DIMENSIONAL CHANGES IN THE AGEING CORNEA

CHRISTINE LESLEY KATHERINE ASTIN

Doctor of Philosophy

ASTON UNIVERSITY

July 2005

ASTON UNIVERSITY

DIMENSIONAL CHANGES IN THE AGEING CORNEA

Christine Lesley Katherine Astin

Doctor of Philosophy

2005

Summary

The study investigated the central and peripheral corneal characteristics of groups of subjects from 20 to 90 years of age to assist the understanding of ageing changes in the cornea, and to see whether relationships between ocular parameters were revealed.

After age 45 the corneal horizontal radius of curvature gradually decreased with age. This trend was shown by the Aston University subjects (group B). The effect was very significant for the hospital patients undergoing biometry before cataract extraction operation (group D). Vertical radius of curvature showed a slight decrease with age after age 45, but similar to corneal eccentricity, this showed no significant age effect. Corneal astigmatism progressed from with the rule towards against the rule, particularly after age 60.The shift seemed mainly due to the decreasing horizontal corneal curvature.

In biometry no significant age relation was found for axial length, but a significant relation was found between curvature and axial length in the larger group D. Lens thickness showed a very significant relation to age and to axial length, but no significant relation to corneal curvature. Anterior chamber depth showed a very significant relation to age, lens thickness and axial length, but no significant relation to corneal curvature. A significant age effect was found for corneal thickness decreasing with age for the central, nasal and temporal regions of the right eye.

Analysis of the biometry results indicated the influence of two major factors. Firstly, the natural growth of the eye in youth, leading to greater values of axial length, radius of corneal curvature, lens thickness and anterior chamber depth. Secondly, the typical ageing changes where the increasing lens thickness caused a reduction in anterior chamber depth.

The decrease in corneal thickness with age shown in some corneal regions may be a sign of ageing changes in the tissue proteins and hydration balance.

Key Words: Cornea, ageing, keratoscopy, pachometry, biometry.

ACKNOWLEDGEMENTS

I would like to acknowledge with grateful thanks the assistance and encouragement given by my supervisor Dr Colin Fowler during the course of this research, to Professor Bernard Gilmartin for his valuable advice and to the School of Life and Health Sciences for assistance with fees.

I also acknowledge the support of the medical staff at Birmingham Heartlands Hospital and for their permission to carry out this research on the patients. I acknowledge the assistance given by Mr Huddart and Aston University staff with some of the equipment.

CONTENTS

Page

Page

Page

List of Figures

Page

Page

Page

Page

List of Tables

Page

List of Abbreviations

ANOVA	Analysis of Variance
Astig	Astigmatism
Ecc	Eccentricity
EyeSys	EyeSys Keratoscope
JA	Jack Allen
RE	Right eye
LASIK	Laser in-situ Keratomileusis
LE	Left eye
KH	Keratometry horizontal
KV	Keratometry vertical
IOL	Intraocular lens implant
WTR	With the rule astigmatism
ATR	Against the rule astigmatism
SD	Standard deviation
t	Student statistical t test

Chapter 1

Introduction

1.1 Background Reasons for the Study of the Cornea

The investigation of the ageing aspects of the cornea is important because there is a growing population of older age people (Aston, 1990) and therefore a greater number requiring ocular treatment. Commonly, this treatment is cataract extraction and intraocular lens implant (IOL), but the ocular responses to other types of corneal surgery such as refractive surgery and penetrating keratoplasty are also important.

Patient's expectations are becoming greater. Patients tend to believe that the improvements in modern medical technology will guarantee them increased quality and speed of ocular treatment. Their visual world is nowadays more demanding and older people expect and require good vision for driving, television, reading, using computers, travelling and reading written information in much of everyday life.

As a result of media reporting on legal issues, the general public is becoming more litigation conscious and more likely to complain if their ocular surgery outcome is not totally to their satisfaction. Research into corneal changes would provide the surgeons with more information to assist their plans for new surgical techniques and to help reach more predictable treatment outcomes.

A significant number of contact lens wearers have joined the 'over 45 years of age' population. Contact lens designers and practitioners need to gain more knowledge concerning the corneal parameters in this age group, to help when considering new lens designs and contact lens behaviour on the eye.

Kotulak and Brungardt (1980) were intrigued by the normal physiological changes in the tissues of the human eye from birth to death. They recommended that furthering our knowledge of age-related functional and physiological ocular changes could assist in the understanding both of the normal eye and of those conditions caused by pathology.

Weale (1989) pointed out that various parts of the body exhibit age-related changes at different rates, e.g. memory may be impaired later than is true of accommodation. He emphasised that there are many biochemical changes that play important roles. For example, the elasticity of body tissues is maintained by the presence of elastin, a fibrous constituent of blood vessels, which exhibits a marked avidity for lipids and calcium. Therefore a combination of environmental effects (e.g. ingesting lipids) and the genetically determined constitution of elastin mediates the adverse ageing effects in muscles and the vascular system and hence in the eye.

Corneal ageing studies will contribute to the understanding of the manner in which an older cornea responds in a different manner compared to a younger one, with regard to corneal physiology, to wound healing, to medication and to recovery from surgery.

1.2 Aims for the Literature Search

During research carried out on corneal changes following cataract extraction, the author became interested in the ageing changes in corneal parameters. She has performed biometry in hospital clinics to determine the recommended powers of intraocular lenses for cataract extraction surgery. The corneal curvature of many patients has been measured as part of their ocular examination and contact lens fitting, for a patient age range of 1 to 95 years.

Thus the author decided to pursue a study of several groups of subjects of various ages in order to compare their ocular characteristics, in an attempt to discover differences that may be attributable to normal ageing.

To study all aspects of ocular ageing would be impractical, so the author chose to concentrate on the following parameters: corneal curvature and thickness, both central and peripheral, anterior chamber depth, lens thickness and the axial length of the eye.

Therefore the literature search was planned to encompass publications regarding equipment and measurement methods of these parameters.

Results of previous studies carried out on these topics, especially considering age-related aspects, would be reviewed and the results used in the development and planning of this study.

1.3 Basic Corneal Anatomy

The cornea is a part of the outer fibrous tissue of the eye that is avascular and transparent. The oxygen requirements of the cornea are mostly met from the atmosphere via the anterior corneal surface and most of its other nutritional requirements from the aqueous humour via the posterior corneal surface.

The tears are spread over the cornea by the blinking action of the eyelids, resulting in a smooth anterior corneal surface. The cornea appears oval when viewed from the anterior surface due to a wider limbus superiorly and inferiorly. The average corneal diameters are 12.6 mm horizontally and 11.7 mm vertically. The limbus describes the edge of the cornea as it blends into the sclera.

The position of the cornea is shown in Figure 1.3.1. The cornea consists largely (90%) of the stromal tissue (Figure 1.3.2) covered externally by Bowman's membrane and the epithelium, and internally by Descemet's membrane and the endothelium (Ruskell 1997).

Figure 1.3.1 Cross-Section of the Eye

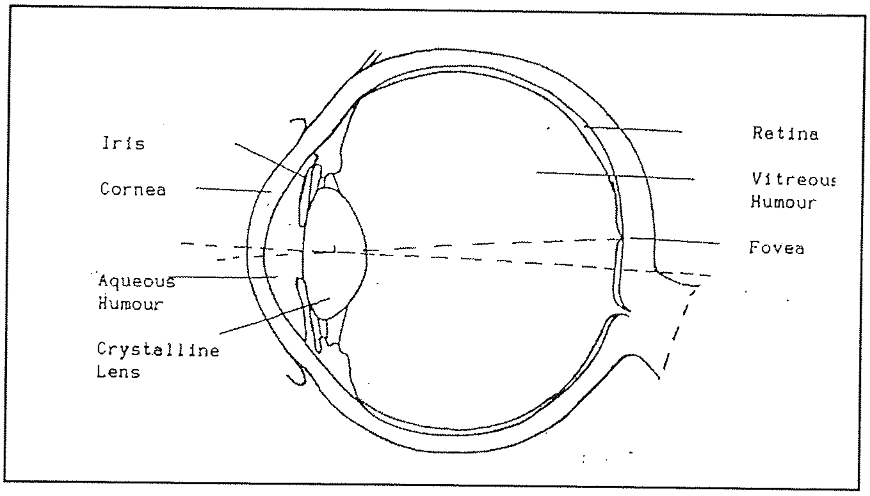
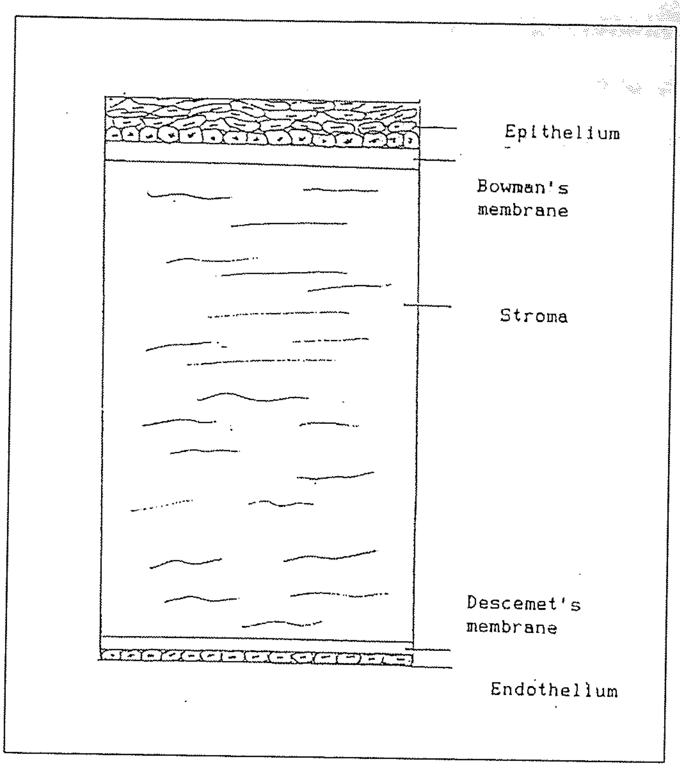


Figure 1.3.2 Diagrammatic Meridional Section of the Human Cornea.



To avoid epithelial damage and local areas of drying the cornea must have an even layer of moisture spread across the surface. This pre-ocular tears layer acts as a refracting surface and is spread by the lids during the blinking action. The tear layer is nearly 7 microns thick and is generally described as having a thin lipid layer of 0.5 microns thickness, provided by the meibomian gland secretions in the lids, and a thick aqueous layer of about 6 microns thickness. The lipid layer inhibits evaporation from the tears and resists epithelial dessication. There is a hydrophilic mucin-rich layer several microns thick on the anterior surface. This associates with the epithelial glycocalyx to maintain the attraction of the aqueous layer to the cornea.

The mean human central corneal thickness is 0.523 mm, SD 0.039, but towards the corneal periphery this value becomes 0.660 mm, SD 0.076, (Martola and Baum 1968). The epithelium may be considered as the anterior continuation of the conjunctiva. The epithelium is 50-100 microns thick and consists of five or six layers of non-keratinised stratified squamous epithelial cells. Every 4 to 5 days the superficial cells gradually fragment and are shed into the tears, being renewed from the basal cells that can undergo

mitosis and renewal (Klyce and Beuerman 1998). The whole epithelium can be renewed about every 7 days. At the limbus the corneal cells gradually become transitional epithelial cells then merge with the conjunctiva. The epithelial cell surface membranes have microscopic projections called microvillae. These assist the mucin layer of the tears to adhere to the extensive fibrillar glycocalyx and maintain good surface wettability.
The superficial cells form tight junctions with their neighbours to provide an effective barrier to substances from the tears. The effectiveness of this barrier can be tested by instilling fluorescein into the tears and observing the uptake or staining by the cells. Normal epithelium does not stain unless damaged by toxins or by trauma, e.g. eye rubbing. In diabetics the basal layer tends to thicken and over the age of 45 years can be duplicated. Their epithelium has weaker adhesion and is susceptible to poor or slowed healing.
The epithelium contains an active transport system for sodium ions from the tears to the corneal stroma, even though normally the superficial epithelial cell membrane restricts sodium ion entry. The deeper epithelial membrane maintains the high potassium and low sodium ion levels in the cells. The swelling of the stromal tissue is due to hydration and is regulated by the epithelial barrier and by the active transport of chlorine ions from the epithelium to the tears.

Bowman's membrane is 8 to 14 microns thick, consisting of random but closely packed collagen fibrils merging with the stroma. Although the fibrils are irregularly arranged the membrane is relatively transparent.

The stroma appears as a set of 300 to 500 lamellae, superimposed on each other and running in layers parallel to the surface. These fibrous layers consist of mucopolysaccharide and collagen fibrils, with thinly scattered keratocyte cells between them. The lamellae extend from limbus to limbus and are orientated at various angles to one another, at less than 90 degrees in the anterior stroma but are nearly aligned in the posterior stroma. The position of these layers is more regular in the posterior stroma and less so in the anterior stroma. Nerve endings tend to be found only in the anterior and central third of the stroma.
There is a low level of production of pro-collagen and collagen. Collagen constitutes about 70% of the dry weight of the cornea and is the structural macromolecule providing tissue transparency and mechanical resistance to intraocular pressure. The concentration

of keratan sulphate seems greatest in the central anterior stroma but is least in the peripheral region, where there is an increased concentration of chondroitin sulphate and increased fibril diameter. Extraction of the extra-cellular material is suspected to occur with corneal oedema, leading to increased spacing between the fibrils. Near the outer portion of the keratocytes membrane small bundles of newly supplied collagen has been found. Basal lamina type of material has been observed gathered in areas round keratocytes, particularly in the corneal periphery.

Descemet's membrane is 5 to 13 microns thick and is an array of collagen bundles. The posterior layer gradually thickens from 2 to 10 microns with age from 10 to 80 years. Focal thickening (guttae) may arise following stimulation of the endothelial cells to produce excess basal lamina materials (Klyce and Beuerman, 1998).

The endothelium is a layer of hexagonal flattened cells 4 to 6 microns thick and 20 microns wide. The endothelium is functionally essential to the cornea because it is involved in the active pump of water to maintain the correct hydration of the stroma. Corneal oedema is when the stroma and surface tissue thicken due to excess hydration. The posterior cell membrane is suspected to have a viscous coating that improves wettability. During normal cell loss in the ageing process and after trauma the endothelial cells have the ability to enlarge, change shape and spread in order to maintain normal function and to cover cell deficiencies. The normal adult cornea has an endothelial cell density of 1,400 to 2,500 cells/mm^2. If the level falls below 700, the endothelium function falters and corneal oedema and misting results (Klyce and Beuerman, 1998).

The anterior surface of the cornea is not uniformly curved. The central one-third of the cornea is called the optical zone and is approximately spherical. The average radius of curvature of the central anterior surface is 7.8 mm, range 7.0 - 8.8 mm, whereas that of the posterior corneal surface is 6.5 mm. The peripheral cornea curvature is flattened to a greater extent nasally than temporally. The posterior surface is more spherical than the anterior surface; therefore the central cornea is thinner (520 microns) than the peripheral cornea (650 or more microns). The corneal refractive index is a variable value between 1.3243 and 1.3339 (mean 1.3304 +/-0.0003) (Klyce and Beuerman, 1998).

1.4 Corneal Transparency

The optical performance of the cornea can be degraded by tissue scarring and by oedema. Dry collagen has a refractive index of 1.550, but the matrix substance has a refractive index of 1.354 so this disparity would produce light scatter and the tissue appear white. Benedek (1971) and Farrell and McCally (1973) investigated this problem. The normal stroma scatters light minimally because its collagen fibrils are uniformly small in diameter (30 nm) and closely spaced (55 nm), whereas the opaque sclera contains fibrils with diameters ranging from 260 - 480 nm. The collagen fibrils are maintained at a fairly steady separation distance of 60 nm by the extracellular matrix.

Increased tissue hydration reduces corneal transparency as the matrix swells and fibril separation increases. This also increases the corneal thickness as described by Maurice (1962) and Benedek (1971). Stevenson et al (1983) demonstrated the nearly linear relationship between osmotic stress and the resulting corneal thickness increase when bathing the cornea in hypotonic saline. The power of the corneal tissue to exclude fluid relates to the metabolic energy available to resist the intrusion of fluid from the aqueous humour in the anterior chamber of the eye. In the normal eye, the active removal of fluid by the epithelium and the endothelium just balances the influx from the limbus and the tears. Interference by means of reduction of oxygen supply, trauma or provision of metabolic toxins leads to increased corneal thickness with associated mistiness of the cornea.

1.5 Mechanisms for Control of Corneal Thickness

Several hypotheses exist as regards the control of corneal hydration and thickness. One main theory is that stromal imbibition pressure must be counteracted to prevent swelling. Tear film evaporation has been proposed as a significant source for this control. The discovery that the cornea is 5% thinner during waking hours than during sleep supports this hypothesis (Holden et al 1985, Klyce and Beuerman 1998). Stromal swelling and epithelial oedema can occur with a large increase in intraocular pressure. Davson (1955) and Harris and Nordquist (1955) demonstrated that corneal hydration is closely linked to the metabolic activity of corneal membranes.

Corneal epithelium and endothelium ion transport processes have been fairly well described by Maurice (1962) and water flow properties of the stroma have been characterised, therefore the combination of these properties is most probable.

The negative charges of glycosaminoglycans are mainly responsible for the presence of sodium and potassium ions in the stroma. When the stroma swells, the diameter of the collagen fibrils stays constant and swelling occurs in the ground substance of the extra-cellular matrix, leading to wider spacing of the fibrils. The corneal structure has mechanical limitations that prevent swelling beyond a certain limit. The ability of fluid to flow through the stromal tissue to even out the differences in hydration is greatest in tissue of near-normal hydration and less in swollen or dehydrated tissue. Therefore normal corneal thickness responds to some changes in the environment e.g. 5% thinning during waking hours, although there may be dry areas in the anterior stroma when surrounding areas are of normal hydration. Generally the stroma is maintained in a relatively dehydrated state compared to the swelling ability. (The hydration is 3.45 parts water to 1 part solid by weight). Stromal swelling pressure is normally about 50 to 60 mm Hg so intraocular pressure (typically 10 to 20 mm Hg) would need to exceed this to cause sub-epithelial oedema. Mild stromal oedema combined with high intraocular pressure can lead to positive stromal imbibition pressure and to the collection of sub-epithelial fluid. The hydration of the anterior stroma is less than that of the posterior stroma ; probably due to variations in the concentrations of glycosaminoglycans at different levels (Klyce and Beuerman 1998, Ruskell 1997, Tripathi, Tripathi and Borisuth 1994).

When the barrier property of the endothelium is compromised, the corneal water balance is overwhelmed by ion and water leakage into the stroma in damaged para-cellular paths. The endothelium has high ionic permeability but low electrical resistance, making investigations into the fluid transport function a problem. The endothelium secretes bicarbonate and sodium into the aqueous humour and this transport system causes an osmotic gradient that balances the swelling tendency of the stroma. As the endothelium deteriorates with ageing, there is leakage of ions and of water and the effectiveness of the ion transport mechanism controlling corneal hydration decreases. Active transport of ions out of the stroma to contrive the osmotic gradient is important to counteract the ion leakage into the stroma by diffusion (Kwok, 1994).

1.6 Corneal Topography

Corneal topographical analysis can evaluate the effects and stability of procedures that change the corneal contour and it is useful in clinical and research work. The uniformity of the anterior corneal surface is an important factor in optical quality. The term 'corneal topography' refers to the topography of the anterior corneal surface and its associated tear film. The corneal surface can be divided into four zones: central, paracentral, peripheral and limbal. The central zone is approximately 4 mm in diameter and overlies the entrance pupil and is important for good image formation on the macula of the retina. The paracentral zone has more effect on the image when the pupil is large. The peripheral zone surface becomes more aspherical and flattens to a greater extent, and the limbal zone is most flat. In vision, the position of the visual axis is more important than the geometric corneal centre (Maloney et al 1993). The vertex normal is the line that connects a fixation point to its perpendicular intersection with the anterior corneal surface. This line is used by all keratoscopes as the centre of the mire images. The topography of the normal cornea has several distinguishing features, e.g. round, oval, bow-tie, irregular (Dingeldein and Klyce 1989, Corbett et al 1994). The contour flattens gradually from the centre to the periphery, with the nasal meridians flattening more than the temporal meridians. This aspherical surface partly corrects ocular spherical aberration. Each cornea has its own individual topographic pattern, usually smooth and without irregular astigmatism.

Corneal astigmatism is defined as that component of the astigmatism of the eye, being the difference in power between the two principal meridians of the corneal curvature (normally at 90 degrees to each other). Refractive astigmatism is the power difference between the two principal meridians of the ocular refraction or total power determination of the eye. Astigmatism is due to the curvature and power of the cornea and of other ocular structures. When the received spherical image is focused at two different planes by the astigmatic surface, a distorted image reaches the retina. The angle between these planes and the horizontal meridian is the angle of axis of the astigmatism. 'With the rule' (WTR) astigmatism is when the more vertical meridian, i.e. that positioned between 60 and 120 degrees, has a steeper curvature (shorter radius of curvature) than that of the horizontal meridian.

The true corneal shape is complex because it is radially asymmetric and aspheric. For many clinical studies this shape is considered as sphero-cylindrical or as part of an ellipse. According to Guillon et al (1986), the locus of any feature can be described by its distance from the corneal centre, and by the meridian on which the feature is situated. Corbett et al (1994) explained the variety of normal shapes existing, usually showing mirror-symmetry to that of the fellow eye. They emphasised that care would be needed when performing videokeratoscopy because a normal cornea may have apparent anomalies due to poor fixation, centring or focusing, or due to tears film irregularities.

Schwiegerling and Greivenkamp (1997) described techniques for removing reference surfaces when studying corneal videokeratoscopic height data. They described the application of their methodology to wave front and ray tracing analysis of corneal aberrations. These aberrations are a measure of refractive error that is beyond conventional sphere and cylinder correction. Wave front aberrometry reveals significant high order aberrations e.g. coma and spherical aberration, can offer an objective measure of visual acuity and can give information on the optical quality of ocular media following surgery or contact lens fitting. Zernike polynomial calculations are used to describe the shape of the measured wave front of the image received on return from the subject's eye.

Chapter 2

The Importance of Studying the Ageing Eye

2.1 Ageing Population

The investigation of ageing aspects of the cornea is important because there is a growing population of people over 45 years of age (Aston 1990). This ageing population will continue to require eye examinations and the demand is likely to rise especially between years 2010 to 2030. The average human life expectancy has grown a great deal during the twentieth century and is now about 75 years. Therefore the need for optical correction for ametropia and presbyopia will increase markedly, according to Haegerstrom – Portnay et al (2002).

Research into ocular changes with ageing is vital for several reasons. Many common age-related changes are not fully understood, particularly in people with healthy eyes. Much understanding of human ocular conditions is based largely on the studies of subjects between 18 and 35 years of age (e.g. Tsilimbaris et al, 1991, Wilson and Klyce, 1991, Wilson et al, 1992, Young et al 1995). Therefore the conclusions may not be as relevant to older subjects. The incidence and prevalence of normal and abnormal visual changes also increase with age.

A significant number of contact lens wearers have joined the 'over 45 years of age' population. This burgeoning presbyopic patient population will be of interest to contact lens designers, manufacturers and practitioners who will need to gain more knowledge concerning the corneal parameters in this age group. Research findings would be useful when creating new lens designs and when assessing contact lens fit and behaviour on the eye. The contact lens suppliers will need to consider lens performance, tolerance and comfort for both older experienced contact lens wearers and first time wearers. The corneal parameters may indicate corneal physiology changes with ageing. These would be relevant in the adaptation to contact lens wear and in the recovery from contact lens related problems.

2.2 Increases in Ocular Treatments

There is much that remains unclear about the development of presbyopic and lens ageing changes. Dubbelman et al (2001) and Koretz and Cook (2001) have shed light on some aspects of lens changes. Although crystalline lens changes may not be preventable, at least a better understanding of why they occur could help with relieving the problems they cause.

The growing population of older age people will lead to a greater number requiring anterior segment surgery and ocular treatment, (Astin 1985, 1986). Commonly, the treatment is cataract extraction and the insertion of intraocular lens implants (IOL). This significant increase has been pointed out by a number of researchers including Batterbury et al, (1991), Jay and Devlin (1990) and Latham and Misson (1997). These studies draw attention for the need to improve efficiency in treatment service. New management methods can be helped by further investigation of the ocular characteristics of the ageing eye. Various operating techniques, designs of IOLs, ocular diagnosis and monitoring methods can be developed using the knowledge gained.

Performing a range of pre-operative measurements is becoming more common. These are necessary to calculate the power of the intraocular lens to be ordered ready for use during the cataract extraction operation. The measurements can also warn the surgeon of potential problems likely with postoperative recovery. The surgeon has the opportunity to adjust the surgical technique taking into account ocular parameters such as corneal thickness, corneal topography and ocular axial length.

The same arguments hold true for other corneal surgery, for example corneal grafts and the expanding field of refractive surgery. For the latter, the aim for a very predictable corneal and optical result is becoming particularly important.

2.3 Previous Research Studies

From the full literature review of ageing changes in the eye, these are a selection of the corneal studies most relevant to this thesis.

Ageing of anterior segment tissues has not been thoroughly investigated, although a considerable volume of studies has concentrated on the crystalline lens and cataract formation, (Pierscionek and Weale 1995). Green (1995) proposed that ocular ageing processes occur as a result of the degradation of enzymes that normally metabolise and detoxify hydrogen and other free radicals. This enzyme loss allows these free radicals to induce irreversible deleterious effects on various ocular tissues, e.g. cataract formation in the lens and the loss of corneal endothelial cells, particularly in the presence of inflammation. Taylor et al (1995) explained that compromises of the function of the crystalline lens with ageing are exacerbated by diminished primary anti-oxidant reserves and by reduced secondary defences. Examples are the proteases that are linked to poor nutrition. Lass et al (1995) showed a decrease in corneal phosphate metabolite with ageing. This indicated an overall decline in high-energy metabolism with ageing.

Meek and Leonard (1993) and Malik and Meek (1996) studied the changes in the corneal stromal inter-fibrillar matrix and collagen fibrils, which gradually become larger and of greater cross-sectional area with ageing. With age, human collagen demonstrates reductions in solubility, elasticity and permeability. This is shown as some degeneration of fibrils. Their results showed that protein glycation, especially the glycation of corneal and scleral collagen, produces significant age related increases in cross-linking and in the collagen intermolecular spacing, but a decrease in inter-fibrillar spacing. They explained that aspirin-like compounds and certain vitamins successfully prevented sugar-induced molecular changes from occurring in corneal and scleral collagen, and so these compounds may have a useful role in the prevention of ageing effects. Daxer et al (1998) also found the corneal collagen fibrils width and length increased with age. This was mostly due to the increased number of collagen molecules and to some expansion of the intermolecular spacing resulting from glycation-induced cross-linking. They suggested that such changes could affect the biomechanical properties of the cornea. Paul and Bailey (1996) explained that initially glycation affects the interactions of collagen with cells and other matrix components. The most damaging effects were caused by the formation of glucose mediated intermolecular cross-links, which decrease the critical flexibility and permeability of the tissue and reduce cell turnover. The vascular system also undergoes glycation modification of the properties of other fibrous connective tissue proteins and elastin. This could restrict circulation and hence reduce the flow of oxygen and nutrients to the corneal stroma via the vascular system.

Weale (1989) and in 1995, Pierscionek and Weale, suggested that ocular parameters decline consistently, indicating that they are under genetic control in keeping with many other biological functions affected by age. They said that age related changes in corneal shape are small and not directly linked to presbyopia. They reported that the optical density of the ocular media increase with age, especially for the crystalline lens. For example, although crystalline lens curvatures become steeper with age, Smith et al (1992) pointed out that the refractive index gradient of the lens alters so the refractive index overall decreases such that there is less myopic power shift than would normally be expected. This lens change, together with an increase in the sagittal thickness in the lens has been reported by Bron et al (2000), who suspected small localised age related variations in protein and water content to be the cause.

With regard to ageing of the corneal epithelium, fluorescein penetration was found to be 12 fold greater in the elderly than in young subjects, according to Nzekwe and Maurice (1994). However, several studies (Chang and Hu 1993) have inferred that this was due to a longer contact time because of poorer quality tear film rather than a true degradation of epithelial cells.

Epithelial fragility increased significantly and progressively with ageing after the age of 40 years, (Millodot and Owens 1984), whilst corneal sensitivity diminished significantly after this age. Descemet's membrane increases in thickness with age.

Previous investigations into corneal endothelium have indicated a decrease fluid pump function associated with cell loss that accompanies normal ageing, (Wilson and Roper-Hall 1982, O'Neal and Polse 1986, Geroski et al 1985). Suda (1984) also showed the increased variability in endothelial cell shape and size (polymegathism) with ageing. Eventually the impairment of endothelial function may lead to some fluid from the aqueous humour seeping into the cornea, disrupting the structured pattern and increasing light scattering. Recovery from corneal thickening caused by oedema is slowed by age according to Siu and Herse (1993) who compared 10 young adults to 10 older adults (average age 69 years), although the amount of oedema in each corneal location measured was not significantly different between their young and older groups. Slow recovery indicates a reduction in efficiency of the corneal function due to ageing. The number of endothelial cells present at birth (3500-4000 cells / mm^2) decreases to 1400-2500 cells / mm^2 by adulthood (Klyce and Beuerman 1998, Kotulak and Brungart 1980), and may fall to 900 cells / mm^2 by the ninth decade, (Waring et al 1992), but this should still maintain adequate corneal dehydration and transparency.

Olsen et al (1984) demonstrated an increase in corneal light scatter with ageing but found no significant change in corneal thickness. Van den Berg and Tan (1994) indicated that as a cornea ages the light scatter increases and therefore the light transmission reduces. Korey et al (1982) confirmed this result, when they reported that the decrease in central corneal endothelial cell density was not accompanied by any significant change in corneal thickness. Dubbelman et al (2001) reported the central corneal thickness (0.57 ± 0.03 mm) did not change significantly with age. This confirmed the results found by Hansen (1971) who measured central corneal thickness as 0.525 mm in subjects over 60 years old, with no significant age effect.

When studying ocular parameters, Ooi and Grosvenor (1995) compared refraction and ocular components of young adults to mature adults. They found no significant age related differences in the mean corneal curvature or in mean axial length. They did find that older adults had shallower anterior chamber depths, and more steeply curved crystalline lenses.

Dubbleman et al (2001) corrected Scheimpflug photographic image for distortion to measure the lens thickness (LT) in 90 subjects of age in the range 16 to 65 years, ($n = 90$, $r = 0.81$, $p<0.0001$). They found an average increase in thickness of 24 microns per year. ($LT = 2.93\ (\pm 0.07) + 0.0236$ mm per year of age. This is much larger than the 13 microns per year given by Koretz et al (1989) who did not correct images fully for the effect of refraction by the cornea and lens. Changes in the refractive index gradient in various parts of the lens have been used in modelling the ageing effects of the crystalline lens. These were used by Koretz et al (2001) and by Smith, Atchison and Pierscionek (1992) and by Atchison and Smith (2000).

Dubbelman et al (2001) reported that the anterior chamber depth decreased with age: AC depth = 3.87 (±0.09) – 0.01 mm per year of age. ($n = 102$, $r = -0.43$, $p < 0.0001$). They found that as the lens thickened, the anterior lens surface approached the cornea and the posterior lens surface became further from the cornea. Koretz et al (1989) and Fontana and Brubaker (1980) agreed with this age related reduction in anterior chamber depth. Dubbelman used 1641 ms^{-1} for the speed of sound in the lens, the value generally accepted for ultrasound.

Studies on corneal astigmatism were reported by Anstice (1971), Baldwin and Mills (1981) and by Ninn-Pedersen (1996). Corbett et al (1994) pointed out that small changes in astigmatism occur throughout life – from WTR in childhood to nearer spherical in middle age, then towards ATR in the older eye. They dismissed short-term shape variations due to lid pressure, to reduced tear evaporation under closed lids, or due to the menstrual cycle, as being rarely of clinical significance. However, long term corneal warping, shown by irregular astigmatism and asymmetry, due to hard contact lenses, had more serious consequences.

Weale (1971) and Lyle (1971) studied many aspects of the ageing eye and was among those who described the changes in corneal astigmatism over the decades from 'with-the-rule' (WTR) where in keratometry the horizontal value is greater than the vertical value, to 'against-the-rule' (ATR) where the vertical value is the greater. This shift in the corneal curvature tended to result mostly from the curvature steepening of the horizontal corneal meridian, (Ehlers and Hansen (1976), Vihlen and Wilson (1983), Kiely et al 1984, Lam et al 1994).

Corbett et al (1994) described a number of diurnal variations of the cornea. For example, the corneal thickness has been found to increase by 3 to 8% after sleeping. The thickness returns to normal thickness within 2 hours after waking then remains steady. The slight 'flattening' effect of the lids on the central corneal curvature during sleep slowly recovers during the day. No apparent significant change was found in corneal asphericity.

A key study in the background of ageing changes in corneal shape is the work carried out by Hayashi et al (1995), who used corneal topography to monitor the changes. They divided their 1,343 sample eyes into 8 groups according to decade of age. On comparing the averaged topography maps of each group, they demonstrated that the younger groups (<20-40) had WTR astigmatism. In the groups of age 50's and 60's, the central steep corneal area became nearer spherical in appearance. The results of groups 70's and 80's indicated ATR astigmatism. This gradual change with age was shown by corneal steepening at the horizontal meridian and by an overall increase in corneal mean refractive power.

Dubbelman et al (2002) studied the vertical corneal meridian of subjects in the age range 16 to 62 years. They found no significant effect on anterior corneal curvature and asphericity, but for the posterior corneal surface there was a trend towards steeper curvature and a significant reduction in asphericity with age.

2.4 Problems in Measuring Ageing Subjects

2.4.1 Keratometry and Keratoscopy

Reduced tear quality, faster tears 'break-up time' therefore a worse optical surface for the mire reflection (Patel and Farrell 1989).

Arthritis in the neck causes problems in keeping the chin on the chinrest and the head steady. With the less steady head position, the operator has to keep refocusing. The measurement takes a longer time, increasing further the risks of error due to tiredness of subject and operation.

Older subjects may find increasing difficultly maintaining reliable fixation on a target point. Therefore misleading measurements may be taken off-centre.

Presbyopia will reduce their accommodation and their ability to clearly see the fixation target. This increases the risks of errors due to poor fixation.

There is a greater likelihood of impaired vision, e.g. due to lens opacities or macular changes, which inhibits accurate target fixation.

In older subjects there is a greater incidence of loose tension lids, skin folds and droopy lids. With a narrower palpebral aperture the target reflections may be restricted, particularly from peripheral and mid-peripheral corneal regions.

2.4.2 A-Scan Ultrasound

To keep the measured eye steady when the cornea is touched by the tip of the ultrasound probe, the operator asks the subject to fixate a distant target using their fellow eye. If vision is poor, fixation stability is reduced, the fellow eye drifts off fixation and so does the eye to be measured. An off-centre measurement may accidentally be taken.

With the narrowing palpebral aperture it is more difficult to get exact centration of the A-Scan probe on the cornea.

The biometer probe works best if there is good lubrication between the probe and cornea. This is more difficult in the ageing subject with poor tear quality, faster tear break-up time and dryness problems. A drop of ocular lubricant, e.g. Guttae Saline, on the probe tip is recommended.

Compared to a handheld ultrasound probe used by an experienced operator a slitlamp mounted probe on microscope table can cause difficulties. These include problems in getting the correct head position if patient is of small stature, or who cannot keep their head steady on the headrest and chinrest or who has a disability or is in a wheelchair, so the slitlamp table cannot be adjusted to the correct position.

Other problems with an elderly subject include head tremor and Parkinson's condition, impaired hearing, slow to follow instructions and also increased health problems that inhibit the subject from keeping appointments.

2.4.3 Pachometry

The problems described above for keratoscopy and keratometry also apply to a pachometer mounted on a slitlamp. Topical anaesthetic has to be instilled before applying the ultrasound probe.

Frequent occurrence of arcus senilis haze in the corneal peripheral region can make the observers view and optical pachometry more difficult.

With ageing there is a greater risk general health problems, e.g. high blood pressure, diabetes and kidney dysfunction, that may cause tissue fluid retention, decreased tear quality and possibly increased corneal thickness. Alvarado et al (1983) proposed that environmental stress over an extended period leads to increased production of basal lamina in older (over age 45) corneas. They found in diabetes and subjects over age 60 evidence of a multi-layer basal lamina, such that the cornea was susceptible to the consequences of poor or delayed epithelial healing. Hence, contact methods of pachometry increase the risk of epithelial trauma.

Chapter 3

Keratometry

3.1 History

The main function of the keratometer is for the measurement of the radius of curvature of the central portion of the anterior surface of the cornea, usually referred to as the optic cap. Helmholtz (1909, cited in 1924) is usually credited with the invention of the keratometer, but Levene (1965, 1977) suggested that Scheiner attempted this in 1619, and that Hare and Ramsden in 1795 were the first to measure corneal radii. According to Helmholtz (1909 translated 1924), Kohlrausch in 1840 and Senff in 1846 performed corneal radius measurements on a living eye. Woodward (1980) noted that Valk in 1897, Tscherning in 1904, and Sorsby in 1961 used keratometry to measure the ocular refracting elements. The cornea is aspheric, so assumptions in the sphericity of the corneal centre led to errors when curvature was measured beyond the apical cap.

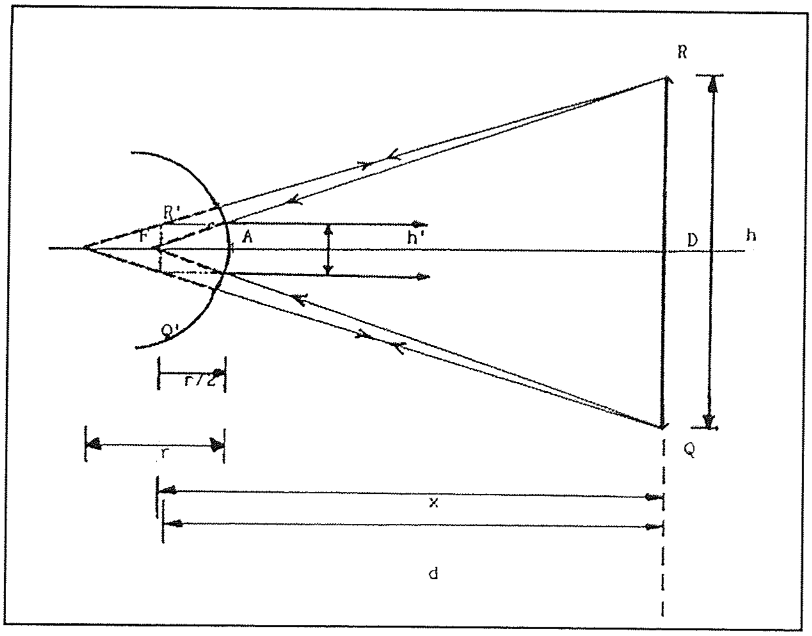
Woodward (1980) described Landolt's (1878) use of a prism for doubling the keratometer image to assist measurement, and the use by Javal and Schiotz (1881) of a Wollaston prism with specially shaped 'mires' as test objects. The advantage of this method over Helmholtz's glass plates doubling method was that the operator obtained a direct reading without further calculations. The work of Helmholtz and others in the development of the keratometer was discussed by Emsley (1952, 1960).

3.2 Keratometer Design

Keratometry measurements are useful in contact lens fitting and aftercare, to assist the calculations of the refracting power of optical elements and intraocular lenses (IOL), to help the diagnosis of condition e.g. keratoconus, and to guide and monitor the progress of ocular surgery (Ruben 1975). Measurements are obtained indirectly from the angular size of the reflected image formed by the cornea of an object of known linear size (h) at a predetermined distance from the image plane (d). The derivation of the radius of curvature is shown in Figure 3.2.1. If the eye was stationary, the image size could be measured directly using a graticule in the eyepiece, but the eye is continually moving with small saccadic movements, so a doubling principle must be incorporated. The two images can then be juxtaposed because even if moving slightly, they do so with the same speed and direction (Figure 3.2.2).

In practice, the object limits are represented by a pair of internally illuminated mires whose magnified corneal images are seen through a short focus telescope as described by Ruben and Woodward (1982) and by Sheridan (1989). This incorporates the doubling device that produces the four images seen in the telescope field, the two central ones being brought into contact or superimposed. To obtain adjacent or superimposed images, either h' may be varied by altering the mire separation h, while the power and position of the doubling device are fixed; or the image size h' and the mire separation h may be fixed while the power of the doubling device, P, or its distance, a, from the image plane is varied.

Figure 3.2.1 Optical Principle of the Keratometer



The optical principle of the keratometer is that r is directly proportional to the image size, h'.

Q and R are the limits of an object of size h

Q' and R' are the limits of the image of size h' formed by the reflection at the anterior corneal surface, F = Principal focus, RQ = Object plane

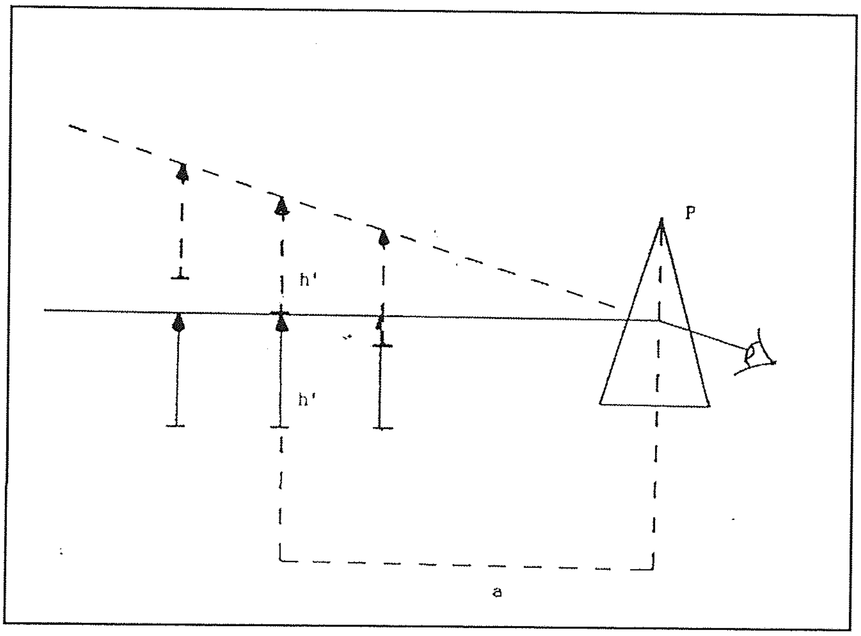
d = Distance between image plane R'Q' and object plane RQ

x = Distance between F and RQ, A = Pole of the cornea, C = Centre of curvature of cornea surface, r = AC and r/2 = AF

$h'/h \quad = \quad (r/2)/x$ but d is approximately equal to x then

$h'/h \quad = \quad (r/2)/d \qquad r = 2dh' / h$

Figure 3.2.2 Image Doubling by Prism



For a prism of power P dioptres fixed halfway in an observation aperture:

H = Image size – seen doubled

a = Distance between plane of the prism and the position at which the doubled image are exactly adjacent to each other.

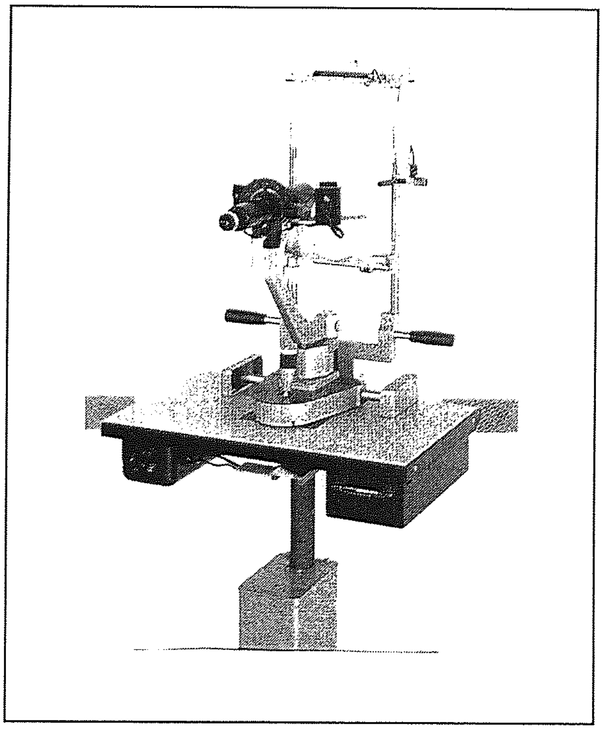
$h'/a = P/100 \ \ldots \ h' = aP/100\text{mm}$

These features distinguish between the 'fixed' and 'variable' doubling types of keratometers. In two position keratometry e.g. using the Javal Schiotz design made by Haag Streit, (Figure 3.2.3) image doubling usually occurs in only one meridian, i.e.

along the line joining the mires, hence the instrument would be rotated about its optical axis in order to align it with each of the principal meridians of the cornea in turn.

A one position keratometer is an instrument in which variable doubling of the mutually perpendicular image is produced by two doubling devices in the corresponding meridians, the distance 'a' being varied as the prism travels along the instrument axis between the objective and the eyepiece. Such an instrument e.g. the American Optical or Bausch and Lomb design, must be rotated about its axis to align the mires with both principal meridians of the cornea (assumed to be perpendicular to each other) and the images in each can be brought into contact without further rotation. The ring mires are used in the Bausch and Lomb keratometer, and are useful for obtaining a measurement even if there is some distortion of the corneal contour. Distortion of the rectangular mires of the Javal Schiotz or Zeiss keratometers more greatly affects measurement. The variable doubling of the image is achieved in the Bausch and Lomb instrument by using four apertures placed between the two objectives of the viewing telescope. Two apertures contain base in and down prisms respectively; the first causes a doubled image horizontally and the second vertically. The doubling is varied by movements of the prisms along the axes. Another two apertures follow the Scheiner principle, i.e. give two closely overlapping direct images unless the telescope is sharply focused.

Figure 3.2.3 Haag-Streit (Javal Schiotz) Keratometer



3.3 Sources of Error in Keratometry

Several factors influencing the keratometer measurement accuracy are functions of the instrument theory and design (Sanders et al 1993). The relationships derived in Figure 3.2.1 are based on paraxial optical theory, shown by Emsley (1960) to oversimplify the situation. Bennett (1966) and Bennett and Rabbetts (1991) adopted a trigonometrical ray-tracing procedure for several spherical surfaces of different radii and showed that as a convex mirror, the cornea focal length would be approximately 4.0 mm, yet the image forming rays from the mires are incident on the cornea at a distance of at least 1.0 mm from the vertex, i.e. over a zone of 2.0 mm diameter. Hence spherical aberration becomes significant and oblique astigmatism is involved since the incident pencils of light are far from being normal to the surface. Bennett estimated that calibration using the paraxial theory gives errors of about 4 to 5 percent in the results. Keratometer manufacturers have incorporated corrections for these errors.

The second of the equations derived using Figure 3.2.1, namely ($r = 2dh'/h$), is an approximation in which d, the separation of object and image, is assumed to be the same as x, the distance of the object from the focal point. This assumption is used in most keratometer designs, the mires being mounted relatively close to the eye. The error is small because of the high reflecting power of the cornea. Emsley (1952) calculated that for a cornea of radius of curvature 8.0 mm, the error amounts to 0.02 mm with the Bausch and Lomb keratometer in which d is 72.0 mm, and that if d is increased to 150.0 mm the error reduces to 0.003 mm. It can be eliminated if the mires are made as targets of a collimating system as in the American Optical design.

Figure 3.2.1 also shows that the light from the mires is reflected, not from the keratometric pole towards which the telescope is directed, but from two small areas either side. The instrument is calibrated on the assumption that these two areas are on a spherical surface and the resulting radius is attributed to the keratometric pole. The measurement becomes incorrect if these two areas where the mire images are reflected differ in curvature from that of the pole or from each other. The error is more serious if the two reflection areas are large or widely separated, or if the keratometric pole is markedly decentred within the optic cap (Mandell 1964). Table 3.3.1 gives the diameter of the corneal reflection area for a single mire as determined by Lehmann (1967), who reported the width of the reflection zones of a Bausch and Lomb instrument to be 0.1 mm, with a separation of about 2.9 mm.

Table 3.3.1 Diameter of Corneal Reflection Areas for a Single Mire

Keratometer	Radius of Curvature 7.00mm	Radius of Curvature 9.00mm	Radius of Curvature 10.00mm
	Diameter of reflection area mm	Diameter of reflection area mm	Diameter of reflection area mm
Bausch and Lomb	0.10	0.10	0.10
Zeiss	0.20	N/a	0.40
Gambs	0.20	N/a	0.40
American Optical	0.10	0.10	0.10
Haag-Streit	0.40	N/a	0.30

The main source of error controlled by the observer is focusing. If the mire images are inaccurately focused in the intended primary image plane, the radius measurement will be incorrect since the object to image separation is then incorrect. These out of focus images may not seem blurred if the observer accommodates. Collimated mires and a fully telecentric viewing system minimise this source of error. The judgement of focus can be assisted by including a Scheiner disc in the viewing system as in the Bausch and Lomb type of keratometer, where the central mire image is seen as double if it is not accurately focused. By keeping errors to a minimum, repeatability of readings should be 0.02 mm. Davis and Dresner (1991) found the results from the keratometer to correlate well with those from the corneal topography system, and were also statistically more accurate for steeper calibration spheres. Calibration errors are very small, and show less influence on a longitudinal study noting changes in values, rather than attempting measurements of absolute values (Stone 1962, Bailey and Carney 1977). Sunderraj (1992) emphasised that although automated keratometers gave a rapid reading, manual keratometry was preferred for accuracy when calculating intra-ocular power. The reliability of keratometry readings was discussed by Brungardt (1969, 1973) and by Butcher and O'Brien (1991). Brungardt (1969) found horizontal variations of 0.074 mm and vertical variations of 0.15 mm. Error is incurred since the measurements are made with respect to the visual axis rather than the corneal apical normal. A portable automatic keratometer is easy to use (Manning and Kloess 1997), but Morlet (1994) found greater measurement error than with a traditional keratometer. The increased reliability and ease of use of the IOLMaster (Sheng et al, 2004) has nowadays led to its popularity over the traditional keratometer .

3.4 Keratometry of the Peripheral Cornea

Mandell (1962 a, 1962 b, 1965) modified a Bausch and Lomb keratometer in order to reduce the mire separation from 64.0 mm to 26.0 mm and he made measurements of the corneal periphery by using the addition of a series of off-axis fixation points. The systems used by Bonnet and Cochet (1960) use one mire for peripheral measurements, which can be obtained from small corneal areas of diameter 0.50 mm. Their measurements of central radii are by the classic method of two mires over a wide corneal chord. The Zeiss keratometer can also give single mire keratometry and has a moveable fixation point and an extended radius scale needed for peripheral measurements.

Several instruments, including the Zeiss, Bausch and Lomb, and American Optical keratometers have topographical attachments whereby the subject's fixation is diverted to points away from the keratometer main axis so that the reflection zones fall on more peripheral portions of the cornea. With the Topogometer attachment for the Bausch and Lomb keratometer and with the Topographical attachment for the American Optical Ophthalmometer (keratometer), the device or varying the fixation is graduated in millimetres from the corneal centre. Full consideration of the position of the centre of rotation of the eye does not seem to have been made in the fixation target and the distances from the keratometer objective to corneal vertex and from the corneal vertex to the centre of ocular rotation. Ludlam and Wittenberg (1966) emphasised the importance of taking into account this rotation centre. The American Optical Company design assumed that the corneal vertex distance from the centre of rotation was 11.3 mm, which was adequate for clinical use.

Keratometry theory also assumes a common axis for the centre of curvature of all parts of the cornea, whereas in practice the centres of curvature for the peripheral zones are offset from the central axis of symmetry. Mandell and St. Helen (1968) used small mire keratometry with peripheral fixation but found this very time consuming and the results insufficiently accurate and reliable. Douthwaite (1987) recommended an instrument adjustment using a method of Drysdale, in order to improve the keratometer for peripheral corneal measurement.

Rosenfield and Portello (1996) said that multi-meridional keratometry gave comparable results to standard two meridian keratometry but could prove to be more valuable in cases of irregular astigmatism e,g. in keratoconus. Alimisi et al (1996) found keratometry to be inaccurate on cases of irregular corneal astigmatism. They recommended the use of quantitative descriptors derived from computer assisted corneal topography.

Zadnik, Mutti and Adams (1992) found keratometry to be more reliable (+/- 0.87D, 0.17 mm) than photokeratoscopy (+/- 2.02D, 0.4 mm) with a statistically significant bias of 0.57D (0.11 mm) flatter corneal curvature reading when using photokeratoscopy. Cuaycong et al (1993) agreed with these findings and suggested that the larger diameter ring mires of the photokeratoscope were reflected by loci further from the corneal apex. The aspheric corneal contour gradually becomes of a flatter curvature the greater is the distance from the apex.

Chapter 4

Keratoscopy

4. 1 Methods of Assessing Corneal Topography

A keratoscope is an instrument that projects multiple concentric rings (mires) onto the anterior corneal surface. In this chapter 'mire' refers both to the projected image and the image reflected from the cornea. Keratoscopy is the direct visual observation of the mires, used to assess the regularity of the corneal topography. Antonio Placido in 1880 designed a disc of alternating black and white concentric circular rings with a small central hole through which the examiner observed the mires formed by reflection from the anterior corneal surface. Following the major modification and further investigation of the keratoscope principle since its introduction in 1896 by Gullstrand, who described this more fully in 1911, sophisticated photokeratoscopes were invented for assessing corneal topography and for photographing images reflected by the cornea. Gullstrand measured the corneal topography by analysing photographic data of the corneal ring images and calculating the radius of curvature of a given sector of the cornea from the target ring separation in the image reflected from that area. A valid corneal measuring device should be accurate, reproducible, easy to operate, able to produce results rapidly, harmless to the cornea, tolerable to subject and operator, and inexpensive. Elliptical distortion of the mires is the classic sign of regular astigmatism, with the short axis of the ellipse relating to the steepest curvature meridian and the long axis of the ellipse relating to the flattest curvature meridian.
Early studies were performed by: Knoll (1961, 1966), Townsley (1967, 1970), Wesley (1969), Mandell et al (1969a, 1969b, 1971) and Rowsey et al (1981, 1983, 1989).

Many of the methods for assessing corneal topography were reviewed by Clark in 1973. He criticised the majority of methods as being invalid or unsuitable. He described the optical methods of autocollimation, interferometry, Moire fringe methods, and stereophotogrammetry. In describing profile and section methods he included direct profile photography, slitlamp photography, cast and template, and fluorescein methods. Attempts with television, sclerokeratometry, and ultrasonography were also mentioned.

Clark (1972) and Maguire (1988) recommended the use of the autocollimated photokeratoscope. Fowler and Dave (1994) reviewed the development of keratoscopy to the recently used computer- assisted videokeratography. Although the latter, e.g. the EyeSys Corneal Topography System, provides an increased number of data points and contour description in the form of colour mapping, there remain problems in the consistent reproduction of measurements (McCarey et al 1992, Mandell 1992, Roberts 1994, Tang et al 2000).

4.2 Keratoscopy

4.2.1 Introduction

The keratoscope provides information about whether the corneal region centred about the visual axis is spherical or toroidal, also about the directions at the principal meridians of a toroidal cornea and at the displacement direction at the corneal apex with respect to the visual axis. Information is given as to whether the peripheral corneal flattening is equal in all meridians and to the presence of any localised surface irregularities, (El Hage 1971, El Hage and Beaulne 1975).

Factors affecting the accuracy of photokeratoscopes include:

Aberrations due to the camera lens,
variation in magnification over the image plane,
the grain size of high-speed film necessarily used that can make precise measurement difficult,
difficulty in the accurate focusing of the camera.

As with most photography, methods of film developing and printing are rarely constant and possible film shrinkage can affect accuracy.
Additional problems are caused by aberrations introduced by oblique reflections from peripheral portions of the cornea.

4.2.2 Advantages and Disadvantages of Photokeratoscopes:

Advantages: -

- Simultaneous acquisition at all the information,
- Provision of hard copy data that may be re-examined,
- Examination allowed of the apical cap within the usual 3.0 mm ring of the cornea,
- The mid-peripheral cornea is examined so early astigmatic errors may be traced,
- Approximately 55% of the total corneal area can be examined compared to 8% using the clinical keratometer,
- Subtle topographic shifts induced by trauma, contact lens wear or progressive corneal dystrophies can be estimated,
- Complex contact lens fitting is assisted by the determination of peripheral corneal curvature and sequential quantitative evaluations of astigmatism are improved.

Disadvantages: -

- Aberrations are introduced due to measurement being around the visual axis rather than the corneal apical normal,
- Some photography experience is required,

- Refocusing of the reflected images produces an inaccurate chord length measurement. This measurement is large if the eye is too near the camera and the measurement is small if the eye is too distant.

4.2.3 Requirements for Reliable Photokeratoscopy

These requirements include instrument stability and the accurate knowledge at the positions of the object and of the camera axis (Riss et al 1992). The working distance of the system should be defined, the depth at focus should be minimal and maximum aperture photography be used. The viewfinder image should be magnified to assist accurate and consistent focusing of the camera and the alignment of the reflecting surface with the camera system must be assessed for each photokeratograph. The instrument must have freedom of movement in the lateral, vertical and anterior—posterior directions, and the movement of the subject's head must be controlled. The resulting turn images should be free from distortion, whether induced by the lens system or by dimensional instability of the film, so effects at processing can be assessed and controlled, Dingeldein et al 1989). The total system should have good accuracy and reproducibility and, regardless of the mathematical expression for the corneal shape, the units should be internally consistent.

When examining the keratoscopy photograph of the cornea the pattern can be interpreted in the same manner as for a contour map (Ruben 1975). If the reflected rings are circular and uniformly spaced, then the cornea is spherical, if elliptical then the cornea is toroidal in shape. If the spacing increases as the ring diameter increases, this indicates that the corneal curve flattens towards its periphery, according to Brungardt (1981) and to Edmund and Sjontoft (1985).

4.2.4 Autocollimation

Drysdale in 1900 showed that the radius of curvature of a convex spherical mirror could be determined by measuring the distance between the two positions where the mirror would reflect an incident converging beam of light back along a similar path. There are the two positions of autocollimation; the first is when the light is focused on the surface while the second position is when the light is focused towards the centre of curvature of the surface. The autocollimation method introduces an error due to the depth of focus, since the lens can be moved a short distance yet the locus seems the same. This error is given by w/2(NA), where w = the wavelength of light and NA is the numerical aperture of the system. The autocollimation instrument operates on the assumption that the surface is spherical beyond the central 1.0 mm diameter region, but except for the central optical cap the cornea is aspherical.

The autocollimating photokeratoscope can be used to measure corneal topography with greater accuracy than with the methods described by Clark (1973). Light from a set of target rings is directed onto a cornea at near normal incidence and the reflected real image from the cornea is projected onto a scale that indicates the radius of the reference sphere. The difference in position between any target point on the photograph and the corresponding point on a photograph taken with a spherical control reflector is used to calculate the departure of the corneal curvature from spherical.

This type of keratoscope allows qualitative and quantitative determination of the surface topography of the cornea by observation of a real image reflected from the cornea rather than of the first Purkinje image (virtual image) as used in a conventional keratoscope. Light from the target rings is directed onto the cornea at near normal incidence, enabling use of a wider corneal image area, whereas light from the flat or cylindrical target of the conventional keratoscope subtends an angle of about 150° at the eye, so the image area is limited. With this photokeratoscope many corneal meridians can be described in terms of asphericity, S, from a reference sphere as a function of the distance from the keratoscope axis. The reference sphere has a radius calculated to most readily fit the central 3.0 mm portion of the corneal diameter.

An example of this type of instrument was the photoelectronic keratoscope, which was developed to improve the accuracy and reproducibility of topography measurement. Schultz (1976), Bibby (1976) and Bibby and Townsley (1976) described this keratoscope and its usefulness in obtaining information regarding the geometric axis displacement relative to the visual axis. The instrument allowed rapid use because the single lens reflex camera enabled the clinician to obtain the correct camera position and focus swiftly. The Polaroid camera gave prompt film development. Other photokeratoscopes with a larger number of target rings were devised (Zabel et al 1989). The photokeratoscope was superceded by the videokeratoscope.

4.3 Videokeratoscopy

4.3.1 Videokeratoscopy Methods

Automated analysis of corneal surfaces used computers to map the cornea, record the position of the mires and use mathematical relationships for the three dimensional reconstruction of corneal shape to help clinical assessment. The systems that incorporate a video camera for data collection are called videokeratoscopes. Examples are the Computed Anatomy TMS-1 (Gormley et al 1988) and the EyeSys from EyeSys Laboratories. The image of the mires is captured by video frame, digitised and analysed by computer (Doss et al 1981, El Hage 1989). Dingeldein and Klyce (1989) and Klyce and Dingeldein (1990) developed graphic presentation systems in attempts to provide accurate and easily assimilated information regarding corneal topography. Their advantage over previous keratoscopes is the ability to make measurements from the central cornea and to present data in a simple format with reasonable accuracy. A number of papers have described these measurements, e.g. Douthwaite and Sheridan (1989), Hannush et al (1990), Wilson et al (1992), Fowler and Dave (1994), Hubbe and Foulks (1994), Zadnik et al (1995), Belin et al (1996) and Douthwaite and Pardhan (1998). The transverse spatial resolution of the instrument should be able to detect surface irregularities that would affect visual function. Their effect is likely to vary from centre to the periphery of the cornea.

Most videokeratoscopes use an internally illuminated cone of concentric ring mires with a central light spot for the subject to fixate. The mires cover the cornea over a diameter of 11.0 mm, excluding the central 0.3 mm diameter region and the paralimbal 1.0 mm region. Some systems, e.g. the Topographic Modeling System (TMS-1) described by Brenner (1997), have 30 to 40 narrow rings with steeply curved cones which position closely to the subjects eye, allowing assessment of wider corneal peripheral zones even on steep corneas. Certain systems have a long working distance between the cornea and the cone and are therefore liable to a smaller error given by misalignment of the cone position relative to the cornea. However, shadows caused by the subjects brow, lids and nose may obscure part of the mire pattern.

The mires cone is aligned by correctly positioning relative to the cornea, and the cone is slowly moved towards the cornea until the mires are focussed. Modern systems incorporate auto-alignment and auto-focus features used after the initial positioning of the instrument. Klyce (1984), Wilson and Klyce (1991) and Wilson et al (1992) explained that this aims to improve the measurement reliability when the video camera records the reflected image. The single video image is stored and automatically digitised, using electronic detection of the mires to a good accuracy (error is less than

0.25 D, 0.05mm). Most systems use the fixation light reflection or the centre of the inner mire as the central reference point. Rectangular co-ordinates are found for each data point where a semi-meridian intersects the mire. Many systems have 15 to 38 circular mires and 256-360 equally spaced semi-meridians (so approximately 11000 data points). The accuracy of the final reconstruction depends on the data point density.

Mathematical formulae known as reconstruction algorithms convert the data to topographical information by attempting to calculate the corneal surface contour at each point of reflection. These formulae cannot guarantee accuracy. Firstly the normal corneal shape is complex, varies between individuals and there is no known mathematical formula that exactly describes it. The algorithm tends to be more accurate for the central corneal region that is more spherical. Secondly there is the assumption that light from one meridian on a particular mire falls on the same meridian at the plane of the film capturing the image. Averaging techniques are incorporated to 'smooth out' irregularities due to artefacts but these can also lead to reduced detection of the true corneal surface irregularities.

When calibrated on spherical test objects, videokeratoscopes tend to give a computational accuracy of 0.15 D (0.03 mm), which is well within clinical tolerance. However, when measuring aspheric surfaces similar to those of the normal cornea, the accuracy decreases rapidly in the peripheral regions, e.g. beyond the 8.0 mm diameter limit this becomes approximately 3.00 D (0.60 mm). This results from the spherical bias in the assumptions as the system calculates the tangential radius of curvature. Heath et al (1991) claimed that the Corneal Modelling System gave accurate topography values on spherical and toroidal corneas and that those on aspherical surfaces were less accurate but clinically acceptable. Tripoli et al (1996) were among those who tried new algorithms to address this problem.

Accuracy is also decreased when there is significant surface irregularity or when the curvature is very steep (> 46 D, 7.34 mm) or very flat (<38 D, 8.88 mm).

Most placido disc based systems require a critical focus to be accurate. Both the TMS (Computed Anatomy Inc New York) and the Corneal Analysis System (EyeSys

Laboratories, Houston, Texas) are focused in the horizontal meridian (Young et al 1995). On highly astigmatic or irregular corneas the non-horizontal meridians are partly out of focus and susceptible to errors. Antalis et al (1993) compared these two corneal topography instruments and found many similarities in measurements across various curvatures. The TMS sometimes gave higher readings. Certain patients found that the glare from the wide illuminated target rings of the EyeSys led to some discomfort and they preferred the smaller, more dimly lit TMS rings. However, the closely spaced ring mires of the TMS tended to merge on steep surfaces so were more difficult to analyse. As the EyeSys depth of focus is approximately three times that of the TMS-1, the image focussing was easier to obtain with the EyeSys. Koch et al (1989, 1992) claimed the EyeSys instrument provided an accuracy and reproducibility of values of 0.25D (0.05 mm) on spherical test surfaces and similar results for several aspherical surfaces. They emphasised the advantages of the EyeSys with regard to easy operation, convenience and rapid data analysis and display.

Two groups, Wilson and Klyce (1991) and Alvi et al (1997) gave interesting overviews of topographic analysis and how this can reveal topographic alterations not previously discernable. They explained how information given by the colour-coded maps can be augmented by quantitative descriptors e.g. asymmetry index, surface irregularity index and simulated keratometry. These descriptors were used by Stevenson (1992) and Rabinowitz et al (1998).

Corbett et al (1994, 1995) and Dave (1998a) also gave good overviews of many of the corneal topography systems, most of which are based on the principle of reflection (videokeratoscopy). Corbett advised how to present the topographic data in the most useful form. Artefacts can be edited out before the image is processed and alignment can be checked by super-imposing the topographic map onto the videokeratoscopy image. The resolution of the map may be altered by adjusting the colour scales. Grids can be over laid to help estimate the size and location of abnormal corneal areas. A few modern systems use projection methods (raster-stereography, Moire interference and laser interferometry). The latter directly measuring corneal height so can be used in the 'tailor-made' treatment of irregular astigmatism.

Most modern keratoscopes use colour coded maps of corneal surface powers as described by Maguire et al (1987) and by Applegate and Howland (1995). These maps indicate the surface power distribution for easier recognition of corneal shape patterns and irregularities.

Other methods of topographic analysis include interferometry and raster-stereography. Studies on interferometry (Corbett et al 1995) show higher resolution of corneal topography but the application to the range of corneal shapes is limited because this method compares small differences between two surfaces. Raster-stereography uses a projected grid of light to illuminate fluorescein-dyed tear film on the ocular surface and the pattern is photographed at a defined angle (Arffa et al 1989). However it can be less sensitive than keratoscopy in the detection of subtle topographic changes. This is because it uses a projected technique rather than the keratoscopes reflection technique that amplifies topography distortions. Warniki et al (1988), Baker (1990) and Naufal et al (1997) described raster-stereography but noted that this is less successful in clinical applications.

The OrbScan topography-pachymetry system uses a slit image scanning device to measure anterior and posterior corneal surface curvatures and thickness (Cairns and McGhee 2005). This instrument is becoming used more often but the high cost puts this beyond the budget of many clinicians.

4.3.2 Normal Corneal Topography

Classifications of normal and abnormal corneal topography that are based on the recognition of topographical map patterns have been described by Klyce and Wilson (1989), Bogan et al (1990) and Alvi et al (1997). They found 22% round, 21% oval, 17% regular astigmatism and 32% asymmetric astigmatism, but no significant relationship of pattern to age. These results were similar to those of Kanpolat et al (1997). Rasheed et al (1998) claimed consistent results with good inter-observer reliability for their 'ten category' classification system of the TMS-1 absolute scale. Holladay (1997b) used topography categories to produce a diagnostic guide. Camp et al (1990) studied the relationship between topography and optical performance.

Maloney et al (1993) determined basic corneal image forming properties on a variety of cornea including those of irregular contour where keratometry was unreliable. Wilson and Klyce (1994) declared the videokeratoscope to be useful when screening subjects requesting refractive surgery. Abnormal corneal topography was found in 33% of the 106 eyes of their study, many related to contact lens induced corneal warpage. This may help to explain why some subjects experience irregular astigmatism following refractive surgery and require refitting with contact lenses (Astin et al 1996).

Potvin et al (1996) compared keratometry with the EyeSys and the TMS systems and found that (the angle kappa) variability in the fixation angle had a greater effect on results when using the EyeSys system, probably due to the longer distance between the mires cone and the cornea. They found the inter-observer variability to be 0.25-0.50 D (0.10 mm), and that the 'smoothing out' process in the curvature calculations led to smaller astigmatism values. Dave et al (1998b) found high repeatability of the EyeSys on test surfaces.

Hannush et al (1990) claimed that over the central 3.0 mm diameter corneal region the Corneal Modeling System (videokeratoscope) gave only 83% reproducibility of values compared to the keratometer, although it was still better than the Corneoscope (photokeratoscope) system. However, the videokeratoscope provides corneal topography information in a visually more useful manner.

Tang et al (2000) tested the accuracy of three placido-disc videokeratoscopes and generally found good accuracy on aspheric surfaces although the TMS showed a weakness on multiple curvature surfaces.

Davis and Dresner (1991) measured calibration spheres and fourteen eyes and found keratometry to be statistically more accurate and precise when measuring steep curvatures (>45D, <7.50 mm). On humans the keratometer showed very good correlation with the topography instrument but the latter demonstrated greater variability of values especially on steep curves. Tsilimbaris et al (1991) found no significant difference in values between the Corneal Analysis System (EyeSys) and the keratometer. The measurement repeatability of a keratometer, a photokeratoscope and a videokeratoscope was studied by Zadnik et al (1995). They concluded that over the central cornea region the keratometer was best and the videokeratoscope was a close second, although over the mid-periphery region the repeatability soon became worse.

Errors due to defocusing were studied by Oltrup et al (1997). Most systems guide the operator by means of various adjustment marks. However, involuntary misalignments may occur and influence image acquisition and data evaluation (defocusing in the range of 1.5 mm gives result deviation of less than 0.05 mm, 0.25D). They concluded that automated compensation in the videokeratoscope actively compensates for these errors, enhances precision and avoids artefacts.

Dave, Ruston and Fowler (1998b), when evaluating the EyeSys videoscope, compared measurements from a keratometer with those obtained from the EyeSys corneal analysis system. Their keratometry values from the two instruments were not interchangeable, possibly due to different areas of the cornea. Their results on 14 keratometry subjects and 10 EyeSys subjects indicated a similarity for repeatability. However, the EyeSys analysis showed variations in repeatability along the superior and nasal meridians. They suggested that this spatial dependency could be attributed to interference by the ocular adnexa in those areas. When this research team (1998c) studied the precision and repeatability of the EyeSys on a series of 12 aspherical convex surfaces, they found a high correlation between the actual and the measured values of the central radii. The instrumental bias was small and the limits of agreement were narrow, indicating clinically acceptable accuracy. The overall accuracy of the peripheral radii was also good. The repeatability for the aspherical surfaces was high (SD=0.01mm). However, for surfaces whose eccentricity was 0.50 the bias increased to +0.049, with maximum error of 0.110mm, therefore they concluded that for rapidly flattening surfaces the value accuracy decreases but is still clinically acceptable.

Pavlopoulos et al (1995) described how topical lubricants gave misleading results for normal eyes but could be of benefit when assessing irregular corneal surfaces.

The EyeSys videokeratoscope study by Douthwaite et al (1999) (n= 98) showed a group average apical corneal radius of 7.93mm (horizontal) and 7.78mm (vertical). The group average corneal asphericity (p-value) was 0.76 (horizontal) and 0.82 (vertical), and both the apical radius and the p-value were similar when comparing the right and left eyes in the two principal meridians. The median age of the group was 36 years (range 20-59) and no apparent association was found between age and either apical radius or p-value. The apical radii of the males were longer than those of females but the p-values were the same. The corneal asphericity did not show any apparent association with corneal curvature.

Douthwaite (2002) studied three methods of linear regression analysis on data displayed by the EyeSys videokeratoscope. Various errors were found which varied depending on the asphericity (p-value) and on the tilt of the surface under observation. He recommended one analysis method for the most accurate prediction of apical radius. A separate method considering each semi-meridian was preferred for the prediction of p-value and surface tilt.

Chapter 5

Pachometry

5.1 Pachometry – Description and History

5.1.1 Introduction

The pachometer is an instrument designed and calibrated to measure materials of refractive index 1.376. It is used for in vivo measurements of human corneal thickness, - a complex task since the cornea is a mobile, fairly transparent medium with transparent fluids bounding it anteriorly and posteriorly. Pachometry is a useful measure of the physiological stability of the cornea, since factors affecting the corneal metabolism, even in localised areas, alter the hydration and hence thickness of the cornea in those areas. Pachometry is becoming more widely used in monitoring eye conditions, e.g. after surgery. The two main methods are optical and ultrasonic.

5.1.2 History

Early studies on corneal thickness tended to give rather high values e.g. 0.90 mm, Thomson (1912), 0.80 mm, Salzmann (1912) both cited by Von Bahr (1948). This was probably because those measurements were mostly on cadaver corneas which had undergone post-mortem swelling and decreased clarity. The first measurements on living eyes were attributed to Blix (1879) who found values of 0.48 to 0.58 mm. Values in the range 0.46 mm to 0.51 mm were found by Gullstrand in 1909, and a mean value of 0.53 mm found by Sobanski in 1934 (cited by Von Bahr 1948).

Various optical methods of pachometry have been described by Ehlers and Hansen (1971), Woodward (1980), Button (1986) and Brennan et al (1989). Blix used the method of successive focusing on the specular reflexes from the anterior and posterior corneal surfaces. The distance moved by the apparatus along the axis between the two microscopes is the apparent corneal thickness. If the refractive index and radius of curvature of the cornea are known, the real thickness may be calculated. Problems with this method included the possibility of corneal movements between measurements, also the large difference in brightness between the two reflective images.

Gullstrand (1911) made simultaneous observations of the specular reflexes and used a weak light source for the anterior corneal surface and a bright one for the posterior surface. Von Bahr (1948) used rotating parallel glass plates to superimpose the two reflections instead of using a travelling microscope. The successive focusing technique was later developed into a commercial instrument, claiming an accuracy of +/- 0.01mm by Maurice and Giardini (1951).

Another method is to measure the apparent thickness of the optical section of the cornea, as seen by diffuse reflection of the stromal layers. Dependent upon the angle of incidence of the light and observation angle, the real thickness may be calculated in different ways from the measured apparent thickness (Juillerat and Koby 1928). The measurement is simplified if the surfaces are observed simultaneously. By applying this principle (Figure 5.1.2.1), Jaeger (1952) developed a commercial instrument for the use with the Haag-Streit Slitlamp model 900 (Figure 5.1.2.2).

Figure 5.1.2.1 Jaeger's Pachometry Method

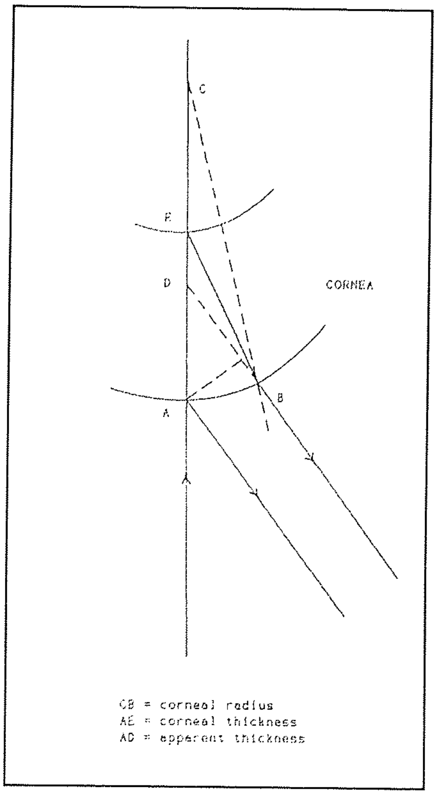


Figure 5.1.2.2 Haag-Streit Slitlamp

Using this apparatus, Mishima and Hedbys (1968), and Lavergne and Kelecom (1962) found reliable results with a standard error of only +/- 0.003 mm. The two currently commercially available instruments use different principles. The Maurice-Giardini pachometer aligns the specular reflexes from the two corneal surfaces, and the Jaeger designed attachment measures the apparent thickness of the optical section.

5.2 Factors Affecting Reliability of Measurements

5.2.1 Instrument Reliability Factors

Corneal thickness measurements have been attempted by a number of researchers, with increasing attention on improving accuracy, (Molinari 1982, Molinari and Bunds 1983, Snyder 1984). Molinari noted that the clinician has difficulty achieving high accuracy because of standardisation problems including slit width and angle of incidence, accuracy of focusing during the measurements, fixation instability, the observer's judgement and the need to determine the same point on the cornea for sequential measurements. Edmund and La Cour (1986) noted variation from pachometer adjustment of 0.013 mm. Difficulties can arise if measuring abnormal eyes with irregular surfaces, poor transparency, disturbed epithelial or endothelial layers, photophobia, blepharospasm or nystagmus.

Several problems were noted by Mishima (1968) as follows:

a) Illumination

A bright slit beam and dim room illumination, as used in the current study, help the observer to judge the focusing of the slit beam, since the contrast is improved. Such little change in result has been found from changing the light wavelength, that single wavelength beams have been abandoned and the usual white slitlamp beam used. Slit width and the focusing of the microscope limit the accuracy of the optical section methods, (Olsen et al 1980a,b). The Haag-Streit microscope has relatively low magnification (10 times), and has fewer problems.

b) Observer

Experience and dexterity with the pachometer enables swifter, more accurate measurements to be taken before the subject tires. Inter ocular error of up to 0.02 mm may occur with differences in judging the optical section edge profile as shown by Patel (1981), Hirji and Larke (1978a & b). The current study used the same experienced operator for all the pachometry. Olsen et al (1980 a & b) found this optical method gave high intra-observer reproducibility of readings and concluded that corneal thickness can be determined with good accuracy.

c) Tear Layer

Sometimes fluorescein is put into tear layer so that this layer is more easily distinguished, and the contrast improved when focusing on the epithelium (Crook 1979). Care is taken not to stimulate lacrimation, which would increase tear layer thickness and confuse the observer. Farrell and McCally (1973) indicated the problems associated with alignment setting of the optical section.

d) Electronics

If the pachometer read-out system relies on electronics then these must be checked and ensured reliable. In the current study time was allowed for the electronics to stabilise before readings were taken.

e) Regional Fixation Targets

These are steady, not glaring but easily visible without the need for focusing or good visual acuity. For all sequential measurements, they must have the same position as originally located. Their position from the centre is not so distant that the subject's eye is under strain to maintain fixation.

5.2.2 Ultrasonic versus Optical Pachometry Method

Salz et al (1983) Gordon et al (1990) and Wheeler et al (1992) claimed that the ultrasonic method gives more repeatable results and the following advantages: high reproducibility, negligible inter observer and inter ocular variations, simple operation and needs minimal patient co-operation. However, the ultrasonic measuring system has several disadvantages, Gritz and McDonnell (1990). Measurements are only considered accurate to +/- 0.05 mm of tissue thickness, and since valid measurement of variations involves error if there is misalignment of the sound beam with the visual axis then this error could increase to +/- 0.10 mm (Coleman and Carlin 1967).

There is physical contact between the probe and the cornea, requiring the cornea to be anaesthetized. The probe tip must be cleaned and sterilized immediately before use (Mandell, Polse and Bonanno 1988), although some highly resistant organisms may remain. A disposable tip cover could help hygeine. The exact corneal area measured is difficult to localise and a wider range of measurements is obtained. This reflects the uncertainty of repositioning the ultrasound probe at the same point on the corneal surface for each reading (Patel and Stevenson 1994).

A number of independent clinical comparisons indicated reasonable correlation between optical and ultrasound pachometry so that the two methods may be generally considered to be equivalent but not identical. Doughty and Zaman (2000) reported group- averaged values for central corneal thickness of 0.530 mm (SD 0.029) using the slitlamp method and of 0.544 mm (SD 0.034) using the ultrasound method. Binder et al (1977), Giasson and Forthomme (1992) and Yebra-Pimentel et al (1998) claimed that for clinical purposes, optical and ultrasonic pachometry techniques were comparable. Nissen et al (1991) and Patel (1987) agreed with their conclusion but pointed out that the difference between the results obtained by optical method and ultrasound method increases with increasing corneal hydration. Ultrasonic methods tend to give thicker readings if set at higher velocity, depending on corneal temperature. Improvements to the accuracy of optical pachometry have been made by the following: Donaldson (1966), Mishima and Hedbys (1968), Ehlers and Sperling (1977), Mandell, Polse and Bonanno (1988), and Stevenson (1989). Stucchi et al (1993) noted that ultrasonic pachymetry tended to underestimate the value by about 3%. They showed a corneal thickening response to application of topical anaesthetic.

5.2.3 Angle Kappa

This is the angle between the subject's visual axis and the axis that is perpendicular to the cornea as according to Jaeger's principle. Usually in routine clinical pachometry, the patient fixates the incident light so the measurement is made along their visual axis. This often limits pachometry to the central cornea only and also introduces measuring errors proportional to angle 'kappa' (Von Bahr 1948) and a difference between measuring right and left eyes, left eyes having higher readings. This difference has been shown to be statistically significant by Hansen (1971), by Woodward (1980) and by Yebra-Pimentel et al (1998). (As the sum angle kappa increased to 20 then the thickness error increased to +/- 0.02mm). If consistent fixation is maintained, there should be no significant change in angle kappa.

Mishima and Hedbys (1968) designed the modification using two vertically mounted pin lights placed to one side of the incident beam such that their reflections were at an angle equal to the observation angle and were viewed through the eyepiece to allow measurements with the incident light falling perpendicular to the anterior corneal surface. No reduction in standard deviation of the measurements was demonstrated, possibly due to the patients' difficulty in maintaining accurate fixation on a small target near the source of the incident slit beam.

5.3 Variations in Normal Corneal Thickness

These variations can relate to the normal range of thickness or to the region of the cornea. Factors such as age, diurnal variation, menstrual related variation, contact lens wear, tears osmolarity, and intraocular pressure can also affect the corneal thickness, (Feldman et al 1978, Kiely et al 1983 and Cho and Lam 1999).

5.3.1 Age and Gender

Weale (1971) mentioned that the muco-polysaccharides and collagen fibrils in Descemet's membrane develop cross-links with ageing, decreasing elasticity and slowing down corneal swelling in the elderly eye. The review of ocular collagens by Bailey (1987) supported these findings. Although corneal thinning with age was suggested, no significant difference with regard to age or gender was found by Hansen (1971), Von Bahr (1948), Lowe (1969), nor by Siu and Herse (1993). Wolfs et al (1997) involved 352 normal control patients aged over 55 years to improve the statistical significance of the Rotterdam study results. They found a mean central corneal thickness value of 0.537 mm and that for most subjects the right and left eyes gave similar values. No significant relation to gender or age ($p = 0.82$) was revealed. Martola and Baum (1968) found no significant difference between right and left eyes, nor a relation to gender, refractive error nor arcus senilis. Lowe (1969) showed a non-significant trend of 0.002 mm increase in thickness per decade of age.

Central corneal thinning after fifty years of age (0.004 mm per decade) was found by Ehlers et al (1976), Alsbirk (1978) and by Olsen and Ehlers (1984). Kotulak and

Brungardt (1980) and Korey et al (1982) reported that increasing age was associated with a decrease in endothelial cell density, but not with central corneal thickness changes. Wigham and Hodson (1987) found that after 60 years of age the endothelial pump capacity could reduce by 25%, although the endothelial permeability shows no deterioration.

Li et al (1994) and Lam and Douthwaite (1998) reported that with increasing age the central corneal thickness reduces. Doughty and Zaman (2000) analysed a wide range of studies concerning normal corneal thickness. They described those using optical pachometry gave a mean central corneal thickness value of 0.535 mm (SD 0.029) whereas those using ultrasound gave a mean value of 0.544 mm (SD 0.034). Re-analysis suggested a slight decline in values for non-white subjects. Their survey reported the general idea that corneal thickness is independent of other ocular biometric measurements but they mentioned that certain studies e.g. Alsbirk (1978), Foster et al (1998) and Cho and Lam (1999), discovered age dependent corneal thickness reductions in some non-white subjects.

Doughty et al (2002) noted a mean value for central corneal thickness of 0.533 mm (SD 0.033) for European adults aged 32 - 60 years and a mean value of 0.527 mm (SD 0.034) in subjects aged 61– 82 years. Their overall statistical analysis indicated no significant effects due to age or gender.

Polse (1989) and Siu and Herse (1993b), when studying corneal response to contact lens induced hypoxia, found no significant difference with age as regards central and peripheral oedema produced but noted significantly slower recovery from oedema in older corneas ($p = 0.004$). O'Neal and Polse (1986) suggested that this phenomenon was related to ageing changes in endothelial morphology. Ageing appeared to influence the oedema production and recovery mechanisms of both the central and mid-peripheral cornea in similar ways. However, Bonanno and Polse (1985) and Herse et al (1993) reported that a smaller oedema response occurs in the extreme peripheral region. They proposed that this limitation of the corneal swelling was due to restraint caused by the tight interweaving of collagen fibres and the structural tissue continuity with the sclera at the limbus.

5.3.2 Diurnal Variation

Investigators of diurnal variation in cornea include Mandell and Fatt (1965), Hirji and Larke (1978) and Friedman (1973). Kiely et al (1982b) found corneal thickness increased at night when eyelids were closed, was maximal on awakening and decreased by afternoon. The diurnal thickness changes appeared to correlate with the diurnal pattern of corneal curvature. They found that corneal radius of curvature tended to decrease during the day, whereas contour asphericity remained constant. The latter showed low correlation with central corneal thickness, although some subjects parameters varied widely.

Care was taken in the study described in this thesis to perform pachometry at the same time of day for each patient and several hours after their waking. Harper et al (1996) noted on human corneas an overnight thickness increase of 5.5% (SD 2.9%) and diurnal increase of 7.2% (SD 2.8%) with individual variations. This pattern is mirrored by the diurnal fluctuations in corneal sensitivity, which is lowest in the morning and apparently related to the reduced oxygen availability due to lid closure during sleep (Lawrenson 1997).

5.3.3 Menstrual Related Variation

Kiely, Carney and Smith (1983) in a study of six women found corneal thickening at ovulation time, when oestrogen levels were raised, followed by corneal thinning. Feldman et al (1978) found similar results. No significant changes in corneal thickness were found by El Hage and Beaulne (1973) nor by Hirji and Larke (1978). In some patients, menstrual cycle changes involve water retention in the tissues, which could explain the increasing corneal thickness. Doughty and Zaman (2000) on reviewing many corneal thickness studies noted studies on women gave an overall average central corneal thickness value of 0.554 mm whereas the gender independent average value was 0.535 mm. They suggested that hormonal effects could have influenced the outcome. Certain general health problems e.g. hypertension and diabetes can cause similar effects. To avoid these influences in the current study, patients with such conditions or pre-menopause were excluded from the group undergoing pachometry.

5.3.4 Osmotic Effect

Although they could not find a significant relationship between tears tonicity and corneal thickness, Chan and Mandell (1975) and Stevenson (1983) showed a relationship to the hypotonicity of eye bathing solutions. Holden et al (1985) discovered that the corneal thickness change was almost twice the value for the central than for the peripheral region. They proposed that this was due to a difference in the structure and/or hydration characteristic of the cornea at the limbus as shown by Maurice (1969, 1972), by Borcherding et al (1975) and by Hodson et al (1981). Perfusion of hydrogen peroxide that damages the corneal endothelial cells was suspected.

5.3.5 Intraocular Pressure

Hansen (1971) showed that central corneal thickness may increase significantly with markedly raised intraocular pressure (IOP). Very high IOP disturbs the endothelium and the corneal hydration balance, leading to stromal swelling, disruption of the lamellar pattern and decreased corneal transparency. Naturally thicker corneas are likely to resist applanation by a tonometer and lead to a higher IOP reading (Dohadwala et al 1998). Wolfs et al (1997) found that ocular hypertensive subjects had slightly greater corneal thickness values and chronic glaucoma subjects had significantly decreased values ($p < 0.001$). Herndon et al (1997) agreed and highlighted that 'normal tension glaucoma' subjects had thinner corneas. Doughty and Zaman (2000), Eysteinsson et al (2002) and Doughty et al (2002) reviewed a number of studies into the relationship between IOP and central corneal thickness (CCT). Considering a mixed sample of 133 studies, analysis indicated that a 10 % increase in CCT would result in a very significant (3.4 (SD 0.9) mm Hg) increase in IOP measurement value ($p< 0.001$). In a smaller group of non-diseased subjects, the result was only 1.1 (SD 0.6) mm Hg ($p = 0.023$). Therefore pachometry was not clinically necessary when tonometry was carried out on healthy eyes but was recommended for corneas of chronically diseased eyes, especially if the tonometry readings were borderline or unusual. Patients with markedly raised IOP were excluded from the current study.

5.3.6 Regional Variation

Human corneal thickness is greater in the peripheral than in the central region and so the response to ocular surgery can differ in various corneal locations. Li et al (1994) explained the superior location as being the thickest and the temporal inferior location as the thinnest of the peripheral locations. This finding was confirmed by Colin et al (1996). Figure 5.4.1.1 shows typical values found by Hirji and Larke (1978). Similar results were obtained by the following: Martola and Baum (1968), Bailey and Carney (1972), Tomlinson (1972), El Hage and Beaulne (1975), Kiely et al (1983), Edmund (1987), Herse et al (1993), Rapuano et al (1993) Gromacki and Barr (1994), Longanesi et al (1996) and Cho and Cheung (2000, 2002).

Siu and Herse (1993b) found the following mean corneal thickness values: central 0.530 mm, mid-peripheral 0.583 mm and peripheral region 0.705 mm. Doughty and Zaman (2000) described corneal thickness studies where the overall mean value for central region was 0.538 mm and peripheral region (beyond 4.0 mm optical zone) the mean was 0.672 mm. They emphasised that discrepancies between studies occurred as the exact positions of the peripheral sites were not often defined. Some reports were of non-white subjects where there was a slight tendency towards thinner corneas: e.g. group averaged mean for central site was 0.533 mm (SD 0.02) and for peripheral site was 0.657 mm (SD 0.07).

Colin et al (1996) drew attention to certain subjects where there was corneal thinning associated with decreased radius of curvature in the infero-temporal corneal region.

Martola and Baum (1968) and Siu and Herse (1993a) reported that the difference between the central and peripheral thickness values decreases progressively with age. They suggested that this tendency could lead to some elderly subjects over age 80 having no measurable thickness difference between these regions.

5.4 Use of Pachometry on Abnormal Eyes and After Surgery

5.4.1 To Judge Treatment Effect

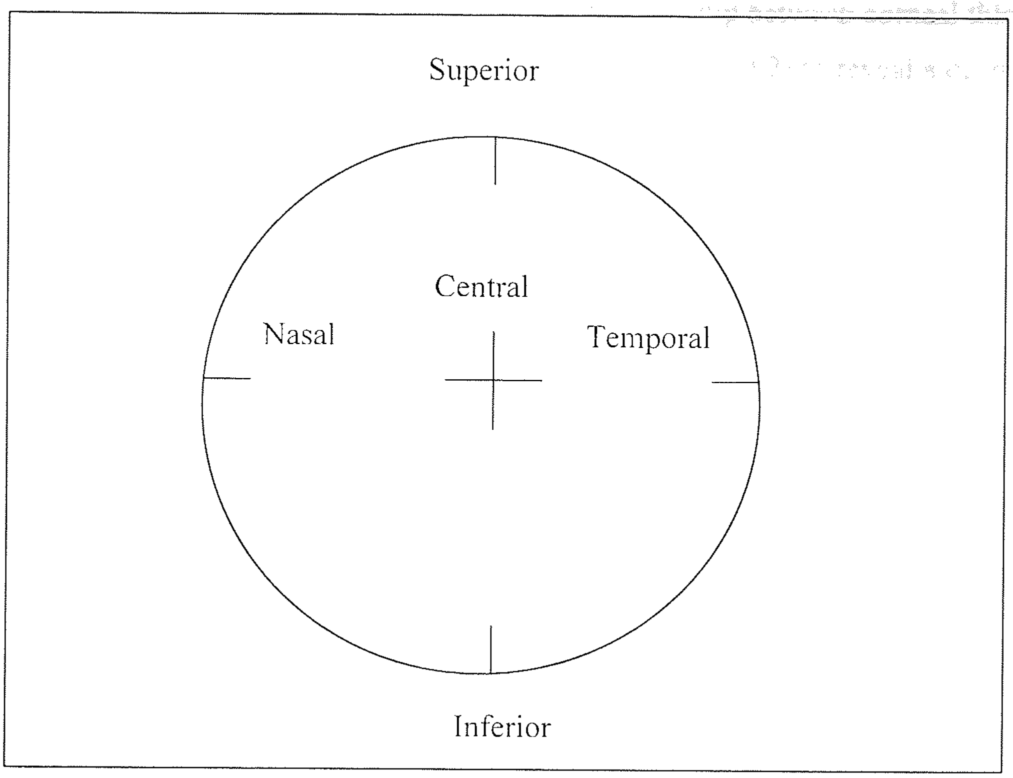
Providing pre-treatment measurements are correctly taken, change in corneal thickness can indicate corneal oedema (Stone 1975). Pachometry is a valuable indicator of the corneal response to treatment, whether surgery, topical medication or contact lens wear (Guillon and Morris 1982). Cheng et al (1988) found greater and more widespread corneal oedema after IOL implantation. Corneal oedema is more likely to occur if the endothelium is damaged e.g. by trauma during surgery or by intermittent contact between the IOL and the endothelium (Dohlman and Hyndiuk 1972, Cheng et al 1988).

Vannas et al (1985), comparing stabilised postoperative eyes to normal control eyes, found the former to have less corneal thickness increase under hypoxic stress but the same as the control eyes when under osmotic stress. This indicates that corneal surgery affects the corneal epithelium physiology in a manner proportional to the angular size of the surgery incision.

Holden et al (1980, 1982) suggested that this decrease in corneal swelling of the aphakic eye results from a decrease in the overall metabolic activity of the epithelium.

Pachometry is useful to monitor contact lens related corneal thickening, e.g. Harris et al (1975), Hirji and Larke (1979), Holden et al (1980), Guillon and Morris (1982), Nilsson and Morris (1983), Snyder and Schoessler (1983), Holden et al (1985) and Bonnano and Polse (1985)

Figure 5.4.1.1 Regional Corneal Thickness



Region	Degrees	Mean Thickness	SD
S	30	0.69	0.03
C	30	0.55	0.04
I	30	0.65	0.04
N	30	0.69	0.04
T	30	0.63	0.04

{After Hirji and Larke (1978)}

5.4.2 Diagnostic Aid

Pachometry is useful when monitoring corneal thickness changes due to ocular disturbances e.g. those causing corneal decompensation. Pachometry can assist diagnosis, as in cases of keratoconus, (Mandell and Polse 1969, De Cunha and Woodward (1993)) and of Terrians dystrophy.

Preoperative pachometry is valuable to the surgeon as a guide to incision depth e.g. in corneal transplant resection, and in reflective surgery such as radial keratotomy.

Fatt and Harris (1973) attempted to find the relationship between corneal thickness and refractive index. Pachometry was found by Hovding (1992) to reveal a central cornea thinning associated with kerato-conjunctivitis sicca.
Herndon et al (1997) revealed that in ocular hypertension eyes the central corneal thickness was significantly greater (0.606 mm) than that of normal eyes (0.56 mm). Argus (1995) and Doughty and Zaman (2000) agreed with these findings.

5.5 Aims for this study

The proposed study will investigate the central and peripheral corneal characteristics of groups of subjects in the 43 to 90 years age range. These will be compared to the similar characteristics of younger subjects measured and as reported by other researchers.
The study will compare the results in a) to g) below to help the understanding of ageing corneal changes and whether relationships between the ocular parameters are revealed. The results will be discussed to further the knowledge concerning the ageing eye.

a) Review previous studies regarding ageing changes in ocular parameters.
b) Calibrate the equipment to be used to measure the curvature of the central cornea (keratometry, keratoscopy) and that of the periphery (videokeratoscopy). Also to calibrate equipment used for measuring central and peripheral corneal thickness (pachometry).
c) After a pilot study on a group (A) of 'over age 43' subjects, to measure the corneal contour and corneal thickness of a group (B) of subjects, aged 43 years or greater, from a random population of staff and patients attending Aston University clinics.
d) Measure corneal contours of a group (C) of young subjects aged 20 to 32 years taken from Aston University student population.
e) Measure ocular parameters of a group (D) of subjects, aged 43 years or greater, from a population of patients on a hospital waiting list for cataract surgery. Also to repeat the measurements on a group (E) of young subjects aged 20 to 40, to compare the results. These parameters are central corneal curvature, anterior chamber depth, lens thickness and axial length of the eyeball.

Chapter 6

Experimental Procedures and Subject Groups

6.1 Keratometry

At Aston University, the Rodenstock two position keratometer of the Haag Streit (Javal Schiotz) style (see Chapter 3.2) was chosen for central corneal curvature measurements. A two-position design gave the advantage of allowing more correct focusing of each of the corneal meridians. A small study of the accuracy and repeatability of a number of keratometers in the department had been carried out by an optometry student (Huddart 1998). This revealed that the Rodenstock keratometer gave the most accurate corneal curvature readings and was therefore selected for this study.

To ascertain the accuracy and repeatability of readings with this keratometer, measurements were performed on eight calibration spheres, twelve Jack Allen Perspex calibration contact lenses and lastly on a set of ten aspheric buttons which had previously been accurately characterised by the specialists Precision Company Ltd. using Talysurf equipment. Ten measurements were taken on each item and the mean and standard deviation calculated.

The calibration measurements are shown in Tables 6.1.1, 6.1.2 and 6.1.3, (all in mm).

Table 6.1.1 Rodenstock Keratometry (KH) versus Calibrated Spheres

Sphere	Calibrated Value	KH average	SD
A	5.50	5.50	0.007
D	7.15	7.16	0.013
E	7.59	7.39	0.019
F	7.89	7.84	0.011
B	8.00	8.01	0.008
G	8.50	8.40	0.009
H	8.80	8.75	0.025
C	11.00	11.02	0.018

Table 6.1.2 Rodenstock Keratometry versus Jack Allen Calibrated Contact Lenses

Measure					Lens	Code						
Number	JA1	JA2	JA3	JA4	JA5	JA6	JA7	JA8	JA9	JA10	JA11	JA12
1	6.90	7.05	7.00	7.00	7.80	7.80	7.80	7.80	8.50	8.50	8.50	8.50
2	6.90	7.05	7.00	7.00	7.78	7.80	7.82	7.81	8.50	8.51	8.51	8.52
3	6.95	7.00	7.00	7.00	7.82	7.80	7.80	7.82	8.51	8.50	8.50	8.51
4	6.95	7.00	7.00	7.00	7.80	7.80	7.80	7.81	8.50	8.50	8.50	8.50
5	7.00	7.00	7.05	7.00	7.80	7.80	7.80	7.80	8.50	8.50	8.51	8.51
6	7.00	7.05	7.02	7.00	7.80	7.80	7.80	7.80	8.50	8.50	8.50	8.50
7	7.00	7.00	7.02	7.02	7.80	7.80	7.80	7.80	8.50	8.51	8.49	8.52
8	7.00	7.00	7.01	7.01	7.82	7.80	7.80	7.81	8.49	8.50	8.50	8.51
9	6.95	7.05	7.00	7.00	7.80	7.80	7.80	7.80	8.50	8.50	8.50	8.50
10	7.00	7.05	7.02	7.00	7.80	7.80	7.80	7.80	8.50	8.50	8.49	8.50
Mean K	**6.97**	**7.03**	**7.01**	**7.00**	**7.80**	**7.80**	**7.80**	**7.81**	**8.50**	**8.50**	**8.50**	**8.51**
SD	0.041	0.026	0.016	0.007	0.011	0.000	0.006	0.007	0.005	0.004	0.007	0.008
JA K value	**7.00**	**7.00**	**7.00**	**7.00**	**7.80**	**7.80**	**7.80**	**7.80**	**8.50**	**8.50**	**8.50**	**8.50**

Table 6.1.3 Rodenstock Keratometry versus Talysurf Radius of Curvature on Calibrated Buttons

	Button Number									
Measure	**No.1**	**No. 2**	**No. 3**	**No. 4**	**No. 5**	**No. 6**	**No. 7**	**No. 8**	**No. 9**	**No. 11**
Number	**741208**	**821200**	**741205**	**821205**	**701208**	**781205**	**781200**	**701200**	**821208**	**781208**
1	7.39	8.15	7.4	8.22	7.00	7.80	7.75	6.95	8.19	7.90
2	7.4	8.15	7.45	8.24	7.05	7.80	7.75	6.95	8.19	7.90
3	7.39	8.15	7.41	8.25	7.00	7.85	7.76	6.96	8.20	7.80
4	7.4	8.15	7.4	8.21	7.01	7.85	7.77	6.96	8.18	7.79
5	7.41	8.15	7.45	8.23	7.02	7.86	7.75	6.95	8.19	7.87
6	7.4	8.15	7.42	8.21	7.03	7.85	7.75	6.95	8.19	7.85
7	7.4	8.16	7.42	8.22	7.01	7.85	7.76	6.95	8.18	7.82
8	7.4	8.16	7.41	8.21	7.02	7.83	7.77	6.95	8.19	7.85
9	7.39	8.14	7.4	8.23	7.01	7.84	7.75	6.96	8.19	7.79
10	7.41	8.16	7.41	8.23	7.02	7.80	7.77	6.97	8.20	7.80
Mean	**7.399**	**8.152**	**7.417**	**8.225**	**7.017**	**7.833**	**7.758**	**6.955**	**8.190**	**7.837**
SD	0.007	0.006	0.018	0.013	0.014	0.023	0.009	0.007	0.006	0.041

The readings indicated that for fixed objects with regular reflective surfaces the operator obtained an acceptable degree of accuracy.

The range possible is 5.50 mm - 9.50 mm, but most human corneal readings are in the range 7.00 - 9.00 mm. Figures 6.1.1, 6.1.2 and 6.1.3 show the close correlation of the readings with the verified measurements.

Figure 6.1.1 Rodenstock Keratometry versus Manufacturer's Calibrated Value

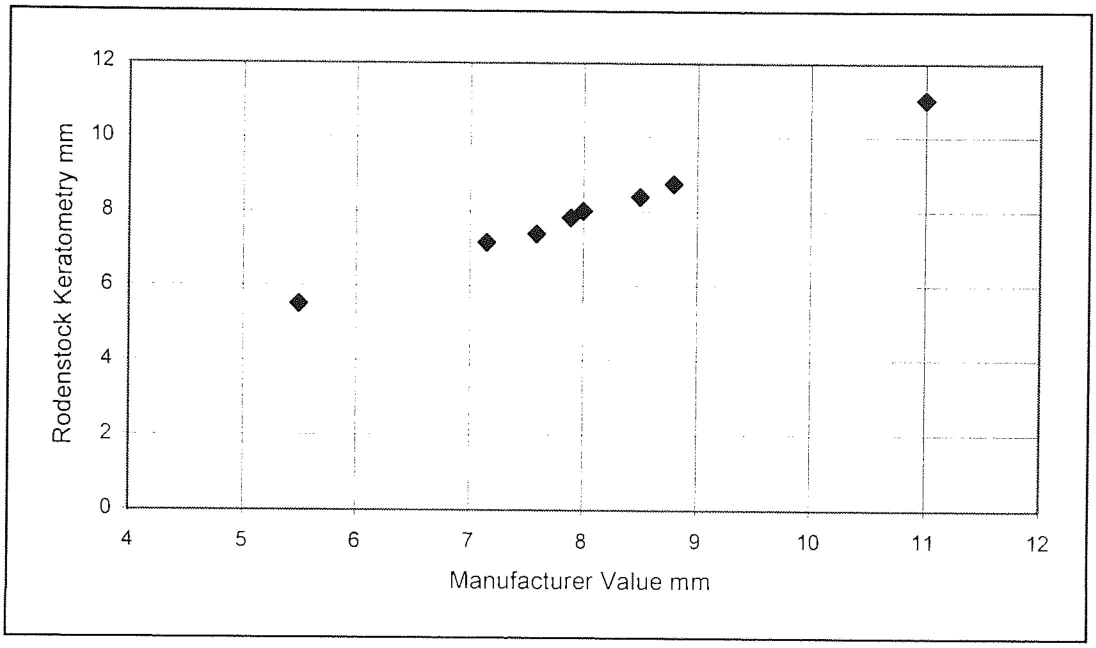


Figure 6.1.2 Rodenstock Keratometry versus Jack Allen Standard Contact Lens Value

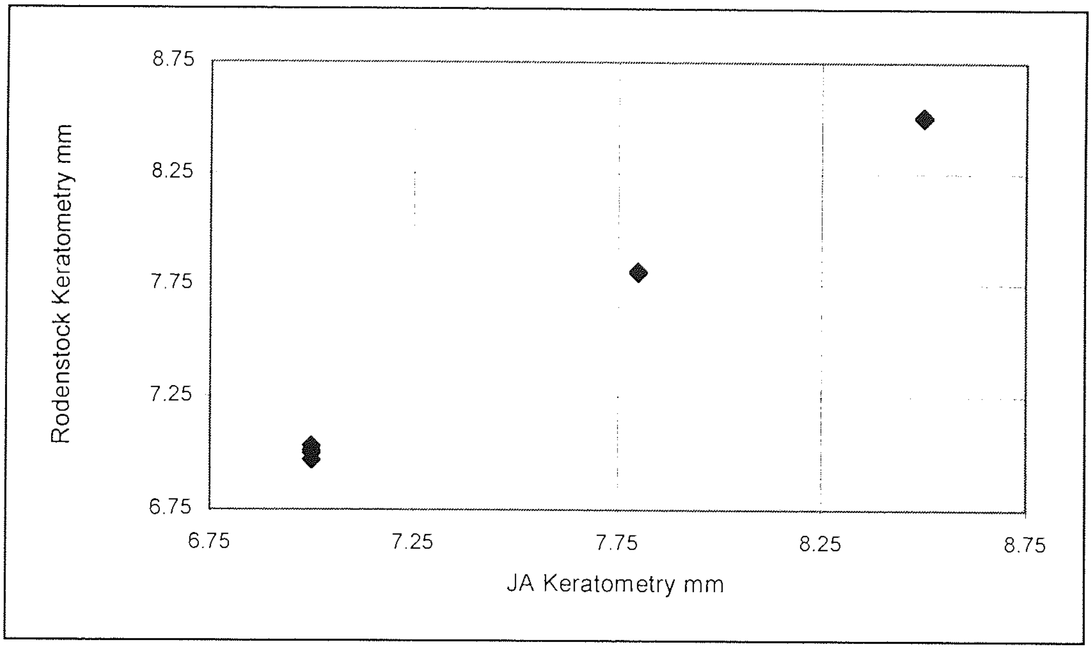
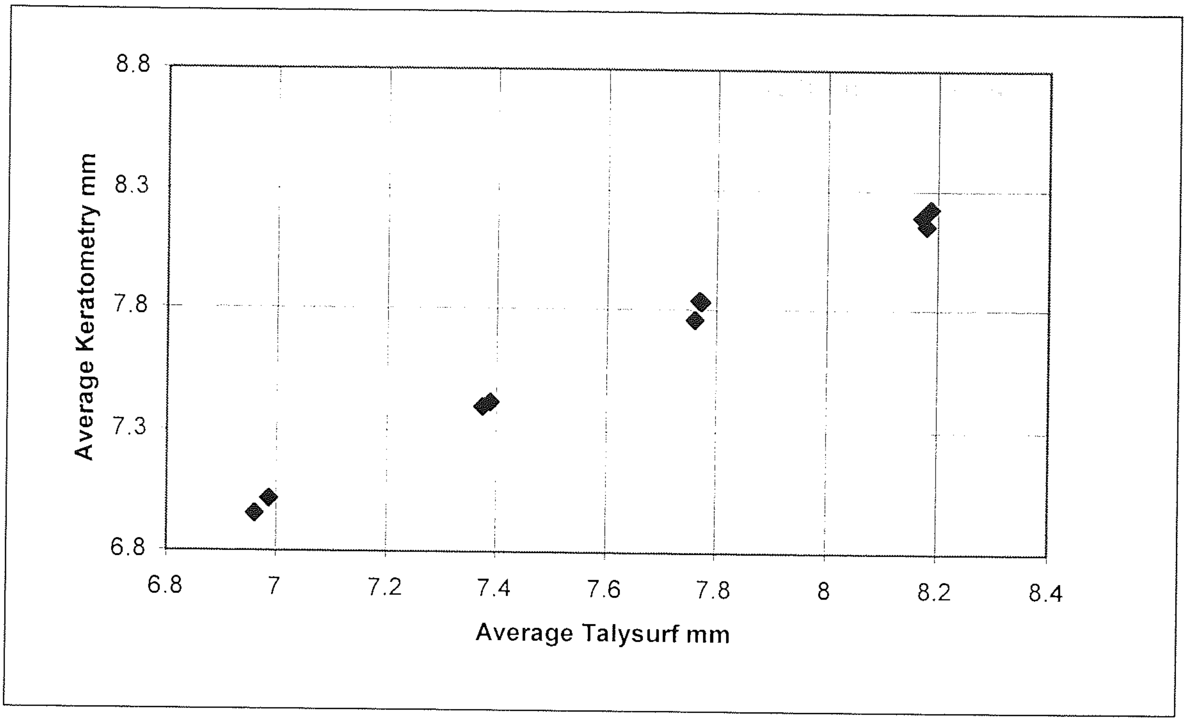


Figure 6.1.3 Rodenstock Keratometry versus Talysurf Keratometry (Buttons)



The operator had many years of experience in performing keratometry and was the sole observer in the study.

When measuring the subjects, three readings were taken in each of the two principal corneal meridians and the mean value found. Butcher and O'Brien (1991) recommend this method to improve the reliability of the technique. Care was taken to explain the procedure to each subject, obtain their permission to perform the measurements and to reduce experimental errors (see Chapter 3.3). A few of the younger subjects had been wearing soft contact lenses, and those contact lens wearers had removed the lenses a minimum of 12 hours prior to the measurements, as lenses may cause corneal irregularity.

6.2 Keratoscopy

Central and peripheral corneal curvatures were measured using the EyeSys videokeratoscope (Software version 3.1). This machine was calibrated using the special calibration model eye surfaces provided with the instrument and also the set of ten aspheric buttons. The measurements were repeated ten times for each model eye or button, repositioning and re-focusing for each measurement. The EyeSys readings for average central radius of curvature are shown in Table 6.2.1.

Table 6.2.1 EyeSys Central Keratometry versus Talysurf Radius Average

Button	**Talysurf**		**EyeSys**	
Number	**R_{av} mm**	**SD**	**Central R_{av} mm**	**SD**
8	6.961	0.024	7.063	0.008
5	6.986	0.002	7.061	0.011
1	7.375	0.001	7.457	0.008
3	7.389	0.002	7.484	0.017
7	7.76	0.004	7.837	0.009
10	7.769	N/a	7.871	N/a
6	7.772	0.002	7.88	0.012
9	8.171	0.003	8.267	0.015
2	8.179	0.002	8.244	0.015
4	8.187	0.002	8.262	0.006

The accuracy of the EyeSys was found to be ± 0.10 mm on measuring central corneal radius of curvature. The EyeSys tended to give readings approximately 0.10 mm greater than that measured by Talysurf.

The peripheral contours were described in terms of eccentricity value (e) of the nearest matching elliptical curve. The calibration of the model eyes and buttons showed that the EyeSys gave eccentricity values corresponding to (1 – Talysurf value). The Talysurf readings are given in Table 6.2.2.

Table 6.2.2. Eccentricity: EyeSys versus Talysurf

Button Number	Talysurf Eccentricity	(1 – Talysurf Eccentricity)	EyeSys Eccentricity	EyeSys SD
8	0.994	0.006	0.000	0.000
5	0.788	0.212	0.403	0.007
1	0.779	0.221	0.405	0.005
3	0.467	0.533	0.659	0.010
7	0.988	0.012	0.006	0.019
10	0.774	0.226	0.406	0.012
6	0.472	0.528	0.668	0.004
9	0.772	0.228	0.409	0.009
2	0.993	0.007	0.030	0.036
4	0.473	0.527	0.657	0.005

Note different terminologies between Talysurf and EyeSys.

Figures 6.2.1 and 6.2.2 indicate the correlation of the EyeSys readings with those given by Talysurf.

Figure 6.2.1 EyeSys Radius of Curvature (mean) versus Talysurf Radius

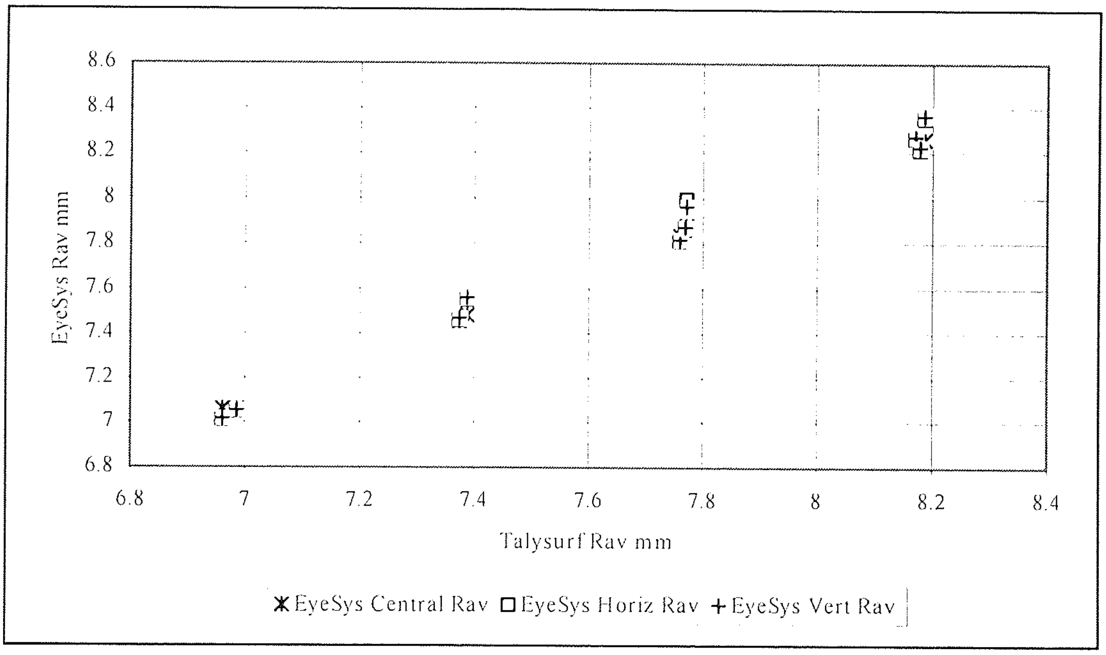
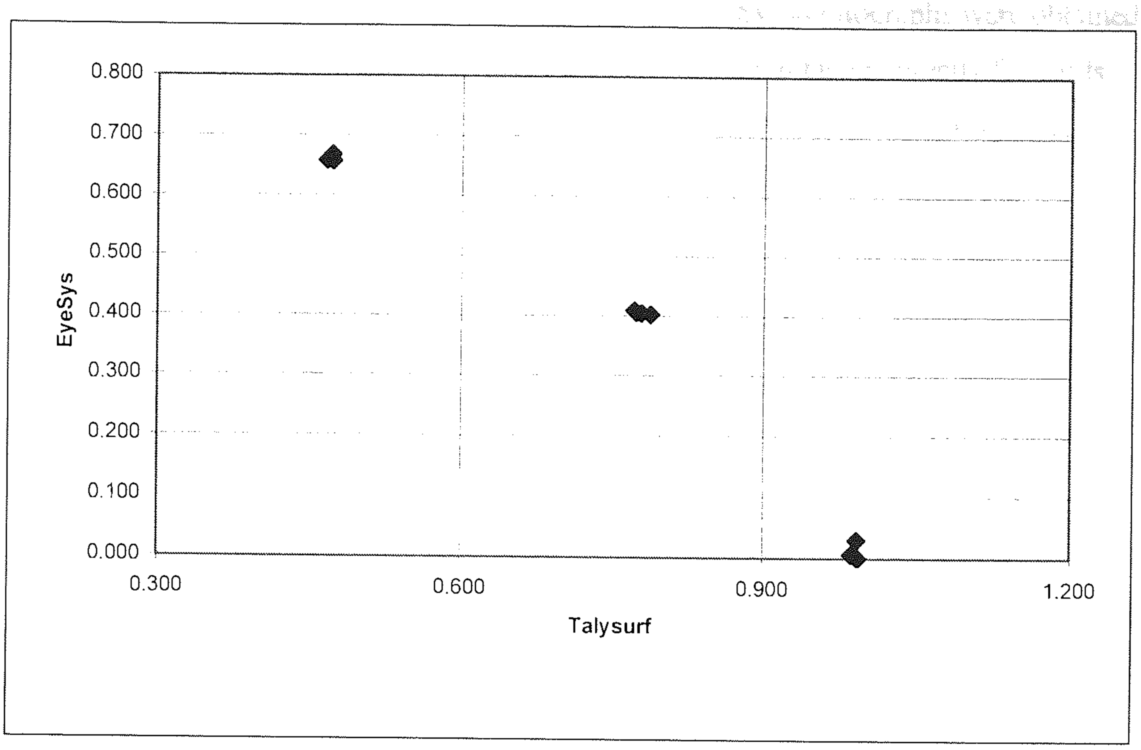


Figure 6.2.2. Eccentricity with EyeSys versus with Talysurf for Buttons



Therefore, for spherical surfaces (button 8) the EyeSys value and the (1-Talysurf) value are very close. However, for aspheric surfaces where the curvature gradually becomes flatter from the centre to the periphery, the EyeSys does not follow the equation as closely and the eccentricity reading is less reliable.

Care was taken to explain the procedure to each subject, to obtain their permission to take the EyeSys measurements and to minimise experimental errors. The test room was quiet and without windows. Moderate diffuse background room illumination was used to help the subject to relax and to avoid glare and discomfort. The subject's chair, the headrest and chinrest were adjusted to assist their comfort, co-operation and steady head position. The subject was instructed to maintain fixation on the central fixation target, whilst they took a full blink and stared wide. The blink helped to provide a smooth optical reflecting surface of tears over the cornea. The wide palpebral aperture aimed to maximise the corneal reflecting area.

To avoid the nose and eyebrows from encroaching too much in the region of the keratoscope mires reflection the subject was requested to turn their head slightly to one side then to fixate the central observation target. This method allowed more of the keratoscope ring mires to be 'captured' by the system and hence provide more information concerning the corneal periphery. To determine if this head position led to measurement errors a small check was carried out on two subjects. EyeSys keratographs were obtained on both with the head turned position and with a head position facing directly forwards. On scrutiny of the radius of curvature readings for various corneal zones no significant differences were found, so the first head position was considered acceptable.

6.3 Pachometry

The Haag Streit optical pachometer was calibrated using a set of ten monocurve polymethylmethacrylate (PMMA) contact lenses that had been made to specified thickness and curvatures. Ten measurements of each lens were performed and the readings compared to those found using a micrometer (Table 6.3.1). The accuracy and reproducibility of the readings was good on these inanimate objects.

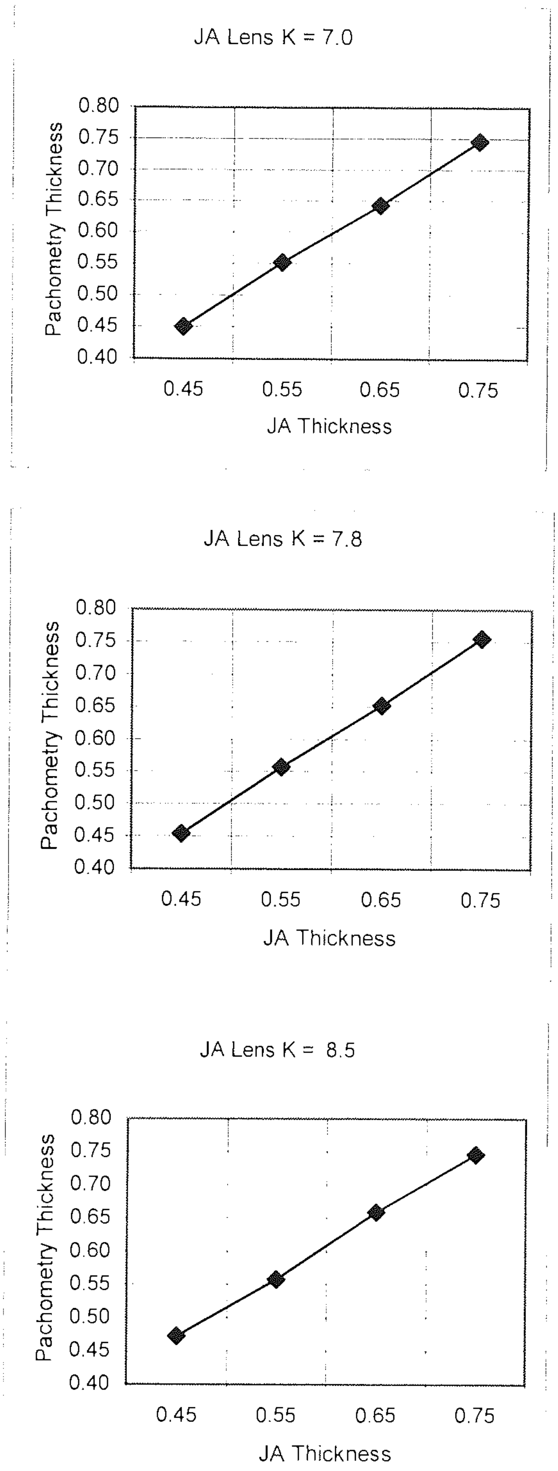
Table 6.3.1. Thickness Measurements of Calibration Lenses

Measure Number	JA1	JA2	JA3	JA4	JA5	JA6	JA7	JA8	JA9	JA10	JA11	JA12
1	0.445	0.555	0.645	0.745	0.450	0.560	0.650	0.765	0.470	0.560	0.665	0.745
2	0.450	0.555	0.645	0.750	0.455	0.550	0.660	0.755	0.475	0.560	0.660	0.750
3	0.455	0.555	0.635	0.745	0.455	0.560	0.660	0.760	0.455	0.550	0.645	0.745
4	0.450	0.555	0.650	0.750	0.460	0.560	0.660	0.750	0.470	0.560	0.670	0.740
5	0.450	0.545	0.640	0.745	0.460	0.565	0.640	0.755	0.480	0.555	0.660	0.750
6	0.445	0.545	0.640	0.750	0.460	0.545	0.655	0.745	0.480	0.560	0.670	0.750
7	0.450	0.555	0.650	0.740	0.455	0.560	0.645	0.750	0.480	0.560	0.670	0.750
8	0.455	0.550	0.640	0.745	0.450	0.560	0.655	0.760	0.460	0.560	0.660	0.750
9	0.450	0.550	0.650	0.745	0.450	0.560	0.655	0.755	0.480	0.560	0.645	0.740
10	0.450	0.555	0.640	0.740	0.450	0.555	0.650	0.760	0.470	0.550	0.650	0.740
Mean	**0.450**	**0.552**	**0.644**	**0.746**	**0.455**	**0.558**	**0.653**	**0.756**	**0.472**	**0.558**	**0.660**	**0.746**
SD	0.003	0.004	0.005	0.004	0.004	0.006	0.007	0.006	0.009	0.004	0.010	0.005
JA thickness	0.45	0.55	0.65	0.75	0.45	0.55	0.65	0.75	0.45	0.55	0.65	0.75
JA K value	7.00	7.00	7.00	7.00	7.80	7.80	7.80	7.80	8.50	8.50	8.50	8.50

The pachometry reading versus JA Lens thickness is plotted in Figure 6.3.1 for the three keratometry values and shows that there is a good agreement between the pachometry values and those of the manufacturer of the lenses.

These calibrated lenses were used to check the instrument prior to measurements on patients.

Figure 6.3.1 Pachometry Thickness versus Manufacturer's Value



Brennan et al (1989) found that an explicit solution to the equation giving the true corneal thickness in terms of the apparent (measured) thickness from a pachometer cannot be established. They concluded that the results obtained by different experimenters were not necessarily comparable because no corrections had been applied or different approximations were required. They demonstrated a linear relation between the inverse of true thickness to the inverse of apparent thickness for a range of approximations of parameters such as refractive index and angle of light incident to the cornea.

The primary aim of the work in this thesis was the investigation of corneal thickness trends with age and with corneal curvature, therefore the need for absolute values of true corneal thickness was less important. Given the difficulty in establishing the accurate values of the parameters required to use the equations described by Brennan et al (1989), the author chose to use the raw data for analysis of trends, taking the pachometry readings direct from the scale without applying a correction factor.

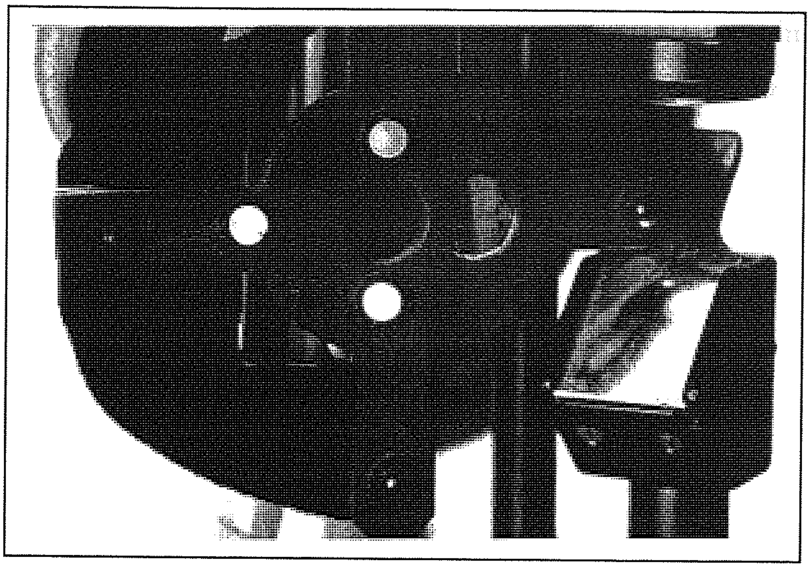
As described above, the pachometer readings for the JA calibration lenses showed good agreement with the manufacturers values. However, on comparing the mean human corneal thickness results with those obtained by other researchers e.g. Siu and Herse (1993), Doughty and Zaman (2000), Lam and Douthwaite (1998) and Cho and Cheung (2002) it was estimated that on the human subjects the pachometer readings probably required a correction factor of minus 0.1 mm.

The pachometry measurements proved to be more difficult to perform on elderly subjects than on young subjects. The test room was set up in an attempt to carry out the procedure at a quiet time to avoid distraction. The room lights were dimmed to improve the contrast of the corneal image seen through the slitlamp.

The procedure was explained to the subject, their spectacles removed and they were requested to keep their head steady on the chin rest and headrest of the slitlamp. They were advised to steadily fixate each illuminated target in turn when requested and to maintain the head position during the measurements.

The target plate is shown in Figure 6.3.2. The targets were bright yellow LED display lights to facilitate fixation. Positions on the plate are referred to as: superior (S), inferior (I), nasal (N) and temporal (T).

Figure 6.3.2 Target Plate



For the central position, the subject was instructed to fixate directly ahead, halfway between the vertically separated Donaldson green LED lights. The plate had to be held such that the white slitlamp beam passed through an aperture at the correct angle of incidence to reach the cornea. Three readings of corneal thickness were taken at each fixation position. Five readings were preferred but few of the subjects were able to maintain steady fixation long enough to do the extra measurements.

Subjects with glaucoma or ocular hypertension were excluded from the study. Herndon et al (1997) reported that the central corneal thickness in ocular hypertensive eyes is significantly greater than normal.

Although Scheimpflug photography was considered for the assessment of corneal thickness, the optical pachometer was more accessible and could be performed faster and with least nuisance for the subject. Kampfer et al (1989) said that the photographic method incurs 'camera distortion' and refractive distortion of the ocular image hence the measurements do not correctly correspond to those in the human eye. Dubbelman et al (2001) noted that the Scheimpflug photography method tended to under-estimate values for corneal thickness, lens thickness and anterior chamber depth. They suggested that this was due to the greater depth of focus and the wider separation of the illumination and observation systems compared to the Jaeger optical method.

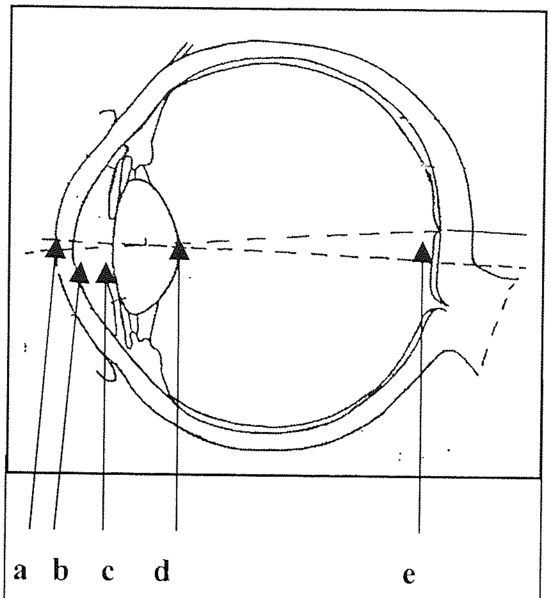
6.4 Biometry

Biometry involves ultrasound in the measurement of ocular dimensions. Ultrasound may be defined as a sound wave with a frequency equal or greater than 10 kilohertz, which will only travel through a liquid or solid substance. This electromagnetic wave follows the same properties as light in that it can be focused, reflected and refracted. The ultrasound wave is generated and detected with a transducer, a thin disc of piezo-electric material e.g. quartz. This can expand as a voltage is passed over it and be made to oscillate and produce an ultrasound beam. The speed at which the ultrasound waves travel away from the transducer is known as the propagation velocity. This is usually fast in a solid but slower in softer materials that are more readily compressed. The ultrasound waves are reflected at boundaries between media that possess differing mechanical characteristics, e.g. anterior and posterior corneal surfaces, anterior and posterior lens surfaces and the retina. The echoes from each tissue boundary arrive back at the transducer and generate an output voltage, which is displayed on an oscilloscope. The A-scan is the pattern of the time-amplitude display. The distance between the tissue boundaries can be calculated if the wave velocity and the time between echoes are known.

Lam et al (2002) declared that for measurements of anterior chamber depth ultrasound biometry was superior to optical methods in terms of precision and repeatability, although the new Anterior Segment Analysis System of the EAS-1000 instrument was useful for screening a large number of eyes as it was non-invasive.

Biometry was performed on the cornea of each subject using the Vision Care 3M EchoRule ultrasonic biometer (10MHz). Measurements were made on right and left eyes, unless the eye had previously undergone ocular surgery or significant trauma. Topical anaesthetic drops (0.4% Benoxinate, unpreserved, in unit dose Minims) were instilled onto the ocular surface and allowed two minutes to take effect. The biometer was used to measure anterior chamber depth, lens thickness and axial length of the eye, see (Figure 6.4.1). The Zeiss IOLMaster was not available at this time.

Figure 6.4.1 Eye



Anterior chamber depth = b to c
Lens thickness = c to d
Axial length = a to e

Prior to the measurements the biometer had been set up according to the manufacturers instructions and calibrated on the test rod to ensure acceptable reproducibility of axial length measurement (+/- 0.15 mm).

Before using the equipment the probe tip was disinfected according to the hospital protocol by wiping the tip with a fresh swab and medical alcohol and allowing the probe surface to dry in air for several minutes. At this stage, research on the difficulty of eradicating prions had not been fully understood. Non-contact methods would be advised for future studies.

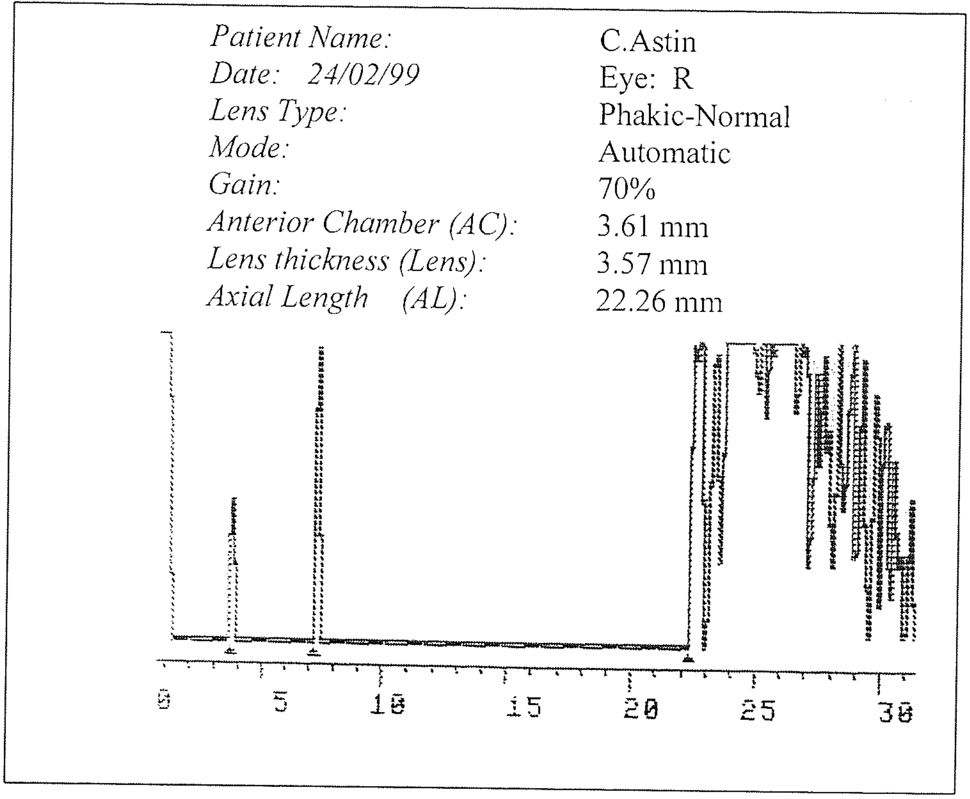
The ultrasound transducer probe was approximately 30 mm long, attached by an electric cord to the biometry machine, placed on a steady table. The thin probe had a smooth flat endplate, which gently touched the subjects central corneal region. The natural ocular surface moisture bridged the narrow gap between the probe and the corneal epithelium, facilitating the transmission of the ultrasound pulses from the probe into the eye, with the echo being received by the probe. If the subject had insufficient tear fluid to maintain this contact layer, a drop of topical unpreserved saline (Minim) was placed on the probe tip to act as the interface fluid. The subject was comfortably seated at a suitable height and was instructed to fixate an illuminated target at a distance, and not to move during the measurement. Shum et al (1993) studied biometric ocular changes during accommodation and found decreased anterior chamber depth, thickening of the crystalline lens and a mean increase in axial length of 0.06 mm. Therefore, a distance target was chosen to discourage accommodation. The operator held the transducer parallel to the subject's visual axis and with the probe tip in light contact with the central cornea. Contact with the eyelids was avoided. Whelehan et al (1996) found no significant difference in axial length measurements between handheld and slitlamp-mounted methods. The handheld method was easier, particularly for elderly subjects who would struggle to maintain correct head postures at the slitlamp.

In the automatic mode, the instrument software automatically assessed the measurement scans acquired and screened out potentially erroneous readings. When a valid reading was acquired, a beep noise sounded. After two consecutive valid readings were obtained, the machine 'froze' the scan from the second reading and displayed the average of the two axial length measurements. A double beep noise was the audible signal that the machine had 'frozen' a reading. The machine was pre-set such that the axial length measurements of two consecutive validated scans had to fall within 0.1 mm, otherwise the machine would not 'freeze' the reading. The acquired reading and scan was printed out, an example is given in Figure 6.4.2.

In hospitals the biometer was commonly used in the calculation of the intraocular implant power for cataract extraction surgery. The non-contact method of the Zeiss IOLMaster is becoming the preferred instrument in recent years.

Particular attention was paid to the anterior chamber depth, lens thickness and axial length readings, to check for indications of accidental corneal indentation or poor alignment of the probe, (Holladay et al 1997). Suitable biometry training is important, as noted by Hovding et al (1994) who claimed that inter-observer discrepancy for the accuracy of pre-operative axial length measurement is the main cause of unpredicted post-operative refractive errors. The fellow eye was measured and in most cases the axial length values of right and left eyes were expected to be within 0.2 mm of each other. The values and the variability of the keratometry readings were taken into account when deciding to accept a biometry measurement. The scan printout was checked to ensure that the instrument had triggered an echo on the correct peaks.

Figure 6.4.2 Biometry Reading and Scan



Patient Name: C.Astin
Date: 24/02/99 Eye: R
Lens Type: Phakic-Normal
Mode: Automatic
Gain: 70%
Anterior Chamber (AC): 3.61 mm
Lens thickness (Lens): 3.57 mm
Axial Length (AL): 22.26 mm

The manufacturers specification quoted for this biometer is shown in Table 6.4.1 below:

Table 6.4.1 Biometer specification

Instrument accuracy (on axial length)		+/-0.025 mm
Subject measurement accuracy		+/-0.100 mm
Biometry ranges:	Anterior chamber depth	2.0 – 5.5 mm
	Lens thickness	2.7 – 7.5 mm
	Axial length	15.0 – 32.0 mm
Calibrated sonic velocities:	Vitreous and aqueous	1533 m/sec
	Lens	1641 m/sec
Transducer ultrasound frequency:		10 MHz
Measurement update rate:		8 per second
Operating Temperature range:		0° C – 40°C

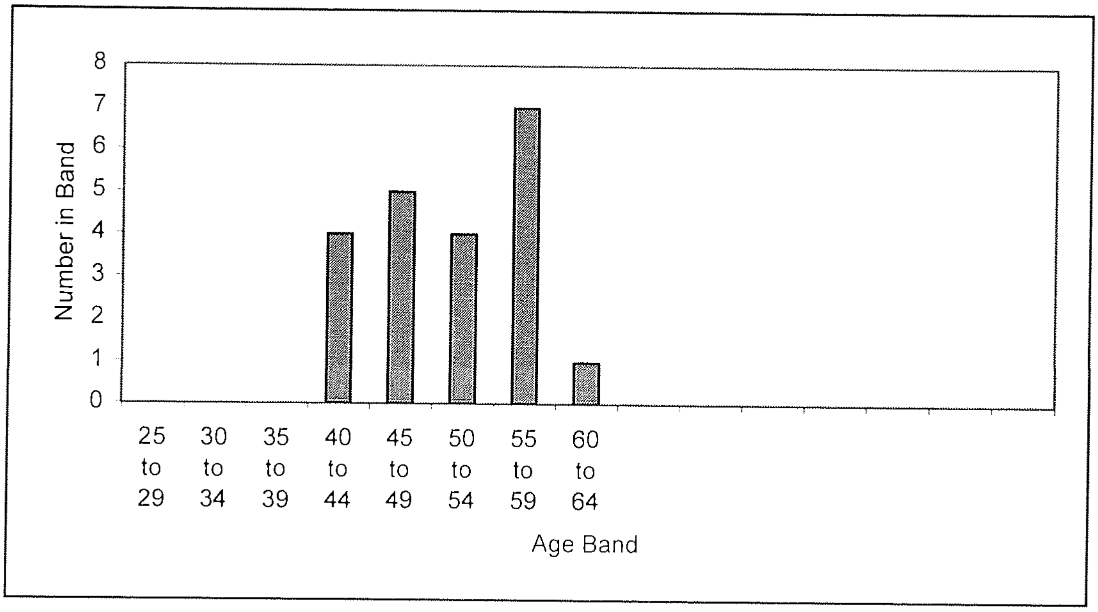
6.5 Aston University Older Subjects - Group A

This group consisted of subjects over the age of 42 years and were recruited from university staff. They were chosen because they were over age 40, healthy, had satisfactory visual acuity and were co-operative with the experimental procedures. This group were used to check procedures and were then incorporated into group B.

Number = 21 (16 males, 5 females)

Mean age = 50.86 (SD 5.92)

Figure 6.5.1 Age Distribution – Group A



6.6 Aston University Older Subjects - Group B

This group included randomly gathered patients who attended the university department to allow students to gain clinical experience. Their health was variable and their age profile was a nearer match to those of group D.

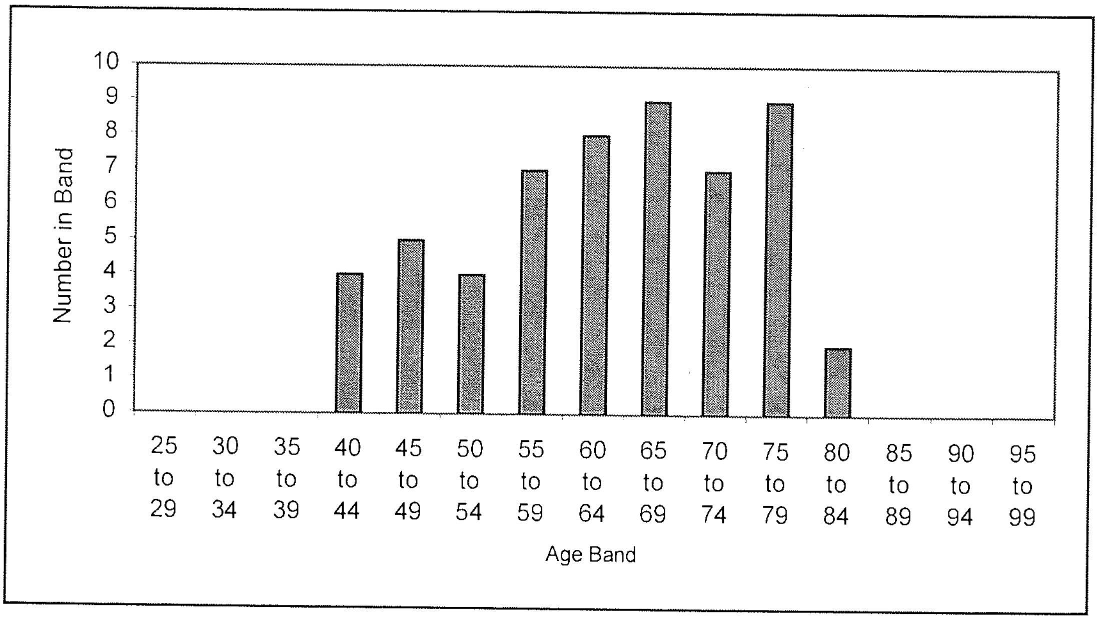
Number = 54, comprising of 32 males, 22 females.

Mean age = 62.84 (SD 11.21)

Refraction estimates (spherical equivalent):

Right Eye mean= +0.30 (SD 1.77), Left Eye mean = +0.28 (1.90)

Figure 6.6.1 Age Distribution – Group B



6.7 Aston University Subjects - controls - Group C

This group was gathered from students in the department to act as 'controls', as a normal young sample for comparison with the older group. They were healthy, had satisfactory visual acuity and were co-operative with the experimental procedures.

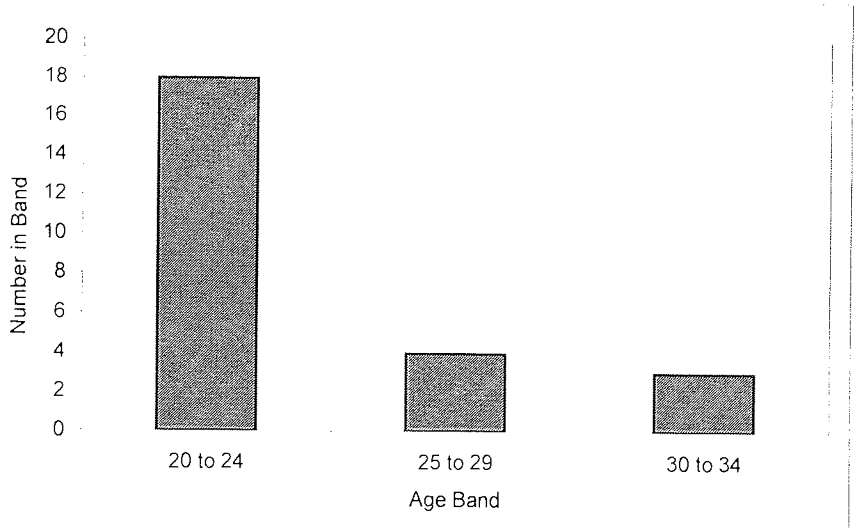
Number = 25, comprising of 7 males, 18 females.

Mean age = 23.56 (SD 3.78)

Refraction estimates (spherical equivalent):

Right Eye mean= -1.42 (SD 2.55), Left Eye mean = -1.52 (SD 2.79)

Figure 6.7.1 Age Distribution – Group C



6.8 Birmingham Heartlands Hospital Subjects - Group D

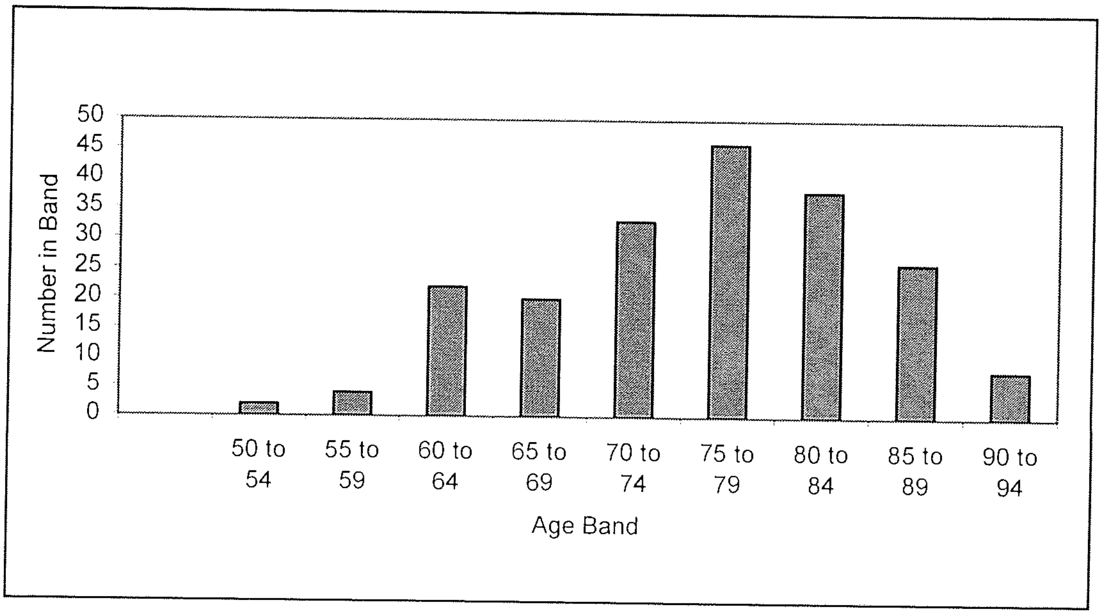
This group were consecutive patients attending the hospital department for assessment before their cataract operation was scheduled. Their health and co-operation were variable.

Number = 201, comprising of 81 males, 120 females.

Mean age = 75.9 (SD 8.46)

No reliable refraction results were obtainable for these subjects.

Figure 6.8.1 Age Distribution – Group D



6.9 Birmingham Heartlands Hospital Subjects - Controls - Group E

This group were recruited from the hospital department staff and had agreed to act as controls.

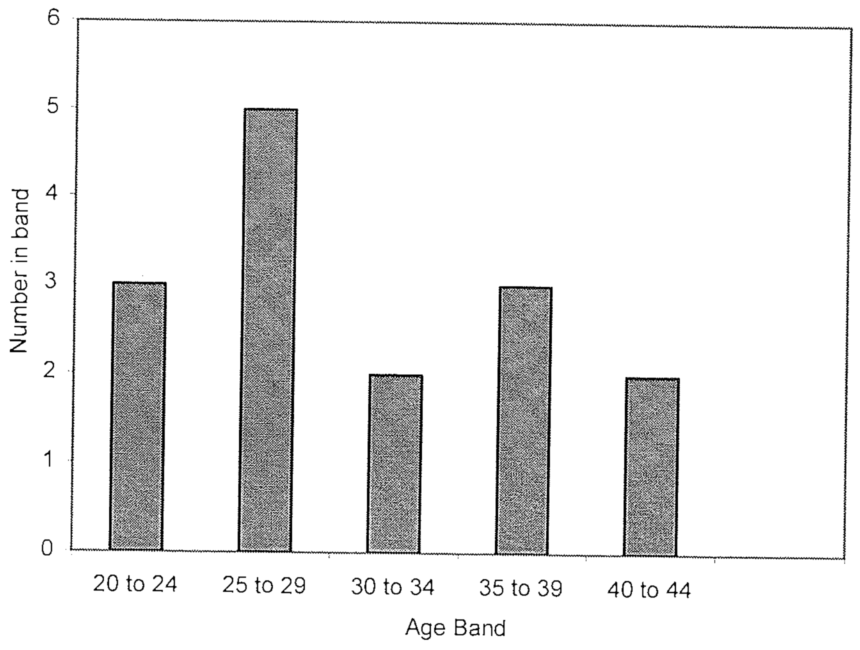
Number = 15, comprising of 5 males, 10 females.

Mean age = 30.46 (SD 6.875)

Refraction estimates (spherical equivalent):

Right Eye mean= -0.688 (SD 3.081), Left Eye mean = -0.719 (SD 2.941)

Figure 6.9.1 Age Distribution – Group E



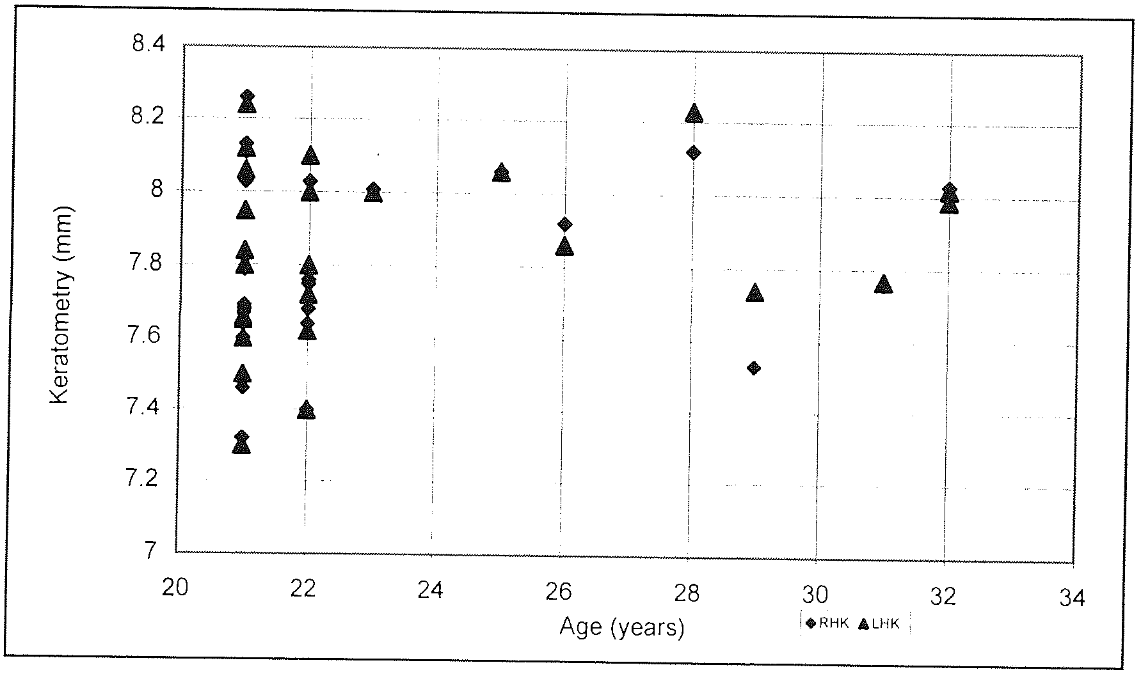
Chapter 7

Results and Discussion of Keratometry and Keratoscopy Study

7.1 Horizontal Keratometry

The measurement of horizontal keratometry using the Rodenstock keratometer on young subjects at Aston University (Group C) used as controls showed no significant relation to age within the group, (Figure 7.1.1) for the age range 21 to 32.

Figure 7.1.1 Horizontal Keratometry versus Age for Group C



By 'T' tests, there was no significant difference ($p > 0.05$) in mean values of horizontal keratometry between the right eye and the left eye, so the values of these two eyes were combined to provide a larger sample (Table 7.1.1).

Table 7.1.1. Horizontal Keratometry for Group C

	RHK	LHK	R+L HK
Mean	7.82	7.84	7.83
SD	0.25	0.25	0.25

The mean value for the combined results was 7.83 mm (SD 0.25), range 7.30 to 8.26 mm, and this value is close to the 7.79 mm (SD 0.26) quoted by Kiely et al (1984) for an adult.

Similarly, the EyeSys measurements for Group C followed the same pattern, the results having a mean value of 7.88 mm (SD 0.25), but the values are slightly greater than those found using the Rodenstock keratometer (Table 7.1.2). This is probably because the circular ring target mires of the EyeSys were reflected from points on the cornea slightly further from the centre, i.e. where the corneal surface curvature begins to flatten.

Figure 7.1.2 RE, LE EyeSys Horizontal Keratometry versus Age for Group C

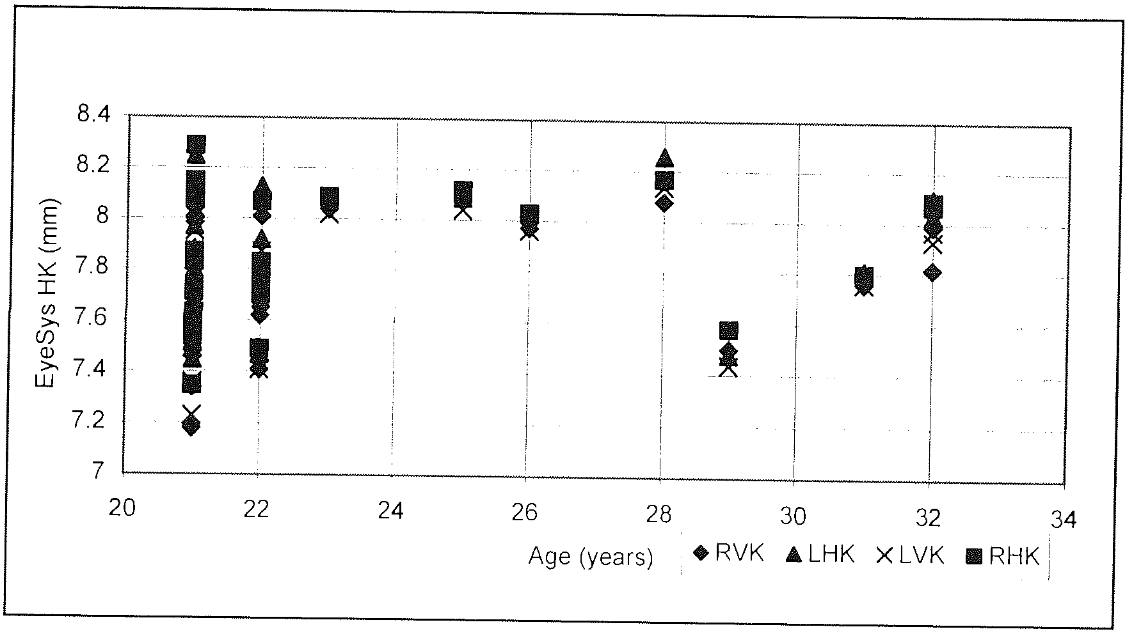


Table 7.1.2 EyeSys Horizontal Keratometry for Group C

	RHK	LHK	R+L HK
Mean	7.88	7.88	7.88
SD	0.25	0.25	0.25

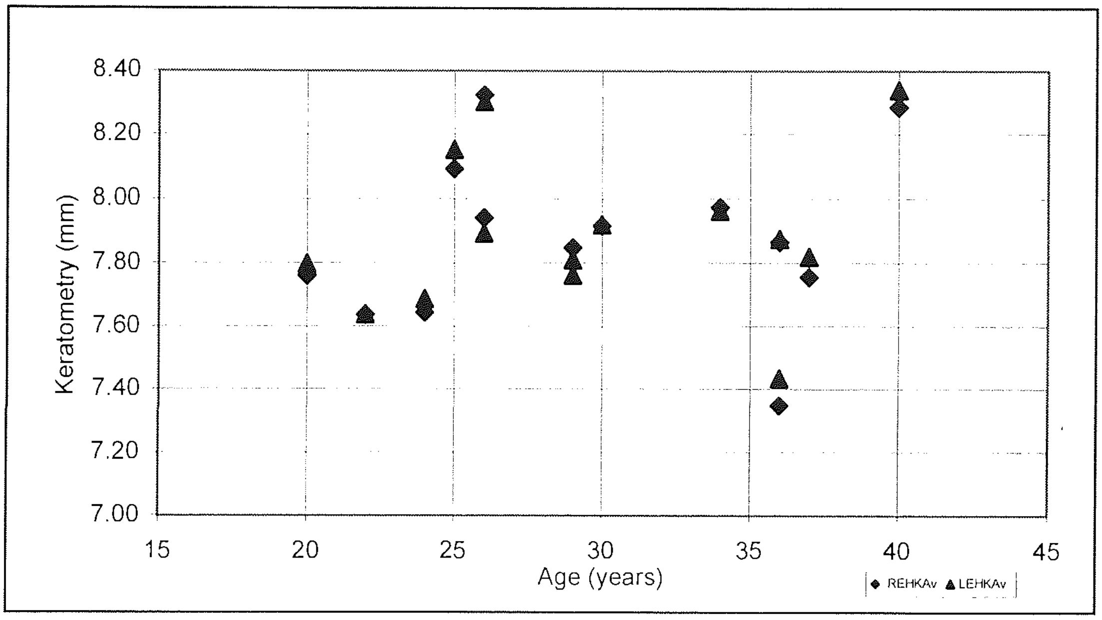
The measurement of horizontal keratometry using a Rodenstock keratometer on the hospital control Group E subjects showed no relation to age (Figure 7.1.3) for subjects between 20 and 42 years. There was no significant difference between the means of the right and left eyes so the values were combined. The mean value was 7.86 mm (SD

0.25) and the range 7.35 to 8.32 mm. This mean value of 7.86 mm for right and left eyes combined for Group E was similar to the value of 7.83 mm found for Group C.

Table 7.1.3 Horizontal Keratometry for Group E

	RHK	LHK	R+L HK
Mean	7.86	7.87	7.86
SD	0.25	0.25	0.25

Figure 7.1.3 Horizontal Keratometry versus Age for Group E



There was no significant relation found between keratometry and spectacle prescription because the majority of these subjects had a minimal prescription. Often both eyes of a subject had almost identical values of keratometry.

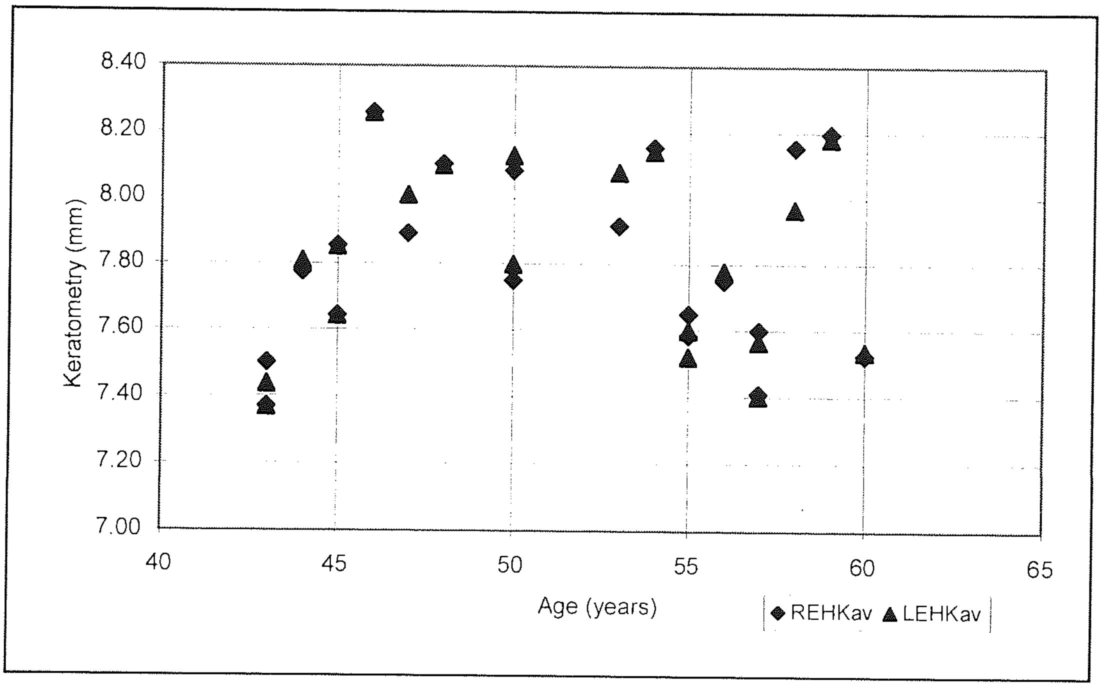
The horizontal keratometry values of the older groups were considered next. The Aston University older subjects (Group A) used initially to check procedures showed values with no significant difference between right and left eyes so these values were combined.

Table 7.1.4 Horizontal Keratometry for Group A

	RHK	LHK	R+L HK
Mean	7.80	7.80	7.80
SD	0.26	0.28	0.27

The mean keratometry was 7.80 mm (SD 0.27), range = 7.20 to 8.20 mm. There was no significant relation to age ($p > 0.05$) by linear regression. Figure 7.1.4 shows the graph.

Figure 7.1.4 Horizontal Keratometry versus Age for Group A.



The Aston University older subjects (Group B) keratometry results were studied. There was no significant difference between the values of the right and left eyes so these were combined. The mean keratometry was 7.72 mm (SD 0.25), and the range was 7.24 to 8.26 mm (Table 7.1.5).

Table 7.1.5 Horizontal Keratometry for Group B

	RHK	LHK	R+L HK
Mean	7.73	7.72	7.72
SD	0.24	0.26	0.25

Although at first Group B seemed to have smaller values than those of Group A, analysis determined no significant difference in their means when taken as a whole, which may have been due to the greater variability of the values of Group B. ANOVA of horizontal keratometry versus age gave RE $p = 0.06$, LE $p = 0.02$. When considering the results of the subjects over 60 years of age there was a small but clear trend for smaller values of horizontal keratometry with increasing age as shown in Figure 7.1.5. These results were analysed separately as shown in Table 7.1.6.

Table 7.1.6 Horizontal Keratometry for Group B over 60 years

	RHK	LHK	R+L HK
Mean	7.69	7.66	7.67
SD	0.22	0.23	0.22

The regression line of the 'age over 60' subgroup values gave the equation:

$$RHK = 8.17 - 0.0069*age$$

$$LHK = 8.36 - 0.0100*age$$

The analysis suggests that after the age of 60 the horizontal keratometry gradually decreases (* = per year of age).

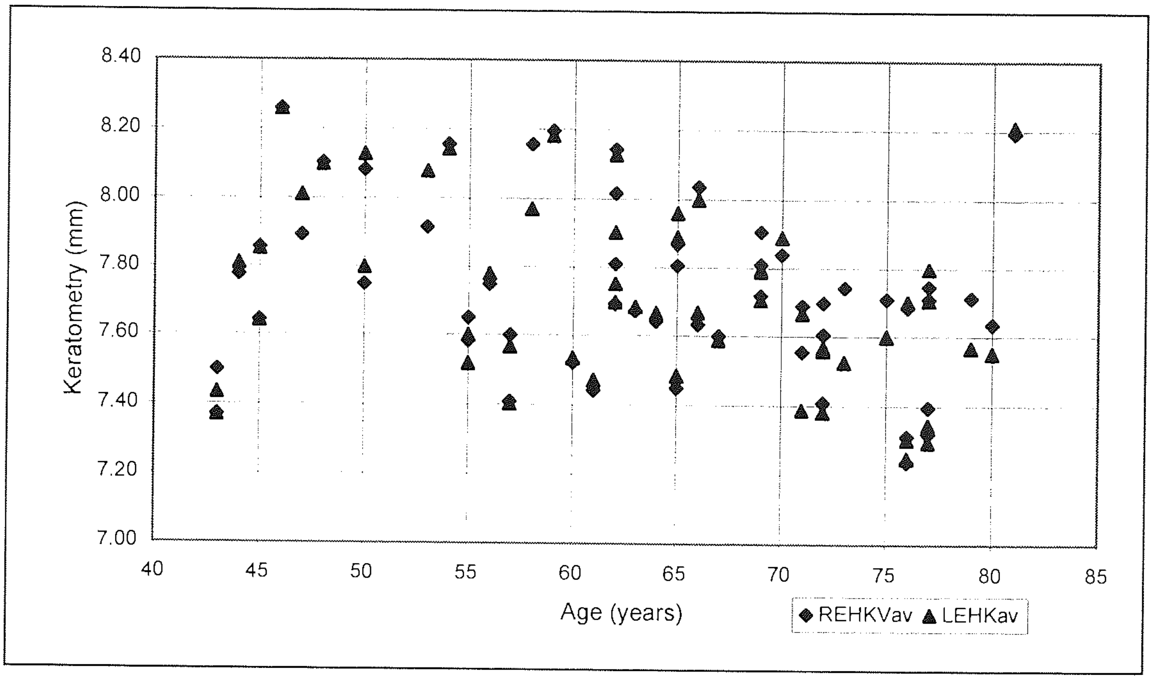
Table 7.1.7 Horizontal Keratometry for Group B age under 60 years

	RHK	LHK	R+L HK
Mean	7.81	7.82	7.82
SD	0.26	0.28	0.27

This small subgroup ($n = 20$) was aged between 43 and 59 years, and had mean horizontal keratometry value of 7.82 mm compared to the 7.67 mm of the over 60 years subgroup. This indicated that there was a decrease in horizontal keratometry with age.

The graph in Figure 7.1.5 indicates that up to age 60 the keratometry values were fairly steady but with variation. This trend was shown more easily when the two youngest subjects aged 43 who had low keratometry values were excluded.

Figure 7.1.5 Horizontal Keratometry versus Age for Group B



Similarly, the horizontal keratometry results given by the EyeSys for Group B showed no significant change related to age up to the age of 60 years as shown in Figures 7.1.6 and 7.1.7, with a decrease in value from age 60 years onwards.

Figure 7.1.6 RE EyeSys Horizontal Keratometry versus Age for Group B

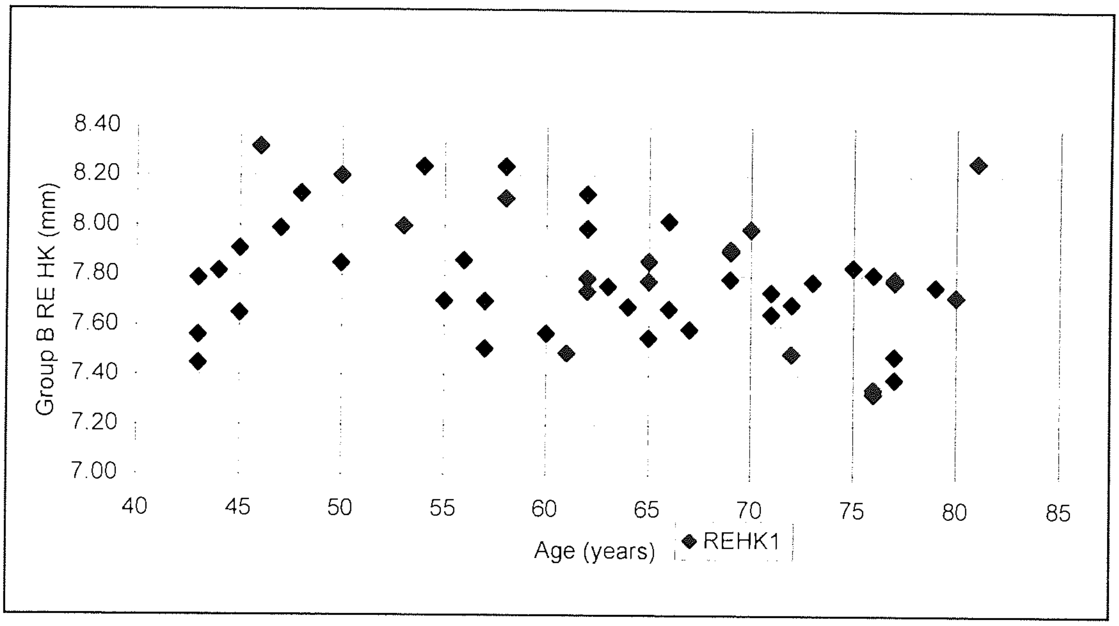
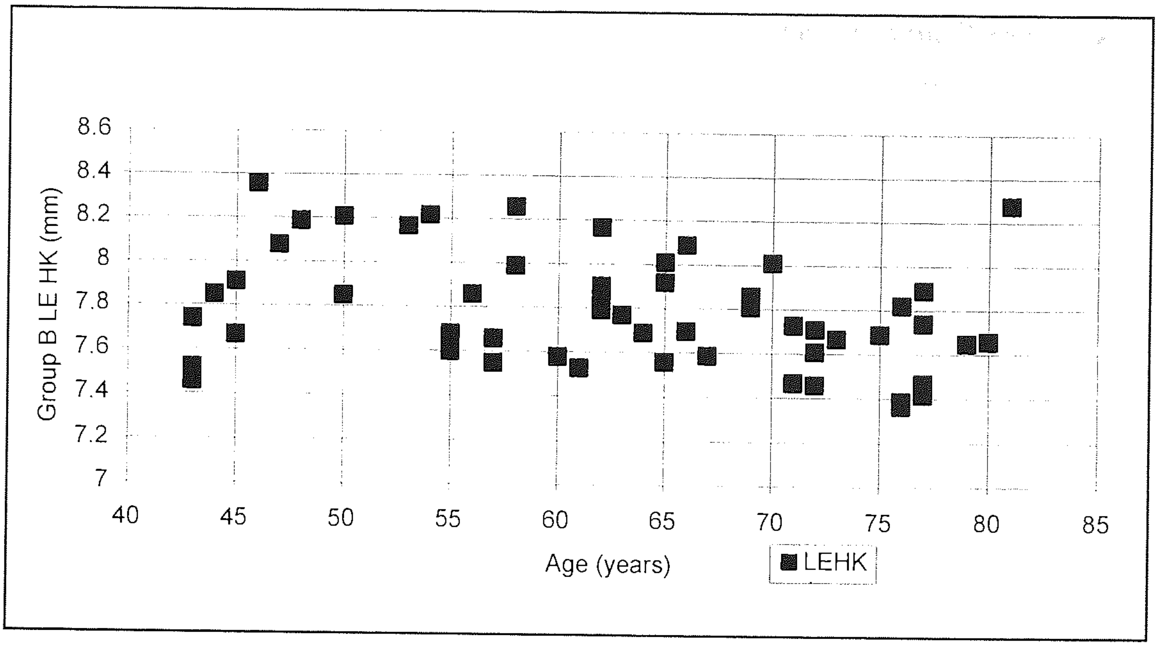


Figure 7.1.7 LE EyeSys Horizontal Keratometry versus Age for Group B



The mean value of 7.79 mm as shown in Table 7.1.8 is similar to that given using the Rodenstock keratometer of 7.73 mm.

Table 7.1.8 EyeSys Horizontal Keratometry for Group B

	RHK	LHK	R+L HK
Mean	7.79	7.79	7.79
SD	0.24	0.25	0.24

The subgroup of over 60 years was re-assessed and found to have smaller values than Group B as a whole.

Table 7.1.9 EyeSys Horizontal Keratometry for Group B over 60

	RHK	LHK	R+L HK
Mean	7.74	7.73	7.73
SD	0.21	0.22	0.21

The regression line for values of subjects over 60 years of age was:

RHK = 7.977 - 0.0035*age

LHK = 8.210 – 0.0068*age

This indicates a slow but definite reduction in horizontal keratometry with age after 60. The EyeSys gave slightly larger values (mean 0.07 mm higher) than the Rodenstock keratometer, probably because it used ring mire targets which were reflected by a slightly off-centre portion of the cornea where the surface curvature began to flatten.

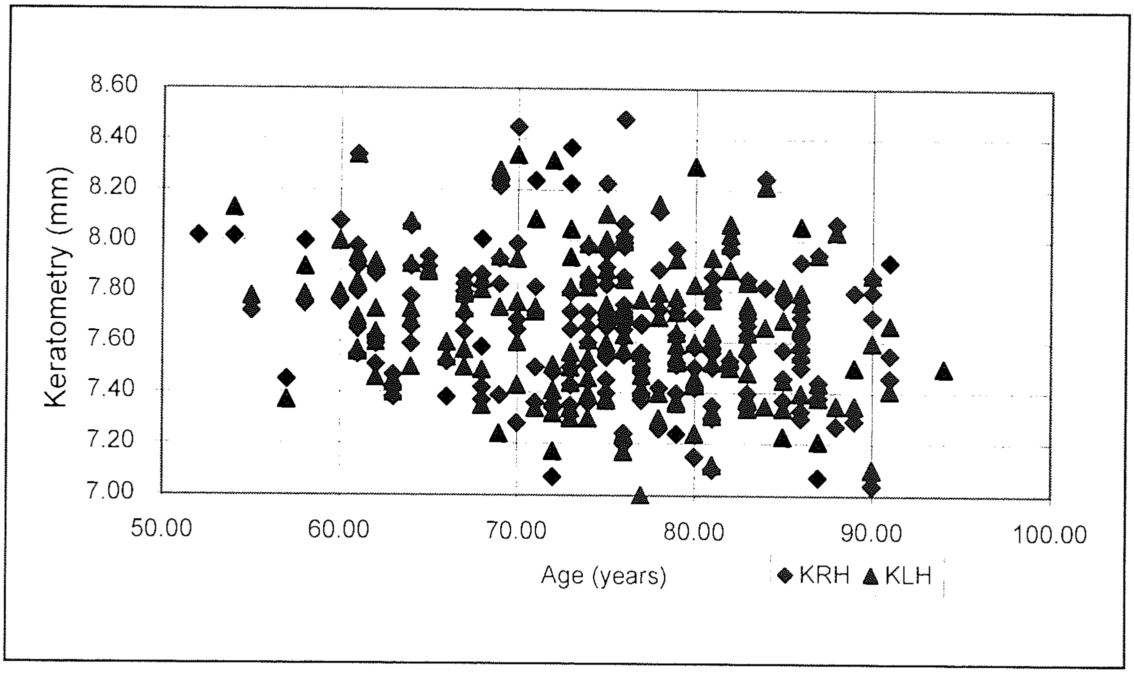
Table 7.1.10 Horizontal Keratometry for Group B under 60

	RHK	LHK	R+L HK
Mean	7.89	7.89	7.89
SD	0.26	0.28	0.27

The EyeSys results further support the conclusion that horizontal keratometry decreases with age.

Birmingham Heartlands Hospital subjects (Group D) were next considered. Usually there was no significant difference found in horizontal keratometry values between right and left eyes although the eye with the denser cataract often demonstrated more variable measurements. This increased variability may have been related to the poorer vision and to less reliable fixation by the eye with more dense cataract. When the target fixation drifted a measurement may have been made of an slightly off-centre portion of the cornea. Group D results are shown in Figure 7.1.8.

Figure 7.1.8 Horizontal Keratometry versus Age for Group D.



There was very little difference in the mean values (shown in Table 7.1.11 and 7.1.12) between the whole Group D and the 'over 60 years' subgroup because only 7 subjects were below age 60 years.

For Group D the analysis demonstrated a very significant relation between horizontal keratometry and age (RE $p = 0.0001$, LE $p = 0.0036$). The horizontal keratometry values gradually decreased with age. The linear regression equations were:

$RE = 8.377 - 0.0095*age$, $LE = 8.173 - 0.0068*age$.

The p values obtained from ANOVA analysis of keratometry with age are given in Table 7.2.13.

Table 7.1.11 Horizontal Keratometry for Group D

	RHK	LHK	R+L HK
Mean	7.66	7.66	7.66
SD	0.29	0.27	0.28

Table 7.1.12 Horizontal Keratometry for Group D over 60

	RHK	LHK	R+L HK
Mean	7.66	7.66	7.66
SD	0.29	0.27	0.28

Thus, comparing the horizontal keratometry results of the young Aston subjects of Group C to those of Groups A, B, D and E, it can be seen that:

- 'T' tests showed that there was no significant difference between the mean values of Groups A and C ($p > 0.05$)

- Analysis using ANOVA indicated that the variability of values for Group A was slightly greater than that of Group C, but this was not significant ($p > 0.05$)

- The subjects between age 40 and 60 years had a mean horizontal keratometry value lying between that of the younger and over 60 years subjects.

- The trend for Group B just missed significance for the right eye ($p = 0.06$), but was significant for the left eye (p = 0.02). The trend was of smaller values of horizontal keratometry (steeper corneal curvature) with increasing age.

- Group D demonstrated this trend much more significantly (RE p = 0.0001, LE p = 0.004) using linear regression.

- Using ANOVA to compare Group D and Group E subjects at Birmingham Heartlands Hospital there was found to be a reduction in the mean horizontal keratometry with increasing age. The variability was much greater in Group D.

- Although it is suspected that this greater variability in Group D was related to age, it may have been exaggerated by the subjects' poorer visual acuity, poorer target fixation and poorer physical condition as the older patients were attending a cataract assessment clinic.

- Although proof of the significance of the relationship between horizontal keratometry and age was hindered by the increased variability of values obtained from older patients, regression analysis showed a very significant relationship, RE p = 0.0001, LE p = 0.0036 (see Table 7.2.13).

Reading (1984) and Hayashi et al (1993) suggested that the horizontal keratometry gradually becomes steeper with age. This suggestion is verified by the results from this study, and is particularly true after 60 years of age. Hayashi et al (1995) using topographical analysis demonstrated a marked keratometry decrease (corneal curvature becomes steeper) at the horizontal meridians with age. They described that for age 20, 30 and 40 years the cornea exhibited with the rule (WTR) astigmatism, age 50, 60 there was a shift towards spherical curvature and for ages 70 and 80 there was against the rule (ATR) astigmatism. In the analysis of their averaged map, the mean refractive corneal power increased with age, i.e. corneal curvatures became steeper, and in the age 60s the horizontal meridian tended to be steeper than the vertical meridian which verifies the shift in astigmatism towards ATR astigmatism, i.e. horizontal keratometry has a smaller value than has vertical keratometry.

Ooi and Grosvenor (1995) studied the ageing eye to investigate the mechanisms of emmetropization, e.g. a myopic shift caused by some parameter changes tends to be balanced by other hypermetropic changes. They found no age related differences in the corneal radius of curvature (average of horizontal and vertical), but they did not study the meridians separately. In addition, their older group of mature adults were in the range 49 to 61 years.

Doughty et al (2000) when measuring a group of 'over 60 year old' subjects noted mean horizontal keratometry of 7.65 mm (SD 0.29) and mean vertical keratometry of 7.58 mm (SD 0.29) but found no relation to gender or corneal thickness.

7.2 Vertical Keratometry

The vertical keratometry values of Group C Aston young subjects are shown in Figure 7.2.1.

Figure 7.2.1 Vertical Keratometry versus Age for Group C

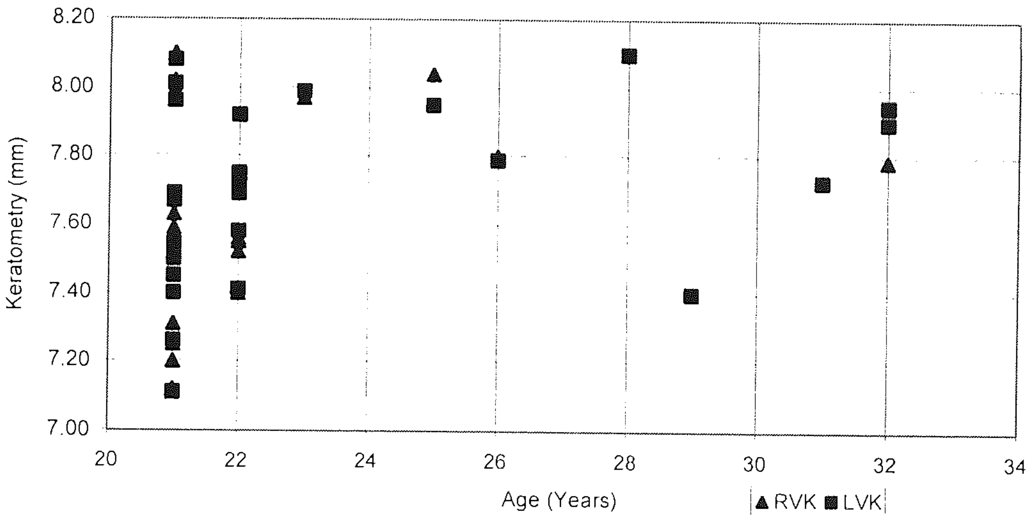


Table 7.2.1 Vertical Keratometry for Group C

	RVK	LVK	R+L VK
Mean	7.67	7.70	7.69
SD	0.29	0.27	0.28

No significant relationship of vertical keratometry to age was found within this group. The mean value shown in Table 7.2.1 is less than that for the horizontal keratometry of the same group. This finding compares fairly well with the results of Kiely et al (1984) who also studied a young group of students (n = 196) and found a vertical keratometry of 7.69 (SD 0.28) mm.

The vertical keratometry results using the EyeSys are shown in Figure 7.2.2 and have a similar distribution but greater mean value to those found with the Rodenstock keratometer. The greater mean value is again probably due to the EysSys measuring the cornea at points further from the corneal apex.

Figure 7.2.2 RE, LE EyeSys Vertical Keratometry versus Age for Group C

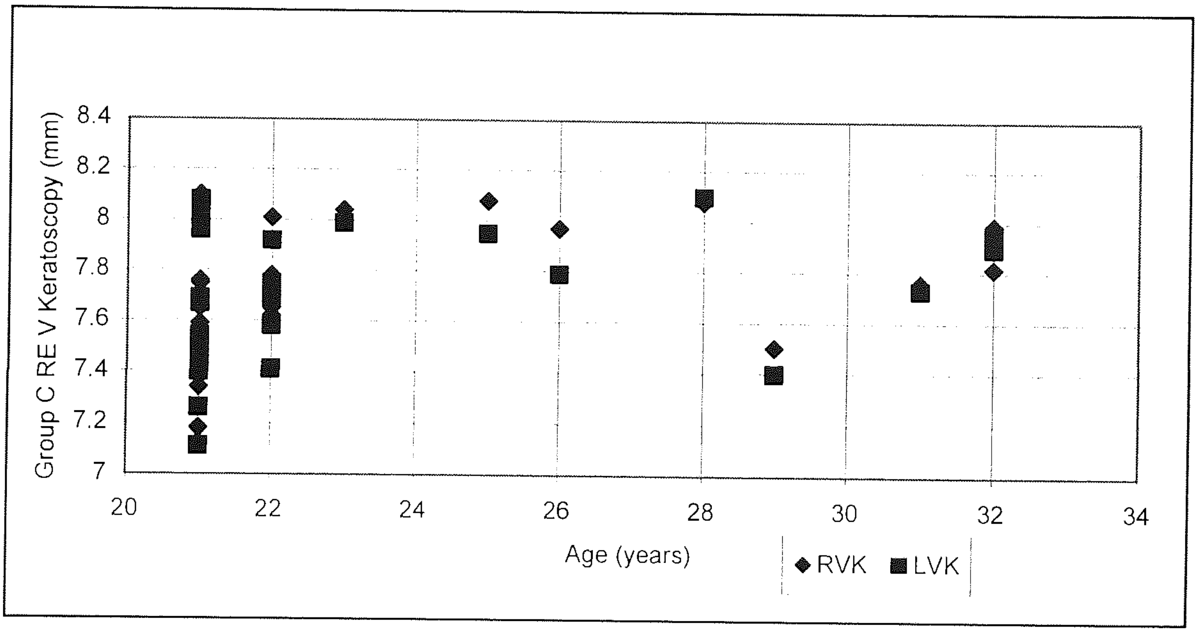


Table 7.2.2 EyeSys Vertical Keratometry for Group C

	RVK	LVK	R+L VK
Mean	7.75	7.77	7.76
SD	0.26	0.26	0.26

No significant difference was found between the mean vertical keratometry values found by Rodenstock keratometry and EyeSys keratoscopy.

The young subjects Group E results are shown in Figure 7.2.3. The mean values are shown in Table 7.2.3.

Figure 7.2.3 Vertical Keratometry versus Age for Group E

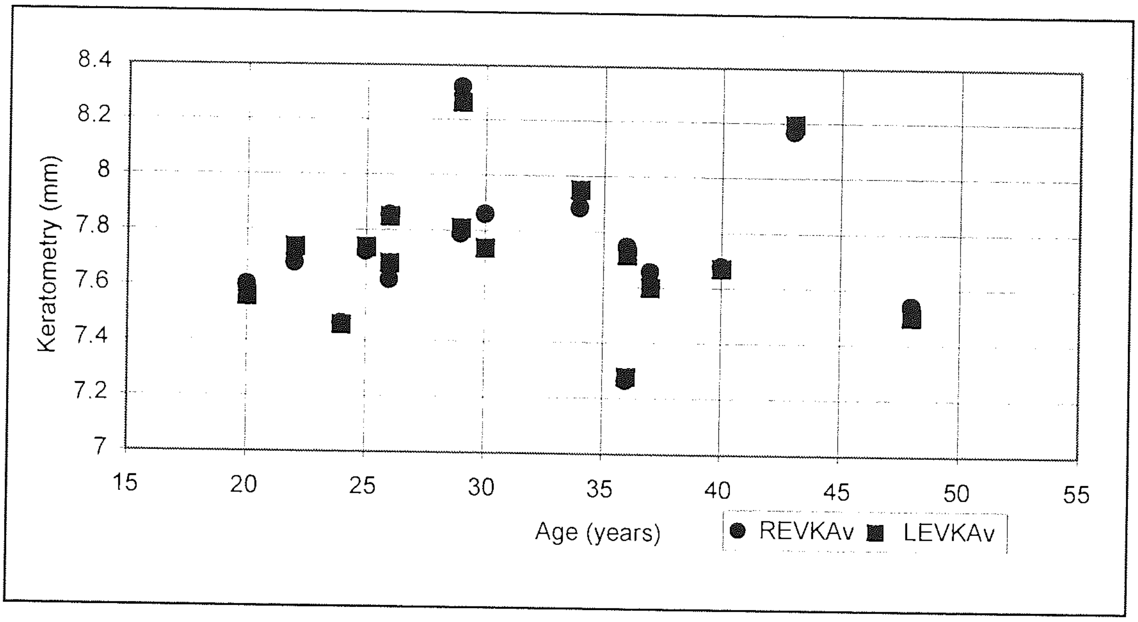


Table 7.2.3 Vertical Keratometry for Group E

	RVK	LVK	R+L VK
Mean	7.76	7.76	7.76
SD	0.26	0.25	0.25

As expected the vertical keratometry mean value was less than the mean value of horizontal keratometry. The mean value for Group E vertical keratometry was not significantly different ($p > 0.05$) from that of Group C. The linear regression analysis found no significant relationship with age for Group E, (RE $p = 0.46$, LE $p = 0.47$).

The vertical keratometry values of the subjects in Aston Group A were analysed and no significant difference was found between the right and left eyes so the results were combined. The results shown in Figure 7.2.4 suggest that the values increased slightly between ages 43 to 50 then gradually decreased as age 60 was approached. However, no significant relation was found to age considering the group as a whole.

Figure 7.2.4 Vertical Keratometry versus Age for Group A

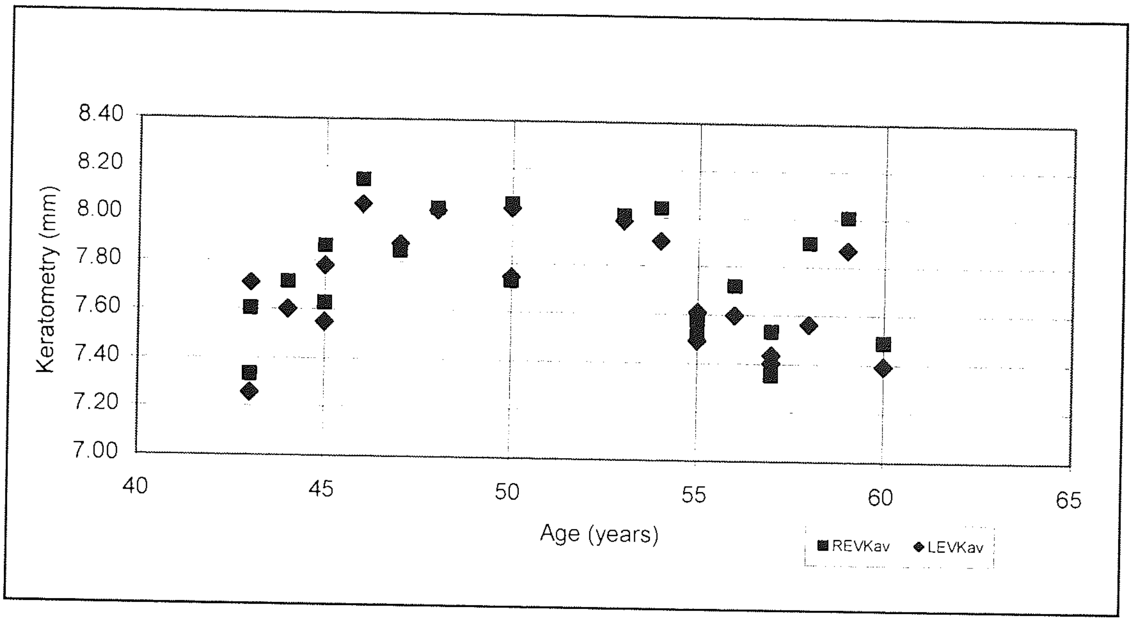


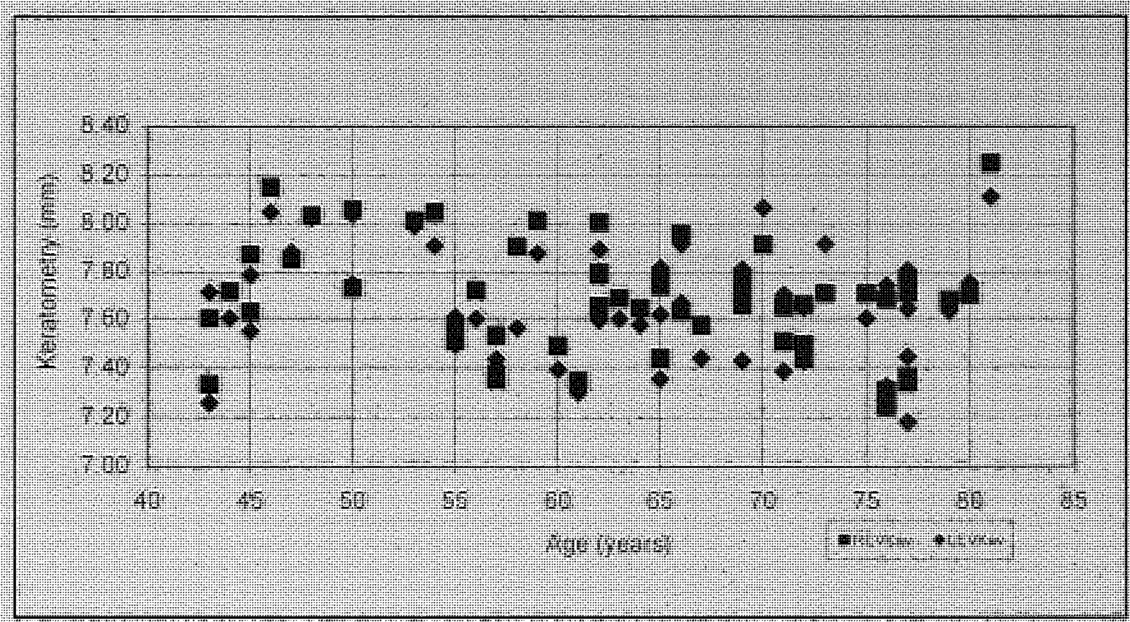
Table 7.2.4 Vertical Keratometry for Group A

	RVK	LVK	R+L VK
Mean	7.69	7.69	7.69
SD	0.24	0.23	0.24

The mean and standard deviation values were very similar to those obtained for the Group C young Aston University subjects. This suggests that there was no definable change in vertical keratometry with age up to age 60 years. The mean vertical keratometry value was less than the horizontal keratometry value, so with the rule (WTR) astigmatism was still present.

When the values for the larger Group B were examined, see Figure 7.2.5, linear regression indicated a slight trend towards decreasing value as age 80 was approached, but the variability was such that no significant relation was demonstrated.
ANOVA analysis showed RE p = 0.28, LE p = 0.26.

Figure 7.2.5 Vertical Keratometry versus Age for Group B



The mean values of right and left eyes as shown in Table 7.2.5 were very similar so they were combined. Compared to the young Group C, the older and wider age range of Group B had a mean value of 0.04 mm lower, and standard deviation 0.05 lower. To investigate further the trend of decreasing value with age, the sub group of subjects over 60 years of age were re-analysed. The trend was demonstrated. For example, the mean value was now 7.61 mm as shown in Table 7.2.6, which was 0.07 mm less than that of Group C. However, the variability of the values prohibited a significant relationship being shown.

Table 7.2.5 Vertical Keratometry for Group B

	RVK	LVK	R+L VK
Mean	7.64	7.65	7.65
SD	0.23	0.23	0.23

Table 7.2.6 Vertical Keratometry for Group B over 60

	RVK	LVK	R+L VK
Mean	7.61	7.62	7.61
SD	0.23	0.23	0.23

When the sub group of under 60 year old subjects was reviewed, the mean value was found to be 7.70 as shown in Table 7.2.7, which is only 0.01 mm greater than the mean for the young subjects in Group C. There was no significant difference ($p > 0.05$) found between these two groups.

Table 7.2.7 Group B under 60 Vertical Keratometry

	RVK	LVK	R+L VK
Mean	7.70	7.70	7.70
SD	0.24	0.23	0.23

Similarly, the vertical keratometry EyeSys measurements for the whole of Group B shown in Figures 7.2.6 and 7.2.7 gave no definite trend with age for either eye and were combined, see Table 7.2.8.

Figure 7.2.6 RE EyeSys VK versus Age for Group B

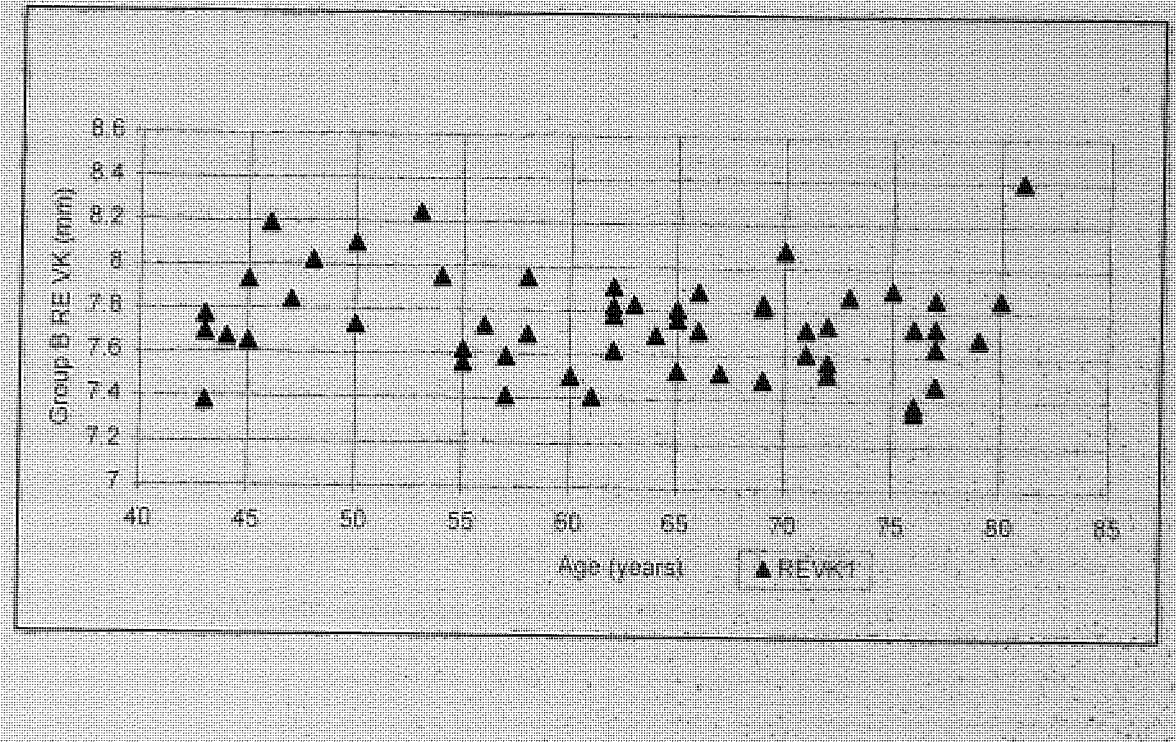


Figure 7.2.7 LE EyeSys VK versus Age for Group B

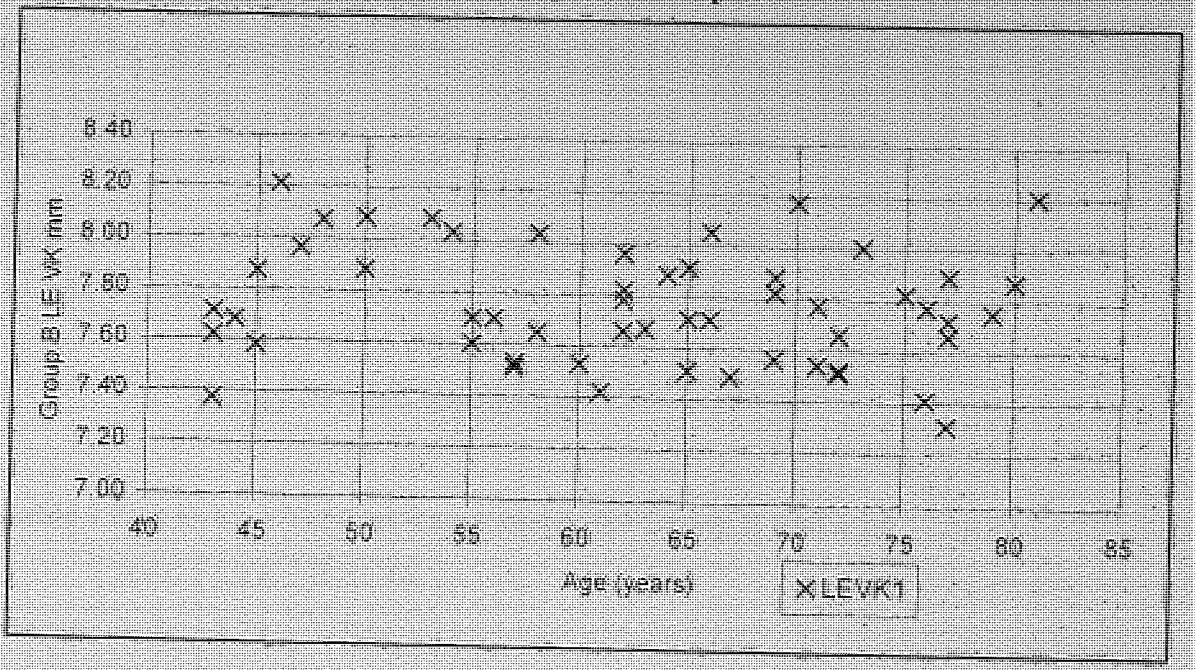


Table 7.2.8 EyeSys Vertical Keratometry for Group B

	RVK	LVK	R+L VK
Mean	7.74	7.75	7.75
SD	0.22	0.22	0.22

When measurements using the Rodenstock keratometer were compared to those from the EyeSys, the mean values for the vertical keratometry given by the EyeSys were greater, but in this case by only 0.10 mm.

When the subgroup of over 60 year olds were re-analysed, the mean was 0.02 mm smaller than that of the whole group as shown in Table 7.2.9 whereas the Rodenstock keratometer showed a 0.03 mm reduction. The result of the corneal curvature becoming steeper may affect the cornea to a greater extent at the apex than slightly off centre. However, no significant difference between the means was revealed.

Table 7.2.9 EyeSys Vertical Keratometry for Group B over 60

	RVK	LVK	R+L VK
Mean	7.72	7.72	7.72
SD	0.21	0.21	0.21

Regression analysis for the subgroup 'age over 60' EyeSys vertical keratometry showed a slight increase with age:

REVK = 7.375 + 0.0049*age

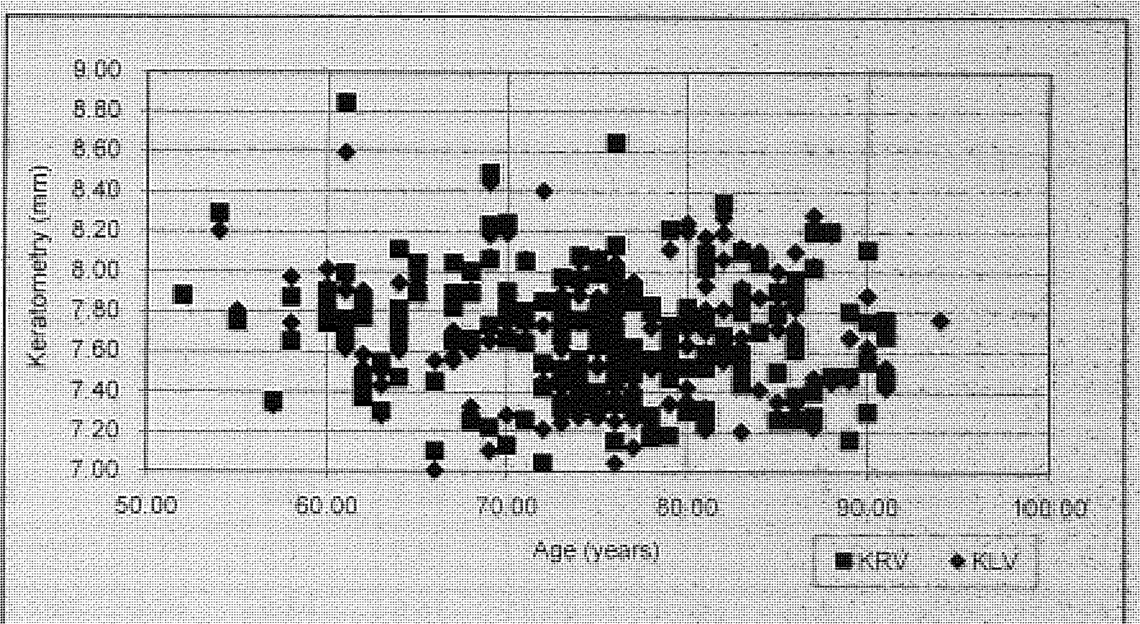
LEVK = 7.587 + 0.0020*age

Table 7.2.10 EyeSys Vertical Keratometry for Group B under 60

	RVK	LVK	R+L VK
Mean	7.79	7.79	7.79
SD	0.24	0.24	0.24

The vertical keratometry of the subjects in Birmingham Heartlands Hospital Group D were analysed.

Figure 7.2.8 Vertical Keratometry versus Age for Group D



There was much variability in the measurements. Analysis indicated a slight reduction in vertical keratometry with age, but this was not significant (RE $p = 0.10$, LE $p = 0.44$). The mean values are shown in Table 7.2.11. These are smaller values (steeper curve) than those found for Group E, but no significant difference in the mean values between Groups D and E was demonstrated ($p < 0.05$).

Table 7.2.11 Vertical Keratometry for Group D

	RVK	LVK	R+L VK
Mean	7.69	7.69	7.69
SD	0.31	0.30	0.30

The sub group over 60 years was re-analysed and a steeper mean value was found as shown in Table 7.2.12. This follows the suspected trend towards a steeper curvature but was not proven to be significantly different from the mean of group D, nor from the mean value of the young subjects of group E.

Linear regression indicated the slight trend towards smaller values but this was not proved to be significant ($p > 0.05$).

Table 7.2.12 Vertical Keratometry for Group D over 60

	RVK	LVK	R+L VK
Mean	7.69	7.69	7.69
SD	0.31	0.30	0.30

ANOVA analysis of the keratometry versus age is shown in Table 7.2.13. The p values of significant relation are shown in bold.

Table 7.2.13. ANOVA Analysis of Keratometry versus Age

Group	RHK	LHK	RVK	LVK	EyeSys RHK	EyeSys LHK	EyeSys RVK	EyeSys LVK
B	0.06	**0.02**	0.28	0.26	0.06	**0.03**	0.48	0.44
B >60	0.27	0.12	0.45	0.77	0.56	0.27	0.41	0.75
B <60	0.54	0.64	0.41	0.76	0.42	0.56	0.78	0.98
D >60	**0.0005**	**0.008**	0.17	0.64	XX	XX	XX	XX
E	0.37	0.28	0.46	0.47	XX	XX	XX	XX

Overall, from the analysis of the results of the horizontal and vertical keratometry measurements made on the Rodenstock keratometer and the EyeSys keratometer the following conclusions can be drawn:

- Differences between right and left eyes were generally small
- Horizontal keratometry decreased with age, as corneal curvature became steeper.
- Vertical keratometry did not appear to change until after age 60, when the values gradually decreased. This may be linked to reduction in eyelid tension with age.
- The range of values increased with age and the variability of the values seemed greater for the older subjects from the Group B. This was probably because on average they were older and their vision and target fixation was limited.

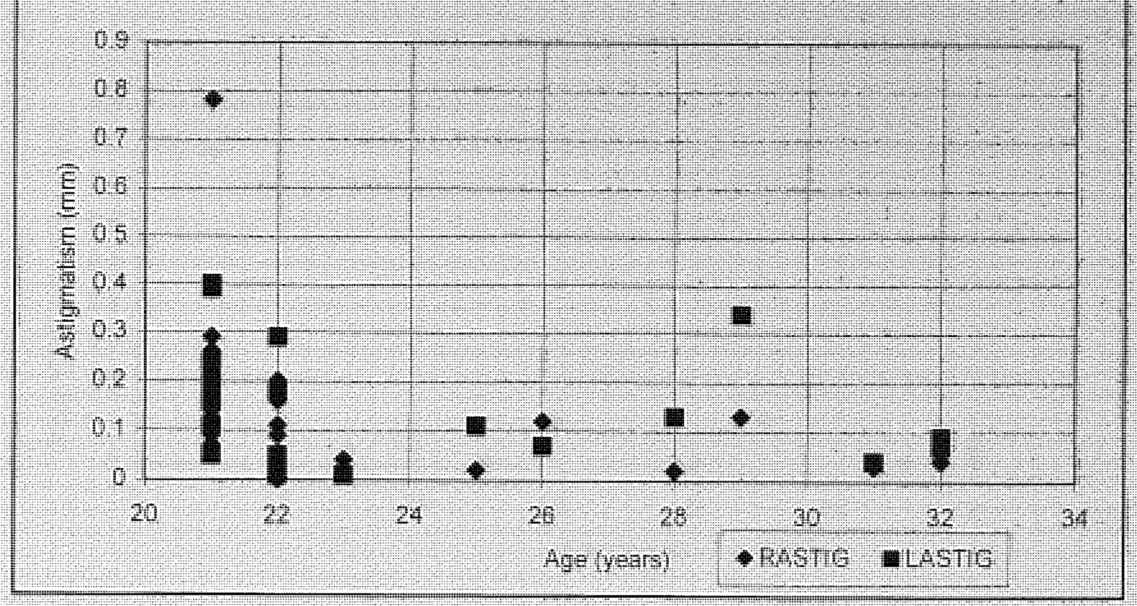
The Rodenstock keratometry values were smaller than those obtained using the EyeSys, most likely because the former instrument measured corneal curvature nearer to the naturally steeper corneal apex position.

7.3 Astigmatism

Astigmatism in this instance is defined as horizontal keratometry value minus vertical keratometry value, (HK – VK) and a positive result is described as "with the rule" (WTR) and a negative result as "against the rule" (ATR).

The corneal astigmatism for the young group C was assessed first (Figure 7.3.1).

Figure 7.3.1 Astigmatism versus Age for Group C



Even if the outlier value of 0.77 mm in the RE was disregarded the plot of the results suggested a slight reduction in astigmatism with age, although not proven to be significant for Group C.

The mean values for RE and LE did not differ significantly so they were combined.

Table 7.3.1 Astigmatism for Group C

	RAstig	LAstig	R+L Astig
Mean	0.14	0.14	0.14
SD	0.15	0.11	0.13

Similarly, the astigmatism measurements using the EyeSys in both RE and LE showed a similar trend towards astigmatism reduction with age (Figures 7.3.2. and 7.3.3).

Figure 7.3.2 RE EyeSys Astigmatism versus Age for Group C

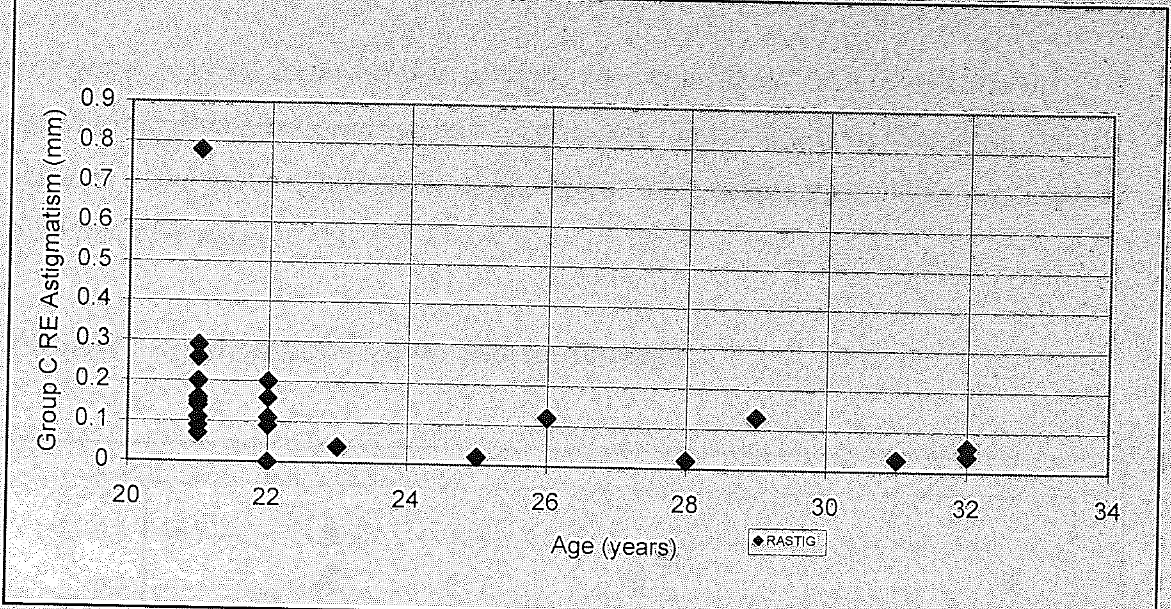


Figure 7.3.3 LE EyeSys Astigmatism versus Age for Group C

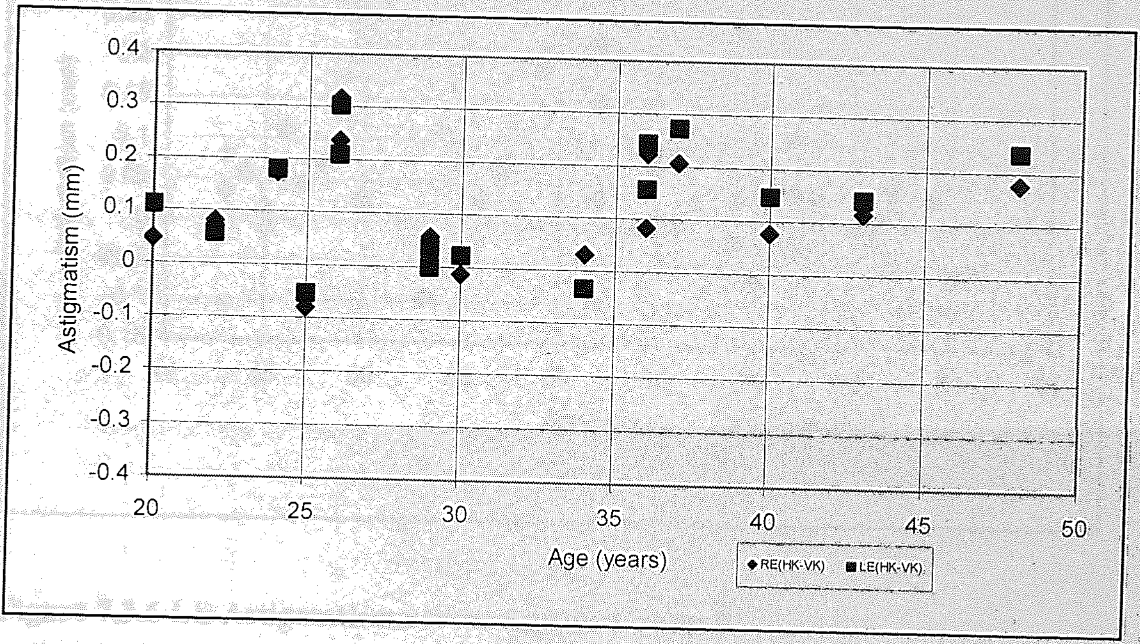
The astigmatism values given by the EyeSys (Table 7.3.2) were slightly lower and less variable than those given by the Rodenstock keratometer.

Table 7.3.2 EyeSys Astigmatism for Group C

	RAstig	LAstig	R+L Astig
Mean	0.12	0.10	0.11
SD	0.11	0.08	0.09

The young subjects in the hospital group E were considered next. There was no significant relation between age and astigmatism. The majority of this group and all subjects in the group C had positive values, i.e. WTR astigmatism. This result agrees with that of Weale (1971).

Figure 7.3.4 Astigmatism versus Age for Group E



Values for RE and LE were similar so they were combined as shown in Table 7.3.3. There was no significant difference between the means of Group C and Group E.

Table 7.3.3 Astigmatism for Group E

	RAstig	LAstig	R+L Astig
Mean	0.10	0.12	0.11
SD	0.11	0.11	0.11

Attention was now turned to the results of the older groups.

As mentioned before, Group A of the pilot study was incorporated into Group B for analysis, and again the measurements analysed for over 60 and under 60 years subgroups.

For both RE and LE (Figures 7.3.5 and 7.3.6) there appeared to be no significant age relationship. However, after age 60 there was a trend towards negative values, i.e. towards ATR astigmatism, with increasing age. This also appeared to be true for the values obtained by the EyeSys, as shown in Figures 7.3.7 and 7.3.8.

Figure 7.3.5 RE Astigmatism versus Age for Group B

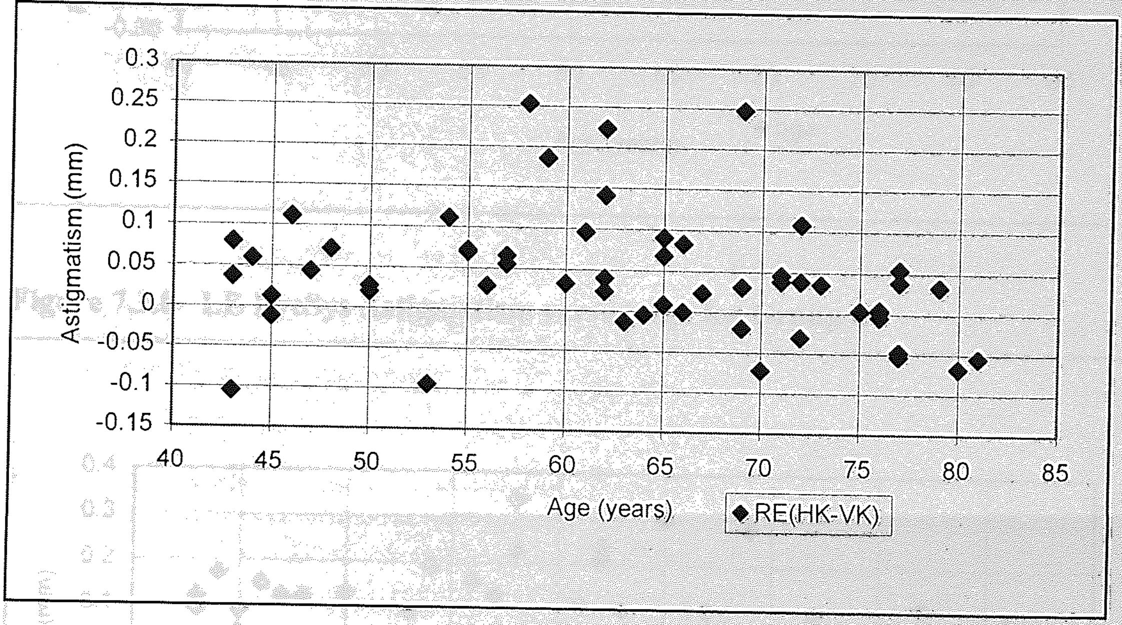


Figure 7.3.6 LE Astigmatism versus Age for Group B

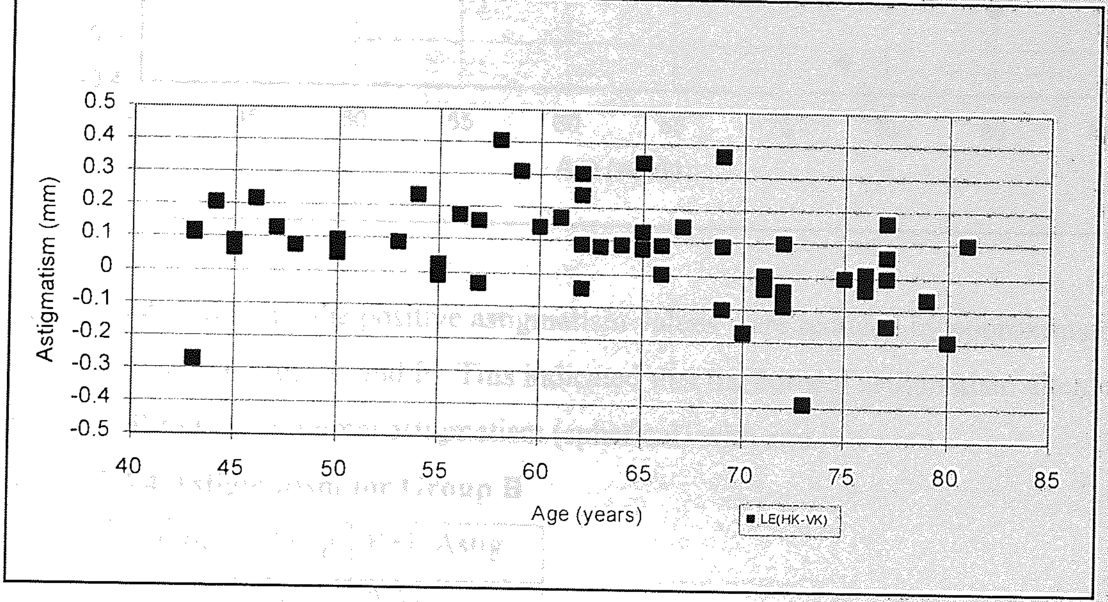


Figure 7.3.7 RE EyeSys Astigmatism versus Age for Group B

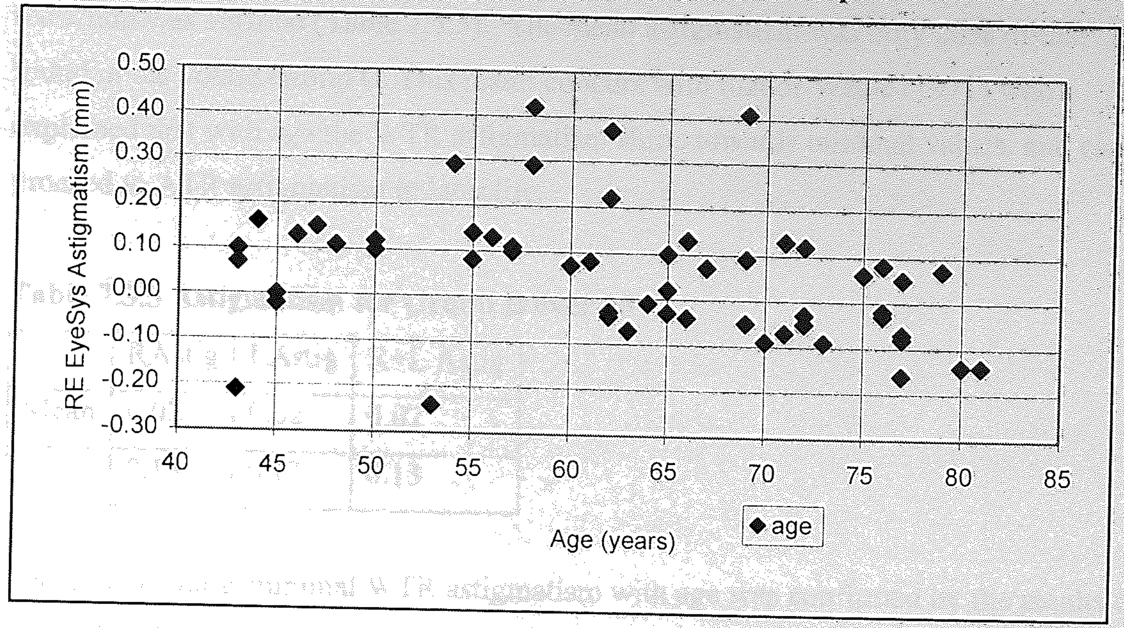


Figure 7.3.8 LE EyeSys Astigmatism versus Age for Group B

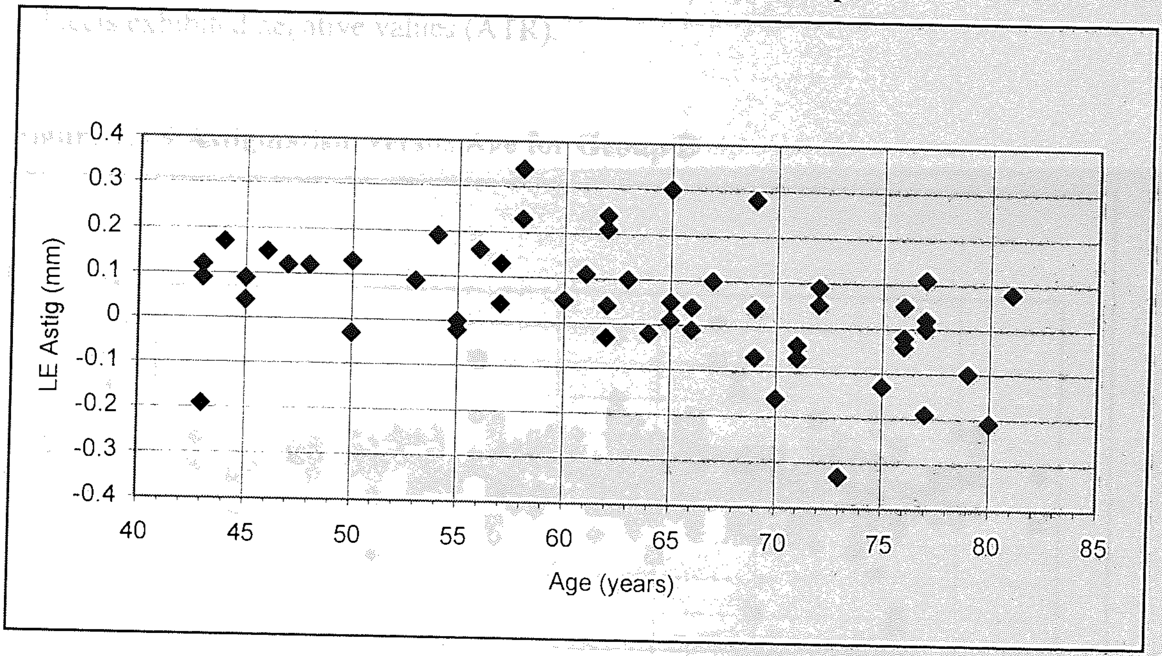


Table 7.3.4 shows that the positive astigmatism values were reduced by approximately 50% of those of Groups C and E. This indicated that the corneal astigmatism altered from WTR towards minimal astigmatism (spherical) with age.

Table 7.3.4 Astigmatism for Group B

	RAstig	LAstig	R+L Astig
Mean	0.05	0.05	0.05
SD	0.14	0.13	0.13

When the sub group of over 60 years was analysed, this trend towards astigmatism reduction was verified (Table 7.3.5). The mean astigmatism was only 20% of that found in the young Group C. This result concurs with that of Weale (1971) who explained that with age the WTR astigmatism shifts towards minimal values, and can proceed to ATR astigmatism in later life.

Table 7.3.5 Astigmatism for Group B over 60

	RAstig	LAstig	R+L Astig
Mean	0.02	0.02	0.02
	0.13	0.13	0.13

The trend towards minimal WTR astigmatism with age was confirmed by the results of Group D. These are shown in Figure 7.3.9. The mean result indicated that the astigmatism value is low and is similar to the Group B over 60 years. Many of the subjects exhibited negative values (ATR).

Figure 7.3.9 Astigmatism versus Age for Group D

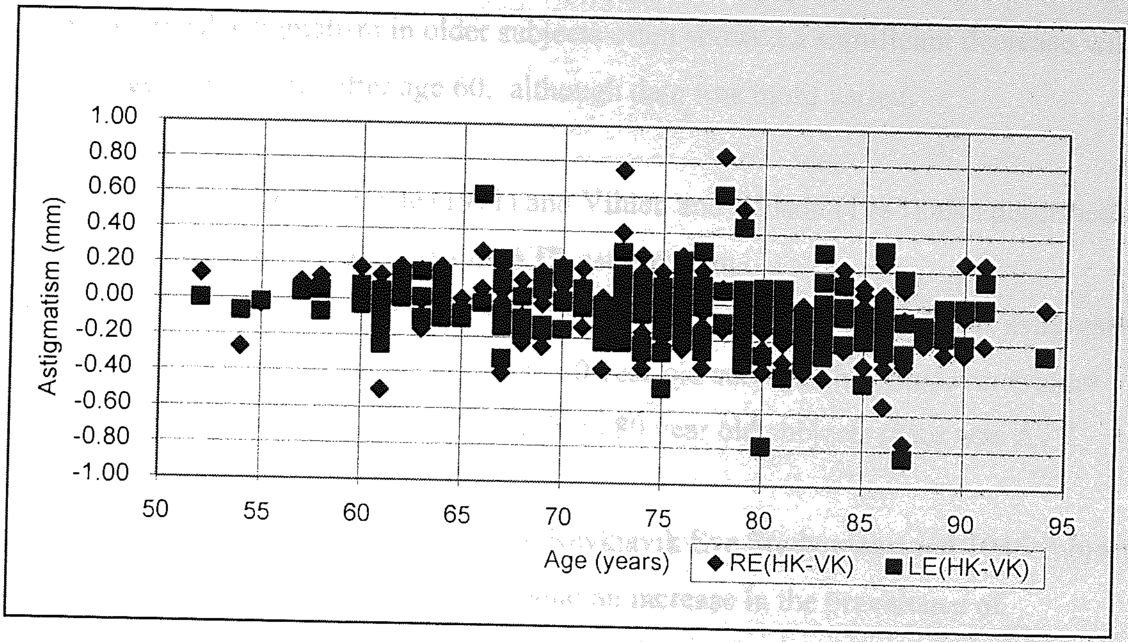


Table 7.3.6 Astigmatism for Group D

	RAstig	LAstig	R+L Astig
Mean	0.02	0.03	0.03
SD	0.19	0.18	0.18

ANOVA analysis of astigmatism and of eccentricity versus age is shown in Table 7.3.7. The p values indicating a significant relation are shown in bold. The positive relation between astigmatism and age was shown to be very significant for Group D.

Table 7.3.7. ANOVA Analysis of Astigmatism and Eccentricity versus Age

Group	R Astig	L Astig	EyeSys R Astig	EyeSys L Astig	R Eccentricity	L Eccentricity
B	0.23	0.025	**0.047**	**0.006**	0.78	0.48
B >60y	**0.008**	**0.004**	**0.02**	**0.008**	0.25	0.78
B <60y	**0.026**	0.119	0.052	0.15	0.13	0.14
D	**0.005**	**0.004**	XX	XX	XX	XX
E	0.75	0.46	XX	XX	XX	XX

Overall, from the analysis of the astigmatism results (p values given in Table 7.3.7) the following conclusions were drawn:

- Corneal astigmatism moved from WTR towards ATR with age
- Corneal astigmatism in older subjects often showed a significant decrease with age, particularly after age 60, although data was more varied.

Ninn – Pederson (1995), Weale (1971) and Vihlen and Wilson (1983) also described the shift in astigmatism with age towards ATR astigmatism.

Hayashi et al (1995) using the TMS video-keratoscopy system, found WTR astigmatism in 'under 40 year old' subjects. For 50 to 60 year old subjects the corneal curvature pattern tended towards spherical and for 70 to 80 year old subjects there was ATR astigmatism.

Haegerstrom –Portnay et al (2002) in the Reykjavik Eye Study measured 1045 subjects including 78 of age 80 or older. They found an increase in the prevalence of astigmatism with age and a change from about 50% WTR, 50% ATR astigmatism at age 50 to a preponderance of ATR astigmatism particularly in the very old group. They reported that the 'under 65' subjects had little astigmatism, whereas of the older subjects about 50% had at least 1.00D of ATR astigmatism. The amount of astigmatism grew larger and more varied in the older group.

7.4 Eccentricity

The eccentricity described the peripheral corneal curvature and was given by the EyeSys video-keratoscopy. Therefore it was only available for Aston subjects. There was no apparent relation between eccentricity and age (RE p = 0.78, LE p = 0.48). Figure 7.4.1

Figure 7.4.1 RE EyeSys Eccentricity versus Age for Group C

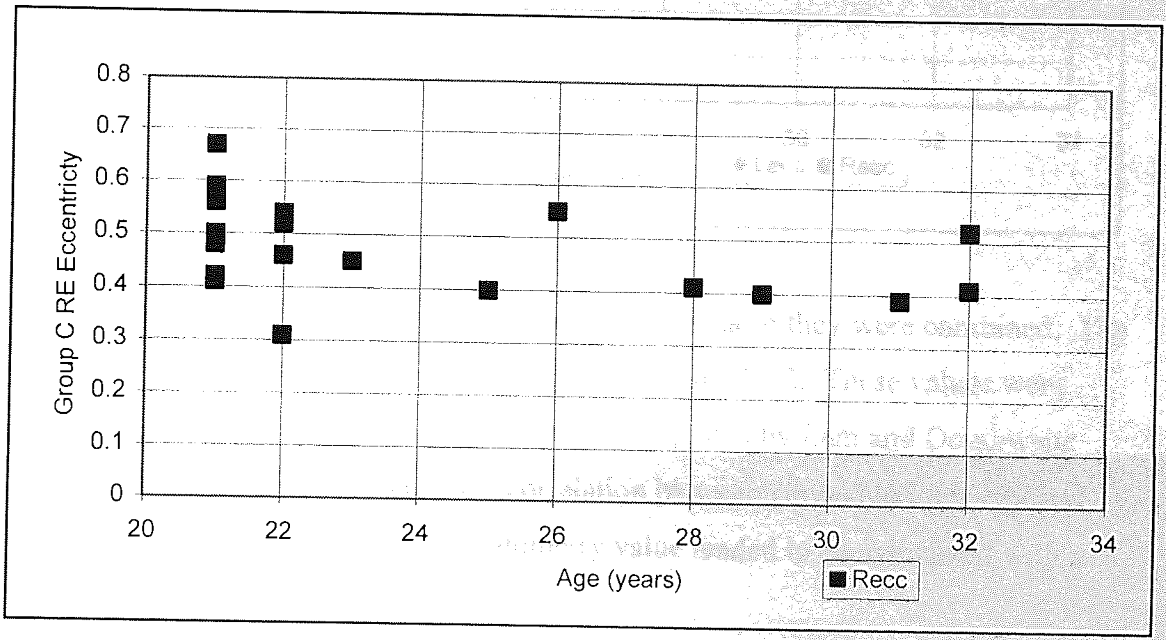


Figure 7.4.2 LE EyeSys Eccentricity versus Age for Group C

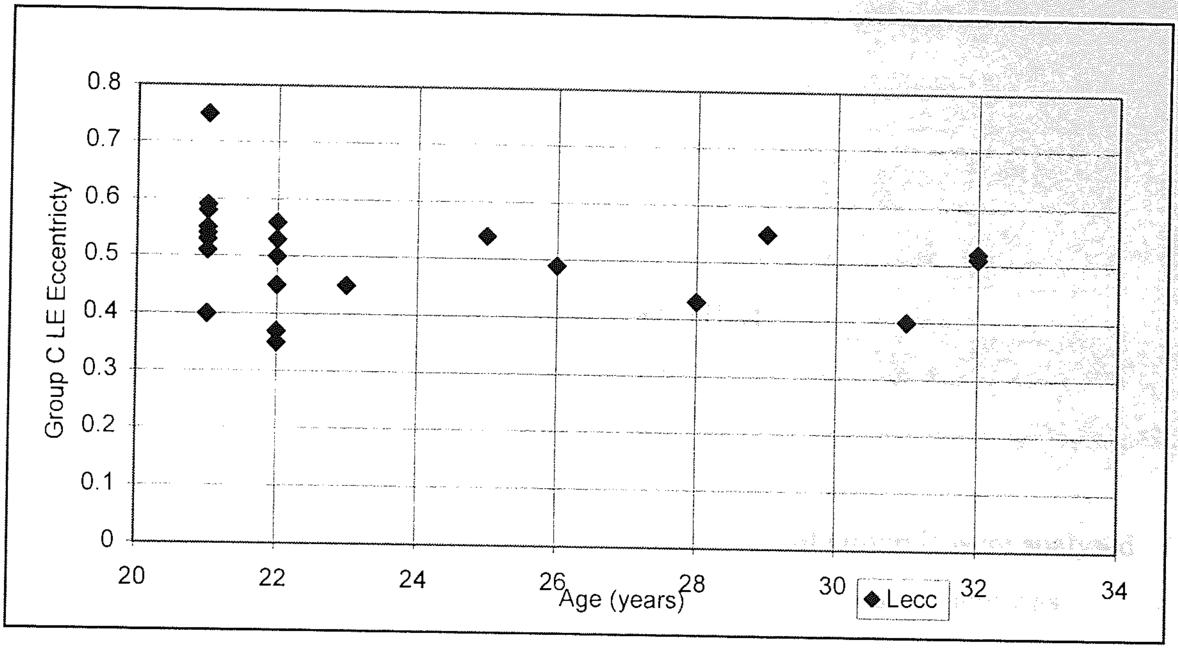
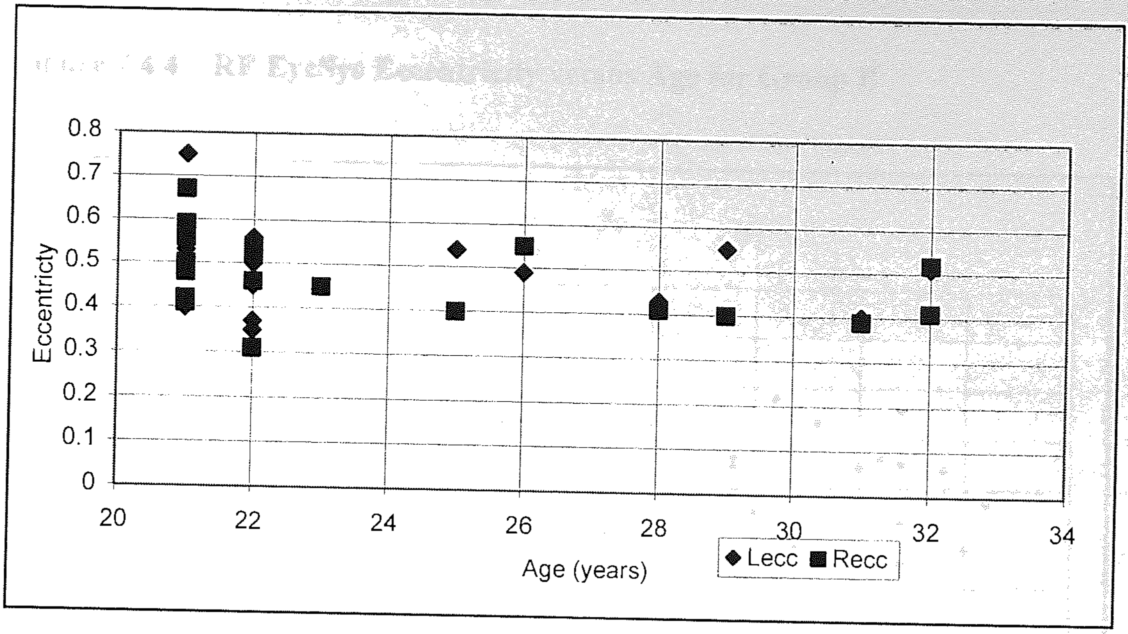


Figure 7.4.3 RE, LE, EyeSys Eccentricity versus Age for Group C



There was no significant difference between fellow eyes so they were combined. The mean eccentricity values are shown in Tables 7.4.1 and 7.4.2. These values were smaller than those (horizontal 0.82, vertical 0.87) quoted by Lam and Douthwaite (1996), who reported a weak negative correlation between corneal eccentricity and central keratometry, i.e. a smaller keratometry value tended to be associated with a larger eccentricity value.

Table 7.4.1 Eccentricity for Group C

	R Ecc	L Ecc	R+L Ecc
Mean	0.48	0.51	0.50
SD	0.08	0.08	0.08

ANOVA indicated that there was no significant age effect.

Regression equation: R Ecc = 0.45 + 0.0004 *age

L Ecc = 0.57 – 0.001 *age

The eccentricity values (Figure 7.4.2.) for the older subjects of Group B were analysed and no significant age relation was found (RE $p = 0.78$, LE $p = 0.48$). There was increased variability of values and the range expanded to 0.22 – 0.80 mm for this older group. Table 7.4.2 shows the mean values and the larger value of standard deviation.

Figure 7.4.4 RE EyeSys Eccentricity versus Age for Group B

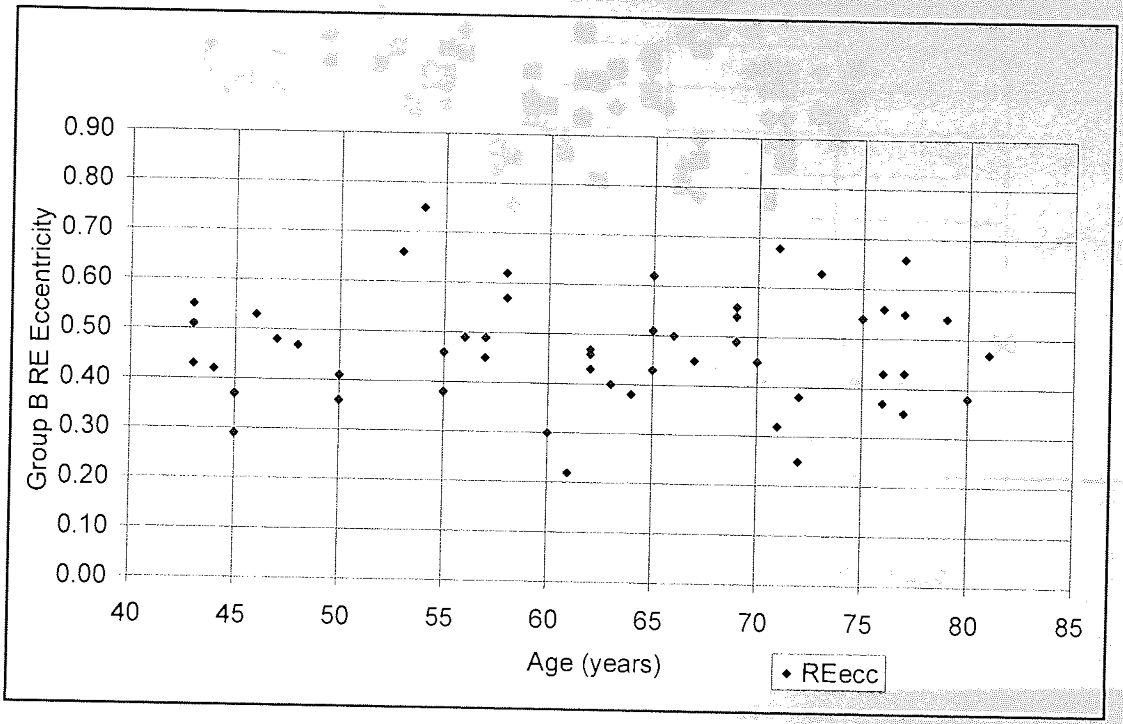


Figure 7.4.5 LE EyeSys Eccentricity versus Age for Group B

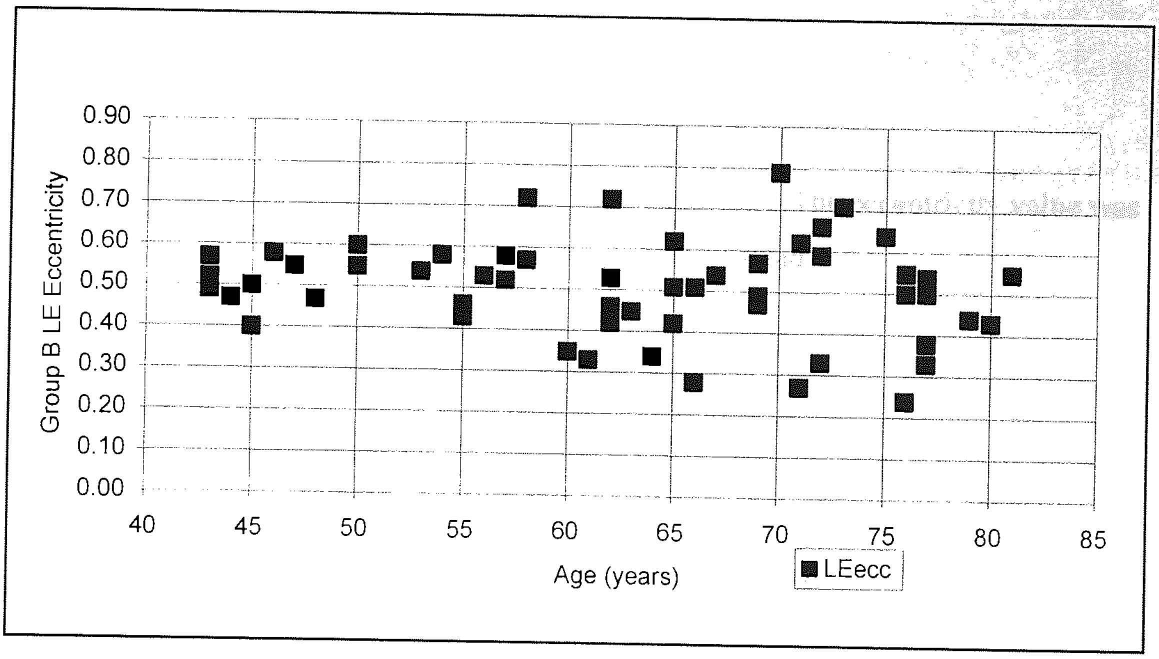
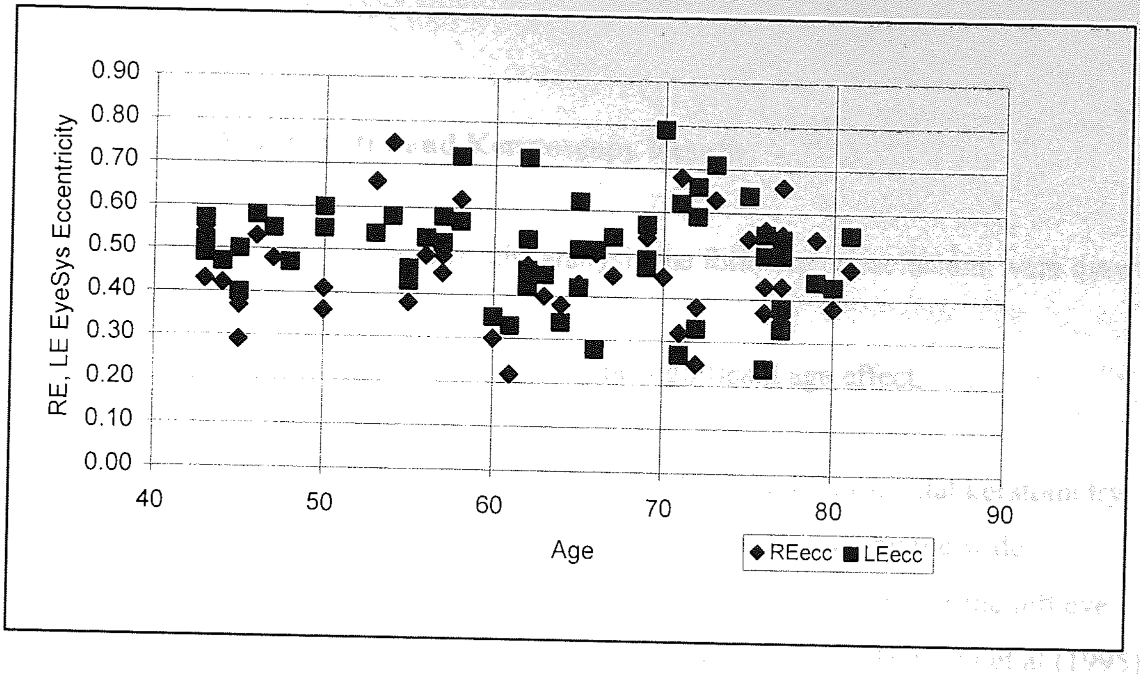


Figure 7.4.6 RE and LE EyeSys Eccentricity versus Age for Group B



Linear regression on these results indicated no significant relation to age.

Table 7.4.2 Eccentricity for Group B

	R Ecc	L Ecc	R+L Ecc
Mean	0.47	0.50	0.49
	0.11	0.12	0.11

Regression equation: R Ecc = 0.446 + 0.0004*age
L Ecc = 0.567 – 0.0010*age

Table 7.4.3 gives the mean results for the over 60 subjects. The eccentricity value was only slightly reduced but the variability remained fairly steady.

Table 7.4.3 Eccentricity for Group B over 60

	R Ecc	L Ecc	R+L Ecc
Mean	0.46	0.49	0.47
	0.11	0.13	0.12

Regression equation: $R\ Ecc = 0.446 + 0.0004*age$

$L\ Ecc = 0.567 - 0.0010*age$

7.5 Discussion Keratometry and Keratoscopy Results

Overall from the keratometry results analysis the following conclusions were drawn:

- The younger Groups E and C showed no significant age effect.
- Group B demonstrated the trend of gradual decrease in horizontal keratometry with age. Although proof of the relationship was hindered by the wide variability of the values, the trend was shown to be significant for the left eye (RE $p = 0.06$, LE $p = 0.02$). The result agrees with those of Hayashi et al (1995) and other researchers.
- In the larger Group D the relationship was very significant (RE $p = 0.0001$, LE $p = 0.0036$).
- Differences between RE and LE corneal curvature values were small.
- Keratometry and EyeSys keratometry were comparable but keratometry gave slightly smaller values as it measured near to the steeper corneal apex position.
- Vertical keratometry was generally steady up until age 60 when the values gradually decreased. No significant relation was found.
- Corneal astigmatism changed from WTR to ATR with age, mainly due to the horizontal keratometry decreasing. The astigmatism reduction with age was significant in Group D, (RE $p = 0.005$, LE $p = 0.004$).
- No significant relation was found between corneal eccentricity and age, keratometry or astigmatism.

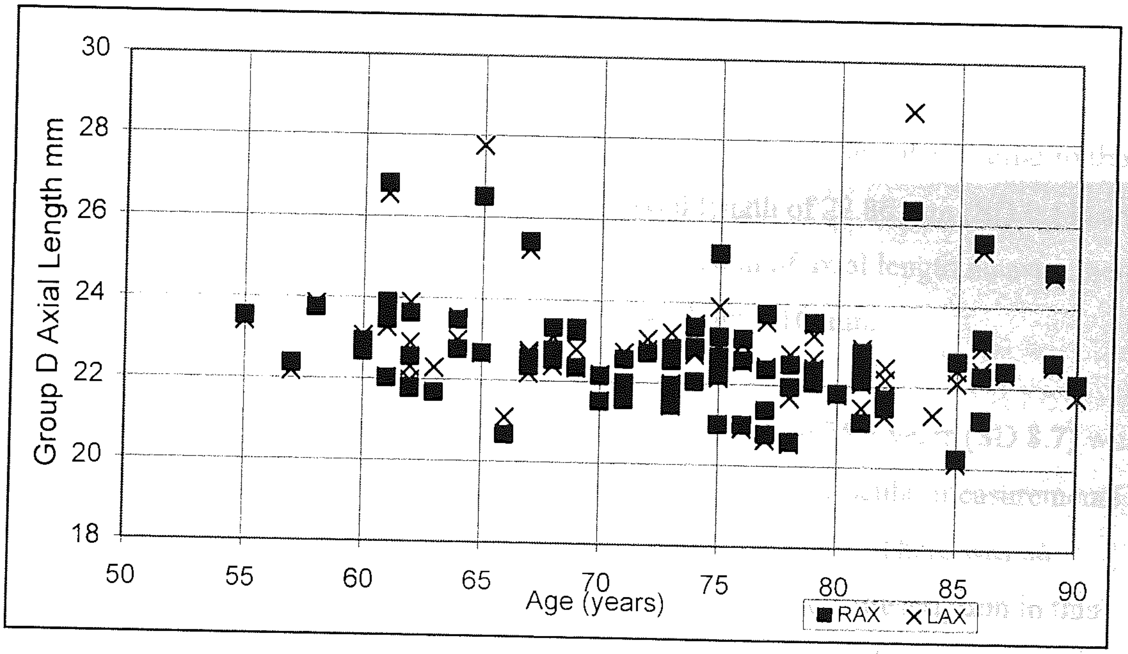
Chapter 8

Results and Discussion of Biometry Study

8.1 Axial Length

Due to limited access to subjects and equipment at Aston University the biometry measurements were only carried out at Birmingham Heartlands Hospital. The measurements only apply to the subjects in Groups D and E (hospital patients and staff). The biometry printout gives measurement values for axial length, lens thickness and the anterior chamber depth of the eye under examination. Figure 8.1.1 shows the plot of the axial length against age for Group D.

Figure 8.1.1. Axial Length versus Age for Group D



There was no significant relationship found between these two parameters (RE p = 0.17, LE p = 0.19). This result was not surprising because other studies have reported that the natural growth of the eye axial length tends to stabilise at approximately age 22 years as reported by Ooi and Grosvenor et al (1995). Goss et al (1997) described refractive error, axial length and height as a function of age in young myopic subjects.

Table 8.1.1 Axial Length for Group D.

	RAXL	LAXL	R+L AXL
Mean	22.65	22.74	22.68
SD	1.24	1.29	1.27

Table 8.1.2 Axial Length for Group D over 60.

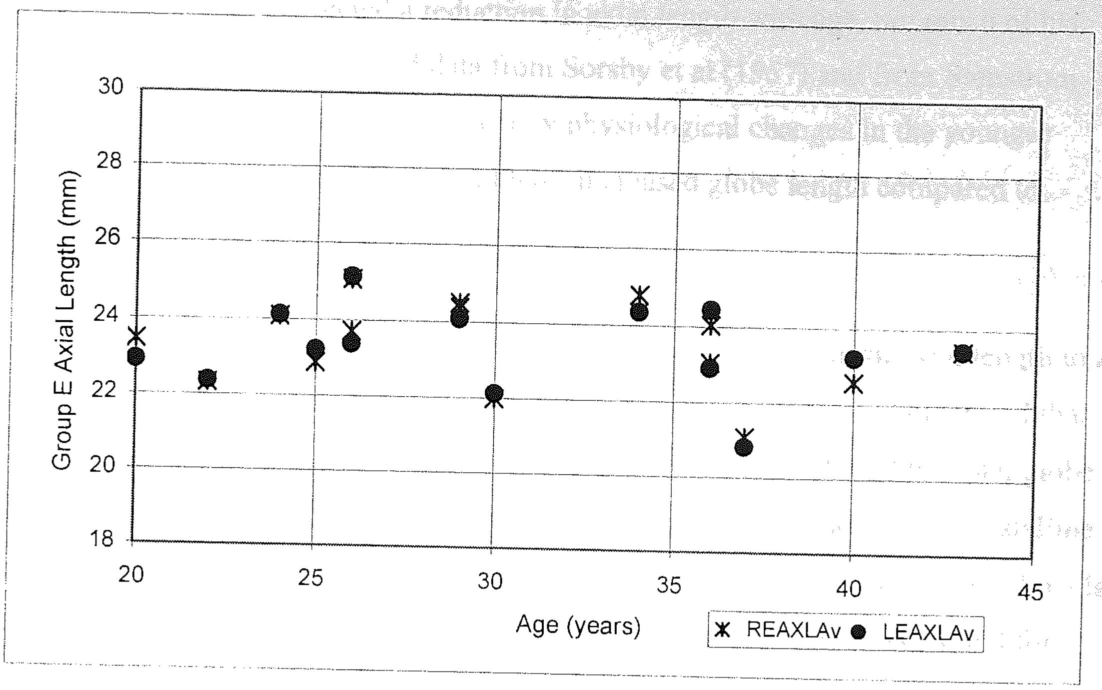
	RAXL	LAXL	R+L AXL
Mean	22.63	22.72	22.68
SD	1.26	1.30	1.28

As shown in Table 8.1.1 the mean axial length value of the 200 subjects was 22.68 mm (SD 1.27), and Table 8.1.2 for the 194 subjects over 60 years of age shows very similar results. The range of values was 20.10 – 28.70 mm.

These results agree with those of Goss et al (1997). The results are also similar to those of Koch Jensen et al (1995) who found a mean axial length of 22.86 mm (SD 0.11) and range of 20.56 – 26.41 mm. They described the precision of axial length measurements obtained with commercial A-scan equipment to be about 0.10 mm.

The subjects were a random selection of patients of mean age 75.5 years (SD 8.7) with a variety of refractive errors. They were attending the clinic for ocular measurements while they were on the waiting list for cataract extraction surgery. There was no significant relationship found between axial length and spectacle prescription in this varied group. Partly this was because many of the subjects had undergone a myopic shift in their spectacle prescription resulting from the increasing nuclear sclerosis of their crystalline lens, leading to cataract. Therefore their prescription gradually became more dependent on crystalline lens characteristics rather than on axial length of the eye. As their lens opacities became severe enough to warrant their referral for cataract extraction, their vision became poor and their refraction result became less reliable. A few subjects with long axial length values did demonstrate the higher degrees of myopia expected. Figure 8.1.2 shows a plot of axial length against age for the Group E (hospital staff).

Figure 8.1.2. Axial Length versus Age for Group E



For Group E no significant age relationship was found, (RE p = 0.50, LE p = 0.72). Although this was a relatively small group of randomly selected staff members the subjects were co-operative, reliable, had good vision and fixation ability, and were less susceptible to measurement errors than were the older subjects.

Table 8.1.3 Axial Length for Group E

	RAXL	LAXL	R+L AXL
Mean	23.42	23.38	23.40
SD	1.11	1.08	1.10

The mean axial length for both eyes was 23.40 mm (SD 1.10) and the range was 20.80 – 25.30 mm. Most of their refractive errors were small and showed no relation to their axial length. The 0.72 mm difference in mean axial length between the two age groups was similar to the 0.60 mm difference found by Grosvenor (1987) when he re-analysed the studies of Sorsby and co-workers (1946, 1957).

Analysis showed no significant ($p < 0.05$) difference in mean axial length between Groups D and E, but Group D had a wider range and variability. This was understandable because Group D had a wider range of ages and ocular conditions.

In 1987, Grosvenor suspected a reduction in axial length with age, but much of his report was the re-analysis of old data from Sorsby et al (1957) and from Stenstrom (1948). It is possible that he was misled by physiological changes in the younger population who tended to be taller and have increased globe length compared to subjects born in the early 1900s.

Ooi and Grosvenor (1995) also found no significant relation of mean axial length to age, in subjects over the age of 20 years. Leighton and Tomlinson (1972) suggested that axial length may decrease slowly with age. Koretz et al (1989) found the total globe axial length to be independent of age, and that the posterior surface of the crystalline lens remains fixed in position relative to the cornea and retina. In their study of ocular parameters in the elderly in Norway, Midelfart, Short and Aamo (1994) found for women a slightly shorter mean axial length of 22.98 ± 1.31 mm and a mean steeper corneal curvature of 7.74 mm (43.64 ± 1.61D) than the similarly aged men, who had a mean axial length of 23.33 ± 0.97 mm, and a mean corneal curvature of 7.87 mm (42.88 ± 1.52D). These differences between the means were not significant.

Groups D and E were analysed with regard to discovering a possible relationship between axial length measurements and keratometry values. Figures 8.1.3 to 8.1.5 show axial length plotted against horizontal keratometry for Groups D and E respectively.

Figure 8.1.3 RE Axial Length versus Horizontal Keratometry for Group D

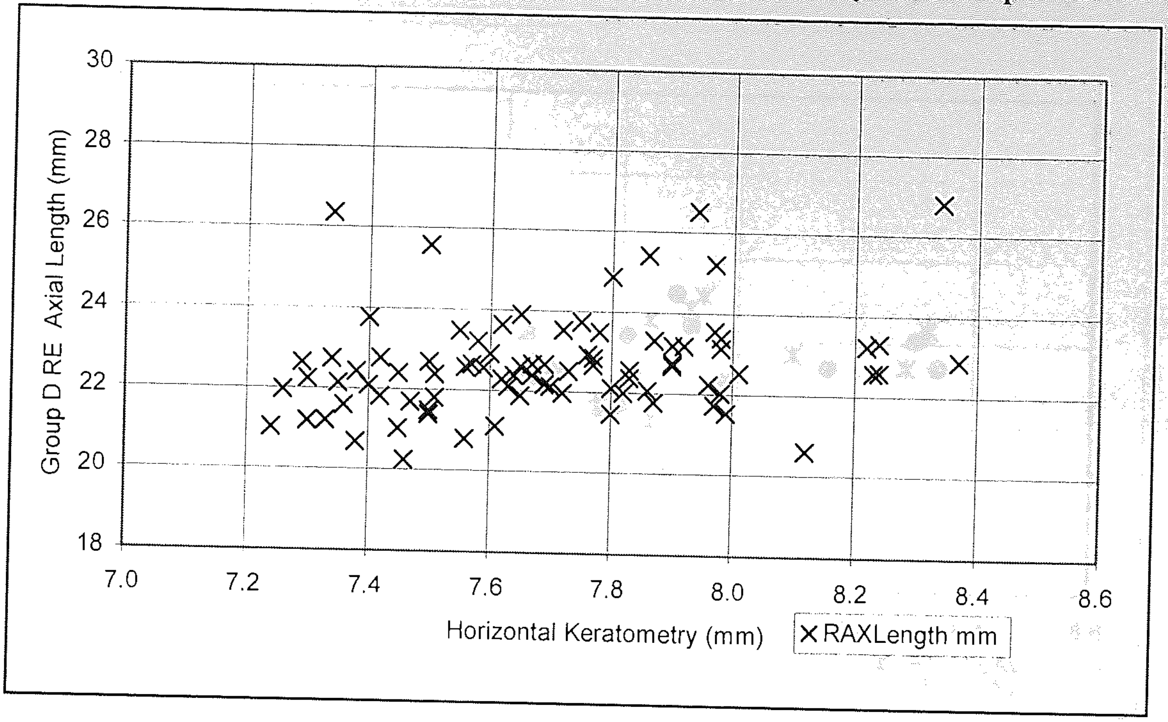


Figure 8.1.4 LE Axial Length versus Horizontal Keratometry for Group D

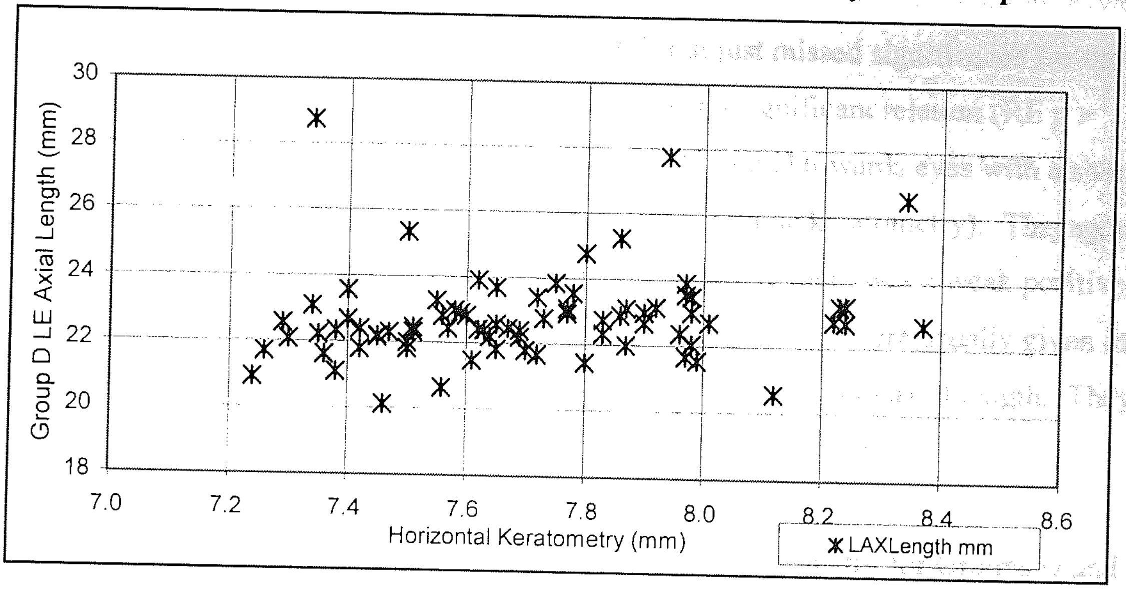
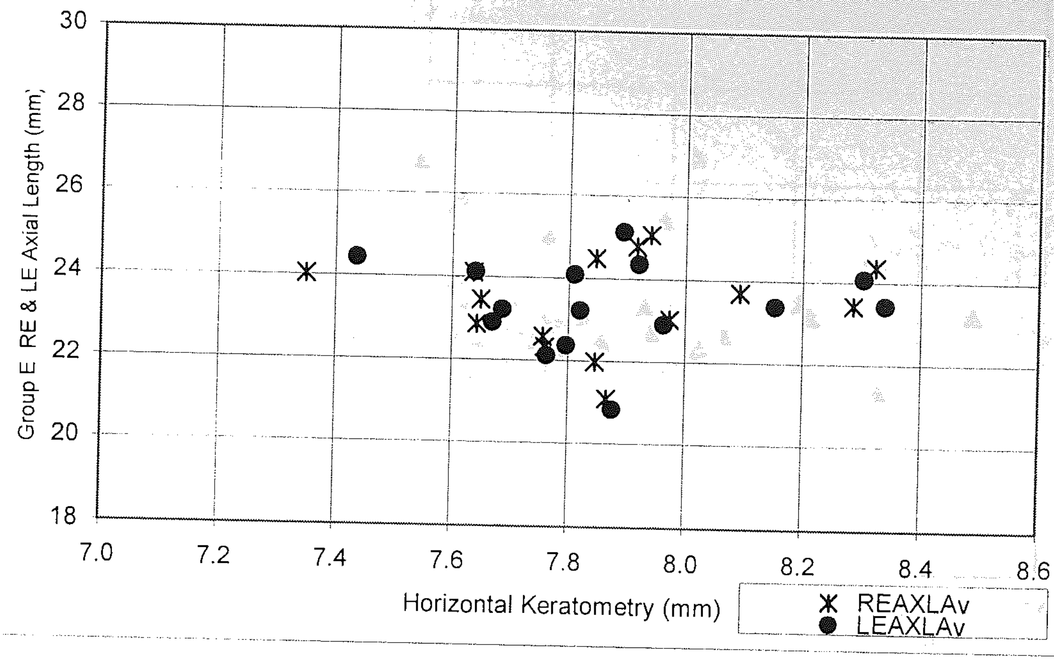


Figure 8.1.5 RE, LE Axial Length versus Horizontal Keratometry for Group E



For Group D, ANOVA analysis of the data points showed a significant relation between the two parameters for right eye, (RE $p = 0.0067$) but just missed significance for the left eye (LE $p = 0.061$). For the Group E, there was no significant relation (RE $p > 0.05$, LE $p > 0.05$). There appeared to be a trend in Group D towards eyes with a shorter axial length having steeper corneal curvatures (smaller value keratometry). This agrees with Goss et al (1997) who described several studies where there was a weak positive correlation between keratometry and axial length. Their studies were usually given in the form of a negative value correlation of corneal power related to axial length. They suggested that this contributed to the 'emmetropization' of the eye.

Figures 8.1.6 to 8.1.8 show axial length versus vertical keratometry for Groups D and E.

Figure 8.1.6 RE Axial Length versus Vertical Keratometry for Group D.

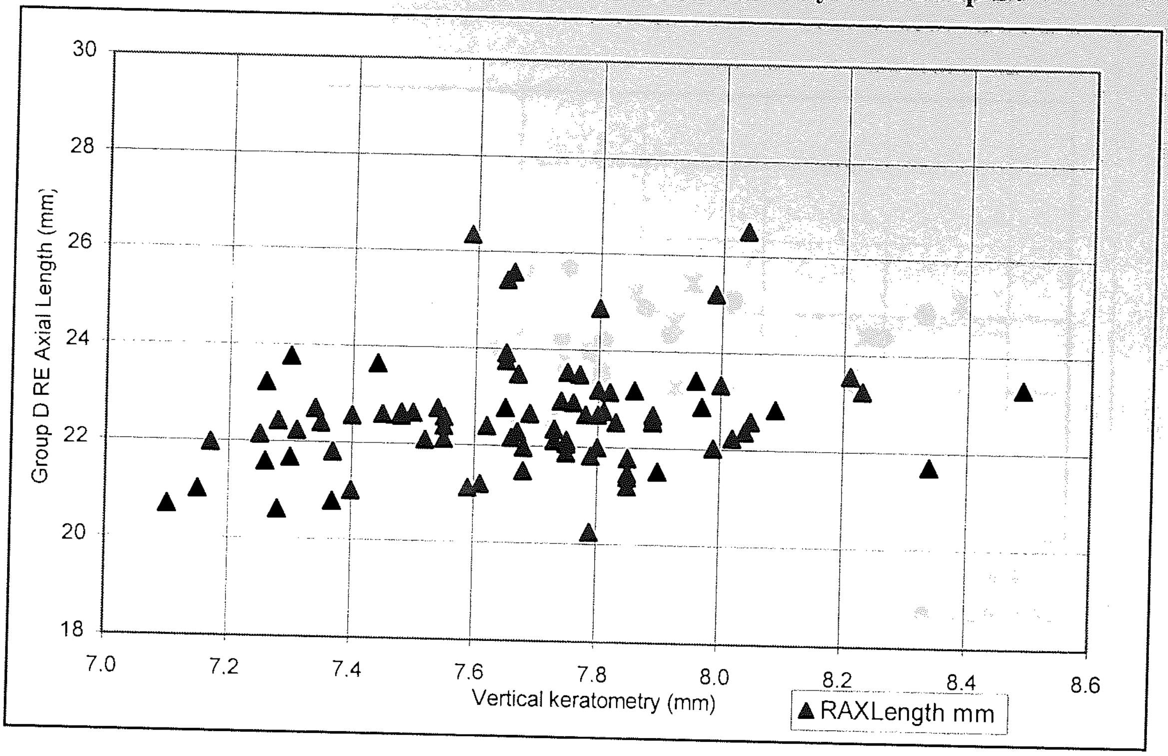


Figure 8.1.7 LE Axial Length versus Vertical Keratometry for Group D.

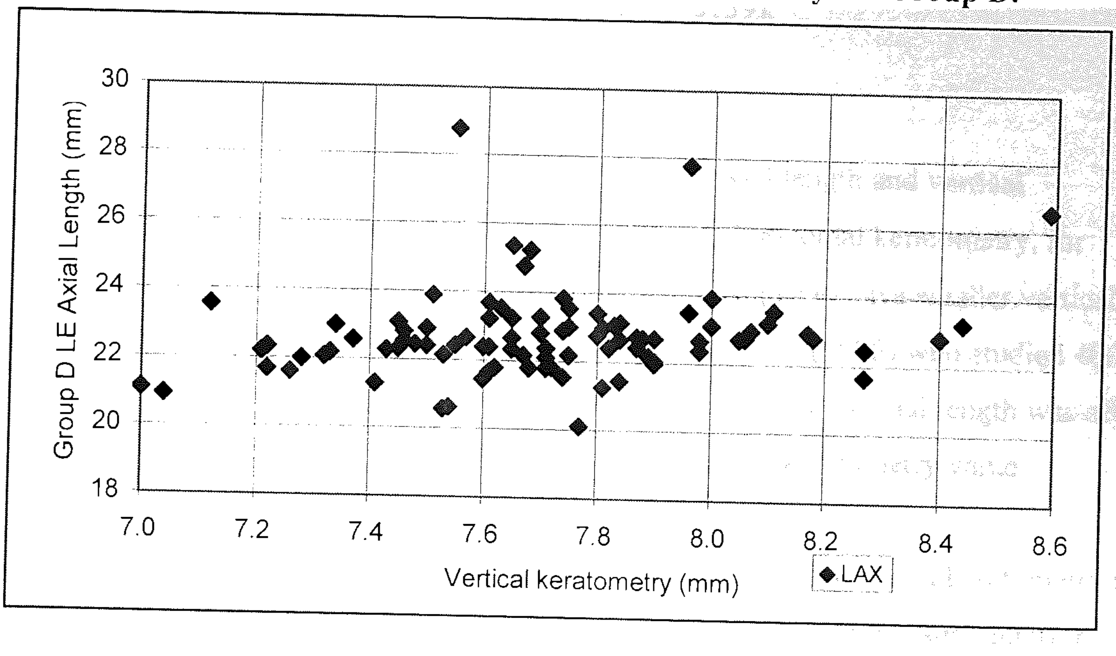
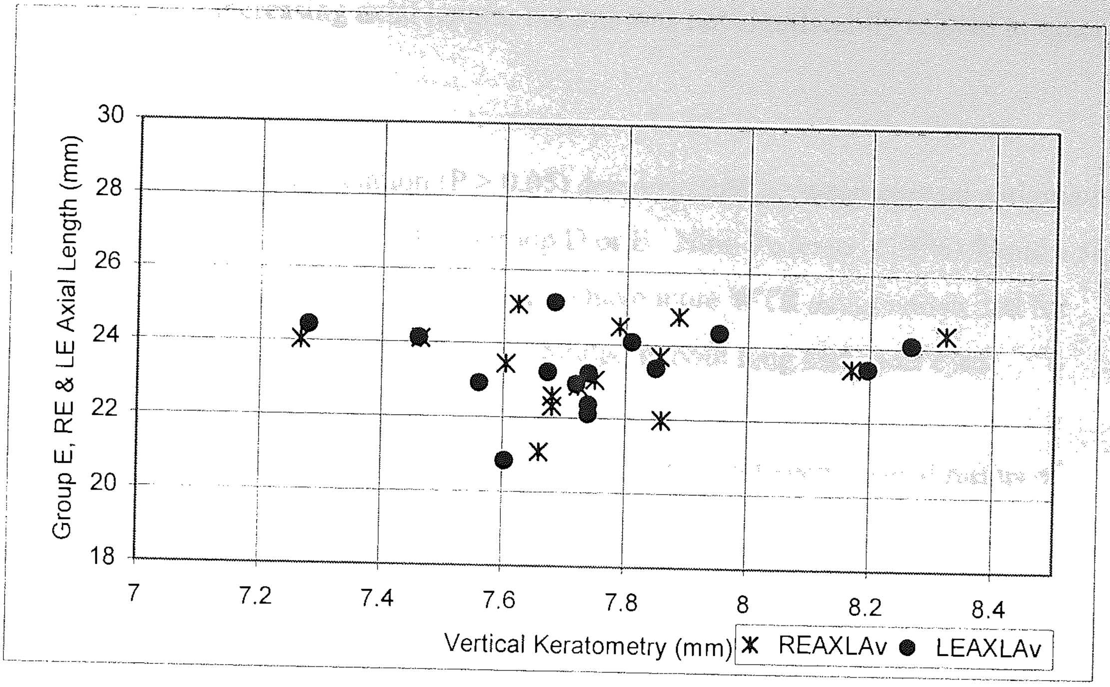


Figure 8.1.8 RE, LE Axial Length versus Vertical Keratometry for Group E.



For Group D, there was a significant relationship demonstrated between axial length and vertical keratometry, (RE p = 0.0006, LE p = 0.0139).

Regression: RE = 10.836 + 1.535* age

LE 14.067 + 1.123* age

No significant relation ($p > 0.05$) was found between axial length and vertical keratometry values in Group E. In a similar manner to horizontal keratometry, for Group D there was a trend for eyes with shorter axial lengths to have smaller vertical keratometry readings. This agrees with the results of Sayeah (1996) who studied 400 cases of cataract subjects and found that in myopia an increase in axial length was often compensated by a decrease in corneal power, i.e. increased keratometry value.

Carney et al (1997) used the Topographic Modeling System (TMS) and keratometry to assess corneal curvatures, and used a hand-held biometric ruler to measure anterior chamber depth, lens thickness and axial length. They found a low but statistically significant positive correlation ($p < 0.05$) between corneal asphericity and the refractive error expressed in mean sphere power; also they found a weak correlation ($p < 0.05$) between corneal asphericity and axial length. Corneal asphericity versus keratometry showed no significant relation. They concluded that the cornea tended to flatten less rapidly (i.e. lower eccentricity value) in the periphery with increase of myopia, also associated with increasing axial length, but this was not significantly related to other ocular factors.

There was no significant relation ($P > 0.05$) demonstrated between corneal astigmatism and the axial length values of either Group D or E. Ninn-Pedersen (1996) found a slight tendency for eyes with a long axial length to have more WTR astigmatism and for greater variability in astigmatism to be exhibited in both long and short eyes.

Ooi and Grosvenor (1995) found no significant relation between corneal radius of curvature (average of horizontal and vertical) and age.

8.2 Lens Thickness

The crystalline lens thickness values for groups D and E were next considered. Graphs of lens thickness versus age are given in Figures 8.2.1 and 8.2.2.

Figure 8.2.1 RE, LE Lens Thickness versus Age for Group D over 60

Figure 8.2.2 RE, LE Lens Thickness versus Age for Group E

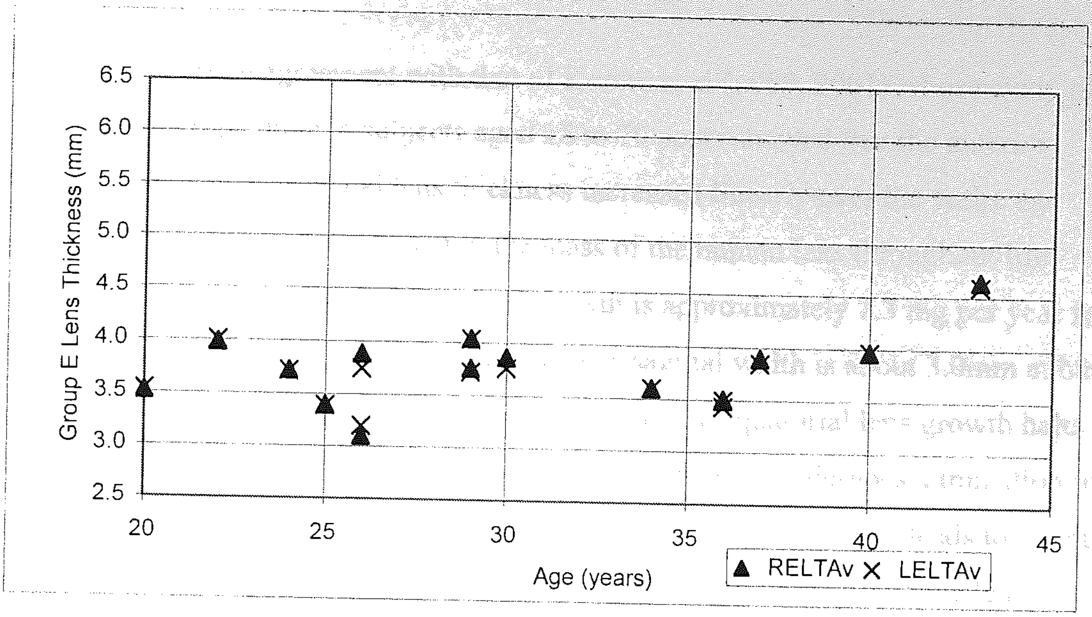


Table 8.2.1 Lens Thickness versus Age for Group D

	RELT	LELT	RE+LE LT
Mean	4.45	4.35	4.40
SD	0.80	0.80	0.80

Table 8.2.2 Lens Thickness versus Age for Group E

	RELT	LELT	RE+LE LT
Mean	3.76	3.74	3.75
SD	0.36	0.34	0.35

Figures 8.2.1 and 8.2.2 show a plot of lens thickness against age for Groups D and E respectively. The mean lens thickness value for Group D was 4.40 mm (SD 0.80) as shown in Table 8.2.1. The thickness range was 2.90 – 6.10 mm. A significant difference was revealed between the mean values of groups D and E ($p < 0.01$). For group E no significant age effect was demonstrated (RE $p = 0.10$, LE $p = 0.11$), but there was a very significant positive relationship ($p < 0.0001$) between lens thickness and age for the 'over 60 years' subgroup of Group D.

Regression: RE = 0.6798 + 0.502* age.

LE = 1.1406 + 0.043* age.

The graph in Figure 8.2.1 indicates this trend of lens thickness increasing with age.

These results are in agreement with that of Koretz et al (1989, 2001) whose study of 100 normal emmetropic human subjects aged 18 to 70 years showed that the anterior chamber depth decreased and lens thickness increased linearly over the entire age group. Component cells are added to the mass of the human lens throughout life. According to Patel and Bron (2001) lens growth is approximately 1.3 mg per year from age 10 to 90 years. They explained that the lens sagittal width is about 3.0mm at birth gradually increasing to 5-6 mm. After age 20 years the equatorial lens growth halts but there is continued growth in the sagittal direction. The lens undergoes a transition from lens development to maintenance of the lens function. This transition leads to a reduced anterior radius of curvature and therefore an increased optical power of the lens, although there are some compensatory refractive changes occurring, as previously suggested by Leighton and Tomlinson (1972).

There appears to be a rapid decrease in lens water content across the lens from the superficial cortex to the nucleus, causing a refractive index gradient according to Pierscionek and Weale (1995). They explained that the gradient decreases with age which gives rise to a reduction in the refractive power of the lens. The complexity of lens fibre organisation increases with age. Four zones of discontinuity have been proposed, some of which become thicker and have increased light scattering with age. All the four zones are in the cortical region and as the zones increase with age therefore estimated ages have been assigned to each zone. Koretz et al (1994) demonstrated a bi-modal increase in scattering by the innermost zones of the lens with age.

Zadnik et al (1992) explained that the natural lens sclerosis gradually increases with the ageing process, leading to increased lens thickness and refractive index changes. Green (1995) explained that as the tissue ages, free radicals cause irreversible chemical changes in the crystalline lens matrix, reducing elasticity and transparency. These features are linked to the age-related increasing lens sclerosis.

On examining the results for Group E, there was no apparent relationship to age. All of the subjects of Group E were pre-presbyopic and they had reasonable flexibility of their crystalline lens to alter lens curvature for accommodation to focus on near objects. As shown in Table 8.2.2 the mean lens thickness value for Group E was = 3.75 mm (SD 0.35). One subject in Group E was over age 40 years and had a lens thickness of 4.60 mm, much greater than the mean lens thickness for this younger group. This result indicates that this subject could be experiencing the first stages of developing lens sclerosis.

On comparing the results for Groups D and E, there was a significant difference between the two means (t test, P = 0.03). It is suggested that this supports the hypothesis that the lens becomes thicker with age. ANOVA was used to compare the variability of the two mean values. This proved significant (p = 0.05) and Group D had more variability of values, probably because they were an older, less healthy group. Group D understandably showed greater variability because there was a larger subject number across a wide range of ages and of stages in cataract development.

The possibility of lens thickness being related to other ocular parameters was considered.

Ooi and Grosvenor (1995) found significant age related changes. They found thicker lenses and shallower anterior chamber depths with age but no significant changes in average keratometry or axial length. They suggested that a decrease in the gradient – index of the lens occurred with increasing age, acting as an emmetropizing mechanism to compensate for the curve steepening of the anterior and posterior lens surfaces. In their review of lens senescence, Weale (1988) and Pierscionek and Weale (1995) describe the increasing thickness of the crystalline lens and its loss of light transmittance in the later decades of life. These aspects were attributed to sclerosing tissue changes of the lens. Hemenger et al (1995) and Savage et al (1993) suggest that changes in the refractive index of the various layers in the lens lead to the age changes of increased ocular lens thickness and increased lens surface curvature.

Figure 8.2.3 RE Lens Thickness versus Axial Length for Group D

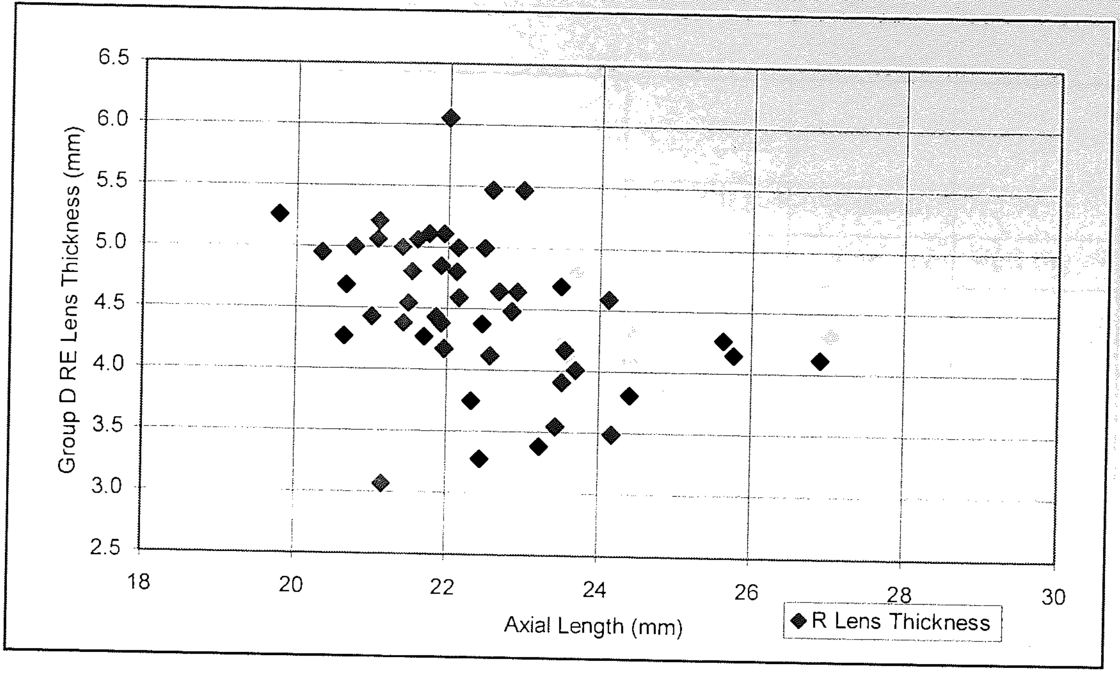


Figure 8.2.4 LE Lens Thickness versus Axial Length for Group D

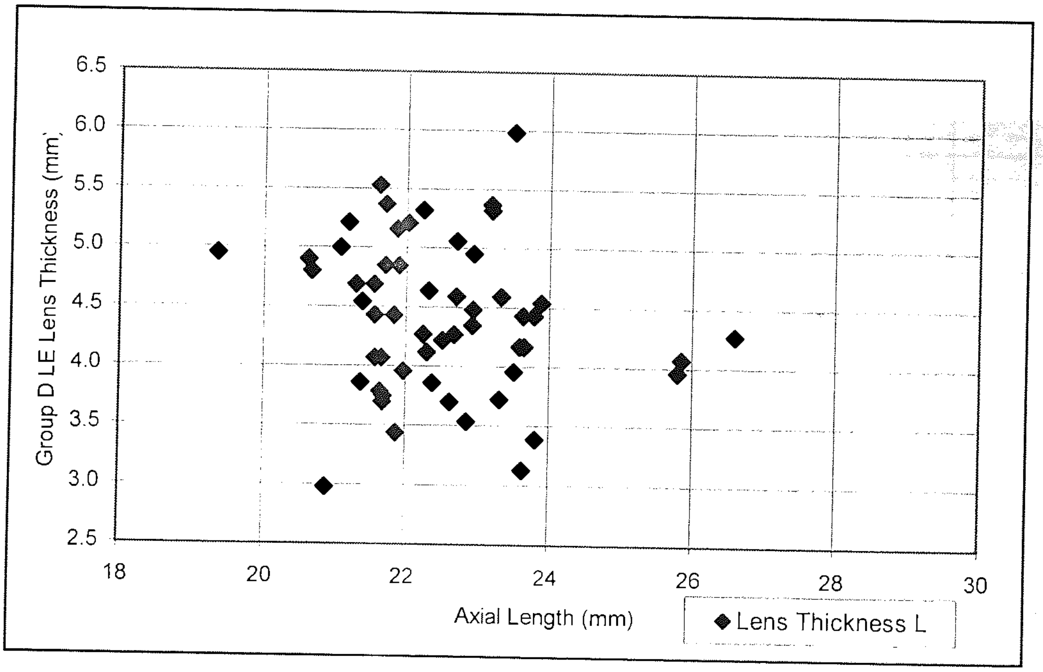


Figure 8.2.5 RE Lens Thickness versus Axial Length for Group E

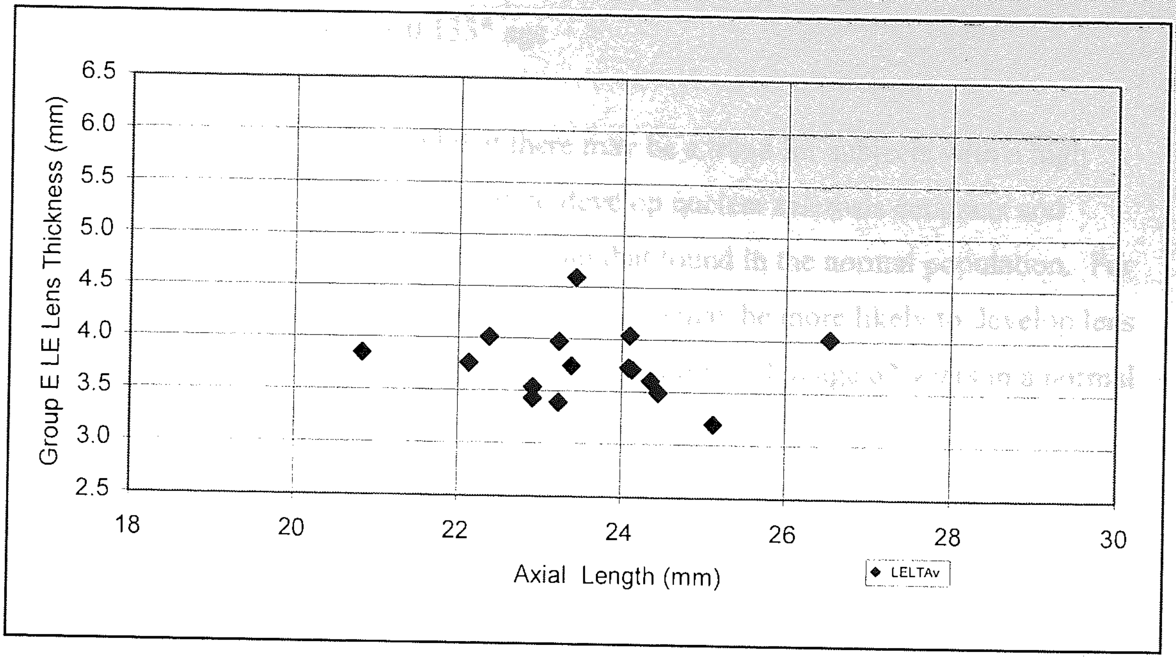
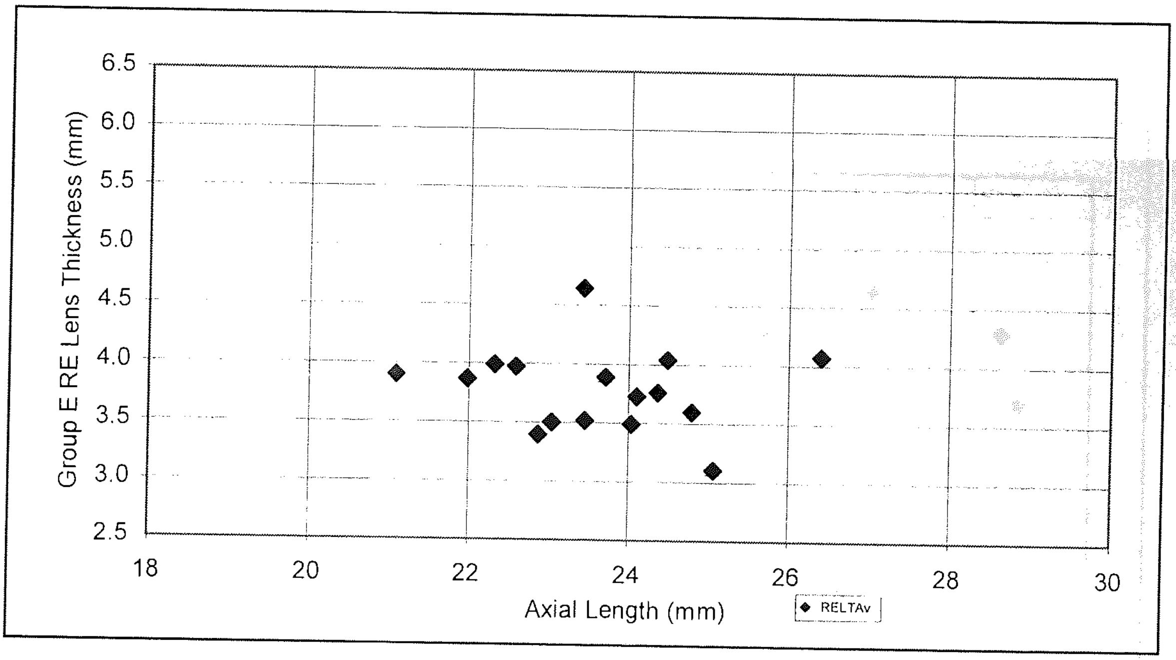


Figure 8.2.6 LE Lens Thickness versus Axial Length for Group E



Figures 8.2.3 to 8.2.6 shows the plot of right eye and left eye lens thickness versus axial length for Groups D and E respectively.

There was no significant relationship found between these two parameters in Group E. For the older Group D, ANOVA indicated a very significant relationship:

(RE $p = 0.0002$, LE $p < 0.0001$).

Regression: RE = 1.717 + 0.124* age

LE = 1.403 + 0.133* age

Batterbury et al (1991) suggested that there may be a trend for subjects with a high degree of myopia and long axial length to develop nuclear sclerosis cataracts and therefore, a thicker lens at an earlier age than that found in the normal population. For example, myopic subjects of high power refraction may be more likely to develop lens sclerosis cataracts at age approximately 58 years compared to age 63 years in a normal population.

However, due to the wide variability in the subjects and their lens condition this conclusion could not be proved in this study.

Lens thickness was re-considered with respect to horizontal keratometry as shown in Figures 8.2.7 to 8.2.10 for Groups D and E respectively.

Figure 8.2.7 RE Lens Thickness versus Horizontal Keratometry for Group D.

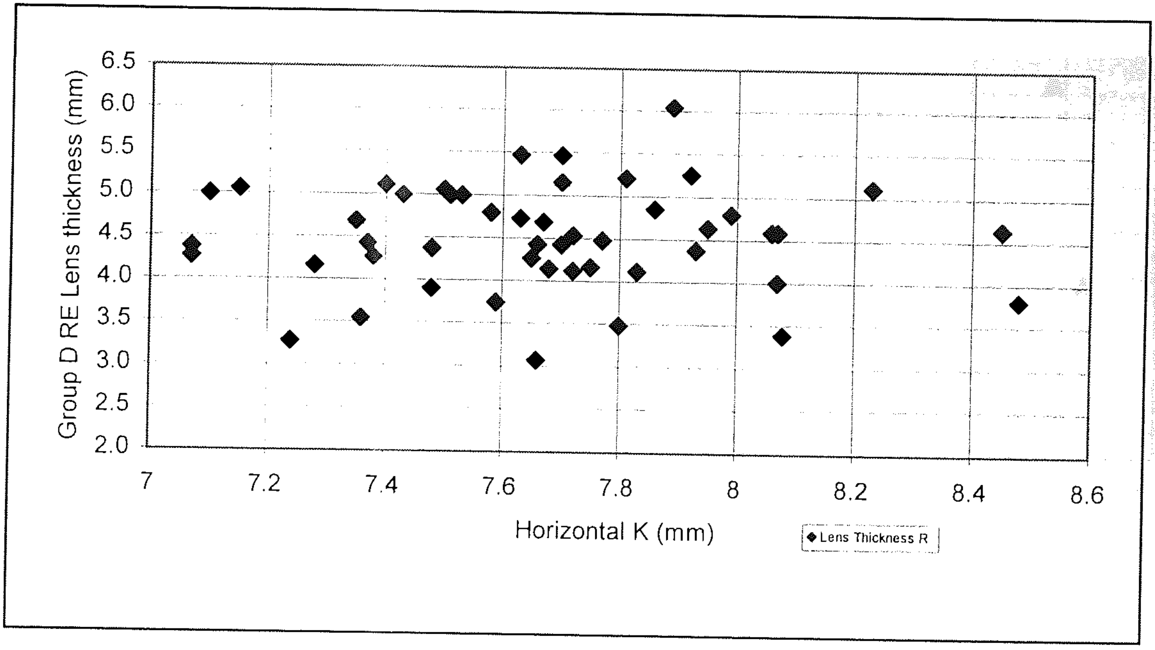


Figure 8.2.8 LE Lens Thickness versus Horizontal Keratometry for Group D.

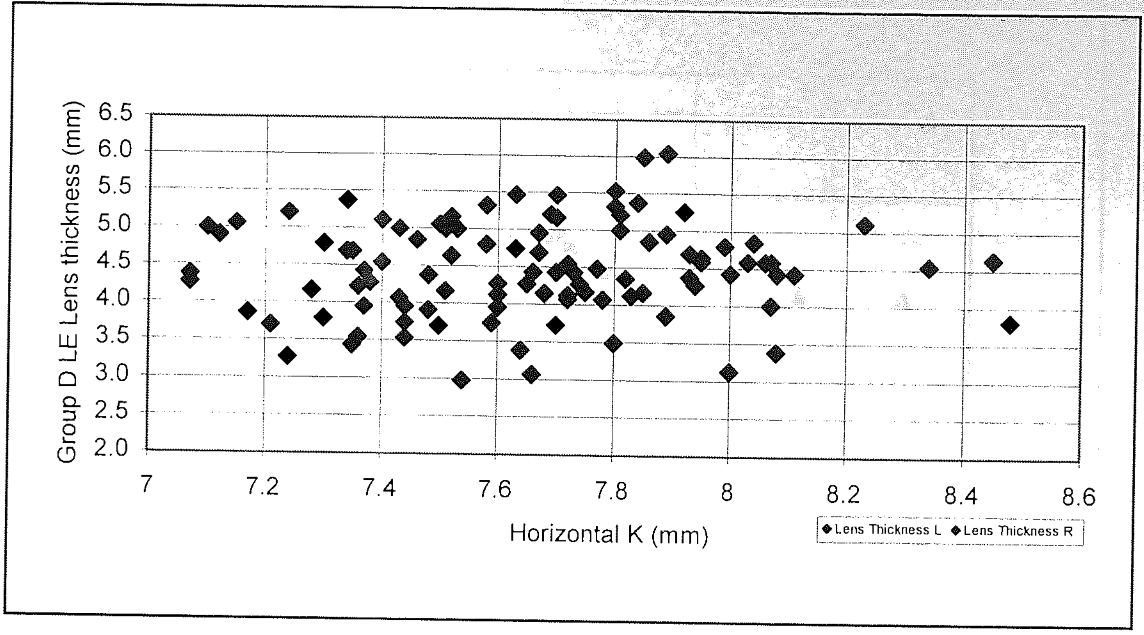


Figure 8.2.9 RE Lens Thickness versus Horizontal Keratometry for Group E.

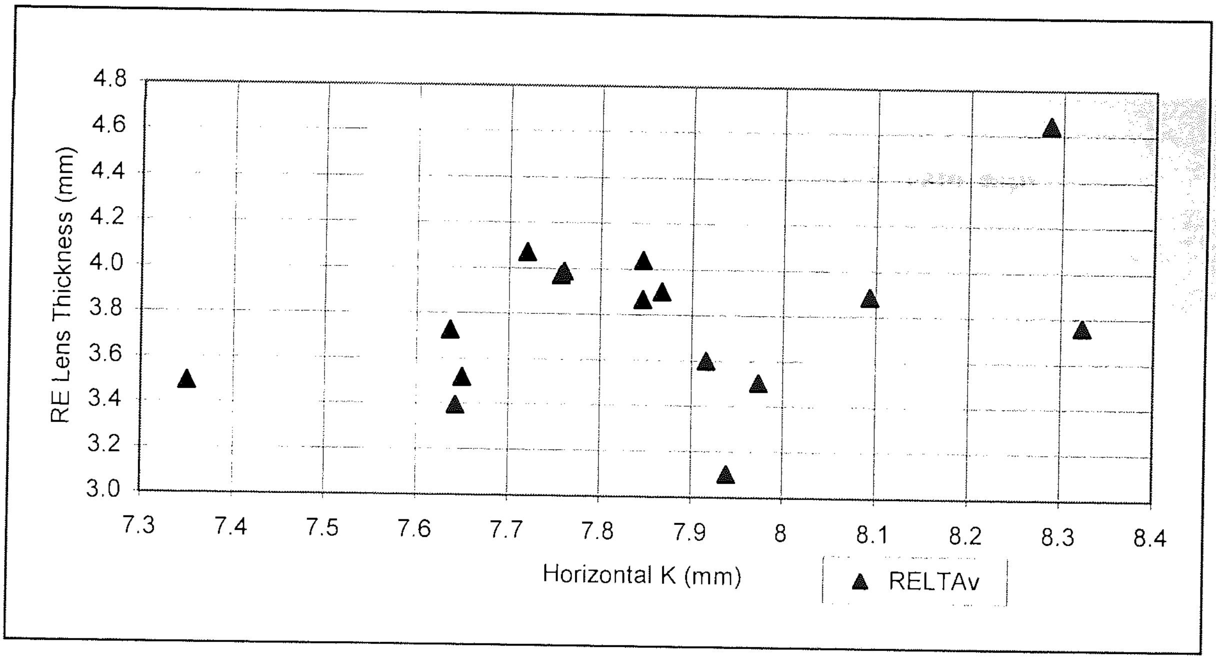
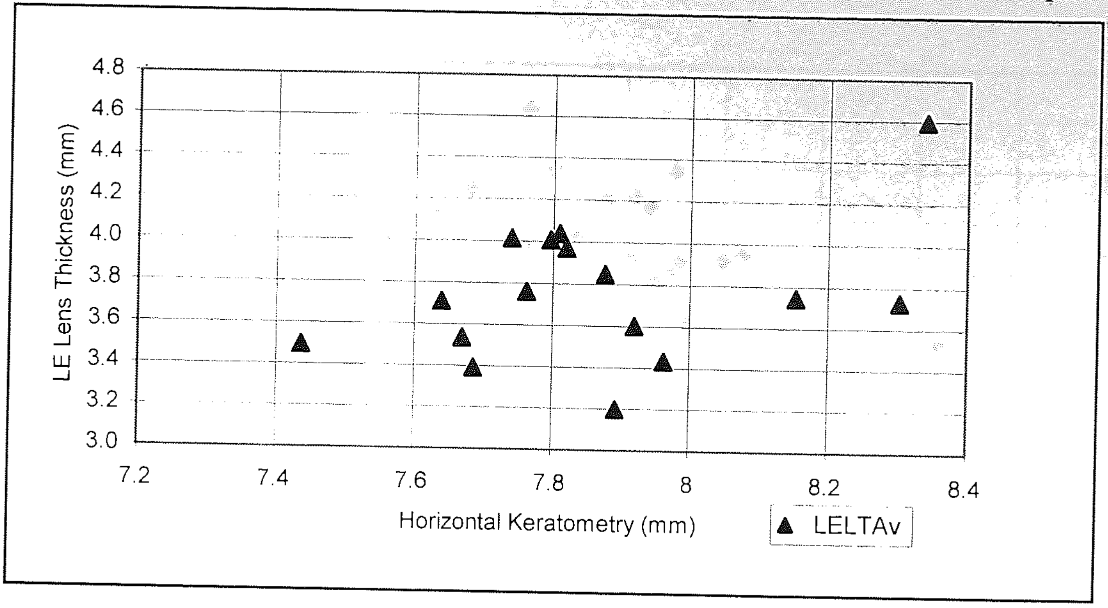


Figure 8.2.10 LE Lens Thickness versus Horizontal Keratometry for Group E.



For both groups D and E there was no significant relationship found between lens thickness and keratometry, and between lens thickness and keratoscopy.

ANOVA gave the following results:

Table 8.2.3 ANOVA Lens Thickness versus Keratometry and Keratoscopy

	Group D RE p	Group D LE p	Group E RE p	Group E LE p
Horizontal keratometry	0.89	0.29	0.11	0.09
Vertical keratometry	0.91	0.37	0.07	0.09

It is suggested that although lens thickness increases with age, the changes in corneal curvature with age are only of a small amount and therefore do not show a correlation.

Similarly, lens thickness was re-considered with respect to vertical keratometry as shown in Figures 8.2.11 to 8.2.14 for Groups D and E respectively.

Figure 8.2.11 RE Lens Thickness versus Vertical Keratometry for Group D.

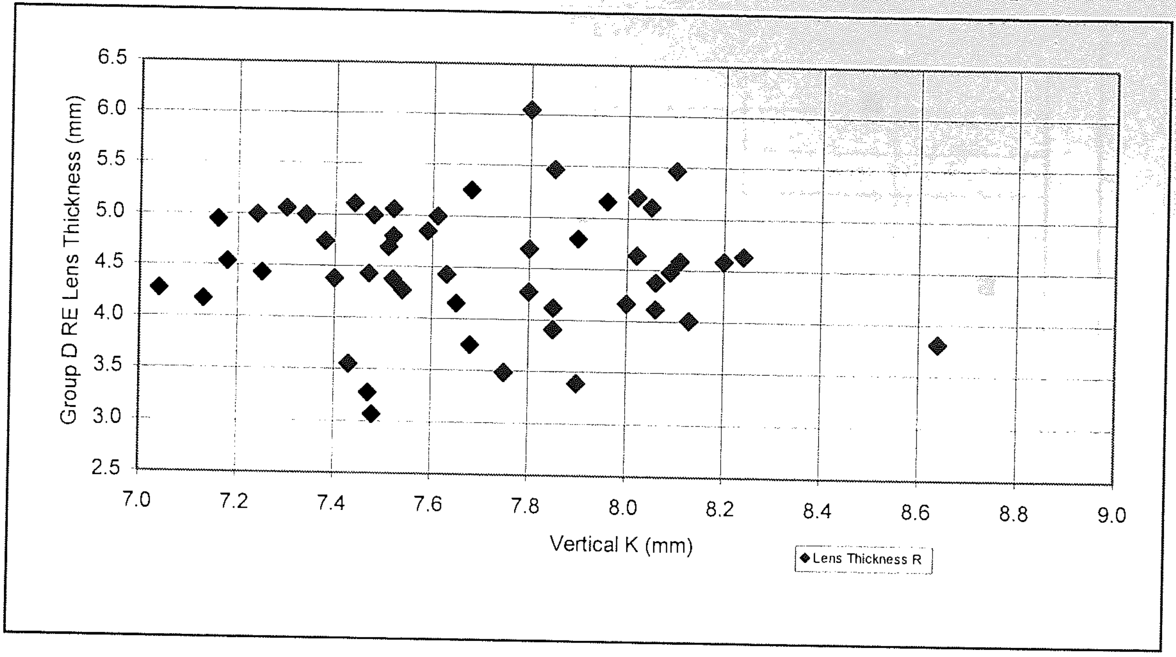


Figure 8.2.12 LE Lens Thickness versus Vertical Keratometry for Group D.

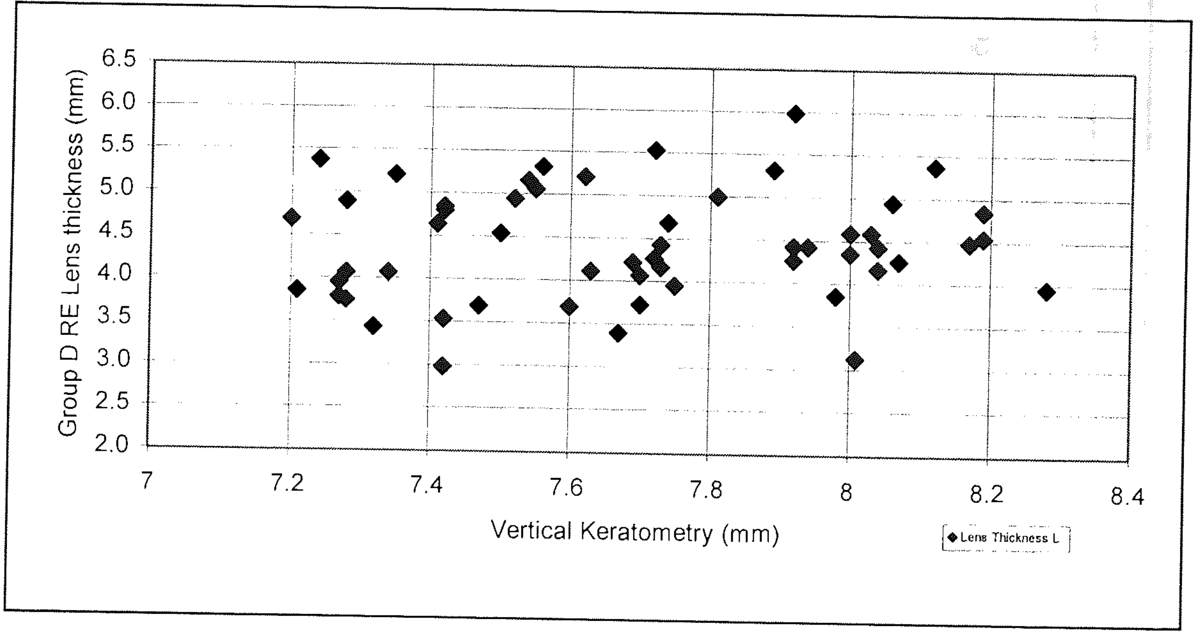


Figure 8.2.13 RE Lens Thickness versus Vertical Keratometry for Group E.

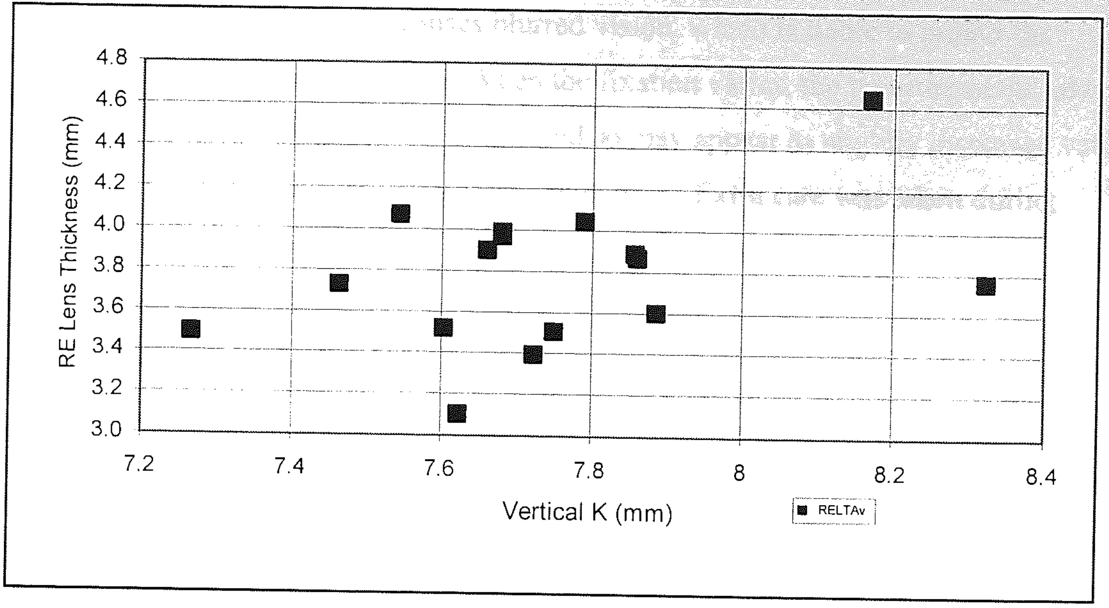
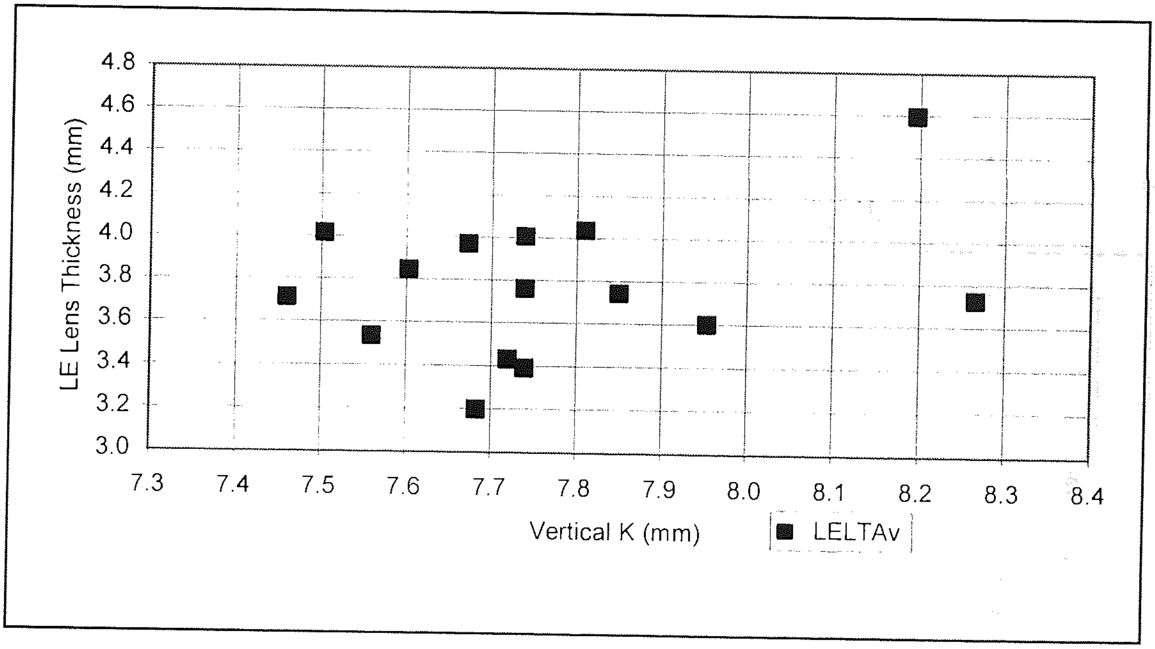


Figure 8.2.14 LE Lens Thickness versus Vertical Keratometry for Group E.



There was no significant relationship demonstrated between the lens thickness and vertical keratometry or between lens thickness and corneal astigmatism in either Group D or E. This agrees with results from other studies (Dubbelman et al 2001).

This finding indicates that the increasing lens thickness of the developing lens sclerosis cataract progresses independently from the slight ageing changes of the corneal curvature.

However, such hypotheses are difficult to demonstrate in this elderly population because the insipient cataract causes blurred vision, which is liable to impair the accuracy of the subject's fixation. When the fixation varies, the measurements taken may not be exactly along the visual axis and so may appear as slightly increased values for corneal curvature and for natural lens thickness. Extra care was taken during measurements to keep this source of error to a minimum.

8.3 Anterior Chamber Depth

For the cases where the biometry printout gave a value for the anterior chamber depth the values were studied to see if they revealed indications of change when compared to other ocular parameters. The graphs of anterior chamber depth for Group D and E show a trend towards decreased values with age, even from age 30 years. These results concur with those of Cook et al (1994) and of Dubbelman et al (2001). Koretz et al (1989) explained that the reduction in the anterior chamber depth is directly due to the increasing lens thickness with age.

Figure 8.3.1 Anterior Chamber Depth versus Age for Group D.

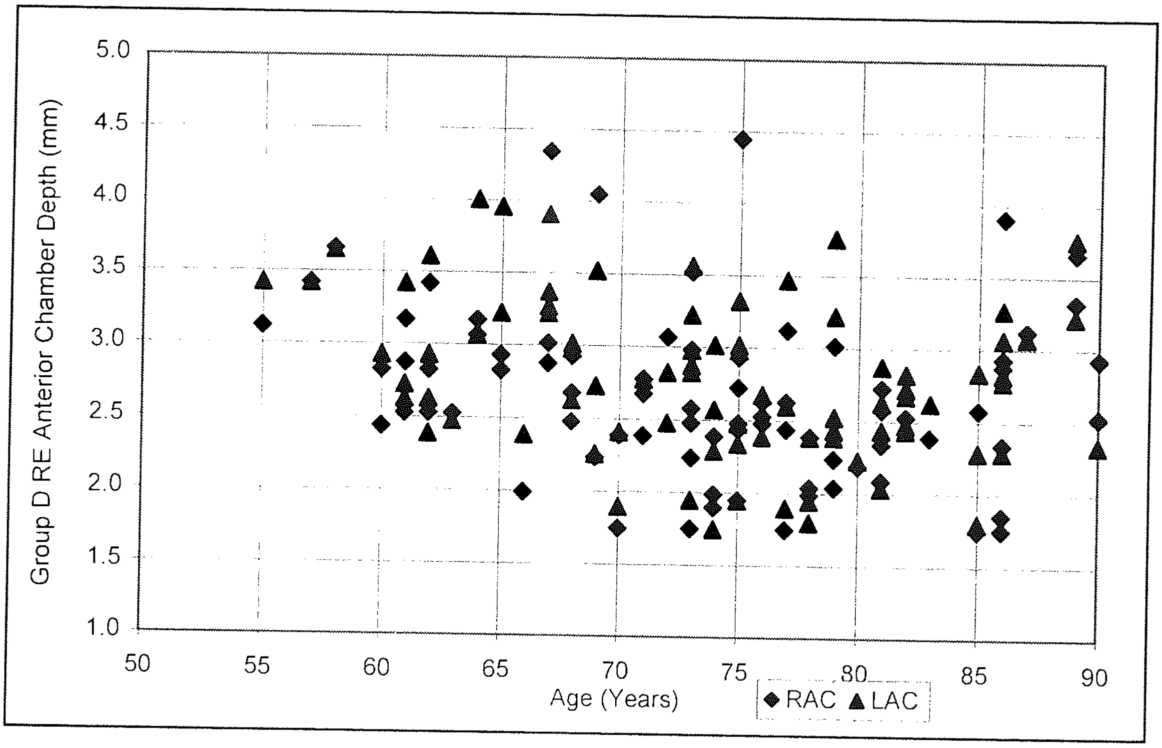
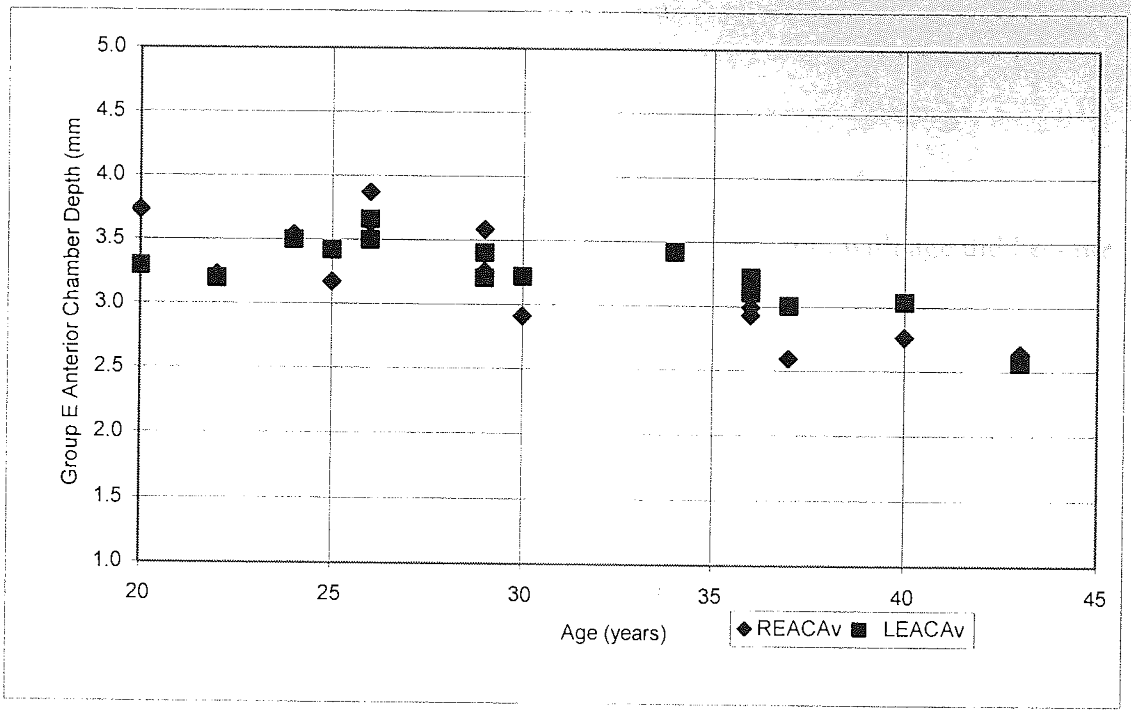


Figure 8.3.2 Anterior Chamber Depth versus Age for Group E.



Figures 8.3.1 and 8.3.2 show the graph of anterior chamber depth versus age for several subjects of Groups D and E respectively. The mean values (mm) are given in Table 8.3.1:

Table 8.3.1 Anterior Chamber Depth for Groups D and E.

	Group D RE	Group D LE	Group E RE	Group E LE
Mean	2.63	2.76	3.21	3.25
SD	0.56	0.51	0.40	0.26

T tests indicated there was a significant difference ($p = 0.049$) between the mean values for Group D (2.69 SD 0.53) and for Group E (3.23 SD 0.33), although the wider variability of Group D values reduced the significance. As suspected, the younger subjects had greater values.

Although the Group D points on the graph are rather widely scattered and show a greater variability, the graph shows a trend of anterior chamber depth decreasing with age. Analysis indicated that for the left eye the relationship just missed significance (RE p = 0.27, LE p = 0.05).

Regression: RE = 3.25 - 0.008* age

LE = 3.77 – 0.014* age

For Group E, the tendency for decreased anterior chamber depth with age did become significant (RE p = 0.0007, LE p = 0.0028).

Regression: RE = 4.61 - 0.046* age

LE = 4.08 – 0.027* age.

This trend of the reduction of the anterior chamber depth with age was also pointed out by Fontana and Brubaker (1980), who proposed that this was because the iris muscle tone reduced with age, thereby allowing the iris to flop forward and reduce the anterior chamber angle. However they considered the distance between the iris and the rear of the cornea, whereas this study used biometry to measure the distance from the centre of the posterior corneal surface and the anterior surface of the crystalline lens.

For the young Group E subjects the graph indicates the gradual age-related reduction in anterior chamber depth appeared to begin at about age 25 years.
The ANOVA test revealed a significant difference (p=0.04) between the two groups and therefore indicated that the hypothesis where anterior chamber depth decreases with age was correct. The author suggests that this reduction in the depth occurs because the lens becomes thicker with age and the anterior lens surface moves forward. The relationship between the anterior chamber depth and lens thickness was investigated.

Koretz et al (1989) found that the respective slopes and intercepts of the age dependent linear decline in the anterior chamber depth were essentially the same for measurements made independently by optical pachometry, A-Scan ultrasonography and slitlamp Scheimpflug photography. This helped to confirm that the anterior chamber depth does decrease with age and that the change is not an artefact of the measurement method. They concluded that the decrease in anterior chamber depth resulted from natural lens thickening and they explained that the position of the anterior lens surface moves towards the cornea whilst the posterior lens surface position hardly changes.

Figure 8.3.3 RE Anterior Chamber Depth versus Lens Thickness for Group D

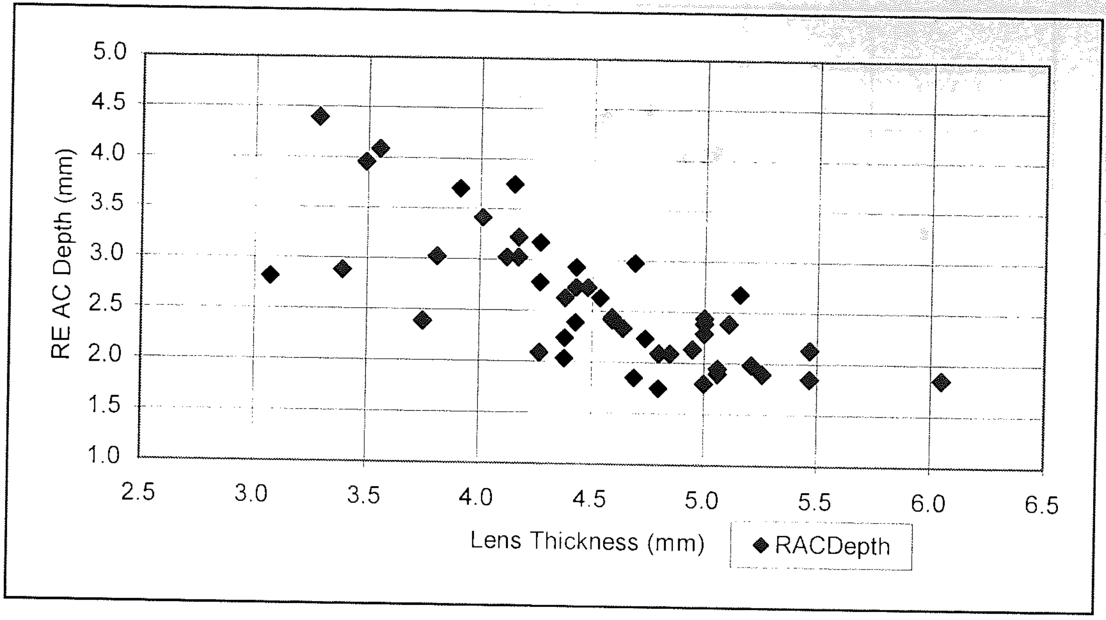


Figure 8.3.4 LE Anterior Chamber Depth versus Lens Thickness for Group D

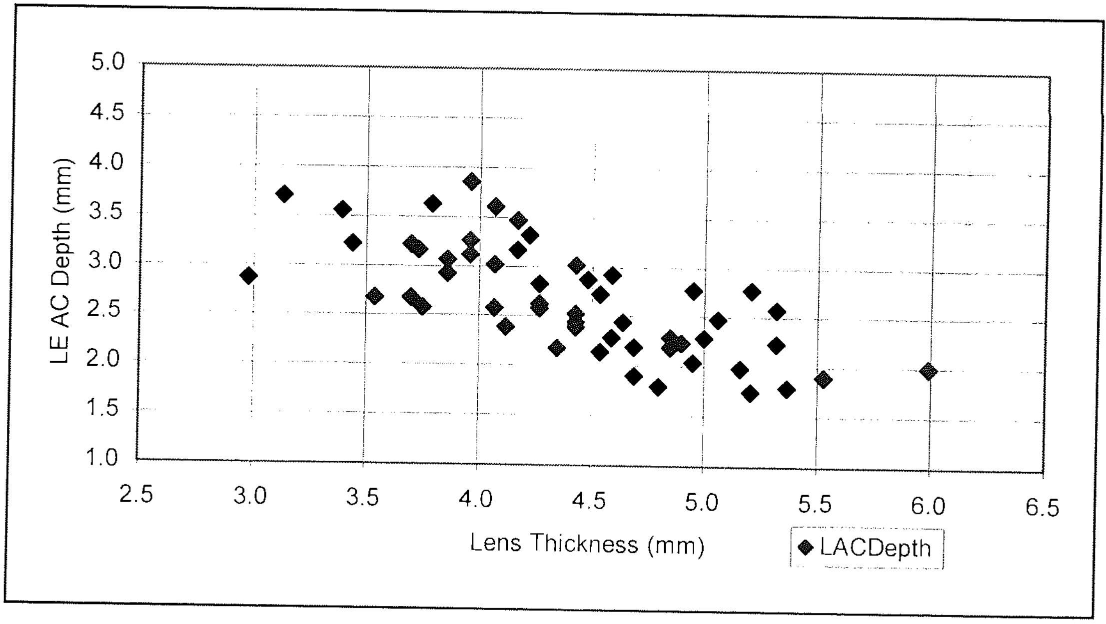


Figure 8.3.5 RE Anterior Chamber Depth versus Lens Thickness for Group E

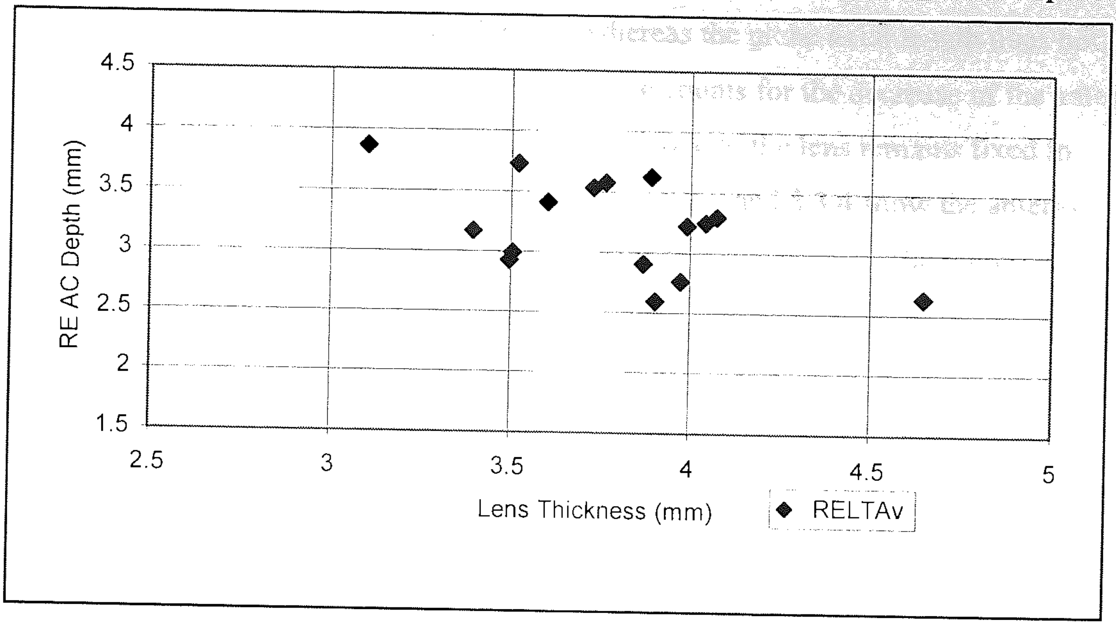
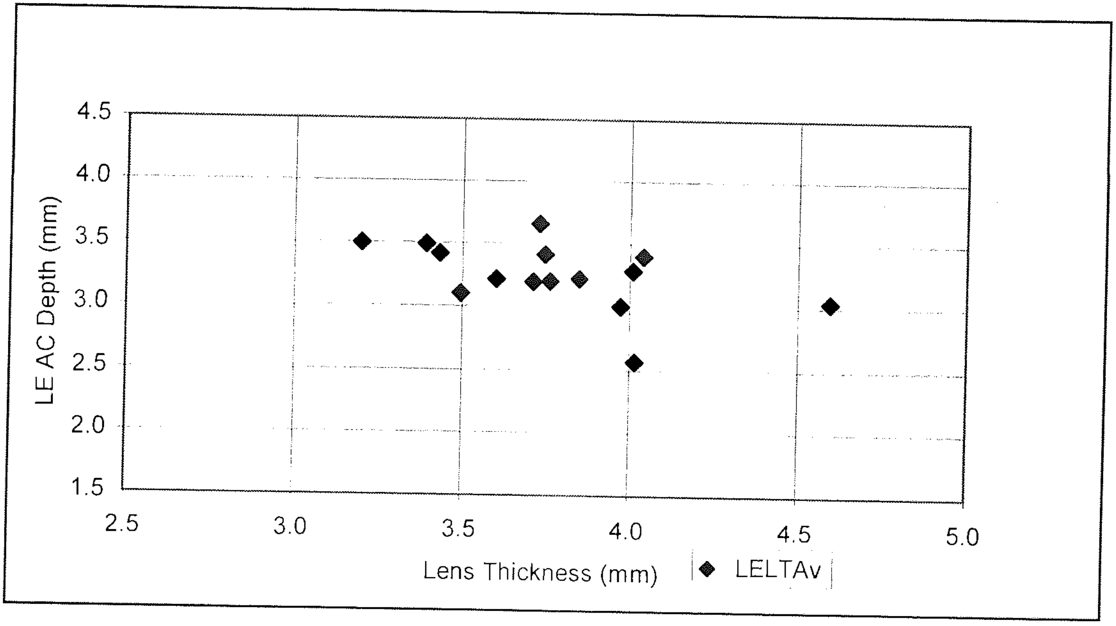


Figure 8.3.6 LE Anterior Chamber Depth versus Lens Thickness for Group E



Analysis of the results for Group D indicated a trend for a reduction in anterior chamber depth related to increased lens thickness, but the data variability inhibited the significance (RE $p = 0.11$, LE $p = 0.11$).

However, for Group E, a significant relationship was shown between these parameters (RE $p = 0.034$, LE $p = 0.0008$), where a smaller anterior chamber depth was accompanied by a greater lens thickness.

Koretz et al (1989) in their studies on the ageing of the anterior segment concluded that the human lens grows throughout adult life, whereas the globe axial length does not. They said the thickening of the lens completely accounts for the decrease of the anterior chamber depth with age, but that the posterior surface of the lens remains fixed in position relative to the cornea and retina. Figures 8.3.3 and 8.3.4 show the anterior chamber depth versus lens thickness plot supports this suggestion. The regression line also indicates this trend.

Next, the anterior chamber depth values were considered with relation to corneal curvature. The graphs of anterior chamber depths versus keratometry values are given in Figures 8.3.7 to 8.3.10.

Table 8.3.2. Anterior Chamber Depth Versus Keratometry - p values

	REHK	LEHK	REVK	LEVK
Group D	0.947	0.918	0.591	0.612
	0.845	0.331	0.964	0.425

There was no significant relation found between anterior chamber depth and keratometry for either group.

Figure 8.3.7. RE Anterior Chamber Depth versus Horizontal Keratometry for Group D

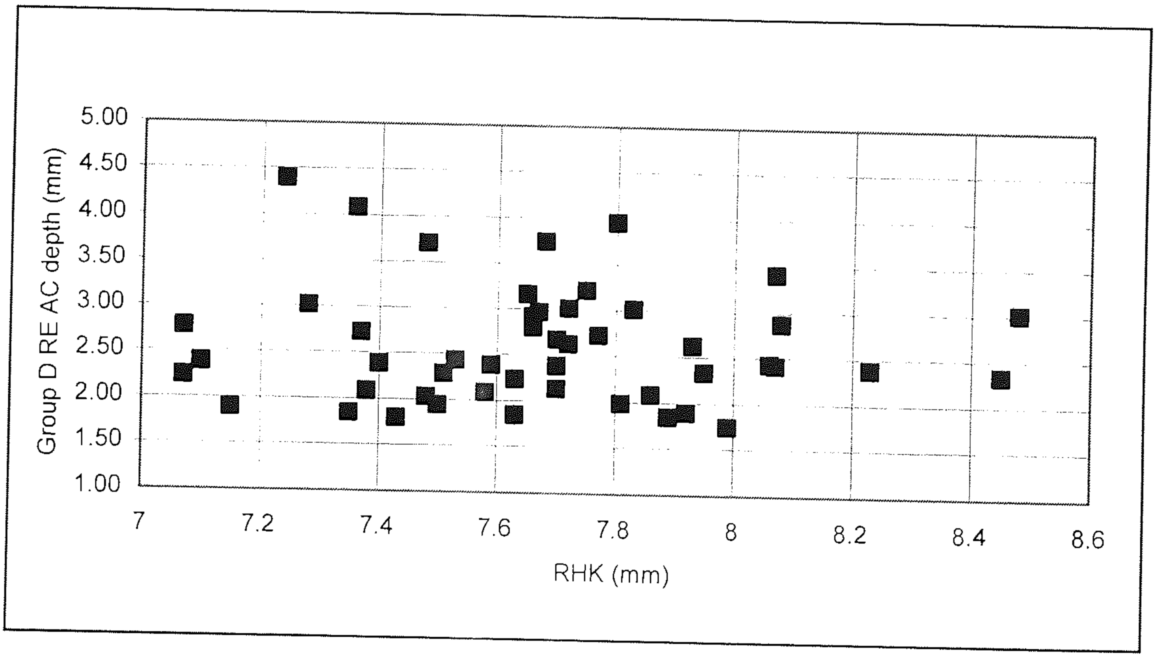


Figure 8.3.8. LE Anterior Chamber Depth versus Horizontal Keratometry for Group D

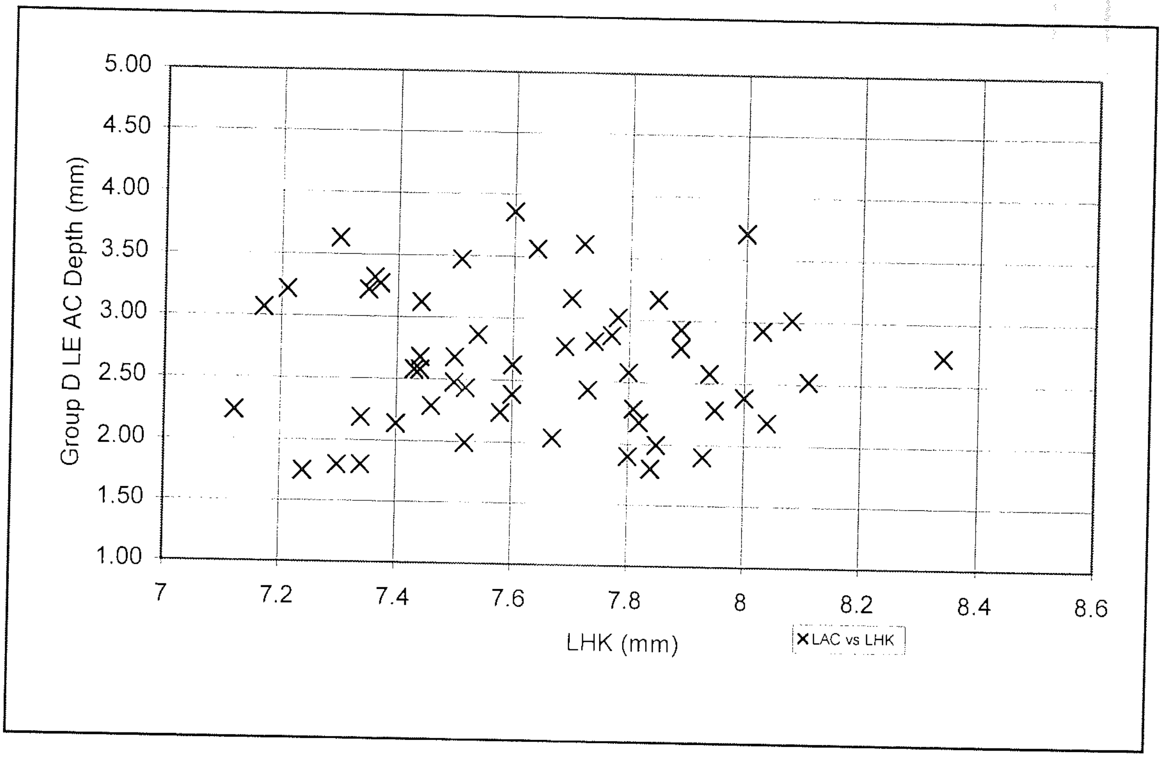


Figure 8.3.9. RE Anterior Chamber Depth versus Horizontal Keratometry for Group E

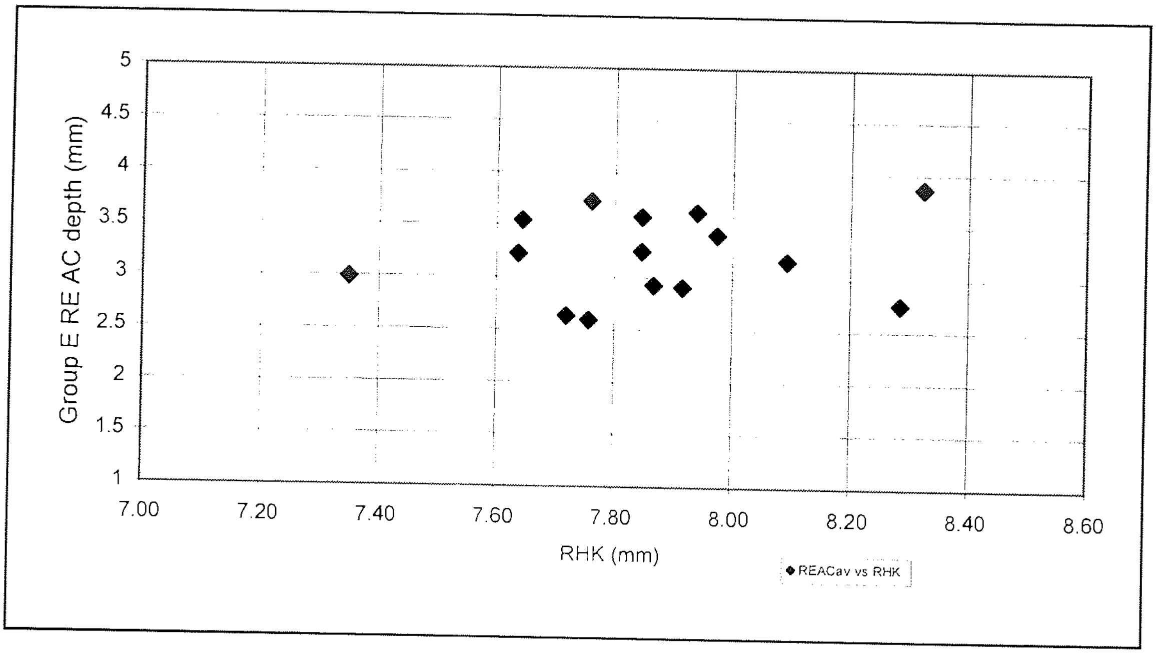


Figure 8.3.10. LE Anterior Chamber Depth versus Horizontal Keratometry for Group E

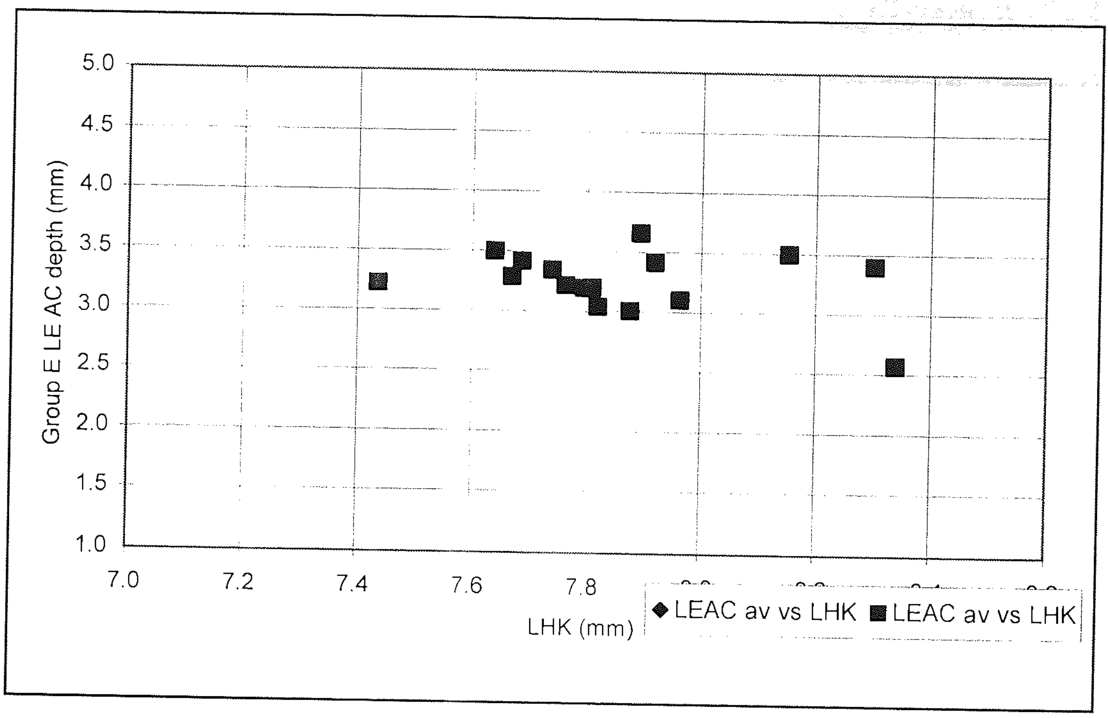


Figure 8.3.11. RE Anterior Chamber Depth versus Vertical Keratometry for Group D

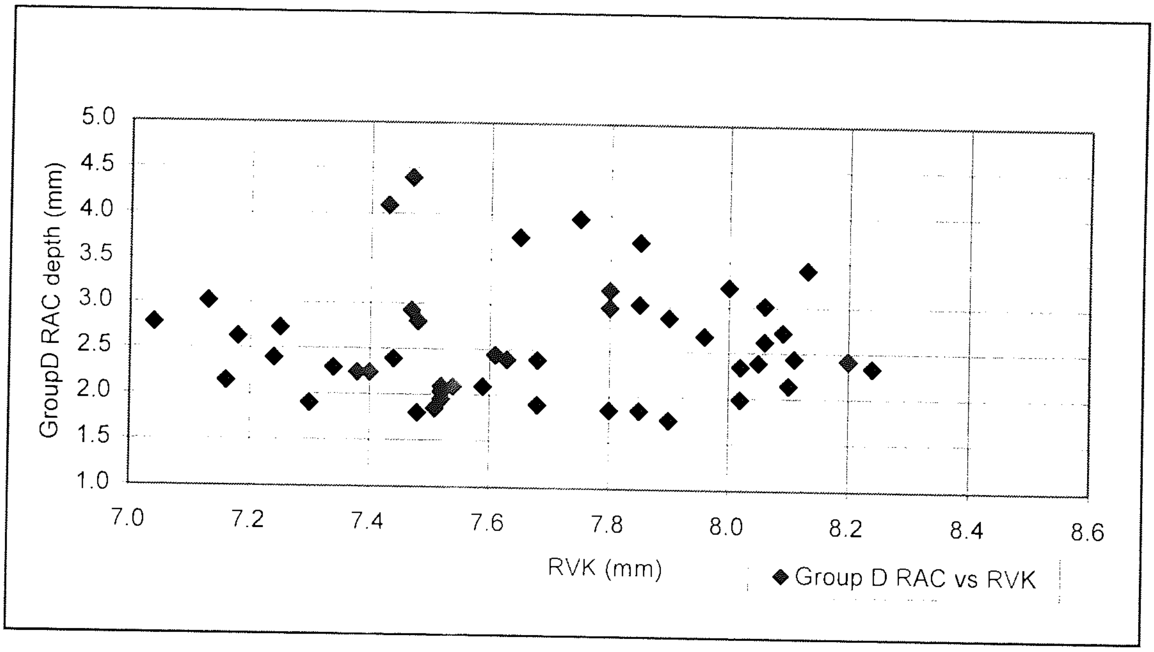


Figure 8.3.12. LE Anterior Chamber Depth versus Vertical Keratometry for Group D

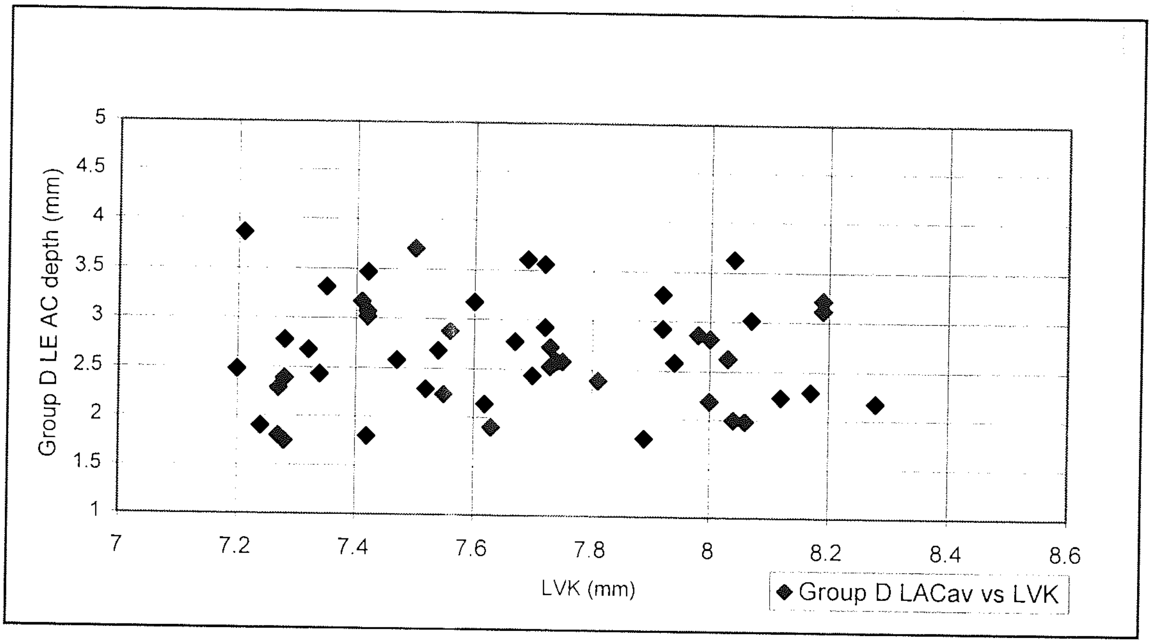


Figure 8.3.13. RE Anterior Chamber Depth versus Vertical Keratometry for Group E

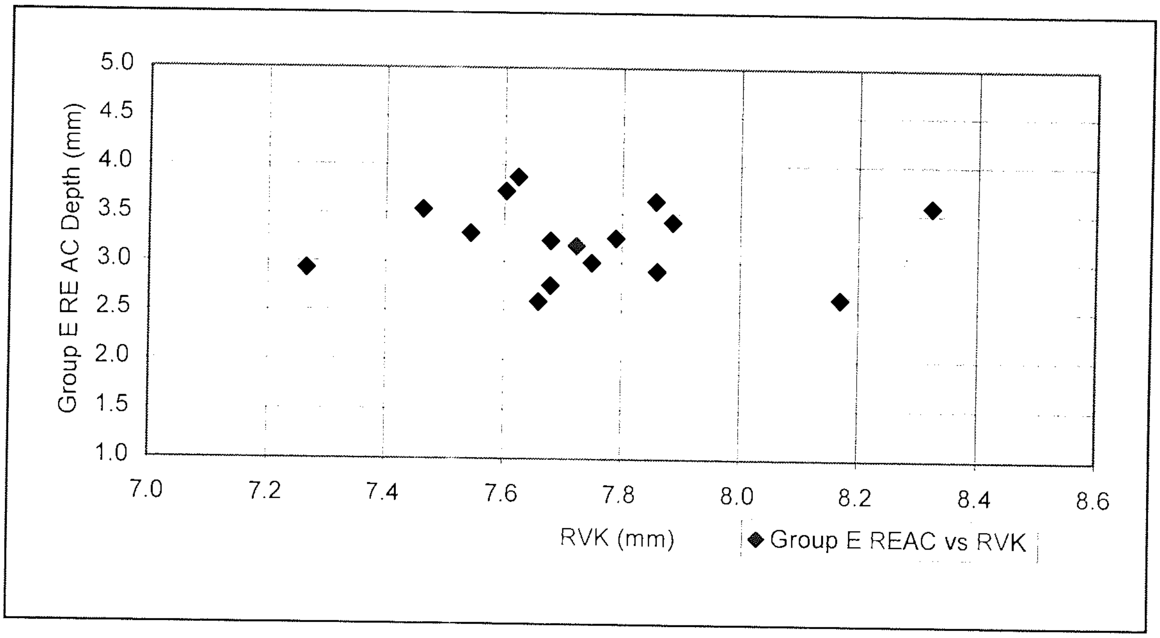
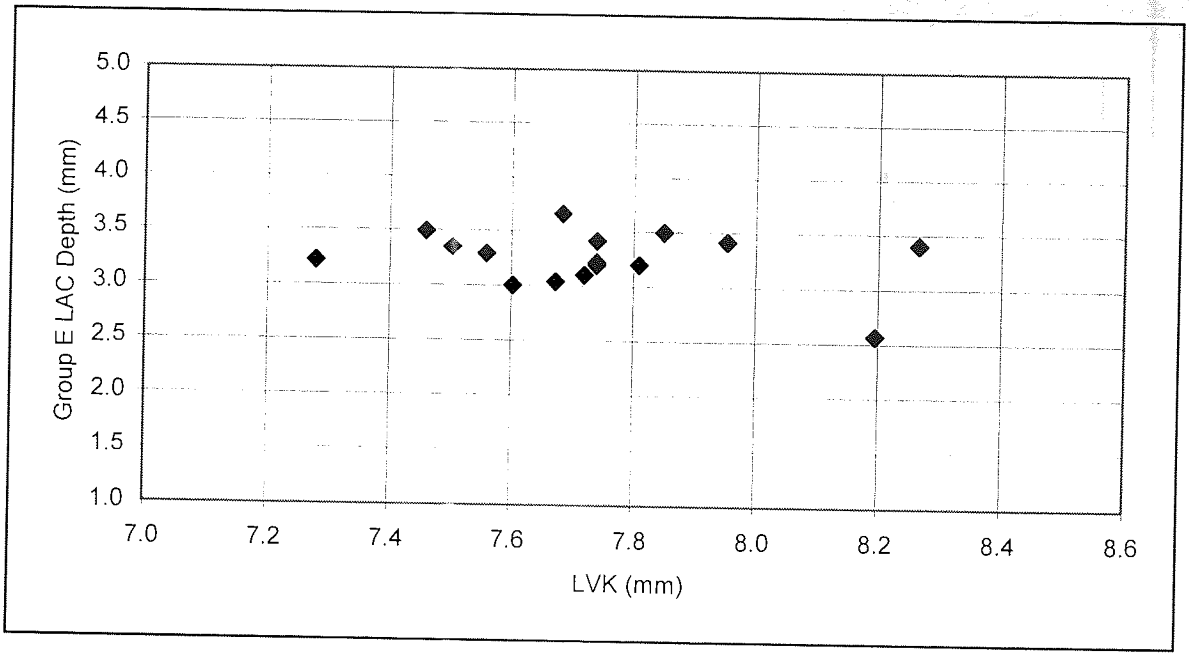


Figure 8.3.14. LE Anterior Chamber Depth versus Vertical Keratometry for Group E



Figures 8.3.13 and 8.3.14 plot anterior chamber depth versus vertical keratometry.

The values of anterior chamber depth and axial length were next compared.

Figure 8.3.15. RE Anterior Chamber Depth versus Axial Length for Group D

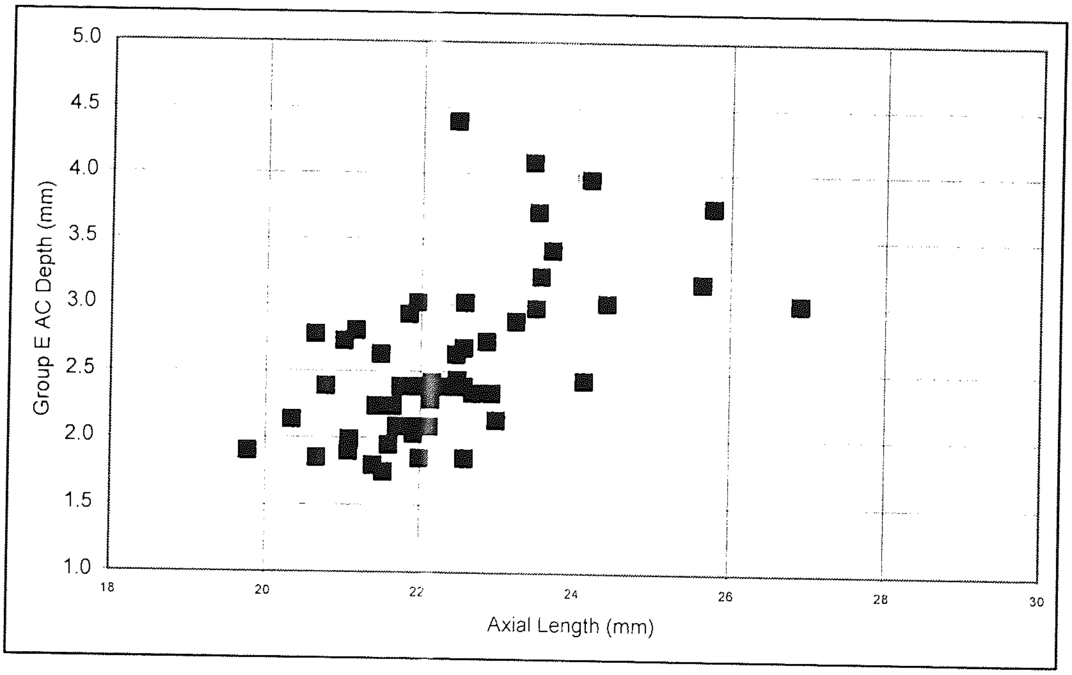


Figure 8.3.16. LE Anterior Chamber Depth versus Axial Length for Group D

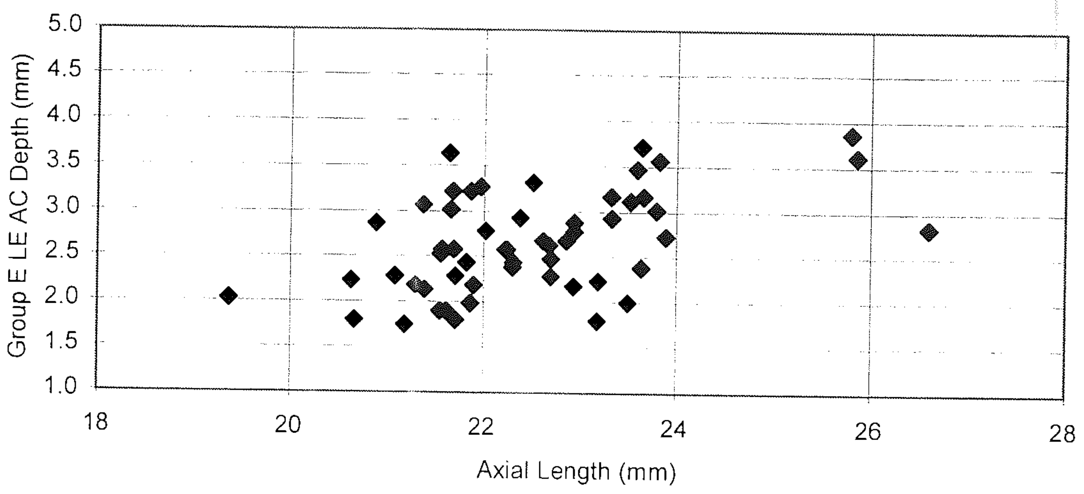


Figure 8.3.17. RE Anterior Chamber Depth versus Axial Length for Group E

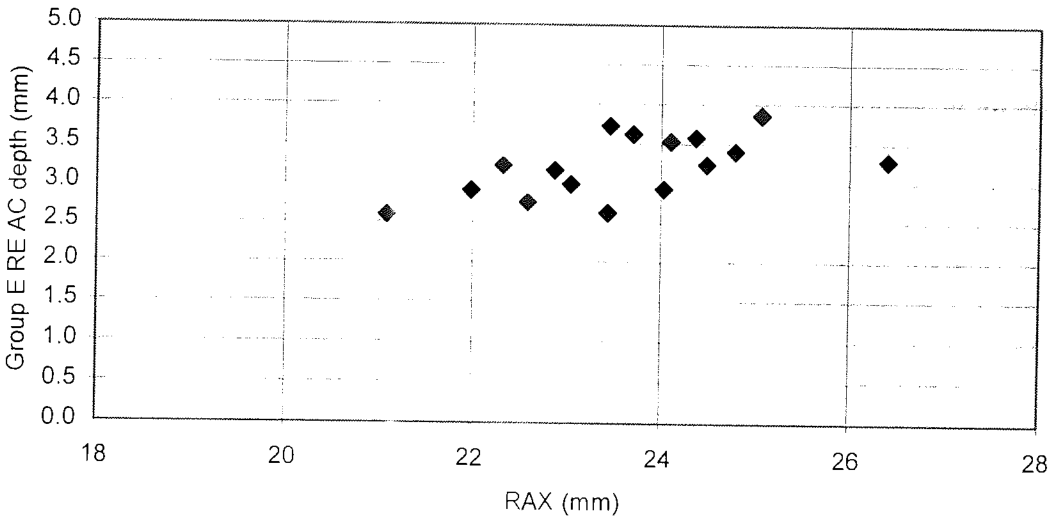
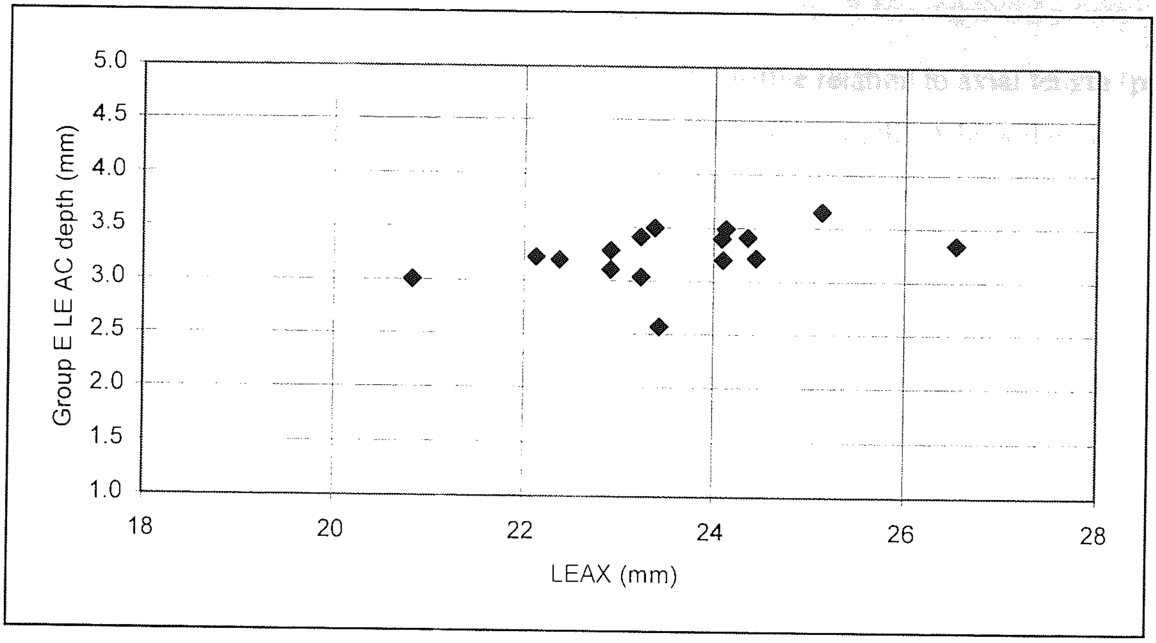


Figure 8.3.18. LE Anterior Chamber Depth versus Axial Length for Group E



Figures 8.3.15 to 8.3.18 plots anterior chamber depth versus axial length for right and left eyes of Groups D and E. respectively. For Group D, there was a very significant ($p < 0.0001$) positive relationship found between these two parameters. There appeared to be a trend for eyes with larger axial length values to also have relatively larger anterior chamber depth values, possibly because of growth during teenage years.

Regression: RE = 0.005 + 0.118 mm, LE = 0.166 + 0.124 mm.

For Group E the relationship was less significant (RE $p = 0.0056$, LE $p = 0.0800$).

8.4 Discussion of Biometry Results

Overall, the study of biometry showed:

- No significant relation of axial length to age or astigmatism
- The results in Groups D and E showed the trend of a positive relationship between axial length and both horizontal and vertical keratometry. In the larger Group D this relationship was shown to be significant. (VK: RE $p = 0.0007$, LE $p = 0.014$).These results agree with Sayeah (1996) and Goss (1997). It is likely that the association of greater values for axial length and keratometry arose during the natural period of body growth in youth. This appears to lead towards emmetropization in the eye. as proposed by Grosvenor (1987).

- Lens thickness showed a trend of increasing with age, which in Group D was proved to be very significant (p< 0.0001)
- Lens thickness also showed a very significant positive relation to axial length (p = 0.0002). This may be due partly to natural growth in youth. A hypothesis is suggested that some of the increased lens thickness and sclerosis with age occurs more often in eyes with a long axial length.
- No relationship was found between lens thickness and corneal curvature.
- Anterior chamber depth showed a trend of a positive relation to axial length, significantly shown by p = 0.0001. This trend is possibly due to natural eye growth in youth.
- Anterior chamber depth was significantly related to lens thickness. As described by Koretz et al (1989), the lens becomes thicker due to ageing changes in the tissue proteins, the anterior lens surface approaches the cornea and the anterior chamber depth decreases.
- No significant relation was found between anterior chamber depth and corneal curvature.

Chapter 9

Results and Discussion of Corneal Thickness Study

9.1 Introduction

The corneal thickness measurement (pachometry) was performed on the older Group B, using the Haag-Streit pachometer. The measurements were not carried out on Groups D and E because there was no pachometer available at the time at the clinic at Birmingham Heartlands Hospital. Access to the slitlamp microscope upon which the pachometer was fitted at Aston University was severely restricted and the measurements had to be performed quickly with only 3 or 5 measurements averaged rather than the 10 that would have been preferable.

The pachometer was not fixed to a digital read-out instrument, therefore the corneal thickness values had to be read directly from the pachometer scale that only gave measurement readings to 0.01 mm rather than to 0.005 mm possible on some other instruments.

The subjects were in the older age range so not surprisingly problems were encountered with subjects not maintaining a steady head position and steady fixation of the target. As a result of the reduction in near focussing ability after age 45 (presbyopia), their unaided near vision was generally poor and therefore there was reduced stability of fixation.

The combination of the problems with the instrument and the older subjects led to increased variability in the results and a reduction in the significance of the trends found in the results. The subjects were measured several hours after waking to allow the corneas to stabilise at their normal thickness value, having recovered from the natural overnight swelling. Kiely et al (1982) showed that the diurnal variation of thickness settled at about 3 hours after waking.

The corneal regions were measured in the following order: superior, central, inferior nasal, temporal (SCINT). The subject was instructed to fixate the target lights in turn and the upper lid was lifted gently, to reveal the superior corneal region when the subject looked downwards.

9.2 Superior Region Corneal Thickness

For Group B the mean value for superior region corneal thickness (SCT) was 0.774 mm (SD 0.04), the range was 0.68 to 0.88 mm.

Table 9.2.1 Superior Region Corneal Thickness for Group B

	RE SCT	LE SCT	RE + LE SCT
Mean	0.773	0.776	0.774
SD	0.04	0.04	0.04

Siu and Herse (1993a) obtained 0.705 mm for the mean peripheral corneal thickness value. Herse et al (1993) found 0.695 mm (SD 0.05) for the superior region.

Figure 9.2.1 Superior Corneal Thickness versus Age for Group B

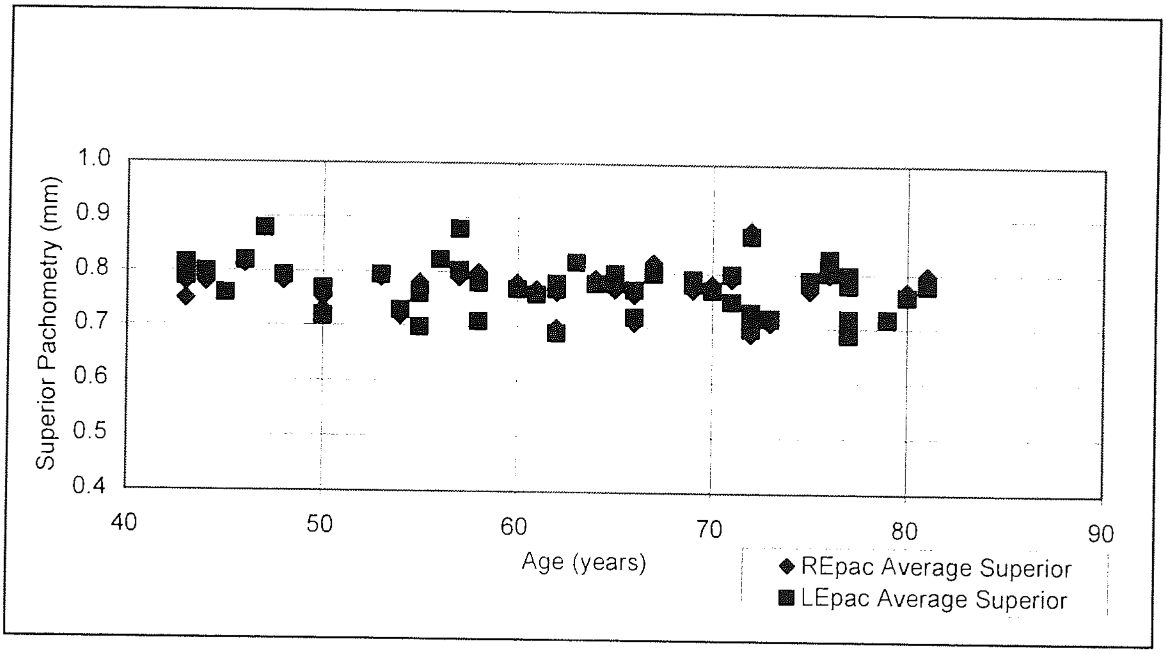


Figure 9.2 .1 shows the plot of superior region corneal thickness versus age. The results indicate a very slightly decreasing value of superior corneal thickness with age, but this is not a significant change (RE p = 0.33, LE p = 0.19).

Regression line: SRE = 0.805 – 0.0005*age

SLE = 0.820 – 0.00069*age

One hypothesis for the gradual decrease in corneal thickness with age was that after many years of fluid transport through the stroma, there was a reduction in the volume of inter-cellular matrix. Lowe (1969) found a slight increase of 0.002 mm per decade of age, and he explained that the corneal metabolism and the water pump from the stromal tissues slow down with age. He suggested that these changes lead to an increased accumulation of water in the cornea and a resultant increased stromal thickness. However, there was variability in the measurements because the corneal thickness increased from the centre towards the limbus and when some subjects did not maintain accurate steady fixation the measurement was sometimes performed at a point nearer to the limbus than was expected. This position led to some slightly greater than expected values being noted.

Siu and Herse (1993a) on using ultrasound in several corneal locations to estimate corneal thickness of subjects in the age range 16 to 75 years found no significant difference (p = 0.64) with age. They obtained a mean value of 0.705 mm for the peripheral cornea, whereas Cho and Cheung (2002) obtained 0.624 mm (SD 0.03) but this was only for the mid periphery. Gromacki and Barr (1994) found the superior peripheral thickness to be 0.75 mm (SD 0.04).

9.3 Central Region Corneal Thickness

For Group B the mean value was 0.688 mm (SD 0.04) as shown in Table 9.3.1, the range was 0.60 to 0.82 mm.

Table 9.3.1 Central Region Corneal Thickness for Group B

	RE CCT	LE CCT	RE + LE CCT
Mean	0.688	0.689	0.0.689
SD	0.0450	0.046	0.0455

Donaldson (1966) reported a mean value of 0.522 mm (SD 0.41) and Mishima (1968) a value of 0.518 mm. Gromacki and Barr (1994) obtained a mean value for the central corneal thickness of 0.56 ± 0.02 mm whereas Polse et al (1989) obtained a mean value of 0.537 mm using an ultrasound pachometer. Wolfs (1997) also obtained a mean value of 0.537 mm with no significant relation to age and Cho and Cheung (2002) used an OrbScan pachometer and gave a mean of 0.545 mm (SD 0.05). Herndon (1997) obtained a mean of 0.561 mm (SD 0.03) and Dohadwala (1998) obtained 0.553 mm (SD 0.23). Doughty and Zaman (2000) using a meta-analysis of many previous studies found 0.536 mm (SD 0.031) with a range of 0.473 – 0.597 mm. Doughty et al (2002) measured subjects in the age range 32 – 62 years and found a mean value of 0.533 mm (SD 0.33) and for 61 – 82 years 0.527 mm (SD 0.03). They found that linear regression analysis gave no significant relation with age (p = 0.085). Lam and Douthwaite (1998) measured mean corneal thickness and found a general thinning of corneal thickness for all regions, but no relationship to gender or to age. Previous workers have therefore in general reported lower mean values of central corneal thickness than found in this study.

Figure 9.3.1 Central Corneal Thickness versus Age for Group B

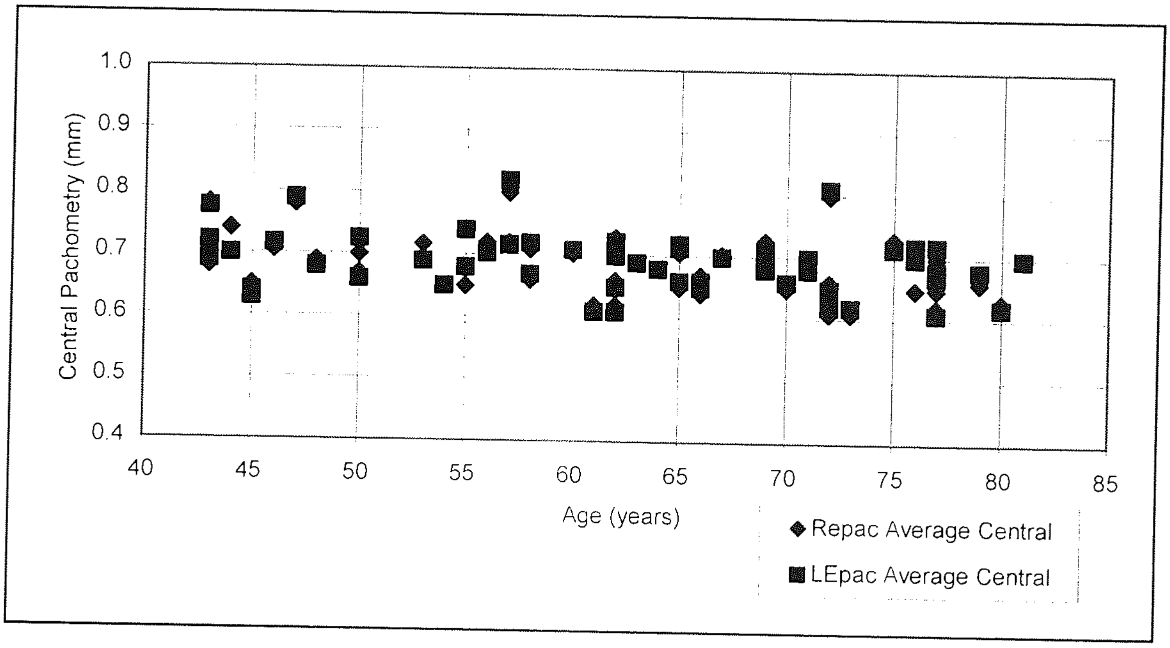


Figure 9.3.1 shows the graph of central corneal thickness versus age that indicated the trend that corneal thickness decreased slightly with age, but although there was a large

variability in values this was proven significantly for the RE but not for the LE, (RE $p = 0.046$, LE $p = 0.146$).

Regression line: $CRE = 0.757 - 0.0011*age$

$CLE = 0.741 - 0.0008*age$

Ehlers (1976) reported a decrease in thickness with age of 0.004 mm per decade. Alsbirk (1978) reported for Greenland Eskimos a reduction of 0.007 mm per decade for males and 0.003 mm per decade for females ($p < 0.001$) and Cho and Lam (1999) also found a 0.003 mm decrease for females ($p = 0.008$). Korey et al (1982) found no significant relation with age, as did Olsen and Ehlers (1984). Polse et al (1989) found that in their study of 16 subjects (8 subjects mean age 24 and 8 subjects mean age 72) the older age corneas were slightly thicker than those of the younger subjects (0.537 ± 0.029 versus 0.516 ± 0.03 mm), and the recovery from hypoxic stress and corneal hydration control decreased with age.

In contrast to that study, Li et al (1994) using a larger number of subjects ($n = 100$), found no significant difference in the central corneal thickness in comparisons between RE and LE, between male and female, among various degrees of refractive power and among various age groups. However, their study showed with increasing age the corneal thickness gradually became thinner. They described how from the central to the peripheral region the corneal thickness gradually increased, but this varied depending on the region studied e.g. superior region was thickest and temporal/inferior region thinnest of the peripheral regions.

On normal subjects of mean age 60 years, the study by Guillon and Morris (1982) using optical pachometry described the mean central thickness value as 0.518 ± 0.02 mm. (range 0.462 to 0.561 mm)

9.4 Inferior Region Corneal Thickness

As shown in Table 9.4.1 for Group B the mean inferior thickness value was 0.755 mm (SD 0.04) and the range was 0.66 to 0.84 mm.

Table 9.4.1 Inferior Region Corneal Thickness for Group B

	RE ICT	LE ICT	RE + LE ICT
Mean	0.753	0.756	0.755
SD	0.039	0.040	0.040

Siu and Herse (1993) measured the inferior region mid periphery thickness and found it was 0.005 mm thinner in the 69 years old group compared to the age 20 years group, but the difference was not significant (p = 0.92). Gromacki and Barr (1994) found a value of 0.85 mm (SD 0.03). Lam and Douthwaite (1998) found no significant difference in thickness between the four peripheral quadrants of the cornea.

Figure 9.4.1 Inferior Corneal Thickness versus Age for Group B

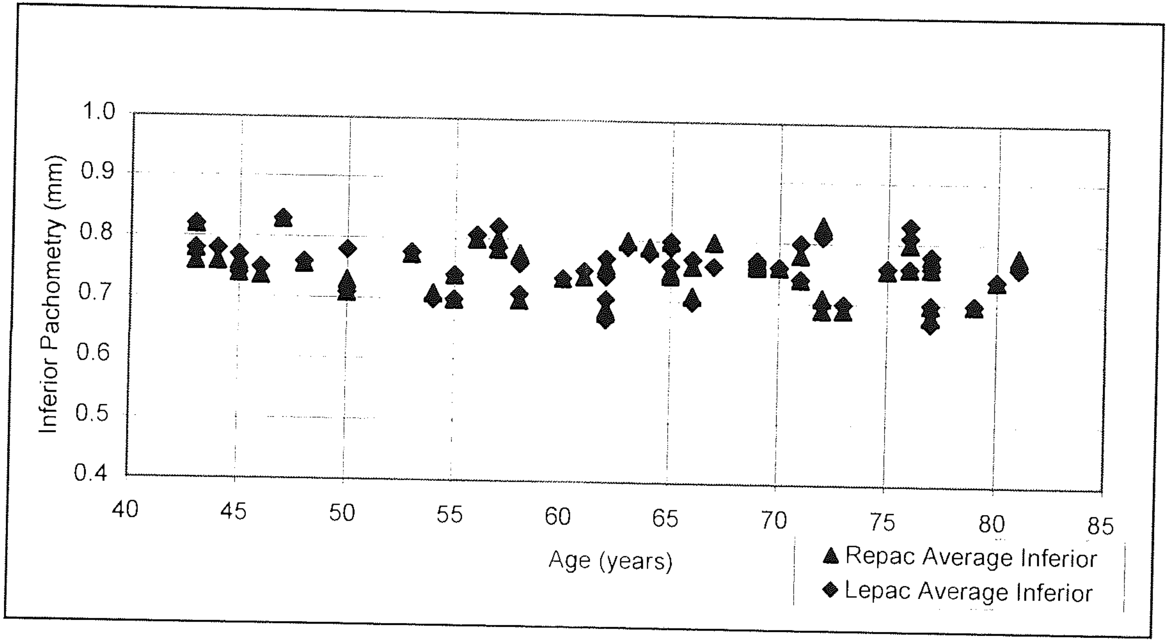


Figure 9.4.1 is the plot of inferior region corneal thickness versus age. Again there is no definite trend shown, possibly due to the large variability in measurements. There was no significant age effect (RE p = 0.395, LE p = 0.170)

Regression line: IRE = 0.779 – 0.00041*age

ILE = 0.799 – 0.00068*age

9.5 Nasal Region Corneal Thickness

For Group B the mean thickness was 0.76 mm (SD 0.041) as shown in Table 9.5.1, the range was 0.67 to 0.85 mm.

Table 9.5.1 Nasal Region Corneal Thickness for Group B

	RE NCT	LE NCT	RE + LE NCT
Mean	0.760	0.765	0.763
SD	0.041	0.041	0.041

Gromacki and Barr (1994) found a thickness value of 0.85 mm (SD 0.03).
The nasal region thickness tended to be a greater value than the mean value of the corresponding point of the temporal region, most likely because the visual axis was usually displaced slightly nasally from the corneal geometric axis. Therefore the nasal measurement was likely to be slightly nearer to the limbus and of greater value than that for the temporal region.

Figure 9.5.1 Nasal Corneal Thickness versus Age for Group B

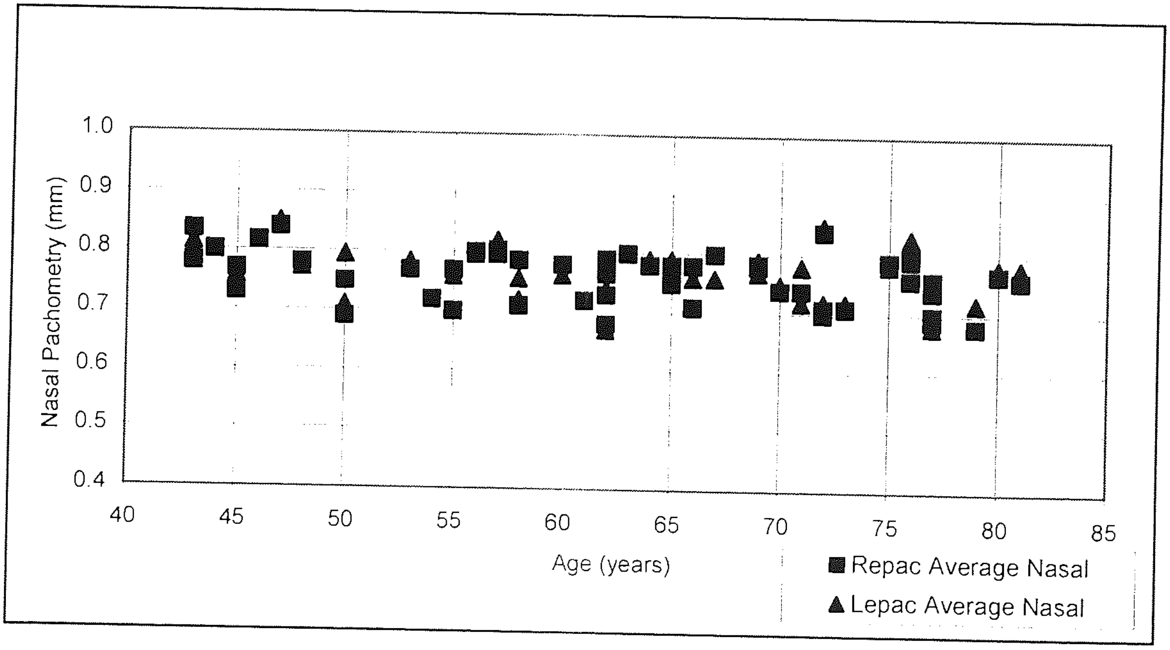


Figure 9.5.1 shows the graph of nasal region corneal thickness versus age and shows a significant age effect for the RE (RE $p = 0.045$) but not for the LE (LE $p = 0.138$).

Regression line: $NRE = 0.823 - 0.0010*age$

$NLE = 0.811 - 0.0007*age$

Herse et al (1993) used ultrasound pachometry on 23 subjects of mean age 21.5 years to study topographic pachometry changes in corneal response to contact lens induced hypoxia. They found that the greater thickness increases occurred in the central and temporal regions, with least swelling in the nasal and inferior peripheral corneal regions.

9.6 Temporal Region Corneal Thickness

For Group B the mean value was 0.74 mm (SD 0.04) as shown in Table 9.6.1, the range was 0.60 to 0.80 mm. This measurement had high variability due to some visual fixation instability. Gromacki and Barr (1994) found 0.83 ± 0.04 mm.

Table 9.6.1 Temporal Region Corneal Thickness for Group B

	RE TCT	LE TCT	RE + LE TCT
Mean	0.743	0.748	0.745
SD	0.043	0.043	0.043

Figure 9.6.1 Temporal Corneal Thickness versus Age for Group B

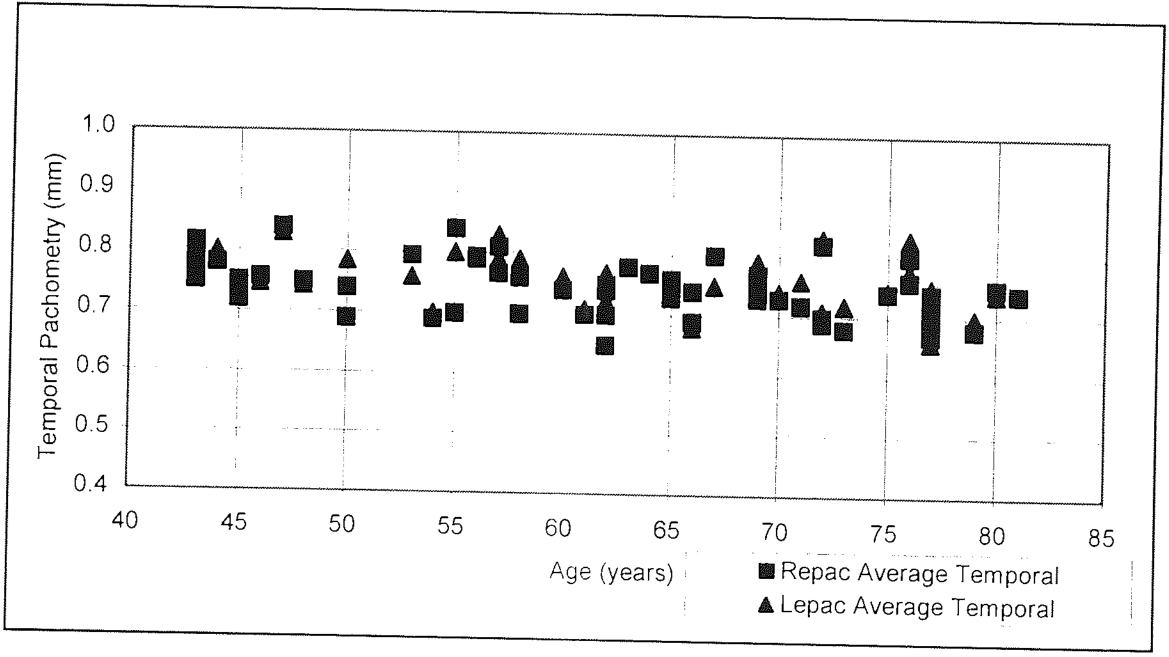


Figure 9.6.1 shows the plot of temporal corneal thickness versus age. A significant relationship was revealed for the RE (RE $p = 0.026$), but not for the LE (LE $p = 0.064$).

Regression line: $TRE = 0.816 - 0.0012*age$

$TLE = 0.809 - 0.00097*age$

9.7 Discussion of the results of Corneal Thickness related to Age

There was a significant difference ($p < 0.05$) between the mean central corneal measurement and that of any of the peripheral corneal regions. This result was expected since in human eye studies over all age ranges the central cornea was found to be the thinnest corneal region Herse et al (1993), Holden et al (1985), Gromacki and Barr (1994) and Cho and Cheung (2002). Patel (1987) suggested that the refractive index of the cornea varies in different corneal regions and this can influence pachometry.

The mean corneal thickness values obtained for the five corneal regions are summarised in Table 9.7.1.

Table 9.7.1 Mean Corneal Thickness (Group B)

Region	Mean (mm)	SD
Superior	0.774	0.04
Central	0.688	0.04
Inferior	0.755	0.04
Nasal	0.763	0.04
Temporal	0.745	0.04

The direct pachometry readings found for Group B were greater than those reported in the references quoted in each section. Although the values may be slightly less accurate than those from more modern instruments, this alone is insufficient to explain the difference. The calibration using the standard contact lens was repeated at regular intervals and achieved reproducible results. The conclusion has to be that the instrument required a calibration factor of approximately minus 0.1 mm for human eyes as described in Chapter 6. The direct pachometry readings were used for analysis.

Therefore, as shown in Table 9.7.2, analysis of the results revealed that there was no significant age effect for corneal thickness except for the right eye central, nasal and temporal regions. For the 'under age 60' subgroup there was no significant age effect for any corneal region, and for the 'over age 60' subgroup also there was no significant age effect (Table 9.7.3).

Eysteinsson et al (2002) measured 925 subjects aged 50 or over using non-contact techniques. They measured central corneal thickness (CCT) and found the mean value in males was 0.528 mm (SD 0.04) and in females the mean was 0.526 mm (SD 0.04), which did not differ significantly. They found no relationship between age and CCT, and none between gender and CCT, but said that the analysis of CCT and intraocular pressure (IOP) suggested that higher IOP values were found with thicker corneas.

Cho and Cheung (2002) measured 33 young adults using the Orbscan pachometer. This had the advantages of being non-invasive and fairly quick to use and provided both corneal thickness (CT) and topography data. Their repeatability study showed that only central corneal thickness measurements were repeatable (± 3%). For peripheral locations the confidence interval was nearer to ± 5%. Therefore they recommended that Orbscan pachometry be used only for central corneal measurements. Marsich and Bullimore (2000) also found the repeatability of the Orbscan for central measurements to be good and to be comparable with optical and with ultrasound pachometry. However, they too found large variability in the peripheral measurements.

The results of Cho and Cheung gave the following values:

Location:	Superior	Central	Inferior	Nasal	Temporal.
Mean:	0.624	0.545	0.615	0.637	0.614
SD:	0.032	0.046	0.038	0.035	0.034

They reported that the central cornea was significantly thinner than the peripheral cornea and the nasal cornea was significantly thicker than the other 4 locations. Gritz and McDonnell (1990) also reported that the nasal cornea was thicker than the temporal cornea but not a significant difference. They explained that because the corneal anatomical centre is slightly temporal to the visual axis (i.e. a positive angle kappa) then the pachometry of the nasal location is actually more peripheral than a temporal location of the same distance from the visual axis. Hence this discrepancy is to be expected.

Cho and Cheung (2002) found the superior cornea to be significantly thicker than the inferior region. The Orbscan tended to give values 25 microns greater than with ultrasound. However, Herse et al (1993) using ultrasound pachometry on 23 subjects found superior corneal thickness value of 695 ± 50 microns being slightly thinner than the 713 ± 61 microns of the inferior location. A possible explanation is that the ultrasound probe touched the cornea at a location nearer to the limbus during the inferior measurement. The increasing thickness as the limbus is approached could lead to the greater value.

ANOVA analysis of corneal thickness versus age is shown in Table 9.7.2. Those p values that suggest a significant relationship between these factors are given in bold.

Table 9.7.2. Mean Corneal Thickness, Standard Deviation and p values from the Analysis of Corneal Thickness versus Age.

Region	**Right Eye Mean**	**Right Eye SD**	**Right Eye p**	**Left Eye Mean**	**Left Eye SD**	**Left Eye p**
Superior	0.773	0.044	0.330	0.776	0.043	0.188
Central	0.688	0.045	**0.046**	0.689	0.046	0.146
Inferior	0.753	0.039	0.395	0.756	0.040	0.169
Nasal	0.760	0.041	**0.045**	0.765	0.041	0.138
Temporal	0.743	0.043	**0.026**	0.748	0.043	0.064

TABLE 9.7.3 Analysis of Corneal Thickness versus Age for Group B, Central, Nasal and Temporal Regions (RE p values).

REGION	**Group B RE**	**Group B under 60 RE**	**Group B over 60 RE**
	n = 55	n = 20	n = 35
Central	**0.046**	0.987	0.806
Nasal	**0.045**	0.233	0.454
Temporal	**0.026**	0.671	0.842

9.8 Relationship of Corneal Thickness to Other Parameters

9.8.1 Corneal Thickness Relationship with Keratometry and EyeSys Keratometry

Central corneal thickness was plotted against keratometry, and also against the EyeSys keratometry value gained using the EyeSys equipment as shown in Figures 9.8.1.1 – 9.8.1.8. For the central corneal region there was no significant relationship found between these parameters. This was also true for the EyeSys keratometry readings obtained with the EyeSys keratoscope. No significant relation was found between the thickness in each of the peripheral locations and keratometry or EyeSys keratometry.

Rapuano et al (1993) found a trend for the central thickness decrease as the average keratometry reading decreased (became steeper), but the change was not statistically significant. Carney et al (1997) and Eysteinsson et al (2002) found no significant relation to corneal radius of curvature. Kiely et al (1982b) when studying diurnal variations on 21 subjects found a good correlation between keratometry and central corneal thickness in that they both changed at a similar rate as the day progressed. The corneal asphericity remained constant, and had a minimal correlation with central corneal thickness.

Figure 9.8.1.1 RE Central Corneal Thickness versus Horizontal Keratometry

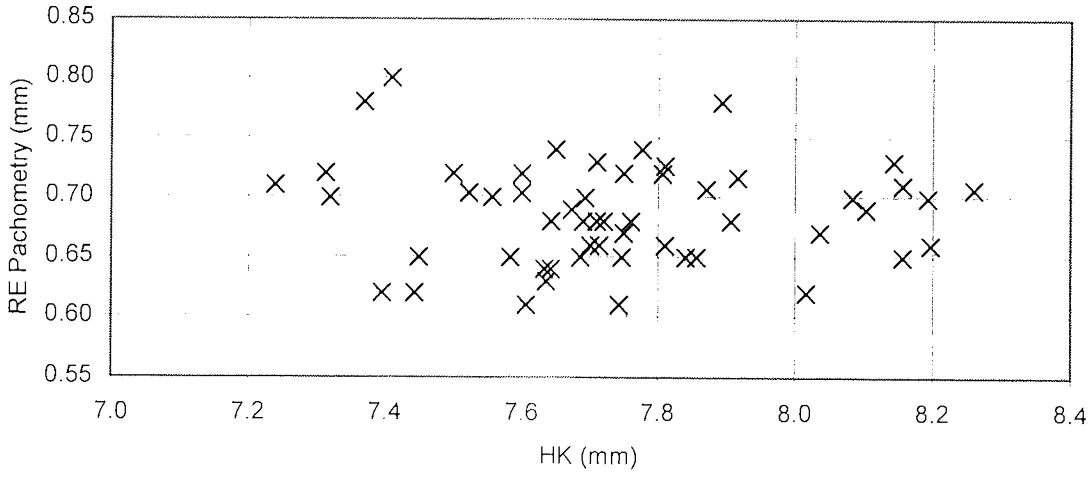
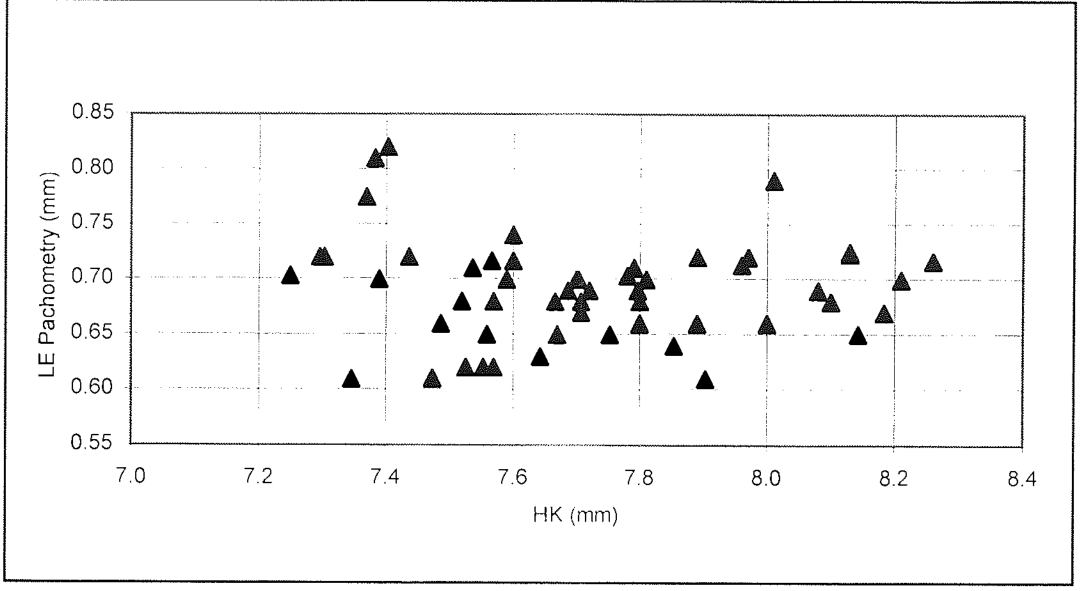


Figure 9.8.1.2 LE Central Corneal Thickness versus Horizontal Keratometry



ANOVA analysis indicated no significant relationship between corneal thickness in any of the peripheral corneal regions and keratometry, EyeSys keratometry, corneal astigmatism or corneal eccentricity value.

Table 9.8.1. Corneal Thickness versus Keratometry

Region	REHK	LEHK	REVK	LEVK	EyeSys REHK	EyeSys LEHK	EyeSys REVK	EyeSys LEVK
Superior	0.293	0.648	0.502	0.645	0.458	0.787	0.605	0.642
Central	0.451	0.694	0.555	0.524	0.620	0.845	0.550	0.569
Inferior	0.195	0.628	0.406	0.650	0.298	0.697	0.514	0.613
Nasal	0.496	0.802	0.696	0.958	0.618	0.951	0.765	0.919
Temporal	0.281	0.400	0.550	0.510	0.402	0.477	0.675	0.433

Table 9.8.2. Corneal Thickness versus Astigmatism and Eccentricity

Region	RE Astig	RE EyeSy Astig	RE Ecc	LE Astig	LE EyeSy Astig	LE Ecc
Superior	0.145	0.663	0.929	0.934	0.602	0.409
Central	0.474	0.821	0.428	0.771	0.400	0.223
Inferior	0.073	0.479	0.741	0.887	0.719	0.878
Nasal	0.280	0.793	0.753	0.611	0.977	0.403
Temporal	0.074	0.456	0.770	0.660	0.810	0.450

Figure 9.8.1.3 RE Central Corneal Thickness versus EyeSys Horizontal Keratometry

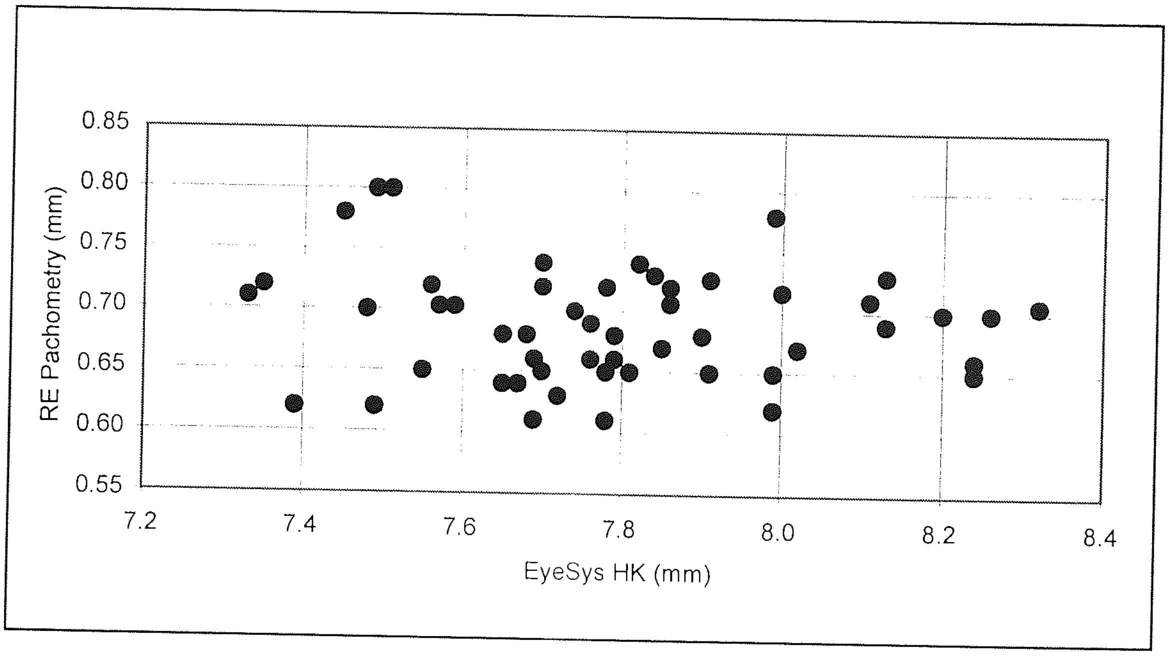


Figure 9.8.1.4 Left Eye Central Corneal Thickness versus EyeSys Horizontal Keratometry

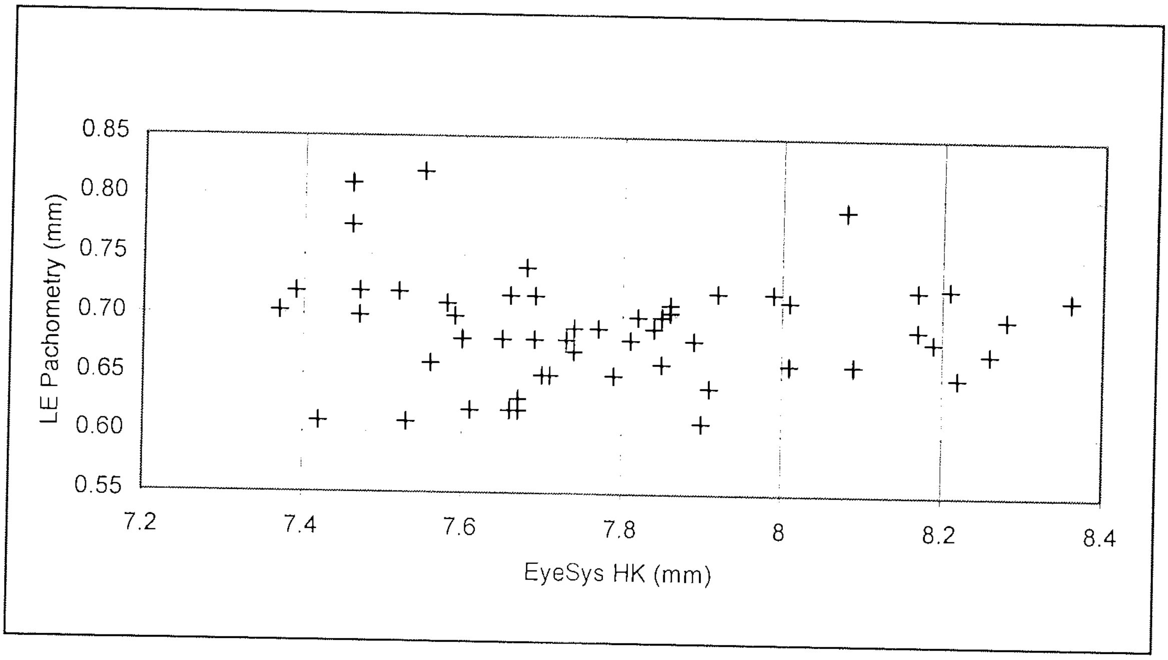


Figure 9.8.1.5 RE Central Corneal Thickness versus Vertical Keratometry

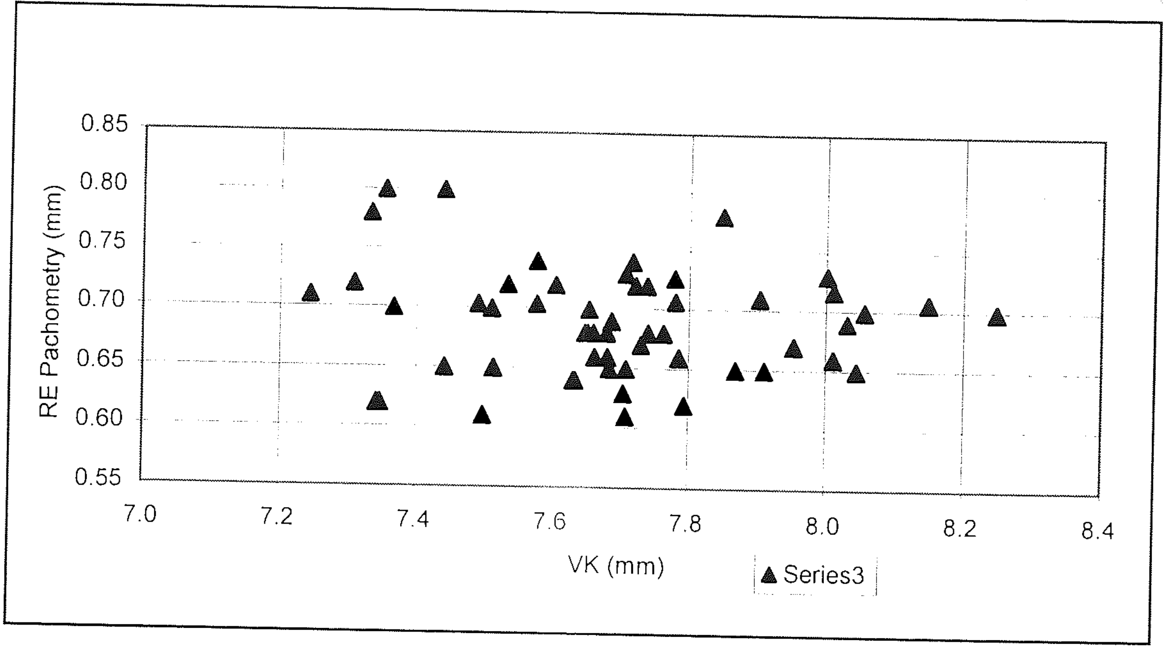


Figure 9.8.1.6 LE Central Corneal Thickness versus Vertical Keratometry

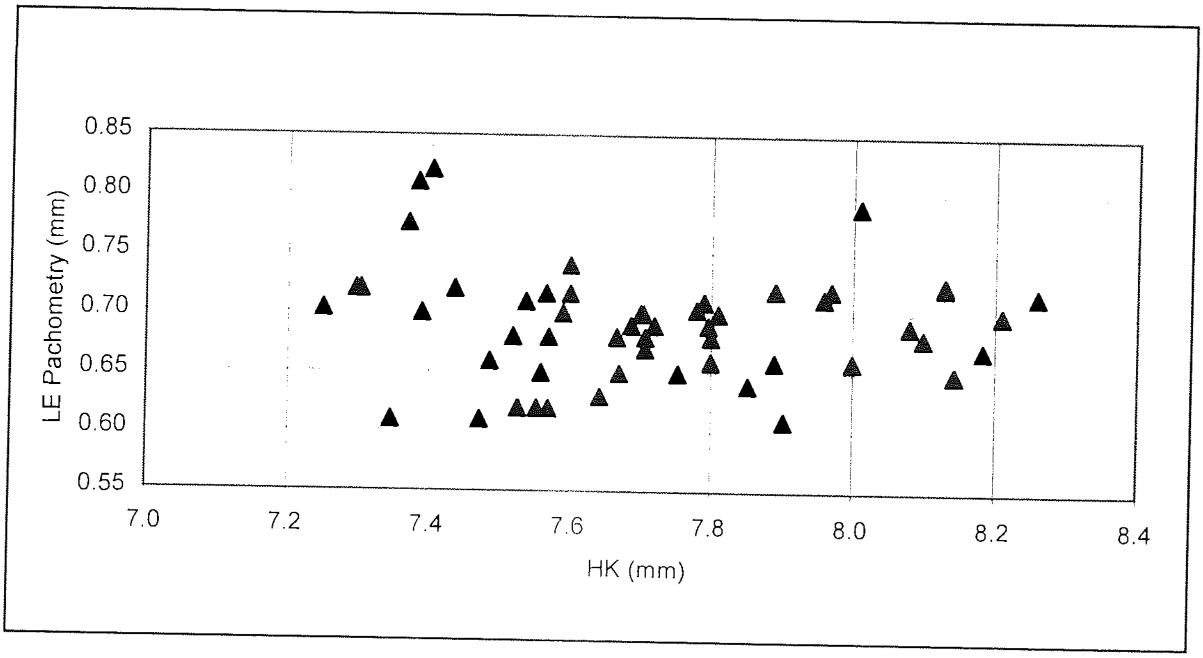


Figure 9.8.1.7 RE Central Corneal Thickness versus EyeSys Vertical Keratometry

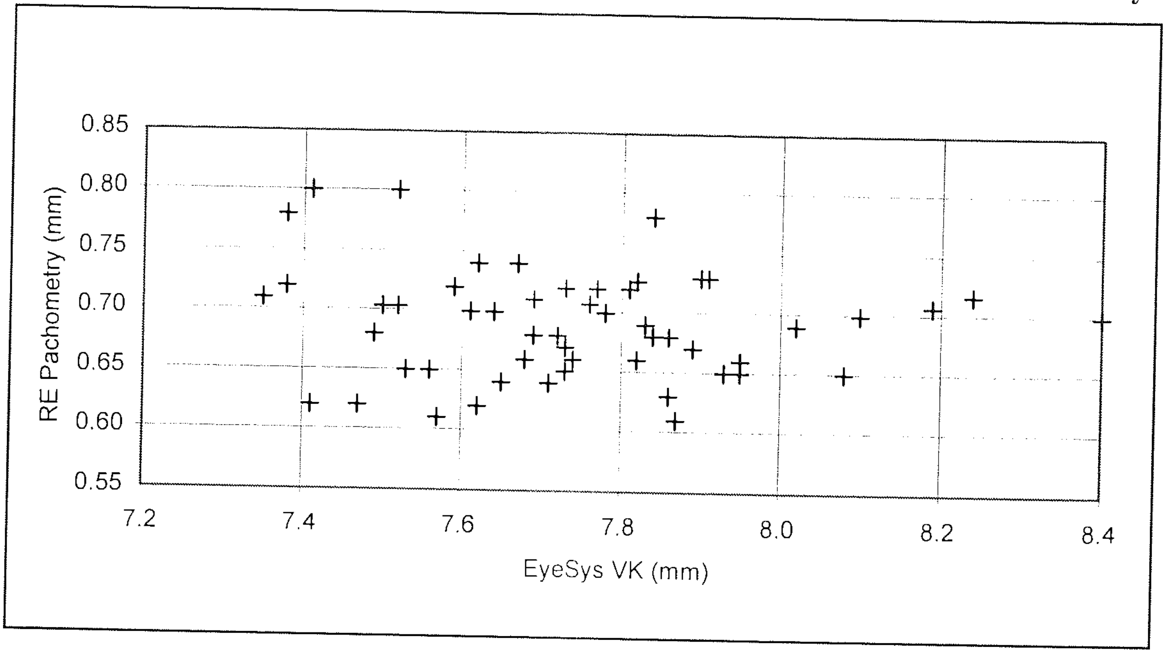
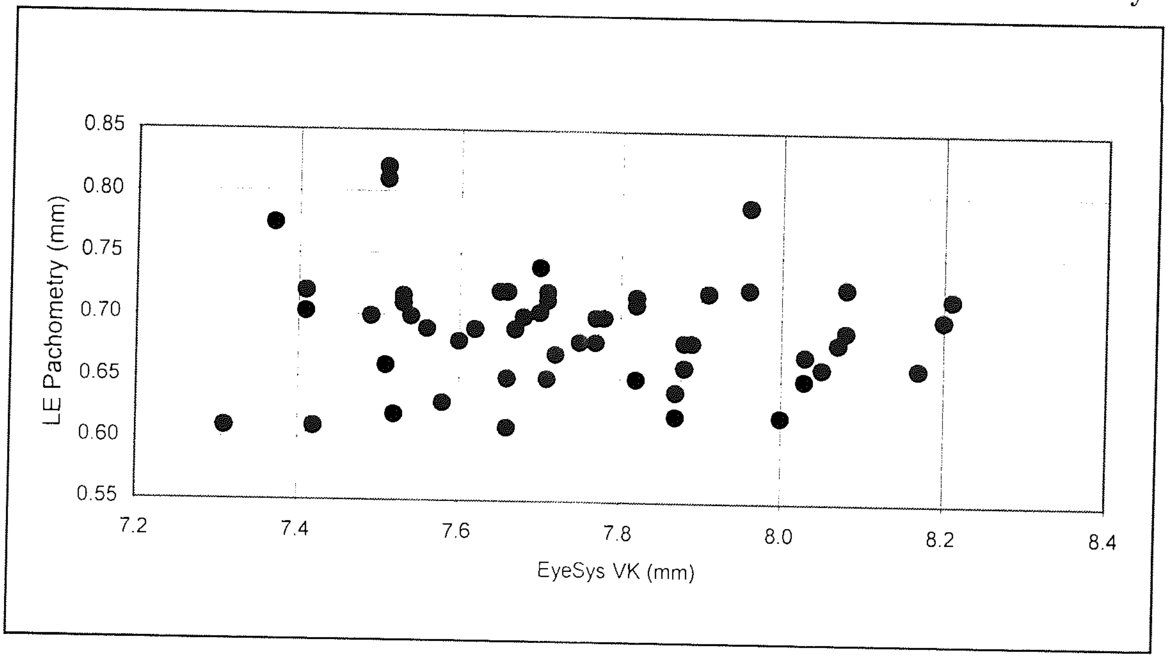


Figure 9.8.1.8 LE Central Corneal Thickness versus EyeSys Vertical Keratometry



9.9 Discussion of Pachometry Results

Overall the following conclusions were drawn:

- Central corneal thickness was significantly ($p<0.05$) less than that measured at the four peripheral regions.

- No significant difference was found between the mean values of the four peripheral regions. Temporal region thickness was the lowest value of these.

- In agreement with Lam and Douthwaite (1998), a general corneal thinning with age was found for all regions. However, they reported no significant relation to age, whereas this study found a significant decrease due to age for the central ($p = 0.046$), nasal ($p = 0.045$) and temporal (0.026) corneal regions.

- No significant relation was found between corneal thickness and keratometry, EyeSys keratometry, corneal astigmatism or eccentricity. These results concur with those of Carney et al (1997) and Eysteinsson et al (2002) who also found no relationship. Rapuano et al (1993) found a trend for central corneal thickness increase when mean keratometry value decreased, but this was not significantly proven.

Chapter 10

Conclusions

10.1 Conclusions

10.1.1. Dimensional Changes in the Ageing Cornea.

This study has investigated the central and peripheral corneal characteristics of groups of subjects from age 20 to 90 years of age to assist the understanding of ageing changes in the cornea and whether relationships between the ocular parameters are revealed.

The study measured horizontal and vertical keratometry and corneal astigmatism using both a traditional keratometer and the EyeSys keratoscope. The latter also gave values for the eccentricity of the peripheral corneal curvature.

Topographical corneal thickness was assessed using the optical pachometer on an older subject group B (Aston University subjects, n = 54, mean age = 62.8). Ocular biometry was performed on subject Groups D and E at a hospital. Group D attended for clinical assessment prior to cataract surgery (n = 201, mean age = 75.9). Group C were younger subjects (n = 15, mean age = 30.5). The results were used to investigate effects of age on corneal curvature, axial length of the eye, lens thickness and anterior chamber depth.

Keratometry

Similar corneal curvature changes were found both by the keratometer and by the EyeSys keratoscope.

Horizontal keratometry was fairly steady up to age 45 years then the value gradually decreased (curvature became steeper) with age. The trend was shown by Group B (RE $p = 0.06$, LE $p = 0.02$) and became a very significant age effect for Group D (RE $p = 0.0001$, LE $p = 0.004$).

Regression equation: RE = 8.377 – 0.0095*age

LE = 8.173 – 0.0068* age.

Vertical keratometry was fairly steady up to age 45 years then showed a slight decrease with age, especially after age 60. The trend was not significantly proved. One theory is that lid tension decreases after age 60 and provides less pressure on the vertical meridian of the globe.

Many previous studies have tended to consider only the average of horizontal and vertical keratometry readings and therefore this trend was hidden.

Corneal astigmatism showed the trend with age from WTR (HK > VK) towards minimal astigmatism then further to ATR (HK < VK), with this trend accelerating after age 60 years. The age effect on astigmatism was shown by Group B (RE $p = 0.227$, LE $p = 0.025$) and became very significant when the values from the subgroup of 'over age 60' subjects were analysed (RE $p = 0.008$, LE $p = 0.004$).

The Group D astigmatism results also showed a very significant age effect (RE $p = 0.0046$, LE $p = 0.0044$).

Regression equation: Group B = 0.13 WTR – 0.005 mm *age

Group D = 0.10 WTR – 0.008 mm *age

A further analysis of the horizontal keratometry data from all the subject groups combined ($n = 272$) was conducted. ANOVA showed a very significant relationship ($p< 0.0001$) between the decrease in keratometry and the increase in age.

These results corresponded well with those of Weale (1971), Hansen (1971), Reading (1984), Hayashi et al (1995) and Dubbelman et al (2001).

Keratoscopy

Corneal peripheral topography (eccentricity) showed a minor trend towards a decreased value with age, but this was not a significant effect (RE $p = 0.78$, LE $p = 0.48$).

Keratometry, EyeSys keratometry and corneal astigmatism analysis was impeded by the wide variation in individuals, which reduced the significance of the observed changes with age. No relation was found between the corneal curvature parameters.

Biometry

Axial Length

In agreement with the results of Ooi and Grosvenor (1995) and Koretz et al (1989), no significant relation to age was found for axial length of the eye. Grosvenor (1987) found smaller values in an older age subject group compared to a young group and suggested that reduction of axial length with age could be a natural emmetropizing occurrence. However, he had re-analysed early data from studies by Stenstrom (1948) and Sorsby et al (1957). It was possible that other factors influenced the characteristics of the two groups, as proposed by Weale (1989). For example, the younger subjects had greater body height and ocular axial length because they been healthier during their growth years than those subjects born in the early 1900's.

An emmetropizing trend was found in the positive relationship between axial length and keratometry, where greater length occurs with greater keratometry value, although only shown significantly in the older Group D.
For horizontal keratometry: RE $p = 0.0067$, LE $p = 0.061$.
For vertical keratometry: RE $p = 0.0006$, LE $p = 0.014$.
These results agree with Sayeah et al (1996) and with Goss et al (1997).
There was no significant relation to corneal astigmatism.

Lens Thickness

The trend was found of greater lens thickness with age in both groups and in Group D this showed a very significant relationship (RE $p < 0.0001$, LE $p < 0.0001$). This confirms the conclusions of Koretz et al (1989) and Ooi and Grosvenor (1995).

No significant relation was found with keratometry or with corneal astigmatism.
A trend indicating a positive relation between lens thickness and axial length was found. This probably indicated general ocular changes in the natural growth years. For the Group D this relation was very significant (RE $p = 0.0002$, LE $p < 0.0001$).

Anterior Chamber Depth

No significant relation was found to keratometry, but there was a trend in Group D for a positive relationship with axial length (RE $p = 0.006$, LE $p = 0.001$), which probably indicated changes in the natural growth period.

In agreement with the results of Koretz et al (1989), a significant relation was found between anterior chamber depth and lens thickness (RE $p = 0.035$, LE 0.0008). As Koretz et al explained, when the crystalline lens becomes thicker with age the anterior surface of the lens approaches the cornea, thus reducing the anterior chamber depth.

In Group D, a very significant relation was found between anterior chamber depth and axial length (RE $p < 0.0001$, LE $p < 0.0001$). Again this most likely demonstrated natural changes during the growth period.

Corneal Thickness

Central corneal thickness had a lower value than at the superior, inferior, nasal and temporal peripheral regions. This result agreed with that of many other studies e.g. Hansen (1971), Hirji and Larke (1978), Polse et al (1989), Siu and Herse (1993) and Cho and Cheung (2002).

Both right and left eyes showed the trend for decreasing central corneal thickness with age.

For the right eye results, ANOVA analysis revealed a significant relationship as central corneal thickness decreased with age ($p = 0.046$).

Regression equation: $RE = 0.756 - 0.0011 * age$.

Many previous workers (Ehlers et al 1971, Siu and Herse 1993b, Cho and Lam 1999) found the trend of central corneal thickness decreasing with age but were unable to prove the discovery to be statistically significant.

Alsbirk (1978) did find a significant decrease in corneal thickness ($p < 0.001$) of 0.007 mm per decade of age in males, but his study was on Greenland Eskimos and could not be presumed to apply to European subjects.

According to O'Neal and Polse (1986) and to Siu and Herse (1993) the older age cornea had a similar thickness and oedema response to hypoxia as that of a young corne, but was slower to recover from oedema. They suggested that this resulted from corneal metabolism slowing with age.

Corneal peripheral thickness results were analysed and compared to other parameters. In agreement with the conclusions of Lam and Douthwaite (1998), no significant difference was found in the mean corneal thickness between each of the four peripheral regions: superior, inferior, nasal and temporal.

Most earlier research studies on corneal peripheral thickness reported that no age effect was found whatever the region of the peripheral cornea measured. However, the study described in this thesis found a significant age effect in the right eye at both the nasal (RE $p = 0.045$) and temporal (RE $p = 0.026$) peripheral regions.

Regression equation: RE = Nasal: 0.823 - 0.0010* age,
RE = Temporal: 0.816 – 0.0012* age.

However, following the statistical analysis of the measurements in this study the mean value at each position was greater than those reported by the majority other researchers. This was because the direct readings from the pachometer scale were used in this study and no estimated correction factors applied.

Corneal thickness was found to have no significant relation to keratometry, EyeSys keratometry, or corneal eccentricity.

10.2 Suggestions for Application of the Results in the Treatment of Ageing Eyes

Knowledge concerning corneal contours and the manner in which astigmatism changes towards ATR, could be used in designing contact lenses for the older age wearer.

When considering refractive surgery to reduce corneal astigmatism, the age effect on astigmatism should be remembered, to avoid enhancing the ATR shift.

The depth to which LASIK is performed should also take into account the trend for corneal thinning with age. This trend may help in the studies of degenerative and thinning conditions of the cornea, of corneal thickness and intraocular pressure measurements and when developing better pachometers.

Information regarding the typical astigmatism found per age group could help when planning cataract extraction and IOL implantation, to reduce astigmatism and improve visual acuity, thereby leading to more rapid patient rehabilitation.

Chapter 11

Further work proposals

When future researchers wish to investigate the corneal and other ocular biometric changes due to ageing, I would recommend the following points for consideration.

11.1 Literature search

An up-to-date search in appropriate ophthalmology and vision/ocular science journals would be needed. This may help when choosing which type of equipment to use (Brenner 1997, Campbell 1997, Mattioli and Tripoli 1997). Other studies can give advice on methods of equipment calibration and use (Gonzalez-Meijome et al 2004). A detailed overview of the technology involved in corneal topography measurement was given by Applegate and Howland (1995). They discussed the fundamental limitations of the operating principles, hardware and data analysis. Other studies recommend certain methods to avoid and extrinsic factors that may affect the reliability of measurements: Giasson and Forthomme (1992), Jeandervin and Barr (1998), Hollingsworth et al (2001) and Douthwaite (2003). Some research papers describe the correct calibration of an instrument and the normal ocular parameters on groups of healthy young (e.g. age 20-30 years) subjects according to that instrument (Dave, Ruston and Fowler 1998 a, b), (Kinge and Midelfart 2000). These papers can help when re-calibrating the actual instrument used in further work on ageing changes.

Although it would be tempting to just quote their results for a young subject group, the measurements could be repeated on a similar young aged group to affirm similar results. If there proved to be a significant difference in results, one would seek an explanation.

For example this may be due to a slight measurement bias in an individual instrument, or inter-observer variation or due to subtle differences in the subjects characteristics. Occasionally, publications may be found involving measurements similar to those of the proposed study: for example those given by Lam and Chan (1999), Pardhan and Beesley (1999), and Gudmundsdottir (2001). By critical review, the reader may learn how to improve their own study design or to consider including the measurement of some extra parameters. For example, aiming to measure a larger number of subjects or using new exclusion criteria.

11.2 Experimental Design

Advice would be obtained on setting up and calibrating the new equipment. New ideas on reducing the variability of measurements would be sought. For example, reliable instruments which could easily be set for the observer (eyepiece, table height) and to a typical subject (chin rest comfortable for older age subject) and in a protected environment (not jolted by moving equipment about or other workers re-using it or splashing it). Examples would be 'lock on position' eye trackers or instrument features to improve the stability of vision fixation on a target. Several researchers have carried out studies recommending various modern models of corneal topography measurement systems e.g. Brenner (1997), Campbell (1997) and Mattioli and Tripoli (1997). Better choice of subjects and suitable allocation of measurement time would be advantageous, so there would be less risk of error related to rushing the measurement time or to the subject having prior ocular disturbance such as eye rubbing or fatigue.

11.3 Choice of Subjects

I would recommend that all the ocular parameter measurements would be carried out on the same instrument by the same observer, to reduce the risk of inter-operator and inter machine variability. When studying subjects aged from 20 to 80 years, it would be useful to allot them to age bands of ten years. About 20 to 50 subjects would be in each age band. Advice would be obtained and power equations done to estimate the best number for each age band to help with statistical analysis. Beyond age 80, reliable subjects without other eye problems would be more difficult to find. An even distribution of male to females would be preferred (Midelfart and Aamo 1994). The subjects would agree to the study protocol in advance, receive sufficient explanation of what measurements if any were invasive and be reassured regarding the confidentiality of personal data. Non-invasive methods would be preferred, e.g. rather than using a probe for ultrasonic pachometry method, measuring by optical pachometry. The Zeiss IOLMaster is a helpful non-invasive instrument for biometry and central corneal curvature measurement, as recommended by Eleftheriadis (2003), Atchison and Smith (2004) and Sheng et al (2004). Dunne et al (2005) preferred a different non-invasive phakometric method when studying the cornea and the crystalline lens.

11.4 Subjects Exclusion Criteria

These could include the following, to avoid the risk of prior ocular influence:

1. Contact lens wear,
2. Ocular surgery,
3. Prolonged use of topical medication,
4. Glaucoma,

5. Clinical dry eye.
6. Thyroid eye disease,
7. Ocular infection or inflammation,
8. Residual corneal scarring or deformation due to previous ocular disturbance
9. Ectasia such as keratoconus.
10. General health problems, e.g. diabetes, Parkinson's disease

In the previous study, many of the group of 'over age 60 years' had cataracts and therefore were undergoing ocular biometry for clinical as well as research reasons. However, the presence of cataract is liable to decrease vision and make fixation and eye position stability less reliable. This reason could have increased the data variability. Therefore, where possible, it would be better to avoid those subjects who have significant cataract or to consider them in a separate study.

11.5 Choice of Parameters

Although the previous study indicated a slight trend towards the reduction in corneal asphericity with age, the trend was not significant and did not show any relationship to the other parameters measured. This would suggest that it would not be worth measuring peripheral corneal curvature in a future study until a much more accurate measurement method becomes available.

The EyeSys keratoscope is a typical example of an automatic instrument where the manufacturer uses undisclosed algorithms to calculate corneal asphericity. Also there remains the problem of ensuring the measurement of the geographic corneal centre as the EyeSys aligns with the visual axis instead. Although the Orbscan keratoscope sounds attractive, I have reservations about the validity of the peripheral measurements particularly in moderate to high amounts of astigmatism and on older subjects with reduced vision and poor fixation. Cairns and McGhee (2005) reviewed the advantages and limitations of the Orbscan keratoscope.

Keratometry would still be recommended, although more modern corneal curvature assessment equipment would be comparable.

Ocular biometry could be done using the ZeissIOL Master, which has the advantage of being non-invasive, so does not disturb the eye or risk cross-infection. Future studies need equipment that more accurately finds the correct corneal centre locus for topography and pachometry.

In addition to corneal curvature measurements, axial length, lens thickness, anterior chamber depth and central corneal thickness should be measured. More modern equipment should be able to assess these parameters with a greater degree of accuracy. Ocular refraction could be included to help understand the influence of corneal parameters on refractive error (Carney et al 1997). It may be possible to measure all of these at various set central and peripheral corneal loci, to draw up a more accurate picture of these ocular structures.

Analysis of these measurements would help to study the results of ocular growth and refraction as recommended by Dubbelman et al (2001), and also to investigate ocular changes related to ageing as advised by Hosny and Alio (2000), Arta et al (2002) and Fujikado et al (2004).

The results may give a further detailed insight into hypotheses such as:

1. Do myopes with long axial length tend to have greater lens thickness? (The study showed a significant increase in lens thickness related to axial length.

2. Does the age-related increase in lens thickness accelerate in these myopes and be linked to lens sclerosis in ageing myopes? The Tanjong Payar 2003 survey tried to discover ocular relationships to axial length.
3. Does the cornea thin slightly with age? The study here showed some significant thinning with age at the nasal, central and temporal regions of the cornea of the right eye. Daxer (1998) attempted to find ageing changes in corneal fibrils.

If the age-related trend in corneal thinning can be proven, this would be of particular interest to researchers studying the relation between corneal thickness, rigidity and intra-ocular pressure measurement (Foster et al (1998), Doughty and Zaman (2000), Doughty et al (2002), and Shimmyo et al (2003)).

11.6 Analysis of Results

Before analysis, some investigators may prefer to adjust their videokeratoscope data in an attempt to reduce errors, as recommended by Klein (1997 a, b,) and by Barsky, Klein and Garcia (1997), and by Schwiegerling and Greivenkamp (1997).

Linear regression can be used initially to see if there are simple relationships between parameters (Douthwaite 2002). However, ANOVA and multi-variate analysis would be useful as there are several parameters and influences that may affect each other (Armstrong, Eperjesi and Gilmartin 2002, 2005).

A further avenue of statistical analysis could explore the data comparing male and female subjects (Cho and Lam 1999, Wong and Wong 2002) or various ethnic groups to estimate whether certain ocular changes occur earlier or progress faster (Goto, Klyce and Zheng 2001, Wong and Foster 2001, Mallen et al 2005).

The results described in Chapter 10 of this study, when all the subject groups were combined, indicated that the corneal curvature changes were gradual between ages 20 and 60, and showed a more rapid progress after age 60 years. This tended to be true for the decrease in radius of curvature, for the increase in lens thickness, for the decrease in anterior chamber depth, and possibly for the slight decrease in axial length. Therefore, when analysing the results of future studies of these parameters, in addition to trying to plot a function line or curve to the overall results of the 20 to 60 years group, it may be useful to break the age group into smaller age bands e.g. 20 to 40 years and compare to an older group e.g. 60 to 80 years. These analyses may reveal more significant differences between the two age groups and identify age-related changes. The increased variability of data in older age band subjects also needs to be analysed.

REFERENCES

Alimisi, S., Miltsakakis, D., Klyce, S. (1996) Corneal topography for IOL calculations. J. Refract. Surg. **12** (2). S.309 - 311.

Alsbirk, P.H. (1978) Corneal thickness: 1. Age variation, sex difference and oculometric correlations. Acta Ophthalmologica. **56**. 95 - 104.

Alvarado, J, Murphy, C. and Juster, R. (1983) Age-related changes in the basement membrane of the human corneal epithelium. Invest. Ophthalmol. Vis. Sci. **24**. 1015-1018

Alvi, N.P., McMahon, T.T., Devulappally, J., Chen, T.C., Vianna, M.A. (1997) Characteristics of normal corneal topography using the EyeSys corneal analysis system. J. Cataract. Ref. Surg. **23**. 849 - 855.

Anstice, J. (1971) Astigmatism - its components and their changes with age. Am. J. Optom. **48**. (1) 1001 - 1006.

Antalis, J.J., Lembach, R.G., Carney, L.G. (1993) A comparison of the TMS-1 and the Corneal Analysis System for the evaluation of abnormal corneas. C.L.A.O.J. **19**. 58 - 63.

Applegate, R. and Howland, H. (1995) Non-invasive measurement of corneal topography. I.E.E.E. Eng. Med. Biol. **14**. 30-42.

Arffa, R.C., Warnicki, J.W, Rehkopf, P.G. (1989) Corneal topography using rasterstereography. Ref. Corneal Surg. **5**. (6) 414 - 417.

Argus, W.A. (1995) Ocular hypertension and central corneal thickness. Ophthalmology **102**. (12) 1810 - 1812.

Armstrong, R.A., Eperjesi, F. and Gilmartin, B. (2002) The application of analysis of variance (ANOVA) to different experimental designs in optometry. Ophthalmic. Physiol. Opt. **22**. 248-256.

Armstrong, R.A., Eperjesi, F. and Gilmartin, B. (2005) The use of correlation and regression methods in optometry. Clin. Exp. Optom. **88**. (2) 81-88.

Arta, L et al. (2002) Contribution of corneal and internal surfaces to the change in ocular aberration with age. J. Opt. Soc. Am. Ass. Opt. Image Sci. Vis **19** (1) 13-43.

Astin, C.L.K. (1985) Astigmatism in aphakia. The Optician. **11**. Jan.14 - 15.

Astin, C.L.K (1986) Review of the 2nd year- of the aphakia clinic at Moorfields Eye Hospital contact lens department. J. Br. Contact Lens. Assoc. **9**. 14 - 18.

Astin, C.L.K., Gartry, D.S., Steele, A.D.McG. (1996) Contact lens fitting after photorefractive keratectomy. Br. J. Ophthalmol. **80**. 597-603.

Aston, S.J. (1990) The graying of America: Optometric Considerations Introduction. Optometry and Vision Sci. **67** (5) 313 - 314.

Atchison, D.A and Smith, G. (2000) Optics of the Human Eye. Oxford. Butterworth-Heinemann. 222. 30.

Atchison, D.A., Smith, G. (2004) Possible errors in determining axial length changes during accommodation with the IOLMaster. Optom. Vis. Sci. **81**. (4) 283-286.

Atkins, A.D. and Roper-hall, M.J. (1985) Control of postoperative astigmatism. Br. J. Ophthalmol. **69**. 348 - 351.

Azar, D., Salvat, M. and Benson, A. (1996) Corneal topography, ultrasound biomicroscopy and scatterometry. Current Opinion in Ophthalmol. **7**. (4) 83-93.

Bailey, I.L. and Carney, L.G. (1972) Corneal thickness proportional to shape changes. J. Am. Optom. Assoc. **43** (6). 669 - 672.

Bailey, I.L. and Carney, L.G. (1977) A survey of corneal curvature changes from contact lens wear. The Contact Lens J. **6** (1) 3 - 13.

Bailey, A.J. (1987) Structure, function and ageing of the collagens of the eye. Eye. **1** (2) 175 - 183.

Baker, P.C. (1990) Holographic contour analysis of the cornea. **61**. In: Masters B.R. (ed) Non-invasive diagnostic techniques in ophthalmology. Publisher: Springer Verlag. New York.

Baldwin, W.R. and Mills, D. (1981) A longitudinal study of corneal astigmatism and total astigmatism. Am. J. Optom. Physiol. Opt. **58** (3) 206 - 211.

Barsky, B.A., Klein, S.A., and Garcia, D.D. (1997) Gaussian power with cylinder vector field representation for corneal topography maps. Optom.Vis. Sci. **74**. (11) 917-925.

Batterbury, M., Khaw, P.T., Hands, R., et al. (1991) The cataract explosion: the changing pattern of diagnoses of patients attending an ophthalmic outpatient dept. Eye. **5**. 369 - 372.

Belin, M.W. and Ratliffe, C.D. (1996) Evaluating data acquisition and smoothing functions of currently available videokeratoscopes. J. Cataract Ref. Surg. **22**. 421 - 426.

Benedek, G.B. (1971) Theory of transparency of the eye. Appl. Optics. **10**. 459 - 472.

Bennett, A.G. (1966) The calibration of keratometers. The Optician. **151** (3913) 317 - 322.

Bennett, A.G. (1966) Optics of contact lenses. 4th Ed. Assoc. Disp. Opticians. London. 16 - 19.

Bennett, A.G. and Rabbetts, R. (1991). What radius does the conventional keratometer measure? Ophthalmic. Physiol. Opt. **11**. 239 - 247.

Bibby, M.M. (1976) Computer assisted photokeratoscopy and contact lens design. The Optician. **171**. (4423) 31 - 44, **171** (4424) 11 - 17, **171** (4426) 15 - 17.

Bibby, M.M. and Townsley, M.G. (1976) The Wesley-Jessen System 2000 photokeratoscope. Contact Lens Forum. **1**. 37 - 45.

Binder, P.S., Kohler, J.A. and Rorabaugh, D.A. (1977) Evaluation of an electronic corneal pachometer. Invest. Ophthalmol. Vis. Sci. **8**. 855 - 858.

Bishara, S.A., Goya, V. and Rand, W.J. (1988) Cataract and ocular parameters: sexual comparison. Ann. Ophthalmol. **20**. 73 - 74.

Blix, M. (1879) Oftalmometriska studies. Uppsala Lakareforenigs Farhandiglingar. **15**. 349 - 420.

Bogan, S.J., Waring III, G.O., Ibrahim, O., Drews, C., Curtis, L. (1990) Classification of normal corneal topography based on computer assisted videokerotography. Arch. Ophthalmol. **108**. 945 - 949.

Bonanno, J., Polse, K.A. (1985) Central and peripheral corneal swelling accompanying softlens extended wear. Am. J. Optom. Physiol. Opt. **62** (2) 74 - 81.

Bonnet, R. and Cochet, P. (1960) Nouvelle methode D'Ophthalmometrie topographique. Bull. Mem Soc. Fr. Ophtalmol. **73**. 687 - 716.
(translated by Eagle, E. (1962) Am. J. Optom. **39**. 227 - 251)

Borcherding, M.S., Black, L.J., Sittig, R.A., Bizzell, J.W., Green, M. and

Weinstein, H.G. (1975) Proteoglycans and collagen fibre organisation in human corneoscleral tissue. Exp. Eye Res. **21**. 59 - 70.

Brennan N.A., Smith G., Macdonald J.A., and Bruce A.S. (1989) Theoretical principles of optical pachometry. Ophthalmic. Physiol. Optics. **9** (3) 247 - 254.

Brenner, D. (1997) Modeling the cornea with the Topographic Modeling System videokeratoscope. Optom Vis. Sci. **74** (11) 895 - 898.

Bron, A.J., Vrensen, G.F., Koretz, J, Maraini, G., Harding, J.J. (2000) The ageing lens. Ophthalmologica. **214**. 86-104.

Brungardt, T. (1969) Reliability of keratometer readings.
Am. J. Optom. Arch. Am. Acad. Optom. **46** (9) 686 - 691.

Brungardt, T. (1973) Reliability of keratometry readings - an addenda.
Am. J. Optom. Arch. Am. Acad. Optom. **50** (9) 736 - 737.

Brungardt, T.F. (1981) A corneal topographical model and fitting conclusions.
Am. J. Optom. Physiol. Optics. **58** (2) 136 - 138.

Butcher, J.M. and O'Brien, C. (1991) The reproducibility of biometry and keratometry measurements. Eye. **5** (6) 708 - 711.

Button, N.F. (1986) Clinical manifestations of corneal metabolic disturbances during contact lens wear. 2 vol., Doctoral Thesis. Glasgow College of Technology.

Cairns, G., McGhee, C.N. (2005) Orbscan computerized topography: attributes, applications, and limitations. J. Cataract Refract. Surg. **31**. (1) 205-220.

Camp J.J., Maguire, L.J., Cameron, B.M., Robb, R.A. (1990) A computer model , for the evaluation of the effect of corneal topography on optical performance. Am. J. Ophthalmol. **109** (4) 379 - 386.

Campbell, C. (1997) Reconstruction of the corneal shape with the MasterVue corneal topography system. Optom. Vis. Sci. **74**. (11) 899-905.

Carney, L.G. (1975) Effect of hypoxia on central and peripheral corneal thickness and corneal topography. Aust. J. Optom. **58**. 61 – 65.

Carney, L.G., Mainstone, J.C. and Henderson, B.A. (1997) Corneal topography and myopia: a cross sectional study. Invest. Ophthalmol. Vis. Sci. **38** (2) 311 - 320.

Chan, T.L. and Mandell, R. (1975) Corneal thickness change from bathing solutions. Am. J. Optom. Physiol. Optics. **52**. 467 - 469.

Chang, S.W. and Hu, F.R. (1993) Changes in corneal autofluorescence and corneal epithelium barrier function with aging. Cornea**.** **12** (6) 493 - 499.

Cheng, H., Bates, A.K, Wood, L. and McPherson, K. (1988) Positive correlation of corneal thickness and endothelial cell loss - serial measurements after cataract surgery. Arch. Ophthalmol. **106**. 920 - 922.

Cho, P., and Lam, C., (1999) Factors affecting the central corneal thickness of Hong Kong Chinese. Curr. Eye Research. **18**. 368 - 374.

Cho, P., and Cheung, S.W., (2002) Repeatability of corneal thickness measurements made by a scanning slit topography system. Ophthalmic.Physiol.Opt. **22**. 505 - 510.

Cho, P., and Cheung, S.W. (2000) Central and peripheral corneal thickness measurements with the TOPCON Specular Microscope SP-2000P.
Curr. Eye Res. **21** (4) 799 - 807.

Clark, B.A.J. (1972) Autocollimating keratoscope. J. Opt. Soc. Am. **62**. 169.

Clark, B.A.J. (1973a) Systems for describing corneal topography. Aust J. Optom. **56** (48). 49 - 56.

Clark, B.A.J. (1973b) Conventional keratoscopes - a critical review. Aust. J. Optom. **56**. 141 - 155.

Clark, B.A.J. (1974) Mean topography of normal corneas. Aust. J. Optom. **57**. 107.

Coleman, D.J. and Carlin, B. (1967) A new system for visual axis measurement in the human eye using ultrasound. Arch. Ophthalmol. **77**. 124 - 127.

Colin, J., Sale, Y., Malet, F., Cochener, B. (1996) Inferior steepening is associated with thinning of the inferotemporal cornea. J. Refract. Surg. **12** (6) 697 - 699.

Cook, C.A., Koretz, J.F., Pfahnl, A., Hyun, J., Kaufman, P.L. (1994) Aging of the human crystalline lens and anterior segment. Vision Res. **34**. 2945 - 2954.

Corbett, M.C., O'Brart, D.P., Saunders, D.C., Rosen, E.S. (1994)
a) The assessment of corneal topography, b) The topography of the normal cornea. Eur. J. Implant Ref. Surg. **6**. 98 - 105 and 286 - 297.

Corbett, M.C, O'Brart, D.P, Stultiens, B.A., Jongsma, F.H, Marshall, J. (1995) Corneal topography using a new moiré image-based system. Eur. J. Implant. Ref. Surg. **7**. 353 - 370.

Crook, T.G. (1979) Fluorescein as an aid in pachometry. Am. J. Optom. Physiol. Optics. **56** 124 - 127.

Cuaycong, M.J., Gay, C.A., Emery, J., Haft, E.A., Koch, D.D. (1993) Comparison of the accuracy of computerized videokeratography and keratometry for use in intraocular lens calculations. J. Cataract Refract. Surg. **19**. suppl. 178 -181.

Dave, T. (1998a) Current developments in the measurement of corneal topography. Contact lens and Anterior Eye **21** (suppl.) S13 - S30.

Dave, T., Ruston, D., and Fowler, C. (1998b) Evaluation of the EyeSys model II computerised videokeratoscope. Part 1: Clinical assessment. c) Part 2: The repeatability and accuracy in measuring convex aspheric surfaces. Optom. Vis. Sci. **75** (9) 647-662.

Dave, T., Rushton, D., Fowler, C. (1998c) Evaluation of the EyeSys model II computerised videokeratoscope. Part II: The repeatability and accuracy in measuring convex aspheric surfaces. Optom.Vis.Sci. **75** (9) 656 - 662.

Davis, L.J. and Dresner, M.S. (1991) A comparison of the EH-270 corneal topographer with conventional keratometry. C.L.A.O.J. **17** (3) 191 - 196.

Davson, H. (1955) The hydration of the cornea. Biochem. J. **59**. 24.

Daxer, A, Misof, K, Grabner, B, Ettl, A, Fratzl, P. (1998) Collagen fibrils in the human corneal stroma: structure and aging. Invest. Ophthalmol. Vis. Sci. **39** (3) 644 - 648.

De Cunha, D.A. and Woodward, E.G. (1993) Measurement of corneal topography in keratoconus. Ophthalmic Physiol. Opt. **13** (4) 377 - 382

Dingeldein, S.A. and Klyce, S.D. (1989) The topography of normal corneas. Arch. Ophthalmol. **107**. 512 - 518.

Dingeldein, S.A., Klyce, S.D. and Wilson, S.E. (1989) Quantitative description of corneal shape derived from computer assisted analysis of photokeratographs. Refract.Corneal Surg. **5**. 372.

Dohadwala, A., Munger, R., Damji, K., (1998) "Positive correlation between Tono-Pen IOP and central corneal thickness. Ophthalmology **105**. 1849 - 1854.

Dohlman, C.H. and Hyndiuk, R.A. (1972.) Subclinical and manifest corneal oedema after cataract extraction. Symposium on the Cornea. Trans. New Orleans. Àcad. Ophthalmol. Mosby. **2** (11) 214 - 231.

Donaldson, D.D. (1966) A new instrument for the measurement of corneal thickness. Arch. Ophthalmol. **76**. 25 - 31.

Doss, J.D., Hutson, R.L., Rowsey, J.J. and Brown, R.D. (1981) Method for calculation of corneal profile and power distribution. Arch. Ophthalmol. **99**. 1261 - 1265.

Doughty, M.J. and Zaman, M.L. (2000) Human corneal thickness and its impact on intraocular pressure measures: a review and meta-analysis approach. Survey of Ophthalmol **44** (5) 367 - 408.

Doughty, M.J., Laiquzzaman, M., Muller, A., Oblak, E., Button, N. (2002) Central corneal thickness in European (white) individuals especially children and the elderly, and assessment of its possible importance in clinical measures of intraocular pressure. Ophthalmic. Physiol. Opt. **22**. (6) 491 - 504.

Douthwaite, W.A. (1987) A new keratometer. Am. J. Optom. Physiol. Optics. **64** (9) 711 - 115.

Douthwaite, W.A. and Sheridan, M. (1989) The measurement of the corneal ellipse for the contact lens practitioner. Ophthalmic. Physiol.Opt. **9**. 239 - 242.

Douthwaite,W.A. (1995) EyeSys corneal topography measurements applied to calibrated ellipsoidal surfaces. Br. J. Ophthalmol. **79**. 797 - 801.

Douthwaite, W.A., Pardhan, S., Burek, H. (1996) Extent and effect of surface tilt on the data display of the EyeSys videokeratoscope. Br. J. Ophthalmol. **80**. (10) 986 - 993.

Douthwaite, W.A., Pardhan, S. (1998) Surface tilt measured with the EyeSys videokeratoscope: Influence on corneal asymmetry. Invest. Ophthalmol. Vis. Sci. **39**. 1727 - 1735.

Douthwaite, W.A., Hough, T., Edwards, K, Notay, H. (1999) The EyeSys videokeratoscopic assessment of apical radius and p-value in the normal human cornea. Ophthalmic. Physiol. Opt. **19** (6) 467 - 474.

Douthwaite, W.A. (2002) Application of linear regression to videokeratoscopy data for tilted surfaces. Ophthalmic. Physiol. Opt. **22**. 46 - 54.

Douthwaite,W.A. (2003) The asphericity, curvature and tilt of the human cornea measured using a videokeratoscope. Ophthalmic. Physiol. Opt. **23**. 141-150.

Dubbelman,M. (2001) The shape of the aging human lens, curvature, equivalent refractive index and the lens paradox. Vision Research. **41**. (14) 1867-1877.

Dubbelman, M., Van der Heijde, G.L., Weeber, H.A. (2001) The thickness of the aging human lens obtained from corrected scheimpflug images.
Optom.Vis.Sci. **78**. 411 - 416.

Dubbelman, M., Weeber, H.A., Van der Heijde, R.G., Volker-Dieben, H. (2002) Radius and asphericity of the posterior corneal surface determined by corrected scheimpflug photography. Acta Ophthalmologica. **80**. 379 - 383.

Dunne, M.C., Davies, L.N., Mallen, E.A., Kirschkamp, T. and Barry, J.C. (2005) Non-invasive phakometric measurement of corneal and crystalline lens alignment in human eyes. Ophthalmic. Physiol. Opt. **25**. 143-152.

Edmund, C. and Sjontoft, E. (1985) The central- peripheral radius of the normal corneal curvature. A photokeratoscopic study. Acta. Ophthalmologica. **107**. 512.

Edmund, C. and La Cour, M. (1986) Some components affecting the precision of corneal thickness measurement performed by optical pachometry.
Acta Ophthalmologica. **64**. 499 - 503.

Edmund, C. (1987) Determination of the corneal thickness profile by optical pachometry. Acta Ophthalmologica **65** (2) 147 -152.

Ehlers, N. and Hansen, F.K. (1971) On the optical measurement of corneal thickness. Acta Ophthalmologica. **49**. 65 - 81.

Ehlers, N. and Hansen, F.K. (1976) Further data on biometric correlations of central corneal thickness. Acta Ophthalmologica. **54**. 774 - 778.

Ehlers, N. and Sperling, S. (1977) A technical improvement of the Haag-Streit pachometer. Acta Ophthalmologica. **55**. 333 - 335.

Eleftheriadis, H. (2003) IOLMaster biometry refractive results of 100 consecutive cases. Br.J.Ophthalmol. **87**. (8) 960-963.

El Hage, S.G. (1971) Suggested new methods for photokeratoscopy- a comparison of their validities. Am. J. Optom. **40**. 897 - 912.

El Hage, S.G. (1989) A computerised corneal topographer for use in refractive surgery. Refract. Corneal Surg. **5**. 418 - 424.

El Hage, S.G. and Beaulne, G.C. (1973) Changes in central and peripheral corneal thickness with menstrual cycle.
Am. J. Optom. Arch. Am. Acad. Optom. **11**. 863 - 871.

El Hage, S.G. and Beaulne, G.C. (1975) Relationship between changes in corneal configuration and thickness. Am. J. Optom. Physiol. Optics. **52**. 823 - 833

Emsley, H. H. (1952) Visual Optics. Butterworths. London 5th Edition **1**. 309 - 331

Emsley, H. H. (1960) Revival of the keratometer. The Optician **139**. 585 - 589.

Eysteinsson, T., Jonasson, F., Sasaki, H., Arnarsson, A. et al (2002) Central corneal thickness, radius of the corneal curvature and intraocular pressure in normal subjects using non-contact techniques: Reykjavik Eye Study. Acta Ophthalmologica Scandinavica. **80**. 11-15.

Farrell, R.A. and McCally, R.L. (1973) On corneal transparency and its loss with swelling. J. Opt. Soc. Am. **66** (4). 342 – 345.

Fatt, I. and Harris, M.G. (1973) Refractive index of the cornea as a function of its thickness. Am. J. Optom. Arch. Am. Acad. Optom. **50**. 383 - 386.

Feldman, F., Bain, J., and Matuk, A.R. (1978) Daily assessment of ocular and hormonal variables throughout the menstrual cycle. Arch. Ophthalmol. **96**. 1835 - 1838.

Fontana, S.T. and Brubaker, R.F. (1980) Volume and depth of the anterior chamber in the normal aging human eye. Arch. Ophthalmol. **98**. 1803 -1808.

Foster, P.J., Baasanhar, J., Alsbirk, P.H. et al (1998) Central corneal thickness and intraocular pressure in a Mongolian population. Ophthalmology. **105**. 969 - 973.

Fowler, C.W. and Dave, T.N. (1994) Review of past and present techniques of measuring corneal topography. Ophthalmic Physiol. Optics. **14**. 49 - 58.

Friedman, M.H. (1973) Unsteady aspects of corneal thickness control.
Exp. Eye Res. **15** (5). 645 - 658.

Fry, G.A. (1975) Analysis of photometric data.
Amer. J. Optom. and Physiol. Optics. **52**. 305 - 312.

Fujikado, T., Kuroda, T., Ninomiya, S., Maeda, N., Tano, Y, Oshika, T., Hirohara, Y., Mihashi, T. (2004) Age-related changes in ocular and corneal aberrations. Am. J. Ophthalmol. **138** (1) 143-146.

Geroski, D.H., Matsuda, M., Yee, R.W., Edelhauser, H.F. (1985) Pump function of the

human corneal endothelium; Effects of age and corneal guttata.
Ophthalmology. **92** (6) 759-763.

Giasson, C. and Forthomme, D. (1992) Comparison of central corneal thickness measurements between optical and ultrasound pachometers.
Optom. Vis. Sci. **66** (3) 236 – 241.

Gonzalez-Meijome, J.M., Jorge, J., Queiros, A., Almeida, J. and Parafita, M. (2004) A comparison of the ARK-700A autokeratometer and the Medmont E300 corneal topographer when measuring peripheral corneal curvature. Ophthalmic Physiol. Opt. **24** 391-398.

Gordon, A., Boggess, E.A., Moliari, J.F. (1990) Variability of ultrasonic pachometry.
Optom. Vis. Sci. **67** (3) 162 - 165.

Gormley, D.J., Gersten, M., Koplin, R.S. and Lubkin, V. (1988) Corneal modeling.
Cornea. 7. 30 - 35.

Goss, D.A., Van Veen, H.G., Rainey, B.B., Feng, B. (1997) Ocular components measured by keratometry, phakometry and ultrasonography in emmetropic and myopic optometry students. Optom. Vis. Sci. **74** (7) 489 - 495.

Goto, T, Klyce, S.D., Zheng, X., Maeda, N., Kuroda, T., Ide, C. (2001) Gender and age related differences in corneal topography. Cornea. **20** (3) 270-276.

Green, K. (1995) Free radicals and aging of anterior segment tissues of the eye: a hypothesis. Ophthalmic Research **27** suppl. (1) 143 - 149.

Gritz, D.C. and McDonnell, P.J. (1990) Comparison of a computer-assisted laser pachometer with two ultrasonic pachometers in normal corneas.
Refract. Corneal. Surg. **6**. 9 - 14.

Gromacki, S.J. and Barr, J.T (1994) Central and peripheral corneal thickness in keratoconus and normal patient groups. Optom. Vis. Sci. **71** (7) 437 - 441

Grosvenor, T. (1987) Reduction in axial length with age: an emmetropizing mechanism for the adult eye. Am. J. Optom. Physiol. Opt. **64** (9) 657-663

Gudmundsdottir, D. (2001) With the rule astigmatism is not the rule in the elderly. Acta Ophthalmologica Scandinavia. **78** (6) 642-646.

Guillon, M. and Morris, J. (1982) Corneal evaluation of prospective aphakic wearers of contact lenses. Br. J. Ophthalmol. **66**. 520 - 523.

Guillon, M., Lydon, D., Wilson, C. (1986) Corneal topography – a clinical model. Ophthalmic Physiol. Optics. **6** (1) 47 - 56.

Gullstrand, A. (1911) In: Southall, J.P.(ed) (1962) Helmholtz's treatise in physiological optics Vols I , II. Publisher Dover. New York. Translated by Ludlum, S.M. (1966) Am. J. Optom. **43**. 143.

Haegerstrom-Portnay, G., Schneck, M., Brabyn, J., Lott, L. (2002) Development of refractive errors into old age. Optom. Vis. Sci. **79** (10) 643 - 649.

Hannush, S.B., Crawford, S.L., Waring, G.O.III., Gemmill, M.C., Lynn, M.J., Nizam, A. (1990) Reproducibility of normal corneal power measurements with a keratometer, photokeratoscope and video imaging system. Arch. Ophthalmol. **108**. 539 - 544.

Hansen, F.K. (1971) A clinical study of the normal human central corneal thickness Acta Ophthalmologica. **49**. 83 - 89.

Hardten, D.R and Lindstrom, L. (1993) Corneal complications of cataract extraction and intraocular lens implantation. Current Opinion in Ophthalmol. **4** (4) 99 - 105.

Harper, C.L., Boulton, M.E., Bennett, D., Marcyniuk, B., Jarvis-Evans, J.H., Tullo, A.B., Ridgway, A.E. (1996) Diurnal variations in human corneal thickness.

Br. J. Ophthalmol. **80** (12) 1068 - 1072.

Harris, J.H. and Nordquist, L.T. (1955) The hydration of the cornea: 1.The transport of water from the cornea. Am. J. Ophthalmol. **40**. 100.

Harris, M.G., Sarver, M.D., Polse, K.A. (1975) Corneal curvature and refractive error changes associated with wearing hydrogel contact lenses.
Am. J. Optom. Physiol. Optics. **52**. 313 - 319.

Hayashi, K., Masumoto, M, Fujino, S., Hayashi. F. (1993) Changes in corneal astigmatism with aging. Acta Societatis Ophthalmol. Japonicae. **97** (10) 1193 - 1196.

Hayashi, K., Hayashi, H., Hayashi, F. (1995) Topographical analysis of the changes in corneal shape due to aging. Cornea. **14** (5) 527 - 532.

Heath, G.G., Gerstman, D.R., Wheeler, W.H., Soni, P.S., Horner, D.G.
(1991) Reliability and validity of videokeratoscopic measurements.
Optom. Vision Sci. **68** (12) 946 - 949.

Helmholtz, H.von (1909)* Handbuch der physiologischen optik. Translated as Helmholtz's Treatise on Physiological optics by Southall, J.P.C. (1924)
Banta.Menasha. Wisconsin. USA. **1** (2) 301 - 335.

Hemenger, R.P., Garner, L.F., Ooi, C.S. (1995) Change with age of the refractive index gradient of the human ocular lens. Invest. Ophthalmol. Vis. Sci. **36** (3) 703 - 707.

Herndon, L.W., Choudhari, S.A., Cox, T., Damji, K.F., Shields, M.B., Allingham, R.R. (1997) Central corneal thickness in normal, glaucomatous and ocular hypertensive eyes. Arch. Ophthalmol. **115** (9) 1137 - 1141.

Herse, P, Akakura, N., Ooi, C.S. (1993) Topographical corneal oedema.
Acta Ophthalmologica. **71**. 539 - 543.

Hirji, N.K. and Larke, J.R. (1978) Thickness of human cornea measured by topographic pachometry. Am. J. Optom. Physiol. Optics. **55** (2) 97 - 100.

Hirji, N.K. and Larke, J.R (1978) Is corneal pachometry worth the effort? The Optician. Aug. 16. 14 - 18.

Hirji, N.K. and Larke, J.R. (1979) Corneal thickness in extended wear of soft contact lenses. Br. J. Ophthalmol. **63** (4) 274 - 276.

Hirsch, M.J. (1959) Changes in astigmatism after the age of forty. Am. J. Optom. Arch. Am. Acad. Optom. **36** (8) 395 - 405.

Hodson, S., Wigham, C., Williams, L., Mayes, K.R. and Graham, M.V. (1981) Observations on the human cornea in vitro. Exp. Eye. Res. **42**. 353 - 360.

Holden, B.A., Mertz, G.W. and Guillon, M. (1980) Corneal swelling response of the aphakic eye. Invest. Ophthalmol'. Vis. Sci. **19** (11) 1394 - 1397.

Holden, B.A., Polse, K.A., Fonn, D., Mertz, G.W. (1982) Effects of cataract surgery on corneal function. Invest. Ophthalmol. Vis. Sci. **22** (3) 343 - 350.

Holden, B.A., Menalley, J.J., Metz, G.W. and Swarbrick, H.A. (1985) Topographical corneal oedema. Acta Opthalmologica. (Copenhagen) **63**. 684 - 691.

Holden, B.A., Sweeney, D.F., Efron, N., Vannas, A. and Nilsson, K.T. (1985) Effects of long term extended contact lens wear on the human cornea. Invest. Ophthalmol. Vis. Sci. **26**. 1489 - 1501.

Holladay, J.T. (1997) Standardizing constants for ultrasonic biometry, keratometry and intraocular lens power calculations. J. Cataract Ref. Surg. **23** (9) 1356 -1370.

Holladay, J.T. (1997) Corneal topography using the Holladay diagnostic summary. J. Cataract. Refract. Surg. **23**. 209 - 211.

Hollingsworth, J., Perez-Gomez, I., Mutalib, H.A., Efron, N. (2001) Population study of normal cornea using an in-vivo slit scanning confocal microscope. Optom. Vis. Sci. **78** (10) 706-711.

Hosny, M., Alio, J.L., Claramonte, P., Attia, W.H., Perez-Santonja, J.J. (2000) Relationship between anterior chamber depth, refraction, corneal diameter and axial length. J. Refract. Surg. **16** (3) 336-340.

Hovding, G. (1983) A clinical study of the association between thickness and curvature of the central cornea. Acta Ophthalmologica **61**. 461-466.

Hovding, G. (1992) The central corneal thickness in kerato-conjunctivitis sicca. Acta Ophthalmologica **70**. 108 - 110.

Hovding, G., Natvik, C., Sletteberg, O. (1994) The refractive error after implantation of a posterior chamber intraocular lens. The accuracy of intraocular lens power calculation in a hospital practice. Acta Ophthalmologica. **72** (5) 612 - 616.

Huddart, T.M. (1998) The accuracy and reproducibility of keratometry readings. Elective studies submission, Aston University.

Hubbe, R.E. and Foulks, G.N. (1994) The effect of poor fixation on computer-assisted topographic corneal analysis. Ophthalmology **101**. 1745 - 1748

Jaeger, W. (1952) Tiefenmessung der menschlichen vorderkammer mit planparallelen platten. (zusatsgerat zur spaltlampe).
Albrecht Graefes Arch. Clin. Exp. Ophthalmol. **153**. 120 - 131.

Javal and Schiotz (1881) Annales d'Ocul. **86** (6) 5 - 20.

Jay, J.L. and Devlin, M.L. (1990) The increasing frequency of surgery for cataract. Eye. **4**. 127 - 131.

Jeandervin, M. and Barr, J. (1998) Comparison of repeat videokeratography: repeatability and accuracy. Optom. Vis. Sci. **75**. (9) 663-669.

Juillerat. and Koby, F. (1928). Determination de l'epaisseur de la cornee sur le vivant au moyen de la lampe a fente. Rev. Generale d'Ophthalmol. **42** (6) 203 - 227.

Kampfer, T., Wegener, A., Dragomirescu, V., Hockwin, O. (1989) Improved biometry of the anterior eye segment. Ophthalmic Res. **21** (3) 239 - 248.

Kanpolat, A., Simsek, T. Alp, N.M. (1997) The evaluation of normal corneal topography in emmetropic eyes with computer-assisted video keratography. C.L.A.O. J. **23** (3) 168 - 171.

Kiely, P.M., Smith, G. and Carney, L.G. (1982a) The mean shape of the human cornea. Optica Acta. **29**. 1027 - 1040.

Kiely, P.M., Carney, L.G., Smith, G. (1982b) Diurnal variations of corneal topography and thickness. Am. J. Optom. Physiol. Optics. **59** (12) 976 - 982.

Kiely, P.M., Carney, L.G., Smith, G. (1983) Menstrual cycle variations at corneal topography and thickness. Am. J. Optom. Physiol. Optics. **60** (10) 822 - 829.

Kiely, P.M., Smith, G. and Carney, L.G. (1984) Meridional variations of corneal shape. Am. J. Optom. Physiol. Optics. **61** (10) 619 - 626.

Kinge, S and Midelfart, A. (2000) Biometric changes in eyes of Norwegian University students, a three year longitudinal study. Acta Ophthalmologica. **77** (6) 648-652.

Klein, S.A. (1997a) Axial curvature and the skew ray error in corneal topography. Optom.Vis.Sci. **74**. (11) 931-944.

Klein, S.A. (1997b) Corneal topography reconstruction algorithm that avoids the skew ray ambiguity and the skew ray error. Optom.Vis.Sci. **74**. 11. 945-962.

Klyce, S.D. (1984). Computer assisted corneal topography. High resolution graphic presentation and analysis of keratoscopy. Invest. Ophthalmol. Vis. Sci. **25**. 1426 - 1435.

Klyce, S.D.,and Wilson, S.E. (1989) Methods of analysis of corneal topography. Ref.Corneal.Surg. **5**. 368-371.

Klyce, S.D. and Dingeldein, S.A. (1990) Corneal Topography. **61**. In: Non-invasive diagnostic techniques in ophthalmology. (ed: Masters B) Springer-Verlag. New York.

Klyce, S.D. and Beuerman, R.W. (1998) Structure and function of the cornea. In The Cornea. Eds. Kaufman H.E., Barron, B.B., McDonald, M.B. and Waltman, S.R. Churchill Livingston, New York. **1**. 3-50.

Knoll, H.A. (1961) Corneal contours in the general population as revealed by the photokeratoscope. Am. J. Optom. Arch. Am. Acad. Optom. **36**. 389 - 397.

Knoll, H.A. (1966) Mathematical model of the human eye.
Int. Eyecare. **2**. 54 - 55.

Koch, D.D., Foulks, G.N., Moran, C.T. and Wakil, J.S. (1989) The corneal EyeSys system: Accuracy, analysis and reproducibility of first generation prototype.
Refract. Corneal. Surg. **5**. 424.

Koch, D.D., Wakil, J.S., Samuelson, S.W. and Haft, E.A. (1992) Comparison of the accuracy and reproducibility of the keratometer and the EyeSys Corneal Analysis System, model 1. J. Cataract. Refract. Surg. **18**. 342 - 347.

Koch- Jensen, P., Rask, R., Olsen, T. (1995) Video controlled M-mode biometry. Acta Ophthalmologica. Scandinavia. **73**. 61 - 65.

Kohlrausch (1840) cited by Helmholtz in Handbuch der Physiologischer Optik (1909). Translated In Treatise on Physiological Optics. Ed Southall, J.P.
Dover publ. London. (1962).

Koretz, J.F., Kaufman, P.L., Neider, M.W., Goeckner, P.A. (1989) Accommodation and presbyopia in the human eye – aging of the anterior segment.
Vision Res. **29** (12) 1685 -1692.

Koretz, J.F., Cook, C.A., Kuszak, J.R. (1994) The zones of discontinuity in the human lens: Development and distribution with age. Vision Res. **34** (22) 2955 - 2962.

Koretz, J.F. and Cook, C.A. (2001) Aging of the optics of the human eye: lens refraction models and principal plane locations. Optom.Vis.Sci. **78**. 396 - 404.

Korey, M., Geiser, D., Kass, M.A., Waltman, S.R., Gordon, M. and Becker B. (1982) Central corneal endothelial cell density and central corneal thickness in ocular hypertension and primary open-angle glaucoma.
Am. J. Ophthalmol. **94** (5). 610 - 616.

Kotulak, J.C. and Brungart, T. (1980) Age-related changes in the cornea. J. Am. Optom. Assoc. **51** (8) 761 - 765.

Kremer F. B., Walton, P., Gensheimer, G. (1985) Determination of corneal thickness using corneal pachometry. Ann. Ophthalmol. **17**. 506 - 507.

Kwok, S.L. (1994) Corneal physiology and biophysics. In: Contact Lens Practice. Eds: Ruben, M. and Guillon, M. Chapman & Hall, London. **4**. (14) 239-268.

Lam, C.S., Goh, W., Tang, Y., Tsui, K., Wong, W.C., Man, T.C. (1994) Changes in refractive trends and optical components of Hong Kong Chinese aged over 40 years.
Ophthalmic Physiol.Opt. **14** (4) 383 - 384.

Lam, A.K. and Douthwaite, W.A. (1994) Derivation of corneal flattening factor, p-value. Ophthalmic Physiol. Opt. **14**. 423 - 427.

Lam, A.K. and Douthwaite, W.A. (1998) The corneal thickness profile in Hong Kong Chinese. Cornea **17**. 384 - 388.

Lam, A. and Chan, C. (1999) The aging effect on corneal curvature and the validity of Javal's rule on Hong Kong Chinese. Curr.Eye.Res. **18** (2) 83-90.

Lass, J.H., Greiner, J.V., Merchant, T.E., Glonek, T. (1995) The effects of age on phosphatic metabolites of the human cornea. Cornea. **14** (1) 89 - 94.

Latham, K. and Devlin, M.L. (1991) The increasing frequency of surgery for cataract. Eye. **5**. 369 - 372.

Latham, K. and Misson, M.L. (1997) Patterns of cataract referral in the West Midlands. Ophthalmic Physiol. Opt. **17**. 300 - 306

Lavergne, G. and Kelecom, J. (1962) Application cliniques de la mesure de l'epaisseur de la cornee. Bull. Soc. Belge. Ophtalmol. **131**. 323 - 333.

Lawrenson, J.G. (1997) Corneal sensitivity in health and disease. Ophthalmic. Physiol. Opt. **17** (1) S.17 - S.22.

Lehmann, S.P, (1967) Corneal areas utilised in keratometry.
The Optician. **154** (3989) 261 – 264.

Leighton, D. and Tomlinson, A. (1972) Changes in axial length and other dimensions of the eyeball with increasing age. Acta Ophthalmologica **50**. 815-826.

Levene, J.R. (1965) The true inventors of the keratoscope.
Br. J. History. Sci. **2**. 324 - 342.

Levene, J.R. (1977) Clinical refraction and visual science. Butterworths. London. **5**. 128 - 129.

Li, J., Zhou, F., Zhou, S. (1994) Research on corneal thickness at multi-points in normal and myopic eyes. Chinese J. Ophthalmol. **30** (6) 445 - 448.

Loganesi, L., Cavallini, G.M., Toni, R. (1996) A morphometric study by ultrasonic pachymetry and computer-assisted topographic videokeratoscopy.
Acta Anatomica 157 (1) 73 - 79.

Lowe, R.F. (1969) Central corneal thickness. Ocular corrections in normal eyes and those with primary angle-closure glaucoma. Br. J. Ophthalmol. **53**. 824 - 826.

Ludlam, W.M, and Wittenberg, S. (1966) Measurements of the ocular dioptric elements using photographic methods.
Am. J. Optom. Arch. Am. Acad. Optom. **43**. 249 - 267,

Ludlam, W.M., Wittenberg, S., Rosenthal, P., Harris, M. (1967)
Photographic analysis of the ocular dioptric components. Am. J. Optom. **44**. 276 - 296.

Lyle, W.M. (1971) Changes in corneal astigmatism with age.
Am. J. Optom. Arch. Am. Acad. Optom. **48**. 467 - 478.

Maguire, L.J., Singer, D.E. and Klyce, S.D. (1987) Graphic presentation of computer-analyzed keratoscope photographs. Arch. Ophthalmol. **105**. 223 - 230.

Maguire, L.J. (1988) Corneal topography. The Cornea, Churchill Livingstone Inc. USA. **34**. 897 - 909.

Malik, N.S. and Meek, K.M. (1996) Vitamins and analgesics in the prevention of collagen ageing. Age and Ageing. **25** (4) 279-284.

Mallen, E.A., Gammoh, Y., Al-Bdour, M., Sayegh, F.N. (2005) Refractive error and ocular biometry in Jordanian adults. Ophthalmic Physiol. Opt **25**. (4) 302-309.

Maloney, R.K., Bogan, S.J., Waring G.O. III (1993) Determination of corneal image-forming properties from corneal topography. Am. J. Ophthalmol. **115** (1) 31 - 41.

Mandell, R.B. (1962a) Reflection point ophthalmometry. Am. J. Optom. Arch. Am. Acad. Optom. **39** (10) 513 - 537.

Mandell, R.B. (1962b) Methods to measure the peripheral corneal curvature. Part 3. Ophthalmometry. J. Am. Optom. Assoc. **33**. 869 - 892.

Mandell, R.B. (1964) Corneal area utilized in keratometry. Am. J. Optom. **41**. 50.

Mandell, R.B. (1965) Corneal topography. Contact Lens Practice - Basic and Advanced, Thomas. Springfield. Illin. USA. Ch.3.

Mandell, R.B, and Fatt, I. (1965). Thinning of the human cornea on awakening. Nature. **208**. 292 - 293.

Mandell, R.B, and St. Helen, R. (1968) Stability of the corneal contour. Am. J. Optom. **45**. 797 - 806.

Mandell, R.B. and Polse, K.A, (1969) Keratoconus: spatial variation of corneal thickness as a diagnostic test. Arch. Ophthalmol. **82**. 162 - 168.

Mandell, R.B. and York, M.A. (1969) A new calibration system for photokeratoscopy. Am. J. Optom. Arch. Am. Acad. Optom. **46**. 410 - 420.

Mandell, R.B, and St.Helen, R. (1971) Mathematical model of the corneal contours. Br. J. Physiol. Optics. **26** (3) 183 - 186.

Mandell, R.B., Polse, K.A., Bonanno, J. (1988) Reassessment of optical pachometry. The Cornea: Trans World Congress on Cornea III. Ed. Cavanagh, H.D. Raven Press Ltd. New York. Chp **35**. 201- 205.

Mandell, R.B. (1992) The enigma of the corneal contour.
C.L.A.O. J. **18** (4) 267 - 273.

Manning, C.A., Kloess, P.M. (1997) Comparison of portable automated Keratometry and manual Keratometry for intraocular lens calculation.
J. Cataract Refract. Surg. **23** (8) 1213 - 1216.

Marsich, M.W., Bullimore, M.A. (2000) The repeatability of corneal thickness measures. Cornea. **19**. 792 - 795.

Martola, D.M. and Baum, J.L. (1968) Central and peripheral corneal thickness, Arch. Ophthalmol. **79**. 28 - 30.

Mattioli, R. and Tripoli, N.K. (1997) Corneal geometry reconstruction with the Keratron videokeratographer. Optom.Vis.Sci. **74**. (11) 881-894.

Maurice, D,M. and Giardini, A,A. (1951) A simple optical apparatus for measuring the corneal thickness and the average thickness of the human cornea.
Br. J. Ophthalmol. **35**. 169 - 177.

Maurice, D.M. (1962) Clinical physiology at the cornea.
Int. Ophthalmol. Clinic. **2**. 561 - 572.

Maurice, D.M. (1969) The cornea and sclera. The Eye. Ed.: Davson. New York Acad.Press, **1**. (521) 26 - 29.

Maurice, D.M. (1972) The location of the fluid pump in the cornea. J. Physiol. **221**. 43.

McCarey, B.E., Zurawski, C.A., O'Shea, D.S. (1992) Practical aspects of a corneal topography system. C.L.A.O. J. **18** (4) 248 - 254.

Meek, K.M. and Leonard, D.W. (1993) Ultrastructure of the corneal stroma: a comparative study. Biophysical J. **64** (1) 273-280.

Midelfart, A., Aamo, B. (1994) Ocular parameters in the elderly in Norway. Acta Ophthalmologica. **72** (1) 61 - 66.

Millodot, M. and Owens, H. (1984) The influence of age on the fragility of the cornea. Acta Ophthalmologica. **62** (5) 819 - 824.

Mishima, S. and Hedbys, B.O. (1968). Measurement of corneal thickness with the Haag-Streit pachometer. Arch. Ophthalmologica. **80**. 710 - 113.

Mishima, S. (1968) Corneal thickness. Surv. Ophthalmol. **13**. 57 - 96.

Molinari, J.F. (1982) A review of pachometry. Am. J. Optom. Physiol. Optics. **59** (11) 912 - 917,

Molinari, J.F. and Bunds, T. (1983) Pachometry: A comparison between touch and overlap method. Am. J. Optom. Physiol. Optics. **60** (1) 61 - 66.

Morlet, N. (1994) Clinical utility of the Barrett keratoscope with astigmatic dial. Ophthalmic Surg. **25**. 150 - 153.

Morrow, G.L. and Stein, R.M. (1992) Evaluation of corneal topography, past, present and future trends. Can. J. Ophthalmol. **27**. 214 - 225.

Naufal, S.C., Hess, J.S., Friedlander, M.H., Granet, N.S. (1997) Rasterstereography-based classification of normal corneas. J. Cataract Ref. Surg. **23**. 222 - 230.

Nilsson, S.E. and Morris, J.A. (1983) Corneal thickness response to high and low water content lenses in aphakic eyes. Br. J. Ophthalmol. **61** (5) 311 - 319.

Ninn-Pedersen, K. (1996) Relationships between pre-operative astigmatism and corneal optical power, axial length, intraocular pressure, gender and patient age.
J. Refract.Surg. **12** (4) 472 - 482.

Nissen, J., Hjortdal, J.O., Ehlers, N., Frost-Larsen, K., Sorensen, T. (1991) A clinical comparison of optical and ultrasonic pachometry.
Acta Ophthalmologica. **69**. 659 - 663.

Nzekwe, E.U. and Maurice, D.M. (1994) The effect of age on the penetration of fluorescein into the human eye. J. Ocular. Pharmacol. **10** (3) 521 - 523.

Olsen, T., Nielsen, C.B., Ehlers, N. (1980a) On the optical measurement of corneal thickness: optical principle and sources of error. Acta Ophthalmologica **58**. 760 - 766.

Olsen, T., Nielsen, C.B., Ehlers, N. (1980b) On the optical measurement of corneal thickness: the measuring conditions and sources of error.
Acta Ophthalmologica **58**. 775 - 784.

Olsen, T. and Ehlers, N. (1984) The thickness of the human cornea as determined by a specular method. Acta Ophthalmologica. **62** (6) 859 - 871.

Oltrup, T., Jean, B., Bende, T. (1997) Automated compensation of defocusing errors in videokeratography. C.L.A.O.J. **23** (3) 157 - 160.

Ooi, C.S. and Grosvenor, T. (1995) Mechanisms of emmetropization in the ageing eye. Optom. Vis. Sci. **72** (2) 60 - 66.

O'Neal, M.R. and Polse, K.A. (1986) Decreased endothelial pump function with aging. Invest. Ophthalmol. Vis. Sci. **27** (4) 457 - 463.

Pardhan, S. and Beesley, J. (1999) Measurement of corneal curvature in young and old normal subjects. J.Refract.Surg. **15** (4) 469-474.

Patel, S. (1981) Some theoretical factors governing the accuracy of corneal thickness measurement. Ophthalmic. Physiol. Optics. **1**. 193 - 203.

Patel, S. (1987) Refractive index of the mammalian cornea and its influence during pachometry. Ophthalmic. Physiol. Optics. 7 (4) 503 - 506.

Patel, S. and Farrell, J.C. (1989) Age related changes in personal tear film stability Optom.Vis. Sci. 1989. **66**. 175.

Patel, S. and Stevenson, R.W. (1994) Clinical evaluation of a portable ultrasonic and a standard optical pachometer. Optom.Vis.Sci. **71** (1) 43 - 46.

Patel, C.K. and Bron, A.J. (2001) The ageing eye. Optometry Today. 27 - 31.

Paul, R.G., Bailey, A.J. (1996) Glycation of collagen:the basis of its central role in the late complications of ageing and diabetes.
Int. J. Biochem. Cell Biol. **28** (12) 1297 - 1310.

Pavlopoulos, G.P., Horn, J., Feldman, S.T. (1995) The effect of artificial tears on computer-assisted corneal topography in normal eyes and after penetrating keratoplasty. Am. J. Ophthalmol. **119** (6) 712 - 722.

Pierscionek, B.K., Weale, R.A. (1995) The optics of the eyelens and lenticular senescence: a review. Doc. Ophthalmologica. **89** (4) 321 - 335.

Placido (1880) Novo instrumento de esploracao de cornea. Periodico d'Ofthalmologica Practica. Lisbon. **5**. 27-30. cited by Woodward, E.G. (1980) Keratoconus, the disease and its progression. Doc. Thesis. City Univ. London.

Pointer, J.S. (1996) Age-distribution of patients attending for an optometric examination. Br J. Optom. Disp. **4**, 89 - 92.

Polse, K.A., Brand. R., Mandell, R., Vastine, D., Demartini, D., Flom, R. (1989) Age differences in corneal hydration control. Invest. Ophthalmol. Vis. Sci. **30** (3) 392 - 399.

Potvin, R.J., Fonn, D., Sorbara, L. (1996) In vivo comparison of corneal topography and keratometry systems. Int. C. L. Clinic. **23**. 20 - 25.

Rabinowitz, Y., Yang, H., Brickman, V. et al (1996) Videokeratography database of normal human corneas. Br. J. Ophthalmol. **80**. 610 - 616.

Rapuano, C.J., Fishbough, J.A., Strike, D.J. (1993) Nine point corneal thickness measurement and keratometry reading in normal corneas using ultra-sound pachymetry. Insight. **18** (4) 10 - 22.

Rasheed, K., Rabinowitz, Y., Remba, D., Remba, M. (1998) Interobserver and intraobserver reliability of a classification scheme for corneal topographic patterns. Br. J. Ophthalmol. **82**. 1401 - 1406.

Reading, V.M. (1984) Astigmatism following cataract surgery. Br. J. Ophthalmol. **68**. 97 - 104.

Riss, I., Dupuy, B. and Hostyn, F. (1992.) Critical analysis and optimal use of the photokerato-analyser (PKA). Eur. J. Impl. Ref. Surg. **4**. 37 – 41.

Roberts, C. (1994) The accuracy of 'power' maps to display curvature data in corneal topography systems. Invest.Ophthalmol.Vis.Sci. **35**. 3525-3532.

Roddy, KC. and Goss, D.A. (1988). Reliability of corneal topography measurements with the Corneascope and Comparator. Int. C. L. Clin. **15** (9) 287 - 290.

Rosenfield, M., Portello, J.K. (1996) Multi-meridional keratometry . Ophthalmic. Physiol. Optics. **16** (1) 83 - 85.

Rowsey, J.J., Reynolds, A.E. and Brown, R. (1981) Corneal topography - a corneascope. Arch. Ophthalmol. **99**. 1093 - 1100.

Rowsey, J.J. and Isaac, M.S. (1983) Corneoscopy in keratorefractive surgery.
Cornea. **2**. 133 - 142.

Rowsey, J.J., Monlux, R., Balyeat, H.D. et al (1989) Accuracy and reproducibility of Kerascanner analysis in PERK corneal topography.
Curr. Eye Res. **8** (7) 661 - 674.

Ruben, M. (1975) Introduction to kerato-topography and keratometry.
Contact Lens Practice, Visual, Therapeutic and Prosthetics, Balliere Tyndall. London. **6**. 97 - 103.

Ruben, M. and Woodward, E.G. (1982) Revision clinical optics.
Macmillan Press, Hong Kong. Section **4**. 112 - 118.

Ruskell, G.L. (1997) Anatomy and physiology of the cornea and related structures. In: Contact Lenses. Eds. Phillips, A.J.and Speedwell, L. Butterworths. London. **2**. 17-49.

Salz, J.J., Azen, S.P., Berstein, J., Caroline, P., Villasensor, R.A., Schanzlin, D.J. (1983) Evaluation and comparison of sources of variability in the measurement of corneal thickness with ultrasonic and optical pachymeters.
Ophthalmic. Surg. **14** (9) 150 - 754.

Sanders, D.R., Gills, J.P. and Martin, R.G. (1993) When keratometric measurements do not accurately reflect corneal topography. J. Cataract Refract. Surg. **19**. suppl. 131-135.

Savage, G.L., Haegerstrom-Portnay, G., Adams, A.J., Hewlett, S.E. (1993) Age changes in the optical density of human ocular media. Clin.Vis. Sci. **8**. 97 - 108.

Sayegh, F.N. (1996) The correlation of corneal refractive power, axial length, and the refractive power of the emmetropizing intraocular lens in cataractous eyes. German J. Ophthalmol. **5** (6) 328 - 331.

Schultz, D.N. (1976) Asymmetry of central and peripheral corneal astigmatism measured by photokeratoscopy. Am. J. Optom. Physiol. Opt. **54** (11) 776 - 781.

Schwiegerling, J. and Grevenkamp, J.E. (1997) Using corneal height maps and polynomial decomposition to determine corneal aberrations. Optom. Vis. Sci. **74**. (11) 906-916.

Sheng, H., Bottjer, C.A., Bullimore, M.A. (2004) Ocular component measurement using the Zeiss IOLMaster. Optom. Vis. Sci. **81**. (1) 27-34.

Sheridan, M. (1989) Keratometry and slitlamp biomicroscopy. Contact Lenses. 3rd Edition. Ed. Phillips, A.J. and Stone, J. Butterworths, London, **6**. 23 - 249.

Shum, P.J., Ko, L.S., Ng, C.L., Lin, S.L. (1993) A biometric study of ocular changes during accommodation. Am J. Ophthalmol. **115** (1) 76 - 81.

Siu, A.W. and Herse, P.R. (1993a) The effect of age on human corneal thickness: Statistical implications of power analysis. Acta. Ophthalmol. **71**. 51 - 56.

Siu, A.W. and Herse, P.R. (1993b) The effect of age on the oedema response of the central and mid-peripheral cornea. Acta Ophthalmologica. **71** (1) 57 - 61.

Smith, G., Atchison, A., Pierscionek, B.K. (1992) Modelling the power of the ageing human eye. J. Opt.Soc. Am. **9**. 2111 - 2117.

Snyder, A.C. and Schoessler, J.P. (1983) Corneal thickness changes associated with daily and extended contact lens wear. Am. J. Optom. Physiol. Optics. **60** (10) 830 - 838

Snyder, A.C. (1984) Optical pachometry measurements: reliability and variability. Am. J. Optom. Physiol. Optics. **61** (6) 408 - 413.

Sorsby, A., Benjamin, J., Davey, M., Sheridan, M., Tanner, J. (1957) Emmetropia and its aberrations. London. Her Majesty's Stationery Office.

Stevenson, R., Vaja, N., Jackson, J. (1983) Corneal transparency changes resulting from osmotic stress. Ophthalmic. Physiol. Optics. **3** (1) 33 - 39.

Stevenson, R.W. (1989) Automated recording and analyses of pachometrv data: a technical note. Contact Lens J. **17** (10) 323 - 326

Stevenson, R.W. (1992) Corneal topographic modelling systems. Optician. **11**. 16 - 22.

Stenstrom, S. (1948) Investigation of the variation and the correlation of the optical elements of human eyes. Part III.
Am. J. Optom. Am Arch Acad..Optom **25**. 40 - 350.

Stone, J. (1962) The validity of existing methods of measuring corneal contour compared with suggested new methods. Br. J. Physiol. Optics. **19** (4) 205 - 230.

Stone, J. (1975) The measurement of corneal thickness. The Contact Lens J. **5** (5) 14-19.

Stucchi, C.A., Gennari, G., Aimino, G., di Darlo, I., Bauchiero, L. (1993) Systematic error in computerised pachymetry. Ophthalmologica **207**. 208 - 244.

Suda, T. (1984) Mosaic pattern changes in human corneal endothelium with age. Japan J. Ophthalmol. **28** (4) 331 - 338.

Sunderraj, P. (1992) Clinical comparison of automated and manual keratometry in preoperative ocular biometry. Eye. **6**. 60 - 62.

Tang, W., Collins, M.J., Carney, L., Davis, B. (2000) The accuracy and precision performance of four videokeratoscopes in measuring test surfaces.
Optom. Vis. Sci. **77** (9) 483 - 491.

Tanjong Pagar Survey (2003) Refractive errors, axial ocular dimensions and age related cataract. Invest. Ophthalmol. Vis. Sci. **44** (4) 1479-1485.

Taylor, A., Jacques, P.F., Epstein, E.M. (1995) Relations among aging, antioxidant status and cataract. Am. J. Clinical Nutrition **62** (6) 1439 - 1447.

Tomlinson, A. (1972) A clinical study of the central and peripheral thickness variation at the human cornea. Acta. Ophthalmol. **50** (1) 73 - 82.

Townsley, M.G. (1967) New equipment and methods for determining the contour of the human cornea. Contacto. **11**. 72 - 81.

Townsley, M.G. (1970) New knowledge of the corneal. contour. Contacto. **14.** 38 - 43.

Tripathi, B.J., Tripathi, R.C., Borisuth, N.S. (1994) Microscopic anatomy and ultrastructure of the corneal, conjunctiva and lids. In: Contact Lens Practice. Eds: Ruben, M. and Guillon, M. Chapman & Hall. London. **12** 195-224.

Tripoli, N.K., Cohen, K.L., Obla, P., Coggins, J.M., Holmgren, D.E. (1996) Height measurement of astigmatic test surfaces by a keratoscope that uses plane geometry surface reconstruction . Am. J. Ophthalmol. **121**. 668 - 676.

Tsilimbaris, M.K., Vlachonikolis, I.G., Siganos, D., Makridakas, G., Pallikaris, I.G. (1991) Comparison of keratometric readings as obtained by Javal ophthalmometer and corneal analysis system (EyeSys). Refract. Corneal Surg. **7**. 368 - 373.

Van den Berg, T.J. and Tan, K.E. (1994) Light transmittance of the human cornea from 320 to 700 nm for different ages. Vision Res. **34** (11) 1453 - 1456.

Vannas, A., Holden, B.A., Sweeney, D.F., Polse, K. (1985) Surgical incision alters the swelling response of the human cornea. Invest. Ophthalmol. Vis. Sci. **26**. 864 - 868.

Vihlen, F.S., Wilson, G. (1983) The relation between eyelid tension, corneal toricity and age. Invest. Ophthalmol. Vis Sci. **24**. 1367 – 1373.

Von Bahr, G. (1948) Measurements of the thickness of the cornea. Acta. Ophthalmologica. **26**. 247 - 265.

Waring, G.O.III, Hannush, E.B., Bogan, S.J., Maloney, R.K. (1992) Classification of corneal topography with videokeratography. Corneal topography - measuring and modifying the cornea. Ed. Schanzlin, D.J., Robin, J.B. Springer-Verlag. New York Inc. **6**. 47 – 73.

Warniki, J.W., Rehkopf, P.G., Curtin, D.Y., Burns, S.A., Arffa, R.C., Stuart, A.C. (1988) Corneal topography using computer analysed rasterstereographic images. Appl. Optics. **27** (6).1135 - 1140.

Weale, R.A. (1971) The ageing eye. The Scientific Basis of Medicine. Ann. Reviews. **14**. 244 - 260.

Weale,R.A. (1989) Presbyopia toward the end of the 20th century. Surv. Ophthalmol. **34**. 15 - 30.

Wesley, N.K. (1969) Practical application and new concepts with the Photoelectronic keratoscope. Contacto. **13**. 32 - 43.

Wheeler, N.C., Morantes, C.M., Kristensen, R.M., Pettit, T.H., Lee, D.A. (1992) Reliability coefficients of three corneal pachymeters. Am. J. Ophthalmology **113** (6) 645 - 651.

Whelehan, I.M., Heyworth, P., Tabaudeh, H., McGuigan, S., Foss, A.J. (1996) A comparison of slitlamp supported versus hand-held biometry. Eye. **10** (4) 514 - 516.

Wigham, C.G. and Hodson, S.A. (1987) Physiological changes in the cornea of the ageing eye. Eye. **1** (2) 190 - 196.

Wilson, R.S. and Roper-Hall, M.J. (1982) Effect of age on the endothelial cell count in the normal eye. Br. J. Ophthalmol. **66**. 513 - 515.

Wilson, S.E. and Klyce, S.D. (1991) Advances in the analysis of corneal topography. Surv. Ophthalmol. **35**. 269 - 277.

Wilson, S.E., Klyce, S.D. (1991) Quantitative descriptors of corneal topography: A clinical study. Arch. Ophthalmol. **109**. 349 - 353.

Wilson, S.E., Wong, S.Y., Klyce, S.D. (1992) Quantification and mathematical analysis of photokeratoscopic images. **1**. In: Corneal Topography, Measuring and modifying the cornea. (eds. Schanzlin, D.J., Robin, J.B.) Springer Verlag. New York.

Wilson, S.E., Verity, S.M., Conger, D.L. (1992) Accuracy and precision of the corneal analysis system and the topographical modelling system. Cornea. **11** (1) 28 - 35.

Wilson, S.E., Klyce, S.D. (1994) Screening for corneal topographic abnormalities before refractive surgery. Ophthalmology. **101** (1) 147 - 152.

Wolfs, R.C., Klaver, C.C., Vingerling, J.R., Grobbee, D.E., Hoffman, A., de Jong, P. (1997) Distribution of central corneal thickness and its association with intraocular pressure. The Rotterdam Study. Am. J. Ophthalmol. **123** (6) 767 - 772.

Wong, D, Foster, D. (2001) Variations in ocular biometry in an adult chinese population in Singapore. Invest Ophthalmol. Vis. Sci. 42 (1) 73-80.

Wong, A.C., Wong, C.C., Yuen, S.S., Hui, S.P. (2002) Correlation study of central corneal thickness measurement on Hong Kong Chinese using optical coherence tomography, Orbscan and ultrasound. Eye. **16** (6) 715-721.

Woodward, E.G. (1980) Keratoconus, the disease and its progression. Doc. Thesis. City University. London.

Yebra-Pimentel, E., Giraldez, M.J., Gonzalez, J., Perez, M.V., Parafita, M. (1998) Changes in corneal thickness after daily and extended wear of hydrogel lenses: a comparison of optical and ultrasound pachometry. Int. C. L. Clinic **25**. 21 - 22.

Young, J.A., Talamo, J.H., Siegel, I.M. (1995) Contour resolution of the EyeSys corneal analysis system. J. Cataract Refract. Surg. **21**. 404 - 406.

Zabel, R.W., Tuft, S.J., Fitzke, F.W., Marshall, J. (1989) Corneal topography; A new photokeratoscope. Eye. **3**. 298 - 301.

Zadnik, K., Mutti, D.O., Adams, A.J. (1992) The repeatability of measurement of the ocular components. Invest. Ophthalmol. Vis. Sci. **33** (7) 2325 - 2333.

Zadnik, K., Friedman, N.E., Mutti, D.O. (1995) Repeatability of corneal topography: the corneal field. J. Refract. Surg. **11**. 119 - 125.

Appendix

Statistical Analyses

A.1 Introduction

The large quantity of data collected in this study, nearly 8000 measurements taken from 295 subjects, required careful analysis to try and determine the effect of age on the various parameters measured. In addition, several parameters were compared to each other to investigate whether a relationship between them could be demonstrated.

The name, reference number, age and gender of each subject were recorded. On Aston University subjects (Groups B and C) keratometry, EyeSys keratometry, EyeSys eccentricity measurement were carried out in horizontal and vertical corneal meridians. The hospital subjects (Groups D and E) underwent keratometry in horizontal and vertical meridians and had biometry, i.e. ultrasound measurements, which gave ocular axial length, anterior chamber depth and lens thickness. From the keratometry measurements the corneal astigmatism was calculated. Subjects in Group B had their corneal thickness measured using a pachometer in five corneal regions: Superior, Central, Inferior, Nasal and Temporal. (SCINT).

Data was initially entered into a series of Microsoft Excel spreadsheets. Scatter plots were generated of each parameter versus age to gain an idea of the spread of the results and to assist in the decision of how to analyse the data.

A.2 Statistical Methods

A number of statistical methods could be applied to the data. Reference was made to a number of papers describing the application of statistical methods to optometric measurements, in particular the series by Armstrong et al. (2000, 2002, 2004, 2005) and to the textbooks by Hayslett and Murphy (1979), Walpole (1982), and Hulley and Cummings (1988). The benefits and restrictions of some of the analysis methods were considered.

The analysis methods include:

1. Student's 't' test – comparison of means of two sample groups
2. Pearson's correlation – applicable to linear relationships between parameters X and Y
3. Regression coefficient - measure of change in Y per unit of X
4. Standard error of regression coefficient – enables 't' test of regression coefficient
5. Prediction of confidence interval for mean Y at specific X
6. Prediction of confidence interval for individual Y at specific X
7. ANOVA – analysis of variance, to estimate the degree of variation of a particular measurement and to estimate the differences between subject groups.
8. Post-hoc tests following ANOVA

A.2.1 Student's 't' Test

The Student's 't' test compares the mean values and the variation of individual measurements, i.e. standard deviations, of two data sets. For example comparing a young age group to an older age group.
To determine whether there is a significant difference between the means of two samples in an experiment, knowledge is required of the degree of variability of the difference between two sample means. For each sample the mean is calculated and the difference between the two means represents the effect of the treatment given to one of the samples. In this study the 'treatment' is ocular ageing. If the distributions of the 1st and 2nd sample means are normally distributed, then the distribution of the differences between pairs of means taken from these two populations will also be normally distributed. When 't' is calculated, the difference between the two sample means is converted so that it becomes a member of the 't' distribution. Then one must judge the probability of obtaining a 't' value of this size by chance. The computer software provides the p probability corresponding to this calculated 't' value, taking into account the degrees of freedom.

If the two samples are related, e.g. the same subjects are used in both samples but in the second sample they have undergone a treatment, then the 't' statistic more likely relates to changes incurred by the treatment only. However, if the samples are unrelated, i.e. two different subject groups are measured, it is more difficult to prove whether the second sample data are different due to the treatment or to natural individual variations. If the variation amongst experimental subjects is large, this variation will increase the standard error of the difference and also lower the value of 't', even if the difference between the group means appears relatively large.

In an unpaired experimental design, the subjects are allocated at random and without restriction to the control and treated sample groups. Compared to a paired design, this arrangement allows for a larger number of degrees of freedom and therefore a smaller value of 't' is required to demonstrate a significant difference between means. A paired design, e.g. subjects put into logical pairs according to gender, race, age or ocular characteristics, can be used where a significant reduction in the standard error is likely to be achieved. If there is no reduction in the standard error due to pairing, a paired design gives a disadvantage because it has a smaller number of degrees of freedom.

Sometimes prior knowledge can help to guide the choice of analyses. For example, if the null hypothesis states that the treatment has no effect on a variable, then an increase or a decrease in value would refute this hypothesis. Therefore a two-tailed 't' test would be appropriate. However, if a hypothesis specifies that a positive effect is necessary to refute the null hypothesis, then a one tailed 't' test would be appropriate.

A.2.2 Linear Regression

Another method is to attempt to fit a regression line or a formula that describes the data. Linear regression compares many data points of a parameter and relates them to a variable, e.g. age, to ascertain whether a linear relationship can be demonstrated. The magnitude of the association between the parameter and the variable in a sample can be expressed as the regression coefficient. The angle of slope of the best-fit regression line gives an indication of how strong the influence of age is on the parameter. The closer this slope is to zero the less the influence of age on the parameter is shown.

Once the assumption of linearity of regression is justified, to avoid laborious calculations the linear regression equation is preferred over a non-linear regression curve to predict estimates of parameter values.

A.2.3 ANOVA

The analysis of the variance is termed ANOVA. The ANOVA method comprises different variations. The appropriate variation should be selected for specific experimental conditions. In an analysis of variance of a one-way design the method calculates the confidence interval for the difference between two means. The basis of an ANOVA is the testing of the difference between two mean squares. To compare between treatments and error mean squares, the sums of squares are divided by the appropriate degrees of freedom (DF), where DF of a quantity is the number of parameters estimated from the data that are used to calculate the quantity (Armstrong and Eperjesi (2002)). The variance ratio, F, is calculated by dividing the treatment mean square by the error mean square. The 'p' value is the probability of obtaining the study results by chance, if the null hypothesis is true. The null hypothesis (e.g. that age has no influence on the parameter) is rejected in favour of the alternative (e.g. age does influence the parameter) if the p value is less than the predetermined level of statistical significance, usually $p = 0.05$. If the p value is greater than 0.05 there may be a relationship between age and the parameter, but the association is small compared to that which could occur by chance. The relationship is then termed 'not significant'.

There are a series of ANOVA variations of increasing complexity likely to be considered when analysing data in optometric research (Armstrong et al 2002). The simplest is ANOVA one-way, then ANOVA two-way in randomised blocks, ANOVA three-way, factorial ANOVA, factorial ANOVA, split-plot design and factorial ANOVA repeated measures design. For this study the appropriate ANOVA variation was in most cases the ANOVA one-way method.

A.2.4. Post-hoc Tests

Finally the requirement for post-hoc tests was considered. These are analyses not explicitly planned at the start of a study but are suggested during the examination of the data, e.g. multiple comparison procedures, sub-group analyses and planned comparisons. Multiple comparison tests are procedures for the detailed examination of differences between a set of means, usually after a hypothesis that they are all equal has been rejected. These tests are used to check that a particular analysis has not suffered from a Type 1 error by rejecting the null hypothesis when it is true or a Type 2 error by accepting the null hypothesis when a real difference is present. A major distinction between techniques is how they control the possible inflation of the Type 1 error.

The following are methods of making post-hoc tests:

Fisher PLSD, Student-Newman-Keuls and Bonferroni tests require equal numbers of replicates in each treatment group.
Tukey-Kramer HSD, Tukey-Compromise, Spjotvoll-Stoline and Duncan's Multiple Range Test cope with unequal replicates but require equal Mean Squares.
Dunnett's Test, Games/Howell and Scheffe's S Test are robust methods, which can be used with non-equal subject group numbers and with heterogeneous variances.

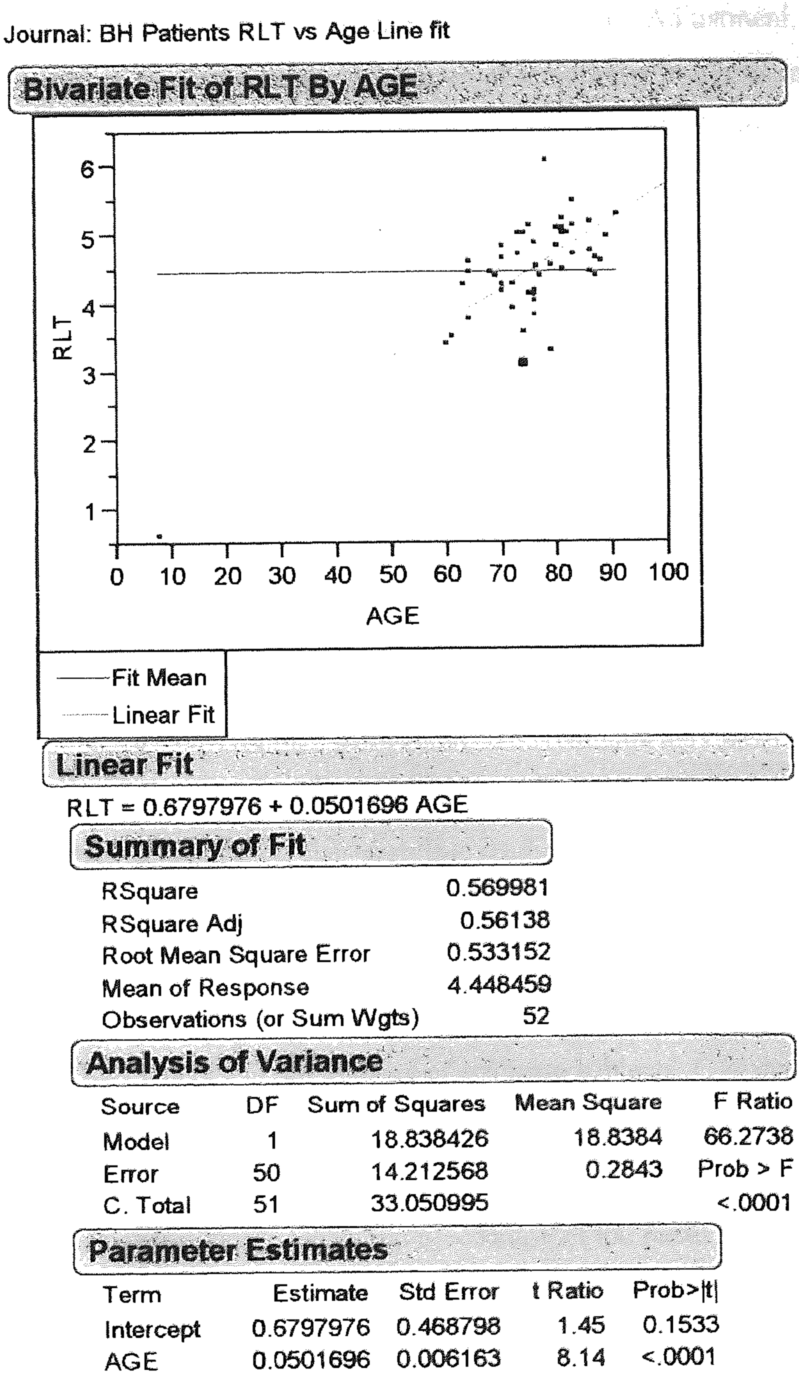
Armstrong et al (2000) recommend the application of Scheffe's method for the multiple comparison between treatment means but advise the use of Dunnett's method when several treatments are compared with a control mean.

A.3 Statistical Methods Applied

The following methods were selected as those suited for the analysis of the bulk of the data: Student 't' test, linear regression and ANOVA one-way.
Generally, following consideration of the scatter plot of parameter versus age, the three tests were carried out. Sets of data from the Excel spreadsheets were imported into the statistical software package JMP Professional Edition, Version 5, produced by the SAS Institute Inc, Cary, N.C., United States of America. The software allowed the data to be analysed by the selected method and the results presented as graphs or tables.

A typical JMP print out from the analysis of subjects over 60 years of age, right eye lens thickness versus age, for Group D is shown in Figure A.3.1.

Figure A. 3.1. Typical JMP printout



```
Journal: BH Patients RLT vs Age Line fit

Bivariate Fit of RLT By AGE

Linear Fit
RLT = 0.6797976 + 0.0501696 AGE

Summary of Fit
RSquare                          0.569981
RSquare Adj                       0.56138
Root Mean Square Error           0.533152
Mean of Response                 4.448459
Observations (or Sum Wgts)             52

Analysis of Variance
Source     DF   Sum of Squares   Mean Square    F Ratio
Model       1        18.838426       18.8384    66.2738
Error      50        14.212568        0.2843   Prob > F
C. Total   51        33.050995                   <.0001

Parameter Estimates
Term        Estimate    Std Error   t Ratio   Prob>|t|
Intercept   0.6797976   0.468798       1.45     0.1533
AGE         0.0501696   0.006163       8.14     <.0001
```

In the JMP printout:

Fit mean is the mean of all measurements. Linear fit gives an equation of linear regression. Summary of fit gives the R square, R square Adjustment, Root Mean Square Error, mean of response and the number of observations. Analysis of variance gives the Degrees of Freedom, Sum of Squares, Mean Square and the F ratio. Parameter estimates gives the standard error, t ratio and probability.

In total 252 analyses were carried out on the data gathered in this study: each parameter considered versus age and versus other parameters.

A.3.2 Calibration, Reproducibility and Groups

In Chapter 6 is a description of the equipment calibration and how the measurement reproducibility was checked. As recommended by Butcher and O'Brien (1991) three readings for keratometry were made because the repeatability of the readings was good. For pachometry, five readings were taken. Ten readings may have been preferable to try to reduce the effect of errors, but this number led to subjects' fatigue and the variability of the later readings increased, therefore five readings were made.

Armstrong and Eperjesi (2002) quote the useful approximate formula by Ridgman for estimating the degree of precision (or power) being obtained in a particular experiment: $r = (2C\sqrt{2}/R)^2$ where r is the number of replicate subjects, C is the coefficient of variation defined as the standard deviation expressed as a percentage of the mean, and R is the percentage difference detectable in an experiment, i.e. the difference between a treatment and a control mean expressed as the percentage of the mean of the whole experiment. The formula can be used to give a guide as to how many subjects should be recruited for a study. Alternatively, a reasonable approximation is to consider the number of degrees of freedom of the error term, and aim to achieve at least fifteen degrees of freedom. The measurement accuracy of each ocular parameter being tested influences the choice of subject numbers in a group.

For example, using the Ridgman approach, at least twenty two subjects in each group should have been measured for keratometry and eight for lens thickness (see Table A.3.2.1).

Table A.3.2.1 Example of Ridgman calculation to determine number of replicates

	Group E		Group D		Whole Group		Ridgman values		
	Mean	SD	Mean	SD	Mean	SD	C	R	r
HK	7.86	0.25	7.66	0.28	7.70	0.33	4.29	-2.6	22
LT	3.75	0.35	4.40	0.80	4.344	0.639	14.7	15	8

Preferably all of the parameters should have been measured on each subject. However, the restrictions on subject and measurement time availability meant that this ideal situation could not be realised and unfortunately the group sizes were not matched. There were two options to cope with this problem. Firstly the data set for a particular measurement with the lowest number of subjects could be selected and subjects from each decade of age selected at random until the same number had been achieved. Secondly, a more preferable solution was to use all measurements gathered in the knowledge that the result would give a reasonable indication of relationship, not necessarily a definitive statistically proven one.

A.3.3 Student's 't' Test

Student's 't' tests were used in the initial assessments of the data. For all the parameters, the mean values of right eye (RE) and left eye (LE) were compared in each group of subjects. As no significant difference was found for all of the parameters the data from both eyes could be combined and averaged. Examples (page 91) are given in Chapter 7. The 't' test was also carried out to compare mean values from different instruments, e.g. keratometry using the Rodenstock keratometer and the EyeSys keratoscope. Although the EyeSys always gave slightly larger values the test showed no significant difference between the means (Chapter 7, pages 92, 102).

The 't' test was used to compare the mean values of parameters in several subject groups. Usually no significant difference was found between young age groups (Groups C and E). On comparing young versus older age groups there was a difference between the keratometry means but the variability of the data prevented a significant effect being revealed. However for anterior chamber depth (ACD, page 143) and for lens thickness (LT, page 132) a significant difference was shown between the young and older age groups. This helped to show that with increasing age lens thickness increased and anterior chamber depth decreased. The 't' test was limited in that it could only indicate the differences between two group means and could not provide information on how the parameter values gradually changed with increasing age.

A.3.4 Linear Regression

Linear regression analysis was used on the data of all the groups initially to search for a relationship between a parameter and age. In a number of cases no significant relationship was proved. When this analysis was re-applied to the sub-group (Group B over age 60), the regression equation demonstrated that horizontal keratometry decreased with age (page 95). The same result was shown using the EyeSys (page 97). When linear regression was applied to the horizontal keratometry values of Group D, equations indicating a significant gradual reduction with age were obtained,
e.g. for the right eye (RE) keratometry = 8.377 - 0.0095*age,
e.g. for the left eye (LE) keratometry = 8.173 - 0.0068*age (page 99).
Normally these expressions would be written in the form: Y = M X + C,
e.g. (RE) k = (-0.0095 * age in years) + 8.377, (LE) k = (-0.0068* age in years) + 8.173.
In this study the notation format from the JMP printouts was copied to avoid errors in rearranging the format. For vertical keratometry a similar trend was indicated but the variability in the data prevented demonstration of a significant effect. This was also true for astigmatism and for corneal eccentricity.

On studying the biometry results, linear regression was tried on the axial length (AXL) data. No significant relation to age was found (page 123). However, when comparing axial length with keratometry, both horizontal and vertical keratometry showed a trend of smaller values linked to smaller values of axial length. For vertical keratometry the regression equation for RE was (RE) AXL = 10.836 + 1.535*k mm (page 130).

When linear regression was applied to lens thickness (LT) data, in Group D 'over 60 years' a significant positive relationship was found, as given by the following equation: (RE) LT = 0.6798 + 0.502*age (page 132). This analysis, when used on lens thickness versus axial length, showed a significant relationship given by the regression equation (RE) LT = 1.717 + 0.124*AXL mm (page 137).

The method was used on anterior chamber depth (ACD) data and for young Group E showed a relationship with age, giving the regression equation RE = 4.61 – 0.046*age (page 144). Group D showed a similar trend. Some significant relationships came to light when linear regression was carried out on the plots of ACD versus lens thickness (page 146) and on ACD versus axial length. For example, for Group D, the regression equation gives RE = 0.005 + 0.118*AXL mm (page 153).

A.3.5 ANOVA One-way

The most instructive analysis was ANOVA one-way and it was used on all parameters to determine whether the parameter showed a significant relationship to age. On several occasions this was repeated on data from a sub-group, e.g. subjects over age 60. Sometimes a more definite trend was shown but at other times the smaller number of subjects inhibited the significance of the relationship being revealed. ANOVA not only demonstrated which parameters were related to each other but also gave a suggestion of the strength of the relationship. For example a significant relationship ($p = 0.0001$) was shown between lens thickness and age for the Group D 'over 60' age subgroup (page 132).

ANOVA analysis of keratometry versus age is described in Chapter 7, and summarised in Table 7.2.13 (page 109). The 'p' values indicating where a significant relation was found are shown in bold. The table indicates that the larger number of subjects in Group D helped ANOVA to demonstrate the strong relationship between keratometry and age ($p = 0.005$).

ANOVA Analysis of Keratometry versus Age (Table 7.2.13.)

Group	RHK	LHK	RVK	LVK	EyeSys RHK	EyeSys LHK	EyeSys RVK	EyeSys LVK
B	0.06	**0.02**	0.28	0.26	0.06	**0.03**	0.48	0.44
B >60	0.27	0.12	0.45	0.77	0.56	0.27	0.41	0.75
B <60	0.54	0.64	0.41	0.76	0.42	0.56	0.78	0.98
D >60	**0.0005**	**0.008**	0.17	0.64	XX	XX	XX	XX
E	0.37	0.28	0.46	0.47	XX	XX	XX	XX

XX = No value was obtained because the EyeSys instrument was not available at the hospital where groups D and E were measured.

A strong relationship was also found when plotting corneal astigmatism versus age. Table 7.3.7, which summarised several ANOVA analysis ‘p’ values (page 117), was useful to demonstrate the very significant relationship between these two parameters ($p = 0.005$).

ANOVA Analysis of Astigmatism and Eccentricity versus Age (Table 7.3.7.)

Group	R Astig	L Astig	EyeSys R Astig	EyeSys L Astig	R Eccentricity	L Eccentricity
B	0.23	0.025	**0.047**	**0.006**	0.78	0.48
B >60y	**0.008**	**0.004**	**0.02**	**0.008**	0.25	0.78
B <60y	**0.026**	0.119	0.052	0.15	0.13	0.14
D	**0.005**	**0.004**	XX	XX	XX	XX
E	0.75	0.46	XX	XX	XX	XX

XX = No value was obtained because the EyeSys instrument was not available at the hospital where groups D and E were measured.

Use of ANOVA was able to demonstrate that for Group D (272 subjects) these parameters had a very significant relationship. Group B as a whole did show the trend of reducing astigmatism with age, which was shown more effectively when the group was split into two age subgroups.

ANOVA analysis was applied to the comparisons of all the parameters to investigate whether some interactions could be revealed. For example, this analysis showed a very significant positive relationship between lens thickness and axial length in Group D, RE p = 0.0002, LE p = 0.0001 (page 136).

The results in Groups D and E showed the trend of a positive relationship between axial length and both horizontal and vertical keratometry. In the larger Group D this relationship between axial length and vertical keratometry was shown to be significant. (VK: RE p = 0.0007, LE p = 0.014). Lens thickness showed a trend of increasing with age, which in Group D was proved to be very significant (p< 0.0001)

ANOVA method was useful to show the relationship between decreasing anterior chamber depth (ACD) and increasing lens thickness in Group E (page 146). ANOVA also demonstrated the high degree of significance (p = 0.0001) in the relationship between ACD and axial length of Group D.

When corneal thickness data was analysed using ANOVA one-way, as shown in Table 9.7.3, only for the right eye in central, nasal and temporal corneal regions was a significant (p<0.05) relationship found (page 165). For example, the analysis showed a 'p' value for Group B that gave an indication of a relationship with age, which was not shown in the two sub groups, Group B <60 years and Group B >60 years, probably because of the smaller number of subjects. This is an example of insufficient numbers of subjects in each decade of age to give reliable analysis in the smaller subgroups. The larger number of subjects in Group D >60 years showed a significant relationship with age.

ANOVA Analysis of Corneal Thickness versus Age for Group B, Central, Nasal and Temporal Regions (RE p values) (Table 9.7.3.)

REGION	**Group B** **RE**	**Group B under 60** **RE**	**Group B over 60** **RE**
	n = 55	n = 20	n = 35
Central	**0.046**	0.987	0.806
Nasal	**0.045**	0.233	0.454
Temporal	**0.026**	0.671	0.842

The ‘p’ values indicating where a significant relation was found are shown in bold.

A.4 Discussion

Some of the main difficulties with the study were the diverse characteristics of the various groupings. This result was partly due to the problems of recruiting appropriate numbers of reliable subjects in each of the age decade bands at the university and the hospital. The data analyses and comparison of groups were hindered by the uneven match in the subject numbers. This limited the interpretation of the data analyses.

The ANOVA one-way method is more accurate when the number of subjects in each decade is close, and the reliability decreases the greater is the difference in numbers in each decade. However, if the numbers are dissimilar, it is permissible to compare the means from the decades using the student ‘t’ test to infer that age causes a change. This does not claim to describe the exact line of change in terms of a formula. ANOVA one-way gave the best insight into the relationships between parameters and age, and between parameters.

Future researchers studying the effect of age on the eye should measure larger numbers of subjects, get a better balance of the numbers of subjects in each age group, preferably matched for age and gender, such that subsequent statistical analyses have a greater confidence level.

A.7 Statistics References

Hayslett, H.T. and Murphy, P. (1979) Statistics Made Simple. W.H. Allen. London.

Walpole, R.E. (1982) Introduction to Statistics (3^{rd} Edit). Collier Macmillan. London.

Hulley, S.B. and Cummings, S.R. (1988) Designing Clinical Research. Williams and Wilkins. Baltimore. USA.

Armstrong, R.A., Slade, S.V., Eperjesi, F. (2000) Ophthal. Physiol Optics. **20** 235 - 241

Armstrong, R.A., Eperjesi, F. (2000) Optometry Today Jan 16. 36 - 40

Armstrong, R.A., Eperjesi, F. (2001) Optometry Today Jan 26. 27 - 31

Armstrong, R.A., Eperjesi, F. (2002) Optometry Today May 31. 34 - 37

Armstrong, R.A., Eperjesi, F., Gilmartin, B. (2002) Ophthal Physiol. Optics. **22** 248 - 256

Armstrong, R.A., Eperjesi, F. (2004) Optometry Today March 12. 33 – 36

Armstrong, R.A., Eperjesi, F., Gilmartin, B. (2005) Clinical and Experimental Optometry. **88** 2: 81 – 88