CORNEAL BIOMECHANICAL PROPERTIES AND THE DEVELOPMENT OF MYOPIA

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Corneal Biomechanical Properties and the Development of Myopia

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CERTIFICATE OF ORIGINALITY

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_____ (Signed)

Wong Yin Zhi (Name of student)

I dedicate this dissertation to K.W Wong and Cikgu Lim F.L

for their continued support and unconditional love.

Abstract

Title of thesis: **Corneal biomechanical properties and the development of myopia**

Chief supervisor: **Dr. Andrew K.C. LAM**

Cornea is a viscoelastic tissue. Collagen fibrils and ground substance in the corneal stroma are the contributors of corneal biomechanical properties. Clinical measurement of these properties is available using Ocular Response Analyzer (ORA). Corneal hysteresis (CH) and corneal resistance factors (CRF) are corneal biomechanical parameters obtainable using ORA.

Myopia is a major refractive error that affects people worldwide. It garners research interest because of its pathological complications and direct socioeconomic costs. Although low CH is associated with a long eyeball, its role in axial elongation is unclear. This study comprises three experiments to investigate the influence of corneal curvature on CH and CRF (Study I), the associations of CH and CRF with other corneal parameters (Study II), and to monitor the changes in CH and CRF with axial elongation in children (Study III).

The ORA measurement relies on an infrared reflection along the horizontal corneal meridian, but provides CH and CRF for the whole cornea. Study I (n=95) investigated the influence of corneal curvature and astigmatism and meridional differences on CH and CRF measurements, when inter-subject variation on CH and CRF was eliminated. We measured CH and CRF at the default position, and with 10°, 20°, and 30° head tilts. CH and CRF were similar in different amounts of corneal

astigmatism. Only CRF had a marginal association with corneal astigmatism (r^2=0.04, p=0.047). CH and CRF were the lowest along the horizontal meridian compared with the other meridians, but the difference was clinically small. CH and CRF were unaffected by corneal curvature and astigmatism.

No previous studies have considered the contribution of corneal stroma on CH and CRF. Study II (n=80) investigated the associations among CH, CRF, axial length (AL), corneal volume (CV), corneal curvature, full stromal thickness (FST), and epithelial and Bowman's thicknesses (Epi+BT) between high myopes and emmetropes. We first confirmed the good inter-observer reproducibility of FST and Epi+BT measurements with confocal microscopy. High myopes exhibited a longer AL and lower CH compared with emmetropes. FST (standard coefficient, β=0.591) and CV (β=0.575) had a stronger association than did AL (β=-0.1) for CH.

Study III involved observing the changes in CH and CRF with axial elongation in children. We conducted school-based vision screenings (n=1199) to measure the CH, CRF, AL, corneal curvature, and astigmatism. Apart from cross-sectional analysis, we conducted 1-year cohort (n=269) and 2-year cohort (n=144) studies to monitor the changes of these parameters. Chinese children had lower CH compared with other ethnic groups. Chinese children in local and international schools shared similar CH and CRF. Among Chinese children, CH increased by an average of 0.21mmHg and 0.28mmHg, and after axial elongation by an average of 0.23mm and 0.46mm, annually and biennially, respectively. However, whether a change in CH is a cause or a result of axial elongation remains inconclusive.

In summary, corneal curvature, corneal astigmatism, and Epi+BT had minimal influence on CH and CRF. FST and CV had a stronger association with low CH than did the long AL. CH was found to have increased after axial elongation.

Publications arising from the thesis

Paper published:

Wong YZ, Lam AK. The roles of cornea and axial length in corneal hysteresis among emmetropes and high myopes: A pilot study. Curr Eye Res.2014 May 28:1--8. [Epub ahead of print]

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Wong YZ, Lam AK. Influence of corneal astigmatism, corneal curvature and meridional differences on corneal hysteresis and corneal resistance factor. *Clin Exp Optom*. 2011;94:418--424.

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Table of Contents

List of Tables

List of Figures

List of Abbreviations

ACD	Anterior chamber depth
Adjusted R^2	Adjusted coefficient of determination
AGEs	Advanced glycosylation end products
AL	Axial length
ANOVA	Analysis of variance
BSV	Best signal value
CA	Corneal astigmatism
CCT	Central corneal thickness
CH	Corneal hysteresis
Corvis ST	Corneal Visualization Scheimpflug Technology
CRF	Corneal resistance factor
CT	Corneal thickness
CV	Corneal volume
CXL	Corneal cross-linking with Ultraviolet-A and Riboflavin
D	Diopter
DM	Diabetes mellitus
EndoT	Endothelial thickness
Epi+BT	Epithelial and Bowman's layer thickness
ETDRS	Early Treatment Diabetic Retinopathy Study
FST	Full stromal thickness
GAT	Goldmann applanation tonometry
HbA1c	Glycated haemoglobin
IOP	Intraocular pressure
IOPcc	Corneal-compensated intraocular pressure
IOPg	Goldmann-correlated intraocular pressure
LASIK	Laser in situ keratomileusis

LASEK	Laser-assisted sub-epithelial keratectomy
logMAR	Logarithmic minimum angle of resolution
MeanK	Mean keratometry
n/a	Not available
OCT	Optical coherence tomography
ORA	Ocular Response Analyzer
P1	First corneal applanation
P2	Second corneal applanation
r	Pearson's correlation coeeficient
R^2	Coefficient of determination
SD	Standard deviation
SE	Spherical equivalent
SPSS	Statistical Package for the Social Sciences
VA	Visual acuity
WS	Waveform score

Chapter 1 Introduction

1.1 Myopia

Myopia is a "refractive condition of the eye in which the images of distant objects are focused in front of the retina when the accommodation is relaxed. Thus distance vision is blurred" (Millodot, 2009). It is caused by a mismatch between the total ocular refractive power and axial length (AL), particularly in corneal refractive power and vitreous chamber depth (Scott and Grosvenor, 1993).

Myopia is an eye focusing disorder rather than an ocular disease per se, but high myopia can be pathological and also a leading cause of visual impairment (Saw *et al*., 2005b). It always garners research interest and raises public concerns because of its consistently increasing prevalence. Myopia is affecting young people worldwide, especially in the Asian Chinese population. Myopia is highly prevalent in mainland China, Hong Kong, Taiwan, as well as Singapore (Pan *et al*., 2012). The pathology and degeneration associated with high myopia, for instance, glaucoma and retinal complications, are well known for being the root cause of blindness. In addition, it also has an enormous socioeconomic impact on society (Lim *et al*., 2009b).

1.2 Ocular rigidity in myopia

Ocular rigidity may refer to "the resistance of the coats of the eye to indentation" (Millodot, 2009). In brief, ocular rigidity could indicate the combined biomechanical properties of the whole eye wall, including the cornea, sclera, choroid, and ocular blood circulation, although the sclera is believed to be the principal contributor to ocular rigidity (Friberg and Lace, 1988; Dastiridou *et al*., 2009).

The ocular rigidity coefficient was first introduced by Friedenwald (1937), and it is still widely used. A formula was derived based on an observation between the changes in intraocular pressure (IOP) to ocular volume in enucleated eyes. A critical assumption in deriving this formula is as follows: for an eye of a certain size, a proportional change in pressure varies with a proportional change in volume. Although numerous theories, methods, and units on ocular rigidity have been introduced (Detorakis and Pallikaris, 2013), these methods were either involved complex calculations or they were difficult to be performed clinically (Ytteborg, 1960a; Ytteborg, 1960b; Eisenlohr *et al*., 1962; Silver and Geyer, 2000). Moreover, most ocular rigidity studies have initially been performed in enucleated eyes or pathological eyes.

Based on the pressure-volume relationship, small eyes were postulated to have a high ocular rigidity, because they had a smaller ocular volume (Perkins, 1981). Thus, this suggested that high myopic eyes with a large ocular volume or long AL would yield a low ocular rigidity (Friendenwald, 1937). A decreased ocular rigidity coefficient was found in enucleated myopic eyes, thereby supporting this notion (Perkins, 1981).

Pallikaris *et al*. (2005) and Dastiridou *et al*. (2013) have investigated the association between ocular rigidity and AL by using direct manometry. They found a decreased ocular rigidity with longer AL. Only Dastiridou *et al*. (2013) found a significant association between ocular rigidity and AL (r = 0.0641), but the association was weak. Although direct manometry can be used to monitor ocular rigidity, it is an invasive procedure that can be conducted during ocular surgery. Hence, it is unsuitable for general clinical practice.

Clinically, the ocular rigidity coefficient can be assessed indirectly by measuring a change in volume or AL at two different IOPs. Differential tonometry and combined applanation-indentation tonometry have commonly been used for the clinical measurement of ocular rigidity. In differential tonometry, two IOP readings were measured using two different plunger-weights, for instance, 5.5gm and 10.0gm, in a Schiotz tonometer. In combined tonometry, Goldmann applanation tonometry (GAT) was applied before Schiotz tonometry with 10.0gm weights (Friedenwald, 1949; Friedenwald, 1957). The two IOP readings obtained either from differential tonometry or combined tonometry were used to determine the ocular rigidity coefficient. However, differential tonometry is time consuming and easily affected by reading errors compared with combined applanation-indentation tonometry.

Castrén and Pohjola (1961) found that ocular rigidity is lower in myopes than in non-myopes. Bonomi *et al*. (1982) found lower ocular rigidity in the greater myopic eyes of 137 anisometropic subjects with unilateral high myopia. They did not account for the inter-ocular corneal differences. On the other hand, Wong and Yap (1991) found similar ocular rigidity in myopic, low myopic, and emmetropic or hyperopic young Chinese adults. Although this study involved subjects with a spherical equivalent (SE) of up to -11D, the number of subjects with an SE greater than -6D was relatively small. Furthermore, they did not evaluate the AL of their subjects. Sergienko and Shargorogska (2012) measured the ocular rigidity of 86 young adults whose ages were matched to that of the participants in the Wong and Yap (1991) study. They used a pneumotonometer with an indentation of a modified

metal tubing weight of 30g, instead of Schiotz differential tonometry. Their results demonstrated that emmetropic and hyperopic eyes had lesser axial elongation compared with that of myopic eyes when the IOP was artificially elevated. Furthermore, higher myopia was associated with greater axial elongation during IOP elevation, which indicated that subjects with greater AL had lower ocular rigidity. However, the IOP reading from the pneumatonometer was easily affected by the measured position; therefore, it might be less reliable for readings (Moses and Grodzki, 1979). Meanwhile, McMonnies (2014) also postulated that IOP fluctuations can play a vital role in axial elongation. The IOP spikes may cause irrevocable effect on axial length, especially in eyeball with weak susceptibility to force. Difficulty in monitoring IOP continuously is still the key stumbling block to prove this hypothesis. Therefore, the role of IOP spikes in axial elongation is still remaining unknown until further confirmation.

Schmid and co-workers (2003) measured the ocular rigidity of 20 myopic and 20 age- and gender-matched non-myopic children. They found an overlap in ocular rigidity values between the myopic and non-myopic groups. They also could not find a significant difference in eye wall stress between the 2 groups. Furthermore, they found no change in ocular rigidity with a refractive error shift, and suggested that eye wall stress in children was lower than in adults.

1.3 Corneal biomechanical properties

The cornea is a transparent and mechanically tough tissue that provides two-thirds of ocular refractive power. Corneal biomechanical properties include resistance of the cornea to mechanical stress. They are related to the response of the cornea towards alterations to its configuration (Luce, 2005). Corneal biomechanical properties are

crucial parameters that protect and stabilize the cornea, and maintain its clarity (Liu and Roberts, 2005).

The corneal epithelium is the outermost layer, and consists of five to six cellular layers. Overall, a full-thickness cornea is consistently 1%-3% stiffer than a cornea without the epithelium (Elsheikh *et al*., 2008a). Therefore, Elsheikh *et al*. (2008a) suggested that the removal of the corneal epithelium has minimal influence on corneal biomechanical properties. Similar to the corneal epithelium, the corneal endothelium also has low mechanical stiffness. It consists of single-layered cells.

The stroma is the corneal mechanical scaffold, and plays an essential role in maintaining the corneal shape and transparency. It comprises water, collagen fibrils, and ground substance, for instance, glycosaminoglycans, keratocytes, and proteoglycans (Levin *et al*., 2011). Collagen fibrils are believed to be the predominant load-bearing elements of the cornea. Collagen fibril bundles comprise the stromal lamella. Approximately 200 to 400 lamellae are superimposed upon one another to form a considerably complex anisotropic network that provides tensile strength to the cornea (Müller *et al*., 2004). When stromal elasticity was measured parallel to the direction of the collagen fibrils, the results were stronger than measuring it perpendicularly to the arrangement of the collagen fibrils (Levin *et al*., 2011). Ground substance is a gel-like material that fills the space between the lamellae and binds them. Although ground substance serves a more complex physiological function than providing corneal mechanical properties, it plays a role in corneal viscosity (Hendrickson, 2009). Therefore, stromal biomechanical properties do not merely depend on collagen fibrils, but on the arrangement and interaction among collagen fibrils, cells, and the ground substance.

Bowman's layer is another corneal layer that consists of collagen fibrils. It is an acellular layer composed of collagen fibrils. However, the function of Bowman's layer remains debatable. Seiler *et al*. (1992) found that Bowman's layer does not

make a significant contribution to the corneal biomechanical properties. The absence of Bowman's layer only caused a 6.7% reduction in corneal stiffness.

The cornea is a viscoelastic tissue consisting of both viscosity and elasticity when deformation occurs. Elasticity can refer to time-dependent energy storage. It allows the cornea to recover to its original state after an external force is removed. Viscosity can refer to time-dependent energy dissipation. It prevents the cornea from deformation by external stress (Levin *et al*., 2011). When an external force is applied on the cornea, corneal deformation occurs as a function of time in the direction of the applied force. An internal force from the opposite direction simultaneously helps it regain its original shape after the external force is removed. Because of this viscoelastic characteristic, the cornea is a mechanotransducer that could protect itself against mechanical stress through energy absorption and dissipation.

Traditionally, biological tissues were evaluated by the stress-strain relationship, tangent Young's modulus, Poisson's ratio, hysteresis, stress relaxation, creep, and shear strength. Elasticity can be represented by Young's modulus. It is calculated from the slope of the stress-strain curve (Buzard, 1992). Stress is the external force applied on the surface, whereas the strain is the deformation from the applied force. A pure elastic material has a linear stress-strain relationship. The cornea is viscoelastic tissue, instead of pure elastic tissue, and therefore, it exhibits a non-linear J-shaped stress-strain curve (Levin *et al*., 2011) (**Figure 1.1**). A greater slope in the stress-strain curve indicates a higher corneal modulus, and it corresponds to a stiffer cornea, whereas a lower corneal modulus is indicative of a weaker cornea. Young's modulus of the cornea has spanned a wide range, from 0.159 to 57MPa (Woo *et al*., 1972; Andreassen *et al*., 1980; Nash *et al*., 1982; Hoeltzel *et al*., 1992; Seiler *et al*., 1992; Hjortdal, 1996; Orssengo and Pye, 1999; Djotyan *et al*., 2001; Zeng *et al*., 2001; Wollensak *et al*., 2003; Elsheikh *et al*., 2007). Because these studies have been conducted in different experimental settings, the data have further been filtered within the physiological IOP range (< 25mmHg). However, Young's

modulus of the cornea still varied highly, from 0.25MPa to 9MPa (Hjortdal, 1996; Djotyan *et al*., 2001; Zeng *et al*., 2001; Elsheikh *et al*., 2007).

Hysteresis, creep, and stress relaxation are the primary phenomena for describing corneal viscoelasticity. The loading and unloading curves of the cornea do not overlap. Hysteresis is the dissipated energy that corresponds to the difference in the area between the loading and unloading curves (**Figure 1.2**). The mechanical properties show that low hysteresis indicates a higher bounce, and thus, a rapid return to the original shape. Conversely, high hysteresis demonstrates energy dissipation, instead of energy storing; hence, it takes longer to return to the original state after deformation (Broman *et al*., 2007).

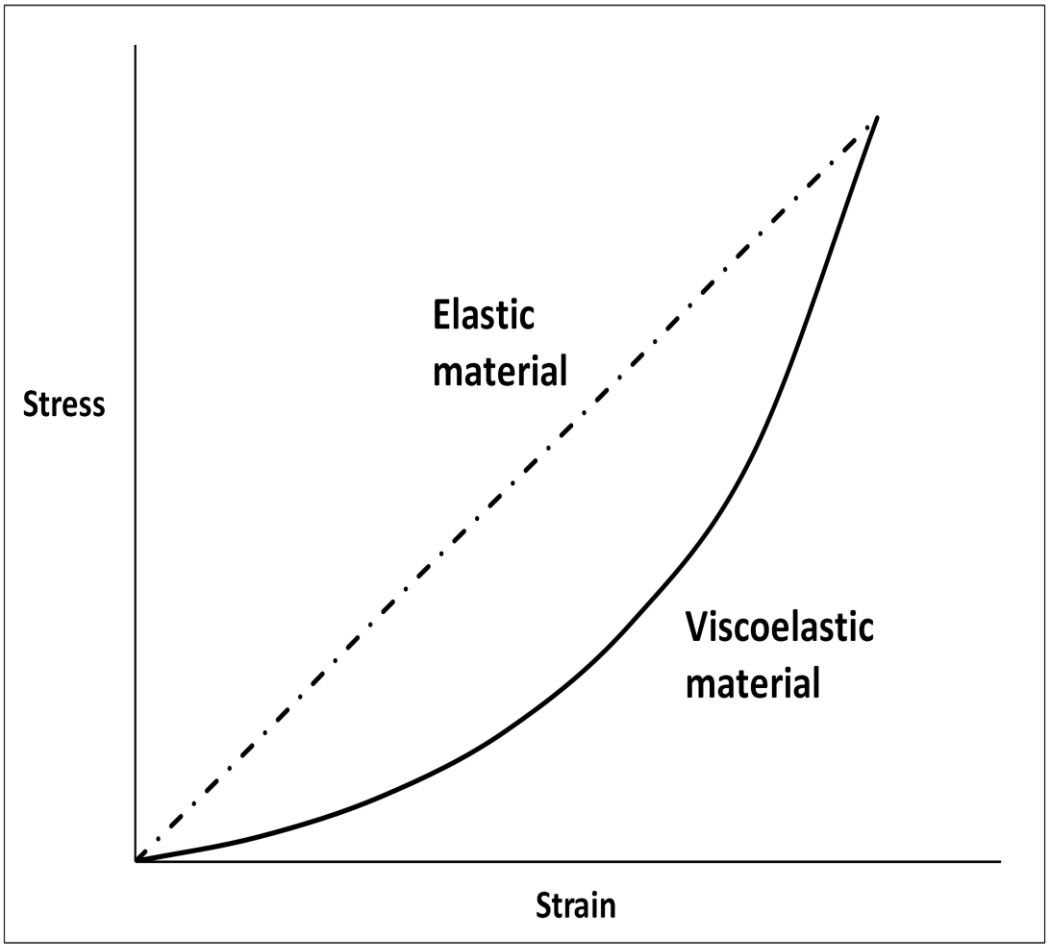

Figure 1.1 The stress-strain curves of elastic and viscoelastic material. Viscoelastic material such as the cornea assumes a J-shaped curve, instead of a linear elastic curve.

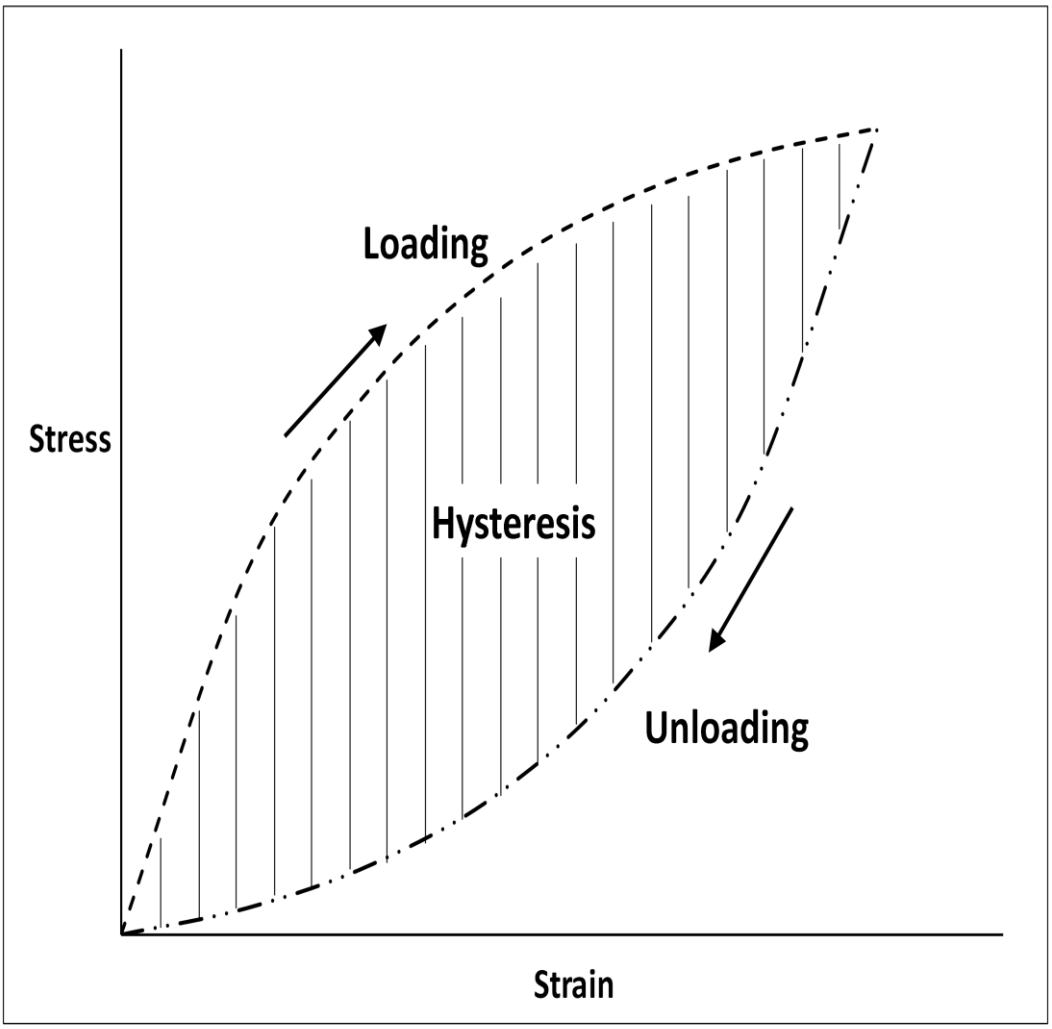

Figure 1.2 The loading and unloading stress-strain curves of the cornea. Because the loading and unloading curves of the cornea are not overlapping, the area between the two curves represents corneal hysteresis.

1.3.1 *Ex vivo* / *In vitro* corneal biomechanical properties measurements

Corneal biomechanical properties have traditionally been studied through experimental settings. Strip extensiometry and inflation testing are the two major experimental techniques for investigating the stress-strain relationship of the cornea. Both techniques have their advantages and disadvantages in accessing the corneal biomechanical properties. Strip extensiometry is suitable for studying corneal anisotropy and viscoelasticity, whereas inflation testing is suitable for monitoring the global corneal biomechanical properties.

Strip extensiometry is a simple laboratory method for obtaining the biomechanical properties of tissues. The corneal flap is extracted before the stress and strain are calculated based on the load and elongation data obtained using an extensometer. It has been used widely to investigate Young's modulus of the cornea (Andreassen *et al*., 1980; Nash *et al*., 1982; Hoeltzel *et al*., 1992; Seiler *et al*., 1992; Zeng *et al*., 2001; Wollensak *et al*., 2003). A reduced modulus was found in the keratoconic cornea by using this method (Andreassen *et al*., 1980). The drawbacks of strip extensiometry include a neglect of initial corneal curvature and the heterogeneity of the corneal thickness, the disruption of the corneal structure, misalignment of the fibrils with the orientation of the corneal flap, and a possible misplacement of the clamp. These are critical because the cornea does not bear the same tensile load once it is cut to a strip (Pinsky and Datye, 1991).

Inflation testing is usually conducted using either a corneal button or the whole eyeball. External pressure is applied on the corneal surface, and the corneal deformation is monitored. The pressure-deformation data are calculated to obtain the stress and strain data. One advantage of the inflation test over strip extensiometry is

that the whole globe can be used during the test to mimic a more anatomical and physiological condition (Kobayashi *et al*., 1973; Hjortdal and Jensen, 1995; Bryant and McDonnell, 1996). Using donor corneas over 50 years old, Elsheikh *et al*. (2007) found that Young's Modulus increased with age by more than 10% per decade.

The major limitations of *ex vivo* corneal biomechanical properties measurement are the restriction of experimental techniques and the challenge of corneal preservation. Corneal swelling, loss of tear film and optical clarity, as well as temperature and tissue degradation could alter the corneal biomechanical properties (Hjortdal, 1995). In addition, donor corneas have usually been obtained from old patients or from eyes with different ocular diseases.

1.3.2 *In vivo* corneal biomechanical properties measurements

The Ocular Response Analyzer (ORA; Reichert Ophthalmic Instruments, Depew, New York, USA) was first introduced in 2005 (Luce, 2005) to measure the corneal biomechanical properties *in vivo.*

The ORA functions with a similar principle to the conventional non-contact air puff tonometer. Short and calibrated air pressure is applied through a metered collimated air pump to deform the central 3mm cornea within 20ms. It uses dynamic bidirectional corneal applanation technology to applanate the cornea twice during each measurement. The air pressure rises steadily, and the air pump shuts down after the first corneal applanation (P1). The cornea continues moving inward to a concave state before starting to return to its original shape. The air pressure is re-recorded when the cornea reaches a second applanation (P2) (**Figure 1.3**). The changes in

reflected light during corneal deformation are detected using an infrared electro-optical system, and a signal plot is generated at the end of the measurement.

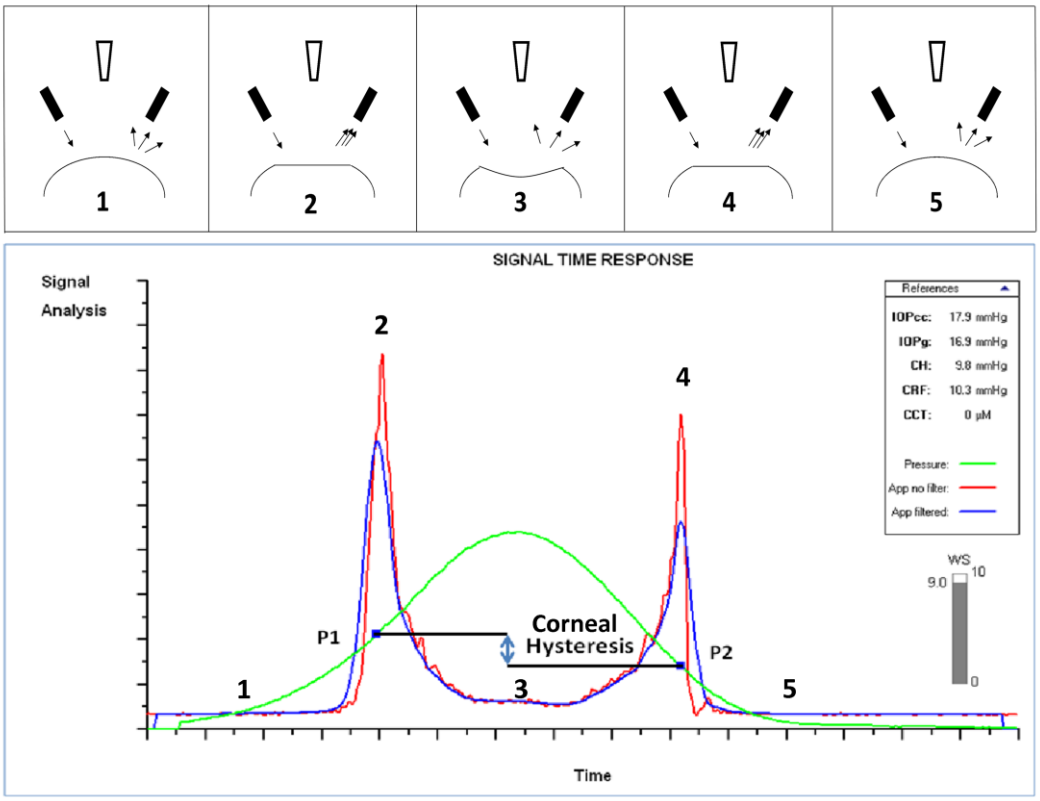

Figure 1.3 The mechanism of the Ocular Response Analyzer (ORA). (1) Calibrated air pressure is emitted by the ORA to deform the cornea. (2) The air pressure rises and shut down until the cornea is applanated, and the first applanated peak is detected. (3) Because of inertia, the air pressure further increases, and pushes the cornea to a concave state. (4) The air pressure decreases gradually until the second corneal applanation, and the second applanated peak is detected. (5) The cornea returns to its original shape. The reflected light reaches the infrared receiver maximally when the cornea is applanated. Thus, two applanation peaks are detected during the corneal applanation. P1 represents the intraocular pressure for the loading (first applanation), whereas P2 is the unloading pressure (second applanation).

The ORA provides four primary parameters that are derived from the two corneal applanations (P1 and P2). They include Goldmann-correlated intraocular pressure (IOPg), corneal-compensated intraocular pressure (IOPcc), corneal hysteresis (CH) and the corneal resistance factor (CRF). IOPg is an average of P1 and P2. IOPcc is a novel algorithm incorporated that adopted the corneal factors in IOP calculations (P2 - 0.43P1) (Medeiros and Weinreb, 2006). CH is the difference between P1 and P2 (P1 - P2). Because some energy is absorbed by the cornea, the unloading pressure (P2) is always lower than P1 (loading). CH is believed to reflect the ability of the cornea in energy absorption. The CRF is expressed empirically to maximize its dependence on the central corneal thickness (CCT). It is calculated as P1 - *k*P2, where *k* is a constant (Luce, 2005). The CRF is claimed to represent the overall resistance of the cornea.

The ORA primary parameters have been confirmed to have attainable repeatability and reliability in adults (Monero-Montañés *et al*., 2008) and children (Hon *et al*., 2012). Using the software version 2.0 or above, 37 ORA waveform parameters are available for providing the deformation response of the cornea. It renders the changes in the cornea for the area, height, width, slope, and noise of the signal waveforms during the deformation. Of the 37 waveform parameters, 23 parameters describe the upper 75% of the applanation peak, and the other 14 are obtained from the upper 50% of the applanation peak. These waveform parameters may contain valuable information regarding corneal biomechanical properties, but most of them had low repeatability (Landoulsi *et al*., 2013).

Corneal Visualization Scheimpflug Technology (Corvis ST; Oculus, Wetzlar, Germany) is a new non-contact tonometer that has incorporated an ultrafast Scheimpflug camera to capture the corneal deformation. Four thousand three hundred and thirty images with an image resolution of up to 640 x 480 pixels are captured along the 8.5mm horizontal meridian of the cornea within 100ms (Hong *et al*., 2013). It records the corneal deformation process and provides detailed information, for example, the time to reach maximum deformation, maximum

deformation amplitude, corneal length, corneal velocity, and highest corneal concavity during the deformation. It was unavailable when our study started in 2009. Details of the Corvis ST are hence not covered in this thesis.

Other prototypes can provide *in vivo* corneal biomechanical properties measurements. Measuring corneal biomechanical properties by using ultrasound (Wang *et al*., 1996) and indentation topography (Grabner *et al*., 2005) have been recommended since 1996 and 2005, respectively. Although the measurements obtainable using an ultrasound was proposed over a decade ago, it has a few limitations such as a low resolution in the measurement of corneal biomechanical properties. Quantitative ultrasonic spectroscopy is a quantitative ultrasound method that is commonly used for other medical purposes. He and Liu (2009) modified and introduced it for corneal thickness and corneal biomechanical properties measurements. Furthermore, the development of high-resolution and high-speed spectral domain optical coherence tomography (OCT) garnered research interest on corneal biomechanical properties measurement obtained using the OCT synchronized with air-pulse applanation (Dorronsoro *et al*., 2012). Brillouin optical microscopy is another device which is being developed for corneal biomechanical properties measurements (Scarcelli *et al*., 2012). It uses Brillouin scattering to evaluate the interaction between phonons in cornea. The change in energy of the photon corresponds to a shift in frequency in the Brillouin spectrum in order to determine the elastic moduli of cornea (Scarcelli and Yun, 2012).

1.4 Factors affecting corneal hysteresis and the corneal resistance factor

The ORA was the only instrument available for *in vivo* corneal biomechanical measurements at the beginning of our study. Innumerable studies, including clinic- and population-based studies, have been conducted to evaluate CH and the CRF in a wide range of aspects. This section covers the factors affecting the two major ORA parameters, CH and the CRF. Because these parameters represent the biomechanics of the cornea, they were expected to have an association with some corneal parameters as well as other variables that could affect the biomechanical properties.

Age

Because CH and the CRF represent the corneal viscoelasticity, they decrease slowly with age (Foster *et al*., 2011; Narayanaswamy *et al*., 2011). CH decreased by 0.34 mmHg per decade, whereas the CRF decreased to 0.31mmHg per decade for subjects over 40 years old (Foster *et al*., 2011). These results were in line with the experimental conclusion, where a decreased hysteresis was found in older donor corneal buttons (Elsheikh *et al*., 2008b). However, other studies have yielded contradictory results. No association has been found between CH and the CRF with age (Kirwan *et al*., 2006; Chang *et al*., 2010). Although no direct CH and CRF comparison is available between children and adults, apparently the average CH and CRF in children (age ≤ 18 years old) were higher than in adults (**Table 1.1**). The significant association between age and CH and the CRF has typically only been demonstrated in studies with a larger age range or involved older subjects (Foster *et al*., 2011; Narayanaswamy *et al*., 2011). CH and the CRF either rarely change in the

young population, or they decrease slowly; which is the reason studies with a narrow age range could not detect the change.

Gender

Regardless of the few studies that have found females to have a slightly higher CH and CRF compared with males (Fontes *et al*., 2008; Narayanaswamy *et al*., 2011; Radhakrishnan *et al*., 2012), most studies have suggested that CH and the CRF are unaffected by gender (Kamiya *et al*., 2008; Lim *et al*., 2008; Chang *et al*., 2010; Plakitsi *et al*., 2011). However, CH and the CRF are affected by the menstrual cycle. A thicker CCT but decreased CH and CRF were observed during ovulation (Goldich *et al*., 2011). Nonetheless, there is a lack of large-scale population-based studies to support these findings. Sen *et al*. (2014) reported that CH and the CRF were unaffected by hormonal changes during pregnancy.

Circadian rhythm and diurnal variations

CH is typically a stable parameter when measured during office hours (Laiquzzaman *et al*., 2006), and even throughout the day, after the resolution of overnight corneal edema (Kida *et al*., 2006, Lau and Pye, 2012). It was diurnally stable, irrespective of whether it has been measured in normal adults (Kida *et al*., 2006; Laiquzzaman *et al*., 2006; Shen *et al*., 2008b; Lau and Pye, 2012) or glaucoma patients (Villas-Bôas *et al*., 2009). According to 24 hours observations, the greatest fluctuation in CRF has been recorded between the last measurements before sleeping and measurements immediately after awakening (Shen *et al*., 2008b; Lau and Pye, 2012). However, Villas-Bôas *et al.* (2009) stated that the CRF was relatively stable during office hours in both normal and glaucoma subjects.

Table 1.1 Corneal hysteresis (CH) and the corneal resistance factor (CRF) in children, adults and different ethnic groups.

Studies	Age (Year) mean ± SD (range)	Gender	Population	CH (mmHg) mean ± SD	CRF (mmHg) mean ± SD
Children (≤ 18 years old)					
Kirwan *et al*. (2006)	n/a (4-18)	22 male (n = 42)	Caucasian	12.50 ± 1.35	n/a
Lim *et al*. (2008)	13.97 ± 0.89 (12-15)	138 male (n = 271)	Mixed ethnicity	11.78 ± 1.55	11.81 ± 1.71
Song *et al*. (2008)	14.70 ± 0.80 (10-14)	n/a (n = 1153)	Chinese	10.70 ± 1.64	n/a
Chang *et al*. (2010)	12.02 ± 3.19 (7-18)	37 male (n = 63)	Chinese	10.85 ± 1.33	11.03 ± 1.46
Huang *et al*. (2011)	8.60 ± 2.08 (7-12)	340 male (n = 651)	Chinese	10.40 ± 2.20	11.20 ± 2.10
Huang *et al*. (2013)	10.47 ± 1.00 (n/a)	303 male (n = 571)	Chinese	10.98 ± 1.78	11.46 ± 1.69
Bueno-Gimeno *et al*. (2014)	10.84 ± 3.05 (6-17)	135 male (n = 293)	Spanish	12.12 ± 1.71	12.30 ± 1.89
Adults					
Fontes *et al*. (2008)	45.09 ± 20.58 (18-90)	53 male (n = 150)	Brazilian	10.17 ± 1.82	10.14 ± 1.80
Kamiya *et al*. (2008)	39.10 ± 14.50 (19-68)	13 male (n = 43)	Japanese	10.20 ± 1.30	n/a
Foster et al. (2011)	n/a (48-91)	1831 male (n = 4184)	British	10.00 ± 1.64	10.22 ± 1.74
Narayanaswamy *et al*. (2011)	55.30 ± 8.40 (44-83)	554 male (n = 1136)	Chinese	10.60 ± 1.50	10.10 ± 1.60
Radhakrishnan *et al*. (2012)	n/a (18-65)	41 male (n = 117)	Mixed ethnicity	10.80 ± 1.52	10.67 ± 1.64
Rosa *et al*. (2014)	43.1 ± 15.4 (19-82)	58 male (n = 105)	Italian	10.26 ± 1.49	10.38 ± 1.64

n/a = not available.

Other corneal parameters

Modest to strong positive associations have been found between CH and the CRF with the CCT (Shah *et al*., 2006; Fontes *et al*., 2008; Kamiya *et al*., 2008; Lim *et al*., 2008; Franco and Lira, 2009; Plakitsi *et al*., 2011). Assuming that a thicker CCT has more collagen fibrils and ground substance, which contribute to the corneal biomechanical properties, the logical extension from this assumption would be that the cornea with a greater corneal volume (CV) has greater CH and CRF. Sedaghat *et al*. (2012) reported that a CV at the 7- and 5-mm diameters had the strongest positive correlation with CH (r = 0.44) and CRF (r = 0.58), respectively. Rosa *et al*. (2014) also found a positive correlation between CV at the 10-mm diameter with CH and the CRF (r = 0.32 and r = 0.34, respectively).

Because the corneal stroma is the load-bearing structure of the cornea, corneal histomorphology in the stroma is believed to have a relation with CH and the CRF, because cells are the fundamental units for studying corneal biomechanical properties. Hurmeric *et al*. (2010) discovered negative correlations among stromal keratocyte density, CH, and the CRF. They postulated that a high keratocyte density compensates for a decrease in corneal biomechanical strength.

Corneal biomechanical properties are pivotal for maintaining the corneal shape. Hence, the corneal geometry concerning corneal curvature and corneal astigmatism could be affected by CH and the CRF. Nevertheless, the influence of corneal curvature on CH and CRF has remained debatable. Various studies have demonstrated that corneal curvature only had minimal influence on CH and the CRF (Fontes *et al*., 2008; Kamiya *et al*., 2008; Chang *et al*., 2010). Rosa *et al*. (2014) found that CH and the CRF were lower in flatter corneas; nonetheless, the associations were weak (r = 0.16 and r = 0.13, respectively). Narayanaswamy *et al*. (2011) stated that CH and the CRF decreased by 0.96mmHg and 0.80mmHg,

respectively, in adults, with every millimeter increase in corneal radius (standardized coefficients (β) = -0.963 and β = -0.771, respectively, $p < 0.001$). Lim *et al*. (2008) found that, with every millimeter increase in the corneal radius, CH and the CRF decreased by 1.28mmHg and 1.08mmHg, respectively, in children.

Axial length and Myopia

The sclera and cornea are derived from the mesoderm, which comprises collagen fibrils to form the ocular fibrous tunic (Nickla and Wallman, 2010). Moreover, a decreased scleral thickness and scleral rigidity have been reported in myopes (McBrien *et al*., 2009; Sergienko and Shargorogska, 2012). Myopia-related corneal biomechanical changes remain inconclusive, although numerous studies have been conducted to evaluate the corneal biomechanical properties in myopes (Lim *et al*., 2008; Shen *et al*., 2008a; Song *et al*., 2008; Chang *et al*., 2010; Xu *et al*., 2010; Jiang *et al*., 2011; Narayanaswamy *et al*., 2011; Plakitsi *et al*., 2011; Altan *et al*., 2012; Kara *et al*., 2012). Despite a few apparently contradictory findings having reported no association between CH, SE, and AL (Lim *et al*., 2008; Shah *et al*., 2014), most studies have revealed a weak negative association among corneal biomechanical properties with AL (r^2 = 0.017 to 0.260) (Song *et al*., 2008; Chang *et al*., 2010; Huang *et al*., 2011; Narayanaswamy *et al*., 2011; Altan *et al*., 2012; Kara *et al*., 2012) and SE (r^2 = 0.044 to 0.176) (Shen *et al*., 2008a; Jiang *et al*., 2011; Plakitsi *et al*., 2011; Bueno-Gimeno *et al*., 2014).

The literature has also reported lower CH in myopes compared with emmetropes who had a similar CCT (Shen *et al*., 2008a; Bueno-Gimeno *et al*., 2014; Del Buey *et al*., 2014). Moreover, lower CH was also observed in highly myopic eyes compared with that in the fellow eye of anisometropic subjects who exhibited a similar CCT between the two eyes (Xu *et al*., 2010). These researchers suggested that axial elongation may result in lower CH, possibly because of a change in corneal collagen

fibrils (Xu *et al*., 2010), or that corneal biomechanical properties can be an indicator of scleral or ocular biomechanical properties (Song *et al*., 2008). Narayanaswamy *et al*. (2011) found that, for a 1mm increase in AL, CH decreased by 0.17mmHg in adults. CH decreased by 0.24mmHg to 0.51mmHg for every 1mm increase in AL in children (Song *et al*., 2008; Chang *et al*., 2010; Bueno-Gimeno *et al*., 2014). Moreover, CH was found to reduce by 2.05mmHg for every 1mm increase in AL in highly myopic children (SE > -6.00D) (Bueno-Gimeno *et al*., 2014). This indicated that the corneal biomechanical properties of high myopes might different from emmetropes and low myopes.

Corneal refractive surgeries

CH and the CRF decreased significantly after corneal refractive surgery. Using the preoperative CH and CRF as a reference, CH decreased by 11% to 22% after myopic laser in situ keratomileusis (LASIK) (Ortiz *et al*., 2007; Pepose *et al*., 2007; Chen *et al*., 2008; Hamilton *et al*., 2008; Kamiya *et al*., 2009a; Kamiya *et al*., 2009b; Qazi *et al*., 2009; Shah *et al*., 2009; Shah and Laiquzzaman, 2009; Chen *et al*., 2010). The CRF dropped even more significantly by 20% to 40% after LASIK (Ortiz *et al*., 2007; Pepose *et al*., 2007; Chen *et al*., 2008; Hamilton *et al*., 2008; Kamiya *et al*., 2009a; Kamiya *et al*., 2009b; Qazi *et al*., 2009; Shah *et al*., 2009; Shah and Laiquzzaman, 2009; Chen *et al*., 2010).

The reduction in CH and CRF in post- epi-LASIK and laser-assisted sub-epithelial keratectomy (LASEK) was 17%-21% and 22%-31%, respectively, which was comparable to post-LASIK values (Qazi *et al*., 2009; Ryan *et al*., 2011). CH decreased by approximately 15%-23%, whereas the CRF decreased by 18%-29% after surface ablation (Hamilton *et al*., 2008; Kamiya *et al*., 2009a). The decreased CH and CRF are likely due to the decreased CCT, which was caused by the laser corneal ablation. Because the CRF was derived to have a maximal correlation with the CCT, the reduction in CCT caused a greater drop in CRF compared with CH.

Keratoconus and keratectasia

Histopathologic alterations to the keratoconic cornea include a decreased quantity of normal collagen fibrils and collagen fibril diameter, increased proteoglycans density and area, and a weaker cohesion between collagen fibrils and ground substance (Meek *et al*., 2005; Akhtar *et al*., 2008; Gefen *et al*., 2009). These changes further cause lamellae displacement and slippage (Meek *et al*., 2005; Akhtar *et al*., 2008; Gefen *et al*., 2009). A weaker distensibility and rigidity with greater elasticity in the keratoconic cornea has been well documented in *in vitro* studies (Andreassen *et al*., 1980).

CH and the CRF could be the surrogate markers of the etiopathogenesis of certain corneal diseases, for example, keratoconus and keratectasia (Spörl *et al*., 2009; Schweitzer *et al*., 2010), which could also cause complications after corneal refractive surgery. The CRF in keratoconus patients was 16% to 33% lower than in controls (Shah *et al*., 2007; Fontes *et al*., 2010; Saad *et al*., 2010; Fontes *et al*., 2011; Galletti *et al*., 2012). Conversely, CH in keratoconus patients was 10% to 24% lower than in controls (Shah *et al*., 2007; Fontes *et al*., 2010; Saad *et al*., 2010; Fontes *et al*., 2011; Galletti *et al*., 2012). The CRF typically decreased more considerably than CH in keratoconus patients compared with controls. Nonetheless, because of the overlapping CH and the CRF between the normal and keratoconic corneas, these parameters had low sensitivity and specificity to differentiate the normal cornea from the keratoconus suspect cornea (Saad *et al*., 2010), mild keratoconic cornea (Fontes *et al*., 2010), and CCT-matched keratoconic cornea (Fontes *et al*., 2011).

Corneal cross-linking with Ultraviolet-A and Riboflavin

Corneal cross-linking with Ultraviolet-A and Riboflavin (CXL) has been widely attempted to slow the progression of keratoconus, and eventually reduce the need for a keratoplasty. Its efficacy has been proven after 2 to 6 years of long-term follow-up studies without a relevant adverse effect (Raiskup-Wolf *et al*., 2008; Caporossi *et al*., 2010; Hashemi *et al*., 2013).

Corneal rigidity increased by more than 300% (Wollensak *et al*., 2003) and corneal Young's modulus increased by a factor of more than 4 in *in vitro* studies using stress-strain measurements (Wollensak *et al*., 2003; Knox Cartwright *et al*., 2012). Nonetheless, several *in vivo* studies could not find significant changes in CH and the CRF after CXL for 6 months to 2 years (Goldich *et al*., 2009b; Sedaghat *et al*., 2010; Spoerl *et al*., 2011; Gkika *et al*., 2012; Goldich *et al*., 2012). The mechanism underlying CXL in slowing down the progression of keratoconus is still yet to be fully understood. The effect of CXL may be contributed by the stromal ground substance of the cornea (Søndergaard *et al*., 2013). Notwithstanding, CH and CRF may represent the viscoelastic properties of the cornea, which are related to the ground substance and also their interactions with corneal collagen fibrils, rather than corneal collagen stiffness (Spörl *et al*., 2009). Another possibility is that CH and the CRF did not demonstrate significant changes after CXL because they may be influenced by other ocular structures such as the sclera, and thus, it may have scleral or ocular biomechanical properties (Reinstein *et al*., 2011).

Diabetes Mellitus

Increased protein glycosylation because of hyperglycemia in diabetes mellitus (DM) produces advanced glycosylation end products (AGEs), which could enhance collagen cross-linking, thereby stiffening the cornea (Brownlee *et al*., 2001). Few *in vitro* studies have been in agreement with glucose-mediated corneal stiffening (Monnier *et al*., 1988; Sady *et al*., 1995), and have shown that Young's Modulus of the cornea increased by 15% and 30% in low- and high-glucose-concentrated corneas, respectively, compared with controls (Ni *et al*., 2011).

Although higher CH and CRF have been postulated in DM patients, the findings from the literature remain inconclusive (Goldich *et al*., 2009a; Hager *et al*., 2009; Sahin *et al*., 2009; Castro *et al*., 2010; Kotecha *et al*., 2010). Moreover, similar CH and CRF values have been observed in Type 1 DM children and their age-, gender-matched controls (Kara *et al*., 2013; Nalcacioglu-Yuksekkaya *et al*., 2014). CH and the CRF has not been associated with the duration of DM (Hager *et al*., 2009; Sahin *et al*., 2009; Kotecha *et al*., 2010; Kara *et al*., 2013; Nalcacioglu-Yuksekkaya *et al*., 2014) for up to 24 years (Kotecha *et al*., 2010). Studies have shown a significant association between CH and the CRF with glycated haemoglobin (HbA1c) (Narayanaswamy *et al*., 2011; Scheler *et al*., 2012), whereas other studies have found no association between HbA1c and CH and the CRF (Sahin *et al*., 2009; Kotecha *et al*., 2010; Kara *et al*., 2013; Nalcacioglu-Yuksekkaya *et al*., 2014). HbA1c is another diagnostic indicator for DM, which is formed when glucose binds to hemoglobin. It could show the average blood glucose levels of DM patients for up to 6 to 12 weeks (Rochman, 1980). A higher level of HbA1c implies a greater possibility of developing diabetes-related complications.

DM is a complex systemic disease with two types (Type 1 and Type 2 DM). It is also associated with other systemic diseases, for example, hypertension, high

cholesterol, and renal disease. Thus, this might increase the difficulty in investigating the influence of CH and the CRF on DM patients, because DM-related systemic diseases may also affect the readings of CH and the CRF.

Soft contact lens wear, Orthokeratology and corneal edema

González-Méijome *et al*. (2008) postulated that a cornea with a lower rigidity is easier for reshaping and yields a faster outcome and recovery in orthokeratology treatment. Although several studies have found a decreased CRF after orthokeratology (Chen *et al*., 2009; Yeh *et al*., 2013), the mechanism behind it has yet to be confirmed. It could be related to the response of corneal remodeling during treatment. By contrast, the CRF was higher in long-term soft contact lens wearers, even after ceasing lens wear for at least 24 hours prior to the measurement (Cankaya *et al*., 2012). The CRF was shown to be a weak predictor for changes in CCT in the edema cornea, but CH was unable to quantify the changes, even if the contact lens-induced corneal edema was greater than 10% (Lu *et al*., 2007).

Connective tissue disorders

CH and the CRF are also influenced by other systemic diseases, especially connective tissue disorders, for example, Marfan syndrome, systemic lupus erythematosus (SLE), rheumatoid arthritis and scleroderma (**Table 1.2**). Lower CH and CRF were observed in Marfan patients with ectopia lentis (Kara *et al*., 2012). Moreover, lower CH and CRF were also observed in SLE patients compared with a control group, although the 2 groups had a similar CCT and AL (Yazici *et al*., 2011). Prata *et al*. (2009) found a decreased CH in rheumatoid arthritis patients, but they did not report the CRF findings. Collagen is the main structural protein of connective

tissue. Meanwhile, collagen fibrils comprise more than 70% of the dry weight of the cornea (Levin *et al*., 2011). The CRF typically undergoes greater changes than CH. Nonetheless, the involved control and experimental groups in these studies had wide ranges and large overlaps of CH and CRF. Whether CH and the CRF are sensitive parameters for detecting the corneal changes in these diseases remains unknown.

Table 1.2 Corneal hysteresis (CH) and corneal resistance factors (CRF) in patients with connective tissue disorders.

Studies	Systemic Diseases	CH (mmHg) mean ± SD (range)		CRF (mmHg) mean ± SD (range)	
		Control group	**Study group**	**Control group**	**Study group**
Prata *et al*. (2009)	Rheumatoid arthritis	10.3 ± 1.2 (9.8-10.9) n = 20	9.5 ± 1.4 (8.9-10.1) n = 11	n/a	n/a
Emre *et al*. (2010)	Scleroderma	9.5 ± 1.2 n = 29	9.8 ± 1.7 n = 29	9.2 ± 1.4	10.0 ± 1.5
Yazici *et al*. (2011)	Systemic lupus erythematosus	11.3 ± 1.3 (9.6-13.8) n = 30	10.2 ± 0.6 (9.2-11.6) n = 30	11.9 ± 1.5 (9.8-14.1)	9.7 ± 1.1 (7.8-11.4)
Kara *et al*. (2012)	Marfan Syndrome	11.1 ± 1.2 (8.3-13.1) n = 38	With ectopia lentis 9.9 ± 1.2 (8.6-11.7) n = 17	11.0 ± 1.3 (9.2-14.2)	With ectopia lentis 8.2 ± 1.8 (5.3-10.8)

n/a = not available.

Intraocular pressure

It has been well documented that the CCT could affect the clinical measurements of IOP. A thicker cornea is postulated to require greater force for applanation, and thus, tends to overestimate the IOP measurement, whereas a thinner cornea is likely to underestimate the IOP measurement (Bhan *et al*., 2002).

Recent reports have indicated that IOP measurements could be influenced by CH and the CRF (Hager *et al*., 2007; Kotecha, 2007; Touboul *et al*., 2008). A cornea with lower CH and CRF tends to be associated with an underestimated IOP (Touboul *et al*., 2008). The CRF has been reported to be a better parameter for predicting changes in GAT, instead of the CCT (Lau and Pye, 2012). CH remained steady within the physiological range of the IOP from 10 to 21mmHg (Tao *et al*., 2013), but had a weak and negative association with the IOP when the IOP was higher than the normal range (Ang *et al*., 2008; Kamiya *et al*., 2008).

The IOP obtained using dynamic contour tonometry was less dependent on corneal properties (Punjabi *et al*., 2006), and closer to intracameral IOP values (Boehm *et al*., 2004), whereas IOPcc was comparable to dynamic contour tonometry (Renier *et al*., 2010). A recent study reported that the IOP between glaucoma-treated patients and CCT-matched normal controls using GAT was substantially lower than when using IOPcc and dynamic contour tonometry (Costin *et al*., 2014). However, IOPcc was not affected by CCT, but by CH (Oncel *et al*., 2009; Del Buey *et al*., 2014). The limitation of correlating IOPcc to CH and the CRF was that they were all derived from the same raw applanation pressures, despite IOPcc being supposedly less affected by corneal biomechanics than GAT (Medeiros and Weinreb, 2006).

Glaucoma

CH and the CRF have recently garnered interest in glaucoma research after numerous studies have reported a decrease in CH with glaucomatous disc changes (Bochmann *et al*., 2008; Wells *et al*., 2008; Vu *et al*., 2013) and visual field defect progression (Congdon *et al*., 2006). The recovery of CH was observed after glaucoma treatment, although CH was still lower in treated glaucomatous eyes than in normal eyes (Sun *et al*., 2009). CH was suggested to be related to a thinner lamina cribosa (Congdon *et al*., 2006; Kirwan *et al*., 2006), and this was supported by the findings of lower CH in glaucomatous eyes with an acquired pit of the optic nerve. This is because the acquired pit of the optic nerve indicates a defect of the lamina cribosa (Bochmann *et al*., 2008). In addition, Lim and co-workers (2009a) found lower CH and CRF in healthy children with narrower retinal arterioles. Nonetheless, no association was found between CH with optic disc features in a large-scale cohort (Carbonaro *et al*., 2014).

1.5 Intraocular pressure and myopia

Researchers have indicated that the IOP and the mechanical properties of the eye wall influence ocular elongation (Tomlinson and Philips, 1970; Pruett, 1988; Edwards and Brown, 1993; Schmid *et al*., 2003). Numerous studies have been conducted to observing the relationship between the IOP and myopia, and have included children and adults as subjects. However, the findings of previous studies remain controversial. A higher IOPcc has been reported in high myopes (Altan *et al*., 2012; Del Buey *et al*., 2014). Moreover, a significant association has also been found between AL and IOPcc (Song *et al*., 2008; Altan *et al*., 2012).

Edwards and her co-workers performed two studies with different research designs to investigate the association between the IOP and SE in Chinese children. They measured the IOP by using a non-contact tonometer. The retrospective study involved 30 myopes and 30 age-matched non-myopes. Myopic children had a significantly higher IOP than non-myopic children (Edwards and Brown, 1993). In the later prospective study, they found the opposite results (Edwards *et al*., 1993). No significant association emerged between the IOP and SE in 123 Chinese children aged between 6 and 7 years. The later study was limited by an imbalanced sample size in the myopic (n = 13) and non-myopic (n = 93) groups. This might have affected the statistical power of data analysis.

Quinn *et al*. (1995) found a slightly higher IOP in myopic eyes than in non-myopic eyes. They measured the IOP by using a pneumatonometer on 321 children aged from 1 month to 19 years. Cycloplegic refraction was performed to observe the association between the IOP and refractive error. However, Lee and co-workers (2004) found contradictory results when they monitored the IOP, AL, and SE in children. Cycloplegic refraction and non-contact tonometry were performed on 636 Chinese children aged 9 to 11 years. The IOP was similar in children with different refractive statuses. In addition, the IOP had no association with AL or SE.

Jensen (1992) found that children with a higher baseline IOP (> 16mmHg) had a faster rate of axial elongation than children with an IOP ≤ 16mmHg in a 2-year longitudinal study. The IOP of 49 Danish children aged 9 to 12 years was measured using a Goldmann tonometer. Edwards and Brown (1996), Goss and Caffey (1999), and Schmid *et al*. (2003) have observed changes in the IOP in children during the refractive error shift, and they also could not find increments of IOP in myopes compared with non-myopes or children with greater refractive error shifts. These studies have postulated that an increased IOP occurs after the onset of myopia.

1.6 Conclusion

CH and the CRF have been introduced as corneal biomechanical properties approximately a decade ago. Different corneal parameters, ocular diseases, and systemic diseases have been documented to be associated with CH and the CRF. Numerous studies have found decreased CH in myopes. Nevertheless, our understanding of CH and the CRF and the development of myopia remains far from complete, and a gap must be filled, specifically, the roles of CH and the CRF in corneal geometry, corneal stromal thickness, and axial elongation.

Chapter 2 Aims of the study

2.1 Knowledge gaps

The cornea has a complex collagen fibres scaffold that contributes to the corneal biomechanical properties. This complex system increases the measurement difficulties of the corneal biomechanical properties. The ORA was the only available device for the *in vivo* measurement of corneal biomechanical properties when we started this study in 2009. CH and the CRF are corneal biomechanical parameters that can be assessed using the ORA. The measurement of corneal biomechanical properties, especially the stromal properties, is influenced by the direction of the lamellae. The ORA measurement relies on the reflection of an infrared beam from the corneal surface. The infrared transmitter and receiver of the ORA are placed laterally, and such a measurement provides CH and the CRF for the 'entire' cornea.

The ORA is a dynamic bidirectional applanation non-contact tonometer. Because of the potential interactions between the corneal biomechanical properties and corneal geometry, evaluating the within-subject corneal curvature variation on CH and the CRF is warranted. Previous studies have evaluated the association of ORA parameters with corneal curvature, but they have been based on corneal curvatures from different subjects, and inter-subject variation could be the confounding factor to different CH and CRF results.

Because the corneal stroma is the main contributor of the corneal biomechanical properties, it is critical to determine the influence of corneal stroma on CH and the CRF. Nonetheless, research investigating the association between corneal sublayer thickness such as stromal thickness with CH and the CRF has been scant. A stromal thickness examination is not a routine clinical measurement in ophthalmic practice, and therefore, whether it is a clinically valuable parameter remains unknown.

CH and the CRF have been found to decrease in long eyeballs, and the long eyeball is mainly caused by a deepening of the vitreous chamber from an extension of the scleral tissue. Researchers have postulated that the association between a decreased CH and CRF and scleral extension in a long eyeball is due to the cornea and sclera being derived from the same primitive tissue, the mesoderm. Corneal biomechanical properties should be greatly associated with the corneal stromal thickness. However, no study has investigated decreased CH and CRF after considering both corneal stromal thickness and AL.

Despite a recent abundance of studies reporting that CH has a weak association with AL and decreased CH in high myopes, whether the decreased CH is a factor for, or an outcome of axial elongation requires further investigation and clarification. A longitudinal study to monitor the changes of CH and the CRF and axial elongation can fill this knowledge gap.

2.2 Research Questions

Research Question 1:

What is the effect of corneal curvature on the ORA measurements when considering the inter-subject variation on CH and the CRF?
Null hypothesis: The ORA measurement is not affected by corneal curvature when considering the inter-subject variation on CH and the CRF.

Research Question 2:

What is the contribution of the corneal stromal thickness on lower CH in myopes compared with the effect of AL?
Null hypothesis: Lower CH in myopes is not caused by corneal stromal thickness, but by a long AL.

Research Question 3:

How do the ORA parameters change during axial elongation?
Null hypothesis: The ORA parameters do not change during axial elongation.

2.3 Aims and objectives of the study

To answer the research questions, this study investigates the association of ORA parameters with other corneal and ocular parameters. In addition, the long-term purpose is to monitor the changes of the ORA parameters with axial elongation in children.

The specific aims and objectives are as follows.

Aim 1:

To understand the influence of corneal curvature, corneal astigmatism, and corneal meridional differences on CH and the CRF.

Objectives:

We obtained data on the corneal biomechanical properties and corneal curvature by using the ORA and a corneal topographer. The ORA measurement was obtained at the recommended position, as well as at the superotemperal 10°, 20°, and 30°. We also calculated the corneal powers at those specific meridians and corneal astigmatism. We studied the associations between corneal powers with CH and CRF at different meridians.

Aim 2:

To investigate the association among ORA parameters, AL, and other corneal parameters, included corneal sublayer thickness in emmetropes and high myopes.

Objectives:

We assessed the corneal biomechanical properties, corneal thickness, corneal sublayer thickness, AL, corneal curvature, and CV by using the ORA, a confocal

microscope, a partial coherence interferometer, and a rotating Scheimpflug camera system. We developed a method to reduce the inter-observer variation of confocal microscopy. We compared the differences of emmetropic and highly myopic eyes.

Aim 3:

To observe the changes of ORA parameters with axial elongation in children.

Objectives:

We conducted vision screenings in different local and international schools in different districts of Hong Kong. We assessed the corneal biomechanical properties, AL, corneal curvature, corneal astigmatism and SE by using the ORA, a partial coherence interferometer and a handheld automatic kerato-refractometer. The same measurement protocol was used in the cross-sectional study, 1-year cohort and 2-year cohort to study the changes in CH and CRF as well as other ocular parameters with axial elongation.

Chapter 3 Influence of corneal astigmatism, corneal curvature, and meridional differences on corneal hysteresis and the corneal resistance factor

3.1 Introduction

The cornea is a transparent and mechanically tough tissue. The restrictions of corneal biomechanical properties measurement had either been *in vitro* (Förster *et al*., 1994; Hjortdal, 1995; Zeng *et al*., 2001) or involving complicated calculations (Buzard, 1992; Carnell and Vito, 1992). The ORA is a non-contact tonometer capable of *in vivo* measurement of corneal biomechanical properties, in terms of CH and the CRF. Various studies have been conducted to investigate these parameters as they may play a role in the etiology of some ocular diseases (Spörl *et al*., 2009; Abitbol *et al*., 2010; Schweitzer *et al*., 2010). These parameters also had been found to be significantly reduced after refractive surgery (Pepose *et al*., 2007; Chen *et al*., 2008; Shah *et al*., 2009) and to influence the measurement of IOP (Liu and Roberts, 2005; Hager *et al*., 2007).

Because CH and the CRF are derived from the applanated pressures and IOP measurement could be influenced by corneal astigmatism (Mark and Mark, 2003; Rask and Behndig, 2006), there had been one study investigating the correlation between CH and corneal astigmatism (Broman *et al*., 2007). The alignment of the infrared electro-optical system in the ORA is placed laterally, thus the ORA may monitor the inward and outward corneal deformation mainly along the horizontal meridian only. Because of the specific arrangement of collagen fibrils, cornea exhibits different elastic modulus at different regions (Kotecha, 2007). The corneal biomechanical properties may be different along different corneal meridians. There were limited studies investigated the association between corneal biomechanical properties and corneal curvature (Broman *et al*., 2007; Franco and Lira, 2009), further, they used the average corneal curvature only.

The purpose of this study was to observe the influence of corneal curvature, corneal astigmatism, and corneal meridional differences on CH and the CRF.

3.2 Methodology

3.2.1 Subjects

Ninety-five normal subjects (50 male and 45 female) with a mean age of 23.9 ± 4.0 years (range from 19 to 40 years old) were enrolled in this study. They were staff and students of the University. Exclusion criteria included rigid contact lens wear of any kind (such as orthokeratology), history of ocular surgery, corneal disease or trauma, and use of any ocular medication or pregnancy. This study was approved by

the ethics committee of the University and was carried out with due regard to the tenets of the Declaration of Helsinki. Details of the study were given to subjects before informed consent was obtained.

3.2.2 Procedures

The measurements included corneal topography by the Medmont E300 system (Medmont; Melbourne, Australia) and corneal biomechanical properties by the ORA, in a random sequence. For corneal topography, three valid readings with ratings at least 80 were used (Cho *et al*., 2002). Each of these three readings was first converted to its vector form (M, J_0 and J_{45}) for averaging and then converted back to its spherocylindrical form for analysis (Thibos *et al*., 1997).

For corneal biomechanical properties, measurements were obtained at the recommended (default) position as well as positions with different amounts of head tilt. Subject was required to wear a headband which sewed with protractor and spirit level to indicate the head position. Subject was instructed to seat in front of the instrument and to fixate at the internal blinking green light. For measurement at the default position, the indicator should be aligned with the reading of the protractor at 90° (**Figure 3.1**). After proper alignment of the head position, three consecutive ORA measurements with waveform score of at least 3.5 were obtained (Lam *et al*., 2010). Corneal biomechanical properties along the superotemporal to inferonasal (called superotemporal in the following context) meridians from different amounts of head tilt (they were 10°, 20°, and 30°, respectively) were also measured. To achieve this, subject was required to tilt the head 10°, 20°, and 30° to the right (for right eye measurement) and 10°, 20°, and 30° to the left (for left eye measurement) respectively. The amount of head tilt was checked from the headband (**Figures 3.2a & 3.2b**). Eyelids were lifted up if necessary to obtain a valid reading. The ORA measurements at different meridians were performed randomly to avoid bias.

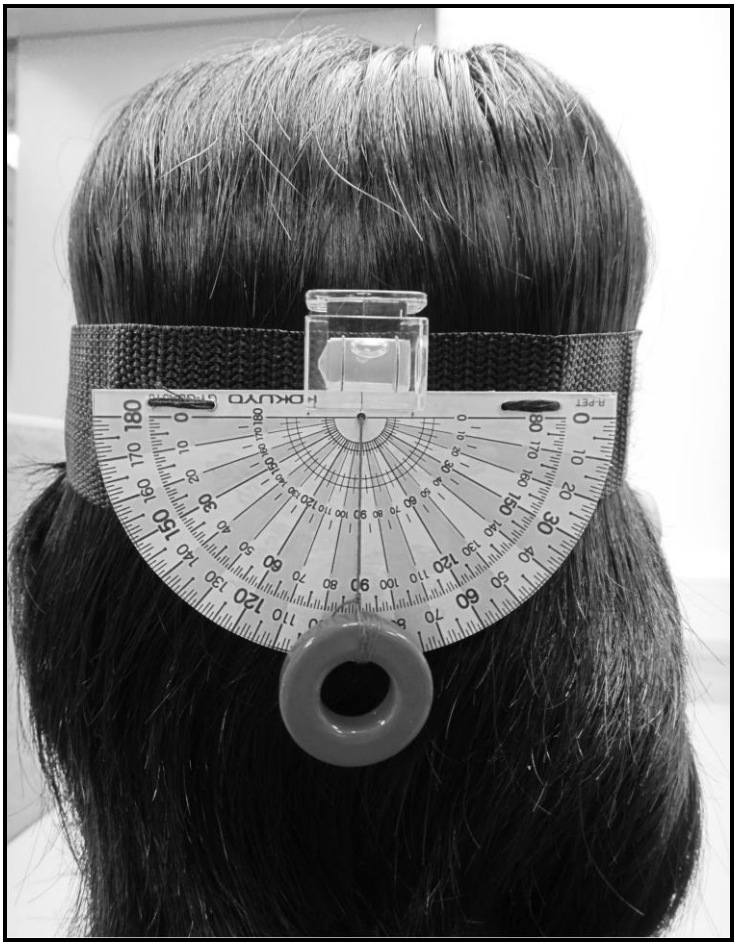

Figure 3.1 Default head position of a subject, the indicator is aligned with the reading of the protractor at 90°.

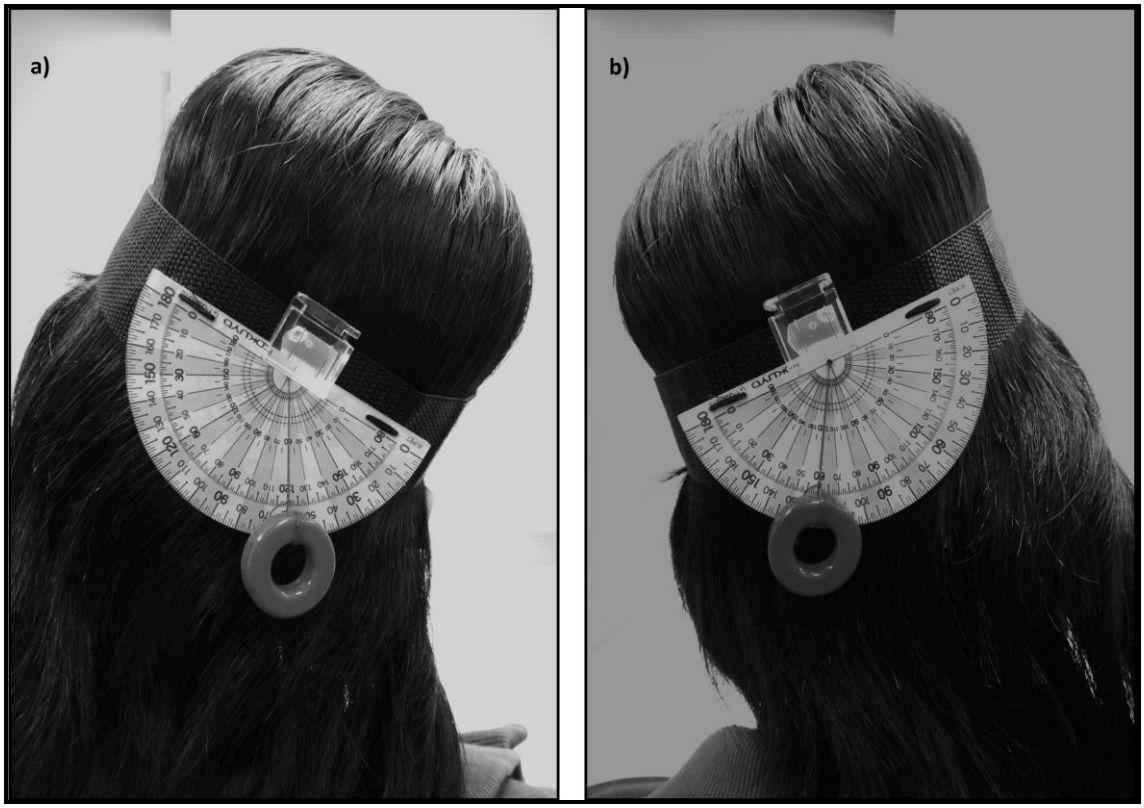

Figure 3.2 (a) A 30° head tilt to the right for right eye measurement. The indicator is aligned with the reading of 120° at the protractor. (b) A 30° head tilt to the left when measuring the left eye. The indicator is aligned with the reading of the protractor at 60°.

3.3 Statistical analysis

The within-subject variation of CH was 0.80mmHg (Lam and Chen, 2007; Lu *et al*., 2007) and the standard deviation of CH in normal young adult Chinese was 1.50mmHg (Lam *et al*., 2007). To investigate if corneal astigmatism affects CH, it requires 38 subjects in each of the low and high astigmatic group (determined from the average corneal astigmatism of the sample) to detect a difference of at least 0.80mmHg at a power of 90% with significance level of 0.05. Both eyes were measured and the eye with a higher amount of corneal astigmatism was used for analysis. The corneal powers along horizontal, and 10°, 20°, and 30° at superotemporal were calculated from the Medmont results (Thibos *et al*., 1997). The Kolmogorov-Smirnov test was used to assess the normality of the data. Parametric (paired t-tests, unpaired t-tests, and repeated measures analysis of variance, ANOVA) and non-parametric (Wilcoxon matched pairs tests) tests were used to analyse the data as appropriate. Whenever significant differences were found in repeated measures-ANOVA, post hoc tests with Bonferroni corrections were performed to minimize any Type I error. Pearson correlation and regression were used to investigate the relationships of CH and CRF with corneal astigmatism and corneal power. A value of $p < 0.05$ was considered significant in all statistical tests. All analyses were conducted using SPSS software version 15 (SPSS Inc., Chicago, Illinois, USA) and SigmaPlot software version 11 (Systat Software Inc., San Jose, California, USA).

3.4 Results

There was no significant difference between the two eyes in SE and corneal curvature in terms of M, J_0 and J_{45} (paired t-tests, $p > 0.05$). Forty-two right eyes and 53 left eyes were involved in the following analysis. The SE and corneal curvature of these 95 eyes are shown in **Table 3.1**. With-the-rule corneal astigmatism was found in 92 eyes and three eyes had against-the-rule corneal astigmatism. The CRF at the default position had a significant but weak positive correlation with corneal astigmatism ($r^2 = 0.04$, $p = 0.047$). The correlation between CH at the default position and corneal astigmatism was not significant ($r^2 = 0.01$, $p = 0.39$).

When the whole sample (95 eyes) was divided into two groups using the mean corneal astigmatism (1.51D) as the cutoff, there were 57 eyes with corneal astigmatism less than 1.51D and 38 eyes with corneal astigmatism at 1.51D or higher. CH and the CRF between these two groups were similar (Unpaired t-tests, CH, $p = 0.48$, CRF, $p = 0.15$).

The corneal measurements along different meridians were tabulated in **Table 3.2**. There was significant difference in CH at different meridians (Repeated measures-ANOVA, $F = 4.908$, $p < 0.01$). Post hoc test showed that CH at the default position was significantly lower. Although there was an increasing trend in the CRF from obliquity, there was no significant difference in the CRF at different meridians (Repeated measures-ANOVA, $F = 1.138$, $p = 0.33$).

Table 3.1 Demographic data of 95 eyes.

Parameters	mean ± standard deviation (range)
Spherical equivalent (D)	–4.07 ± 3.14 (–14.75 to 2.50)
M component (D)	43.46 ± 1.50 (39.43 to 48.37)
J_0 component (D)	0.69 ± 0.42 (–0.52 to 1.91)
J_{45} component (D)	–0.04 ± 0.26 (–0.75 to 0.53)
Corneal astigmatism (D)	1.51 ± 0.77 (0.40 to 3.82)

Table 3.2 Corneal hysteresis (CH), corneal resistance factor (CRF), and corneal power along different corneal meridians. The mean ± standard deviation (SD) is shown, with the range in parenthesis.

	Default position mean ± SD (range)	10-degree mean ± SD (range)	20-degree mean ± SD (range)	30-degree mean ± SD (range)
CH (mmHg)	10.86 ± 1.30 (7.23 to 13.77)	11.09 ± 1.26* (7.20 to 13.80)	11.17 ± 1.36** (7.90 to 14.67)	11.10 ± 1.29* (7.23to 13.77)
CRF (mmHg)	10.75 ± 1.43 (6.73 to 14.00)	10.81 ± 1.41 (6.83 to 13.87)	10.86 ± 1.47 (7.43 to 14.17)	10.88 ± 1.42 (6.77 to 14.00)
Corneal power (D)	42.77 ± 1.43 (38.82 to 47.15)	42.82 ± 1.43** (38.80 to 47.27)	42.95 ± 1.44** (38.87 to 47.48)	43.15 ± 1.46** (39.00 to 47.77)

*significant difference compared with default position (post hoc test, $p < 0.05$)
**significant difference compared with default position (post hoc test, $p < 0.01$)

The corneal power was significantly increased from horizontal towards 30° superotemporal (Repeated measures-ANOVA, $F = 114.998$, $p < 0.01$). CH was significantly correlated with corneal power at all meridians (**Table 3.3**). The association was weak which accounted for 6% of the difference in CH solely by corneal power ($r^2 = 0.06$). The correlation between the CRF and corneal power was significant at 30° superotemporal only but the correlation was very weak ($r^2 = 0.08$). The correlations were not significant at other meridians after the Bonferroni correction.

Table 3.3 Correlations between corneal hysteresis (CH), the corneal resistance factor (CRF), and corneal power at different meridians.

	CH (mmHg)	CRF (mmHg)
Default position	$r^2 = 0.06$, $p = 0.01$	$r^2 = 0.03$, $p = 0.11$
10-degree	$r^2 = 0.06$, $p = 0.02$	$r^2 = 0.04$, $p = 0.07$
20-degree	$r^2 = 0.06$, $p = 0.02$	$r^2 = 0.07$, $p = 0.01$
30-degree	$r^2 = 0.06$, $p = 0.02$	$r^2 = 0.08$, $p < 0.01$

With Bonferroni correction, it should reach 0.0125 to be significant.

3.5 Discussion

The ORA can measure corneal biomechanical properties in terms of CH and the CRF. There had been some reports on these corneal parameters in different ethnic and age groups recently (Shah *et al*., 2006; Lam *et al*., 2007; Lim *et al*., 2009a). The ORA is a dynamic bidirectional applanation device which uses air puff to flatten the central 3 mm corneal region. The pressure required to flatten this pre-determined area could be affected by corneal astigmatism similar to that in the GAT (Mark and

Mark, 2003; Rask and Behndig, 2006). In the current study, a weak and marginally significant correlation was found between corneal astigmatism and the CRF. To our knowledge, this is the first study investigating the association between corneal astigmatism and the CRF. The correlation between corneal astigmatism and CH was not significant. We further divided our sample into high and low corneal astigmatism subgroups using their mean results. These two subgroups had similar CH and the CRF. The effect of corneal astigmatism on the GAT may not be applicable in the ORA because GAT requires the entire corneal area (3.06 mm diameter) to form two semi-circular rings. Rings formed will not be circular but elliptical in astigmatic cornea. Hagishima and co-workers (2010) found positive correlation between corneal astigmatism and the GAT-IOP but the effect of corneal astigmatism on the ORA-IOP was not significant. They did not investigate corneal biomechanical properties in their study. Broman *et al*. (2007) could not find any significant correlation between corneal astigmatism and CH. However, their sample included eyes with glaucoma. From the current study, it appeared that the measurement of CH and the CRF was not affected by corneal astigmatism. Although the highest corneal astigmatism of our sample was 3.82D, the number of subjects with astigmatism greater than 1.51D was limited.

Since the ORA infrared emitter and receiver are placed laterally, CH and the CRF measured may represent the corneal biomechanical properties along the horizontal meridian. We further our investigation by measuring these parameters with subjects having different amounts of head tilt. Superotemporal meridian was selected and measured up to 30° because oriental eyes tend to have narrow palpebral aperture (Lam and Loran, 1991). Some of our subjects required to have their eyelids lifted up using eyelid retractor. Both CH and the CRF were measured the lowest along the horizontal meridian (**Table 3.2**) and showed an increasing trend from obliquity. On average, the greatest regional CH difference was 0.31mmHg (CH-default versus CH-20°). Clinically, such a difference is not significant because the within-subject CH

variation could be up to 0.80mmHg (Lu *et al*., 2007; Lam and Chen, 2007). The CRF difference could not reach statistical significance.

Broman *et al*. (2007) found positive correlation between corneal curvature and CH. They used average corneal curvature instead of neither the flattest nor the steepest meridians. Franco and Lira (2009) could not demonstrate any significant correlation between corneal radius and CH and the CRF. They did not specify which corneal meridian and probably they used the mean corneal radius. The corneal powers along different measurement meridians were also calculated in the current study. Majority of our subjects had with-the-rule corneal astigmatism (except three subjects), the corneal power was calculated the greatest along 30° superotemporal. A stronger air puff should be needed to flatten a more curved cornea (Mark and Mark, 2003). Since CH and the CRF are derived from applanated pressures, we wonder if the viscoelastic damping property (that is CH) or the corneal resistance (CRF) would also be affected. Although the correlations between each of these biomechanical properties (CH and CRF) and the calculated corneal power along any particular meridian were significant, they were all very weak (**Table 3.3**). The influence of corneal power on CH and the CRF could not be demonstrated. Interestingly, the coefficient of determinations (r^2) was similar for CH along different meridians while there was an increasing trend for the CRF. The measurement of CH and the CRF becomes increasingly popular especially in corneal refractive surgery (Ortiz *et al*., 2007; Pepose *et al*., 2007; Chen *et al*., 2008; Hamilton *et al*., 2008; Shah *et al*., 2009). Almost all previous studies had documented a greater CRF drop than CH after the surgical procedure (Ortiz *et al*., 2007; Pepose *et al*., 2007; Chen *et al*., 2008; Hamilton *et al*., 2008). Could the CRF be a more sensitive parameter than CH? This requires further investigation.

Although the calculated corneal power represented the curvature of that particular meridian, the measured CH and CRF were not exactly along these meridians due to compensatory torsional eye movement from head tilt. Lam *et al*. (2000) reported an

average of 6.5° compensatory eye rotations during a 90° head tilt. Therefore, the measured CH and the CRF with 30° head tilt may only represent corneal biomechanical properties along 24° superior temporal, not exactly 30° superior temporal.

The current study is limited by the lack of corneal thickness measures. Both CH and the CRF had been found higher in thicker cornea (Franco and Lira, 2009). The earlier model of the ORA did not come with a chin rest. Subjects, especially children might have difficulty to maintain an upright head posture following the recommendation of manufacturer. However, from the current study, it showed that the measured CH and CRF was less likely to be affected by head tilt during the measurement.

3.6 Conclusion

Both CH and the CRF were measured lower along the horizontal meridian but the difference was small and clinically insignificant. Higher CH and CRF were also found in steeper cornea, but the influence of corneal curvature and corneal astigmatism on CH and the CRF were negligible. CH and the CRF were not affected by corneal curvature even the inter-subject variations were taken into account.

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Chapter 4 The roles of cornea and axial length in corneal hysteresis among emmetropes and high myopes

4.1 Introduction

Myopia is caused by axial elongation, particularly in the vitreous chamber (McBrien and Gentle, 2003). Previous studies have reported that scleral thickness and scleral rigidity are reduced in myopes (McBrien *et al*., 2009; Sergienko and Shargorogska, 2012). A thin sclera in a myopic eye is associated with the thinning of collagen fibrils bundles, and with abnormal and deranged collagen fibrils in the sclera (Rada *et al*., 2006).

The introduction of the ORA, a dynamic bidirectional corneal applanation device, has enabled the clinical measurement of some corneal biomechanical properties. CH and the CRF are 2 corneal biomechanical properties that can be obtained using the ORA (Kotecha, 2007). CH represents the ability of cornea to absorb and dissipate energy, whereas the CRF expresses the overall resistance of the cornea (Kotecha, 2007). The IOP parameters from the ORA include IOPg and IOPcc.

Numerous studies had been conducted to observe the changes in corneal biomechanical properties in myopes (Lim *et al*., 2008; Shen *et al*., 2008a; Song *et al*., 2008; Chang *et al*., 2010; Xu *et al*., 2010; Jiang *et al*., 2011; Narayanaswamy *et al*., 2011; Plakitsi *et al*., 2011; Altan *et al*., 2012; Kara *et al*., 2012). However, the results were contradictive. Several studies had demonstrated a significant and negative association among corneal biomechanical properties with AL and SE (Shen *et al*., 2008a; Song *et al*., 2008; Chang *et al*., 2010; Xu *et al*., 2010; Jiang *et al*., 2011; Narayanaswamy *et al*., 2011; Plakitsi *et al*., 2011; Altan *et al*., 2012; Kara *et al*., 2012), whereas there was study could not reveal any significant correlation among these variables (Lim *et al*., 2008). In addition, some studies had considered only the refractive errors, in terms of SE without including the AL (Shen *et al*., 2008a; Jiang *et al*., 2011; Plakitsi *et al*., 2011).

Corneal hysteresis and the CRF are concomitant with the CCT, with a thick cornea giving a high CH and CRF (Lim *et al*., 2008; Song *et al*., 2008; Chang *et al*., 2010; Plakitsi *et al*., 2011). Corneal stroma contributes to over 80% of the total corneal thickness (Levin *et al*., 2011). The presence of collagen fibrils in the stroma are primarily attributed to the corneal biomechanical properties (Kotecha, 2007). The reduced corneal biomechanical properties in the corneal ectasia (Kozobolis *et al*., 2012), after corneal refractive surgeries (Hamilton *et al*., 2008) and in Marfan patients is possibly related to the changes in the corneal stroma (Kara *et al*., 2012). We would hypothesize a stronger association between stromal thickness and the CH and CRF than AL does in myopia. Stromal thickness and AL are worth investigating together. A study recently reported that the CV at the 7-mm zone and 5-mm zone were also strongly correlated with CH and CRF, respectively (Sedaghat *et al*., 2012).

The corneal stromal thickness can be obtained from a confocal microscope with z-ring attachment (Chan *et al*., 2011). This is an invasive procedure as it can cause corneal staining (Chan *et al*., 2012). The measurement of corneal stromal thickness relies on the skills of practitioner to select the appropriate frames (Chan *et al*., 2011).

Because corneal stromal thickness is not commonly measured clinically, we would first confirm its reproducibility (Section 4.2.2.2) before applying it in our experiment.

The current study aimed to study the associations between CH and CRF with corneal stromal thickness, CV, and AL, in order to observe the contribution of different factors on CH and the CRF.

4.2 Methodology

4.2.1 Subjects

Forty young (from 18 to 35 years old) emmetropes and 40 age-matched high myopes were recruited for this study. Emmetropes were control subjects and defined as the SE within ± 0.50D and high myopes had an SE of at least -6D and an AL of more than 26mm. The exclusion criteria included a history of corneal surgery, corneal disease, glaucoma, or systematic diseases. The amount of corneal astigmatism was not considered as an exclusion criterion because corneal astigmatism minimally affects the ORA measurements (Wong and Lam, 2011). Participants who wore soft contact lenses were required to stop wearing them for a minimum of 24 hours before the examination. Informed consent was obtained from the participants after knowing the study protocol. This study was approved by the Ethics Committee at The Hong Kong Polytechnic University and adhered to the Declaration of Helsinki.

4.2.2 Procedures

All participants underwent a comprehensive eye examination, which included visual acuity (VA) measurement using a logarithmic minimum angle of resolution (logMAR) chart, subjective refraction, slit-lamp biomicroscopy, and funduscopy. The SE of the refractive error was defined as the sum of the spherical refractive error and half of the astigmatism of the subjective refraction. The slit-lamp biomicroscopy was performed to exclude any corneal disease. The ORA was used to measure the corneal biomechanical properties and IOP. Only IOPcc was considered in the current study because IOPg is affected by corneal properties such as CCT, and IOPcc is not (Lam *et al*., 2007). IOPcc also demonstrated high concordance with dynamic contour tonometry (Costin *et al*., 2014). Three ORA readings with a smooth signal and a waveform score of 3.5 or above were obtained from each eye (Lam *et al*., 2010). Five AL and anterior chamber depth (ACD) measurements were obtained using a partial coherence interferometer (IOLMaster; Carl Zeiss, Jena, Germany). The corneal topography (in terms of mean keratometry reading, MeanK) and CV were measured using a rotating Scheimpflug camera-based corneal topographer (Pentacam; Oculus, Dutenhofen, Germany) at a 25-scan mode in triplicate. The CV at the 3-mm, 5-mm, 7-mm, and 10-mm zones were used for analyses (Sedaghat *et al*., 2012). Corneal thickness was measured using a confocal microscope.

To control for potential diurnal variations (e.g. IOP), all of the measurements were obtained between 4:00 PM and 8:00 PM. Confocal microscopy was always conducted last because of the invasive nature of the procedure. All measurements were performed on both eyes. However, only the more emmetropic eyes in the control group and the more myopic eyes in the high myopic group were studied.

4.2.2.1 Confocal microscopy

Corneal thickness was measured using the Confoscan 4 confocal microscope (CS4; Nidek Technologies, Padova, Italy) with the z-ring attachment. The z-ring adapter was placed in front of a 40x objective lens and the light intensity of the CS4 was set between 75 and 78. It scanned through the cornea from the posterior to the anterior surfaces and recorded 25 video frames per second. A maximum of 350 frames were captured for each scan and the minimum step distance was 4μm between frames. During the scan, the z-ring and the tip of the objective lens were immersed in an ophthalmic gel (Lacryvisc® unidose, Alcon Laboratories, Kaysersberg, France). Subjects were required to wear a pair of bandage contact lenses. A drop of 0.5% proparacaine (Alcaine, Alcon Laboratories, Hong Kong, China) was instilled into each eye prior to the scan. The Etafilcon A soft hydrogel contact lens was used as the bandage contact lens (58% water content, base curve 9.0mm, diameter 14.2mm, power -3D and centre thickness 0.084mm) to prevent corneal staining and improve the image quality without affecting the corneal sublayer measurement (Chan *et al*., 2012). The examiner advanced the z-ring until the coupling gel contacted with the cornea through the contact lens and the auto-alignment mode was initiated once the stroma was observed. For most of the subjects, the fully automated feature was used for final alignment and landing of the z-ring on the central cornea. Manual landing was performed on some of the subjects when the automatic mode took longer than the average time to land. Three completed scans through the cornea with satisfactory image quality were obtained from each eye. After completing the scan, the bandage lenses were removed followed by slit-lamp biomicroscopy to ensure that good corneal health was maintained.

The corneal and corneal sublayer pachymetry in CS4 were measured by selecting the corresponding corneal structural frames from the light intensity profile manually

(Brugin *et al*., 2007; McLaren *et al*., 2007; Chan *et al*., 2011; Chan *et al*., 2012). A light intensity profile and the peak locations for corneal structures of interest are shown in **Figure 4.1**. Corneal thickness (CT) was defined as the distance between the epithelium and endothelium (Li *et al*., 1997). Concisely, the epithelial and Bowman's layer thickness (Epi+BT) was the distance between epithelium and the most anterior keratocytes, thus Bowman's layer was included (Li *et al*., 1997). Meanwhile, full stromal thickness (FST) was the distance between the most anterior keratocytes and the most posterior keratocytes (McLaren *et al*., 2010). On the other hand, endothelial thickness (EndoT) was the distance between the most posterior keratocytes and the endothelium, which included the Descemet's membrane.

4.2.2.2 Reproducibility study

There are detailed descriptions about corneal sublayer pachymetry using CS4 with z-ring in the literatures (Brugin *et al*., 2007; McLaren *et al*., 2007; Chan *et al*., 2011; Chan *et al*., 2012), but discrepancy could still happen between observers following the written guideline. This was because there could be in- and out-focus frames on the same peak of the light intensity profile (Chan *et al*., 2011), further some corneal frames could also be obscured by other corneal structures. All these factors could induce variation in frame selection.

A reproducibility study involved 34 subjects at the mean age of 24.4 ± 3.1 years old were performed in order to observe and describe the method of reducing inter-observer variation during corneal sublayer pachymetry. None of the subjects had any history of ocular surgery, ocular disease, systemic disease, pregnancy or rigid lens wear. Subjects included four soft contact lens wearers who ceased wearing contact lenses for a minimum of 24 hours before the examination. Subjects underwent slit-

lamp biomicroscopy before and after performing confocal microscopy in order to ensure their corneal integrity.

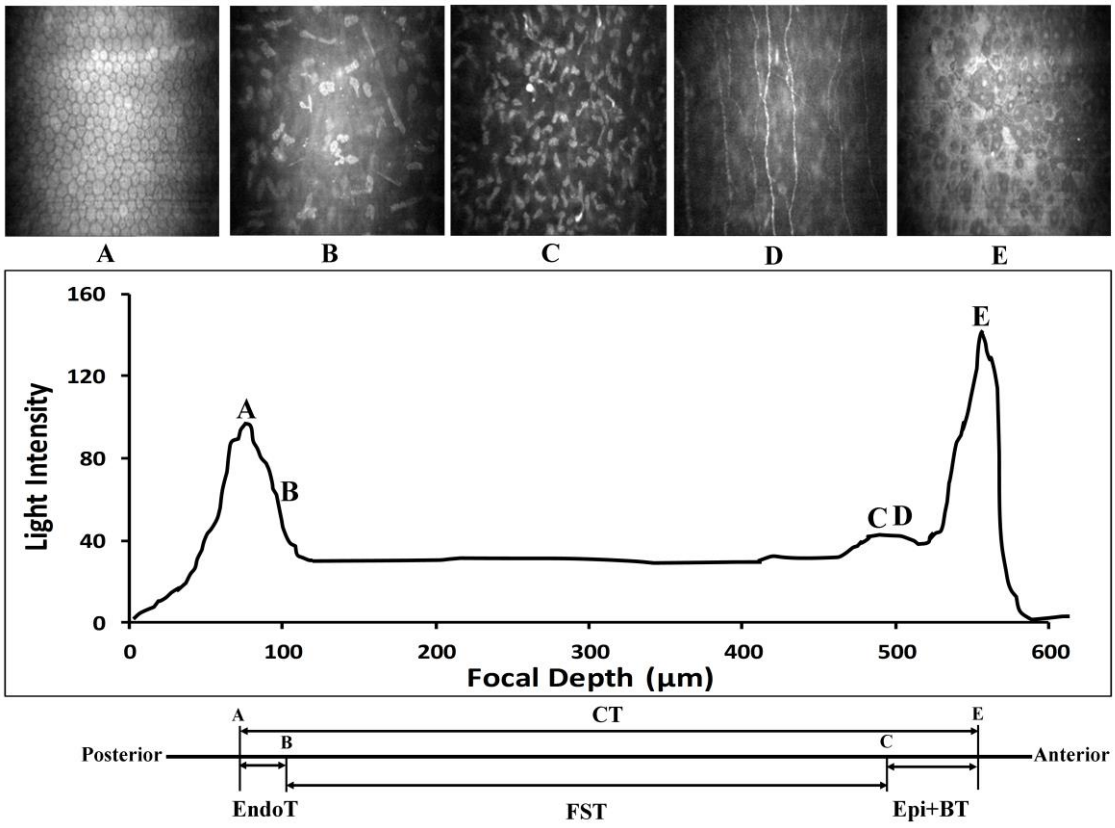


Figure 4.1 The corneal structure frames (**A-E**) and their corresponding peaks or locations at a light intensity profile.

A = Endothelium
B = The most posterior keratocytes
C = The most anterior keratocytes
D = Subbasal nerve plexus
E = Epithelium
CT = Corneal thickness, from A to E
EndoT = Endothelial thickness (included Descemet's membrane), from A to B
FST = Full stromal thickness, from B to C
Epi+BT = Epithelial and Bowman's layer thickness, from C to E

One pass from each scan with a better quality was selected by a masked and the same observer for pachymetry measurements. The average reading of three passes from three independent scans was used for analysis. In order to study the inter-observer variation, two masked observers performed the corneal sublayer measurement (measurement 1) by using the written protocols (Li *et al*., 1997). First, the peaks of the corresponding corneal structures were detected. Then, the best in-focus image for the interested corneal structures was identified. After that, the z-axis position of the corneal frame was ascertained. Last, the distance between the selected frames was calculated.

The two observers then randomly selected some corneal frames from the CS4 and went through them together following the above protocols. During this jointed session, observers came up with consensus on the criteria for identifying some corneal frames in doubt. A typical example was that the most sharply focused corneal frame was not corresponding to the peak of the light intensity profile, but one frame behind or in front of it. Another example was corneal frame obscured by other corneal structures. The two masked observers reassessed the corneal sublayer measurement of the same set of scans independently using the revised criteria (measurement 2). Again, the observers were masked during the corneal sublayer measurement. In measurement 2, all selected corneal frames were free from the obscuration of the other corneal structure. The most anterior in-focus frame of epithelium and the most anterior keratocytes were chosen while the most posterior in-focus frame of endothelium and the most posterior keratocytes were selected. Eight subjects who did not meet these criteria in either one of the three scans were excluded in the measurement 2.

Thirty-four subjects were included in the analysis of measurement 1 (24 males and 10 females), whereas only 26 subjects were included in measurement 2 (17 males and 9 females). Measurements of the right eye were used for analyses as there was no significant difference between the left and right eyes (paired t-test, $p > 0.05$). The

CT, corneal sublayer thickness as well as the inter-observer variation in the two measurements are shown in **Table 4.1**. CT yielded significant inter-observer difference in measurement 1, but no significant difference was found in measurement 2 (paired t-test, $p = 0.0014$ and $p = 0.41$, respectively). EndoT remained significant between observers throughout the study (paired t-test, $p < 0.0001$ and $p = 0.02$, respectively). No significant difference was found between observers in Epi+BT and FST for the two measurements (paired t-test, $p > 0.05$).

Inter-observer difference significantly reduced in CT ($1.93 \pm 3.23\mu m$ versus $0.28 \pm 1.75\mu m$ in measurements 1 and 2, respectively; Wilcoxon signed-rank test, $p = 0.014$) and EndoT ($4.01 \pm 4.82\mu m$ versus $0.86 \pm 1.81\mu m$ in measurements 1 and 2, respectively; paired t-test, $p = 0.03$) using the revised criteria. Although there was no significant inter-observer difference in the two measurements in FST (paired t-test, $p = 0.53$) and Epi+BT (Wilcoxon signed-rank test, $p = 0.39$), the SD of the inter-observer difference was reduced by half in measurement 2 (**Table 4.1**). The reduction in SD was significant in CT and all the three corneal sublayers (F-tests, all $p < 0.01$). The 95% confidence limits of the inter-observer difference for CT, FST, Epi+BT, and EndoT were 6.33µm, 13.74µm, 6.55µm, 9.45µm (measurement 1); and 3.43µm, 3.33µm, 3.41µm, and 3.55µm (measurement 2), respectively. The 95% limits of agreement (LoA) plots of CT and corneal sublayer pachymetry for the two measurements are shown in **Figure 4.2**.

FST is a clinically useful parameter which solely consisted of stromal structures measured from the most anterior keratocytes to the most posterior keratocytes. Additionally, FST also had consistent findings between the two measurements as well as low inter-observer difference. The findings from the reproducibility study suggested that observers should go through some corneal frames together to reduce inter-observer variation in CT and corneal sublayer pachymetry. This could assist observers to choose the frame following the same criteria and reduce examiner bias.

Table 4.1 The corneal and corneal sublayer thickness measured by the two observers in measurements 1 and 2.

		Thickness (µm, mean ± standard deviation)			
		Cornea	Full stroma	Epithelium and Bowman's layer	Endothelium
Measurement 1 n = 34	Observer A	546.20 ± 26.64	459.11 ± 25.87	66.65 ± 8.33	20.44 ± 5.02
	Observer B	544.26 ± 26.21	461.21 ± 24.94	66.63 ± 7.63	16.43 ± 5.24
	Difference (95% CI)	1.93 ± 3.23** (0.80 to 3.06)	-2.10 ± 7.01 (-4.55 to 0.35)	0.02 ± 3.34 (-1.15 to 1.19)	4.01 ± 4.82** (2.33 to 5.69)
Measurement 2 n = 26	Observer A	551.85 ± 22.45	464.64 ± 21.17	68.56 ± 8.05	18.64 ± 5.47
	Observer B	551.56 ± 22.50	464.58 ± 21.73	69.21 ± 7.88	17.78 ± 4.80
	Difference (95% CI)	0.28 ± 1.75 (-0.42 to 0.99)	0.06 ± 1.70 (-0.63 to 0.75)	-0.64 ± 1.74 (-1.34 to 0.06)	0.86 ± 1.81* (0.13 to 1.59)

CI= Confidence limits
* $p < 0.05$, ** $p < 0.01$

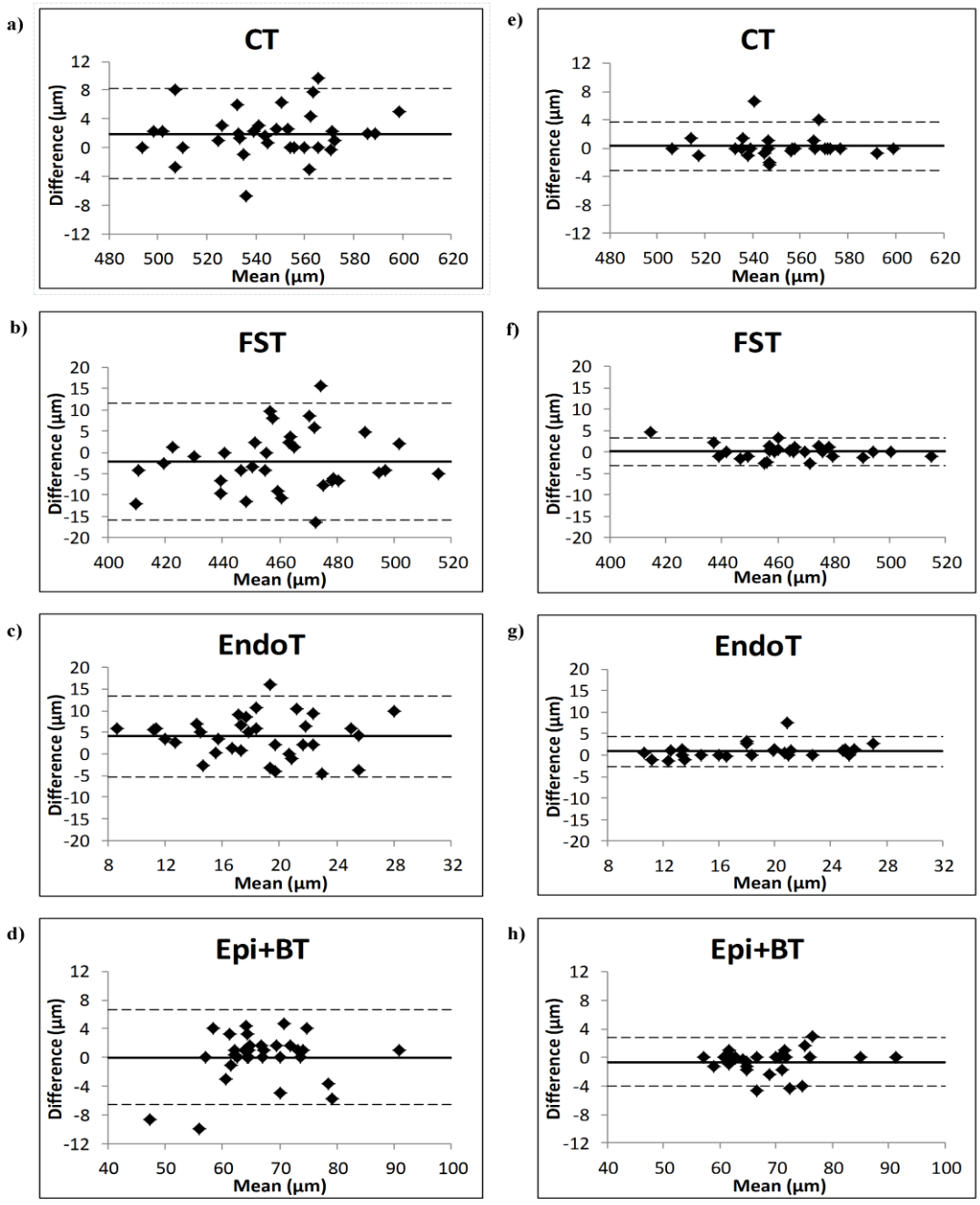


Figure 4.2 The Bland and Altman plots of the corneal and corneal sublayer thickness for measurement 1, n = 34 ((a), (b), (c), and (d)) and measurement 2, n = 26 ((e), (f), (g), and (h)). The dashed lines show the upper and lower 95% limits of agreement while the continuous line expresses the mean differences.

4.2.2.3 Inclusion criteria for confocal microscopy

Three cornea scans were obtained from each eye and only one pass from each scan was selected for pachymetry. The average reading obtained from the 3 passes performed in 3 independent scans was used for analysis. The CS4 readings involving strong eye movement, difficulty in detecting the peaks of the corresponding corneal structures, out-focus corneal frame, or the corneal frame obscured by other corneal structures were excluded from the analysis. Only 30 young emmetropes (20 male, 10 female) and 27 high myopes (18 male, 9 female) produced valid CS4 readings. The following analyses were based on these 57 participants.

4.3 Statistical analysis

A sample size calculation was conducted using G*Power version 3.1.2 (Faul *et al*., 2009). According to the findings from Altan *et al*. (2012) and Shen *et al*. (2008a), an average sample size of 38 was required for each group in order to reach the statistical power of 80% and an alpha level of 0.05. Forty subjects were recruited for each group. No previous study compared the stromal thickness between emmetropes and high myopes. Normal Chinese had stromal thickness of 465 ± 21μm (**Table 4.1**). Assuming a 5% difference between the two groups, it required 19 subjects in each group to reach a 90% power with a significance level of 5%.

The normality of the variables was tested using the Kolmogorov-Smirnov test. The differences between the 2 groups were evaluated using independent sample t-tests. Linear regressions were performed to assess the relationships between the ORA parameters (CH, CRF, and IOPcc) and other variables. Subsequently, multiple linear

regressions were employed to further explore the association between CH and CRF with variables demonstrating significant correlations in the univariate analysis. The data were expressed in mean ± SD, and $p < 0.05$ was determined to be statistically significant. AL rather than SE and ACD was included in the multivariate analysis because of the high correlations. The collinearity among dependent variables was accessed in the multivariate linear regressions. The multivariate analysis was done on FST and CV separately due to the high collinearity among these two parameters. All statistical analyses were 2-tailed and performed using SPSS 16.0 (SPSS, Inc., Chicago, Illinois, USA).

4.4 Results

Significant differences were observed in AL, SE, and ACD between the emmetropic and high myopic groups. The mean SE was 0.00 ± 0.26D in the emmetropes and -8.57 ± 1.78D in the high myopes. No emmetropic eye contained an AL over 25mm (23.71 ± 0.79mm). The AL of the highly myopic eyes was 27.34 ± 0.90mm. The demographic data for these 2 groups is presented in **Table 4.2**.

By combining the results derived from the 2 groups, eyes with a longer AL were determined to be more myopic ($r^2 = 0.870$, $p < 0.001$) and exhibited a deeper ACD than those with a shorter AL ($r^2 = 0.347$, $p < 0.001$). The univariate regressions of CH and the CRF with other variables are displayed in **Table 4.3**. CH was closely correlated with all the corneal variables, including FST ($r^2 = 0.368$, $p < 0.001$) (**Figure 4.3**), and CV from the 3-mm to 10-mm zones ($r^2 = 0.251$-0.391, $p < 0.001$), but not with the MeanK and Epi+BT ($p > 0.05$). In addition, CH was significantly correlated with the SE ($r^2 = 0.094$, $p = 0.020$) and AL ($r^2 = 0.112$, $p = 0.011$) (**Figure 4.4**), but not with the ACD ($r^2 = 0.040$, $p = 0.137$). Significant correlations were observed between the CRF and FST, and CV from the 3-mm to 10-mm zones ($r^2 = 0.424$, $r^2 = 0.408$ and $r^2 = 0.201$-0.346, respectively; $p < 0.001$). No significant correlation was observed between gender and CH and CRF ($p > 0.05$). IOPcc demonstrated a positive correlation with AL ($r^2 = 0.206$, $p < 0.001$), but was independent from FST ($r^2 < 0.001$, $p = 0.959$). The associations among IOPcc, CV, and MeanK were not significant.

Table 4.2 Demographic data between emmetropes (30 eyes) and high myopes (27 eyes), and the independent sample t-test results.

Parameters	Emmetropes	High myopes	p - value
Age (Year)	23.13 ± 3.29	22.78 ± 2.59	0.655
Spherical equivalent, SE (D)	0.00 ± 0.26	-8.57 ± 1.78	< 0.001
Axial length, AL (mm)	23.71 ± 0.79	27.34 ± 0.90	< 0.001
Anterior chamber depth, ACD (mm)	3.49 ± 0.19	3.78 ± 0.23	< 0.001
Mean keratometry reading, MeanK (D)	42.85 ± 1.50	43.54 ± 1.04	0.050
Corneal hysteresis (mmHg)	11.11 ± 1.25	10.17 ± 1.38	0.009
Corneal resistant factor (mmHg)	10.55 ± 1.42	10.31 ± 1.80	0.577
Corneal-compensated IOP (mmHg)	13.91 ± 2.49	16.50 ± 3.05	0.001
Corneal volume, CV at 3mm (mm^3)	4.14 ± 0.15	4.07 ± 0.20	0.153
Corneal volume, CV at 5mm (mm^3)	12.13 ± 0.45	11.90 ± 0.59	0.109
Corneal volume, CV at 7mm (mm^3)	26.08 ± 1.06	25.63 ± 1.29	0.151
Corneal volume, CV at10mm (mm^3)	63.68 ± 3.38	62.72 ± 3.31	0.285
Full stromal thickness, FST (μm)	462.80 ± 19.05	455.42 ± 29.88	0.266
Epithelium and Bowman's layer thickness, Epi+BT (μm)	67.22 ± 6.43	65.41 ± 6.12	0.281

The multivariate analysis involving FST, AL, and IOPcc showed that FST was determined to have stronger association (β = 0.591) than IOPcc (β = -0.415) for CH (adjusted R^2 = 0.508). There was no significant association with AL (β = -0.105) (model 1, **Table 4.4**). It estimated CH = -0.206 + (0.033 x FST) + (-0.159 x IOPcc) + (-0.072 x AL). Whereas model 2 involved CV at 3mm zone demonstrating CH = -3.858 + (4.421 x CV3) + (-0.135 x IOPcc) + (-0.063 x AL). CH had modest positive association with CV-3mm (β = 0.575), followed by negative association with IOPcc (β = -0.297), but no significant association with AL (β = -0.091) (adjusted R^2 = 0.482) (model 2, **Table 4.4**). The Tolerance of all selected dependent variables was above 0.75; hence the effect of multicollinearity is minimal.

Table 4.3 The univariate analysis of corneal hysteresis and the corneal resistance factor.

	Corneal Hysteresis				Corneal Resistance Factor			
Factor	*r*	R^2	*Slope*	*Intercept*	*r*	R^2	*Slope*	*Intercept*
Age	-0.034	0.001	-0.016	11.027	-0.092	0.008	-0.050	11.581
SE (D)	0.307	0.094*	0.095	11.050	0.042	0.002	0.015	10.498
AL (mm)	-0.334	0.112*	-0.230	16.507	-0.051	0.003	-0.040	11.464
ACD (mm)	-0.199	0.040	-1.080	14.582	0.066	0.004	0.414	8.935
MeanK (D)	-0.011	< 0.001	0.010	10.251	0.002	< 0.001	0.002	10.360
CV 3-mm (mm^3)	0.625	0.391***	4.803	-9.063	0.588	0.346***	5.238	-11.076
CV 5-mm (mm^3)	0.609	0.371***	1.594	-8.495	0.560	0.314***	1.698	-9.979
CV 7-mm (mm^3)	0.576	0.332***	0.672	-6.720	0.515	0.265***	0.697	-7.578
CV 10-mm (mm^3)	0.501	0.251***	0.207	-2.411	0.448	0.201***	0.214	-3.105
FST (μm)	0.606	0.368***	0.034	-4.832	0.638	0.408***	0.041	-8.471
Epi+BT (μm)	0.074	0.005	0.016	9.594	-0.011	< 0.001	-0.003	10.619
IOPcc (mmHg)	-0.402	0.161**	-0.183	13.428	0.240	0.057	0.126	8.529

SE = spherical equivalent, AL = axial length, ACD = anterior chamber depth, MeanK = mean keratometry reading, CV = corneal volume, CCT = central corneal thickness, FST = full stromal thickness, Epi+BT = epithelial and Bowman's thickness, IOPcc = corneal-compensated intraocular pressure.
* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

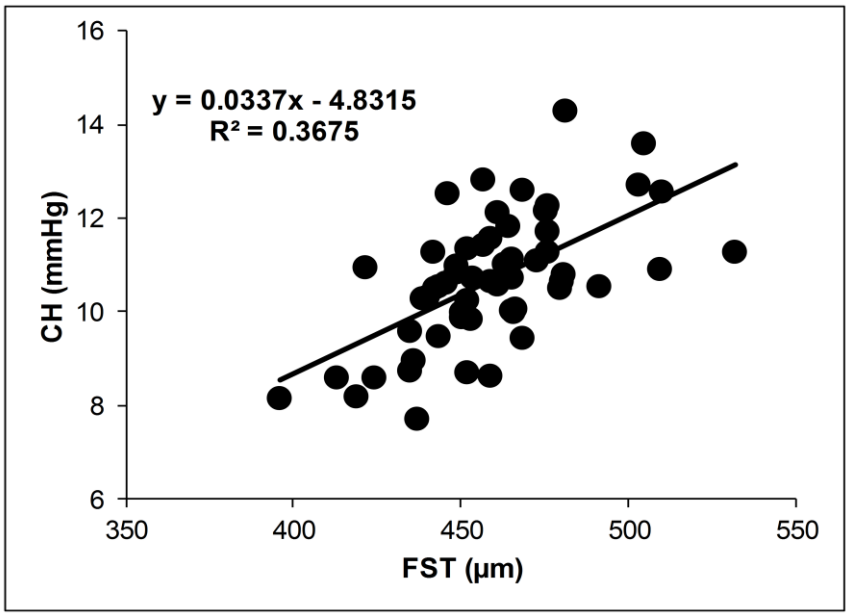


Figure 4.3 Corneal hysteresis (CH) as a function of full stromal thickness (FST). CH is increasing with thicker FST ($r^2 = 0.368$, $p < 0.001$).

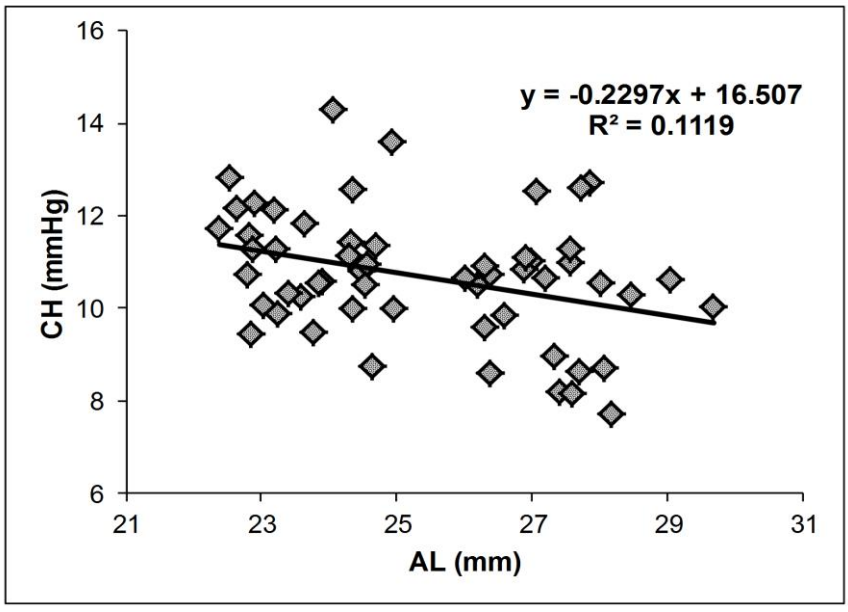


Figure 4.4 Corneal hysteresis (CH) as a function of axial length (AL). CH is decreasing with longer AL ($r^2 = 0.112$, $p = 0.011$).

Table 4.4 Multiple linear regressions of potential predictors for corneal hysteresis (CH) (n = 57).

Model 1
CH *(adjusted R^2 = 0.508)*
CH = -0.206 + (0.033 x FST) + (-0.159 x IOPcc) + (-0.072 x AL)

Parameters	Partial coefficients (β)	Standardized coefficient (β)
AL (mm)	-0.135	-0.105
FST (μm)	0.652	0.591***
IOPcc (mmHg)	-0.350	-0.415**

Model 2
CH (adjusted R^2 = 0.482)
CH = -3.858 + (4.421 x CV3) + (-0.135 x IOPcc) + (-0.063 x AL)

Parameters	Partial coefficients (β)	Standardized coefficient (β)
AL (mm)	-0.114	-0.091
CV3 (mm3)	0.628	0.575***
IOPcc (mmHg)	-0.354	-0.297**

AL= axial length, CV3= corneal volume at 3mm zone, FST= full stromal thickness, IOPcc= corneal-compensated intraocular pressure.
* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

4.5 Discussion

Because the corneal biomechanical properties could be accessed clinically, innumerable studies have investigated the association between CH and CRF and various corneal properties, including CCT (Lim *et al*., 2008; Song *et al*., 2008; Chang *et al*., 2010; Plakitsi *et al*., 2011), corneal curvature (Lim *et al*., 2008; Wong and Lam, 2011), keratocytes density (Hurmeric *et al*., 2010), and CV (Sedaghat *et al*., 2012). Our study evaluated the association between CH and CRF and most corneal properties, particularly the corneal sublayer thickness and CV between emmetropes and high myopes. We discovered significant positive associations between FST, and CH and CRF. No significant association between Epi+BT and CH and CRF was observed. Therefore, the association with CCT reported in previous studies could be primarily attributed to the corneal stroma. FST is a valuable variable because it is a measure of stromal thickness, which renders information on the corneal collagen layer. This is the first clinical study in which corneal biomechanical properties and the corneal sublayer thickness were investigated together.

In the present study, high myopes produced a significantly lower CH compared with that of the emmetropes. Although corneal biomechanical properties are believed to be dominated by the corneal stroma (Kotecha, 2007), the difference in corneal sublayer thickness did not achieve statistical significance. However, the univariate analysis which involved all subjects demonstrated significant association between low CH and long AL, and between low CH and thin FST. The multivariate analysis indicated that CH variation was dependent on FST rather than on axial elongation (standardized coefficient, β displayed in **Table 4.4**). Hurmeric *et al*. (2010) discovered negative correlations among stromal keratocyte density, CH, and CRF. Researchers have postulated that a high cell density occurs to compensate for the

decrease in corneal biomechanical strength. We did not measure the keratocyte density, but the FST reflected the keratocyte density (Patel *et al*., 2001). The corneal stroma, particularly the anterior stroma, is crucial for maintaining the corneal shape (Müller *et al*., 2001). The FST is a reproducible parameter and has been suggested to be more clinically useful than other corneal sublayer (Section 4.2.2.2). Besides confocal microscopy, corneal sublayer thickness can also be measured using non-invasive high resolution optical coherence tomography (OCT) (Hutchings *et al*., 2010). This technology may be able to replace confocal microscopy in measuring stromal keratocyte density (Karimi *et al*., 2011).

Similar to the results of previous studies, CH was significantly lower in high myopes despite both refractive groups exhibiting similar MeanK and CCT (Shen *et al*., 2008a; Song *et al*., 2008; Chang *et al*., 2010; Xu *et al*., 2010). AL, instead of the SE, was used in the analysis, as AL could indirectly reflect ocular rigidity, whereas the SE was the combined effect of corneal power, lens power, and AL. Our results were also consistent with previous findings that reported lower CH in highly myopic eye compared with that of the fellow eye in anisometropic subjects who exhibited a similar CCT between the 2 eyes (Xu *et al*., 2010). They suggested that axial elongation may result in lower CH possibly because of a change in corneal collagen fibrils. The present study revealed that CH had a stronger correlation with FST than that with AL, which concurs with this postulation (**Table 4.4**) (**Figures 4.3** and **4.4**). Cornea as a viscoelastic material contains both viscous and elastic characteristics. During the deformation, energy dissipation occurred due to viscosity while the energy storing was due to elasticity (Levin *et al*., 2011). Generally, emmetropic cornea had higher CH which reflected a higher loss of energy; conversely, high myopic cornea had lower CH which stored more energy. Although cornea with lower CH does not mean being more elastic, reduced tensile strength and increased elasticity were observed in myopic sclera (McBrien *et al*., 1991), similar changes may also appear in cornea due to their same origin (mesoderm) (Nickla and Wallman, 2010).

There was no study concurrently assessing the association of corneal curvature, corneal sublayer thickness, and CVs with CH and CRF. Despite the expected positive association between CH and CRF with FST and CVs, there was no significant association between MeanK and CH and CRF. Further, corneal epithelium had minimal impact on CH and CRF. This finding had good concordance with Elsheikh *et al*. (2008). They suggested that corneal epithelium, compared with corneal stroma, could be ignored in predicting the corneal biomechanical properties. However, our regression models could only account for about 50% of the CH variance, no matter using FST or CV as the independent variables, leaving another half of the total variance unexplained. Therefore, besides the corneal properties, CH might also have association with other ocular variables.

Numerous researchers have speculated that CH is associated with other ocular variables. Previous studies have reported that CH affects the alteration of the glaucomatous optic disc morphology (Wells *et al*., 2008; Vu *et al*., 2013) and the structural changes in matrix proteoglycans in diabetic patients (Scheler *et al*., 2012). In the same time, CH and the CRF were also observed to be lower in Marfan patients with ectopia lentis (Kara *et al*., 2012). Researchers have considered the biomechanical changes in the ocular connective tissues renders more extensible eye wall which were related to the elongation of the axial dimension (Rada *et al*., 2006). A long AL was postulated to produce high scleral tension across the lamina cribrosa, although IOP was the same (Perera *et al*., 2010). Furthermore, the extracellular matrix collagens at the corneal stroma were continual with the sclera and lamina cribrosa (McBrien and Gentle, 2003). In addition, a substantial decline in AL after performing trabeculectomy on eyes with low pre-operative CH has been recently reported (Huang *et al*., 2012). These findings were consistent with the prediction that the lower the CH, the more extensible is the eye. CH might convey information on the biomechanical properties of components other than the cornea, and it could represent the response of the entire eye wall because the tissue is continual.

Studies on the CRF are scant. The CRF is derived to optimize the correlation with CCT. Similarly to CCT and IOP, CRF demonstrated a diurnal variation (Shen *et al*., 2008b). Therefore, researchers have suggested that the CRF is related to corneal hydration (Shen *et al*., 2008b). Because the CRF is strongly correlated with CCT, a larger drop in the CRF was observed compared with that of CH after refractive surgery (Hamilton *et al*., 2008). In the current study, the association between AL and CRF was not significant, which is similar to the results of previous studies (Chang *et al*., 2010; Huang *et al*., 2011), and CRF was dependent on FST (**Table 4.3**). The significant correlation between FST and CRF may support the hypothesis that CRF represents the overall resistance of the cornea, which depends highly on the corneal stroma.

In addition to the corneal biomechanical properties, we also reported the IOPcc findings derived from the ORA. Similar to previous studies, IOP was positively associated with the degree of myopia or AL (Xu *et al*., 2010; Jiang *et al*., 2011; Altan *et al*., 2012). The high IOP readings exhibited in high myopes might be related to the changes in ocular biomechanical properties. IOPcc was used because it was not affected by corneal curvature and CCT (Oncel *et al*., 2009; Wong and Lam, 2011). The study design could be further improved by incorporating other tonometry procedures. The limitation of comparing IOPcc to CH and CRF was that they were all derived from the same raw applanation pressures, despite IOPcc being supposedly less affected by corneal biomechanics than GAT (Medeiros and Weinreb, 2006). In addition, we could not determine a significant association between IOPcc and FST. A recent study found similar IOP between glaucoma-treated patients and CCT-matched normal controls using GAT, and GAT was substantially lower than both IOPcc and dynamic contour tonometry (Costin *et al*., 2014). The IOP obtained using dynamic contour tonometry was less dependent on corneal properties (Punjabi *et al*., 2006) and closer to intracameral IOP values (Boehm *et al*., 2004). IOPcc is possibly

a more sensitive parameter that accurately reflects the IOP values, particularly in high myopia (Xu *et al*., 2010; Altan *et al*., 2012).

Using corneal tomography such as Pentacam enables the concurrent evaluation of corneal topography and pachymetry. Thus, the CV at various disc zones can be obtained using a cross-sectional analysis. Recent studies have proposed that analyzing the CV, CH, and CRF can assist in detecting corneal ectasia (Kozobolis *et al*., 2012), monitoring the changes in the cornea after refractive surgeries (Suzuki *et al*., 2006; Diniz *et al*., 2010), and contact-lens-induced edema (Lam *et al*., 2010). To elucidate the association between corneal biomechanical properties and CV further, we performed regression analyses of the CH, CRF, and CV at various disc zones. We determined that the strongest correlation among CV, CH, and CRF was at the 3-mm zone, instead of at the 7-mm and 5-mm zones, as indicated in a previous study (Sedaghat *et al*., 2012). In addition, no association was found between CV and IOPcc. CV is a relatively new parameter that warrants further study to establish the association between CV and corneal biomechanical properties, and with ocular variables.

4.6 Conclusion

The strength of this study was the simultaneous inclusion of most corneal properties. However, we were unsure whether the microstructure could be varied in the same FST because the stromal keratocyte density was not evaluated in this study. Since the sample size was small, with only 57 valid subjects in total, further study with a larger sample size is recommended to confirm the findings of this study. In accordance with other studies, CH exhibited a reduced trend in high myopes but the correlation between CH and AL was very weak. Because the ORA does not visualize the

corneal deformation, future studies could be conducted using the Corvis ST or OCT combined with air-puff applanation, in order to observe the deformation of cornea and FST. Most recent studies were limited by the cross-sectional design used. Therefore, we suggest that a longitudinal study to be conducted to monitor the changes in corneal biomechanical properties in children in order to understand their role in the myopia progression.

In addition, CS4 with z-ring is suitable to be used for evaluating the CT and FST. FST is a valuable parameter to observe the changes of corneal stroma. Inter-observer discrepancy in corneal sublayer pachymetry could be reduced by going through some corneal frames together rather than solely relying on any written criteria.

Papers published:

Wong YZ, Lam AKC. Improving interobserver variation in corneal sublayer pachymetry using Confoscan4 with z-ring. *Eye Contact Lens*. 2013;39:214--219.

Wong YZ, Lam AK. The roles of cornea and axial length in corneal hysteresis among emmetropes and high myopes: A pilot study. Curr Eye Res.2014 May 28:1--8. [Epub ahead of print]

Conference presentation:

Wong YZ, Lam AKC. The inter-observer variation of corneal sublayer pachymetry using slit-scanning confocal microscopy. The 8th Asia Cornea and Contact Lens Conference 2012, Hong Kong SAR. Oral presentation.

Chapter 5 Longitudinal changes of ORA parameters in children

5.1 Introduction

Myopia has sparked public concerns in the last few decades because its prevalence is increasing dramatically, particularly among children in East Asia (Lam *et al*., 2012). The increasing prevalence is affecting the Western population as well (Vitale *et al*., 2008; Vitale *et al*., 2009). The complications and degeneration caused by pathological myopia are well documented (Saw *et al*., 2005b). Because of its prevalence, the global burden of myopia on education and socioeconomic factors is rising and significant (Lim *et al*., 2009b).

Because of the growing concerns regarding myopia, innumerable myopia-related research has been conducted to study its cause and mechanisms as well as approaches to slow myopia progression. Scleral biomechanical properties could be a surrogate marker for axial elongation in myopia progression. However, the ocular rigidity assessments are either invasive or time consuming, and hence, inconvenient for clinical examination (Detorakis and Pallikaris, 2013). Thus, the measurement of corneal biomechanical properties using the automatic and non-contact ORA has attracted considerable interest, especially that of corneal researchers. It had been

postulated that the scleral biomechanical properties could be estimated using corneal biomechanical properties, because the cornea and sclera consist of similar collagen fibrils. In addition, both of them are derived from the mesoderm (Nickla and Wallman, 2010).

Numerous studies have been conducted to observe the role of corneal biomechanical properties in myopia. Song *et al*. (2008), Chang *et al*. (2010) and Huang *et al*. (2011) had found a significant reduction in CH in children and adults with longer AL, but Lim *et al*. (2008) could not demonstrate the association between CH and AL in Singaporean children. Studies on the CRF and AL are sparse. Xu *et al*. (2010) found a similar CRF between the two eyes of highly myopic anisometropes. Similar to other studies, they found that CH was significantly lower in highly myopic eyes compared with the fellow emmetropic eyes, although the two eyes had a similar CCT.

The ORA had attainable repeatability in children (Hon *et al*., 2012). Several studies have reported the corneal biomechanical properties of children aged 4 to 18 years (Kirwan *et al*., 2006; Lim *et al*., 2008; Song *et al*., 2008; Chang *et al*., 2010; Huang *et al*., 2011; Huang *et al*., 2013; Bueno-Gimeno *et al*., 2014). Nevertheless, other studies have been limited to being clinic based, which may lead to a sample bias. Moreover, they were cross-sectional studies with no information on the longitudinal changes of corneal biomechanical properties. Regardless of whether the decreased CH is the cause for, or the consequence of myopia development, knowledge of its role in axial elongation can yield an improved understanding of myopia development. Information on interactions between axial elongation and corneal biomechanical properties is currently unavailable. To fill this knowledge gap, a longitudinal study is crucial to observe the changes in corneal biomechanical properties with axial elongation.

This study investigates the changes in AL, CH and the CRF in Chinese children in Hong Kong through a longitudinal study.

5.2 Methodology

5.2.1 Subjects

This prospective cohort vision screening program commenced at the end of 2009 in an attempt to evaluate the longitudinal changes of the corneal biomechanical properties in children. Vision screening was initially offered to 14 primary schools for children studying at their Primary 4. These children would be followed annually for 2 more years. Both local and international schools from Hong Kong Island, Kowloon and New Territories were invited to have children of different ethnicities participate in the study. Three local and five international schools accepted our invitation at the beginning of the study. A local and an international school withdrew from the program after the baseline measurements because of their busy curricula. Vision screenings were conducted during school hours on weekdays, except for in a local school where the vision screenings were held on Saturday.

Information sheet and consent forms were distributed to parents or guardians of the children through the schoolteachers. Written parental consent forms and children's verbal consent were obtained before the measurements started every year. This study was conducted in accordance with the Declaration of Helsinki and endorsed by the Human Subject Ethics Subcommittee of the Hong Kong Polytechnic University. A vision screening report was given to parents after each vision screening. A referral was made if the children failed the vision screening (**Appendix A**).

We classified the ethnicity of the children into four categories. This included Chinese, Other Asian (comprising Indian, Japanese, Malay, Korean, and Middle Eastern), Caucasian, and Mixed (comprising children with parents from two or more ethnic groups) (Bradford, 2006). Their ethnicity was confirmed by asking the children, school nurse or teacher prior to the examination.

5.2.2 Procedures

We evaluated habitual visual acuity monocularly using the Early Treatment Diabetic Retinopathy Study (ETDRS) charts at 4m. Tong *et al*. (2004) recommended a threshold of 0.26 logMAR as the most efficient cutoff for myopia screenings, with a sensitivity and specificity of 92% and 88%, respectively. Distant and near heterophoria was screened using cover tests, followed by measurements using the Howell phoria cards at 3m and 33cm, respectively. Stereopsis was measured using Titmus Fly Stereotest. We assessed color vision by using the Ishihara test. All of the tests were performed between 9:00 AM and 4:00 PM, and following the standard protocol of each test (Ferris *et al*., 1982; Wong *et al*., 2002; Carlson and Kurtz, 2004).

Corneal curvature and the refractive error data were obtained using a handheld automatic kerato-refractometer (Nidek ARK-30; Nidek Co. Ltd, Aichi, Japan). The ARK-30 was used in its automatic and AI mode, so that the acquisition was determined by the machine based on the reliability of the findings (Nidek, 2000). The SE from the non-cycloplegic autorefraction was calculated for analysis. It was defined as the sum of the spherical component and half of the astigmatism component. Non-cycloplegic autorefraction, instead of cycloplegic refraction, was conducted because cycloplegic refraction is time consuming and infeasible for large-scale vision screenings. Both local and international schools in Hong Kong have

busy curricula. Their normal teaching schedules would be affected if too much time was spent on vision screenings, which could discourage their participation. Furthermore, the drawbacks of cycloplegic refraction include temporary blurred near vision and potential systemic side effects (Ma *et al*., 2013). A combination of non-cycloplegic autorefraction and the uncorrected visual acuity test could provide good specificity (91%) and sensitivity (84%) in screening myopia (Ma *et al*., 2013). Corneal curvature was analyzed as the mean corneal power of the two principal meridians (MeanK) and corneal astigmatism.

The IOLMaster was used to measure the AL. Valid AL readings referred to measurements with a signal-to-noise ratio > 2.0 and a variation of less than 0.1mm from three to five measurements (Lam *et al*., 2001). The average AL was calculated for analysis. The IOLMaster was calibrated every time before it was moved to the school for vision screening.

Intraocular pressure and corneal biomechanical properties were assessed using the ORA. The ORA generated four primary parameters: IOPg, IOPcc, CH, and the CRF and 37 waveform parameters. Only the four primary ORA parameters were included in the analysis. Those 37 waveform parameters were not used because of poor repeatability in people younger than 30 years (Landoulsi *et al*., 2013). Four consecutive measurements were obtained from each child. The ORA software (version 2.0) provided an indicator called the waveform score (WS), and the best signal value (BSV) was identified automatically after four consecutive measurements. The BSV was the ORA measurement with the highest WS. Lam *et al*. (2010) first recommended that WS be at least 3.5 to increase the measurement precision. Mandalos *et al*. (2013) showed that WS ≥ 6.0 is reliable for ORA measurements. Hon *et al*. (2012) reported repeatable CH and CRF measurements in children by using the ORA. A preliminary study involving 101 children was conducted to confirm the usefulness of the BSV. One eye from each child was used in our preliminary study. Three consecutive ORA measurements with WS ≥ 6.0 were

included. The mean values of the ORA results from the three measurements were compared with the BSV results. No significant difference was found in all of the ORA parameters between the mean and BSV values (paired t-tests, $p > 0.05$). Strong correlations (correlation of determination, $r^2 > 0.8$) were demonstrated between the mean and BSV values of all four ORA parameters (**Figure 5.1**). The Bland and Altman plots presented good agreement (95% limits of agreement: ± 2.3mmHg for IOPg and IOPcc; ± 1.2mmHg for CH and CRF) in all of the ORA parameters (**Figure 5.2**). Our results were in accordance with a report by Goebels *et al*. (2012), who did not find any significant difference between the mean values of the four measurements (WS ≥ 4.0) and the BSV.

In the current study, valid ORA measurements were considered as either three ORA readings with WS ≥ 3.5, or a WS of the BSV ≥ 6.0. When WS was ≥ 3.5, the average results were used for analysis. The BSV results were used for analysis when the WS of the BSV ≥ 6.0.

After the baseline measurements, annual vision screening took place for 2 consecutive years. Children with systemic diseases, ocular diseases, a history of corneal surgery and contact lens wear during the vision screening assessment were excluded from the analysis. Other exclusion criteria included ORA reading failures to meet the mentioned criteria. If the Principle of Pascal is applicable to the eyeball, the IOP should be the same inside the whole globe. According to the Law of Laplace, wall tension is proportionated by pressure and the radius of curvature. Therefore, eyes with a greater IOP require greater external forces for applanation (Levin *et al*., 2011). To minimize the potential effect of the IOP on the ORA measurements, children with an IOP greater than 21mmHg were excluded from our analysis.

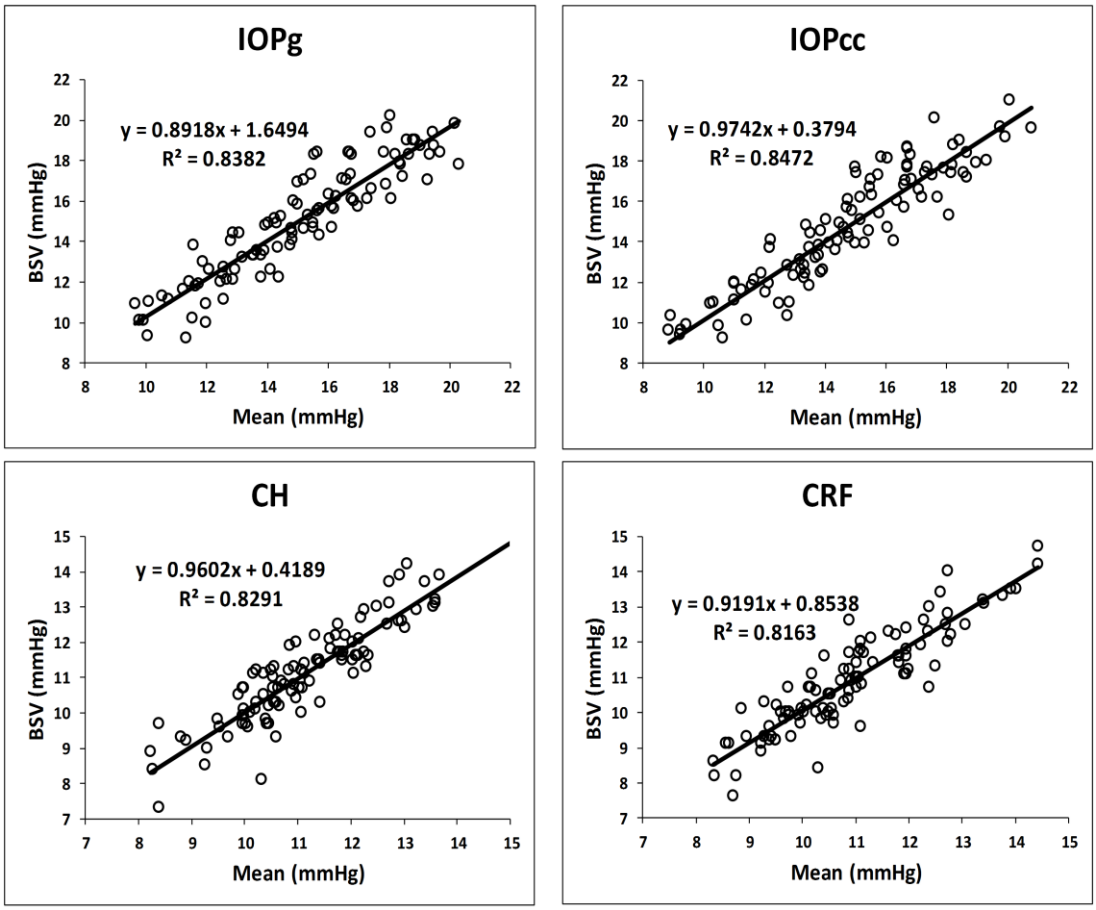


Figure 5.1 Correlations between the mean value from three measurements and the best signal value (BSV) for Goldmann-correlated intraocular pressure (IOPg), corneal-compensated intraocular pressure (IOPcc), corneal hysteresis (CH), and the corneal resistance factor (CRF).

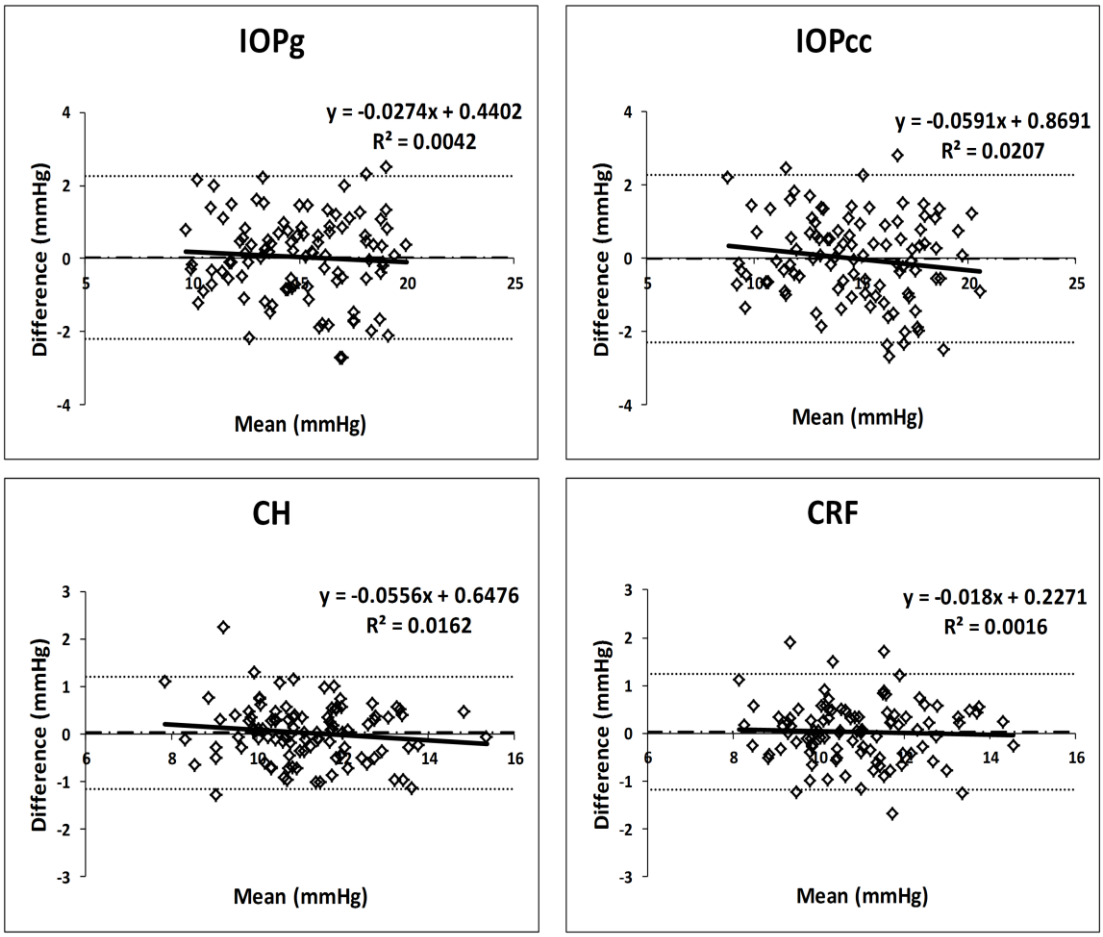


Figure 5.2 The Bland and Altman plots of the mean value and the best signal value (BSV) for Goldmann-correlated intraocular pressure (IOPg), corneal-compensated intraocular pressure (IOPcc), corneal hysteresis (CH), and the corneal resistance factor (CRF). The dotted lines present the upper and lower limits of agreement, the dashed line shows the mean differences, and the solid line represents the trend line.

A total of 1255 children enrolled and completed the baseline measurements **(Table 5.1)**. After the first and the second annual follow-up visits, 941 and 883 subjects remained, respectively. However, some children did not provide valid ORA readings every visit. Hence, all the data were sorted and grouped into three studies.

Cross-sectional study

This study represented children with all of the valid measurements for a comprehensive analysis of their refractive errors, ocular biometry and corneal biomechanical properties. The majority were from the baseline visit, with a few of them from the follow-up visits because of incomplete baseline measurement results. They comprised 1,199 children.

One-year cohort study

This cohort represented Chinese children (details explained in Section 5.3) with all the valid measurements in two consecutive visits. It mainly consisted of children enrolled at the baseline visit with their first-year follow-up. Some children who did not have valid measurements at the baseline visit, but had valid measurements at the two follow-up visits, were also included. Only children with a positive mean difference of AL (indicating axial elongation) were involved in the analysis. The total number of children in this 1-year cohort study was 269.

Two-year cohort study

This cohort represented Chinese children (details explained in Section 5.3) with all the valid measurements at the baseline visit as well as in the second annual follow-up. Similar to the 1-year cohort study, only children with a positive mean difference of AL were included in the analysis. They comprised 144 children.

Table 5.1 The details of the study sample.

Sample	Number of children
Baseline	1,255
First follow-up visit	941
Second follow-up visit	883
Cross-sectional Study	1,199
One-year cohort Study (Baseline versus First follow-up visit or First follow-up visit versus Second follow-up visit)	269
Two-year cohort Study (Baseline versus Second follow-up visit)	144

5.3 Statistical analysis

Distributions of all the data were assessed using the Kolmogorov-Smirnov test. AL was used in the analysis to represent a change in refractive status. SE was not used, because only non-cycloplegic auto-refraction was performed. Hence, the value of SE was reported but not involved in the analysis. Either the parametric or non-parametric test was performed depending on the data distribution. One eye from each child with valid ORA readings was selected for analysis. If both eyes had valid ORA readings, the right eye was considered. Data were presented in mean ± SD, and the statistical analyses were performed using SPSS 16.0 (SPSS, Inc., Chicago, Illinois, USA). All statistical tests were two-tailed, and a p - value less than 0.05 was considered to be statistically significant.

In the cross-sectional study, children were further divided into subgroups based on their ethnicity, school, and quartiles of AL. One-way ANOVA or the Kruskal-Wallis test with the Bonferroni post hoc test were employed to evaluate the influence of ethnicity and AL quartiles, and the Student's t-test or Mann-Whitney test was used to investigate the influence of schools on the refractive status and corneal biomechanical properties.

The 1-year cohort study was a 1-year longitudinal study, whereas the 2-year cohort study involved an interval of 2 years. Both studies included data obtained from two visits. The mean differences of the ocular parameters were calculated by subtracting the first-visit results from those of the second visit. The paired t-test or Wilcoxon signed-rank test was applied to observe the changes in ocular parameters throughout 1 year or 2 years. One-way ANOVA or the Kruskal-Wallis test with the Bonferroni post hoc test were performed to investigate the mean differences of the corneal

biomechanical properties, IOPs, and ocular biometry in different AL quartiles and CH quartiles. To eliminate the effect of ethnicity on CH and the CRF, only Chinese children were included in the analysis of the 1- and 2-year cohort studies.

The changes in ocular parameters were defined as the value of the follow-up visit minus the baseline value. Univariate regression was performed to evaluate the relationships between the changes of CH and the CRF with other ocular parameters. As an additional approach to evaluating the changes of CH at different levels of axial elongation, children were stratified based on their axial elongation at 0.10mm intervals for the 1-year cohort and 0.20mm intervals for the 2-year cohort. This is because Chinese children were found to have an annual axial elongation of 0.20mm (Xiang *et al*., 2012); therefore, half of the annual rate (0.10mm) and half of the biennial rate (0.20mm) were adopted as the interval size for the 1-year and 2-year cohorts, respectively. To assess whether the changes in CH during axial elongation were affected by the IOP, the mean changes of IOPcc at different levels of axial elongation were also monitored to determine whether a significant result would emerge from the changes in CH.

Another thorough approach was adapted to assess the axial elongation at different levels of CH changes. Children were further divided based on their changes in CH at 0.80mmHg intervals for the 1- and 2-year cohorts. This is because the within-subject variation of CH was found to be 0.80mmHg (Lu *et al*., 2007).

5.4 Cross-sectional Study

5.4.1 Results

5.4.1.1 Demographic characteristics of the population

Of the 1,199 children aged 8 to 15 years (mean ± SD: 10.55 ± 1.26 years), 652 were boys and 547 were girls, with 900 right eyes and 299 left eyes examined. Their demographic characteristics and the ocular variables are presented in **Table 5.2**. The frequency distributions of the CH, CRF, and AL of 1,199 eyes are illustrated in **Figure 5.3**, **5.4,** and **5.5**, respectively.

Table 5.2 Demographic characteristics of 1,199 eyes from 1,199 children.

Variables	**Total mean ± SD (range)**
Age (Year)	10.55 ± 1.26 (8 to 15)
Gender	652 boys
Eye	900 right eyes
Habitual visual acuity (logMAR)	0.12 ± 0.20 (-0.60 to 1.20)
Spherical equivalent (Diopter)	-2.20 ± 1.90 (-13.25 to 4.00)
Mean corneal power (Diopter)	43.12 ± 1.40 (38.18 to 47.71)
Corneal astigmatism (Diopter)	1.02 ± 0.59 (-0.87 to 4.52)
Axial length (mm)	23.83 ± 1.01 (21.24 to 27.64)
Corneal hysteresis (mmHg)	11.29 ± 1.47 (6.30 to 16.60)
Corneal resistance factor (mmHg)	11.18 ± 1.54 (6.80 to 16.70)
Goldmann-correlated intraocular pressure (mmHg)	15.54 ± 2.63 (6.37 to 21.00)
Corneal-compensated intraocular pressure (mmHg)	15.06 ± 2.65 (6.50 to 21.00)

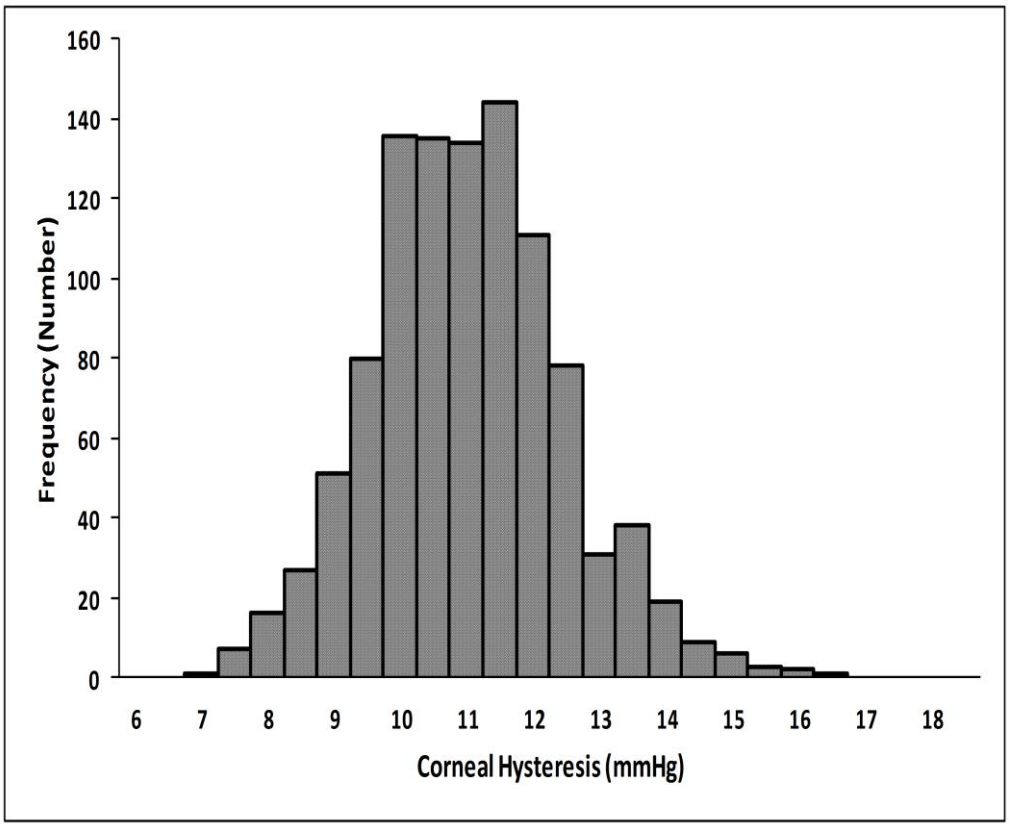


Figure 5.3 Distribution of corneal hysteresis in 1,199 children (mean ± standard deviation, 11.29 ± 1.47mmHg).

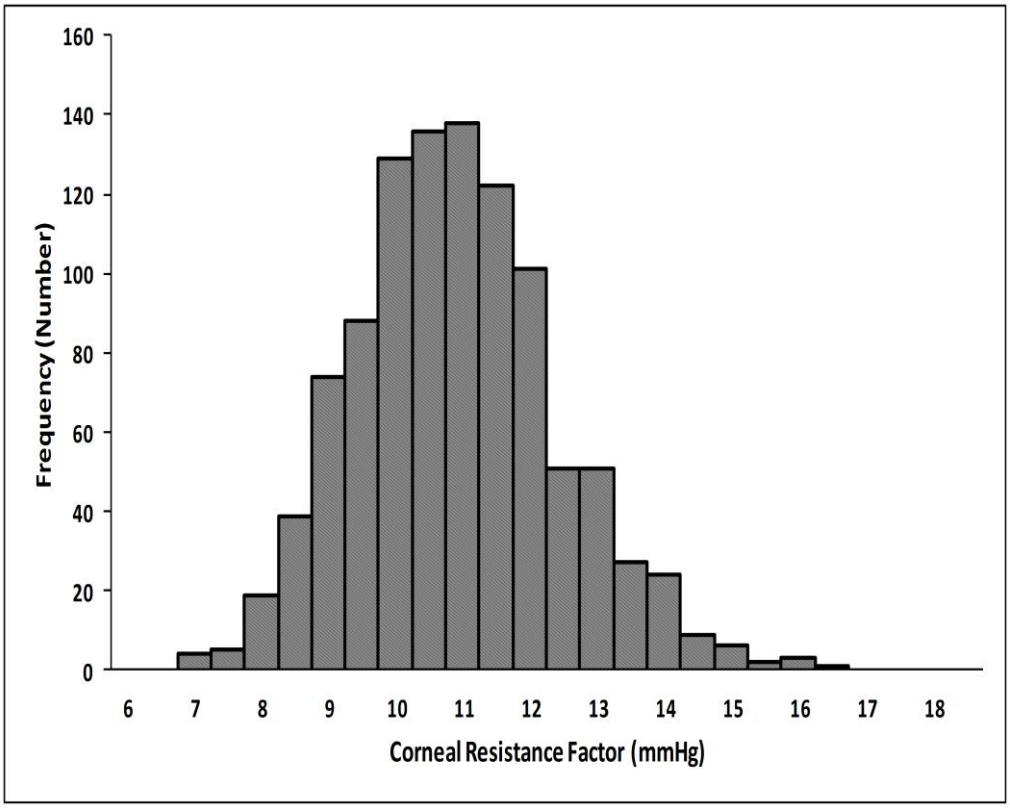


Figure 5.4 Distribution of the corneal resistance factor in 1,199 children (mean ± standard deviation, 11.18 ± 1.54mmHg).

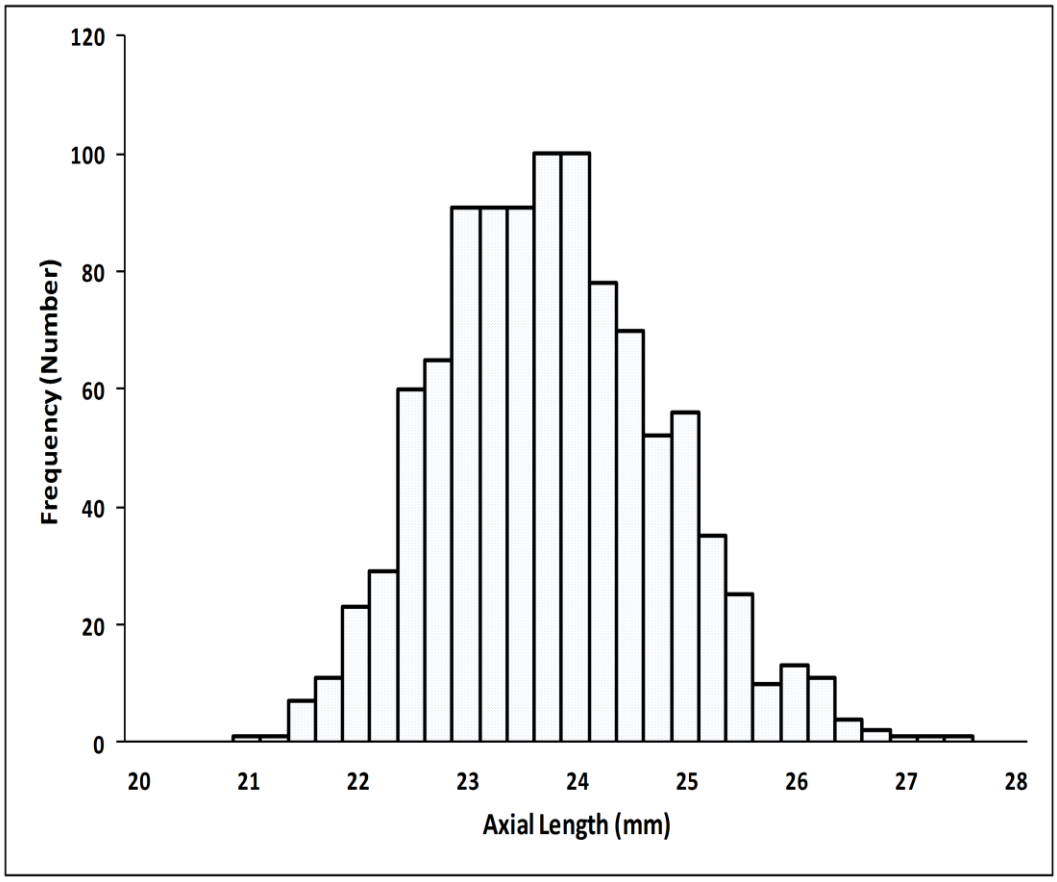


Figure 5.5 Distribution of axial length in 1,199 children (mean ± standard deviation, 23.83 ± 1.01mm).

Both CH and the CRF had a weak positive association with MeanK ($r^2 = 0.019$, $p < 0.001$ and $r^2 = 0.007$, $p = 0.003$, respectively) (**Table 5.3**). In addition, CH and the CRF had a weak association with AL ($r^2 = 0.040$, $p < 0.001$ and $r^2 = 0.006$, $p = 0.004$, respectively) (**Figures 5.6** and **5.7**). The associations with corneal astigmatism were not significant ($p > 0.05$). IOPcc was significantly associated with CH only ($r^2 = 0.277$, $p < 0.001$).

Table 5.3 Linear regressions of corneal hysteresis and the corneal resistance factor with mean corneal power (MeanK), corneal astigmatism (CA), axial length (AL), and corneal-compensated intraocular pressure (IOPcc).

Variables	**Corneal hysteresis**			**Corneal resistance factor**		
	Beta	R^2	p - value	Beta	R^2	p - value
MeanK	0.139	0.019	$p < 0.001$	0.084	0.007	$p = 0.003$
CA	-0.019	0.000	$p = 0.514$	-0.009	0.000	$p = 0.752$
AL	-0.200	0.040	$p < 0.001$	-0.082	0.006	$p = 0.004$
IOPcc	-0.526	0.277	$p < 0.001$	-0.015	0.000	$p = 0.611$

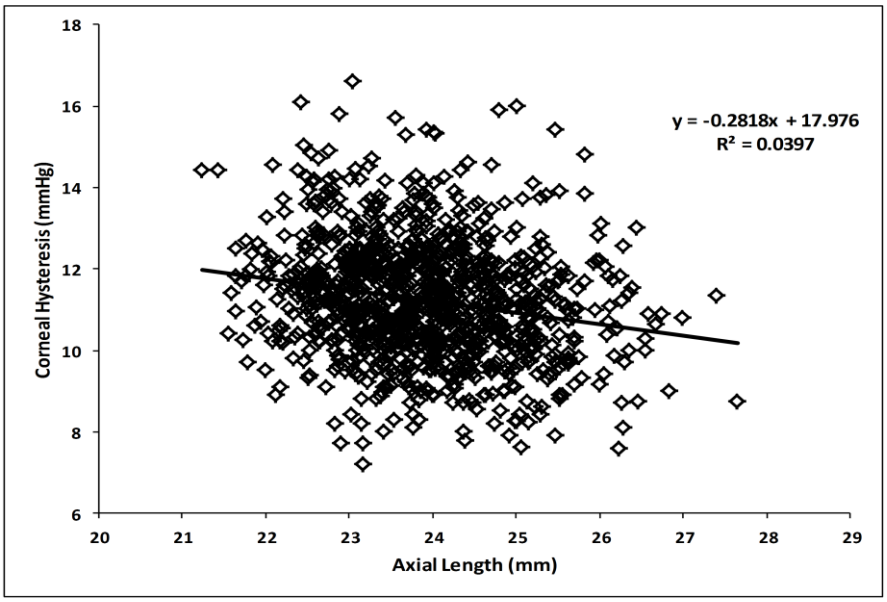


Figure 5.6 Corneal hysteresis as a linear function of axial length in the scatter plot.

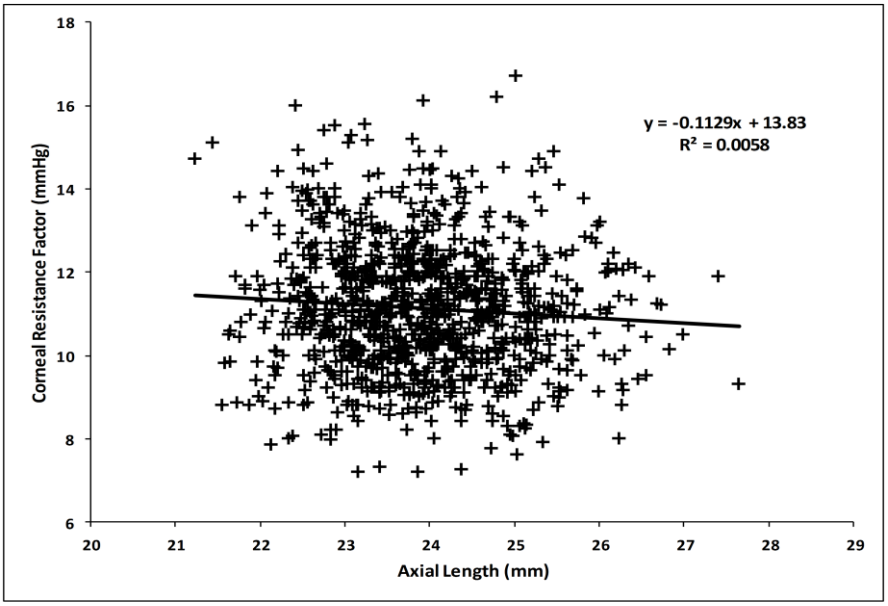


Figure 5.7 The corneal resistance factor as a linear function of axial length in the scatter plot.

5.4.1.2 Different schools

We recruited 644 children from three local schools and 555 children from five international schools. Children in local schools had a longer AL (23.97 ± 1.05mm versus 23.65 ± 0.94mm, Student's t-test, $p < 0.001$), lower CH (11.20 ± 1.42mmHg versus 11.39 ± 1.52mmHg, Student's t-test, $p = 0.025$) and higher corneal astigmatism (1.08 ± 0.61D versus 0.94 ± 0.55D, Mann-Whitney test, $p < 0.001$) than those studied in international schools (**Table 5.4**). Children in local schools had a slightly higher IOPcc than children in international schools (15.20 ± 2.69mmHg versus 14.89 ± 2.60mmHg, Student's t-test, $p = 0.049$). MeanK, the CRF, and IOPg values were similar between local and international schools (Student's t-test, $p > 0.05$).

Chinese students in local and international schools were also compared (**Table 5.5**). Chinese students in local schools had a longer AL (23.97 ± 1.06mm versus 23.78 ± 0.96mm, Student's t-test, $p = 0.003$), steeper MeanK (43.19 ± 1.43D versus 42.98 ± 1.36D, Student's t-test, $p = 0.020$) and greater corneal astigmatism (1.09 ± 0.61D versus 0.99 ± 0.55D, Mann-Whitney test, $p = 0.016$) than Chinese students in international schools. Chinese students in both local and international schools had similar CH, CRF, IOPg, and IOPcc values (Student's t-test, $p > 0.05$).

Table 5.4 Distribution and characteristics of children in local and international schools.

Variables	Local school mean ± SD (range)	International school mean ± SD (range)	p - value
Sample size	644	555	
Eye	496 Right eyes	404 Right eyes	
Age (Year)	11.00 ± 1.20 (9.00 to 15.00)	10.03 ± 1.12 (8.00 to 12.00)	p < 0.001
Gender	337 boys	315 boys	p = 0.125
Mean corneal power (Diopter)	43.19 ± 1.43 (39.12 to 47.71)	43.03 ± 1.36 (38.18 to 47.37)	p = 0.055
Corneal astigmatism (Diopter)	1.08 ± 0.61 (0.12 to 4.52)	0.94 ± 0.55 (-0.87 to 3.50)	p < 0.001
Axial length (mm)	23.97 ± 1.05 (21.44 to 27.64)	23.65 ± 0.94 (21.24 to 26.83)	p < 0.001
Corneal hysteresis (mmHg)	11.20 ± 1.42 (7.57 to 16.60)	11.39 ± 1.52 (6.80 to 16.57)	p = 0.025
Corneal resistance factor (mmHg)	11.12 ± 1.49 (7.20 to 16.70)	11.25 ± 1.59 (6.30 to 16.33)	p = 0.152
Goldmann-correlated intraocular pressure (mmHg)	15.59 ± 2.67 (7.17 to 21.00)	15.47 ± 2.58 (6.37 to 20.90)	p = 0.453
Corneal-compensated intraocular pressure (mmHg)	15.20 ± 2.69 (7.80 to 21.00)	14.89 ± 2.60 (6.50 to 20.90)	p = 0.049

Table 5.5 Distribution and characteristics of Chinese children in local and international schools.

Variables	Chinese in local school mean ± SD (range)	Chinese in international school mean ± SD (range)	p - value
Sample size	641	388	n/a
Eye	493 Right eyes	276 Right eyes	n/a
Age (Year)	11.00 ± 1.20 (9.00 to 15.00)	10.08 ± 1.11 (8.00 to 12.00)	$p < 0.001$
Gender	334 boys	228 boys	$p = 0.038$
MeanK (Diopter)	43.19 ± 1.43 (39.12 to 47.71)	42.98 ± 1.36 (38.18 to 47.00)	$p = 0.020$
CA (Diopter)	1.09 ± 0.61 (0.12 to 4.52)	0.99 ± 0.55 (0.00 to 3.38)	$p = 0.016$
AL (mm)	23.97 ± 1.06 (21.44 to 27.64)	23.78 ± 0.96 (21.24 to 26.83)	$p = 0.003$
CH (mmHg)	11.20 ± 1.42 (7.57 to 16.60)	11.32 ± 1.49 (7.20 to 16.10)	$p = 0.199$
CRF (mmHg)	11.11 ± 1.49 (7.20 to 16.70)	11.16 ± 1.58 (7.20 to 16.10)	$p = 0.606$
IOPg (mmHg)	15.58 ± 2.68 (7.17 to 21.00)	15.42 ± 2.59 (7.73 to 20.50)	$p = 0.323$
IOPcc (mmHg)	15.20 ± 2.70 (7.80 to 21.00)	14.93 ± 2.56 (6.50 to 20.53)	$p = 0.111$

MeanK = mean corneal power, CA = corneal astigmatism, AL = axial length, CH = corneal hysteresis, CRF = corneal resistance factor, IOPg = Goldmann-correlated intraocular pressure, IOPcc = corneal-compensated intraocular pressure, n/a = not available.

5.4.1.3 Different ethnic groups

The sample was further classified into four subgroups according to their ethnicity. The majority were Chinese (n = 1,029), followed by 95 Mixed and 39 Caucasian and 36 Other Asian (**Table 5.6**). The Chinese group had a significantly longer AL compared with other ethnic groups (Chinese: 23.90 ± 1.02mm; Mixed group: 23.46 ± 0.82mm; Other Asian: 23.31 ± 0.93mm; Caucasian: 23.29 ± 0.71mm) (**Figure 5.8**). The four ethnic groups had similar IOPg, IOPcc, CRF and MeanK values ($p > 0.05$).

The Chinese group had the lowest CH (11.24 ± 1.45mmHg), followed by Other Asian (11.46 ± 1.45mmHg), Mixed (11.50 ± 1.39mmHg), and Caucasian (11.91 ± 2.01mmHg), in an increasing trend (**Figure 5.9**). However, only the Chinese and Caucasian categories yielded a significant CH difference in the post hoc tests. The Chinese group had a higher corneal astigmatism (1.05 ± 0.59D) than Mixed and Caucasian groups (0.87 ± 0.55D and 0.54 ± 0.26D, post hoc test, $p < 0.01$, respectively). **Table 5.7** shows the association between CH and AL in different ethnic groups. Only the Chinese group showed a significant association between CH and AL ($r^2 = 0.040$, post hoc test, $p < 0.001$). No significant association was found between CH and AL in the Other Asian ($r^2 = 0.073$, $p > 0.05$), Caucasian ($r^2 = 0.008$, $p > 0.05$), and Mixed ($r^2 = 0.008$, $p > 0.05$) groups.

Table 5.6 Distribution and characteristics of children in different ethnic groups. Chinese was the reference group for other groups.

	Chinese mean ± SD (range) (n = 1029)	**Other Asian mean ± SD (range) (n = 36)**	**Caucasian mean ± SD (range) (n = 39)**	**Mixed mean ± SD (range) (n = 95)**
MeanK (Diopter)	43.11 ± 1.41 (38.18 to 47.71)	43.44 ± 1.25 (40.00 to 46.00)	43.05 ± 1.26 (40.75 to 45.75)	43.07 ± 1.41 (40.38 to 47.37)
CA (Diopter)	1.05 ± 0.59 (0.00 to 4.52)	1.02 ± 0.68 (0.12 to 3.50)	0.54 ± 0.26** (0.00 to 1.00)	0.87 ± 0.55** (-0.87 to 3.25)
AL (mm)	23.90 ± 1.02 (21.24 to 27.64)	23.31 ± 0.93** (21.78 to 25.42)	23.29 ± 0.71** (21.75 to 24.76)	23.46 ± 0.82** (21.59 to 25.92)
CH (mmHg)	11.24 ± 1.45 (7.20 to 16.60)	11.46 ± 1.45 (8.40 to 14.43)	11.91 ± 2.01* (6.30 to 16.33)	11.50 ± 1.39 (7.60 to 15.27)
CRF (mmHg)	11.13 ± 1.52 (7.20 to 16.70)	11.52 ± 1.48 (8.40 to 14.73)	11.61 ± 2.02 (6.80 to 16.57)	11.37 ± 1.43 (7.37 to 14.30)
IOPg (mmHg)	15.52 ± 2.65 (7.17 to 21.00)	16.18 ± 2.78 (9.20 to 20.90)	15.22 ± 2.37 (11.07 to 20.30)	15.59 ± 2.54 (6.37 to 20.73)
IOPcc (mmHg)	15.10 ± 2.65 (6.50 to 21.00)	15.42 ± 2.86 (9.07 to 20.90)	14.10 ± 2.58 (8.93 to 20.20)	14.88 ± 2.63 (8.50 to 19.80)

MeanK = mean corneal power, CA = corneal astigmatism, AL = axial length, CH = corneal hysteresis, CRF = corneal resistance factor, IOPg = Goldmann-correlated intraocular pressure, IOPcc = corneal-compensated intraocular pressure.
post hoc test, * $p < 0.05$, ** $p < 0.01$.

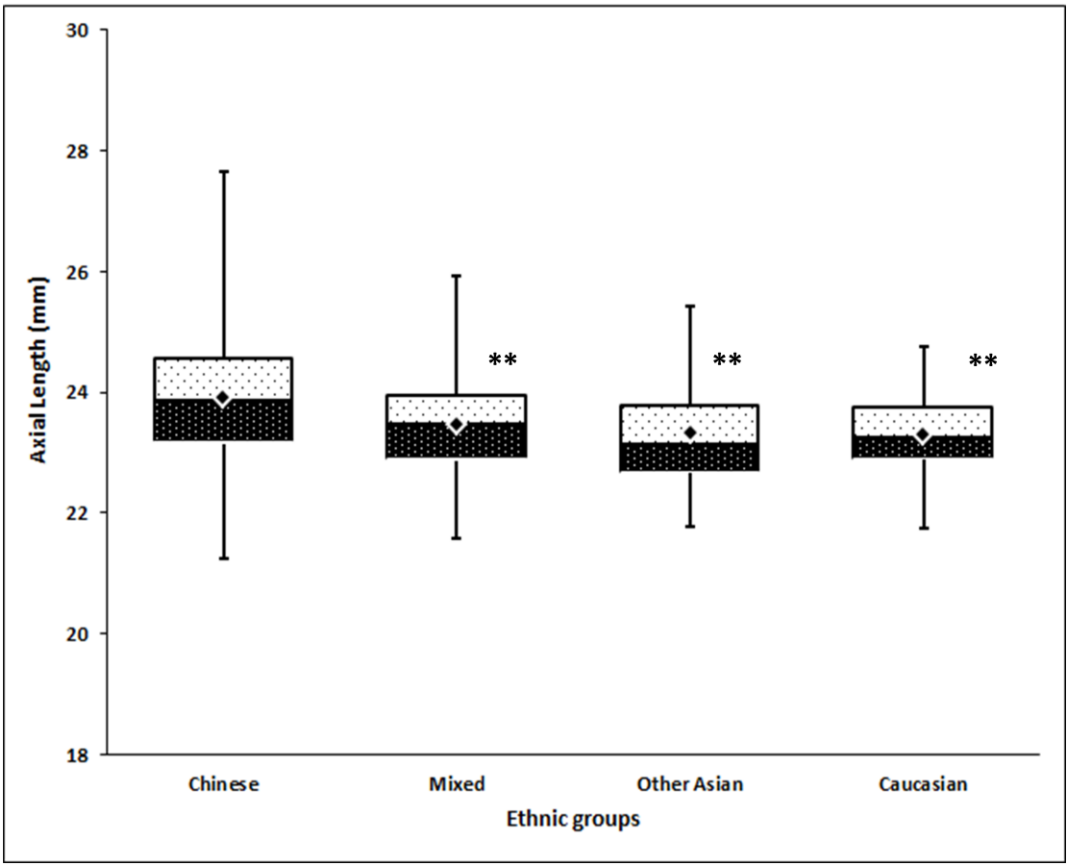


Figure 5.8 Box and whisker plots of axial length in Chinese, Other Asian, Caucasian, and Mixed children. The box corresponds with the lower quartile, the upper quartile and the median, whereas the whiskers represent the minimum and maximum axial length, and the diamonds express the mean.

** $p < 0.01$

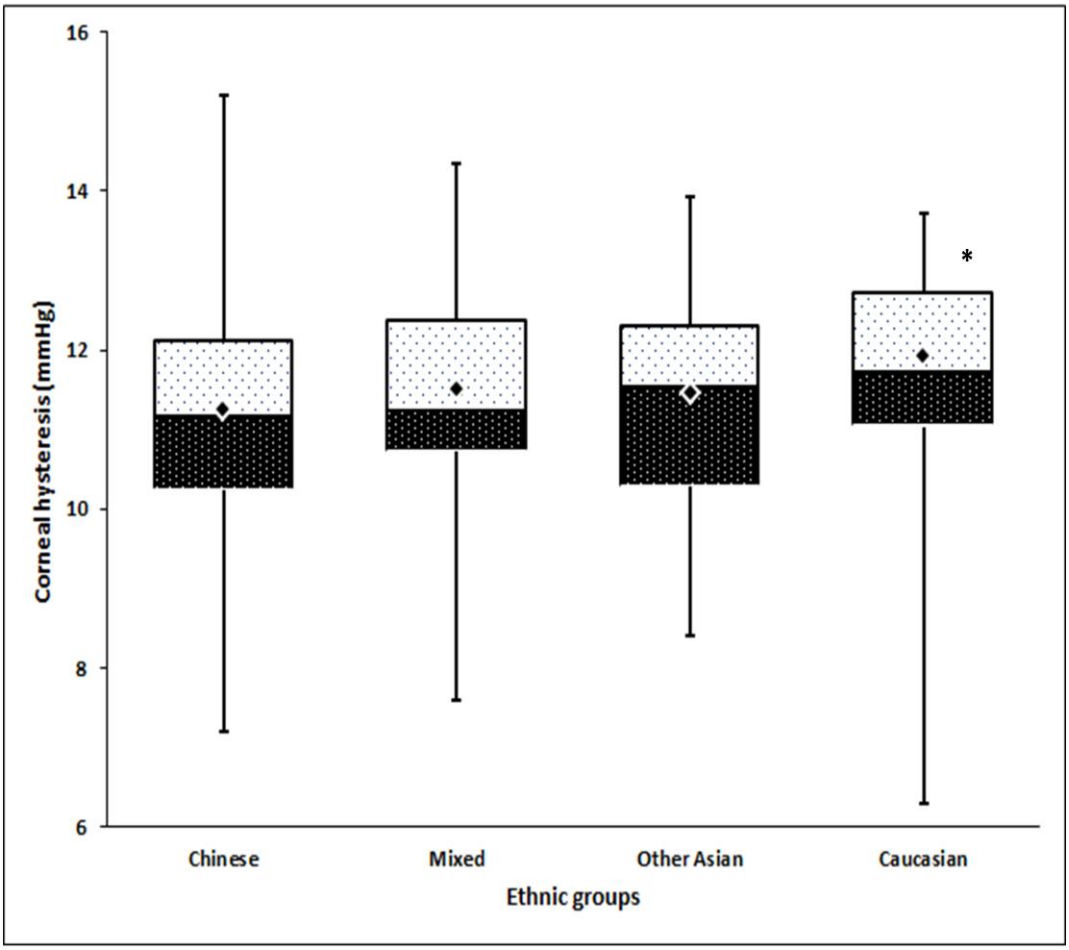


Figure 5.9 Box and whisker plots of corneal hysteresis in Chinese, Other Asian, Caucasian, and Mixed children. The box corresponds with the lower quartile, the upper quartile and the median, whereas the whiskers represent the minimum and maximum corneal hysteresis, and the diamond expresses the mean.

* $p < 0.05$

Table 5.7 Linear regressions between corneal hysteresis and axial length in different ethnic groups.

	Corneal hysteresis versus Axial length		
	Beta	R^2	p - value
Chinese	-0.199	0.040	p < 0.001
Other Asian	-0.271	0.073	p = 0.110
Caucasian	-0.088	0.008	p = 0.594
Mixed	-0.087	0.008	p = 0.401

5.4.1.4 Different axial length quartiles

Children were divided into four equal subgroups based on the quartiles of AL. There were 301 children in the first quartile (21.24 to < 23.10), 299 children in the second quartile (23.10 to < 23.77), 300 children in the third quartile (23.77 to < 24.48) and 299 children in the fourth quartile (24.48 to < 27.64) quartile. The first quartile was the reference for the other three quartiles. Children with AL longer than the first quartile had a flatter cornea (one-way ANOVA, post hoc test, p < 0.001), lower CH (one-way ANOVA, post hoc test, p < 0.001), a lower CRF (one-way ANOVA, post hoc test, p = 0.013), and higher IOPg and IOPcc (one-way ANOVA, post hoc test, p < 0.001) (**Table 5.8**). Although a significant difference emerged in corneal astigmatism in different quartiles (Kruskal-Wallis test, p = 0.001), the difference was not significant according to the post hoc test.

Table 5.8 Distribution and characteristics of children in different quartiles of axial length. The first quartile was the reference group for the other quartiles.

	Axial Length (mm) mean ± SD (range)				
	1st Quartile (21.24 to < 23.10) (n = 301)	**2nd Quartile (23.10 to < 23.77) (n = 299)**	**3rd Quartile (23.77 to < 24.48) (n = 300)**	**4th Quartile (24.48 to < 27.64) (n = 299)**	**p - value**
MeanK (Diopter)	44.14 ± 1.28 (40.81 to 47.71)	43.20 ± 1.10** (40.00 to 46.73)	42.67 ± 1.26** (39.81 to 46.06)	42.45 ± 1.31** (38.18 to 46.25)	p < 0.001
CA (Diopter)	1.06 ± 0.61 (0.12 to 4.52)	0.97 ± 0.58 (-0.87 to 3.58)	0.95 ± 0.57 (0.12 to 3.50)	1.09 ± 0.58 (0.00 to 3.25)	p = 0.001
CH (mmHg)	11.70 ± 1.41 (7.70 to 16.60)	11.29 ± 1.44** (6.30 to 16.33)	11.25 ± 1.47** (7.60 to 15.40)	10.91 ± 1.46** (7.57 to 16.23)	p < 0.001
CRF (mmHg)	11.40 ± 1.64 (7.37 to 16.50)	11.09 ± 1.44 (6.80 to 15.53)	11.21 ± 1.55 (7.20 to 16.10)	11.01 ± 1.48* (7.60 to 16.70)	p = 0.013
IOPg (mmHg)	15.10 ± 2.90 (6.37 to 20.90)	15.25 ± 2.55 (9.10 to 21.00)	15.76 ± 2.57* (8.60 to 21.00)	16.05 ± 2.39** (7.17 to 21.00)	p < 0.001
IOPcc (mmHg)	14.24 ± 2.60 (7.80 to 20.90)	14.80 ± 2.71* (6.50 to 20.37)	15.29 ± 2.55** (8.57 to 21.00)	15.90 ± 2.48** (7.80 to 20.97)	p < 0.001

MeanK = mean corneal power, CA = corneal astigmatism, CH = corneal hysteresis, CRF = corneal resistance factor, IOPg = Goldmann-correlated intraocular pressure, IOPcc = corneal-compensated intraocular pressure.
post hoc test, * $p < 0.05$, ** $p < 0.01$.

5.4.2 Discussion

This study had a sample greater than 1,000. Among them, 85.82% of the children were Chinese (n = 1,029), followed by 7.92% of Mixed, 3.25% of Caucasian, and 3% of Other Asian children. Our samples were recruited from both local and international schools in different regions in Hong Kong. The majority of studies on corneal biomechanical properties are clinic based, or have been conducted with a limited number of schools. A clinic-based study could be affected by a sample selection bias, and a large-scale study with a larger sample size could achieve greater insight into the population.

To date, few large-scale school-based studies have been conducted on corneal biomechanical properties in children. Lim and co-workers (2008) reported CH and the CRF in Singapore children. Their subjects were composed of a mixed ethnicity, with more than 30% non-Chinese. They showed a slightly higher CH (11.78 ± 1.55mmHg versus 11.29 ± 1.47mmHg) and CRF (11.81 ± 1.71mmHg versus 11.18 ± 1.54mmHg) compared with the current study. However, they had 271 subjects only, all of who were recruited from one school. Song *et al*. (2008) reported CH from more than 1,000 Chinese children aged 10 to 14 years. They reported CH (10.70 ± 1.64mmHg) that was slightly lower than in our Chinese subjects (11.24 ± 1.45mmHg). Huang and co-workers (2011) measured CH and the CRF from 651 Chinese children. Their mean CH (10.4 ± 2.2mmHg) was lower than our current findings, but their mean CRF (11.2 ± 2.1mmHg) was similar. A later study by the same researchers also found a slightly lower mean CH (10.98 ± 1.78mmHg) and CRF (11.46 ± 1.69mmHg) among 571 children (Huang *et al*., 2013). Among these studies, standard deviations for CH and the CRF were the highest from Huang and co-workers (2011), which was more than 2.0mmHg.

Similar to previous studies (Song *et al*., 2008; Chang *et al*., 2010; Bueno-Gimeno *et al*., 2014), a negative but weak association was found between CH and AL. Lim *et al*. (2008) and Shah *et al*. (2014) could not find a significant association, probably because of the mixed ethnicity of their study population. Previous studies have demonstrated that corneal curvature had minimal influence on CH and the CRF, including in Chinese, Caucasian and mixed ethnicity subject groups (Chang *et al*., 2010; Fontes *et al*., 2008; Lim *et al*., 2008; Kamiya *et al*., 2008; Bueno-Gimeno *et al*., 2014). The current study found a higher CH and CRF with a steeper cornea, but the association was weak. In addition, no association was observed between corneal astigmatism and CH and the CRF. These results were in accordance with our earlier study, which involved subjects with corneal astigmatism even higher than 3.00D (Chapter 3).

Our IOP results were in agreement with the results obtained by Edwards and Brown (1993) and Quinn *et al*. (1995). Both studies had found a higher IOP in myopes. Edwards and Brown (1993) used a non-contact tonometer, and Quinn *et al*. (1995) measured the IOP by using a pneumatonometer. By contrast, Edwards *et al*. (1993) and Lee *et al*. (2004) had found opposing results in their prospective study. Edwards and co-workers found two completely contradictory results in different sample populations, although they had used similar research protocols. They found a higher IOP in 30 myopes in one selected sample (Edwards and Brown, 1993), but they did not find any correlation between the IOP and the degree of myopia in their population-based study (Edward *et al*., 1993). The controversial results could be due to unequal myopic subjects in the two studies. Only 13 myopic subjects were recruited in their population-based study. Lee *et al*. (2004) did not consider that the IOP and refractive error were linked in children. However, they defined high myopia as SE $\geq$ -3D, which was different from the consensus of high myopia (SE > -6D). Moreover, some studies that have used the ORA have found a higher IOPcc in myopes (Xu *et al*., 2010; Jiang *et al*., 2011; Radhakrishnan *et al*., 2012; Bueno-

Gimeno *et al*., 2014). Our current findings also indicate that longer eyeballs have a higher IOP (**Table 5.8**).

Lam and co-workers (2004) found a prevalence of myopia close to 90% in local school children, whereas it was only 62% in international schools. Although we did not include SE in our analysis, we demonstrated that the mean AL of international school children was approximately 0.32mm shorter than in local school children. This may indicate one diopter difference in refractive error between the two groups (Levin *et al*., 2011). We could not compare our results with those obtained by Lam *et al*. (2004), because they did not report the amount of myopia in their subjects. Children in international schools also had a slightly higher CH and lower corneal astigmatism compared with local school children. It is difficult to determine whether the differences in AL, CH and corneal astigmatism were due to different academic environments in local and international schools. Lam *et al*. (2004) found a prevalence of myopia in over 80% of Chinese students in international schools, similar to Chinese students in local schools. Perhaps it is not simply the difference in academic environment contributing to the differences.

Because 30% of children from the international schools were non-Chinese, they could be a silent contributing factor to the difference in ocular parameters between local and international schools. When Chinese students in local schools and international schools were compared, they had similar CH, CRF, and IOPcc. However, Chinese students in local schools showed a longer AL, steeper cornea, and higher corneal astigmatism compared with Chinese students in international schools (**Table 5.5**). Lam *et al*. (2004) found that Chinese children studying in local schools had a slightly higher prevalence of myopia (87.2%) compared with Chinese children studying in international schools (82.8%). Chinese students in local schools are significantly older than those in international schools (**Table 5.5**). Children are eligible to enter local primary education once they reach 6 years of age (specifically, 5 years and 8 months), and most international schools begin admission at 5 years of

age. The difference in ocular biometry as well as refractive status could be due to their difference in age. In conclusion, these two groups of Chinese students shared similar CH and CRF, regardless of whether they were studying in local or international schools.

With wave after wave of immigrants as well as globalization, the world is increasingly becoming a melting pot. Hong Kong is a cosmopolitan metropolis where East meets West. Regardless of the Chinese population, sizeable communities of people from different ethnic backgrounds live in Hong Kong. The number of people from a multiethnic background is increasing globally, and they are further becoming the mainstream in society. The Mixed children in this study were included with the characters of different ethnic groups. All of their findings were ranked in the middle among all of the ethnic groups. However, our findings on the influence of ethnicity on CH and the CRF were only preliminary, because of the limited sample size and predominant Chinese population in this study.

Earlier studies have shown inter-ethnic differences in the IOP (Leske *et al*., 1997), CCT (Lazreg *et al*., 2008) and corneal biomechanical properties (Haseltine *et al*., 2012). Considering that numerous studies have proposed heterogeneity in the prevalence of myopia (Lam *et al*., 2004; Pan *et al*., 2013a; Pan *et al*., 2013b), ethnicity could be the surrogate risk factor for it. Only AL, CH, and corneal astigmatism reached a significant inter-ethnic difference. As expected, Chinese children had the longest AL and highest corneal astigmatism compared with other ethnic groups. This result is probably due to the higher prevalence of myopia in the Chinese category. Chinese students also had the lowest CH among the four groups, whereas the Caucasian group had the highest CH. Caucasian children in this study had a similar CH compared with those in Shah *et al*. (2014), who had similar age range (**Table 5.9**). Nevertheless, our Caucasian children had a lower CH compared with those examined by Kirwan *et al*. (2006) and Bueno-Gimeno *et al*. (2014) (11.91 ± 2.01mmHg versus 12.50 ± 1.35mmHg; 12.12 ± 1.71mmHg, respectively) (**Table**

5.9). These studies involved subjects as young as 4 years (Kirwan *et al*., 2006) and 6 years (Bueno-Gimeno *et al*., 2014), who might contribute to a higher CH. The four ethnic groups had similar MeanK, CRF, and IOPs.

AL, instead of SE, was used in our analysis, because AL is a parameter postulated to have a direct association with ocular rigidity and the pathological complications of myopia, whereas SE is a combined effect from corneal power, lens power, and AL. Only Chinese children showed a significant association between AL and CH (**Table 5.7**). Does this result suggest that a lower CH could be the precursor of a longer AL in Chinese children, and vice versa? The current findings could be potentially related with the asymmetric sample size among different ethnic groups. The sample size was much larger in Chinese than in other ethnic groups. Alio *et al*. (2010) found that a greater change in CH was associated with a longer AL after cataract surgery. Children were further divided into four equal subgroups based on the quartiles of AL to better understand the trend of different ocular parameters in different AL groups. A decreasing trend was observed in CH and MeanK, whereas IOPs exhibited an increasing trend from the first to the fourth quartile of AL. It was again confirmed that MeanK was flatter, CH was lower and IOPs were higher in longer AL. Nevertheless, for a 1 mm increase in AL, CH and the CRF decreased by only 0.28 mmHg and 0.11 mmHg, respectively.

Table 5.9 Corneal hysteresis (CH) and the corneal resistance factor (CRF) in different ethnic groups.

Studies	Age (Year) mean ± SD (range)	Gender	Population	CH (mmHg) mean ± SD	CRF (mmHg) mean ± SD
Kirwan *et al*. (2006)	n/a (4-18)	22 male (n = 42)	Caucasian	12.5 ± 1.35	n/a
Fontes *et al*. (2008)	45.09 ± 20.58 (18-90)	53 male (n = 150)	Brazilian	10.17 ± 1.82	10.14 ± 1.80
Kamiya *et al*. (2008)	39.1 ± 14.5 (19-68)	13 male (n = 43)	Japanese	10.2 ± 1.3	n/a
Lim *et al*. (2008)	13.97 ± 0.89 (12-15)	138 male (n = 271)	Mixed ethnicity	11.78 ± 1.55	11.81 ± 1.71
Song *et al*. (2008)	14.7 ± 0.8 (10-14)	n/a (n = 1153)	Chinese	10.7 ± 1.64	n/a
Chang *et al*. (2010)	12.02 ± 3.19 (7-18)	37 male (n = 63)	Chinese	10.85 ± 1.33	11.03 ± 1.46
Foster et al. (2011)	n/a (48-91)	1831 male (n = 4184)	British	10.00 ± 1.64	10.22 ± 1.74
Huang *et al*. (2011)	8.6 ± 2.08 (7-12)	340 male (n = 651)	Chinese	10.4 ± 2.2	11.2 ± 2.1
Narayanaswamy *et al*. (2011)	55.3 ± 8.4 (44-83)	554 male (n = 1136)	Chinese	10.60 ± 1.50	10.10 ± 1.60
Radhakrishnan *et al*. (2012)	(18-65)	41 male (n = 117)	Mixed ethnicity	10.80 ± 1.52	10.67 ± 1.64
Huang *et al*. (2013)	10.47 ± 1.00	303 male (n = 571)	Chinese	10.98 ± 1.78	11.46 ± 1.69
Bueno-Gimeno *et al*. (2014)	10.84 ± 3.05 (6-17)	135 male (n = 293)	Spanish	12.12 ± 1.71	12.30 ± 1.89
Rosa *et al*. (2014)	43.1 ± 15.4 (19-82)	58 male (n = 105)	Italian	10.26 ± 1.49	10.38 ± 1.64
Shah *et al*. (2014)	11.51 ± 0.50 (10-12)	96 male (n = 96)	Mixed ethnicity	11.80 ± 1.70	11.90 ± 1.80

n/a = not available

With the probability of *in vivo* measurements of corneal biomechanical properties, numerous works that have covered a wide scope of areas have been conducted to investigate CH and the CRF, although their roles as the biomechanical properties of cornea have yet to be confirmed. As mentioned in the literature review, CH and CRF decreased significantly after refractive surgery (Pepose *et al*., 2007; Chen *et al*., 2008; Shah *et al*., 2009). CH and CRF could be the surrogate marker in the etiology of corneal ectasia (Spörl *et al*., 2009; Schweitzer *et al*., 2010) and glaucoma (Kotecha, 2007). The corneal biomechanical properties were postulated to represent the biomechanical properties of the lamina cribrosa or ocular biomechanical properties because of tissue continuity. Although the average CH and CRF have been found to have decreased in these studies, the standard deviations were high between the study and control groups. Similar findings have been observed in other myopia studies (**Table 5.10**). A large overlap of CH and the CRF in different quartiles of AL was observed in the present study.

Another possible reason for the large overlap of CH could be the types of myopia. Although several types of myopia exist, clinically, simple myopia and degenerative myopia are the two types related to axial elongation. In addition, the ultimate cause of the pathological complications of degenerative myopia is an exaggerated axial elongation. Song *et al*. (2008) suggested that children with lower CH had a weakened cornea or sclera, and tend to experience faster myopia progression. In children, a similar AL but different corneal biomechanical properties could be the cause of the large overlap of CH and the CRF in different AL quartiles. CH might be able to be used to predict the progression of axial elongation in children. For instance, children with a lower CH but shorter AL might experience faster myopia progression and be at a higher risk of developing degenerative myopia in the future, whereas children with greater CH but longer AL might experience slower myopia progression in the future. They might develop only simple myopia, instead of degenerative myopia.

Table 5.10 A comparison of corneal hysteresis (CH) and the corneal resistance factor (CRF) in myopes and non-myopes.

Studies	Population (Age, year)	CH (mmHg) mean ± SD		CRF (mmHg) mean ± SD	
		Myopes	Control	Myopes	Control
Shen *et al.* (2008a)	Chinese (11-65)	9.93 ± 1.73 (n = 45)	11.11 ± 1.49 (n = 90)	8.26 ± 2.04	8.57 ± 1.63
Xu *et al.* (2010)	Chinese (18-63)	10.0 ± 1.6 (n = 23)	11.2 ± 1.5 (n = 55)	8.4 ± 2.1	8.5 ± 1.7
Jiang *et al.* (2011)	Chinese (11-65)	Low myopes 11.24 ± 1.47 (n = 34) Moderate myopes 10.49 ± 0.89 (n = 18) High myopes 10.05 ± 1.66 (n = 55)	Non-myopes 11.13 ± 1.45 (n = 65)	Low myopes 8.88 ± 1.74 Moderate myopes 8.40 ± 1.48 High myopes 8.46 ± 1.98	Non-myopes 8.56 ± 1.60
Plakitsi *et al.* (2011)	Caucasian (19-48)	Moderate myopes 10.10 (n = 33) High myopes 10.20 (n = 30)	Low myopes and emmetropes 11.00 (n = 32)	Moderate myopes 9.70 High myopes 10.60	Low myopes and emmetropes 10.60

Altan *et al.* (2012)	Turkish (n/a)	AL ≥ 26mm 9.6 ± 2.3 (n = 83)	AL < 26mm 10.8 ± 1.6 (n = 82)	AL ≥ 26mm 9.9 ± 2.2	AL < 26mm 10.7 ± 2.1
Bueno-Gimeno *et al*. (2014)	Spanish (6-17)	Myopes 11.55 ± 1.45 (n = 100)	Emmetropes 12.56 ± 1.68 (n = 99) Hyperopes 12.25 ± 1.84 (n = 94)	Myopes 11.93 ± 1.85	Emmetropes 12.63 ± 1.91 Hyperopes 12.32 ± 1.89
Del Buey *et al*. (2014)	Spanish (20-56)	Low myopia 11.00 ± 1.25 (n = 47) Moderate myopia 10.52 ± 1.54 (n = 72) High myopia 10.35 ± 1.33 (n = 33)	Emmetropia 11.08 ± 0.98 (n = 25)	Low myopia 10.63 ± 1.39 Moderate myopia 10.34 ± 1.64 High myopia 10.36 ± 1.46	Emmetropia 11.07 ± 1.06

AL = axial length, n/a = not available.

The ORA is the simplest and a noninvasive approach for assessing corneal biomechanical properties in children. It was the only readily available approach that provided a clinical measurement of corneal biomechanical properties when this study started in 2009. Because of the apparent paucity of information obtained from cross-sectional studies, evidence is insufficient to prove whether CH and the CRF have the potential to aid with screening, early detection, and diagnosis of degenerative myopia. Whether CH and the CRF are promising addenda to axial elongation will remain unclear until longitudinal studies are performed. The following sections cover the longitudinal findings from the 1- and 2-year cohort studies.

5.5 Longitudinal Studies

5.5.1 Results

5.5.1.1 Results: One-year cohort

In total, 269 Chinese children completed the 1-year cohort study. The first eye examination was performed with an age range of 8 to 14 years, and a mean age of 10.36 ± 1.22 years. Demographic data are listed in **Table 5.11**.

AL increased from 23.92 ± 0.97mm to 24.15 ± 1.02mm (mean difference 0.23 ± 0.17mm, paired t-test, $p < 0.001$). CH increased from 11.10 ± 1.46mmHg to 11.31 ± 1.41mmHg (mean difference 0.21 ± 1.23mmHg, paired t-test, $p = 0.006$). Corneal astigmatism increased from 0.91 ± 0.52D to 0.97 ± 0.53D (mean difference 0.06 ± 0.25D, Wilcoxon signed-rank test, $p < 0.001$). Decreases in IOPg (mean difference -0.44 ± 2.11mmHg, paired t-test, $p = 0.001$) and IOPcc (mean difference -0.61 ± 2.33mmHg, paired t-test, $p < 0.001$) were significant, whereas the CRF and MeanK remained similar throughout the year (paired t-test, $p > 0.05$).

Table 5.11 A summary of demographic data, ocular components, and the mean difference of the ocular components after 1 year (n = 269).

	First visit mean ± SD (range)	**Second visit mean ± SD (range)**	**Mean difference mean ± SD (range)** (= Second visit – First visit)	**p - value**
Age (Year)	10.36 ± 1.22 (8.00 to 14.00)	11.36 ± 1.22 (9.00 to 15.00)	n/a	n/a
Gender	154 boys	n/a	n/a	n/a
Eye	214 right eyes	n/a	n/a	n/a
CH (mmHg)	11.10 ± 1.46 (7.00 to 16.00)	11.31 ± 1.41 (7.80 to 15.30)	0.21 ± 1.23 (-4.80 to 4.27)	0.006
CRF (mmHg)	10.99 ± 1.50 (6.80 to 15.70)	11.03 ± 1.41 (7.60 to 15.70)	0.04 ± 1.17 (-4.40 to 3.60)	0.544
IOPg (mmHg)	15.44 ± 2.44 (7.17 to 20.73)	15.00 ± 2.39 (7.87 to 20.60)	-0.44 ± 2.11 (-5.80 to 5.80)	0.001
IOPcc (mmHg)	15.18 ± 2.51 (7.30 to 20.97)	14.57 ± 2.52 (8.93 to 20.53)	-0.61 ± 2.33 (-6.63 to 6.70)	< 0.001
AL (mm)	23.92 ± 0.97 (21.32 to 27.33)	24.15 ± 1.02 (21.35 to 27.46)	0.23 ± 0.17 (0.00 to 1.02)	< 0.001
MeanK (Diopter)	42.86 ± 1.29 (39.81 to 46.28)	42.88 ± 1.29 (39.88 to 46.31)	0.02 ± 0.27 (-3.00 to 1.69)	0.245
CA (Diopter)	0.91 ± 0.52 (0.21 to 3.50)	0.97 ± 0.53 (0.12 to 3.63)	0.06 ± 0.25 (-1.24 to 1.37)	< 0.001
VA (logMAR)	0.15 ± 0.23 (-0.14 to 0.98)	0.14 ± 0.23 (-0.20 to 1.02)	0.00 ± 0.21 (-0.78 to 0.84)	0.877
SE (Diopter)	-2.57 ± 1.82 (-9.75 to 3.94)	-2.87 ± 1.82 (-9.69 to 3.25)	-0.30 ± 1.15 (-3.94 to 4.62)	< 0.001

CH = corneal hysteresis, CRF = corneal resistance factor, IOPg = Goldmann-correlated intraocular pressure, IOPcc = corneal-compensated intraocular pressure, AL = axial length, MeanK = mean corneal power, CA = corneal astigmatism, VA = visual acuity, SE = spherical equivalent, n/a = not available.

The changes in CH were not associated with axial elongation (r^2 = 0.00, p > 0.05), even when the data were further subdivided according to the initial CH quartiles. Axial elongation was not associated with changes in other ORA parameters (CRF: r^2 = 0.00; IOPg: r^2 = 0.002; IOPcc: r^2 = 0.001, all p > 0.05). However, MeanK became flatter with axial elongation (r^2 = 0.029, p = 0.005), but such corneal flattening was not associated with either the changes in CH or the CRF (r^2 = 0.005 and r^2 = 0.004, p > 0.05). The changes in CH were associated with the changes in IOPcc (r^2 = 0.404, p < 0.001), but the changes in CRF were not (r^2 = 0.014, p = 0.051).

Children were stratified into 4 groups according to AL quartiles, with a similar number of children in each group. The mean changes in CH, CRF, IOPg, IOPcc (all one-way ANOVA, p > 0.05) and MeanK (Kruskal-Wallis test, p > 0.05) did not show a significant difference in all four quartiles (**Table 5.12**). However, the mean difference in corneal astigmatism at the third quartile was significantly greater than in the first quartile (one-way ANOVA, post hoc test, p = 0.01). The children were subdivided into 4 groups by their initial CH quartiles (**Table 5.13**). Axial elongation in each quartile was non-significant (Kruskal-Wallis test, p > 0.05).

Table 5.12 Mean cumulative 1-year differences of corneal hysteresis (CH), the corneal resistance factor (CRF), Goldmann-correlated intraocular pressure (IOPg), corneal-compensated intraocular pressure (IOPcc), mean corneal power (MeanK), and corneal astigmatism (CA) in quartiles of axial length (n = 269). The first quartile was the reference group for the other quartiles.

	Axial Length at First visit (mm) mean ± SD (range)				
1-year differences (= Second visit – First visit)	**1st Quartile (21.32 to < 23.23) (n = 67)**	**2nd Quartile (23.23 to < 23.79) (n = 68)**	**3rd Quartile (23.79 to < 24.52) (n = 67)**	**4th Quartile (24.52 to < 27.33) (n = 67)**	**p - value**
CH (mmHg)	0.27 ± 1.39 (-4.80 to 3.00)	0.30 ± 1.06 (-1.90 to 3.80)	0.26 ± 1.20 (-2.50 to 4.27)	-0.01 ± 1.24 (-3.00 to 2.27)	0.431
CRF (mmHg)	0.00 ± 1.20 (-4.40 to 2.40)	0.16 ± 1.08 (-2.03 to 3.10)	0.14 ± 1.23 (-2.50 to 3.60)	-0.12 ± 1.18 (-3.13 to 2.50)	0.503
IOPg (mmHg)	-0.77 ± 2.30 (-4.83 to 5.80)	-0.33 ± 2.09 (-5.00 to 4.03)	-0.29 ± 2.03 (-5.80 to 4.10)	-0.38 ± 2.01 (-5.27 to 3.43)	0.523
IOPcc (mmHg)	-0.96 ± 2.76 (-6.63 to 6.70)	-0.61 ± 2.16 (-6.23 to 3.50)	-0.55 ± 2.11 (-5.40 to 4.00)	-0.32 ± 2.26 (-5.27 to 5.23)	0.463
MeanK (Diopter)	-0.03 ± 0.40 (-3.00 to 0.56)	0.01 ± 0.15 (-0.44 to 0.38)	0.08 ± 0.25 (-0.41 to 1.69)	0.02 ± 0.19 (-0.48 to 0.38)	0.465
CA (Diopter)	0.00 ± 0.26 (-1.24 to 0.63)	0.03 ± 0.21 (-0.33 to 0.62)	0.12 ± 0.29* (-0.38 to 1.37)	0.11 ± 0.23 (-0.37 to 0.87)	0.010

* post hoc test, $p < 0.05$

Table 5.13 Mean cumulative 1-year differences of axial length in quartiles of corneal hysteresis (n = 269). The first quartile was the reference group for the other quartiles.

	Corneal Hysteresis at First visit (mmHg) mean ± SD (range)				
1-year differences (= Second visit – First visit)	**1st Quartile (7.00 to < 10.15) (n = 67)**	**2nd Quartile (10.15 to < 11.10) (n = 63)**	**3rd Quartile (11.10 to < 12.05) (n = 72)**	**4th Quartile (12.05 to < 16.00) (n = 67)**	**p - value**
Axial length (mm)	0.24 ± 0.18 (0.00 to 1.02)	0.26 ± 0.16 (0.04 to 0.78)	0.24 ± 0.16 (0.02 to 0.66)	0.20 ± 0.16 (0.00 to 0.70)	0.13

To account for the effect of axial elongation on the mean changes of CH, the children were subdivided into groups at 0.10mm intervals based on their axial elongation. However, different AL elongations had no significant association with different CH changes (one-way ANOVA, $p > 0.05$) (**Table 5.14**). By contrast, to account for the effect of CH changes on axial elongation, children were stratified to different groups at 0.80mmHg intervals based on their mean CH changes. Axial elongation showed a similar tendency in different mean CH changes (one-way ANOVA, $p > 0.05$) (**Table 5.15**).

Table 5.14 Mean cumulative 1-year differences of corneal hysteresis (CH) in different groups of axial elongation (n = 269). Mean CH changes exhibit a similar tendency in different axial elongation groups (p = 0.735).

	Mean axial elongation (mm) mean ± SD (range)					
1-year differences (= Second visit – First visit)	**0.00 to 0.099 (n = 56)**	**0.100 to 0.199 (n = 81)**	**0.200 to 0.299 (n = 52)**	**0.300 to 0.399 (n = 40)**	**0.400 to 0.499 (n = 20)**	**≥ 0.500 (n = 20)**
Changes of CH (mmHg)	0.23 ± 1.43 (-4.80 to 4.27)	0.31 ± 1.12 (-2.77 to 2.93)	0.22 ± 1.05 (-2.03 to 2.47)	0.07 ± 1.20 (-3.00 to 2.47)	-0.13 ± 1.53 (-2.77 to 3.80)	0.33 ± 1.21 (-2.97 to 1.80)

Table 5.15 Mean cumulative 1-year differences of axial elongation in different mean corneal hysteresis changes (n = 269). Axial elongation exhibits a similar tendency in different mean corneal hysteresis changes (p = 0.347).

	Mean corneal hysteresis changes (mmHg) mean ± SD (range)					
1-year differences (= Second visit – First visit)	**< -1.600 (n = 18)**	**-1.600 to -0.801 (n = 27)**	**-0.800 to -0.001 (n = 69)**	**0.000 to 0.799 (n = 72)**	**0.800 to 1.599 (n = 48)**	**≥ 1.600 (n = 35)**
Axial elongation (mm)	0.26 ± 0.17 (0.02 to 0.56)	0.22 ± 0.15 (0.01 to 0.56)	0.21 ± 0.14 (0.00 to 0.70)	0.24 ± 0.16 (0.03 to 0.78)	0.27 ± 0.21 (0.02 to 1.02)	0.21 ± 0.15 (0.00 to 0.63)

5.5.1.2 Results: Two-year cohort

A total of 144 Chinese children were included in this 2-year cohort study. The first examination was conducted at an age ranging between 8 and 12 years, with a mean age of 9.27 ± 0.89 years. **Table 5.16** shows the demographic data of this 2-year cohort.

The children typically had a significantly longer AL (mean difference 0.46 ± 0.30mm, paired t-test, $p < 0.001$), increased CH (mean difference 0.28 ± 1.22mmHg, paired t-test, $p = 0.007$) and greater corneal astigmatism (mean difference 0.10 ± 0.36D, Wilcoxon signed-rank test, $p = 0.002$) in 2 years (**Table 5.16**). A decrease in IOPg and IOPcc (mean difference -0.45 ± 2.29mmHg and -0.69 ± 2.46mmHg, respectively; paired t-test, $p = 0.019$ and $p = 0.001$, respectively) occurred, but with a similar CRF and MeanK (mean difference 0.10 ± 1.20mmHg and 0.01 ± 0.23D, respectively, paired t-test, $p > 0.05$).

Children were classified into quartiles based on their AL at the first visit. All four quartiles had similar changes in CH, CRF, IOPg, and IOPcc (One-way ANOVA, $p > 0.05$) (**Table 5.17**). Changes in corneal astigmatism at the fourth quartile were significantly higher than in the first quartile (Kruskal-Wallis test, $p = 0.001$, post hoc test, $p = 0.003$). Children were subdivided into 4 groups based on their initial CH quartiles (**Table 5.18**). The mean difference of AL in each quartile was non-significant (Kruskal-Wallis test, $p > 0.05$).

Table 5.16 A summary of demographic data, ocular components, and the mean difference of the ocular components in 2 years (n = 144).

	First visit mean ± SD (range)	**Second visit mean ± SD (range)**	**Mean difference mean ± SD (range)** (= Second visit – First visit)	**p - value**
Age (Year)	9.27 ± 0.89 (8.00 to 12.00)	11.52 ± 0.81 (10.00 to 14.00)	n/a	n/a
Gender	74 boys	n/a	n/a	n/a
Eye	116 right eyes	n/a	n/a	n/a
CH (mmHg)	11.09 ± 1.28 (7.40 to 14.40)	11.37 ± 1.46 (7.20 to 16.90)	0.28 ± 1.22 (-2.47 to 5.57)	0.007
CRF (mmHg)	10.95 ± 1.43 (6.80 to 15.30)	11.05 ± 1.55 (7.00 to 16.60)	0.10 ± 1.20 (-2.70 to 4.87)	0.325
IOPg (mmHg)	15.33 ± 2.45 (7.73 to 21.00)	14.88 ± 2.47 (8.20 to 20.80)	-0.45 ± 2.29 (-4.60 to 6.80)	0.019
IOPcc (mmHg)	15.10 ± 2.28 (8.80 to 20.50)	14.41 ± 2.41 (8.57 to 20.60)	-0.69 ± 2.46 (-7.10 to 6.70)	0.001
AL (mm)	23.63 ± 0.92 (21.44 to 26.98)	24.09 ± 1.03 (21.78 to 27.72)	0.46 ± 0.30 (0.00 to 2.36)	< 0.001
MeanK (Diopter)	42.77 ± 1.32 (40.00 to 46.08)	42.78 ± 1.27 (40.12 to 46.00)	0.01 ± 0.23 (-0.72 to 0.56)	0.508
CA (Diopter)	0.94 ± 0.55 (0.00 to 3.26)	1.04 ± 0.58 (0.12 to 3.13)	0.10 ± 0.36 (-1.07 to 1.50)	0.002
VA (logMAR)	0.14 ± 0.22 (-0.40 to 1.04)	0.10 ± 0.20 (-0.18 to 1.00)	-0.04 ± 0.22 (-1.00 to 0.60)	0.032
SE (Diopter)	-2.03 ± 1.59 (-8.00 to 1.06)	-2.70 ± 1.85 (-9.00 to 1.32)	-0.68 ± 1.37 (-4.00 to 3.13)	< 0.001

CH = corneal hysteresis, CRF = corneal resistance factor, IOPg = Goldmann-correlated intraocular pressure, IOPcc = corneal-compensated intraocular pressure, AL = axial length, MeanK = mean corneal power, CA = corneal astigmatism, VA = visual acuity, SE = spherical equivalent, n/a = not available.

Table 5.17 Mean cumulative 2-year differences of corneal hysteresis (CH), the corneal resistance factor (CRF), Goldmann-correlated intraocular pressure (IOPg), corneal-compensated intraocular pressure (IOPcc), mean corneal power (MeanK) and corneal astigmatism (CA) in quartiles of axial length (n = 144). The first quartile was the reference group for the other quartiles.

	Axial Length at First visit (mm) mean ± SD (range)				
2-year differences (= Second visit – First visit)	**1st Quartile (21.44 to < 23.04) (n = 36)**	**2nd Quartile (23.04 to < 23.58) (n = 36)**	**3rd Quartile (23.58 to < 24.16) (n = 36)**	**4th Quartile (24.16 to < 26.98) (n = 36)**	**p - value**
CH (mmHg)	0.43 ± 1.29 (-2.20 to 3.70)	0.10 ± 1.44 (-2.30 to 5.57)	0.44 ± 1.08 (-2.47 to 2.90)	0.15 ± 1.04 (-2.20 to 1.93)	0.508
CRF (mmHg)	0.32 ± 1.44 (-2.70 to 3.20)	-0.04 ± 1.30 (-2.30 to 4.87)	0.19 ± 0.96 (-2.20 to 1.80)	-0.07 ± 1.03 (-2.60 to 2.23)	0.465
IOPg (mmHg)	-0.13 ± 2.57 (-4.50 to 6.80)	-0.42 ± 2.41 (-4.20 to 4.90)	-0.62 ± 2.22 (-4.60 to 5.37)	-0.64 ± 1.99 (-4.17 to 4.20)	0.772
IOPcc (mmHg)	-0.58 ± 2.40 (-6.40 to 6.00)	-0.46 ± 2.81 (-5.60 to 6.70)	-1.00 ± -2.53 (-7.10 to 5.40)	-0.71 ± 2.11 (-4.87 to 3.27)	0.818
MeanK (Diopter)	-0.01 ± 0.21 (-0.72 to 0.38)	-0.02 ± 0.26 (-0.55 to 0.49)	0.01 ± 0.17 (-0.47 to 0.31)	0.08 ± 0.26 (-0.55 to 0.56)	0.253
CA (Diopter)	0.04 ± 0.45 (-1.07 to 1.50)	0.00 ± 0.24 (-0.38 to 0.63)	0.06 ± 0.27 (-0.50 to 0.63)	0.29 ± 0.40** (-0.41 to 1.38)	0.001

** post hoc test, $p < 0.01$

Table 5.18 Mean cumulative 2-year differences of axial length in quartiles of corneal hysteresis (n = 144). The first quartile was the reference group for the other quartiles. The mean difference of axial length is similar in different initial corneal hysteresis quartiles (p = 0.32).

	Corneal Hysteresis at First visit (mmHg) mean ± SD (range)			
2-year differences (= Second visit – First visit)	**1st Quartile (7.40 to < 10.20) (n = 37)**	**2nd Quartile (10.20 to < 11.13) (n = 35)**	**3rd Quartile (11.13 to < 11.90) (n = 39)**	**4th Quartile (11.90 to < 14.40) (n = 33)**
Axial length (mm)	0.50 ± 0.27 (0.05 to 1.06)	0.44 ± 0.28 (0.04 to 1.09)	0.51 ± 0.39 (0.07 to 2.36)	0.38 ± 0.22 (0.00 to 1.14)

The changes in CH were not associated with axial elongation ($r^2 = 0.015$, $p > 0.05$), although the data were further subdivided according to the initial CH quartiles. Nevertheless, the changes in CRF were weakly associated with axial elongation ($r^2 = 0.037$, $p = 0.022$). Axial elongation was not associated with the changes in MeanK, IOPg and IOPcc ($r^2 = 0.00$, $r^2 = 0.024$ and $r^2 = 0.003$, respectively, $p > 0.05$). In addition, the association between the changes in CH and in IOPcc was significant ($r^2 = 0.361$, $p < 0.001$), although the changes in CRF were not ($r^2 = 0.001$, $p > 0.05$). Neither the changes in CH or CRF were associated with changes in MeanK ($r^2 = 0.004$ and $r^2 = 0.001$, respectively, $p > 0.05$).

Children were stratified into 5 groups based on their axial elongation at a 0.20mm interval to observe the mean changes of CH at different levels of axial elongation. Only children with an axial elongation ≥ 0.80mm had a greater CH increase (one-way ANOVA, post hoc test, $p = 0.008$) compared with that in children who had an axial elongation between 0.200mm and 0.399mm (**Table 5.19**). No significant difference in the mean IOPcc changes was observed in the 5 groups (one-way ANOVA, $p > 0.05$).

Considering the effect of CH changes, children were classified into 6 groups to evaluate the axial elongation at different levels of CH changes. No significant axial elongation was noted (one-way ANOVA, $p > 0.05$) (**Table 5.20**).

Table 5.19 Mean cumulative 2-year differences of corneal hysteresis (CH) and corneal-compensated intraocular pressure (IOPcc) in different groups of axial elongation (n = 144). The mean CH changes are highest when the mean axial elongation ≥ 0.80mm (p = 0.019). The mean IOPcc changes are similar in different levels of axial elongation (p = 0.374).

	Mean axial elongation (mm) mean ± SD (range)				
2-year differences (= Second visit – First visit)	**0.000 to 0.199 (n = 25)**	**0.200 to 0.399 (n = 49)**	**0.400 to 0.599 (n = 31)**	**0.600 to 0.799 (n = 23)**	**≥ 0.800 (n = 16)**
Changes of CH (mmHg)	0.40 ± 1.07 (-2.20 to 3.20)	-0.01 ± 1.37 (-2.47 to 5.57)	0.28 ± 0.83 (-1.10 to 2.40)	0.16 ± 1.42 (-2.20 to 3.70)	**1.16 ± 0.95 (-0.40 to 2.90)
Changes of IOPcc (mmHg)	-1.30 ± 2.40 (-6.40 to 3.70)	-0.46 ± 2.56 (-5.60 to 6.70)	-0.44 ± 2.20 (-3.30 to 5.40)	-0.31 ± 2.29 (-3.50 to 4.20)	-1.43 ± 2.87 (-7.10 to 3.50)

** post hoc test, $p < 0.01$

Table 5.20 Mean cumulative 2-year differences of axial elongation in different mean corneal hysteresis changes (n = 144). The axial elongation is similar in different groups of mean CH changes (p = 0.233).

	Mean corneal hysteresis changes (mmHg) mean ± SD (range)					
2-year differences (= Second visit – First visit)	**< -1.600 (n = 9)**	**-1.600 to -0.801 (n = 12)**	**-0.800 to -0.001 (n = 34)**	**0.000 to 0.799 (n = 52)**	**0.800 to 1.599 (n = 18)**	**≥ 1.600 (n = 19)**
Axial elongation (mm)	0.44 ± 0.23 (0.09 to 0.76)	0.37 ± 0.18 (0.08 to 0.71)	0.46 ± 0.38 (0.10 to 2.36)	0.41 ± 0.26 (0.00 to 1.14)	0.60 ± 0.31 (0.15 to 1.09)	0.51 ± 0.32 (0.05 to 1.06)

5.5.2 Discussion

Numerous studies have observed the association between corneal biomechanical properties and AL (Kirwan *et al*., 2006; Lim *et al*., 2008; Song *et al*., 2008; Chang *et al*., 2010). Nonetheless, majority of them were limited to cross-sectional studies. The drawback of cross-sectional study is that the inter-subject variation on CH and CRF could be a confounding factor masking the association between corneal biomechanical properties and AL. The actual cause-and-effect relationship between AL and corneal biomechanical properties had been unclear. There has been one longitudinal study monitoring the changes of CH and CRF and AL over two years (Shah *et al*., 2014). Although Shah and co-workers found significant increase in AL, no significant changes were found in CH and CRF. Their study was limited by involving 58 boys only and their mixed ethnicity.

The current study found a significant increase in CH in both the 1- and 2-year cohort studies (**Tables 5.11** and **5.16**, respectively). The average increase in CH was 0.21mmHg for 1 year and 0.28mmHg for 2 years, but the standard deviation was large, with up to 1.23 mmHg. Despite no association having been found between the axial elongation and CH changes, it was still an unsatisfactory rationale for establishing a causal relationship between the two variables. If a lower CH in myopes is the cause of axial elongation, children with a lower baseline CH should have greater axial elongation, although the opposite seems to be true, because axial elongation was the lowest (1-year cohort, 0.20mm and 2-year cohort, 0.38mm) in children with the highest initial CH (**Tables 5.13** and **5.18**, respectively). However, it could not achieve statistical significance. Moreover, axial elongation was also similar, regardless of the changes in CH (**Tables 5.15** and **5.20**, respectively). Hence, axial elongation is unlikely to be influenced by CH.

Numerous cross-sectional studies have reported a low CH in high myopia (Shen *et al*., 2008a; Song *et al*., 2008; Chang *et al*., 2010; Xu *et al*., 2010; Jiang *et al*., 2011; Narayanaswamy *et al*., 2011; Altan *et al*., 2012; Bueno-Gimeno *et al*., 2014). If lower CH in myopes is a compensation of axial elongation, a greater axial elongation is expected, in addition to a greater drop in CH. By contrast, a greater increase in CH was noted in children with greater axial elongation (**Table 5.19**). Therefore, a lower CH in myopes is improbable to be the compensation of axial elongation. A lower CH may be postulated as a higher bounce cornea, and therefore, it could return to its original state quickly after the removal of external force. Conversely, a higher CH indicates greater energy dissipation and a lower bounce cornea (Broman *et al*., 2007). Hence, a cornea with a higher CH returns to its original shape slowly. A significant increase in CH was found in the 2-year cohort study, but not in the 1-year cohort study (**Table 5.14**). Whether the change in CH is caused to cope with an accelerated axial elongation remains unknown.

Clinically, the association between ocular rigidity and myopia remains inconclusive. Castrén and Pohjola (1961) and Bonomi *et al*. (1982) found a substantial decline in ocular rigidity in myopes. Wong and Yap (1991) and Schmid and co-workers (2003) have found no significant difference in ocular rigidity between myopic and non-myopic Chinese populations. Schmid and co-workers (2003) also reported no significant reduction in ocular rigidity during myopia progression.

Numerous studies have suggested that CH and CRF measurements could have other applications. This is supported by the association between CH and changes in glaucomatous disc morphology (Wells *et al*., 2008; Vu *et al*., 2013) and structural alterations in matrix proteoglycans in poorly controlled diabetic patients (Scheler *et al*., 2012). Furthermore, a significantly decreased AL in lower-CH patients was found after an IOP reduction caused by a trabeculectomy (Huang *et al*., 2012). All of these findings have attempted to show that a lower CH is related to a more extensible eye. Although we did not find a greater axial elongation from eyes with a low initial

CH (Tables **5.13** and **5.18**), the opposite was observed (the smallest axial elongation from eyes with the highest initial CH). What remains unknown is/are the cause(s) of increased CH from both the 1- and 2-year cohort studies.

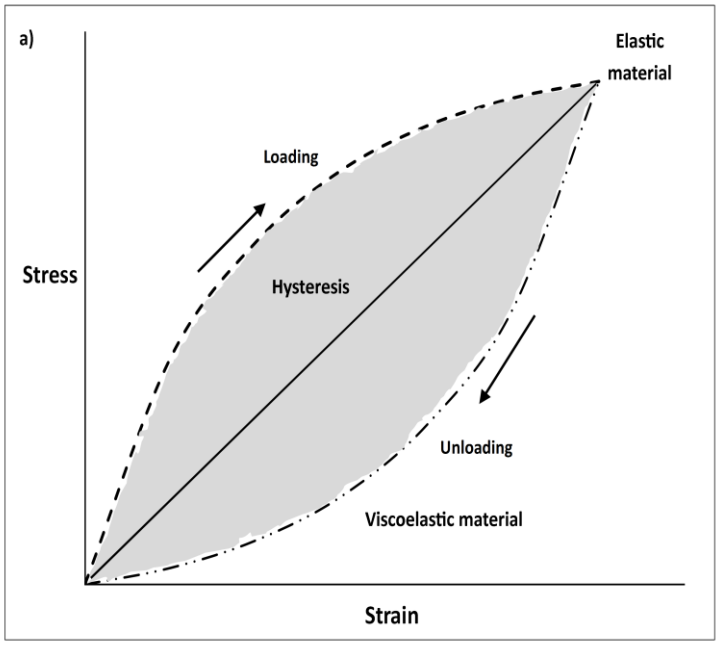
The cornea is a viscoelastic material that has both the characteristics of elasticity and viscosity (Kotecha, 2007). When stress is applied on viscoelastic material, deformation occurs, and energy is dissipated. Elasticity involves energy storage during the recovery of deformation, whereas viscosity involves energy dissipation to resist corneal deformation. This relationship could be plotted using a stress-strain curve. The term "hysteresis" refers to the energy dissipated under the loading and unloading curves, and it is a primary phenomenon for describing the viscoelastic material. CH is called corneal hysteresis because it is derived from the differences in pressure during the first and second applanations (Luce, 2005). CH may therefore represent only the dissipated energy during the two applanated states at a preset applanation (strain) (**Figure 5.10**).

IOPs decreased in both the 1- and 2-year cohort studies. These results contradicted the findings of previous longitudinal studies. Edwards and Brown (1996) found a rise in IOP in children with myopia development. They included 2 groups of subjects, one with preexisting myopia, and the other without myopia at the beginning of the study. The IOP rise was significant in the non-myopic group only, and IOP changes were non-significant in subjects with preexisting myopia. Goss and Caffey (1999) monitored the changes in refractive error and the IOP of children. For children becoming myopic, a trend in IOP increase was demonstrated, but the change was non-significant. The mean axial elongation doubled (0.46mm) in the 2-year cohort study compared with the 1-year cohort study (0.23mm) in our sample. However, the reduction in IOPcc was similar (0.69mmHg and 0.61mmHg, respectively). The IOP reduction was independent of the initial AL (**Tables 5.12 and 5.17**) and different levels of axial elongation (**Tables 5.19**). It is unlikely that any real association exists between IOP changes and axial elongation. Despite the tendency to decrease at a

high IOP, CH remained steady in an IOP range between 10mmHg and 25mmHg (Tao *et al*., 2013). Only children with IOPcc within the normal physiological range (≤ 21mmHg) were included in the analysis; thus, the influence of IOPcc on CH was minimal.

Myopia most often develops in children aged between 8 and 14 years (Zadnik, 1997). Therefore, children attending Primary 4 were recruited in this study. The average annual axial elongation for Western children between 6 and 14 years of age was between 0.02mm and 0.16mm (Fledelius *et al*., 2014) and for Western emmetropes it was 0.10mm (Zadnik *et al*., 2004). Nevertheless, the average annual axial elongation in Chinese children was 0.20mm (Xiang *et al*., 2012), and Singaporean children aged between 7 and 9 years had a range of 0.10 to 0.45mm (Saw *et al*., 2005a). Our results were in accordance with previous works on Asian populations, at an annual and biennial rate of 0.23mm and 0.46mm, respectively. Saw *et al*. (2005a) did not find significant changes in corneal curvature in their 3-year longitudinal study. The current results were in accordance with their findings.

One limitation of this study is the lack of CCT measurements. CH and the CRF have been stated to have a modest association with the CCT. According to our findings reported in Chapter 4, we found that high myopes had a lower CH compared with emmetropes, although the two groups had a similar corneal thickness. In addition, CH and the CRF were not comparable to other corneal biomechanical properties, such as Young's modulus. Future studies could be conducted using more recent methods such as the Corvis ST or OCT combined with an air pulse to assess the corneal biomechanical properties. An analysis of the ORA waveform parameters should also be considered. It is still too early to form the conclusion that the ORA is merely a deluxe tonometer. The analysis of waveform parameters conducted using the ORA during corneal deformation was found to be useful for keratoconus screening (Mikielewicz *et al*., 2011). Therefore, the waveform parameters may help enhance our understanding of how the cornea changes during axial elongation.



b)

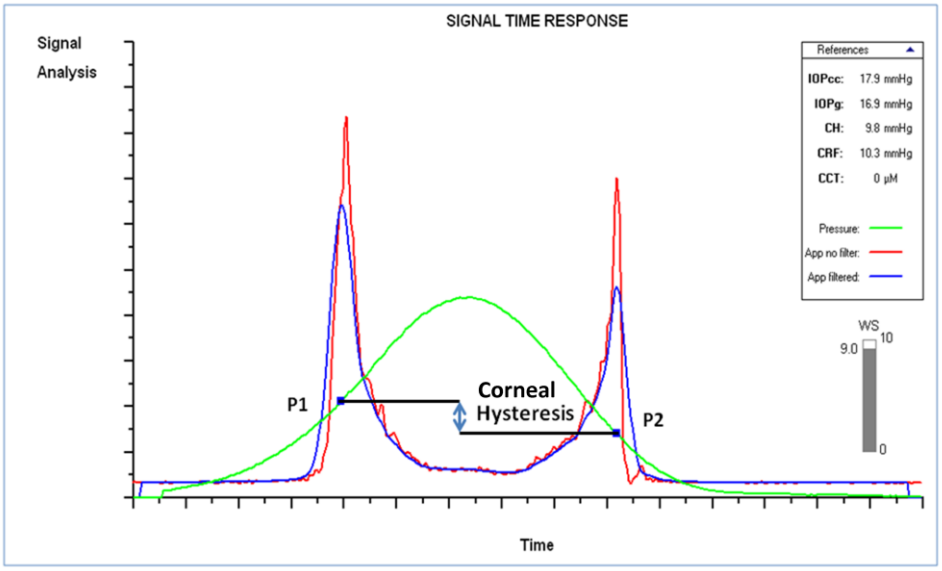


Figure 5.10 The stress-strain curve indicates (a) the area of hysteresis, and (b) the applanation and pressure plot of the Ocular Response Analyzer indicate the definition of corneal hysteresis.

5.6 Conclusion

This is the first longitudinal study to monitor both CH and the CRF in children at an age of fast myopia progression. Weak associations between CH and AL and between the CRF and AL were confirmed from the cross-sectional study. Moreover, our findings fill some knowledge gaps. Chinese children had a lower CH compared with other ethnic groups. Nonetheless, Chinese students in local and international schools had a similar CH and CRF. Although CH was found to have increased after axial elongation in both the 1- and 2-year cohort studies, our analysis did not conclude whether CH is a cause or a result of axial elongation.

Conference presentation:

Wong YZ, Lam AKC. Corneal biomechanical properties among healthy Chinese, Indian and Caucasian: A pilot study. ARVO Annual Meeting 2013, Seattle USA. Poster presentation.

Chapter 6 Summary and future work

6.1 Summary

The continuously proliferating population of myopes (Pan *et al*., 2012), with the significant detrimental effect of pathological myopia (Saw *et al*., 2005b), and the socioeconomic impact of myopia (Lim *et al*., 2009b) have raised the concern of the public and researchers, thus leading to extensive interest on myopia research. However, the mechanism of axial elongation is complex, and remains inconclusive.

The cornea is a transparent and mechanically tough tissue that provides clarity and protection against external force. Methods for measuring corneal biomechanical properties have been clinically available for approximately a decade. A lower CH has been found in myopic eyes with a similar CCT (Shen *et al*., 2008a; Bueno-Gimeno *et al*. 2014; Del Buey *et al*., 2014). One study postulated that the corneal biomechanical properties might reflect the biomechanical properties of the sclera, because the two tissues are formed from the continuous extracellular matrix (Song *et al*., 2008). Therefore, studying the corneal biomechanical properties might enhance our understanding of the mechanism of myopic axial elongation.

Three experiments were conducted for this study to evaluate the association between corneal biomechanical properties and myopia. We first investigated the associations

of CH and the CRF with other corneal and ocular parameters. The parameters from the ORA and AL in children were assessed and monitored annually and biennially.

Experiment 1 involved investigating the influence of corneal curvature, corneal astigmatism, and corneal meridional differences on CH and the CRF. The ORA measurements were obtained at the default recommended position, as well as at the superotemperal 10°, 20°, and 30°. Experiment 2 was designed to investigate the association among ORA parameters, AL, and other corneal parameters, including corneal sublayer thickness and CV in emmetropes and high myopes. We first developed a method to reduce the inter-observer variation of corneal sublayer thickness measurements by using a confocal microscope before beginning Experiment 2. Experiment 3 involved observing the changes of ORA parameters with axial elongation in children. Vision screenings were conducted in local primary schools and international schools. The corneal biomechanical properties, AL, corneal curvature, corneal astigmatism and SE were assessed. The same measurement protocol was used in the cross-sectional, 1-year cohort, and 2-year cohort studies to monitor changes in these parameters with axial elongation.

The cornea has different biomechanical properties at different regions because of the arrangement of collagen fibrils (Kotecha, 2007). The measurement of the IOP using GAT is affected by corneal curvature and corneal astigmatism (Mark and Mark, 2003). CH and the CRF are derived from the applanated pressures, and thus, the measurements of CH and the CRF could be affected by corneal curvature and corneal astigmatism. Moreover, the infrared emitter and receiver of the ORA are placed laterally; they might detect the corneal deformation along the horizontal meridian only. Both CH and the CRF were measured lower along the horizontal meridian, but the difference was clinically non-significant. A higher CH and CRF were also found in the steeper cornea, but the influence of corneal curvature and corneal astigmatism on CH and the CRF was negligible. CH and CRF measurements

were not affected by corneal curvature, even after considering the inter-subject variations.

We demonstrated that FST is a suitable parameter for clinically monitoring corneal changes. The corneal stroma comprises more than 80% of the total corneal thickness, and it consists of collagen fibrils and ground substance, which play a crucial role in corneal biomechanical properties. High myopes had a lower CH, although they had a similar FST, CV, and corneal curvature compared with age-matched emmetropes. Multivariate analysis indicated that CH variations were dependent on FST, rather than on AL. However, our multivariate models involving FST or CV, AL, and IOPcc could only account for approximately 50% of the CH variance, leaving another half of the total variance unexplained. Therefore, CH might also have an association with other ocular variables. Another possibility of the unexplained CH variance could be that CH is a more complex parameter to be explained by simple anatomic thickness or volume. FST or CV alone may be unable to reflect the whole corneal viscoelasticity. CH may have a relationship or be a factor in interactions between corneal collagen fibrils and ground substance.

The CRF had a strong positive association with FST. It is derived to correlate maximally with CCT (Luce, 2005). Hence, changes in CCT would have a greater effect on the CRF compared with that of CH. Both CH and the CRF were unaffected by the corneal epithelium. By contrast, IOPcc is another parameter generated by the ORA to have a minimal association with CCT (Luce, 2005). Moreover, no association has been found between IOPcc and CCT in previous studies (Medeiros and Weinreb, 2006; Lam *et al*., 2007), and the current study also found that IOPcc does not have an association with FST and CV.

The cross-sectional studies involving 1,199 children further confirmed that eyes with a longer AL have a flatter corneal curvature, lower CH, and higher IOP. There was heterogeneity in myopia and corneal biomechanical properties. Chinese children had

a longer AL, lower CH, and higher corneal astigmatism compared with other ethnic groups. We studied four ethnic groups, which included Chinese, Caucasian, Other Asian, and Mixed children. All of the ethnic groups had a similar corneal curvature, CRF and IOP. All of the ocular findings of the Mixed children were ranked in the middle among all ethnic groups. Chinese children in local schools had a similar CH, CRF and IOPcc compared with Chinese children in international schools, who had a shorter AL, flatter cornea, and lower corneal astigmatism.

Annual and biennial rates of axial elongation in Hong Kong Chinese children were 0.23mm and 0.46mm, respectively. An increased CH was observed during axial elongation, but whether the increased CH is a physiological response to cope with an accelerated axial elongation is unknown. Previous findings have attempted to show that the reduced CH was related to a more extensible eye (Alió *et al*., 2010; Huang *et al*., 2012; Kara *et al*., 2012). Greater axial elongation from eyes with a low initial CH was not found in the current study. Conversely, a marked increase in CH was found in eyes with greater axial elongation. However, corneal stiffness and viscoelasticity are not directly related (Glass *et al*., 2008).

The reason for the association between low CH and long AL from previous studies remains unknown. CH might be associated with other ocular variables because of tissue continuity. The extracellular matrix collagen at the corneal stroma is continual with the sclera and lamina cribrosa. Thus, it might convey the information of these ocular components because of tissue continuity. CH was found to be associated with connective tissue disorders (Emre *et al*., 2010; Yazici *et al*., 2011; Kara *et al*., 2012). The weaker scleral structure of highly myopic eyes might be reflected and quantified in some way through the biomechanical analysis of the cornea. However, the wide overlapping and inter-subject differences of CH could make CH analysis difficult.

6.2 Future work

The measurement of corneal biomechanical properties is challenging because of the complex structures of collagen fibrils. The usefulness of CH and the CRF as the corneal biomechanical properties remains unclear. CH and the CRF are derived from the two corneal applanation pressures, P1 and P2, during corneal deformation. They are detected through the reflection from the infrared emitter and receiver (Luce, 2005). The derivation of CH, that is, the difference between P1 and P2, varies from the universal acceptance that "hysteresis" refers to energy dissipation under the loading and unloading curves.

Future studies should involve the analysis of ORA waveform parameters, which seem useful for screening keratoconus (Mikielewicz *et al*., 2011) and for characterizing the corneal response after refractive surgeries (Zarei-Ghanavati *et al*., 2012; Wu *et al*., 2014). A recent study suggested that hyperopic eyes were stiffer than myopic eyes after evaluating the waveform parameters, although CH was similar between the two groups (Roberts *et al*., 2014). Another novel device, the Corvis ST, also provides some corneal biomechanical properties information, for example, the corneal deformation amplitude and time to first applanation. These new parameters have been investigated to study the corneal biomechanics (Han *et al*., 2014; Shen *et al*., 2014; Tian *et al*., 2014).

Future work could investigate the association between CH and CRF with corneal histomorphology. This is because cells are the fundamental units in forming tissue. Understanding the micro- and nano-mechanical behavior of a cell could enhance our comprehension of the overall mechanical properties of the cornea. Numerous studies have demonstrated the impact of heterogeneity in corneal diseases. Therefore, a

population-based study that involves a large sample and different ethnic groups is recommended to understand the role of ethnicity in CH and the CRF. Longitudinal studies are crucially required to establish a causal-effect relationship of corneal biomechanical properties and axial elongation.

Appendix A

Appendix 1. Referral criteria for the vision screening program.

1. Referral was made if any one of the following conditions was met.

    - Any eye with habitual visual acuity worse than 0.24 (logMAR).
    - Any heterotropia detected during cover test.
    - Stereopsis worse than 80 seconds of arc.
    - Intraocular pressure greater than 21mmHg.

2. Child missing 4 plates or more during color vision test was considered failure. No referral was made but parents were notified.

References

Abitbol O, Bouden J, Doan S, Hoang-Xuan T, Gatinel D. Corneal hysteresis measured with the Ocular Response Analyzer in normal and glaucomatous eyes. *Acta Ophthalmol*. 2010;88:116-119.

Akhtar S, Bron AJ, Salvi SM, Hawksworth NR, Tuft SJ, Meek KM. Ultrastructural analysis of collagen fibrils and proteoglycans in keratoconus. *Acta Ophthalmol*. 2008;86:764-772.

Alió JL, Aqdeppa MC, Rodríquez-Prats JL, Amparo F, Pinero DP. Factors influencing corneal biomechanical changes after microincision cataract surgery and standard coaxial phacoemulsification. *J Cataract Refract Surg*. 2010;36:890-897.

Altan C, Demirel B, Azman E, Satana B, Bozkurt E, Demirok A, Yilmaz OF. Biomechanical properties of axially myopic cornea. *Eur J Ophthalmol.* 2012;22:S24-S28.

Andreassen TT, Simonsen AH, Oxlund H. Biomechanical properties of keratoconus and normal corneas. *Exp Eye Res*. 1980;31:435-441.

Ang GS, Bochmann F, Townend J, Azuara-Blanco A. Corneal biomechanical properties in primary open angle glaucoma and normal tension glaucoma. *J Glaucoma*. 2008;17:259-262.

Bhan A, Browning AC, Shah S, Hamilton R, Dave D, Dua HS. Effect of corneal thickness on intraocular pressure measurements with the pneumatonometer, Goldmann applanation tonometer, and Tono-Pen. *Invest Ophthalmol Vis Sci*. 2002;43:1389-1392.

Bochmann F, Ang GS, Azuara-Blanco A. Lower corneal hysteresis in glaucoma patients with acquired pit of the optic nerve (APON). *Graefes Arch Clin Exp Ophthalmol*. 2008;246:735-738.

Boehm A, Weber A, Pillunat LE, Koch R, Spoerl E. Dynamic contour tonometry in comparison to intracameral IOP measurements. *Invest Ophthalmol Vis Sci*. 2004;45:3118-3121.

Bonomi L, Mecca E, Massa F. Intraocular pressure in myopic anisometropia. *Int Ophthalmol*. 1982;5:145-148.

Bradford, B. Who are the 'Mixed' ethnic group? Article London: Office for National Statistics, 2006. Available at: http://www.statistic.gov.uk/articles/nojournal/Mixed_ethnic_groups_pdf.pdf. Accessed January 03, 2014.

Broman AT, Congdon NG, Bandeen-Roche K, Quigley HA. Influence of corneal structure, corneal responsiveness, and other ocular parameters on tonometric measurement of intraocular pressure. *J Glaucoma*. 2007;16:581-588.

Brownlee M. Biochemistry and molecular cell biology of diabetic complications. *Nature*. 2001;414:813-820.

Brugin E, Ghirlando A, Gambato C, Midena E. Central corneal thickness: z-ring corneal confocal microscopy versus ultrasound pachymetry. *Cornea*. 2007;26:303-307.

Bryant MR, McDonnell PJ. Constitutive laws for biomechanical modeling of refractive surgery. *J Biomech Eng*. 1996;118:473-481.

Bueno-Gimeno I, Españ̃a-Gregori E, Gene-Sampedro A, Lanzagorta-Aresti A, Piñ̃ero-Llorens DP. Relationship among corneal biomechanics, refractive error, and axial length. *Optom Vis Sci*. 2014;91:507-513.

Buzard KA. Introduction to biomechanics of the cornea. *Refract Corneal Surg*. 1992;8:127-138.

Cankaya AB, Beyazyildiz E, Ileri D, Ozturk F. The effect of contact lens usage on corneal biomechanical parameters in myopic patients. *Cornea*. 2012;31:764-769.

Caporossi A, Mazzotta C, Baiocchi S, Caporossi T. Long-term results of riboflavin ultraviolet a corneal collagen cross-linking for keratoconus in Italy: the Siena eye cross study. *Am J Ophthalmol*. 2010;149:585-593.

Carbonaro F, Hysi PG, Fahy SJ, Nag A, Hammond CJ. Optic disc planimetry, corneal hysteresis, central corneal thickness, and intraocular pressure as risk factors for glaucoma. *Am J Ophthalmol*. 2014;157:441-446.

Carlson and Kurtz. *Clinical procedures for ocular examination*. 3rd ed. NY: McGraw Hill Professional; 2004.p7-p63.

Carnell PH, Vito RP. A model for estimating corneal stiffness using an indenter. *J Biomech Eng*. 1992;114:549-552.

Castrén JA, Pohjola S. Refraction and scleral rigidity. *Acta Ophthalmol (Kbh)*. 1961;39:1011-1014.

Castro DP, Prata TS, Lima VC, Biteli LG, de Moraes CG, Paranhos A Jr. Corneal viscoelasticity differences between diabetic and nondiabetic glaucomatous patients. *J Glaucoma*. 2010;19:341-343.

Chan KY, Cheung SW, Lam AK, Cho P. Corneal sublayer thickness measurements with the Nidek ConfoScan 4 (z Ring). *Optom Vis Sci*. 2011;88:E1240-E1244.

Chan KY, Cheung SW, Cho P. Nidek ConfoScan 4 (z-ring) measurements over soft contact lenses. *Eye Contact Lens.* 2012;38:80-85.

Chang PY, Chang SW, Wang JY. Assessment of corneal biomechanical properties and intraocular pressure with the Ocular Response Analyzer in childhood myopia. *Br J Ophthalmol*. 2010;94:877-881.

Chen D, Lam AK, Cho P. A pilot study on the corneal biomechanical changes in short-term orthokeratology. *Ophthalmic Physiol Opt*. 2009;29:464-471.

Chen MC, Lee N, Bourla N, Hamilton DR. Corneal biomechanical measurements before and after laser in situ keratomileusis. *J Cataract Refract Surg*. 2008;34:1886-1891.

Chen S, Chen D, Wang J, Lu F, Wang Q, Qu J. Changes in ocular response analyzer parameters after LASIK. *J Refract Surg*. 2010;26:279-288.

Cho P, Lam AK, Mountford J, Ng L. The performance of four different corneal topographers on normal human corneas and its impact on orthokeratology lens fitting. *Optom Vis Sci*. 2002;79:175-183.

Congdon NG, Broman AT, Bandeen-Roche K, Grover D, Quigley HA. Central corneal thickness and corneal hysteresis associated with glaucoma damage. *Am J Ophthalmol*. 2006;141:868-875.

Costin BR, Fleming GP, Weber PA, Mahmoud AM, Roberts CJ. Corneal biomechanical properties affect Goldmann applanation tonometry in primary open-angle glaucoma. *J Glaucoma*. 2014;23:69-74.

Dastiridou AI, Ginis HS, De Brouwere D, Tsilimbaris MK, Pallikaris IG. Ocular rigidity, ocular pulse amplitude, and pulsatile ocular blood flow: the effect of intraocular pressure. *Invest Ophthalmol Vis Sci*. 2009;50:5718-5722.

Dastiridou AI, Ginis H, Tsilimbaris M, Karyotakis N, Detorakis E, Siganos C, Cholevas P, Tsironi EE, Pallikaris IG. Ocular rigidity, ocular pulse amplitude, and pulsatile ocular blood flow: the effect of axail length. *Invest Ophthalmol Vis Sci*. 2013;54:2087-2092.

Del Buey MA, Lavilla L, Ascaso FJ, Lanchares E, Huerva V, Cristóbal JA. Assessment of corneal biomechanical properties and intraocular pressure in myopic Spanish health population. *J Ophthalmol*. 2014 Feb 25:905129. [Epub ahead of print] DOI: 10.1155/2014/905129.

Detorakis ET, Pallikaris IG. Ocular rigidity: biomechanical role, in vivo measurements and clinical significance. *Clin Experiment Ophthalmol*. 2013;41:73-81.

Diniz CM, Hazarbassanov RM, Yamazaki E, Murata C, Mallmann F, Campos M. Pentacam Scheimpflug evaluation of corneal volume after LASIK. *J Refract Surg.* 2010;26:600-604.

Djotyan GP, Kurtz RM, Fernandez DC, Juhasz T. An analytically solvable model for biomechanical response of the cornea to refractive surgery. *J Biomech Eng*. 2001;123:440-445.

Dorronsoro C, Pascual D, Pérez-Merino P, Kling S, Marcos S. Dynamic OCT measurement of corneal deformation by an air puff in normal and cross-linked corneas. *Biomed Opt Express*. 2012;3:473-487.

Edwards MH, Brown B. Intraocular pressure in a selected sample of myopic and nonmyopic Chinese children. *Optom Vis Sci*. 1993;70:15-17.

Edwards MH, Chun CY, Leung SS. Intraocular pressure in an unselected sample of 6- to 7-year-old Chinese children. *Optom Vis Sci*. 1993;70:198-200.

Edwards MH, Brown B. IOP in myopic children: the relationship between increases in IOP and the development of myopia. *Ophthalmic Physiol Opt*. 1996;16:243-246.

Eisenlohr JE, Langham ME, Maumenee AE. Manometric studies of the pressure-volume relationship in living and enucleated eyes of individual human subjects. *Br J Ophthalmol*. 1962;46:536-548.

Elsheikh A, Wang D, Brown M, Rama P, Campanelli M, Pye D. Assessment of corneal biomechanical properties and their variation with age. *Curr Eye Res*. 2007;32:11-19.

Elsheikh A, Alhasso D, Rama P. Assessment of the epithelium's contribution to corneal biomechanics. *Exp Eye Res*. 2008a;86:445-451.

Elsheikh A, Wang D, Rama P, Campanelli M, Garway-Heath D. Experimental assessment of human corneal hysteresis. *Curr Eye Res*. 2008b;33:205-213.

Emre S, Kayikçioğlu O, Ateş H, Cinar E, Inceoğlu N, Yargucu F, Pirildar T, Oksel F. Corneal hysteresis, corneal resistance factor, and intraocular pressure measurement in patients with scleroderma using the reichert ocular response analyzer. *Cornea*. 2010;29:628-631.

Faul F, Erdfelder E, Buchner A, Lang AG. Statistical power analyses using G*Power 3.1: tests for correlation and regression analyses. *Behav Res Methods*. 2009;41:1149-1160.

Ferris FL, Kassoff A, Bresnick GH, Bailey I. New visual acuity charts for clinical research. *AM J Ophthalmol*. 1982;94:91-96.

Fledelius HC, Christensen AS, Fledelius C. Juvenile eye growth, when completed? An evaluation based on IOL-Master axial length data, cross-sectional and longitudinal. *Acta Ophthalmol.* 2014;92:259-264.

Fontes BM, Ambrósio R Jr, Alonso RS, Jardim D, Velarde GC, Nosé W. Corneal biomechanical metrics in eyes with refraction of -19.00 to +9.00 D in healthy Brazilian patients. *J Refract Surg*. 2008;24:941-945.

Fontes BM, Ambrósio R Jr, Jardim D, Velarde GC, Nosé W. Corneal biomechanical metrics and anterior segment parameters in mild keratoconus. *Ophthalmology*. 2010;117:673-679.

Fontes BM, Ambrósio R Jr, Velarde GC, Nosé W. Ocular response analyzer measurements in keratoconus with normal central corneal thickness compared with matched normal control eyes. *J Refract Surg*. 2011;27:209-215.

Foster PJ, Broadway DC, Garway-Heath DF, Yip JL, Luben R, Hayat S, Dalzell N, Wareham NJ, Khaw KT. Intraocular pressure and corneal biomechanics in an adult British population: the EPIC-Norfolk eye study. *Invest Ophthalmol Vis Sci*. 2011;52:8179-8185.

Förster W, Kasprzak H, von Bally G. Measurement of elastic modulus of the central bovine cornea by means of holographic interferometry. Part II. Results. *Optom Vis Sci*. 1994;71:27-32.

Franco S, Lira M. Biomechanical properties of the cornea measured by the Ocular Response Analyzer and their association with intraocular pressure and the central corneal curvature. *Clin Exp Optom*. 2009;92:469-475.

Friberg TR, Lace JW. A comparison of the elastic properties of human choroid and sclera. *Exp Eye Res*. 1988;47:429-436.

Friedenwald JS. Contribution to the theory and practice of tonometry. *Am J Ophthalmol*. 1937;20:985-1024.

Friedenwald JS. Clinical significance of ocular rigidity in relation to the tonometric measurement. *Trans Am Acad Ophthalmol Otolaryngol*. 1949;53:262-264.

Friedenwald JS. Tonometer calibration; an attempt to remove discrepancies found in the 1954 calibration scale for Schiotz tonometers. *Trans Am Acad Ophthalmol Otolaryngol.* 1957;61:108-222.

Galletti JG, Pförtner T, Bonthoux FF. Improved keratoconus detection by ocular response analyzer testing after consideration of corneal thickness as a confounding factor. *J Refract Surg*. 2012;28:202-208.

Gefen A, Shalom R, Elad D, Mandel Y. Biomechanical analysis of the keratoconic cornea. *J Mech Behav Biomed Mater*. 2009;2:224-236.

Gkika M, Labiris G, Giarmoukakis A, Koutsogianni A, Kozobolis V. Evaluation of corneal hysteresis and corneal resistance factor after corneal cross-linking for keratoconus. *Graefes Arch Clin Exp Ophthalmol*. 2012;250:565-573.

Glass DH, Roberts CJ, Litsky AS, Weber PA. A viscoelastic biomechanical model of the cornea describing the effect of viscosity and elasticity on hysteresis. *Invest Ophthalmol Vis Sci*. 2008;49:3919-3926.

Goebels SC, Seitz B, Langenbucher A. Precision of ocular response analyzer. *Curr Eye Res*. 2012;37:689-693.

Goldich Y, Barkana Y, Gerber Y, Rasko A, Morad Y, Hartstein M, Avni I, Zadok D. Effect of diabetes mellitus on biomechanical parameters of the cornea. *J Cataract Refract Surg*. 2009a;35:715-719.

Goldich Y, Barkana Y, Morad Y, Hartstein M, Avni I, Zadok D. Can we measure corneal biomechanical changes after collagen cross-linking in eyes with keratoconus?--a pilot study. *Cornea*. 2009b;28:498-502.

Goldich Y, Barkana Y, Pras E, Fish A, Mandel Y, Hirsh A, Tsur N, Morad Y, Avni I, Zadok D. Variations in corneal biomechanical parameters and central corneal thickness during the menstrual cycle. *J Cataract Refract Surg*. 2011;37:1507-1511.

Goldich Y, Marcovich AL, Barkana Y, Mandel Y, Hirsh A, Morad Y, Avni I, Zadok D. Clinical and corneal biomechanical changes after collagen cross-linking with riboflavin and UV irradiation in patients with progressive keratoconus: results after 2 years of follow up. *Cornea*. 2012;31:609-614.

González-Méijome JM, Villa-Collar C, Queirós A, Jorge J, Parafita MA. Pilot study on the influence of corneal biomechanical properties over the short term in response to corneal refractive therapy for myopia. *Cornea*. 2008;27:421-426.

Goss DA, Caffey TW. Clinical findings before the onset of myopia in youth: 5. Intraocular pressure. *Optom Vis Sci*. 1999;76:286-291.

Grabner G, Eilmsteiner R, Steindl C, Ruckhofer J, Mattioli R, Husinsky W. Dynamic corneal imaging. *J Cataract Refract Surg*. 2005;31:163-174.

Hager A, Schroeder B, Sadeghi M, Grossherr M, Wiegand W. The influence of corneal hysteresis and corneal resistance factor on the measurement of intraocular pressure. *Ophthalmologe*. 2007;104:484-489.

Hager A, Wegscheider K, Wiegand W. Changes of extracellular matrix of the cornea in diabetes mellitus. *Graefes Arch Clin Exp Ophthalmol*. 2009;247:1369-1374.

Hagishima M, Kamiya K, Fujimura F, Morita T, Shoji N, Shimizu K. Effect of corneal astigmatism on intraocular pressure measurement using ocular response analyzer and Goldmann applanation tonometer. *Graefes Arch Clin Exp Ophthalmol*. 2010;248:257-262.

Hamilton DR, Johnson RD, Lee N, Bourla N. Differences in the corneal biomechanical effects of surface ablation compared with laser in situ keratomileusis using a microkeratome or femtosecond laser. *J Cataract Refract Surg*. 2008;34:2049-2056.

Han Z, Tao C, Zhou D, Sun Y, Zhou C, Ren Q, Roberts CJ. Air puff induced corneal vibrations: theoretical simulations and clinical observations. *J Refract Surg*. 2014;30:208-213.

Haseltine SJ, Pae J, Ehrlich JR, Shammas M, Radcliffe NM. Variation in corneal hysteresis and central corneal thickness among black, Hispanic and white subjects. *Acta Ophthalmol*. 2012;90:e626-e631.

Hashemi H, Seyedian MA, Miraftab M, Fotouhi A, Asgari S. Corneal collagen cross-linking with riboflavin and ultraviolet a irradiation for keratoconus: long-term results. *Ophthalmology*. 2013; 120:1515-1520.

He X, Liu J. A quantitative ultrasonic spectroscopy method for noninvasive determination of corneal biomechanical properties. *Invest Ophthalmol Vis Sci.* 2009;50:5148-5154.

Hendrickson T. *Massage for Orthopedic Conditions*. Philadelphia, PA: Lippincott Williams&Wilkins; 2009:9.

Hjortdal JØ. Extensibility of the normo-hydrated human cornea. *Acta Ophthalmol Scand*. 1995;73:12-17.

Hjortdal JØ, Jensen PK. In vitro measurement of corneal strain, thickness, and curvature using digital image processing. *Acta Ophthalmol Scand*. 1995;73:5-11.

Hjortdal JØ. Regional elastic performance of the human cornea. *J Biomech*. 1996;29:931-942.

Hoeltzel DA, Altman P, Buzard K, Choe K. Strip extensiometry for comparison of the mechanical response of bovine, rabbit, and human corneas. *J Biomech Eng*. 1992;114:202-215.

Hon Y, Cheung SW, Cho P, Lam AK. Repeatability of corneal biomechanical measurements in children wearing spectacles and orthokeratology lenses. *Ophthalmic Physio Opt*. 2012;32:349-354.

Hong J, Xu J, Wei A, Deng SX, Cui X, Yu X, Sun X. A new tonometer - the Corvis ST tonometer: clinical comparison with noncontact and Goldmann applanation tonometers. *Invest Ophthalmol Vis Sci*. 2013;54:659-665.

Huang C, Zhang M, Huang Y, Chen B, Lam DS, Congdon N. Corneal hysteresis is correlated with reduction in axial length after trabeculectomy. *Curr Eye Res*. 2012;37:381-387.

Huang Y, Huang C, Li L, Qiu K, Gong W, Wang Z, Wu X, Du Y, Chen B, Lam DS, Zhang M, Congdon N. Corneal biomechanics, refractive error, and axial length in Chinese primary school children. *Invest Ophthalmol Vis Sci*. 2011;52:4923-4928.

Huang Y, Lin S, Ma D, Wang Z, Du Y, Lu X, Zhang M. Corneal biomechanical properties and associated factors in school-age children. *Eye Sci*. 2013;28:34-39.

Hurmeric V, Sahin A, Ozge G, Bayer A. The relationship between corneal biomechanical properties and confocal microscopy findings in normal and keratoconic eyes. *Corneal*. 2010;29:641-649.

Hutchings N, Simpson TL, Hyun C, Moayed AA, Hariri S, Sorbara L, Bizheva K. Swelling of the human cornea revealed by high-speed, ultrahigh-resolution optical coherence tomography. *Invest Ophthalmol Vis Sci.* 2010;51:4579-4584.

Jensen H. Myopia progression in young school children and intraocular pressure. *Doc Ophthalmol*. 1992;82:249-255.

Jiang Z, Shen M, Mao G, Chen D, Wang J, Qu J, Lu F. Association between corneal biomechanical properties and myopia in Chinese subjects. *Eye (Lond).* 2011;25:1083-1089.

Kamiya K, Hagishima M, Fujimura F, Shimizu K. Factors affecting corneal hysteresis in normal eyes. *Graefes Arch Clin Exp Ophthalmol*. 2008;246:1491-1494.

Kamiya K, Shimizu K, Ohmoto F. Comparison of the changes in corneal biomechanical properties after photorefractive keratectomy and laser in situ keratomileusis. *Cornea*. 2009a;28:765-769.

Kamiya K, Shimizu K, Ohmoto F. Time course of corneal biomechanical parameters after laser in situ keratomileusis. *Ophthalmic Res.* 2009b;42:167-171.

Kara N, Bozkurt E, Baz O, Altinkaynak H, Dundar H, Yuksel K, Yazici AT, Demirok A, Candan S. Corneal biomechanical properties and intraocular pressure measurement in Marfan patients. *J Cataract Refract Surg*. 2012;38:309-314.

Kara N, Yildirim Y, Univar T, Kontbay T. Corneal biomechanical properties in children with diabetes mellitus. *Eur J Ophthalmol*. 2013;23:27-32.

Karimi AH, Wong A, Bizheva K. Automated detection and cell density assessment of keratocytes in the human corneal stroma from ultrahigh resolution optical coherence tomograms. *Biomed Opt Express.* 2011;2:2905-2916.

Kida T, Liu JH, Weinreb RN. Effect of 24-hour corneal biomechanical changes on intraocular pressure measurement. *Invest Ophthalmol Vis Sci*. 2006;47:4422-4426.

Kirwan C, O'Keefe M, Lanigan B. Corneal hysteresis and intraocular pressure measurement in children using the reichert ocular response analyzer. *Am J Ophthalmol*. 2006;142:990-992.

Knox Cartwright NE, Tyrer JR, Marshall J. In vitro quantification of the stiffening effect of corneal cross-linking in the human cornea using radial shearing speckle pattern interferometry. *J Refract Surg*. 2012;28:503-508.

Kobayashi AS, Staberg LG, Schlegel WA. Viscoelastic properties of human cornea. *Experimental Mechanics*. 1973;13:497-503.

Kotecha A. What biomechanical properties of the cornea are relevant for the clinician? *Surv Ophthalmol*. 2007;52:S109-S114.

Kotecha A, Oddone F, Sinapis C, Elsheikh A, Sinapis D, Sinapis A, Garway-Heath DF. Corneal biomechanical characteristics in patients with diabetes mellitus. *J Cataract Refract Surg*. 2010;36:1822-1828.

Kozobolis V, Sideroudi H, Giarmoukakis A, Gkika M, Labiris G. Corneal biomechanical properties and anterios segment parameters in forme fruste keratoconus. *Eur J Ophthalmol.* 2012;22:920-930.

Laiquzzaman M, Bhojwani R, Cunliffe I, Shah S. Diurnal variation of ocular hysteresis in normal subjects: relevance in clinical context. *Clin Experiment Ophthalmol*. 2006;34:114-118.

Lam A, Chen D, Chiu R, Chui WS. Comparison of IOP measurements between ORA and GAT in normal Chinese. *Optom Vis Sci*. 2007;84:909-914.

Lam AK, Chan R, Pang PC. The repeatability and accuracy of axial length and anterior chamber depth measurements from the IOLMaster. *Ophthalmic Physiol Opt*. 2001;21:477-483.

Lam AK, Chen D. Effect of ocular massage on intraocular pressure and corneal biomechanics. *Eye (Lond)*. 2007;21:1245-1246.

Lam AK, Chen D, Tse J. The usefulness of waveform score from the ocular response analyzer. *Optom Vis Sci*. 2010;87:195-199.

Lam AK, Wong YZ , Cheng SY. Corneal volume measures for monitoring contact lens induced corneal swelling: a pilot study. *Clin Exp Optom.* 2010;94:93-97.

Lam AKC, Chung E, Kho J, Wong S. Digital measurement of torsional eye movement due to postural change and its effect on reading performance. *Curr Eye Res.* 2000;21:763-766.

Lam CS, Goldschmidt E, Edwards MH. Prevalence of myopia in local and international schools in Hong Kong. *Optom Vis Sci*. 2004;81:317-322.

Lam CS, Lam CH, Cheng SC, Chan LY. Prevalence of myopia among Hong Kong Chinese schoolchildren: changes over two decades. *Ophthalmic Physiol Opt.* 2012;32:17-24.

Lam CSY, Loran DFC. Designing contact lenses for oriental eyes. *Journal of The British Contact Lens Association*. 1991;14:109-114.

Landoulsi H, Saad A, Haddad NN, Guilbert E, Gatinel D. Repeatability of Ocular Response Analyzer waveform parameters in normal eyes and eyes after refractive surgery. *J Refract Surg*. 2013;29:709-714.

Lau W, Pye DC. Associations between diurnal changes in Goldmann tonometry, corneal geometry, and ocular response analyzer parameters. *Cornea*. 2012;31:639-644.

Lazreg S, Meslié N, Praud D, Delcourt C, Kamoun H, Chahbi M, Leoni-Meslié S, Smadja D, Trattler W, Touboul D, Colin J. Comparison of corneal thickness and biomechanical properties between North African and French patients. *J Cataract Refract Surg*. 2013;39:425-430.

Lee AJ, Saw SM, Gazzard G, Cheng A, Tan DT. Intraocular pressure associations with refractive error and axial length in children. *Br J Ophthalmol*. 2004;88:5-7.

Leske MC, Connell AM, Wu SY, Hyman L, Schachat AP. Distribution of intraocular pressure. The Barbados Eye Study. *Arch Ophthalmol*. 1997;115:1051-1057.

Levin LA, Nilsson Siv FE, Ver Hoeve J, Wu SM, Alm A, Kaufman PL. *Adler's physiology of the eye.* 11th ed. St. Louis: Saunders Elsevier; 2011.p3-p108.

Li HF, Petroll WM, Møller-Pedersen T, Maurer JK, Cavanagh HD, Jester JV. Epithelial and corneal thickness measurements by in vivo confocal microscopy through focusing (CMTF). *Curr Eye Res.* 1997;16:214-221.

Lim L, Gazzard G, Chan YH, Fong A, Kotecha A, Sim EL, Tan D, Tong L, Saw SM. Cornea biomechanical characteristics and their correlates with refractive error in Singaporean children. *Invest Ophthalmol Vis Sci*. 2008;49:3852-3857.

Lim L, Cheung N, Gazzard G, Chan YH, Wong TY, Saw SM. Corneal biomechanical properties and retinal vascular caliber in children. *Invest Ophthalmol Vis Sci*. 2009a;50:121-125.

Lim MC, Gazzard G, Sim EL, Tong L, Saw SM. Direct costs of myopia in Singapore. *Eye (Lond)*. 2009b;23:1086-1089.

Liu J, Roberts CJ. Influence of corneal biomechanical properties on intraocular pressure measurement: quantitative analysis. *J Cataract Refract Surg*. 2005;31:146-155.

Lu F, Xu S, Qu J, Shen M, Wang X, Fang H, Wang J. Central corneal thickness and corneal hysteresis during corneal swelling induced by contact lens wear with eye closure. *Am J Ophthalmol*. 2007;143:616-622.

Luce DA. Determining in vivo biomechanical properties of the cornea with an ocular response analyzer. *J Cataract Refract Surg*. 2005;31:156-162.

Ma Y, He X, Zou H, Lu L, Qu X, Zhu J. Myopia screening: combining visual acuity and noncycloplegic autorefraction. *Optom Vis Sci*. 2013;90:1479-1485.

Mandalos A, Anastasopoulos E, Makris L, Dervenis N, Kilintznis, Topouzis F. Inter-examiner reproducibility of Ocular Response Analyzer using the waveform score quality index in healthy subjects. *J Glaucoma*. 2013;22:152-155.

Mark HH, Mark TL. Corneal astigmatism in applanation tonometry. *Eye*. 2003;17:617-618.

McBrien NA, Moqhaddam HO, Reeder AP, Moules S. Structural and biochemical changes in the sclera of experimentally myopic eyes. *Biochem Soc Trans*. 1991;19:861-865.

McBrien NA, Gentle A. Role of sclera in the development and pathological complications of myopia. *Prog Retin Eye Res.* 2003;22:307-338.

McBrien NA, Jobling AI, Gentle A. Biomechanics of the sclera in myopia: extracellular and cellular factors. *Optom Vis Sci*. 2009;86:E23-E30.

McLaren JW, Nau CB, Patel SV, Bourne WM. Measuring corneal thickness with the ConfoScan 4 and z-ring adapter. *Eye Contact Lens*. 2007;33:185-190.

McLaren JW, Bourne WM, Patel SV. Automated assessment of keratocyte density in stromal images from the ConfoScan 4 confocal microscope. *Invest Ophthalmol Vis Sci.* 2010;51:1918-1926.

McMonnies CW. An examination of the baropathic nature of axial myopia. *Clin Exp Optom*. 2014;97:116-124.

Medeiros FA, Weinreb RN. Evaluation of the influence of corneal biomechanical properties on intraocular pressure measurements using the ocular response analyzer. *J Glaucoma.* 2006;15:364-370.

Meek KM, Tuft SJ, Huang Y, Gill PS, Hayes S, Newton RH, Bron AJ. Changes in collagen orientation and distribution in keratoconus corneas. *Invest Ophthalmol Vis Sci*. 2005;46:1948-1956.

Mikielewicz M, Kotliar K, Barraquer RI, Michael R. Air-pulse corneal applanation signal curve parameters for the characterization of keratoconus. *Br J Ophthalmol*. 2011;95:793-798.

Millodot M. Dictionary of optometry and visual science. 7th ed. UK: Butterworth-Heinemann; 2009.

Monero-Montañés J, Maldonado MJ, García N, Mendiluce L, García-Gómez PJ, Seguí-Gómez M. Reproducibility and clinical relevance of the ocular response analyzer in nonoperated eyes: corneal biomechanical and tonometric implications. *Invest Ophthalmol Vis Sci*. 2008;49:968-974.

Monnier VM, Sell DR, Abdul-Karim FW, Emancipator SN. Collagen browning and cross-linking are increased in chronic experimental hyperglycemia. Relevance to diabetes and aging. *Diabetes*. 1988;37:867-872.

Moses RA, Grodzki WJ Jr. The pneumatonography. A laboratory study. *Arch Ophthalmol*. 1979;97:547-552.

Müller LJ, Pels E, Vrensen GF. The specific architecture of the anterior stroma accounts for maintenance of corneal curvature. *Br J Ophthalmol.* 2001;85:437-443.

Müller LJ, Pelsa E, Schurmans LRHM, Vrensen GFJM. A new three-dimensional model of the organization of proteoglycans and collagen fibrils in the human corneal stroma. *Experimental Eye Research*. 2004;78:493-501.

Nalcacioglu-Yuksekkaya P, Sen E, Cetinkaya S, Bas V, Aycan Z, Ozturk F. Corneal biomechanical characteristics in children with diabetes mellitus. *Int Ophthalmol*. 2014 Jan 23 [Epub ahead of print] DOI 10.1007/s10792-014-9899-7

Narayanaswamy A, Chung RS, Wu RY, Park J, Wong WL, Saw SM, Wong TY, Aung T. Determinants of corneal biomechanical properties in an adult Chinese population. *Ophthalmology*. 2011;118:1253-1259.

Nash IS, Greene PR, Foster CS. Comparison of mechanical properties of keratoconus and normal corneas. *Exp Eye Res*. 1982;35:413-424.

Ni S, Yu J, Bao F, Li J, Elsheikh A, Wang Q. Effect of glucose on the stress-strain behavior of ex-vivo rabbit cornea. *Exp Eye Res*. 2011;92:353-360.

Nickla DL, Wallman J. The multifunctional choroid. *Prog Retin Eye Res*. 2010;29:144-168.

Nidek: Handheld Auto Ref/ Keratometer 30 Manual. 2000. Nidek.

Oncel B, Dinc U, Orge F, Yalvac B. Comparison of IOP measurement by ocular response analyzer, dynamic contour, Goldmann applanation, and noncontact tonometry. *Eur J Ophthalmol*. 2009;19:936-941.

Orssengo GJ, Pye DC. Determination of the true intraocular pressure and modulus of elasticity of the human cornea in vivo. *Bull Math Biol*. 1999;61:551-572.

Ortiz D, Piñero D, Shabayek MH, Arnalich-Montiel F, Alió JL. Corneal biomechanical properties in normal, post-laser in situ keratomileusis, and keratoconic eyes. *J Cataract Refract Surg*. 2007;33:1371-1375.

Pallikaris IG, Kymionis GD, Ginis HS, Kounis GA, Tsilimbaris MK. Ocular rigidity in living human eyes. *Invest Ophthalmol Vis Sci*. 2005;46:409-414.

Pan CW, Ramamurthy D, Saw SM. Worldwide prevalence and risk factors for myopia. *Opthalmic Physiol Opt*. 2012;32:3-16.

Pan CW, Klein BE, Cotch MF, Shrager S, Klein R, Folsom A, Kronmal R, Shea SJ, Burke GL, Saw SM, Wong TY. Racial variations in the prevalence of refractive errors in the United States: the multi-ethnic study of atherosclerosis. *Am J Ophthalmol*. 2013a;155:1129-1138.

Pan CW, Zheng YF, Anuar AR, Chew M, Gazzard G, Aung T, Cheng CY, Wong TY, Saw SM. Prevalence of refractive errors in a multiethnic Asian population: the Singapore epidemiology of eye disease study. *Invest Ophthalmol Vis Sci*. 2013b;54:2590-2598.

Patel S, McLaren J, Hodge D, Bourne WM. Normal human keratocyte density and corneal thickness measurement by using confocal microscopy in vivo. *Invest Ophthalmol Vis Sci.* 2001;42:333-339.

Pepose JS, Feigenbaum SK, Qazi MA, Sanderson JP, Roberts CJ. Changes in corneal biomechanics and intraocular pressure following LASIK using static, dynamic, and noncontact tonometry. *AM J Ophthalmol*. 2007;143:39-47.

Perera SA, Wong TY, Tay WT, Foster PJ, Saw SM, Aung T. Refractive error, axial dimensions, and primary open-angle glaucoma: the Singapore Malay Eye Study. *Arch Ophthalmol.* 2010;128:900-905.

Perkins ES. Ocular volume and ocular rigidity. *Exp Eye Res*. 1981;33:141-145.

Pinsky PM, Datye DV. A microstructurally-based finite element model of the incised human cornea. *J Biomech*. 1991;24:907-922.

Plakitsi A, O'Donnell C, Miranda MA, Charman WN, Radharishnan H. Corneal biomechanical properties measured with the Ocular Response Analyser in a myopic population. *Ophthalmic Physiol Opt*. 2011;31:404-412.

Prata TS, Sousa AK, Garcia Filho CA, Doi LM, Paranhos A Jr. Assessment of corneal biomechanical properties and intraocular pressure in patients with rheumatoid arthritis. *Can J Ophthalmol*. 2009;44:602.

Pruett RC. Progressive myopia and intraocular pressure: what is the linkage? A literature review. *Acta Ophthalmol Suppl*. 1988;185:117-127.

Punjabi OS, Kniestedt C, Stamper RL, Lin SC. Dynamic contour tonometry: principle and use. *Clin Experiment Ophthalmol.* 2006;34:837-840.

Qazi MA, Sanderson JP, Mahmoud AM, Yoon EY, Roberts CJ, Peopose JS. Postoperative changes in intraocular pressure and corneal biomechanical metrics Laser in ditu keratomileusis versus laser-asisted subepithelial keratectomy. *J Cataract Refract Surg*. 2009;35:1774-1788.

Quinn GE, Berlin JA, Young TL, Ziylan S, Stone RA. Association of intraocular pressure and myopia in children. *Ophthalmology*. 1995;102:180-185.

Rada JA, Shelton S, Norton TT. The sclera and myopia. *Exp Eye Res.* 2006;82:185-200.

Radhakrishnan H, Miranda MA, O'Donnell C. Corneal biomechnical properties and their correlates with refractive error. *Clin Exp Optom*. 2012;95:12-18.

Raiskup-Wolf F, Hoyer A, Spoerl E, Pillunat LE. Collagen crosslinking with riboflavin and ultraviolet-A light in keratoconus: long-term results. *J Cataract Refract Surg*. 2008;34:796-801.

Rask G, Behndig A. Effects of corneal thickness, curvature, astigmatism and direction of gaze on Goldmann applanation tonometry readings. *Ophthalmic Res*. 2006;38:49-55.

Reinstein DZ, Gobbe M, Archer TJ. Ocular biomechanics: measurement parameters and terminology. *J Refract Surg*. 2011;27:396-397.

Renier C, Zeyen T, Fieuws S, Vandenbroeck S, Stalmans I. Comparison of ocular response analyzer, dynamic contour tonometer and Goldmann applanation tonometer. *Int Ophthalmol*. 2010;30:651-659.

Roberts CJ, Reinstein DZ, Archer TJ, Mahmoud AM, Gobbe M, Lee L. Comparison of ocular biomechanical response parameters in myopic and hyperopic eyes using dynamic bidirectional applanation analysis. *J Cataract Refract Surg*. 2014;40:929-936.

Rochman H. Hemoglobin A1c and diabetes mellitus. *Ann Clin Lab Sci*. 1980;10:111-115.

Rosa N, Lanza M, De Bernardo M, Signoriello G, Chiodini P. Relationship between corneal hysteresis and corneal resistance factor with other ocular parameters. *Semin Ophthalmol*. 2014 Feb 7. [Epub ahead of print] DOI: 10.3109/08820538.2013.874479

Ryan DS, Coe CD, Howard RS, Edwards JD, Bower KS. Corneal biomechanics following epi-LASIK. *J Refract Surg.* 2011;27:458-464.

Saad A, Lteif Y, Azan E, Gatinel D. Biomechanical properties of keratoconus suspect eyes. *Invest Ophthalmol Vis Sci*. 2010;51:2912-2916.

Sady C, Khosrof S, Nagaraj R. Advanced Maillard reaction and crosslinking of corneal collagen in diabetes. *Biochem Biophys Res Commun*. 1995;214:793-797.

Sahin A, ayer A, Ozge G, Mumcuoğlu T. Corneal biomechanical changes in diabetes mellitus and their influence on intraocular pressure measurements. *Invest Ophthalmol Vis Sci*. 2009;50:4597-4604.

Saw SM, Chua WH, Gazzard G, Koh D, Tan DT, Stone RA. Eye growth changes in myopic children in Singapore. *Br J Ophthalmol*. 2005a;89:1489-1494.

Saw SM, Gazzard G, Shih-Yen EC, Chua WH. Myopia and associated pathological complications. *Ophthalmic Physiol Opt*. 2005b;25:381-391.

Scarcelli G, Pineda R, Yun SH. Brillouin optical microscopy for corneal biomechanics. *Invest Ophthalmol Vis Sci*. 2012;53:185-190.

Scarcelli G, Yun SH. In vivo Brillouin optical microscopy of the human eye. *Opt Express*. 2012;20:9197-9202.

Scheler A, Spoerl E, Boehm A. Effect of diabetes mellitus on corneal biomechanics and measurement of intraocular pressure. *Acta Ophthalmol*. 2012;90:e447-e451.

Schmid KL, Li RW, Edwards MH, Lew JK. The expandability of the eye in childhood myopia. *Curr Eye Res*. 2003;26:65-71.

Schweitzer C, Roberts CJ, Mahmoud AM, Colin J, Maurice-Tison S, Kerautret J. Screening of forme fruste keratoconus with the ocular response analyzer. *Invest Ophthalmol Vis Sci*. 2010;51:2403-2410.

Scott R, Grosvenor T. Structural model for emmetropic and myopic eyes. *Ophthalmic Physiol Opt*. 1993;13:41-47.

Sedaghat M, Naderi M, Zarei-Ghanavati M. Biomechanical parameters of the cornea after collagen crosslinking measured by waveform analysis. *J Cataract Refrac Surg*. 2010;36:1728-1731.

Sedaghat MR, Sharepoor M, Hassanzadeh S, Abrishmani M. The corneal volume and biomechanical corenal factors: Is there any correlation? *J Res Med Sci*. 2012;17:32-39.

Seiler T, Matallana M, Sendler S, Bende T. Does Bowman's layer determine the biomechanical properties of the cornea? *Refract Corneal Surg*. 1992;8:139-142.

Sen E, Onaran Y, Nalcacioglu-Yuksekkaya P, Elgin U, Ozturk F. Corneal biomechanical parameters during pregnancy. *Eur J Ophthalmol*. 2014;24:314-319.

Sergienko NM, Shargorogska I. The sclera rigidity of eyes with different refractions. *Graefes Arch Clin Exp Ophthalmol*. 2012;250:1009-1012.

Shah S, Laiquzzaman M, Cunliffe I, Mantry S. The use of the Reichert ocular response analyser to establish the relationship between ocular hysteresis, corneal resistance factor and central corneal thickness in normal eyes. *Cont Lens Anterior Eye*. 2006;29:257-262.

Shah S, Laiquzzaman M, Bhojwani R, Mantry S, Cunliffe I. Assessment of the biomechanical properties of the cornea with the ocular response analyzer in normal and keratoconic eyes. *Invest Ophthalmol Vis Sci*. 2007;48:3026-3031.

Shah S, Laiquzzaman M. Comparison of corneal biomechanics in pre and post-refractive surgery and keratoconic eyes by Ocular Response Analyser. *Cont Lens Anterior Eye*. 2009;32:129-132.

Shah S, Laiquzzaman M, Yeung I, Pan X, Roberts C. The use of the Ocular Response Analyser to determine corneal hysteresis in eyes before and after excimer laser refractive surgery. *Cont Lens Anterior Eye*. 2009;32:123-128.

Shah A, Low S, Garway-Heath DF, Foster PJ, Barton K. Iris concavity, corneal biomechanics and their correlations to ocular biometry in a cohort of 10-12 year old UK school boys: baseline data. *Invest Ophthalmol Vis Sci*. 2014;55:3303-3310.

Shah A, Lascaratos G, Garway-Heath DF, et al. Longitudinal study of it is concavity, corneal biomechanics, and correlations to ocular biometry in a cohort of 10- to 12-year-old UK schoolboys: 2-year follow-up data. *Invest Ophthalmol Vis Sci*. 2014;55:4645-4650.

Shen M, Fan F, Xue A, Wang J, Zhou X, Lu F. Biomechanical properties of the cornea in high myopia. *Vision Res*. 2008a;48:2167-2171.

Shen M, Wang J, Qu J, Xu S, Wang X, Fang H, Lu F. Diurnal variation of ocular hysteresis, corneal thickness, and intraocular pressure. *Optom Vis Sci*. 2008b;85:1185-1192.

Shen Y, Chen Z, Knorz MC, Li M, Zhao J, Zhou X. Comparison of corneal deformation parameters after SMILE, LASEK, and femtosecond laser-assisted LASIK. *J Refract Surg*. 2014;30:310-318.

Silver DM, Geyer O. Pressure-volume relation for the living human eye. *Curr Eye Res*. 2000;20:115-120.

Song Y, Congdon N, Li L, Zhou Z, Choi K, Lam DS, Pang CP, Xie Z, Liu X, Sharma A, Chen W, Zhang M. Corneal hysteresis and axial length among Chinese secondary school children: the Xichang Pediatric Refractive Error Study (X-PRES) report no.4. *Am J Ophthalmol*. 2008;145:819-826.

Søndergaard AP, Ivarsen A, Hjortdal J. Corneal resistance to shear force after UVA-riboflavin cross-linking. *Invest Ophthalmol Vis Sci*. 2013;54:5059-5069.

Spoerl E, Terai N, Scholz F, Raiskup F, Pillunat LE. Detection of biomechanical changes after corneal cross-linking using Ocular Response Analyzer software. *J Refract Surg*. 2011;27:452-457.

Spörl E, Terai N, Haustein M, Böhm AG, Raiskup-Wolf F, Pillunat LE. Biomechanical condition of the cornea as a new indicator for pathological and structural changes. *Ophthalmologe*. 2009;106:512-520.

Sun L, Shen M, Wang J, Fang A, Xu A, Fang H, Lu F. Recovery of corneal hysteresis after reduction of intraocular pressure in chronic primary angle-closure glaucoma. *Am J Ophthalmol*. 2009;147:1061-1066.

Suzuki H, Takahashi H, Hori J, Hiraoka M, Igarashi T, Shiwa T. Phacoemaulsification associated corneal damage evaluated by corneal volume. *Am J Ophthalmol.* 2006;142:525-528.

Tao C, Han Z, Sun Y, Zhou C, Roberts C, Zhou D, Ren Q. Corneal hysteresis with intraocular pressure of a wide range: a test on porcine eyes. *J Refract Surg*. 2013;29:850-854.

Thibos LN, Wheeler W, Horner D. Power vectors: an application of Fourier analysis to the description and statistical analysis of refractive error. *Optom Vis Sci*. 1997;74:367-375.

Tian L, Huang YF, Wang LQ, Bai H, Wang Q, Jiang JJ, Wu Y, Gao M. Corneal biomechanical assessment using corneal visualization scheimpflug technology in keratoconic and normal eyes. *J Ophthalmol*. 2014;2014:147516.

Tomlinson A, Phillips CI. Applanation tension and axial length of the eyeball. *Br J Ophthalmol*. 1970;54:548-553.

Tong L, Saw SM, Chan ES, Yap M, Lee HY, Kwang YP, Tan D. Screening for myopia and refractive errors using LogMAR visual acuity by optometrists and a simplified visual acuity chart by nurses. *Optom Vis Sci*. 2004;81:684-691.

Touboul D, Roberts C, Kérautret J, Garra C, Maurice-Tison S, Saubusse E, Colin J. Correlations between corneal hysteresis, intraocular pressure, and corneal central pachymetry. *J Cataract Refract Surg*. 2008;34:616-622.

Villas-Bôas FS, Doi LM, Sousa AK, Melo LA Jr. Correlation between diurnal variation of intraocular pressure, ocular pulse amplitude and corneal structural properties. *Arq Bras Oftalmol*. 2009;72:296-301.

Vitale S, Ellwein L, Cotch MF, Ferris FL 3rd, Sperduto R. Prevalence of refractive error in the United States, 1999-2004. *Arch Ophthalmol*. 2008;126:1111-1119.

Vitale S, Sperduto RD, Ferris FL 3rd. Increased prevalence of myopia in the United States between 1971-1972 and 1999-2004. *Arch Ophthalmol*. 2009;127:1632-1639.

Vu DM, Silva FQ, Haseltine SJ, Ehrlich JR, Radcliffe NM. Relationship between corneal hysteresis and optic nerve parameters measured with spectral domain optical coherence tomography. *Graefes Arch Clin Exp Ophthalmol*. 2013; 251:1777-1783.

Wang H, Prendiville PL, McDonnell PJ, Chang WV. An ultrasonic technique for the measurement of the elastic moduli of human cornea. *J Biomech*. 1996;29:1633-1636.

Wells AP, Garway-Heath DF, Poostchi A, Wong T, Chan KC, Sachdev N. Corneal hysteresis but not corneal thickness correlates with optic nerve surface compliance in glaucoma patients. *Invest Ophthalmol Vis Sci*. 2008;49:3262-3268.

Wollensak G, Spoerl E, Seiler T. Stress-strain measurements of human and porcine corneas after riboflavin-ultraviolet-A-induced cross-linking. *J Cataract Refract Surg*. 2003;29:1780-1785.

Wong E, Yap MKH. Factors affecting ocular rigidity in the Chinese. *Clin Exp Optom*. 1991;74:156-159.

Wong EP, Fricke TR, Dinardo C. Interexaminer repeatability of a new, modified prentice card compared with established phoria tests. *Optom Vis Sci*. 2002;79:370-375.

Wong YZ, Lam AK. Influence of corneal astigmatism, corneal curvature and meridional differences on corneal hysteresis and corneal resistance factor. *Clin Exp Optom.* 2011;94:418-424.

Woo SL, Kobayashi AS, Lawrence C, Schlegel WA. Mathematical model of the corneo-scleral shell as applied to intraocular pressure-volume relations and applanation tonometry. *Ann Biomed Eng*. 1972;1:87-98.

Wu D, Wang Y, Zhang L, Wei S, Tang X. Corneal biomechanical effects: Small-incision lenticule extraction versus femtosecond laser-assisted laser in situ keratomileusis. *J Cataract Refract Surg*. 2014;40:954-962.

Xiang F, He M, Morgan IG. Annual changes in refractive errors and ocular components before and after the onset of myopia in Chinese children. *Ophthalmology*. 2012;119:1478-1484.

Xu S, Xu A, Tao A, Wang J, Fan F, Lu F. Corneal biomechanical properties and intraocular pressure in high myopic anisometropia. *Eye Contact Lens*. 2010;36:204-209.

Yazici AT, Kara N, Yüksel K, Altinkaynak H, Baz O, Bozkurt E, Demirok A. The biomechanical properties of the cornea in patients with systemic lupus erythematosus. *Eye (Lond)*. 2011;25:1005-1009.

Yeh TN, Green HM, Zhou Y, Pitts J, Kitamata-Wong B, Lee S, Wang SL, Lin MC. Short-term effects of overnight orthokeratology on corneal epithelial permeability and biomechanical properties. *Invest Ophthalmol Vis Sci*. 2013;54:3902-3911.

Ytteborg J. The effect of intraocular pressure on rigidity coefficient in the human eye. *Acta Ophthalmol*. 1960a;38:54-561.

Ytteborg J. Further investigations of factors influencing size of rigidity coefficient. *Acta Ophthalmol*. 1960b;38:643-657.

Zadnik K. The Glenn A. Fry Award Lecture (1995). Myopia development in childhood. *Optom Vis Sci*. 1997;74:603-608.

Zadnik K, Mutti DO, Mitchell GL, Jones LA, Burr D, Moeschberger ML. Normal eye growth in emmetropic schoolchildren. *Optom Vis Sci*. 2004;81:819-828.

Zarei-Ghanavati S, Ramirez-Miranda A, Yu F, Hamilton DR. Corneal deformation signal waveform analysis in keratoconic versus post-femtosecond laser in situ keratomileusis eyes after statistical correction for potentially confounding factors. *J Cataract Refract Surg*. 2012;38:607-614.

Zeng Y, Yang J, Huang K, Lee Z, Lee X. A comparison of biomechanical properties between human and porcine cornea. *J Biomech*. 2001;34:533-537.