calculation; however, there was no control for the effect that genetics or environmental factors might have on the ocular blood flow.

Anisomyopic patients (myopic patients with a difference in prescription between the two eyes) represent an interesting subject cluster to investigate the features of myopia, as the less myopic eye can act as a control. The fact that both eyes have been exposed to the same genetic and environmental conditions ensures reduced bias in the analysis (Gilmartin 2004).

The choroidal pulsatile ocular blood pulse has been described to be affected by refractive error, axial length and ocular volume. In a study by Logan and colleagues (Logan, Gilmartin and Cox

2002), 14 anisomyopes were evaluated and significant relationships between the inter-eye differences in refractive error, ocular pulse volume and ocular pulse amplitude were reported. These differences in pulsatile ocular volume and amplitude have been suggested to represent an existing difference in the blood circulation of the paired anisomyopic eyes. However, this finding is affected by the assumptions entailed by the pulsatile ocular blood flow calculations. OBFA calculates the total pulsatile ocular blood flow (POBF) from the IOP variation with time as a ratio of pressure and volume values at each pulse (Langham, *et al.* 1989).

To date only the pulsatile blood flow and the blood velocity of the ophthalmic artery have been assessed in anisomyopic subjects. Thus, this study was design to evaluate, not only the ocular haemodynamics across several vascular beds (pulsatile, retrobulbar and microretinal), but additionally, the regulatory features of the ocular circulation in anisomyopic eyes.

6.3 Aims and objectives

The aim of this study was to evaluate the true resting state ocular blood flow and haemodynamic autoregulation during stress testing in the different vessel beds of the human myopic eye. In order to eliminate the effects of factors such as tissue type or the contribution of the systemic circulation, anisomyopes were selected as a model, using the less myopic as a control.

6.4 Methods

The current chapter is subdivided into two sections, the first of which describes the true resting state ocular blood flow of the human myopic eye by comparing the two eyes of anisomyopic patients (N=14). In the second section, the vascular regulatory profile is investigated using a stress test on a subgroup of anisomyopes (n=7).

6.4.1 Study Sample: Recruitment and Exclusion Criteria

Power statistics revealed that a sample size with a statistical power of 80%, allocation ratio 1:1 and a significant value of 5% (α=0.05) required a sample ranging from 78 to 120 (78 for the CDI values, 108 for the OBFA readings and 120 for HRF measurements) to show statistical significance between the retrobulbar blood velocity in anisomyopes). However, due to the low prevalence of anisomyopes, it was not possible to reach the sample size suggested by power statistics and only 14 anisomyopes were recruited from the student and staff population of Aston University. Both eyes of 14 healthy anisomyopic volunteers were evaluated for the first part of the study.

Fourteen healthy anisomyopic volunteers were recruited from whom the MSE between the two eyes differed by 1.50D in one subject and by ≥ 2.00D in thirteen subjects. Their ages ranged from 20 to 78 years (mean age 36.92 ± 17.73y), and the axial anisometropia expressed as mean spherical equivalent (MSE) was 2.39D ± 0.57 (range 1.50 D to 3.32D).

To assess the ocular vascular response in human anisomyopia, 7 healthy anisomyopic volunteers whose refraction differed by at least 1.50D between the two eyes participated in the study. The MSE difference was ≥ 2.00D in six subjects and only one had MSE difference of 1.50D. Their ages ranged from 20 to 54 years (mean age 33.42 ± 13.74), and the axial anisometropia expressed as mean spherical equivalent (MSE) was 2.25D ± 0.63 (range 1.50 D to 3.32D).

All the participants were healthy volunteers, which was confirmed by the author by investigation the fundus using Volk lens (90D), recording blood pressure and a detailed recording of systemic and ocular history and symptoms. Exclusion factors included any ocular disorder, diabetes, hypotension, hypertension or any other systemic disorder or medication likely to affect the systemic or ocular vasculature. Those subjects exhibiting any sign of myopic fundus changes (lacquer cracks, staphylomas, chorioretinal atrophy, lattice degeneration or pavingstone degeneration) were not included in the study. Since no blood test was performed, all of the previous were self-reported by the participants. A corrected visual acuity of 6/9 or better was required together with astigmatism of less than 1.5 diopters cylinder, which reduced the percentage of astigmatic patients being taken as myopic subjects after MSE calculation

6.4.2. Ethical Approval and Informed Consent

Written and verbal information about the exact procedures to be performed during the visit was given to all the subjects prior to data collection. The participants were encouraged to ask any questions and to clarify any doubts they might have before signing the written consent. The volunteers were requested to sign two copies of the written consent, one of which was given to them for their own records.

All investigations were approved by the Ethical Review Committee and conformed to the declaration of Helsinki (appendix 2.1).

6.4.3 Study Sample: Dietary Restrictions

Alcohol, nicotine and caffeine containing products have been reported to affect the flow of blood in the eye (Domino, *et al.* 2004; Gdovinova 2001). To ensure the reliability of the data, the

participants recruited for this study were asked to refrain from smoking, or consuming alcohol and caffeine containing products for 12 hours prior to the study.

6.4.4. Experimental Protocol and Investigations

Chapter six is subdivided into two main sections. Section one describes the differences in resting retrobulbar, pulsatile choroidal and retinal microcirculation between the two eyes of anisomyopic patients using CDI, OBFA and HRF. The second section evaluates the vascular regulatory differences between anisomyopic eyes measured using hypercapnia as a stress test.
The instruments and experimental procedure followed for both studies were the same as those followed in chapter 4 (section 4.4.4).

6.4.5 Statistical analysis

Data are presented as mean ± SD. For the first study, Student's paired *t*-test was used to assess the differences in ocular biometric and vascular parameters between the more and less myopic eyes. Bonferroni correction was performed when associated parameters calculated from the same piece of equipment were compared. To account for the disparity between anisomyopic eyes, the differences in ocular biometric, vascular and refractive parameters were converted into percentage differences. Pearson's linear correlation analysis was used to determine the relationship between the inter-ocular biometrics and blood flow.
Two-tailed paired Student's *t*-test was used to test for significance between baseline and hypercapnic conditions in the second study for the more and less myopic eyes. Bonferroni correction was performed when associated parameters calculated from the same piece of equipment were compared. One-way repeated measures ANOVA allowed changes in variable measured on the same scale under three or more different occasions to be ascertained. Thereby, one-way repeated measures ANOVA was used to evaluate potential differences of each patient's systemic blood pressure prior to each ocular blood flow measurement. Two-way between-groups ANOVA was used to assess the differences in response from the less and more myopic eye to hypercapnia.
Statistical significance was defined as $p<0.05$, unless Bonferroni correction was applied, in which case the new significant p value was calculated.

6.5 Results

6.5.1 Differences between the more myopic and less myopic eyes

Data obtained from the more myopic eye was compared to that of the less myopic eye for each volunteer (table 6.1).

Two sets of data analysis were performed in this first study, the first of which evaluated the differences between the paired eyes of each participant using a paired t-test, whereas the second analysis assessed the potential relationship between the inter-ocular differences in biometric (axial length, anterior chamber depth and corneal thickness), refractive (MSE) and vascular parameters using Pearson's correlation test.

	Less myopic	More myopic	p
MSE (Dioptres)	-3.73 ± 2.15	-5.04 ± 3.32	*0.041
Gender	6m; 9f	6m; 9f	n/a
Axial length (mm)	25.08 ± 0.73	25.49 ± 1.23	0.09
ACD (mm)	3.51± 0.48	3.56 ±0.46	ns
CT (μm)	565.00 ± 49.02	566.14 ± 48.35	ns
IOP (mmHg)	15.45 ± 3.18	14.88 ± 3.32	ns

Table 6.1. *Summary of the refractive and biometric features of the anisomyopic eyes assessed (explanation of abbreviations: ACD, anterior chamber depth; CT, corneal thickness; IOP, intraocular pressure) * significant value p<0.05 ns: not significant n/a: not applicable*

The eyes of the 14 anisomyopes were grouped and compared by the degree of myopia; the less myopic eye of each volunteer was compared to the more myopic eye. The results showed that both groups differed significantly in refractive error (p=0.041), but not in axial length (p=0.09), anterior chamber depth (p=0.17), corneal thickness (p=0.83) or intraocular pressure (p=0.28) (table 6.1). No differences were found in ocular perfusion pressure (p=0.46) between the less and more myopic eyes.

The pulsatile ocular blood flow (table 6.2), retrobulbar circulation (table 6.3) or microretinal circulation did not show significant differences between the more and less myopic eyes (table 6.4).

	More myopic	Less myopic	p
OBFiop (mmHg)	12.47 ± 4.71	12.59 ± 4.28	ns
OBFa (mmHg)	2.89 ± 1.05	2.63 ± 0.93	ns
OBFv (μl)	7.11 ± 2.51	6.65 ± 1.90	ns
OBFr	66.00 ± 14.16	64.00 ±7.22	ns
POBF (μl/min)	966.40 ± 288.14	917.10 ± 289.78	ns

Table 6.2. *Pulsatile ocular blood flow outcomes for the less and more myopic eyes (explanation of abbreviations: OBFiop, intraocular pressure; OBFa, amplitude of ocular pulse; OBFv, volume of ocular pulse; OBFr, rate of ocular pulse; POBF, pulsatile ocular blood flow) ns: not significant difference)*

Vessel	Parameter	More myopic	Less myopic	p
OA	**PSv (m/s)**	0.436 ± 0.166	0.443 ± 0.183	ns
	EDv (m/s)	0.124 ± 0.07	0.122 ± 0.07	ns
	RI	0.718 ± 0.09	0.734 ± 0.085	ns
	Ratio	5.100 ± 1.75	5.009 ± 1.22	ns
CRA	**PSv (m/s)**	0.125 ± 0.02	0.129 ± 0.03	ns
	EDv (m/s)	0.042 ± 0.009	0.045 ± 0.01	ns
	RI	0.674 ± 0.05	0.652 ± 0.06	ns
	Ratio	3.149 ± 0.56	2.966 ± 0.58	ns
SPCA	**PSv (m/s)**	0.146 ± 0.03	0.157 ± 0.05	ns
	EDv (m/s)	0.060 ± 0.02	0.069 ± 0.03	ns
	RI	0.594 ± 0.08	0.570 ± 0.08	ns
	Ratio	2.575 ± 0.64	2.41 ± 0.43	ns

Table 6.3*.Results obtained at the retrobulbar vessels measured with CDI in the less and more myopic eyes (explanation of abbreviations: PSv, peak systolic velocity; EDv, end diastolic velocity; RI, resistance index) ns: not significant difference*

	Parameter	More myopic	Less myopic	P
MAX	**Volume (AU)**	22.50 ± 6.50	20.64 ± 5.92	ns
	Flow (AU)	418.58 ± 136.30	389.32 ± 165.47	ns
	Velocity (AU)	1.45 ± 0.44	1.35 ± 0.53	ns
MIN	**Volume (AU)**	36.22 ± 39.43	39.64 ± 44.42	ns
	Flow (AU)	213.36 ± 140.51	215.83 ± 167.44	ns
	Velocity (AU)	54.48 ± 101.81	59.48 ± 112.12	ns

Table 6. 4*. Maximum and minimum values of volume, flow and velocity measured in the retinal capillaries using HRF of the less and more myopic eyes (ns: not significant difference; AU: arbitrary units)*

6.5.2 Relationship between biometric parameters and resting vascular status

In order to analyse the relationship between the biometric parameters and the resting vascular status, the correlations between the more and less myopic eye difference in ocular biometric, refractive and vascular parameters were calculated.

The differences in refractive error given as MSE between each pair of anisomyopic eyes correlated with the differences in systolic and diastolic blood velocity at the short posterior ciliary arteries (SPCA) (R=0.60, p=0.02; R=0.56, p=0.037), and with the minimum blood volume, flow and velocity of the retinal microcirculation (R=-0.85, p<0.001; R=0.54, p=0.047; R=-0.93, p<0.001) (figure 6.1). The differences in AL between the two eyes correlated significantly with the same parameters as MSE (table 6.5). The significant correlations found between the differences in anterior chamber depth, corneal thickness IOP and ocular blood flow parameters are summarised in table 6.5.

Parameter	MSE R value	p	AL R value	p	ACD R value	p	CT R value	p	IOP R value	P
SPCA PSv	0.60	*0.02	-0.61	*0.02	-0.20	ns	-0.22	ns	0.53	ns
SPCA EDv	0.56	*0.037	-0.52	*0.05	-0.16	ns	-0.32	ns	0.48	0.08
HRF min vol	-0.85	*<0.001	0.81	*<0.001	0.31	ns	0.18	ns	-0.80	*0.001
HRF min flow	0.54	*0.047	-0.54	*0.048	-0.47	ns	-0.47	ns	0.40	ns
HRF min vel	0.15	ns	-0.30	ns	-0.31	ns	-0.54	*0.04	0.02	ns
HRF max vol	-0.61	ns	-0.82	*0.014	-0.61	ns	-0.81	*0.008	-0.77	*0.014

Table 6.5 *Correlations between the differences in ocular biometrics and ocular blood flow in anisomyopes. SPCA: short posterior ciliary arteries, PSv: peak systolic velocity, EDv: end diastolic velocity, HRF min flow: minimum blood flow of the retinal capillaries, HRF min vel: minimum blood velocity of the retinal capillaries, HRF max vol: maximum blood volume of the retinal capillaries (ns: no significant difference * statistically significant)*

To account for the disparity between anisomyopic eyes, the differences in ocular biometric, vascular and refractive parameters were converted into percentage differences. The percentage difference in axial length between the anisometropic eyes correlated with the percentage difference in diastolic blood velocity at the ophthalmic artery (OA) (R=-0.64, p=0.024) and systolic blood velocity at the SPCA for the retrobulbar vessels (R=-0.64, p=0.026).

The percentage difference in refractive error between eyes did not correlate significantly with any ocular vascular parameter; whereas the percentage difference in anterior chamber depth correlated significantly with the percentage difference in microretinal blood volume (R=0.59, p=0.04), and the percentage change in IOP correlated with that of diastolic blood velocity at the central retinal artery (CRA), diastolic blood velocity at the SPCA and retinal capillary blood volume (R=-0.6, p=0.04) (table 6.6).

% difference	MSE R value	P	AL R value	P	ACD R value	P	CT R value	P	IOP R value	P
OA EDv	-0.08	ns	-0.64	*0.02	-0.39	ns	0.15	ns	-0.10	ns
CRA EDv	-0.43	ns	0.19	ns	0.11	ns	0.02	ns	0.63	*0.028
SPCA PSv	-0.14	ns	-0.64	*0.026	-0.44	ns	-0.37	ns	-0.55	ns
HRF min vol	0.09	ns	0.83	*0.001	0.59	0.04	0.26	ns	0.29	ns
HRF min flow	-0.34	ns	-0.97	<0.001	-0.56	ns	-0.41	ns	-0.30	ns
HRF min vel	0.34	ns	0.76	*0.004	0.34	ns	0.28	ns	0.157	ns
HRF max vol	0.041	ns	-0.67	0.017	-0.54	ns	-0.41	ns	-0.60	0.04

Table 6.6 *Correlations between the percentage differences in ocular biometrics and ocular blood flow in anisomyopes. OA: ophthalmic artery, CRA: central retinal artery, SPCA: short posterior ciliary arteries, PSv: peak systolic velocity, EDv: end diastolic velocity, HRF min flow: minimum blood flow of the retinal capillaries, HRF min vel: minimum blood velocity of the retinal capillaries, HRF max vol: maximum blood volume of the retinal capillaries (ns: no significant difference * statistically significant)*

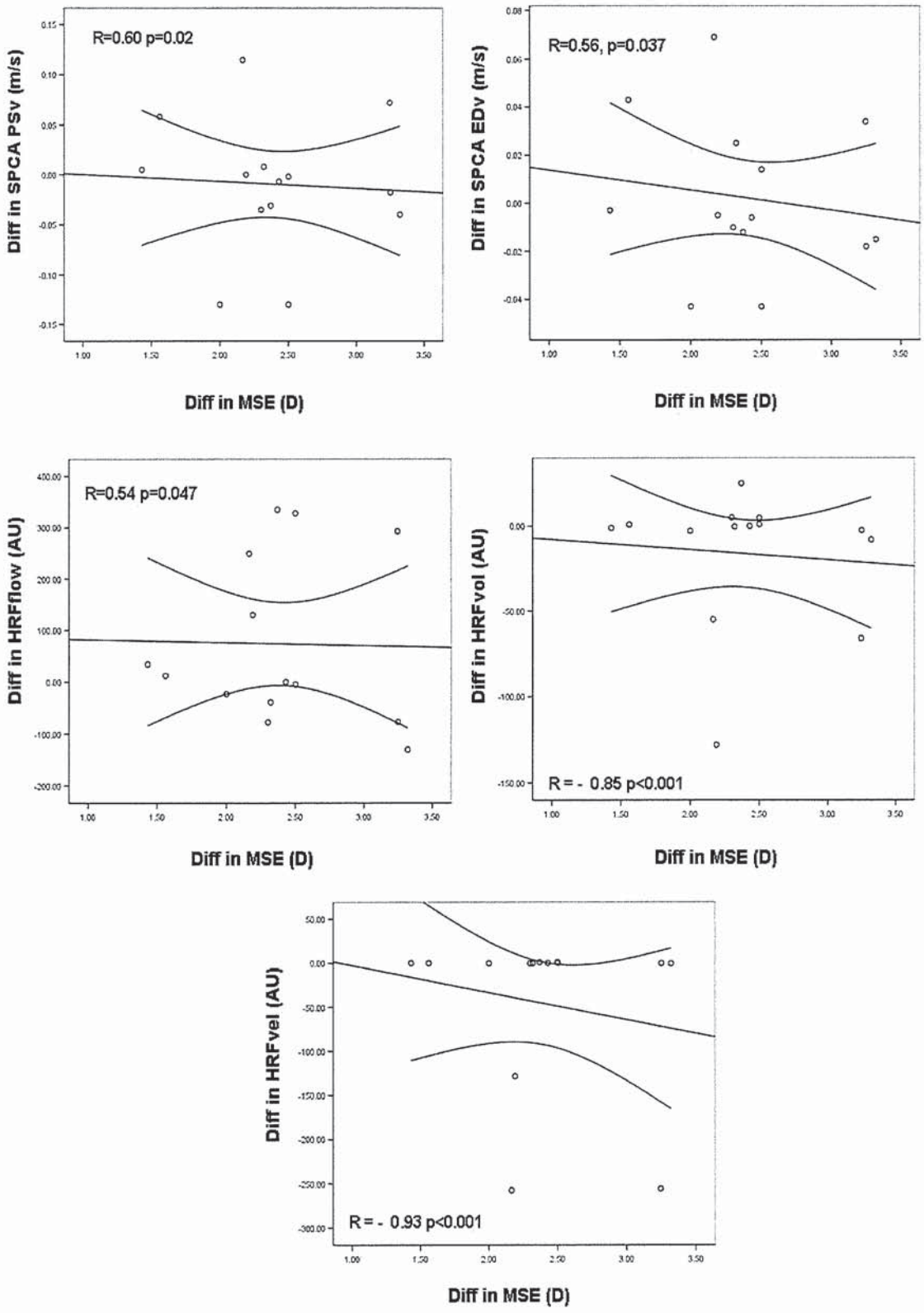


Figure 6.1 *Significant correlations between the differences in MSE between each pair of anisomyopic eyes and ocular blood velocity*

6.6 Ocular vascular response in human anisomyopia

The vascular response to hypercapnia from the more myopic eye was compared to that of the less myopic eye. In order to understand the response to hypercapnia and since only a subgroup of anisomyopes underwent this study, the biometric features of the anisomyopes evaluated are summarised in table 6.7. The more and less myopic eyes were found to differ significantly only in axial length (p=0.038).

	Less myopic	More myopic	p
MSE	-3.24 ± 2.97	-5.49 ± 3.28	ns
Gender	4m; 3f	4m; 3f	n/a
Axial length (mm)	24.97 ± 0.68	25.84 ± 0.71	* 0.038
ACD (mm)	3.65± 0.31	3.73 ±0.30	ns
CT (μm)	578.85 ± 41.83	589.00 ± 31.17	ns
IOP (mmHg)	15.14 ± 5.27	14.35 ± 3.59	ns

Table 6.7. *Summary of the average biometric parameters during baseline in the less and more myopic eyes (explanation of abbreviations: ACD, anterior chamber depth; CT, corneal thickness; IOP, intraocular pressure) ns: not significant * statistically significant*

6.6.1 Mask baseline measurements

At baseline, the systemic pulse rate, as measured using the OBFA device, was significantly higher in the more myopic group at baseline (p=0.026), however, the analysis of the retrobulbar circulation and microretinal circulation did not exhibit significant differences between the more and less myopic groups (table 6.8, table 6.9 and table 6.10).

	More myopic	Less myopic	p
OBFiop (mmHg)	16.08 ± 6.484.71	15.26 ± 4.28	ns
OBFa (mmHg)	3.12 ± 0.55	3.16 ± 0.92	ns
OBFv (μl)	5.72 ± 1.41	5.58 ± 0.96	ns
OBFr	64.60 ± 2.70	58.20 ±4.94	* 0.026
POBF (μl/min)	795.40 ± 195.08	685.60 ± 130.60	ns

Table 6.8. *Pulsatile ocular blood flow outcomes for the more and less myopic eyes (explanation of abbreviations: OBFiop, intraocular pressure; OBFa, amplitude of ocular pulse; OBFv, volume of ocular pulse; OBFr, rate of ocular pulse; POBF, pulsatile ocular blood flow) * significant value; ns: no significant difference*

Vessel	Parameter	More myopic	Less myopic	p
OA	**PSv (m/s)**	0.517 ± 0.151	0.504 ± 0.234	ns
	EDv (m/s)	0.142 ± 0.058	0.119 ± 0.047	ns
	RI	0.711 ± 0.111	0.745 ± 0.104	ns
	Ratio	3.98 ± 1.61	4.56 ± 1.70	ns
CRA	**PSv (m/s)**	0.118 ± 0.01	0.127 ± 0.03	ns
	EDv (m/s)	0.043 ± 0.008	0.047 ± 0.02	ns
	RI	0.590 ± 0.146	0.641 ± 0.09	ns
	Ratio	2.794 ± 0.46	2.940 ± 0.70	ns
SPCA	**PSv (m/s)**	0.165 ± 0.06	0.124 ± 0.03	ns
	EDv	0.067 ± 0.02	0.050 ± 0.01	ns
	RI	0.582 ± 0.04	0.580 ± 0.09	ns
	Ratio	2.464 ± 0.246	2.501 ± 0.587	ns

Table 6.9. *Results obtained at the retrobulbar vessels using CDI for the more and less myopic eyes (explanation of abbreviations: PSv, peak systolic velocity; EDv, end diastolic velocity; RI, resistance index) ns: no significant difference*

	Parameter	More myopic	Less myopic	p
MAX	**Volume (AU)**	19.75 ± 4.27	23.71 ± 7.70	ns
	Flow (AU)	412.41 ± 135.84	400.79 ± 144.48	ns
	Velocity (AU)	1.36 ± 0.34	1.38 ± 0.45	ns
MIN	**Volume (AU)**	14.62 ± 6.10	13.96 ± 2.74	ns
	Flow (AU)	294.19 ± 28.05	230.89 ± 35.48	ns
	Velocity	1.05 ± 0.44	0.73 ± 0.31	ns

Table 6.10. *Maximum and minimum values of volume, flow and velocity measured in the retinal capillaries using HRF for the more and less myopic eyes .ns:no significant difference*

6.6.2 Isoxic Hypercapnia (IH) Condition

6.6.2.1 Hypercapnia characteristics

BP did not show significant changes throughout the hypercapnic session (table 6.11). The end tidal carbon dioxide concentration (pCO_2) increased from 38.30 mmHg at baseline to 44.62 mmHg during hypercapnia (16.50 % increase) ($p<0.001$). The oxygen concentration increased proportionally to CO_2 rise and varied significantly from 96.71 % at baseline to 97.70% during hypercapnia ($p=0.001$). A summary of the data describing changes in end tidal carbon dioxide and oxygen saturation is given in table 6.12.

recording	SBP (mmHg)	DBP (mmHg)	Heart rate (ppm)	MAP (mmHg)	OPP
1	120.14 ± 12.54	77.28 ± 12.88	68.00 ± 8.26	91.57 ± 11.67	48.73 ± 8.13
2	123.28 ± 12.98	74.42 ± 9.94	66.57 ± 8.52	90.71 ± 7.13	48.16 ± 6.26
3	120.28 ± 11.35	74.42 ± 9.84	62.00 ± 8.44	89.71 ± 8.75	47.49 ± 5.00

Table 6.11. *. Summary of systemic blood pressure readings at baseline taken prior to 1. CDI, 2. OBFA, 3. HRF (explanation of abbreviations: SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial pressure; OPP, ocular perfusion).There were no significant differences between the three sets of BP measurements*

	Baseline(mean ± sd)	Hypercapnia (mean ± sd)	p
End tidal CO_2 (mmHg)	38.30 ± 8.84	44.62 ± 9.57	* 0.001
O_2 saturation levels (%)	96.71 ± 1.25	97.70 ± 1.16	* 0.001

Table 6.12. *Changes in CO_2 and O_2 from baseline to hypercapnic conditions in anisomyopic subjects *statistically significant*

	Baseline	Hypercapnia	P
SBP (mmHg)	119.57 ± 20.84	126.14 ± 14.22	ns
DBP (mmHg)	73.42 ± 11.04	78.00 ± 8.56	ns
pulse rate (bpm)	65.57 ± 9.99	68.42 ± 9.67	* 0.029
MAP (mmHg)	88.80 ± 12.47	94.04 ± 9.77	ns
	less myopic		
OPP	46.89 ± 8.71	50.38 ± 7.18	ns
	more myopic		
OPP	45.15 ± 8.82	48.65 ± 7.92	ns

Table 6.13 *Systemic BP changes from baseline to hypercapnia *p<0.05 ns:not significant*

6.6.2.2 Systemic response to hypercapnia

The response to hypercapnia given by the systemic blood pressure in anisomyopes only showed a significant increase in pulse rate (p=0.002) (table 6.13).

6.6.2.3 Vascular response to hypercapnia

The less myopic eyes did not show any significant changes in the retrobulbar, pulsatile or microretinal circulation between the mask baseline and hypercapnia condition.

The response to hypercapnia from the more myopic eyes did not exhibit any significant variations in pulsatile ocular blood flow or microretinal circulation. The SPCAs, however, showed an increase in blood velocity (p=0.029) and a reduction in resistance index (p=0.022) (figure 6.2).

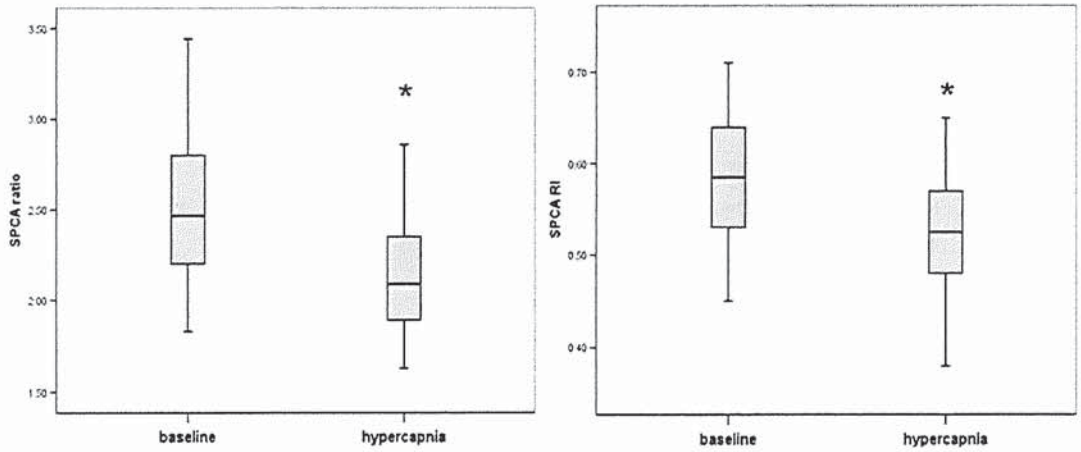


Figure 6.2 *Box plots showing the significant decrease in the SPCA PSv and EDv ratio and the significant decrease in resistance index (* statistically significant)*

6.6.3 Relationship between ocular biometry and changes in ocular haemodynamics during hypercapnia

The percentage changes in retrobulbar, pulsatile and microretinal circulation during hypercapnia did not correlate significantly with the refractive error or the axial length of the eyes evaluated (appendix 1.7), nor with the corporal indices recorded: height, weight and body mass index (BMI) (appendix 1.8).

6.6.4 Interaction between anisomyopia and response to hypercapnia

The difference in ocular vascular response to hypercapnia was analysed for the less myopic and the more myopic eyes performing 2-way ANOVA with refractive group and breathing condition as independent variables.

The response to hypercapnia in blood volume of the retinal capillaries measured using HRF differed significantly between the less and more myopic eyes. The microretinal blood volume in the more myopic eyes increased significantly during hypercapnia, whereas the less myopic eyes did not (p=0.034) (figure 6.3).

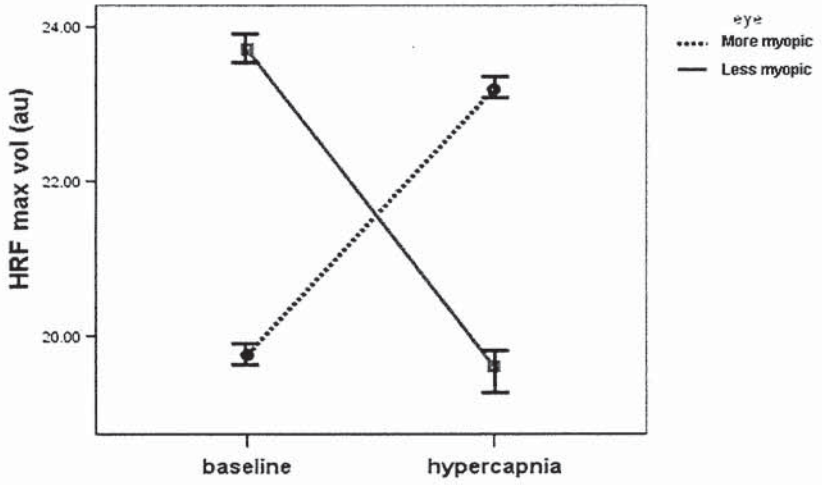


Figure 6.3 *Graph showing the significant interaction in hypercapnia responses of the retinal circulation blood volume in the less and more myopic eyes*

6.6.5 Differences in hypercapnia-induced percentage change in ocular vascular response between pairs of anisomyopic eyes

The inter-eye differences in the ocular blood flow changes induced by hypercapnia between each pair of anisomyopic eyes at mask baseline and during hypercapnia were calculated as percentage changes, and these percentage changes were assessed for correlation with inter-eye differences in axial length and refractive error. The inter-eye percentage response to hypercapnia between anisomyopic eyes (PDAE) in the OA resistance index correlated significantly with axial length (R=-0.80, p=0.03) (figure 6.4). The correlation between the ocular pulse rate PDAE and refractive error approached the level of significance (R=0.81, p=0.09), as did the correlation between the ophthalmic artery systolic velocity PDAE and axial length (R=-0.72, p=0.06); however, none of the two correlations reached the level of significance.

The differences in microretinal blood velocity and flow percentage change during hypercapnia between each pair of anisomyopic eyes correlated significantly with the difference in AL and MSE between anisomyopic eyes (R=0.91, p=0.004; R=-0.82, p=0.023; R=0.92, p=0.004; R=-0.86, p=0.01; R=0.76, p=0.04) (figure 6.5).

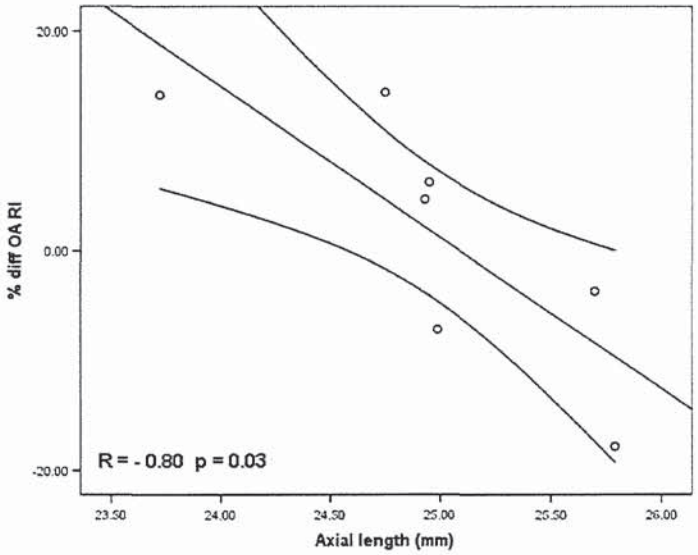


Figure 6.4 *Significant correlation between the percentage difference response during hypercapnia between anisomyopic eyes in OA RI (ophthalmic artery resistance index) and axial length (mm).*

6.7 Discussion

This study evaluated the profile of the ocular blood flow in human myopia by assessing the vascular features of anisomyopic eyes. The biometric and vascular characteristics from the more and less myopic eyes were compared, and significant differences were found in refractive error, but not in axial length, anterior chamber depth, corneal thickness or intraocular pressure.

No differences were shown in the resting ocular perfusion pressure or ocular haemodynamics between the more and less myopic eyes. The response to hypercapnia from the less myopic eyes showed an increase in pulse rate, with no difference in ocular blood flow measurements; whereas in the more myopic eyes there was a trend towards an increase in SPCAs blood velocity and a decrease in SPCAs resistance index that did not reach the level of significance after Bonferroni correction.

The inter-eye percentage change differences during hypercapnia in microretinal blood velocity and flow and OA resistance index correlated with the inter-eye difference in AL and MSE.

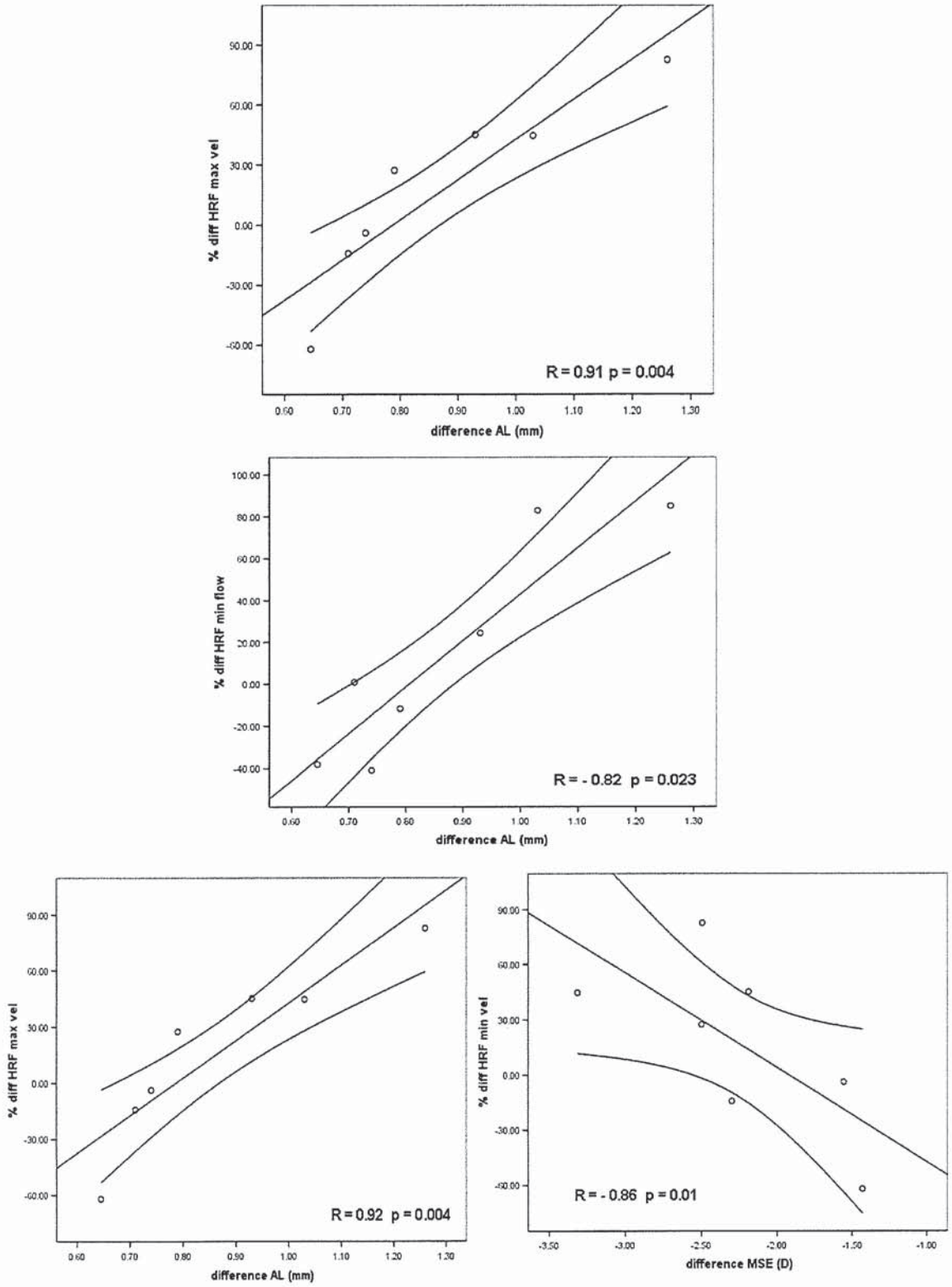


Figure 6.5 *Significant correlations between the differences in microretinal blood velocity and flow percentage change (% diff HRF) during hypercapnia between each pair of anisomyopic eyes correlated significantly with the difference in AL and MSE between anisomyopic eyes*

6.7.1 Ocular vascular profile of human anisomyopia

Chapter 3 and chapter 4 of this thesis assessed the vascular features of human myopia by comparing a matched sample of myopes and emmetropes. To ensure unbiased outcomes, data were frequently matched for age, gender and ethnicity. However, this did not guarantee the control of genetic or environmental conditions. Anisomyopic patients provide a reliable sample to investigate the features of myopia, as the less myopic eye can act as a control.

Chapter three of this thesis reported a lower CRA blood velocity and pulsatile ocular blood flow, accompanied by a significantly higher CRA resistance index in myopes with MSE greater than 5.00D. Furthermore, the degree of myopia correlated significantly with the blood velocity at the CRA and the amplitude of the ocular pulse. For this study, 28 eyes of 14 anisomyopes were evaluated in a paired manner; therefore, the analysis was controlled for systemic blood pressure, weight and height. No significant differences in pulsatile ocular blood flow, retrobulbar or microretinal circulation were found.

A study by Logan and colleagues (Logan, Gilmartin and Cox 2002) evaluated the vascular pulsatile profile of 14 anisomyopes and found a significant relationship between the inter-eye differences in refractive error, ocular pulse volume and ocular pulse. The differences in pulsatile ocular volume and amplitude were suggested to represent an existing difference in the blood circulation of the paired anisomyopic eyes.

The lack of POBF significant difference between anisomyopic eyes found in the present study does not agree with the significant decrease in the more myopic eyes reported by Lam and colleagues, or Logan and co-workers, which may be due to the known relationship between pulsatile ocular blood flow and axial length (Lam, *et al.* 2003; Logan, Gilmartin and Cox 2002; Morgan, *et al.* 2002; Ravalico, *et al.* 1997; Shih, *et al.* 1991; To`mey, *et al.* 1981). In the current study the more myopic and the less myopic eyes did not differ significantly in axial length, suggesting that the nature of the difference between the anisomyopic eyes assessed was not of axial origin. Thereby, discrepancy between studies may be related to the lack of significant difference between the axial length of the more myopic and the less myopic eyes. Lam and colleagues (Lam, *et al.* 2003) also assessed the blood circulation in the ophthalmic artery and reported no difference between paired OA readings, which agreed with the results obtained in the present study. Blood velocity, flow and volume of the retinal microvessels did not show significant differences between the more myopic and less myopic eyes, which suggested that the flow of blood did not differ in anisomyopic eyes. Additionally, as already mentioned in chapter four, the capillary network is known to be vulnerable to raised IOP, and due to the role of IOP on the perfusion features of the eye, similar

IOPs will most probably lead to similar retinal microcirculation. In this study, anisomyopes were not found to differ significantly in IOP, a reason why the retinal microcirculation may have appeared to be unaffected in paired anisomyopic eyes.

The less and more myopic eyes differed in MSE, but not in axial length, which explain the lack of significant difference between inter-eye ocular haemodynamics in anisomyopes. These results suggested that the sample group that underwent resting ocular blood flow analysis comprised both refractive and axial anisomyopia, and not just axial. The difference in refraction between the two eyes of 2 participants aged 65 and 78 was not accompanied by differences in axial length, possibly suggesting changes in lens index with age that resulted in altered MSE.

It was interesting, however, to observe that differences in axial length and refractive error between each pair of anisomyopic eyes showed a significant correlation with the differences in blood velocity at the short posterior ciliary arteries (SPCA), and retinal microcapillaries. Thereby, larger MSE and AL differences are related to larger differences in retrobulbar blood velocity. Chapter four did not find significant differences in blood velocity between the OA of myopes and emmetropes. Despite the lack of significant difference found in the present study between paired anisomyopic eyes, these correlations suggest a relationship between axial length, MSE and the blood circulation in the retrobulbar and microretinal vessels. Being aware of the limitations of correlation analysis, it is important to highlight that the relationship described in the present study between MSE, AL and retinal microcirculation, appeared supported by the results reported in chapter 4 (section 4.6.4) in which the microretinal response to hypercapnia also correlated significantly with AL.

The difference in AL and MSE calculated as absolute values between the more and less myopic eyes correlated with the microretinal and SPCAs blood velocity, whereas the correlations between percentage difference in AL and MSE correlated with OA, SPCAs and retinal capillaries blood velocity. This suggested that the dioptric difference between the more and less myopic eyes had a greater effect on the difference in ocular haemodynamics than the dioptric power of the less myopic eye. Thereby, the difference in blood velocity between an anisomyope whose right eye MSE was -2.00D and left eye MSE is -4.00D was similar to the difference in another subject whose right eye MSE was -6.00D, whereas the left eye MSE is -8.00D.

6.7.2 Vascular response to hypercapnia in anisomyopia

The less and more myopic eyes of the anisomyopes recruited for evaluation of the ocular vascular autoregulation differed significantly in axial length. There were no significant differences in ocular

perfusion pressure or in pulsatile, retrobulbar or microretinal circulation between the more and less myopic eyes at baseline.
The response to hypercapnia from the retrobulbar vessels in the more myopic eyes showed an increase in SPCAs blood velocity and a reduction in SPCAs resistance index (RI) that did not reach significance after Bonferroni correction. The vascular parameters in the OA and CRA did not reveal any significant changes. Thus, the trend towards an increase in SPCA PSv accompanied by a decrease in SPCA RI suggested a trend towards an increase in SPCA blood flow during hypercapnia in the more myopic eyes only; with no variations approaching the level of significance in the less myopic eyes. Hence, the more myopic eyes showed a higher ocular vascular reactivity to changes in oxygenation than the less myopic eyes. These results are in accordance with those obtained in chapter 4 in which only the myopic eyes exhibited significant changes in ocular blood flow during increased pCO_2. The control of confounding factors normally affecting the responses to a provocation test was ensured not only due to the anisomyopic nature of the subjects, but also due to the protocol followed, which assessed one eye after the other in each subject.

Additionally, the difference in response given between the less and more myopic eyes in the OA was found to correlate with the difference in axial length, which suggested that the myopic OA experienced axial length-related changes during hypercapnia. However, since no significant changes were found during hypercapnia in the OA, these changes are not of clinical significance.

Therefore, the retrobulbar response to hypercapnia reported in the present study suggested a regulatory mechanism in the SCPAs of the more myopic eyes of anisomyopes. Similar results have been reported by Hosking *et al* (2006) after assessing the retrobulbar blood velocities in a sample of 16 normals and 12 glaucoma patients. The glaucoma participants exhibited a significant increase in the CRA and SPCAs blood flow after increasing pCO_2, which suggested a greater vascular reactivity in this vascular bed in glaucoma patients. Similar to the results reported in the present study, no changes were observed in the OA of glaucoma patients. It is interesting to observe a comparable response to hypercapnia in the eyes of glaucomatous patients and in the more myopic eyes of anisomyopes. This resemblance may be indicative of related ocular vascular regulatory profiles in myopia and glaucoma, which in turn is of significance since myopes are known to be at higher risk of suffering from glaucoma (Jonas, Peter and Wido 2002; Medeiros and Weinreb 2002; Grodum, Heijl and Bengtsson 2001; Mitchell, *et al.* 1999; Chihara, Liu and Dong 1997).

Hypercapnia did not induce statistically significant changes on the microretinal circulation, which may appear to contradict the findings obtained in chapter four. Chapter four described an increase in microvascular blood flow and velocity in myopes during the hypercapnic condition, which was

found to correlate with AL. Despite not finding evidence for vascular reactivity in the microretinal circulation of myopes, the inter-eye response to hypercapnia given by the retinal capillaries in anisomyopes correlated significantly with the inter-eye differences in axial length and refractive error, which supported the findings reported in chapter 4 and additionally highlighted the significant effect of axial length and refractive error on the vascular regulation of the microretinal circulation. Thus, the significant correlation found between inter-eye axial length, refractive error and retinal capillary circulation suggested that the microretinal vascular reactivity in longer eyes is greater than that in shorter eyes, which in turn agreed with the lack of vascular reactivity found in emmetropes compared with myopes in chapter 4.

6.8 CONCLUSION

The perfusion characteristics of human anisomyopia were evaluated in this study to provide a wider understanding of the ocular haemodynamics in the human myopic eye. The differences in axial length and refractive error between anisomyopic eyes were found to correlate significantly with the differences in blood velocity at the ophthalmic artery, short posterior ciliary arteries, and retinal microcapillaries, whose response to hypercapnia also correlated significantly with axial length and MSE. These results highlight the relationship between ocular haemodynamics and axial length, as larger eyes showed significantly lower ocular blood flow. The role that axial length appeared to play in the ocular haemodynamics was emphasised by the relationship between the vascular reactivity in the retinal capillaries and axial length and refractive error, which suggested a greater vascular reactivity in larger eyes.

Further work is needed using a larger sample size and in addition the comparison of ocular blood flow using a matched isomyopic group as an additional control condition.

CHAPTER SEVEN
BLOOD FLOW AND ITS AUTOREGULATION IN THE CHOROID OF HUMAN MYOPIC EYES

7.1 Abstract

Purpose: The choroidal circulation has been implicated as having an effect on myopic eye growth, but remains largely unexplored in humans. The purpose of this investigation was to evaluate the choroidal circulation characteristics and vascular autoregulatory properties of the choroid in human myopia.

Methods: The choroidal circulation was assessed at the central foveal avascular zone using Laser Doppler Flowmetry (LDF) on myopes and emmetropes. Subfoveal choroidal velocity (LDFvel), volume (LDFvol) and flow (LDFflow) were recorded at 2 sessions: baseline (B1, breathing room air) and isoxic hypercapnia (end tidal $pCPO_2$ increased approximately 15% above B1 with constant O_2 supply). For the baseline condition (B1) to establish resting choroidal flow, the sample comprised one eye of each of 51 healthy volunteers grouped by refractive error: a) 23 emmetropes with MSE ±0.05 D (mean MSE of -0.006 ±0.47; mean age 35.60 ± 14.94years), b) 28 myopes with spherical equivalent (MSE) ≥ -1.00D (average MSE-3.80 ± 2.12D; mean age 35.60 ± 14.94years). To determine the autoregulation characteristics, 28 participants underwent the isoxic hypercapnia (IH) condition; however, only data from 10 myopes and 10 emmetropes were analysed to ensure the quality of the match. The myopes' age ranged from 20 to 54 years (average age 30.90 ± 12.16 years) and their average MSE was -3.90 ± 2.06 (refractive range -1.68 to -7.94 D). Emmetropes were 18 to 54 years old (average age 33.10± 10.37 years) and with mean MSE of -0.21 ± 0.30 (refractive range -0.50 to +0.12 D). Both groups were matched for age, gender and ethnicity. Blood pressure (BP) and intraocular pressure (IOP) were measured in order to establish the mean arterial pressure (MAP) and ocular perfusion pressure (OPP), and body mass index (BMI) was also recorded.

Results: Resting Condition (B1): There were no differences in BP, MAP, BMI or OPP between the groups. The myopia group exhibited lower choroidal blood velocity (p=0.033). Isoxic Hypercapnia (IH): BP, IOP, OPP and MAP did not change significantly during hypercapnia. Myopes exhibited a significant increase in choroidal blood velocity that exceeded statistically that of emmetropes (p=0.03).

Conclusions: These results suggested that, compared to the emmetropic eye, the myopic eye had a significantly lower resting choroidal flow velocity which was highly responsive to CO_2.

7.2 Introduction

The choroid represents the main source of energy and blood supply to the retina. As previously described (see section 1.10.1.2), the choroid comprises a rich network of vessels located below the retinal pigment epithelium (RPE) and is known to provide metabolites and nourishment to the outer retina, as well as to dissipate the heat generated by the light absorbed in retinal photopigments.

The question of a mechanism to convey the information gathered by the retina onto the sclera to direct ocular growth stressed the importance of the investigation of the choroid in animal studies, as it is a layer located between the retina and the sclera. Studies by Wallman (1995) showed how the choroid in the chick eye increased in thickness in response to myopic defocus. Consequently, a thinning and thickening of the choroid as a compensatory mechanism aiming to relocate the blurred retinal image and offset changes in axial elongation in animal deprivation myopia and myopia induced by lens defocus have been reported (Wallman and Winawer 2004). These thickness changes may be due to changes in choroidal blood flow (Fitzgerald, Wildsoet and Anton 2001) or changes in vascular permeability (Pendrak, *et al.* 1997).

Despite the importance of the choroidal layer in myopia, little information is available regarding the features of choroidal blood flow in the human eye. The regulatory features of the retinal and the choroidal layers have been investigated in the past, and the vascular regulatory profile of the retina has been reported to lack autonomic innervation (Ye, Laties and Stone 1990; Ferrari-Dileo, Davis and Anderson 1989; Laties 1967); whereas the choroidal circulation was initially suggested to lack local regulatory mechanisms (Bettman and Fellows 1956). One of the reasons why choroidal circulation was suggested to be free from metabolic regulation is the high rate of blood flow through the choroid, which may prevent the choroidal bed from being sensitive to small metabolite changes (Delaey and Van de Voorde 2000). However, recent animal studies (Kiel 1999; Kiel and van Heuven 1995; Kiel and Shepherd 1992) and human investigations (Riva, *et al.* 1994) have shown some level of choroidal autoregulation. Riva and colleagues first suggested the ability of the choroid to autoregulate after experimentally inducing a rapid and a slower increase in intraocular pressure (IOP). Lack of a constant linear relationship between ocular perfusion pressure and choroidal blood flow was reported and when slower increases in IOP were induced, resulting in small changes in OPP, no changes in choroidal blood flow were found, pointing towards some kind of local regulation. Additionally, the time response to the experimentally induced variations in IOP was reported to appear as a neural regulatory response (Riva, *et al.* 1997).

Different techniques have been used to evaluate the regulatory properties of the choroid, such as postural changes (Longo, Geiser, and Riva, 2004), induced changes in IOP (Lovasik et al., 2003), OPP and arterial blood pressure (Longo, Geiser and Riva 2004; Hafez, *et al.* 2003; Hardy, *et al.* 2001; Longo, Geiser and Riva 2000; Reiner, Shih and Fitzgerald 1995).

The instrument used to measure the choroidal blood flow in this study was first described in 1994, and it was designed to provide a non-invasive method of assessing the choroidal blood flow in the human eye. The principle has been described in this thesis (see section 1.11.7.1). In summary, LDF provides semi quantitative measurements of blood velocity, volume and flow derived from the analysis of frequency changes induced by moving red blood cells. The change in the laser frequency when the laser falls on moving red blood cells is calculated by an exponential function from which blood volume, velocity and flow are given. The reliability described for the parameters assessed have been assessed as 93% for the velocity, 74.6% for the volume and 77.5% for the flux (Gugleta, *et al.* 2002). LDF has also been used to demonstrate a regulatory response from the choroid after an increase in ocular perfusion pressure induced by isometric exercise. Both eyes of 11 participants with ages ranging from 18 to 57 years were assessed after 90 seconds static in squatting position. The ocular perfusion pressure increased by 67% whereas the mean choroidal blood flow increased by only 12%, which suggested an increase in choroidal vascular resistance to compensate for the increase in mean arterial pressure and limit to 12% the potential increase in choroidal blood flow. However, this possible mechanism of autoregulation appeared to fail with larger changes in perfusion pressure (Riva, *et al.* 1997).

Chapter three of this thesis described a significantly lower central retinal artery (CRA) blood velocity and pulsatile blood flow, accompanied by a significantly higher CRA resistance index (RI) in myopes, which suggested a lower pulsatile and CRA blood flow in human myopia. POBF is widely accepted as a tool for choroidal assessment and it calculates the pulsatile ocular blood flow from the variations in IOP with the cardiac cycle. However, as previously explained in section 1.11.2, the calculation of the total pulsatile blood flow relies on certain assumptions which limit the interpretation of the data obtained. Thus, in the present study the choroidal blood flow was recorded using LDF, which provided a direct measure of the choroidal blood flow, to overcome the limitations of the OBFA system.

In view of the vascular findings reported in the previous chapters of this thesis, which are supported by the implication of the choroid in animal models of myopia and considering that choroidal blood flow represents 85% of the total blood supply of the eye, the investigation of the choroidal circulation in myopia is of intrinsic interest. Thus, this study was designed to address

the existing gap in the literature regarding the choroidal blood flow features of the human myopic eye.

7.3 Aims and objectives

The aim of this study was to evaluate the resting choroidal vascular profile in human myopia and, additionally, the vascular regulatory properties of the choroid using a mild provocation test.

7.4 Methods

7.4.1 Study Sample: Recruitment and Exclusion Criteria

Fifty-one healthy volunteers participated in this study. All volunteers were recruited from the student and staff population of Aston University. Twenty-eight myopes with mean spherical equivalent (MSE) ≤ -1.00D comprised the study group (average MSE -3.80 ± 2.12D; range: -1.00 to -9.00; mean age 35.60 years ± 14.94, range: 20-74). The control group consisted of 23 emmetropes with MSE ±0.05 D (mean MSE of -0.006 ±0.47; mean age 35.60 years ± 14.94, range: 18-78). All the three groups were matched for age, gender and ethnicity.

To assess the choroidal vascular response to hypercapnia in human myopia, the sample comprised two subgroups of volunteers: a) an emmetropic group comprising 10 participants with mean age 33.10± 10.37 years (range from 18 to 54 years) and with mean MSE of -0.21 ± 0.30D (refractive range -0.50 to +0.12 D), b) a myopic group formed by 10 participants with mean age 30.90 ± 12.16 years (range from 20 to 54 y) and whose mean MSE was -3.90 ± 2.06D (refractive range -1.68 to -7.94 D). All the three groups were matched coincidently for age, gender and ethnicity (i.e. each myope was individually matched for age, gender and ethnicity with a subject from each refractive group).

All the participants were healthy volunteers, a fact that was confirmed by the author by investigation the fundus using Volk lens (90D), recording blood pressure and a detailed recording of systemic and ocular history and symptoms. Exclusion factors included any ocular disorder, diabetes, hypotension, hypertension or any other systemic disorder or medication likely to affect the systemic or ocular vasculature. Since no blood test was performed, all of the previous were self-reported by the participants. Those subjects exhibiting any sign of myopic fundus changes (lacquer cracks, staphylomas, chorioretinal atrophy, lattice degeneration or pavingstone degeneration) were not included in the study. A corrected visual acuity of 6/9 or better was required together with astigmatism of less than 1.5 diopters cylinder, which reduced the percentage of astigmatic patients being taken as myopic subjects after MSE calculation. None of the subjects included in the study were smokers.

Alcohol, nicotine and caffeine containing products have been reported to affect the flow of blood in the eye (Domino, *et al.* 2004; Gdovinova 2001). To ensure the reliability of the data, the participants recruited for this study were asked to refrain from smoking and consuming alcohol and caffeine containing products 12 hours prior to the study.
Exercising is to increase the mean arterial pressure (MAP); however the maximal BP falls rapidly on stopping exercise (Tzemos, Lim and MacDonald 2002). Therefore, since the total duration of the experiment was approximately 1hour and the vascular readings were performed approximately in the last 30 minutes, the potential effect of exercising on the systemic circulation was minimal. Thus, the subjects were not asked to refrain from exercising before the appointment.

7.4.2. Ethical Approval and Informed Consent

Written and verbal information about the exact procedures to be performed during the visit was given to all the subjects prior to data collection. The participants were encouraged to ask any questions and to clarify any doubts they might have before signing the written consent. The volunteers were requested to sign two copies of the written consent, one of which was given to them for their own records. All investigations were approved by the Ethical Review Committee and conformed to the declaration of Helsinki (appendix 2.1).

7.4.3 Study Sample: Dietary Restrictions

Alcohol, nicotine and caffeine containing products have been reported to affect the flow of blood in the eye (Domino, *et al.* 2004; Gdovinova 2001). To ensure the reliability of the data, the participants recruited for this study were asked to refrain from smoking and consuming alcohol and caffeine containing products for 12 hours prior to the study.

7.4.4. Experimental Protocol and Investigations

Chapter seven was subdivided into two main sections. Section one describes the resting choroidal blood flow features; whereas the second section evaluates the vascular response to hypercapnia from the choroidal layer.

The experimental design of the study was the following (see section 1.9.1 and section 1.9.2 for detailed explanation of the instruments used):

- Autorefraction (Shin-Nippon NVision-K 5001 Autorefractor, Japan) and biometry data (IOLMaster, Carl Zeiss Jena, Germany Zeiss) were taken once written consent was obtained from the volunteers.
- Proxymetacaine HCl 0.5% (Minims®, Chauvin Pharmaceuticals) was instilled as anaesthetic corneal thickness was measured (DGH-550 Pachette 2, DGH technology, Pennsylvania, US)

and intraocular pressure (IOP) were recorded (Tono-Pen®XL, Medtronic Xomed, Inc., Minnesota, USA).

- The choroidal blood flow was measured using the Laser Doppler Flowmeter (LDF) (Riva method, Institut de Recherche en Ophtalmologie, Switzerland). The instillation of one drop of tropicamide (Tropicamide 0.5% Minims®, Chauvin Pharmaceuticals) facilitated the correct visualisation of the macula, and accurate measurement of choroidal circulation.
- Systemic blood pressure (UA-767 Fully Automatic Blood Pressure Monitor BHS A/A, A & D Co. Ltd, Oxon, UK) was recorded with an automated sphygmomanometer.
- Weight and height were then measured on all the participants.

The protocol followed to assess the resting state of the choroidal blood flow is described in table 7.1. Similarly, the protocol designed to evaluate the choroidal autoregulatory response to hypercapnia is summarised in table 7.2.

Protocol- Resting state of the choroidal blood flow
Autorefraction (Shin-Nippon NVision-K 5001 Autorefractor, Japan)
Ocular biometric analysis (IOLMaster, Carl Zeiss Jena, Germany Zeiss)
Instillation of proxymetacaine HCl 0.5% (Minims®, Chauvin Pharmaceuticals)
Measurement of corneal thickness (DGH-550 Pachette 2, DGH technology, Pennsylvania, US)
Instillation of tropicamide 0.5% (Minims®, Chauvin Pharmaceuticals)
Choroidal blood flow measurement (Laser Doppler Flowmeter, LDF, Riva method, Institut de Recherche en Ophtalmologie, Switzerland).
Measurement of systemic blood pressure (UA-767 Fully Automatic Blood Pressure Monitor BHS A/A, A & D Co. Ltd, Oxon, UK).
Measurement of weight and height

Table 7.1 *Summary of the protocol followed to assess the resting state of the choroidal blood flow*

Protocol- Choroidal response to hypercapnia
Baseline measurements *
Autorefraction (Shin-Nippon NVision-K 5001 Autorefractor, Japan)
Ocular biometric analysis (IOLMaster, Carl Zeiss Jena, Germany Zeiss)
Instillation of tropicamide 0.5% (Minims®, Chauvin Pharmaceuticals)
Measurement of systemic blood pressure (* UA-767 Fully Automatic Blood Pressure Monitor BHS A/A, A & D Co. Ltd, Oxon, UK).
Continuous recording of oxygen and carbon dioxide breathing saturation (**Capnocheck Plus Capnograph, Smiths Medical, Waukesha, Wisconsin, USA).
Normal room air breathing through face mask with non-rebreathing inlet valve of a gas mask connected to a mixing chamber (1014 series non-rebreathing mask, Hans Rudolph Inc., Missouri, USA
Achievement of baseline condition by breathing normal room air for approximately 5 minutes until the patient's respiration rate stabilized.
Measurement of systemic blood pressure *
Choroidal blood flow measurement (Laser Doppler Flowmeter, LDF, Riva method, Institut de Recherche en Ophtalmologie, Switzerland).
Measurement of systemic blood pressure *
3 minute-rest with mask on & CO_2, O_2 and BP recording
Hypercapnia measurements
Measurement of systemic blood pressure *
Continuous recording of oxygen and carbon dioxide breathing saturation **
Increase in pCO_2 by 15% (performed by adding CO_2 to room air in a mixing chamber)
Measurement of systemic blood pressure *
Choroidal blood flow measurement (Laser Doppler Flowmeter, LDF, Riva method, Institut de Recherche en Ophtalmologie, Switzerland).
Measurement of systemic blood pressure *
Removal of breathing mask & 5-minute rest
Measurement of systemic blood pressure to ensure adequate recovering *
Instillation of proxymetacaine HCl 0.5% (Minims®, Chauvin Pharmaceuticals)
Measurement of corneal thickness (DGH-550 Pachette 2, DGH technology, Pennsylvania, US)
Confirmation of corneal integrity using slitlamp after the instillation of fluorescein (Fluorets strips, Chauvin Pharmaceutical)

Table 7.2 *Summary of the protocol followed to assess the choroidal response to hypercapnia (*throughout the session, a fan was used to ensure adequate room air ventilation for baseline measurement and for hypercapnic conditions)*

The protocol followed to record choroidal blood flow was the same for the baseline and hypercapnia conditions. To direct the laser to the macula, where choroidal measurements were taken, the patient was asked to fixate for 60 seconds on the measuring laser. The heart rate of the patient was recorded using an ear infrared plethysmometer, and the average time course of each LDF parameter during the cardiac cycle was obtained by averaging over a period T of 60 seconds, taking into account the phase of the heart cycle.

The parameters obtained were foveolar choroidal blood flow velocity, LDFvel (kHz), relative foveal choroidal blood volume, LDFvol (arbitrary units, au) and relative choroidal blood flow, LDFflow (au). The data was analysed using software specifically designed to evaluate Doppler signalling from ocular tissue (NeXT Software, Inc, Redwood City, CA, USA). From the 60 seconds recording performed, only the data showing stable measurements were chosen for analysis, and the results were averaged.

7.4.5 Statistical analysis

Data are presented as mean ± SD. One-way ANOVA was used to assess the differences in choroidal blood parameters between emmetropes and myopes.

Bonferroni correction was performed since associated vascular parameters were calculated from the same piece of equipment. Pearson's correlation test was used to evaluate the relationship between choroidal blood parameters and ocular biometric parameters such as axial length, anterior chamber depth, corneal thickness and vitreous chamber volume.

Refractive error, age, body mass index and blood pressure parameters were also assessed for potential correlation with choroidal blood circulation using Pearson's correlation test.

Two-tailed paired Student's *t*-test was used to test for significance between baseline and hypercapnic conditions in each group.

Statistical significance was defined as $p<0.05$, unless Bonferroni correction was applied, in which case the new significant p value was calculated.

7.5 Results

7.5.1 Resting State Choroidal Perfusion Characteristics

There were no significant differences in age, gender, height, weight and BMI between myopes and emmetropes.

Moreover, systemic blood pressure, MAP and OPP did not differ significantly between the two groups (table 7.3).

From the ocular biometric parameters assessed, myopes exhibited longer axial length (p<0.001) and anterior chamber depth (p=0.043) and greater corneal thickness (p=0.039) (table 7.3).

Choroidal parameters were defined as the average values of the parameters obtained during the 60 seconds of the measurement. The choroidal blood velocity showed a significant reduction in the myopic group (p=0.033) (table 7.4).

	Emmetropes	Myopes	p
MSE (D)	- 0.006 ± 0.47	-3.80 ± 2.12	* <0.001
Gender	17f; 11 m	13f; 10m	n/a
Age (years)	42.75 ± 16.94	35.60 ± 14.94	ns
Systolic BP (mmHg)	114.30 ± 15.97	113.26 ± 16.40	ns
Diastolic BP (mmHg)	75.26 ± 13.96	71.78 ± 12.25	ns
Pulse rate (bpm)	71.51 ± 12.16	73.60 ± 12.69	ns
MAP (mmHg)	91.92 ± 13.26	90.42 ± 14.43	ns
OPP (mmHg)	47.40 ± 8.40	46.64 ± 10.01	ns
Height (m)	1.68 ± 0.09	1.66 ± 0.08	ns
Weight (kg)	72.18 ± 16.41	69.53 ± 13.78	ns
BMI	25.21 ± 4.63	24.88 ± 4.22	ns
Axial length (mm)	23.56 ± 0.72	24.92 ± 1.10	* <0.001
ACD (mm)	3.34 ± 0.29	3.55 ±0.40	* 0.043
CT (μm)	535.06 ± 40.68	558.39 ± 36.84	* 0.039
IOP (mmHg)	13.92 ± 4.10	14.25 ± 3.94	ns

Table 7.3. *Summary of systemic and ocular characteristics for emmetropes and myopes prior to choroidal blood flow assessment (explanation of abbreviations: MAP, mean arterial pressure; OPP, ocular perfusion pressure; BMI, body mass index; ACD, anterior chamber depth; CT, corneal thickness; IOP, intraocular pressure; m: males; f: females); *statistically significant*

	Emmetropes	Myopes	p
LDFvel (kHz)	1.80 ± 0.52	1.39 ± 0.79	*0.033
LDFvol (au)	0.73 ± 0.53	0.65 ± 0.57	ns
LDFflow (au)	1.51 ± 1.33	1.16 ± 1.47	ns
LDF DC	0.63 ± 0.17	0.59 ± 0.19	ns

Table 7.4. *Choroidal blood flow parameters for the emmetropic and myopic group (explanation of abbreviations: LDFvel: choroidal blood velocity; LDFvol: choroidal blood volume; LDFflow: choroidal blood flow; LDF DC: choroidal direct current or noise of the recording) ns: not significant difference *statistically significant*

7.5.1.1 Relationship between systemic and choroidal blood flow

Choroidal blood volume correlated significantly with the ocular perfusion pressure (OPP) and mean arterial pressure (MAP) (R=-0.31, p=0.029; R=-0.30, p=0.033). No other significant correlations were found between choroidal parameters and blood pressure parameters, or choroidal parameters and ocular biometric parameters, corporal biometric parameters or age (appendix 1.9).

When the correlations were analysed independently for myopes and emmetropes, body weight correlated with choroidal blood velocity in myopes (R=-0.45; p=0.032) and body mass index correlated with choroidal blood volume in emmetropes (R=0.40; p=0.034) (figure 7.1).

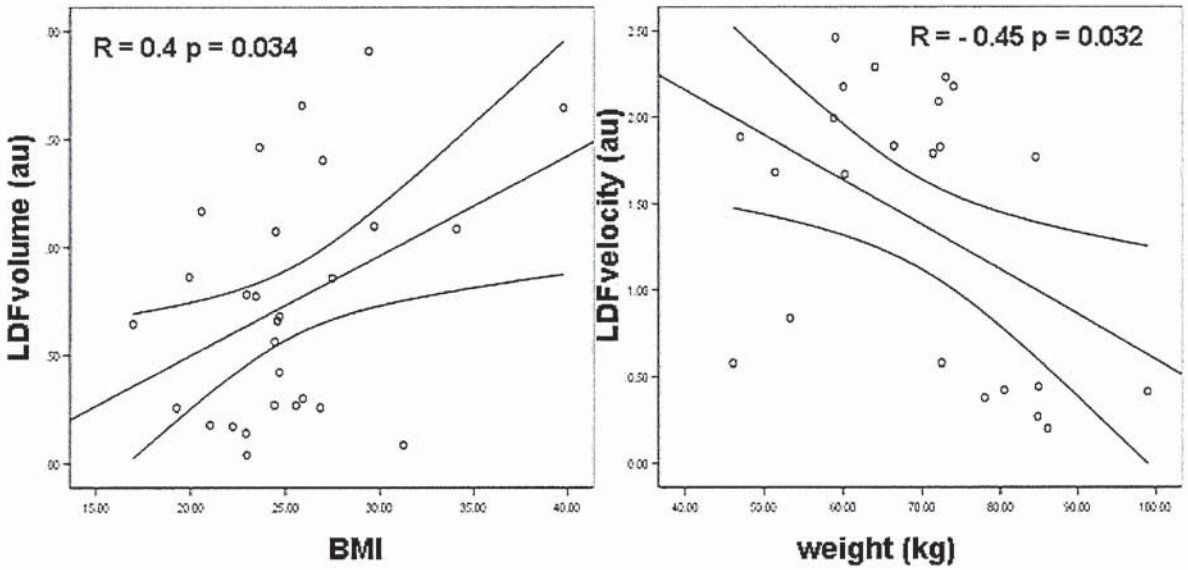


Figure 7.1 *Significant correlations between LDF volume and body mass index in emmetropes (p=0.034), and LDF velocity and weight in myopes (p=0.032).*

7.5.2 Choroidal autoregulatory response to hypercapnia in human myopia

There were no significant differences in age, gender, height, weight and BMI mass index between myopes and emmetropes. Moreover, systemic blood pressure, MAP and OPP at baseline did not differ significantly between the two refractive groups (table 7.5).

From the ocular biometric parameters assessed (AL, corneal thickness, ACD and IOP), AL and ACD differed significantly between the two refractive groups (p<0.001; p=0.046) (table 7.5).

	Emmetropes	Myopes	p
N	10	10	n/a
MSE (D)	-0.20 ± 0.30	-3.90 ± 2.06	*<0.001
Gender	6m; 4f	6m; 4f	n/a
Age (years)	33.10 ± 10.37	30.90 ± 12.16	ns
Systolic BP (mmHg)	112.25 ± 15.76	121.60 ± 17.26	ns
Diastolic BP (mmHg)	78.25 ± 20.17	72.90 ± 13.43	ns
Pulse rate (bpm)	73.06 ± 9.52	70.10 ± 8.43	ns
MAP (mmHg)	96.08 ± 16.76	96.06 ± 15.80	ns
OPP (mmHg)	49.18 ± 9.24	48.46 ± 9.62	ns
Height (m)	1.68 ± 0.08	1.69 ± 0.06	ns
Weight (kg)	66.18 ± 13.96	71.60 ± 14.30	ns
BMI	23.16 ± 3.57	24.82 ± 4.27	ns
Axial length (mm)	23.66 ± 0.74 *	25.41 ± 0.86 *	*<0.001
ACD (mm)	3.45 ± 0.26 *	3.71 ±0.26 *	* 0.046
CT (μm)	522.80 ± 25.47	546.58 ± 53.71	ns
IOP (mmHg)	14.88 ± 3.94	15.78 ± 3.06	ns

Table 7.5. *Summary of systemic and ocular characteristics by refractive group (explanation of abbreviations: MAP, mean arterial pressure; OPP, ocular perfusion pressure; BMI, body mass index; ACD, anterior chamber depth; CT, corneal thickness; IOP, intraocular pressure; SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial pressure; OPP, ocular perfusion pressure; ; m: males; f: females)* statistically significant*

7.5.2.1 Mask baseline

Choroidal blood flow parameters did not show any significant difference (table 7.6).

	Emmetropes	Myopes	p
LDFvel (kHz)	1.61 ± 0.59	1.50 ± 0.71	ns
LDFvol (au)	0.89 ± 1.27	0.67 ± 0.61	ns
LDFflow (au)	1.02 ± 1.03	1.28 ± 1.47	ns
LDF dc	0.65 ± 0.17	0.62 ± 0.17	ns

Table 7.6 *Choroidal perfusion parameters for the emmetropic and myopic group during mask baseline condition B2. (explanation of abbreviations: LDFvel: choroidal blood velocity; LDFvol: choroidal blood volume; LDFflow: choroidal blood flow) ns: no significant difference*

7.5.2.2 Hypercapnic condition

The end tidal carbon dioxide concentration in emmetropes increased significantly from 38.05 mmHg at baseline to 44.54 mmHg during hypercapnia (17.21% increase) ($p<0.001$). Myopes end tidal concentration changed significantly from 36.60 mmHg at baseline to 43.00 mmHg (17.48% increase) ($p<0.001$) (table 7.7). For all subjects, the saturation of oxygen increased parallel to CO_2 rise, which ensured constant levels of O_2 during gas perturbation. Blood oxygen saturation varied significantly from 96.88% at baseline to 97.88% during hypercapnia in emmetropes ($p<0.001$) and from 97.11 to 98.20 in myopes ($p<0.001$). A summary of the data describing changes in end tidal carbon dioxide and oxygen saturation is given in table 7.7.

	Baseline	Hypercapnia	p
emmetropes			
End tidal CO_2 (mmHg)	38.05	44.54	* <0.001
O_2 saturation levels (%)	96.88	97.88	* <0.001
myopes			
End tidal CO_2 (mmHg)	36.60	43.00	* <0.001
O_2 saturation levels (%)	97.11	98.20	*<0.001

Table 7.7. *Changes in CO_2 and O_2 from baseline to hypercapnic conditions for each refractive group*
** statistically significant*

	Baseline	Hypercapnia	p
emmetropes			
SBP (mmHg)	115.33 ± 12.81	118.67 ± 12.55	ns
DBP (mmHg)	74.33 ± 13.69	78.22 ± 19.09	ns
Heart rate (bpm)	66.33 ± 11.05	66.55 ± 8.29	ns
MAP	88.00 ± 12.75	91.70 ± 16.66	ns
OPP	47.14 ± 5.35	49.02 ± 10.26	ns
myopes			
SBP (mmHg)	119.44 ± 20.28	121.11 ± 19.42	ns
DBP mmHg)	71.44 ± 13.73	73.77 ± 13.33	ns
Heart rate (bpm)	61.88 ± 5.71	64.44 ± 10.81	ns
MAP	86.48 ± 14.61	89.55 ± 14.53	ns
OPP	43.70 ± 7.36	45.00 ± 8.77	ns

Table 7.8. *Systemic BP change from baseline to hypercapnia: overall analysis and by refractive group. SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial pressure; OPP, ocular perfusion pressure.*

7.5.2.3 Systemic response to hypercapnia

The systemic blood pressure parameters did not change significantly during hypercapnia. A summary of the BP, MAP and OPP readings during baseline and hypercapnia is provided in table 7.8.

Ocular vascular response to hypercapnia

Emmetropes did not exhibit an increase in choroidal blood velocity, volume or flow, whereas myopes exhibited a significant increase in choroidal blood velocity during hypercapnia (table 7.9, figure 8.2).

	Baseline	Hypercapnia	p
	emmetropes		
LDFvel (kHz)	1.61 ± 0.59	1.50 ± 0.52	ns
LDFvol (au)	4.89 ± 1.27	5.69 ± 1.21	ns
LDFflow (au)	1.02 ± 1.03	1.13 ± 1.23	ns
LDF dc	0.65 ± 0.17	0.63 ± 0.11	ns
	myopes		
LDFvel (kHz)	1.50 ± 0.71	1.66 ± 0.71	*0.03
LDFvol (au)	0.67 ± 0.61	0.68 ± 0.54	ns
LDFflow (au)	1.28 ± 1.47	1.43 ± 1.49	ns
LDF dc	0.62 ± 0.17	0.66 ± 0.17	ns

Table 7.9. *LDF results of choroidal blood velocity (LDFvel), choroidal blood volume (LDFvol) and choroidal blood flow (LDFflow) during hypercapnia by refractive group. ns: not significant *statistically significant*

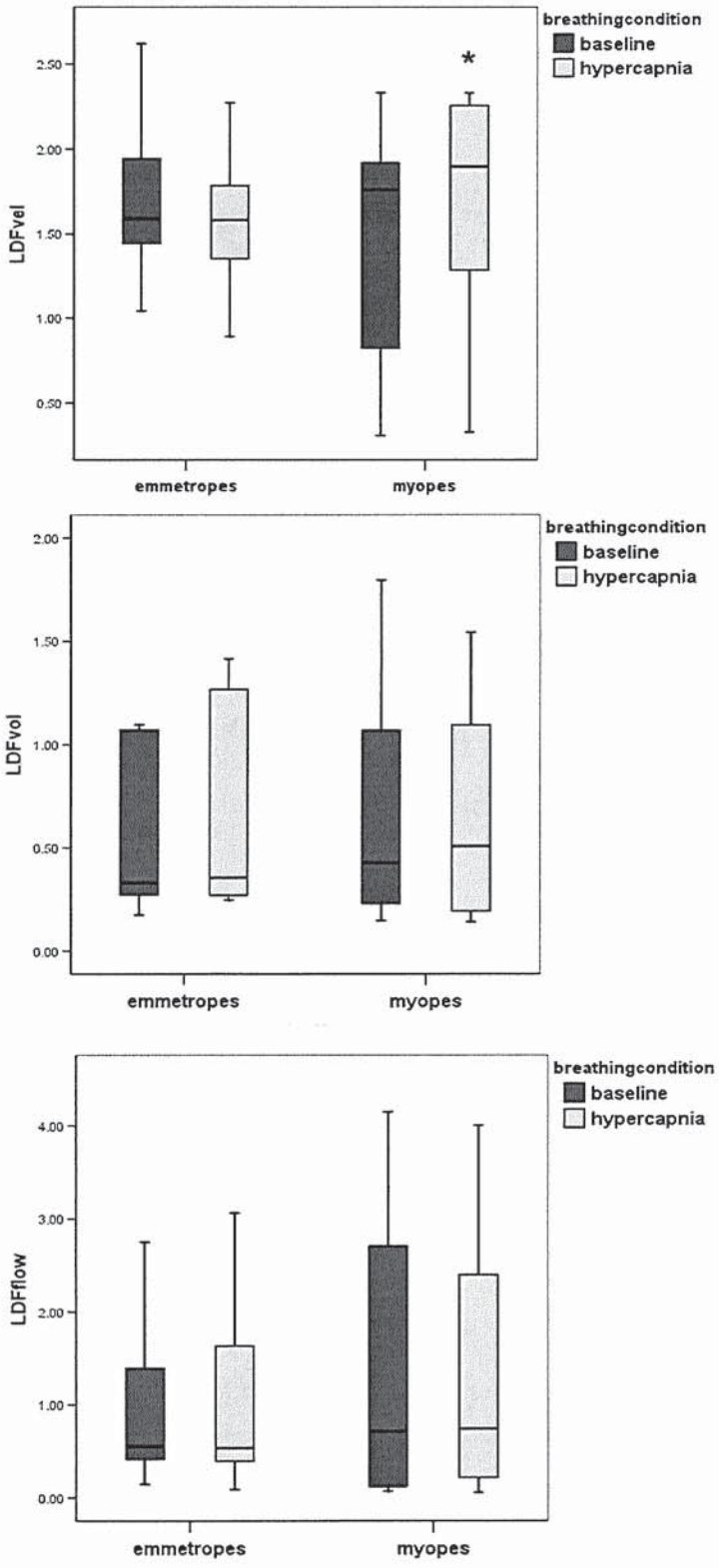


Figure 7.2 *Choroidal responses assessed with LDF: hypercapnia resulted in a significant increase in choroidal blood velocity in the myopic group (*statistically significant)*

7.5.3 Relationship between ocular biometric parameters and systemic and choroidal blood parameters during hypercapnia

Hypercapnia-induced percentage changes in choroidal blood velocity, blood volume and blood flow did not correlate significantly with the hypercapnia-induced percentage changes in blood pressure for the emmetropic group (appendix 1.10). However, when the myopic group was assessed for correlations between the percentage change induced by hypercapnia on choroidal vasculature and systemic blood pressure, choroidal blood volume correlated significantly with percentage change in SBP (R=0.83, p=0.005), DBP (R=0.69, p=0.038), OPP (R=0.79, p=0.01) and MAP (R=0.69, p=0.038) (figure 7.3).

Overall analysis showed a significant correlation between the hypercapnia-induced percentage change in choroidal blood flow and mean spherical equivalent (R=-0.47, p=0.03), and only in myopes, significant correlation was found between the hypercapnia-induced percentage change in choroidal blood volume and axial length (R=0.64, p=0.045) (figure 7.4, figure 7.5).

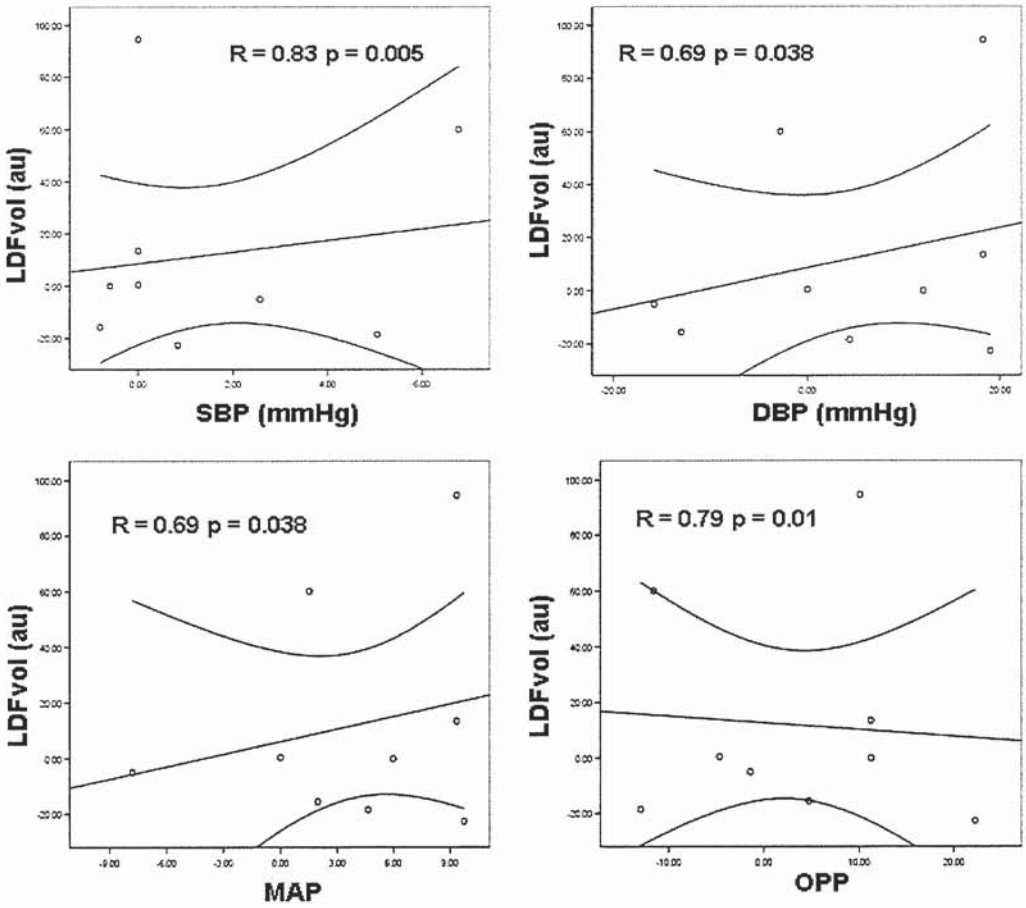


Figure 7.3 *Scatter plots showing relationship between choroidal flow measures and systemic and ocular perfusion characteristics in myopes*

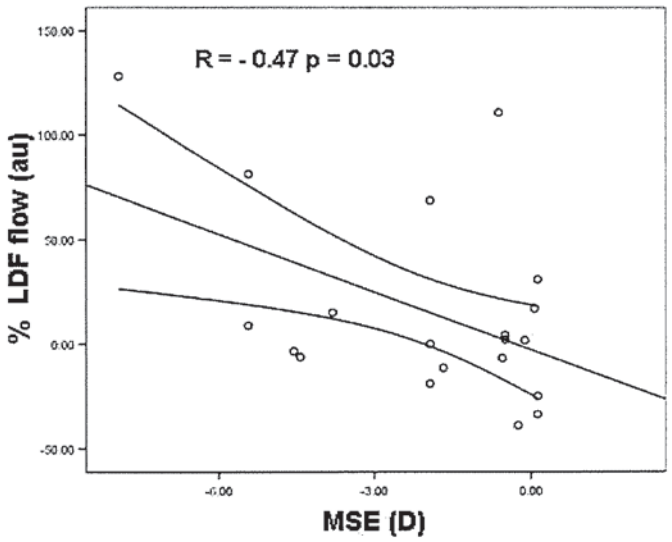


Figure 7.4 *Scatterplot showing the relationship between the percentage change induced by hypercapnia on choroidal blood flow and mean spherical equivalent (MSE) (p=0.03)*

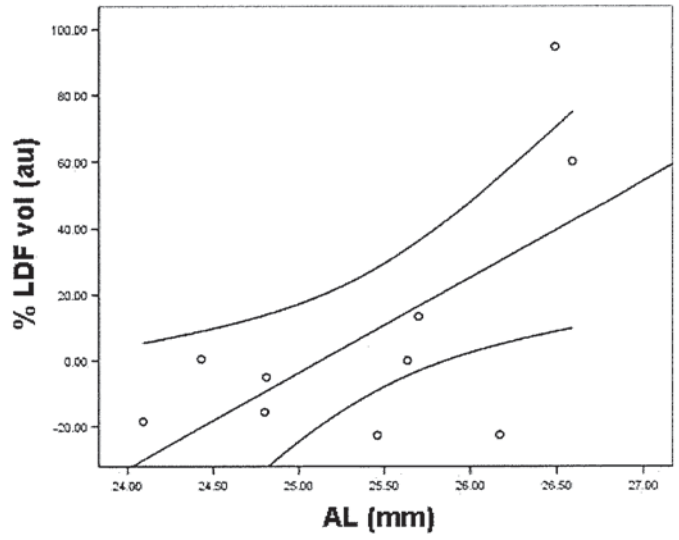


Figure 7.5 *Significant correlations between the percentage change induced by hypercapnia on choroidal blood volume and axial length (AL) (p=0.045)*

7.6 Discussion

This study evaluated the resting vascular profile of the choroidal circulation in human myopia using the laser Doppler flowmeter (LDF) by comparing a group of myopes with a matched sample of emmetropes. There were no significant differences in age, gender, height, weight, BMI and systemic blood pressure, MAP or OPP between myopes and emmetropes, who only differed

significantly in axial length, anterior chamber depth, refractive error and corneal thickness. The blood velocity in the choroid of myopes appeared significantly decreased compared to that from emmetropes. The volume of blood in the choroid correlated significantly with the ocular perfusion pressure and the mean arterial pressure in myopes only. No other significant correlations were found between the choroidal circulation and the ocular or principle biometric parameters and age. When the autoregulatory features of the choroid were assessed by increasing the percentage of carbon dioxide concentration ($pCPO_2$), myopes showed a significant increase in choroidal blood velocity during hypercapnia that differed statistically from the percentage change induced by hypercapnia in emmetropes. Moreover, the choroidal blood flow response to hypercapnia was found to correlate with the MSE. Additionally, in myopes a significant correlation was found between response of the choroidal blood volume and axial length, MAP and OPP.

7.6.1 Features of the choroidal circulation in myopia

The results of this study revealed significantly lower choroidal blood velocity in eyes a with low to high degree of myopia (-1.00D to -9.00D). No differences in systemic blood pressure readings were found between emmetropes and myopes, which suggested a similar systemic cardiovascular profile in both refractive groups. However, differences in choroidal blood velocity were found between myopes and emmetropes. Moreover, LDF provides semi quantitative measurements of blood velocity (LDFvel), volume (LDFvol) and flow (LDFflow), all of which are related to each other by the relationship LDFflow = K x LDFvel x LDFvol (where K represents an instrumental constant derived from the analysis of frequency changes induced by moving red blood cells). Assuming constant hematocrit (proportion of volume occupied by the red blood cells), the changes in ChBVol and ChBF are proportional to changes in actual blood volume and flow, respectively (Longo, Geiser and Riva 2000). Therefore, since LDF readings were taken at the foveal region, the trend in myopic eyes towards reduced blood velocity points towards an actual decrease in choroidal blood velocity.

Structural features of myopic eyes describe an eye with an elongated vitreous chamber (Jones. *et al*, 2005), which in the case of highly myopic eyes can result in breaks in the Bruch's membrane, retinal pigment epithelium and choriocapillaris, all of which are attributed to the progressive elongation of the ocular posterior segment mentioned previously. The structural features of the myopic posterior segment that show an increased axial length and a prolate shape have been confirmed in a study by Logan and co-workers (Logan, *et al.* 2004). From the same research group, some highly myopic eyes were also found to have an oblate shape using ocular MRI to generate 3-D images of the whole eye (Singh, Logan and Gilmartin 2006). Owing to the elongated myopic vitreous chamber and its prolate shape, the management of the central vision in myopia

alone may lead to a hyperopic state of the peripheral retina (Charman 2005; Schmid 2003). The correction of central vision only in myopia leads to a relative peripheral hyperopia, which may stimulate an active emmetropisation mechanism. Such emmetropisation may in turn trigger ocular growth, leading to the progression of myopia, which in turn could be related to the myopic choroidal thinning and reduced choroidal blood flow reported in animal models of myopia. When form-deprived, young chicks were induced with axial myopia, the increased axial growth of the eye was accompanied by choroidal thinning and decreased choroidal blood flow (Fitzgerald, Wildsoet and Anton 2001). In contrast, during the early part of the recovery process, the choroid thickened, shifting the retina towards the new plane of focus. In chick eyes recovering from form deprivation myopia, a large increase in choroidal blood flow preceded an increase in choroidal thickness, which suggested that an increase in choroidal blood flow might trigger choroidal expansion (Fitzgerald, Wildsoe and Anton 2001; Wildsoet and Wallman 1995). However, it is necessary to highlight that the mechanisms involved in form deprivation myopia in animals may differ from those involved in human emmetropisation (Bartmann, *et al.* 1994) and additionally, it remains unexplored whether the mechanisms that result in form deprivation myopia in animal studies are similar to those involved in the onset and progression of myopia in humans.

These findings reported previously support the results of the present study, and additionally support the hypothesis of a reduction in choroidal blood velocity in human myopia. However, it must be clarified that only the subfoveal choroid was evaluated; therefore, the findings only apply to the central macular area, or foveal avascular zone. To date, the features of the peripheral choroid have not been assessed due to lack of appropriate instrumentation.

Interestingly, the choroidal blood velocity, volume or flow did not exhibit any significant correlation with the degree of myopia or axial length. This may represent the need for a bigger sample size with greater range of myopia to assess more accurately the relationship between the degree of myopia and the reduction in choroidal blood flow. Power statistics revealed that a sample size with a statistical power of 80%, allocation ratio 1:1 and a significant value of 5% (α=00.05) required a sample of 31 participants to show statistical significance between the choroidal blood velocity of emmetropes and myopes. The sample size of this study approached this value, not reaching the exact number due to the inherent difficulty of recruiting volunteers for a study in which enriched CO_2 breathing is required. The sample size required to find significant differences between the choroidal volume and flow of myopes and emmetropes, also keeping a statistical power of 80%, allocation ratio 1:1 and a significant value of 5% (α=00.05) was found to be 300 and 700 participants respectively (sample size calculations performed following Simple Interactive Statistical Analysis, SISA), which differs largely from the sample size recruited in the present study. However, Polska *et al* (2004) assessed the reproducibility of the LDF readings

throughout a 12 hour period and in support of our findings, they reported a higher variability for the choroidal volume parameter than for the choroidal velocity. Thus, due to the known relationship between choroidal velocity, volume and flow, a large part of the variability in choroidal flow is known to be due to the spread of the volume data (Polska, *et al.* 2004). Since choroidal blood velocity provides a narrower measurement variability, it is more likely to find statistical differences in choroidal blood velocity, than in flow or volume, which agrees with our results.

The reduction in choroidal blood velocity found in myopes followed similar pattern to the significantly lower pulsatile ocular blood amplitude and CRA blood velocity. OBFA assesses the pulsatile blood flow in the large choroidal vessels, whereas the LDF signal originates from the choriocapillaris, where the pulsatility is much lower than in the main choroidal vessels. Thus, myopia appeared to exhibit attenuation in both the pulsatile and non-pulsatile component of the choroidal blood flow, and additionally, a decrease in retrobulbar blood velocity.

The choroidal blood velocity and volume exhibited a correlation trend with the body weight and body mass index. Myopic eyes are known to have significantly larger axial lengths, and similarly, height has been found to correlate with myopia. However, this does not mean that taller subjects are at higher risk of myopia, but that height appears as a confounding factor for axial elongation.
The weak trend found between BMI and choroidal blood flow may be due to the role that collagen plays as main support protein in ligaments, tendons, bones and skeletal muscles. Collagen is additionally the major component of the extracellular matrix of the blood vessel walls (Hughes and Schachter 1994), and interestingly, the inhibition of nitric oxide (NO, primary relaxing factor released from endothelial cells, see section 1.12.2.8 for more information on NO) has previously been shown to increase vascular collagen metabolism (Myers and Tanner 1998). Therefore, NO vasodilation-mediated increase in blood velocity will decrease vascular collagen metabolism, which in turn has been linked to a reduction in the formation of collagen I in obesity (Papakitsou, *et al.* 2004).

7.6.2 Choroidal regulatory response to hypercapnia

During the baseline condition, the systemic and ocular vascular features did not differ between emmetropes and myopes. However, myopes showed a significant increase in choroidal blood velocity during hypercapnia that differed statistically from the percentage change induced by hypercapnia in emmetropes.

Early papers evaluating choroidal blood flow (Bill, Sperber and Ujiie 1983) had shown a linear decrease and increase in choroidal blood flow with decreased and increased ocular perfusion pressure respectively, which was initially interpreted as lack of vascular regulation in the choroidal bed. It was argued that the reason as to why the choroid did not keep a constant blood flow with changes in perfusion pressure could be the choroidal capability to nourish the retina even at reduced blood flow levels. However, subtle reductions in choroidal blood flow have been shown to impair retinal function and, additionally, manipulation of blood pressure triggered a compensatory mechanism of choroidal blood flow, which questioned the lack of regulatory choroidal mechanisms (Cheng, *et al.* 2001; Riva, *et al.* 1997).

Postural changes affect the systemic circulation such that when the body moves from the standing to the supine position a significant decrease in diastolic blood pressure and heart rate occur. The choroidal response to postural changes has been assessed by Longo and colleagues (Longo, Geiser and Riva 2004) and it exhibited a significant increase in IOP and choroidal blood velocity. The OPP percentage change induced by the postural variation was found to be similar to that of the choroidal velocity, which suggested a passive choroidal response to postural changes. However, the 43% increase in OPP induced by stationary biking in a paper by Lovasik and collegues (Hafez, *et al.* 2003) occurred parallel to an increase in LDF flow that remained within 10% of its basal value, suggesting some kind of blood flow regulation that enables the choroid to keep the values close to its basal readings. These studies highlight the importance of not only analysing changes in LDF velocity, volume and flow after a provocation test, but also the importance of evaluating the temporal features of the choroidal response. The increase in choroidal circulation after biking remained within 10% of the basal values; therefore, if only the increase in choroidal blood flow had been evaluated, an equivocal conclusion suggesting lack of choroidal regulation would have been drawn.

The vascular reactivity to hypercapnia in the choroidal circulation of myopes described in the present study is supported by findings reported by Geiser and colleagues (2000), in which increased pCO_2 on the choroidal circulation of 16 healthy subjects resulted in increased blood velocity (Geiser, *et al.* 2000).

Therefore, the studies reviewed above suggest a regulatory capacity of the choroid and support the findings of the present study. However, it was interesting to observe that the increase in choroidal blood velocity at the subfoveal level occurred only in myopes. This vascular reactivity in myopia correlated with the ocular refractive status and with axial length such that the increase in choroidal velocity response to hypercapnia reduced with increasing axial length or refractive error. Previous chapters of this thesis found a correlation between axial length and the response to

hypercapnia in the pulsatile, retrobulbar and microretinal circulation. The myopic eyes assessed in chapter 4 suggested that eyes with increasing axial length respond to hypercapnia with significantly increased pulsatile ocular blood flow and lower microretinal blood velocity (section 4.5.4), whereas in anisomyopes the inter-eye difference in axial length and refractive error correlated positively with the inter-eye difference in microretinal blood velocity. These differences in vascular reactivity may be due to differences in regional reactivity and they indicate large variation in the responses of the different ocular vascular beds.

No changes were observed in perfusion pressure of myopes during hypercapnia; therefore, the increase in blood velocity suggested a local metabolic response in the choroid of myopes. Interestingly, the systemic response to hypercapnia in emmetropes did not show a correlation with the hypercapnia-induced ocular response, whereas in myopes, changes in choroidal blood volume induced by hypercapnia correlated significantly with hypercapnia-induced changes in blood pressure, ocular perfusion pressure and mean arterial pressure. These findings describe a large effect of the arterial and ocular perfusion pressure under stress conditions in myopes.
There was, however, no evaluation of the temporal component of the regulatory response, a limitation to the interpretation of the autoregulatory response in myopia and the size of the sample assessed was smaller than the one given by the power calculation.

7.7 CONCLUSION

The profile of the subfoveal choroidal circulation in human myopia was described in this chapter. Myopes were found to have a significantly lower resting choroidal blood velocity measured using LDF, which suggested an actual decrease in choroidal blood velocity in myopia ranging from -1.00 to -9.00D. However, no correlation was found between refractive error or axial length and choroidal blood velocity, volume or flow, which reflects lack of linear correlation at baseline between the degree of myopia and the flow of blood in the choroid.

Evaluation of the autoregulatory features of the human myopic eye exhibited significant difference in the response to hypercapnia compared to emmetropes. Emmetropes did not exhibit any vascular reactivity to hypercapnia; whereas in myopes there was a significant increase in LDF velocity during hypercapnia which differed significantly from the response given by emmetropes.

These results suggest that the myopic eye has a reduced resting choroidal flow velocity which is highly responsive to CO_2.

Future work could include the assessment of a larger sample size to confirm the findings reported in this chapter by the assessment of the temporal behaviour of the choroidal regulatory response to hypercapnia and additionally by evaluating the relationship between the physiological (choroidal blood flow) and functional (visual fields) response to hypercapnia.

CHAPTER EIGHT
CHOROIDAL THICKNESS IN THE MYOPIC MACULA: REPRODUCIBILITY AND RELATIONSHIP TO OCULAR ANATOMY AND CHOROIDAL BLOOD FLOW

8.1 Abstract

Purpose: The aim of this study was to assess the relationship between axial length, mean spherical equivalent (MSE), choroidal blood flow and choroidal thickness in healthy human eyes, for which it was also necessary to evaluate the within-visit repeatability and between-visit reproducibility of choroidal thickness measurements obtained at the macula using SD OCT 'Copernicus' (SOCT).

Methods: To assess the repeatability and reproducibility of the SOCT, measurements of choroidal thickness were obtained at each of 2 visits at the macular region of 12 healthy volunteers (mean age 26.66±6.44 years; range 18-39 years). The scan used was a radial macular scan that comprised 15 radial line scans of 7mm in length arranged in a star-like pattern with the centre on the fovea. Each radial scan was spaced 12 degrees from each other. The within-visit repeatability of the choroidal measurements was assessed by calculating the coefficient of variability (CoV) and coefficient of repeatability (CoR); whereas the coefficient of reproducibility (CoRepro) and intraclass correlation coefficient (ICC) determined the reproducibility between visits. A subgroup of 10 volunteers (mean age 35.72 ± 13.19 years; range 23 to 57 years) with mean MSE -1.10 ± 1.79 D (range 0.38 to -4.56D) were evaluated to assess the potential relationships between choroidal thickness, choroidal blood flow parameters (measured using Laser Doppler Flowmetry, LDF), mean spherical equivalent (MSE) and axial length (AL). Paired Student's *t*-test was used to assess the differences between the choroidal thickness readings from the two visits.

Results: The within-visit analysis resulted in a CoV of 0.80% and a CoR of 2.97%. The between-visit CoRepro was 2.44%, and ICC was 99%. There was no significant difference between the choroidal thickness measurements at the two visits (p=0.22).

Choroidal thickness showed a significant negative correlation with axial length (R=-0.60 p=0.05).

Conclusions: The SOCT provides repeatable and reproducible *in-vivo* measurements of choroidal thickness in normal subjects. The thickness of the choroidal layer at the macular correlated negatively with axial length such that macular choroidal thickness decreased with increasing axial length.

8.2 Introduction

Optical coherence tomography (OCT) allows *in-vivo* imaging of the retinal structures. OCT is based upon the measurement of echo time delay and intensity of backscattered or backreflected light, performs non-contact, non-invasive, high resolution, cross-sectional imaging of the internal ocular microstructures in biological tissues. The inherently high resolution of OCT imaging permits visualization of the morphology of individual retinal layers, thus facilitating the diagnoses of a wide range of retinal pathologies. The microstructure of the various retinal layers can be differentiated in the OCT image and correlates with the well-known morphology of the retina in the foveal and parafoveal region (Huang, *et al.* 1991).

OCT uses low-coherence interferometry to perform ultrahigh-resolution time and distance measurements for imaging. OCT can be performed without physical contact to the eye, thereby minimising patient discomfort during examination. Imaging using light rather than sound, as in the case of ultrasound, provides a significantly higher spatial resolution of the order of 8-10 microns, which is 10 to 20 times finer than standard ultrasound imaging.

OCT terminology refers to an A-scan as a one-dimensional array of information of the retinal tissues, which allows the visualization of the different layers within the retina. OCT machines use consecutive A-scans to build a two-dimensional scan, called B-scan (figure 8.1). Successive B-scans arranged together result in a 3D-scan. The resolving power of an optical system is referred to as resolution, which for OCT systems is expressed in the longitudinal and transverse directions. Longitudinal resolution, also called axial resolution, is a measure of the smallest feature that can be seen in an image. Longitudinal resolution is determined by the distance between two adjacent focal images and it is based on the physical properties of the light source, such as wavelength.

The capability to produce cross-sectional images allows OCT the calculation of optic disc diameters, cup sizes and retinal thickness using expert algorithms. These algorithms determine automatically the points at which the specific area to measure finishes using edge detection strategies. However, for a clinical instrument to be considered reliable for the calculation of diameters, areas and thickness it must be assessed for repeatability and reproducibility.

Several papers have reported good repeatability of OCT in the measurement of retinal thickness. Muscat and colleagues found the Humphrey OCT to give repeatable readings of macular retinal thickness. Data from twenty healthy volunteers were used to calculate the coefficient of reproducibility between sessions, which was found to be 1.51% (CoRepro). Twenty healthy subjects were used to assess the within-session coefficient of repeatability (CoR), which was found

to range from 1% to 2% (Muscat, *et al.* 2002). Massin and co-workers had previously assessed the reproducibility of retinal thickness calculation in an older version of the same OCT instrument by performing six radial scans on 10 healthy volunteers and 10 eyes of 10 diabetic patients with clinically significant macular oedema. Measurement of reproducibility was tested taking 3 series of scans performed by 2 different observers on 2 different days. The repeatability intraclass correlation coefficient (ICC) was greater than 0.89 in healthy subjects and greater than 0.98 in diabetic subjects, with reproducibilities (CoRepro) ranging from 5% to 6% (Massin, *et al.* 2001). OCT therefore appeared as a reliable tool to measure retinal thickness in both healthy subjects and diabetic patients with macular oedema.

OCT has also been used to describe the structural features of various ocular conditions. Idiopathic juxtafoveolar retinal telangiectasis has recently been shown to be characterised by intraretinal cystoid spaces without foveal thickening and disruption of the inner segment/outer segment photoreceptor junction line by OCT imaging, and foveal thinning was reported in the later stages of the pathology (Gaudric et al., 2006). Furthermore, the intraretinal changes associated with macula oedema were studied retrospectively in 78 patients using the Humphrey 2000 OCT. The aetiologies found to be related to the oedema were diabetic retinopathy, central retinal vein occlusion, pseudophakia, posterior uveitis and retinitis pigmentosa. Additionally, visual acuity correlated with macular thickness in diabetic retinopathy (Catier, *et al.* 2005).

Until recently, the only OCT technique available was time-domain (TD) OCT, which provided information about the depth of the retina from sequence sampling over time. To improve the resolution of the image given by the OCT different approaches have been attempted; the quality of the image was optimized (Sander, *et al.* 2005), and different light sources were used (Drexler 2004). However, it has not been until the recent introduction of spectral-domain (SD) OCT that the imaging speed, sensitivity and resolution have been significantly improved (Wojtkowski, *et al.* 2004). The axial resolution of time domain OCT systems that are commercially available is approximately 10μm; whereas OCT systems used for research purposes can achieve ultra high resolution of up to 1μm. With recent improvements using spectral interferometry (SD OCT) in clinical settings, axial resolutions the order of 6μm.

The difference between TD and SD OCT lies principally in the acquisition procedure. TD OCT collects the signal as a function of time, by moving the reference mirror used in interferometry; whereas SD OCT scans on the basis of a theoretical function dependent on a variable "k". SD OCT works by using the spectrometer as detector (instead of the mirror), or varying the wavelength of the light source (van Velthoven, *et al.* 2007). SD OCT imaging speed is, thus, higher due to the lack of moving parts that characterises TD OCT (Chen, *et al.* 2005). This increase in capturing

speed additionally allows the generation of 3D images, which enhances the clinical use of SD OCT by potentially producing higher reproducibility and repeatability coefficients in the calculation of retinal thickness, and early detection of ocular pathologies (Wojtkowski, *et al.* 2004).

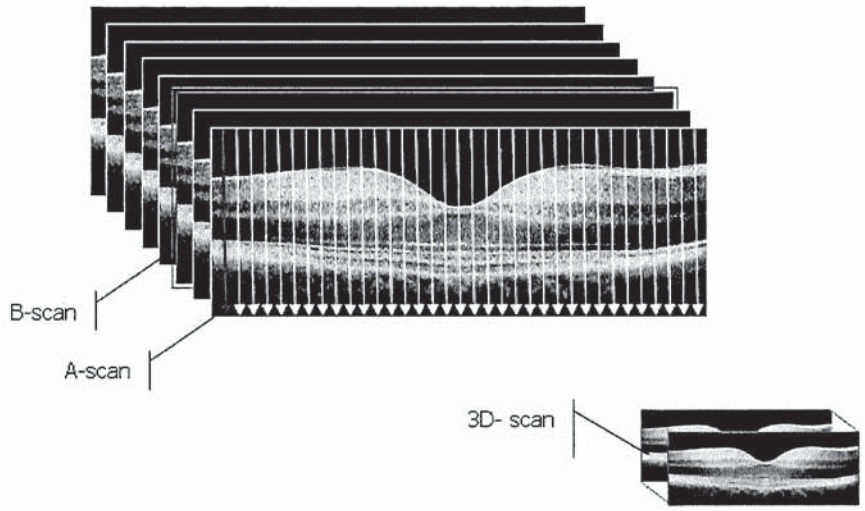


Figure 8.1 *Two and three dimensional reconstruction of the retinal tissue structure. A-scan (1D), B-scan (2D) and 3D-scan (3D) are shown to form the retinal morphology. Reproduced from Bansal D, Benavente-Perez A (2006) Optical Coherence Tomography: Terminology and interpretations. Optometry Today 20th Oct, with permission from Optopol Technology Sp. z o.o.(Optopol SA)*

The elongation of the posterior segment of the eye (Logan, *et al.* 2004) is one of the main structural features of the myopic eye, and highly myopic eyes are at higher risk of suffering from fundus complications such as chorioretinal atrophy and choroidal neovascularization in the macula. These features suggest the potential participation of the choroid in the development of fundus complications, which could be related to the reported thinning of the choroidal layer in induced-myopia studies in animals (Wildsoet and Wallman 1995). Additionally, the choroid has been found to thicken after the recovery phase of form deprivation myopia in chick eyes, which supports the existence of an active choroidal mechanism of visual growth control (Fitzgerald, Wildsoe and Anton 2001).

In view of these findings, and considering that choroidal blood flow represents 85% of the total blood supply of the eye, assessment of the choroidal circulation and choroidal thickness in myopia is of intrinsic interest.

8.3 Aims and objectives

The aim of this study was to assess the relationship between axial length, mean spherical equivalent (MSE), choroidal blood flow and choroidal thickness in healthy human eyes, for which it was also necessary to evaluate the within-visit repeatability and between-visit reproducibility of choroidal thickness measurements obtained at the macula using SD OCT 'Copernicus' (SOCT).

8.4 Repeatability and reproducibility of choroidal thickness using SD OCT Copernicus (SOCT)

8.4.1 Study Sample and Recruitment Criteria

Twelve healthy volunteers were recruited from the students and staff at Aston University. Details of the subject sample are given in table 8.1.

Since a physiological correlation is known to exist between the two eyes of the same subject, only one eye randomly chosen from each subject was evaluated (Kimura, *et al.* 2003).

Sample size	Gender	Eye tested	Age (years)
12	8 females 5 males	5 left eyes 7 right eyes	26.66±6.44 (range 18-40)

Table 8.1 *Characteristics of the volunteers involved in the SOCT repeatability study*

All the participants were healthy volunteers, which was confirmed by ophthalmologic investigation of the fundus, blood pressure measurements and detailed recording of systemic and ocular history and symptoms. Exclusion factors included any ocular disorder, diabetes, hypotension, hypertension or any other systemic disorder or medication likely to affect the systemic or ocular vasculature. A corrected visual acuity of 6/9 or better was required together with astigmatism of less than 1.5 diopters cylinder, which reduced the percentage of astigmatic patients being taken as myopic subjects after MSE calculation.

8.4.2. Ethical Approval and Informed Consent

Written and verbal information about the exact procedures to be performed during the visit was given to all the subjects prior to data collection. The participants were encouraged to ask any questions and to clarify any doubts they might have before signing the written consent. The volunteers were requested to sign two copies of the written consent, one of which was given to

them for their own records. All investigations were approved by the Ethical Review Committee and conformed to the declaration of Helsinki. (appendix 2.2).

8.4.3. Experimental Protocol and Investigations

This first part of the study evaluated the repeatability of the choroidal measurements using SOCT, prior to the assessment of the relationship between choroidal thickness, choroidal blood flow, MSE and axial length.

The measurement of choroidal thickness was performed using the SOCT, which was designed and built following studies at the Physics Institute of University of Nicolaus Copernicus in Torun, Poland (Wojtkowski, *et al.* 2005; Wojtkowski, *et al.* 2004). The SOCT is a non-invasive method that uses a light source wavelength of 830 nm. The reference arm terminates by the mirror held in a fixed position. The interference signal from the interferometer was detected by a spectrometer (reflecting holographic grating with 1800 lines per mm), which provides a transversal (axial) resolution of 6 μm and a tangential resolution of 10 μm with an examination speed of 26,000 A-scans per second. The maximum number of A-scans per B scan is 7,000, and all head movements motorized and controlled from computer screen. It also allows direct fundus preview during scanning (Wojtkowski, *et al.* 2005; Wojtkowski, *et al.* 2004). Due to the high resolution of the SOCT, and despite not having been designed for that purpose, the choroidal layer can also be visualised.

The collection of data was performed on two consecutive days to evaluate the within-visit and between-visit reproducibility. Since diurnal variations in choroidal thickness have been reported in animal studies (Papastergiou, *et al.* 1998), the two visits were performed at approximately the same time on consecutive days to minimize potential diurnal changes in human choroidal thickness. In total 12 macular radial scans were taken per patient per day, from which ten were chosen based on best image quality.

In order to calculate the choroidal thickness, and based on previous OCT studies assessing the repeatability of the retinal thickness measurement (Muscat, *et al.* 2002; Massin, *et al.* 2001), the scan of choice was the radial scan. The radial macular scan comprised 15 radial line scans of 7mm in length arranged in a star-like pattern with the centre on the fovea. Each radial scan was spaced 12 degrees from each other (figure 8.2) and contained 2500 A-scans (compared to 128 A-scans per 1.92 seconds of the OCT3).

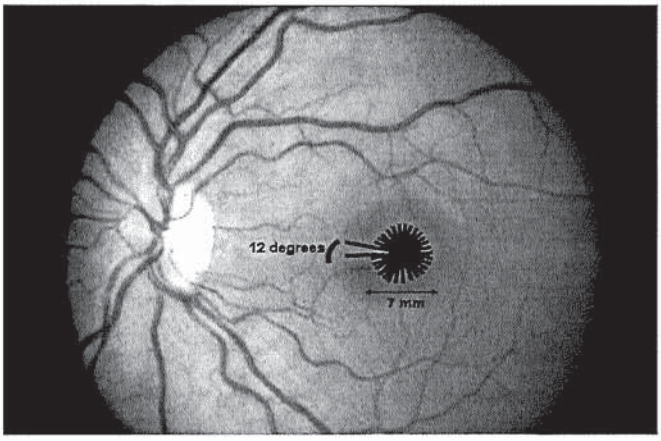


Figure 8.2 *Example of the SOCT macular scan protocol used in this study. The central macular was scanned with 15 radial lines, each of which was 7mm wide and differed from the previous by 12 degrees.*

For the present study, the choroidal thickness was only measured at the centre of the fovea. The calculation of the choroidal thickness was performed manually using the calipers provided by the instrument as shown in figure 8.3; the foveal area was first delimited using a horizontal marker (figure 8.3, line 1) that was later used as a reference to draw a perpendicular line that crossed the centre of the fovea (figure 8.3, line 2). Line 2 was then used to measure choroidal thickness using the caliper option provided by the instrument.

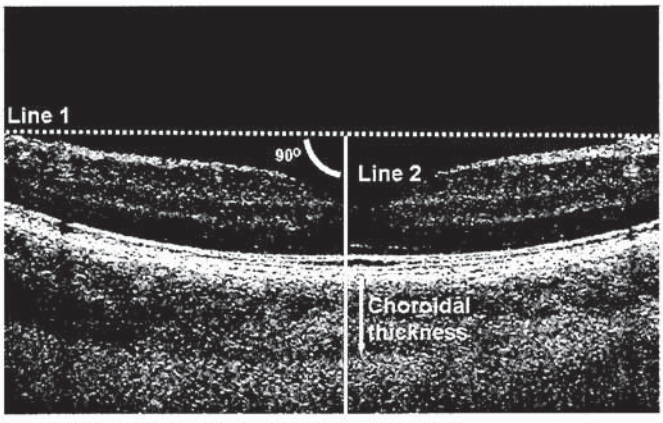


Figure 8.3 *Protocol followed for the calculation of choroidal thickness ensuring the accurate positioning of the callipers.*

In order to obtain the best quality scans, the patient was asked to rest chin and forehead on the machine chinrest and forehead bar directing their gaze towards a fixation light of adjustable position. No adjustment of the scan position was required. The scanning time of the SD OCT

Copernicus varies from 0.01 seconds if the scan of choice is a 2-D scan (512 A-scans), to 1.2 seconds if the scan protocol is a 3-D scan (50 B-scans with 800 A-scans).

8.4.4 Statistical analysis

8.4.4.1 Distribution of choroidal thickness measurements

The readings obtained using SOCT were tested for normality using the Shapiro-Wilk test in the statistical software package SPSS.

8.4.4.2 Number of images

The number of images required per visit per patient was calculated by plotting the standard deviation of 2, 3,4,5,6,7,8,9 and 10 images against the number of images. The number of images ideally required per patient was identified as the point at which the graph reached a plateau, as further images after this point did not provide additional information.

8.4.4.3 Repeatability within-visit

For each macular scan, the coefficient of repeatability (CoR) and coefficient of variation (CoV) were used to determine the repeatability of the choroidal thickness within-visit, based on the recommendations of the British Standard Institution (BSI).

The CoV was calculated as the standard deviation of the measurements divided by the mean. The CoV is usually expressed as a percentage.

The CoR was calculated as the standard deviation of the difference of each measurement from the mean of the repeated measurements, divided by the total average measurement. CoR is usually expressed as a percentage.

8.4.4.4 Reproducibility between-visit

For each macular scan, the coefficient of reproducibility (CoRepro) represents the capability of the instrument to provide repeatable choroidal thickness measurements using the same method on different conditions (or different visits, as is the case).

The CoRepro was calculated as the standard deviation of the differences between pairs of readings obtained in different sessions, divided by the average of the means of each pair of readings (Muscat, *et al.* 2002).

The intraclass correlation coefficient (ICC) is a statistic that estimates the proportion of variance as the ratio of the between-class minus the within-class variance divided by the sum of the two kinds of variance (formula 8.1)

$$ICC = (\text{within variance} - \text{between variance})/(\text{within variance} + \text{between variance}) \quad (8.1)$$

The ICC was calculated using the statistical software package SPSS. The total sums of squares and the sums of squares between subjects calculated during ANOVA were used to determine the ICC. Paired Student's *t*-test was used to assess the differences between the choroidal thickness readings from the two visits.

8.4.5 Results

8.4.5.1 Measurements of choroidal thickness

Ten radial scans comprising 12 radial line scans were taken for each participant. The choroidal thickness for each radial location and total choroidal thickness are given in table 8.2. as mean and sd.

Macular location (degrees)	Choroidal thickness (μm)
0	448.9758 ± 74.55281
12	453.7244 ±81.56276
24	450.1368 ±81.98565
36	447.1753 ±82.66755
48	446.4603 ±82.22659
60	446.4179 ±81.97005
72	445.9751 ±81.08199
84	443.5928 ±84.34752
96	447.7558 ±84.33706
108	448.7956 ±87.77564
120	445.3803 ±83.59598
132	452.2853 ±86.77099
144	452.2889 ±82.29183
156	450.2687 ±82.58536
168	447.9018 ±78.95654
Mean choroidal thickness	448.4756 ±81.98861

Table 8.2. *Summary of total mean and sd of the choroidal thickness at each macular location*

8.4.5.2 Distribution of choroidal thickness measurements

The Shapiro-Wilk test showed a p value equal to p=0.30, which indicated that the measurement of choroidal thickness were normally distributed.

8.4.5.3 Number of images

The mean standard deviation of 2, 3, 4, 5, 6, 7, 8, 9 and 10 scans was plotted against the number of images (figure 8.4). The number of images ideally required per patient was identified as the point at which the graph reached a plateau, as further images after this point did not provide additional information. Figure 8.4 shows a difference of approximately 0.75μm in macular choroidal thickness between the first four images. Since the thickness of the choroid measured in this study ranged from 440 to 470 μm and from image number 4 the standard deviations differed only by 0.50 μm, the number of images taken for this study was 6, to ensure the quality of the measurements.

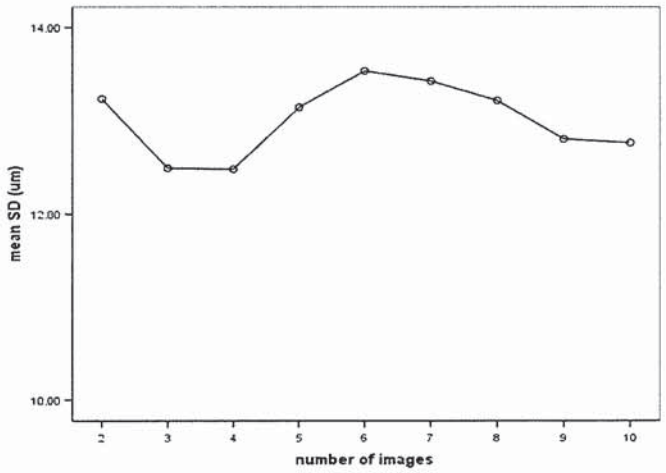


Figure 8.4 *Graph showing change in mean SD as a function of the number of scans*

8.4.5.4 Within-visit repeatability of choroidal thickness measurements

The coefficient of variability (CoV) and coefficient of repeatability (CoR) were calculated for the mean of the choroidal thickness measurements at the centre of the fovea. The coefficient of variability (CoV) was 0.80%, and the coefficient of repeatability (CoR) was 2.97%

8.4.5.4 Between-visit reproducibility of choroidal thickness measurements

The coefficient of reproducibility (CoRepro), calculated for the mean of the choroidal thickness measurements at the centre of the fovea, was 2.44%. Paired Student's *t*-test was used to assess differences between the choroidal thickness readings from the two visits, and the result showed no significant differences between them (p=0.22). The intraclass correlation coefficient (ICC) was 99%.

A Bland and Altman plot was used to represent the mean choroidal thickness differences for each participant between visits as a function of the mean choroidal thickness (figure 8.5).

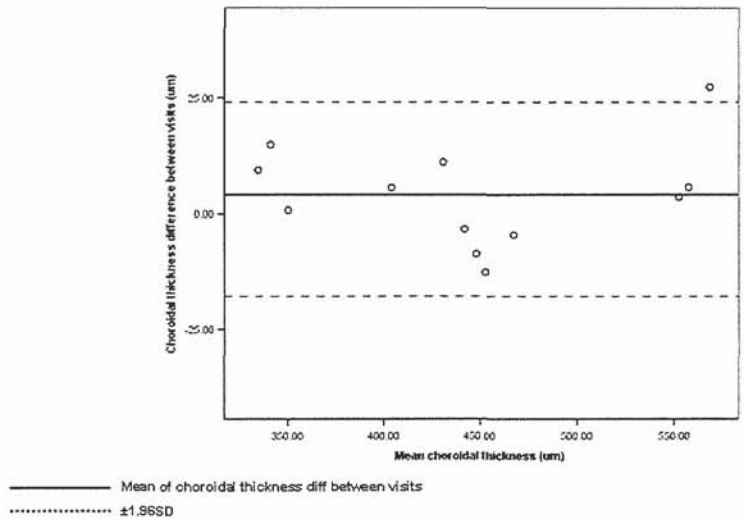


Figure 8.5 *Bland and Altman plot representing the intersession reproducibility of choroidal thickness measurement. Mean choroidal thickness for each subject is plotted against the difference in retinal thickness between the two visits. Ninety-five per cent of the values fell within 2SD of the mean*

8.5 Relationship between axial length, refractive error, choroidal blood flow and choroidal thickness

8.5.1 Study Sample

A sample of 11 volunteers was recruited to assess the relationship between axial length, refractive error, choroidal blood flow and choroidal thickness from the students and staff at Aston University. Details of the subject sample are given in table 8.3.

	mean ± sd
gender	5 females 6 males
age	35.72 ± 13.19
MSE (D)	-1.10 ± 1.79
AL (mm)	23.80 ± 0.97
Choroidal thickness (μm)	463.78 ± 80.56
Choroidal blood velocity (AU)	1.75 ± 0.69
Choroidal blood volume (AU)	0.71 ± 0.62
Choroidal blood flow (AU)	1.57 ± 1.62

Table 8.3 *Summary of the characteristics of the participants involved in the study*

8.5.2 Recruitment Criteria, Ethical Approval, Informed Consent and Dietary Restrictions

The recruitment criteria, ethical approval, informed consent and dietary restrictions were the same as those in section 8.4.

8.5.3. Experimental Protocol and Investigations

Data collection was initially designed to take place within the same day. However, due to logistical reasons, data could be only collected on two separate days, the second being after one month gap. Choroidal blood flow was recorded the first day, whereas the choroidal thickness measurements were taken the second day. Readings of axial length and refractive error were obtained on both dates, from which an average was calculated.

The protocol followed for each day was as follows:

First day:

- Autorefraction (*Shin-Nippon NVision-K 5001* Autorefractor, Japan) (section 1.9.1) and biometry data (*IOLMaster*, Carl Zeiss Jena, Germany Zeiss) (section 1.9.2).
- Choroidal blood flow parameters (choroidal blood velocity, ChBVel, choroidal blood volume, ChBVol, and choroidal blood flow , ChBF) were recorded using the Laser Doppler Flowmeter (LDF, Riva method, Institut de Recherche en Ophtalmologie, Switzerland). The instillation of one drop of tropicamide (Tropicamide 0.5% Minims®, Chauvin Pharmaceuticals) facilitated the correct visualisation of the macula, and accurate measurement of choroidal circulation.

Second day

- Autorefraction (*Shin-Nippon NVision-K 5001* Autorefractor, Japan) (section 1.9.1) and biometry data (*IOLMaster*, Carl Zeiss Jena, Germany Zeiss) (section 1.9.2).
- Measurement of choroidal thickness using SD OCT SD OCT 'Copernicus' (SOCT). Since the minimum number of images required per patient was six (section 8.5.6), 6 macular scans were taken on each patient, all of which consisted of 15 radial line scans. An average choroidal thickness from the total 90 scans for each participant was calculated.

8.5.4 Statistical analysis

Pearson product-moment correlation (R) was used to evaluate the relationship between axial length (AL), mean spherical equivalent (MSE), choroidal blood parameters (ChBVel, ChBVol and ChBF) and choroidal thickness (ChT).

8.5.5 Results

The relationship between axial length, MSE, choroidal blood flow parameters and choroidal thickness was evaluated using bivariate analysis. Choroidal thickness was found to correlate significantly with axial length (R=-0.60 p=0.05) (figure 8.7). A summary of the measurements is given in table 8.4.

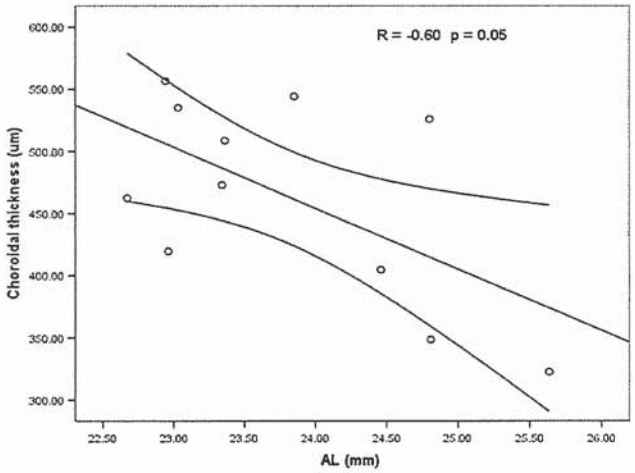


Figure 8.6 *Scatter plot showing a significant negative linear trend between axial length and choroidal thickness*

	Choroidal thickness R value	p
Axial length (mm)	-0.60	* 0.05
MSE (D)	0.31	ns
ChBVel (kHz)	-0.33	ns
ChBVol (au)	-0.38	ns
ChBF (au)	-0.35	ns

Table 8.4 *Correlations between choroidal thickness and axial length, mean spherical equivalent (MSE), ChBVel (choroidal blood velocity), ChBVol (choroidal blood volume) and ChBF (choroidal blood flow) (* significant value)*

8.6 Discussion

This study evaluated the repeatability and reproducibility of the new SD OCT 'Copernicus' for the measurement of choroidal thickness. The results provided described a highly repeatable and reproducible instrument whose repeated scans gave consistent measurements of choroidal

thickness. Additionally, the choroidal thickness at the macular correlated negatively with axial length.

The Bland and Altman plot provides an additional qualitative way of analysing the between-visit reproducibility of an instrument (Bland and Altman 1986) in which the width of the upper and lower limits is also an indicator for the reproducibility of the measurements. In the present study only one measurement fell outside the 95% confidence interval, which supported the reproducibility of the instrument. The coefficients of variability, repeatability and reproducibility obtained in the present study using the spectral domain OCT for the measurement of choroidal thickness were 0.80%, 2.97% and 2.44% respectively. The ICC was found to be 99%. These results indicated that the SOCT could be used for the calculation of choroidal thickness *in-vivo* and that it provided consistent choroidal thickness measures.

Hougaard and coworkers evaluated the factors affecting the measurement of RNFL thickness using Stratus OCT (Hougaard, *et al.* 2006) on 178 participants with ages ranging between 20 and 80 years of age. Age, refractive error (MSE), axial length (AL), and lens nuclear colour and opalescence were the factors showing a significant effect on RNFL thickness, whereas IOP, optic disc size or gender did not have significant effect. Multivariate evaluation of the age, MSE, AL and lens colour and opalescence indicated age and MSE as the factors with larger effect on the calculation of RNFL thickness. RNFL thickness decreased by 2.6-2.9 μm per decade, and increased by 1.5-1.8 μm per positive MSE diopter (Hougaard, *et al.* 2006). Similarly, a more recent study by Budenz and colleagues (Budenz, *et al.* 2007) also reported age, ethnicity, axial length, and optic disc area as having a significant effect on RNFL thickness, measured using Stratus OCT.

The apparent relationship between AL, MSE and RNFL thickness has been assessed recently in a group of myopes in a study by Hoh and coworkers (Hoh, *et al.* 2006). One hundred and thirty-two young males with myopia (MSE ranging from -0.50 to -14.25 diopters) were evaluated using concentric circular scans on the optic disc with scan diameters of 3.40 mm, 4.50 mm, and 1.75mm. Mean peripapillary RNFL thickness was not found to correlate with MSE or axial length, suggesting that the mean peripapillary RNFL thickness does not vary with increasing myopia. However, unpublished data from Workman (Workman 2006) suggested that macular retinal thickness and RNFL thickness correlate significantly with axial length. Sixty-six and sixty-four subjects were assessed in two studies in which the relationship between axial length, macular thickness and RNFL thickness were assessed respectively. The protocol followed was multiple radial line scans for the macular thickness and circular scans centered on the optic nerve for the RNFL thickness calculation. Interestingly, the correlation between macular and RNFL thickness only showed significance in the parafoveal region located 6mm away from the central fovea. These

results suggested an effect of axial length on the macular retinal thickness that became apparent at increased distances from the fovea. The RNFL thickness correlated significantly with axial length at the superior, nasal and inferior locations of the optic disc margin (Workman 2006).

Axial length, thus, appeared to have an effect on the retinal thickness, which, however, is not agreed unanimously. Furthermore, the choroid has been suggested to be part of the signal cascade of ocular growth between the retina and the sclera. If the thickness of the choroid decreases in animals induced with myopia, this may be accompanied by a retinal thinning, which is supported by the decrease in retinal thickness with increasing axial length described in the unpublished data by Workman or those from Luo and colleagues, in which decreased retinal macular thickness was found in Singaporean myopic children (Luo, *et al.* 2006).

The significant negative relationship between choroidal thickness and axial length in the human eye may play an important role in the aetiology of myopia, since the choroidal layer may thin to reallocate the defocused myopic image. This is cautiously hypothesised since the choroidal thinning and decrease in choroidal blood flow described in animal studies have been shown to be transient changes followed by scleral stretch and scleral thinning (Wildsoet and Wallman 2005). Thereby, results like those described by Hougaard, Hoh and Workman (Hoh, *et al.* 2006; Hougaard, *et al.* 2006; Workman 2006) added to the findings reported in chapter 7 of this thesis (section 7.5) describing a decreased choroidal blood velocity in myopes, together with the previously reported thinning of the choroidal layer and decrease in choroidal blood flow in animal deprivation myopia (Fitzgerald, Wildsoe and Anton 2001; Wildsoet and Wallman 1995) prompted the evaluation of the relationship between the *in-vivo* measurement of choroidal thickness, axial length, MSE. and choroidal blood parameters in the human eye.

For this study, six macular radial scans in turn comprising 15 radial line scans were taken per patient on 11 participants (90 images analysed per participant, 990 images in total). The results showed a significant negative correlation trend between macular choroidal thickness and axial length. No significant correlation was found between macular choroidal thickness and MSE or choroidal blood flow. Our results are in accordance with those from animal studies, in which a significant relationship between choroidal thickness and axial length was reported after form deprivation myopia (Fitzgerald, Wildsoe and Anton 2001; Wildsoet and Wallman 1995). The thinning and thickening mechanisms observed in animals induced with myopia have also been linked to changes in ultrastructural changes in choroidal lymphatics, as shown by the contribution of the lymphatic choroidal sinusoids to the expansion of the choroid in the recovery from induced myopia (Junghans, *et al.* 1999). Also important in understanding the results of the present study are the morphological changes undergone by the choriocapillaris of 40 chick eyes induced with

form deprivation myopia (Hirata and Negi 1998). Scanning electron microscopy (SEM) and Transmission electron microscopy (TEM) described less dense choriocapillaris with wider and more irregular intercapillary meshes than those of the control eyes, whose dense networks exhibited round and oval intercapillary meshes. Thus, myopic eyes appear to exhibit significantly lower capillary density and capillary diameter and significantly greater distance between adjacent intercapillary meshes than the control chick eyes (Hirata and Negi 1998).

The apparent thinning of the choroid in human myopic eyes may be a response of the mechanism controlling the vascular supply to a decrease in metabolic needs, which may result in decreased choroidal blood flow, leading to a reduction in retinal thickness as a consequence. If the retinal thickness reduces, as shown by previous papers, the retinal vascular supply may regulate by decreasing the blood supply to the retinal layer, which is mainly supplied by the CRA. In this thesis, the CRA blood velocity was found to decrease and the resistance index was found to increase with increasing myopia (see section 3.1.5), which supports the results found in the present study.

It is, however, contradictory, to observe lack of significant correlation between the thickness and the blood flow of the choroid, when the choroidal blood velocity measured using LDF showed a reduction in myopic subjects (chapter 7). The explanation to this finding may lie on the reported diurnal rhythm changes in axial elongation and choroidal thickness in chick (Papastergiou, Schmid, Riva et al, 1998) and marmoset eyes (Nickla et al., 2002). Those rhythms in axial and choroidal dimensions have been found to be circadian (driven by an endogenous clock), and, therefore, the changes in choroidal blood flow may possibly follow a different pattern from those in choroidal thickness. Thus, the recording the choroidal thickness and choroidal blood flow on different days may have been contributed to the lack of significant correlation between the parameters, which additionally be influenced by the small sample size.

8.7 Conclusion

The SOCT provided repeatable and reproducible *in-vivo* measurements of choroidal thickness in normal subjects. The thickness of the choroidal layer at the macular appeared to correlate negatively with axial length such that macular choroidal thickness decreased with increasing axial length. This finding was of especial interest with respect to the reduced blood velocity in the retrobulbar, choroidal and pulsatile ocular blood flow in human myopia described in this thesis, as it may be part of the causative or resulting cascade of events related to the changes in choroidal thickness reported in degenerative human myopia and in the eye growth of induced myopia in animals.

CHAPTER NINE
GENERAL DISCUSSION

Current thinking regarding an altered ocular circulation as a cause or consequence of myopia and as a risk factor for co-morbidity with other ocular diseases such as glaucoma remains equivocal. This thesis has investigated the ocular perfusion features of healthy human eyes in the retrobulbar, microretinal and choroidal beds to provide a vascular perspective of myopia and to further the current understanding of the relationship between non-pathological myopia and the development of posterior fundus complications. Due to a lack of available data regarding the haemodynamics of the human myopic eye and the existence of factors known to affect blood circulation, this thesis was structured into several studies to assess independently the resting state vascular profile of human myopia, the regulation responses to stress testing and the effect of age and smoking on ocular blood flow. Additionally, the relationship between choroidal thickness and choroidal blood flow, axial length and refractive error explored an aspect of special relevance as choroidal thinning in eyes with greater axial lengths may increase their risk to degenerative complications in the fundus.

The present study assessed the ocular perfusion features of the myopic eye using several instruments to measure various vascular factors in different ocular vascular beds that are part of a continuous vessel network and therefore influence each other profoundly. This study provided an overall picture of how the resting state flow varies as it passes through the eye, assessing those factors known to affect flow and whether aspects such as biometry or autonomic regulation are functional in all ocular vascular beds of all eyes.

9.1 The human myopic eye: vascular profile and the effect of age on ocular perfusion characteristics

There is evidence of reduced choroidal blood flow in studies assessing animals induced with myopia (Wildsoet and Wallman 1995; Fitzgerald, et al. 2001) and in human studies evaluating eyes with degenerative myopia (Dimitrova et al., 2002; Akyol et al, 1996). Moreover, myopic patients appear to have a two- to three-fold increased risk of developing glaucoma (Mitchell et al., 1999) and they are at a higher risk of ocular complications in the posterior pole (Mitchell et al. 1999; Swann, 2002). Although data from animal studies and investigations on human pathological myopia suggested the perfusion features of the myopic eye differs from an

emmetropic eye, a detailed analysis of the ocular circulation in moderate degrees of human myopia was required.
The studies presented in chapter 3 of this thesis investigated the ocular haemodynamic characteristics of the healthy human myopic eye by assessing the retrobulbar, pulsatile and microretinal blood circulation in emmetropes, low myopes and high myopes. A significant decrease in the pulsatile ocular blood flow (POBF) and central retinal artery (CRA) blood velocity was found in high myopes compared to emmetropes. The decrease in CRA blood velocity was accompanied by a significant increase in its blood resistance index.

A decrease in pulsatile flow and retrobulbar blood velocity could have been thought to be caused by differences in blood pressure (BP) or age between the three refractive groups. However, in this study the three refractive groups were matched for age, which resulted in a lack of significant difference between the systemic blood pressure of emmetropes, low myopes and high myopes. Therefore, the decrease in POBF and CRA velocity in high myopes did not appear to be related to differences in blood pressure.

Reduced POBF in myopes has been described previously by several investigators (Lam, et al. 2002; Logan, et al. 2002; Shih, et al. 1991; To`mey, et al. 1981) and it has been suggested that the reduction is due to the rigidity and volume assumptions of the derived calculation of pulsatile blood flow. However, this thesis also found a significantly lower CRA blood velocity in high myopes that was accompanied by an increase in resistance index, which based on the Poiseuille's law of flow analysis, suggests a reduction in vessel radii in myopia, as previously reported in a study by Shimada and colleagues (Shimada et al., 2004) and ultimately in CRA blood flow.

Knowing the limitations of POBF, it is however misleading to draw conclusions such as those by Lam and colleagues, who, due to the lack of agreement between the results obtained using POBF and CDI, reported that the reduction observed in POBF in anisomyopes was due to the scleral rigidity and eye volume assumptions (Lam et al., 2003). The location of the retrobulbar vessels and the technique used for their assessment differ pronouncedly from those of the OBFA, which analyses ocular pulsatile blood flow. Thus, interpretation of the outcomes obtained with different vascular instruments should be made from both a technical, anatomical and clinical perspective.
There was no significant difference between the resting microretinal circulation of myopes and emmetropes.

The significant negative correlation found between axial length with POBF and CRA blood velocity is an additional factor to support the hypothesis of reduced ocular blood flow at both the choroidal level (pulsatile blood flow) and retrobulbar level (CRA) in human myopia.

9.2 The effect of age on the perfusion features of the human eye

To further the understanding of human myopia the nature of how the haemodynamics of ocular blood flow alters in the ageing eye must also be taken into consideration. Ageing is associated with several well-recognised changes in the cardiovascular system such as an increase in systolic and diastolic blood pressures, reduction in cerebral blood flow and velocity and increase in vessel rigidity among others (Fleg 1986; Kannel and Gordan 1978).

The studies of chapter 3 showed higher systolic (SBP) and diastolic blood pressures (DBP) and a higher intraocular pressure (IOP) accompanied by higher ophthalmic artery (OA) end diastolic and peak systolic velocities (OA EDv, OA PSv) and lower POBF in the older group. Therefore, increased age appeared to show higher IOP, lower POBF and retrobulbar flow. The decrease in OA blood velocities may have been due to a decreasing vessel area with age, which would explain the increased susceptibility of the senescent eye to ocular vascular pathologies.

A decrease in POBF with age has previously been reported by Ravalico and co-workers (Ravalico et al., 1997) and by Geyer and colleagues (Silver and Geyer 2000). Age has been shown previously to correlate significantly with scleral rigidity, which may suggest that the reduction in POBF found with age may be due to an increase in scleral rigidity. However, the present study did not find a significant difference between the corneal thickness of the young (below the age of 30 years) and the older group (above the age of 40), which suggested that the scleral rigidity did not change with age and that the pulsatile ocular blood flow was reduced with advancing age. This finding was supported by a study by Lam and colleagues, in which multiple regression analysis indicated that the most influential factor affecting POBF was ageing (Lam, et al. 2003). The pulsatile component of ocular blood flow, known to represent approximately 90% of the choroidal circulation, thus appeared significantly decreased in high myopes and additionally was reduced with advancing age, which may suggest an interaction effect of myopia and age on the pulsatile blood flow of the human eye, by which the drop in POBF was greater in older myopes than in older emmetropes.

The higher OA EDv found in those participants above the age of 40 years does not fit the systemic profile of the ageing circulatory system. However, since the blood velocity in the OA correlated significantly with the systemic blood pressure and BP was higher in the older group, this may explain the increase in OA blood velocity. Therefore, an increase in the OA blood velocity may just represent a decrease in vessel area, which would in turn be associated with the decrease in vessels radii known to occur with age. Additionally it may represent the known systemic adaptation to the biological changes that occur with age such that when the walls of the vessels start thickening, the pressure in the vessels rises to ensure the minimum supply of blood (Lakatta, Gerstenblith and

Weisfeldt 1997). The current results were supportive of the concept that a similar protection mechanism occurs in the ocular circulation of elderly people.

In summary, results from the present study showed that the perfusion characteristics of the human myopic eye differed significantly from those of the emmetropic eye. High myopia was characterised by significantly lower CRA blood velocities and POBF and higher CRA resistance index (RI) with no apparent differences in microretinal circulation. Additionally, the pulsatile and retrobulbar flow were significantly reduced with age suggesting a reduction in the choroid and retrobulbar vessels perfusion. These results may also infer a decreasing vessel area with age, which would explain the increased susceptibility of the senescent eye to ocular vascular pathologies. However, there was no interaction effect between the decrease in flow with age that associated with increasing myopia, possibly due to the increase with age of OA velocity, which opposed the reduction found in myopia.

9.3 Vascular autoregulation in human myopia

To ensure the correct supply of nutrients to the human eye in the face of changes in perfusion pressure, the eye has been shown to be able to alter specific vascular parameters such that the resulting ocular blood flow remains constant (Riva, Logean and Falsini 2005; Longo, Geiser and Riva 2004; Hafez, *et al.* 2003; Riva, *et al.* 1997; Aaslid, *et al.* 1989; Riva, Grunwald and Petrig 1986). A detailed analysis of the ocular haemodynamics of the human myopic eye should include the assessment of its vascular regulatory properties; therefore, the studies presented in chapter 4 of this thesis assessed the ocular vascular regulatory response of the retrobulbar, pulsatile and microretinal circulation in myopes, which remained unexplored to date, by comparing it to the vascular response in emmetropes.

A minimally invasive method of stress testing involving gas perturbations to induce hypercapnia was adopted in order to reflect as much as possible the normal physiological vascular autoregulatory responses.

The systemic response to hypercapnia in emmetropes contrasted with the systemic response given by myopes, as shown by the lack of significant variations in systolic or diastolic blood pressure response to hypercapnia in emmetropes compared to the significant increase in BP and mean arterial pressure in myopes. This may represent a potential systemic dysregulation in the autonomic nervous system in the aetiology of myopia. The studies presented on form deprivation myopia by Raviola and Wiesel (Raviola and Wiesel 1985; Wiesel and Raviola 1977) first suggested

the involvement of the nervous system in the development of ocular growth in animal models of myopia, since alteration of the visual perception, and not the local effect of the closed lids, resulted in induced myopia in the eyes of macaques. However, the major relationship between the autonomic nervous system and myopia to date has come from the assessment of ocular accommodation. Near work induced transient myopia and the issue of accommodation adaptation, known to be of sympathetic origin, has been proposed as one of the factors involved in the development of myopia and the two theories proposed are a potential deficit in sympathetic innervation, or a deficit in both sympathetic and parasympathetic system (Chen, Schmid and Brown 2003). However, a recent paper by Mallen and colleagues found no significant differences in the relative access percentages to sympathetic inhibition between emmetropes and myopes (Mallen, Gilmartin and Wolffsohn 2005), which suggested that emmetropes and myopes cannot be differentiated based only on percentage access to sympathetic inhibition.

At the ocular level, hypercapnia did not result in any changes in pulsatile ocular blood flow or retinal capillaries blood flow in emmetropes; however, the OA blood velocity increased significantly. Despite previous papers describing the vasodilation effect of hypercapnia on the retrobulbar circulation (Chung, et al. 1999a; Hosking, et al. 2004; Luksch, et al. 2002; Roff, et al. 1999; Roff Hilton, et al. 2003; Schemetterer, et al. 1996; Schmetterer, et al. 1997), the increase in OA PSv observed in emmetropes should strictly be interpreted as OA vasodilation during hypercapnia only if the increase in blood velocity is accompanied by a decrease in resistance index. Since no RI changes were observed, the increase in OA PSv in emmetropes might have been the result of the systemic effect of blood pressure on the OA, found to correlate significantly in one of the studies in chapter 3. Myopes did not exhibit any retrobulbar changes during hypercapnia despite showing an apparent lower basal CRA blood flow, which suggested that myopes and emmetropes did not differ in retrobulbar vascular reactivity.

Despite not finding a significant retrobulbar response to hypercapnia in myopes, their pulsatile and microretinal circulation increased significantly during hypercapnia, thus showing an active vascular reactivity of the retinal microvasculature and pulsatile flow in myopes that agreed with previous research on healthy volunteers (Nemeth et al. 2002; Movaffaghy et al. 1998; Riva et al. 1981; Robinson et al. 1986; Michelson et al. 1994). Additionally, since regional differences in the retinal microvasculature of healthy volunteers have been reported previously (Chung et al., 1999), the known anatomical differences between the myopic and the emmetropic retina and the differences in shape regulation may possibly be related to differences in ocular vascular regulation.

Additionally, despite the lack of significant differences in vascular reactivity to hypercapnia in the old and young groups, age correlated with the percentage change in OA PSv, SPCA PSv and SPCA EDv such that the percentage change in velocity increased with increasing age, which may be indicative of a progressive reduction in vessel diameter with age. The eyes of older people, thus, appeared to respond to the oxygenation changes induced by hypercapnia, as shown by the significant increase in blood velocity, but they did not seem to be capable of maximum vasodilation as suggested by the lack of significant changes during hypercapnia in the older group, all of which might be possibly due to the age-related arteriosclerosis known to affect the rigidity of the vessels.

Axial length correlated with the response to hypercapnia given by the pulsatile and microretinal circulation indicating an increased response to hypercapnia in longer eyes. Additionally, the interaction found between the effect of axial length and age on the response to hypercapnia given by the retrobulbar vessels suggested that the increase in blood velocity response with age may appeared exacerbated by axial length.

In summary, myopic and emmetropic eyes appear to differ in vascular reactivity, with myopic eyes exhibiting a larger response to hypercapnia than emmetropes. The significant correlation between the increased retrobulbar blood velocities as a response to hypercapnia with age may be indicative of a protective mechanism occurring parallel to the decrease in vessel diameter occurring with age. Axial length correlated with the response to hypercapnia given by the pulsatile and microretinal circulation indicating an increased response to hypercapnia in longer eyes.

9.4 The effect of smoking on ocular blood flow

Smoking is a risk factor for systemic and peripheral vascular conditions, and it has been associated with a two to four-fold increased risk of coronary heart disease and a higher risk of stroke and stroke related mortality (Lakier 1992). Retinal ischemia, anterior ischemic optic neuropathy and Graves ophthalmopathy have also been linked to smoking (Solberg, Rosner and Belkin 1998). However, at present, most of the literature available has assessed the acute effects of smoking on ocular haemodynamics, therefore leaving a gap regarding its chronic effect on the ocular blood flow with the evaluation of the systemic circulation as a potential variable, and including a larger washout period.

Chapter 5 of this thesis evaluated the chronic effect of smoking on the resting vascular perfusion and regulatory features of the human eye. No differences in resting BP, mean arterial pressure

(MAP), ocular perfusion pressure (OPP) or ocular vascular perfusion were found between smokers and non-smokers; nor the studies presented in the ocular response to hypercapnia in smokers differ from those in non-smokers. Thus, the resting state and autoregulatory ocular haemodynamics of young smokers with 3 to 10 years smoking history did not appear to differ significantly from that of non-smokers, possibly due to the young age of the smokers (age range: 19 to 31 years).

Hypercapnia induced a significant change in the systemic blood pressure only in smokers (significant increase in SBP, DBP, MAP and OPP). Since OPP changed significantly in the smoking group, the confounding effect of the change in OPP on the response to hypercapnia was controlled by including it as covariate in an ANCOVA analysis. No significant changes were observed in the ocular circulation of smokers or non-smokers. However, the Fagerström Tolerance Questionnaire (FTQ) score, which is known to provide an objective measurement of the degree of physical dependence to smoking (Fagerström, 1978) and whose scores correlate significantly with nicotine blood levels and chronic nicotine intake (Pomerleau et al. 1990), was found to correlate significantly with the hypercapnia percentage change increase in OA and CRA blood velocities and retinal microcirculation, suggesting that the vascular reactivity of the retrobulbar and microretinal circulation increased with higher levels of nicotine intake possibly due to the decreased O_2 binding affinity at resting conditions. Additionally, there was a significant interaction between the effect of axial length and the FTQ score on the response to hypercapnia given by the retinal microvessels by which the increase in microretinal velocity given as a response to hypercapnia by smokers with larger eyes appeared higher than the increase in microretinal velocity in the eyes of smokers with smaller axial lengths. These results agreed with the correlation found between axial length and the response to hypercapnia given by the pulsatile and microretinal circulation that suggested an increased response to hypercapnia in longer eyes.

In summary, the basal ocular haemodynamics between young smokers and non-smokers did not appear to differ significantly. However, the vascular reactivity to hypercapnia was significantly related to the exposure of nicotine and it appeared largely affected by the systemic response. Thus, young healthy smokers may be characterised by a systemic hypoxia, which would explain the large systemic and ocular response given to hypercapnia to compensate for the lower O_2 under normal smoking conditions.

It was interesting to find that the systemic response to hypercapnia for the smokers was similar to that of myopes, which suggested that both groups needed to make systemic changes to cope with relatively small changes in pCO_2.

9.5 Ocular blood flow and vascular regulation in anisomyopia

Anisomyopia provided a good model to study the features of myopia, as it is free from the genetic and environmental background influences that inevitably affect the onset and progression of myopia in different subjects, and the less myopic eye acted as a control. The possibility of evaluating the inter-eye differences increased the statistical power of the sample, it not being necessary to assess a sample as big as in a non-paired analysis. The inter-eye differences in refractive error and ocular biometrics have been reported to correlate with the inter-eye differences in ocular pulse volume and ocular pulse amplitude (Logan, Gilmartin and Cox 2002). However, as explained previously, OBFA readings are affected by the assumptions entailed by the pulsatile ocular blood flow calculations (Langham, *et al.* 1989). To date only the pulsatile blood flow and the blood velocity of the ophthalmic artery have been assessed in anisomyopic subjects. Thereby, the retrobulbar and microretinal circulation, and the regulatory mechanisms of the retrobulbar, pulsatile and microretinal blood flow remained unexplored.

The studies presented in chapter 6 investigated the true resting state ocular blood flow and haemodynamic autoregulation during stress testing in the different vessel beds of the human anisomyopic eye using the less myopic eye as a control, in order to eliminate the effects of factors such as tissue type or the contribution of the systemic circulation. The less and more myopic eyes differed in MSE, but not in axial length. Therefore, the extent of myopic difference may not have been enough to exhibit ocular blood flow asymmetry, suggesting that the sample group recruited for the basal study consisted of a mixture of refractive and axial anisomyopia. This was supported by the difference in refraction consequently observed between the two eyes of 2 participants aged 65 and 78 years, which was not accompanied by differences in axial length, possibly suggesting changes in lens index with age that resulted in altered MSE.

Despite the lack of significant differences between the ocular perfusion of the more and less myopic eyes in anisomyopes, the inter-eye differences in axial length and refractive error between anisomyopic eyes correlated significantly with the inter-eye differences in the SPCA blood velocity and microretinal blood flow such that larger differences in AL were related with larger differences in retrobulbar and microretinal circulation. These results supported the hypothesis proposed in previous chapters that suggested a significant lower retrobulbar blood flow in myopes, which also had significantly larger eyes, and added the possibility of finding a lower microretinal circulation.

The assessment of the response to hypercapnia in anisomyopes revealed no significant response to hypercapnia by the systemic pressure. However, the more myopic eyes showed an increased SPCAs blood velocity and decreased SPCAs resistance index under no significant changes in OPP,

which suggested a potential vasodilation in the SPCAs. The inter-eye differences in axial length and refractive error between anisomyopic eyes were found to correlate significantly with the inter-eye response to hypercapnia calculated as a percentage change in retrobulbar vessels and retinal microcapillaries. These correlations emphasised the relationship described previously between axial length and both the resting state vascular haemodynamics and the vascular reactivity to hypercapnia.

The greater sensitivity to hypercapnia described in both the myopic and smokers' eyes in the previous studies appeared stressed by a significant hypercapnic response given only by the more myopic eyes in anisomyopes, and suggested a different mechanism (posterior segment anatomy or O_2 exchange respectively), which appeared effective in young patients, but may be linked to increased risk of vascular compromise with age once the vessels are no longer able to respond as required.

Additionally, since the two eyes of each anisomyope were supplied with the same level of increased pCO_2, the question as to the differences in vascular reactivity between myopes and emmetropes were due to differences in pCO_2 concentration was addressed. Therefore, the evaluation of anisomyopes in this study not only provided a control of the genetic and environmental confounding effects, but also helped to reduce the effects that different CO_2 concentrations would have on the analysis of vascular reactivity.

9.6 Choroidal blood flow and vascular autoregulation in myopia

The investigation of myopic growth in animal studies has reported variations in choroidal blood flow and choroidal thickness as part of an active choroidal mechanism of visual growth control. Shih and co-workers reported a decrease in choroidal blood flow after inducing myopia in chick eyes using goggles (Shih et al., 1993), and an increase in choroidal blood flow was described in the recovery phase from form deprivation myopia in chick eyes (Wilsoet and Wallman, 1995).

Additionally, the existence or lack of vascular autoregulation in the choroid has been a controversial topic for the past fifty years. Early studies failed to report vascular regulation in the choroid (Bettman and Fellows, 1956; Friedman and Chandra, 1972; Alm and Bill, 1972; Friedman, 1970; Yu et al., 1988); whereas recent studies on human eyes have raised the possibility of the existence of vascular choroidal regulation (Riva, *et al.* 1997). Riva and colleagues first described some level of choroidal autoregulation after experimentally inducing a rapid increase in IOP and

measuring choroidal blood flow with Laser Doppler Flowmetry (LDF) (Riva, *et al.* 1994). Despite the reported lack of a constant linear relationship between ocular perfusion pressure and choroidal blood flow, when slower increases in IOP were induced, no changes in choroidal blood flow were found, which pointed towards some kind of local regulation. Thus, the choroid appeared to exhibit some degree of vascular regulation.

In view of these previous findings on animal myopia and choroidal regulation, the assessment of the choroidal circulation and regulatory characteristics in the human myopic eye was of particular interest and it was a field that remained unexplored.

Studies presented in chapter 7 of this thesis evaluated the resting choroidal vascular profile in human myopia using the Laser Doppler Flowmeter (LDF). Additionally, the vascular choroidal response to hypercapnia in myopes was compared to that in emmetropes to provide information on the choroidal regulation features of the human myopic eye. The results described a significantly lower resting choroidal blood velocity in myopes compared to emmetropes and their response to hypercapnia described a significant increase in choroidal blood velocity that exceeded statistically that of emmetropes, which was consistent with earlier findings.

The LDF provides repeatable measurements of choroidal blood flow, volume and velocity. The LDF readings were collected at the foveal region for this study, thereby, the data obtained aimed to describe the choroidal circulation. No significant difference between the BP of myopes and emmetropes was found; thereby, the decrease in LDF blood velocity found in myopes suggested an actual trend towards reduced blood velocity in myopic eyes. No significant differences in choroidal blood volume or flow were reported, which was thought to be due to the narrower variability reported for the LDF blood velocity parameter.

The reduction in choroidal blood velocity found in myopes thus followed a similar pattern to the decrease in ocular pulse amplitude described in chapter three. Since the OBFA assesses the pulsatility of blood flow in the large choroidal vessels, and the LDF signal originates from the choriocapillaris, where the pulsatility is much lower than in the main choroidal vessels, the human myopic eye appeared to exhibit attenuation in both the pulsatile and non-pulsatile component of the choroidal blood flow, and additionally, a significantly lower retrobulbar blood velocity. These findings are of particular importance due to the agreement found between different techniques that evaluated different vascular bed in the human myopic eye.

Myopes were also found to exhibit a significant increase in choroidal blood velocity during hypercapnia that differed statistically from the percentage change induced by hypercapnia in

emmetropes. No changes were observed in blood pressure during hypercapnia; therefore, the increase in choroidal blood velocity in myopes suggested the existence of a local choroidal response.

In summary, the results suggested that the myopic eye had a reduced resting choroidal flow velocity which was highly responsive to CO_2 in the young patients.

9.7 The relationship between choroidal blood flow, choroidal thickness, refractive error and axial length

The myopic eye is characterised by an elongation of the posterior segment of the eye (Jones. et al, 2005; Logan, Gilmartin, Wildsoet et al., 2004) and it is also at higher risk of developing fundus complications, which suggests that the thinning of the choroidal layer reported in animal studies in myopia (Wildsoet and Wallman 1995) may be involved in the myopic eye growth. Despite this evidence, the relationship between choroidal blood flow and thickness, refractive status and axial length in myopic eyes had not been explored to date.

The studies presented in chapter 8 of this thesis provided an assessment of the relationship between axial length, mean spherical equivalent (MSE), choroidal blood flow and choroidal thickness in healthy human eyes. The measurements of choroidal thickness were performed using the SD OCT' Copernicus', (SOCT), which allowed the visualisation of the choroidal layer. Since the instrument had not been previously used to calculate choroidal thickness, the repeatability and reproducibility of the instrument were evaluated. The results described a coefficient of variability (CoV) of 0.80%, and a coefficient of repeatability (CoR) of 2.97%. The coefficient of reproducibility (CoRepro), calculated for the mean of the choroidal thickness measurements at the centre of the fovea, was 2.44%. Thus, the SOCT provides repeatable measurements of choroidal thickness, which allowed its use in this study.

The choroidal thickness at the macular was found to correlate significantly, following a negative trend, with axial length. This relationship was of interest for understanding the findings reported in this thesis, as choroidal thickness played an important role in the myopic growth induced in animal studies (Hougaard et al., 2006; Hoh et al, 2006).

It was contradictory to observe a lack of significant correlation between the thickness and the blood flow in the choroid, despite the fact that a positive correlation between choroidal blood flow and choroidal thickness was hypothesised. The explanation for this may relate to the reported

diurnal rhythm changes in axial elongation and choroidal thickness demonstrated in animal research (Papastergiou, Schmid, Riva et al, 1998; Nickla et al., 2002). The lack of significant correlation between choroidal thickness, flow and volume may also be due to the fact that the choroidal volume may not change with thickness changes. Since the choroidal velocity is the LDF variable that exhibits higher repeatability, it appears necessary to assess factors that may influence velocity. Choroidal thickness appears largely involved with choroidal blood flow; however the overall choroidal volume may exhibit a stronger relationship with choroidal blood flow, which would explain the absence of significant correlation between choroidal thickness and blood flow. Additionally, the recording of the choroidal thickness and choroidal blood flow was performed on different days for this study, which may have also contributed to the lack of significant correlation between the parameters.

9.8 Additional discussion

To view the results of this study in conjunction with the findings reported in the previous chapters, the thinning of the choroid due to the elongation of the posterior segment in the human myopic eye may be a response by the vascular supply to a decrease in metabolic needs, leading to a decreased choroidal blood flow. This in turn may result in a reduction in retinal thickness as a consequence. If the retinal thickness reduces, as shown by previous papers (Hoh, *et al.* 2006; Hougaard, *et al.* 2006; Luo, *et al.* 2006; Workman 2006), the retinal vascular supply may induce a decrease in the blood supply to the retinal layer, which is mainly supplied by the CRA. In this thesis, the CRA blood velocity was found to decrease and the resistance index was found to increase with increasing myopia, thus the results reported in the present investigations are in agreement with the findings from other studies.

It was interesting to observe an absence of vascular reactivity in each of the emmetropic, young and non-smokers groups compared to the apparent oversensitive response to hypercapnia given by myopes, older participants and smokers, who exhibited significantly larger reactivity in the retrobulbar, choroidal and microretinal circulation with increasing axial length. The increase in pCO_2 supplied to the participants was not clinically different between the different studies in this thesis but it induced systemic and ocular changes in myopes only, older participants and smokers, which may suggest that these subjects reacted to small changes in oxygenation in a systemic and ocular manner to which emmetropic, young and non-smokers did not appear to be affected. The vascular reactivity to hypercapnia in myopes, smokers and older people might have been due to anatomical features of the posterior segment, decrease in O_2 exchange and changes in vascular physiology with age respectively. It could also be argued that it was the emmetropic, young and

non-smoker participants which were the ones not responding effectively to the changes in oxygenation. Several studies have reported a reduced vasodilatory response to changes in pCO_2 in otherwise healthy subjects with peripheral vasospasm (Gugleta, *et al.* 2005). Subjects with vasospasm are generally described to suffer from cold extremities and they have been reported to exhibit ocular vascular dysregulation (Gugleta, *et al.* 2005; Gherghel, *et al.* 1999). However, they also appear to exhibit BP changes in response to hypercapnia (Gugleta, *et al.* 2005). Therefore, the absence of changes in systemic blood pressure during hypercapnia in the emmetropic, non-smokers and young groups did not fully support the hypothesis that these subjects might have been vasospastic. However, the protocol followed in the present investigations did not include the recording of history of cold extremities, therefore preventing the dismissal of this hypothesis.

Hypercapnia can be used to test the vascular metabolic regulation due to the induced decrease in O_2 and it has been shown to result in vasodilation in several vascular beds of the eye. Therefore, the participation of metabolic regulators in the oversensitivity to hypercapnia observed in myopes may be a topic of interest. Nitric oxide (NO) (section 1.12.2.8) has been reported to act a vasodilator and of special interest is the fact that nitric oxide synthase has been observed within the vascular endothelium of the retina and choroid. Nitric oxide probably acts as a choroidal vasodilator of parasympathetic origin in the eye (Yamamoto, *et al.* 1993), which has been supported by findings describing the presence of neural NO synthase in retinal amacrine cells and photoreceptor cells from a variety of species (Yamamoto, *et al.* 1993). Additionally the presence of nitric oxide synthase molecules has been reported in the choroidal vasculature of humans using immunochemistry (Flugel, *et al.* 1994). It is of interest to observe that injected L-NAME (NO synthase inhibitor) in chick eyes resulted in an inhibition of form deprivation myopia and lens-induced myopia (Fujikado, *et al.* 2001; Fujikado, *et al.* 1997). The results of the present investigations suggest an over-reactive metabolic mechanism in myopia. Therefore, if NO is known to be a metabolic regulator whose inhibition allows recovery of chick eyes from form deprivation myopia, and the metabolic vascular reactivity appears to be over-sensitive in myopes, NO may play a role in the vascular reactivity of myopic eyes.

9.9 Suggestions for further work

The results presented in this thesis showed a reduction in both the resting vascular state and regulatory features of low to moderate healthy myopic eyes and smokers. The highly responsive vascular regulatory capacity of the human myopic eye to changes in pCO_2 was shown to be effected by age, which may predispose the ageing myopic eye to ocular disease. However, there are some unanswered questions that have resulted in the suggestion of the following future work:

1. There was no significant difference between the microretinal circulation in myopes and emmetropes. Since the HRF was used to assess the perfusion features of the retinal capillaries around the macular area, these results may be interpreted as a similar haemodynamic profile in the macular retinal capillaries of myopes and emmetropes. No data was obtained for the peripapillary capillaries. The CRA blood velocity, which is known to supply the superficial ONH, was significantly lower in myopes. Due to increased vulnerability to raised intraocular pressure of the retinal capillaries, future work could be directed towards the assessment of this capillary bed, which will further the understanding of the results described in this thesis.
2. Future work on the ocular haemodynamics of smokers could include the assessment of older smokers with a longer smoking history, with higher FTQ scores as the perfect vascular autoregulatory response found in the young smokers of the present study is probably compromised with age, which would provide further evidence of the chronic effect of smoking on the ocular blood flow and its interaction with the effect of age.
3. The assessment of a bigger sample of anisomyopes with greater inter-eye difference in MSE and axial length would provide a wider picture of the ocular haemodynamics of anisomyopes.
4. The choroidal blood flow in myopia constitutes a topic that remains mainly unexplored in humans, thus, future work could include the assessment of the choroidal haemodynamics in a larger sample size, including the assessment of the temporal behaviour of the choroidal regulatory response to hypercapnia and additionally evaluating the relationship between the physiological (choroidal blood flow) and functional (visual fields) response to hypercapnia.
5. A longitudinal assessment of the changing aspects of the choroidal haemodynamics and physiology in relation to longitudinal changes in refractive error would allow evaluation of the degree of the association between choroidal blood flow and choroidal thickness in human myopia

9.10 Final conclusion

This thesis demonstrated a reduced resting ocular pulse amplitude and retrobulbar blood flow in human myopia, associated with an apparent oversensitivity to the vasodilatory effects of hypercapnia, which may be due to differences in the anatomy of ocular vascular beds or in differences in retinal shape. In young smokers, normal resting state vascular characteristics were present; however there also appeared to be increased reactivity to hypercapnia, possibly due to relative chronic hypoxia. The systemic circulation in myopes and smokers over-reacted similarly

to hypercapnia suggesting that physiologic differences are not confined to the eye. Age also showed a negative effect on autoregulatory capacity in otherwise normal eyes. Collectively, these findings suggest that myopes and smokers require greater autoregulatory capacity to maintain appropriate oxygenation of retinal tissue, and since the capacity for such regulation reduces with age, these groups are at greater risk of insufficient autoregulation and relative hypoxia with age.

REFERENCES

- Aaslid R, Lindegaard KF, Sorteberg W, Nornes H. 1989. Cerebral autoregulation dynamics in humans. Stroke 20(1):45-52.
- Adams A, Baldwin W, Biederman I, Curtin B, Ebenholtz S, Goss D, Hutchison G, Seddon J, Wallman J, Vision Co. 1989. Myopia Prevalence and Progression. Washington DC: National Academy Press.
- Agnew C, Williams A. 1877. Near sightedness in the public schools. New York. Report nr 12. 34-36 p.
- Ahlborg G, Lundberg JM. 2001. Endothelin-1: increased plasma clearance, pulmonary affinity and renal vasoconstriction in young smokers. Clin Physiol 21(6):693-703.
- Akyol N, Kukner A, Ozdemir T, Esmerligil S. 1996. Choroidal and retinal blood flow changes in degenerative myopia. Can J Ophthalmol. 31(3):113-119.
- Alm A BA. 1972. The oxygen supply to the retina: Effects of changes in intraocular and arterial blood pressures and in arterial PO2 and PCO2 on the oxygen tension in the vitreous body of the cat. Acta Physiol Scand, 84:261.
- Alm A. 1992. Ocular circulation. In: WM H, editor. Adler´s physiology of the eye. St. Louis: Mosby. p 198-227.
- Amstrong RA, Slade SV and Eperjesi F. 2000. An introduction to analysis of variance (ANOVA) with special reference to data from clinical experiments in optometry. Ophthal Physiol Opt. 20(3):235-241.
- Araki M. 1975. Anatomical study of the vascularization of the optic nerve (author's transl). Nippon Ganka Gakkai Zasshi 10(79):101-109.
- Astrup P, Kjeldsen K, Wanstrup J. 1970. Effects of carbon monoxide exposure on the arterial walls. Ann N Y Acad Sci 174(1):294-300.
- Atchison DA, Pritchard N, White SD, Griffiths AM. 2005. Influence of age on peripheral refraction. Vision Res 45(6):715-720.
- Aydin A, Wollstein G, Price L, Schuman J. 2003. Evaluating pulsatile ocular blood flow analysis in normal and treated glaucomatous eyes. Am J Ophthalmol 136(3):448-453.
- Azuara-Blanco A, Harris A, Cantor LB, Abreu MM, Weinland M. 1998. Effects of short term increase of intraocular pressure on optic disc cupping. Br J Ophthalmol 82(8):880-883.
- Balacco-Gabrieli C, Mastrandea G, Acquafredda A, Stefania O, Ciufreda A, Cavallo L. 1986. Congenital cataract and evolutive myopia. Relationship with hypophyseal-adrenal cortical axis function. Ophthalmic Paediatr Genet. 7(1):63-67.

- Bansal D, Benavente-Perez A (2006) Optical Coherence Tomography: Terminology and interpretations. Optometry Today 20th Oct
- Bardiger M, Stock A. 1972. The effects of sucrose-containing diets low in protein on ocular refraction in the rat. Proc Nutr Soc 31.
- Bartmann M, Schaeffel F, Hagel G, Zrenner E. 1994. Constant light affects retinal dopamine levels and blocks deprivation myopia but not lens-induced refractive errors in chickens. Vis Neurosci. 11(2):199-208.
- Basu S. 2005. Comparison of IOL power calculations by the IOLMaster vs theoretical calculations. Eye 20(1):90-97.
- Baxter G, Williamson T. 1995. Colour Doppler Imaging of the eye: normal ranges, reproducibility, and obsever variation. J Ultrasound Med 14(2):91-96.
- Bayerle-Eder M, Wolzt M, Polska E, Langenberger H, Pleiner J, Teherani D, Rainer G, Polak K, Eichler H, Schmetterer L. 2000. Hypercapnia-induced cerebral and ocular vasodilation is not altered by glibenclamide in humans. Am J Physiol Regul Integr Comp Physiol 278(6):1667-1673.
- Becker B. 1954. Decrease in intraocular pressure in man by a carbonic anhydrase inhibitor, diamox; a preliminary report. Am J Ophthalmol. 37(1):13-15.
- Benedito S, Prieto D, Nielsen PJ, Berg Nyborg NC. 1991. Role of the endothelium in acetylcholine-induced relaxation and spontaneous tone of bovine isolated retinal small arteries. Exp Eye Res 52(5):575-579.
- Benowitz NL, Jacob P, Jones RT, Rosenberg J. 1982. Interindividual variability in the metabolism and and cardiovascular effects of nicotine in man. J Pharmacol Exp Ther. 221:368-372.
- Berglund G, Wilhelmsen L. 1975. Factors related to blood pressure in a general population sample of Swedish men. Acta Med Scand. 198(4):291-298.
- Berne R, Levy M. 1997. Cardiovascular physiology. Mosby, editor. Missouri.
- Bettman J, Fellows V. 1956. Factors influencing the blood volume of the choroid and retina. Trans Am Acad Ophthalmol Otolaryngol 60(6):791-804.
- Bill A, Sperber G, Ujiie K. 1983. Physiology of the choroidal vascular bed. Int Ophthalmol 6(2):101-107.
- Bland J, Altman D. 1986. Statistical methods for assessing agreement between two methods of clinical measurement. Lancet:307-310.
- Block F, McDonald J. 1992. Sidestream versus mainstream carbon dioxide analyzers. J Clin Monit 8:139-141.
- Bonomi L, Mecca E, Massa F. 1982. Intraocular pressure in myopic anisometropia. Int Ophthalmol 5,(Issue 3,):Pages 145-148.

- Bourne RR, Alsbirk PH. 2006. Anterior chamber depth measurement by optical pachymetry: systematic difference using the Haag-Streit attachments. Br J Ophthalmol 90(2):142-145.
- Brand C, Burkhardt E, Schaeffel F, Choi J, Feldkaemper M. 2005. Regulation of Egr-1, VIP, and Shh mRNA and Egr-1 protein in the mouse retina by light and image quality. Mol Vis 11:309-320.
- Branson R, Hess D, Chatburn R. 1994. Noninvasive respiratory monitoring equipment. Respiratory care equipment. Philadelphia: Lippincott. p 184-216.
- Brasil O, Brasil M, Japiassu R, Biancardi A, Souza D, Oliveira R, Moraes HJ. 2006. Fundus changes evaluation in degenerative myopia. Arq Bras Oftalmol 69(2):203-206.
- Broadway DC, Drance SM, Parfitt CM, Mikelberg FS. 1998. The ability of scanning laser ophthalmoscopy to identify various glaucomatous optic disc appearances. Am J Ophthalmol. 125(5):593-604.
- Bron A, Tripathi R, Tripathi B. 1997. Wolff's anatomy of the eye and orbit. London: Chapman & Hall Medical.
- Brown N, Koretz J, Bron A. 1999. The development and maintenance of emmetropia. Eye 13 (1):83-92.
- Buchanan J, Phillis J. 1993. The role of nitric oxide in the regulation of cerebral blood flow. Brain Res 610(2):248-255.
- Budenz DL, Anderson DR, Varma R, Schuman J, Cantor L, Savell J, Greenfield DS, Patella VM, Quigley HA, Tielsch J. 2007. Determinants of Normal Retinal Nerve Fiber Layer Thickness Measured by Stratus OCT. Ophthalmology In Press, Corrected Proof.
- Buerk DG, Riva CE, Cranstoun SD. 1996. Nitric Oxide Has a Vasodilatory Role in Cat Optic Nerve Head during Flicker Stimuli. Microvas Res 52(1):13-26.
- Bullimore M, Fusaro R, Adams C. 1998. The repeatability of automated and clinician refraction. Optom Vis Sci 75 617-622.
- Bullimore M, Gilmartin B, Royston J. 1992. Steady-state accommodation and ocular biometry in late-onset myopia. Doc Ophthalmol. 80(2):143-155.
- Bullimore M, Gilmartin B. 1987. Tonic accommodation, cognitive demand, and ciliary muscle innervation. Am J Optom Physiol Opt. 64:499-503.
- Bullimore M, Gilmartin B. 1987. Aspects of tonic accommodation in emmetropia and late-onset myopia. Am J Optom Physiol Opt. 64(7):499-503.
- Burnett RT, Dales RE, Brook JR, Raizenne ME, Krewski D. 1997. Association between ambient carbon monoxide levels and hospitalizations for congestive heart failure in the elderly in 10 Canadian cities. Epidemiology 8(2):162-167.

- Campisi R, Czernin J, Schoder H, Sayre JW, Marengo FD, Phelps ME, Schelbert HR. 1998. Effects of Long-term Smoking on Myocardial Blood Flow, Coronary Vasomotion, and Vasodilator Capacity. Circulation 98(2):119-125.
- Carel R, Korczyn A, Rock M, Goya I. 1984. Association between ocular pressure and certain health parameters. Ophthalmology. 91(4):311-314.
- Castren J. 1955. The significance of prematurity on the eye with reference to retrolental fibroplasia. Acta Ophthalmol Supp.44.
- Catier A, Tadayoni R, Paques M, Erginay A, Haouchine B, Gaudric A, Massin P. 2005. Characterization of Macular Edema From Various Etiologies by Optical Coherence Tomography. Am J Ophthalmol 140(2):200.e201-200.e209.
- Chakravarthy U, Gardiner TA. 1999. Endothelium-derived agents in Pericyte function/dysfunction. Prog Retin Eye Res. 18(4):511-527.
- Chakravarti S, Paul J, Roberts L, Chervoneva I, Oldberg A, Birk DE. 2003. Ocular and Scleral Alterations in Gene-Targeted Lumican-Fibromodulin Double-Null Mice. Invest. Ophthalmol. Vis. Sci. 44(6):2422-2432.
- Charman W. 2005. Aberrations and myopia. Ophthalmic Physiol Opt. 25(4):285-301.
- Charman WN, Jennings JA. 2006. Longitudinal changes in peripheral refraction with age. Ophthalmic Physiol Opt 26(5):447-455.
- Chegwidden W, Carter N. 2000 Introduction to the carbonic anhydrases. Exs 90
- Cheitlin MD. 2003. Cardiovascular Physiology-Changes With Aging. Am J Cardiol 12(1):9-13.
- Chen C, Cohen B, Diamond E. 1985 Genetic and environmental effects on the development of myopia in Chinese twin children. Ophthalmic Paediatr Genet. 6(1-2):353-359.
- Chen JC, Schmid KL, Brown B. 2003. The autonomic control of accommodation and implications for human myopia development: a review. Ophthalmic Physiol Opt. 23(5):401-422.
- Chen TC, Cense B, Pierce MC, Nassif N, Park BH, Yun SH, White BR, Bouma BE, Tearney GJ, de Boer JF. 2005. Spectral Domain Optical Coherence Tomography: Ultra-high Speed, Ultra-high Resolution Ophthalmic Imaging. Arch Ophthalmol 123(12):1715-1720.
- Chen Z, Gu Q, Kaufman PL, Cynader MS. 1998. Histochemical mapping of NADPH-diaphorase in monkey and human eyes. Curr Eye Res. 17(4):370-379.
- Cheng C-Y, Liu CJ, Chiou H-J, Chou JC, Hsu W-M, Liu J-H. 2001. Color Doppler imaging study of retrobulbar hemodynamics in chronic angle-closure glaucoma. Ophthalmology 108(8):1445-1451.
- Chihara E, Liu X, Dong J. 1997. Severe myopia as a risk factor for progressive visual field loss in primary open-angle glaucoma. Ophthalmologica. 211:66-71.

- Chua W-H, Balakrishnan V, Chan Y-H, Tong L, Ling Y, Quah B-L, Tan D. 2006. Atropine for the Treatment of Childhood Myopia. Ophthalmology 113(12):2285-2291.
- Chung H, Harris A, Evans D, Kagemann L, Garzozi J, Martin B. 1999a. Vascular Aspects in the Pathophysiology of Glaucomatous Optic Neuropathy. Surv Ophthalmol. 43,(Supplement 1):Pages S43-S50.
- Chung H, Harris A, Halter P, Roff E, Garzozi J, Hosking S, Martin B. 1999b. Regional differences in retinal vascular reactivity. Invest Ophthalmol Vis Sci. 40(10):2448-2453.
- Ciulla T, Harris A, Chung H, Danis R, Kagemann L, McNulty L, Pratt L, Martin B. 1999. Color Doppler imaging discloses reduced ocular blood flow velocities in nonexudative age-related macular degeneration. Am J Ophthalmol. 128(1):75-80.
- Clifford PS, Buckwalter JB, Hamann JJ. 2002. Attenuated sympathetic vasoconstriction in contracting muscles: just say NO. J Physiol (Lond).2001.015743 540(1):2-.
- Cordain L, Eaton SB, Sebastian A, Mann N, Lindeberg S, Watkins BA, O'Keefe JH, Brand-Miller J. 2005. Origins and evolution of the Western diet: health implications for the 21st century. Am J Clin Nutr 81(2):341-354.
- Cryer PE, Haymond MW, Santiago JV, Shah SD. 1976. Norepinephrine and epinephrine release and adrenergic mediation of smoking-associated hemodynamic and metabolic events. N Engl J Med 295(11):573-577.
- Curtin B. 1988. Anterior chamber anomalies and pathologic myopia. Acta Ophthalmologica 185(111-3).
- Cuypers M, Kasanardjo J, Polak B. 2000. Retinal blood flow changes in diabetic retinopathy measured with the Heidelberg scanning laser Doppler flowmeter. Graefes Arch Clin Exp Ophthalmol 238(12):935-941.
- Dallinger S, Bobr B, Findl O, Eichler H-G, Schmetterer L. 1998. Effects of Acetazolamide on Choroidal Blood Flow. Stroke 29(5):997-1001.
- Dallinger S, Findl O, Strenn K, Eichler H, Wolzt M, Schmetterer L. 1998. Age dependence of choroidal blood flow. J Am Soc Geriatr Dent46(4):484-487.
- Davies L, Wolffsohn J, Gilmartin B. 2005. Cognition, ocular accommodation, and cardiovascular function in emmetropes and late-onset myopes. Investigative Ophthalmology & Visual Science 46(5):1791-1796.
- Dawber T, Kannel W. 1961. Susceptibility to coronary heart disease. Mod Concepts Cardiovasc Dis 30::671-676.
- Delaey C, Van de Voorde J. 2000. Regulatory Mechanisms in the Retinal and Choroidal Circulation. Ophthalmic Res 32(6):249-256.
- Denninger JW, Marletta MA. 1999. Guanylate cyclase and the NO/cGMP signaling pathway. Biochimica et Biophysica Acta (BBA) - Bioenergetics 1411(2-3):334-350.

- Derby H. 1877. A report on the percentage of nearsight in the class of 1880 at Harvard College. Boston Medical and Surgical Journal 18:337.
- Diether S, Schaeffel F. 1997. Local Changes in Eye Growth induced by Imposed Local Refractive Error despite Active Accommodation. Vision Res. 37(6):659-668.
- Dimitrova G, Tamaki Y, Kato S, Nagahara M. 2002. Retrobulbar circulation in myopic patients with or without myopic choroidal neovascularisation. Br J Ophthalmol 86(7):771-773.
- Domino E, Zubieta J, Guthrie S, Ni L. Nicotine effects on regional cerebral blood flow and glucose in awake resting tobacco smokers; 2004; North Washington St, Alexandria.
- Drexler W. 2004. Ultrahigh-resolution optical coherence tomography. J Biomed Opt9(1):47-74.
- Duijm H, van den Berg T, Greve E. 1997. A comparison of retinal and choroidal hemodynamics in patients with primary open-angle glaucoma and normal-pressure glaucoma. Am J Ophthalmol. 123(5):644-656.
- Dunphy E, Stoll M, King S. 1968. Myopia among Americal male graduate students. Am J Ophthalmol. 65:518-522.
- Edwards M. 1996. Do variations in normal nutrition play a role in the development of myopia? Optom Vis Sci. 73(10):638-643.
- Edwards R, McElduff P, Harrison R, Watson K, Butler G, Elton P. 2006. Pleasure or pain? A profile of smokers in Northern England. Public Health.
- Ehlers N, Hjortdal J. 2004. Corneal thickness: measurement and implications. Exp Eye Res. 78(3):543-548.
- Eleftheriadis H. 2003. IOLMaster biometry: refractive results of 100 consecutive cases. Br J Ophthalmol 87(8):960-963.
- Embleton S, Hosking S, Roff Hilton E, Cunliffe I. 2002. Effect of senescence on ocular blood flow in the retina, neuroretinal rim and lamina cribrosa, using scanning laser Doppler flowmetry. Eye 16(2):156-162.
- Ergul A. 2002. Endothelin-1 and endothelin receptor antagonists as potential cardiovascular therapeutic agents. Pharmacotherapy.22(1):54-65.
- Fagerstrom K. 1978. Measuring degree of physical dependence to tobacco smoking with reference to individualization of treatment. Addict Behav. 3(3-4):235-241.
- Faraci FM, Breese KR, Heistad DD. 1994. Cerebral vasodilation during hypercapnia. Role of glibenclamide- sensitive potassium channels and nitric oxide. Stroke 25(8):1679-1683.
- Farbrother JE, Kirov G, Owen MJ, Pong-Wong R, Haley CS, Guggenheim JA. 2004. Linkage Analysis of the Genetic Loci for High Myopia on 18p, 12q, and 17q in 51 U.K. Families. Invest. Ophthalmol. Vis. Sci. 45(9):2879-2885.

- Farrell C, Stewart P, Farrell C, Del Maestro R. 1987. Pericytes in human cerebral microvasculature. Anat Rec 218(4):466-469.
- Fearnley S. 1995. Pulse Oximetry. Practical procedures 5(2):1.
- Feke G, Riva C. 1978. Laser Doppler measurements of blood velocity in human retinal vessels. J Opt Soc Am 68(4):526-531.
- Ferrari-Dileo G, Davis E, Anderson D. 1989. Biochemical evidence for cholinergic activity in retinal blood vessels. Invest Ophthalmol Vis Sci 30(3):473-477.
- Ferry J, Cerri R, Cohen P. 1999. Insulin-Like Growth Factor Binding Proteins: New Proteins, New Functions. Horm Res. 51:53-67.
- Findl O, Rainer G, Dallinger S, Dorner G, Polak K, Kiss B, Georgopoulos M, Vass C, Schmetterer L. 2000. Assessment of optic disk blood flow in patients with open-angle glaucoma. Am J Ophthalmol. 130(5):589-596.
- Findl O, Strenn K, Wolzt M, Menapace R, Vass C, Eichler H, Schmetterer L. 1997. Effects of changes in intraocular pressure on human ocular haemodynamics. Current Eye Research. 16(10):1024-1029.
- Fischer A, McGuire J, Schaeffel F, Stell W. 1999. Light- and focus-dependent expression of the transcription factor ZENK in the chick retina. Nature Neuroscience 2(8):706-712.
- Fitzgerald M, Wildsoe C, Anton R. 2001. Temporal Relationship of Choroidal Blood Flow and Thickness Changes during Recovery from Form Deprivation Myopia in Chicks. Exp Eye Res. 74(5):561-570.
- Fitzgerald MEC, Tolley E, Jackson B, Zagvazdin YS, Cuthbertson SL, Hodos W, Reiner A. 2005. Anatomical and functional evidence for progressive age-related decline in parasympathetic control of choroidal blood flow in pigeons. Exp Eye Res. 81(4):478-491.
- Flammer J, Orgul S. 1998. Optic nerve blood-flow abnormalities in glaucoma. Prog Retin Eye Res. 17(2):267-289.
- Flammer J, Orgüla S, Costab V, Orzalesic N, Krieglsteind G, Metzner Serrae K, Renardf J, Stefánssong E. 2002. The impact of ocular blood flow in glaucoma. Prog Retin Eye Res. 21(4): 359-393.
- Fledelius H. 1982. Ophthalmic changes from age of 10 to 18 years. A longitudinal study of sequels to low birth weight. IV. Ultrasound oculometry of vitreous and axial length. Acta Ophthalmol (Copenh) 60:403-411.

- Fledelius H. 1982. Ophthalmic changes from age of 10 to 18 years. A longitudinal study of sequels to low birth weight. IV. Ultrasound oculometry of vitreous and axial length. Acta Ophthalmol (Copenh) 60:403-411.

- Fleg J. 1986. Alterations in cardiovascular structure and function with advancing age. Am J Cardiol.12(57):5.

- Flugel C, Tamm ER, Mayer B, Lutjen-Drecoll E. 1994. Species differences in choroidal vasodilative innervation: evidence for specific intrinsic nitrergic and VIP-positive neurons in the human eye. Invest Ophthalmol Vis Sci. 35(2):592-599.
- Forrester J, Dick A, McMenamin P, Lee W. 2001. The Eye: Basic Sciences in Practiced: Saunders.
- Forte P, Copland M, Smith LM, Milne E, Sutherland J, Benjamin N. 1997. Basal nitric oxide synthesis in essential hypertension. The Lancet 349(9055):837-842.
- Foster PJ, Baasanhu J, Alsbirk PH, Munkhbayar D, Uranchimeg D, Johnson GJ. 1998. Central corneal thickness and intraocular pressure in a Mongolian population. Ophthalmology 105(6):969-973.
- Francois J. 1961. Heredity in ophthalmology. St. Louise: C.V Mosby company.
- Fredrick D. 2001. Myopia: was mother right about reading in the dark? Br J Ophthalmol 85(5):509-510.
- Fredrick DR. 2002. Myopia. BMJ. 324:1195-1199.
- Fujikado T, Kawasaki Y, Fujii J, Taniguchi N, Okada M, Suzuki A, Ohmi G, Tano Y. 1997. The effect of nitric oxide synthase inhibitor on form-deprivation myopia. Curr Eye Res 16(10):992-996.
- Fujikado T, Tsujikawa K, Tamura M, Hosohata J, Kawasaki Y, Tano Y. 2001. Effect of a nitric oxide synthase inhibitor on lens-induced myopia. Ophthalmic Res. 33(2):75-79.
- Furchgott RF, Zawadzki JV. 1980. The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. Nature 288(5789):373-376.
- Gardin JM, Arnold AM, Bild DE, Smith V-E, Lima JAC, Klopfenstein HS, Kitzman DW. 1998. Left ventricular diastolic filling in the elderly: the cardiovascular health study. Am J Cardiol 82(3):345-351.
- Gardiner P. 1956 Observations on the food habits of myopic children. Br Med J. 22(4994)(32):699-700.
- Garner L. 1983. Mechanisms of accommodation and refractive error. Ophthalmic Physiol Opt 3(3):287-293.
- Gavrilov L, NS G. 2006. Reliability Theory of Aging and Longevity. Handbook of the Biology of Aging. San Diego, CA, USA: Elsevier Academic Press.
- Gay A, Rosenbaum A. 1996. Retinal artery pressure in asymmetric diabetic retinopathy. Arch Ophthalmol 75:758.
- Gdovinova Z. 2001. Blood flow velocity in the middel cerebral artery in heavy alcohol drinkers. Alcohol and Alcoholism 36(4):346-348.
- Geiser M, Riva C, Dorner G, Diermann U, Luksch A, Schmetterer L. 2000 Response of choroidal blood flow in the foveal region to hyperoxia and hyperoxia-hypercapnia. Curr Eye Res 21(2):669-676.

- Gerhard J. 1983. Myopia in premature infants. Bulletin Of The Society of Opththalmology Fr 83(2):221-223.
- Gherghel D, Orgul S, Dubler B, Lubeck P, Gugleta K, Flammer J. 1999. Is vascular regulation in the central retinal artery altered in persons with vasospasm? Arch Ophthalmol. 117(10):1359-1362.
- Gillies WE, Brooks AMV, Scott M, Ryan L. 1999. Comparison of colour Doppler imaging of orbital vessels in elderly compared with young adult patients. Aust N Z J Ophthalmol27(3-4):173-175.
- Gilmartin B. 2004. Myopia: precedents for research in the twenty-first century. Clin Exp Ophthalmol 32(3):305-324.
- Goadsby PJ. 1994. Nitric oxide is not the sole determinant of hypercapnic or metabolically driven vasodilation in the cerebral circulation. J Auton Nerv Syst.49(1): 67-72.
- Goh W, Lam C. 1994. Changes in refractive trends and optical components of Hong Kong Chinese aged 19-39 years. Ophthal. Physiol. Opt. 14:378-382.
- Goldschmidt E. 1968. On the etiology of myopia. An epidemiological study. Acta Ophthalmol 98(1).
- Goss D, Cox V, Herrin-Lawson G, Nielsen E, Dolton W. 1990. Refractive error, axial length, and height as a function of age in young myopes. Optom Vis Sci. 67(332-8).
- Gow AJ. 2005. Nitric Oxide, Hemoglobin, and Hypoxic Vasodilation. Am. J. Respir. Cell Mol. Biol. 299 32(6):479-482.
- Graham B, Judge S. 1999. Normal development of refractive state and ocular component dimensions in the marmoset (Callithrix jacchus). Vision Res. 39(2):177-187.
- Grice K. 1997. Myopic progression differs between children in Boston and Singapore
- Grodum K, Heijl A, Bengtsson B. 2001. Refractive error and glaucoma. Acta Ophthalmol Scand. 79(6):560-566.
- Groenink M, Langerak SE, Vanbavel E, van der Wall EE, Mulder BJM, van der Wal AC, Spaan JAE. 1999. The influence of aging and aortic stiffness on permanent dilation and breaking stress of the thoracic descending aorta. Cardiovasc Res. 43(2):471-480.
- Groh M, Michelson G, Langhans M, Harazny J. 1996. Influence of age on retinal and optic nerve head blood circulation. Ophthalmology 103(3):529-534.
- Gronlund M, Andersson S, Aring E, Hard A, Hellstrom A. 2006. Ophthalmological findings in a sample of Swedish children aged 4-15 years. Acta Ophthalmologica Scandinavica 84(2):169-176.
- Grossniklaus HE, Green WR. 1992. Pathologic findings in pathologic myopia. Retina 12(2):127-133.
- Grosvenor T, Scott R. 1991. Comparison of refractive components in youth-onset and early adult-onset myopia. Optom Vis Sci 68(204-9).

- Grunwald J, Hariprasad S, DuPont J. 1998. Effect of aging on foveolar choroidal circulation. Archives of Ophthalmology 116(2):150-154.
- Gugleta K, Orgul S, Flammer I, Gherghel D, Flammer J. 2002. Reliability of Confocal Choroidal Laser Doppler Flowmetry. Invest. Ophthalmol. Vis. Sci. 43(3):723-728.
- Gugleta K, Orgul S, Hasler P, Flammer J. 2005. Circulatory Response to Blood Gas Perturbations in Vasospasm. Invest. Ophthalmol. Vis. Sci. 46(9):3288-3294.
- Gunvant P, Baskaran M, Vijaya L, Joseph IS, Watkins RJ, Nallapothula M, Broadway DC, O'Leary DJ. 2004. Effect of corneal parameters on measurements using the pulsatile ocular blood flow tonograph and Goldmann applanation tonometer. Br J Ophthalmol 88(4):518-522.
- Gunvant P, Watkins R, Broadway D, O'Leary D. 2004. Repeatability and Effects of Sequential Measurements with POBF Tonograph. Optom Vis Sci. 81(10):794-799.
- Gupta R, Das J, Vaitheeswaran K, Kumar Puri S. 2002. Effect of age on blood flow velocities in glaucoma. AIOC Proceedings:252-253.
- Gwiazda J, Bauer J, Thorn F, Held R. 1995. A dynamic relationship between myopia and blur-driven accommodation in school-aged children. Vision Res. 35(9):1299-1304.
- Gwiazda J, Gricea K, Thorna F. 1999. Response AC/A ratios are elevated in myopic children. Ophthalmic Physiol Opt. 19(2):173-179.
- Gwiazda J, Marsh-Tootle WL, Hyman L, Hussein M, Norton TT. 2002. Baseline Refractive and Ocular Component Measures of Children Enrolled in the Correction of Myopia Evaluation Trial (COMET). Invest. Ophthalmol. Vis. Sci. 43(2):314-321.
- Haddy F, Vanhoutte P, Feletou M. 2006. Role of potassium in regulating blood flow and blood pressure. Am J Physiol Regul Integr Comp Physiol 290(3):R546-552.
- Haefliger IO, Dettmann E, Liu R, Meyer P, Prunte C, Messerli J, Flammer J. 1999. Potential Role of Nitric Oxide and Endothelin in the Pathogenesis of Glaucoma. Surv Ophthalmol. 43(Supplement 1):S51-S58.
- Hafez A, Bizzarro R, Rivard M, Trabut I, Lovasik J, Kergoat H, Lesk M. 2003. Reproducibility of retinal and optic nerve head perfusion measurements using scanning laser Doppler flowmetry. Ophthalmic Surg Lasers Imaging 34(5):422-432.
- Haigis W, Lege B, Miller N, Schneider B. 2000. Comparison of immersion ultrasound biometry and partial coherence interferometry for intraocular lens calculation according to Haigis. Graefe's Archive for Clinical and Experimental Ophthalmology 238(9):765-773.
- Hara K. 1991. Effects of cigarette smoking on ocular circulation chronic effect on choroidal circulation. Nippon Ganka Gakkai Zasshi 95(10):939-943.
- Hardy P, Lamireau D, Hou X, Dumont I, Abran D, Nuyt AM, Varma DR, Chemtob S. 2001. Major role for neuronal NO synthase in curtailing choroidal blood flow autoregulation in newborn pig. J Appl Physiol 91(4):1655-1662.

- Harris A, Harris M, Biller J, Garzoz H, Zarfty D, Ciulla T, Martin B. 2000. Aging affects the retrobulbar circulation differently in women and men. Arch Ophthalmol. 118(8):1076-1080.
- Harris A, Ishii Y, Chung H, Jonescu-Cuypers C, McCranor L, Kagemann L, Garzozi H. 2003. Blood flow per unit retinal nerve fibre tissue volume is lower in the human inferior retina. Br J Ophthalmol. 87:184-188.
- Harris A, Joos K, Kay M, Evans D, Shetty R, Sponsel W, Martin B. 1996. Acute IOP elevation with scleral suction: effects on retrobulbar haemodynamics. Br J Ophthalmol 80(12):1055-1059.
- Harris A, Kagemann L, Cioffi GA. 1998. Assessment of Human Ocular Hemodynamics. Surv Ophthalmol.42(6):509-533.
- Hashemi H, Yazdani K, Mehravaran S, Fotouhi A. 2005. Anterior chamber depth measurement with a-scan ultrasonography, Orbscan II, and IOLMaster. Optom Vis Sci. 82(10):900-904.
- Havelius U, Hansen F. 2005. Ocular Vasodynamic Changes in Light and Darkness in Smokers. Invest. Ophthalmol. Vis. Sci. 46(5):1698-1705.
- Hayreh S. 1996. Blood supply of the optic nerve head. Ophthalmologica. 210(5):285-295.
- Hayreh SS. 2001. Blood Flow in the Optic Nerve Head and Factors that may Influence it. Prog Retin Eye Res. 20(5):595-624.
- Heath S, Robledo R, Beggs W, Feola G, Parodo C, Rinaldi A, Contu L, Dana D, StambolianD, Siniscalco M. 2001 Human Heredity. A novel approach to search for identity by descent in small samples of patients and controls from the same mendelian breeding unit: a pilot study on myopia. Hum Hered 52(4):183-190.
- Hellstrom M, Kalen M, Lindahl P, Abramsson A, Betsholtz C. 1999. Role of PDGF-B and PDGFR in recruitment of vascular smooth muscle cells and pericytes during embryonic blood vessel formation in the mouse. Development 126(14):3047-3055.
- Henningfield J, Schuh L, Jarvik M. 1995. Pathophysiology of tobacco dependence. Psychopharmacology: The Fourth Generation of Progress. New York: Raven Press,Ltd.
- Hillen T, Nieczaj R, Munzberg H, Schaub R, Borchelt M, Steinhagen-Thiessen E. 2000. Carotid atherosclerosis, vascular risk profile and mortality in a population-based sample of functionally healthy elderly subjects: the Berlin Ageing Study. J Intern Med 247(6):679-688.
- Hirata A, Negi A. 1998. Morphological changes of choriocapillaris in experimentally induced chick myopia. Graefes Arch Clin Exp Ophthalmol. 236(2):132-137.
- Hoh S-T, Lim MCC, Seah SKL, Lim ATH, Chew S-J, Foster PJ, Aung T. 2006. Peripapillary Retinal Nerve Fiber Layer Thickness Variations with Myopia. Ophthalmology 113(5):773-777.

- Honmura S. 1968. Studies on the relationship between ocular tension and myopia. II. Ocular tension, ocular rigidity, aqueous outflow and aqueous secretion in myopic eyes. Nippon Ganka Gakkai Zasshi. 72(6):688-696.
- Hosking S, Embleton S, Cunliffe I. 2001a. Application of a local search strategy improves the detection of blood flow deficits in the neuroretinal rim of glaucoma patients using scanning laser Doppler flowmetry. Br J Ophthalmol 85(11):1298-1302.
- Hosking S, Embleton S, Kagemann L, Chabra A, Jonescu-Cuypers C, Harris A. 2001b. Detector sensitivity influences blood flow sampling in scanning laser Doppler flowmetry. Graefes Arch Clin Exp Ophthalmol. 239(6):407-410.
- Hosking SL, Evans DW, Embleton SJ, Houde B, Amos JF, Bartlett JD. 2001. Hypercapnia invokes an acute loss of contrast sensitivity in untreated glaucoma patients. Br J Ophthalmol 85(11):1352-1356.
- Hosking SL, Harris A, Chung HS, Jonescu-Cuypers CP, Kagemann L, Roff Hilton EJ, Garzozi H. 2004. Ocular haemodynamic responses to induced hypercapnia and hyperoxia in glaucoma. Br J Ophthalmol 88(3):406-411.
- Hougaard J, Ostenfeld C, Heijl A, Bengtsson B. 2006. Modelling the normal retinal nerve fibre layer thickness as measured by Stratus optical coherence tomography. Graefes Arch Clin Exp Ophthalmol 244(12).
- Howlett MHC, McFadden SA. 2006. Form-deprivation myopia in the guinea pig (Cavia porcellus). Vision Res46(1-2):267-283.
- Huang D, Swanson EA, Lin CP, Schuman JS, Stinson WG, Chang W, Hee MR, Flotte T, Gregory K, Puliafito CA and others. 1991. Optical coherence tomography. Science 254(5035):1178-1181.
- Huber KK, Adams H, Remky A, Arend KO. 2006. Retrobulbar haemodynamics and contrast sensitivity improvements after CO2 breathing. Acta Ophthalmologica Scandinavica 84(4):481-487.
- Hughes AD, Schachter M. 1994. Hypertension and blood vessels. Br Med Bull 50(2):356-370.
- Hughes S, Gardiner T, Hu P, Baxter L, Rosinova E, Chan-Ling T. 2006. Altered pericyte-endothelial relations in the rat retina during aging: Implications for vessel stability. Neurobiology of Aging 27(12):1838-1847.
- Hung L-F, Wallman J, Smith EL, III. 2000. Vision-Dependent Changes in the Choroidal Thickness of Macaque Monkeys. Invest. Ophthalmol. Vis. Sci. 41(6):1259-1269.
- Ignarro LJ, Buga GM, Wood KS, Byrns RE, Chaudhuri G. 1987. Endothelium-derived relaxing factor produced and released from artery and vein is nitric oxide. Proceedings Of The National Academy Of Sciences Of The United States Of America 84(24):9265-9269.Invest Ophthalmol Vis Sci 38:4539.

- Iuvone P, Tigges M, Stone R, Lambert S, Laties A. 1991 Effects of apomorphine, a dopamine receptor agonist, on ocular refraction and axial elongation in a primate model of myopia. Invest Ophthalmol Vis Sci 32(5):1674-1677.
- Jaffe GJ, Caprioli J. 2004. Optical coherence tomography to detect and manage retinal disease and glaucoma. Am J Ophthalmol 137(1):156-169.
- Jensen H. 1992. Myopia progression in young school children and intraocular pressure. Doc Ophthalmol. 82(3):249-255.
- Jiang B. 1995. Parameters of accommodative and vergence systems and the development of late-onset myopia. Invest Ophthalmol Vis Sci 36(8):1737-1742.
- Jin N, Stjernschantz J. 2000. Regional Blood Flow in the Myopic Chick Eye during and after Form Deprivation: a Study with Radioactively-labelled Microspheres. Exp Eye Res 71(3):233-238.
- Johnson P, Henrich H. 1975. Metabolic and myogenic factors in local regulation of the microcirculation. Fed Proc 34(11):2020-2024.
- Jonas J, Peter M, Wido M. 2002. Anisometropia and degree of optic nerve damage in chronic open-angle glaucoma. Am J Ophthalmol 134(4): 547-551.
- Jones LA, Mitchell GL, Mutti DO, Hayes JR, Moeschberger ML, Zadnik L. 2005. Comparison of ocular component growth curves among refractive error groups in children. Invest Ophthalmol Vis Sci. 46(7):2317-27.
- Jonescu-Cuypersa C, Chungb H, Kagemannb L, Ishiib Y, Zarfatib D, Harris A. 2001. New neuroretinal rim blood flow evaluation method combining Heidelberg retina flowmetry and tomography. Br J Ophthalmol 85:304-309.
- Joos KM, Kay MD, Pillunat LE, Harris A, Gendron EK, Feuer WJ, Steinwand BE. 1999. Effect of acute intraocular pressure changes on short posterior ciliary artery haemodynamics. Br J Ophthalmol 83(1):33-38.
- Junghans B, Crewther S, Liang H, Crewther D. 1999. A role for choroidal lymphatics during recovery from form deprivation myopia? Optom Vis Sci. 76(11):796-803.
- Kaiser HJ, Schoetzau A, Flammer J. 1997. Blood flow velocity in the extraocular vessels in chronic smokers. Br J Ophthalmol 81(2):133-135.
- Kannel W, Dawber T, Kagan A, Revotskie N, Stokes Jr. 1961. Factors of risk in the development of coronary heart disease--six year follow-up experience. The Framingham Study. Ann Intern Med. 55(33-50).
- Kannel W, Gordan T. 1978. Evaluation of cardiovascular risk in the elderly: the Framingham study. Bull N Y Acad Med. 54(6):573-591
- Kannel WB, Lebauer EJ, Dawber TR, McNamara PM. 1967. Relation of Body Weight to Development of Coronary Heart Disease: The Framingham Study. Circulation 35(4):734-744.

- Karatzi K, Papamichael C, Karatzis E, Papaioannou TG, Voidonikola PT, Lekakis J, Zampelas A. 2007. Acute Smoking Induces Endothelial Dysfunction in Healthy Smokers. Is This Reversible by Red Wine's Antioxidant Constituents? J Am Coll Nutr 26(1):10-15.
- Kaufman P, Alm A. 2003. Adler's Physiology of the Eye. St. Louis, Missouri: Mosby.
- Kellogg DL, Jr., Pergola PE, Piest KL, Kosiba WA, Crandall CG, Grossmann M, Johnson JM. 1995. Cutaneous Active Vasodilation in Humans Is Mediated by Cholinergic Nerve Cotransmission. Circ Res 77(6):1222-1228.
- Kennedy R, Dyer J, Kennedy M, Parulkar S, Kurland L, Herman D, McIntire D, Jacobs D, Luepker R. 2000. Reducing the progression of myopia with atropine: a long term cohort study of Olmsted County students. Binocul Vis Strabismus 15(3):281-304.
- Kerr J, Nelson P, O'Brien C. 2003. Pulsatile ocular blood flow in primary open-angle glaucoma and ocular hypertension. Am J Ophthalmol 136(6):1106-1113.
- Kiel JW, Shepherd AP. 1992. Autoregulation of choroidal blood flow in the rabbit. Invest. Ophthalmol. Vis. Sci. 33(8):2399-2410.
- Kiel JW, van Heuven WA. 1995. Ocular perfusion pressure and choroidal blood flow in the rabbit. Invest. Ophthalmol. Vis. Sci. 36(3):579-585.
- Kiel JW. 1999. Modulation of Choroidal Autoregulation in the Rabbit. Exp Eye Res69(4):413-429.
- Kim JW, Park CG, Hong SJ, Park SM, Rha SW, Seo HS, Oh DJ, Rho YM. 2005. Acute and chronic effects of cigarette smoking on arterial stiffness. Blood Pressure 14(2):80-85.
- Kimura I, Shinoda K, Tanino T, Ohtake Y, Mashima Y, Oguchi Y. 2003. Scanning laser Doppler flowmeter study of retinal blood flow in macular area of healthy volunteers. Br J Ophthalmol 87(12):1469-1473.
- Kiowski W, Linder L, Stoschitzky K, Pfisterer M, Burckhardt D, Burkart F, Buhler F. 1994. Diminished vascular response to inhibition of endothelium-derived nitric oxide and enhanced vasoconstriction to exogenously administered endothelin-1 in clinically healthy smokers. Circulation 90((1):27-34.
- Kiss B, Dallinger S, Findl O, Rainer G, Eichler H, Schmetterer L. 1999. Acetazolamide-induced cerebral and ocular vasodilation in humans is independent of nitric oxide. Am. J. Physiol. 276(6, Part 2):R1661-R1667.
- Klabunde R. 2004. Cardiovascular Physiology Concepts: Lippincott Williams & Wilkins.
- Klatt K, Langenbucher A, Seitz B, Nguyen N. 2006. Accommodation ability under the aspect of refractive, demographic, and biometric parameters. Ophthalmologe.
- Klein R, Klein BEK, Linton KLP, DeMets DL. 1993. The Beaver Dam Eye Study: The Relation of Age-related Maculopathy to Smoking. Am. J. Epidemiol. 137(2):190-200

- Kleinman MT, Davidson DM, Vandagriff RB, Caiozzo VJ, Whittenberger JL. 1989. Effects of short-term exposure to carbon monoxide in subjects with coronary artery disease. Arch. Environ Health 44(6):361-369.
- Kleinstein R, Jones L, Hullett S, Kwon S, Lee R, Friedman N, Manny R, Mutti D, Yu J, Zadnik K and others. 2003. Refractive error and ethnicity in children. Arch Ophthal 121(8):1141-1147.
- Knobloch WH, Leavenworth NM, Bouchard TJ, Eckert ED. 1985. Eye findings in twins reared apart. Ophthalmic Paediatr Genet. 5 (1-2):59-66.
- Kolodjaschna J, Berisha F, Lung S, Schima H, Polska E, Schmetterer L. 2005. Comparison of the Autoregulatory Mechanisms between Middle Cerebral Artery and Ophthalmic Artery after Thigh Cuff Deflation in Healthy Subjects. Invest. Ophthalmol. Vis. Sci. 46(2):636-640.
- Kontos HA. 1989. Validity of cerebral arterial blood flow calculations from velocity measurements. Stroke 20(1):1-3.
- Koss MC. 1999. Functional role of nitric oxide in regulation of ocular blood flow. Eur J Pharmacol Volume 374(Issue 2):Pages 161-174.
- Lakatta E, Gerstenblith G, Weisfeldt M. 1997. The aging heart: structure, function, and disease. In: Braunwald E, editor. A Textbook of Cardiovascular Medicine. Philadelphia: Heart Disease. p 1687-1703.
- Lakatta E. 1994. Aging effects on the vasculature in health: risk factors for cardiovascular disease. American Journal of Geriatric Cardiology 3(11-17).
- Lakier J. 1992. Smoking and cardiovascular disease. Am J Med Genet 93 (1A):8S-12S.
- Lam A, Chan S, Chan B, Chan H. 2003. The effect of axial length on ocular blood flow assessment in anisometropes. Ophthalmic Physiol Opt 23(4): 315.
- Lam A, Wong S, CSY L, Chi-To T. 2002. The effect of myopic axial elongation and posture on the pulsatile ocular bloof flow in the young normal subjects. Optom Vis Sci 79(5).
- Lam C, Goh W. 1991. The incidence of refractive errors among schoolchildren in Hong Kong and its relationship with the optical components. Clin Exp Optom 74:97-103.
- Lam D, Tam P, Fan D, Baum L, Leung Y, Pang C. 2003 Familial high myopia linkage to chromosome 18p. Ophthalmologica 217(2):115-118.
- Lam DSC, Lee WS, Leung YF, Tam POS, Fan DSP, Fan BJ, Pang CP. 2003b. TGF{beta}-Induced Factor: A Candidate Gene for High Myopia. Invest. Ophthalmol. Vis. Sci. 44(3):1012-1015.
- Lane B. 1982. Myopia prevention and reversal: new data confirms the interaction of accommodative stress and deficit-inducing nutrition. J Int Acad Prev Med 17-30.
- Langham M, Farrell R, O'Brien V, Silver D, Schilder P. 1989. Blood flow in the human eye. Acta Ophthalmol Suppl. 191:9-13.

- Langham M, Kramer T. 1990. Decreased choroidal blood flow associated with retinitis pigmentosa. Eye 4 (Pt 2):374-381.
- Langham ME, Grebe R, Hopkins S, Marcus S, Sebag M. 1991. Choroidal blood flow in diabetic retinopathy. Exp Eye Res 52(2):167-173.
- Langhans M, Michelson G, Groh MJM. 1997. Effect of breathing 100% oxygen on retinal and optic nerve head capillary blood flow in smokers and non-smokers. Br J Ophthalmol 81(5):365-369.
- Larsen J. 1971. The sagittal growth of the eye. IV. Ultrasonic measurement of the axial length of the eye from birth to puberty. Acta Ophthalmol (Copenh) 49(873-86).
- Laties A. 1967. Central retinal artery innervation. Absence of adrenergic innervation to the intraocular branches. Arch Ophthalmol 77(3):405-409
- Lee J, Fang P, Yang I, Chen C, Lin P, Lin S, Kuo H, Wu P. 2006 Prevention of myopia progression with 0.05% atropine solution. J Ocul Pharmacol Ther. 22(1):41-46.
- Lekskul M, Aimpun P, Nawanopparatskul B, Bumrungsawat S, Trakulmungkijkarn T, Charoenvanichvisit J, Herunpattarawong T, Suksangthong P, Jaiprasat T, Rattananantapat M. 2005. The correlations between Central Corneal Thickness and age, gender, intraocular pressure and refractive error of aged 12-60 years old in rural Thai community. J Med Assoc Thai. 88(3):S175-179.
- Leuenberger UA, Gray K, Herr MD. 1999. Adenosine contributes to hypoxia-induced forearm vasodilation in humans. J Appl Physiol 87(6):2218-2224.
- Levinsohn G. 1929. Die Entstehung der Kurzsichtigkeit Arch. Augenheilk. 100:138-163.
- Lietz-Partzsch A, Griesser S, Flammer J, Haefliger I. 2001. Decreased Heidelberg Retina Flowmeter (HRF) parameter flow at the papilla shortly after smoking a cigarette. Klin Monatsbl Augenheilkd.(5):332-334.
- Lin L, Shih Y, Lee Y, Hung P, Hou P. 1996. Changes in ocular refraction and its components among medical students--a 5-year longitudinal study. Optom Vis Sci. 73(7):495-498.
- Lin LL, Shih YF, Tsai CB, Chen CJ, Lee LA, Hung PT, Hou PK. 1999. Epidemiologic study of ocular refraction among school children in Taiwan in 1995. Optom Vis Sci. 76(5):275-281.
- Liu CJ, Chiou H-J, Chiang S-C, Chou JC, Chou Y-H, Liu J-H. 1999. Variations in ocular hemodynamics in patients with early and late glaucoma. Acta Ophthalmologica Scandinavica 77(6):658-662.
- Logan NS, Gilmartin B, Dunne MC. 1995. Computation of retinal contour in anisomyopia. Ophthalmic Physiol Opt. 15(5):263-6.
- Logan NS. 1997. Ocular biometric investigation of anisometropia. PhD, Aston University, Birmingham, UK

- Logan NS, Gilmartin B, Cox W. Ocular volume and blood flow in human anisomyopia; 2002. IOVS conference abstract
- Logan NS, Gilmartin B, Wildsoet CF, Dunne MCM. 2004. Posterior Retinal Contour in Adult Human Anisomyopia. Invest. Ophthalmol. Vis. Sci. 45(7):2152-2162.
- Logan NS, Davies LN, Mallen EA, Gilmartin B. 2005. Ametropia and ocular biometry in a UK university student population. Optom Vis Vis Sci. 82(4):261-6.
- Longo A, Geiser M, Riva C. 2004. Posture Changes and Subfoveal Choroidal Blood Flow. Invest. Ophthalmol. Vis. Sci. 45:546-551.
- Longo A, Geiser M, Riva CE. 2000. Subfoveal Choroidal Blood Flow in Response to Light-Dark Exposure. Invest. Ophthalmol. Vis. Sci. 41(9):2678-2683.
- Luft WA, Ming Y, Stell WK. 2003. Variable Effects of Previously Untested Muscarinic Receptor Antagonists on Experimental Myopia. Invest. Ophthalmol. Vis. Sci. 44(3):1330-1338.
- Luksch A, Garhofer G, Imhof A, Polak K, Polska E, Dorner GT, Anzenhofer S, Wolzt M, Schmetterer L. 2002. Effect of inhalation of different mixtures of O2 and CO2 on retinal blood flow. Br J Ophthalmol 86(10):1143-1147.
- Luksch A, Polak K, Beier C, Polska E, Wolzt M, Dorner G, Eichler H, Schmetterer L. 2000. Effects of Systemic NO Synthase Inhibition on Choroidal and Optic Nerve Head Blood Flow in Healthy Subjects. Invest. Ophthalmol. Vis. Sci 41:3080-3084.
- Luo H-D, Gazzard G, Fong A, Aung T, Hoh ST, Loon S-C, Healey P, Tan DTH, Wong T-Y, Saw S-M. 2006. Myopia, Axial Length, and OCT Characteristics of the Macula in Singaporean Children. Invest. Ophthalmol. Vis. Sci. 47(7):2773-2781.
- Maddock R, Millodot M, Leat S, Johnson C. 1981 Accommodation responses and refractive error. 20 3(387-91).
- Maepea O. 1992. Pressure in the anterior ciliary artery, choroidal veins and choriocapillaries. Ex Eye Res 54:731.
- Mahmud A, Feely J. 2003. Effect of Smoking on Arterial Stiffness and Pulse Pressure Amplification. Hypertension 41(1):183-187.
- Mallen E, Gilmartin B, Wolffsohn J. 2005. Sympathetic innervation of ciliary muscle and oculomotor function in emmetropic and myopic young adults. Vision Res 45:1641-1651.
- Mann SJ, James GD, Wang RS, Pickering TG. 1991. Elevation of ambulatory systolic blood pressure in hypertensive smokers. A case-control study. JAMA 265(17):2226-2228.
- Mansberger S, Harris A, Arend O. 1993. Small PCO2 changes significantly alter retinal flow indices as measured by the scanning laser ophthalmoscope. Invest Ophthalmol Vis Sci 34:1395.
- Martinez A, Gonzalez F, Capeans C, Perez R, Sanchez-Salorio M. 1999. Dorzolamide effect on ocular blood flow. Invest. Ophthalmol. Vis. Sci. 40(6):1270-1275.

- Massin P, Vicaut E, Haouchine B, Erginay A, Paques M, Gaudric A. 2001. Reproducibility of retinal mapping using optical coherence tomography. Arch Ophthalmol 119(8):1135-1142.
- Mathis U, Schaeffel F. 2006. Glucagon-related peptides in the mouse retina and the effects of deprivation of form vision. Graefe's archive for clinical and experimental ophthalmology.
- Matsumura H, Hirai H. 1999. Prevalence of myopia and refractive changes in students from 3 to 17 years of age. Surv Ophthalmol. 44(Suppl 1):S109-115.
- Mc Neil N. 1955. Patterns of visual defects in children. Br J Ophthalmol 39:688-701.
- McBrien N, Millodot M. 1986 Amplitude of accommodation and refractive error. Invest. Ophthalmol. Vis. Sci 27(7):1187-1190.
- Medeiros F, Weinreb R. 2002. Medical backgrounders: glaucoma. Drugs Today (Barc) 38(8):563-570.
- Melin A, Bonnet P, Eder V, Antier D, Obert P, Fauchier L. 2005. Direct implication of carbon monoxide in the development of heart failure in rats with cardiac hypertrophy subjected to air pollution. Cardiovascular Toxicology 5(3):311-320.
- Michael C. 1999. Functional role of nitric oxide in regulation of ocular blood flow. Eur J Pharmacol 374(2): 161-174.
- Michelson G, Groh M, Grundler A. 1994. Regulation of ocular blood flow during increases of arterial blood pressure. Br J Ophthalmol 78(6):461-465.
- Michelson G, Schmauss B, Langhans M, Harazny J, Groh M. 1996. Principle, validity, and reliability of scanning laser Doppler flowmetry. J Glaucoma. 5(2):99-105.
- Mirza A, Eder V, Rochefort GY, Hyvelin JM, Machet MC, Fauchier L, Bonnet P. 2005. CO inhalation at dose corresponding to tobacco smoke worsens cardiac remodeling after experimental myocardial infarction in rats. Toxicol Sci 85(2):976-982.
- Mitchell P, Hourihan F, Sandbach J, Wang J. 1999. The relationship between glaucoma and myopia. The blue mountains eye study. Ophthalmology 106(10): 2010-2015.
- Monfrecola G, Riccio G, Savarese C, Posteraro G, Procaccini E. 1998. The Acute Effect of Smoking on Cutaneous Microcirculation Blood Flow in Habitual Smokers and non-smokers. Dermatol 197(2):115-118.
- Morgado P, Chen H, Patel V, Herbert L, Kohner E. 1994. The acute effect of smoking on retinal blood flow in subjects with and without diabetes. Ophthalmology 101(7):1220-1226.
- Morgan IG. 2003. The biological basis of myopic refractive error. Clin Exp Optom. 86(5):276-288.
- Morgan IG, Rose K. 2005. How genetic is school myopia?. Prog Retin Eye Res. 24(1):1-38.
- Morgan A, Harper J, Hosking S, Gilmartin B. 2002. The effect of corneal thickness and corneal curvature on pneumatonometer measurements. Curr Eye Res. 25(2):107-112.

- Mori F, Konno S, Hikichi T, Yamaguchi Y, Ishiko S, Yoshida A. 2001. Factors affecting pulsatile ocular blood flow in normal subjects. Br J Ophthalmol 85:529-530.
- Mori F, Konno S, Hikichi T, Yamaguchi Y, Ishiko S, Yoshida A. 2001. Factors affecting pulsatile ocular blood flow in normal subjects. Br J Ophthalmol 85:529-530.
- Movaffaghy A, Lochhead J, Riva CE, Harding SP, Petrig BL, Molyneux ME, Taylor TE. 2002. Feasibility of LDF Measurements of Optic Nerve Head Blood Flow in Children with Cerebral Malaria. Microvas Res64(2):247-253.
- Muscat S, Parks S, Kemp E, Keating D. 2002. Repeatability and Reproducibility of Macular Thickness Measurements with the Humphrey OCT System. Invest. Ophthalmol. Vis. Sci. 43(2):490-495
- Mutti DO, Hayes JR, Mitchell GL, Jones LA, Moeschberger ML, Cotter SA, Kleinstein RN, Manny RE, Twelker JD, Zadnik K. 2007. Invest Ophthalmol Vis Sci. 48(6):2510-19.
- Mutti DO, Mitchell GL, Hayes JR, Jones LA, Moeschberger ML, Cotter SA, Kleinstein RN, Manny RE, Twelker JD, Zadnik K. 2006. Accommodative Lag before and after the Onset of Myopia. Invest. Ophthalmol. Vis. Sci. 47(3):837-846.
- Mutti DO, Mitchell GL, Moeschberger ML, Jones LA, Zadnik K. 2002. Parental Myopia, Near Work, School Achievement, and Children's Refractive Error. Invest Ophthalmol Vis Sci 43(12):3633-3640.
- Myers PR, Tanner MA. 1998. Vascular Endothelial Cell Regulation of Extracellular Matrix Collagen : Role of Nitric Oxide. Arterioscler Thromb Vasc Biol 18(5):717-722.
- Nagel E, Vilser W, Lanzl I. 2002. Functional analysis of retinal vessel diameter reaction to artificially raised intraocular pressure in glaucoma patients with and without dorzolamide therapy. Vasa. 31(4):230-234.
- Nagy Z, Kricsfalvi P, Hargitai M, Molnar L. 2006. Vascular risk factors in healthy population in the repeated screening examinations. Orv Hetil. 147(35):1675-1684.
- Narkiewicz K, van de Borne PJH, Hausberg M, Cooley RL, Winniford MD, Davison DE, Somers VK. 1998. Cigarette Smoking Increases Sympathetic Outflow in Humans. Circulation 98(6):528-534.
- Nemeth J, Knezy K, Tapaszto B, Kovacs R, Harkanyi Z. 2002. Different autoregulation response to dynamic exercise in ophthalmic and central retinal arteries: a color Doppler study in healthy subjects. Graefes Arch Clin Exp Ophthalmol. 240(10):835-840.
- Nemeth J, Michelson G, Harazny J. 2001. Retinal Microcirculation Correlates With Ocular Wall Thickness, Axial Eye Length, and Refraction in Glaucoma Patients. J Glaucoma 10:390-395.
- Nesterov A, Bervin A, Katsnelson L. 1978. Intraocular pressure. Moscow: MIR Publishers.
- Newell DW, Aaslid R, Lam A, Mayberg TS, Winn HR. 1994. Comparison of flow and velocity during dynamic autoregulation testing in humans. Stroke 25(4):793-797.

- Nicolela MT, Walman BE, Buckley AR, Drance SM. 1996. Various glaucomatous optic nerve appearances. A color Doppler imaging study of retrobulbar circulation. Ophthalmology. 103(10):1670-9.
- Nicolela MT, Walman BE, Buckley AR, Drance SM. 1996. Ocular hypertension and primary open-angle glaucoma: a comparative study of their retrobulbar blood flow velocity. J Glaucoma. 5 (5):308-10
- Niden AH, Schulz H. 1965. The ultrastructural effects of carbon monoxide inhalation on the rat lung. Virchows Arch Pathol Anat Physiol Klin Med 339(4):283-292.
- Nilsson M, Stenlund H, Bergstrom E, Weinehall L, Janlert U. 2006. It takes two: reducing adolescent smoking uptake through sustainable adolescent-adult partnership. Int J Adolesc Med Health 39(6):880-886
- Niwa Y, Harris A, Kagemann L, Yamamoto T, Matsubara M, Takahashi D, Kitazawa Y. 1999. A New System to Supply Carbon Dioxide Safely to Glaucoma Patients. Japanese Journal of Ophthalmology 43(1):16-19.
- Ofri R, Millodot S, Shimoni R, Horowitz I, Ashash E, Millodot M. 2001. Development of the refractive state in eyes of ostrich chicks (Struthio camelus). Am J Vet Res 62(5):812-815.
- Ohno-Matsui K, Yoshida T, Futagami S, Yasuzumi K, Shimada N, Kojima A, Tokoro T, Mochizuki M. 2003. Patchy atrophy and lacquer cracks predispose to the development of choroidal neovascularisation in pathological myopia. Br J Ophthalmol 87(5):570-573.
- Olivetti G, Melissari M, Capasso JM, Anversa P. 1991. Cardiomyopathy of the aging human heart. Myocyte loss and reactive cellular hypertrophy. Circ Res 68(6):1560-1568
- Olivier M. 2004. Knobloch syndrome: Novel mutations in COL18A1, evidence for genetic heterogeneity, and a functionally impaired polymorphism in endostatin. Human Mutation 23(1):77-84.
- Olver JM. 1990. Microvascular study of the retrolaminar optic nerve in man: the possible significance in anterior ischemic optic neuropathy. Eye 4:7.
- Pacella R, McLellan J, Grice K, Del Bono E, Wiggs J, Gwiazda J. 1999. Role of genetic factors in the etiology of juvenile-onset myopia based on a longitudinal study of refractive error. Optom Vis Sci 76(6):381-386.
- Paluru PC, Nallasamy S, Devoto M, Rappaport EF, Young TL. 2005. Identification of a Novel Locus on 2q for Autosomal Dominant High-Grade Myopia. Invest. Ophthalmol. Vis. Sci. 46(7):2300-2307.
- Papakitsou EF, Margioris AN, Dretakis KE, Trovas G, Zoras U, Lyritis G, Dretakis EK, Stergiopoulos K. 2004. Body mass index (BMI) and parameters of bone formation and resorption in postmenopausal women. Maturitas 47(3):185-193.

- Papastergiou GI, Schmid GF, Riva CE, Mendel MJ, Stone RA, Laties AM. 1998. Ocular Axial Length and Choroidal Thickness in Newly Hatched Chicks and One-year-old Chickens Fluctuate in a Diurnal Pattern that is Influenced by Visual Experience and Intraocular Pressure Changes. Exp Eye Res66(2):195-205.
- Park C, Pardhasaradhi K, Gianotti C, Villegas E, Krishna G. 1994. Human Retina Expresses both Constitutive and Inducible Isoforms of Nitric Oxide Synthase mRNA. Biochem Biophys Res Commun205(1):85-91.
- Parnell R. 1951. Sight of undergraduates. British Journal of Ophthalmology 35:467-472.
- Passos-Bueno M, Marie S, Monteiro M, Neustein I, Whittle M, Vainzof M, Zatz M. 1994. Knobloch syndrome in a large Brazilian consanguineous family: confirmation of autosomal recessive inheritance. Am J Med Genet 15(52):2.
- Patton N, Maini R, MacGillivary T, Aslam TM, Deary IJ, Dhillon B. 2005. Effect of Axial Length on Retinal Vascular Network Geometry. Am J Ophthalmol140(4):648.e641-648.e647.
- Paulson OBS, S; Edvinsson, L. 1990. Cerebral autoregulation. Cerebrovascular And Brain Metabolism Reviews 2(2): 161-192.
- Pendrak K, Nguyen T, Lin T, Capehart C, Zhu X, Stone RA. 1997. Retinal dopamine in the recovery from experimental myopia. Curr Eye Res 16(2):152 - 157.
- Penn A, Currie J, Snyder C. 1992. Inhalation of carbon monoxide does not accelerate arteriosclerosis in cockerels. Eur. J. Pharmacol. Environ. Toxicol. Pharmacol. Sect. 228(2-3):155-164.
- Petropoulos I, Pournaras C. 2005. Effect of indomethacin on the hypercapnia-associated vasodilation of the optic nerve head vessels: an experimental study in miniature pigs. Ophthalmic Res 37(2):59-66.
- Pillunat L, Lang G, Harris A. 1994. The visual response to increased ocular blood flow in normal pressure glaucoma. Surv Ophthalmol. 38(Suppl:S139-47;):S147-148.
- Polska E, Kircher K, Ehrlich P, Vecsei P, Schmetterer L. 2001. RI in central retinal artery as assessed by CDI does not correspond to retinal vascular resistance. Am J Physiol Heart Circ Physiol 280:H1442-H1447.
- Polska E, Polak K, Luksch A, Fuchsjager-Mayrl G, Petternel V, Findl O, Schmetterer L. 2004. Twelve hour reproducibility of choroidal blood flow parameters in healthy subjects. Br J Ophthalmol 88(4):533-537.
- Pomerleau CS, Pomerleau OF, Majchrzak MJ, Kloska DD, Malakuti R. 1990. Relationship between nicotine tolerance questionnaire scores and plasma cotinine. Addictive Behaviors 15(1):73-80.
- Pourcelot L. 1975. Indications de l'ultrasonographie Doppler dans l'etude des vaisseaux peripheriques. Rev. Practicien 25:4671-4680.

- Pournaras CJ, Logean E, Riva CE, Petrig BL, Chamot SR, Coscas G, Soubrane G. 2006. Regulation of Subfoveal Choroidal Blood Flow in Age-Related Macular Degeneration. Invest. Ophthalmol. Vis. Sci. 47(4):1581-1586.
- Priebe HJ. 2000. The aged cardiovascular risk patient. Br. J. Anaesth. 85(5):763-778.
- Primatesta P, Falaschetti E, Gupta S, Marmot MG, Poulter NR. 2001. Association Between Smoking and Blood Pressure: Evidence From the Health Survey for England. Hypertension 37(2):187-193.
- Pruett R. 1988. Progressive myopia and intraocular pressure: what is the linkage? A literature review. Acta Ophthalmol Suppl 185:117-127.
- Pugh K, Wei J. 2001. Clinical Implications of Physiological Changes in the Aging Heart. Drugs & Aging 18(4):263-276.
- Quinn G, Berlin J, Young T, Ziylan S, Stone R. 1995. Association of intraocular pressure and myopia in children. Ophthalmology 102(2):180-185.
- Rabsilber TM, Khoramnia R, Auffarth GU. 2006. Anterior chamber measurements using Pentacam rotating Scheimpflug camera. J Cataract Refract Surg 32(3):456-459.
- Rai G. 2007. Accommodation and Intraocular pressure. Birmingham, UK: Aston University.
- Rai GK, Gilmartin B, Wolffsohn JW. 2007 The effect of accommodation on pulsatile ocular blood flow. ARVO 2007 Annual meeting.
- Ramrattan RS, van der Schaft TL, Mooy CM, de Bruijn WC, Mulder PG, de Jong PT. 1994. Morphometric analysis of Bruch's membrane, the choriocapillaris, and the choroid in aging. Invest. Ophthalmol. Vis. Sci. 35(6):2857-2864.
- Rassam S, Patel V, Kohner E. 1993. The effect of acetazolamide on the retinal circulation. Eye 7 (5):697-702.
- Ravalico G, Pastori G, Croce M, Toffoli G. 1997. Pulsatile ocular blood flow variations with axial length and refractive error. Ophthalmologica. 211(5):271-273.
- Ravalico G, Toffoli G, Pastori G, Crocé M, Calderini S. 1996. Age-related ocular blood flow
- Raviola E, Wiesel T. 1985. An animal model of myopia. N Engl J Med 312(25):1609-1615.
- Ray W, O'Day D. 1985. Statistical analysis of multi-eye data in ophthalmic research. Invest Ophthalmol Vis Sci. Aug;. Links
- Reiner A, Shih Y, Fitzgerald M. 1995. The relationship of choroidal blood flow and accommodation to the control of ocular growth. Vision Res 35(9):1227-1245.
- Remington L. 1997. Clinical Anatomy of the Visual System: Butterworth Heinemann.
- Renkin E, Michel C. 1984. Handbook of Physiology: The Cardiovascular System Volume, Microcirculation. Bethesda M, editor: American Physiological Society.

- Ringelstein E, Van Eyck S, Mertens I. 1992. Evaluation of cerebral vasomotor reactivity by various vasodilating stimuli: comparison of CO2 to acetazolamide. Cereb Blood Flow Metab 34(2):398-410
- Riva C, Cranstoun S, Grunwald J, Petrig B. 1994. Choroidal blood flow in the foveal region of the human ocular fundus. Invest Ophthalmol Vis Sci 35(13)::4273-4281.
- Riva C, Grunwald J, Petrig B. 1986. Autoregulation of human retinal blood flow. An investigation with laser Doppler velocimetry. Invest. Ophthalmol. Vis. Sci. 27(12):1706-1712.
- Riva C, Hero M, Titze P, Petrig B. 1997. Autoregulation of human optic nerve head blood flow in response to acute changes in ocular perfusion pressure. Graefes Arch Clin Exp
- Riva C, Ross B, Benedek G. 1972. Laser Doppler measurements of blood flow in capillary tubes and retinal arteries. Invest Ophthalmol Vis Sci 11(11):936-944.
- Riva C. 1990. Rhythmic changes in velocity, volume and blood flow in the optic nerve head tissue. Microvasc Res 40:36-45.
- Riva CE, Logean E, Falsini B. 2005. Visually evoked hemodynamical response and assessment of neurovascular coupling in the optic nerve and retina. Prog Retin Eye Res24(2):183-215.
- Robinson B. 1999. Factors associated with the prevalence of myopia in 6-year-olds. Optom Vis Sci. 76(5):266-271.
- Robinson F, Petrig B, Riva C. 1985. The acute effect of cigarette smoking on macular capillary blood flow in humans. Invest Ophthalmol Vis Sci 26(5):609-613.
- Rochtchina E, Mitchell P, Wang JJ. 2002. Relationship between age and intraocular pressure: the Blue Mountains Eye Study. Clinical and Experimental Ophthalmology 30(3):173-175.
- Roff E, Harris A, Sung-Chung H, Hosking S, Morrison A, Halter P, Jagemann L. 1999. Comprenhensive assessment of retinal, choroidal and retrobulbar haemodynamics during blood gas perturbation. Graefe's Arch Clin Exp Ophthalmol 237:984-990.
- Roff Hilton EJ, Hosking SL, Cubbidge RP, Morgan AJ. 2003. Regional variability in visual field sensitivity during hypercapnia. Am J Ophthalmol136(2):272-276.
- Rose L, Yinon U, Belkin M. 1974. Myopia induced in cats deprived of distance vision during development. Vision Res. 14(10):1029-1032.
- Rose LT, Moshegov CN. 2003. Comparison of the Zeiss IOLMaster and applanation A-scan ultrasound: biometry for intraocular lens calculation. Clin Exp Ophthalmol 31(2):121-124.
- Rosenfield M, Ciuffreda K. 1994. Cognitive demand and transient nearwork-induced myopia. Optom Vis Sci 71(6):381-385.

- Rosenfield M, Cohen A. 1996 Repeatability of clinical measurements of the amplitude of accommodation. Ophthalmic Physiol Opt 16(3):247-249.
- Rosenfield M, Gilmartin B. 1988. Disparity-induced accommodation in late-onset myopia. Ophthalmic Physiol Opt 8(3):353-355.
- Rosenfield M,Gilmartin B. 1998. Myopia and Nearwork. Oxford: Butterworth-Heinemann.
- Rosner M, Laor A, Belkin M. 1995 Myopia and stature: findings in a population of 106,926 males. Eur J Ophthalmol. 5(1):1-6.
- Rouviere H. 1976. Précis d'anatomie et de dissection.
- Sander B, Larsen M, Thrane L, Hougaard JL, Jorgensen TM. 2005. Enhanced optical coherence tomography imaging by multiple scan averaging. Br J Ophthalmol 89(2):207-212.
- Santodomingo-Rubido J, Mallen EAH, Gilmartin B, Wolffsohn JS. 2002. A new non-contact optical device for ocular biometry. Br J Ophthalmol 86(4):458-462.
- Satomura S. 1957. Ultrasonic Doppler method for the inspection of cardiac function. J. Acoust Soc Am 29:1181-1185.
- Saunders KJ, McCulloch DL, Shepherd AJ, Wilkinson AG. 2002. Emmetropisation following preterm birth. Br J Ophthalmol 86(9):1035-1040.
- Saw S. 2006. How blinding is pathological myopia?.Br J Ophthalmol. 90(5):525-6.
- Saw S, Chua W, Gazzard G, Koh D, Tan D, Stone R. 2005. Eye growth changes in myopic children in Singapore. Br J Ophthalmol. 89(11):1489-1494. .
- Saw S, Gazzard G, KG A-E, Tan D. 2002. Myopia: Attempts to arrest progression. Br J Ophthalmol86:1306-1311.
- Saw S, Katz J, Schein O, Chew S, Chan T. 1996. Epidemiology of myopia. Epidemiol Rev 18(2):175-187.
- Saw S-M, Gazzard G, Shih-Yen EC, Chua W-H. 2005. Myopia and associated pathological complications. Ophthalmic Physiol Opt25(5):381-391.
- Schachar R, Cudmore D, Black T. 1993a. Experimental support for Schachar's hypothesis of accommodation. Ann Ophthalmol 25(11):404-409.
- Schachar R, Cudmore D, Torti R, Black T, Huang T. 1994. A physical model demonstrating Schachar's hypothesis of accommodation. 26 1(4-9).
- Schachar R, Huang T, Huang X. 1993b. Mathematic proof of Schachar's hypothesis of accommodation. Ann Ophthalmol 25(1):5-9.
- Schaefer O. 1971. When the Eskimo Comes to Town. Nutrition Today 6:8-16.
- Schaefer O. 1977. Changing dietary patterns in the Canadian North: Health, social and economic consequences. J Can Diet Assoc 38:17-25.
- Schaeffel F, Burkhardt E. 2005. Pupillographic evaluation of the time course of atropine effects in the mouse eye. Optom Vis Sci. 82(3):215-220.

- Schaeffel F, Glasser A, Howland H. 1988. Accommodation, refractive error and eye growth in chickens. 28(5):639-657.
- Schaeffel F, Simon P, Feldkaemper M, Ohngemach S, Williams R. 2003. Molecular biology of myopia. Clin Exp Optom 86(5):295-307.
- Schaeffel F, Troilo D, Wallman J, Howland H. 1990. Developing eyes that lack accommodation grow to compensate for imposed defocus. Vis Neurosci 4(2):177-183.
- Schemetterer L, Lexer F, FindlL O, Graselli U, Eichler H, Woltz M. 1996. The Effect of Inhalation of Different Mixtures of O2and CO2on Ocular Fundus Pulsations. Exp Eye Res 63(4):351-355.
- Schmetterer L, Findl O, Strenn K, Graselli U, Kastner J, Eichler HG, Wolzt M. 1997. Role of NO in the O2 and CO2 responsiveness of cerebral and ocular circulation in humans. The Am J Physiol 273(6): 2005-R2012.
- Schmetterer L, Wolzt M, Lexer F, Alschinger C, Gouya G, Zanaschka G, Fassolt A, Eichler H, Fercher A. 1995. The effect of hyperoxia and hypercapnia on fundus pulsations in the macular and optic disc region in healthy young men. Exp Eye Res 61(6):685-690.
- Schmetterer L. 1998. Noninvasive investigations of the normal ocular circulation in humans. Invest Ophthalmol Vis Sci, 39:1210.
- Schmid G. 2003. Variability of retinal steepness at the posterior pole in children 7-15 years of age. Curr Eye Res27(1):61-68.
- Schorderet M, Sovilla J, Magistretti P. 1981. VIP-and glucagon-induced formation of cyclic AMP in intact retinae in vitro. European journal of clinical pharmacology. 71(1):131-133.
- Scott E. 1956. Nutrition of Alaskan Eskimos. Nutrition Reviews 14:1-3.
- Seidemann A, Schaeffel F, Guirao A, Lopez-Gil N, Artal P. 2002. Peripheral refractive errors in myopic, emmetropic, and hyperopic young subjects. J Opt Soc Am A Opt Image Sci Vis 19(12):2363-2373.
- Seltner R, Stell W. 1995. The effect of vasoactive intestinal peptide on development of form deprivation myopia in the chick: a pharmacological and immunocytochemical study. Vision Res 35(9):1265-1270.
- Seltzer C. 1974. Effect of smoking on blood pressure. Am Heart J. 87(5):558-564.
- Shaikh A, Siegwart JJ, Norton T. 1999 Effect of interrupted lens wear on compensation for a minus lens in tree shrews. Optom Vis Sci 76(5):308-315.
- Sheng H, Bottjer C, Bullimore M. 2004. Ocular component measurement using the Zeiss IOLMaster. Optom Vis Sci. 81(1):27-34.
- Shih Y, Fitzgerald M, Norton T, Gamlin P, Hodos W, Reiner A. 1993a. Reduction in choroidal blood flow occurs in chicks wearing goggles that induce eye growth toward myopia. Curr Eye Res. 12(3):219-227.

- Shih Y, Horng I, Yang C, Lin L, Peng Y, Hung P. 1991. Ocular pulse amplitude in myopia. J Ocul Pharmacol. 7(1):83-87.
- Shimada N, Ohno-Matsui K, Harino S, Yoshida T, Yasuzumi K, Kojima A, Kobayashi K, Futagami S, Tokoro T, Mochizuki M. 2004. Reduction of retinal blood flow in high myopia. Graefes Arch Clin Exp Ophthalmol. 242(4):284-288.
- Shimmyo M, Ross AJ, Moy A, Mostafavi R. 2003. Intraocular pressure, Goldmann applanation tension, corneal thickness, and corneal curvature in Caucasians, Asians, Hispanics, and African Americans. Am J Ophthalmol 136(4):603-613.
- Shiose Y. 1984. The aging effect on intraocular pressure in an apparently normal population. Arch Ophthalmol 102(6):883-887
- Silver D, Geyer O. 2000. Pressure-volume relation for the living human eye. Curr Eye Res 20(2):115-120.
- Singh KD, Logan NS, Gilmartin B. 2006. Three-Dimensional Modeling of the Human Eye Based on Magnetic Resonance Imaging. Invest. Ophthalmol. Vis. Sci. 47(6):2272-2279.
- Simple Interactive Statistical Analysis, SISA.Sep-May2005< http://home.clara.net/sisa/>.
- Smith Er, Hung L, Kee C, Qiao Y. 2002. Effects of brief periods of unrestricted vision on the development of form-deprivation myopia in monkeys. Invest Ophthalmol Vis Sci 43(2):291-299.
- Smith Er, Maguire G, Watson J. 1980. Axial lengths and refractive errors in kittens reared with an optically induced anisometropia. Invest Ophthalmol Vis Sci 19(10):1250-1255.
- Smith W, Assink J, Klein R, Mitchell P, Klaver CCW, Klein BEK, Hofman A, Jensen S, Wang JJ, de Jong PTVM. 2001. Risk factors for age-related macular degeneration: Pooled findings from three continents. Ophthalmology 108(4):697-704.
- Solberg Y, Rosner M, Belkin M. 1998. The Association Between Cigarette Smoking and Ocular Diseases. Surv Ophthalmol 42(6):535-547.
- Sorsby A, Benjamin B, Davey J, Sheridan M, Tanner J. 1957. Emmetropia and its aberrations: a study of the correlations of the optical components of the eye. London. Report nr 293.
- Sorsby A, Sheridan M, Leary G. 1961. Refraction and its components during the growth of the eye from the age of three. London: HMSO. Report nr 301.
- Spencer J, Giussani D, Moore P, Hanson M. 1991. In vitro validation of Doppler indices using blood and water. J Ultrasound Med. 10(6):305-308.
- Stefansson E, Pedersen D, Jensen P, la Cour M, Kiilgaard J, Bang K, Eysteinsson T. 2005. Optic nerve oxygenation. Prog Retin Eye Res 24(3):307-332.
- Steigerwalt RJ, Laurora G, Incandela L, Cesarone M, Belcaro G, De Sanctis M. 2000. Ocular and orbital blood flow in cigarette smokers. Retina. 20(4):394-397.

- Stern MD, Lappe DL, Bowen PD, Chimosky JE, Holloway GA, Jr, Keiser HR, Bowman RL. 1977. Continuous measurement of tissue blood flow by laser-Doppler spectroscopy. Am J Physiol Heart Circ Physiol 232(4):H441-448.
- Stewart PA. 1994. Blood barriers in the rat: correlation of ultrastructure with function. J. Comp.Neurol. 340:566-576.
- Stone RA, Laties AM, Raviola E, Wiesel TN. 1988. Increase in Retinal Vasoactive Intestinal Polypeptide after Eyelid Fusion in Primates. Proc Natl Acad Sci U S A 85(1):257-260.
- Stone RA, Lin T, Laties AM, Iuvone PM. 1989. Retinal Dopamine and Form-Deprivation Myopia. Proc Natl Acad Sci U S A 86(2):704-706.
- Sullivan P. 2005 Saturday December 3. Dr. Thomas R. Dawber, Heart Researcher, Dies. Washington Post;B06.
- Swann PG, Schmid KL. 2002. Fundus changes in myopia - an overview. Optometry Today 42(6):33-35.
- Tamaki Y, Araie M, Nagahara M, Tomita K, Matsubara M. 2000 The acute effects of cigarette smoking on human optic nerve head and posterior fundus circulation in light smokers. Eye. 14 (1):67-72. .
- Tan DTH, Lam DS, Chua WH, Shu-Ping DF, Crockett RS. 2005. One-year multicenter, double-masked, placebo-controlled, parallel safety and efficacy study of 2% pirenzepine ophthalmic gel in children with myopia. Ophthalmology 112(1):84-91.
- Tano Y. 2002. Pathologic myopia: where are we now? Am J Ophthalmol134(5):645-660.
- Tarczy-Hornoch K, Ying-Lai M, Varma R, the Los Angeles Latino Eye Study G. 2006. Myopic Refractive Error in Adult Latinos: The Los Angeles Latino Eye Study. Invest. Ophthalmol. Vis. Sci.47(5):1845-1852.
- Teakle E, Wildsoet C, Vaney D. 1993. The spatial organization of tyrosine hydroxylase-immunoreactive amacrine cells in the chicken retina and the consequences of myopia. Vision Res 33(17):2383-2396.
- Teikari J, O'Donnell J, Kaprio J, Koskenvuo M. 1991. Impact of heredity in myopia. Hum Hered 41(3):151-156.
- Thorburn A, Brand J, O'Dea K, Spargo R, Truswell A. 1987. Plasma glucose and insulin responses to starchy foods in Australian aborigines: a population now at high risk of diabetes. Am J Clin Nutr. 46(2):282-285.
- Tian R, Vogel P, Lassen NA, Mulvany MJ, Andreasen F, Aalkjær C. 1995. Role of Extracellular and Intracellular Acidosis for Hypercapnia-Induced Inhibition of Tension of Isolated Rat Cerebral Arteries. Circ Res 76(2):269-275.
- Tiecks FP, Lam AM, Aaslid R, Newell DW. 1995. Comparison of Static and Dynamic Cerebral Autoregulation Measurements. Stroke 26(6):1014-1019

- To`mey K, Faris B, Jalkh A, Nasr A. 1981. Ocular pulse in high myopia: a study of 40 eyes. Ann Ophthalmol 13:569-571.
- Troilo D, Judgeb S. 1993. Ocular development and visual deprivation myopia in the common marmoset (Callithrix jacchus). Vision Res 33(10):1311-1324.
- Troilo D, Nickla DL, Wildsoet CF. 2000a. Choroidal Thickness Changes during Altered Eye Growth and Refractive State in a Primate. Invest. Ophthalmol. Vis. Sci. 41(6):1249-1258.
- Troilo D, Nickla DL, Wildsoet CF. 2000b. Form Deprivation Myopia in Mature Common Marmosets (Callithrix jacchus). Invest. Ophthalmol. Vis. Sci. 41(8):2043-2049.
- Troilo D, Wallman J. 1991. The regulation of eye growth and refractive state: an experimental study of emmetropization. Vision Res 31(7-8):1237-1250.
- Tutaj M, Brown CM, Brys M, Marthol H, Hecht MJ, Dutsch M, Michelson G, Hilz MJ. 2004. Dynamic cerebral autoregulation is impaired in glaucoma. Journal of the Neurological Sciences 220(1-2):49-54.
- Tzemos N, Lim PO, MacDonald TM.2002.Is exercise blood pressure a marker of vascular endothelial function?. Q J Med. 95:423-429.
- Ustymowicz A, Krejza J, Tarasow E, Mariak Z, Proniewska-Skretek E. 1999. Variations of Doppler blood flow parameters in central retinal artery in relation to position of sample volume. Klin Oczna. 101(6):441-444.
- Ustymowicz A, Tarasowa E, Krejzaa J, Skretek-Proniewskaa E, Szulca S, Stankiewicza A. 1997. Retrobulbar Haemodynamic In Patients with IOP Elevation -- An Investigation by Colour Doppler Imaging. Eur J Ultr 6(2): 43.
- Van Alphen GWHM. 1961. On emmetropia and ametropia. Ophthalmologica. 142 (Suppl):1.
- Van Alphen GWHM. 1986. Choroidal stress and emmetropisation. Vision Res. 26(5):723-34.
- Van Alphen and Graebel WP. 1991. Elasticity of tissues involved in accommodation. Vision Res. 31(7-8):1417-38.
- Van Camp G, Snoeckx R, Hilgert N, van den Ende J, Fukuoka H, Wagatsuma M, Suzuki H, Smets R, Vanhoenacker F, Declau F and others. 2006. A new autosomal recessive form of Stickler syndrome is caused by a mutation in the COL9A1 gene. Am J Hum Genet 79(3):449-457.
- Van Velthoven MEJ, Faber DJ, Verbraak FD, van Leeuwen TG, de Smet MD. 2007. Recent developments in optical coherence tomography for imaging the retina. Prog Retin Eye Res 26(1):57-77.
- Vardavas C, Kafatos A. 2006. Smoking policy and prevalence in Greece: an overview. Eur J Public Health

- Vilchez S, Matthiessen E, Klemm M, Zeitz O. Volumentric color-Doppler imaging in glaucoma; 2004; Berlin.
- Vogel A, Dick HB, Krummenauer F. 2001. Reproducibility of optical biometry using partial coherence interferometry : intraobserver and interobserver reliability. J Cataract Refract Surg 27(12):1961-1968.
- Wallman J, Gottlieb M, Rajaram V, Fugate-Wentzek L. 1987. Local retinal regions control local eye growth and myopia. Science 3(237(4810):):73-77.
- Wallman J, Winawer J. 2004. Homeostasis of eye growth and the question of myopia. Neuron 43(4):447-468.
- Walls G. 1942. The Vertebrate Eye and Its Adapative Radiation. Bloomfield Hills: IL.
- Wangsa-Wirawan N, Linsenmeier R. 2003. Retinal oxygen: fundamental and clinical aspects. Arch Ophthalmol. 121:547-557.
- Ware J. 1813. Observations realtive to the near and distant sight of different persons. Philosophical transactions of the Royal Society of London 1:31.
- Warwick R. 1976. The eyeball. Saunders, editor. Philadelphia: Warwick R.
- Watanabe T, Suzuki N, Shimamoto N, Fujino M, Imada A. 1990. Endothelin in myocardial infarction. Nature 344(6262):114.
- Wei J. 1992. Age and the cardiovascular system. New England Journal of Medicine 327(24):1735-1739.
- Weih L, Mukesh B, McCarty C, Taylor H. 2001. Association of demographic, familial, medical, and ocular factors with intraocular pressure. Arch Ophthalmol 119(6):875-880.
- Weir FW, Fabiano VL. 1982. Re-evaluation of the role of carbon monoxide in production or aggravation of cardiovascular disease processes. J. Occup Med. 24(7):519-525.
- Wells P. 1990. Blood flow: insights from ultrasound. Proc Inst Mech Eng [H] 204(1):1-20.
- Welzel J. 2001. Optical coherence tomography in dermatology: a review. Skin Research and Technology 7(1):1-9.
- Werbach M. 2003 Myopia and diet - nearsightedness - Nutritional Influences on Illness. Townsend letter for doctors and patients.
- Wickremasinghe S, Foster PJ, Uranchimeg D, Lee PS, Devereux JG, Alsbirk PH, Machin D, Johnson GJ, Baasanhu J. 2004. Ocular Biometry and Refraction in Mongolian Adults. Invest. Ophthalmol. Vis. Sci. 45(3):776-783.
- Widdows K. 1990. Myopia and the control of eye growth. Ciba Foundation symposium, editor. Wiley: Chichester
- Wiesel TN, Raviola E. 1977. Myopia and eye enlargement after neonatal lid fusion in monkeys. Nature 266(5597):66-68.
- Wildsoet C, Wallman J. 1995 Choroidal and scleral mechanisms of compensation for spectacle lenses in chicks. Vision Res35(9):1175-1194.

- Wildsoet C. 1997. Active emmetropization--evidence for its existence and ramifications for clinical practice. Ophthalmic Physiol Opt. 17(4):279-290.
- Williams C. Myopia, Reading and School Attainment at Age 7. In: Optics OaP, editor; 2006; Singapore. Blackwell
- Williamson T, Lowe G, Baxter G. 1995. Influence of age, systemic blood pressure, smoking, and blood viscosity on orbital blood velocities. Br J Ophthalmol. 79(1):17-22.
- Wilson T, Strang R, Mackenzie E. 1977. The response of the choroidal and cerebral circulation to changing arterial PCO2 and azetolamide in the baboon. Invest. Ophthalmol. Vis Sci., 16:576.
- Wilson TM. 1977. The response of the choroidal and cerebral circulation to changing arterial PCO2 and azetolamide in the baboon. Invest. Ophthalmol. Vis Sci., 16.
- Wimpissinger B, Resch H, Berisha F, Weigert G, Polak K, Schmetterer L. 2003. Effects of Isometric Exercise on Subfoveal Choroidal Blood Flow in Smokers and Nonsmokers. Invest. Ophthalmol. Vis. Sci. 44(11):4859-4863.
- Wimpissinger B, Resch H, Berisha F, Weigert G, Schmetterer L, Polak K. 2005. Response of retinal blood flow to systemic hyperoxia in smokers and nonsmokers. Graefe's Archive Clin Exp Ophthalmol 243(7):646-652.
- Winn B, Gilmartin B, Mortimer LC and Edwards NR. 1991. The effect of mental effort on open- and closed-loop accommodation. *Ophthal. Physiol. Opt.* 11:335-339.
- Winkelmann BR, Boehm BO, Nauck M, Kleist P, auml, rz W, Verho NK, Ranjith N, Kneissl G. 2001. Cigarette Smoking is Independently Associated with Markers of Endothelial Dysfunction and Hyperinsulinaemia in Non-diabetic Individuals with Coronary Artery Disease. Curr Med Res Opin 17:132-141.
- Wistrand P, Schenholm M, Lönnerholm G. 1986. Carbonic anhydrase isoenzymes CA I and CA II in the human eye. Investigative Ophthalmology & Visual Science 27(3):419-428.
- Wojtkowski M, Bajraszewski T, Gorczynska I, Targowski P, Kowalczyk A, Wasilewski W, Radzewicz C. 2004. Ophthalmic imaging by spectral optical coherence tomography. Am J Ophthalmol 138(3):412-419.
- Wojtkowski M, Srinivasan V, Fujimoto JG, Ko T, Schuman JS, Kowalczyk A, Duker JS. 2005. Three-dimensional Retinal Imaging with High-Speed Ultrahigh-Resolution Optical Coherence Tomography. Ophthalmology 112(10):1734-1746.
- Wolf S, Arend O, Haase A. 1995. Retinal hemodynamics in patients with open-angle glaucoma. Ger J Ophthalmol 4:279-282.
- Wong T, Foster P, Johnson G, Seah S. 2002. Education, socioeconomic status, and ocular dimensions in Chinese adults: the Tanjong Pagar Survey. Br J Ophthalmol 86(9):963-968.
- Wong T, Klein B, Klein R, Knudtson M, Lee K. 2003. Refractive errors, intraocular pressure, and glaucoma in a white population. Ophthalmology 110(1): 211-217.

- Wong T. Is the pattern of refractive errors in adults an age-related or cohort effect? In: Eong K, Maheshwar B, editors; 2006; Singapore. Blackwell. p 53.
- Wong TY, Foster PJ, Hee J, Ng TP, Tielsch JM, Chew SJ, Johnson GJ, Seah SKL. 2000. Prevalence and Risk Factors for Refractive Errors in Adult Chinese in Singapore. Invest. Ophthalmol. Vis. Sci. 41(9):2486-2494.
- Woo W, Lim K, Yang H, Lim X, Liew F, Lee Y, Saw S. 2004 Refractive errors in medical students in Singapore. Singapore Med J 45(10):470-474.
- Workman H. 2006. The effect of senescence and pathology on the structural and functional performance of the visual pathway. Birmingham, UK: Aston University.
- Wu H, Seet B, Yap E, Saw S, Lim T, Chia K. 2001. Does education explain ethnic differences in myopia prevalence? A population-based study of young adult males in Singapore. Optometry and Vision Sciences 78(4):234-239.
- Wu M, Edwards M. 1999 The effect of having myopic parents: an analysis of myopia in three generations. Optom Vis Sci. 76(6):341-342.
- Yamamoto R, Bredt DS, Snyder SH, Stone RA. 1993. The localization of nitric oxide synthase in the rat eye and related cranial ganglia. Neurosci 54(1):189-200.
- Yang Y, Hulbert M, Batterbury M, Clearkin L. 1997. Pulsatile ocular blood flow measurements in healthy eyes: reproducibility and reference values. J Glaucoma. 6(3):175-179.
- Ye XD, Laties AM, Stone RA. 1990. Peptidergic innervation of the retinal vasculature and optic nerve head. Invest. Ophthalmol. Vis. Sci. 31(9):1731-1737
- Yoshida T, Mori M, Nimura Y, G H, Taka Gishi S, Nakanishi K, Satomura S. 1961. Analysis of heart motion with ultrasonic Doppler method and its clinical application. Am Heart J. 61:61-75.
- Young T, Deeb S, Ronan S, Dewan A, Alvear A, Scavello G, Paluru P, Brott M, Hayashi T, Benegas N and others. 2004. X-linked high myopia associated with cone dysfunction. 122(6):897-908.
- Young T. 2004. Dissecting the genetics of human high myopia: a molecular biologic approach. Transactions of the American Ophthalmological Society. 102(423-45).
- Zadnik K, Manny R, Yu J, Mitchell G, Cotter S, Quiralte J, Shipp M, Friedman N, Kleinstein R, Walker T and others. 2003. Ocular component data in schoolchildren as a function of age and gender.Collaborative Longitudinal Evaluation of Ethnicity and Refractive Error (CLEERE) Study Group. Optom Vis Sci. 80(3):226-236.
- Zagvazdin YS, Fitzgerald ME, Sancesario G, Reiner A. 1996. Neural nitric oxide mediates Edinger-Westphal nucleus evoked increase in choroidal blood flow in the pigeon. Invest Ophthalmol Vis Sci 37(4):666-672.

- Zeiher AM, Schachinger V, Minners J. 1995. Long-term Cigarette Smoking Impairs Endothelium-Dependent Coronary Arterial Vasodilator Function. Circulation 92(5):1094-1100.
- Zeitz O, Vilchez SE, Matthiessen ET, Richard G, Klemm M. 2005. Volumetric colour Doppler imaging: a useful tool for the determination of ocular blood flow in glaucoma patients? Eye 20(6):668-673.
- Zhong X, Ge J, Smith EL, III, Stell WK. 2004. Image Defocus Modulates Activity of Bipolar and Amacrine Cells in Macaque Retina. Invest. Ophthalmol. Vis. Sci. 45(7):2065-2074.
- Zhu X, Park TW, Winawer J, Wallman J. 2005. In a Matter of Minutes, the Eye Can Know Which Way to Grow. Invest. Ophthalmol. Vis. Sci. 46(7):2238-2241

APPENDICES

APPENDIX 1. Experimental data

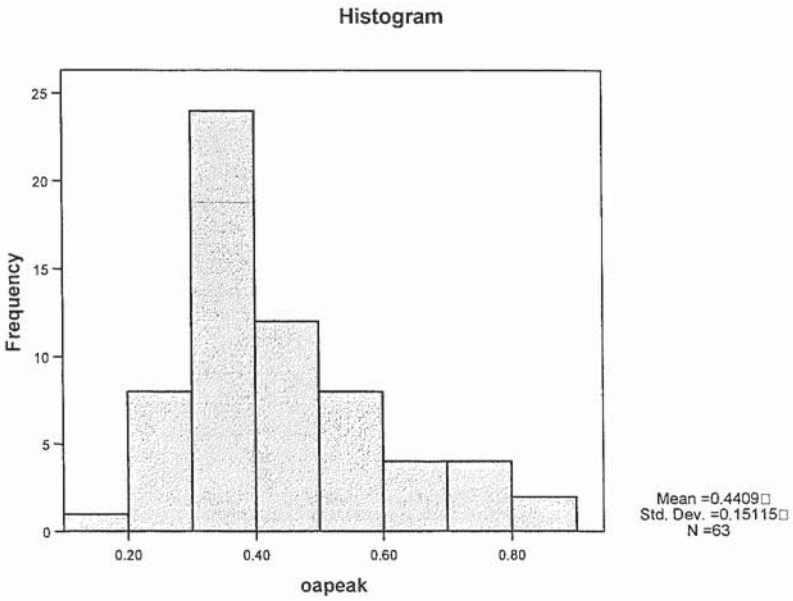
Appendix 1.1

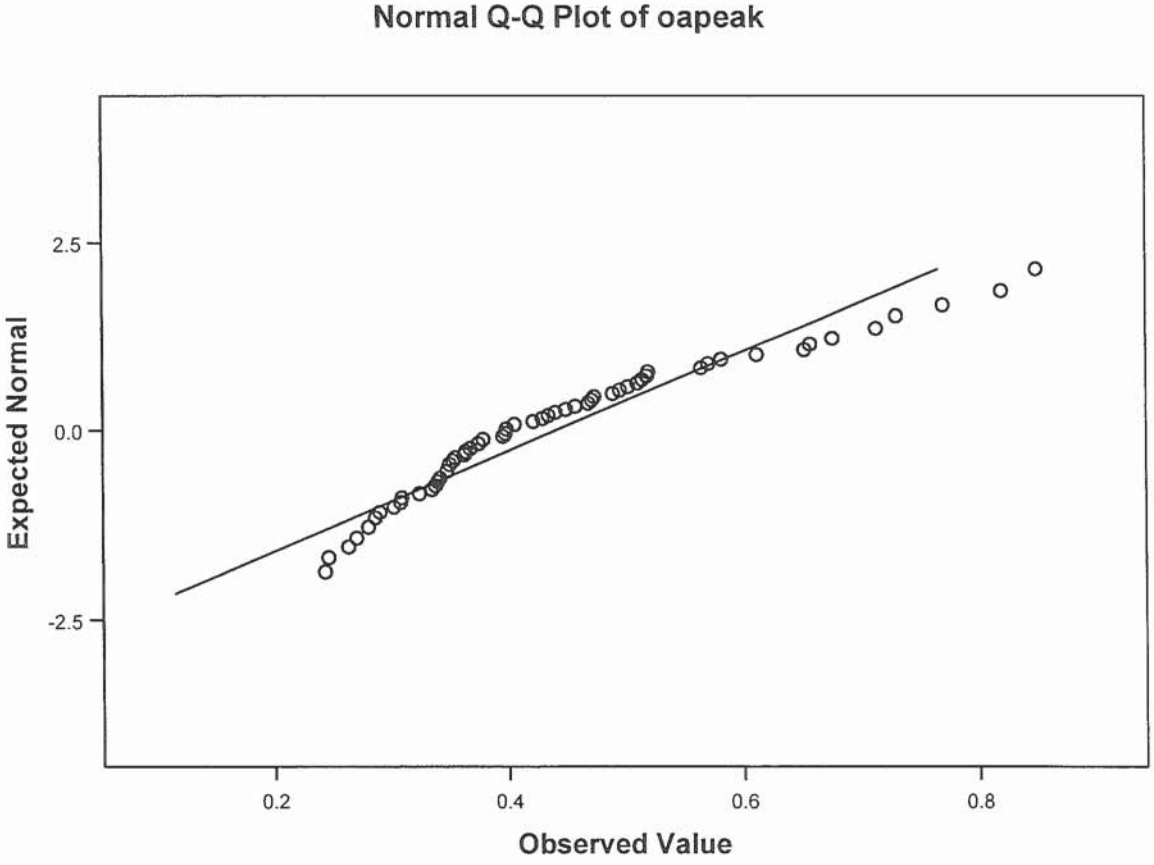
Vitreous chamber repeatability calculation

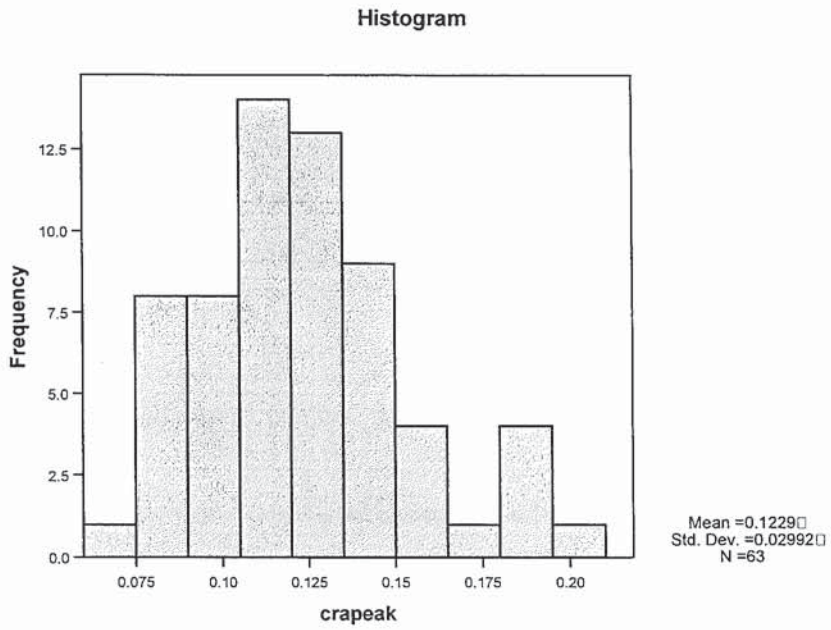
DAY 1

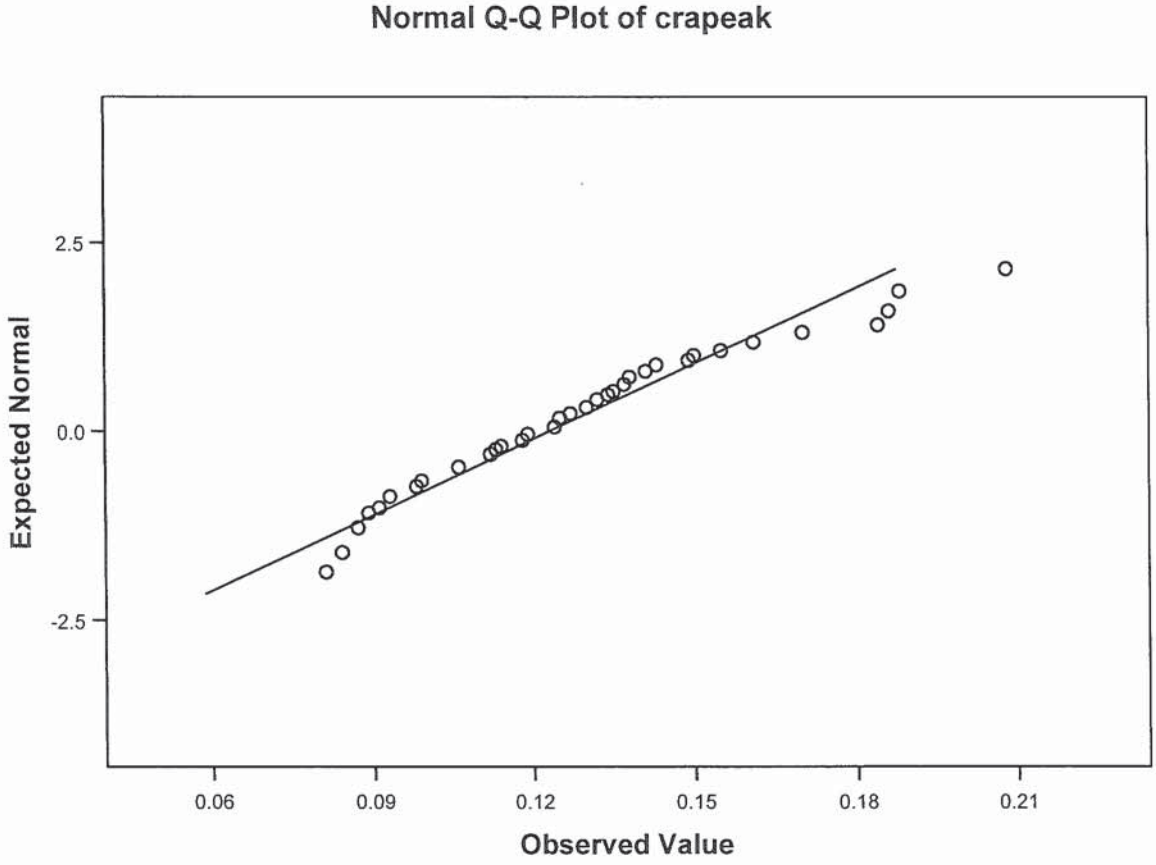
VCV visit2a	VCV visit2b	VCV visit2c	VCV visit2d	VCV visit2e	mean2
6.23	6.57	6.12	6.34	6.56	6.364
5.33	5.54	4.91	5.31	5.56	5.33
5.77	5.22	5.49	4.99	5.19	5.332
6.21	6.55	5.8	6.32	6.43	6.262
4.37	4.58	4.26	4.35	4.57	4.426
6.05	5.5	5.94	5.27	5.52	5.656
6.24	6.58	5.82	6.35	6.55	6.308
4.87	5.08	4.59	4.85	4.96	4.87
5.71	5.16	5.3	4.93	5.15	5.25
5.3	5.64	5.19	5.41	5.66	5.44
5.2	5.41	5.09	5.18	5.38	5.252
5.69	5.14	5.27	4.91	5.02	5.206
4.56	4.9	4.28	4.67	4.89	4.66
5.15	5.36	4.74	5.13	5.38	5.152
5.7	5.15	5.59	4.92	5.12	5.296
4.92	5.26	4.81	5.03	5.14	5.032
4.69	4.9	4.27	4.67	4.89	4.684
5.12	4.57	4.84	4.34	4.59	4.692
5.14	5.48	4.73	5.25	5.45	5.21
5.64	5.85	5.53	5.62	5.73	5.674
6.11	5.56	6	5.33	5.55	5.71
5.74	6.08	5.32	5.85	6.1	5.818
5.79	6	5.51	5.77	5.97	5.808

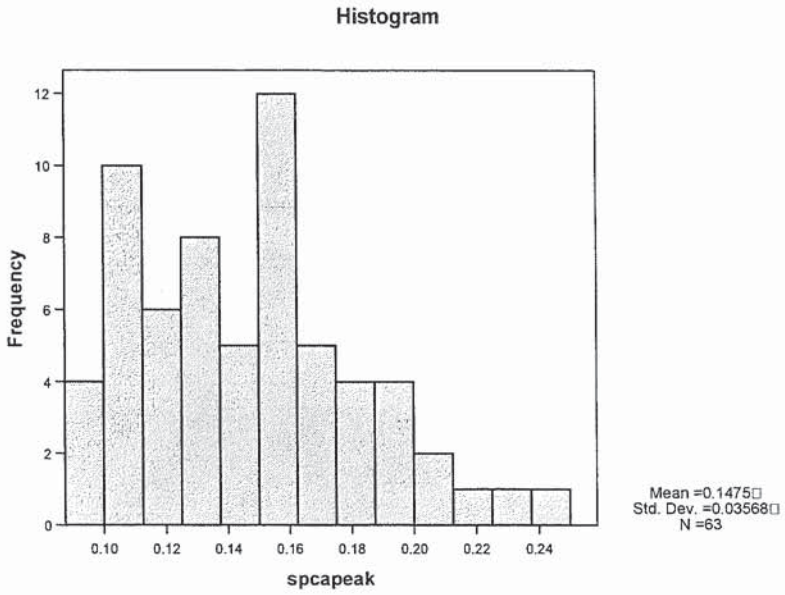
5.67	6.01	5.56	5.78	6	5.804
4.37	4.58	3.96	4.35	4.57	4.366
VCV visit1a	**VCV visit1b**	**VCV visit1c**	**VCV visit1d**	**VCV visit1e**	**mean**
6.37	6.14	6.35	6.11	6.34	6.262
5.71	5.24	5.45	5.2	5.42	5.404
6.03	5.68	5.89	5.63	5.84	5.814
5.93	6.12	6.33	6.09	6.32	6.158
5.31	4.28	4.49	4.24	4.46	4.556
6.45	5.96	6.17	5.91	6.12	6.122
4.87	6.15	6.36	6.12	6.35	5.97
5.71	4.78	4.99	4.74	4.96	5.036
5.46	5.62	5.83	5.57	5.78	5.652
5.15	5.21	5.42	5.18	5.41	5.274
5.18	5.11	5.32	5.07	5.29	5.194
5.74	5.6	5.81	5.55	5.76	5.692
5.18	4.47	4.68	4.44	4.67	4.688
4.33	5.06	5.27	5.02	5.24	4.984
5.87	5.61	5.82	5.56	5.77	5.726
4.91	4.83	5.04	4.8	5.03	4.922
4.56	4.6	4.81	4.56	4.78	4.662
4.79	5.03	5.24	4.98	5.19	5.046
5.86	5.05	5.26	5.02	5.25	5.288
5.33	5.55	5.76	5.51	5.73	5.576
6.33	6.02	6.23	5.97	6.18	6.146
4.48	5.65	5.86	5.62	5.85	5.492
6.09	5.7	5.91	5.66	5.88	5.848
5.97	5.58	5.79	5.55	5.78	5.734
4.1	4.28	4.49	4.24	4.46	4.314

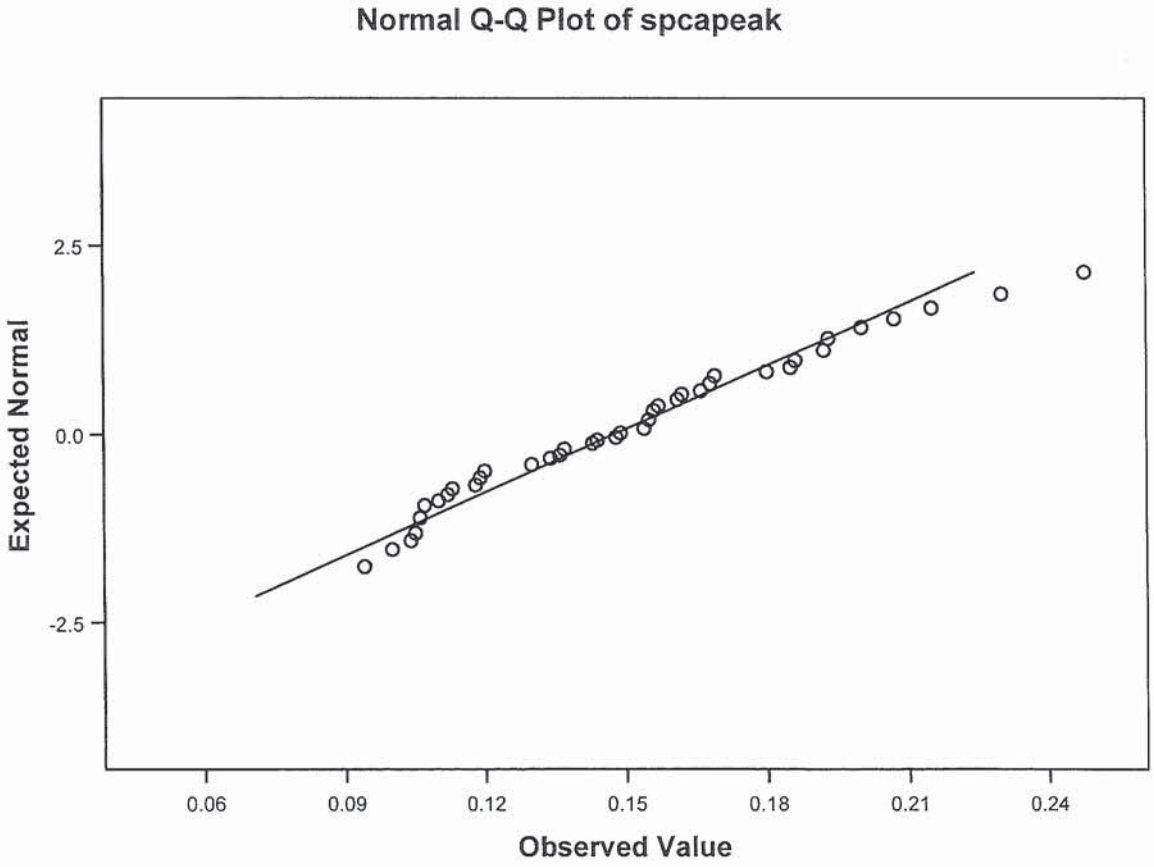

Histogram
Frequency
25
20
15
10
5
0
0.20
0.40
0.60
0.80
oapeak
Mean =0.4409
Std. Dev. =0.15115
N =63

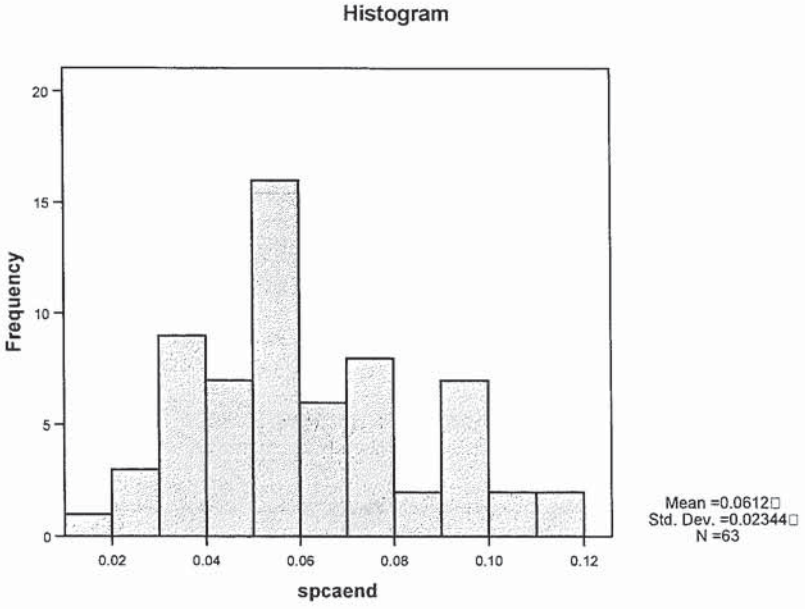

Normal Q-Q Plot of oapeak
Expected Normal
2.5
0.0
-2.5
0.2
0.4
0.6
0.8
Observed Value

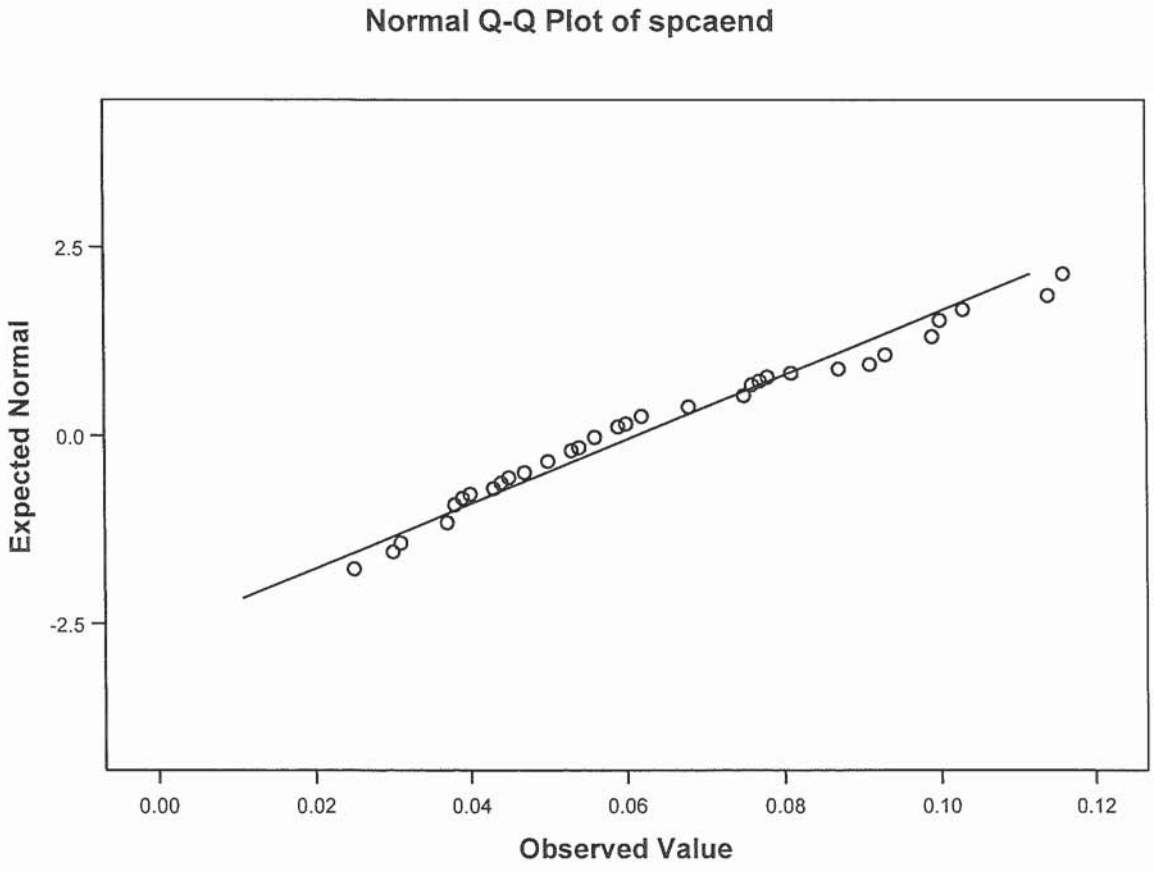

Histogram
Frequency
12.5
10.0
7.5
5.0
2.5
0.0
0.075
0.10
0.125
0.15
0.175
0.20
crapeak
Mean =0.1229
Std. Dev. =0.02992
N =63


Normal Q-Q Plot of crapeak
Expected Normal
2.5
0.0
-2.5
0.06
0.09
0.12
0.15
0.18
0.21
Observed Value


Histogram
Frequency
spcapeak
Mean =0.1475
Std. Dev. =0.03568
N =63


Normal Q-Q Plot of spcapeak
Expected Normal
Observed Value


Histogram
Frequency
spcaend
Mean =0.0612
Std. Dev. =0.02344
N =63


Normal Q-Q Plot of spcaend
Expected Normal
Observed Value

Appendix 1.3

Correlation between age and ocular blood flow

	Mean	Std. Deviation	N
age	37.0229	16.84840	118
obf	1069.1348	343.53189	118
oapeak	.4697	.19282	118
oaend	.1238	.08889	118
oari	1.3261	6.21760	118
oaratio	4.4222	1.36600	118
oaaccel	.2251	.15546	118
crapeak	.1146	.03006	118
craend	.0467	.05623	118
crari	1.3115	6.20698	118
craratio	2.9132	.84676	118
craaccel	.0401	.02814	118
spcapeak	.1449	.04149	118
spcaend	.0675	.05343	118
spcari	.5866	.19029	118
spcarati	2.4979	.85519	118
spcaacce	.0836	.45390	118
hrfmaxvo	38.7357	163.29527	118
hrfmaxfl	419.6053	184.04044	118
hrfmaxve	1.6566	2.59136	118
dcmax	102.8484	40.80181	118
hrfminvol	23.0235	49.27332	118
hrfminfl	246.1676	119.22720	118
hrfminvel	1.1194	1.74462	118
dcmin	106.8663	34.21322	118

Appendix 1.4
One-way ANOVA between 3 age groups

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
age									
	below 40	48	24.8333	4.16844	0.60166	23.6229	26.0437	19	38
	between 40 and 60	34	49.3824	5.61392	0.96278	47.4236	51.3411	42	59
	above 60	14	69.5714	6.80175	1.81784	65.6442	73.4986	60	78
Total	96	40.0521	17.39169	1.77503	36.5282	43.576	19	78	
mes	below 40	48	-2.1853	2.81892	0.40688	-3.0038	-1.3668	-9.37	0.57
	between 40 and 60	34	-2.6588	3.14559	0.53946	-3.7564	-1.5613	-9.31	1.32
	above 60	14	-0.0886	1.80319	0.48192	-1.1297	0.9526	-3.25	2.5
	Total	96	-2.0472	2.91929	0.29795	-2.6387	-1.4557	-9.37	2.5
axialeng	below 40	48	24.2269	1.17015	0.1689	23.8871	24.5667	22.14	26.59
	between 40 and 60	34	24.9165	1.26503	0.21695	24.4751	25.3579	23.05	27.93
	above 60	14	23.6629	1.28974	0.3447	22.9182	24.4075	21.93	26.33
	Total	96	24.3889	1.28511	0.13116	24.1285	24.6493	21.93	27.93
acd	below 40	48	3.5965	0.34083	0.0492	3.4975	3.6954	2.33	4.19
	between 40 and 60	34	3.495	0.37679	0.06462	3.3635	3.6265	2.9	4.55
	above 60	14	3.2564	0.3056	0.08168	3.08	3.4329	2.89	3.78
	Total	96	3.5109	0.36456	0.03721	3.4371	3.5848	2.33	4.55
pachymet	below 40	48	553.7667	40.52119	5.84873	542.0005	565.5328	456	632
	between 40 and 60	34	560.4706	43.24016	7.41563	545.3834	575.5578	478	657
	above 60	14	564.1429	44.15333	11.80047	538.6495	589.6362	495	660
	Total	96	557.6542	41.77649	4.2638	549.1895	566.1189	456	660
obfiop1	below 40	34	10.5471	2.59197	0.44452	9.6427	11.4514	6.8	18.8
	between 40 and 60	29	13.5345	4.40813	0.81857	11.8577	15.2112	7	22.2
	above 60	6	10.7183	4.87147	1.98877	5.606	15.8306	4.3	18.3
	Total	69	11.8175	3.89582	0.469	10.8817	12.7534	4.3	22.2
obfpa1	below 40	33	3.0576	1.15732	0.20146	2.6472	3.4679	1.4	7.7
	between 40 and 60	29	3.131	1.18053	0.21922	2.682	3.5801	0.9	7.1
	above 60	6	2.9167	0.93684	0.38246	1.9335	3.8998	1.7	4.2
	Total	68	3.0765	1.13639	0.13781	2.8014	3.3515	0.9	7.7
obfpv1	below 40	33	8.403	2.60066	0.45272	7.4809	9.3252	4	16.5
	between 40 and 60	29	7.431	3.23288	0.60033	6.2013	8.6608	2.9	16.8
	above 60	6	6.9	3.09192	1.26227	3.6552	10.1448	4	11.7
	Total	68	7.8559	2.9358	0.35602	7.1453	8.5665	2.9	16.8
obfpr1	below 40	33	65.4697	12.86221	2.23902	60.909	70.0304	26	94
	between 40 and 60	29	64.069	9.38818	1.74334	60.4979	67.64	44	90
	above 60	6	66.5	8.38451	3.42296	57.701	75.299	58	82
	Total	68	64.9632	11.03533	1.33823	62.2921	67.6344	26	94
obf	below 40	35	1184.3857	287.73011	48.63527	1085.547	1283.2245	592	1729
	between 40 and 60	29	983.069	382.11338	70.95667	837.7208	1128.4171	420	2028
	above 60	8	930.375	341.00018	120.56177	645.2917	1215.4583	531	1627
	Total	72	1075.0764	346.98461	40.89253	993.539	1156.6138	420	2028
iop	below 40	48	13.4785	3.34998	0.48353	12.5057	14.4512	7	20.5
	between 40 and 60	34	15.5627	3.74489	0.64224	14.2561	16.8694	7	21.33
	above 60	14	15.3405	3.98759	1.06573	13.0381	17.6428	5	20.2
	Total	96	14.4882	3.69256	0.37687	13.74	15.2364	5	21.33
oapeak	below 40	47	0.4344	0.14013	0.02044	0.3932	0.4755	0.18	0.85
	between 40 and 60	31	0.515	0.20235	0.03634	0.4408	0.5893	0.23	0.93
	above 60	14	0.4423	0.2927	0.07823	0.2733	0.6113	0.22	1.34
	Total	92	0.4628	0.19255	0.02007	0.4229	0.5026	0.18	1.34
oaend	below 40	47	0.0964	0.04586	0.00669	0.083	0.1099	0.03	0.24
	between 40 and 60	31	0.1614	0.08152	0.01464	0.1315	0.1913	0.05	0.33

	above 60	14	0.0942	0.05137	0.01373	0.0646	0.1239	0.04	0.19
	Total	92	0.118	0.06781	0.00707	0.1039	0.132	0.03	0.33
oari	below 40	47	0.7764	0.06674	0.00973	0.7568	0.796	0.59	0.9
	between 40 and 60	30	0.6913	0.07851	0.01433	0.662	0.7206	0.51	0.86
	above 60	14	0.7693	0.07374	0.01971	0.7267	0.8119	0.67	0.9
	Total	91	0.7473	0.0813	0.00852	0.7303	0.7642	0.51	0.9
oaratio	below 40	47	4.9698	1.56084	0.22767	4.5115	5.4281	2.46	9.67
	between 40 and 60	31	3.4935	1.04285	0.1873	3.111	3.8761	2.05	7.11
	above 60	13	4.54	1.5606	0.43283	3.5969	5.4831	3.05	8.6
	Total	91	4.4055	1.54525	0.16199	4.0837	4.7273	2.05	9.67
oaaccel	below 40	47	0.2156	0.13717	0.02001	0.1753	0.2558	0	0.63
	between 40 and 60	31	0.1977	0.15349	0.02757	0.1414	0.254	0	0.6
	above 60	13	0.16	0.1812	0.05026	0.0505	0.2695	0.03	0.6
	Total	91	0.2015	0.14901	0.01562	0.1705	0.2326	0	0.63
crapeak	below 40	47	0.1206	0.02701	0.00394	0.1126	0.1285	0.08	0.19
	between 40 and 60	31	0.1151	0.03485	0.00626	0.1023	0.1279	0.06	0.2
	above 60	14	0.1266	0.03853	0.0103	0.1043	0.1488	0.06	0.2
	Total	92	0.1196	0.03156	0.00329	0.1131	0.1262	0.06	0.2
craend	below 40	47	0.0436	0.01512	0.0022	0.0391	0.048	0.03	0.09
	between 40 and 60	31	0.0435	0.01677	0.00301	0.0373	0.0496	0.02	0.09
	above 60	14	0.0453	0.01457	0.00389	0.0369	0.0537	0.03	0.08
	Total	92	0.0438	0.01546	0.00161	0.0406	0.047	0.02	0.09
crari	below 40	47	0.6398	0.08583	0.01252	0.6146	0.665	0.45	0.8
	between 40 and 60	31	0.624	0.07794	0.014	0.5954	0.6526	0.43	0.77
	above 60	14	0.6471	0.06696	0.0179	0.6085	0.6858	0.5	0.74
	Total	92	0.6356	0.08027	0.00837	0.619	0.6522	0.43	0.8
craratio	below 40	47	2.9466	0.7481	0.10912	2.7269	3.1662	1.82	5
	between 40 and 60	31	2.7835	0.58982	0.10593	2.5672	2.9999	1.75	4.33
	above 60	14	2.9386	0.56784	0.15176	2.6107	3.2664	2	3.91
	Total	92	2.8904	0.67047	0.0699	2.7516	3.0293	1.75	5
craaccel	below 40	47	0.0556	0.0749	0.01093	0.0336	0.0775	0	0.5
	between 40 and 60	31	0.0308	0.02253	0.00405	0.0226	0.0391	0	0.1
	above 60	14	0.0301	0.03179	0.0085	0.0117	0.0484	0	0.11
	Total	92	0.0433	0.05749	0.00599	0.0314	0.0553	0	0.5
spcapeak	below 40	47	0.1526	0.04246	0.00619	0.1401	0.165	0.09	0.27
	between 40 and 60	31	0.1511	0.04389	0.00788	0.135	0.1672	0.08	0.26
	above 60	14	0.1369	0.04222	0.01128	0.1125	0.1612	0.07	0.21
	Total	92	0.1497	0.04279	0.00446	0.1408	0.1585	0.07	0.27
spcaend	below 40	47	0.0645	0.02344	0.00342	0.0576	0.0714	0.02	0.12
	between 40 and 60	31	0.066	0.02843	0.00511	0.0556	0.0765	0.03	0.15
	above 60	14	0.0527	0.02274	0.00608	0.0396	0.0658	0.03	0.1
	Total	92	0.0632	0.02527	0.00263	0.058	0.0685	0.02	0.15
spcari	below 40	47	0.584	0.11207	0.01635	0.5511	0.6169	0.39	0.85
	between 40 and 60	31	0.5723	0.09043	0.01624	0.5391	0.6054	0.28	0.77
	above 60	14	0.6086	0.10748	0.02872	0.5465	0.6706	0.41	0.8
	Total	92	0.5838	0.10409	0.01085	0.5622	0.6054	0.28	0.85
spcarati	below 40	47	2.6574	1.0487	0.15297	2.3495	2.9654	1.65	6.5
	between 40 and 60	31	2.4423	0.55848	0.10031	2.2374	2.6471	1.38	4.37
	above 60	14	2.7864	0.97513	0.26061	2.2234	3.3494	1.7	5
	Total	92	2.6046	0.90006	0.09384	2.4182	2.791	1.38	6.5
spcaacce	below 40	47	0.0483	0.03969	0.00579	0.0366	0.06	0	0.16
	between 40 and 60	31	0.0442	0.03149	0.00566	0.0326	0.0557	0.01	0.15
	above 60	14	0.0293	0.02526	0.00675	0.0147	0.0439	0.01	0.1
	Total	92	0.044	0.03546	0.0037	0.0367	0.0514	0	0.16
eyevolum	below 40	38	5.4253	0.87647	0.14218	5.1372	5.7134	4.17	7.35
	between 40 and 60	31	5.5994	0.88863	0.1596	5.2734	5.9253	4.4	7.73
	above 60	14	5.2571	0.719	0.19216	4.842	5.6723	4.34	6.77
	Total	83	5.4619	0.85581	0.09394	5.2751	5.6488	4.17	7.73

weight	below 40	47	69.4745	15.71405	2.29213	64.8606	74.0883	46.1	115
	between 40 and 60	34	74.7794	13.40354	2.29869	70.1027	79.4561	52.5	108.7
	above 60	14	74.4464	10.92077	2.9187	68.141	80.7519	60	91.4
	Total	95	72.1058	14.39675	1.47708	69.173	75.0386	46.1	115
height	below 40	47	1.7004	0.10164	0.01483	1.6706	1.7303	1.5	1.95
	between 40 and 60	34	1.6968	0.08668	0.01487	1.6665	1.727	1.55	1.93
	above 60	14	1.6586	0.10776	0.0288	1.5964	1.7208	1.55	1.92
	Total	95	1.6929	0.09751	0.01	1.6731	1.7128	1.5	1.95
bodymass	below 40	47	23.8447	4.00219	0.58378	22.6696	25.0198	17.78	39.79
	between 40 and 60	34	25.7433	3.36757	0.57753	24.5683	26.9183	18.88	34.09
	above 60	14	25.1123	7.73629	2.06761	20.6455	29.5791	0.13	34.26
	Total	95	24.711	4.56963	0.46883	23.7801	25.6419	0.13	39.79
systloc	below 40	47	109.0638	15.19211	2.216	104.6033	113.5244	82	155
	between 40 and 60	34	117.9412	16.19192	2.77689	112.2915	123.5908	96	167
	above 60	14	124.5714	20.13307	5.38079	112.9469	136.1959	90	164
	Total	95	114.5263	17.18166	1.7628	111.0262	118.0264	82	167
diastoli	below 40	47	64.9362	11.01265	1.60636	61.7027	68.1696	44	94
	between 40 and 60	34	77.4118	9.71758	1.66655	74.0211	80.8024	53	100
	above 60	14	76.3571	10.66704	2.85089	70.1982	82.5161	64	107
	Total	95	71.0842	12.07306	1.23867	68.6248	73.5436	44	107
pulserat	below 40	47	71.7766	9.94868	1.45116	68.8556	74.6976	49	112
	between 40 and 60	34	67.8529	9.07254	1.55593	64.6874	71.0185	53	86
	above 60	14	69.2143	10.59157	2.83072	63.0989	75.3297	55	86
	Total	95	69.9947	9.80732	1.00621	67.9969	71.9926	49	112
map	below 40	47	90.4539	16.38905	2.39059	85.6419	95.2659	60	141.67
	between 40 and 60	34	94.2941	12.35059	2.11811	89.9848	98.6034	76	123.67
	above 60	14	96.5593	20.93589	5.59535	84.4713	108.6473	52.16	142.67
	Total	95	92.728	15.8515	1.62633	89.4989	95.9572	52.16	142.67
opp	below 40	47	47.1267	11.38443	1.66059	43.7841	50.4693	28.56	84.44
	between 40 and 60	34	47.3588	8.79747	1.50875	44.2892	50.4284	32.67	72.44
	above 60	14	48.7659	14.52541	3.88208	40.3792	57.1526	19.04	79.81
	Total	95	47.4513	10.95841	1.12431	45.219	49.6837	19.04	84.44
hrfmaxvo	below 40	41	24.7434	9.12757	1.42549	21.8624	27.6244	9.45	51.03
	between 40 and 60	30	22.2083	7.0539	1.28786	19.5744	24.8423	12.31	43.18
	above 60	9	22.5889	6.8428	2.28093	17.3291	27.8487	11.48	32.91
	Total	80	23.5504	8.16814	0.91323	21.7326	25.3681	9.45	51.03
hrfmaxfl	below 40	42	436.9476	190.72677	29.42978	377.513	496.3823	128.55	995.98
	between 40 and 60	31	375.2152	121.15558	21.76018	330.7749	419.6554	219.55	712.46
	above 60	9	404.8122	132.14369	44.0479	303.2376	506.3869	173.7	555.79
	Total	82	410.0827	162.53209	17.94867	374.3705	445.7949	128.55	995.98
hrfmaxve	below 40	42	1.5105	0.58	0.0895	1.3297	1.6912	0.48	2.93
	between 40 and 60	31	1.289	0.36486	0.06553	1.1552	1.4229	0.8	2.03
	above 60	9	1.4144	0.43761	0.14587	1.0781	1.7508	0.64	1.93
	Total	82	1.4162	0.49929	0.05514	1.3065	1.5259	0.48	2.93
hrfminvo	below 40	42	16.6133	6.60787	1.01962	14.5542	18.6725	6.89	38.42
	between 40 and 60	31	13.9297	4.90433	0.88084	12.1308	15.7286	7.5	25.05
	above 60	9	14.7456	5.432	1.81067	10.5702	18.921	9.16	24.03
	Total	82	15.3938	5.96342	0.65855	14.0835	16.7041	6.89	38.42
hrfminfl	below 40	42	271.5986	127.36274	19.6525	231.9095	311.2876	83.21	692.54
	between 40 and 60	31	225.6213	86.25506	15.49187	193.9827	257.2599	92.24	464.4
	above 60	9	249.8011	118.59378	39.53126	158.6419	340.9604	130.21	448.68
	Total	82	251.8245	113.23145	12.50432	226.9448	276.7042	83.21	692.54
hrfminve	below 40	42	0.9788	0.45258	0.06983	0.8378	1.1199	0.06	2.11
	between 40 and 60	31	0.7935	0.31031	0.05573	0.6797	0.9074	0.24	1.67
	above 60	9	0.8944	0.40491	0.13497	0.5832	1.2057	0.48	1.58
	Total	82	0.8995	0.40385	0.0446	0.8108	0.9883	0.06	2.11

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
age	Between Groups	26276.615	2	13138.307	497.071	0
	Within Groups	2458.125	93	26.431		
	Total	28734.74	95			
mes	Between Groups	67.342	2	33.671	4.219	0.018
	Within Groups	742.271	93	7.981		
	Total	809.613	95			
axialeng	Between Groups	18.105	2	9.053	6.066	0.003
	Within Groups	138.789	93	1.492		
	Total	156.894	95			
acd	Between Groups	1.267	2	0.633	5.185	0.007
	Within Groups	11.359	93	0.122		
	Total	12.626	95			
pachymet	Between Groups	1584.547	2	792.273	0.449	0.64
	Within Groups	164216.61	93	1765.77		
	Total	165801.16	95			
obfiop1	Between Groups	147.619	2	73.809	5.508	0.006
	Within Groups	884.446	66	13.401		
	Total	1032.065	68			
obfpa1	Between Groups	0.251	2	0.126	0.095	0.91
	Within Groups	86.271	65	1.327		
	Total	86.522	67			
obfpv1	Between Groups	20.596	2	10.298	1.202	0.307
	Within Groups	556.872	65	8.567		
	Total	577.468	67			
obfpr1	Between Groups	45.826	2	22.913	0.184	0.833
	Within Groups	8113.332	65	124.82		
	Total	8159.158	67			
obf	Between Groups	831202.05	2	415601.03	3.716	0.029
	Within Groups	7717078.8	69	111841.72		
	Total	8548280.8	71			
iop	Between Groups	98.366	2	49.183	3.821	0.025
	Within Groups	1196.961	93	12.871		
	Total	1295.327	95			
oapeak	Between Groups	0.128	2	0.064	1.762	0.178
	Within Groups	3.245	89	0.036		

	Total	3.374	91			
oaend	Between Groups	0.088	2	0.044	11.868	0
	Within Groups	0.33	89	0.004		
	Total	0.418	91			
oari	Between Groups	0.14	2	0.07	13.606	0
	Within Groups	0.454	88	0.005		
	Total	0.595	90			
oaratio	Between Groups	40.982	2	20.491	10.368	0
	Within Groups	173.918	88	1.976		
	Total	214.901	90			
oaaccel	Between Groups	0.032	2	0.016	0.719	0.49
	Within Groups	1.966	88	0.022		
	Total	1.998	90			
crapeak	Between Groups	0.001	2	0.001	0.669	0.515
	Within Groups	0.089	89	0.001		
	Total	0.091	91			
craend	Between Groups	0	2	0	0.076	0.927
	Within Groups	0.022	89	0		
	Total	0.022	91			
crari	Between Groups	0.007	2	0.003	0.527	0.592
	Within Groups	0.579	89	0.007		
	Total	0.586	91			
craratio	Between Groups	0.535	2	0.267	0.59	0.557
	Within Groups	40.372	89	0.454		
	Total	40.907	91			
craaccel	Between Groups	0.014	2	0.007	2.225	0.114
	Within Groups	0.286	89	0.003		
	Total	0.301	91			
spcapeak	Between Groups	0.003	2	0.001	0.748	0.476
	Within Groups	0.164	89	0.002		
	Total	0.167	91			
spcaend	Between Groups	0.002	2	0.001	1.476	0.234
	Within Groups	0.056	89	0.001		
	Total	0.058	91			
spcari	Between Groups	0.013	2	0.006	0.582	0.561
	Within Groups	0.973	89	0.011		
	Total	0.986	91			
spcarati	Between Groups	1.411	2	0.706	0.868	0.423
	Within Groups	72.308	89	0.812		

	Total	73.719	91			
spcaacce	Between Groups	0.004	2	0.002	1.571	0.214
	Within Groups	0.111	89	0.001		
	Total	0.114	91			
eyevolum	Between Groups	1.224	2	0.612	0.832	0.439
	Within Groups	58.834	80	0.735		
	Total	60.058	82			
weight	Between Groups	645.162	2	322.581	1.575	0.212
	Within Groups	18837.882	92	204.76		
	Total	19483.044	94			
height	Between Groups	0.02	2	0.01	1.035	0.359
	Within Groups	0.874	92	0.01		
	Total	0.894	94			
bodymass	Between Groups	73.764	2	36.882	1.796	0.172
	Within Groups	1889.095	92	20.534		
	Total	1962.859	94			
systloc	Between Groups	3211.565	2	1605.782	6.021	0.003
	Within Groups	24538.119	92	266.719		
	Total	27749.684	94			
diastoli	Between Groups	3527.068	2	1763.534	15.947	0
	Within Groups	10174.258	92	110.59		
	Total	13701.326	94			
pulserat	Between Groups	313.721	2	156.861	1.654	0.197
	Within Groups	8727.526	92	94.864		
	Total	9041.247	94			
map	Between Groups	531.957	2	265.978	1.06	0.351
	Within Groups	23087.428	92	250.95		
	Total	23619.384	94			
opp	Between Groups	29.437	2	14.718	0.12	0.887
	Within Groups	11258.726	92	122.377		
	Total	11288.163	94			
hrfmaxvo	Between Groups	120.709	2	60.355	0.902	0.41
	Within Groups	5150.057	77	66.884		
	Total	5270.766	79			
hrfmaxfl	Between Groups	68250.507	2	34125.254	1.301	0.278
	Within Groups	2071500.6	79	26221.527		
	Total	2139751.1	81			
hrfmaxve	Between Groups	0.875	2	0.437	1.788	0.174
	Within Groups	19.318	79	0.245		
	Total	20.193	81			
hrfminvo	Between Groups	132.7	2	66.35	1.908	0.155
	Within Groups	2747.85	79	34.783		
	Total	2880.55	81			
hrfminfl	Between Groups	37744.285	2	18872.142	1.49	0.232
	Within Groups	1000785.9	79	12668.176		
	Total	1038530.2	81			
hrfminve	Between Groups	0.613	2	0.306	1.921	0.153
	Within Groups	12.598	79	0.159		
	Total	13.211	81			

Appendix 1.5

One-way ANOVA between myopes and emmetropes

BASELINE analysis of ocular blood flow

Instrument	Parameter	Emmetropes	Myopes	P *
POBF	OBFiop	11.42 ± 3.73	13.12 ± 4.08	ns
	OBFa	3.65 ± 1.34	3.07 ± 0.82	ns
	OBFv	8.91 ± 3.00	8.90 ± 9.71	ns
	OBFr	57.36 ± 14.86	63.44 ± 11.00	ns
	POBF	1098 ± 255.10	930.08 ± 273.78	ns
CDI	OA PSv	0.498 ± 0.19	0.493 ± 0.20	ns
	OA EDv	0.129 ± 0.07	0.118 ± 0.05	ns
	OA RI	0.745 ± 0.07	0.747 ± 0.09	ns
	OA ratio	4.33 ± 1.41	4.47 ± 1.57	ns
	CRA PSv	0.122 ± 0.03	0.113 ± 0.03	ns
	CRA EDv	0.046 ± 0.01	0.045 ± 0.01	ns
	CRA RI	0.625 ± 0.08	0.597 ± 0.08	ns
	CRA ratio	2.75 ± 0.60	2.61 ± 0.59	ns
	SPCA PSv	0.156 ± 0.05	0.143 ± 0.04	ns
	SPCA EDv	0.063 ± 0.02	0.065 ± 0.02	ns
	SPCA RI	0.575 ± 0.09	0.543 ± 0.07	ns
	SPCA ratio	2.50 ± 0.74	2.24 ± 0.34	ns
HRF	Max Volume	21.64 ± 6.23	20.40 ± 4.97	ns
	Max Flow	387.37 ± 99.68	374.17 ± 95.69	ns
	Max Velocity	1.35 ± 0.58	1.30 ± 0.31	ns
	Min Volume	19.36 ± 5.68	19.87 ±7.98	ns
	Min Flow	249.06 ± 108.97	245.92 ± 92.58	ns
	Min Velocity	0.86 ± 0.38	0.88 ± 0.30	ns

Appendix 1.6

Correlation between FTQ score and ocular blood flow

Parameter	FTQ score R value	p
OBFiop (mmHg)	-0.048268567	ns
OBFa (mmHg)	-0.079421204	ns
OBFv (ul)	-0.10170922	ns
OBFr	-0.001399957	ns
POBF (ul/min)	-0.145261601	ns
IOP (mmHg)	-0.070396354	ns
OA PSv (m/s)	0.298366751	ns
OA Edv (m/s)	0.276393605	ns
OA RI	-0.200668022	ns
OA ratio	-0.256950682	ns
CRA PSv (m/s)	-0.237775614	ns
CRA ED (m/s)	0.115358906	ns
CRA RI	-0.21504707	ns
CRA ratio	-0.12058087	ns
SPCA PSv (m/s)	-0.332720859	ns
SPCA Edv (m/s)	-0.210911756	ns
SPCA RI	0.04785412	ns
SPCA ratio	0.016303118	ns
HRFmax vol (AU)	0.146502466	ns
HRF max flow (AU)	0.110134668	ns
HRF max vel (AU)	0.145145612	ns
HRF min vol (AU)	0.090801282	ns
HRF min flow (AU)	0.058580239	ns
HRF min vel (AU)MINVE	-0.039614965	ns
Weight (kg)	-0.039011976	ns
Height (m)	-0.161664908	ns
Body Mass Index	0.048894784	ns
SBP (mmHg)	-0.226679421	ns
DBP (mmHg)	-0.145247927	ns
pulse (bpm)	0.180160528	ns
MAP (mmHg)	-0.138153201	ns
OPP	-0.073447881	ns

Appendix 1.7

Correlation between percentage response to hypercapnia, MSE and AL in anisomyopia

Parameter (%)	MSE R value	p	AL R value	p
OBFiop	-0.332076107	ns	0.091039654	ns
OBFa	-0.242427443	ns	0.550198873	ns
OBFv	0.002900724	ns	0.391091731	ns
OBFr	-0.089518869	ns	-0.114062635	ns
POBF	0.28620246	ns	0.000828747	ns
IOP	0.120402905	ns	-0.164072505	ns
OA PSv	0.003272392	ns	-0.198183381	ns
OA Edv	0.254133497	ns	-0.063102477	ns
OA RI	0.250268953	ns	-0.06075163	ns
OA ratio	0.358126745	ns	-0.454120352	ns
CRA PSv	-0.036612952	ns	-0.131909221	ns
CRA ED	0.021084508	ns	0.062715618	ns
CRA RI	0.281653354	ns	-0.113975185	ns
CRA ratio	0.414956958	ns	-0.209837289	ns
SPCA PSv	0.371155242	ns	-0.15129464	ns
SPCA Edv	-0.171513028	ns	0.05362202	ns
SPCA RI	-0.101478026	ns	0.048297192	ns
SPCA ratio	0.522438983	ns	-0.377684976	ns
HRFmax vol	0.399320907	ns	-0.153450534	ns
HRF max flow	0.235044729	ns	-0.208090548	ns
HRF max vel	0.114559977	ns	0.12705016	ns
HRF min vol	0.155350505	ns	-0.501608294	ns
HRF min flow	-0.33911925	ns	-0.020649677	ns
HRF min vel	-0.039614965	ns	0.476547288	ns

Appendix 1.8

Correlation analysis between percentage response to hypercapnia, height, weight and BMI in anisomyopia

Parameter (%)	Height R value	p	Weight R value	p	BMI R value	p
OBFiop	0.30	ns	-0.34	ns	0.30	ns
OBFa	0.28	ns	-0.22	ns	0.28	ns
OBFv	-0.20	ns	0.04	ns	-0.20	ns
OBFr	-0.25	ns	0.01	ns	-0.26	ns
POBF	-0.23	ns	0.14	ns	-0.24	ns
IOP	0.12	ns	0.10	ns	0.12	ns
OA PSv	-0.21	ns	0.14	ns	-0.22	ns
OA Edv	-0.12	ns	0.08	ns	-0.12	ns
OA RI	-0.33	ns	0.05	ns	-0.33	ns
OA ratio	-0.21	ns	-0.05	ns	-0.21	ns
CRA PSv	0.05	ns	-0.05	ns	0.05	ns
CRA ED	0.02	ns	0.26	ns	0.02	ns
CRA RI	0.15	ns	-0.21	ns	0.15	ns
CRA ratio	0.11	ns	-0.27	ns	0.11	ns
SPCA PSv	0.15	ns	-0.25	ns	0.15	ns
SPCA Edv	0.09	ns	0.11	ns	0.09	ns
SPCA RI	0.06	ns	-0.22	ns	-0.21	ns
SPCA ratio	-0.04	ns	-0.21	ns	0.05	ns
HRFmax vol	-0.04	ns	0.05	ns	0.02	ns
HRF max flow	-0.16	ns	0.02	ns	0.15	ns
HRF max vel	-0.20	ns	0.15	ns	0.11	ns
HRF min vol	-0.26	ns	-0.27	ns	0.25	ns
HRF min flow	-0.24	ns	-0.25	ns	0.32	ns
HRF min vel	0.12	ns	0.10	ns	0.40	ns

Appendix 1.9

Correlation analysis between systemic and ocular features with choroidal blood flow

Parameter	ChB vel R value	p	ChB vol R value	p	Ch B flow R value	p
age (years)	0.10	ns	-0.09	ns	-0.08	ns
weight (kg)	-0.21	ns	0.18	ns	0.06	ns
height (m)	-0.25	ns	0.10	ns	-0.04	ns
BMI	-0.10	ns	0.16	ns	0.11	ns
MSE (D)	0.12	ns	0.04	ns	0.07	ns
AL (mm)	-0.12	ns	-0.06	ns	-0.13	ns
ACD (mm)	-0.06	ns	-0.20	ns	-0.16	ns
IOP (mmHg)	0.15	ns	-0.03	ns	-0.02	ns
CT (um)	0.07	ns	0.06	ns	0.04	ns
SBP (mmHg)	0.07	ns	-0.04	ns	0.02	ns
DBP (mmHg)	0.03	ns	-0.17	ns	-0.08	ns
pulse rate	-0.13	ns	-0.10	ns	-0.11	ns
MAP (mmHg)	0.07	ns	-0.31	0.03	-0.19	ns
OPP (mmHg)	-0.02	ns	-0.30	0.03	-0.20	ns

Appendix 1.10

Correlation analysis between choroidal and systemic circulation

Choroidal parameter	SBP	p	DBP	p	Pulse rate	p	MAP	p	OPP	p
LDFavevel	0.55	ns	0.06	ns	-0.09	ns	0.17	ns	-0.50	ns
LDFavevol	0.15	ns	0.26	ns	0.39	ns	0.22	ns	-0.07	ns
LDFaveflow	0.44	ns	0.22	ns	0.26	ns	0.25	ns	-0.33	ns

APPENDIX 2. Applications for ethical approval

ASTON UNIVERSITY

MEMORANDUM

REGISTRY & PLANNING SERVICES

DATE: 24 September 2004

TO: Dr Sarah Hosking,
Life & Health Sciences

FROM: John Walter,
Head of Registry & Planning Services

SUBJECT: **Project 05/H: Inter-eye autoregulatory and visual responses in both young and mature subjects**

I am pleased to inform you that a Sub-Group of the University's Ethics Committee has, on behalf of the Committee, approved the above project proposal as revised in response to the comments of the Sub-Group.

The Sub-Group asked me to convey to the Project Team its appreciation of the quality and diligence of your response to the Sub-Group's comments on the initial submission. The documentation now provides a clear and detailed statement of the research to be undertaken, a comprehensive risk assessment and statement of action to minimise the impact of any potential risks identified, and clear and comprehensive information for volunteers.

The details of the investigation will be placed on file. You should notify me of any difficulties experienced by the volunteer subjects, and any significant changes which may be planned for this project in the future.

Secretary to the Ethics Committee

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:

INTER-EYE AUTOREGULATORY AND VISUAL RESPONSES IN BOTH A SAMPLE OF YOUNG AND MATURE SUBJECTS.

Outline Scientific Purpose/Objectives for Project and Potential Benefits:

This study will explore the effect of hypercapnia (increment in CO_2 partial pressure) on the haemodynamic and visual field responses of both young and mature subjects.

The results will allow us to further our understanding about the already known relationship between myopia and glaucoma, based on vascular concepts.

Investigator(s):	**Department/address:**	**Telephone:**
(First name should be a member of Aston's Academic staff who ill act as main contact)		
Dr. Sarah Hosking	Neurosciences Research Institute	
	Life and Health Sciences	5172
Dr. Nicola Logan	Neurosciences Research Institute	
	Life and Health Sciences	5154
Ms. Alexandra Benavente-Perez	Neurosciences Research Institute	
	Life and Health Sciences	5182

A

Details of sponsoring/collaborating organisation (if any)

1. Name: N/A
2. Does the sponsoring/collaborating organisation provide insurance? ~~YES~~/NO*
3. If drugs are used, do any require a clinical trials certificate or clinical trials exemption certificate?

   ~~YES~~/NO*

*If yes, please provide a copy of the certificate

B
Summary of Project

1 Starting date: immediate following ethical approval

2 Duration: 3 years

3 Location: Optometry, Life and Health Sciences, Aston University

4 Physical procedures:
(See sheet "Explanation of technical terms" attached)

Autorefraction (*Shin-Nippon 5001*, Japan)

Systemic Blood Pressure and heart rate (*Digital BP monitor*, PMS Instruments)

Intraocular pressure assessment (IOP) (*Goldmann method*)

Axial length measurement (*IOLMaster*, Zeiss)

Ocular Blood Flow Pneumotonometry (*OBFA*, Paradigm Medical Instruments Inc.)

Colour Doppler Imaging (*Sequoia CDI System*, Siemens Acuson)

Laser Doppler flowmetry (*Riva method*)

Heidelberg Retinal flowmeter (*HRF*)

Response Gas Analyser (*Capnograph Plus V1.06*, BCI Inc., Waukesha, Wisconsin, USA)

Wrist sphygmomanometer (*Omron Rx*, Omron Matsusaka Co. Ltd, Japan)

Humphrey Visual Field Analyser (*HFA*; Zeiss-Humphrey Inc.)

Mask with non rebreathing inlet valve of gas connected to a mixing chamber

Pulse oximeter (option included within the response gas analyser).

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:

- Topical anaesthetic, proxymetacaine hydrochloride 0.5% in a single dose applicator (Minims®, Chauvin Pharmaceuticals).
- Fluorescein strips (indication dye) (Chauvin Pharmaceuticals).
- Tropicamide 0.5% (topical cycloplegic and mydriatic) in a single dose applicator (Minims®, Chauvin Pharmaceuticals
- Normal room air will be supplied through a gas mask connected to a mixing chamber
- Room air mixed with CO_2 will be supplied through a gas mask connected to a mixing chamber. The procedure will be as follows:

- The carbon dioxide end tidal will be manually increased due to the addition of CO_2 from a 100% carbon dioxide tank to a mixing chamber. The readings will be taken once the patient reaches a state level of 13% CO_2 end tidal above the room breathing conditions for 3 minutes. A rapid response gas analyser (*Capnograph Plus V1.06*, BCI Inc., Waukesha, Wisconsin, USA) will be

used to monitor the O_2 and CO_2 end tidal from the gas obtained form the initial portion of expired air stream.

6 Psychological assessment:

None

7. Questionnaires: (only to be completed when project contains questionnaire(s) which fall within the types of questionnaire requiring HSEC approval [Guidelines D (3)])

No

C
Volunteers

1. Number of volunteers to be used:
The volunteers will be subdivided into two main categories: volunteers over 40 years old and those between 18 and 30. The group >40 will in turn be subdivided into emmetropic (no prescription) and myopic subjects. The younger group will be put into emmetropic, myopic and anisomyopic (patients with at least 2 dioptre difference in refractive error between the two eyes). Each of these groups mentioned above will be formed by 30 subjects.

Mature sample (>40 years):
Emmetropic ____ 30 patients
Myopic _____ 30 patients

Young sample (18-30 years):
Emmetropic ____ 30 patients
Myopic _____ 30 patients
Anisomyopic____ 30 patients

2. Over what time span? 3 years

3. Age of volunteers:

As indicated above, two age-groups will be recruited: subjects over 40 years old and volunteers between 18 and 30 years old.

4. Sex of volunteers: Male and female

5. Source: Aston University (students and staff)
Optometry clinics (patients)

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 patients will be healthy volunteers

8. Will any volunteers be excluded and if so, on what grounds?

Patients will be excluded if they have any ocular disorders other than anisometropia. A corrected visual acuity of 6/9 or better will be required together with astigmatism of less than 2.5 dioptres cylinder.
Subjects whose anisometropia is secondary to surgical procedures will be excluded.
Pregnant women or planning to get pregnant will also be excluded from the study.

9. Is the activity of the volunteer to be restricted in any way either before or after the procedure? (eg diet, driving)

Subjects will be requested to refrain from alcohol, smoking, nicotine, extreme exercise and caffeine- containing products, and dietary supplements 12 hours before appointments.
The procedure requires the instillation of the cycloplegic and mydriatic tropicamide 0.5%. This will, at most, cause mild visual blurring (at near and possibly distance) for a few hours. The subject may also suffer from mild glare due to the dilated pupil. Following the instillation of tropicamide 0.5% subjects will be advised not to drive a motor vehicle or ride a bicycle for 6 hours after the appointment. Moving machinery should also not be operated for 6 hours after the appointment.

10. Consent: Please attach a copy of the consent form you intend to use, detailing how procedures and hazards will be explained.
Consent form 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 the patient result from the instillation of the diagnostic drugs, ocular contact with tonometry probe, ultrasound imaging of the eye, repeated measures with the *IOLMaster* and systemic side-effects after the supply of CO_2:

- Instillation of proxymetacaine 0.5% during OBFA and IOP measurement.
- Instillation of tropicamide 0.5% for laser Doppler flowmetry and autorefraction
- Corneal damage with Goldmann tonometry and OBFA measurement

- Ultrasound imaging of the eye (CDI)
- More than 20 readings with the *IOLMaster* taken on the same eye in one day
- CO_2 partial pressure higher than 15% above baseline levels

No hazards have been related to the use of HRF, response gas analyser, wrist sphygmomanometer, or Humphrey Visual Field Analyser (HFA).

All of the remaining instruments used are non-invasive, commercially available and not considered to carry any risk to health or sight.

2. What levels of risk are associated with these hazards?

- Ocular adverse reactions to proxymetacaine are rare. Sensitivity reactions have been reported in repeated instillation of the drug. The allergic reaction manifests as marked epithelial stippling and slight stromal oedema. Conjunctival hyperaemia, slight puffiness of the eyelids, pain and lacrimation have also been reported in allergic reaction to proxymetacaine. Systemic effects of proxymetacaine are minimal if small volumes of drug are used.
- Instillation of tropicamide 0.5% causes mydriasis and cycloplegia, and the subjects may experience slight blurring of vision and photophobia(glare for distance and near). Ocular adverse reactions are rare. They consist of stinging and possible irritation, redness and discomfort. On instillation the volunteer experiences transient stinging, lasting less than 1 minute. There is a minimal risk of acute angle-closure glaucoma associated with use of tropicamide. This condition has an incidence of 1 in 10000 people over the age of 40, the risk is close to zero for people under the age of 40 [1].
- Contact tonometry is a routine optometry technique and the risk of corneal damage is minimal when carried out by an experienced optometrist.
- The hazard related to the hypercapnic condition of this study is minor. Normal air contains roughly 2% CO_2.The concentration of CO_2 exhaled during normal breathing is approximately 3-5%. The hypercapnic stage reached by the subjects involved in this study would not be higher than the one we all experience during sleeping, or while rebreathing exhaled air through a paper bag in an anxious attack. These levels of CO_2 concentration do not differ from the ones experienced by a subject during a strenuous exercise. This test is currently used worldwide as vascular stressing test in neurology and cardiology. No side effects or hazards have been reported apart from mild dizziness, light headedness, or headache in 1/50 of the population (Dr. D Evans, School of Optometry, University of Alabama at Birmingham, USA).
- Maintaining CO_2 at a fixed level can create problems because the same concentration of CO_2 can alter the levels of CO_2 partial pressure (pCO_2 levels) differently in different individuals. These effects on the human health range from the stimulation of respiration at 5% pCO_2 to unconsciousness after few minutes to exposure at 7% /10 pCO_2. For this reason, the increment of end tidal carbon dioxide will be done in relation to the known individual pCO_2 baseline. The

capnograph's oximeter option will constantly monitor and display the pCO_2 values of the patient. The pulse oximeter alarm allows us to set it at high pitch over/under a desirable pCO_2, therefore we will be able to control the CO_2 levels at any time (*Capnograph Plus V1.06*, BCI Inc., Waukesha, Wisconsin, USA).

3. How do you propose to control the risks associated with these hazards?

- Any risks can be minimised by using the minimum amount of the drug.
- The puncta can be occluded as a preventative measure against systemic absorption.
- Risks can be minimised by preparing for adverse systemic side effects and by quick recognition of the signs and visual acuity check.
- CO_2 will be supplied making sure that the levels of O_2 in blood are not affected (isoxic hypercapnia). The respiratory end-tidal CO_2 level of each subject will be raised just 13% above baseline to room air in a mixing chamber. This procedure has been described as safe (Niwa et al., 1999). Therefore, by limiting the *Pa* CO_2 levels to less than 15% above normal baseline, the possibility of undesirable side effects is minimised.
- The subjects will be warned of the procedure and possible effects; therefore, if they experience slightly dizziness or headache they must remove the mouth piece and breathe the normal room air. The investigators will be controlling the end tidal carbon dioxide to immediately remove the mouthpiece from the patient in the unfortunate event that the CO_2 levels reach 15%. In any of the cases indicated above the patient will be asked to lie on a bed available on the room, and rest for at least 30 minutes. Side effects do not last longer than that amount of time, and have no long term effects.
- To ensure that such levels are maintained the respiratory rate and end-tidal CO_2 levels will be continuously measured. The pulse oximeter will unable us to constantly control the heart rate and the oxygen saturation. Blood pressure will be assessed with the automated wrist sphygmomanometer

4. What criteria have you used to determine whether the risks are acceptable?

The ophthalmic literature suggests that the risks posed are minimal. Furthermore, all investigators have extensive clinical experience in these procedures.

- Proxymetacaine 0.5% is routinely instilled in clinical optometric practice, precautions will be taken to minimise risk.
- Tropicamide 0.5% is regularly used in optometry practice and in ophthalmology departments of hospitals, precautions will be taken to minimise risks.
- The BMUS Safety Guidelines (2000) have been referred to, which clearly state the safety criteria to minimise hazards.
- The safety of the system employed to supply CO_2 to patients to investigate the vasodilatory effect of this gas on orbital blood flow has been described (Niwa et al., 1999). Due to the close monitoring of the end-tidal percentage of CO_2 and the sufficient supply of oxygen, this procedure

was tolerated by all the subjects studied. Therefore, it was concluded that this system supplies CO_2 in a safe and controlled manner.

5. Is there any precedent for these experiments? If so, please give details with references if possible.

The flow of blood through the eye is essential for the maintenance of visual function and for the nourishment of eye tissues. Impaired vascular circulation is believed to be involved in the pathology of the retina, the optic nerve head and the choroid [2, 3].

The intraocular pressure (IOP) and the ocular pulse are generally believed to be symmetrical in a pair of eyes, and loss of symmetry may be an indication of ocular or cerebrovascular disease [4]. The ocular pulse has been found to be influenced by a variety of factors including posture [5], glaucoma [6], heart rate [7], age [8] and axial length [9].

Similarly differences in the ocular pulse and ocular blood flow have been noted in different refractive groups with myopes demonstrating a lower value than emmetropes or hyperopes [10-12]. Our research team has shown inter-eye differences in choroidal blood flow in anisometropes (persons whose eyes have an inter-eye refractive difference of 2D or more) [13]. Another study agreed with these finding but found no differences in ocular blood supply in the ophthalmic arteries [14].

The relationship between choroidal blood flow and the development of myopia has been studied in animal models of myopia. Unilaterally induced myopia in chicks resulted in axial elongation of the eye and a reduction in blood flow in the myopic eye compared to the contralateral eye [15, 16]. The increase in axial length is also accompanied by choroidal thinning and recovery to form-deprivation myopia in chick eyes is associated with choroidal thickening, which later regresses. Studies utilising laser Doppler flowmetry have shown a large increase in choroidal blood flow precedes the increase in choroidal thickness in chick eyes[17].

Anisometropic subjects have been previously used in a study by this group, as they offer an opportunity to investigate potential ocular differences in eyes with a significant difference in refractive error [18]. By comparing right and left eyes in one individual, ocular differences may be addressed without consideration of inter-subject variability.

Unlike other studies, which have focused on one component of the ocular vasculature, this study will comprehensively assess all areas, providing us with a greater understanding of the effects of refractive error, and more specifically myopia, on the human eye. The Riva laser Doppler flowmetry will enable us to measure retinal flow at the microvascular level at the optic nerve head and blood flow at the choroid, POBF will provide a global measure of pulsatile flow and an interpretation of choroidal perfusion and colour Doppler imaging will enable us to measure quantitatively the blood flow velocities of the retrobulbar arteries that supply the eye.

The Heidelberg Retinal Flowmeter (HRF) merges two sophisticated techniques: confocal laser scanning and scanning laser Doppler flowmetry (SLDF). The result is a new kind of instrument that provides non-invasive, two-dimensional mapping of the retinal micro-circulation. This technology

measures blood flow, volume and velocity, while CDI measures only blood velocity. The technique of HRF is a non-invasive procedure and has been previously used as main instrument to assess ocular blood flow and as complement to the CDI observations [20-22]

Many studies have been lately interested in the effect of hypercapnia on ocular blood flow [19-22]. Therefore, the effects of blood flow gas perturbation on vascular perfusion are well documented. Studies by this group already assessed the effect of vasodilatory stimuli on the retina of young healthy volunteers using CO_2 as stressing test [23, 24]. Variations in the responses of the retina to this test and others (hyperoxia) have been observed to vary across the retina. This finding correlates with the observation that neuroretinal rim damage occurs earlier in the inferior area of the optic nerve. We have also reported a difference in responsiveness to hypercapnia between the superior and inferior part of the retina [21].

6. Has this project been considered/is it being considered by any other Ethical Committee? If so, please give details and decision made.

No

Previous related Aston University human sciences ethical committee submissions

Reference

01B The effect of inhalation of increased carbon dioxide levels on the higher harmonics of the intraocular pressure pulse (CO_2 used)
01D Screening of refractive error and axial length October 2001
01F Relative peripheral refractive error and retinal shape in young adults
02Y The effect of refractive error o ocular haemodynamics
99E Inter-eye differences in pulsatile ocular blood flow

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 or Supervisor of Student ..... date.....

Investigator..... date.....

Investigator..... date.....

Investigator..... date.....

Investigator..... date.....

Head of School..... date.....
(or nominee)

The following should be attached:

* volunteer consent form
* insurance certificate (if available)
* clinical trials certificate or clinical trials exemption certificate (if appropriate)
* experimental protocol

PATIENT INFORMATION SHEET AND CONSENT FORM

Project title

INTER-EYE AUTOREGULATORY AND VISUAL RESPONSES IN BOTH YOUNG AND MATURE SUBJECTS.

Research Workers and Department Responsible

Dr Sarah Hosking — Optometry, Life and Health Sciences, Aston University

Dr Nicola Logan

Ms Alexandra Benavente-Perez

Explanation of any possible hazards and the procedures to be used.

Participation in this research is voluntary and you are free to withdraw at any time.

If you decide to take part in our study you will be given this information sheet. You will be asked to sign it.

Our research consists of two visits, in which we will ask you to undergo the following tests:

- You will be first asked to go through a routine eye test where your visual field will also be examined to allow us evaluate how well your eyes see (these are normal tests like the ones at your optometrists).

- We will then measure your blood pressure. We will place an inflatable cuff around your arm and we will listen to your heart.

- Eye imaging methods will be used to calculate the blood flow in your eyes (it will measure how fast the blood flows and what are your blood pulsations). We have different instruments to measure blood flow in the eye. Two of these methods will require an eye drop. We will put a harmless eye drop in your eyes that will let us take a photograph of the back of your eyes. The drop makes the pupil large so that we get a better view of the back of your eye. The other eye drop that we use will slightly numb your eye so that you will not feel the test. For another test, we will ask you to close your eyes, and we will place some jelly on your closed eyelid. The feeling is quite pleasant and relaxing. With a scan on this jelly we will obtain readings of the speed of the blood in your eyes. The scan we have (ultrasound) is exactly the same as the one used for pregnant women, and is harmless to your eyes.

- The tests we have mentioned above will be repeated again on a different day while you breathe normal room air mixed with CO_2 through a mask. The air you will be breathing will not cause any harm to you, however, if you feel any kind of dizziness, headedness or headache at any point, we will stop the experiment immediately. The mask will be removed to allow you to breathe normal room air, and these effects will disappear. The concentration of carbon dioxide you will be breathing is similar to the one we experiment while sleeping, or during exhausting exercise. This technique has been done before in our laboratory, and it is safe.

Extra information about the drops to used:

- The drops used will numb your eyes and will last for about 20 minutes. It is best to avoid windy or dusty rooms and you should not rub your eyes during that time because you will be unaware of dust or fragments in your eyes.
- After we have placed the dilation drops in your eyes, you may experience some dazzling in bright sunshine or in artificial light for a few hours. We therefore advise you not to drive a motor vehicle or ride a bicycle to and from this appointment or for 6 hours after the appointment. Moving machinery should also not be operated for 6 hours after the appointment. Please bring sunglasses or a hat to the sessions.

Prior to participation

You are encouraged to ask any questions at any point of the study (before, during, after...).

Subjects will be requested to refrain from alcohol, smoking, nicotine, extreme exercise and caffeine-containing products, and dietary supplements for 12 hours before appointments.

If you need any further information, please, don't hesitate to contact Ms. Alexandra Benavente on 07834776616.

Confidentiality of information

The confidentiality of personal information and the anonymity of all subjects involved in this investigation will be preserved in the following way:
Your identity will be recorded against the findings but will not be stored on computer. Your identity is needed in case we wish to contact you at a later date. This information will be kept for six weeks.

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 the above project and I understand that I am free to withdraw at any time.

Signed: .....

Print Name: .....

Date: .....

Signature of researcher: .....

ASTON UNIVERSITY

MEMORANDUM

REGISTRY & PLANNING SERVICES

DATE: 23 November 2007

TO: Dr Nicola Logan,
Life and Health Sciences

FROM: Mr JG Walter
Academic Registrar

SUBJECT: **Application for Human Science Ethical Committee approval of the project "Screening of refractive error and axial length." (Ref 01/D)**

I am writing to inform you that a Sub-Group of the University's Ethics Committee has approved the proposed changes to the above project, on behalf of the Ethics Committee.

The details of the investigation will be placed on file. You should notify me of any difficulties experienced by the volunteer subjects, and any significant changes which may be planned for this project in the future.

Secretary to the Human Science Ethical Committee

To : **John Walter**
Secretary to the Ethics Committee

19th September 2006

Subject : Amendment of research project involving human volunteers ref. number 01D

Dear Mr. Walter,

I am writing to amend a research project that has previously been approved in 2001 and amended in September 2003. The reference number of the project is **01D.** The project title was "Screening of refractive error and axial length October 2003".

Two instruments were initially included in the study, an autorefractor that allowed the objective measurement of the subject's refractive error, and an IOLMaster, to calculate axial length and curvature of the anterior surface of the eye (cornea).

We would like to amend the project with the inclusion of the following changes as annotated by an asteric:

Project title: * "Screening of refractive error, axial length and retinal thickness".

Researchers involved: Dr Nicola Logan (Optometry, Life and Health Sciences)
* Dr. Sarah Hosking (Optometry, Life and Health Sciences),
Ms. Alexandra Benavente Perez (Optometry, Life and Health Sciences).
* Dr. Dheeraj Bansal (Birmingham Optical Group)
Professor Bernard Gilmartin (Optometry, Life and Health Sciences)

Instruments used: Autorefractor
IOLMaster
* Spectral Optical Coherence Tomography (SOCT Copernicus) (see enclosed information on SC

Please, do not hesitate to contact me for further clarification or additional information.

Thank you very much in advance for your help.

Best regards,

Dr Nicola Logan (principal investigator of project ref.01D)

SPECTRAL OPTICAL COHERENCE TOMOGRAPHY SYSTEM (SOCT) 'COPERNICUS'

Features

Optical tomography is a non-invasive technology of cross-sectional imaging of the retina. SOCT is a commercially available OCT machine that uses frequency domain OCT, also called spectral tomography, which improves imaging speed, while the reduced losses during a single scan improve the signal to noise proportional to the number of detection elements. These features provide higher resolution of the retinal layers assessed, and a decrease testing time than time domain OCT (e.g . OCT3).
SOCT displays 2-D and 3-D images.

OCT is a NHS approved machine, currently operational in many UK hospitals including: Sunderland Eye Infirmary, Royal Eye Infirmary in Leicester and City Hospital in Birmingham among others.

Construction of the device was based on studies from Physics Institute of University of Nicolaus Copernicus in Torun, Poland, which constitutes one of the world leading scientific centres in spectral optical tomography. The first images of the human eye from a prototypical device built in Physics Institute UMK were received in 2001, parallel to clinical research undertaken by Prof. Kaluzny at the Eye Clinic of the Medical Academy in Bydgoszcz, Poland (see references below).

Technical Specification

- Non-invasive method
- Light source wavelength: 830 nm, 50 nm half bandwidth
- Transversal (axial) resolution: 6 um
- Tangential resolution: 10um
- Axial scanning window: 2mm
- Examination speed: 20'000 A scans per second
- B scan width: 6mm
- Max number of A scans per B scan: 7'000
- Device dimensions: 600x400x400 (W x H x D)
- All head movements motorized (using electrical actuators) and controlled from computer screen.
- Direct fundus preview during scanning.

Protocol

- The patient is asked to rest chin and forehead on the machine chinrest and forehead bar directing their gaze towards a fixation light of adjustable position. The operation takes from 0.4 seconds to 1.2 seconds, time that varies depending on scan of choice.

Manufacturer: Optopol Technology Sp. z o.o.
42-400 Zawiercie POLAND ul. Zabia 42
telephone: 0048 326 709 173
e-mail: zpum@optopol.com
www.optopol.com

UK & Ireland Distributor: Birmingham Optical Group Ltd
583 Moseley Road Birmingham B12 9BL
TEL: 0845 230 3020
FAX: 0845 230 8703

Safety guidelines

Does the product contain a source of ionising radiation or is it capable of emitting ionising radiation? NO

Is there a recommended maximum number of uses? NO

Are decontamination/reprocessing instructions supplied? YES

Are there any restrictions on detergent/disinfectant types? NO

Does reprocessing require the use of specified equipment? NO

References:

1. Maciej Wojtkowski, Rainer Leitgeb, Andrzej Kowalczyk, Adolf F. Fercher, In vivo human retinal imaging by Fourier domain optical coherence tomography, Journal of Biomedical Optics 7(3), 457–463, July 2002.

2. Maciej Wojtkowski, Tomasz Bajraszewski, Iwona Gorczyn´ Ska, Piotr Targowski, Andrzej Kowalczyk, Wojciech Wasilewski, And Czesław Radzewicz, Ophthalmic Imaging by Spectral Optical Coherence Tomography, American Journal of Ophthalmology 2004;138; 412–419.

3. A Szkulmowska, M Wojtkowski, I Gorczynska, T Bajraszewski, M Szkulmowski, P Targowski, A Kowalczyk and J J Kaluzny, Coherent noise-free ophthalmic imaging by spectral optical coherence tomography, Journal of Physics D: Application Physics 38 (2005) 2606–2611.

4. Anna Szkulmowska, Marta Cyganek, Piotr Targowski, Andrzej Kowalczyk, Jakub J. Kałużny, Maciej Wojtkowski, James G. Fujimoto, Standard Resolution Spectral domain Optical Coherence Tomography In Clinical Ophthalmic Imaging, Ophthalmic Technologies XV, Proc. of SPIE Vol. 5688.

5. Anna Szkulmowska, Iwona Gorczynska, Tomasz Bajraszewski, Andrzej Kowalczyk, Jakub Kaluzny, Maciej Wojtkowski, James G. Fujimoto, The applicability of standard resolution Spectral Optical Coherence Tomography for examination of the eye pathologies, Coherence Domain Optical Methods and Optical Coherence Tomography in Biomedicine IX, Proc. of SPIE Vol. 5690.

6. Maciej Wojtkowski, Vivek Srinivasan, James G. Fujimoto, Tony Ko, Joel S. Schuman, Andrzej Kowalczyk, Jay S. Duker, Three-dimensional Retinal Imaging with High-Speed Ultrahigh-Resolution Optical Coherence Tomography, Ophthalmology 2005;112:1734–1746.

ASTON UNIVERSITY
THE SENATE ETHICS COMMITTEE

CONSENT FORM FOR VOLUNTEER SUBJECTS

PROJECT TITLE

Screening of refractive error, axial length and retinal thickness

RESEARCH WORKERS AND DEPARTMENT RESPONSIBLE

Dr Nicola Logan, Optometry, School of Life and Health Sciences
Dr Sarah Hosking, School of Life and Health Sciences
Ms Alexandra Benavente Perez, Optometry, School of Life and Health Sciences
Dr Dheeraj Bansal, IT manager, Birmingham Optical Group

EXPLANATION OF ANY POSSIBLE HAZARDS AND THE PROCEDURES TO BE USED

1. As part of our ongoing research programme we would be grateful if you would participate in our screening of the new students at Aston to evaluate the percentage of refractive error among the new students for the academic year 2006-2007. The length and size of the eye are known to be related to the refractive error. We would also like to find out if the thickness of the eye is also related to refractive error.

2. The procedure uses standard consulting room instrumentation (an autorefractor),all commercially available machines used widely by optometrists in every day practice, and takes only a few minutes. The second and third instruments are used mainly by optometrists who work in hospitals and will take both a measurement of the length and the thickness of the eye. Again this will take only a few minutes. The procedure is NOT a complete eye examination. There are no known hazards of the procedures involved.

3. Participation in the programme is not a requirement of your University course and you are free to withdraw at any time.

4. A relatively small number of students may, at a later date, be asked to participate in additional experiments. Full details of the experiments will be given at that time. Again participation is not a requirement of your University course and you are free to withdraw at any time.

CONFIDENTIALITY OF INFORMATION

The confidentiality of personal information and the anonymity of all subjects involved in this investigation will be preserved in the following way

Your identity will be recorded against the refractive findings but will not be stored on computer. Your identity is needed in case we wish to contact you at a later date. The information will be kept for six weeks.

VOLUNTEER'S STATEMENT

I have read and understand 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 the above project and I understand that I am free to withdraw at any time.

Signed: .....

Dated: .....

APPENDIX 3. Refereed published abstracts of conference proceedings

Appendix 3.1

American Academy of Optometry Conference (2005) December. San Diego, California, US.

Vascular profile of myopia in a young student population

Alexandra Benavente-Perez, Nicola Logan, Sarah Hosking
Research Institute, Aston University, Birmingham, B4 7ET, UK.
Email:benavena@aston.ac.uk

Purpose: The majority of studies that have assessed blood flow in myopic eyes have been performed on animals, with comparatively little research in the field of vascular behaviour related to the human myopic eye. The purpose of this project is to analyse the vascular profile in a young myopic group and to compare it against an emmetropic sample matched for age, gender and ethnicity, to help understand the pathogenesis of myopia in humans.

Method: Ocular Blood Flow Analyser (OBF), Colour Doppler Imaging (CDI), and Heidelberg Retinal Flowmeter (HRF) were used to measure the behaviour of blood in one eye of a myopic group containing 21 subjects (10 females, 11 males, mean equivalent sphere (MES) -5.38± 2.25, age range 19-27) and compared to that in an emmetropic group of 21 subjects (11 females, 10 males, MES – 0.19± 0.34, age range 19-33). One- way ANOVA was used to assess the differences in blood flow and velocity between the groups, with a post-hoc analysis to correct for correlated factors such as blood pressure, eye volume, axial length, corneal thickness, intraocular pressure (IOP) and body mass index (BMI).

Results: Myopes were found to have a decreased choroidal blood flow compared with emmetropes ($p=0.03$ for OBF and $p=0.04$ for CDI). When these values were corrected for BMI and IOP, the level of significance increased ($p<0.001$ and $p=0.017$).

Conclusion: The reduction in choroidal blood flow seen in myopic eyes may suggest a vascular aetiology in myopia. If additional physiological changes to the blood flow occur with age, this may lead to an impaired vascular system and an increased susceptibility to other ocular vascular related condition.

Appendix 3.2

11th International Myopia Conference (2006).August. Singapore.

Reduced retrobulbar blood flow and ocular pulsatility in human myopia

Alexandra Benavente-Perez, Sarah L. Hosking, Nicola S. Logan,
School of Life and Health Sciences
Aston University, Birmingham, B4 7ET, UK.
Email:benavena@aston.ac.uk

Objectives

To assess the ocular vascular profile of human myopia.

Methods

Ocular blood flow in the retrobulbar (Colour Doppler Ultrasound, CDI) and retinal (Heidelberg Retina Flowmeter, HRF) vessels and ocular pulsatility (Ocular Blood Flow Analyser, POBF) were determined in 86 healthy volunteers grouped into 3 mean sphere equivalent (MSE) bands: i) high myopes (n=26), MSE ≥ -5.00D (average MSE -6.86D±1.54; mean age 26.76 years ± 10.92, range: 18-59); ii) low myopes (n=30) MSE -1.00 to -4.50D (mean MSE -2.26D ± 1.00; mean age 32.65 years ± 15.35, range: 18-73); iii) controls (n=30) MSE ≤-0.75 (mean MSE -0.14 ±0.32; mean age 33.16 years ± 15.31, range: 18-71). Groups were matched for age, gender and ethnicity.

Axial length (AL), vitreous chamber volume (VCV), corneal thickness, blood pressure, intraocular pressure, and body mass index were recorded to evaluate potential correlations with ocular blood flow parameters.

One-way ANOVA (Tukey post-hoc) and Pearson´s correlation test assessed the differences between the three groups evaluated and the strength of the relation between MSE and vascular parameters.

Results

Pulsatile ocular blood flow amplitude (POBFa), POBF volume (POBFv) and total POBF were significantly reduced in high myopes compared to emmetropes (p=0.024; p=0.025; p=0.017). Higher resistance index (ri) in the Central Retinal Artery (CRA) together with an increased systolic/diastolic velocity ratio were found in high myopes compared to low myopes (p=0.002; p=0.008). MSE correlated with AL, VCV, POBFa, POBFv, total POBF and CRAri (p<0.001; p<0.001; p=0.027; p=0.023; p=0.01; p=0.031), implying a correlation between AL increase and OBFa reduction (R^2= 0.38; p=0.001).

Conclusions

Retrobulbar blood flow and ocular pulsatility are reduced in the human myopic eye such that ocular flow reduction correlates with the axial length increase observed in myopia. These findings suggest that CRA and choroidal blood flow are reduced in myopia, but the retinal microvasculature is unaffected.

Appendix 3.3

Association for Research in Vision and Ophthalmology (ARVO) Annual Meeting (2007) May. Florida, US

Defective haemodynamic autoregulation associated with autonomic dysregulation in human myopia

Alexandra Benavente-Perez[1], Sarah L. Hosking[1,2], Nicola S. Logan[1]

1. School of Life and Health Sciences, Aston University, Birmingham, UK.

2. Department of Optometry and Visual Science, City University, London, UK

Email:benavena@aston.ac.uk

Objectives

To evaluate the vascular responses to CO2 provocation in human myopia.

Methods

The sample comprised 1 eye of each of 66 healthy volunteers, subdivided into two refractive groups by mean square error (MSE): 33 emmetropes with MSE ± 0.50D (mean MSE 0.08 ± 0.41; mean age 40.27 ± 16.13 years, range 18-78) and 33 myopic subjects MSE -1.00 to -9.30D (mean MSE -4.51 ± 2.56; mean age 36.78± 15.00 years, range 20-73). Groups were matched for age, gender and ethnicity. Ocular perfusion was assessed using CDI for the ophthalmic artery (OA), central retinal artery (CRA) and short posterior ciliary arteries (SPCA), Heidelberg Retinal Flowmeter (HRF) for retinal microcirculation and the Ocular Blood Flow Analyser (OBFA) for pulsatile ocular blood flow (POBF), at each of 2 sessions: baseline (B1, breathing room air) and during isoxic hypercapnia (end tidal $pCPO_2$ increased 15% above B1 with constant O_2 supply). Blood pressure (BP), intraocular pressure (IOP), and body mass index (BMI) were also recorded.

Two-tailed paired student t-test was used to test for significance between baseline and hypercapnic conditions.

Results

At baseline no differences in age, BMI, systemic BP, mean arterial pressure (MAP) or ocular perfusion pressure (OPP) were found between myopes and emmetropes. During hypercapnia, emmetropes experienced an increase in OA PSv and OA RI (p=0.008; p=0.019), while in myopes there was a significant increase in MAP (p=0.023) ocular pulse volume (p=0.038) and POBF (p=0.011) .

Conclusions: These data suggest that ocular haemodynamic autoregulation is defective, even in low-moderate degrees of myopia. Further, the basis of these vascular defects may lie in autonomic dysregulation in myopic patients.

PROPERTY IRREGULARITY REPORT
CLEVER HANDLING SERVICES TEL 0034 928860506
HORARIO: TODOS LOS DIAS DE 08H00 A 22H00
OPEN EVERY DAY FROM 08H00 TO 22H00
DESPUES DE 05 DIAS POR FAVOR CONTACTE CON SU COMPANIA AEREA
AFTER 05 DAYS PLEASE CONTACT WITH YOUR AIRLINE

(0151-907022)

FILE REFERENCE	[illegible]25AUG10/2111GMT
NAME	- SURESH/CHOHAN/
TITLE/INITIALS	- SC/
FLIGHT/DATE	- U27167/25AUG/
NUMBER OF BAGS	- 1
TICKET NUMBER	- ETKD
COLOUR/TYPE	- MC22HWX
TAG NUMBER	- XH16301X/

UTE CLEGE-IBERIA
FUERTEVENTURA
CIF U-84796135

0034-928860506

0129 81004

ARGUS