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OPTICAL AND STRUCTURAL OCULAR CHANGES DURING INCIPIENT PRESBYOPIA

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Doctor of Philosophy

ASTON UNIVERSITY

December 2014

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Summary

The primary aim of this thesis was to investigate the *in vivo* ocular morphological and contractile changes occurring within the accommodative apparatus prior to the onset of presbyopia, with particular reference to ciliary muscle changes with age and the origin of a myopic shift in refraction during incipient presbyopia.

Commissioned semi-automated software proved capable of extracting accurate and repeatable measurements from crystalline lens and ciliary muscle Anterior Segment Optical Coherence Tomography (AS-OCT) images and reduced the subjectivity of AS-OCT image analysis.

AS-OCT was utilised to document longitudinal changes in ciliary muscle morphology within an incipient presbyopic population (n=51). A significant antero-inwards shift of ciliary muscle mass was observed after 2.5 years. Furthermore, in a subgroup study (n=20), an accommodative antero-inwards movement of ciliary muscle mass was evident. After 2.5 years, the centripetal response of the ciliary muscle significantly attenuated during accommodation, whereas the antero-posterior mobility of the ciliary muscle remained invariant.

Additionally, longitudinal measurement of ocular biometry revealed a significant increase in crystalline lens thickness and a corresponding decrease in anterior chamber depth after 2.5 years (n=51). Lenticular changes appear to be determinant of changes in refraction during incipient presbyopia. During accommodation, a significant increase in crystalline lens thickness and axial length was observed, whereas anterior chamber depth decreased (n=20). The change in ocular biometry per dioptre of accommodation exerted remained invariant after 2.5 years.

Cross-sectional ocular biometric data were collected to quantify accommodative axial length changes from early adulthood to advanced presbyopia (n=72). Accommodative axial length elongation significantly attenuated during presbyopia, which was consistent with a significant increase in ocular rigidity during presbyopia.

The studies presented in this thesis support the Helmholtz theory of accommodation and despite the reduction in centripetal ciliary muscle contractile response with age, primarily implicate lenticular changes in the development of presbyopia.

Key words: accommodation, ciliary muscle, crystalline lens, myopia, presbyopia

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CHAPTER 1

Optical and structural ocular changes during accommodation, presbyopia and myopia development

1.1 Introduction

Current presbyopia theories are derived from our understanding of the mechanism of accommodation in young eyes. However, after at least a century of investigation, the mechanism of accommodation, particularly throughout the incipient phase of presbyopia, remains equivocal.

This chapter reviews the most recent research pertaining to the optical and structural ocular changes that occur with accommodation, age and the development of myopia. The structure of the eye is described and current theories of accommodation and presbyopia are discussed. The literature review highlights the paucity of research over the incipient presbyopic years, and particularly the need for longitudinal data over this time period.

1.2 Accommodation

Accommodation is defined as the dynamic change in refractive power of the eye to focus on objects at different distances (Atchison, 1995; Millodot, 2008). Fig. 1.1 shows the structure of the eye, including the accommodative apparatus.

1.2.1 Anatomy of the accommodative apparatus

1.2.1a Crystalline Lens

The crystalline lens is a transparent bi-convex fibrous tissue, which is responsible for approximately one third of the total refractive power of the eye when viewing a distant object (Manns *et al.*, 2004).

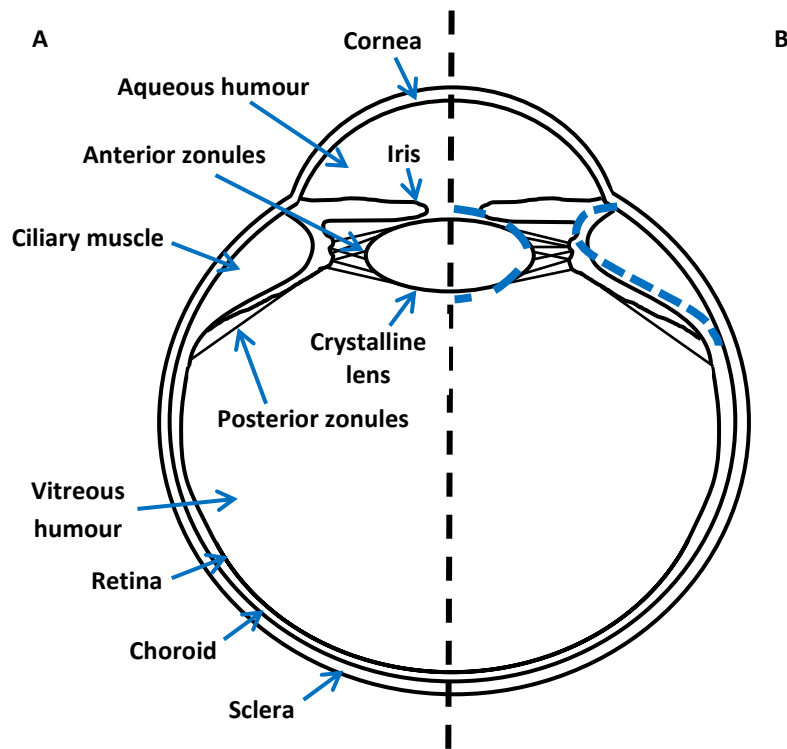


Fig. 1.1. Schematic transverse cross-section of the human accommodative apparatus in a disaccommodated (A) and accommodated state (B) according to the Helmholtz theory. In the disaccommodated eye (A), the ciliary muscle is relaxed, the anterior zonules are stretched and the crystalline lens is flat. During accommodation (B), the ciliary muscle contracts (moving anteriorly and centripetally, as shown by the dashed blue lines), reducing anterior zonular tension and allowing the crystalline lens to take up a more powerful shape (thicker and more spherical as shown by the dashed blue lines).

The crystalline lens develops from an invagination of surface ectoderm, forming the hollow lens vesicle, which is gradually permeated by primary lens fibres extending across the crystalline lens equator (Cohen, 1965; Hoar, 1982). The crystalline lens can be broadly split into two distinct zones: the nucleus and the cortex. The oldest fibres (including fibres present at birth) reside within the nucleus and the overlying fibres form the cortex (Dubbelman *et al.*, 2003). The crystalline lens continues to grow throughout life (Koretz *et al.*, 1989; Smith *et al.*, 1992; Cook *et al.*, 1994; Glasser and Campbell, 1999; Strenk *et al.*, 1999; Dubbelman *et al.*, 2001a; Dubbelman *et al.*, 2001b; Koretz *et al.*, 2001; Dubbelman *et al.*, 2003; Jones *et al.*, 2007; Atchison *et al.*, 2008; Richdale *et al.*, 2008; Doyle *et al.*, 2013; Richdale *et al.*, 2013), corresponding to an increase in thickness of both the nucleus and the cortex. U-shaped lens fibres are formed by mitosis of

anterior epithelial cells at the crystalline lens equator (Kaufman and Alm, 2003). To maintain transparency, the fibre cells lose their organelles, elongate and migrate to cover the crystalline lens underneath the surface epithelium, forming an increasingly ellipsoidal crystalline lens shape. The crystalline lens fibres are uniformly packed into hexagonal patterns (viewed as cross-section) and are bonded to other fibres produced from around the circumference of the crystalline lens equator by Y-sutures during gestation and increasingly more complex junction patterns during infancy, adolescence and adulthood (Kuszak *et al.*, 2004). A uniform distribution of crystallin protein surrounds the fibres, thus promoting lens transparency (Kuszak, 1995). The nucleus has a higher refractive index (1.406) than the surrounding cortex (1.386) due to a higher concentration of proteins (Fagerholm *et al.*, 1981; Atchison *et al.*, 2008).

1.2.1b Capsule

The capsule surrounds the crystalline lens and is responsible for transmitting extra-lenticular forces from the ciliary muscle, via the zonules (Fincham, 1937; Glasser and Campbell, 1998; Koopmans *et al.*, 2003). A key property of the capsule is its elasticity; permitting consecutive moulding and restoration of the crystalline lens shape to occur rapidly and accurately.

The capsule is formed embryonically from the basement membrane of surface ectoderm, which later invaginates to create the crystalline lens (Cohen, 1965). The capsule is the thickest basement membrane in the body and chiefly comprises of long, flexible three-dimensional lattice networks of type IV collagen (Timpl *et al.*, 1981; Krag and Andreassen, 2003). Fisher and Pettet (1972) reported that the capsule is thickest around the crystalline lens equator, with the anterior and posterior capsule representing one-half and one-sixth of the equatorial capsule thickness, respectively. Whereas, Travers (1990) and Fincham (1937) found the capsule was thickest in the mid-periphery, forming a thicker annulus around the centre of the anterior and posterior capsule. Nevertheless, the literature is in agreement that the capsule is thinnest posteriorly (Fincham, 1937; Fisher and Pettet, 1972; Travers, 1990). The anterior capsule surface area grows in

accordance with the age-related increase in crystalline lens volume (Fincham, 1937; Fisher and Pettet, 1972; Travers, 1990).

1.2.1c Zonules

The zonules connect the ciliary muscle to the crystalline lens and capsule, relaxing and contracting based on ciliary muscle tonus (Rohen, 1979).

The zonules are derived embryonically from loose bundles of fibres from the vitreous framework (Hoar, 1982). The zonules are tubular fibrils that form sheets of bundles arranged radially from the ciliary body (Raviola, 1971). The zonular plexus consists of two different fibre types: main and tension. The main fibres are further divided into anterior and posterior fibres. The main anterior zonules are responsible for suspending the crystalline lens and are flexible enough to permit dynamic changes in crystalline lens size and shape. The main anterior zonular insertion sites are within the ciliary processes (non-pigmented ciliary epithelium) and the crystalline lens capsule, close to the crystalline lens epithelium (Raviola, 1971; Rohen, 1979). The insertion sites of the main posterior zonules are the ciliary processes and the *pars plana* (Glasser, 2008). Following the cessation of ciliary muscle contraction, the posterior zonules are thought to help restore the ciliary muscle to its original position (Glasser, 2008). The tension zonular fibres link and anchor the main fibres to the ciliary epithelium and are thought to act as a fulcrum, facilitating dynamic changes in main fibre tension (Rohen, 1979).

1.2.1d Ciliary body

The ciliary body is part of the uveal tract, which forms embryonically from the mesenchyme surrounding the two vesicles that bud off the forebrain (Nickla and Wallman, 2010). The ciliary body connects to the peripheral iris anteriorly and the choroid posteriorly. The ciliary body runs continuously with the sclera from the scleral spur to the *ora serrata* (junction between ciliary body and retina). The anterior section of the ciliary body is the *pars plicata*, which consists of 70 to 80 highly-vascular folds of non-pigmented ciliary epithelium (ciliary processes), which are

responsible for aqueous humour secretion (Cole, 1977; Atchison *et al.*, 1995). The posterior section of the ciliary body is the *pars plana*, which extends from the ciliary processes to the *ora serrata*. The ciliary body comprises six layers: the supraciliary lamina, ciliary muscle, stroma, basal lamina, epithelium and internal limiting membrane (Aiello *et al.*, 1992). The ciliary muscle lies beneath the ciliary processes and constitutes approximately two-thirds of the ciliary body mass (Atchison, 1995; Remington, 2005).

Ciliary muscle

The ciliary muscle is a multi-unit muscle, made up of bundles of smooth muscle cells surrounded by connective tissue cells (Ishikawa, 1962). The smooth muscle bundles form three distinct fibre types: longitudinal, radial and circular, as shown in Fig. 1.2. Longitudinal fibres run parallel to the sclera from the scleral spur to the posterior visible limit of the ciliary muscle. Radial fibres run perpendicular to longitudinal fibres and circular fibres encircle the ciliary muscle aperture and are the closest fibres to the crystalline lens (Ishikawa, 1962; Pardue and Sivak, 2000). The radial fibre cells contain the most mitochondria organelles (Ishikawa, 1962), whereas the tips of the longitudinal fibre cells contain the fewest mitochondria and more myofibrils (Flügel *et al.*, 1990), possibly facilitating faster contraction and providing greater stiffness than the rest of the fibres (Rohen, 1979). The ciliary muscle connective tissue is mainly made up of collagen fibrils and fibroblasts (Ishikawa, 1962). The ciliary muscle is thicker temporally than nasally (Aiello *et al.*, 1992; Glasser *et al.*, 2001; Sheppard and Davies, 2010a).

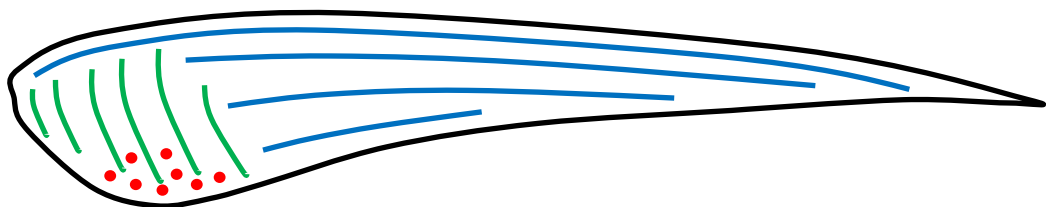


Fig. 1.2. Schematic transverse cross-section of the human ciliary muscle. The blue horizontal lines represent the longitudinal fibres, the green vertical lines represent the radial fibres and the red spots represent the circular fibres. *In vivo*, the muscle fibres are intermingled and the diagram above represents where the fibres are most prevalent.

Ciliary muscle contractile response

Contraction of the ciliary muscle causes a centripetal (inwards, towards the centre of the eye) and anterior (towards the cornea) movement of ciliary muscle mass (Flügel *et al.*, 1990; Tamm *et al.*, 1991; Tamm *et al.*, 1992a; Croft *et al.*, 2006; Sheppard and Davies, 2010a). The longitudinal fibres are responsible for the anterior shift in muscle mass during contraction, whereas the radial and circular fibres are responsible for the inward movement of muscle mass during contraction, with the circular fibres acting as a sphincter (Pardue and Sivak, 2000). It is likely a small proportion of the longitudinal fibres become radially or circularly orientated during accommodation (Pardue and Sivak, 2000). The contractile response is thought to be greater temporally than nasally, possibly in order to align the lenticular axes during convergence (Sheppard and Davies, 2010a).

Ciliary muscle innervation

The accommodative reflex arises from near vision blur imposed on the retina, which is detected at the visual cortex (Atchison, 1995; Charman, 2008). The ciliary muscle is innervated by the autonomic nervous system, which is responsible for involuntary control of smooth muscle, cardiac muscle and certain glands (Cogan, 1937). Contraction and relaxation are typically controlled by two antagonistic divisions of the autonomic nervous system: the parasympathetic and sympathetic nervous systems. The role of the parasympathetic nervous system (supplied from the oculomotor cranial nerve) in ciliary muscle contraction has been well established and accepted. However, the role of the sympathetic nervous system (supplied from the preganglionic cervical nerve) in ciliary muscle relaxation has proved more contentious (Cogan, 1937; Törnqvist, 1966; Toates, 1972; Gilmartin *et al.*, 1984; Gilmartin and Hogan, 1985).

Horner's syndrome (caused by paralysis of the cervical sympathetic nerve) results in an increase in amplitude of accommodation of the affected eye, which has been extensively cited as clinical evidence that the ciliary muscle is also innervated by the sympathetic nervous system (Horner, 1869; Cogan, 1937). Törnqvist (1966) reported a hypermetropic refractive shift (caused by relaxation of the ciliary muscle) in response to artificial stimulation of the preganglionic cervical

sympathetic nerve in monkeys, which persisted with application of adrenergic alpha-receptor antagonists and was eliminated by application of beta-receptor antagonist drugs. Thus, Törnqvist hypothesised that relaxation of the ciliary muscle, and therefore disaccommodation, may be driven by the action of the neurotransmitter noradrenaline on beta-receptors present in the ciliary muscle. Indeed, the action of the sympathetic nervous system in humans is mediated principally by stimulation of beta-2 receptors, which are also present in the human ciliary muscle (Wax and Molinoff, 1987; Zetterström and Hahnenberger, 1988). However, later work by Törnqvist (1967) stimulating the preganglionic cervical nerve in tandem with the oculomotor nerve found the level of sympathetic relaxation was directly proportional to the level of parasympathetic contraction and maximal ciliary muscle contraction occurred after 1 to 2 seconds in response to parasympathetic stimulation. Similarly, accommodation driven by a near vision stimulus was maximal after 1 second, prior to a 370 millisecond initial latency period in human patients (Campbell and Westheimer, 1960; Kasthurirangan and Glasser, 2006). However, sympathetic stimulation alone was not sufficient to provide the rapid disaccommodation required to view the dynamic visual environment, taking up to 40 seconds to maximally relax with simultaneous parasympathetic background activity in monkeys (Törnqvist, 1967). Therefore, Törnqvist re-postulated that the role of the sympathetic nervous system in driving disaccommodation is not likely to be significant. Later animal research by Hurwitz *et al.* (1972) utilising isoproterenol (adrenergic non-selective beta-receptor agonist) and propranolol (adrenergic beta-receptor antagonist) supported this theory.

More recent research has investigated the passive accommodative resting point of the human eye (open-loop accommodation) and the accommodative changes driven by near stimuli (closed-loop accommodation) after instillation of various pharmacological agents to elicit the role of the sympathetic nervous system. Culhane *et al.* (1999) and Winn *et al.* (2002) reported the sympathetic nervous system has a significant effect on closed-loop accommodation at low to mid-temporal frequencies (<0.3 Hz, sinusoidally oscillating between 2.00 and 4.00 D), however the

accommodative response at higher temporal frequencies was unaffected, thus supporting previous research indicating sympathetic inhibition is small (less than 2.00 D) and slow (Törnqvist, 1967; Gilmartin, 1986). Gilmartin and colleagues (1984; 1985) and Mallen *et al.* (2005a) investigated the effects of timolol (adrenergic non-selective beta-receptor antagonist), betaxolol (adrenergic selective beta-1 receptor antagonist), isoprenaline (adrenergic non-selective beta-receptor agonist) and tropicamide (muscarinic non-selective receptor antagonist) and concluded that sympathetic innervation is unlikely to have a significant role in modulating accommodation under open or closed-loop conditions. In addition, only one in three individuals are likely to have access to sympathetic inhibition during sustained near vision (Gilmartin *et al.*, 2002; Mallen *et al.*, 2005a; Vasudevan *et al.*, 2009). Therefore, the parasympathetic nervous system provides the primary innervation to control contraction and relaxation of the ciliary muscle.

In addition to extrinsic innervation by the autonomic nervous system, the ciliary muscle may also be innervated by intrinsic nitric oxide synthase (NOS)-positive nerve cells (*Plexus gangliosis ciliaris*) present within the inner ciliary muscle fibres (Tamm *et al.*, 1995). Nitric oxide (NO) is a major chemical messenger molecule regulating smooth muscle contractility and is thought to contribute to disaccommodation and accommodative fluctuations during sustained near vision (Wiederholt *et al.*, 1994; Tamm *et al.*, 1995). Recent research also indicates that there are sensory receptors (proprioceptors) within the ciliary muscle which may be able to modulate the contraction of the circular muscle portion locally via a self-contained reflex arc (Flügel-Koch *et al.*, 2009). However, innervation of the ciliary muscle is dominated by the parasympathetic nervous system, therefore the intrinsic nerve cells are likely to have a minor impact on overall ciliary muscle contractility, perhaps merely fine-tuning changes in the accommodative response (Flügel-Koch *et al.*, 2009).

1.2.1e Choroid

The choroid forms the posterior part of the uveal tract, connecting anteriorly to the ciliary body and running adjacent to the sclera. The choroid is a vascular tissue, which is innervated by the ciliary arteries and is responsible for supplying nutrients and removing waste from the outer retinal layers, the prelaminar portion of the optic nerve and the fovea (Hayreh, 1969; Lütjendrecoll, 2006; Jablonski *et al.*, 2007). The choroidal vasculature provides approximately 65% of the ocular oxygen supply (Alm and Bill, 1973) and is responsible for approximately 35% of the aqueous humour drainage via the uveoscleral pathway (Nickla and Wallman, 2010). The choroidal vasculature also thermoregulates the posterior eye by dissipating heat (Parver *et al.*, 1991). The choroid is black in colour due to intrinsic melanocytes and is responsible for absorbing stray light penetrating beyond the retina and retinal pigment epithelium and therefore prevents light from reaching the sclera. In addition, the choroid also consists of collagenous and elastic connective tissue (Nickla and Wallman, 2010).

1.2.2 Historical theories of accommodation

Evidence of accommodation was first discovered by Scheiner's double pin-hole experiment in the seventeenth century (cited by Young, 1801; Fincham, 1937; Atchison, 1995). Scheiner observed that an object viewed through two pin-holes appeared single, however once Scheiner directed his attention to an object at a different distance, the original object appeared doubled (physiological diplopia), thus demonstrating the action of a variable focusing mechanism within the eye. Descartes later hypothesised that variable ocular focus was instigated by changes in the curvature of the crystalline lens, with an increase in crystalline lens curvature required to focus objects at near (cited by Fincham, 1937; Atchison, 1995). Other early theories suggested that accommodation was driven by an increase in corneal curvature or an increase in axial length. However, Young (1801) concluded that the magnitude of changes in corneal curvature and axial length were not significant enough to be responsible for the accommodative response, therefore supporting Descartes' hypothesis that changes in the crystalline lens are key. Early Purkinje

imaging research confirmed that accommodation corresponded to an increase in crystalline lens curvature (Cramer, 1851; von Helmholtz, 1855).

Cramer (1851) was one of the first scientists to include the ciliary muscle in his theory of accommodation. Cramer suggested that contraction of the ciliary muscle exerts tension on the choroid, which compresses the vitreous against the posterior surface of the crystalline lens and pushes the crystalline lens forwards. The forward movement is restricted peripherally due to the presence of the iris, which causes a forward bulge of the crystalline lens centrally through the iris aperture. Alternate accommodation theories also suggested the role of the iris is crucial, bringing about a change in focus due to the increase in depth of focus accompanying pupil miosis or by the force of ciliary muscle contraction increasing aqueous pressure between the iris and lens, flattening the peripheral crystalline lens and causing the central crystalline lens region to bulge forwards (Crawford *et al.*, 1990). However, the presence of accommodation in aniridic individuals proves the iris is not vital for accommodation (von Graefe, 1861, cited by Fincham, 1937; Atchison, 1995). Additionally, accommodation has also been observed in eyes following a vitrectomy, suggesting that the force from the choroid on the vitreous is not necessary to move the crystalline lens forwards during accommodation (Fisher, 1983). The choroid may however have a role in restoring the ciliary muscle to its relaxed position following cessation of accommodation (Gullstrand, 1909 cited by von Helmholtz, 1924).

Later accommodation theories also considered the role of the zonules. Helmholtz (1855) theorised that the crystalline lens moves forwards and becomes more curved during accommodation due to relaxation of the zonules during ciliary muscle contraction. However, Tscherning (1909), building on Cramer's theory, hypothesised that equatorial zonular tension increases during accommodation, increasing the crystalline lens diameter and leading to steepening of the central part of the anterior crystalline lens (due to the greater curvature and resistance to stretching of the nucleus), with peripheral crystalline lens flattening. Fincham (1937) observed that the crystalline lens is displaced in the direction of gravity during accommodation,

thus providing evidence that zonule tension actually decreases during accommodation, in support of the theory of Helmholtz (1855).

Helmholtz (1855) also suggested that the change in shape and position of the posterior crystalline lens surface is minor and the crystalline lens equatorial diameter reduces during accommodation. Helmholtz proposed that the increase in anterior crystalline lens surface curvature is facilitated by the peripheral pressure provided by the iris. Following the evidence of accommodation in aniridic patients (Fincham, 1937), Fincham augmented the theory of Helmholtz to remove the contribution of the iris in the accommodative response. Fincham also discussed the role of the capsule in accommodation, believing the capsule imparted a variable compressive force according to its thickness overlying the crystalline lens. Therefore, during accommodation, the equatorial crystalline lens, where the capsule is thick, is compressed, causing a reduction in crystalline lens diameter and the anterior central crystalline lens, where the capsule is relatively thin, steepens by bulging forwards. However, mathematical modelling has suggested the capsule imparts force evenly over the entire crystalline lens surface (Fisher, 1969a; Koretz and Handelman, 1982; 1983). Nevertheless, the literature is in agreement that both the anterior and posterior crystalline lens surfaces increase in curvature during accommodation (Cramer, 1851; von Helmholtz, 1855; Tscherning, 1909; Fincham, 1937; Koretz and Handelman, 1982; Koretz and Handelman, 1983; Schachar, 1992; Schachar *et al.*, 1996).

1.2.3 Current theories of accommodation

Current understanding is based on the theory of accommodation posed by Helmholtz (1855), and supplemented by the work of Fincham (1937) to discount the role of the iris and Gullstrand (1909), who included the role of the choroid in disaccommodation (cited by von Helmholtz, 1924). In summary, to view an object at optical infinity, the ciliary muscle is relaxed and the crystalline lens is flattened by zonular tension (Fig. 1.1A). In order to view near targets, the ciliary muscle contracts, moving anteriorly and centripetally, reducing zonular tension and allowing the capsule to reform the crystalline lens to a thicker and more spherical shape, reducing the crystalline lens

diameter (Fig. 1.1B). Following cessation of accommodation, the elastic recoil of the choroid returns the ciliary muscle to its relaxed position.

An alternative, controversial theory of accommodation, originally proposed by Tscherning (1909), has been renewed by Schachar (1992). Here, equatorial zonular tension is increased during accommodation, causing an increase in crystalline lens diameter and a steepening of the central anterior crystalline lens surface. However, this theory has been widely discredited by the large research base that supports the Helmholtz theory of accommodation (Brown, 1973; Shum *et al.*, 1993; Pierscionek *et al.*, 1995; Drexler *et al.*, 1997; Garner and Yap, 1997; Koretz *et al.*, 1997; Wilson, 1997; Koretz *et al.*, 2002; Dubbelman *et al.*, 2003; Baikoff *et al.*, 2004; Dubbelman *et al.*, 2005; Ostrin *et al.*, 2006; Hermans *et al.*, 2007; Tsorbatzoglou *et al.*, 2007; Kasthurirangan *et al.*, 2011; Sheppard *et al.*, 2011; Richdale *et al.*, 2013).

In agreement with the Helmholtz theory, *in vivo* ocular imaging studies have confirmed the crystalline lens diameter decreases during accommodation (Brown, 1973; Wilson, 1997; Hermans *et al.*, 2007; Kasthurirangan *et al.*, 2011). Furthermore, the capsule moulds the anterior surface of the crystalline lens into a more curved, spherical shape during accommodation and the central steeper zone suggested by Schachar (1992) is not apparent *in vivo* (Baikoff *et al.*, 2004), thus indicating the zonules are in fact relaxed during accommodation. The posterior crystalline lens surface and the anterior and posterior borders of the nucleus also become steeper (radii of curvature decrease) during accommodation (Dubbelman *et al.*, 2005). The change in anterior crystalline lens surface curvature is greater than the posterior surface change (Garner and Yap, 1997; Koretz *et al.*, 2002; Baikoff *et al.*, 2004; Dubbelman *et al.*, 2005), as Helmholtz suggested. The posterior axial movement of the posterior crystalline lens surface during accommodation is thought to be between 3 to 5 times smaller than the anterior movement of the anterior crystalline lens surface (Drexler *et al.*, 1997; Dubbelman *et al.*, 2005), and may vary according to the magnitude of accommodative demand (Beers and van der Heijde, 1996; Gibson, 2008). The poor response of the posterior crystalline lens surface during accommodation may be due to the

resistance provided by the vitreous or restriction by the capsule (Ziebarth *et al.*, 2005; Charman, 2008).

The resultant increase in crystalline lens thickness and corresponding reduction in anterior chamber depth during accommodation is due solely to an increase in axial thickness of the nucleus of the crystalline lens (Brown, 1973; Koretz *et al.*, 1997; Dubbelman *et al.*, 2003; Hermans *et al.*, 2007; Kasthurirangan *et al.*, 2011). The change in central crystalline lens thickness per dioptre of accommodation exerted is thought to be between 0.043 and 0.080 mm (Garner and Yap, 1997; Koretz *et al.*, 1997; Ostrin *et al.*, 2006; Bolz *et al.*, 2007; Richdale *et al.*, 2008; Sheppard *et al.*, 2011; Richdale *et al.*, 2013) and remains constant with age (Koretz *et al.*, 1997).

Mathematical modelling and *in vivo* ocular imaging have reported that the cross-sectional area of the crystalline lens decreases and the overall volume increases with accommodation (Gerometta *et al.*, 2007; Zamudio *et al.*, 2008; Sheppard *et al.*, 2011). Crystalline lens compression and a rapid influx of water (refractive index 1.33) via osmosis across the capsule have been cited as possible sources of the increase in volume during accommodation (Candia *et al.*, 2010; Sheppard *et al.*, 2011). Unfortunately, the exact nature of the change in the average refractive index of the crystalline lens during accommodation is still not fully understood (Gullstrand, 1911; Garner and Smith, 1997; Dubbelman *et al.*, 2005; Jones *et al.*, 2007; Hermans *et al.*, 2008; Kasthurirangan *et al.*, 2008), which may have otherwise helped to indicate the cause of the reported volumetric increase.

As dictated by both the Helmholtz and Schachar theories, the crystalline lens shape is altered by ciliary muscle contraction to produce the accommodative response. The ciliary muscle ring diameter reduces during accommodation due to an anterior and centripetal movement of muscle mass during contraction (Kano *et al.*, 1999; Stachs *et al.*, 2002; Strenk *et al.*, 2006; Sheppard and Davies, 2010a; Kasthurirangan *et al.*, 2011; Sheppard and Davies, 2011; Lewis *et al.*, 2012; Lossing *et al.*, 2012; Richdale *et al.*, 2013).

1.2.4 Types of accommodation

To reflect differences in accommodative stimuli, Heath (1965) subdivided accommodation into 4 categories: reflex, convergence, proximal and tonic.

1.2.4a Reflex accommodation

Reflex accommodation describes the dynamic response of the eye to overcome retinal blur in order to focus an object at near (Heath, 1956; Charman, 2008). Reflex accommodation is the most frequently investigated type of accommodation in ophthalmic research.

1.2.4b Convergence accommodation

Convergence, accommodation and pupil miosis form the near vision triad. The neuronal pathways that control each component of the near vision triad are distinct but interrelated (Kothari *et al.*, 2009). Therefore, isolated stimulation of convergence results in an accommodative response; a phenomenon known as convergence accommodation (Heath, 1956).

1.2.4c Proximal accommodation

Accommodation in response to knowledge or expectation of an object's close proximity is known as proximal accommodation (Heath, 1956; Rosenfield and Ciuffreda, 1991). Overcoming proximal accommodation is a challenge when designing instruments to measure objective distance refraction in an enclosed space due to the inherent increase in total power of the eye during proximal accommodation (Hennessy, 1975; Smith, 1983; Rosenfield and Ciuffreda, 1991; Sheppard and Davies, 2010b).

1.2.4d Tonic accommodation

Typically, the accommodative system operates as a negative feedback closed-loop system, whereby accommodation is exerted in response to the retinal blur produced by objects at near. In the absence of negative feedback, the accommodative response reaches a resting point known as open-loop or tonic accommodation. Tonic accommodation can be observed while asking a patient to observe complete darkness, a bright empty field, a Gaussian target or viewing through small

artificial pupils (Gilmartin *et al.*, 1984; Gilmartin and Hogan, 1985; Rosenfield *et al.*, 1993; Gilmartin *et al.*, 2002; Mallen *et al.*, 2005a; Morrison *et al.*, 2010). The magnitude of this accommodative response is approximately 1.00 D, although large intersubject variation is evident (Heath, 1956; Rosenfield *et al.*, 1993). Originally thought to be a result of tonic ciliary muscle contraction due to the dual innervation of the ciliary muscle (Gilmartin *et al.*, 1984; Gilmartin and Hogan, 1985), tonic accommodation has been shown to be associated with multiple, non-optical stimuli, for example mental and auditory inputs (Bullimore and Gilmartin, 1987; Rosenfield and Ciuffreda, 1991; Rosenfield *et al.*, 1993).

1.3 Presbyopia

Presbyopia is diagnosed clinically when the clarity of a patient's near vision is insufficient to meet their requirements due to a reduction in their amplitude of accommodation (Atchison, 1995). The onset of presbyopia usually occurs between 42-48 years of age in European individuals (Millodot, 2008). The ability to accommodate becomes essentially non-existent by the age of approximately 55 years (Donders, 1864; Duane, 1922).

Theories exploring the cause of presbyopia can broadly be divided into two viewpoints: lenticular and extra-lenticular.

1.3.1 Lenticular presbyopia theories

As discussed previously in section 1.2.1a, the crystalline lens continues to grow throughout life due to the progressive addition of new lens fibres (Koretz *et al.*, 1989; Smith *et al.*, 1992; Cook *et al.*, 1994; Glasser *et al.*, 1999; Strenk *et al.*, 1999; Dubbelman *et al.*, 2001a; Koretz *et al.*, 2001; Dubbelman *et al.*, 2003; Jones *et al.*, 2007; Atchison *et al.*, 2008; Richdale *et al.*, 2013). There is a corresponding increase in protein content throughout the crystalline lens, however the overall protein concentration does not change with age (Sato, 1972).

The increase in axial crystalline lens thickness per year of life is thought to be between 0.019 and 0.031 mm/year (Koretz *et al.*, 1989; Dubbelman *et al.*, 2001a; Wojciechowski *et al.*, 2003; Koretz

et al., 2004; Allouch *et al.*, 2005; Shufelt *et al.*, 2005; Atchison *et al.* 2008; Kasthurirangan *et al.* 2011; Richdale *et al.* 2013). The equatorial diameter of the crystalline lens also appears to increase with age (Fea *et al.*, 2005; Jones *et al.*, 2007; Atchison *et al.*, 2008; Kasthurirangan *et al.*, 2011). The crystalline lens surface radii of curvature decrease with age, becoming steeper (Lowe and Clark, 1973; Atchison *et al.*, 2008), with the greatest change observed across the anterior surface (Brown *et al.*, 1974; Dubbelman *et al.*, 2001b; Koretz *et al.* 2001; Koretz *et al.*, 2004).

The increase in crystalline lens thickness and the steepening of the anterior and posterior surfaces with age might suggest the crystalline lens becomes progressively more powerful, creating a more myopic refractive error. However, due to changes in the gradient refractive index of the crystalline lens with age, the equivalent power of the crystalline lens actually decreases with age; this is known as the crystalline lens paradox (Brown *et al.*, 1974; Hemenger *et al.*, 1995; Dubbelman *et al.*, 2001b; Koretz *et al.*, 2004; Jones *et al.*, 2005; Atchison *et al.*, 2008). The refractive indices of the nucleus (1.406) and cortical (1.386) regions of the crystalline lens do not change significantly with age, however, the nucleus increases in size with age, causing the gradient between high and low refractive indices to become steeper (Jones *et al.*, 2005; Kasthurirangan *et al.*, 2008). Overall, the average refractive index of the crystalline lens actually decreases with age (Dubbelman *et al.*, 2001b; Atchison *et al.*, 2008).

Furthermore, the flexibility of the crystalline lens also changes with age. A more than three-fold increase in the overall resistance of the *in vitro* human crystalline lens to compressive forces over the life-span has been observed (Glasser *et al.*, 1999). Pierscionek (1995) and Glasser and Campbell (1998) found that older lenses did not undergo significant changes in focal length in response to simulated zonular tension and relaxation *in vitro*. The stiffness of the nucleus and cortex increase at different rates with age, becoming similar between the ages of 35 to 45 years (Hey *et al.*, 2004; Weeber *et al.*, 2007). The nucleus is stiffer than the cortex in old lenses, whereas the cortex is stiffer than the nucleus in young lenses (Fisher, 1971; Pau and Kranz, 1991; Weeber and Eckbert, 2004; Hey *et al.*, 2004; Weeber *et al.*, 2007). Fisher (1973) investigated the structural

origins of the changes in crystalline lens stiffness, finding no change in the water content with age and suggesting increased lens fibre adhesions may be responsible. Increasing rigidity of the crystalline lens has been cited as the main cause of presbyopia in humans (Heys *et al.*, 2004; Weeber *et al.*, 2007; Glasser, 2008).

The change in crystalline lens shape is vital to produce the accommodative response, thus the aforementioned age-related changes in crystalline lens size, shape, structure and flexibility has led to the crystalline lens becoming central to several presbyopia theories.

1.3.1a Hess-Gullstrand presbyopia theory

The Hess-Gullstrand presbyopia theory builds on the Helmholtzian theory of accommodation, suggesting that the ciliary muscle maintains its contractile ability throughout life, and the contractile effort required to exert a unit change in accommodation remains constant with age. However, decreasing lenticular flexibility would cause an increasing proportion of ciliary muscle contraction to become latent with age (Hess, 1901, cited by Alpern, 1962; Gullstrand 1911; Atchison, 1995; Mordi and Ciuffreda, 2004; Charman, 2008).

In support of this theory, cross-sectional studies have reported the anterior (Sheppard and Davies, 2011) and centripetal (Strenk *et al.* 1999; 2006; Sheppard and Davies, 2011; Richdale *et al.*, 2013) contractile response of the ciliary muscle is not significantly attenuated with age, despite significant ciliary muscle morphological changes with age (increased thickness and anterior displacement of muscle mass). Transient axial length elongation evident during accommodation, which has been linked to ciliary muscle contraction (Shum *et al.*, 1993; Drexler *et al.*, 1998; Uozato *et al.*, 2003; Mallen *et al.*, 2006; Read *et al.*, 2010a; Maheshwari *et al.*, 2011; Woodman *et al.*, 2011; Woodman *et al.*, 2012), has been observed during near vision in a patient with advanced presbyopia (Hollins, 1974). Furthermore, continued pupil miosis (part of the near vision triad with accommodation and convergence) has also been observed in response to levels of accommodation greater than a patient's maximum amplitude of accommodation (Alpern and

David, 1958). Therefore, it is feasible the ciliary muscle contractile response may persist into advanced presbyopia despite its diminished capacity to instigate changes in crystalline lens shape.

Atchison *et al.* (1994) suggested that the slackening of the zonules, in response to ciliary muscle contraction, would increase with age due to the age-related increase in crystalline lens rigidity preventing a decrease in crystalline lens equatorial diameter during accommodation. Therefore, Atchison *et al.* hypothesised that the greater zonule laxity in the presbyopic eye would permit a larger movement of the crystalline lens with gravity. However, the largest gravity-driven movements of the crystalline lens were reported amongst their pre-presbyopic cohort, which Atchison and colleagues suggested undermined the Hess-Gullstrand theory of presbyopia. Crystalline lens movements were measured indirectly based on the change in the subjective near point of accommodation, with the near point moving closer in pre-presbyopic patients and becoming more remote in presbyopic patients during down-gaze and downward head positioning. However, later mathematical modelling by Davies *et al.* (2010) found that the forward movement of the crystalline lens during accommodation actually reduces the overall power of the accommodative response, therefore the work of Atchison and colleagues (1994) may actually support the Hess-Gullstrand theory by showing increased levels of zonular laxity during presbyopia. However, zonular laxity is an indirect measurement of ciliary muscle contraction and the zonules themselves may undergo age-related changes in elasticity (Assia *et al.*, 1991; van Alphen and Graebel, 1991; Abolmaali and Schachar, 2007).

1.3.1b Duane-Fincham presbyopia theory

Similarly to the Hess-Gullstrand theory, Fincham (1937) also attributed presbyopia development to an increase in crystalline lens rigidity. However, Fincham suggested that the ciliary muscle contractile force required to exert a unit change in accommodation actually increased with age in order to mould the stiffer crystalline lens. The ciliary muscle is therefore maximally contracted at the near point of accommodation. Duane (1922) reported topical instillation of atropine produced a more rapid reduction in accommodation in older patients, and therefore, in contrast to the

Hess-Gullstrand theory, alleged that the ciliary muscle becomes weaker with age, as supported by Donders (1864). Rhesus monkey studies have also reported ciliary muscle contractibility weakens with increasing age (Bito and Miranda, 1989; Tamm *et al.*, 1992b), however the majority of human eye studies have shown that ciliary muscle contractibility does not weaken with age (Fincham, 1955; Fisher, 1977; 1986; Strenk *et al.*, 1999; 2006; Sheppard and Davies, 2011; Richdale *et al.*, 2013).

The Hess-Gullstrand theory predicts that the accommodation-convergence relationship will remain unchanged throughout life. However, an increase in the AC/A ratio (convergence stimulated by 1 dioptre of accommodation) and a decrease in the CA/C ratio (accommodation stimulated by 1 prism dioptre of convergence) with age has been reported (Fincham, 1955; Bruce *et al.*, 1995; Rosenfield *et al.*, 1995; Baker and Gilmartin, 2002); indicating the ciliary muscle effort required to produce a unit change in accommodation increases with age. However, the age-dependency of tonic accommodation and tonic vergence, which may also influence AC/A and CA/C ratio measurements, is currently unclear (Ramsdale and Charman, 1989; Ciuffreda *et al.*, 1993; Bruce *et al.*, 1995; Ciuffreda *et al.*, 1997; Mordi and Ciuffreda, 1998; Baker and Gilmartin, 2002).

Contrary to the Duane-Fincham theory, the binocular amplitude of accommodation is greater than the monocular amplitude of accommodation (Duane, 1922), suggesting the ciliary muscle is not maximally contracted at the near point under monocular conditions. However, the increase in amplitude of accommodation binocularly may be an artefact arising from the inherent increase in depth-of-focus accompanying pupil miosis, which is likely to be enhanced during binocular vision (Atchison, 1995). Nevertheless, recent cross-sectional research reporting the contractility of the ciliary muscle does not change with age (Strenk 1999; 2006; Sheppard and Davies, 2011; Richdale *et al.*, 2013) refutes the Duane-Fincham theory and supports the Hess-Gullstrand theory of presbyopia.

1.3.1c Geometric presbyopia theory

In addition to increasing lenticular rigidity, the change in size and shape of the crystalline lens with age has also been attributed to the development of presbyopia. The geometric theory suggests the axial increase in crystalline lens mass and reduction in the radii of curvature causes the zonular insertion area to widen around the lens equator, increasing the distance between the anterior and posterior zonules (Farnsworth and Shyne, 1979), pulling the ciliary muscle antero-inwards (Pardue and Sivak, 2000) and reducing the magnitude of the parallel vector force the zonules can impart on the crystalline lens equator. Therefore, contraction and relaxation of the zonules will gradually have less of an impact on crystalline lens shape with age. The geometric theory has been mathematically modelled (Koretz and Handelman, 1986), but no research has proved the efficiency of zonule action is reduced with age.

Strenk *et al.* (2005) modified the geometric theory to consider the uveal tract. Strenk and colleagues suggested continuous anterior crystalline lens growth and movement pushes the pupillary margin forwards. The applied force travels down the iris root and across the rest of the uvea, causing an antero-inwards movement. The age-related reduction in circumlental space (the distance between the ciliary muscle inner apex and the crystalline lens equator) reduces zonular tension in the absence of accommodation, allowing the crystalline lens to take-up a thicker, more curved shape and therefore reducing the change in crystalline lens shape possible during accommodation. Indeed, the relocation of the anterior uveal tract to a more posterior position once the presbyopic crystalline lens has been removed supports this hypothesis (Strenk *et al.*, 2010). The relative contribution of changes in crystalline lens size and shape compared to the increase in crystalline lens rigidity in the development of presbyopia is currently unknown.

1.3.1d Schachar presbyopia theory

An alternative and controversial presbyopia theory has been proposed by Schachar (1992). Schachar hypothesised that equatorial zonular tension is increased during accommodation, causing the central anterior crystalline lens surface to steepen, as discussed in section 1.2.3.

However, with age, Schachar suggested weakened zonular tension, caused by equatorial growth of the crystalline lens, renders the zonules unable to impart enough force to drive a change in crystalline lens shape. Based on this theory, Schachar developed a novel mode of presbyopic correction called scleral expansion. The surgical procedure involves implanting polymethylmethacrylate arches into the sclera overlying the ciliary muscle to stretch the sclera radially outwards by 0.5 to 1.5 mm in order to restore zonular tension. Schachar performed scleral expansion surgery on human presbyopic subjects (Schachar, 1992) and animal subjects (Schachar et al., 1993) and concluded the ability to accommodate was restored. However, Mathews (1999) found no evidence of accommodation in Schachar's scleral expansion patients post-operatively when measured with an objective optometer, thus undermining Schachar's theory.

1.3.2 Extra-lenticular presbyopia theories

1.3.2a Ciliary muscle presbyopia theory

Croft *et al.* (2006) found iridectomised presbyopic Rhesus monkeys demonstrated a 20% loss of centripetal ciliary muscle movement and a 67% loss of anterior movement when compared to young Rhesus monkeys during Edinger-Westphal stimulated accommodation. Neider *et al.* (1990) suggested the reduced response may be due to configurational changes rather than reduced ciliary muscle contractibility. However, Lütjen-Drecoll *et al.* (1988) reported that the age-related increase in connective tissue within and adjacent to the ciliary muscle, observed in both human and monkey specimens, would not have a significant impact on the mobility of the ciliary muscle. Ciliary muscle contraction in the ageing monkey eye is largely restored when the ciliary muscle posterior tendon attachments, which become thicker and are surrounded by an increasing amount of collagen fibrils with age (Tamm *et al.*, 1991), are cut *in vitro* (Tamm *et al.* 1992a). Indeed, decreased flexibility of ciliary muscle posterior tendons, restricting ciliary muscle action during accommodation, has been posed as a dominant factor in the development of presbyopia in Rhesus monkeys (Tamm *et al.*, 1992b). More recent research has reported the accommodative centripetal ciliary muscle contractile response reduces with age in Rhesus monkey and human

subjects (Croft *et al.*, 2013a), which is contradictory of the results from earlier studies reporting no significant attenuation of the ciliary muscle contractile response with age (Strenk 1999; 2006; Sheppard and Davies, 2011; Richdale *et al.*, 2013). However, Croft *et al.*'s (2013a) results were based on a small sample of 12 human patients aged between 19 and 65 years and accommodation was stimulated pharmacologically, which does not represent the natural accommodative response *in vivo* (Koepl *et al.*, 2005). The role of the ciliary muscle in the development of presbyopia therefore remains unclear.

1.3.2b Capsular presbyopia theory

Ageing of the human anterior crystalline lens capsule is associated with an increase in its thickness, focused particularly at the anterior zonular insertion sites (Fisher, 1969b; Fincham, 1937; Fisher and Pettet, 1972; Travers, 1990), whereas the thickness of the posterior capsule remains constant with age (Fisher and Pettet, 1972). Fisher (1969b) and Glasser (1999) reported the flexibility of the capsule decreased with age, becoming less extensible (Krag *et al.*, 1997). Glasser and Campbell (1999) observed a marked change in *in vitro* crystalline lens shape and power when the capsule was removed from young crystalline lenses, whereas little change was observed when the capsule was removed from older crystalline lenses, thus suggesting capsular force has little effect on crystalline lens shape after the age of approximately 50 years. However, Ziebarth *et al.* (2008) reported the age-related changes in capsular elasticity and thickness do not have a significant impact on its performance. Moreover, the capsular force required to mould the crystalline lens increases with age due to the increased rigidity of the crystalline lens itself (Ziebarth *et al.*, 2008). Therefore it is unlikely the capsule has a significant role to play in the development of presbyopia.

1.3.2c Zonular presbyopia theory

As discussed previously as part of the geometric theory (section 1.3.1c), the ciliary muscle's contractile force applied to the capsule and crystalline lens may reduce with age due to the anterior migration of the zonular insertion sites, which increases the distance between the

anterior and posterior zonules (Farnsworth and Shyne, 1979). The force transmitted via the zonules to the crystalline lens is proportional to the thickness of the capsule (Krag and Andreassen, 2003), therefore the age-related increase in capsular thickness, particularly at the zonular sites of insertion, may compensate for the reduced force applied.

However, an age-related decrease in the elasticity of the zonules may reduce the magnitude of the change in zonular tension during ciliary muscle contraction and relaxation, thus dampening the force transmitted to the capsule and crystalline lens (Assia *et al.*, 1991; Abolmaali and Schachar, 2007). The authors based this theory on the age-related elastic behaviour of the zonules under maximum tension (Assia *et al.*, 1991; Abolmaali and Schachar, 2007), however due to the decrease in ciliary muscle ring diameter (Strenk *et al.*, 2006; Kasthurirangan *et al.*, 2011) and increase in crystalline lens equatorial diameter with age (Fea *et al.*, 2005; Jones *et al.*, 2007; Atchison *et al.*, 2008; Kasthurirangan *et al.*, 2011) reducing circumlental space, it is unlikely the zonules would be stretched to these limits. Van Alphen and Graebel (1991) reported zonular elasticity was independent of age at 10% elongation, which more closely simulates the physiological response *in vivo*. Furthermore, at age 43 years, the force required to instigate a 10% crystalline lens elongation is approximately 22 times greater than that required for a 10% elongation of the zonules (van Alphen and Graebel, 1991); suggesting the decreased flexibility of the crystalline lens is much more likely to be instrumental in the development of presbyopia than changes in the zonules.

1.3.2d Choroidal presbyopia theory

The choroid is thought to be instrumental in relocating the ciliary muscle to a more posterior position following cessation of ciliary muscle contraction (Gullstrand, 1909 cited by Helmholtz, 1924). *In vitro* research has reported that the flexibility of the choroid decreases with age (van Alphen, 1991; Ugarte *et al.*, 2006), thus perhaps reducing its ability to restore the ciliary muscle to its relaxed position. However, the *in vivo* relocation of the anterior uveal tract post-cataract surgery to a more youthful, posterior position suggests the choroid may retain its elasticity with

age (Strenk *et al.*, 2010), despite the choroid becoming thinner with age (Manjunath *et al.*, 2010). Therefore the choroid is unlikely to be instrumental in the development of presbyopia.

Despite the numerous and varied theories for the development of presbyopia, the literature is in agreement that the development of presbyopia is likely to be multifactorial, involving a number of interdependent structures (Gilmartin, 1995), with perhaps the most significant factor being the increase in crystalline lens stiffness with age (Heys *et al.*, 2004; Glasser, 2008).

1.4 Patterns of refractive error changes during presbyopia development

1.4.1 Introduction

Cross-sectional studies investigating refractive error changes with age have shown a general hypermetropic shift in refraction occurs between the ages of 35 and 65 years, and a myopic shift thereafter due to the onset of crystalline lens nuclear sclerosis (Tassman, 1932; Brown, 1938; Slataper, 1950; Hirsch, 1958; Saunders, 1981; Fledelius, 1983; Fledelius and Stubgaard, 1986; Sperduto *et al.*, 1996, McCarty *et al.*, 1997; Kempen *et al.*, 2004; Atchison *et al.*, 2008; Fig. 1.3). However the period of incipient presbyopia (typically 35 to 45 years of age) was not sampled in many cases.

Longitudinal retrospective studies have reported a hypermetropic shift in refraction above the ages of 35 to 40 years (Saunders, 1986; Grosvenor and Skeates, 1999; Lee *et al.*, 2002), as shown in Fig. 1.3. The data were acquired retrospectively from routine sight test records and therefore no measures of ocular biometry were obtained. Nevertheless, the authors hypothesise that the origins of the putative hypermetropic refractive shift may be due to the manifestation of previously latent hypermetropia (Goss *et al.*, 1985) and the crystalline lens paradox, where the decrease in average refractive index of the crystalline lens is more significant to the overall power of the crystalline lens than the increase in thickness and curvature (Saunders, 1981; Grosvenor and Skeates, 1999; Mutti and Zadnik, 2000).

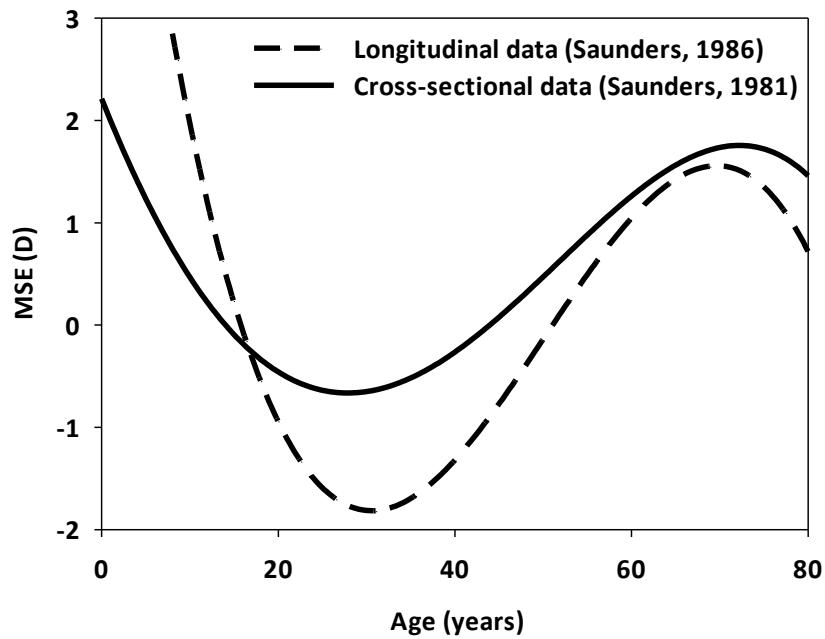


Fig. 1.3. Longitudinal (dashed line) and cross-sectional (solid line) mean spherical equivalent refraction data plotted according to age. Redrawn from Saunders (1981; 1986).

Grosvenor and Skeates (1999) isolated retrospective longitudinal myopic patient data and reported the hypermetropic shift in refraction (≥ 0.50 D) was less prevalent amongst the myopic cohort (19%), when compared to the prevalence in patients who were emmetropic (54%) and hypermetropic (62%) at baseline. Most of the myopic patients remained stable (66%) or became more myopic by ≥ 0.50 D (15%) 10 to 26 years after the age of 40 years. A total of 2% of hypermetropes and 3% of emmetropes became more myopic over the same period. Informal clinical observation supports Grosvenor and Skeates' (1999) results. Pointer and Gilmartin (2011) published a retrospective longitudinal study focused on the period of incipient presbyopia, including data from 39 patients, aged 35 to 44 at baseline, reviewed over 4 to 10 years. The study found that 20% of myopes became between 0.50 to 0.75 D more myopic during this period, as shown in Fig. 1.4.

Our current understanding of the changes that occur during presbyopia does not explain why a myopic shift might be observed. Myopia onset and progression has been linked to changes in the cornea (Goss *et al.*, 1985), ciliary muscle (Bailey *et al.*, 2008), crystalline lens (Lee *et al.*, 2002) axial length (McBrien and Adams, 1997), peripheral refraction (Hoogerheide *et al.*, 1971) and retinal

contour (Schmid, 2011), which will each be discussed in detail in the subsequent sections. Pointer and Gilmartin (2011) suggested changes in ocular structures may work alone or in concert to produce the overall myopic shift in refraction and indicated that prospective longitudinal studies are required to investigate its origin.

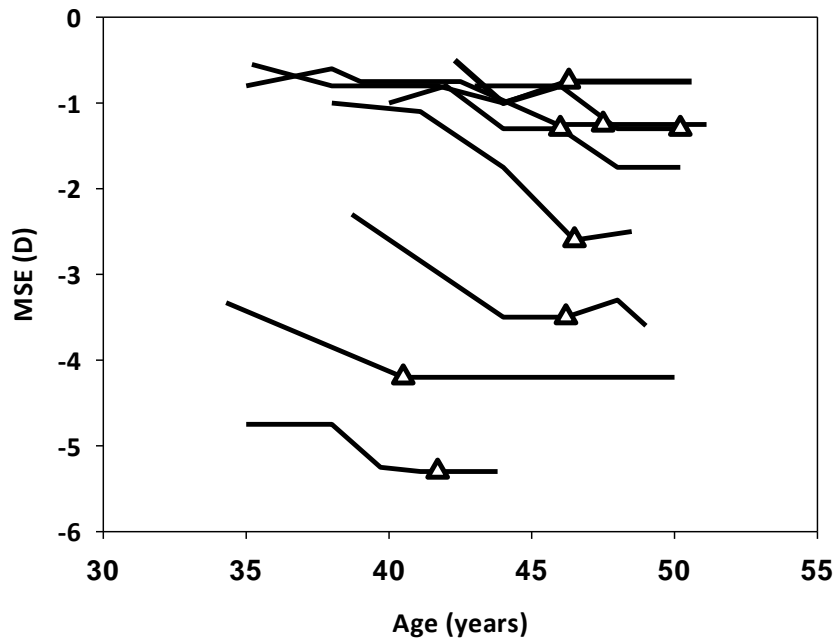


Fig. 1.4. Longitudinal refractive data collected by Pointer and Gilmartin (2011) for the 20% of myopes who became more myopic during incipient presbyopia. The triangles represent where near vision correction was first prescribed, but not necessarily dispensed. Redrawn from Pointer and Gilmartin (2011).

1.4.2 Corneal changes during myopia development

With axial length elongation, the cornea loses power in early life (Sorsby *et al.*, 1962; Mutti *et al.*, 2005). From approximately 20 years of age, a gradual increase in corneal power (Exford, 1965; Baldwin and Mills, 1981; Fledelius and Stubgaard, 1986; Hayashi *et al.*, 1995) and a decrease (steepening) in anterior and posterior corneal radii of curvature occurs (Hayashi *et al.*, 1995; Dubbelman *et al.*, 2006).

The cornea represents the major refractive surface of the eye (Gipson, 2007). However, the differences in overall corneal power between emmetropes, myopes and hypermetropes have been found to be negligible in both children (Mutti *et al.*, 2012) and adults (McBrien and Millodot, 1987; Bullimore *et al.*, 1992), suggesting changes in corneal power are not responsible for changes

in refractive error. No correlation between refractive error and corneal thickness has been observed (Pedersen *et al.*, 2005; Fam *et al.*, 2006; Oliveira *et al.*, 2006; Shen *et al.*, 2008). However, corneal hysteresis is reduced in high myopia (> 6.00 D), indicating the structural organisation of the cornea may be compromised in high myopia (Shen *et al.*, 2008; Jiang *et al.*, 2011; Altan *et al.*, 2012).

Topography research has shown a propensity for the anterior corneal surface to flatten less rapidly in the periphery (becoming less prolate; see Fig. 1.5) with increasing myopia in both children (Horner *et al.*, 2000; Davis *et al.*, 2005) and adults (Carney, 1997; Budak *et al.*, 1999). However, a consensus has not been reached as to whether changes in central corneal radii of curvature (Sorsby *et al.*, 1962; Goss and Erickson, 1987; McBrien and Millodot, 1987; Bullimore *et al.*, 1992; Garner *et al.*, 1992; Grosvenor and Scott, 1993; Scott and Grosvenor, 1993; Goss and Jackson, 1995; McBrien and Adams, 1997; Horner *et al.*, 2000; Davis *et al.*, 2005; Dubbelman *et al.*, 2006; Mutti *et al.*, 2012) or asphericity (Carney, 1997; Budak *et al.*, 1999; Horner *et al.*, 2000; Davis *et al.*, 2005; Dubbelman *et al.*, 2006) are associated with myopic progression in children or adults. Nevertheless, previous authors have utilised the axial length: corneal radius ratio to monitor changes in the average central anterior corneal radius of curvature in relation to the axial length.

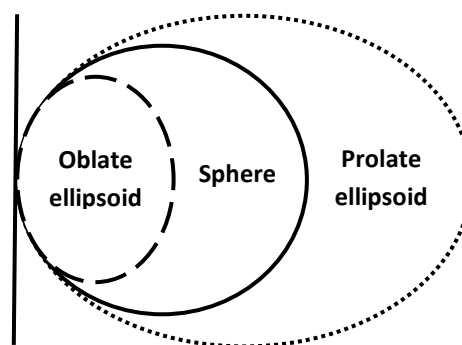


Fig. 1.5. An oblate ellipsoidal shape (squashed sphere) is represented by the dashed lines, a sphere is represented by the solid outline and a prolate ellipsoid (stretched sphere) is represented by the spotted line.

1.4.2a Axial length: corneal radius ratio

The ratio of axial length to corneal radius (AXL:CR) is commonly calculated at the beginning of longitudinal studies and is used as an indicator of a patient's risk of a myopic shift in refraction. For example, an eye with an axial length of 24 mm and an average anterior corneal radius of curvature (measured by keratometry) of 8 mm would have an AXL:CR ratio of 3:1.

Goss and Jackson (1995) found that 88% of emmetropic children who became myopic had baseline AXL:CR ratios of 3:1 or greater, whereas 95% of emmetropic eyes with AXL:CR ratios of less than 3:1 did not become myopic; suggesting an AXL:CR ratio of 3 and above is a risk factor for the development of childhood-onset myopia, a theory supported by Grosvenor (1988). Grosvenor and Goss (1998) later suggested that the observed relationship may be due to greater corneal powers (steeper radii of curvature) being a risk factor for myopia onset in children. The AXL:CR ratio may be of limited value when considering myopic progression during adulthood because McBrien and Adams (1997) found that emmetropic adults (age range 21 to 63 years) who became myopic during the course of the 2 year study did not have significantly different AXL:CR ratios to those subjects who remained emmetropic. Further longitudinal research is required to investigate whether the AXL:CR ratio could be a useful predictor for adult-onset myopia.

1.4.3 Ciliary muscle changes during myopia development

The anterior ciliary muscle thickness (thickness measurement 1 mm from the scleral spur and the point of maximum ciliary muscle thickness; Fig. 1.6) has been reported to be greater in the eyes of hypermetropic children (Pucker *et al.*, 2013), whereas the posterior ciliary muscle thickness (thickness measurements 2 mm and 3 mm from the scleral spur; Fig. 1.6) has been found to be greater in the eyes of myopic children (Bailey *et al.*, 2008; Schultz *et al.*, 2009; Pucker *et al.*, 2013) and adults (Oliveira *et al.*, 2005; Muftuoglu *et al.*, 2009; Jeon *et al.*, 2012; Buckhurst *et al.*, 2013; Kuchem *et al.*, 2013). Sheppard and Davies (2010a) found longer axial length measurements (typically associated with myopia) correlated positively with longer ciliary muscle length values in adults aged 19 to 34 years. Therefore, it is not surprising myopic ciliary muscles are thicker more

posteriorly as the ciliary muscle of emmetropic and hypermetropic eyes terminate (reaching a point of minimum thickness) more anteriorly. The finding that myopic eye ciliary muscles are thinner anteriorly when compared to emmetropic and hypermetropic eyes suggests the increase in myopic ciliary muscle length may be due to posterior relocation of muscle mass, rather than hypertrophy of the tissue during ocular axial length growth. However, when considering distances from the scleral spur as a percentage of the total length of the ciliary muscle, the thickness measurements at 25%, 50% and 75% of the total ciliary muscle length are not dependent on axial length or refractive error (Sheppard and Davies, 2010a). Furthermore, when considering longitudinal ciliary muscle changes in children, a myopic shift in refraction is not associated with a change in ciliary muscle thickness (Bailey *et al.*, 2011) and the more myopic eye of adults with anisometropia (>1.00 D) has a similar ciliary muscle thickness profile to the contralateral eye (Kuchem *et al.*, 2013). Therefore, it appears likely that an increase in ciliary muscle length during myopia progression is accompanied by an increase in ciliary muscle mass, which prevents the muscle from thinning anteriorly and maintains a constant thickness.

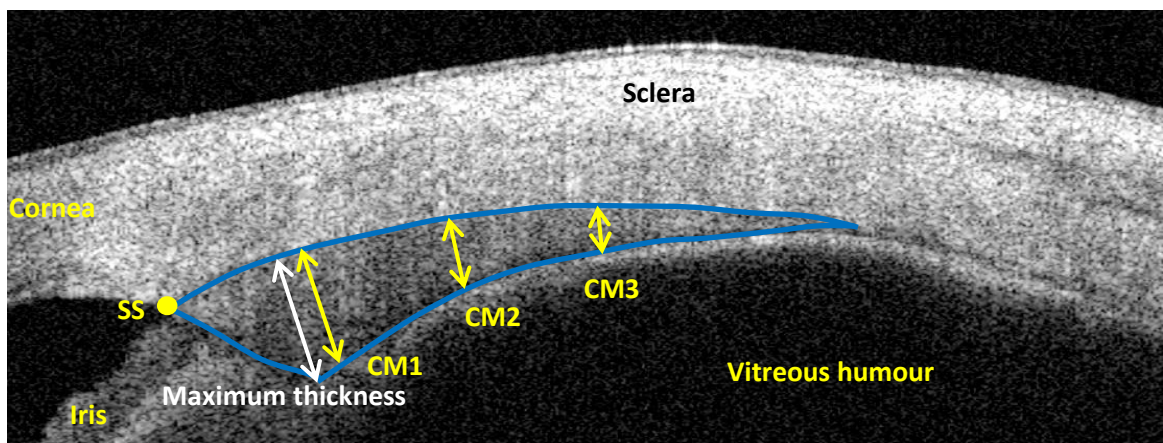


Fig. 1.6. Visante Anterior-Segment Optical Coherence Tomography (AS-OCT) image of a human ciliary muscle section. The ciliary muscle is outlined in blue. SS= scleral spur, CM1= thickness 1 mm posteriorly from the SS, CM2= thickness 2 mm posteriorly from the SS, CM3= thickness 3 mm posteriorly from the SS.

Jeon *et al.* (2012) reported reduced ciliary muscle movement during accommodation in young adult eyes with longer axial lengths and increased ciliary muscle thickness. However the objective accommodative response was not measured; failing to elucidate whether the change in crystalline

lens thickness per unit of accommodative response was lower in eyes with longer axial lengths (Davies *et al.*, 2010) or whether a larger lag of accommodation was evident in the myopic cohort (Abbott *et al.*, 1998; Rosenfield *et al.*, 2002; Mutti *et al.*, 2006). However, Sheppard and Davies (2010a) and Lewis *et al.* (2012) found no dependence of the ciliary muscle accommodative response on axial length or ciliary muscle baseline thickness, respectively.

There is a paucity of longitudinal research investigating ciliary muscle changes with age and the potential influence structural changes may have on accommodative dynamics during myopia progression.

1.4.3a Near work induced transient myopia

A link has been established between high levels of near work induced transient myopia (thought to be caused by the failure of the ciliary muscle to relax following cessation of near vision) and myopia progression (Vera-Díaz *et al.*, 2002; Wolffsohn *et al.*, 2003a; Wolffsohn *et al.*, 2003b; Vasudevan and Ciuffreda, 2008). Hence incipient presbyopes may be at increased risk of a permanent myopic shift in refraction due to near work induced transient myopia resulting from a progressively slower ability to modify the accommodative response to focus from one fixation distance to another (accommodative inertia), which is associated with smaller amplitudes of accommodation.

Furthermore, the reported myopic shift in refraction during incipient presbyopia could be due to ciliary muscle spasm in response to repeatedly straining to focus at near. However, the refractive shift observed by Pointer and Gilmartin (2011) in Fig. 1.4 appears to have persisted for the duration of the study period (4 to 10 years after the initial myopic shift), even after near vision correction was prescribed, thus indicating that ciliary muscle spasm is unlikely to be responsible for the myopic shift in refraction. Longitudinal ciliary muscle imaging and observing the permanency of the myopic shift in refraction following instillation of a cycloplegic agent will help

to investigate whether the cause of the myopic shift during incipient presbyopia is ciliary muscle spasm.

1.4.4 Crystalline lens changes during myopia development

During childhood, the crystalline lens thins axially and flattens in coordination with axial length elongation in an attempt to maintain an emmetropic refractive error (Zadnik *et al.*, 1995; Mutti *et al.*, 2012). The reported crystalline lens thinning and corresponding decrease in crystalline lens power has been suggested to be a result of equatorial eye growth in tandem with axial length growth (Zadnik *et al.*, 1995), compaction of the crystalline lens nucleus and steepening of the crystalline lens refractive index gradient (Iribarren *et al.*, 2012). Myopia occurs when the crystalline lens exhausts its ability to compensate for axial length elongation, possibly due to cessation of equatorial scleral growth or mechanical restriction resulting from ciliary muscle hypertrophy (Mutti *et al.*, 2012). In accordance, myopes have been reported to have thinner crystalline lenses than age-matched emmetropes and hypermetropes (McBrien and Millodot, 1987; Zadnik *et al.* 1995; McBrien and Adams, 1997). Evidence of crystalline lens thinning in an attempt to offset axial length elongation has also been reported during myopia onset in clinical microscopists aged between 21 to 63 years (McBrien and Adams, 1997), despite the commonly reported increase in crystalline lens thickness during adulthood (Koretz *et al.*, 1989; Smith *et al.*, 1992; Cook *et al.*, 1994; Glasser *et al.*, 1999; Strenk *et al.*, 1999; Dubbelman *et al.*, 2001a; Koretz *et al.*, 2001; Dubbelman *et al.*, 2003; Jones *et al.*, 2007; Atchison *et al.*, 2008; Richdale *et al.*, 2013). However, the significant decrease in crystalline lens power typically reported in conjunction with crystalline lens thinning in children (Zadnik *et al.*, 1995; Mutti *et al.*, 2012) was not observed amongst the adult cohort (McBrien and Adams, 1997) and the data for incipient presbyopes was not teased out. It is important to note the crystalline lens and axial length changes reported here are not mutually exclusive, and without the axial length elongation, the crystalline lens shape is unlikely to thin.

Alternatively, considering the crystalline lens in isolation, a myopic shift may be instigated by an increase in crystalline lens thickness, curvature and/or average refractive index, thus increasing the power of the crystalline lens, as has been reported during transient hyperglycemia in patients with diabetes mellitus (Løgstrup *et al.*, 1997; Bron *et al.*, 1998; Furushima *et al.*, 1999; Wiemer *et al.*, 2008; Charman, 2012). As discussed in section 1.3.1, the age-related increase in crystalline lens thickness and decrease in radii of curvature are offset by a reduction in the average refractive index of the crystalline lens due to the steeper gradient between high and low refractive indices. However, if the changes in crystalline lens shape were not entirely compensated for by a change in refractive index gradient, the power would increase and a myopic shift in refraction would occur. Unfortunately, research to-date investigating the refractive index has included emmetropes only (Kasthurirangan *et al.*, 2008) or has not divided the cohort according to refractive error (Jones *et al.*, 2005; Dubbelman *et al.*, 2005). Further research investigating longitudinal crystalline lens changes during myopia development in incipient presbyopia is required.

1.4.5 Axial length changes during myopia development

Cross-sectional (McBrien and Millodot, 1987; Grosvenor and Scott, 1991; Bullimore *et al.*, 1992) and longitudinal (Grosvenor and Scott, 1993; McBrien and Adams, 1997; Mutti *et al.*, 2012) research has implicated axial length elongation, particularly vitreous chamber elongation, as the origin of myopic shifts in refraction during both childhood and adulthood. An axial length elongation of between 0.33 and 0.35 mm/D has been reported in adult subjects experiencing myopic shifts in refraction (Deller *et al.*, 1947; Atchison *et al.*, 2004). However, minimal data are available specifically during the period of incipient presbyopia.

Goss *et al.* (1990) found that axial elongation stabilised earlier in myopic females (between the ages of 14.6 and 15.3 years) than in myopic males (between the ages of 15.0 and 16.7 years), and tended to coincide with cessation of the growth of the body in height. After this age, myopic progression is typically considerably slower, however still appears to be due to vitreous chamber

elongation (McBrien and Adams, 1997; Lin *et al.*, 1999). Early onset myopes are likely to progress to much higher levels of myopia than those who develop myopia after puberty (Blegvad, 1927; Mäntyjärvi, 1985). A consistent pattern in axial length change with age has not been reported (Leighton and Tomlinson, 1972; Grosvenor, 1987; Koretz *et al.* 1989; Ooi and Grosvenor, 1995; Atchison *et al.*, 2008).

In the search to determine the mechanism driving continued axial length elongation in myopia, a connection has been made between high levels of near work and myopia onset and progression in both children (Mutti *et al.*, 2002; Saw *et al.*, 2002) and adults (Goldschmidt, 1968; Simensen and Thorud, 1994; McBrien and Adams, 1997; Maheshwari *et al.*, 2011). Central hypermetropic blur (produced by a lag of accommodation; Hung *et al.*, 1995; Gwiazda *et al.*, 2004) and transient axial length elongation during accommodation (Drexler *et al.*, 1998; Mallen *et al.*, 2006) have been suggested as possible triggers for this association.

1.4.5a Transient axial length elongation during accommodation

Transient axial length elongation during accommodation is thought to be driven by the force applied to the equatorial choroid by ciliary muscle contraction, necessitating posterior pole elongation to maintain a constant ocular volume (Drexler *et al.*, 1998; Mallen *et al.*, 2006), as shown in Fig. 1.7. In support of this theory, Croft *et al.* (2013b) reported that the equatorial choroid and retina moved centripetally with Edinger-Westphal stimulated accommodation in Rhesus monkeys. Indeed, van Alphen (1961; 1986) suggested the ciliary muscle and choroid behave as a single elasto-musculous sheet around the eye; moulding the sclera to accommodate ciliary muscle tonus.

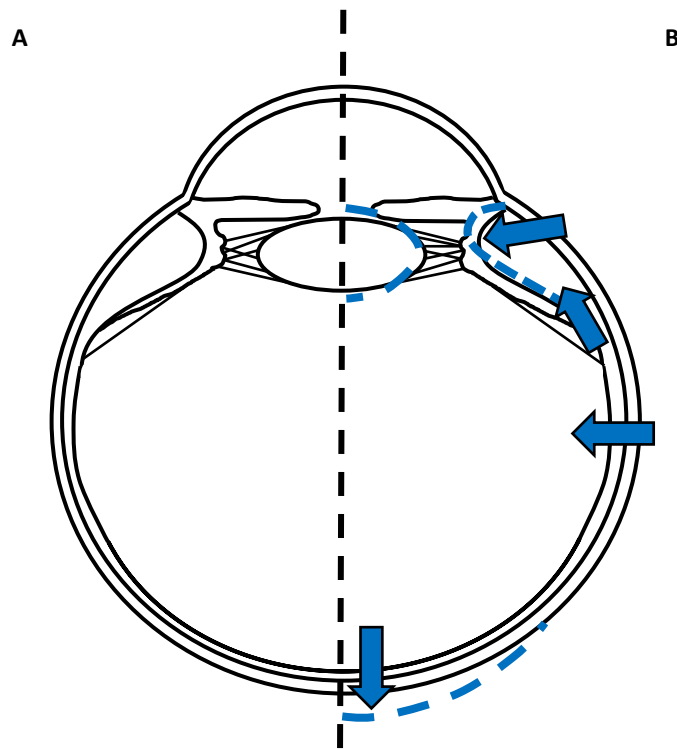


Fig. 1.7. Schematic transverse cross-section of the human accommodative apparatus in an disaccommodated (A) and accommodated state (B). During accommodation, the ciliary muscle moves forward and inward (as shown by the dashed blue lines and arrows), pulling the equatorial choroid inwards and resulting in posterior pole elongation in order to maintain a constant ocular volume.

Hitherto, studies reporting transient axial length elongation in response to short periods of accommodation in young adults have failed to reach a consensus as to whether myopes experience a larger degree of axial length elongation than emmetropes (Drexler *et al.*, 1998; Mallen *et al.*, 2006; Read *et al.*, 2010a; Woodman *et al.*, 2011; Woodman *et al.*, 2012). However, posterior choroidal thinning in response to accommodation is evident in myopic patients only (Woodman *et al.*, 2012). The degree of choroidal thinning observed did not entirely offset the magnitude of the axial length elongation observed. Nonetheless the choroid thins as myopia progresses (Ikuno and Tano, 2009), therefore frequent transient choroidal thinning during accommodation could precipitate a permanent myopic shift in refraction.

Choroidal (van Alphen and Graebel, 1991; Ugarte *et al.*, 2006) and scleral (Friberg and Lacey, 1988; Pallikaris *et al.*, 2005) stiffness has been reported to increase with age, therefore potentially muting transient axial length elongation during accommodation. Nevertheless, the relocation of

the anterior uveal tract post-cataract surgery to a more youthful position suggests the choroid may retain its elasticity with age (Strenk *et al.*, 2010) and therefore it is reasonable to assume that transient axial length changes are still evident during presbyopia. In support of this theory, Hollins (1974) demonstrated transient axial elongation during accommodation in one advanced presbyopic patient. However, a larger sample is required to investigate whether this mechanism is still evident during incipient presbyopia. Longitudinal follow-up is required to reveal whether the mechanism is attenuated with age and if the patients who experience a myopic shift in refraction display the largest transient axial length changes during accommodation.

1.4.5b Central hypermetropic blur during accommodation

Central hypermetropic defocus imposed at near due to a lag of accommodation has also been suggested as a possible cause for the connection between myopic progression and near work (Hung *et al.*, 1995; Gwiazda *et al.*, 2004). Indeed, artificially imposed hypermetropic defocus has been shown to stimulate axial elongation in young monkeys (Hung *et al.*, 1995). Transient bidirectional axial length changes also occur in response to 60 minutes exposure to hypermetropic and myopic defocus in young human subjects, although no differences in axial length and choroidal thickness changes between emmetropes and myopes have been observed (Read *et al.*, 2010a). Myopic children with high accommodative lags corrected with progressive addition spectacle lenses demonstrate a significantly reduced rate of myopia progression when compared to myopes corrected with single vision lenses (Gwiazda *et al.*, 2004), supporting the theory that central hypermetropic defocus may stimulate myopia progression.

However, the link between larger lags of accommodation and myopia progression in adulthood has not been consistently shown (Abbott *et al.*, 1998; Rosenfield *et al.*, 2002). Nonetheless, it is plausible to expect that the degree of accommodative lag (and therefore hypermetropic defocus) at near will increase during incipient presbyopia due to the age-related reduction in amplitude of accommodation, therefore potentially increasing the risk of a myopic shift in refraction during incipient presbyopia. Longitudinal analysis according to average time spent working at near daily

is required to investigate the possible link between near work and myopia progression during incipient presbyopia.

1.4.6 Peripheral refraction changes during myopia development

In addition to central retinal blur, peripheral retinal blur has also been suggested to play an important role in myopia development and progression (Hoogerheide *et al.*, 1971; Millodot, 1981; Mutti *et al.*, 2000; Seidemann *et al.*, 2002; Schmid, 2003; Wallman and Winawer, 2004; Atchison *et al.*, 2005a; Atchison *et al.*, 2006; Mutti *et al.*, 2007; Chen *et al.*, 2010; Sankaridurg *et al.*, 2010; Taberner *et al.*, 2011).

Peripheral retinal blur is typically measured by calculating mean spherical equivalent (MSE, derived in equation 1.1), orthogonal astigmatism (J_{180} , derived in equation 1.2) and oblique astigmatism (J_{45} , derived in equation 1.3) vectors from off-axis sphere(S)/cylinder(C)/axis(θ) refraction measurements (Thibos *et al.*, 1997). Peripheral refraction is thought to be influenced by a combination of off-axis optical aberrations and the curvatures of the crystalline lens surfaces and retinal contour (Hoogerheide *et al.*, 1971; Millodot, 1984; Guirao and Artal, 1999; Schmid, 2003; Verkicharla *et al.*, 2012) and is not thought to change significantly with age (Atchison *et al.*, 2005a; Chen *et al.*, 2010) or accommodation (Calver *et al.*, 2007; Davies and Mallen, 2009; Taberner and Schaeffel, 2009a).

$$MSE = S + \frac{C}{2} \quad 1.1$$

$$J_{180} = -C \cos(2\theta)/2 \quad 1.2$$

$$J_{45} = -C \sin(2\theta)/2 \quad 1.3$$

Animal research has shown that defocus or deprivation imposed on the peripheral retina can affect axial growth of the macula region, and *vice versa* (Wallman *et al.*, 1987; Smith *et al.*, 2005; 2007; Huang *et al.*, 2009; Smith *et al.*, 2009; 2013), suggesting a mutually dependent relationship between the central and peripheral retina. Wallman and Winawer (2004) hypothesised that the

mechanism for axial length elongation in human eyes could be driven by homeostatic signals from the hypermetropic peripheral retina, overriding the signals from the emmetropic/myopic central retina to cease elongation. Although the density of neurons is much greater in the central retina, the peripheral retina represents a much larger area and, overall, there are a larger number of neuron signals produced by the peripheral retina. In addition, Ho *et al.* (2012) measured greater electrical retinal response to defocus imposed on the paracentral retina when compared with defocus imposed on the central retina. Therefore, due to spatial summation, the overall peripheral signal will drive axial length elongation until a comparative area of the central retina becomes myopic and counteracts the peripheral signal. Wallman and Winawer (2004) proposed that conventional myopic spectacle correction would prevent the retina from reaching this balance by maintaining a clear image at the fovea, and thus axial length elongation would continue.

However, under-correction of myopia with spectacle lenses (imposing myopic defocus centrally and reducing the area of peripheral retina exposed to relative hypermetropia) increases the rate of myopia development in children when compared to children who are optimally corrected (Chung *et al.*, 2002; Adler and Millodot, 2006). Furthermore, correcting peripheral refraction in spectacle lenses has proved unsuccessful in reducing the rate of myopia progression (Sankaridurg *et al.*, 2010), possibly due to difficulties ensuring the peripheral refractive error is corrected during eye movements. Nevertheless, dual focus contact lens designs, correcting central myopia and reducing peripheral hypermetropia, have produced promising results. When compared to single vision contact lenses, dual focus contact lens designs appear to reduce myopic progression in children (Anstice and Philips, 2011; Sankaridurg *et al.*, 2011), therefore supporting Wallman and Winawer's (2004) theory.

Multiple authors have reported that horizontal relative peripheral refraction is more hypermetropic and myopic in myopic and emmetropic eyes, respectively (Hoogerheide *et al.*, 1971; Millodot, 1981; Mutti *et al.*, 2000; Seidemann *et al.*, 2002; Schmid, 2003; Logan *et al.*, 2004;

Wallman and Winawer, 2004; Atchison *et al.*, 2006; Mutti *et al.*, 2007; Davies and Mullen, 2009; Berntsen *et al.*, 2010; Chen *et al.*, 2010; Sankaridurg *et al.*, 2010; Tabernero *et al.*, 2011). However, great variability has been observed within refractive error groups (Verkicharla *et al.*, 2012; Ehsaei *et al.*, 2013) and beyond 45° eccentricity, the relative peripheral profile has been reported to shift from myopia to hypermetropia in some patients (Gustafsson *et al.*, 2001). No consensus has been reached regarding the dependency of vertical peripheral refraction on refractive error group classification (Seidemann *et al.*, 2002; Schmid, 2003; Atchison *et al.*, 2006; Marthur *et al.*, 2009; Berntsen *et al.*, 2010; Chen *et al.*, 2010; Ehsaei *et al.*, 2011).

Regarding myopia onset, longitudinal research of young pilots aged 18 years and above found that emmetropic and hypermetropic patients with horizontal relative peripheral hypermetropia had a 40% chance of developing myopia within the next few years, whereas those with peripheral myopia had a 4% chance (Hoogerheide *et al.*, 1971). Later studies involving children found that relative peripheral hypermetropia did not have a consistent influence on the risk of myopia onset (Mutti *et al.*, 2007; Mutti *et al.*, 2011; Sng *et al.*, 2011), leading to the suggestion that relative peripheral hypermetropia is a consequence of, rather than determinant of, a myopic central refractive error (Mutti *et al.*, 2007; Mutti *et al.*, 2011; Sng *et al.*, 2011; Verkicharla *et al.*, 2012).

Irrespective of the role peripheral refraction may have in the onset and development of central refractive development, it is informative when considering how the eye grows. Researchers have predicted the shape of the retinal contour based on peripheral refraction values, suggesting that relative peripheral myopia, as typically observed in emmetropic and hypermetropic eyes, indicates an oblate retinal contour (see Fig. 1.5), whereas relative hypermetropia, as commonly observed in myopic eyes, indicates a prolate retinal contour (Mutti *et al.*, 2000). The retinal contour has been reported to become progressively more prolate and irregular with myopia progression (Mutti *et al.*, 2000; Logan *et al.*, 2004; Tabernero and Schaeffel, 2009b), typically with the longest eye length in the nasal retina (Logan *et al.*, 2004). Atchison *et al.* (2006) and Marthur *et al.* (2009) found that the degree of horizontal relative peripheral hypermetropia in myopic

adults did not continue to increase significantly in myopic eyes > 4.00 D. Therefore, it is possible that myopic ocular growth occurs in two stages; after the retinal contour has become maximally prolate (Fig. 1.8C,D), uniform global or equatorial expansion of the eye (Fig. 1.8A,B) is responsible for further myopic progression.

However, the degree of correlation between the retinal contour measured indirectly (by peripheral refraction) and directly (by measuring peripheral eye length) decreases with increasing eccentricity, presumably due to the influence of anterior segment optics affecting peripheral refraction measurements more significantly in the periphery (Ehsaei *et al.*, 2013). Therefore direct measurements of peripheral eye length are preferable to investigate how the retinal contour changes during refractive development.

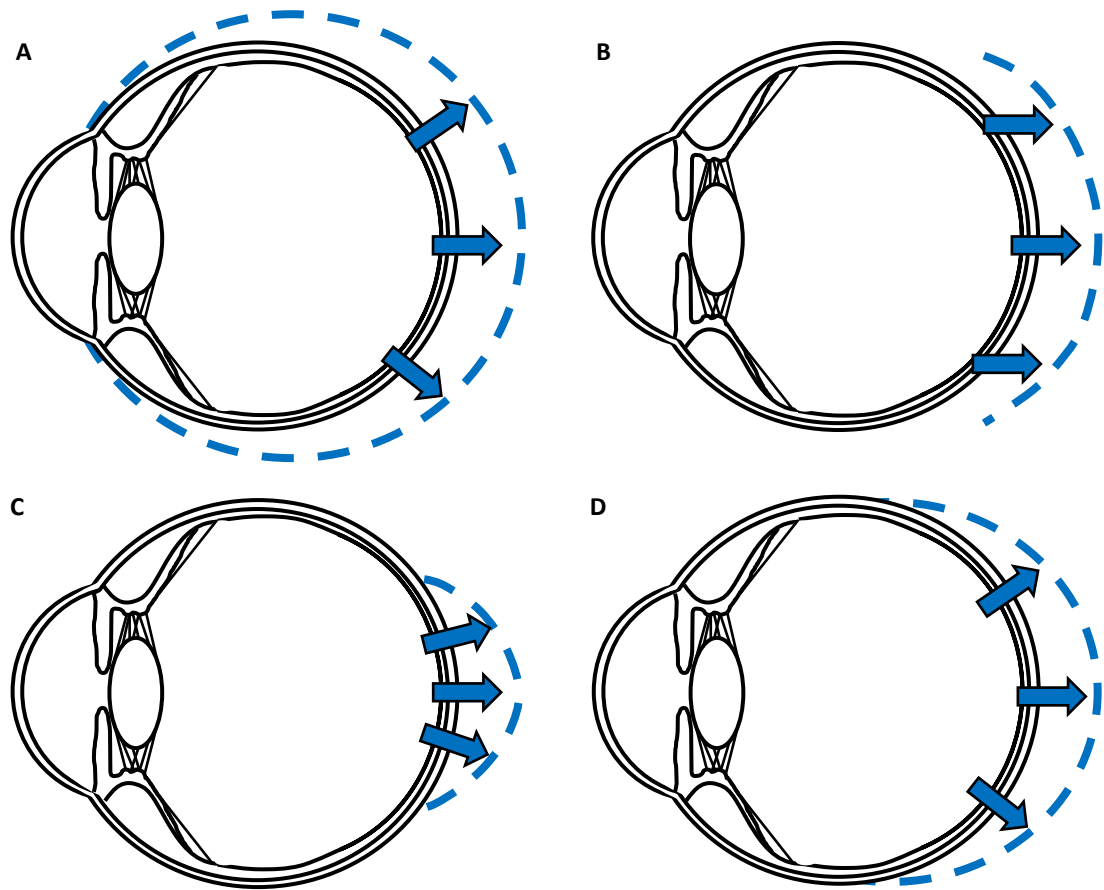


Fig. 1.8. Simplified diagrams representing possible patterns of myopic eye growth. The dashed blue lines and arrows represent the possible growth patterns of global expansion (A), equatorial expansion (B), posterior pole expansion (C) and axial expansion (D). Relative peripheral refraction and retinal contour would be expected to remain stable in models A and B, whereas relative peripheral refraction would become more hypermetropic in the periphery and the retinal contour would become steeper in C and D. Redrawn from Strang *et al.* (1998) and Verkicharla *et al.* (2012).

1.4.7 Retinal contour changes during myopia development

Longitudinal research measuring peripheral eye length in children aged 7 to 11 years old reported a correlation between shorter temporal eye lengths (steeper temporal retinal curvatures) and myopia progression (Schmid, 2011). Schmid (2011) therefore suggested that the shape of the retinal contour plays an important part in triggering eye growth. Schmid also observed that relative peripheral eye length did not change significantly despite axial length elongation. Similar to the results of peripheral refraction studies (Mutti *et al.*, 2000; Logan *et al.*, 2004; Tabernero

and Schaeffel, 2009b), peripheral eye length measurements have indicated that the horizontal retinal contour is oblate in shape, and becomes progressively more prolate and irregular with myopia progression (Schmid, 2003; Gray *et al.*, 2005; Schmid, 2011; Ehsaei *et al.*, 2013), typically with the longest eye lengths measured in the nasal retina (Ehsaei *et al.*, 2013). However, peripheral eye length is likely to be overestimated by optical biometers due to oblique transmission through the crystalline lens (Atchison and Charman, 2011; Verkicharla *et al.*, 2012). A semi-customised eye model has been developed to correct peripheral eye length measurements for inaccuracies arising from oblique transmission through the eye by incorporating individual patient's corneal topography and applying a fixed crystalline lens shape model (Faria-Ribeiro *et al.*, 2014). However, even the corrected retinal contour values still provide only an estimate of the true retinal contour because the actual crystalline lens curvatures and refractive index distribution of each patient is not incorporated into the model.

In order to avoid optical distortion and capture data from cross-sections of the entire eye simultaneously, more recent research has utilised MRI to analyse eye shape. MRI data also provide a more accurate indication of the asphericity of the posterior eye because it maps a much larger area than can be profiled using current optical biometry methods. Moreover, optical biometry measures of eye length typically include the retina thickness (to the retinal pigment epithelium), whereas MRI studies can isolate the posterior border between the vitreous and the anterior retinal surface. However, the spatial resolution provided by MRI scanners is typically poorer (0.15 to 1.00 mm) than can be achieved by optical biometers (Sheppard *et al.*, 2011; Gilmartin *et al.*, 2013).

In accordance with previously discussed findings (McBrien and Millodot, 1987; Grosvenor and Scott, 1991; Bullimore *et al.*, 1992; Grosvenor and Scott, 1993; McBrien and Adams, 1997; Mutti *et al.*, 2012), Atchison *et al.*'s (2004) MRI data reported that the axial length of myopic eyes is elongated compared to emmetropic eyes. Furthermore, the height and width of myopic eyes is also greater than measured in emmetropic eyes, although the disparity is not as significant as the

difference between axial lengths (Deller *et al.*, 1947; Wang *et al.*, 1994; Atchison *et al.*, 2004). Deller *et al.* (1947) reported that axial length increased at twice the rate of the changes in height and width in the myopic eye. It is not surprising that the dimensions of height and width are more similar between emmetropic and myopic eyes than axial length measurements because, as previously mentioned, the crystalline lens fails to thin (thought to be instigated by crystalline lens stretching as a result of equatorial eye growth) sufficiently to compensate for axial length elongation in myopia (Zadnik *et al.*, 1995; Mutti *et al.*, 2012). Indeed, Fischer *et al.* (2008) provided *in vivo* evidence that the mechanism promoting equatorial growth of the chick eye is independent of the process triggering axial length elongation.

Regarding the horizontal vitreous chamber shape measured by MRI devices, an oblate to prolate growth pattern has been observed across a large sample of children from early infancy to adolescence (Ishii *et al.*, 2011; Lim *et al.*, 2011). Whereas, emmetropic and myopic eyes have been reported to be oblate in adulthood, albeit to a lesser degree in myopic eyes (Cheng *et al.*, 1992; Atchison *et al.*, 2005b; Gilmartin *et al.*, 2013), thus indicating myopic vitreous chamber shape approximates a sphere and may be best represented by a global or equatorial expansion myopic eye growth model (Fig 1.8A,B). However, similarly to peripheral refraction measurements, considerable intersubject variation has been reported (Atchison *et al.*, 2004; Atchison *et al.*, 2005b; Ehsaei *et al.*, 2013) and one myopic growth model (Fig. 1.8) has not been able to classify the resultant vitreous chamber shape of all myopic patients adequately (Cheng *et al.*, 1992; Atchison *et al.*, 2004; Lim *et al.*, 2011). Nevertheless, Gilmartin and colleagues (2013) suggested that the more spherical vitreous chamber shape observed in myopic eyes when compared to emmetropic eyes may act as a biomechanical limitation, restricting further axial elongation and myopia progression. Therefore, significantly oblate myopic eyes may be at greater risk of myopic progression than myopes with less oblate shapes. Prolate vitreous chamber shapes are likely to be specific to high myopia and pathological myopia only (Moriyama *et al.*, 2011; Moriyama *et al.*, 2012; Ohno-Matsui *et al.*, 2012).

Gilmartin *et al.* (2013) also remarked that symmetry was observed between the two eyes of each patient for the vertical (superior to inferior) vitreous chamber shape, whereas asymmetry was reported with respect to the horizontal (temporal to nasal) vitreous chamber shape between the eyes. However, the profiles were reversed for temporal to nasal quadrants, that is to say, the right temporal and left nasal quadrants and left nasal and right temporal quadrants were symmetrical. Therefore, Gilmartin *et al.* (2013) hypothesised that binocular growth may be coordinated by signals produced beyond the optic chiasm, where the nasal and temporal fibres from each eye partially decussate so that the fibres in the left optic tract represent the right visual field and the fibres in the right optic tract represent the left visual field. Alternatively, Gilmartin and colleagues (2013) suggested that the reported horizontal growth pattern might be controlled by the diffusion gradient of signalling molecules (Wolpert, 2011).

Regardless of whether the shape of the posterior eye triggers axial length growth (Schmid, 2011), considering the pattern of eye growth longitudinally is important to understand how myopic eye growth may differ from emmetropic eye growth. To-date, limited research has longitudinally investigated the changes in retinal contour and/or vitreous chamber shape during myopia progression and the period of incipient presbyopia has been neglected.

1.5 Summary

The literature is in agreement that the development of presbyopia is likely to be multifactorial, involving a number of interdependent structures (Gilmartin, 1995), with perhaps the most significant input arising from the increase in crystalline lens stiffness with age (Heys *et al.*, 2004; Glasser, 2008). However, despite at least a century of investigation, the exact mechanisms of accommodation and presbyopia remain equivocal. Furthermore, the origin of the putative myopic shift in refraction during incipient presbyopia is unknown (Pointer and Gilmartin, 2011). Review of current research has highlighted the gap in research over the incipient presbyopic years, and particularly the need for longitudinal data over this period to fully investigate the optical and structural ocular changes that occur.

1.6 Aims of thesis

The aim of this thesis is to document the *in vivo* optical and structural ocular changes that occur during incipient presbyopia over a longitudinal period using the latest imaging techniques. Changes in accommodative dynamics will also be profiled during this phase of presbyopia. In particular, the possible causes of the putative myopic shift in refraction during incipient presbyopia will be explored.

CHAPTER 2

Instrumentation and techniques

2.1 Introduction

The following chapter outlines the technical specification and operation of instruments appropriate for profiling optical and structural ocular changes. Furthermore, bespoke instrument attachments and commissioned software developed for ophthalmic research and analysis in the subsequent chapters are also described.

2.2 Objective refraction

Autorefractors were first introduced over 40 years ago to provide an objective measurement of ocular refraction (Bullimore *et al.*, 2000). Autorefraction now forms part of the pre-screening service in most general optometric practices and is often used as a starting point for refraction by optometrists (Mallen *et al.*, 2001; Sheppard and Davies, 2010b). However, despite poor patient satisfaction gleaned from prescribing the autorefraction result (Bullimore *et al.*, 1996; Strang *et al.*, 1998), autorefraction is more repeatable than subjective refraction and is therefore more appropriate for research studies investigating refractive error progression (Bullimore *et al.*, 1998; Walline *et al.*, 1999).

2.2.1 Grand Seiko WAM-5500 Auto Ref/Keratometer

The Grand Seiko WAM-5500 Auto Ref/Keratometer (Grand Seiko Co. Ltd., Hiroshima, Japan) is a binocular open-field device, which can measure refraction, pupil size and keratometry. The binocular open-field design minimises instrument-induced myopia triggered by proximal accommodation, which is typical of closed-view devices (Hennessy, 1975; Smith, 1983; Rosenfield and Ciuffreda, 1991; Sheppard and Davies, 2010b; Section 1.2.4c). The instrument has been previously described and validated by Sheppard and Davies (2010b).

A 5.6 inch colour monitor facilitates accurate instrument alignment and allows patient fixation to be monitored throughout data acquisition. Objective refraction is calculated by multiple meridian

digital analysis of an infra-red measurement ring, which is reflected off the retina and brought into focus by an internal motorised lens rack. The WAM-5500 autorefractor can measure refraction in the range of ± 22.00 DS and ± 10.00 DC in increments of 0.01, 0.12 or 0.25 D for power and increments of 1° for cylindrical axis. The intrasession repeatability, calculated from the standard deviation of 5 measurements acquired in 1 session, for the spherical component is ± 0.09 D, ± 0.14 D for the cylindrical component, ± 0.09 D for MSE, ± 0.07 D for J_{180} and ± 0.06 D for J_{45} (Sheppard and Davies, 2010b). The intersession bias for the spherical component is -0.04 ± 0.26 D, -0.07 ± 0.29 D for the cylindrical component, -0.07 ± 0.26 D for MSE, -0.04 ± 0.16 D for J_{180} D and -0.01 ± 0.14 D for J_{45} . The difference between WAM-5500 objective refraction and subjective refraction is $+0.04 \pm 0.41$ D for the spherical component, -0.10 ± 0.34 D for the cylindrical component, -0.01 ± 0.38 D for MSE, 0.04 ± 0.17 D for J_{180} and 0.00 ± 0.15 D for J_{45} (Sheppard and Davies, 2010b). Sheppard and Davies (2010b) found that the differences between objective and subjective refraction were statistically significant for the cylindrical component and J_{180} only, however these were not clinically significant differences. Similarly to other autorefractor instruments, the WAM-5500 underestimates hypermetropia in young adults, possibly due to the ability of young adults to use their large amplitude of accommodation in order to clearly focus the target (Mallen *et al.*, 2001; Davies *et al.*, 2003; Sheppard and Davies, 2010b).

Pupil diameter is measured in 0.1 mm increments (minimum 2 mm), simultaneously with objective refraction, via automatic detection of the iris boundary and subsequent superimposition of a circle of best-fit. Keratometry is determined from analysis of the diameter of an additional infra-red ring reflected off the cornea, which is measured in 3 meridians, each separated by 60° . The instrument can measure corneal radii in the range of 5.0 to 10.0 mm (0.01 mm steps) and corneal power in the range of 33.75 to 67.50 D. Compared to Javal-Schiotz measurements, WAM-5500 keratometry values are steeper on average by -0.05 ± 0.07 mm and -0.06 ± 0.08 mm in the horizontal and vertical meridians, respectively (Sheppard and Davies, 2010b).

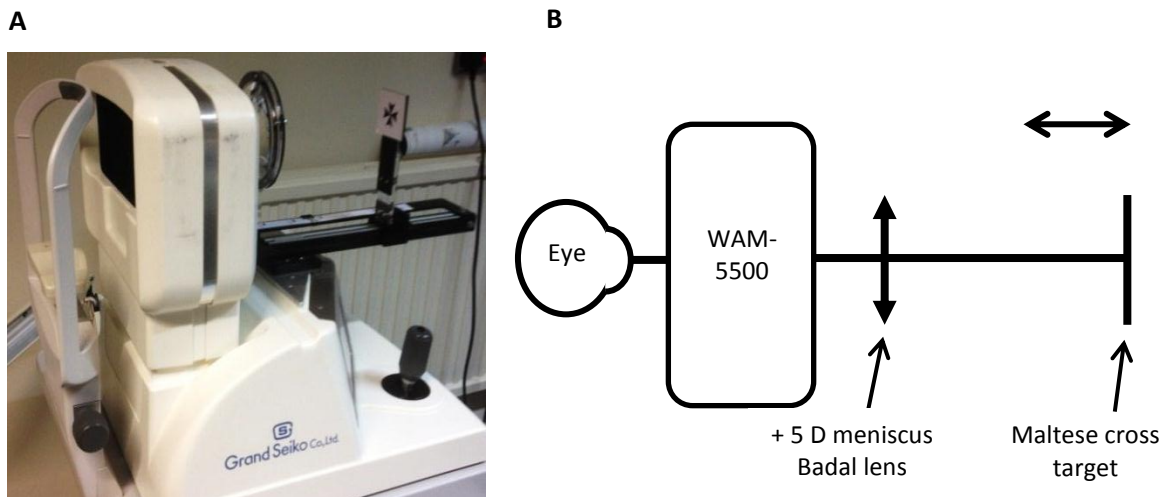


Fig. 2.1. A) Photograph of the WAM-5500 autorefractor with bespoke Badal lens system attachment. B) Schematic diagram of the WAM-5500. The Maltese cross target (subtending 6.8° horizontally and vertically) is positioned 20 cm from the Badal lens in order to stimulate 0.00 D of accommodation. The distance between the Badal lens and Maltese cross target can be reduced to stimulate accommodation.

2.2.1a Dynamic mode

In addition to conventional static autorefraction capabilities and similarly to the Shin-Nippon SRW-5000 autorefractor (Grand Seiko Co. Ltd., Hiroshima, Japan), the WAM-5500 can also operate in dynamic (Hi-Speed) mode, providing continuous recordings of MSE and pupil size (Wolffsohn *et al.*, 2001; Sheppard and Davies, 2010b; Win-Hall *et al.*, 2010). The operator only needs to press the button on the joystick once to initialise data collection and can monitor alignment and patient fixation by observing the 5.6 inch colour monitor display. Data are recorded at a rate of 5 Hz and can be exported via an RS-232 cable to an external computer using the WAM communication system (WCS-1) software (Sheppard and Davies, 2010b). No data are recorded if the instrument moves out of alignment with the eye or if the patient blinks. The root-mean-square of the refractive fluctuations (noise) of dynamic mode is 0.0050 ± 0.0005 D (Sheppard and Davies, 2010b). In order to measure dynamic accommodation, a Badal lens system with a moveable accommodative target can be mounted in front of the viewing window of the WAM-5500 autorefractor (Fig. 2.1) to allow different levels of accommodation to be stimulated

consecutively whilst ensuring the accommodative target appears to remain the same size and contrast (Wolffsohn *et al.*, 2003b).

2.3 Ocular biometry

Ocular biometry is the measurement of axial distances between ocular structures. Traditionally, A-scan ultrasonography was utilised routinely in hospital ophthalmology departments to calculate the power of an intraocular lens (IOL) for implantation following phacoemulsification of a cataract. However, 54% of suboptimal refractive outcomes in 584 patients following cataract surgery were due to inaccuracies in axial length measurements by ultrasound devices (Olsen, 1992). The remaining 8% and 38% were due to corneal power miscalculations and post-operative anterior chamber depth estimation errors, respectively (Olsen, 1992). Advances in biometric instrumentation have been driven by the necessity for more accurate power calculations of intraocular lenses.

With the advent of optical biometers approximately 20 years ago, acoustic biometers have largely been superseded in hospital practice and research (Tehrani *et al.*, 2003). Optical biometers demonstrate enhanced accuracy, repeatability (Lam *et al.*, 2001), speed and improved post-operative refractive outcome in cataract patients (Rose and Moshegov, 2003) when compared with ultrasonography. Moreover, patients also prefer optical biometers due to their non-contact design, absence of requirement for topical anaesthesia and pupil dilation and eliminated risk of corneal infection when compared with ultrasound biometry devices (Findl *et al.*, 1998).

2.3.1 LenStar LS-900

The LenStar LS-900 (Haag-Streit, Koeniz, Switzerland) is a non-contact biometer (Fig. 2.2), which is able to simultaneously measure keratometry, central corneal thickness (CT), anterior chamber depth (ACD), crystalline lens thickness (LT) and axial length (AXL; the distance between the anterior cornea and the retinal pigment epithelium). The LenStar utilises coherent superposition of light waves produced by an 820 µm superluminescent diode with a Gaussian-shaped spectrum

(low coherence reflectometry) to measure optical path length, which is converted to a geometric length based on an average refractive index value for the eye (Read *et al.*, 2010a; O'Donnell *et al.*, 2011). The measurement range of the LenStar for CT is 0.30 to 0.80 mm, ACD is 1.50 to 5.50 mm, LT is 0.50 to 6.50 mm and AXL is 14.00 to 32.00 mm, all measured in 0.01 mm increments. The corneal radius of curvature measurement range is between 5.00 and 10.50 mm in 0.01 mm increments.

The LenStar has been described and validated by Buckhurst *et al.* (2009) and Cruysberg *et al.* (2010), who found measurement variation between the LenStar and the IOLMaster (Carl Zeiss Meditec, California, USA), A-scan applanation ultrasonographer OcuScan (Alcon Laboratories, Texas, USA) and Visante AS-OCT (Carl Zeiss Meditec, California, USA) to be statistically significant but not clinically significant. The standard deviation of the intra-session and inter-session bias, respectively, of the LenStar is ± 0.003 mm and ± 0.001 mm for corneal thickness, ± 0.051 mm and ± 0.013 mm for anterior chamber depth, ± 0.089 mm and ± 0.024 mm for crystalline lens thickness and ± 0.016 mm and ± 0.006 mm for axial length (Buckhurst *et al.*, 2009).

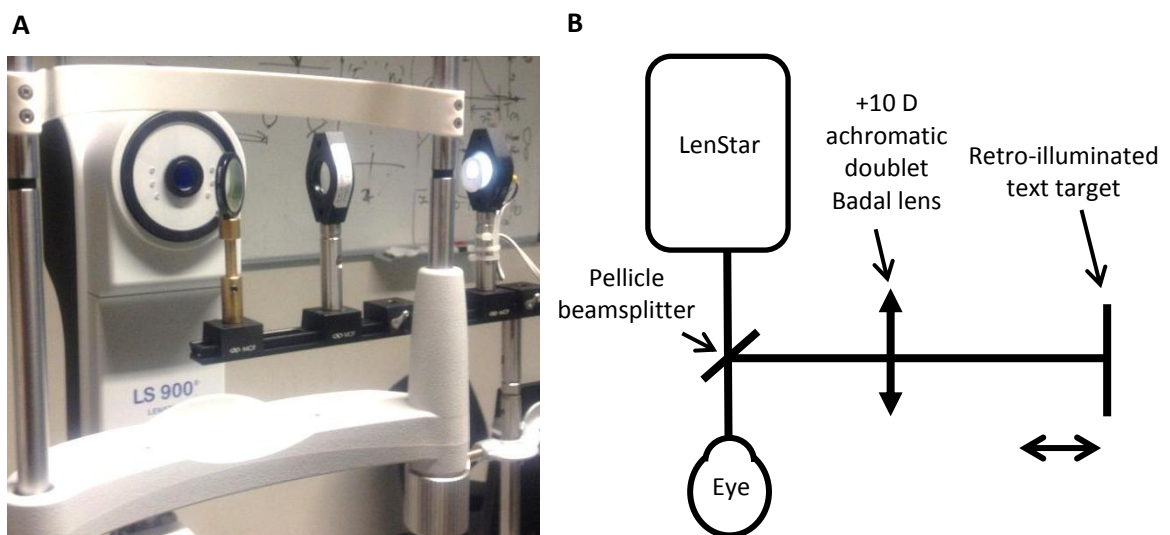


Fig. 2.2. A) Photograph of the LenStar LS 900 with Badal lens and pellicle beamsplitter bespoke attachment. B) Schematic diagram of the LenStar LS 900. The letter grid target (subtending 4.3° vertically and 3.4° horizontally) is positioned 10 cm from the Badal lens in order to stimulate 0.00 D of accommodation. The distance between the Badal lens and Maltese cross target can be reduced to stimulate accommodation.

In order to correct ametropia and stimulate a range of accommodative levels whilst measuring biometry, it is possible to mount a Badal optometer and a beamsplitter to the LenStar (Alderson *et al.*, 2012), as shown in Fig. 2.2. The bespoke attachment featured in Fig. 2.2 incorporates a mobile, retro-illuminated high contrast (90%) 5x5 grid of 0.8 logMAR letters and a 92% transmission, 8% reflection pellicle beamsplitter (Edmund Optics, New Jersey, USA). The pellicle beamsplitter is positioned at an angle of 45° to allow simultaneous viewing of the LenStar measurement beam and the perpendicular retro-illuminated text target.

2.3.1a Calculation of the axial length error induced during accommodation

The LenStar uses an average ocular refractive index to convert an optical path length (OPL) to a geometric axial length (Read *et al.*, 2010a). Therefore, to correct for the potential over-estimation of axial length due to an increase in crystalline lens thickness (LT) during accommodation (Atchison and Smith, 2004), the error (E) needs to be calculated for each patient to provide corrected axial length values. Atchison and Smith's (2004) calculations are based on the assumption that the true geometric length of the eye does not elongate during accommodation, therefore vitreous chamber depth (VCD) during accommodation is calculated by subtracting the accommodated anterior segment length (ASL) from the disaccommodated axial length ($AXL_{\text{disaccommodated}}$). The segmentation of the crystalline lens can be modified according to age (in years) using equations 2.1, 2.2 and 2.3, derived by Dubbelman *et al.* (2003), facilitating more accurate comparison between non-aged-matched groups. Anterior cortex thickness (ACT), nucleus thickness (NT) and posterior cortex thickness (PCT) thereafter are considered as separate structures in order to calculate optical path length (OPL) in equation 2.4. In line with previous publications (Read *et al.*, 2010a; Woodman *et al.* 2011; Woodman *et al.* 2012), equations 2.1 to 2.7 make no adjustment for changes in the relative proportions of the crystalline lens taken up by the anterior cortex, nucleus and posterior cortex thickness during accommodation because, despite the well-established thickening of the nucleus during accommodation (Brown, 1973; Koretz *et al.*, 1997; Dubbelman *et al.*, 2003), the exact nature of the change in refractive index

during accommodation is still not fully understood (Dubbelman *et al.*, 2005; Jones *et al.*, 2007; Hermans *et al.*, 2008). Optical path length (OPL) and the average refractive index of the disaccommodated eye (n_{ave}) are calculated using the refractive indices specified by Gullstrand's No. 1 (exact) eye with shell lens (equations 2.4 and 2.5). Equation 2.6 calculates the error (E), which is subtracted from the geometric axial length reported by the LenStar to provide corrected axial length values (equation 2.7).

$$ACT = LT * \frac{(0.51 + 0.012 * \text{age})}{(0.51 + 0.012 * \text{age}) + (2.11 + 0.003 * \text{age}) + (0.33 + 0.0082 * \text{age})} \quad 2.1$$

$$NT = LT * \frac{(2.11 + 0.003 * \text{age})}{(0.51 + 0.012 * \text{age}) + (2.11 + 0.003 * \text{age}) + (0.33 + 0.0082 * \text{age})} \quad 2.2$$

$$PCT = LT * \frac{(0.33 + 0.0082 * \text{age})}{(0.51 + 0.012 * \text{age}) + (2.11 + 0.003 * \text{age}) + (0.33 + 0.0082 * \text{age})} \quad 2.3$$

$$OPL = (CT * 1.376) + (ACD * 1.336) + (ACT * 1.386) + (NT * 1.406) + (PCT * 1.386) + (VCD * 1.336) \quad 2.4$$

$$n_{av} = \left[\left(\frac{CT}{AXL} \right) * 1.376 \right] + \left[\left(\frac{ACD}{AXL} \right) * 1.336 \right] + \left[\left(\frac{ACT}{AXL} \right) * 1.386 \right] + \left[\left(\frac{NT}{AXL} \right) * 1.406 \right] + \left[\left(\frac{PCT}{AXL} \right) * 1.386 \right] + \left[\left(\frac{VCD}{AXL} \right) * 1.336 \right] \quad 2.5$$

$$E = \frac{OPL}{n_{ave}} - AXL_{disaccommodated} \quad 2.6$$

$$AXL_{corrected} = AXL_{accommodated} - E \quad 2.7$$

2.3.1b Calculation of the axial length error induced by age

In addition, the aforementioned error calculation can also be applied to correct disaccommodated axial length for the age-related increase in crystalline lens thickness, particularly important in longitudinal biometry studies.

As previously discussed, equations 2.1 to 2.3 allow for age-related changes in the thickness of the anterior cortex, nucleus and posterior cortex. The current age of the patient at each longitudinal study visit must be inputted into equations 2.1 to 2.3 for subsequent calculation of the potential error at each study visit. Optical path length (OPL) and the average refractive index of the eye (n_{ave}) are calculated using the refractive indices specified by Gullstrand's No. 1 (exact) eye with shell lens (equations 2.4 and 2.5). The average refractive index of the eye calculated at baseline is used in equation 2.6 for all future study visits. Similarly to section 2.3.1a, vitreous chamber depth (VCD) at each visit is calculated by subtracting the new anterior segment length (ASL) from the baseline axial length ($AXL_{disaccommodated}$). The error (equation 2.6) is then subtracted from the new measured AXL (equation 2.7).

The corrected AXL values only represent an estimation of the induced error because the ocular average refractive index value the LenStar employs is proprietary information.

2.3.2 Visante Anterior Segment Optical Coherence Tomographer (AS-OCT)

The LenStar is analogous to A-scan ultrasonography, collecting one-dimensional data, whereas optical coherence tomography (OCT) instruments are comparable to B-scan ultrasonography, acquiring two-dimensional data. Similarly to the LenStar, OCT images are formed using low coherence interferometry. A near-infrared super-luminescent light-emitting diode beam scans the tissue and harnesses the backscattered light to form two-dimensional OCT images (tomograms) based on the magnitude and location of the reflected light from the microstructures within the tissue (Huang *et al.*, 1991). The near-infrared light source utilised in OCT instruments demonstrates good tissue penetration and is able to provide detailed images depicting each ocular layer. A real-time image is presented on the OCT instrument display in a false colour scale, snapshots of which can be acquired for future image analysis. A series of consecutive scans can build up a three-dimensional profile of tissue microstructures. Current OCT instruments available can scan the anterior segment and the posterior segment consecutively. Correction of ametropia and stimulation of accommodation is possible by adjustment of the internal Badal optometer.

The Visante AS-OCT (Carl Zeiss Meditec, California, USA) utilises a 1310 nm super-luminescent LED and produces a lateral resolution of 60 μm and axial resolution of 18 μm for anterior segment imaging (Dada *et al.*, 2007). The scanning speed of the Visante is 4000 axial scans per second, producing 8 image frames per second. The Visante has been validated by Dada *et al.* (2007), who found an excellent correlation between the anterior eye parameters (corneal thickness, anterior chamber depth and peripheral iridocorneal angles) measured using the Visante and P40 ultrasonographer (Paradigm Medical Industries, Utah, USA).

2.3.2a Ciliary muscle image acquisition and analysis

Localisation of the scleral spur is superior in OCT images compared to ultrasonography images due to sharper image definition (Dada *et al.*, 2007). Ciliary muscle image analysis relies on the scleral spur as a reference point for measurements, therefore more recent research has progressed to use OCT devices rather than ultrasonography instruments to image the ciliary muscle (Bailey *et al.*, 2008; Schultz *et al.*, 2009; Sheppard and Davies, 2010a; Sheppard and Davies, 2011; Lossing *et al.*, 2012; Lewis *et al.*, 2012; Buckhurst *et al.*, 2013; Pucker *et al.*, 2013; Richdale *et al.*, 2013).

In order to image the full length of the ciliary muscle using the Visante, patients must avert their gaze to a point external to the central viewing window, because the iris blocks visualisation of the ciliary muscle in primary gaze. Fig. 2.3 shows a bespoke Badal lens system with a moveable Maltese cross target, attached to the forehead rest of the Visante to provide a steady peripheral fixation target, correct for ametropia and stimulate accommodation, as required. The attachment featured in Fig. 2.3 can be angled to the left or right sides of the Visante viewing window to image nasal and temporal ciliary muscle sections of each eye. The minimum level of horizontal eye movement required to view the temporal ciliary muscle of the right eye is 40° from the internal Visante target to ensure the peripheral target is unobstructed by the instrument casing. Fixating externally causes the Visante beam to be directed through the sclera, rather than the cornea, reducing optical distortion due to the flatter scleral plane. It is possible to acquire a complete

ciliary muscle section on high resolution corneal mode (Fig. 2.4), scanning an area of 10 mm in width and 3 mm in depth and resulting in higher magnification than anterior segment mode (scanning an area of 16 mm width and 6 mm in depth). High resolution corneal mode provides an axial resolution of 8 μm .

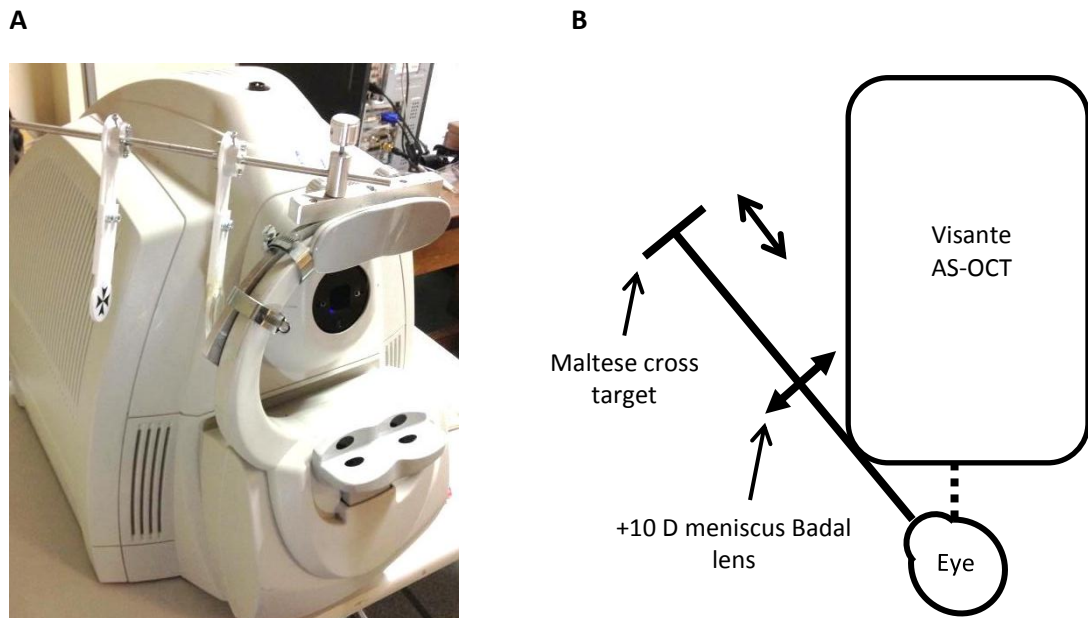


Fig. 2.3. A) Photograph of the Visante AS-OCT with a bespoke Badal lens system attached to the forehead rest. B) Schematic diagram of the Visante. The dashed line represents the path of the OCT beam through the sclera. The Maltese cross target (subtending 6.8° horizontally and vertically) is positioned 10 cm from the Badal lens in order to stimulate 0.00 D of accommodation. The distance between the Badal lens and Maltese cross target can be reduced to stimulate accommodation.

Similarly to ultrasound image analysis, calipers can be superimposed on the OCT images to extract data in millimetres, to two decimal places. During analysis, the Visante internal software outlines the boundaries of the ocular media and applies corrective refractive indices (n) to improve measurement accuracy ($n=1.000$ anterior to the cornea, $n=1.338$ to the cornea, $n=1.343$ posterior to the cornea). However, the Visante also fits the same refractive index adjustments to ciliary muscle images, with no option to alter the magnitude of the tiered refractive index corrections. Therefore, previous authors have applied a refractive index of 1.000 to the entire ciliary muscle image in order to remove erroneous correction factors (Bailey *et al.*, 2008; Schultz *et al.*, 2009;

Sheppard and Davies, 2010a; Sheppard and Davies, 2011). To provide data more closely associated with physiological *in vivo* ciliary muscle parameters, Sheppard and Davies (2010a; 2011) divided caliper measurement results by 1.382, which is the best estimate of the refractive index of the ciliary muscle based on *in vitro* bovine muscle tissue studies using confocal microscopy (Dirckx *et al.*, 2005) and *in vitro* human ventricular muscle studies using OCT (Tearney *et al.*, 1995). However, the refractive indices of the overlying sclera, as well as the ciliary muscle itself, need to be compensated for in order to ensure the magnitude of the measured ciliary muscle parameters are as accurate as possible. Furthermore, the ciliary muscle tissue is not accurately represented by the straight lines of the calipers because the scleral and ciliary muscle tissues are curved, to varying degrees in different patients. Therefore, to improve the accuracy of morphological assessment, data have been exported for analysis with external software (Kao *et al.*, 2011; Lossing *et al.*, 2012; Lewis *et al.*, 2012; Pucker *et al.*, 2013; Richdale *et al.*, 2013).

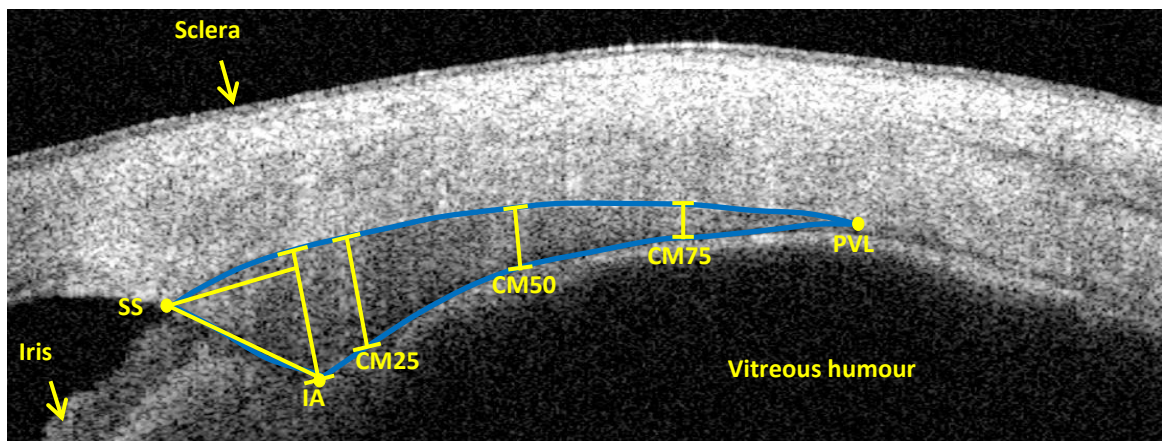


Fig. 2.4. Visante AS-OCT image of a human ciliary muscle section. The ciliary muscle is outlined in blue with superimposed yellow caliper measurements. PVL = posterior visible limit; SS = scleral spur; IA = inner apex; CM25, CM50, CM75 = thickness at 25%, 50% and 75% of total length (SS to PVL); maximum thickness = perpendicular distance from IA to sclera; anterior length = perpendicular distance from line of maximum thickness to SS.

2.3.2b Crystalline lens image acquisition and analysis

Images of the central crystalline lens can be acquired whilst the patient fixates the internal Visante target. Correction of ametropia and stimulation of accommodation is possible by

adjustment of the internal Badal optometer. The equatorial region of the crystalline lens where the zonules insert cannot be visualised because the iris is impermeable to the OCT beam. A white vertical line appears through the centre of the crystalline lens image to signify the OCT beam is directed through the centre of the tissue (Richdale *et al.*, 2008; Lehman *et al.*, 2009; Doyle *et al.*, 2013). In order to save images of the crystalline lens, the Visante's raw image mode must be selected because all other Visante modes require an overlying corneal or scleral surface to be visualised to fit the aforementioned internal refractive index corrections. Unfortunately, calipers cannot be superimposed on images obtained in raw image mode using the updated version of Visante software (2.0 and above), therefore data must be analysed with external software (Lehman *et al.*, 2009; Doyle *et al.*, 2013; Richdale *et al.*, 2013).

2.3.3 Development and validation of semi-automated software to analyse Visante AS-OCT crystalline lens images

In order to extract crystalline lens thickness from images captured by the Visante AS-OCT (versions 2.0 and above), previous authors have exported their data for analysis with external semi-automated software (Lehman *et al.*, 2009; Doyle *et al.*, 2013; Richdale *et al.*, 2013). Lehman *et al.* (2009) derived a pixel to millimetre conversion factor (1.000 pixel = 0.020 mm) by exporting and analysing an image with a superimposed 1 mm caliper line. A refractive index of 1.00 was applied to the image, however the image was not acquired in raw image mode and was exported as a JPEG file. In order to remove geometric distortions induced by the Visante, OCT images need to be exported in binary form (Kao *et al.* 2011). The file format crystalline lens images were exported in and the derivation of the pixel to millimetre conversion factor used by subsequent authors is unclear (Doyle *et al.*, 2013; Richdale *et al.*, 2013). The conversion factors utilised by Doyle *et al.* (1.000 pixel = 0.014 mm) and Richdale *et al.* (1.000 pixel = 0.016 mm) ostensibly also incorporate compensation for the refractive index of the crystalline lens (1.42 and 1.39 by Doyle *et al.* and Richdale *et al.*, respectively). Whereas, Lehman *et al.* (2009) applied a further correction factor to their data to convert the refractive index from 1.000 to 1.388.

To determine an appropriate pixel to millimetre conversion factor for crystalline lens binary files exported from the Visante with the version 3.0 software update applied, this study describes and validates custom-designed software developed in conjunction with the Aston University Engineering Department using Matlab R2012b (The MathWorks Inc., Massachusetts, USA).

2.3.3a Method

Visante AS-OCT crystalline lens imaging

Each patient was asked to wear an eye patch over their left eye and room lights were extinguished. To acquire OCT crystalline lens images, patients placed their chin and forehead against the left-sided Visante supports. Ametropia was corrected by adjustment of the internal Badal optometer and each patient was encouraged to focus on the internal star target throughout image acquisition. The alignment of each patient was adjusted in all three dimensions until the full thickness of the crystalline lens with the vertical white fixation line through the axis was visible, as shown in Fig. 2.5. Three consecutive images were acquired in raw image mode.

In preparation for analysis, crystalline lens images were exported as binary files and resized to 512 x 995 pixels. The semi-automated software measures the pixel thickness between two manually selected points inputted by the user. To ensure the maximum crystalline lens thickness (LT) is measured, the points where the white vertical fixation line (highlighting the centre of the crystalline lens) bisected the anterior and posterior crystalline lens surfaces were manually chosen, as shown in Fig. 2.5. The measured pixel crystalline lens thickness is not displayed during image analysis and is automatically exported to an Excel spreadsheet (Microsoft, Washington, USA), which allows the examiner to be masked to the results during data extraction.

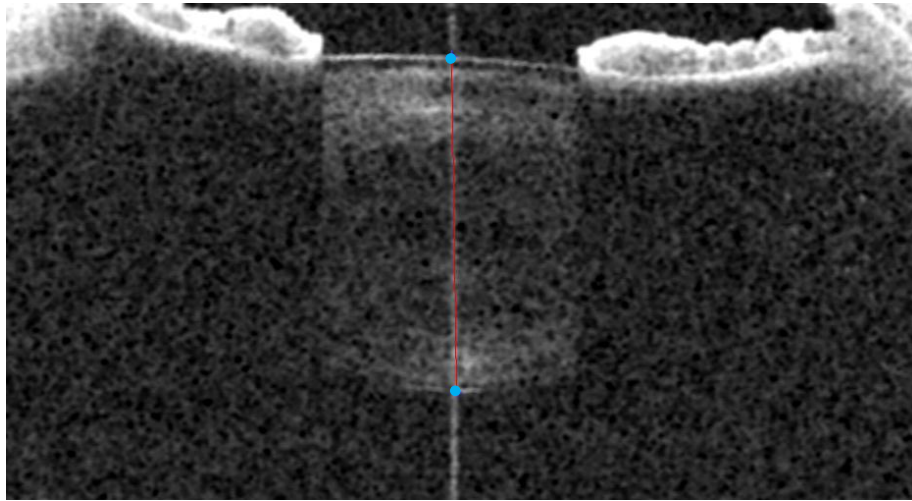


Fig. 2.5. A screenshot of the semi-automated software designed to extract LT from Visante AS-OCT images. The two blue spots indicate the points chosen by the examiner and the red line represents LT.

LenStar crystalline lens measurements

To ensure that Visante LT measurements are comparable to the results of other research (Koretz *et al.*, 1989; Smith *et al.*, 1992; Cook *et al.*, 1994; Glasser *et al.*, 1999; Strenk *et al.*, 1999; Dubbelman *et al.*, 2001a; Koretz *et al.*, 2001; Dubbelman *et al.*, 2003; Jones *et al.*, 2007; Atchison *et al.*, 2008; Richdale *et al.*, 2008; Doyle *et al.*, 2013; Richdale *et al.*, 2013), a pixel to millimetre conversion ratio is required. As mentioned in section 2.3.1, the LenStar is capable of measuring crystalline lens thickness simultaneously with corneal thickness, anterior chamber depth and axial length. Therefore, in order to derive a pixel to millimetre conversion factor, OCT crystalline lens pixel thickness values were compared with LenStar crystalline lens millimetre thickness data obtained on the same day.

Crystalline lens thickness was acquired by the LenStar biometer with a bespoke Badal lens system attachment (Fig 2.2). The attachment featured in Fig 2.2 incorporates a mobile, retro-illuminated high contrast (90%) 5x5 grid of 0.8 logMAR letters and a 92% transmission, 8% reflection pellicle beamsplitter. The pellicle beamsplitter is positioned at an angle of 45° to allow simultaneous viewing of the LenStar measurement beam and the perpendicular retro-illuminated text target (Alderson *et al.*, 2012). Each patient placed their head against the forehead and chin rests and focussed on the letter that appeared closest to the LenStar measurement beam throughout data

collection. Ametropia was corrected by adjusting the distance between the Badal lens and the letter target. The LenStar was moved into alignment with the patient's right eye, guided by the visual display on the attached laptop. The average of three crystalline lens measurements was used for analysis.

Statistical analysis

Linear regression analysis was performed to determine the equation of the best fit line and the value of the gradient was used as the pixel to millimetre conversion factor.

Additionally, the repeatability of crystalline lens OCT imaging and semi-automated software interpretation was determined. Crystalline lens images acquired from 10 patients on two separate occasions (less than 1 week apart) via the protocol described above, were analysed and compared. The bias of the crystalline lens thickness measurements was calculated from the mean difference between visits. To identify whether the level of bias was acceptable for clinical research, a paired t-test was used to determine whether the bias was significantly different from 0. The spread over which 95% of the bias data lies (limits of agreement, LoA) were calculated using equation 2.8.

$$LoA = bias \pm (1.96 * SD \text{ of differences}) \quad 2.8$$

In order to determine the errors arising from patient alignment, the crystalline lens of a single patient's right eye was imaged 10 times at 0.00 D accommodative stimulus during one appointment. The patient was asked to remove and reposition their chin and forehead between the acquisition of each image. In order to isolate the repeatability of the semi-automated software interpretation and analysis of a crystalline lens image, 1 image was analysed 10 times.

2.3.3b Results

Pixels to millimetre conversion factor

The gradient of the best fit line of data from the right eye of 46 patients (mean age 39.1 ± 3.2 years; mean spherical equivalent -1.17 ± 2.09 DS) in Fig 2.6 determined a conversion factor of 93.204 pixels per millimetre (1.000 pixel = 0.011 mm).

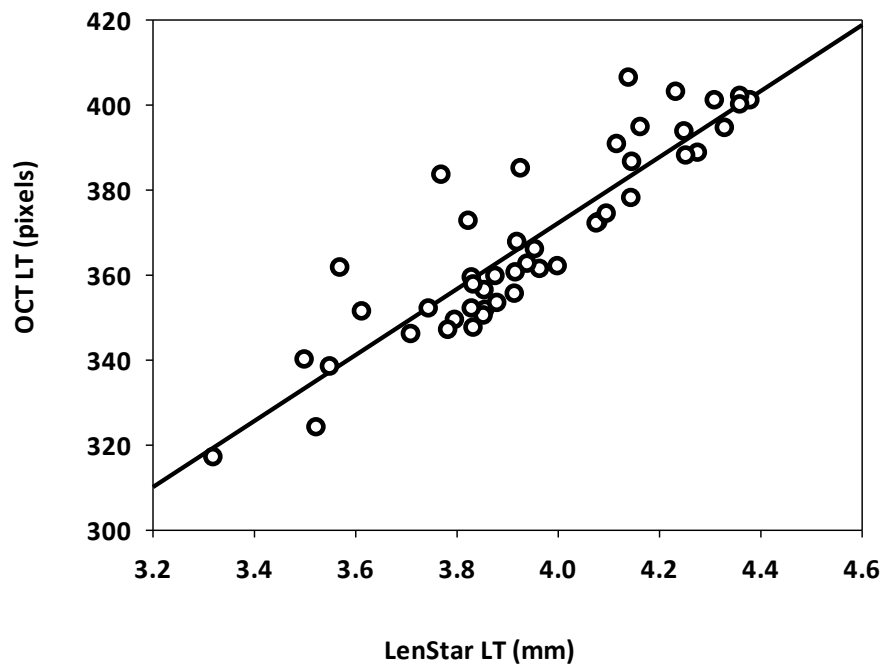


Fig 2.6. LenStar crystalline lens millimetre thickness plotted against Visante OCT crystalline lens pixel thickness data from 46 participants in one visit ($y=93.204x$; $R^2=0.7716$, $p<0.001$). One millimetre = 93.204 pixels.

Repeatability

The bias of the crystalline lens thickness measured in 10 patients (mean age 28.2 ± 5.0 years) across 2 visits, less than 1 week apart, was -0.007 ± 0.023 mm (limits of agreement -0.052 to 0.038 mm). The bias was not significantly different from 0 ($t=-0.836$, $p=0.425$). The mean OCT crystalline lens thickness obtained from imaging 1 patient realigned 10 times was 3.556 ± 0.006 mm. The mean result obtained from analysing 1 image 10 times was 3.550 ± 0.005 mm, suggesting approximately 83% of the difference encountered between visits is likely to be due to the inherent variability associated with manually selecting points for analysis with the bespoke software.

2.3.3c Discussion

Crystalline lens OCT image analysis warrants the development of external software due to the recent Visante software update (version 2.0 and above) disabling the facility to place calipers on crystalline lens OCT images for analysis. The semi-automated software described here is capable of accurately extracting crystalline lens thickness from Visante OCT images and provides repeatable measurements. Furthermore, image analysis is performed remotely to the Visante device on an external computer, ensuring that extracting data does not prohibit other researchers from simultaneously collecting data with the Visante.

The pixel to millimetre conversion ratio of 1.000 pixel: 0.011 mm was determined by comparing Visante AS-OCT pixel LT values to LenStar millimetre LT values (Fig. 2.6). The LenStar uses an average refractive index to convert an optical path length to a geometric length, however the magnitude of the refractive index utilised is proprietary information. Nevertheless, the refractive index used is likely to be less than the average refractive index of the crystalline lens (between 1.39 to 1.42), which was used by previous authors to determine their conversion factors (Doyle *et al.*, 2013; Richdale *et al.*, 2013). Indeed, the conversion factor derived here is smaller than reported by previous studies (Doyle *et al.*, 2013; Richdale *et al.*, 2013), however the conversion factors previously published should be interpreted cautiously due to unclear methods of image extraction and conversion factor derivation. Nevertheless, computing a pixel to millimetre conversion factor based on LenStar LT measurements means OCT and LenStar LT values can be used interchangeably. The LenStar cannot acquire crystalline lens thickness values in all patients (Buckhurst *et al.*, 2009), therefore the Visante can complement LenStar studies investigating biometry, and in those patients where the LenStar acquires all biometric parameters apart from LT, LT values can be supplemented by the Visante measurement.

The bias of LT measured in 10 patients (mean age 28.2 ± 5.0 years) across 2 visits was not significantly different from 0. The limits of agreement reported here (-0.052 to 0.038 mm) were narrower than observed by Doyle *et al.* (2013) for similar age groups reviewed on 2 occasions (18-

29 years: -0.08 to 0.10 mm; 30-39 years old: -0.13 to 0.09). Doyle *et al.* also examined non-cycloplegic eyes, however their analysis was based on 1 OCT image acquired at each visit. Lehman *et al.* (2009) has previously reported the standard deviation of the difference in LT acquired between visits drops by approximately 40% if 3 images are acquired instead of 1, which may account for the differences observed.

The mean OCT LT obtained from imaging 1 patient realigned 10 times (3.556 ± 0.006 mm) and 1 image analysed 10 times (3.550 ± 0.005 mm) suggests approximately 83% of the difference encountered between visits is likely to be due to the inherent variability associated with manually selecting points for analysis with the bespoke software. The remainder of the variation is likely to be due to differences in patient alignment and short-term fluctuations in the tissue parameters. Data were not acquired under cycloplegic conditions, therefore it is possible that the accommodation of the young patients fluctuated whilst viewing the internal 0.00 D target. However, fluctuations in accommodation are likely to represent less than 17% of the variability observed between visits and therefore are unlikely to have a significant impact on the overall repeatability of LT measurements. The impact will also decrease with age due to the decrease in amplitude of accommodation, particularly significant during presbyopia, as demonstrated by the narrowest limits of agreement reported by Doyle *et al.* (2013) in their groups over 50 years of age. Furthermore, the magnitude of any accommodation stimulated is likely to have been similar between the LenStar Badal optometer and Visante internal Badal optometer systems, as reported by Richdale *et al.* (2013), who demonstrated good agreement between Grand Seiko autorefractometer readings with a Badal optometer (synonymous to the LenStar set-up) and the PowerRefractor II (PlusOptix Inc, Florida, USA) results whilst simultaneously acquiring crystalline lens Visante AS-OCT images. Moreover, Badal optometers have been reported to produce similar refractive responses to free-space targets (Stark and Atchison, 1994), indicating that the enclosed Visante bowl is unlikely to have significantly affected the accommodative response and,

therefore, comparing the LT measurements between the two instruments to derive a pixel to millimetre conversion factor is valid.

Future versions of the software may be able to automate the process of measuring LT fully due to the good visibility of the anterior and posterior central crystalline lens surfaces and the high contrast between the crystalline lens borders and surrounding humours. The software could superimpose a series of vertical lines across the OCT image and plot the change in pixel intensity along each line. To define the crystalline lens borders, a 2nd order fourier series could be fitted to the intensity profile and differentiated. The location of the largest peak formed in the differentiated intensity profile corresponds to the point where the line bisects the crystalline lens boundary (the crossing point). This process could be repeated for the top and bottom of each line separately in order to determine crossing points of both the anterior and posterior crystalline lens boundaries on the OCT image. A 2nd order polynomial curve could then be fitted to the crossing points of each boundary and the largest vertical difference between the borders would represent the maximum LT. Furthermore, the radii of curvature of the anterior and posterior surfaces could also be extracted, pending appropriate corrections due to light refraction from the curved cornea and the crystalline lens itself (Dunne *et al.*, 2007; Kim *et al.*, 2009). Using this method, the internal boundaries of the nucleus may also be able to be isolated, creating a segmented profile similar to that observed in Scheimpflug photography (Sparrow *et al.*, 1986; Dubbelman *et al.*, 2003; Dubbelman *et al.*, 2005).

The software presented here is able extract accurate and repeatable measures of LT from Visante OCT crystalline lens images. Examiners require minimal training to operate the system due to the good clarity of the points required for manual selection. The OCT LT values are also suitable to be used interchangeably with LenStar LT measurements and can therefore be used to complement future LenStar biometry studies.

2.3.4 Development and validation of semi-automated software to analyse Visante AS-OCT ciliary muscle images

In order to mitigate the aforementioned limitations of analysing ciliary muscle OCT images using the internal Visante calipers, Kao *et al.* (2011) extracted the Visante OCT ciliary muscle image data in binary form for external analysis. A pixel to millimetre conversion ratio suitable for binary image analysis was calculated by acquiring OCT images of a series of steel spheres with known radii of curvature. A conversion factor of 132.35 pixels per millimetre was determined for high resolution corneal mode images, which was 5% greater than recommended by the manufacturer, therefore Kao *et al.* decided to use the manufacturer guideline of 128 pixels per millimetre.

Kao and colleagues determined the geometric and refractive image distortion present in Visante OCT binary image files by imaging an optical flat (Edmund Optics, Barrington, NJ) and human sclera with attached ciliary muscle tissue *in vitro*. Binary files were found to be free from geometric distortion and the refractive indices of the human sclera and ciliary muscle were found to be very similar to previously reported values (Tearney *et al.*, 1995; Dirckx *et al.*, 2005).

Due to the lack of uniformity of the ciliary muscle outline in AS-OCT images, Kao and colleagues' semi-automated software required manual localisation of the scleral spur before image analysis could commence. Once the sclera and ciliary muscle had been outlined, refractive indices of 1.41 and 1.38 were applied across the y-axis of the scleral and ciliary muscle image sections, respectively. The software produced vertical thickness measures at 1, 2 and 3 mm behind the scleral spur, maximum thickness (Fig. 1.6) and measured the cross-sectional area of the anterior ciliary body. However, the edge detection algorithms were unable to distinguish between the ciliary muscle and the pigmented ciliary epithelium, thus overestimating all ciliary muscle measurements.

To overcome the limitations of Kao *et al.*'s semi-automated algorithm and to address concerns of the subjectivity of identifying the posterior end point of the ciliary muscle (Bailey, 2011), bespoke software was developed in conjunction with the Aston University Engineering Department for use

in the following experimental chapters. The aim of this study is to describe and validate the commissioned semi-automated software and to compare data extracted by the software and the internal Visante calipers. The impact of the addition of a soft contact lens is also determined to establish whether a separate correction factor for contact lenses should be employed.

2.3.4a Method

Semi-automated software analysis of artificial ciliary muscle sections

To ensure the semi-automated software was capable of appropriately applying refractive index corrections and accurately measuring orthogonal and oblique parameters, a series of custom-made rigid gas-permeable lenses (No. 7 Contact Lens Laboratory Ltd, Hastings, UK) of known dimensions were imaged by the Visante OCT. One silicon-acrylate lens ($n = 1.48$) simulated the sclera (L_1 in Fig. 2.7) and 5 fluoro-polymer lenses ($n=1.44$) of varying thickness (0.3, 0.45, 0.6, 0.75, 0.9 mm) each simulated the ciliary muscle (L_2 in Fig. 2.7). Each of the ciliary muscle lenses were of constant thickness. The total diameter of the sclera lens was 10 mm and each ciliary muscle lens was 6 mm. The radius of curvature of the lenses was 12 mm. A ciliary muscle lens and the sclera lens were positioned together, as shown in Fig. 2.7, for image acquisition. For each of the 5 ciliary muscle and sclera lens combinations, 10 OCT images were acquired and exported in binary form.

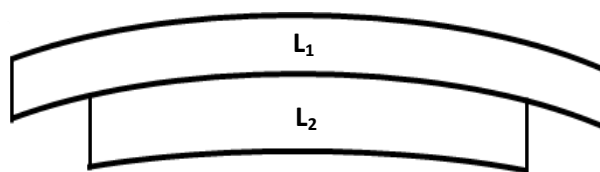


Fig. 2.7 Schematic diagram of two rigid gas-permeable lenses designed to simulate the sclera (L_1) and the ciliary muscle (L_2).

For this exercise only, the ciliary muscle software was adapted to allow 3 points across each of the lens boundaries to be manually selected for subsequent automated polynomial curve (order 2) fitting. The software determines the length of the ciliary muscle lens as the straight-line distance between the first and third points plotted across the scleral/ciliary muscle boundary, therefore the edges of the lens were always picked, as shown in Fig. 2.8. The thicknesses of the ciliary

muscle lenses were measured at 25, 50 and 75% across the lens diameter. Each thickness measurement was measured perpendicular to the scleral/ciliary muscle boundary curve, as shown by the red lines in Fig. 2.8. The refractive indices of the scleral and ciliary muscle lenses (1.48 and 1.44, respectively) were inputted to allow the program to compensate the measurements. All measurements were exported directly to an Excel spreadsheet, masking the examiner to the results during data extraction. The diameter and thickness measurements of the ciliary muscle lenses were also measured 10 times by Vernier calipers and compared to the results produced by the software. The bias of the measurements was calculated from the mean difference between the two techniques. To identify whether the level of bias was acceptable for clinical research, a paired t-test was used to determine whether the bias was significantly different from 0. The spread over which 95% of the data lie (limits of agreement, LoA) was calculated using equation 2.3.

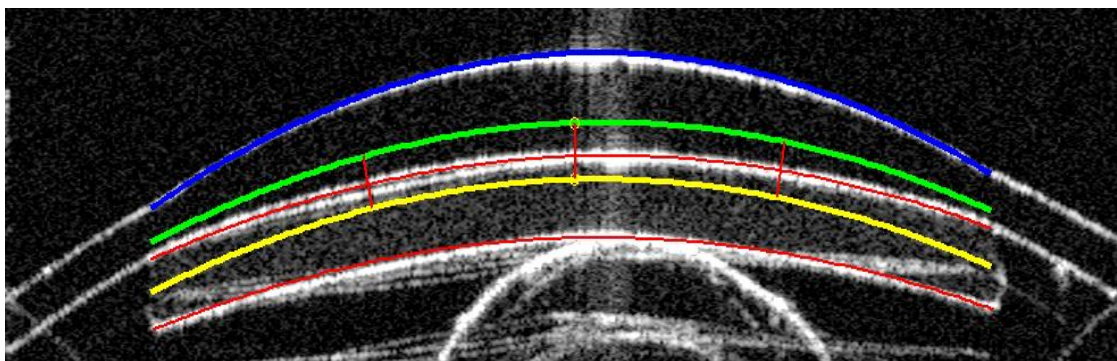


Fig. 2.8. Screenshot from the semi-automated ciliary muscle software adapted for validation using rigid gas-permeable lenses. The lenses were pushed together with a screw, seen at the bottom of the OCT image. The red curves represent the ciliary muscle polynomial curves fitted by the software and the yellow and green lines show the adjustment made for the refractive indices of the lenses. The blue curve highlights the air/lens boundary. The red vertical lines measure the thickness at 25, 50 and 75% across the lens diameter.

Repeatability of semi-automated software analysis of human ciliary muscle

Additionally, the repeatability of human ciliary muscle OCT imaging and semi-automated software interpretation was determined by imaging and analysing the temporal ciliary muscle of 10 patients during two separate appointments, less than 1 week apart. In order to acquire images of

the right eye temporal ciliary muscle for analysis, the left eye of each patient was occluded and ametropia was corrected by adjusting the position of the target within the Badal lens system (Fig. 2.3). Patients were initially aligned in high resolution corneal mode whilst viewing the internal Visante target, and once the vertical white fixation line was observed through the centre of the OCT image, patients were asked to rotate their eyes, with minimal head rotation, to view the external Maltese cross target. Small final adjustments in all three dimensions were made to ensure the patient was aligned prior to the acquisition of data. Three ciliary muscle images were acquired at each visit for subsequent data extraction by the semi-automated software.

The preparation of the ciliary muscle images for analysis was identical to the process used by Kao *et al.*; all images were exported in raw, binary form (refractive index=1) and were imported to Matlab R2012b (The MathWorks Inc., Massachusetts, USA) and resized to 512 x 1280 pixels. Unlike Kao and colleagues' work, the images are not reduced in size for processing. Due to the difficulties localising the outline of the ciliary muscle, the bespoke software requires multiple landmarks to be manually selected before extracting data. Initially, the scleral spur and a point beyond the posterior visible limit must be selected manually (highlighted by yellow dots in Fig. 2.9A). The software then calculates the distance between these two markers and superimposes a vertical line midway, prompting the user to pick the points where the scleral/ciliary muscle and ciliary muscle/pigmented ciliary epithelium boundaries bisect the line. These initial steps identify the area of interest to the software, which then superimposes a block of 10 vertical lines every 0.5 mm between the scleral spur and posterior point chosen. The change in pixel intensity along each line is determined. To define the ciliary muscle border, a 2nd order fourier series is fitted to the intensity profile and differentiated. The location of the largest peak formed in the differentiated intensity profile corresponds to the point where the line bisects the ciliary muscle boundary (the crossing point). This process is repeated for the top and bottom of each line separately in order to determine crossing points of both the superior and inferior ciliary muscle boundaries on the OCT image. A 2nd order polynomial curve is fitted to the crossing points of each boundary.

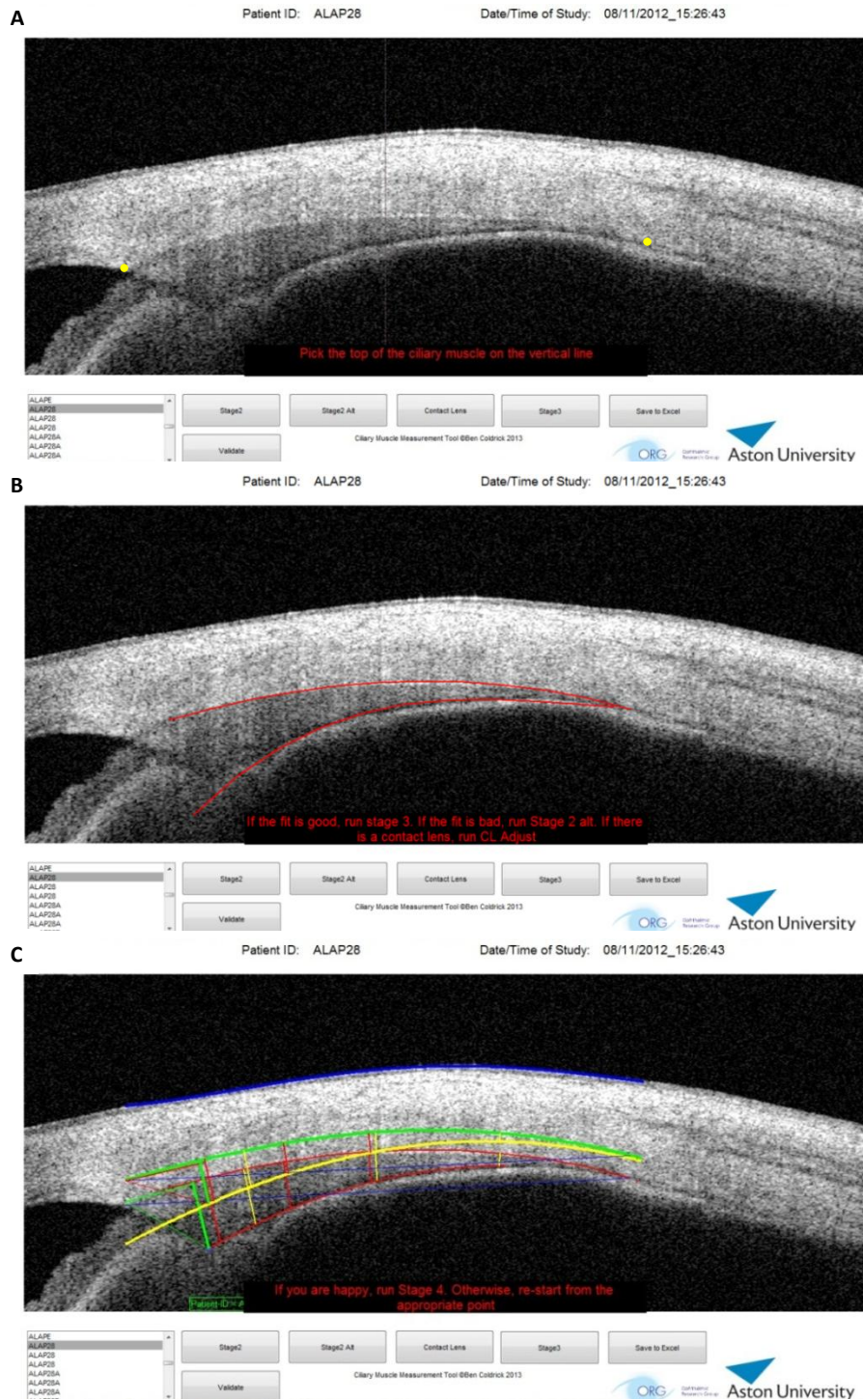


Fig. 2.9. A) Screenshot from the semi-automated software after the scleral spur and a point beyond the posterior visible limit (yellow dots) have been clicked on. The user is required to select where the top and bottom ciliary muscle boundaries are bisected by the superimposed vertical line. B) The software outlines the boundaries of the ciliary muscle and gives the option to manually redefine the lower curve by pressing the 'Stage2 Alt' button. C) After manually selecting the inner apex the software extracts the ciliary muscle data, correcting for the refractive indices of the sclera and ciliary muscle (higher yellow and green curves) and transfers the data to an Excel document.

The ciliary muscle/pigmented ciliary epithelium border is not as easily discriminated as the scleral/ciliary muscle border, therefore the software provides an option to manually pick three points along the boundary to improve the fit of the curve, if the automated fit is not satisfactory (Fig. 2.9B). An unlimited number of iterations are permitted to improve the fit of the curve. Due to relatively poor image clarity around the inner apex of the ciliary muscle, the point of maximum thickness must also be selected manually. Once the fit of the curves to the ciliary muscle borders has been finalised, the OCT image is converted to black and white in order for internal MatLab edge detection algorithms to identify the air/scleral boundary and fit a 2nd order polynomial curve to it. During software development and testing, it was determined that 2nd order curves accurately and satisfactorily fitted the contour of the ciliary muscle and sclera in all 68 patients tested.

Similarly to Kao *et al.*'s work, the software corrects the superimposed contours for the refractive indices of the scleral and ciliary muscle tissue (1.41 and 1.38, respectively), as shown in Fig. 2.9C by the higher yellow and green curves. The posterior visible limit of the ciliary muscle is identified as the point where the curves fitted to the ciliary muscle borders reach minimum separation posteriorly. The software exports the Straight-line TL (straight-line distance between the scleral spur and posterior visible limit), Curved TL (ciliary muscle total length measured along the scleral/ciliary muscle boundary), Max T (maximum thickness), Ant L (anterior length measured perpendicularly from the line of maximum thickness to the scleral spur), SS-IA (distance between scleral spur to the inner apex), CM2 (thickness measured 2 mm from the scleral spur along the scleral curve), CM25 (thickness measured at 25% of the total curved length of the ciliary muscle), CM50 (thickness measured at 50% of the total curved length of the ciliary muscle) and CM75 (thickness measured at 75% of the total curved length of the ciliary muscle) directly to an Excel spreadsheet, allowing the examiner to be masked to the results. Due to the inaccuracies of measuring thickness at fixed distances from the scleral spur, which is likely to represent a different anatomical area of the ciliary muscle between subjects, CM1 (thickness measured 1 mm

behind the scleral spur along the scleral curve) and CM3 (thickness measured 3 mm behind the scleral spur along the scleral curve) were not quantified. However, CM2 was included due to the hypothesis this area may act as a fulcrum point during accommodation, where the net change in thickness is negligible (Lewis *et al.*, 2012). Each image took approximately 4 minutes to analyse and the average ciliary muscle parameters from each set of three images were used for analysis.

The bias of each parameter was calculated from the mean difference between visits. A paired t-test was used to determine whether the bias was significantly different from 0. The spread over which 95% of the bias data lies (limits of agreement, LoA) were calculated using equation 2.3.

In order to determine the errors arising from patient alignment and semi-automated software interpretation, the temporal ciliary muscle of a single patient's right eye was imaged 10 times at 0.00 D accommodative stimulus during one appointment. The patient was asked to remove and reposition their chin and forehead between the acquisition of each image. In order to isolate the repeatability of the semi-automated software interpretation and analysis of a ciliary muscle image, 1 image was analysed 10 times.

Semi-automated software and Visante caliper agreement

The final step for validation is to compare the semi-automated software measurements to those from the traditional method of ciliary muscle Visante OCT image analysis: internal Visante calipers. To ensure the same points are selected for data extraction by both methods, ciliary muscle images of 50 patients were acquired with the same protocol as described previously, and data were extracted by the software and the Visante calipers on separate occasions.

The semi-automated software was designed to measure the ciliary muscle thickness with reference to the curved ciliary muscle length (following the contour of the scleral/ciliary muscle border), whereas the Visante calipers cut across the ciliary muscle to measure its thickness at horizontal distances from the scleral spur. Following the contour of the scleral/ciliary muscle border accounts for intersubject differences in the curvature of the ciliary muscle. Therefore, the

calipers are likely to underestimate the thickness measurements in a more curved ciliary muscle (Bailey *et al.*, 2008; Kao *et al.*, 2011). Due to the aforementioned differences in the origin of thickness measurements from the scleral spur, only the Straight-line TL and Max T measurements were compared between the two methods. The semi-automated software Straight-line TL and the semi-automated software Curved TL values were also compared.

Caliper measurements were acquired by applying a refractive index of 1.00 to the entire image and superimposing internal Visante calipers on to the ciliary muscle image to extract Straight-line TL and Max T measurements. For comparison with the internal Visante caliper measurements, the semi-automated software was adapted to export the raw ciliary muscle measurements with a refractive index of 1.00 applied to the entire image.

The bias was calculated from the mean difference between the techniques and a paired t-test was used to determine whether the bias was significantly different from 0. The spread over which 95% of the bias data lies (limits of agreement, LoA) were calculated using equation 2.8. The agreement of the techniques was also analysed using Bland-Altman plots (Bland and Altman, 1986).

Contact lens induced errors

In order to investigate whether the inclusion of a soft spherical contact lens (*Focus Dailies*, nelfilcon A, 69% water content; refractive index 1.38; Ciba Vision, Georgia, USA) significantly altered the ciliary muscle parameters extracted, the ciliary muscle of 8 myopic patients (MSE -3.16 ± 1.43 D; astigmatism <0.75 D) was imaged with and without a contact lens at 0.00 D accommodative stimulus.

All patients had previous contact lens wear experience, but all had abstained from contact lens wear on the day of the study. Initially, three images of the temporal ciliary muscle were acquired without the contact lens following the same protocol as described previously, reducing the distance between the Badal lens and target to provide a 0.00 D accommodative stimulus for each patient. Contact lens power was chosen based on the right eye MSE derived from 5 repeat

measures of refraction by the WAM-5500 autorefractor. After insertion of the contact lens onto the right eye of each patient, a 10 minute settling period was permitted and visual acuity was checked to ensure it corresponded to the visual acuity attained in their spectacles. Refraction was measured again via the WAM-5500 autorefractor to ensure the residual MSE was $<\pm 0.50$ D with the contact lens *in situ*. Subsequently, three ciliary muscle images were acquired at 0.00 D with the contact lens *in situ*.

The ciliary muscle software was adapted to exclude the thickness of a contact lens from the scleral thickness calculations by converting the image into black and white and prompting the user to encircle the edge of the contact lens (Fig. 2.10B). The outlined scleral border is then corrected to exclude the contact lens (Fig. 2.10C). The bias was calculated from the mean difference of each ciliary muscle parameter with and without a contact lens. A paired t-test was used to determine whether the bias was significantly different from 0. The spread over which 95% of the bias data lies (limits of agreement, LoA) were calculated using equation 2.8.

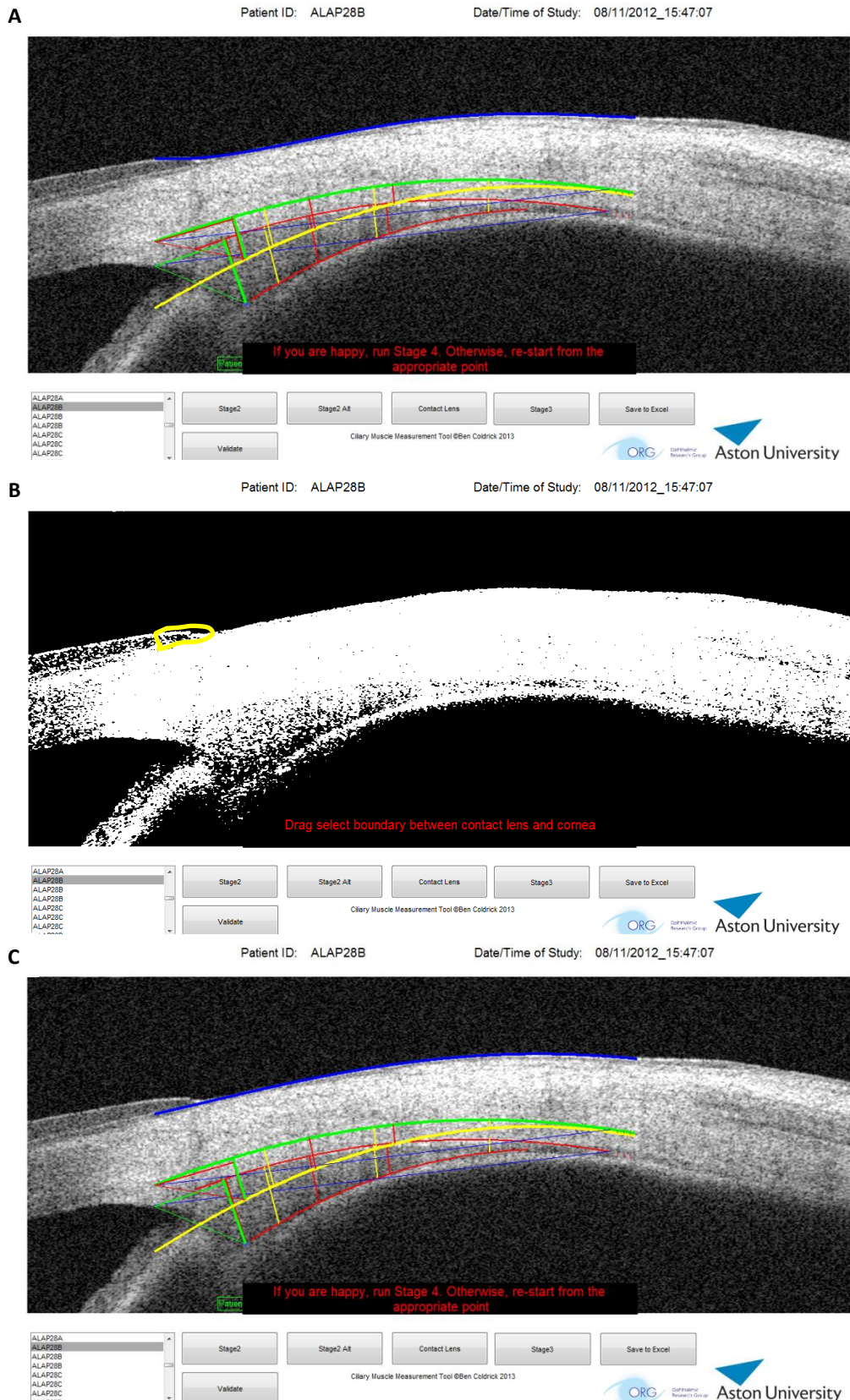


Fig. 2.10. A) Ciliary muscle section with a contact lens *in situ* before the ciliary muscle software has identified the contact lens boundary. B) Following the selection of the 'Contact Lens' button, the ciliary muscle image is converted into black and white and the user is prompted to encircle the edge of the contact lens (highlighted in yellow). C) The outlined scleral border is then corrected to exclude the contact lens.

2.3.4b Results

Artificial ciliary muscle sections

The mean difference between the semi-automated software and the Vernier caliper measurements are displayed in Table 2.1. The bias of CM25, CM50 and CM75 were not significantly different from 0. The difference versus mean Bland-Altman plots (Fig. 2.11) show the bias was not correlated with the magnitude of the measurement. However the total diameter was significantly underestimated by the software ($p=0.001$).

Parameter	Mean difference (mm)	Standard deviation (mm)	Limits of agreement		t	p
			Lower (mm)	Upper (mm)		
Total diameter	0.046	0.092	-0.135	0.226	3.507	0.001
CM25	-0.001	0.017	-0.034	0.032	-0.427	0.671
CM50	-0.003	0.016	-0.034	0.028	-1.458	0.151
CM75	0.000	0.016	-0.031	0.031	-1.810	0.857

Table 2.1. Comparison of parameters obtained from 5 artificial ciliary muscle sections by the semi-automated software and Vernier calipers. A negative mean difference indicates the software values are larger than the Vernier caliper measurements. A bold p value denotes statistical significance.

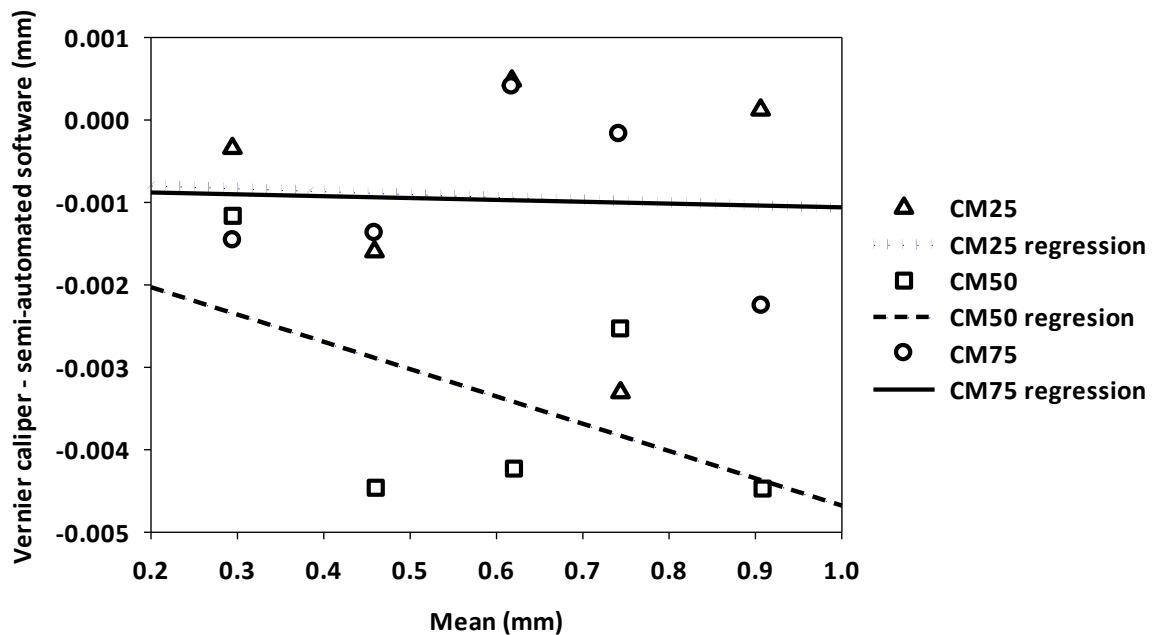


Fig. 2.11. A Bland-Altman difference versus mean plot of the thickness measurements acquired by the semi-automated software and the Vernier calipers. CM25 $y=-0.0004x-0.0007$, $R^2=0.003$, $p=0.931$; CM50 $y=-0.0001x-0.0014$, $R^2=0.288$, $p=0.351$, CM75 $y=-0.0002x-0.0008$; $R^2=0.002$, $p=0.649$.

Repeatability

The bias of ciliary muscle parameters measured in 10 patients across 2 visits are displayed in Table 2.2. The bias of each parameter was not significantly different from 0. Tables 2.3 and 2.4 obtained from 1 patient realigned 10 times and 1 image analysed 10 times, respectively, suggest approximately 60% of the difference encountered between visits is likely to be due to the inherent variability associated with manually selecting points for analysis with the bespoke software.

Parameter	Mean difference (mm)	Standard deviation (mm)	Limits of agreement		t	p
			Lower (mm)	Upper (mm)		
Straight-line TL	-0.016	0.077	-0.166	0.135	-0.569	0.583
Curved TL	-0.017	0.072	-0.157	0.124	-0.736	0.480
Max T	-0.003	0.022	-0.047	0.040	-0.281	0.785
Ant L	0.011	0.059	-0.104	0.126	0.577	0.578
SS-IA	0.007	0.054	-0.099	0.112	0.354	0.731
CM2	-0.007	0.030	-0.066	0.051	-0.761	0.466
CM25	-0.003	0.018	-0.038	0.033	-0.832	0.427
CM50	0.004	0.023	-0.041	0.050	0.535	0.606
CM75	0.009	0.020	-0.030	0.048	1.335	0.215

Table 2.2. Intersession repeatability data of ciliary muscle parameters extracted by the semi-automated software from 2 visits of 10 patients. A negative mean difference indicates the measurement was larger at visit 1.

Parameter	Mean (mm)	Standard deviation (mm)
Straight-line TL	5.164	0.068
Curved TL	5.228	0.075
Max T	0.482	0.014
Ant L	1.182	0.043
SS-IA	1.254	0.052
CM2	0.345	0.025
CM25	0.475	0.016
CM50	0.261	0.015
CM75	0.114	0.015

Table 2.3. Ciliary muscle parameters extracted by the semi-automated software from 10 images acquired from one patient who removed and repositioned their head between acquisitions.

Parameter	Mean (mm)	Standard deviation (mm)
Straight-line TL	5.153	0.043
Curved TL	5.218	0.038
Max T	0.454	0.011
Ant L	1.237	0.021
SS-IA	1.276	0.040
CM2	0.334	0.016
CM25	0.456	0.008
CM50	0.259	0.006
CM75	0.121	0.011

Table 2.4. Ciliary muscle parameters extracted by the semi-automated software from one image analysed 10 times.

Semi-automated software and caliper agreement

The mean difference between the semi-automated software and the Visante internal caliper total Straight-line TL and Max T measurements are displayed in Table 2.5. The bias of each parameter was not significantly different from 0. The data are displayed graphically in Figs. 2.12 and 2.14. The difference versus mean Bland-Altman plots (Figs. 2.13 and 2.15) show the bias was not correlated to the magnitude of the measurement. The mean semi-automated software Curved TL measurements (5.391 ± 0.571 mm) were significantly longer than the mean semi-automated software Straight-line TL measurements (5.301 ± 0.560 mm; $t=-23.356$, $p<0.001$).

Parameter	Mean difference (mm)	Standard deviation (mm)	Limits of agreement		t	p
			Lower (mm)	Upper (mm)		
Straight-line TL	0.011	0.089	-0.163	0.185	0.860	0.394
Max T	-0.005	0.043	-0.089	0.079	-0.864	0.392

Table 2.5. Comparison of ciliary muscle parameters obtained from 50 patients by the semi-automated software and the internal Visante calipers. A negative mean difference indicates the software values are larger than the caliper measurements.

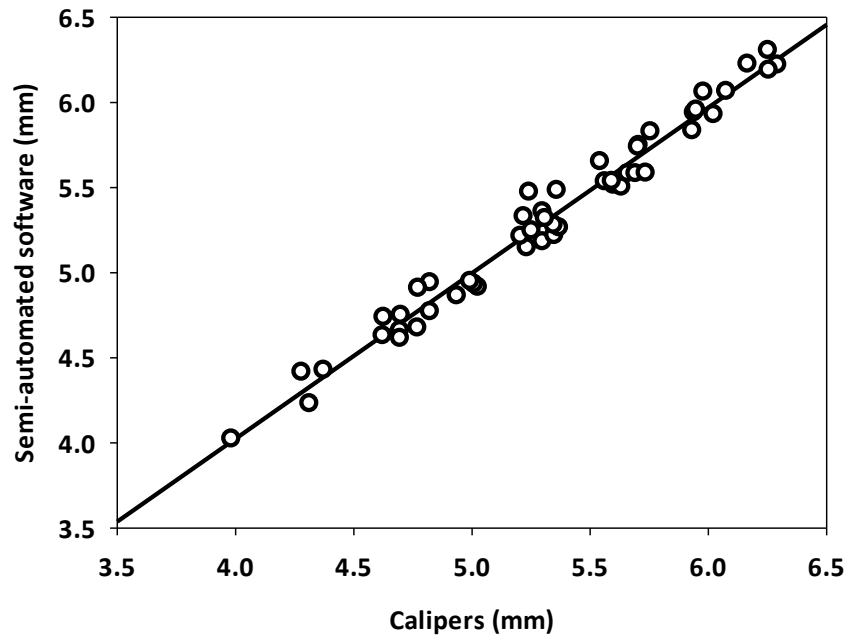


Fig. 2.12. Total straight-line length measured by the semi-automated software and the internal Visante calipers. Regression line $y=0.132+0.973x$, $R^2=0.976$, $p<0.001$.

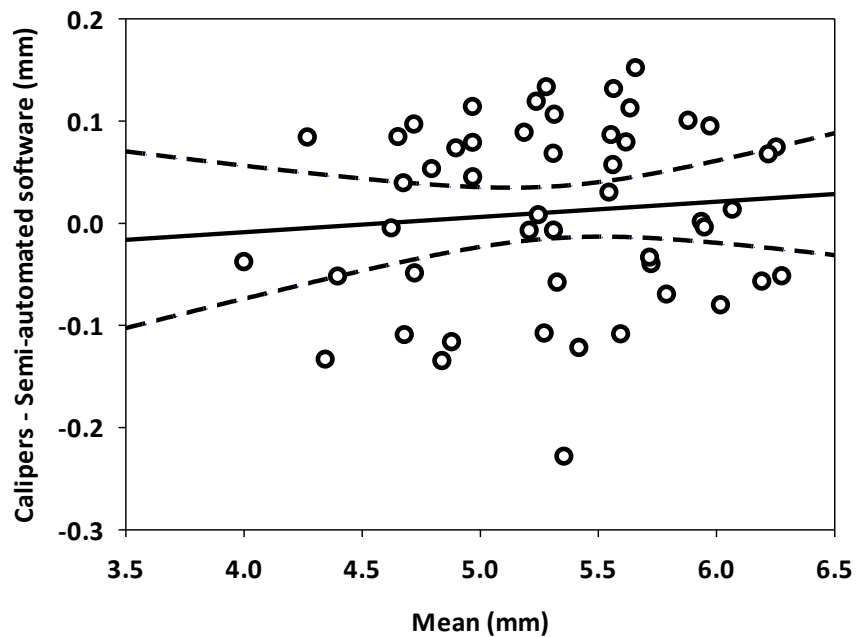


Fig. 2.13. A Bland-Altman difference versus mean plot of the agreement between total straight-line length measured by the semi-automated software and the internal Visante calipers. Regression line $y=-0.068+0.015x$, $R^2=0.009$, $p=0.514$. The dashed lines represent the lower and upper 95% confidence intervals.

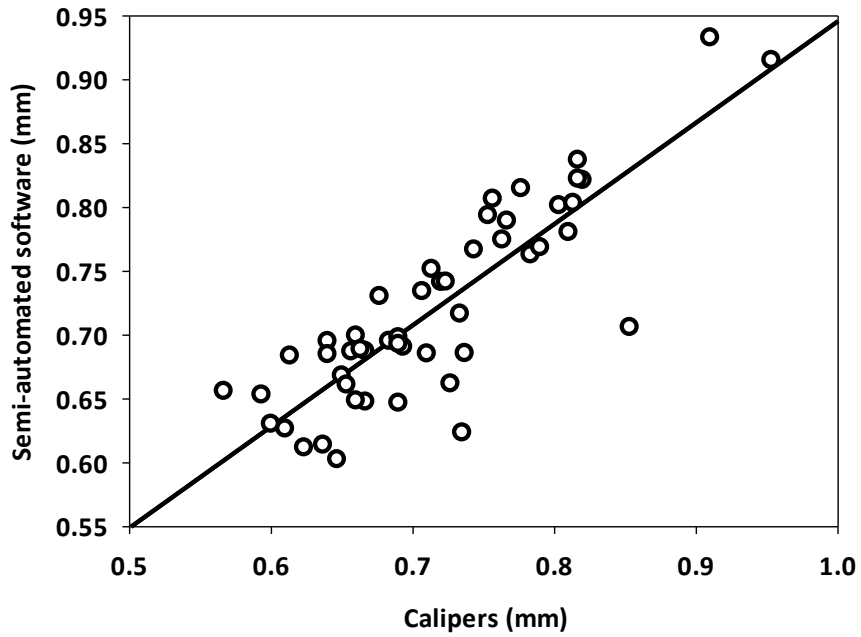


Fig. 2.14. Maximum thickness measured by the semi-automated software and the internal Visante calipers.

Regression line $y=0.152+0.794x$, $R^2=0.732$, $p<0.001$.

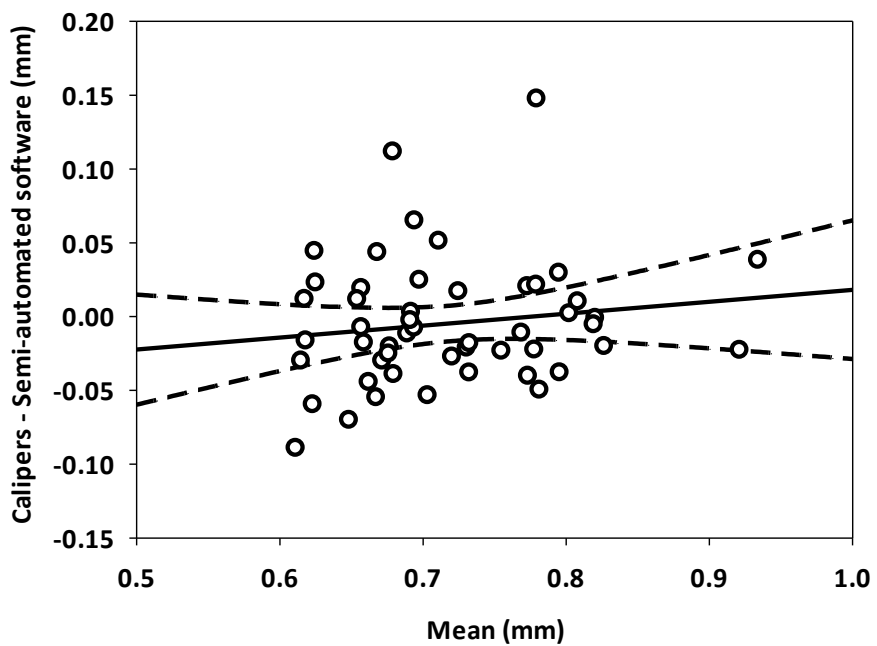


Fig. 2.15. Bland-Altman difference versus mean plot of the agreement between maximum thickness measured by the semi-automated software and the internal Visante calipers. Regression line $y=-0.063+0.081x$, $R^2=0.021$, $p=0.317$. The dashed lines represent the lower and upper 95% confidence intervals.

Contact lens induced errors

The contact lens consistently appeared to overlies the scleral spur only, typically avoiding all other landmarks as shown in Fig. 2.10. The mean difference and limits of agreement between each parameter measured with and without a contact lens *in situ* are displayed in Table 2.6. The bias of each parameter was not significantly different from 0.

Parameter	Mean difference (mm)	Standard deviation (mm)	Limits of agreement		t	p
			Lower (mm)	Upper (mm)		
Straight-line TL	-0.043	0.127	-0.292	0.206	-0.922	0.387
Curved TL	-0.035	0.135	-0.300	0.230	-0.741	0.483
Max T	0.016	0.030	-0.043	0.075	1.546	0.166
Ant L	-0.017	0.066	-0.146	0.112	-0.812	0.444
SS-IA	-0.012	0.070	-0.149	0.125	-0.498	0.634
CM2	0.016	0.029	-0.041	0.073	1.403	0.203
CM25	0.018	0.030	-0.041	0.077	1.498	0.178
CM50	0.016	0.039	-0.060	0.092	1.101	0.307
CM75	0.009	0.032	-0.054	0.072	0.801	0.450

Table 2.6. Comparison of ciliary muscle parameters obtained from 8 patients with and without a contact lens *in situ*. A negative mean difference indicates the parameters are smaller when wearing a contact lens.

2.3.4c Discussion

The semi-automated software described here is capable of accurately outlining the ciliary muscle, applying appropriate refractive index corrections and extracting a variety of repeatable orthogonal and oblique ciliary muscle parameters, thus verifying its suitability for *in vivo* ciliary muscle analysis. Compared to the Visante calipers, the software enables more appropriate measurements of the curved ciliary muscle tissue to be acquired by following the scleral/ciliary muscle contour, rather than cutting horizontally across the ciliary muscle to measure thicknesses with respect to the distance from scleral spur. Image analysis can also be performed remotely to the Visante device on an external computer, ensuring that carrying out data analysis does not prohibit other researchers from simultaneously collecting data with the Visante.

The semi-automated software raw parameters (n=1) compared favourably to internal Visante caliper Straight-line TL and Max T measurements, suggesting the location of the posterior visible

limit, utilised in the total length measurement, is not only evident across a large sample of patients, but can also be consistently identified subjectively and objectively. Nevertheless, the concerns of previous authors over the visibility of the posterior end point of the ciliary muscle are not entirely unfounded (Bailey *et al.*, 2008; Kao *et al.*, 2011); extensive analysis of ciliary muscle images from 68 patients during software development has shown there is large intersubject variability in the visibility of the posterior limit of the ciliary muscle. In order to simplify localisation for the software, the posterior visible limit was defined as the point where the scleral/ciliary muscle and ciliary muscle/pigmented ciliary epithelium contours reached minimum separation posteriorly. The described posterior ciliary muscle model visually appeared to fit all the images analysed, however on average, Straight-line TL was slightly longer when measured by the calipers compared to the software ($+0.011 \pm 0.089$ mm). Therefore this could indicate that the posterior profile of the ciliary muscle is not as sharply demarcated as our model suggests, however the level of bias was not statistically significantly different from 0 ($p=0.394$; Table 2.5), indicating that any error in the model is negligible.

Conversely, Max T was slightly shorter when measured by the calipers compared to the software (-0.005 ± 0.043 mm). Kao *et al.* (2011) also reported the same trend for their thickness measurements, perhaps suggesting that the software was more able to accurately identify the inferior ciliary body border from assessment of pixel intensity. However, the Max T measurement derived from the new semi-automated software relies on the thickest point of the ciliary muscle (inner apex) along the inferior border being manually selected, therefore perhaps the slightly longer measurement arises from the software measuring a distance which is not exactly perpendicular from the scleral/ciliary muscle border. However, the level of bias was not significantly different from 0 (Table 2.5), therefore any errors in determining the perpendicular length are likely to be insignificant.

Orthogonal and oblique thickness measurement accuracy was evidenced by computation of the distance between 2 polynomial curves (order 2) fitted to OCT images of 2 superimposed rigid gas-

permeable lenses (Fig. 2.8). The software consistently overestimated the CM25, CM50 and CM75 thickness measurements when compared to the Vernier caliper measurements. The difference may be due to the artefacts around the lens borders, particularly pronounced at the CM50 point, making the lens borders harder to define. Nevertheless, the bias of each parameter was not significantly different to 0. As expected, the total diameter measurements were significantly underestimated by the software ($p=0.001$) due to difficulties ensuring the scleral and ciliary muscle lenses were perfectly centred, and indeed that the measurement beam scanned across the centre of both lenses. Nonetheless, the validity of horizontal measurements has been confirmed by the good correspondence between the internal Visante caliper and the software Straight-line TL measurements. The thickness measurements obtained are unaffected by the aforementioned alignment issues because all the ciliary muscle lenses were of constant thickness. The scleral lens (L_1 in Fig. 2.7) was a meniscus lens of varying thickness, however the scleral lens correction was continually recalculated based on the measurements of each individual image and therefore would not have influenced the ciliary muscle parameters extracted. The good correspondence between the ciliary muscle lens (L_2 in Fig. 2.7) thickness values measured by the software and Vernier calipers demonstrates the software can appropriately compensate for tiered refractive index levels and the geometric distortion of the exported image in binary form is negligible. These findings also support the conclusions of Kao *et al.* (2011), who reported Visante images exported as binary files are free from geometric distortions and only need to be adjusted for the refractive index of the tissue(s) to be suitable for accurate morphological assessment.

Data obtained from 1 patient realigned 10 times and 1 image analysed 10 times, (Tables 2.3 and 2.4, respectively), suggest approximately 60% of the difference encountered between visits is likely to be due to the inherent variability associated with manually selecting points for analysis with the bespoke software. The remainder of the variation is likely to be due to differences in patient alignment and short-term fluctuations in the tissue parameters. All data were collected without the instillation of a cycloplegic agent, therefore it is feasible accommodation and

therefore ciliary muscle tonus fluctuated whilst the patient attended the 0.00 D accommodative Maltese cross target. However the refractive response to a Badal lens system has been found to be equivalent to free-space targets (Stark and Atchison, 1994), therefore it is unlikely that significant levels of accommodation were stimulated during the short period of data collection.

The variability associated with 1 patient repositioning themselves 10 times (Fig. 2.3) is larger than previously reported for caliper measurements (Sheppard and Davies, 2010a). The lower variability of caliper measurements may be due to the facility of adjusting the contrast of images during caliper analysis, helping to facilitate localisation of key landmarks. However, the bias and variance of the difference in ciliary muscle parameters extracted for 10 patients on 2 separate visits (Table 2.2) was similar to caliper measurement values reported by Sheppard and Davies (2010a). Furthermore, the limits of agreement reported by the software (-0.166 to 0.135 mm) were narrower than for the calipers (-0.228 to 0.193 mm) for the measurement of total straight-line length, suggesting superior repeatability of the localisation of the posterior visible limit by the software. The intersession repeatability of the software developed by Kao and colleagues (2011) was not reported.

The addition of a contact lens did not significantly alter the magnitude of the ciliary muscle parameters extracted by the semi-automated software, therefore a separate contact lens correction is not required to be built into the software. However, the limits of agreement in Table 2.6 are wider than reported for the intersession repeatability of the software in Table 2.2, suggesting that future studies comparing the ciliary muscle accommodative response in myopic patients should acquire all data, including 0.00 D accommodative stimulus, with a contact lens *in situ* to improve the accuracy of comparison between accommodative levels.

Unlike previously developed software (Kao *et al.*, 2011), which measures the full thickness of the ciliary body, the software described here is capable of distinguishing the ciliary muscle from the surrounding tissue, and therefore extracts only ciliary muscle parameters. Consequently, the results of studies implementing the software developed by Kao and colleagues should be

interpreted with caution (Lossing *et al.*, 2012; Lewis *et al.*, 2012; Pucker *et al.*, 2013; Richdale *et al.*, 2013), particularly when comparing with studies reporting results of the ciliary muscle alone. The software described here also has the capacity to extract a variety of additional measurements to Kao *et al.*'s software, including ciliary muscle length, which Kao and colleagues suggested is a vital measurement for presbyopia research.

The newly developed semi-automated software described here reduces, but does not eliminate, the subjective element of ciliary muscle analysis. Due to the non-uniformity of the acquired OCT images, human input is necessary to enable image analysis to commence. The image examiner must be highly trained to extract repeatable results due to the ambiguity of ciliary muscle landmarks in some patients. It is likely that the repeatability of nasal ciliary muscle image analysis is superior to temporal ciliary muscle image analysis because the scleral spur is more easily discernible nasally (Sakata *et al.*, 2008). In order to reduce the complexity of ciliary muscle image analysis in the future, improvements in the tissue image clarity produced by the Visante OCT is required, which could lead to the development of fully automated ciliary muscle analysis software. Perhaps one option may be to improve the resolution of ultrasonography techniques and build in compensation for the speed of sound through the sclera and ciliary muscle. The added benefit of using ultrasonography is that it is able to penetrate the iris and can image the zonules (Ludwig *et al.*, 1999), and in particular the posterior zonules, which may help to serve as a marker of the end point of the ciliary muscle (Kao *et al.*, 2011).

CHAPTER 3

Objective refractive error progression during incipient presbyopia

3.1 Introduction

Age-related changes in refraction have been well documented cross-sectionally and longitudinally during childhood (Zadnik *et al.*, 1993; Mutti *et al.*, 2007), early adulthood (Grosvenor and Scott, 1993; McBrien and Adams, 1997) and presbyopia (Grosvenor and Skeates, 1999; Lee *et al.*, 2002). As shown in Fig. 1.3, infants are typically born with hypermetropia and undergo emmetropisation during early childhood (Saunders *et al.*, 1995; Wood *et al.*, 1995; Ehrlich *et al.*, 1997). After 1 year of age, the frequency distribution of the mean spherical equivalent (MSE, equation 1.1) tends towards a leptokurtic distribution around an emmetropic value with a negative skew, which is also commonly observed in adulthood (Stenstrom, 1948; Sorsby *et al.*, 1960; Wood *et al.*, 1995; Ehrlich *et al.*, 1997; Logan *et al.*, 2005). Logan *et al.* (2011) reported 9.4% of 327 British school children aged 6 to 7 year olds were classified as myopic ($MSE \leq -0.50$ D), whereas 29.4% of 269 British school children aged 12 to 13 year olds were myopic. There is also evidence of myopia onset and progression during adulthood (Saunders, 1981; 1986; McBrien and Adams, 1997). Rahi *et al.* (2011) reported 49% of 2487 randomly selected British adults aged 44 years were myopic ($MSE \leq -0.75$ D), with over 80% of the myopia occurring after the age of 15 years (late onset myopia; McBrien and Millodot, 1987; Bullimore *et al.*, 1992).

Nevertheless, considering the typical change in refraction between the ages of 35 to 65 years, a hypermetropic shift has consistently been documented by cross-sectional (Tassman, 1932; Brown, 1938; Slataper, 1950; Hirsch, 1958; Saunders, 1981; Fledelius, 1983; Fledelius and Stubgaard, 1986; Sperduto *et al.*, 1996; McCarty *et al.*, 1997; Kempen *et al.*, 2004; Atchison *et al.*, 2008) and longitudinal (Saunders, 1986; Grosvenor and Skeates, 1999; Lee *et al.*, 2002) studies, with a myopic shift reported thereafter, possibly due to the onset of crystalline lens nuclear sclerosis. Current understanding of the optical and structural ocular changes that occur during the development of presbyopia suggests the origin of the hypermetropic shift could be the

manifestation of previously latent hypermetropia (Goss *et al.*, 1985), which can no longer be overcome due to a reduction in amplitude of accommodation, or the crystalline lens paradox, where the increase in crystalline lens thickness and curvature is over-compensated for by a reduction in the average refractive index of the crystalline lens (Saunders, 1981; Grosvenor and Skeates, 1999; Mutti and Zadnik, 2000).

However, Pointer and Gilmartin (2011) have presented retrospective data revealing 20% of myopic patients experienced a myopic shift in refraction of 0.50 to 0.75 D between the ages of 35 to 44 years, otherwise classified as the period of incipient presbyopia. Incipient presbyopic patients were largely omitted from the aforementioned adult studies. Despite reporting a hypermetropic shift (≥ 0.50 D) in refraction in emmetropic and hypermetropic individuals after the age of 40 years, Grosvenor and Skeates (1999) isolated retrospective longitudinal myopic patient data to find the hypermetropic shift in refraction was less prevalent amongst myopic patients (19%). In fact, most myopic patients remained stable (66%) or became more myopic by ≥ 0.50 D (15%) after the age of 40 years. The ocular changes driving a myopic shift in refraction during incipient presbyopia are currently unknown. Moreover, it is unclear why myopic individuals appear to be at a greater risk of a myopic shift in refraction than emmetropic individuals during incipient presbyopia. Perhaps the effects of the crystalline lens paradox are less pronounced in myopic eyes due to their thinner crystalline lenses (McBrien and Millodot, 1987; Zadnik *et al.* 1995; McBrien and Adams, 1997) or previous axial elongation acts as a predisposition for future continued axial length elongation. However, informal clinical observation has revealed that a similar proportion of emmetropic and myopic patients experience a myopic shift in refraction during incipient presbyopia.

It is feasible that the putative myopic shift may act as a compensatory mechanism to overcome near vision blur resulting from diminishing levels of accommodation during incipient presbyopia. Indeed, a connection has been made between high levels of near work and myopia onset and progression in both children (Mutti *et al.*, 2002; Saw *et al.*, 2002) and adults (Goldschmidt, 1968;

Simensen and Thorud, 1994; McBrien and Adams, 1997; Maheshwari *et al.*, 2011). The myopic shift may, therefore, be more prevalent in individuals spending long periods of time working at near.

The aim of this study is to provide prospective longitudinal data documenting the natural progression of refraction during incipient presbyopia, and in particular to investigate the incidence of the putative myopic shift in refraction amongst myopic and emmetropic individuals. Refractive changes will also be considered with respect to the amount of time spent working at near daily to explore the link between near work and myopia progression during the incipient phase of presbyopia.

3.1.1 Methods

In order to collect longitudinal data, the study was designed to review participants every 6 months over 2.5 years, thus 6 sessions were completed in total, each time repeating the experimental protocol detailed below. The average recall period for a sight test examination is 2 years, however, a 6 monthly review frequency was chosen to monitor the change in refraction more closely. One optometrist (DL) collected the data at each visit and did not refer to the results from the previous sessions until after each appointment, when the data were inputted into an Excel spreadsheet (Microsoft, Washington, USA).

The study was approved by the Aston University Audiology and Optometry Research Ethics Committee (see Appendix 1 and 5) and was conducted in accordance with the tenets of the Declaration of Helsinki. Informed consent was obtained from all the participants after an explanation of the nature and possible consequences of the study (see Appendix 2 and 6 for a copy of the information sheets and consent forms).

3.1.1a Sample size

To ensure the recruited sample size was appropriate for repeated measures ANOVA analysis (within and between interaction), an effect size (f) of 0.25, an error probability (α) of 0.05 and

required power ($1-\beta$) of 0.80 was inputted into G*Power 3 (Faul *et al.*, 2007) for 6 repeat measurements amongst 2 groups, which produced an overall sample size of 20.

3.1.1b Questionnaire

In order to confirm suitability to participate in the study, each volunteer was asked to complete the questionnaire in Appendix 3. The questionnaire asked each participant to document their date of birth, ethnicity, date of last sight test, medications, previous hospital eye service treatment and current occupation. The British National Formulary (Joint Formulary Committee, 2014) was consulted to ensure none of the medications listed had any potential ocular adverse reactions that might affect the accommodative apparatus. Closed questions were also asked to determine whether any of the participants had been diagnosed with diabetes, anxiety, depression or any eye condition. Each participant was also asked to indicate whether they had undergone photorefractive surgery or anterior chamber lens implantation and whether they wore rigid or soft contact lenses.

The questionnaire also collected information on the lifestyle and occupation of each participant. Due to the established links between high levels of near work and myopia progression in children (Mutti *et al.*, 2002; Saw *et al.*, 2002) and adults (Simensen and Thorud, 1994; McBrien and Adams, 1997), the average daily amount of time spent working at near was determined at baseline. Participants were asked to indicate whether they spent 0, 1-3, 4-7 or 8+ hours working at near or using a computer daily. Low levels of outdoor sun exposure has also been linked with myopia progression (Mutti *et al.*, 2002; Sherwin *et al.*, 2012), therefore each participant was asked whether their job was outdoor-based. Each participant was asked to recheck their completed questionnaire at each subsequent visit and were allowed to alter their responses as required (e.g. if their job had changed).

3.1.1c Visual acuity

Monocular and binocular high contrast logMAR visual acuity was measured at 6 m to ensure each patient could achieve an acuity of 0.1 logMAR or better. Near visual acuity was evaluated with a near logMAR card at 25 cm to ensure each patient could achieve 0.2 logMAR or better at visit 1. At subsequent visits, visual acuity was re-measured to provide an indicator of changes in refraction, accommodation and the development of any pathology, but was not intended to be used as an outcome measure.

3.1.1d Amplitude of accommodation

Monocular subjective amplitude of accommodation was measured using an RAF rule (Richmond Products, New Mexico, USA) via the push-up/pull-down method (McBrien and Millodot, 1986; Wolffsohn *et al.*, 2011). Participants were asked to wear an eye patch over their left eye and try to maintain clear focus of the high contrast N5 print as it was slowly pushed towards them until the point where they first reported sustained blur (push-up amplitude). Subsequently, the first point at which the N5 print became clear when moved away from the eyes (pull-down amplitude) was measured. If a patient was unable to achieve the minimum amplitude of accommodation measurable (2.00 D), a +3.00 D full aperture trial lens was introduced in front of their right eye. The power of the lens was subsequently subtracted from the push-up/pull-down amplitude value obtained. Push-up/pull-down amplitude was measured three times and averaged to calculate the mean amplitude of accommodation for the right eye.

3.1.1e Non-cycloplegic objective refractive error and keratometry

Objective refractive error and keratometry were measured simultaneously by the binocular open-field WAM-5500 autorefractor (Grand Seiko Co. *Ltd.*, Japan), as described in section 2.2.1 (Sheppard and Davies, 2010b). A bespoke +5.00 D Badal lens system with a high contrast Maltese cross target was mounted on the WAM-5500 autorefractor, as pictured in Fig. 2.1. The fixation target was placed at the focal length of the lens (20 cm) in order to measure uncorrected distance refractive error. The left eye of each patient was occluded and patients were asked to focus on

the centre of a Maltese cross as accurately as possible throughout data collection (Stark and Atchison, 1994). Five consecutive measurements were acquired and the average MSE, J_{180} , J_{45} , cylinder (C), axis (θ) and power (P) were calculated using equations 1.1, 1.2, 1.3, 3.1, 3.2 and 3.3, respectively. A refractive shift ≥ 0.50 D in MSE was adopted as the minimum threshold level for statistical significance (Rosenfield and Chiu, 1995). The anterior corneal radii of curvature and toricity were also recorded. The radii of curvature readings from the principle meridians were averaged to determine the mean central keratometry reading.

$$C = -2\sqrt{J_{180}^2 + J_{45}^2} \quad 3.1$$

$$\theta = 0.5 * \tan^{-1}\left(\frac{J_{45}}{J_{180}}\right) \quad 3.2$$

$$P = \sqrt{MSE^2 + J_{180}^2 + J_{45}^2} \quad 3.3$$

3.1.1f Cycloplegic objective refractive error

Participants undergoing a myopic shift in MSE ≥ 0.50 D after 2.5 years were invited to attend a follow-up appointment within 1 month of their final session to measure cycloplegic refraction in order to determine whether the refractive shift was due to a spasm of accommodation. Prior to the instillation of 1 drop of 1% cyclopentolate in the right eye, non-cycloplegic refraction was re-measured by the WAM-5500 autorefractor, the anterior chamber was examined to ensure the angle was grade 3 or 4 (van Herick *et al.*, 1969) and intraocular pressure was measured using a non-contact 7 Auto-Tonometer (Reichert Technologies, New York, USA). Monocular amplitude of accommodation was measured 40 minutes after instillation of cyclopentolate and once the amplitude reduced below 2.00 D (Rosenfield and Linfield, 1986; Ward and Charman, 1986), refraction was measured by the WAM-5500 autorefractor. Five consecutive measurements were acquired and the average MSE, J_{180} and J_{45} were calculated using equations 1.1, 1.2 and 1.3, respectively. Intraocular pressure was measured at the end of the appointment to preclude blockage of the trabecular meshwork.

3.1.1g Statistical analysis

All data were tested for normality using the Shapiro-Wilk test (SigmaPlot Version 12; Systat Software Inc., California, USA). Differences between the baseline age of the emmetropic and myopic groups were determined by a t-test (SPSS Version 21; SPSS Inc., Illinois, USA). In order to determine whether changes in amplitude of accommodation and refraction vectors were significant over the 2.5 year period, repeated measures analysis of variance (ANOVA) testing was performed, comparing the effect of time (within-subject variable) and refractive group classification (between-subject variable). Linear regression analysis was performed to determine whether the baseline MSE, change in amplitude of accommodation and refraction vectors were significantly correlated with baseline age and whether the change in amplitude of accommodation was significantly correlated with the change in refraction.

3.1.2 Results

3.1.2a Participants

In order to allow for attrition during the course of the longitudinal study and to increase the likelihood of enrolling individuals who experience a myopic shift in refraction, 58 participants aged 33 to 45 years old (39.1 ± 3.2 years) were recruited from an Aston University staff and local business volunteer call in March 2012. All of the participants were screened to exclude those with a positive history of ocular or systemic disease. Each participant had visited an optometrist for a full eye examination within 2 years from the baseline appointment and no one had been prescribed a reading addition.

The initial phase of data collection at visit 1 commenced on the 29th March 2012 and the final phase of visit 6 finished on the 24th October 2014. There was no attrition of subjects during the first year of the study, however, due to the development of a cataract following steroid treatment for uveitis (n=1), permanent relocation from Birmingham (n=1) and severe complications following abdominal surgery (n=1), a total of 95% (n=55) of the original cohort completed the 2.5 year study visit. A total of 7% (n=4; mean baseline age 44.6 ± 0.6 years) of participants started

wearing a near addition for reading during the course of the study and were, therefore, excluded from analysis in the proceeding experimental chapters. Of the remaining 51 participants, the average time between visits was 171 ± 7 days, however 6% missed at least 1 study visit due to maternity leave ($n=2$) or job secondment ($n=1$). None of the participants who were pregnant during the study ($n=4$) reported gestational diabetes or any other health or visual issues. Two participants who had bilateral photorefractive surgery over 10 years ago (pre-operative right eye contact lens prescription -2.50 DS and -3.00 DS) and were functionally emmetropic at baseline were included in the myopic group. Photorefractive surgery patients were recruited due to concerns of long-term patient visual satisfaction, considering the risk of a refractive change during the development of presbyopia (Morgan, 1988; Ellingsen *et al.*, 1997; Pointer and Gilmartin, 2011).

Apart from the 2 previous photorefractive surgery patients, participants with a MSE of < -0.75 D were defined as myopic (Sheppard and Davies, 2011). Emmetropes were classified by a MSE of between -0.75 DS and +0.50 D. At baseline, 21 (14 females and 7 males) participants were myopic (MSE -3.25 ± 2.28 DS; baseline age 38.6 ± 3.1 years) and 30 (19 females and 11 males) were emmetropic (MSE -0.17 ± 0.32 DS; baseline age 39.0 ± 2.9 years). No restrictions were made based on the magnitude of cylindrical correction (myopes mean C -0.71 ± 0.71 DC; emmetropes mean C -0.39 ± 0.32 DC). The proportion of the myopic cohort who self-reported becoming myopic before the age of 15 years (early-onset myopes) was 57%. The sample included Afro-caribbean (10%; 1 myope, 4 emmetropes), Chinese (2%; 1 emmetrope), Indian (6%; 1 myope, 2 emmetropes) and white European (82%; 19 myopes, 23 emmetropes) individuals.

The cohort self-reported spending 0 hours (2%; 1 emmetrope who was a security guard), 1 to 3 hours (12%; 1 myope, 5 emmetropes), 4 to 7 hours (55%; 12 myopes, 16 emmetropes) or over 8 hours (31%; 7 myopes, 9 emmetropes) working at near daily. Only 1 emmetropic individual, who was a physical education teacher at a secondary school, reported spending most of the day outside.

3.1.2b Cross-sectional analysis

The baseline average age of the myopic (38.6 ± 3.1 years) and emmetropic (39.0 ± 2.9 years) groups were not statistically significantly different ($t=0.463$; $p=0.646$). Baseline MSE was not correlated with baseline age ($r=0.263$, $p=0.062$; Fig. 3.1), whereas baseline right eye amplitude of accommodation was significantly correlated with baseline age (Fig. 3.2; $r=0.561$, $p<0.001$).

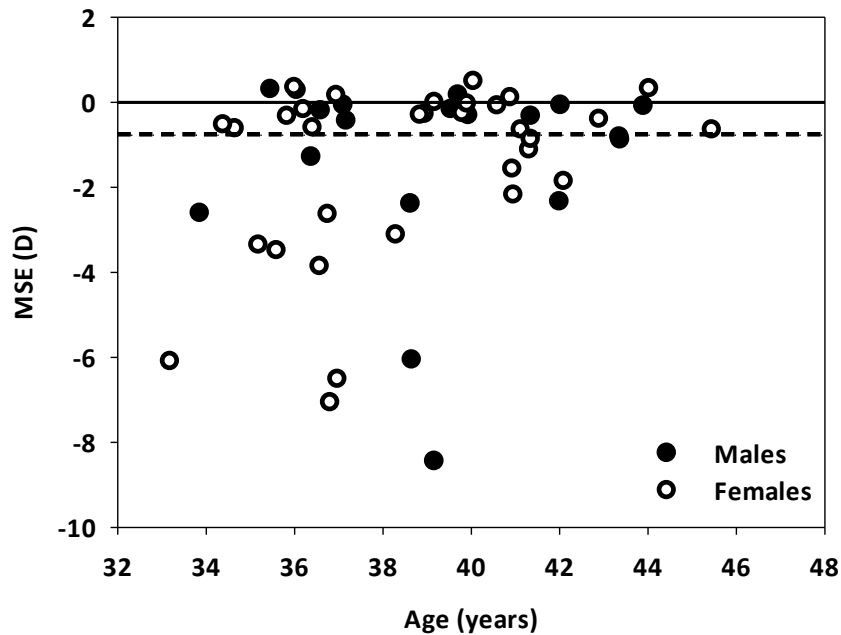


Fig. 3.1. Baseline MSE according to baseline age. Filled circles represent male participants ($n=18$), whereas open circles represent female participants ($n=33$). Participants with a MSE < -0.75 D (represented by the dashed line) were classified as myopic. The solid horizontal line represents a MSE of 0 D. Participants with a MSE between -0.75 D and $+0.50$ D were defined as emmetropic. Baseline MSE was not statistically significantly correlated with baseline age ($y=0.189x-8.772$; $R^2=0.069$; $p=0.062$).

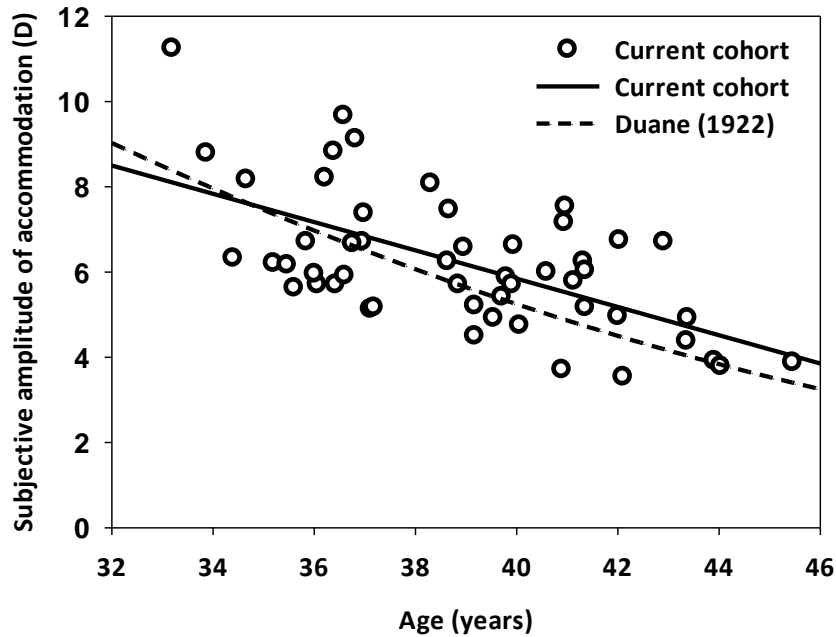


Fig. 3.2 Baseline right eye amplitude of accommodation according to baseline age. The solid line represents the regression line of the data from the current study ($y=-0.330x+18.897$; $R^2=0.314$; $p<0.001$). The dashed line represents the regression line from data taken from Duane (1922).

3.1.2c Longitudinal analysis

Participants in the myopic group experienced a hypermetropic shift (≥ 0.50 D; $n=2$, 10%), myopic shift (≥ 0.50 D; $n=1$, 5%) or had no significant change in MSE (<0.50 D; $n=18$, 85%). A similar proportion of the emmetropic group also experienced a hypermetropic shift (≥ 0.50 D; $n=3$, 10%), myopic shift (≥ 0.50 D; $n=1$, 3%) or had no significant change in MSE (<0.50 D; $n=26$, 87%). The spherocylindrical refraction of the myopic and emmetropic patients undergoing a myopic shift ≥ 0.50 D changed from $-5.72/-1.59 \times 13$ to $-6.63/-1.21 \times 13$ and $+0.15/-0.86 \times 100$ to $-0.66/-0.85 \times 101$, respectively and corresponded with a drop in visual acuity. Three participants with low myopia at baseline (MSE -1.19 ± 0.35 D) became emmetropic (MSE -0.39 ± 0.23 D) and one emmetropic participant, as previously discussed, became myopic during the course of the study.

A repeated measures ANOVA test revealed the change in MSE was not significantly different between the myopic and emmetropic groups ($F=0.150$, $p=0.980$; Fig. 3.3). Furthermore, the overall change in MSE over the 2.5 year period ($+0.10 \pm 0.38$ D) was not statistically significant

($F=1.101$, $p=0.361$). The rate of change in MSE was not correlated to baseline age ($r=0.019$, $p=0.896$).

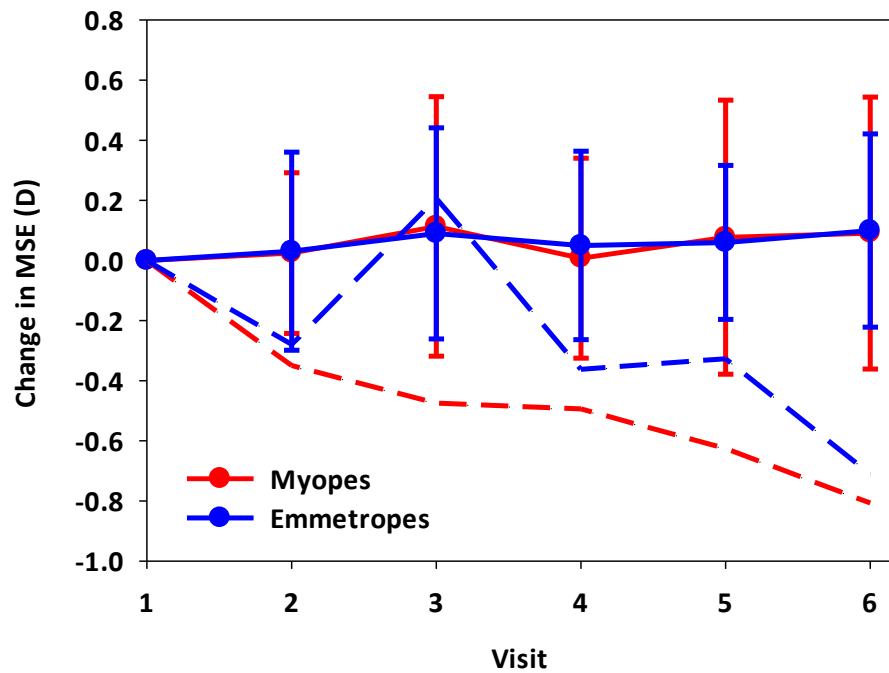


Fig. 3.3. The change in MSE documented at 6 visits over 2.5 years. The dashed lines represent the myopic (red) and emmetropic (blue) patients who became more myopic over the course of the study. The solid lines with error bars (± 1 standard deviation) represent the refractive change observed in the remainder of the myopic and emmetropic cohort.

The overall change in astigmatism vector J_{180} was not found to be statistically significant ($F=1.345$, $p=0.255$). The change in J_{45} was statistically significant ($F=8.085$, $p<0.001$), changing from $+0.02 \pm 0.19$ D at baseline to -0.06 ± 0.19 D at the final visit. Despite a significant difference between baseline J_{45} values ($F=4.506$, $p=0.039$; myopes $+0.07 \pm 0.25$ D; emmetropes -0.03 ± 0.11 D), the relative change in J_{45} observed between the myopic and emmetropic groups was not statistically significant ($F=0.736$, $p=0.597$). Furthermore, the changes in C ($F=0.733$, $p=0.599$), Θ ($F=0.887$, $p=0.491$) and P ($F=0.287$, $p=0.920$) were also found to be invariant over the course of the 2.5 year study. The average angle of the axis (Θ) of astigmatism shifted from 156° at baseline to 144° at the conclusion of the study. The axis of the anterior corneal toricity shifted from 31° to 67° , which

was statistically significant ($F=6.047$, $p<0.001$). The changes in the mean anterior corneal radius of curvature ($F=0.834$, $p=0.527$) and toricity ($F=0.799$, $p=0.496$) were not statistically significant.

The amplitude of accommodation in the right eye significantly decreased over the 2.5 year study ($F=37.219$, $p<0.001$; Fig. 3.4), and was significantly higher in the myopic group than the emmetropic group ($F=7.841$, $p=0.007$). The rate of change over the 2.5 year study was not significantly different between the two refractive groups ($F=1.213$, $p=0.307$) and was not dependent on baseline age ($r=0.045$, $p=0.752$). The change in refractive error was not correlated to the change in amplitude of accommodation ($r=0.028$, $p=0.847$).

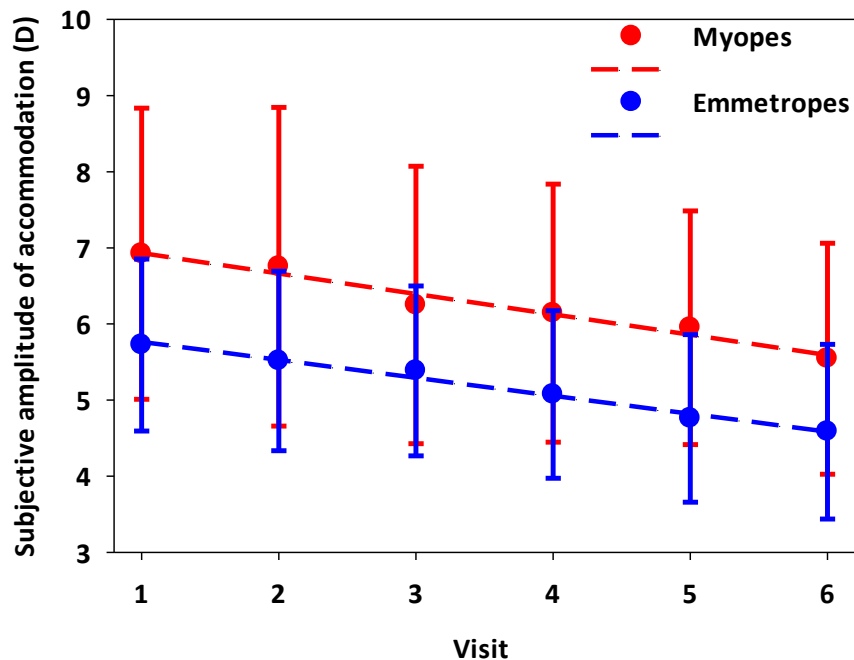


Fig. 3.4. The right eye amplitude of accommodation measured at each visit in myopic (red) and emmetropic (blue) patients with error bars (± 1 standard deviation). The dashed lines represent the regression line of the myopic ($y=-0.269x+7.201$; $R^2=0.969$; $p<0.001$) and emmetropic ($y=-0.236x+6.000$; $R^2=0.986$; $p<0.001$) data.

The two participants who underwent a myopic shift in refraction ≥ 0.50 D were invited to attend a follow-up appointment to undergo cycloplegic refraction. Unfortunately, the Chinese emmetropic participant had a narrow anterior chamber angle (van Herick grade 2) and therefore was not

suitable for instillation of the mydriatic drug. The myopic participant did not consent to the procedure, therefore no cycloplegic measurements were obtained.

3.1.3 Discussion

The present investigation represents the first prospective, longitudinal study to record changes in objective refraction during incipient presbyopia. Overall no significant change in refraction was observed, however a small proportion of participants experienced a significant hypermetropic or myopic shift in refraction (>0.50 D).

Data from previous studies have indicated a hypermetropic shift in refraction would be expected within the age group recruited for this study (Saunders, 1986; Lee *et al.*, 2002), however, the average change in MSE over the course of the 2.5 year study, although hypermetropic, was not found to be statistically significant. It therefore appears that the refractive change during incipient presbyopia is not as uniform as previously suggested and perhaps a myopic shift in refraction is also experienced, as Grosvenor and Skeates (1999) and Pointer and Gilmartin (2011) proposed. Indeed, 4% ($n=2$) of the total cohort underwent a myopic shift in refraction >0.50 D over the 2.5 year study, which constituted 5% of the myopic and 3% of the emmetropic participants. Previous research has indicated that between 15 to 20% of myopes and 3% of emmetropes experience a myopic shift in refraction >0.50 D during incipient presbyopia (Grosvenor and Skeates, 1999; Pointer and Gilmartin, 2011). A hypermetropic shift ≥ 0.50 D was also experienced in the myopic (10%) and emmetropic (10%) groups in the current study, with the remaining participants (85% of myopes and 87% of emmetropes) observing no significant change in MSE (<0.50 D). Grosvenor and Skeates (1999) reported 19% of myopic patients and 54% of emmetropic patients experienced a hypermetropic shift in MSE, whilst the MSE of the majority of myopic patients (66%) remained relatively stable (change <0.50 D). Ellingsen *et al.* (1997) also found the MSE of the majority of myopes did not change ≥ 0.50 D during incipient presbyopia, reporting shifts in MSE of -0.39 ± 0.60 D in 78 patients during their 30s and -0.29 ± 0.56 D in 130 patients during their 40s. The differences between refractive error progression of emmetropic and myopic

individuals reported by previous studies have not been replicated by the current study, possibly as a result of selection bias inherent within the previous retrospective studies. However the data presented by Grosvenor and Skeates (1999) and Pointer and Gilmartin (2011) also mapped refractive changes over a longer period (>4 years), therefore it is possible that, if extended, the current study may find a higher incidence of changes in refraction and differences may emerge according to refractive error group classification. Furthermore, Grosvenor and Skeates' (1999) study quantifies the refractive progression 10 to 26 years after the age of 40 years old, therefore their results are likely to represent refractive changes during presbyopia also.

A longitudinal study of similar length to the current study reported 45% of 322 eyes from 166 clinical microscopists aged 21 to 55 years experienced a myopic shift in MSE (≥ 0.375 D, mean change -0.58 ± 0.04 D) in 2 years (McBrien and Adams, 1997), however, data were not provided specifically on the pattern of refractive change according to age. Nevertheless, it is possible to ascertain from graphical representation of part of the data that 6 eyes belonging to individuals aged 40 to 42 years who were emmetropic (MSE between -0.25 to $+0.625$ D) at the start of the study experienced a myopic shift in refraction (≥ 0.375 D) and were classified as myopic (MSE ≤ -0.375 D) at the end of the 2 year study. The proportion of myopic incipient presbyopes who underwent a myopic shift in refraction is unclear. Despite the suboptimal criteria for a significant change in refraction (Rosenfield and Chiu, 1995), the work of McBrien and Adams (1997) provides longitudinal evidence of a myopic shift within an occupational group who typically spending most of their day working at near. Furthermore, the participants who experienced a myopic shift in refraction in McBrien and Adams' study were older (40 to 42 years) than the individuals who demonstrated a myopic shift in refraction in the present study (37 and 39 years), indicating that the myopic shift also occurs in older incipient presbyopes than observed during the course of the current study.

The present investigation reports a lower prevalence of myopia at baseline (42%) and after 2.5 years (37%) than found by Rahi *et al.* (2011) in a random sample of British 44 year olds selected at

their birth in 1958 (49%). The current cohort was comprised mainly of inner-city office workers, who typically spend a large proportion of their time working at near (55% reported spending 4-7 hours and 33% over 8 hours working at near daily), which has been posed as a dominant risk factor for myopia onset and progression in both children (Mutti *et al.*, 2002; Saw *et al.*, 2002) and adults (Goldschmidt, 1968; Simensen and Thorud, 1994; McBrien and Adams, 1997; Maheshwari *et al.*, 2011). It is therefore surprising that a lower prevalence of myopia was observed in the current study than reported by Rahi *et al.* (2011). Rahi and colleagues (2011) also reported 80% of the myopia was late-onset (after the age of 15 years). A total of 43% of the myopes from the current study were late-onset myopes. Therefore, this could indicate a further myopic shift within this cohort, particularly within the emmetropes, is likely.

The myopic patient (aged 37 years at baseline) who underwent a myopic shift in refraction of 0.71 D after the 2.5 year study was diagnosed as myopic at age 4 (early-onset myopia). Her MSE at visit 1 was -6.52 D and the patient had previously self-reported her refraction had stabilised during her adult years. It has been well-established that early-onset myopes are likely to progress to much higher levels of myopia than those who develop myopia after puberty (Blegvad, 1927; Mäntyjärvi, 1985). The trigger promoting continued myopia progression is unclear, however an increase in vitreous chamber depth has typically been identified as the structural correlate responsible (McBrien and Adams, 1997; Lin *et al.*, 1999).

The emmetropic patient (aged 39 years at baseline) who underwent a myopic shift in refraction of 0.81 D had no previous history of refractive error, although was of Chinese descent and gave birth to her second child 3 months after her second visit. The Chinese ethnicity has the highest prevalence of myopia of all ethnicities (Rose *et al.*, 2008; Lam *et al.*, 2012; Logan *et al.*, 2011) and hitherto the reason for this predisposition is unclear. Furthermore, pregnancy has been linked with myopic shifts in refraction, however the refractive error typically returns to pre-pregnancy levels post-partum (Pizzarello, 2003). The participant from the current study underwent a further myopic shift of 0.33 D from her third to sixth visit and was not diagnosed with gestational

diabetes or any other complications, suggesting her refractive shift is unlikely to be pregnancy-related.

Both participants who experienced a myopic shift were academics who reported spending over 8 hours daily working at near, which supports the theory suggesting high levels of near work are linked with myopia onset and progression in adults (Simensen and Thorud, 1994; McBrien and Adams, 1997). However, 31% of the rest of the cohort (including 1 of the participants who underwent a hypermetropic shift in refraction >0.50 D) also spent over 8 hours daily working at near. It is possible that an increase in hypermetropic blur at near due to an increasing lag of accommodation associated with a reduction in amplitude of accommodation may prompt a myopic shift in refraction in susceptible individuals as a compensatory mechanism to overcome near vision blur. Alternatively, struggling to focus at near may stimulate an accommodative spasm, instigated by a spasm of the ciliary muscle. However, both participants were able to read N5 print comfortably at each visit, maintaining a right eye amplitude of accommodation above 5.25 D and reporting no near visual symptoms throughout the study. Therefore the data do not support the hypothesis that the myopic shift in MSE is triggered as a compensatory mechanism due to near vision blur, and thus suggests that external factors may be responsible. Regardless of the trigger of the myopic shift during incipient presbyopia, the associated structural correlate is currently unclear and requires further investigation.

Considering ocular astigmatism during incipient presbyopia, the changes in the J_{180} , C and Θ presented here failed to reach statistical significance. Nevertheless, the change in J_{45} and the change in the axis of the mean cylinder from 156° to 144° indicate ocular astigmatism changes towards the against-the-rule direction (steeper horizontally) with age, which is agreement with previous studies (Baldwin and Mills, 1981; Goh and Lam, 1994; Gudmundsdottir *et al.*, 2000). The axis of anterior corneal toricity also shifted in the against-the-rule direction from 29° to 64° , thus supporting previous research indicating that the change towards against-the-rule ocular astigmatism with age is due to corneal changes (Anstice, 1971; Baldwin and Mills, 1981; Hayashi

et al., 1995), which may be associated with an age-related reduction in eyelid tension (Grosvenor, 1978; Vihlen and Wilson, 1983; Read *et al.*, 2007a).

Also in line with previous studies (Donders, 1864; Duane, 1922), right eye subjective amplitude of accommodation significantly decreased with age when considered cross-sectionally ($p < 0.001$, Fig 3.2) and longitudinally ($p < 0.001$; Fig. 3.4). The change in right eye amplitude of accommodation was not linked to refractive error progression ($p = 0.847$), possibly indicating that changes in refraction are not governed or coordinated by the age-related structural changes occurring within the accommodative apparatus and may instead be associated with an independent mechanism, for example posterior ocular shell growth. Further work is required to determine whether subjective amplitude of accommodation measured by an RAF rule is sensitive to age-related increases in depth of focus owing to pupil miosis (Birren *et al.*, 1950) and an age-related increase in tolerance to blur (Kline *et al.*, 1999), however these factors are unlikely to have had a significant impact on the results during the course of this study. Nevertheless, quantification of biometric changes occurring in the accommodative apparatus during incipient presbyopia would help to verify whether there is a relationship between a loss in accommodative ability and a change in refractive error.

A possible limitation of the current study is that all objective measurements of refraction were acquired without the prior instillation of a cycloplegic agent. Non-cycloplegic refraction was chosen to ensure involvement in the study did not impair the ability of the cohort, who were mostly Aston University staff, to return to work immediately following the appointment. In addition, the discomfort associated with cycloplegic eye drop instillation and the transient effects may have discouraged participants from attending future review appointments, risking an unacceptably high attrition rate and reducing the power of the study. Unfortunately, current autorefractors typically underestimate hypermetropia due to difficulties relaxing patients' accommodation (Mallen *et al.*, 2001; Davies *et al.*, 2003; Sheppard and Davies, 2010b). However, the individuals who participated in the present investigation were older and had lower amplitudes

of accommodation than patients included in most autorefractor validation studies (Mallen *et al.*, 2001; Davies *et al.*, 2003; Sheppard and Davies, 2010b), therefore it is unlikely that erroneous accommodation would have had a significant impact on the magnitude of the results in the current study.

At the conclusion of the 2.5 year study, participants who underwent a myopic shift in refraction >0.50 D were invited to return for re-measurement of their objective refraction under cycloplegia. The aim of measuring cycloplegic refraction was to determine whether a spasm of accommodation, instigated by ciliary muscle spasm, was responsible for the myopic shift in refraction. Unfortunately, it was not possible to obtain cycloplegic measurements from the 2 participants who experienced a myopic shift during the course of the present study. As well as investigating differences between non-cycloplegic and cycloplegic refraction, future studies should also quantify longitudinal changes in ciliary muscle morphology and axial biometry to investigate the structural correlate responsible for the myopic shift in MSE during incipient presbyopia.

The current study provides the first prospective, longitudinal insight into how refractive error progresses during incipient presbyopia. In conclusion, the incidence of a myopic shift in refractive error during incipient presbyopia does not appear to be as large as previously indicated by retrospective research (Grosvenor and Skeates, 1999; and Pointer and Gilmartin, 2011), however a significant myopic shift in refraction is evident within a small proportion of individuals in this age group, affecting a similar proportion of emmetropic and myopic individuals. The incidence of a hypermetropic shift is greater than the myopic shift, however overall, a significant change in refraction is not evident over 2.5 years during incipient presbyopia. The structural origins of the hypermetropic and myopic shifts in refraction are unclear and require further investigation, particularly focused on the accommodative apparatus in order to determine whether a loss of accommodative ability is associated.

CHAPTER 4

Longitudinal changes in ciliary muscle morphology and contractility during incipient presbyopia

4.1 Introduction

Despite the involvement of the ciliary muscle in accommodation (Sheppard and Davies, 2010a; Lewis *et al.*, 2012; Lossing *et al.*, 2012), presbyopia (Sheppard and Davies, 2011; Richdale *et al.*, 2013) and possibly myopia development (Bailey *et al.*, 2008; Jeon *et al.*, 2012; Buckhurst *et al.*, 2013; Pucker *et al.*, 2013), there is a paucity of *in vivo* ciliary muscle research. Indeed, imaging the ciliary muscle *in vivo* represents a significant challenge due to the obscured position of the ciliary muscle behind the highly pigmented iris. Consequently, the following chapter explores *in vivo* changes in ciliary muscle morphology with age (study one) and accommodation (study two) using anterior segment optical coherence tomography (AS-OCT) in an incipient presbyopic population.

4.2 Study One: A longitudinal investigation of changes in ciliary muscle morphology during incipient presbyopia

The literature is in agreement that the development of presbyopia is complex, involving a number of interdependent structures (Gilmartin, 1995; Atchison, 1995; Charman, 2008), with perhaps the most significant contribution arising from an increase in crystalline lens stiffness with age (Heys *et al.*, 2004; Glasser, 2008). As the driving force for the accommodative response, the ciliary muscle has been implicated in numerous presbyopia theories (Donders, 1864; Duane, 1922; Fincham, 1937; Croft *et al.*, 2013a), primarily in primate models (Bito and Miranda, 1989; Tamm *et al.*, 1992b; Wyatt, 1993; Croft *et al.*, 2006; Croft *et al.*, 2013a). Indeed, significant ciliary muscle structural changes occur with age (Nishida and Mizutani, 1992; Tamm *et al.*, 1992a; Strenk *et al.*, 1999; Pardue and Sivak, 2000; Strenk *et al.*, 2006; Kasthurirangan *et al.*, 2011; Sheppard and Davies, 2011).

Considering human eye data, *in vitro* research has reported the quantity of radial and circular ciliary muscle fibres (both responsible for the centripetal shift in muscle mass during contraction)

increases with age, whereas the proportion of longitudinal fibres (responsible for the anterior shift in muscle mass during contraction) decreases with age (Nishida and Mizutani, 1992; Tamm *et al.*, 1992a; Pardue and Sivak, 2000). Furthermore, additional connective tissue accumulates in the anterior portion of the ciliary muscle, where the radial and circular fibres reside (Nishida and Mizutani, 1992; Tamm *et al.*, 1992a; Pardue and Sivak, 2000). The cumulative effect of these structural changes is an anterior and inwards displacement of ciliary muscle mass. The anterior length (measured perpendicularly from the line of ciliary muscle maximum thickness to the scleral spur; Fig. 2.4) of the ciliary muscle (Tamm *et al.*, 1992a; Pardue and Sivak, 2000) and the total length (distance from scleral spur to ciliary muscle posterior visible limit; Fig. 2.4) of the ciliary muscle (Tamm *et al.*, 1992a) also decrease with age. In addition, the increasingly anterior tension exerted on the ciliary muscle by the anterior movement of the growing crystalline lens may also contribute to the anterior movement of ciliary muscle mass with age (Strenk *et al.*, 1999; Pardue and Sivak, 2000; Atchison *et al.*, 2008; Strenk *et al.*, 2010).

In vivo cross-sectional research supports the findings of *in vitro* studies (Nishida and Mizutani, 1992; Tamm *et al.*, 1992a; Pardue and Sivak, 2000), reporting an anterior (Sheppard and Davies, 2011) and inwards (Strenk *et al.*, 1999; 2006; Kasthurirangan *et al.*, 2011; Sheppard and Davies, 2011) displacement of ciliary muscle mass with age. Unlike the aforementioned *in vitro* studies (Nishida and Mizutani, 1992; Tamm *et al.*, 1992a; Pardue and Sivak, 2000), Sheppard and Davies (2011) investigated age-related ciliary muscle changes with reference to the location of the ciliary muscle section examined and the ametropia of 79 patients, aged 19 to 70 years. The observed age-dependent ciliary muscle changes were particularly evident in emmetropic eyes and nasal-temporal asymmetry was also noted, with thicker ciliary muscle measurements reported temporally throughout the lifespan (Sheppard and Davies, 2011). Sheppard and Davies (2011) reported that total ciliary muscle length remained invariant of age, whereas anterior length decreased nasally and temporally in emmetropic eyes only. Ciliary muscle maximum thickness increased by 0.003 mm/year both nasally and temporally and the distance from the scleral spur to

the innermost ciliary muscle point (inner apex) decreased by 0.005 mm/year nasally and 0.004 mm/year temporally in both myopic and emmetropic eyes. In order to overcome the inherent inaccuracies arising from measuring ciliary muscle thickness at fixed distances from the scleral spur, which is likely to represent different anatomical areas between individuals, Sheppard and Davies (2010a; 2011) also measured the thickness at 25%, 50% and 75% of the total ciliary muscle length. The thickness at 25% of the total ciliary muscle length did not change significantly with age nasally or temporally in myopic or emmetropic individuals. However, the thickness at 50% and 75% of the total length decreased with age temporally in myopic and emmetropic eyes (Sheppard and Davies, 2011).

Despite the limitations of measuring ciliary muscle thickness at fixed distances from the scleral spur, Lewis *et al.* (2012) suggested the CM2 region may act as a fulcrum, where the net change in thickness during accommodation is always zero. Age-related changes in the thickness of this area are therefore of particular interest as the age-related antero-inwards change in ciliary muscle morphology resembles the ciliary muscle movement during accommodation (Flügel *et al.*, 1990; Tamm *et al.*, 1991; Tamm *et al.*, 1992a; Croft *et al.*, 2006; Sheppard and Davies, 2010a; Lewis *et al.*, 2012). Indeed, cross-sectional research has reported an age-related thickening anterior to CM2 and a thinning posterior to CM2 (Sheppard and Davies, 2011). However, longitudinal ciliary muscle changes during adulthood are hitherto undocumented. Longitudinal data provides a more accurate representation of how intrasubject ciliary muscle morphology changes with age and refractive error progression, which is of particular importance considering the reported differences between ciliary muscle morphology in emmetropic and myopic eyes (Oliveira *et al.*, 2005; Bailey *et al.*, 2008; Schultz *et al.*, 2009; Muftuoglu *et al.*, 2009; Sheppard and Davies, 2011; Jeon *et al.*, 2012; Buckhurst *et al.*, 2013; Kuchem *et al.*, 2013; Pucker *et al.*, 2013).

Cross-sectional data have found that the anterior thickness of the ciliary muscle (thickness measurement 1 mm from the scleral spur and the point of maximum ciliary muscle thickness) is greater in hypermetropic children (Pucker *et al.*, 2013) and the posterior thickness of the ciliary

muscle (thickness measurements 2 mm and 3 mm from the scleral spur) is greater in myopic children (Bailey *et al.*, 2008; Schultz *et al.*, 2009; Pucker *et al.*, 2013) and adults (Oliveira *et al.*, 2005; Muftuoglu *et al.*, 2009; Jeon *et al.*, 2012; Buckhurst *et al.*, 2013; Kuchem *et al.*, 2013). However, longitudinal data reviewing children after 1 year showed no significant change in ciliary muscle thickness during myopia progression (Bailey *et al.*, 2011). Similarly, cross-sectional research considering the thickness of the ciliary muscle as a proportion of its total length reported no significant correlation between ciliary muscle thickness and axial ametropia, despite observing that the ciliary muscle was significantly longer in myopic eyes (Sheppard and Davies 2010a; 2011). Further longitudinal research is required in order to elucidate whether changes in ciliary muscle morphology are associated with changes in refraction during adulthood.

The aim of this study is to document *in vivo* changes in ciliary muscle morphology longitudinally during incipient presbyopia and in particular, to elicit whether ciliary muscle changes are associated with changes in ocular refraction within the incipient phase of presbyopia.

4.2.1 Method

The study was approved by the Aston University Audiology and Optometry Research Ethics Committee (see Appendix 1) and was conducted in accordance with the tenets of the Declaration of Helsinki. Informed consent was obtained from all the participants after an explanation of the nature and possible consequences of the study (see Appendix 2 for a copy of the information sheet and consent form). One optometrist (DL) collected the data at each visit and did not refer to the results from the previous sessions until after each appointment, when the data were inputted into an Excel spreadsheet (Microsoft, Washington, USA).

In order to collect longitudinal data to monitor changes in ciliary muscle morphology during incipient presbyopia, the following experimental protocol was repeated every 6 months over 2.5 years.

4.2.1a Participants

The details of the 51 participants recruited for this study have been previously described in section 3.1.2a.

4.2.1b Objective refractive error

Objective refractive error was measured by the binocular open-field WAM-5500 autorefractor (Grand Seiko Co. *Ltd.*, Japan) using the method described in section 3.1.1e. Five consecutive measurements of refraction were acquired and MSE was calculated using equation 1.1.

4.2.1c Visante AS-OCT ciliary muscle imaging

Images of the temporal ciliary muscle of the right eye were acquired using a Visante AS-OCT (Carl Zeiss Meditec, California, USA). Each participant wore an eye patch over their left eye throughout data collection. Patients were asked to place their chin and forehead against the Visante supports and fixate straight-ahead at the centre of the internal green star target. The chin rest was adjusted to align the participant's right eye to allow visualisation of the anterior crystalline lens surface, which was guided by the real-time video stream of the external eye. High resolution corneal mode was selected and once the vertical white fixation line was visible through the centre of the image, which indicated the measurement beam was coincident with the visual axis of the eye, patients were asked to turn their eyes to view the external Maltese cross target through the Badal lens system (angled at 40° to primary position) mounted on the left-hand side of the Visante forehead bar (Fig. 2.3). Patients were permitted to turn their heads very slightly, if required, whilst maintaining constant contact with the chin rest and forehead bar. Since all patients were aligned to the optical axis of the Visante in primary position, only minor vertical alignment adjustments were required once the participant adducted their right eye to view the centre of the external Maltese cross. Horizontal alignment was determined by the real-time image produced on the control screen, which was adjusted to ensure simultaneous visualisation of the scleral spur and ciliary muscle posterior visible limit, as depicted in Fig. 2.4.

Once accurately aligned, the Maltese cross target was moved from the default 10 cm separation from the +10 D Badal lens in order to provide a clear 0.00 D stimulus for myopic participants. The appropriate modification of the distance between the Maltese cross target and Badal lens was based on each participant's MSE, and was confirmed as being appropriate by asking the participant to report when the target first became clear as it was gradually slid closer to the Badal lens. Patients were asked to focus on the centre of the Maltese cross target and keep it as clear as possible throughout data collection. Patients were also asked to keep their head and eyes as still as possible. Three consecutive images of the right eye temporal ciliary muscle were acquired and saved. Only the right eye temporal ciliary muscle was imaged because the ciliary muscle tends to be thicker temporally than nasally and the temporal ciliary muscle shows a greater contractile response during accommodation (Sheppard and Davies, 2010a), therefore changes in ciliary muscle morphology temporally are likely to have a bigger impact on the overall accommodative response.

Each ciliary muscle image was exported from the Visante in binary form for analysis with the custom-designed Matlab R2012b (The MathWorks Inc., Massachusetts, USA) software described in section 2.3.4. The mean values for curved total length (Curved TL), anterior length (Ant L), scleral spur to inner apex (SS-IA), maximum thickness (Max T), the thickness 2 mm posterior to the scleral spur (CM2) and the thickness at 25% (CM25), 50% (CM50) and 75% (CM75) of the curved total length were recorded for each set of images.

4.2.1d Statistical analysis

All data were tested for normality using the Shapiro-Wilk test (SigmaPlot; Systat Software Inc., California, USA). A repeated measures ANOVA test was conducted to determine whether the changes in ciliary muscle parameters were significant over the 2.5 year study, and whether they were dependent on ametropia classification (SPSS, SPSS Inc, Illinois, USA).

Separate multiple linear regression tests were used to investigate whether changes in ciliary muscle morphology were associated with baseline age, the change in amplitude of accommodation or the change in MSE during the course of the study. T-tests were used to determine whether the ciliary muscle changes of the participants who became more myopic ($MSE > 0.50$ D) were significantly different to the changes experienced by the remaining cohort.

4.2.2 Results

Repeated measures ANOVA testing revealed the reduction in curved TL ($F=12.832$, $p<0.001$), Ant L ($F=58.963$, $p<0.001$) and SS-IA ($F=39.279$, $p<0.001$) and the increase in Max T ($F=33.773$, $p<0.001$), CM2 ($F=4.450$, $p=0.001$), CM25 ($F=15.689$, $p<0.001$) and CM50 ($F=16.587$, $p<0.001$) were statistically significant over the 2.5 year study (Table 4.1); indicating ciliary muscle mass moved anteriorly and inwards over the 2.5 year period. However, CM75 did not change significantly during the study ($F=1.758$, $p=0.178$).

The age-related changes in ciliary muscle morphology were not dependent on refractive error group classification for any parameter (curved TL $F=0.428$, $p=0.829$; Max T $F=2.078$, $p=0.069$; Ant L $F=1.901$, $p=0.130$, SS-IA $F=0.697$, $p=0.561$; CM2 $F=1.056$, $p=0.385$; CM25 $F=0.611$, $p=0.692$, CM50 $F=1.202$, $p=0.309$; CM75 $F=0.812$, $p=0.542$; Fig 4.1, Fig 4.2). Curved TL ($F=9.745$, $p=0.003$), Ant L ($F=6.495$, $p=0.014$) and SS-IA ($F=7.211$, $p=0.010$) values were all significantly larger in myopic eyes throughout the study (Max T $F=0.167$, $p=0.684$; CM2 $F=3.249$, $p=0.078$, CM25 $F=0.052$, $p=0.820$; CM50 $F=0.808$, $p=0.373$; CM75 $F=0.476$, $p=0.494$; Fig 4.1, Fig, 4.2). Curved TL and Ant L were directly proportional ($r=0.458$, $p<0.001$).

Post-hoc statistical testing was not carried out because no statistically significant differences were observed between the emmetropic and myopic groups (between-subject ANOVA factor) during the course of the study.

Ciliary muscle parameter	Mean baseline value (mm \pm SD)		Mean change after 2.5 years (mm \pm SD)	Statistical significance (<i>p</i>) of changes after 2.5 years
	Myopes	Emmetropes		
Curved TL	5.606 \pm 0.518	5.250 \pm 0.451	-0.188 \pm 0.258	<0.001
Max T	0.460 \pm 0.051	0.465 \pm 0.037	+0.052 \pm 0.040	<0.001
Ant L	1.199 \pm 0.143	1.109 \pm 0.132	-0.159 \pm 0.122	<0.001
SS-IA	1.266 \pm 0.135	1.179 \pm 0.130	-0.115 \pm 0.112	<0.001
CM2	0.357 \pm 0.048	0.330 \pm 0.048	+0.016 \pm 0.031	0.007
CM25	0.438 \pm 0.048	0.437 \pm 0.049	+0.040 \pm 0.042	<0.001
CM50	0.263 \pm 0.039	0.250 \pm 0.034	+0.029 \pm 0.035	<0.001
CM75	0.121 \pm 0.028	0.125 \pm 0.076	+0.003 \pm 0.066	0.272

Table 4.1. Mean baseline ciliary muscle parameters for the myopic and emmetropic groups and the mean change observed across both groups over 2.5 years. Statistical significance is denoted by a bold *p* value.

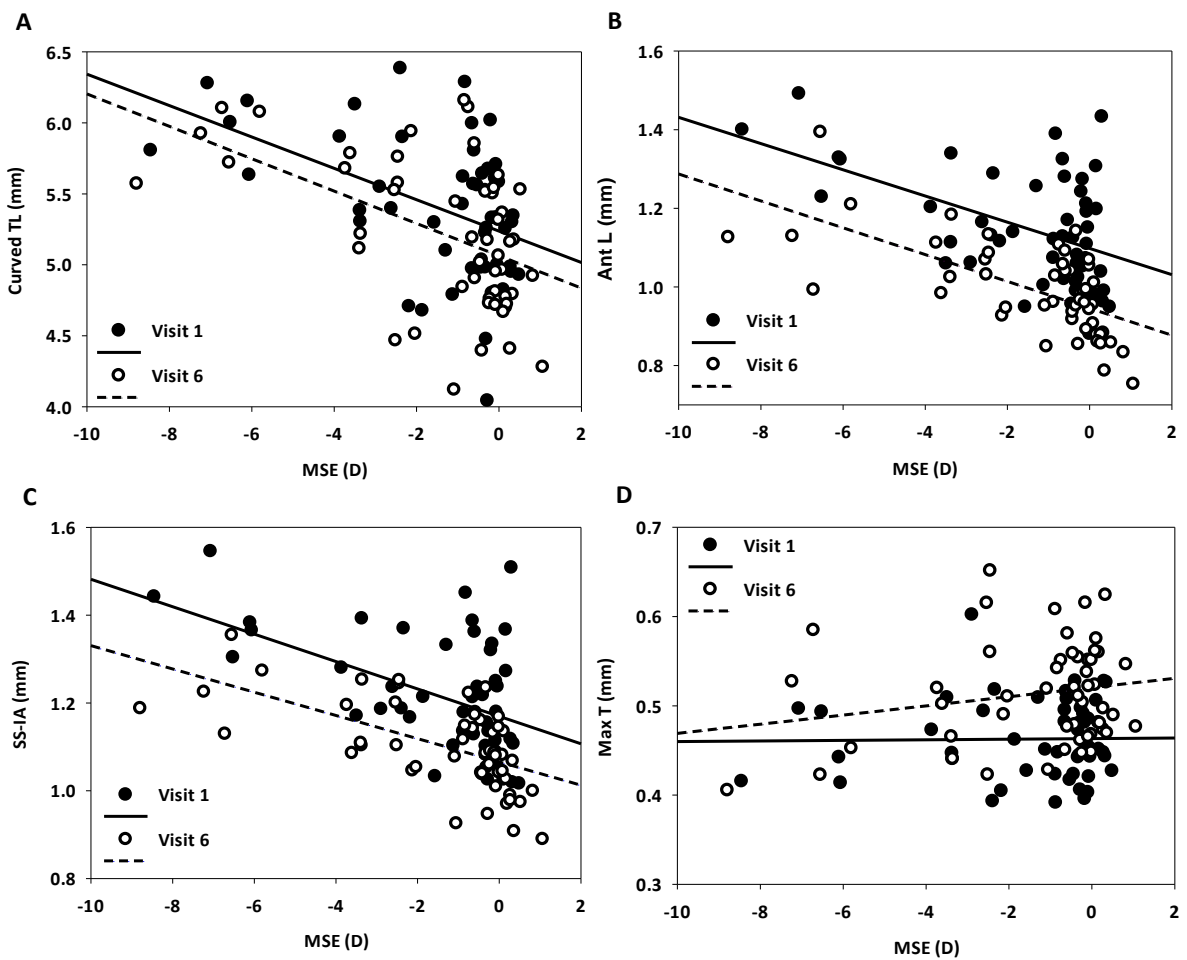


Fig. 4.1. Curved TL (A), Ant L (B), SS-IA (C) and Max T (D) of the temporal ciliary muscle at visit 1 (filled circles and solid regression line) and after 2.5 years at visit 6 (open circles and dashed regression line) according to the MSE measured at visit 1 and 6, respectively.

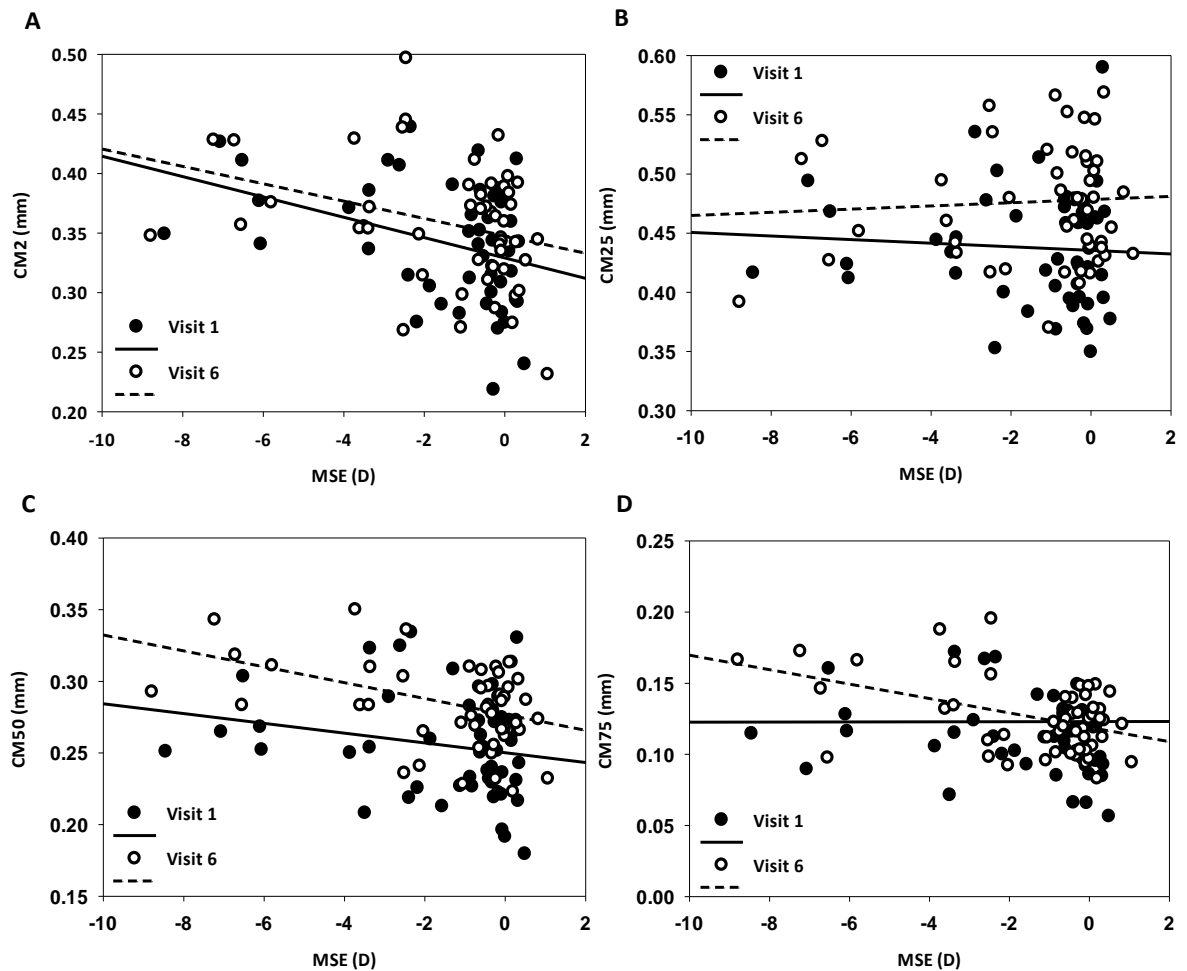


Fig. 4.2. CM2 (A), CM25 (B), CM50 (C) and CM75 (D) of the temporal ciliary muscle at visit 1 (filled circles and solid regression line) and after 2.5 years at visit 6 (open circles and dashed regression line) according to the MSE measured at visit 1 and 6, respectively.

Multiple linear regression analysis showed no relationship between changes in curved TL ($p=0.493$), Max T ($p=0.921$), Ant L ($p=0.731$), SS-IA ($p=0.778$), CM2 ($p=0.731$), CM25 ($p=0.901$), CM50 ($p=0.812$) or CM75 ($p=0.710$) with baseline age ($r=0.225$, $r^2=0.051$). Additionally, no association between the age-related changes in right eye amplitude of accommodation and ciliary muscle parameters were found ($r=0.225$, $r^2=0.051$; curved TL $p=0.658$, Max T $p=0.990$, Ant L $p=0.223$, SS-IA $p=0.120$, CM2 $p=0.451$, CM25 $p=0.739$, CM50 $p=0.937$, CM75 $p=0.328$). Further multiple linear regression analysis revealed changes in MSE were not dependent on curved TL ($r=0.185$, $r^2=0.034$, $p=0.837$), Max T ($p=0.961$), Ant L ($p=0.440$), SS-IA ($p=0.423$), CM2 ($p=0.656$), CM25 ($p=0.858$), CM50 ($p=0.898$) or CM75 ($p=0.732$), as shown in Fig. 4.1 and Fig. 4.2. Moreover, the ciliary muscle change of the 2 participants discussed in Chapter 3 who became more myopic

(>0.50 D) were not significantly different to the remaining cohort (curved TL $t=-1.024$, $p=0.311$; Max T $t=-0.814$, $p=0.419$; Ant L $t=-0.788$, $p=0.435$; SS-IA $t=-1.294$, $p=0.202$; CM2 $t=-0.865$, $p=0.391$, CM25 $t=-1.611$, $p=0.114$; CM50 $t=-0.676$, $p=0.502$; CM75 $t=-0.020$ $p=0.984$).

4.2.3 Discussion

The present investigation is the first to document longitudinal changes in ciliary muscle morphology with respect to changes in ocular accommodative function and refractive error development in an incipient presbyopic population. An age-related antero-inwards shift of ciliary muscle mass was observed, which was independent of baseline ametropia, refractive error progression and the reduction in subjective amplitude of accommodation over the 2.5 year study.

Previous cross-sectional studies considering the thickness of the ciliary muscle at fixed distances from the scleral spur have indicated that myopia is associated with a thicker ciliary muscle posteriorly (2 mm and 3 mm from the scleral spur) in both children (Bailey *et al.*, 2008; Schultz *et al.*, 2009; Pucker *et al.*, 2013) and adults (Oliveira *et al.*, 2005; Muftuoglu *et al.*, 2009; Jeon *et al.*, 2012; Buckhurst *et al.*, 2013; Kuchem *et al.*, 2013). Indeed, a trend emerged for myopic eyes to have a thicker ciliary muscle 2 mm from the scleral spur (CM2) than emmetropic eyes in the work by Sheppard and Davies (2010a; $F=2.84$, $p=0.06$) and the current study ($F=3.249$, $p=0.078$). However, in agreement with the findings of Sheppard and Davies (2010a; 2011), the present investigation also found that the total length of the ciliary muscle is longer in myopic eyes. It is therefore unsurprising that emmetropic patients have a thinner ciliary muscle than myopic patients posteriorly because the ciliary muscle of emmetropic individuals terminates (reaching a minimum thickness) more anteriorly. Furthermore, when considering the thickness of the ciliary muscle as a percentage of the total length, neither the current study, nor the work by Sheppard and Davies (2010a; 2011), found any differences in CM25, CM50 or CM75 values between myopic and emmetropic individuals.

Uniquely, this study reports that in addition to myopic eyes boasting longer ciliary muscle total length and anterior length measurements (Sheppard and Davies, 2010a), the distance from the scleral spur to the inner apex is also significantly longer in myopic eyes. These findings support the proposed model of ciliary muscle growth during axial length elongation and myopia progression suggested by Sheppard and Davies (2010a), whereby antero-posterior stretching of the ciliary muscle, which relocates the inner apex more posteriorly, is accompanied by radial ciliary muscle growth, which ensures the overall thickness of the ciliary muscle does not change. The corresponding change in crystalline lens position, which is connected to the non-pigmented ciliary epithelium by the zonules (Raviola, 1971; Rohen, 1979), is unclear; numerous studies have reported the anterior chamber depth (typically measured axially from the posterior corneal surface to the anterior crystalline lens surface) increases as myopia progresses (Fontana and Brubaker, 1980; Lam *et al.*, 1999; Logan *et al.*, 2005; Chen *et al.*, 2009), albeit at a slower rate than axial length elongation (Lam *et al.*, 1999), however this may be a function of global expansion of the entire eye rather than the posterior relocation of the crystalline lens by the posterior movement of the ciliary muscle inner apex. Superimposing longitudinally acquired T₁-weighted magnetic resonance images of the ciliary muscle and crystalline lens (Richdale *et al.*, 2009) with respect to the crystalline lens centroid would reveal how changes in the position of the ciliary muscle apex influences crystalline lens position.

Regarding the change in refraction observed over the 2.5 year study (as discussed in Chapter 3), there was no correlation between changes in refraction and any ciliary muscle parameter measured. Therefore, changes in ciliary muscle morphology do not appear to be determinant of, or the consequence of, changes in refractive error within an incipient presbyopic population. Indeed, these findings are supported by a longitudinal study in children (Bailey *et al.*, 2011) and intrasubject comparison in anisometropic adults (Kuchem *et al.*, 2013). Additionally, the 2 participants who underwent a myopic shift in MSE (>0.50 D) did not demonstrate an antero-inward movement of ciliary muscle mass beyond the average reported for the cohort, suggesting

a spasm of the ciliary muscle is not responsible for the myopic shift observed during incipient presbyopia.

Considering the anterior length and total length of the ciliary muscle, which are typically longer in myopic eyes, the lack of correlation to a myopic change in refraction could indicate that the age-related reduction in ciliary muscle length negates any elongation occurring as a result of stretching from axial length elongation. Alternatively, perhaps this is indicative that axial length elongation is not responsible for a myopic shift in refraction during incipient presbyopia, and an alternative mechanism, perhaps lenticular based, is responsible for a myopic shift during this period of ageing. Investigating changes in refraction during incipient presbyopia with respect to axial biometry would help to clarify this matter further.

Overall, a significant change in ciliary muscle morphology occurred over the 2.5 year study in all parameters measured apart from CM75. Curved TL, Ant L and SS-IA decreased with age, whereas Max T, CM2, CM25 and CM50 all increased with age (Table 4.1), which is consistent with previous research suggesting the ciliary muscle moves anteriorly (Sheppard and Davies, 2011) and inwards (Strenk *et al.*, 1999; 2006; Kasthurirangan *et al.*, 2011; Sheppard and Davies, 2011) with age. Richdale *et al.* (2013) concluded ciliary muscle thickness was not correlated with age in 26 emmetropic patients aged 30 to 50 years investigated cross-sectionally. However, as previously discussed in section 2.3.4, the semi-automated algorithm employed by Richdale and colleagues to extract ciliary muscle parameters from optical coherence tomography images depicts the ciliary body, not the ciliary muscle, therefore it is possible that intricate ciliary muscle changes would not have been identified.

Despite observing an age-related increase in temporal ciliary muscle Max T, Sheppard and Davies (2011) reported no significant change in CM25 and thinning at CM50 and CM75. The reduction in thickness measured at CM2 was only significant temporally in emmetropic eyes (Sheppard and Davies, 2011), suggesting that an area where no age-related change in ciliary muscle thickness

occurs may lie between CM25 and CM2. Indeed, the current investigation observed the smallest change in thickness at CM2, therefore augmenting the hypothesis that this region may act as a fulcrum point during ageing as well as accommodation (Lewis *et al.*, 2012). However, due to the reduction in total length with age, CM25, CM50 and CM75 represented more anterior ciliary muscle locations in the final visits, therefore it is unsurprising the changes observed in these parameters were greater than observed at a fixed location from the scleral spur (CM2). In order to determine the exact location of the putative fulcrum point in all patients, comprehensive segmentation of superimposed ciliary muscle images acquired longitudinally would be required.

The decrease in posterior ciliary muscle thickness has been attributed to a reduction in the proportion of longitudinal fibres with age (Tamm *et al.*, 1992a; Pardue and Sivak, 2000; Sheppard and Davies, 2011). However, unlike previous studies (Pardue and Sivak, 2000; Sheppard and Davies, 2011), the current study and *in vitro* work by Tamm *et al.* (1992b) found a reduction in total ciliary muscle length with age. Therefore, it is feasible a reduction in ciliary muscle thickness due to age-related attenuation of longitudinal fibres posteriorly may have been masked by an increase in ciliary muscle thickness associated with the anterior movement of the ciliary muscle unit. Indeed, the thickness change at CM75 was not statistically significant, hence indicating that the area most significantly affected by longitudinal fibre attenuation may be close to the posterior visible limit. Thus, the posterior attenuation of longitudinal fibres may also contribute to the reduction in total ciliary muscle length, perhaps elucidating why the reduction in total length (-0.188 ± 0.258 mm) was greater than the reduction in anterior length (-0.159 ± 0.122 mm). Despite longitudinal fibres typically traversing the length of the ciliary muscle, the reduction in thickness more anteriorly is likely to be offset by the age-related build-up of connective tissue and the increase in radial and circular fibres anteriorly (Nishida and Mizutani, 1992; Tamm *et al.*, 1992a; Pardue and Sivak, 2000). Unfortunately, measuring changes in the *in vivo* distribution of fibres within the ciliary muscle is beyond the capability of current imaging techniques.

The present investigation found the rate of change in ciliary muscle morphology during incipient presbyopia was not dependent on baseline ametropia, therefore questioning the theory that the mechanism responsible for co-ordinated ciliary muscle length elongation with AXL elongation is protective of changes in ciliary muscle shape and position during later life (Sheppard and Davies, 2011). Furthermore, the changes in ciliary muscle morphology observed over the current 2.5 year study are larger than reported cross-sectionally by Sheppard and Davies (2011). Within the age-group included in the current study (33 to 45 years), no correlation between changes in ciliary muscle parameters and baseline age emerged. Nevertheless, the reported rate of change in ciliary muscle morphology, particularly total length, does not seem to be sustainable throughout the lifespan. Therefore, it seems likely the rate of change in ciliary muscle morphology is not linear with age, perhaps accelerating during the onset of presbyopia. Further longitudinal studies including a larger age range over an extended period are required to investigate the temporal and spatial characteristics of the changes in ciliary muscle morphology.

A possible limitation of the current study is that the same section of the temporal ciliary muscle may not have been imaged at each visit. However, every effort was made to ensure ciliary muscle alignment was as repeatable as possible by initially aligning the patient in primary position until the vertical white fixation line was visible through the centre of the image, then asking patients to rotate their eyes with minimal head movement to view the external target. Observation of the real-time video image of the anterior eye also guided final adjustments to ensure the Visante beam bisected the centre of the eye transversely. Further positional adjustments were also made if the patient moved during data collection and the average measurements extracted from three images were used for statistical analysis in order to reduce the impact of minor differences in alignment. The repeatability of patient alignment and subsequent ciliary muscle image analysis using the semi-automated algorithm has been investigated in section 2.3.4, and the change in ciliary muscle parameters detailed in Table 4.1 were appreciably larger than the measured bias, indicating that the differences in alignment were unlikely to be significant.

The current study provides the first prospective, longitudinal insight into how ciliary muscle morphology changes during incipient presbyopia. Bespoke software was utilised to extract ciliary muscle parameter measurements from Visante OCT images, which uniquely to all previous studies (Oliveira *et al.*, 2005; Bailey *et al.*, 2008; Muftuoglu *et al.*, 2009; Schultz *et al.*, 2009; Sheppard and Davies, 2010a; Kao *et al.*, 2011; Sheppard and Davies, 2011; Jeon *et al.*, 2012; Lewis *et al.*, 2012; Lossing *et al.*, 2012; Buckhurst *et al.*, 2013; Kuchem *et al.*, 2013; Pucker *et al.*, 2013; Richdale *et al.*, 2013) was able to measure ciliary muscle curved length (following the scleral contour). In conclusion, an antero-inwards shift in muscle mass occurs with age, which is not dependent on ametropia. Further research is required to investigate how the entire circumference of the ciliary muscle changes during the natural ageing process.

4.3 Study Two: A longitudinal investigation of changes in ciliary muscle contractility during incipient presbyopia

The precise role of the ciliary muscle in the decline of accommodative ability and the development of presbyopia remains equivocal. During accommodation, numerous authors have reported the human ciliary muscle thickens anteriorly, moving centripetally (Strenk *et al.*, 1999; 2006; 2010; Kasthurirangan *et al.*, 2011; Sheppard and Davies, 2010a; 2011; Croft *et al.*, 2013a; Richdale *et al.*, 2013), reducing in length (Pardue and Sivak, 2000; Sheppard and Davies, 2010a; 2011) and thinning posteriorly (Jeon *et al.*, 2012; Lewis *et al.*, 2012; Lossing *et al.*, 2012). The ciliary muscle contractile response consequently reduces zonular tension and permits the capsule to mould the crystalline lens into a thicker, more spherical shape (von Helmholtz, 1855; Brown, 1973; Koretz *et al.*, 1997; Dubbelman *et al.*, 2005; Ostrin *et al.*, 2006; Hermans *et al.*, 2007; Tsorbatzoglou *et al.*, 2007; Kasthurirangan *et al.*, 2011). The longitudinal ciliary muscle fibres are responsible for antero-posterior ciliary muscle mobility, whereas the radial and circular fibres are responsible for the centripetal movement of muscle mass during contraction, with the circular fibres acting as a sphincter (Pardue and Sivak, 2000). The contractile response is thought to be greater temporally than nasally, possibly in order to align the lenticular axes during convergence

(Sheppard and Davies, 2010a). However, the impact of the age-related structural changes discussed in section 4.2 on the ciliary muscle contractile response is currently unclear.

Previous research has reported the increase in ciliary muscle thickness during accommodation is weaker in participants with thicker disaccommodated ciliary muscles (Jeon *et al.*, 2012; Croft *et al.*, 2013a). Therefore, owing to the increase in ciliary muscle thickness with age (Sheppard and Davies, 2011; section 4.2), it is feasible the contractile response may diminish. However, the majority of *in vivo* cross-sectional ciliary muscle research supports the Hess-Gullstrand presbyopia theory, reporting no significant attenuation of the ciliary muscle contractile response with age (Strenk *et al.*, 1999; 2006; 2010; Sheppard and Davies, 2011; Richdale *et al.*, 2013), thus primarily implicating crystalline lens changes in the development of presbyopia. Nevertheless, *in vivo* research stimulating the maximum accommodative response by instilling pilocarpine topically has reported that the centripetal ciliary muscle contractile response significantly attenuates with age (Croft *et al.*, 2013a). Indeed, these age-related findings (Croft *et al.*, 2013a) complement those observed in Rhesus monkey studies (Lütjen-Drecoll *et al.*, 1988; Tamm *et al.*, 1992b; Croft *et al.*, 2006; Croft *et al.*, 2013a).

Additionally, Jeon *et al.* (2012) observed reduced centripetal ciliary muscle movement during accommodation in participants with longer axial lengths, thus suggesting a lower ciliary muscle contractile response may be associated with myopia. However, Jeon and colleagues did not measure the magnitude of the objective accommodative response; failing to elucidate whether the change in crystalline lens thickness per unit of accommodative response was lower in eyes with longer axial lengths (Davies, 2010) or whether a larger lag of accommodation was present in myopic participants (Abbott *et al.*, 1998; Rosenfield *et al.*, 2002; Mutti *et al.*, 2006). Furthermore, Sheppard and Davies (2010a) and Lewis *et al.* (2012) found no dependence of the ciliary muscle accommodative response on axial length or ciliary muscle baseline thickness, respectively. Despite these cross-sectional studies, there is a paucity of longitudinal research investigating ciliary

muscle contractile changes with age and the potential influence refractive status may have on accommodative dynamics.

The aim of this investigation is to study the permanency of the *in vivo* ciliary muscle contractile response longitudinally, with particular reference to any differences arising between emmetropic and myopic eyes within the incipient phase of presbyopia.

4.3.1 Method

The study was approved by the Aston University Audiology and Optometry Research Ethics Committee (see Appendix 1) and was conducted in accordance with the tenets of the Declaration of Helsinki. Informed consent was obtained from all the participants after an explanation of the nature and possible consequences of the study (see Appendix 4 for a copy of the information sheet and consent form). One optometrist (DL) collected the data at each visit and did not refer to the results from the previous sessions until after each appointment, when the data were inputted into an Excel spreadsheet (Microsoft, Washington, USA).

In order to collect longitudinal data to monitor changes in ciliary muscle contractility during incipient presbyopia, the following experimental protocol was repeated every 6 months over 2.5 years.

4.3.1a Sample size

The target sample size for repeated measures ANOVA testing (within and between interaction), including an effect size (f) of 0.25, an error probability (α) of 0.05 and required power ($1-\beta$) of 0.80 for 6 repeat measurements amongst 2 groups, was 20 participants (G*Power 3, Faul *et al.*, 2007). Participants from the sample recruited in section 3.1.2a with an amplitude of accommodation >4.50 D and astigmatic error of <0.75 D in their right eye at visit 1 were invited to participate in this further study.

4.3.1b Stimulus response

The change in objective refractive error during accommodation was measured by the binocular open-field WAM-5500 autorefractor (Grand Seiko Co. *Ltd.*, Japan). As described in section 3.2.1e, a bespoke +5.00 D Badal lens system with a 90% high contrast Maltese cross target was mounted on the WAM-5500 autorefractor. The fixation target was placed 20 cm, 8 cm and 2 cm away from the Badal lens in order to stimulate 0.00, 3.00 and 4.50 D of accommodation, respectively. The left eye of each patient was occluded and patients were asked to focus on the centre of the Maltese cross as accurately as possible throughout data collection (Stark and Atchison, 1994). Patients were exposed to the stimulus for 20 seconds prior to the acquisition of data, which is adequate time to achieve the maximal accommodative response (Heron and Winn, 1989). A 1 minute distance-viewing break was permitted between the presentation of each stimulus level. Five consecutive measurements of refraction were acquired and MSE was calculated using equation 1.1.

4.3.1c Visante AS-OCT ciliary muscle imaging during accommodation

Accommodation was stimulated whilst simultaneously imaging the temporal ciliary muscle by adjusting the Badal lens optometer mounted to the Visante forehead rest (as shown in Fig. 2.3). The right eye of all myopic patients was fitted with a soft daily disposable spherical contact lens (*Focus Dailies*, nelfilcon A, 69% water content; Ciba Vision, Georgia, USA). Following a 10 minute settling period, refraction was measured at 0.00 D with the WAM-5500 autorefractor. If the MSE value was $>\pm 0.50$ D, subjective over-refraction was performed to find the optimal contact lens prescription. The right eye visual acuity of each patient was checked to ensure it corresponded to the visual acuity attained in their spectacles.

Patients were positioned and aligned for data collection as described in section 4.2.1b. The Maltese cross target was positioned 10.0, 7.0 and 5.5 cm away from the +10.00 D Badal lens to stimulate 0.00, 3.00 and 4.50 D of accommodation, respectively. Ciliary muscle images were also acquired at the baseline maximum amplitude of accommodation (AA) measured at visit 1 (section

3.1.1d) and at the new maximum AA measured at each subsequent review visit for each patient. Patients were asked to focus on the centre of the Maltese cross target and keep it as clear as possible throughout data collection, whilst also keeping their head and eyes as still as possible. Patients were exposed to the stimulus for 20 seconds prior to acquisition of data and the order the accommodative stimuli were presented in was randomised. Three consecutive images of the ciliary muscle were acquired. In order to ensure the examiner was masked to the accommodative level during image analysis, each accommodative level was saved under a separate patient ID. Whilst changing between patient IDs, the Visante chin-rest moves back to its default position, requiring the patient to be realigned for each accommodative level. Patients were permitted to remove their head from the Visante supports and take a 1 minute distance-viewing break in between accommodative levels.

Each ciliary muscle image was exported from the Visante in binary form for analysis with the custom-designed Matlab R2012b (The MathWorks Inc., Massachusetts, USA) software described in section 2.3.4. The mean values for curved TL, Ant L, SS-IA, Max T, CM2, CM25, CM50 and CM75 were recorded for each set of images.

4.3.1d Statistical analysis

All data were tested for normality using the Shapiro-Wilk test (SigmaPlot; Systat Software Inc., California, USA). Repeated measures ANOVA testing was used to determine whether the changes in ciliary muscle parameters during accommodation (0.00, 3.00 and 4.50 D) were significant at visit 1, and whether any dependency on ametropia classification existed (SPSS, SPSS Inc, Illinois, USA). Repeated measures ANOVAs were also used to investigate whether the change in each ciliary muscle parameter at each accommodative level (3.00, 4.50 D, baseline (visit 1) maximum AA and new maximum AA) was degraded over the course of the study and whether the response per dioptre of accommodation exerted changed significantly.

4.3.2 Results

4.3.2a Participants

From the recruited cohort discussed in section 3.1.2a, 21 patients with an amplitude of accommodation at visit 1 >4.50 D and astigmatic error of <0.75 D in their right eye volunteered to take part in this subgroup study. During the course of the longitudinal study, 1 patient relocated from Birmingham, leaving 13 female and 7 male participants. Of the remaining participants, 10 were emmetropic (-0.25 ± 0.24 D) and 10 were myopic (-3.18 ± 1.27 D). All of the myopic patients had previous contact lens wear experience. The subgroup study baseline age range (34.0 to 41.0 years) was narrower than included in the study in section 4.2 (33.2 to 45.5 years), as shown in Fig. 4.3. The baseline average age of the myopic (37.2 ± 2.1 years) and emmetropic (38.2 ± 2.0 years) groups were not statistically significantly different ($t=1.171$; $p=0.257$). Baseline MSE was not correlated with baseline age ($r=0.252$, $p=0.284$) and the baseline right eye amplitude of accommodation was not significantly correlated with baseline age ($r=0.359$, $p=0.120$).

4.3.2b Cross-sectional analysis

The change in ocular refraction stimulated by the 0.00, 3.00 and 4.50 D accommodative targets was statistically significant at visit 1 ($F=593.034$, $p<0.001$). Myopic participants demonstrated a smaller accommodative lag than emmetropic participants ($F=4.601$, $p=0.031$). The change in ciliary muscle morphology stimulated by the 0.00, 3.00 and 4.50 D accommodative targets was also statistically significant at visit 1 for all parameters measured (Curved TL $F=20.681$, $p<0.001$; Max T $F=31.539$, $p<0.001$; Ant L $F=14.715$, $p<0.001$; SS-IA $F=7.827$, $p=0.002$; CM2 $F=3.291$, $p=0.049$; CM25 $F=20.905$, $p<0.001$; 50 $F=11.712$, $p<0.001$), apart from CM75 ($F=0.358$, $p=0.702$).

The accommodative response of the emmetropic group was significantly greater than the myopic group at the point of Max T ($F=8.778$, $p=0.001$), CM25 ($F=8.223$, $p=0.001$) and CM50 ($F=3.850$, $p=0.031$). The contractile change in Max T ($r=0.159$, $p=0.504$) and Curved TL ($r=0.151$, $p=0.525$) was not dependent on the disaccommodated values measured at visit 1.

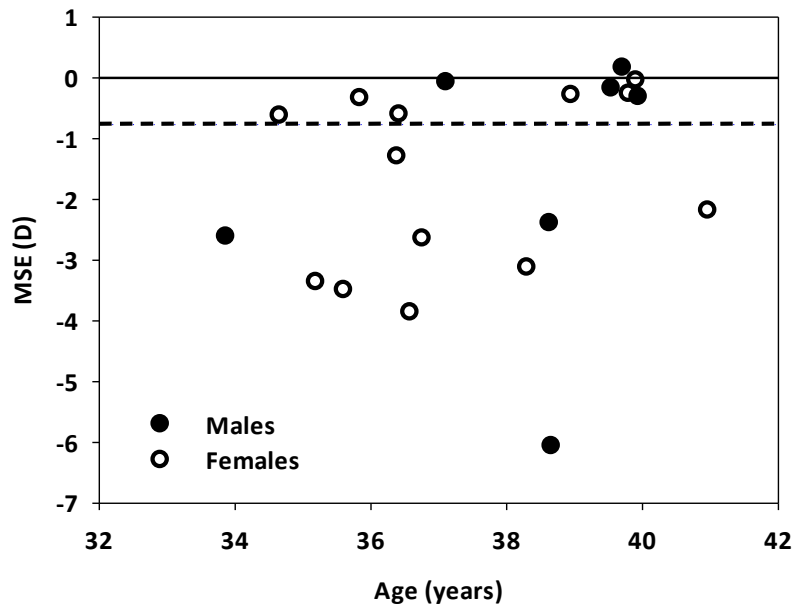


Fig. 4.3. Baseline MSE according to baseline age. Filled circles represent male participants ($n=7$), whereas open circles represent female participants ($n=13$). Participants with a MSE < -0.75 D (represented by the dashed line) were classified as myopic. The solid line represents a MSE of 0.00 D. Participants with a MSE between -0.75 D and $+0.50$ D were defined as emmetropic. Baseline MSE was not statistically significantly correlated with baseline age ($y=0.213x-9.713$; $R^2=0.063$; $p=0.284$).

4.3.2c Longitudinal analysis

Repeated measures ANOVA testing showed that the change in MSE at 0.00 D within the subgroup over the 2.5 year study was not statistically significant ($F=1.321$, $p=0.277$) and had no dependency on ametropia grouping ($F=0.072$, $p=0.979$). The reduction in subjective amplitude of accommodation was statistically significant ($F=20.052$, $p<0.001$; Fig. 4.4), however was not dependent on refractive error ($F=1.353$, $p=0.250$).

Further repeated measures ANOVA tests revealed that the objective accommodative response elicited by the 3.00 D accommodative target decreased significantly over the 2.5 year study ($F=3.898$, $p=0.003$; Fig. 4.5A) and was not dependent on ametropia ($F=1.749$, $p=0.132$). Similarly, the accommodative response elicited by the 4.50 D accommodative target reduced significantly over the course of the study ($F=5.506$, $p=0.001$; Fig 4.5B) and was not dependent on ametropia ($F=1.468$, $p=0.229$), however the magnitude of the accommodative response exerted by the

myopic cohort was significantly greater than the emmetropic response at the 4.50 D level ($F=5.791, p=0.028$).

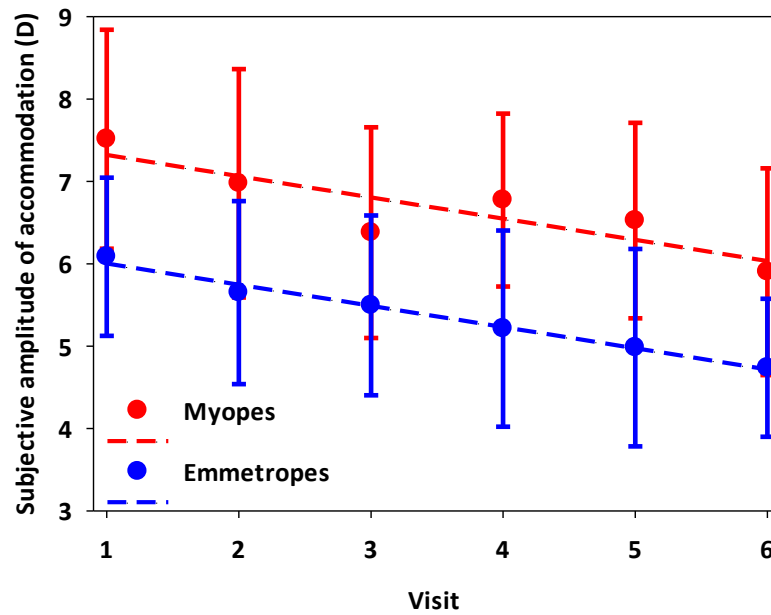


Fig. 4.4. Right eye amplitude of accommodation (with ± 1 standard deviation error bars) measured in myopic (red; $n=10$; mean age 37.2 ± 2.1 years) and emmetropic (blue; $n=10$; mean age 38.2 ± 2.0 years) participants at each visit.

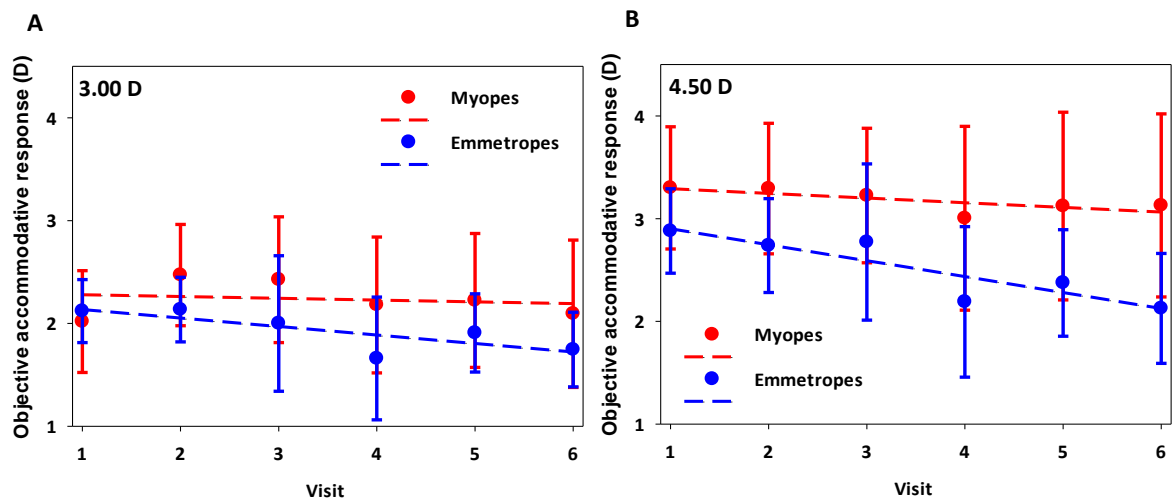


Fig. 4.5. Mean objective accommodative response (with ± 1 standard deviation error bars) measured in myopic (red) and emmetropic (blue) participants whilst viewing a 3.00 D (A) and 4.50 D (B) accommodative target at each visit.

The accommodative change in curved TL ($F=1.031, p=0.405$), Ant L ($F=1.073, p=0.0381$), SS-IA ($F=0.390, p=0.855$) and CM75 ($F=0.910, p=0.478$) whilst focusing on the 3.00 D target was not

significantly attenuated over the 2.5 year study (Fig. 4.6). Whereas, the accommodative change in Max T ($F=5.324$, $p<0.001$), CM2 ($F=4.476$, $p=0.001$), CM25 ($F=6.170$, $p<0.001$) and CM50 ($F=3.434$, $p=0.007$) in response to the 3.00 D target reduced significantly (Fig. 4.6, Fig. 4.7).

The accommodative response of curved TL ($F=0.598$, $p=0.702$), SS-IA ($F=1.354$, $p=0.250$) and CM75 ($F=0.912$, $p=0.408$) whilst focusing on the 4.50 D accommodative target were not significantly degraded over the 2.5 year study (Fig. 4.6). However, the change in Max T ($F=8.929$, $p<0.001$), Ant L ($F=2.503$, $p=0.037$), CM2 ($F=4.641$, $p=0.001$), CM25 ($F=6.734$, $p<0.001$) and CM50 ($F=3.792$, $p=0.004$) significantly decreased (Fig. 4.6, Fig. 4.7).

The emmetropic group demonstrated a greater attenuation of the response at the point of Max T (4.50 D $F=4.509$, $p=0.001$) and CM25 (3.00 D $F=2.457$, $p=0.040$; 4.50 D $F=4.480$, $p=0.001$) than the myopic group (Fig. 4.7).

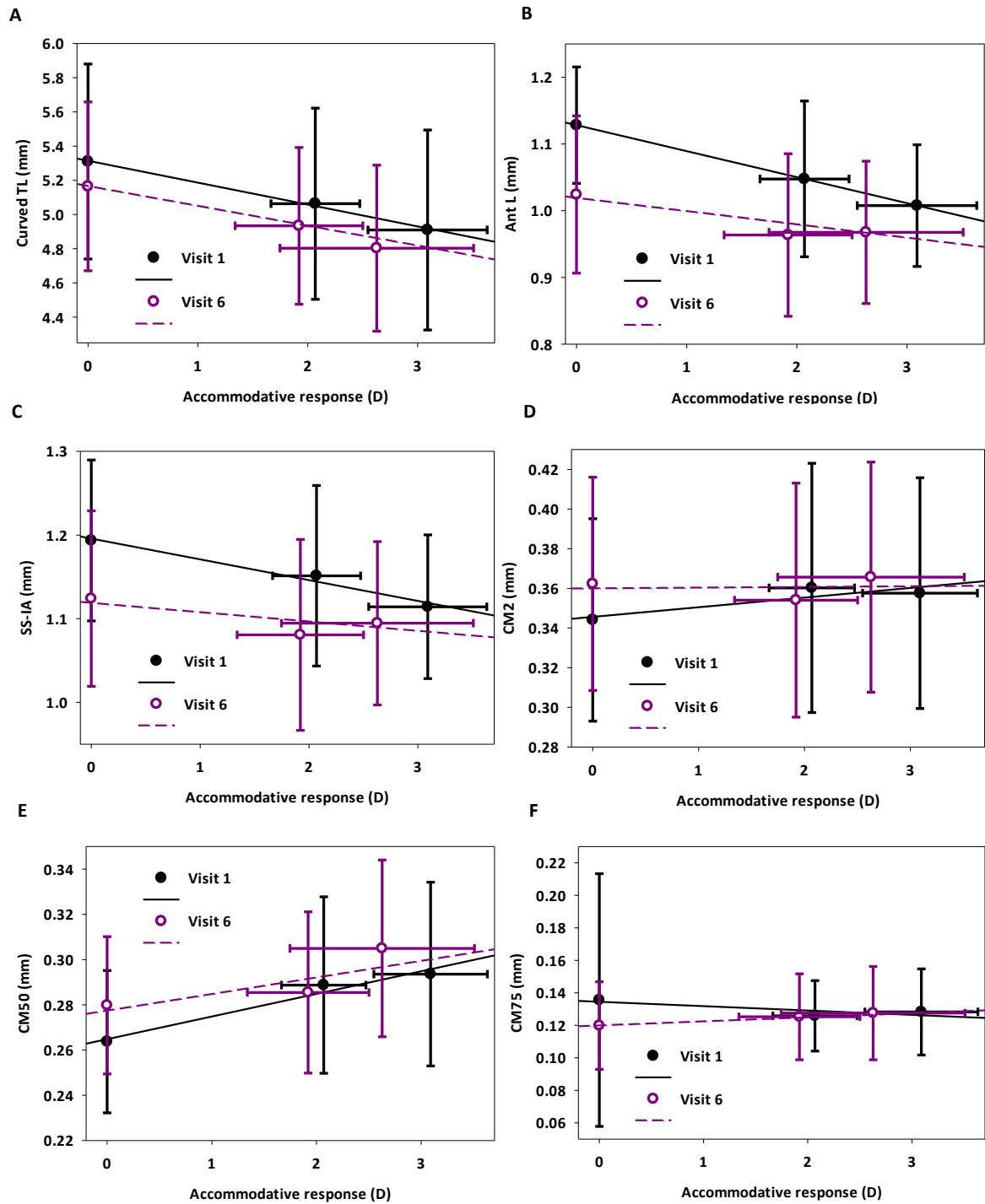


Fig. 4.6. Mean Curved TL (A), Ant L (B), SS-IA (C), CM2 (D), CM50 (E) and CM75 (F) (with ± 1 standard deviation error bars) of the temporal ciliary muscle from all 20 participants at visit 1 (black filled circles and solid regression line) and after 2.5 years at visit 6 (purple open circles and dashed regression line) according to the accommodative response exerted at visit 1 and 6, respectively, to the 0, 3 and 4.50 D targets.

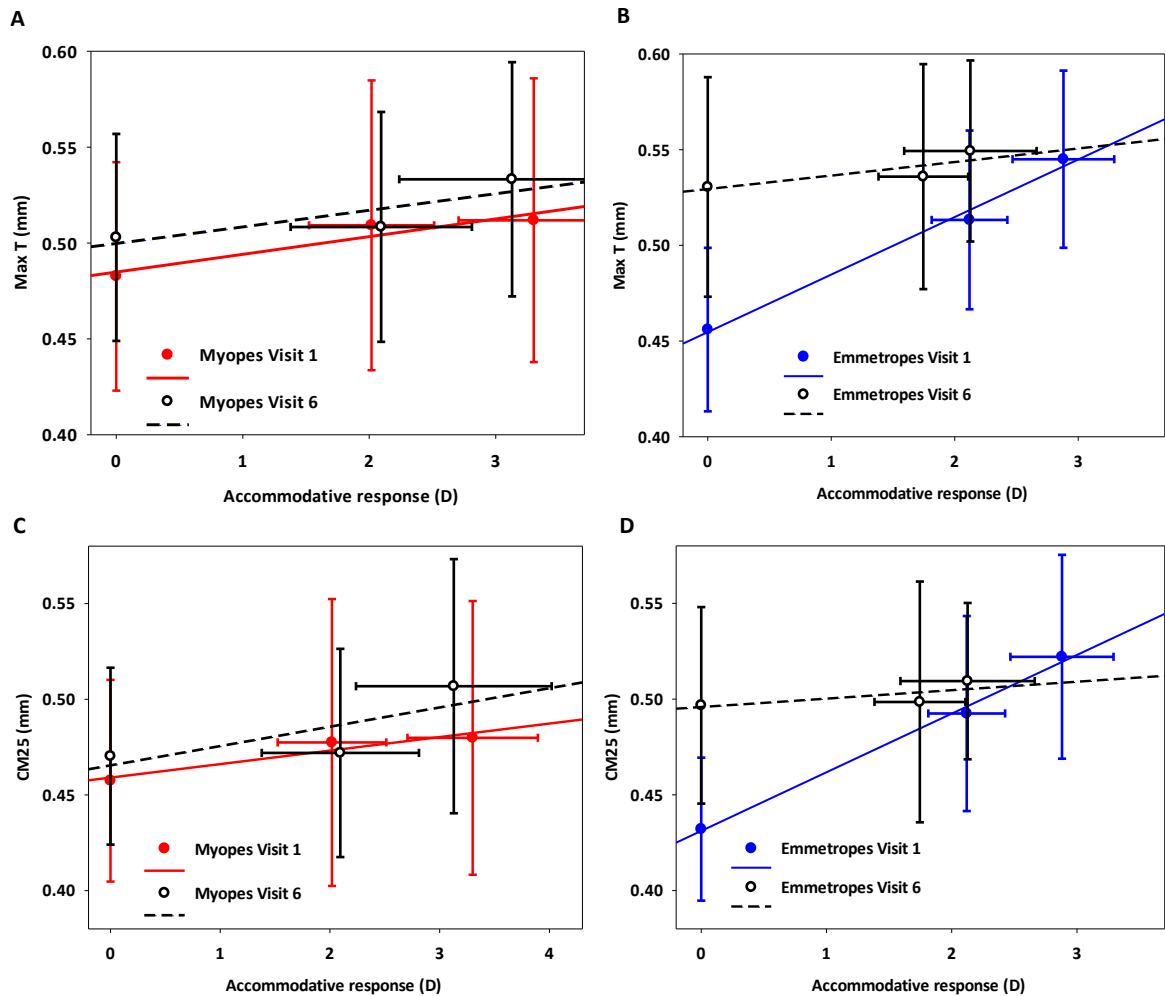


Fig. 4.7. Myopic eye Max T (A) and CM25 (C) (with ± 1 standard deviation error bars) at visit 1 (red filled circles and solid regression line) and after 2.5 years at visit 6 (black open circles and dashed regression line) and emmetropic eye Max T (B) and CM25 (D) at visit 1 (blue filled circles and solid regression line) and after 2.5 years at visit 6 (black open circles and dashed regression line), according to the accommodative response exerted at visit 1 and 6, respectively, to the 0, 3 and 4.50 D targets..

Over the course of the study, the change in Max T ($F=4.006$, $p=0.003$), CM2 ($F=4.159$, $p=0.002$), CM25 ($F=3.384$, $p=0.008$) and CM50 ($F=4.283$, $p=0.002$) whilst each participant focused on a target stimulating their baseline maximum amplitude of accommodation (measured at visit 1) significantly attenuated. The change in the remaining ciliary muscle parameters was not significantly degraded (curved TL $F=0.904$, $p=0.482$; Ant L $F=2.173$, $p=0.065$; SS-IA $F=1.483$, $p=0.204$; CM75 $F=0.831$, $p=0.433$). The emmetropic group response attenuated more than the myopic group response at the point of Max T ($F=3.382$, $p=0.008$) and CM25 ($F=3.381$, $p=0.008$).

The maximum amplitude of accommodation measured at the end of the study (5.32 ± 1.20 D) was significantly smaller ($p < 0.001$) than measured at the initial visit (6.80 ± 1.35 D). Curved TL ($F = 2.392$, $p = 0.044$), Max T ($F = 6.374$, $p < 0.001$), Ant L ($F = 3.361$, $p = 0.025$), CM2 ($F = 3.696$, $p = 0.004$), CM25 ($F = 3.599$, $p = 0.014$) and CM50 ($F = 4.283$, $p = 0.002$) significantly reduced whilst each participant focused on a target placed at their new maximum amplitude of accommodation, as measured at each review visit. The change at SS-IA ($F = 1.483$, $p = 0.204$) and CM75 ($F = 0.774$, $p = 0.571$) was not significantly reduced. The emmetropic group response attenuated more than the myopic group response at the point of Max T ($F = 2.936$, $p = 0.017$).

Considering the contractile response per dioptre of accommodation exerted whilst viewing the 4.50 D accommodative target (Table 4.2), Max T ($F = 7.067$, $p < 0.001$), CM2 ($F = 3.087$, $p = 0.013$) and CM25 ($F = 6.767$, $p < 0.001$) were the only ciliary muscle parameters to attenuate significantly over the 2.5 year study (curved TL $F = 1.171$, $p = 0.322$; Ant L $F = 1.658$, $p = 0.154$; SS-IA $F = 0.734$, $p = 0.528$; CM50 $F = 2.277$, $p = 0.085$; CM75 $F = 1.213$, $p = 0.312$; Table 4.2). The attenuation of the response of the emmetropic participants was greater than the myopic participants at the point of Max T ($F = 3.185$, $p = 0.011$; Fig. 4.8A) and CM25 ($F = 3.899$, $p = 0.003$; Fig. 4.8B).

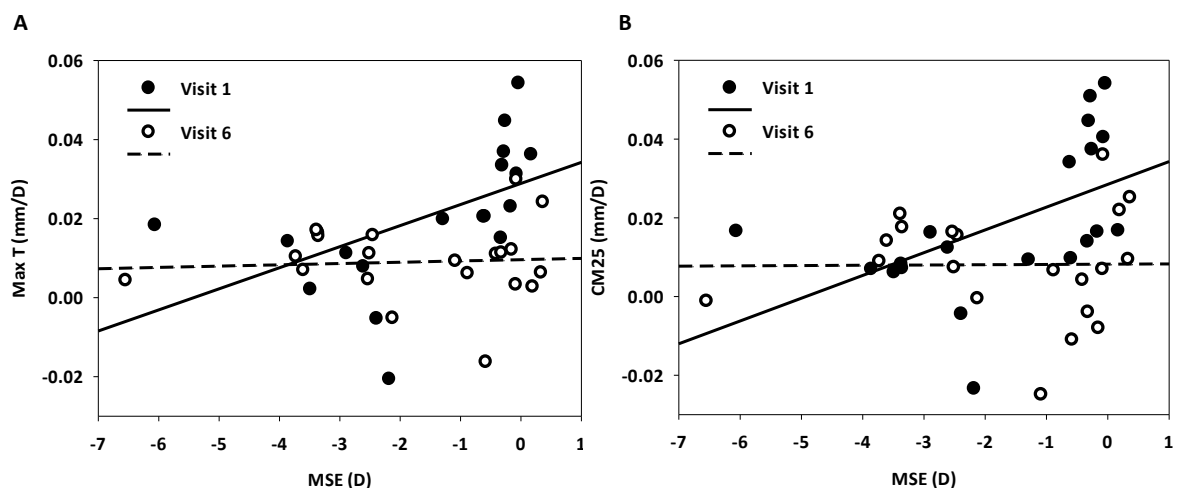


Fig. 4.8. Change in Max T (A) and CM25 (B) per dioptre of accommodative response (measured observing the 4.50 D target) at visit 1 (filled circles and solid regression line) and after 2.5 years at visit 6 (open circles and dashed regression line) according to the MSE measured at visit 1 and 6, respectively. A more negative change indicates a greater attenuation of the accommodative increase in thickness.

Ciliary muscle parameter	Mean change per dioptre of accommodation exerted at baseline (mm ± SD)		Mean change per dioptre of accommodation exerted after 2.5 years (mm ± SD)		Statistical significance (<i>p</i>) of changes over 2.5 years
	Myopes	Emmetropes	Myopes	Emmetropes	
Curved TL	-0.100 ± 0.068	-0.160 ± 0.128	-0.115 ± 0.066	-0.168 ± 0.087	0.322
Max T	+0.011 ± 0.009	+0.032 ± 0.012	+0.008 ± 0.007	+0.009 ± 0.012	<0.001
Ant L	-0.040 ± 0.044	-0.039 ± 0.030	-0.012 ± 0.028	-0.026 ± 0.065	0.154
SS-IA	-0.028 ± 0.032	-0.022 ± 0.028	-0.004 ± 0.022	-0.013 ± 0.054	0.528
CM2	+0.003 ± 0.004	+0.008 ± 0.008	+0.003 ± 0.006	-0.002 ± 0.018	0.013
CM25	+0.009 ± 0.006	+0.032 ± 0.016	+0.011 ± 0.008	+0.006 ± 0.018	<0.001
CM50	+0.005 ± 0.004	+0.015 ± 0.013	+0.008 ± 0.008	+0.011 ± 0.016	0.085
CM75	+0.000 ± 0.007	-0.008 ± 0.032	+0.002 ± 0.007	+0.005 ± 0.011	0.312

Table 4.2. Mean ciliary muscle changes per dioptre of accommodation exerted at baseline and after 2.5 years for the myopic and emmetropic groups individually. The *p* values denote the significance of the change in accommodative response at each parameter over 2.5 years for myopic and emmetropic individuals. Statistical significance is denoted by a bold *p* value.

Multiple linear regression analysis showed no relationship between the changes per dioptre of accommodation exerted from visit 1 to 6 and age-related changes in right eye amplitude ($r=0.645$, $r^2=0.416$; Curved TL $p=0.250$; Max T $p=0.307$; Ant L $p=0.368$; SS-IA $p=0.600$; CM2 $p=0.066$; CM25 $p=0.278$; CM50 $p=0.092$; CM75 $p=0.868$) or changes in MSE ($r=0.749$, $r^2=0.561$; curved TL $p=0.601$; Max T $p=0.480$; Ant L $p=0.309$; SS-IA $p=0.587$, CM2 $p=0.327$, CM25 $p=0.296$, CM50 $p=0.958$, CM75 $p=0.331$).

Further multiple linear regression analysis revealed changes in Curved TL ($p=0.022$; Fig. 4.9A), CM25 ($p=0.048$; Fig. 4.9B) and SS-IA ($p=0.038$; Fig. 4.9C) per dioptre of accommodation exerted from visit 1 to 6 were dependent on baseline age ($r=0.185$, $r^2=0.034$; Max T $p=0.090$; Ant L $p=0.481$, CM2 $p=0.383$, CM50 $p=0.513$; CM75 $p=0.289$).

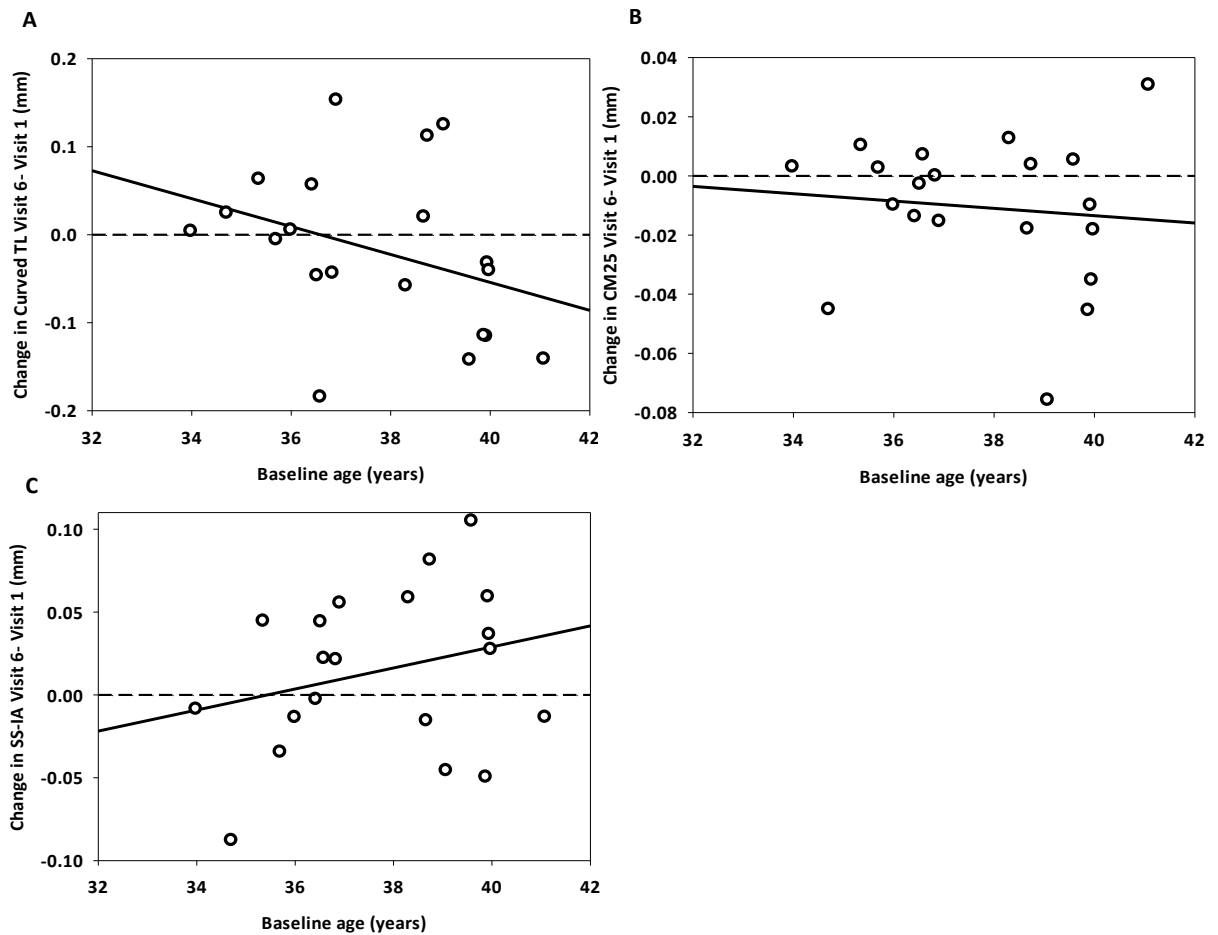


Fig. 4.9. The change in Curved length (A), CM25 (B) and SS-IA (C) per dioptre of accommodation exerted (measured observing the 4.50 D target) between visit 1 and 6, according to baseline age. The dashed horizontal line represents no change (0.00 mm). A more negative change indicates a greater decrease in Curved L (A) and SS-IA (C) and less of an increase in CM25 (B).

4.3.3 Discussion

The present investigation is the first to document an attenuation of the centripetal ciliary muscle contractile response longitudinally with age, whereas the antero-posterior mobility of the ciliary muscle appears to remain invariant with age.

A significant increase in Max T, CM2, CM25 and CM50 and a decrease in Curved TL, Ant L and SS-IA accompanied ocular accommodation, whereas CM75 did not change significantly (Table 4.2).

The cumulative effect of these contractile changes produces an antero-inwards shift of ciliary muscle mass during accommodation, as reported previously (Pardue and Sivak, 2000; Sheppard and Davies, 2010a; Sheppard and Davies, 2011). However, earlier studies investigating children

and young adults have concluded the posterior portion of the ciliary muscle either thins (CM2: Sheppard and Davies, 2010a; CM3: Jeon *et al.*, 2012; Lewis *et al.*, 2012; Lossing *et al.*, 2012) or does not change significantly (CM50, CM75: Sheppard and Davies, 2010a) during accommodation. It is feasible that the hypothesised fulcrum point of ciliary muscle contraction, where the net change during accommodation is always zero (Lewis *et al.*, 2012), relocates more posteriorly due to the significant reorganisation of ciliary muscle fibres with age (Nishida and Mizutani, 1992; Tamm *et al.*, 1992a; Pardue and Sivak, 2000). However, no evidence of this theory has emerged from cross-sectional data investigating individuals aged 30 to 50 years (Richdale *et al.*, 2013) or 19 to 70 years (Sheppard and Davies, 2011). Further longitudinal research is required to quantify how the contractile response varies as a function of ciliary muscle length across the lifespan.

Similarly to the work of Jeon *et al.* (2012), the increase in Max T during accommodation was significantly smaller in myopic than emmetropic individuals. However, some discrepancy was foreseeable due to the shorter vitreous chamber depth typically observed in emmetropic individuals when compared to myopic individuals (McBrien and Adams, 1997), which necessitates greater convergence of light rays entering the eye during accommodation to focus the image of a near object clearly on the relatively closer retinal plane (Davies *et al.*, 2010). The change in crystalline lens thickness per dioptre of accommodation is therefore expected to be lower in myopes than emmetropes (Gibson, 2008). However, the attenuation of the contractile change in Max T and CM25 during the course of the 2.5 year study was significantly greater in the emmetropic participants. Indeed, Jeon *et al.* (2012) and Croft *et al.* (2013a) reported that the contractile response was weaker in participants with thicker disaccommodated ciliary muscles, and whilst further analysis of the subgroup cohort showed no significant difference between the disaccommodated thickness of the myopic and emmetropic participants at baseline (Max T: $r=0.075$, $p=0.752$; CM25: $r=0.024$, $p=0.920$), the disaccommodated age-related increase in ciliary muscle thickness was more pronounced in the emmetropic subgroup participants over the 2.5 year study (Max T: $r=0.535$, $p=0.015$; CM25: $r=0.396$, $p=0.084$; Fig. 4.7). The relationship between

age-related changes in disaccommodated ciliary muscle morphology and ametropia evident in this study are at variance to the longitudinal results reported in section 4.2 on a larger cohort. Therefore, it is likely the attenuation of the centripetal ciliary muscle response with age would be similar in myopic and emmetropic participants who experienced a similar magnitude of increase in disaccommodated ciliary muscle thickness with age. Further investigation is required to confirm these preliminary observations and hypotheses.

The observed attenuation of the centripetal ciliary muscle movement during accommodation opposes the cross-sectional results of the majority of previous authors (Strenk *et al.*, 1999; 2006; 2010; Sheppard and Davies, 2011; Richdale *et al.*, 2013), and supports the findings of Croft *et al.* (2013a). Croft *et al.* (2013a) investigated 12 patients aged between 19 and 65 years, stimulating the maximum contractile ciliary muscle response (the near point of accommodation) by instilling pilocarpine. The pilocarpine-induced thickening of the ciliary muscle apex observed in patients aged 19 to 23 was $+0.34 \pm 0.05$ mm, $+0.15 \pm 0.07$ mm in patients aged 27 to 31 years and $+0.08 \pm 0.01$ in patients aged 49 to 65 years. Notably, incipient presbyopic participants were not included. Pilocarpine acts as a 'super-stimulus' in presbyopic patients (Koepl *et al.*, 2005), therefore it is likely that the true magnitude of contractile attenuation with age may be more profound than reported. Yet, a measurement of ciliary muscle length equivalent to that used by the current study was not acquired by Croft and colleagues.

Similarly to the results of Sheppard and Davies (2011), the reduction in ciliary muscle length during accommodation was not significantly degraded with age. In fact, the change in Curved TL per dioptre of accommodation exerted between visit 1 and 6 was significantly larger in the oldest participants, therefore indicating a component of the anterior contractile movement may become increasingly latent with age. However, the reduction in length does not appear to be linear with accommodation; most of the contractile shortening of total length and anterior length per dioptre of accommodative response occurs between 0.00 to 4.00 D rather than between 4.00 and 8.00 D (Sheppard and Davies, 2010a). This relationship was not reported for the thickness measurements

obtained with respect to the total length (Sheppard and Davies, 2010a). It is therefore possible that a statistically significant age-related attenuation of the accommodative reduction in ciliary muscle length, particularly Ant L, may emerge longitudinally after a larger reduction of the amplitude of accommodation occurs. Additionally, the attenuation of the centripetal ciliary muscle contractile response may be due to the age-related accretion of connective tissue (Nishida and Mizutani, 1992; Tamm *et al.*, 1992a; Pardue and Sivak, 2000), which specifically accumulates around the radial and circular fibres in the ciliary muscle apex, leaving the functionality of the longitudinal fibres largely unaffected and thus preserving the ciliary muscle antero-posterior mobility. The metabolic requirements of the circular and radial fibres may also increase with age (Ishikawa, 1962; Pardue and Sivak, 2000), therefore potentially causing an age-related reduction in the contractile response if the increased metabolic demand is not met. Further longitudinal research including a larger range of patient ages over an extended period is required to investigate how the behaviour of the anterior contractile ciliary muscle movement changes with age. Computer modelling based on *in vivo* ciliary muscle dimensions is also required to investigate how a more rapid reduction of centripetal ciliary muscle contraction compared to the anterior contractile movement may impact the force applied to the crystalline lens via the zonules.

The statistically significant reduction in ciliary muscle centripetal contractile response per dioptre of accommodative demand and per dioptre of accommodation exerted seems counterintuitive. However, it is feasible that the magnitude of centripetal ciliary muscle force required to exert 1 dioptre of accommodation may reduce with age if the anterior accommodative movement of the crystalline lens, which is known to reduce the power of the accommodative response (Davies *et al.*, 2010), declines with age, therefore requiring a smaller change in crystalline lens thickness per dioptre of accommodation exerted and consequently less ciliary muscle centripetal force. Indeed, the anterior movement of the crystalline lens may be diminished with age due to restriction from the posterior iris or age-related changes in the pattern of crystalline lens accommodative expansion. Due to the persistence of the antero-posterior ciliary muscle accommodative mobility

(accommodative reduction in ciliary muscle Curved TL and Ant L) with age, it is unlikely that the ciliary muscle would be responsible for the theorised reduction in anterior crystalline lens movement. Indeed, the theorised augmentation of the change in ocular axial distances with accommodation would act to preserve accommodative ability with age. Further longitudinal research documenting the accommodative position of the crystalline lens is required to explore this hypothesis.

As well as providing an insight into the reduction of the contractile response evident during a naturalistic range of accommodative levels, which are likely to be exerted during habitual near vision, the current study additionally investigated the ciliary muscle contractile response at the near point of accommodation of each patient at each visit. A significant attenuation of the antero-inwards accommodative ciliary muscle response at the near point of accommodation was observed over the 2.5 year study. However, measuring the ciliary muscle response whilst the accommodative target was positioned to stimulate the baseline (visit 1) maximum amplitude of accommodation (6.80 ± 1.35 D) at each review visit displayed that the ciliary muscle was still able to contract to a greater demand than the eye could accommodate to. Only Max T, CM2, CM25 and CM50 significantly attenuated over the course of the 2.5 year study whilst the participant attended a target stimulating their baseline amplitude of accommodation. These findings do not support the hypothesis that the ciliary muscle is maximally contracted at the near point and the ciliary muscle contractile force required to exert a unit change in accommodation increases with age in order to mould the stiffer crystalline lens (Fincham, 1937). However, the results also do not support the Hess-Gullstrand theory, which stipulates that the contractile effort required to exert a unit change in accommodation remains constant with age (Hess, 1901, cited by Alpern, 1962; Gullstrand 1911). Nevertheless, the ability of the ciliary muscle to contract to a larger accommodative stimulus than the eye can accommodate to, indicates that age-related changes in ciliary muscle contractility are unlikely to be primarily responsible for the development of presbyopia and supports a lenticular model for presbyopia development.

Indeed, it is possible that the increasing rigidity of the crystalline lens may reduce the capacity of the ciliary muscle to contract during accommodation. Park *et al.* (2008) reported that the pharmacologically-induced contractility of the human ciliary muscle improved following phacoemulsification and intra-ocular lens (IOL) implantation and, therefore, the age-related stiffening of the crystalline lens restricted ciliary muscle action in humans. A similar restriction of the ciliary muscle contractile response has been reported in Rhesus monkeys (Lütjen-Drecoll *et al.*, 1988; Tamm *et al.*, 1992b; Croft *et al.*, 2006; Croft *et al.*, 2013a), whereby the posterior tendon attachments, which become thicker and are surrounded by an increasing amount of collagen fibrils with age (Tamm *et al.*, 1991), limit the mobility of the ciliary muscle until severed from the ciliary muscle attachment sites (Tamm *et al.* 1992a). However, Strenk *et al.* (2006; 2010) reported IOL implantation did not significantly increase human centripetal ciliary muscle movement in response to an 8.00 D accommodative target. Further research pre- and post-phacoemulsification and IOL implantation whilst stimulating accommodation is required to investigate the impact of dense crystalline lens opacities on ciliary muscle mobility. Nonetheless, a crystalline lens with a dense enough opacity to warrant cataract surgery is likely to be significantly stiffer than the crystalline lens during the development of presbyopia, therefore perhaps clear-lens extraction patients would be better candidates to study to understand the impact that increasing lenticular rigidity may have on ciliary muscle mobility. However, clear-lens extraction patients tend to have high myopia or hypermetropia (Lyle and Jin, 1994) and therefore may not be representative of the general population.

The current study provides the first prospective, longitudinal insight into how ciliary muscle contractility changes during incipient presbyopia. In conclusion, a significant attenuation of the centripetal contractile response was observed, whereas the antero-posterior mobility was preserved. Future studies should investigate the attenuation of the contractile response of the full circumference of the ciliary muscle longitudinally in order to determine whether any asymmetry exists in the attenuation of the contractile response with age.

CHAPTER 5

Longitudinal morphological and accommodative changes in ocular biometry during incipient presbyopia

5.1 Introduction

Axial ocular biometry typically encapsulates central corneal thickness, anterior chamber depth, crystalline lens thickness and axial length measurements. Significant changes in axial ocular biometry occur to propagate the accommodative response (Drexler *et al.*, 1997; Koretz *et al.*, 1997; Dubbelman *et al.*, 2003; Dubbelman *et al.*, 2005; Ostrin *et al.*, 2006; Tsorbatzoglou *et al.*, 2007; Read *et al.*, 2010a; Richdale *et al.*, 2013), presbyopia development (Strenk *et al.*, 1999; Dubbelman *et al.*, 2001b; Dubbelman and van der Heijde, 2001; Jones *et al.*, 2007; Atchison *et al.*, 2008; Richdale *et al.*, 2013) and myopia development (Goss *et al.*, 1985; Zadnik *et al.*, 1995; McBrien and Adams, 1997; Lee *et al.*, 2002; Mutti *et al.*, 2012). However, there is a paucity of longitudinal research specifically documenting the changes in morphology and the attenuation of the accommodative response during the development of presbyopia, particularly with respect to changes in ocular refraction. The proceeding chapter utilises optical low coherence reflectometry to investigate morphological (study one) and accommodative (study two) longitudinal changes occurring in ocular biometry during incipient presbyopia.

5.2 Study One: A longitudinal investigation of morphological changes in ocular biometry during incipient presbyopia

The most rapid changes in ocular biometry are observed during ocular growth in early infancy (Larsen 1971a;b;c;d), however further biometric changes are evident throughout life (McBrien and Adams, 1997; Kinge *et al.*, 1999; Mallen *et al.*, 2005b; Jorge *et al.*, 2007; Atchison *et al.*, 2008; Foster *et al.*, 2010) and are likely to be involved in the development of presbyopia (Atchison, 1995; Gilmartin, 1995; Strenk *et al.*, 2005; Charman, 2008). The literature is in agreement that the development of presbyopia is complex, involving a number of interdependent structures

(Gilmartin, 1995; Atchison, 1995; Charman, 2008), with perhaps the most significant contribution arising from the crystalline lens (Heys *et al.*, 2004; Glasser, 2008).

The age-related progressive addition of new lens fibres perpetuates an increase in crystalline lens equatorial diameter (Fea *et al.*, 2005; Jones *et al.*, 2007; Atchison *et al.*, 2008; Kasthurirangan *et al.*, 2011), a decrease in crystalline lens surface radii of curvature (Lowe and Clark, 1973; Baikoff *et al.*, 2004; Atchison *et al.*, 2008), with the greatest change observed across the anterior surface (Brown *et al.*, 1974; Dubbelman *et al.*, 2001b; Koretz *et al.* 2001; Koretz *et al.*, 2004) and an increase in crystalline lens axial thickness (Koretz *et al.*, 1989; Smith *et al.*, 1992; Cook *et al.*, 1994; Glasser *et al.*, 1999; Strenk *et al.*, 1999; Dubbelman *et al.*, 2001a; Koretz *et al.*, 2001; Dubbelman *et al.*, 2003; Jones *et al.*, 2007; Atchison *et al.*, 2008; Richdale *et al.*, 2013). Cross-sectional research has indicated that the axial crystalline lens thickness increases by between 0.019 to 0.031 mm per year (Koretz *et al.*, 1989; Dubbelman *et al.*, 2001a; Wojciechowski *et al.*, 2003; Koretz *et al.*, 2004; Allouch *et al.*, 2005; Shufelt *et al.*, 2005; Atchison *et al.*, 2008; Kasthurirangan *et al.* 2011; Richdale *et al.*, 2013). The overall thickness increase of the crystalline lens cortex is 7 times greater than the thickness increase of the nucleus with age (Dubbelman *et al.*, 2003). Furthermore, the increase in anterior cortex thickness is between 1.5 to 3 times greater than the increase in posterior cortex thickness with age (Kashima *et al.*, 1993; Cook *et al.*, 1994; Dubbelman *et al.*, 2003) and recent research has indicated that the distance between the sulcus (centre of the crystalline lens nucleus) and cornea remains constant with age (Dubbelman *et al.*, 2003).

Paradoxically, the aforementioned crystalline lens changes have not been reported to result in a myopic shift in refraction due to an increase in the lenticular gradient between high and low refractive indices, thus causing the equivalent refractive index of the crystalline lens to decrease with age (Brown *et al.*, 1974; Dubbelman *et al.*, 2001b; Dubbelman and van der Heijde, 2001; Jones *et al.*, 2005; Atchison *et al.*, 2008; Kasthurirangan *et al.*, 2008; Richdale *et al.*, 2013) and typically resulting in a hypermetropic shift in refraction during presbyopia development (Tassman,

1932; Brown, 1938; Slataper, 1950; Hirsch, 1958; Saunders, 1981; Fledelius, 1983; Fledelius and Stubgaard, 1986; Sperduto *et al.*, 1996, McCarty *et al.*, 1997; Kempen *et al.*, 2004; Atchison *et al.*, 2008). However, retrospective studies have reported that a low proportion of individuals experience a myopic shift in refraction during the development of presbyopia (Grosvenor and Skeates, 1999; Pointer and Gilmartin, 2011). Cross-sectional (McBrien and Millodot, 1987; Grosvenor and Scott, 1991; Bullimore *et al.*, 1992) and longitudinal (Grosvenor and Scott, 1993; Lin *et al.*, 1996; McBrien and Adams, 1997; Kinge *et al.*, 1999) research has reported that vitreous chamber depth and axial length elongation are associated with myopic shifts in refraction during adulthood, however the structural origins of a myopic shift during incipient presbyopia specifically have yet to be investigated. Indeed, no longitudinal data profiling refractive or biometric changes during the development of presbyopia currently exists.

Furthermore, whilst numerous cross-sectional studies have investigated age-related changes in the crystalline lens and ciliary muscle simultaneously, analysis of the results typically isolates each structure and fails to explore any interdependency of the age-related structural changes (Strenk *et al.*, 1999; Richdale *et al.*, 2013). Indeed, it has been hypothesised the increasingly anterior tension exerted on the ciliary muscle by the anterior movement of the growing crystalline lens may contribute to the antero-inwards movement of ciliary muscle mass with age (Strenk *et al.*, 1999; Pardue and Sivak, 2000; Atchison *et al.*, 2008; Strenk *et al.*, 2010). The inverse mechanism has also been suggested, whereby a significant increase in ciliary muscle thickness (reduction in ciliary muscle ring diameter) reduces zonular tension and permits lenticular thickening in the absence of an accommodative stimulus (Strenk *et al.*, 1999).

The aim of this study is to investigate the biometric morphological changes occurring during incipient presbyopia longitudinally, and in particular, to elicit the structural origins of the putative myopic shift in refraction. Interdependency between changes in axial ocular biometry and ciliary muscle morphology (discussed in section 4.2) will also be explored.

5.2.1 Method

The study was approved by the Aston University Audiology and Optometry Research Ethics Committee (see Appendix 1) and was conducted in accordance with the tenets of the Declaration of Helsinki. Informed consent was obtained from all the participants after an explanation of the nature and possible consequences of the study (see Appendix 2 for a copy of the information sheet and consent form). One optometrist (DL) collected the data at each visit and did not refer to the results from the previous sessions until after each appointment, when the data were inputted into an Excel spreadsheet (Microsoft, Washington, USA).

In order to collect longitudinal data to monitor changes in biometry during incipient presbyopia, the following experimental protocol was repeated every 6 months over 2.5 years on all participants. To control for diurnal fluctuations in axial length (Stone *et al.*, 2004; Read *et al.*, 2008; Chakraborty *et al.*, 2011), the allotted appointment time for each participant was kept as similar as possible for each review visit.

5.2.1a Participants

The details of the 51 participants recruited for this study have been previously described in section 3.1.2a.

5.2.1b Objective refractive error

Objective refractive error and keratometry were measured simultaneously by the binocular open-field WAM-5500 autorefractor (Grand Seiko Co. *Ltd.*, Japan), as described in section 3.1.1e. Five consecutive measurements of refraction were acquired and the MSE was calculated using equation 1.1. The anterior corneal radii of curvature and toricity were also recorded. The anterior corneal radii of curvature readings from the principle meridians were averaged to determine the mean anterior corneal radius of curvature.

5.2.1c LenStar biometry

Optical biometry was measured by the LenStar LS-900 (Haag-Streit, AG, Koeniz, Switzerland), with the addition of a bespoke Badal lens system incorporating a 92% transmission, 8% reflection pellicle beamsplitter (Edmund Optics, Barrington, NJ), as described in section 2.3.1. Each participant wore an eye patch over their left eye throughout data collection and room lights were extinguished. Based on each participant's MSE, the mobile, retro-illuminated 5x5 grid of high contrast (90%) 0.8 logMAR letter target was positioned to stimulate 0.00 D of accommodation. To confirm the placement of the target was correct, once positioned on the chin rest and against the forehead bar, each participant was asked to observe the target as it was moved and to report when it first became clear. Subsequently, each participant was asked to concentrate on the letter closest to the LenStar measurement beam and was encouraged to maintain clear focus throughout data collection (Stark and Atchison, 1994). Three consecutive measures of corneal thickness (CT), anterior chamber depth (ACD), crystalline lens thickness (LT) and axial length (AXL) were acquired after patients were exposed to the stimulus for 20 seconds. Anterior segment length ($ASL=CT+ACD+LT$) and vitreous chamber depth ($VCD=AXL-ASL$) were calculated from these values. The average of each parameter was recorded.

Unfortunately, the LenStar biometer was unable to obtain crystalline lens thickness values for all patients. Therefore, it was necessary to substitute the missing parameter with data collected from anterior-segment optical coherence tomography (AS-OCT).

5.2.1d Visante AS-OCT crystalline lens thickness measurements

OCT images were acquired of the crystalline lens by a Visante AS-OCT (Carl Zeiss Meditec, California, USA). Each participant wore an eye patch over their left eye throughout data collection and room lights were extinguished. Patients were asked to position themselves against the chin rest and forehead bar of the Visante and focus on the centre of the internal green star target, keeping it as clear as possible throughout data collection. The internal Badal lens system was adjusted to correct for the ametropia of each participant in order to capture images of the

crystalline lens in a relaxed state (0.00 D accommodative stimulus). Raw image mode was selected because all other Visante modes require simultaneous visualisation of the anterior corneal surface, which is not possible whilst imaging the entire thickness of the crystalline lens. The chin rest was adjusted to align the participant's right eye with the optical axis of the Visante, which was guided by the real-time video stream of the external eye. Images were acquired once the vertical white fixation line through the centre of the image appeared, which indicated the measurement beam was coincident with the visual axis of the eye. Visualisation of the equatorial region of the crystalline lens is obscured by the iris (Fig. 2.5).

Three images of the crystalline lens for each patient were exported in binary form for analysis with the custom-designed Matlab R2012b (The MathWorks Inc., Massachusetts, USA) software described in section 2.3.4. The semi-automated software measured the pixel thickness between two manually selected points inputted by the user. To ensure the maximum crystalline lens thickness (LT) was measured, the points where the white vertical fixation line bisected the anterior and posterior crystalline lens surfaces were manually chosen. A conversion factor of 93.204 pixels per millimetre was used to convert pixel thicknesses into millimetre measurements (section 2.3.3). The mean crystalline lens thickness value obtained from the analysis of three images was recorded for each participant.

5.2.1e Error calculations

The LenStar uses an average refractive index to convert an optical path length into a geometrical axial length, as discussed in section 2.3.1a. Therefore, to compensate for an overestimation of axial length due to the increase in crystalline lens thickness with age, the error was estimated using equations 2.1 to 2.7, as described in section 2.3.1b, to correct the raw axial length values.

5.2.1f Axial length: corneal radius ratio

The ratio of axial length to corneal radius (AXL:CR) was calculated at baseline only to determine whether it is predictive of the risk of a myopic shift in refraction over a longitudinal period (Goss

and Jackson, 1995). The anterior corneal radii of curvature measured at each principle meridian was averaged to determine the mean anterior radius of curvature reading, which was subsequently used as the measurement of corneal radius in the AXL:CR ratio. Therefore, an eye with an axial length of 24 mm and an average anterior corneal radius of curvature of 8 mm would have an AXL:CR ratio of 3:1.

5.2.1g Statistical analysis

All data were tested for normality using the Shapiro-Wilk test (SigmaPlot; Systat Software Inc., California, USA). A repeated measures ANOVA test was conducted to determine whether the changes in axial ocular biometry were significant over the 2.5 year study, and whether they were dependent on ametropia classification (SPSS, SPSS Inc, Illinois, USA).

Separate multiple linear regression tests were used to investigate whether changes in biometry were associated with baseline age, the change in amplitude of accommodation or the change in MSE during the course of the study. T-tests were used to determine whether the morphological changes of the participants who experienced a myopic change in refraction >0.50 D were significantly different to the changes experienced by the remaining cohort.

Multiple linear regression analysis was also used to determine the relationship between changes in ACD, LT, ASL and corrected AXL with changes in the ciliary muscle parameters (discussed in section 4.2).

5.2.3 Results

It was necessary to utilise Visante AS-OCT crystalline lens thickness measurements due to the absence of LenStar LT measurements in 25% of patients at visit 1, 22% at visit 2, 14% at visit 3, 10% at visit 4, 14% at visit 5 and 7% at visit 6.

Repeated measures ANOVA testing revealed changes in ACD ($F=10.745$, $p<0.001$) and LT ($F=12.566$, $p<0.001$) were the only axial biometric parameters to reach statistical significance over

the course of the 2.5 year study (CT $F=0.908$, $p=0.477$; ASL $F=0.791$, $p=0.529$; VCD $F=0.691$, $p=0.651$; raw AXL $F=0.912$, $p=0.438$; corrected AXL $F=0.959$, $p=0.420$; Fig. 5.1). The change over the course of the study was not dependent on refractive error classification (CT $F=1.064$, $p=0.381$; ACD $F=0.721$, $p=0.581$; LT $F=0.727$, $p=0.604$; ASL $F=0.417$, $p=0.790$; VCD $F=0.339$, $p=0.848$; raw AXL $F=1.575$, $p=0.197$; corrected AXL $F=1.503$, $p=0.212$; Fig. 5.1), however, ACD ($F=4.539$, $p=0.039$), VCD ($F=14.131$, $p<0.001$), raw AXL ($F=15.087$, $p<0.001$) and corrected AXL ($F=15.114$, $p<0.001$) measurements were significantly longer in myopic eyes throughout the study (Table 5.1).

Ciliary muscle parameter	Mean baseline value (mm \pm SD)		Mean change after 2.5 years (mm \pm SD)	Statistical significance (p) of changes after 2.5 years
	Myopes	Emmetropes		
CT	0.54 \pm 0.03	0.53 \pm 0.04	-0.01 \pm 0.07	0.477
ACD	3.10 \pm 0.27	2.89 \pm 0.37	-0.14 \pm 0.22	<0.001
LT	3.72 \pm 0.26	3.65 \pm 0.35	+0.18 \pm 0.22	<0.001
ASL	7.36 \pm 0.27	7.07 \pm 0.38	+0.03 \pm 0.24	0.529
VCD	17.30 \pm 1.20	15.63 \pm 0.78	-0.03 \pm 0.24	0.651
Raw AXL	24.66 \pm 1.23	22.70 \pm 0.84	+0.00 \pm 0.04	0.438
Corrected AXL	24.66 \pm 1.23	22.70 \pm 0.84	-0.01 \pm 0.04	0.420

Table 5.1. Mean baseline biometric parameters for the myopic and emmetropic groups and the mean change observed across both groups over 2.5 years. A bold p values denotes statistical significance.

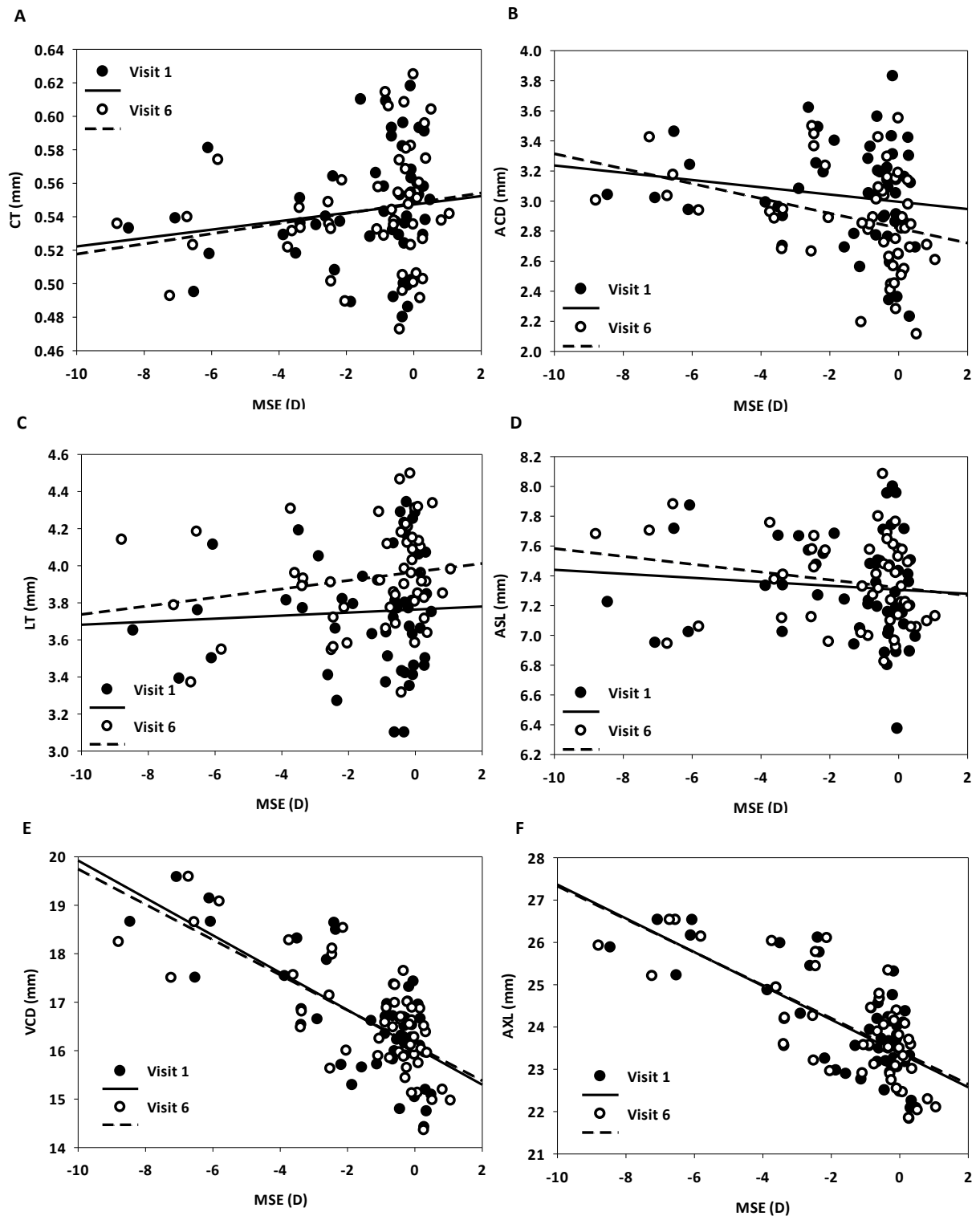


Fig. 5.1. CT (A), ACD (B), LT (C), ASL (D), VCD (E) and corrected AXL (F) at visit 1 (filled circles and solid regression line) and after 2.5 years at visit 6 (open circles and dashed regression line) according to the MSE measured at visit 1 and 6, respectively.

Multiple linear regression analysis showed no relationship between the change in right eye amplitude of accommodation and the change in biometry ($r=0.263$, $r^2=0.069$; CT $p=0.663$; ACD $p=0.300$; LT $p=0.480$; corrected AXL $p=0.508$). Furthermore, the changes in CT ($p=0.514$), ACD

($p=0.859$), LT ($p=0.783$) and corrected AXL ($p=0.423$) were not significantly correlated to the change in MSE over the 2.5 year study ($r=0.141$, $r^2=0.020$). However, the baseline AXL:CR ratio was significantly associated with the change in MSE ($r=0.323$, $p=0.021$; Fig. 5.2A), indicating that participants with a longer baseline AXL and/or steeper baseline anterior corneal curvature are at a greater risk of a myopic shift in refraction during incipient presbyopia. Indeed, the change in MSE was dependent on baseline AXL ($r=0.303$, $p=0.031$; Fig. 5.2B), whereas the baseline mean anterior radius of curvature ($r=0.005$, $p=0.972$) and baseline crystalline lens thickness ($r=0.114$, $p=0.428$) were not significantly associated with the change in MSE. As previously noted in section 3.1.2c, the changes in mean anterior radius of curvature ($F=0.834$, $p=0.527$) and toricity ($F=0.799$, $p=0.496$) were not statistically significant over the course of the study. Baseline AXL was not significantly associated with the change in AXL observed over the 2.5 year study ($r=0.149$, $p=0.296$).

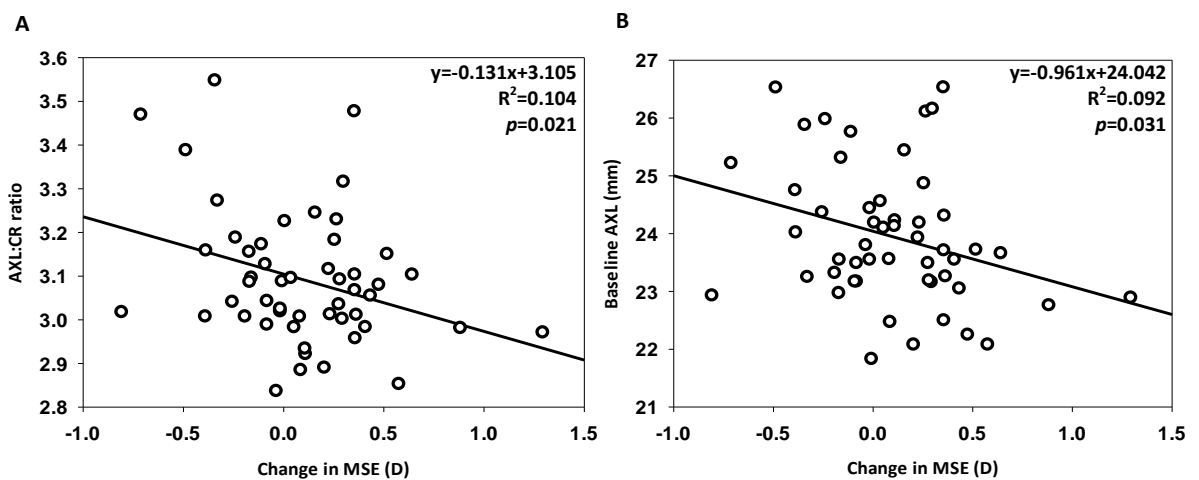


Fig. 5.2. Baseline AXL:CR ratio (A) and baseline AXL (B) according to the change in MSE over the 2.5 year study. The solid line represents the regression line of the data.

Moreover, the biometric changes of the 2 participants discussed in Chapter 3 who became more myopic (MSE >0.50 D) were not significantly different to the remaining cohort (CT $t=-0.254$, $p=0.800$; ACD $t=-0.349$, $p=0.729$; LT $t=-0.724$, $p=0.473$; raw AXL $t=0.774$, $p=0.443$; corrected AXL $t=0.601$, $p=0.550$).

No significant relationship was observed between the changes in CT ($p=0.405$; Fig. 5.3A), ACD ($p=0.924$; Fig. 5.3B) or LT ($p=0.465$; Fig. 5.3C) and baseline age ($r=0.582$, $r^2=0.338$), whereas the

change in AXL was dependent on baseline age ($p < 0.001$; Fig 5.3F). Indeed, there was a trend at baseline for the oldest participants to have shorter AXL measurements ($r = 0.251$, $p = 0.076$). Re-analysing the data after splitting the cohort at age 38.3 years (the age at which the regression line in Fig. 5.3F bisects $y = 0.00$), the change in AXL ($+0.016 \pm 0.037$ mm; $F = 1.353$, $p = 0.249$) and MSE ($+0.09 \pm 0.32$ D; $F = 0.567$, $p = 0.725$) experienced by the younger group (10 myopic and 11 emmetropic individuals) was not statistically significant over the 2.5 year study, however, unlike the change in MSE ($+0.10 \pm 0.42$ D; $F = 0.563$, $p = 0.728$), the change in AXL (-0.024 ± 0.039 mm; $F = 3.334$, $p = 0.033$) observed amongst the older group (11 myopic, 18 emmetropic individuals) was statistically significant. No differences emerged based on the refractive error grouping (≤ 38.3 years MSE: $F = 0.114$, $p = 0.981$, AXL: $F = 0.826$, $p = 0.534$; > 38.3 years MSE: $F = 0.114$, $p = 0.989$, AXL: $F = 2.488$, $p = 0.035$). The change in AXL observed amongst the older age group was not statistically significantly related to changes in ACD ($r = 0.199$, $p = 0.302$) or ASL ($r = 0.067$, $p = 0.728$), which are indirect measures of crystalline lens movement.

The same pattern of significance was also observed when substituting corrected AXL (used throughout the study) for raw AXL (before compensating AXL values for the error calculated in 5.2.1e) in all of the multiple linear regression analysis discussed.

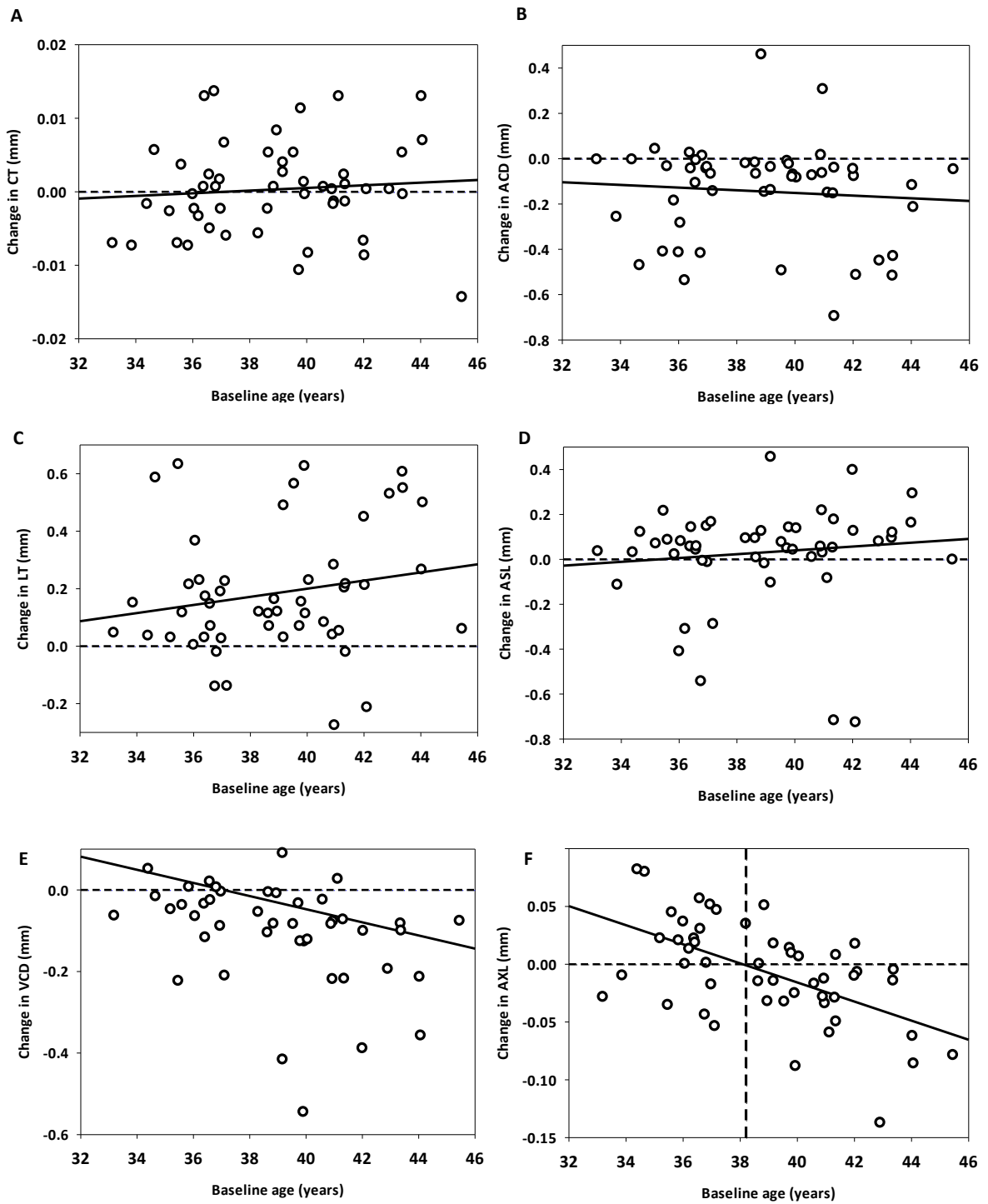


Fig. 5.3. Change in CT (A), ACD (B), LT (C), ASL (D), VCD (E) and corrected AXL (F) over the 2.5 year study according to baseline age. The solid line represents the regression line of the data and the dashed horizontal line represents no change (0.00 mm). The dashed vertical line in F ($x=38.3$ years) splits the cohort at the point where the regression line bisects the $y=0.00$ dashed line.

Similarly to previous reports, ciliary muscle Curved TL ($r=0.593$, $p<0.001$), Ant L ($r=0.575$, $p<0.001$), and SS-IA ($r=0.570$, $p<0.001$) were significantly larger in eyes with longer AXL measurements, whereas Max T ($r=0.129$, $p=0.366$) was not significantly thicker in eyes with longer AXL measurements at visit 1 (Sheppard and Davies, 2010a; 2011; Fig. 5.4). Deeper ACD ($r=0.374$, $p=0.007$) and thinner LT ($r=0.300$, $p=0.032$) measurements were significantly associated with longer Ant L values. A statistically significant relationship emerged between deeper ACD and longer Curved TL measurements ($r=0.308$, $p=0.028$), however no relationship emerged between LT and Curved TL ($r=0.136$, $p=0.342$) at visit 1.

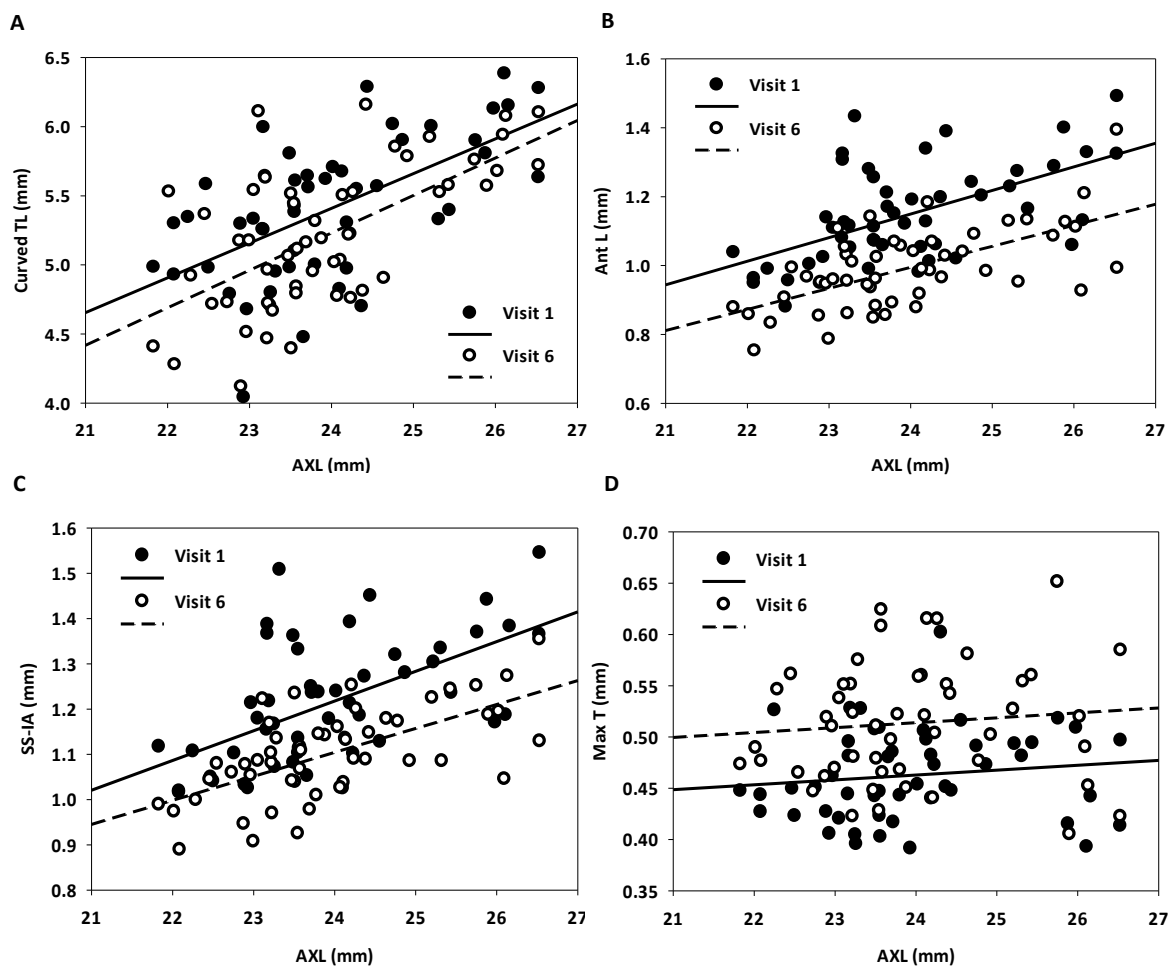


Fig. 5.4. Curved TL (A), Ant L (B), SS-IA (C) and Max T (D) of the temporal ciliary muscle at visit 1 (filled circles and solid regression line) and after 2.5 years at visit 6 (open circles and dashed regression line) according to AXL measured at visit 1 and 6, respectively.

Multiple linear regression analysis was used to investigate whether any interdependency between changes in ocular biometry and ciliary muscle morphology occurred over the 2.5 year study. Changes in Curved TL ($r= 0.379$; ACD $p=0.264$; LT $p=0.466$; AXL $p=0.051$), Ant L ($r=0.299$; ACD $p=0.873$; LT $p=0.064$; AXL $p=0.571$), SS-IA ($r=0.276$; ACD $p=0.985$; LT $p=0.073$; AXL $p=0.988$), Max T ($r=0.179$; ACD $p=0.350$; LT $p=0.369$; AXL $p=0.639$), CM2 ($r=0.253$; ACD $p=0.530$; LT $p=0.174$; AXL $p=0.843$), CM25 ($r=0.088$; ACD $p=0.606$; LT $p=0.972$; AXL $p=0.743$), CM50 ($r=0.085$; ACD $p=0.980$; LT $p=0.749$; AXL $p=0.678$) and CM75 ($r=0.187$; ACD $p=0.620$; LT $p=0.793$; AXL $p=0.231$) were independent of changes in ocular biometry. Of particular interest is the trend that emerged for a relationship between a greater increase in LT and a larger decrease in Ant L with age (Fig. 5.5).

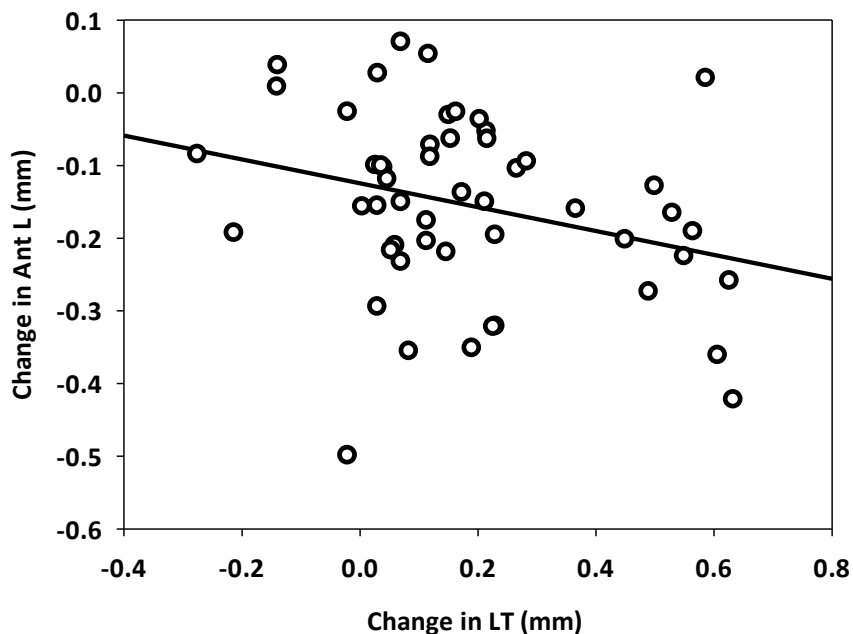


Fig 5.5. The change in Ant L over the 2.5 year study as a function of the change in LT over the 2.5 year study.

5.2.4 Discussion

The present investigation is the first to quantify changes in axial ocular biometry longitudinally with respect to changes in ocular refraction and ciliary muscle morphology within an incipient presbyopic population. A significant decrease in ACD and increase in LT was observed with age. Lenticular optical changes are implicated in the progression of myopia and no statistically significant interdependency between the changes in ciliary muscle morphology and axial biometry were observed over the course of the 2.5 year study.

Similarly to previous studies, myopic participants had significantly longer ACD, VCD and AXL values than emmetropic participants (Fontana and Brubaker, 1980; McBrien and Millodot, 1987; Grosvenor and Scott, 1991; Bullimore *et al.*, 1992; Lam *et al.*, 1999; Logan *et al.*, 2005; Chen *et al.*, 2009). However, no statistically significant correlation between a change in ocular refraction (as discussed in chapter 3) and a change in ocular biometry emerged during the course of the present investigation. Therefore, contrary to the results from previous studies investigating myopia progression during adulthood (Grosvenor and Scott, 1993; Lin *et al.*, 1996; McBrien and Adams, 1997; Kinge *et al.*, 1999), AXL elongation does not appear to be responsible for the myopic shift in refraction observed in the 2 incipient presbyopic participants from the current study. Consequently, it is unlikely changes in the retinal contour and/or the peripheral refraction profile were connected to the observed myopic shift in refraction.

The increase in LT of the 2 participants who became more myopic (>0.50 D) was not significantly larger than observed within the remaining cohort, which suggests a spasm of accommodation was not responsible for the reported myopic shift and thus corroborates the findings in section 4.2.3. Therefore, the results suggest that the myopic shift observed may be due to an increase in the power of the crystalline lens, which contravenes the commonly reported crystalline lens paradox phenomenon (Brown *et al.*, 1974; Hemenger *et al.*, 1995; Dubbelman *et al.*, 2001b; Koretz *et al.*, 2004; Jones *et al.*, 2005; Atchison *et al.*, 2008).

The significant relationship observed between a myopic shift in refraction and eyes with longer baseline AXL values suggests that eyes that have previously undergone significant crystalline lenses thinning in line with AXL elongation (Zadnik *et al.*, 1995; Mutti *et al.*, 1998) may be more susceptible to an increase in lenticular power during incipient presbyopia. The reason for this relationship is currently unclear. Further research is required to investigate whether the hypothesised increase in crystalline lens power is due to a magnified decrease in the radii of curvature of the anterior and posterior crystalline lens surfaces or whether the change in gradient

between high and low refractive indices is muted, or perhaps a combination of both, in eyes experiencing a myopic shift in refraction during incipient presbyopia.

Considering the mean changes occurring in axial biometry over the 2.5 year study, a significant decrease in ACD and increase in LT was observed. Similarly to previous cross-sectional research, consistent age-related changes in CT (Alsbirk, 1978; Cho and Lam, 1999; Doughty and Zaman, 2000; Atchison *et al.*, 2008), VCD (Koretz, 1989; Atchison *et al.*, 2008; Richdale *et al.* 2013) and AXL (Leighton and Tomlinson, 1972; Grosvenor, 1987; Koretz *et al.* 1989; Ooi and Grosvenor, 1995; Dubbelman and van der Heijde, 2001; Atchison *et al.*, 2008; Richdale *et al.* 2013) were not observed.

The change in ACD and LT per year was -0.058 ± 0.086 mm and $+0.074 \pm 0.088$ mm, respectively, and did not result in a statistically significant change in ASL ($+0.012 \pm 0.095$ mm/year). Previous estimates derived from cross-sectional research have suggested the change in ACD and LT is between -0.010 to -0.031 mm/year and $+0.019$ to 0.031 mm/year, respectively (Koretz *et al.*, 1989; Dubbelman *et al.*, 2001a; Wojciechowski *et al.*, 2003; Koretz *et al.*, 2004; Allouch *et al.*, 2005; Shufelt *et al.*, 2005; Atchison *et al.* 2008; Kasthurirangan *et al.* 2011; Richdale *et al.* 2013). Despite evidence suggesting the increase in anterior cortex thickness is between 1.5 to 3.0 times greater than the increase in posterior cortex thickness with age (Kashima *et al.*, 1993; Cook *et al.*, 1994; Dubbelman *et al.*, 2003; Kasthurirangan *et al.* 2011) and the distance between the sulcus (centre of the crystalline lens nucleus) and cornea remains constant with age (Dubbelman *et al.*, 2003), cross-sectional biometry studies investigating a wide range of ages have typically reported the decrease in ACD with age represents approximately 50% of the increase in LT with age (Dubbelman *et al.*, 2001a: 16 to 65 years; Koretz *et al.*, 2004: 18 to 50 years; Allouch *et al.*, 2005: 10 to 95 years; Atchison *et al.*, 2008: 18 to 69 years; Kasthurirangan *et al.* 2011: 19 to 70 years). Whereas, the present longitudinal investigation observed a decrease in ACD with age that represented 78% of the increase in LT, which appears to align more closely with the aforementioned intra-lenticular growth pattern (Kashima *et al.*, 1993; Cook *et al.*, 1994;

Dubbelman *et al.*, 2003). Indeed, Koretz *et al.* (1989) also reported no statistically significant change in ASL occurred during age-related expansion of the crystalline lens after employing an age-dependent correction factor for the velocity of ultrasound through the crystalline lens. However, the validity and necessity of this correction method has been questioned (Dubbelman *et al.*, 2001a; Atchison *et al.*, 2008) and inspection of the raw data reveals a significant increase in ASL with age. Nevertheless, cross-sectional research investigating changes in biometry in participants over 30 years of age have reported ASL does not change significantly with crystalline lens expansion (Wojciechowski *et al.*, 2003: 40 to 79 years; Shufelt *et al.*, 2005: 40 to 80+ years; Richdale *et al.* 2013: 30 to 50 years). Perhaps, therefore, the rate of increase in the posterior cortex declines or the sulcus moves anteriorly with age. Longitudinal studies utilising Scheimpflug photography (Dubbelman *et al.*, 2003) to monitor the structural changes occurring within the ageing crystalline lens are required to investigate the axial laterality of sectorial lenticular expansion.

Furthermore, the magnitude of annual crystalline lens expansion reported by the current study is significantly larger than previously reported by cross-sectional biometry studies (Koretz *et al.*, 1989; Dubbelman *et al.*, 2001a; Wojciechowski *et al.*, 2003; Koretz *et al.*, 2004; Allouch *et al.*, 2005; Shufelt *et al.*, 2005; Atchison *et al.* 2008; Kasthurirangan *et al.* 2011; Richdale *et al.* 2013). However, the majority of studies failed to control their cohort for refractive error (Dubbelman *et al.*, 2001a; Wojciechowski *et al.*, 2003; Koretz *et al.*, 2004; Allouch *et al.*, 2005; Shufelt *et al.*, 2005), despite the well-established link between ametropia progression and changes in biometry (Zadnik *et al.*, 1995; McBrien and Adams, 1997; Mutti *et al.*, 2012). Indeed, even the data from authors who included only emmetropic individuals are unlikely to have compared equivalent eyes across the age-range because of age-related changes in refraction (Koretz *et al.*, 1989; Atchison *et al.* 2008; Richdale *et al.*, 2013), as discussed in section 1.3. Therefore, it is feasible the older participants may have been myopic when they were younger and the younger participants may have been destined to become significantly hypermetropic in old age. Additionally, owing to the

lack of cycloplegic refraction, some of the young emmetropes may have had latent hypermetropia. Further longitudinal data, perhaps collected during routine biennial eye examinations, is required to quantify LT changes over the lifespan, with reference to changes in ocular refraction.

Uniquely, this study reports longitudinal changes in raw and corrected AXL are dependent on baseline age. AXL changes observed amongst the youngest participants (≤ 38.2 years at baseline) were invariant ($+0.016 \pm 0.037$ mm), whereas a statistically significant reduction in AXL (-0.024 ± 0.039 mm) was evident amongst the oldest participants (>38.2 years at baseline). Based on cross-sectional data, Grosvenor (1987) suggested a reduction in AXL may be a mechanism for emmetropisation in the ageing eye. Investigating patients whose ocular refraction was between 0.00 and +2.00 D, Grosvenor reported the mean AXL of patients over the age of 50 years was 23.5 mm, whereas the AXL of patients aged between 20 to 29 years was 24.1 mm. However, a review by Brown *et al.* (1999) suggested the younger participants may have had longer AXL values due to an improvement in nutrition during the twentieth century, which is likely to have resulted in greater systemic growth of the younger participants. Nevertheless, Brown *et al.* conceded it is possible an age-related reduction in AXL may work adjunct to the increase in the refractive index gradient of the crystalline lens to maintain emmetropia or create a hypermetropic shift in refraction. Further research is required to quantify how the retinal contour may change with age.

The data from the present investigation suggest the mechanism causing a reduction in AXL appears to be independent of the measured age-related changes in the accommodative apparatus; failing to show any relationship with changes in ciliary muscle morphology or the movement of the crystalline lens (indirectly measured by changes in ACD and ASL). A significant hypermetropic shift in refraction was not observed amongst the oldest participants, therefore indicating that the increase in lenticular thickness and reduction in radii of curvature are likely to offset the refractive consequences of a reduction in AXL.

Considering potential mechanisms capable of instigating the reduction in AXL, it is feasible that the traction from gradual posterior vitreous detachment at the vitreo-retinal attachment sites (at the fovea and optic disc; Yonemoto *et al.*, 1994; Uchino *et al.* 2001) may remodel the shape of the posterior pole. Indeed, the early stages of posterior vitreous detachments have been observed in patients during the fourth decade of life, which typically progress to complete posterior vitreous detachments in later life (Uchino *et al.* 2001). However a rebound would be expected following the breakage of the attachments around the fovea and optic disc, yet an abrupt change in ocular refraction has not been observed clinically following complete posterior vitreous detachment. Nevertheless, it is feasible the loss of structural support posteriorly due to age-related vitreous humour liquefaction (Sebag, 1987) and detachment posteriorly may cause the shape of the posterior pole to change, possibly reducing AXL. However, the influence of the increase in scleral thickness (Watson and Young, 2004), reduction in choroidal thickness (Manjunath *et al.*, 2010) and increase in posterior shell rigidity (Friberg and Lace, 1988; van Alphen and Graebel, 1991; Ugarte *et al.*, 2006) with age on the shape of the posterior pole is currently unknown. Further research is required to model changes in the posterior pole with reference to changes in retinal, choroidal and scleral thickness and shape to investigate why a reduction in AXL might occur during incipient presbyopia.

Whilst the overestimation of AXL arising from an increase in LT with age was individually adjusted for (showing an age-related reduction in AXL with and without the correction), it is possible an age-related decrease in the refractive index of the ocular media, particularly within the vitreous due to a reduction in density, may artificially depress the measured AXL values. Hitherto, no studies investigating age-related changes in the refractive index of the vitreous humour have been published. Future studies should acquire high-resolution MRI longitudinal data (which is not affected by the optics of the eye) to investigate age-related changes in axial biometry (Richdale *et al.*, 2009). Utilising MRI to investigate changes in axial biometry has the added benefit of visualising the equatorial region of the crystalline lens and the ciliary muscle simultaneously,

allowing the interaction between ocular biometry and ciliary muscle morphology to be explored further.

Supporting the findings reported in section 4.2.3, eyes with longer AXL values, which are typically myopic, demonstrated longer ciliary muscle Curved TL, Ant L and SS-IA measurements, whereas Max T was not significantly thicker in eyes with longer AXL measurements (Sheppard and Davies, 2010a; 2011). A statistically significant interdependency between the measured age-related changes in the ciliary muscle and the crystalline lens did not emerge during the course of the present study. Nevertheless, a trend emerged for a larger decrease in Ant L to accompany a larger increase in LT with age (Fig. 5.5), which supports theories suggesting that age-related changes in the morphology and position of the ciliary muscle and the crystalline lens may be inter-related (Strenk *et al.*, 1999; Pardue and Sivak, 2000; Atchison *et al.*, 2008; Strenk *et al.*, 2010). It is feasible the crystalline lens and ciliary muscle may influence the position of one another more significantly in subsequent years as they continue to enlarge and presbyopia manifests. However, this study does not discount the impact age-related anterior migration of the zonules across the crystalline lens capsule (Farnsworth and Shyne, 1979) may have on ciliary muscle or crystalline lens position. Unfortunately it is not currently possible to image the zonules whilst imaging the ciliary muscle with the Visante AS-OCT and therefore alternative instruments are required to investigate changes in zonular position *in vivo* (Ludwig *et al.*, 1999).

The LenStar was unable to measure the crystalline lens thickness of all 51 subjects across all 6 visits, thus necessitating the inclusion of LT values measured by the Visante AS-OCT. Whereas the LenStar measures anterior and posterior segment axial distances simultaneously, obtaining a snapshot of the axial biometry, the Visante AS-OCT is not able to simultaneously image the full thickness of the crystalline lens with the cornea or retina, therefore only LT was substituted. In an attempt to reduce errors arising from the substitution of LenStar LT values for Visante AS-OCT LT measurements, a pixel to millimetre conversion ratio was used to create an interchangeable LT measurement between the two instruments, as discussed in section 2.3.3. Any inaccuracies

arising due to the substitution of LT are not likely to impact the results of this study because the LenStar was able to measure LT in the majority of patients (75% of patients at visit 1, 78% at visit 2, 86% at visit 3, 90% at visit 4, 86% at visit 5 and 93% at visit 6). The results from the current study and previous clinical experience indicate the LenStar struggles to detect the posterior crystalline lens boundary in younger eyes, possibly due to higher light transmittance (Weale, 1988). Nevertheless, it appears the newer versions of LenStar software have overcome this problem (Read *et al.*, 2010a; Woodman *et al.*, 2011; Woodman *et al.*, 2012).

The current study provides the first prospective, longitudinal insight into how axial ocular biometry changes during incipient presbyopia. In conclusion, a significant increase in LT and a corresponding decrease in ACD occurs with age, which is not dependent on ametropia. Changes in CT, ASL, VCD and AXL were invariant, however the oldest participants demonstrated a significant decrease in AXL, possibly due to age-related changes in the vitreous humour. The lack of dependency of a change in MSE on the parameters measured by the present studies implies lenticular changes are likely to be responsible for the myopic shift in refraction observed within the current study. Further longitudinal research is required to quantify the putative lenticular changes that result in a myopic shift in refraction.

5.3 Study Two: Longitudinal changes in ocular biometry during accommodation in incipient presbyopic individuals

The amplitude of accommodation decreases with age, from approximately 15 D in early infancy to 1 D at 60 years of age (Donders, 1864; Duane, 1922), when presbyopia is well established. During the period of incipient presbyopia, the amplitude of accommodation is typically within the range of 4 to 6 D (Donders, 1864; Duane, 1922). It is thought approximately 80% of the total amplitude of accommodation is accessible during near vision (Wolffsohn *et al.*, 2011), therefore incipient presbyopic patients would be expected to demonstrate significant changes in ocular biometry to accommodate during near vision. However, as discussed in section 5.2.4, significant age-related

changes in biometry occur during incipient presbyopia, which may degrade the magnitude of the accommodative response measured longitudinally.

During accommodation, corneal thickness and curvature do not change (Buehren *et al.*, 2003; Drexler *et al.*, 1997; 1998; Read *et al.*, 2007b; 2010). The force of ciliary muscle contraction drives a reduction in crystalline lens equatorial diameter (Strenk *et al.*, 1999; Sheppard *et al.*, 2011; Richdale *et al.*, 2013), a reduction in the radii of curvature of the anterior and posterior crystalline lens surfaces (Brown, 1973; Pierscionek *et al.*, 1995; Garner and Yap, 1997; Dubbelman *et al.*, 2005) and an increase in crystalline lens axial thickness (Shum *et al.*, 1993; Drexler *et al.*, 1997; Koretz *et al.*, 1997; Dubbelman *et al.*, 2003; Kirschkamp *et al.*, 2004; Ostrin *et al.*, 2006; Hermans *et al.* 2007; Tsorbatzoglou *et al.*, 2007; Richdale *et al.*, 2008; Doyle *et al.*, 2013). The increase in steepness of the crystalline lens surfaces is greater anteriorly than posteriorly (Brown, 1973; Pierscionek *et al.*, 1995; Dubbelman *et al.*, 2005). Moreover, the posterior axial movement of the posterior crystalline lens surface during accommodation is thought to be between 3 to 5 times smaller than the anterior movement of the anterior crystalline lens surface (Drexler *et al.*, 1997; Dubbelman *et al.*, 2005), and may vary according to the magnitude of the accommodative demand (Beers and van der Heijde, 1996; Gibson, 2008). The increase in lenticular thickness during accommodation is produced entirely by an increase in the thickness of the nucleus (Brown, 1973; Koretz *et al.*, 1997; Dubbelman *et al.*, 2003; Hermans *et al.*, 2007; Kasthurirangan *et al.*, 2011), resulting in a decrease in anterior chamber depth (Shum *et al.*, 1993; Drexler *et al.*, 1997; Koretz *et al.*, 1997; Dubbelman *et al.*, 2003; Dubbelman *et al.*, 2005; Ostrin *et al.*, 2006; Tsorbatzoglou *et al.*, 2007; Read *et al.*, 2010a). The change in crystalline lens shape is vital to produce the accommodative response, therefore age-related changes in crystalline lens shape (Koretz *et al.*, 1989; Dubbelman *et al.*, 2001b; Jones *et al.*, 2007; Atchison *et al.*, 2008; Kasthurirangan *et al.*, 2011; Richdale *et al.*, 2013; Section 5.2) and flexibility (Heys *et al.*, 2004; Glasser, 2008) have led to the crystalline lens becoming central to several presbyopia theories. However, despite significant age-related crystalline lens changes, the change in crystalline lens

thickness required to produce 1.00 D of accommodation is thought to remain constant with age (Koretz *et al.*, 1997).

Furthermore, the axial eye length transiently elongates during accommodation (Shum *et al.*, 1993; Drexler *et al.*, 1998; Mallen *et al.*, 2006; Read *et al.*, 2010a; Woodman *et al.*, 2011; Woodman *et al.*, 2012), which is also thought to be driven by ciliary muscle contraction; pulling the equatorial choroid centripetally and therefore necessitating posterior pole elongation to maintain a constant ocular volume (Drexler *et al.*, 1998; Mallen *et al.*, 2006). Hitherto, most studies have reported transient axial length changes in response to short periods of accommodation in young adults only (Shum *et al.*, 1993; Drexler *et al.*, 1998; Mallen *et al.*, 2006; Read *et al.*, 2010a; Woodman *et al.*, 2011; Woodman *et al.*, 2012). However, due to the attenuation of the centripetal ciliary muscle accommodative response (Croft *et al.*, 2013a; section 4.3.3) and the increase in the stiffness of the choroid (van Alphen and Graebel, 1991; Ugarte *et al.*, 2006) and sclera (Friberg and Lace, 1988; Pallikaris *et al.*, 2005) with age, axial length elongation during accommodation may be progressively muted with age. However, relocation of the anterior uveal tract post-cataract surgery to a more youthful, posterior position (Strenk *et al.*, 2010) and evidence of axial length elongation in an advanced presbyope during near vision (Hollins, 1974) suggest the choroid may retain its elasticity with age and transient axial length elongation during accommodation may still occur in incipient presbyopic eyes. Additionally, it has been hypothesised that high levels of transient axial length elongation during accommodation may precipitate permanent axial length elongation and thus a myopic shift in refraction in susceptible eyes (Mallen *et al.*, 2006). Therefore, longitudinal follow-up is required to reveal whether axial length elongation during accommodation is associated with myopia progression, and whether the magnitude of elongation is attenuated with age.

The aim of this study is to profile accommodative biometric changes longitudinally within incipient presbyopic individuals and to determine whether accommodative transient axial length elongation is associated with a myopic shift in refraction during incipient presbyopia.

Interdependency between accommodative changes in ocular biometry and ciliary muscle contractility (discussed in section 4.3.3) will also be explored.

5.3.1 Method

The study was approved by the Aston University Audiology and Optometry Research Ethics Committee (see Appendix 1) and was conducted in accordance with the tenets of the Declaration of Helsinki. Informed consent was obtained from all the participants after an explanation of the nature and possible consequences of the study (see Appendix 4 for a copy of the information sheet and consent form). One optometrist (DL) collected the data at each visit and did not refer to the results from the previous sessions until after each appointment, when the data were inputted into an Excel spreadsheet (Microsoft, Washington, USA).

In order to collect longitudinal data during incipient presbyopia, the following experimental protocol was repeated every 6 months over 2.5 years on all patients.

5.3.1a Participants

The details of the 20 participants recruited for this study have been previously described in section 4.3.2a.

5.3.1b Stimulus response

Change in objective refractive error during accommodation was measured by the binocular open-field WAM-5500 autorefractor (Grand Seiko Co. *Ltd.*, Japan) using the same method described in section 4.3.1b. Five consecutive measurements of refraction were acquired and the accommodative response was calculated using equation 1.1.

5.3.1c LenStar biometry

The LenStar biometer with Badal lens system attachment, as described in section 5.2.1c, was used to stimulate a range of accommodative levels whilst simultaneously measuring biometry. Each participant wore an eye patch over their left eye throughout data collection and room lights were extinguished. The right eye of all the myopic participants was fitted with a soft daily disposable

spherical contact lens (Focus Dailies, nelfilcon A, 69% water content; Ciba Vision, Duluth, GA). The contact lens fit and visual acuity were checked to ensure they were optimal prior to data collection. The LenStar provides accurate measurements of biometry even with a contact lens in place (Buckhurst *et al.*, 2009; Alderson *et al.*, 2012).

In order to provide a 3.00 D and 4.50 D accommodative stimulus, the Badal lens system letter target was moved from 10 cm to 7 cm and 5.5 cm away from the +10.00 D Badal lens, respectively. Each participant was asked to concentrate on the letter closest to the LenStar measurement beam and was encouraged to maintain clear focus throughout data collection (Stark and Atchison, 1994). The presentation order of each accommodative level was randomised and patients were exposed to the stimulus for 20 seconds prior to acquisition of data and were permitted a 1 minute distance-viewing break between stimulus levels. The average of three repeat measures of anterior chamber depth (ACD), crystalline lens thickness (LT) and axial length (AXL) were recorded at the three accommodative levels (0.00, 3.00 and 4.50 D). Corneal thickness (CT) was measured at 0.00 D only because it has been well-established the human CT does not change during accommodation (Drexler *et al.* 1997; 1998; Read *et al.*, 2007b; 2010). Anterior segment length ($ASL=CT+ACD+LT$) and vitreous chamber depth ($VCD=AXL-ASL$) were calculated from these values.

As previously discussed, where the LenStar biometer was unable to obtain crystalline lens thickness values, measures were substituted with data collected from a Visante AS-OCT.

5.3.1d Visante AS-OCT crystalline lens thickness measurements

Accommodated crystalline lens OCT images were acquired by a Visante AS-OCT by modulating the internal Badal lens optometer. Patients were positioned and aligned for data collection as described in section 5.2.1d. Wearing a contact lens made all myopic patients functionally emmetropic, therefore the internal Badal lens optometer was set to 0.00 D to stimulate 0.00 D of accommodation for all patients. Accommodation was stimulated by setting the Badal lens

optometer to -3.00 D and -4.50 D. Three crystalline lens images with the vertical fixation line visible were acquired in raw image mode for each patient and were subsequently exported in binary form for analysis with the custom-designed Matlab R2012b (The MathWorks Inc., Massachusetts, USA) software described in section 2.3.3. The mean crystalline lens thickness value obtained from the analysis of three images was recorded for each participant.

5.3.1e Error calculations

The LenStar uses an average refractive index to convert an optical path length into a geometrical AXL, as discussed in section 2.3.1a. Therefore, to correct for an overestimation of AXL due to the increase in LT with accommodation, the induced error was estimated using equations 2.1 to 2.7, as described in section 2.3.1a. Equations 2.1 to 2.3 also take into consideration the age of the patient, therefore age-related changes in the proportion of the crystalline lens taken up by the anterior cortex, nucleus and posterior cortex (Dubbelman *et al.*, 2003) were also corrected for as the age of each patient increased during the course of the longitudinal study.

5.3.1f Statistical analysis

All data were tested for normality using the Shapiro-Wilk test (SigmaPlot; Systat Software Inc., California, USA). Repeated measures ANOVA testing was used to determine whether the changes in axial biometry during accommodation (0, 3.00 and 4.50 D) were significant at visit 1, and whether any dependency on ametropia classification existed (SPSS, SPSS Inc, Illinois, USA). Repeated measures ANOVAs were also used to investigate whether the change in axial biometry at each accommodative level was degraded over the course of the study and whether the response per dioptre of accommodation exerted changed.

Separate multiple linear regression tests were used to investigate whether accommodative changes in biometry were associated with baseline age, the change in amplitude of accommodation or the change in MSE during the course of the study.

Multiple linear regression analysis was also used to determine the relationship between accommodative changes in ACD, LT, ASL and AXL with the accommodative changes in the ciliary muscle parameters discussed in section 4.2.

5.3.2 Results

5.3.2a Participants

From the recruited cohort discussed in section 3.1.2a, 20 participants (10 myopes, 10 emmetropes) with an amplitude of accommodation >4.50 D and astigmatic error of <0.75 D in their right eye took part in the 2.5 year subgroup study. The details of the subgroup cohort have been previously discussed in section 4.3.2a.

5.3.2b Cross-sectional analysis

The accommodative response stimulated by the 0.00, 3.00 and 4.50 D accommodative targets was statistically significant at visit 1 ($F=593.034$, $p<0.001$) and myopic participants demonstrated a smaller accommodative lag than emmetropic participants ($F=4.601$, $p=0.031$), as reported in section 4.3.2b.

The changes in ACD ($F=28.609$, $p<0.001$; Fig. 5.6), LT ($F=39.007$, $p<0.001$) and AXL ($F=5.417$, $p=0.009$) stimulated by the 0.00, 3.00 and 4.50 D accommodative targets were also statistically significant at visit 1, however changes in ASL ($F=1.070$, $p=0.354$; Fig. 5.6) and VCD ($F=1.111$, $p=0.340$) were not. No differences in accommodative response emerged between the refractive groups (ACD $F=0.860$, $p=0.432$; LT $F=1.112$, $p=0.340$; ASL $F=0.001$, $p=0.999$; VCD $F=0.016$, $p=0.984$; AXL $F=0.757$, $p=0.476$), however VCD ($F=6.636$, $p=0.019$) and AXL ($F=5.417$, $p=0.009$) values were significantly longer in myopic eyes (ACD $F=1.518$, $p=0.234$; LT $F=0.057$, $p=0.814$; ASL $F=0.188$, $p=0.188$).

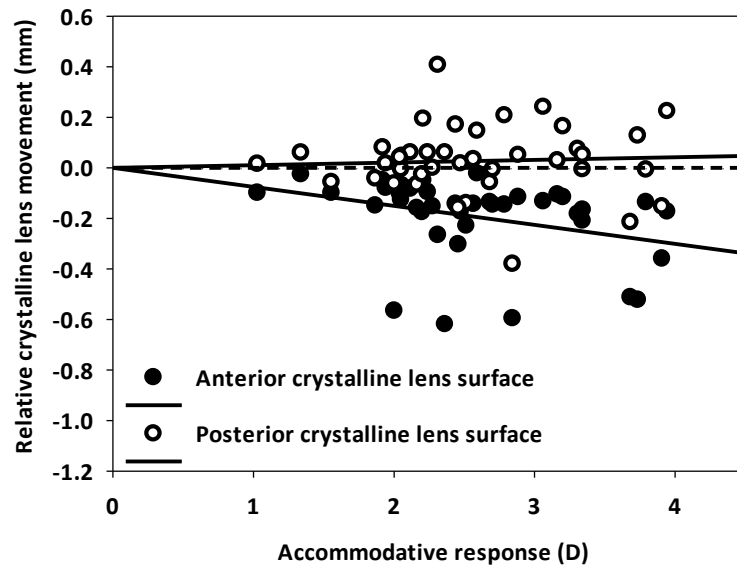


Fig. 5.6. The movement of the anterior crystalline lens surface (change in ACD; filled circles) and posterior crystalline lens surface (change in ASL; open circles) relative to their disaccommodated positions during accommodation to a 3.00 D and 4.50 D target at visit 1. A negative crystalline lens movement indicates the crystalline lens surface has moved anteriorly, whereas a positive movement indicates the crystalline lens surface has moved posteriorly. The dashed horizontal line represents no change (0.00 mm).

5.3.2c Longitudinal analysis

The accommodative refractive response elicited by the 3.00 D and 4.50 D has been discussed previously in section 4.3.2c.

The change in ACD ($F=1.976$, $p=0.137$), LT ($F=1.906$, $p=0.146$), ASL ($F=0.756$, $p=0.498$), VCD ($F=0.676$, $p=0.538$) and AXL ($F=0.448$, $p=0.814$) whilst viewing the 3.00 D accommodative target was not significantly attenuated over the course of the 2.5 year study (Fig. 5.7) and was not dependent on refractive grouping (ACD $F=2.790$, $p=0.055$; LT $F=1.682$, $p=0.148$; ASL $F=1.234$, $p=0.306$; VCD $F=1.337$, $p=0.276$; AXL $F=1.471$, $p=0.208$).

The change in ACD ($F=3.124$, $p=0.039$), LT ($F=3.025$, $p=0.042$) and AXL ($F=2.483$, $p=0.038$) whilst viewing the 4.50 D accommodative target significantly reduced over the 2.5 year study (Fig. 5.7). Whereas, the change ASL ($F=0.682$, $p=0.532$) and VCD ($F=0.604$, $p=0.577$) did not change significantly over the course of the study (Fig. 5.7). None of the changes in axial biometry at 4.50

D were dependent on refractive error (ACD $F=1.744$, $p=0.176$; LT $F=1.682$, $p=0.148$; ASL $F=1.234$, $p=0.306$; VCD $F=1.337$, $p=0.276$; AXL $F=1.471$, $p=0.208$).

The biometric response per dioptre of accommodation exerted to the 4.50 D accommodative target was not significantly attenuated over the 2.5 year study (ACD $F=2.243$, $p=0.097$; LT $F=1.688$, $p=0.178$; ASL $F=1.013$, $p=0.389$; VCD $F=0.8746$, $p=0.463$; AXL $F=1.049$, $p=0.395$; Table 5.2) and did not depend on ametropia (ACD $F=1.164$, $p=0.332$; LT $F=1.483$, $p=0.228$; ASL $F=0.566$, $p=0.619$; VCD $F=0.597$, $p=0.600$; AXL $F=0.970$, $p=0.441$). The magnitude of the change per dioptre of accommodation was not statistically dependent on whether the participant was classified as myopic or emmetropic (ACD $F=0.715$, $p=0.715$; LT $F=3.493$, $p=0.079$; ASL $F=0.703$, $p=0.413$; VCD $F=0.550$, $p=0.468$; AXL $F=0.049$, $p=0.827$). The magnitude of the change in AXL per dioptre of accommodation was not dependent on baseline AXL at visit 1 ($r=0.084$, $p=0.726$).

Axial biometry	Mean change per dioptre of accommodation exerted at Visit 1 (mm \pm SD)		Mean change per dioptre of accommodation exerted at Visit 6 (mm \pm SD)		Statistical significance (p) of changes over 2.5 years
	Myopes	Emmetropes	Myopes	Emmetropes	
ACD	-0.063 \pm 0.020	-0.101 \pm 0.078	-0.051 \pm 0.025	-0.065 \pm 0.026	0.097
LT	+0.066 \pm 0.023	+0.104 \pm 0.079	+0.054 \pm 0.023	+0.073 \pm 0.020	0.178
ASL	+0.003 \pm 0.032	+0.003 \pm 0.063	+0.004 \pm 0.040	+0.008 \pm 0.029	0.389
VCD	+0.002 \pm 0.033	-0.001 \pm 0.063	-0.004 \pm 0.040	-0.004 \pm 0.033	0.463
AXL	+0.005 \pm 0.004	+0.002 \pm 0.006	+0.000 \pm 0.005	+0.004 \pm 0.008	0.395

Table 5.2 Mean changes in axial biometry per dioptre of accommodation exerted (whilst viewing the 4.50 D accommodative target) at visit 1 (baseline) and visit 6 (after 2.5 years) for the myopic and emmetropic groups individually. The p values denote the significance of the change in accommodative response at each parameter over 2.5 years for myopic and emmetropic individuals.

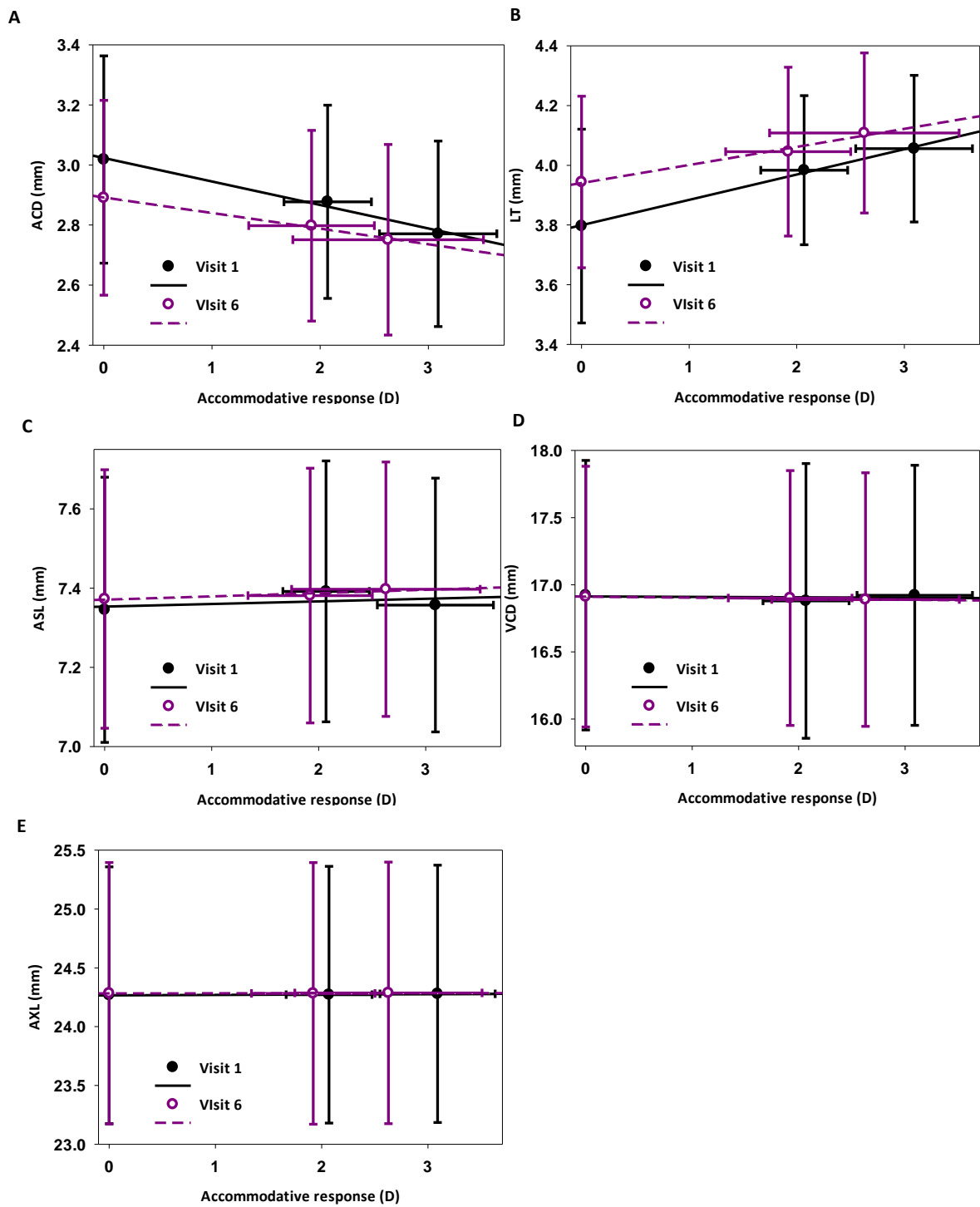


Fig 5.7. Mean ACD (A), LT (B), ASL (C), VCD (D) and AXL (E) (with ± 1 standard deviation error bars) at visit 1 (black filled circles and solid regression line) and after 2.5 years at visit 6 (purple open circles and dashed regression line) according to the accommodative response exerted at visit 1 and 6, respectively, to the 0, 3 and 4.50 D targets..

Multiple linear regression analysis revealed the changes in axial biometry per dioptre of accommodation exerted to the 4.50 D target were not significantly dependent on baseline age ($r=0.523$, $r^2=0.273$; ACD $p=0.331$; LT $p=0.237$; AXL $p=0.400$), the change in amplitude of accommodation ($r=0.326$, $r^2=0.106$; ACD $p=0.244$; LT $p=0.855$; AXL $p=0.889$) or the change in MSE ($r=0.457$, $r^2=0.209$; ACD $p=0.288$; LT $p=0.502$; AXL $p=0.163$) over the course of the 2.5 year study. However, the change in AXL per dioptre of accommodation exerted at baseline was significantly larger in participants who later went on to become myopic ($p=0.024$; Fig. 5.8).

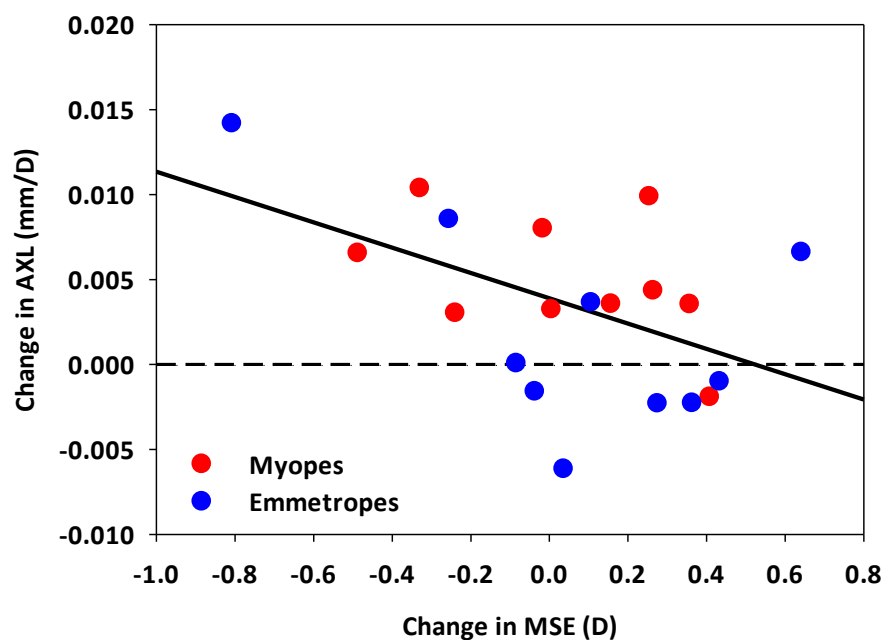


Fig. 5.8. The change in AXL per dioptre of accommodation exerted at baseline according to the change in MSE after 2.5 years. The solid black line represents the regression line ($y=-0.00745x+0.00390$, $r=0.503$, $r^2=0.253$, $p=0.024$) for the myopic (red) and emmetropic (blue) patient data. The dashed horizontal line represents no AXL change (0.000 mm).

The changes in ciliary muscle parameters per dioptre of accommodation exerted to the 4.50 D target were independent of disaccommodated AXL at visit 1 ($r=0.572$, $r^2=0.327$; curved TL $p=0.154$; Max T $p=0.953$; Ant L $p=0.404$; SS-IA $p=0.838$; CM2 $p=0.708$; CM25 $p=0.912$; CM50 $p=0.516$; CM75 $p=0.926$). The attenuation of Max T ($p=0.013$; Fig. 5.9A), SS-IA ($p=0.024$) and CM25 ($p=0.022$) per dioptre of accommodation exerted over the 2.5 year study was significantly associated with the decline in ACD change per dioptre of accommodation exerted ($r=0.778$,

$r^2=0.605$; Curved TL $p=0.243$; Ant L $p=0.325$; CM2 $p=0.055$; CM50 $p=0.205$; CM75 $p=0.134$). The attenuation of Max T ($p=0.003$; Fig. 5.9B), SS-IA ($p=0.029$), CM25 ($p=0.003$) and CM50 ($p=0.045$) per dioptre of accommodation exerted over the 2.5 year study was significantly related to the decline in LT change per dioptre of accommodation exerted ($r=0.822$, $r^2=0.676$; Curved TL $p=0.094$; Ant L $p=0.648$; CM2 $p=0.148$; CM75 $p=0.208$). However, the attenuation of the response of the ciliary muscle parameters per dioptre of accommodation exerted were not significantly related to accommodative changes in ASL ($r=0.722$, $r^2=0.522$; curved TL $p=0.848$; Max T $p=0.923$; Ant L $p=0.489$; SS-IA $p=0.477$; CM2 $p=0.334$; CM25 $p=0.774$; CM50 $p=0.673$; CM75 $p=0.543$), VCD ($r=0.729$, $r^2=0.532$; curved TL $p=0.723$; Max T $p=0.871$; Ant L $p=0.548$; SS-IA $p=0.493$; CM2 $p=0.219$; CM25 $p=0.863$; CM50 $p=0.865$; CM75 $p=0.388$) or AXL ($r=0.543$, $r^2=0.295$; curved TL $p=0.458$; Max T $p=0.762$; Ant L $p=0.609$; SS-IA $p=0.841$; CM2 $p=0.223$; CM25 $p=0.572$; CM50 $p=0.232$; CM75 $p=0.248$) over the 2.5 year study.

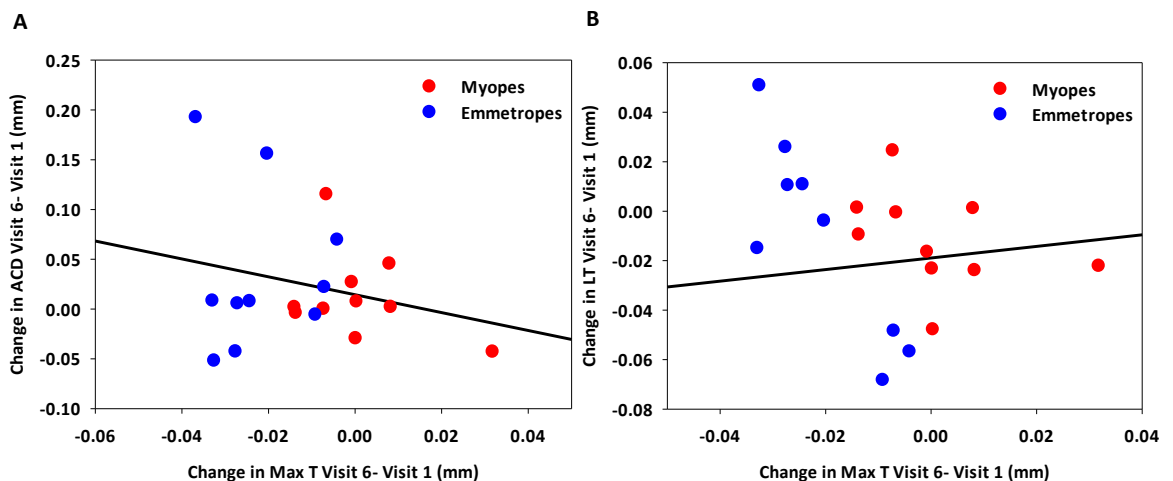


Fig 5.9. The difference between the accommodative change in ACD (A; $p=0.013$) and LT (B; $p=0.003$) from visit 1 to 6 according to the difference between the accommodative change of Max T from visit 1 to 6. The solid black line represents the regression line for the myopic (red) and emmetropic (blue) patient data. The negative gradient of the regression line in A shows a reduction in the accommodative change in ACD is related to a reduction in the accommodative increase in Max T. The positive gradient of the regression line in B shows a reduction in the accommodative increase in LT is associated to the reduction in the accommodative increase in Max T.

5.3.3 Discussion

The present investigation is the first to document an age-related attenuation of changes in ACD, LT and AXL with accommodation in an incipient presbyopic population longitudinally. However, despite significant age-related structural changes in axial biometry (section 5.2), the change in axial biometry per dioptre of accommodation exerted remained invariant with age.

A significant decrease in ACD and an increase in LT and AXL accompanied accommodation, whereas ASL and VCD did not change significantly (Table 5.2). Therefore, the majority of the increase in LT was compensated for by the reduction in ACD (Fig 5.6), which supports previous findings that the movement of the crystalline lens surface and reduction in radii of curvature is significantly greater anteriorly than posteriorly (Brown, 1973; Pierscionek *et al.*, 1995; Drexler *et al.*, 1997; Dubbelman *et al.*, 2005) and the Helmholtz theory of accommodation (von Helmholtz, 1855). Additionally, the increase in LT during accommodation is produced entirely by an increase in the thickness of the nucleus (Brown, 1973; Koretz *et al.*, 1997; Dubbelman *et al.*, 2003; Hermans *et al.*, 2007; Kasthurirangan *et al.*, 2011) and the distance between the sulcus (centre of the crystalline lens nucleus) and the cornea reduces with accommodation by approximately 0.017 ± 0.005 mm per dioptre of accommodative exerted (Ostrin *et al.*, 2006). Therefore, it is unlikely the accommodative change in ASL reflects the absolute magnitude of the accommodative posterior crystalline lens expansion. Consequently, the accommodative change in ASL essentially indicates the change in position of the posterior crystalline lens surface relative to its disaccommodated position. Nevertheless, previous studies have reported the ASL of young adults significantly increases during accommodation (Ostrin *et al.*, 2006; Bolz *et al.*, 2007; Tsorbatzoglou *et al.*, 2007; Read *et al.*, 2010a; Table 5.3), therefore the negligible change in ASL observed during incipient presbyopia might suggest either the mobility of the posterior crystalline lens surface diminishes with age or the anterior movement of the crystalline lens with accommodation increases with age.

Study	Age range (years)	Technique	Change per dioptre of accommodation exerted \pm SD (mm/D)		
			ACD	LT	ASL
Current study Visit 1 n=20	34 to 41	LCR, AS-OCT	-0.082 \pm 0.061	+0.085 \pm 0.060	+0.003 \pm 0.050
Richdale <i>et al.</i> (2013) n=26 Emmetropes	30 to 50	AS-OCT MRI	-	+0.064 +0.065	-
Sheppard <i>et al.</i> (2011) n=19	19 to 30	MRI	-	+0.08 \pm 0.05	-
Richdale <i>et al.</i> (2008) n=22	36 to 50	AS-OCT	-	+0.051 \pm 0.019	-
Bolz <i>et al.</i> (2007) n=10 Myopes	19 to 31	PCI	-0.057	+0.072	+0.013
Bolz <i>et al.</i> (2007) n=10 Emmetropes	19 to 31	PCI	-0.047	+0.063	+0.009
Ostrin <i>et al.</i> (2007) n=22	21 to 30	A-scan US	-0.051 \pm 0.008	+0.067 \pm 0.008	+0.017 \pm 0.005
Garner and Yap (1997) n=11	18 to 28	A-scan US	-0.054	+0.054	-
Koretz <i>et al.</i> (1997) n=42 Emmetropes	18 to 40	Scheimpflug	-0.038 \pm 0.139	+0.043 \pm 0.145	+0.003 \pm 0.174

Table 5.3. Summary of previous research investigating changes in anterior biometry per dioptre of accommodation exerted \pm standard deviation (where possible).

Considering the mobility of the posterior crystalline lens surface, Gibson (2008) reported the movement of the posterior surface during accommodation in patients aged between 18 to 36 years is bi-phasic; that is to say the posterior lenticular movement is negligible until the accommodative demand reaches approximately 2.00 D (eliciting an accommodative response of approximately 1.50 D). The initial static phase is thought to originate from vitreous humour resistance (Gibson, 2008). The mean accommodative response attained by the patients in the present study consistently remained above 2.00 D whilst viewing the 4.50 D target (Fig. 4.5B), therefore it is unlikely the patients described here were limited to the initial, static phase of the bi-phasic movement. However, it is possible age-related lenticular structural changes (Fisher, 1971; Pau and Kranz, 1991; Weeber and Eckbert, 2004; Hey *et al.*, 2004; Weeber *et al.*, 2007) may

perpetuate the static phase of posterior crystalline lens surface movement during accommodation. It is also feasible the age-related anterior migration of the zonules (Farnsworth and Shyne, 1979), decreased flexibility of the posterior crystalline lens capsule (Fisher, 1969b; Krag *et al.*, 1997; Glasser and Campbell, 1999), reduced ciliary muscle centripetal contractile response (section 4.3) and a reduction in vitreous humour density (Sebag, 1987) with age may impact posterior crystalline lens mobility. Further research investigating a wider range of ages is indicated to investigate how the movement of the posterior crystalline lens surface varies per dioptre of accommodation with advancing age. Utilising Scheimpflug photography to investigate crystalline lens changes during accommodation would also allow the accommodative movement of the sulcus to be monitored with age.

The magnitude of the increase in LT and reduction in ACD is almost equal in the current study (Table 5.2), therefore suggesting it is feasible the accommodative anterior movement of the crystalline lens increases with age. Indeed, Koretz *et al.* (1997) reported a trend for the accommodative change in ACD and ASL to increase and decrease, respectively, with age. However, as demonstrated by the large standard deviation values in Table 5.3, significant intersubject variability in the response of the posterior crystalline lens surface occurs. Further research is required to investigate the structural origins of the intersubject discrepancy in the accommodative change in ASL and longitudinal data are required to document intrasubject changes with age. Further analysis of data from the current study reveals no significant association between ciliary muscle contractility and the change in ASL during accommodation ($r=0.548$, $r^2=0.300$; curved TL $p=0.137$; Max T $p=0.816$; Ant L $p=0.366$; SS-IA $p=0.393$; CM2 $p=0.713$; CM25 $p=0.929$; CM50 $p=0.528$; CM75 $p=0.360$), therefore perhaps the architecture of the zonular insertions may be responsible for the intersubject variation. Future studies exploring the link between zonular placement and ASL accommodative changes should utilise high-resolution ultrasonography, which is capable of imaging the zonular apparatus *in vivo* (Ludwig *et al.*, 1999).

However, the trend for the accommodative increase in ASL (visit 1: $+0.003 \pm 0.050$ mm/D, visit 6: $+0.006 \pm 0.035$ mm/D), despite the minor attenuation of the accommodative change in ACD (visit 1: -0.082 ± 0.061 mm/D, visit 6: -0.058 ± 0.026 mm/D) and LT (visit 1: $+0.085 \pm 0.060$ mm/D, visit 6: $+0.063 \pm 0.023$ mm/D) per dioptre of accommodation exerted over the 2.5 year study suggests the accommodative anterior movement of the crystalline lens per dioptre of accommodation exerted may decrease during the development of incipient presbyopia. The accommodative anterior movement of the crystalline is known to reduce the magnitude of the accommodative response by approximately 0.50 D (Davies *et al.*, 2010), therefore it is feasible less ciliary muscle centripetal contractile force is required per dioptre of accommodation exerted (as found in section 4.3.3) because less of the accommodative refractive power is lost by the muted forward movement of the crystalline lens with age. Indeed, a larger decrease in the accommodative changes in ACD and LT were associated with a larger reduction in centripetal ciliary muscle contraction over the 2.5 year study. This mechanism may act to preserve the accommodative response and may be instigated by a restriction of lenticular antero-posterior mobility by the posterior iris or perhaps the impact of age-related anterior migration of the zonules (Farnsworth and Shyne, 1979). Furthermore, age-related changes in the pattern of crystalline lens accommodative expansion may also be instrumental. As discussed in section 4.3.3, due to the persistence of the antero-posterior ciliary muscle accommodative mobility (accommodative reduction in ciliary muscle Curved TL and Ant L) with age, it is unlikely the ciliary muscle would be responsible for the theorised reduction in anterior crystalline lens movement. Mathematical modelling utilising *in vivo* data is required to test the impact of reduced anterior crystalline lens mobility on the magnitude of the overall accommodative refractive response exerted. As identified previously, further longitudinal research documenting the movement of the crystalline lens surfaces and sulcus is also indicated to investigate this hypothesis.

Considering the magnitude of the change in LT per dioptre of accommodation exerted, the value observed by the current study was similar to that reported by previous studies (Garner and Yap,

1997; Koretz *et al.*, 1997; Ostrin *et al.*, 2006; Bolz *et al.*, 2007; Richdale *et al.*, 2008; Sheppard *et al.*, 2011; Richdale *et al.*, 2013; Table 5.3). As discussed in section 4.3.3, due to the shorter vitreous chamber depth typically observed in emmetropic individuals when compared to myopic individuals (McBrien and Adams, 1997), the change in crystalline lens thickness per dioptre of accommodation is expected to be larger in emmetropic individuals than myopic individuals (Gibson, 2008; Davies *et al.*, 2010). Indeed, a trend for myopic participants to experience a smaller change in LT per dioptre of accommodation exerted than emmetropic participants emerged (Table 5.2), however Bolz *et al.* (2007) reported results to the contrary (Table 5.3). Further research is required to substantiate the hypothesis of mathematical modelling studies (Gibson, 2008; Davies *et al.*, 2010).

Similar to the ciliary muscle contractility results discussed in section 4.3.3, a trend for a larger reduction in emmetropic, rather than myopic, accommodative response with age was observed (Table 5.2). Indeed, a larger reduction in accommodative refractive response to a 4.50 D target over the 2.5 year study was evident in emmetropic participants when compared to the myopic participants in Fig 4.5b. Anecdotal and published evidence (Rabbetts and Mallen, 2007) suggests hypermetropic and emmetropic patients manifest presbyopia before myopic patients, however the origin of this phenomenon has been traditionally thought to arise from near vision effectivity of myopic spectacle lenses (reducing the accommodative demand for myopic spectacle wearers; Hunt *et al.*, 2006) or the increased vitreous chamber depth associated with myopia (requiring a smaller change in axial ocular distances to produce accommodation due to the relatively more distant retinal plane; Davies *et al.*, 2010). It is feasible a greater attenuation of the amplitude of accommodation occurring in emmetropic patients during incipient presbyopia may also contribute to this phenomenon. Further research documenting the attenuation of amplitude of accommodation amongst a larger age-range of hypermetropic, emmetropic and myopic patients longitudinally is required to investigate this theory further.

A statistically significant AXL elongation was observed in response to the 4.50 D accommodative target, which attenuated throughout the 2.5 year study and was not dependent on ametropia. The AXL elongation per dioptre of accommodation exerted remained invariant throughout the study, thus suggesting the age-related increase in choroidal (van Alphen and Graebel, 1991; Ugarte *et al.*, 2006) and scleral (Friberg and Lace, 1988; Pallikaris *et al.*, 2005) stiffness was not significant during the course of the study. The mechanism of AXL elongation during accommodation is thought to be controlled by the force of ciliary muscle contraction at the equatorial choroid, which necessitates posterior pole elongation to maintain a constant ocular volume (Drexler *et al.*, 1998; Mallen *et al.*, 2006; Fig. 5.10). However, section 4.3.3 reports centripetal ciliary muscle contraction attenuates with age, therefore it is likely the contractile response of the longitudinal fibres (reducing ciliary muscle length, as shown by the red arrows in Fig 5.10) are primarily responsible for AXL elongation during accommodation.

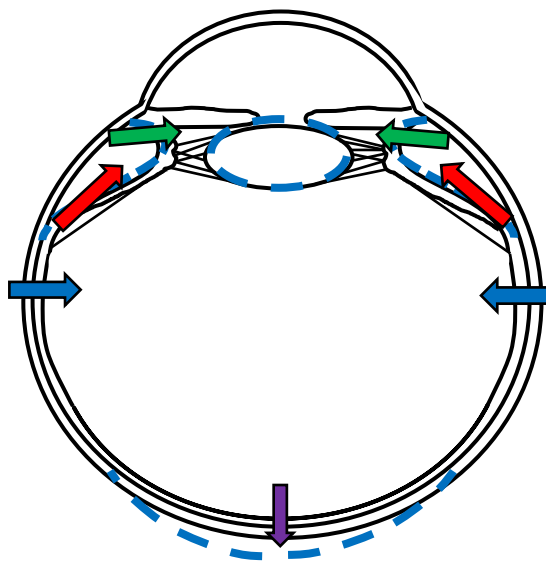


Fig. 5.10. Schematic transverse cross-section of the human eye demonstrating the centripetal (green arrows) and anterior (red arrows) ciliary muscle force during accommodation, which pulls the equatorial choroid inwards (blue arrows) and results in posterior pole elongation (purple arrows) in order to maintain a constant ocular volume.

Alternatively, the accommodative increase in AXL may be due to choroidal thinning as a result of the anterior tension applied by contraction of longitudinal ciliary muscle fibres during accommodation. In support of this theory, Woodman *et al.* (2012) observed choroidal thinning in young adults whilst viewing a 4.00 D accommodative target for 30 minutes, however the degree of choroidal thinning reported did not entirely offset the magnitude of AXL elongation observed. Therefore, it is unlikely choroidal thinning during accommodation was entirely responsible for the observed increase in AXL in the present investigation. Moreover, bidirectional AXL changes occurring in response to optical defocus (Read *et al.*, 2010b) are unlikely to have controlled the AXL changes observed in the current study because the degree of hypermetropic defocus imposed at near (accommodative lag) increased over the course of the study (Fig 4.5B), whereas the AXL elongation at 4.50 D attenuated during the study. Therefore, the most robust and probable theory for the origin of AXL elongation during accommodation appears to be based on the force of ciliary muscle contraction at the equatorial choroid (Drexler *et al.*, 1998; Mallen *et al.*, 2006; Fig. 5.10). Therefore, it would be of interest to investigate whether the AXL change during accommodation is attenuated over the lifespan, when presumably changes in ocular shell rigidity and thickness would become significant (Friberg and Lace, 1988; van Alphen and Graebel, 1991; Watson and Young, 2004; Pallikaris *et al.*, 2005; Ugarte *et al.*, 2006; Manjunath *et al.*, 2010). Indeed, AXL elongation during accommodation has been reported in one presbyopic patient during near vision (Hollins, 1974), however inclusion of a larger cohort is required to document the response of AXL elongation with age comprehensively.

Interestingly, a statistically significant, inversely-proportional relationship between AXL change per dioptre of accommodation exerted and the change in MSE over the 2.5 year study was observed (Fig. 5.8). Previous studies have hypothesised that transient AXL elongation during accommodation may promote myopia progression due to permanent AXL elongation (Mallen *et al.*, 2006). However, as discussed in section 5.2, the observed myopic shift in MSE (>0.50 D) was not accompanied by AXL elongation and was, in fact likely to be attributed to lenticular changes.

The correspondence between changes in lenticular curvature and/or refractive index and AXL changes during accommodation is unclear and requires further longitudinal investigation.

As displayed in Fig. 5.8, some of the participants appeared to experience a reduction in AXL during accommodation, which was also evident before AXL was corrected for age-related and accommodative changes in LT. The apparent reduction of AXL in a minority of patients has not been discussed by previous authors in their young adult cohorts (Drexler *et al.*, 1998; Mallen *et al.*, 2006; Read *et al.*, 2010a; Woodman *et al.*, 2011; Woodman *et al.*, 2012), however the magnitude of the standard deviation of the AXL elongation values reported suggests a reduction in AXL was observed in some patients. The mechanism causing a reduction in AXL during accommodation is unclear, however it is feasible that the AXL may appear to reduce during accommodation if the maximum AXL elongation is not centred on the fovea. An asymmetric accommodative expansion of the posterior pole (Fig. 5.11) may be instigated by the asymmetric ciliary muscle contractile response (greater temporally than nasally; Sheppard and Davies, 2010a; 2011) and/ or disaccommodated posterior pole asymmetry (Logan *et al.*, 2004; Gilmartin *et al.*, 2013). Indeed, there is evidence of spatial distortions in vision accompanying marked accommodation (Blank and Enoch, 1973; Blank *et al.*, 1975; Enoch, 1975) consistent with asymmetric retinal stretch during accommodation. Further research to determine how the retinal contour changes during accommodation and where the point of maximum ocular elongation resides is indicated.

A possible limitation of this study is the inclusion of LT measurements derived from both LenStar and Visante instruments. However, as discussed in section 5.2.4, the pixel thickness measurements of the Visante were calibrated against LenStar measurements (section 2.3.3), therefore minimising any potential disparity. Nonetheless, the LT at each visit was either wholly measured by the LenStar or the Visante to minimise any potential inaccuracies.

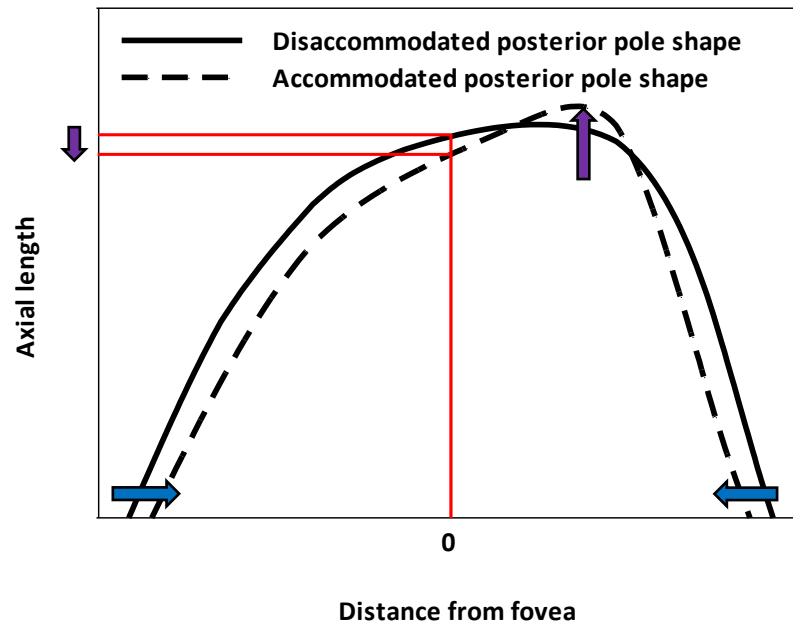


Fig. 5.11. Diagrammatic representation of the shape of a disaccommodated (solid line) and accommodated (dashed line) posterior pole. The x-axis describes the distance from the fovea and the y-axis describes the distance of the posterior pole from the cornea. The blue arrows demonstrate the accommodation-driven inward movement at the equator and the purple arrows show the corresponding pattern of posterior pole elongation. The AXL measured at the central fovea appears to have decreased during accommodation in this scenario.

The current study provides the first prospective, longitudinal insight into how accommodative changes in axial ocular biometry attenuate during incipient presbyopia. In conclusion, the accommodative decrease in ACD and increase in LT and AXL are significantly attenuated with age, however the response per dioptre of accommodation exerted remains constant with age. An age-related reduction in the accommodative anterior movement of the crystalline lens may help to preserve accommodative ability during incipient presbyopia. Further research is required to investigate how accommodative changes in ocular biometry change over the lifespan.

CHAPTER 6

Cross-sectional investigation of changes in ocular biometry during accommodation within a University population

6.1. Introduction

As discussed in section 5.3, the amplitude of accommodation decreases with age, from approximately 15 D in early infancy to 1 D at 60 years of age (Donders, 1864; Duane, 1922), when presbyopia is well established. Significant age-related structural changes in the accommodative apparatus are thought to be responsible for the attenuation of the amplitude of accommodation and therefore the development of presbyopia (Atchison, 1995; Gilmartin, 1995; Strenk *et al.*, 2005; Charman, 2008; Atchison *et al.*, 2008; Section 4.2; Section 5.2). However, whilst age-related accommodative changes in the anterior segment have been investigated cross-sectionally (Koretz *et al.*, 1997; Sheppard and Davies, 2011), published research has not investigated accommodative changes in vitreous chamber depth and axial length with respect to age.

Indeed, a significant transient elongation of axial length during accommodation has been observed in young adults (Shum *et al.*, 1993; Drexler *et al.*, 1998; Mallen *et al.*, 2006; Read *et al.*, 2010a; Woodman *et al.*, 2011; Woodman *et al.*, 2012). It has been hypothesised the mechanism of transient axial length elongation during accommodation may be produced by the force applied to the equatorial choroid by ciliary muscle contraction, thus necessitating posterior pole elongation to maintain a constant ocular volume (Drexler *et al.*, 1998; Mallen *et al.*, 2006; Fig. 5.10). In support of this theory, Croft *et al.* (2013b) reported the equatorial choroid and retina moved centripetally during Edinger-Westphal stimulated accommodation in Rhesus monkeys. However, an *in vivo* anterior scleral study (Pallikaris *et al.*, 2005) and *in vitro* posterior choroidal (van Alphen and Graebel, 1991; Ugarte *et al.*, 2006) and scleral (Friberg and Lace, 1988) studies have reported ocular stiffness increases with age. Van Alphen (1961; 1986) suggested the ciliary muscle and choroid behave as a single elasto-musculous sheet around the eye; moulding the sclera to accommodate ciliary muscle tonus, therefore it is conceivable age-related increases in

ocular stiffness may mute transient axial length elongation during accommodation. Nevertheless, the relocation of the anterior uveal tract post-cataract surgery to a more youthful position in humans suggests the choroid may retain its elasticity with age (Strenk *et al.*, 2010), despite the sclera and choroid becoming thicker (Watson and Young, 2004) and thinner (Manjunath *et al.*, 2010) with age, respectively. Also, previous longitudinal investigation of axial length elongation during accommodation within an incipient presbyopic population, as detailed in section 5.3, has revealed this mechanism does not appear to attenuate over a 2.5 year period. Furthermore, evidence of transient axial length elongation in one advanced presbyopic patient during near vision (Hollins, 1974) also suggests transient axial length elongation during accommodation may persist into advanced presbyopia.

Additionally, it has been hypothesised that high levels of transient axial length elongation during accommodation may precipitate a myopic shift in refraction in susceptible eyes (Mallen *et al.*, 2006). Therefore transient axial length elongation during accommodation may be responsible for the connection between high levels of near work and myopia onset and progression in both children (Mutti *et al.*, 2002; Saw *et al.*, 2002) and adults (Goldschmidt, 1968; Simensen and Thorud, 1994; McBrien and Adams, 1997; Maheshwari *et al.*, 2011). Mallen *et al.* (2006) hypothesised that myopic eyes may be more amenable to transient axial length elongation during accommodation due to reduced ocular rigidity (present due to the stretched, elongated eye characteristic of myopia). Yet, despite reduced posterior choroidal (Ikuno and Tano, 2009) and scleral (Summers-Rada *et al.*, 2006) thickness in myopic eyes compared to emmetropic eyes, no correlation between *in vivo* anterior ocular rigidity and myopia has been reported (Wong, 1991; Schmid *et al.*, 2003; Patel *et al.*, 2011). Moreover, previous research in young adults has produced contradictory results; Drexler *et al.* (1998) reported the magnitude of axial length elongation was larger in emmetropic eyes than myopic eyes, whereas Mallen *et al.* (2006) reported greater elongation in myopic eyes and Read *et al.* (2010b) reported no differences between myopic and emmetropic eye axial length elongation during accommodation.

The aim of this study is to profile accommodative biometric changes from early adulthood to advanced presbyopia and to determine whether any differences exist between the responses of myopic and emmetropic individuals.

6.1.1 Method

The study was approved by the Aston University Audiology and Optometry Research Ethics Committee (see Appendix 1) and was conducted in accordance with the tenets of the Declaration of Helsinki. Informed consent was obtained from all the participants after an explanation of the nature and possible consequences of the study (see Appendix 2 for a copy of the information sheet and consent form).

6.1.1a Sample size calculation

The calculated total sample size for repeated measures ANOVA testing (within and between interaction), including an effect size (f) of 0.25, an error probability (α) of 0.05 and required power ($1-\beta$) of 0.80 for 3 repeat measurements amongst 2 groups, was 28 participants (G*Power 3, Faul *et al.*, 2007).

6.1.1b Amplitude of accommodation

The right eye of all the myopic participants was fitted with a soft daily disposable spherical contact lens (*Focus Dailies*, nelfilcon A, 69% water content; Ciba Vision, Duluth, GA). A minimum settling period of 10 minutes was permitted prior to over-refraction to ensure the contact lens prescription was optimal. The subjective amplitude of accommodation of the right eye was measured using an RAF rule (Richmond Products, New Mexico, USA) via the push-up/pull-down method (McBrien and Millodot, 1986; Wolffsohn *et al.*, 2011), as described in section 3.1.1d. The average of three repeat measurements was recorded.

6.1.1c Stimulus response

Change in objective refractive error during accommodation was measured by the binocular open-field WAM-5500 autorefractor, as described in section 4.3.1b. The fixation target was placed

20cm, 8cm and 2cm away from the Badal lens in order to stimulate 0.00, 3.00 and 4.50 D of accommodation, respectively. Each participant was asked to focus on the centre of the Maltese cross as accurately as possible (Stark and Atchison, 1994) and was exposed to the stimulus for 20 seconds prior to the acquisition of data (Heron and Winn, 1989). A 1 minute distance viewing break was permitted between accommodative levels. Three consecutive measurements of refraction were acquired and the accommodative response was calculated using equation 1.1.

6.1.1d LenStar biometry

LenStar biometry was measured whilst stimulating accommodation, as described in section 5.3.1c. In order to provide a 0.00, 3.00 D and 4.50 D accommodative stimulus, the Badal lens system letter target was placed 10 cm, 7 cm and 5.5 cm away from the +10.00 Badal lens, respectively. The average of three repeat measures of anterior chamber depth (ACD), crystalline lens thickness (LT) and axial length (AXL) were recorded at the three accommodative levels (0.00, 3.00 and 4.50 D). Corneal thickness (CT) was measured at 0.00 D only because it has been well-established the human CT does not change during accommodation (Drexler *et al.* 1997; 1998; Read *et al.*, 2007b; 2010). Anterior segment length ($ASL=CT+ACD+LT$) and vitreous chamber depth ($VCD=AXL-ASL$) were calculated from these values. If the LenStar was unable to acquire LT measurements for a patient, their data was excluded from analysis.

6.1.1e Error calculations

The LenStar uses an average ocular refractive index to convert an optical path length to a geometric AXL (Read *et al.*, 2010a). Therefore, to correct for the potential over-estimation of AXL due to an increase in lens thickness (LT) with accommodation and age, the individual error (E) was calculated for each participant using equations 2.1 to 2.7, as described in section 2.3.1b.

6.1.1f Statistical analysis

Data were tested for normality using the Shapiro-Wilk test (SigmaPlot; Systat Software Inc., California, USA). To assess whether biometry changed significantly with accommodation (within-

subject factor), and to determine its dependency on refractive error (between-subject factor), a repeated measures ANOVA was performed (SPSS, SPSS Inc, Illinois, USA). The relationship between biometry and age was determined by linear regression analysis.

In order to compare the variance of the data between the younger and older participants, a two-tailed variance test was used, which accounts for differences in group sizes. The ANOVA residual mean square indices produced by individual linear regression analysis of the younger and older groups are divided to produce an F ratio. The significance of the difference in variance is determined by a two-tailed F look-up table, according to the degrees of freedom in each group.

6.1.2 Results

6.1.2a Participants

Seventy-two participants aged 18 to 60 years (median age 33.60 years) were recruited from an Aston University staff and student volunteer call. All participants were screened to exclude those with a positive history of ocular/systemic disease and The British National Formulary (Joint Formulary Committee, 2014) was consulted to ensure any prescribed medication did not affect the accommodative response.

Participants were classified as emmetropic ($-0.75 < \text{MSE} \leq +0.50$ D) or myopic ($\text{MSE} \leq -0.75$ D; Sheppard and Davies, 2011) and all subjects had ≤ 0.75 DC astigmatism. Of the total cohort, 37 were myopic (-3.49 ± 1.87 DS) and 35 were emmetropic (-0.16 ± 0.32 DS). The onset of ametropia (established by the participant's recollection) in the majority of the myopic subjects was before the age of 15 years (68%) and all myopes reported their myopia to be stable (defined as myopic progression < 0.50 D in the last 12 months). The myopic and emmetropic groups were 65% and 54% female, respectively. The myopic group contained 20 white European and 17 British Indian individuals, and the emmetropic group contained 21 white European and 14 British Indian individuals.

The emmetropic and myopic groups were balanced for age ($U=607$, $p=0.652$; myopes median age 31.49 years; emmetropes median age 34.67 years) and right eye amplitude of accommodation ($t=0.588$, $p=0.558$; myopes 7.69 ± 3.37 D; emmetropes 7.26 ± 2.83 D). As expected, right eye amplitude of accommodation decreased significantly with age ($r=0.918$, $p<0.001$; Fig. 6.1). Baseline uncorrected MSE was not significantly correlated with age in either group (myopes $r=0.063$, $p=0.701$; emmetropes $r=0.084$, $p=0.621$).

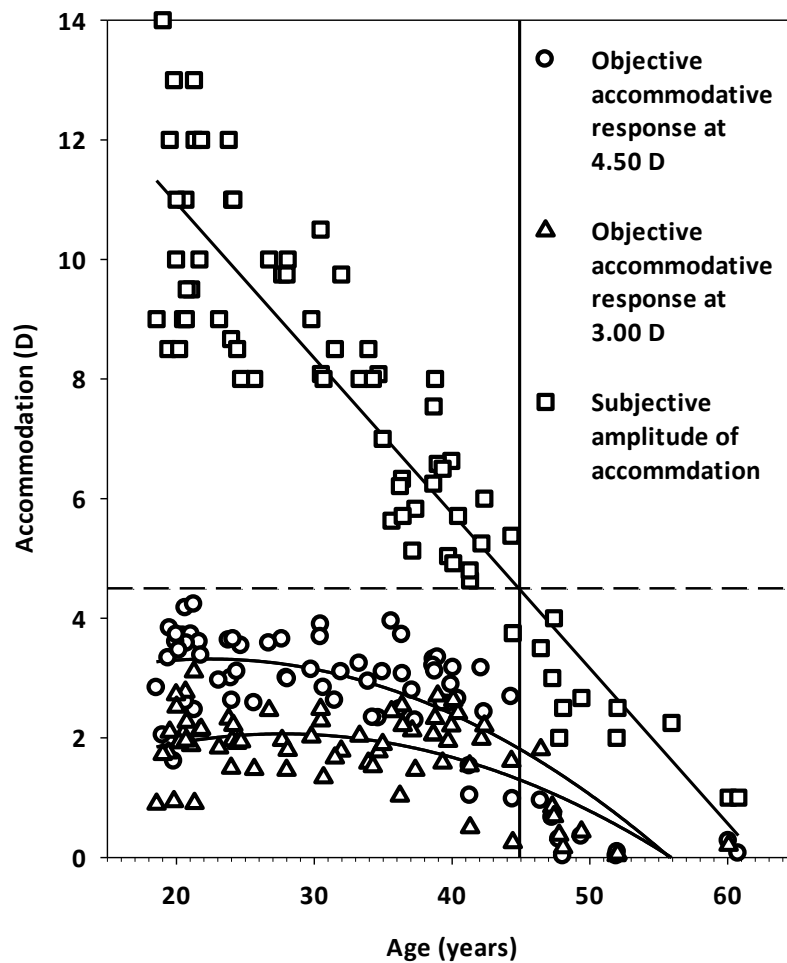


Fig. 6.1. Right eye objective accommodative response to the 3.00 D target (triangles; $y=-0.0026x^2+0.1441x+0.0691$, $R^2=0.539$), 4.50 D target (circles; $y=-0.003x^2+0.134x+1.8137$, $R^2=0.679$) and right eye subjective amplitude of accommodation (squares; $y=-0.257x+15.99$, $R^2=0.829$) according to age. The dashed horizontal line represents 4.50 D of accommodation. The solid vertical line represents the intercept of the subjective accommodation regression line and the dashed 4.50 D of accommodation line ($y=44.9$ years). The objective accommodative response reaches 0.00 D at 56.00 years of age.

6.1.2b Ocular biometry

Disaccommodated VCD was not significantly correlated with age in either group (myopes $r=0.093$, $p=0.584$; emmetropes $r=0.277$, $p=0.108$; Fig.6.2). However, disaccommodated ACD was significantly smaller in the older participants (myopes $r=0.516$, $p=0.001$; emmetropes $r=0.347$, $p=0.041$) and LT (myopes $r=0.707$, $p<0.001$; emmetropes $r=0.602$, $p<0.001$) and ASL (myopes $r=0.417$, $p=0.010$; emmetropes $r=0.375$, $p=0.027$) were significantly larger in the older participants (Fig. 6.2). Older emmetropic individuals had significant longer AXL values than younger emmetropic individuals (myopes $r=0.045$, $p=0.790$; emmetropes $r=0.437$, $p=0.009$; Fig. 6.2).

Repeated measures ANOVA testing showed the decrease in ACD ($F=95.994$, $p<0.001$) and the increase in LT ($F=142.410$, $p<0.001$), ASL ($F=8.239$, $p=0.001$), VCD ($F=5.955$, $p=0.006$) and AXL ($F=11.104$, $p<0.001$) was statistically significant during accommodation. No differences in the accommodative response emerged based on ametropia grouping (ACD $F=0.507$, $p=0.562$; LT $F=3.482$, $p=0.053$; ASL $F=1.342$, $p=0.263$; VCD $F=1.330$, $p=0.266$; AXL $F=0.277$, $p=0.730$). As expected, VCD ($F=68.321$, $p<0.001$) and AXL ($F=63.741$, $p<0.001$) values were consistently longer and ACD ($F=7.653$, $p=0.007$) was consistently deeper in myopic eyes when compared to emmetropic eyes (Fontana and Brubaker, 1980; McBrien and Millodot, 1987; Grosvenor and Scott, 1991; Bullimore *et al.*, 1992; Lam *et al.*, 1999; Logan *et al.*, 2005; Chen *et al.*, 2009). No significant differences emerged between myopic and emmetropic LT ($F=0.285$, $p=0.595$) and ASL ($F=2.340$, $p=0.131$) values.

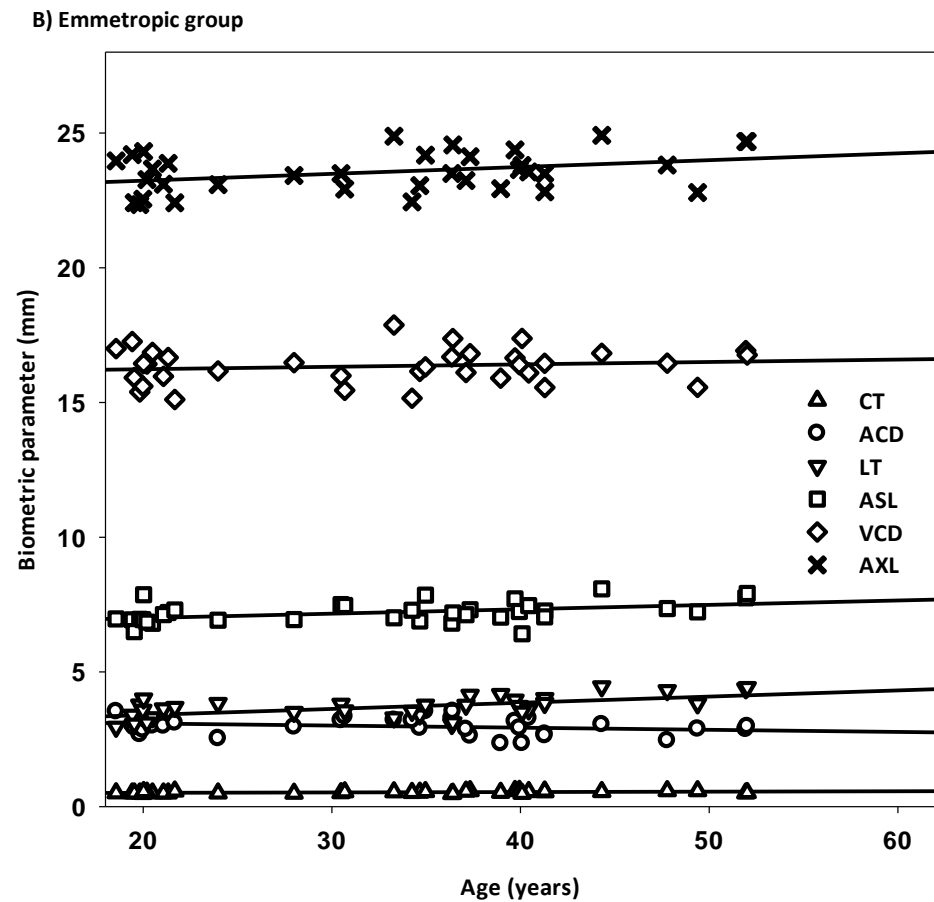
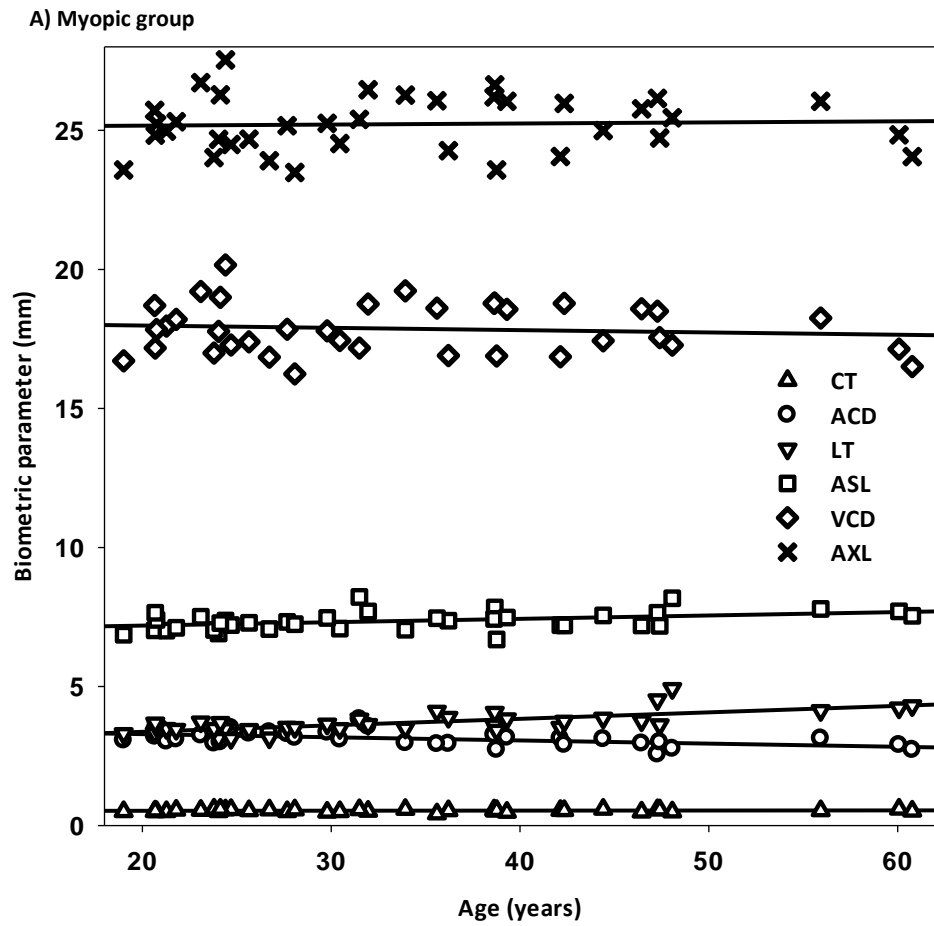


Fig. 6.2. Disaccommodated CT (upward triangles), ACD (circles), LT (downward triangles), ASL (squares), VCD (diamonds) and AXL (crosses) in myopic (A) and emmetropic (B) individuals according to age.

As discussed previously, Gibson (2008) reported the posterior movement of the crystalline lens is negligible until the accommodative demand reaches approximately 2.00 D (eliciting an accommodative response of approximately 1.50 D). Therefore, only participants who demonstrated an accommodative response >1.50 D to the 4.50 D accommodative target were included in calculations to determine the changes in ocular biometry per dioptre of accommodation exerted. The top age limit included in the biometric response per dioptre of accommodation measurements was consequently readjusted to 44 years, however younger participants who failed to accommodate >1.50 D (Fig. 6.1) were excluded. Despite a significant attenuation of the accommodative change in ACD, LT (Fig. 6.3) and AXL (Fig. 6.4) with age according to the magnitude of the accommodative stimulus, the changes per dioptre of accommodation exerted were not dependent on age (Table 6.1). The decrease in ACD per dioptre of accommodation exerted was 0.081 ± 0.053 mm/D, LT increased by 0.109 ± 0.052 mm/D, ASL increased by 0.028 ± 0.057 mm/D, VCD decreased by 0.025 ± 0.059 mm/D and AXL increased by 0.003 ± 0.006 mm/D.

Parameter (mm)	0.00 to 3.00 D accommodative stimulus		0.00 to 4.50 D accommodative stimulus		Per dioptre of accommodation exerted*	
	<i>r</i>	<i>p</i>	<i>r</i>	<i>p</i>	<i>r</i>	<i>p</i>
ACD	0.271	0.021	0.404	<0.001	0.028	0.833
LT	0.412	<0.001	0.509	<0.001	0.051	0.700
ASL	0.105	0.378	0.184	0.121	0.021	0.875
VCD	0.087	0.467	0.154	0.196	0.030	0.821
AXL	0.129	0.279	0.264	0.025	0.105	0.431

Table 6.1. Dependency of accommodative changes in biometry on participant age. VCD and AXL values have been corrected for the increase in LT with age and accommodation. Statistical significance is denoted by a bold *p* value. * denotes results from participants able to accommodate >1.50 D are included only.

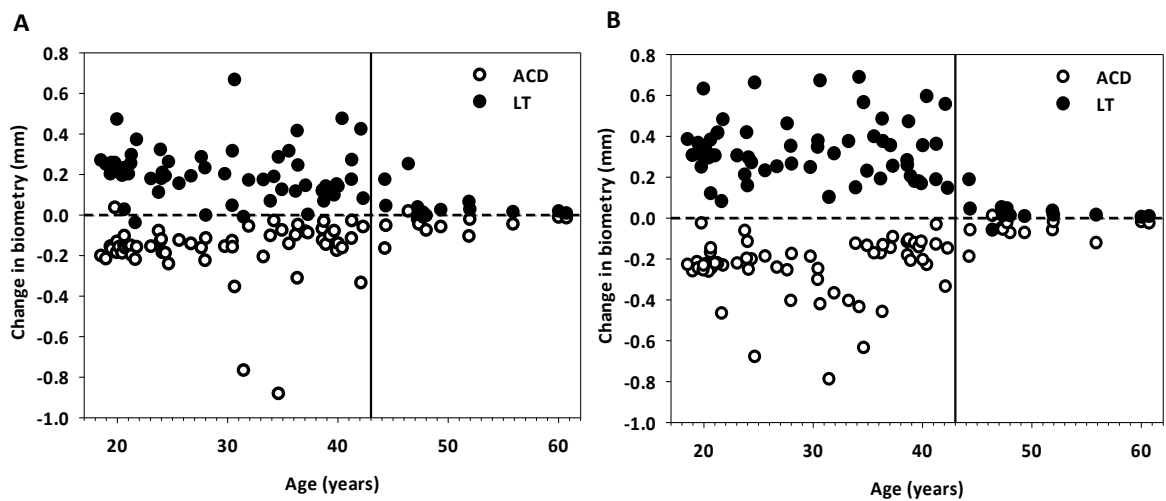


Fig. 6.3. Changes in ACD (open circles) and LT (filled circles) stimulated by the 3.00 D (A) and 4.50 D (B) accommodative stimuli, plotted as a function of age. The dashed horizontal line represents no change in biometry (0.0 mm). The solid vertical line divides the group at age 43 years, highlighting the reduction in both the change in biometry and variance amongst the older participants.

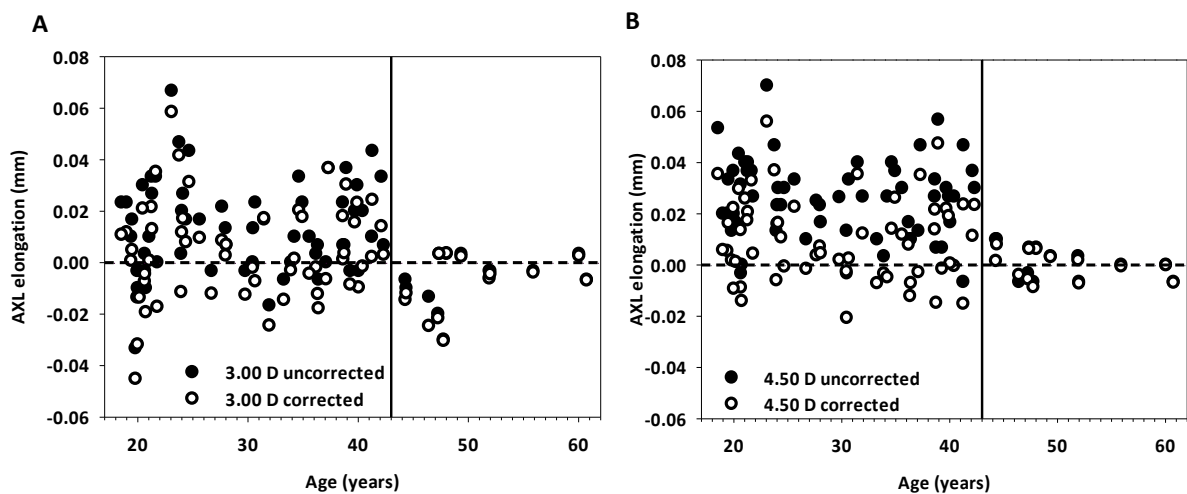


Fig. 6.4. Corrected AXL changes (open circles) at 3.00 D (A) and 4.50 D (B) accommodative stimulus levels. Uncorrected raw AXL (filled circles) values are also presented to demonstrate the difference in magnitude between the corrected AXL values reported in this study and the uncorrected raw AXL values. The dashed horizontal line represents no change in AXL (0.00 mm). The solid vertical line divides the group at age 43 years, highlighting the reduction in variance amongst the older participants.

After visual inspection of the graphical data in Fig. 6.4, the cohort was split into <43 years and \geq 43 years, owing to the dramatic reduction in data spread after age 43 years. The difference in variance of the corrected AXL data between the groups was significant at the 3.00 D ($F=2.983$,

$p < 0.020$) and 4.50 D ($F = 8.852$, $p < 0.002$) accommodative levels. To validate the choice of 43 years as the fulcrum point, the spread of data was analysed in one year increments either side of age 43 years at 3.00 D (41 years $F = 1.848$, $p > 0.100$; 42 years $F = 2.204$, $p < 0.100$; 44 years $F = 2.983$, $p < 0.020$; 45 years $F = 2.454$, $p < 0.050$) and 4.50 D (41 years $F = 2.736$, $p < 0.02$; 42 years $F = 5.055$, $p < 0.002$; 44 years $F = 8.852$, $p < 0.002$; 45 years $F = 8.312$, $p < 0.002$). Between the ages of 43 to 44 years was therefore selected as the fulcrum point.

6.1.3 Discussion

The present study is the first to document the effect of age on AXL elongation during accommodation cross-sectionally. Accommodative changes in ACD, LT and AXL were significantly attenuated with age when considered with respect to the magnitude of the accommodative demand. However, the change in ocular biometry per dioptre of accommodation exerted remained invariant between the ages of 18 to 44 years, as reported previously (Koretz *et al.*, 1997; section 5.3.3).

The magnitude of the accommodative response per dioptre of accommodation exerted is similar to the results previously reported in Table 5.2 within an incipient presbyopic population. The current study also found significant intersubject variation in the change in ASL with accommodation, however, in contrast, the accommodative increase in ASL and decrease in VCD reported by the current study were statistically significant. Further analysis of the data show a non-statistically significant, negative association between the change in ACD ($r = 0.128$ $p = 0.285$), LT ($r = 0.067$ $p = 0.577$) and ASL ($r = 0.020$ $p = 0.093$) with the change in AXL, therefore it is unlikely the posterior crystalline lens movement observed by the present investigation is involved in the mechanism of transient AXL elongation during accommodation.

In accordance with previous studies investigating AXL changes during accommodation (Hollins, 1974; Drexler *et al.*, 1998; Mallen *et al.*, 2006; Read *et al.*, 2010a; Woodman *et al.*, 2011; Woodman *et al.*, 2012; section 5.3.3), a statistically significant AXL elongation with

accommodation was observed in the current investigation, which was proportional to the magnitude of the accommodative stimulus. Section 5.3.3 proposed the anterior contractile force of the ciliary muscle during accommodation is primarily responsible for inducing AXL elongation during accommodation. Indeed, the antero-posterior mobility of the ciliary muscle has not been reported to attenuate with age (Sheppard and Davies, 2011; section 4.3.3), despite a reduction in accommodative refractive response. Therefore, it would be logical to assume AXL elongation during near vision persists with age. However, beyond the age of 43 to 44 years, the AXL change was negligible and the variance of data reduced significantly (Fig. 6.4), despite the preservation of the objective accommodative response until age 56 years (albeit reduced in magnitude compared to the younger cohort; Fig. 6.1). It is feasible that the reduced variance in data and reduced magnitude of change in AXL with accommodation may signify *in vivo* posterior ocular rigidity increases significantly during the presbyopic years. Indeed, previous research has also relied on changes in AXL to determine changes in posterior ocular rigidity (Ebner *et al.*, 2009). Ebner *et al.* (2009) reported ocular rigidity is higher in patients with glaucoma due to a smaller decrease in AXL following a pharmacologically-induced (500mg oral acetazolamide) fall in intraocular pressure when compared to a healthy control group. However, changes in AXL alone are not able to disentangle the relative contributions of each ocular layer to changes in rigidity and thickness, only to infer their cumulative effect. Currently, *in vitro* studies give the best indication of how the properties of the individual ocular layers may vary with age.

In support of the data presented here, an age-related decrease in the elasticity of choroidal and scleral sections has been found by multiple *in vitro* studies (Graebel and van Alphen, 1977; Friberg and Lace, 1988; Ugarte *et al.*, 2006). However, studies of *in vitro* isolated choroidal and scleral sections do not account for the impact of vascular rigidity, perfusion pressure, intraocular pressure, extra-ocular muscles action, post-mortem structural changes, and how these factors may vary with age (Friberg and Lace, 1988). Indeed, the choroid (Manjunath *et al.*, 2010) and its blood vessels (Jablonski *et al.*, 2007) thin and the blood flow reduces with age (Lam *et al.*, 2003),

whereas the sclera becomes thicker with age (Watson and Young, 2004). Unfortunately, it is not currently possible to measure posterior ocular rigidity *in vivo* directly and hitherto, the literature has not reached a consensus as to whether anterior eye rigidity increases with age (Wong and Yap, 1991; Pallikaris *et al.*, 2005).

Indeed, it is feasible the hypothesised increase in ocular shell rigidity with age is accompanied by a reduction in the capacity of the choroid to thin during accommodation, which has been suggested to contribute to the magnitude of AXL elongation in young adults (Woodman *et al.*, 2012). The cumulative effect of these age-related changes may be responsible for the abrupt loss of AXL elongation during accommodation after 43 to 44 years of age. It is possible to extract choroidal thickness measurements from further analysis of the LenStar A-scan peaks, which allows choroidal thickness to be simultaneously captured with CT, ACD, LT and AXL measurements (Woodman *et al.*, 2012). However, Woodman *et al.* (2012) reported resolution was too poor to calculate choroidal thickness in 37% of their cohort and the LenStar may be unable to acquire choroidal thickness measurements in older subjects due to reduced ocular media transparency (Read *et al.*, 2011). Therefore perhaps future studies should utilise optical coherence tomography to investigate accommodative choroidal changes (Margolis and Spaide, 2009), with the additional advantage of capturing data across a larger area of the choroid to profile the spatial change in choroidal thickness with accommodation.

Similarly to the findings of Read *et al.* (2010b) and section 5.3.3, the magnitude of the change in biometry in this study was not dependent on ametropia classification. However, Woodman *et al.* (2012) found that the AXL elongation of young myopes was significantly slower to return to baseline after cessation of a 30 minute 4.00 D near task; this could suggest that the magnitude of AXL elongation during accommodation may be less significant in the mechanism of myopic progression than the time taken for the posterior shell to recoil after cessation of near vision. The delayed recoil may be as a result of the greater vitreous chamber volume in myopic eyes providing a greater resistance to immediate reformation. Alternatively, the choroid in myopic

eyes is known to stretch and consequently thin with AXL growth (Ikuno and Tano, 2009), therefore conceivably its elasticity may also be compromised, which may mute the speed of the recoil response. Further research including longer sustained periods of accommodation and longitudinal follow-up are required to clarify the role of choroidal thickness changes and posterior shell recoil in the development of myopia.

Whilst the mean AXL value increased during accommodation, a minority of participants demonstrated a reduction in AXL (Fig. 6.4), as reported in section 5.3.3. Indeed, once correcting Mallen *et al.*'s (2006) IOLMaster (Carl Zeiss Meditec, Dublin, California) data at 4.00 D by our age-matched mean error value (E) at 4.50 D (0.0152 mm), the standard deviation values reveal a reduction in AXL was evident in a proportion of their cohort (AXL change 0.022 ± 0.026 mm in myopes; 0.011 ± 0.021 mm in emmetropes). However, the error calculations provide only an estimate of the induced error because the average refractive index value the LenStar employs is proprietary information. Therefore, it is possible the calculated error may be overestimating the true error in some cases, superficially depressing the degree of AXL elongation with accommodation. Nevertheless, Fig. 6.4 shows even the raw, uncorrected AXL data show a reduction in AXL in some participants. As discussed in section 5.3.3, an asymmetric elongation of the posterior pole, which is not centred on the fovea may result in an apparent reduction of AXL during accommodation (Fig. 5.11) due to the asymmetric ciliary muscle contractile response (greater temporally than nasally; Sheppard and Davies, 2010a; 2011) and/ or disaccommodated posterior pole asymmetry (Logan *et al.*, 2004; Gilmartin *et al.*, 2013). Indeed, there is evidence of spatial distortions in vision accompanying marked accommodation (Blank and Enoch, 1973; Blank *et al.*, 1975; Enoch, 1975) consistent with asymmetric retinal stretch during accommodation. Further research to determine how the retinal contour changes during accommodation and where the point of maximum ocular elongation resides is indicated.

The current study is the first to investigate how accommodative changes in AXL attenuate with age. In conclusion, the change in ocular biometry per dioptre of accommodation exerted

remained invariant between the ages of 18 to 44 years, however after 43 to 44 years of age, the AXL change was negligible and the variance of data reduced significantly. The findings support the Helmholtz theory of accommodation (von Helmholtz, 1855) and are consistent with a significant increase in ocular rigidity and attenuation of accommodative choroidal thinning during presbyopia. Longitudinal research is required to investigate whether AXL and choroidal changes may precipitate a myopic shift in refraction over the lifespan and whether the choroidal accommodative response is attenuated with age.

CHAPTER 7

Conclusions and indicated future research

7.1 Introduction

Incipient presbyopia represents a unique period of adulthood, where significant changes in the accommodative apparatus result in diminished accommodative ability, eventually falling below the critical level required for comfortable, sustained near vision at a natural working distance and leading to manifest presbyopia. Despite at least a century of research, the exact mechanism of accommodation and the pathogenesis of presbyopia remain equivocal (Gilmartin, 1995; Atchison, 1995; Charman, 2008). Therefore, the primary aim of this thesis was to investigate the *in vivo* ocular morphological and contractile changes occurring within the accommodative apparatus prior to the onset of presbyopia in participants aged 33 to 45 years, with particular reference to ciliary muscle changes with age and the origin of a myopic shift in refraction during incipient presbyopia.

7.2 Refractive progression

Data from previous studies have indicated a hypermetropic shift in ocular refraction would be expected within incipient presbyopia (Saunders, 1986; Lee *et al.*, 2002), however, the prospective longitudinal study described in Chapter 3 (n=51) revealed the average change in refraction within an incipient presbyopic cohort over 2.5 years, although hypermetropic ($+0.10 \pm 0.38$ D), was not statistically significant. Indeed, a small proportion of participants experienced a significant hypermetropic (10% of myopic and 10% of emmetropic individuals) or myopic (5% of myopic and 3% of emmetropic individuals) refractive shift during the course of the study (>0.50 D). However, the incidence of a myopic shift in refraction does not appear to be as large as previously indicated by retrospective research (Grosvenor and Skeates, 1999; and Pointer and Gilmartin, 2011) and emmetropic and myopic individuals appear to be at equal risk.

Changes in refractive error were independent of the measured changes in ciliary muscle morphology (Chapter 4) and ocular biometry (Chapter 5), therefore indicating that the origin of the myopic shift in refraction observed during the course of the study was likely to have been lenticular. Further research is required to identify whether changes in the curvature or refractive index of the crystalline lens may have instigated the observed hypermetropic and myopic shifts in refraction during incipient presbyopia.

7.3 Ciliary muscle morphology and contractility

A bespoke semi-automated algorithm was utilised for Visante AS-OCT ciliary muscle data extraction in Chapter 4, which was validated and found to be accurate and repeatable in section 2.3.4.

During incipient presbyopia, longer anterior length and total ciliary muscle length measurements were observed temporally in myopic eyes, whereas no differences in ciliary muscle thickness arose between myopic and emmetropic individuals. Therefore, it is likely axial eye length growth is accompanied by hypertrophy of the ciliary muscle in order to maintain a constant thickness despite antero-posterior stretching of the ciliary muscle.

Over the course of the 2.5 year study detailed in section 4.2 (n=51), a significant antero-inward shift in temporal ciliary muscle mass was observed, with no differences arising between myopic and emmetropic individuals. It is likely that the age-related structural changes observed are due to an increase in the quantity of apical radial and circular ciliary muscle fibres and a reduction in the proportion of the more posterior longitudinal fibres (Nishida and Mizutani, 1992; Tamm *et al.*, 1992a; Pardue and Sivak, 2000). Furthermore, it is feasible that additional connective tissue accumulating in the anterior portion of the ciliary muscle, where the radial and circular fibres reside, also increased the anterior thickness of the ciliary muscle with age (Nishida and Mizutani, 1992; Tamm *et al.*, 1992a; Pardue and Sivak, 2000). Further longitudinal studies including a larger

age range over an extended period are required to investigate the temporal and spatial characteristics of the changes in ciliary muscle morphology.

In the subgroup study described in section 4.3 (n=20), a significant antero-inwards contractile response was observed during accommodation. The centripetal contractile ciliary muscle response during accommodation was significantly attenuated after 2.5 years, whereas the antero-posterior mobility of the ciliary muscle was unaffected, continuing to move the ciliary muscle anteriorly during accommodation. It is feasible the reduction in centripetal ciliary muscle contraction may be due to the age-related increase of connective tissue surrounding the apical ciliary muscle fibres. However, evidence of greater ciliary muscle contraction to a stimulus beyond the near point indicates the attenuation of centripetal ciliary muscle contraction is unlikely to be primarily responsible for the development of presbyopia and therefore implicates a lenticular model for presbyopia development. Future studies should investigate the attenuation of the contractile response of the full circumference of the ciliary muscle longitudinally in order to determine whether any asymmetry exists in the attenuation of the contractile response with age.

7.4 Ocular biometry and accommodation

Ocular biometry was measured by the LenStar LS-900, which allows simultaneous measurement of corneal thickness, anterior chamber depth, crystalline lens thickness and axial length. With age, a significant increase in crystalline lens thickness and a corresponding decrease in anterior chamber depth was observed (Chapters 5 and 6). Longitudinal research indicated that axial length may decrease with age during incipient presbyopia, possibly due to age-related changes in the vitreous. In section 5.2 (n=51), a trend emerged for a larger decrease in anterior ciliary muscle length to accompany a larger increase in crystalline lens thickness with age, thus suggesting a synergy between antero-posterior crystalline lens and ciliary muscle position. Further longitudinal research is required to investigate the progression of structural ocular changes over the lifespan. The use of high resolution magnetic resonance imaging (MRI) would enable the visualisation of

the entire crystalline lens and the ciliary muscle simultaneously, allowing the interaction between changes in ocular biometry and ciliary muscle morphology to be explored further.

Considering the ocular accommodative response, the anterior chamber depth decreased and crystalline lens thickness and axial length significantly increased (Chapters 5 and 6). The change in anterior segment length with accommodation was not statistically significant during incipient presbyopia, however large intersubject variation was observed (n=20). After 2.5 years, the attenuation of the change in ocular biometry per dioptre of accommodation exerted was not statistically significant. Nevertheless, a trend emerged for a reduction in the accommodative anterior movement of the crystalline lens with age, possibly due to restriction from the posterior iris or age-related changes in the pattern of crystalline lens expansion. The anterior movement of the crystalline lens is known to reduce the magnitude of the accommodative response (Davies *et al.*, 2010), therefore a reduction in accommodative crystalline lens mobility may potentially preserve the accommodative response during incipient presbyopia. Further longitudinal research investigating a wider range of ages is indicated to determine how the movement of the crystalline lens per dioptre of accommodation exerted may vary with advancing age. Utilising Scheimpflug photography would allow the accommodative movement of the sulcus (centre of the crystalline lens nucleus) to be monitored with age. Furthermore, high-resolution ultrasonography would permit visualisation of the zonular architecture, which may impact accommodative antero-posterior crystalline lens mobility.

Cross-sectional research in Chapter 6 (n=72) also reported the change in ocular biometry per dioptre of accommodation exerted remained invariant between the ages of 18 to 44 years. However, after 43 to 44 years of age, the axial length change was negligible and the variance of data reduced significantly. These findings are consistent with a significant increase in ocular rigidity and attenuation of accommodative choroidal thinning during presbyopia. However, the accommodative reduction in AXL observed in a minority of patients aged 18 to 60 years may indicate that the maximum axial length elongation is not centred on the fovea in all individuals,

possibly as a consequence of asymmetric ciliary muscle contractile response (greater temporally than nasally; Sheppard and Davies, 2010a; 2011) and/ or disaccommodated posterior pole asymmetry (Logan *et al.*, 2004; Gilmartin *et al.*, 2013). Further research to determine how the retinal contour changes during accommodation and where the point of maximum ocular elongation resides is indicated.

7.5 Overall conclusion

The studies presented in this thesis are the first to investigate longitudinal optical and structural ocular changes occurring during the incipient phase of presbyopia. The findings support the Helmholtz theory of accommodation and, despite the attenuation of the accommodative centripetal ciliary muscle contractile response with age, primarily implicate lenticular changes in the development of presbyopia. Amidst great interest in techniques to preserve the accommodative response with age (Charman, 2005; Glasser, 2008), this thesis provides invaluable insight into ocular changes occurring during the development of presbyopia, which will help to inform future research and aid the development of techniques to prevent and reverse presbyopia development.

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APPENDICES

A1: Aston University Ethical Committee approval of study AO2012.04



Response from AOREC

28th February 2012

Our Ref: AO2012.04

Project title: Optical and structural adaptation during incipient presbyopia: The Aston Longitudinal Assessment of Presbyopia (ALAP) study

Application Number: AO2012.04

Researchers: Dr Leon Davies, Dr Amy Sheppard, Miss Deborah Laughton
Ophthalmic Research Group

Aston University, B4 7ET

Dear Dr Davies,

On behalf of the Audiology/ Optometry Research Ethics Committee (AOREC), I am pleased to inform you that the AOREC are happy to give approval to the above study and the following documents:

- Laughton_Ethics_Application_Form(v2).doc
- Ciliary structural consent form and guidance notes.doc
- Peripheral consent form and guidance notes.doc
- questionnaire.doc
- Method.docx
- Structural consent form and guidance notes.doc

The details of the investigation will be placed on file. You should notify the Committee of any difficulties experienced by the volunteer subjects, and any significant changes which may be planned for this project in the future.

Yours sincerely,

A handwritten signature in blue ink, appearing to read "M. Dunne".

Dr Mark C. M. Dunne
Vice Chair, AOREC

A2: Participants information sheet and consent form for study AO2012.04 (Main study)

February 2012

Participant Information Sheet

Research workers, school and subject area responsible

Dr Leon N. Davies, Optometry, School of Life & Health Sciences, Aston University
Dr Amy Sheppard, Optometry, School of Life & Health Sciences, Aston University
Miss Deborah Laughton, Optometry, School of Life & Health Sciences, Aston University

Project Title

Structural adaptation during incipient presbyopia: The Aston Longitudinal Assessment of Presbyopia (ALAP) study

Invitation

You are being invited to take part in a research study. Before you decide whether you wish to participate, please take the time to read this information sheet about why the research is being done and what it will involve.

What is the purpose of the study?

To measure how the structure of the eye changes over time in the years leading up to the first prescription of reading spectacles.

Where will the study take place?

Ophthalmic Research Group laboratories, Vision Sciences, Aston University, Birmingham, B4 7ET

What will happen to me if I take part?

By volunteering to participate in this study, you will be invited to attend the Ophthalmic Research Group's laboratories every 6 months over a 2.5 year period, for about half an hour, to sit for a series of non-contact, non-invasive tests.

A short questionnaire will be emailed to you first to check your suitability for the research and to identify your typical work environment. Data collection will start with you being asked to read a distance and near letter chart to measure your vision (wearing contact lenses or spectacles if you require either). Next the ability for your eye to focus at near will be tested using a ruler and a moveable target. At this point, you will be required to remove your contact lenses/take off your spectacles if worn. A measurement of your prescription will be taken next using the *Grand Seiko* autorefractor. Following this, the *Zeiss Visante AS-OCT* will be used to produce an image of your crystalline lens and ciliary muscle. The final instrument (*Haag-Streit Lenstar*) will measure your corneal curvature, central corneal thickness, anterior chamber depth (distance between your cornea and crystalline lens), crystalline lens thickness and axial length (distance between your cornea and retina). These measurements will be taken from one eye only and all instruments use lights to take measurements.

Are there any potential risks in talking part in the study?

There are no known risks involved with the instruments or techniques listed above. All measurements will be taken in accordance with the manufacturers' guidelines by a GOC (General Optical Council- regulatory body in UK) registered Optometrist.

Do I have to take part?

No, you do not have to participate if you do not wish to do so. You are free to withdraw at any time from the project. Your decision to participate (or not) will not influence your ability to participate in any future research.

Expenses and payments

A payment of £60 will be given to you as a thank you for your time.

Will my taking part in this study be kept confidential?

Privacy and confidentiality will be protected vigorously to the extent permissible by law. Your name will be turned into a code, the details of which will be kept on a separate database which will only be accessed by the investigators. Analysis of data by others, including the internal project examiner, will only be undertaken in the coded format to prevent a breach of confidentiality. We cannot, however, guarantee privacy or confidentiality.

What will happen to the results of the research study?

We aim to publish the results of this project. However, there will be no reference to any individual's performance in any publication. After the conclusion of this study, you will be emailed with a chance to request your own data. A copy of the entire thesis (that this study will contribute to) will be available from the British Library.

Who is organising and funding the research?

The project is being conducted by a research team at Aston University. The study is funded by a College of Optometrists Research Scholarship.

Who has reviewed the study?

The research has been submitted and granted approval by the Audiology and Optometry Research Ethics Committee, Aston University.

Who do I contact if something goes wrong or I need further information

Please contact the principal investigator, Dr Leon N. Davies l.n.davies@aston.ac.uk

Who do I contact if I wish to make a complaint about the way in which the research is conducted?

If you have any concerns about the way in which the study has been conducted, then you should contact Secretary of the University Research Ethics Committee on j.g.walter@aston.ac.uk or telephone 0121 204 4665.

Personal Identification Number for this study:

CONSENT FORM

Title of Project

Structural changes during incipient presbyopia

Research Venue

Ophthalmic Research Group laboratories, Vision Sciences, Aston University, Birmingham, B4 7ET

Name of Investigators

Dr Leon N. Davies

Dr Amy Sheppard

Miss Deborah Laughton

Please tick box

- 1. I confirm that I have read and understand the information sheet dated
for this study and have had the opportunity to ask questions.

- 2. I understand that my participation is voluntary and that I am free to withdraw at any time,
without giving any reason, without my legal rights being affected.

- 3. I agree to take part in the above study.

Name of Research Participant Date Signature

Name of Person taking Consent Date Signature

1 copy for research participant
1 copy for investigator

A3: Questionnaire

Date: / /	Researcher:
-----------------	-------------

**ASTON LONGITUDINAL ASSESSMENT OF PRESBYOPIA (ALAP) STUDY
QUESTIONNAIRE**

All questions contained in this questionnaire are confidential.

Participant name:	<input type="checkbox"/> M <input type="checkbox"/> F	DOB:
Ethnicity:	Date of last sight test:	
Do you ever wear reading spectacles (either prescribed or 'ready readers')	Yes <input type="checkbox"/>	No <input type="checkbox"/>

GENERAL HEALTH

Have you been diagnosed with:	Diabetes (any type)	Yes <input type="checkbox"/>	No <input type="checkbox"/>
	Any eye condition	Yes <input type="checkbox"/>	No <input type="checkbox"/>
Are you being treated for:	Anxiety	Yes <input type="checkbox"/>	No <input type="checkbox"/>
	Depression	Yes <input type="checkbox"/>	No <input type="checkbox"/>

List your prescribed drugs:

Name of drug	Strength	Frequency Taken

List any previous hospital eye service treatment:

Year	Reason	Discharged

Have you had refractive surgery or anterior chamber lens implantation?	Yes <input type="checkbox"/>	No <input type="checkbox"/>
Do you wear rigid gas permeable contact lenses?	Yes <input type="checkbox"/>	No <input type="checkbox"/>
Do you wear soft contact lenses? If yes, what modality? (Daily/monthly/extended wear)	Yes <input type="checkbox"/>	No <input type="checkbox"/>

OCCUPATION

What is your occupation? (Full-time/Part-time)				
Is most of your working day spent outside?	Yes <input type="checkbox"/>	No <input type="checkbox"/>		
How many hours per day do you spend working at near/using a computer?	0 <input type="checkbox"/>	1-3 <input type="checkbox"/>	4-7 <input type="checkbox"/>	8 + <input type="checkbox"/>

All information still true?	6 month review (/ /2012)	12 month review (/ /2012)	18 month review (/ /2013)	24 month review (/ /2013)	30 month review (/ /2014)
	Yes <input type="checkbox"/> No <input type="checkbox"/>	Yes <input type="checkbox"/> No <input type="checkbox"/>	Yes <input type="checkbox"/> No <input type="checkbox"/>	Yes <input type="checkbox"/> No <input type="checkbox"/>	Yes <input type="checkbox"/> No <input type="checkbox"/>

A4: Participants information sheet and consent form for study AO2012.04 (Subgroup study)

February 2012

Participant Information Sheet

Research workers, school and subject area responsible

Dr Leon N. Davies, Optometry, School of Life & Health Sciences, Aston University
Dr Amy Sheppard, Optometry, School of Life & Health Sciences, Aston University
Miss Deborah Laughton, Optometry, School of Life & Health Sciences, Aston University

Project Title

Investigation into crystalline lens, ciliary muscle, axial length, peripheral refraction and retinal contour changes during accommodation in incipient presbyopes: The Aston Longitudinal Assessment of Presbyopia (ALAP) study

Invitation

You are being invited to take part in a research study. Before you decide whether you wish to participate, please take the time to read this information sheet about why the research is being done and what it will involve.

What is the purpose of the study?

To measure how the structures within the eye change in the years leading up to the first prescription of reading spectacles.

Where will the study take place?

Ophthalmic Research Group laboratories, Vision Sciences, Aston University, Birmingham, B4 7ET

What will happen to me if I take part?

By volunteering to participate in this study, you will be invited to attend the Ophthalmic Research Group's laboratories every 6 months over a 2.5 year period, for about 30 minutes, to sit for a series of non-contact, non-invasive tests.

First, the curvature of your cornea will be taken using a *Medmont* topographer. If you are short-sighted, you will be fitted with a soft daily disposable contact lens (by an Optometrist) on your right eye only. After the lens has settled, the *Grand Seiko* autorefractor will be used to measure how the prescription of your eye changes as you focus on near targets at different distances away from you and in different locations across your horizontal visual field. The way in which your crystalline lens and ciliary muscle change shape whilst focusing at near will be measured next using the *Zeiss Visante AS-OCT*. The length of your eye will also be measured using the *Haag-Streit Lenstar* as you focus on near targets at different distances in front of you. Finally, the curvature of your retina will be measured while you look at targets in different locations across your horizontal visual field using the *Zeiss IOLMaster*. The contact lens will be removed before you leave.

Are there any potential risks in talking part in the study?

There are no known risks involved with the instruments or techniques listed above. All measurements will be taken in accordance with the manufacturers' guidelines by a GOC (General Optical Council- regulatory body in UK) registered Optometrist. The same Optometrist will also fit the contact lens after checking the front surface of the eye is healthy and suitable.

Do I have to take part?

No, you do not have to participate if you do not wish to do so. You are free to withdraw at any time from the project. Your decision to participate (or not) will not influence your ability to participate in any future research.

Expenses and payments

A payment of £10 will be made after each session.

Will my taking part in this study be kept confidential?

Privacy and confidentiality will be protected vigorously to the extent permissible by law. Your name will be turned into a code, the details of which will be kept on a separate database which will only be accessed by the investigators. Analysis of data by others, including the internal project examiner, will only be undertaken in the coded format to prevent a breach of confidentiality. We cannot, however, guarantee privacy or confidentiality.

What will happen to the results of the research study?

We aim to publish the results of this project. However, there will be no reference to any individual's performance in any publication. After the conclusion of this study, you will be emailed with a chance to request your own data. A copy of the entire thesis (that this study will contribute to) will be available from the British Library.

Who is organising and funding the research?

The project is being conducted by a research team at Aston University. The study is funded by a College of Optometrists Research Scholarship.

Who has reviewed the study?

The research has been submitted and granted approval by the Audiology and Optometry Research Ethics Committee, Aston University.

Who do I contact if something goes wrong or I need further information

Please contact the principal investigator, Dr Leon N. Davies l.n.davies@aston.ac.uk

Who do I contact if I wish to make a complaint about the way in which the research is conducted?

If you have any concerns about the way in which the study has been conducted, then you should contact Secretary of the University Research Ethics Committee on j.g.walter@aston.ac.uk or telephone 0121 204 4665.

Personal Identification Number for this study:

CONSENT FORM

Title of Project

Ciliary muscle and crystalline lens structural adaptation during accommodation in incipient presbyopes

Research Venue

Ophthalmic Research Group laboratories, Vision Sciences, Aston University, Birmingham, B4 7ET

Name of Investigators

Dr Leon N. Davies
Dr Amy Sheppard
Miss Deborah Laughton

Please tick box

- 1. I confirm that I have read and understand the information sheet dated
for this study and have had the opportunity to ask questions.

- 2. I understand that my participation is voluntary and that I am free to withdraw at any time,
without giving any reason, without my legal rights being affected.

- 3. I agree to take part in the above study.

Name of Research Participant Date Signature

Name of Person taking Consent Date Signature

1 copy for research participant
1 copy for investigator

A5 Aston University Ethical approval of amendment to study AO2012.04



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MEMORANDUM

DATE: 13th June 2014

TO: Dr Leon Davies

FROM: Dr. Corinne M Spickett

SUBJECT: AO2012.04: Optical and structural adaptation during incipient presbyopia: The Aston Longitudinal Assessment of Presbyopia (ALAP) study

I am writing to inform you that the minor proposed changes to the above project as described in your email and attachment of 12th June 2014 (namely the additional use of cyclopentolate 1% to relax eye muscles) have been approved.

The Ethic Committee's approval applies only to research conducted in accordance with the amended protocol and documentation approved by the LHS EC; any change to the protocol must be approved by the Committee prior to its implementation.

List of Amended Items approved:

ALAP cyclo information sheet

The details of the investigation will be placed on file. You should notify me of any difficulties experienced by the volunteer subjects, and any significant changes which may be planned for this project in the future.

A handwritten signature in black ink, appearing to read "CM Spickett".

Dr Corinne M. Spickett

Chair of the LHS Research Ethics Committee

A6 Participants information sheet and consent form for study AO2012.04 amendment

June 2014

Participant Information Sheet

Research workers, school and subject area responsible

Dr Leon N. Davies, Optometry, School of Life & Health Sciences, Aston University
Dr Amy Sheppard, Optometry, School of Life & Health Sciences, Aston University
Miss Deborah Laughton, Optometry, School of Life & Health Sciences, Aston University

Project Title

Cycloplegic & non-cycloplegic autorefractometry in incipient presbyopia: The Aston Longitudinal Assessment of Presbyopia (ALAP) study

Invitation

You are being invited to take part in an additional study. Before you decide whether you wish to participate, please take the time to read this information sheet about why the research is being done and what it will involve.

What is the purpose of the study?

The purpose of this study is to measure how the structure and prescription of the eye change over time in the years leading up to the first prescription of reading spectacles. A better understanding of the changes that occur in the ageing eye will help the future development of treatments to prevent the need for reading spectacles.

To ensure the measurement of the spectacle prescription for distance vision is accurate, this study will also compare the prescription in your right eye before and after instillation of a commonly used eye drop (cyclopentolate 1%) to relax the muscle in your eye responsible for focusing at near.

Where will the study take place?

Ophthalmic Research Group laboratories, Vision Sciences, Aston University, Birmingham, B4 7ET

Why have I been chosen?

This study is an extension of the 2.5 year ALAP study you have already agreed to participate in.

What will happen to me if I take part?

By volunteering to participate in this study, you will be invited to attend the Ophthalmic Research Group's laboratories for additional measurements following your 2.5 years ALAP study visit.

Data collection will start with you being asked to read a distance and near letter chart to measure your vision (wearing contact lenses or spectacles if you require either). Next, the ability for your eye to focus at near will be tested using a ruler and a moveable target. At this point, you will be required to remove your contact lenses/take off your spectacles if worn. A measurement of your prescription will be taken next using the *Grand Seiko* autorefractor. Following this, the *Zeiss Visante AS-OCT* will be used to produce an image of your crystalline lens and ciliary muscle. The final

instrument (*Haag-Streit Lenstar*) will measure your central corneal thickness, anterior chamber depth (distance between your cornea and crystalline lens), crystalline lens thickness and axial length (distance between your cornea and retina). These measurements will be taken from your right eye only and will take approximately 15 minutes to complete.

Prior to cyclopentolate 1% eye drop instillation in your right eye, a GOC (General Optical Council-regulatory body in UK) registered optometrist will check the pressure and the depth of your right eye using instrumentation commonly used in daily optometric practice. These tests and eye drop instillation will take approximately 5 minutes to complete. The eye drops take approximately 40 minutes to take action. You will be asked to wait in vision sciences reception while the eye drops are taking action. During this time, your right pupil will become larger and you may notice the vision in your right eye becomes blurred. These effects are transitory and will fade after 12-24 hours. To ensure the eye drop has taken full effect, your ability to focus at near will be tested using a ruler with a movable text target. Once the drops have taken full effect, a measurement of your prescription will be taken using the *Grand Seiko* autorefractor. Before leaving, the pressure inside your eyes will be measured again.

Are there any potential risks in taking part in the study?

The risks associated with taking part in this study are very small. All of the study procedures are routinely undertaken by optometrists and have been shown to have very few side effects and adverse events are extremely rare. As part of the study we will use some eye drops which may cause some minor discomfort and/ or inconvenience. Details of what you should expect and how your activities following the study will be restricted are given below:

When applied to the eye, cyclopentolate 1% eye drops may sting for a few moments. The drops take about 40 minutes to work and around 12 hours to wear off (in some cases up to 24 hours). The resultant large pupils will make you more sensitive to light, whilst distant and near objects may appear slightly blurred. Consequently, you shouldn't perform any activities such as driving and/ or cycling for at least 12 hours after the drops have been instilled. On a bright day, sunglasses may be advisable. It is very unlikely, but should you experience any unusual symptoms such as severe pain and/ or blood shot around the eye and cloudy vision during this period please contact Leon Davies (0121 204 4152) or Deborah Laughton (07760506681) and/ or your optometrist/ GP as you may be experiencing an adverse reaction to the drops.

Do I have to take part?

No, you do not have to participate if you do not wish to do so. You are free to withdraw at any time from the project. Your decision to participate (or not) will not influence your ability to participate in the ALAP study or future research studies.

Expenses and payments

As compensation for your time and any extra travel costs you may incur to avoid driving, in addition to the £10 payment you receive for the ALAP study, an extra payment of £40 will be given to you following completion of the study.

Will my taking part in this study be kept confidential?

Privacy and confidentiality will be protected vigorously to the extent permissible by law. Your name will be turned into a code, the details of which will be kept on a separate database which will only

be accessed by the investigators. Analysis of data by others, including the internal project examiner, will only be undertaken in the coded format to prevent a breach of confidentiality. If you experience a study related health problem, this will be reported to the Research Ethics Committee at Aston University

What will happen to the results of the research study?

We aim to publish the results of this project. However, there will be no reference to any individual's performance in any publication. After the conclusion of this study, you will be emailed with a chance to request your own data. A copy of the entire thesis (that this study will contribute to) will be available from the British Library.

Who is organising and funding the research?

The project is being conducted by a research team at Aston University. The study is funded by a College of Optometrists Research Scholarship.

Who has reviewed the study?

The research has been submitted and granted approval by the Aston University Ethics Committee.

Who do I contact if something goes wrong or I need further information

Please contact the principal investigator, Dr Leon N. Davies l.n.davies@aston.ac.uk

Who do I contact if I wish to make a complaint about the way in which the research is conducted?

If you have any concerns about the way in which the study has been conducted, then you should contact Secretary of the University Research Ethics Committee on j.g.walter@aston.ac.uk or telephone 0121 204 4665.

Personal Identification Number for this study:

CONSENT FORM

Title of Project

Cycloplegic & non-cycloplegic autorefraction in incipient presbyopia: The Aston Longitudinal Assessment of Presbyopia (ALAP) study

Research Venue

Ophthalmic Research Group laboratories, Vision Sciences, Aston University, Birmingham, B4 7ET

Name of Investigators

Dr Leon N. Davies
Dr Amy Sheppard
Miss Deborah Laughton

Please tick box

- 1. I confirm that I have read and understand the information sheet dated
for this study and have had the opportunity to ask questions.

- 2. I understand that my participation is voluntary and that I am free to withdraw at any time,
without giving any reason, without my legal rights being affected.

- 3. I agree to take part in the above study.

Name of Research Participant

Date

Signature

Name of Person taking Consent

Date

Signature

1 copy for research participant
1 copy for investigator