# Effect of atropine on the response of human choroid to retinal image defocus in myopia

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## Abstract

**Purpose:** Both atropine and retinal defocus modify myopia progression in humans. The choroid has been implicated in eye growth and myopia development, particularly in animals. This study investigated the interaction of atropine and imposed retinal defocus on subfoveal choroidal thickness (SFCT) in humans.

**Methods:** Following a preliminary study in 10 young adults, 20 Taiwanese children (aged 6 to 14 years) with myopia (-0.75D to -4.00D) entered the main study. During distance viewing, SFCT was monitored by Optical Coherence Tomography (OCT) in both eyes while 2.00D of hyperopic and myopic retinal defocus (randomly ordered) was imposed in the experimental eye for 60 minutes, with the contralateral Control eye fully corrected for distance. These protocols were repeated before 0.3% atropine treatment and after 1 week, 3 and 6 months of treatment to both eyes.

**Results:** In the preliminary study, hyperopic defocus caused SFCT to thin (maximum at 40-mins) by 10±2µm; p=0.004). Twenty-two hours after instilling 0.5% atropine, baseline SFCT had not changed (p=0.16) and hyperopic defocus failed to thin the choroid (Maximum change in SFCT = +2±2µm, p=0.36). In the main study, before atropine, 60 minutes of myopic and hyperopic defocus induced thicker and thinner choroids respectively (Myopic: 12±2 µm, $p<0.01$, and Hyperopic: 12±2 µm, $p<0.01$), whereas SFCT in Control eyes did not change ($p>0.20$). After 1 week of 0.3% atropine, baseline SFCT (without defocus) in both eyes had increased by ~20µm, $p<0.01$, and 60 minutes of hyperopic defocus failed to cause choroidal thinning ($p>0.05$), although myopic defocus still caused a further increase in SFCT. This additive effect remained after 3 and 6 months of atropine treatment.

**Conclusions:** The normal reduction in SFCT in response to hyperopic retinal defocus is inhibited by atropine, while the normal increase in SFCT with myopic defocus is unaffected. This may imply that choroidal thinning and thickening are mediated by different mechanisms. These changes are superimposed on an increased baseline SFCT caused by atropine. The additive effect of myopic defocus and atropine on choroidal thickening suggests that combining optical and pharmaceutical therapies into dual therapies is likely to provide more effective myopia control than either therapy alone.

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## List of abbreviations

| | |
|---|---|
| AD | Anterior Chamber Depth |
| AL | Axial Length |
| AMD | Age-Related Macular Degeneration |
| ChBF | Choroidal Blood Flow |
| CMO | Cystoid Macular Oedema |
| COMET | Correction of Myopia Evaluation Trial |
| D | Dioptre |
| DF | Dual-Focus |
| EDI | Enhanced Depth Imaging |
| ERG | Electroretinogram |
| GLM | General Linear Model |
| IRMA | Intraretinal Microvascular Abnormalities |
| ICNs | Intrinsic Choroidal Neurons |
| LDF | Laser Doppler Flowmetry |
| LT | Crystalline Lens Thickness |
| MD | Myopic Defocus |
| MRI | Magnetic Resonance Imaging |
| NAVIS-EX | Nidek Advance Vision Information System – Extra |
| NO | Nitric Oxide |
| NOS | Nitric Oxide Synthase |
| OCT | Optical Coherence Tomography |
| Ortho-K | Orthokeratology |
| PAL | Progressive Addition Lenses |
| PFCT | Parafoveal Choroidal Thickness |

| | |
|---|---|
| RGLM | Repeated-Measures General Linear Model |
| RP | Retinitis Pigmentosa |
| RPE | Retinal Pigment Epithelium |
| Rx | Refractive Error / Refraction |
| SD | Standard Deviation |
| SEM | Standard Error of The Mean |
| SER | Spherical Equivalent Refraction |
| SFCT | Subfoveal Choroidal Thickness |
| SD-OCT | Spectral-Domain Optical Coherence Tomography |
| SVL | Single Vision Lenses |
| SS-OCT | Swept-Source Optical Coherence Tomography |
| TD-OCT | Time-Domain Optical Coherence Tomography |
| TEFD-OCT | Time-Encoded Frequency Domain Optical Coherence Tomography |
| TEL | Total Eye Length |
| VA | Visual Acuity |
| VCD | Vitreous Chamber Depth |
| VIP | Vasoactive Intestinal Peptide |
| WDT | Water Drinking Test |

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# 1 Rationale and overview

The overall objective of this research is to provide a better understanding of the way in which atropine eye drops act to inhibit the abnormal eye elongation associated with the progression of myopia in children.

It is well established that the visual environment plays a major role in myopia development and progression. The working model adopted in this research is that certain characteristics of the retinal image initiate signals in the retina which somehow influence the local sclera, causing remodelling or altered scleral growth. This in turn leads to enlargement of the eye and hence the development and progression of myopia in susceptible individuals. The choroid, a vascular bed which lies between the retina and sclera, has been implicated in this signal pathway, particularly in animal studies (Nickla, Wilken et al. 2006, Nickla, Damyanova et al. 2009) in which changes to the choroid appear to precede myopia development. Hyperopic defocus of the retinal image in animal models causes rapid reduction in the thickness of the choroid which is then followed by eye elongation and the development of myopia. Retinal defocus also causes rapid changes to the thickness of the choroid in humans.

Atropine eye drops have been used for decades to slow the progression of myopia in children, particularly in Asia. Recent reviews have concluded that it is the most effective method for slowing progression, at least in the short term (Walline, Lindsley et al. 2011, Huang, Wen et al. 2016). Atropine eye drops act by inhibiting the abnormal elongation of the eye, thus slowing myopia progression e.g. (Chia, Lu et al. 2016). but its site and mode of action remain unclear.

The objective of this study is to investigate the action of atropine on the rapid and predictable changes in thickness of the human choroid that occur in response to hyperopic and myopic retinal image defocus, in individuals (adults and children) with myopia.

The thesis is divided into chapters as follows:

**Chapter 2: Background.** This chapter provides an introduction to the significance and prevalence of myopia; evidence for visually guided refractive development of the eye; myopia control using optical and pharmaceutical methods; anatomy and imaging of the choroid; functions of the choroid, including the effects of retinal defocus on the choroid; the choroid in ocular disease and the effects of pharmacological agents on the choroid. This is then followed by a statement of the Aims of the study.

**Chapter 3: Preliminary study.** This chapter describes the methods and results of a preliminary study investigating the effects of atropine on the thinning of the choroid in response to imposed hyperopic retinal defocus. This study was conducted in 10 young Asian adults at The School of Optometry and Vision Science, The University of Auckland, New Zealand.

**Chapter 4: Study in children: Methods.** This chapter describes in detail the methods used in the main study in children, which was conducted in the Department of Ophthalmology, China Medical University Hospital, Taichung, Taiwan. It includes participant inclusion/exclusion criteria, sample size calculations, details of experimental protocol and equipment, primary and secondary outcome measures, OCT image processing methods, investigator masking and details of statistical analyses.

**Chapter: 5 Results.** This chapter presents the results of the main study in children. It describes measures of subfoveal choroidal thickness (SFCT) made before and after administration of atropine and the effects of atropine on the choroidal changes associated with imposed retinal defocus at baseline, and after 1 week, 3 months and 6 months of atropine treatment. It also describes the changes in secondary outcome measures (ocular biometry, accommodation, pupil diameter etc) associated with atropine administration throughout the study.

**Chapter 6: Discussion.** This chapter summarises and discusses the findings of the main and preliminary studies relating to the effect of atropine on defocus-induced changes to the choroid and on the secondary outcome measures. It emphasises the additive nature of the effects of atropine alone with the effects of defocus on choroidal thickness. It also discusses the limitations to the study and potential future directions for research.

**Chapter 7: Summary and conclusions.** Provides a short summary of the findings and conclusions of the study in the form of bullet-points.

**Chapter 8: References.** Lists all references cited in the thesis in alphabetical order.

**Chapter 9: Appendices.** Appendices include example OCT images (a baseline image of each eye from each participant); Tables showing raw data for primary and secondary outcome measures (e.g. individual absolute choroidal thickness changes in response to retinal image defocus over the 6-month study period for all 20 participants); and Tables of key statistical output (SPSS).

# 2 Background

## 2.1 Myopia significance

Myopia is the leading cause of vision impairment worldwide (Pan, Ramamurthy et al. 2012, Foster and Jiang 2014, Wong and Saw 2016), and is a major health concern particularly in East Asian Countries (Morgan, Ohno-Matsui et al. 2012, Pan, Dirani et al. 2015, Morgan, He et al. 2016, Wu, Huang et al. 2016), but also in western countries like USA (Vitale, Sperduto et al. 2009). The prevalence of myopia is increasing (Hung 2001, Seet, Wong et al. 2001, Shimizu, Nomura et al. 2003, Williams, Bertelsen et al. 2015, McCullough, O'Donoghue et al. 2016) and can be as high as 84% among school children aged 16 to 18 years in Taiwan (Lin, Shih et al. 2004) and further, up to 96.5% in 19 year-old males in Seoul (Jung, Lee et al. 2012). Prevalence in western countries (i.e Australia, West Europe, United States) is typically less, at approximately 16% to 27% (Pan, Ramamurthy et al. 2012).

Myopia of any degree increases the risk of developing sight-threatening diseases such as glaucoma, cataract and myopic maculopathy and retinal detachment (Flitcroft 2012) with risks increasing dramatically in cases of high myopia (Ohno-Matsui, Lai et al. 2016).

### 2.1.1 Evidence for visually guided refractive development of the eye

The presence of myopia suggests that some abnormality has occurred in the refractive development of the eye. Animal models have provided significant insight into the refractive development of the eye and have been used to study the development of refractive error since the discovery (Wiesel and Raviola 1977) that monocular suturing of the eyelids in monkeys caused the eye to grow abnormally long and become myopic relative to the non-sutured eye; a condition known as Form Deprivation Myopia (Wiesel and Raviola 1977, Stone, Lin et al. 1989, Troilo, Nickla et

al. 2000). Since then, studies in many animal species have demonstrated the universality of the finding that in the developing eye, refractive development is an optically guided process. One advantage of studying animal models is that the refractive changes typically occur rapidly. In the case of chicks, significant form deprivation myopia can be induced in a matter of days (Wallman, Wildsoet et al. 1995), although in primates it may take weeks to months e.g. (Smith and Hung 2000). Animals have also been raised wearing plus or minus powered lenses, which result in rapid, predictable changes in eye growth and refractive error (e.g. chick: (Irving, Sivak et al. 1992, Wallman, Wildsoet et al. 1995) and primate (Hung, Crawford et al. 1995, Hung, Wallman et al. 2000, Troilo, Nickla et al. 2000). In particular, hyperopic retinal defocus induced by raising an animal wearing a minus-powered lens over one eye (to shift the focal plane to behind the retina, thus causing hyperopic retinal defocus) causes the eye to elongate and become myopic, whereas plus-powered lens wear (shifting the focal plane anterior to the retina and creating myopic retinal defocus) causes axial eye growth to slow, and the eye to develop hyperopia. Imposition of defocus initiates changes in the eye in a matter of minutes: the choroid becomes thicker in response to myopic defocus and thinner in response to hyperopic defocus in chick (Wallman, Wildsoet et al. 1995). The subsequent changes in scleral growth are observed in days in the chick (Wallman and Winawer 2004).

### 2.1.2 Natural course of refractive change in myopia

During development, birth and growth, most of our body parts actively control their shape and size as necessary (Wallman and Winawer 2004). The refractive error of the eye in a full-term new born child is +3.55 ± 2.39 D (Chen, Xie et al. 2011), and axial length is 17.3 ± 0.90 mm (Lim, Chong et al. 2013). The refractive components (e.g. crystalline lens and corneal curvature) of the eye become flatter as the eye

grows and therefore less optically powerful. The axial length of the eye grows at a rate so as to ensure that images of distant objects fall on the retina (Zadnik, Mutti et al. 2004, Fledelius, Christensen et al. 2014). The axial length of the eye continues to grow into puberty (Gordon and Donzis 1985, Zadnik, Mutti et al. 2004) but its growth rate decreases with increasing age (Fledelius, Christensen et al. 2014). If there is a mismatch between the change in optical power and the increase in axial length, a refractive error develops. Flitcroft (Flitcroft 2013) analysed data from human cross-sectional and longitudinal studies of refractive development of the eye and showed that when normal eye growth fails, the positively skewed leptokurtic distribution of ocular refraction shifts toward more myopia with increasing age. In Asia in particular, myopia is becoming increasingly prevalent (Ding, Shih et al. 2017).

Thorn et al. (Thorn, Gwiazda et al. 2005) modelled the progression of myopia and found that children aged between 10 and 13 years typically undergo the fastest change in refractive error and that myopia progression begins to stabilise at around 15 years of age. Data from children who remain emmetropic indicate that the increase in axial length slows between the ages of 10 and 13 years (Fledelius, Christensen et al. 2014). This implies that in the eyes of children who develop myopia, the axial growth mechanism is disturbed.

### 2.1.3 Risk factors for Myopia Development

Many studies have investigated the risk factors for myopia *development*: these studies have been the subject of recent reviews (Read 2016, Rose, French et al. 2016, Rudnicka, Kapetanakis et al. 2016, Ding, Shih et al. 2017). Many risk factors for myopia development have been identified, including age, gender, time spend outdoors, urbanisation, education, amount of near work performed, parental myopia,

income and education of the parents and ethnicity (Pan, Ramamurthy et al. 2012, Ding, Shih et al. 2017). Previous studies have shown that non-myopic children tend to spent more time outdoors than those children who are myopic (Jones, Sinnott et al. 2007, Dirani, Tong et al. 2009), which suggests that the environment is an important factor for the prevalence of myopia. A recent study (Choi, Yu et al. 2017) investigated the relationship between confined spaces and myopia in Hong Kong children and reported that the axial eye length was significantly longer among children who live within higher population density districts and those in smaller homes. The authors concluded that a constricted living space may be an additional environmental risk for myopia development in children.

In 2010, the Ministry of Education in Taiwan began to apply an 'Outdoors Activity' policy, in an attempt to counter the high incidence of myopia in Taiwanese schoolchildren (www.k12ea.gov.tw). Briefly, the policy 'eye 3010' recommends that children take a 10-minute break after every 30 minutes of near work. In addition, the policy recommends that children spend 120 minutes per day engaged in outdoor activities. This may be implemented in schools (e.g. by having classes outdoors), and by encouraging parents to ensure that their children spend 120 minutes per day outdoors after school and during the weekends.

In addition to studies investigating the risk factors for myopia development with a view to reducing them in the future, there is also increasing interest in exploring interventions to control the *progression* of myopia by optical, pharmaceutical and other methods: these are discussed below.

## 2.2 Optical control of myopia progression

Optical methods typically attempt to slow myopia progression by manipulating retinal defocus. Since retinal defocus is known to affect the human choroid (Read, Collins et al. 2010, Chiang, Phillips et al. 2015), a review of optical control methods is given in this section.

### 2.2.1 Undercorrection

Intentional undercorrection of a myopic refractive error with spectacles or contact lenses has been a popular strategy for attempting to slow myopia progression (Goldschmidt 1990, Saw, Gazzard et al. 2002). The rationale was that undercorrection reduces the accommodative demand while reading (and thus should reduce hyperopic retinal defocus resulting from lag of accommodation) and it would also provide myopic defocus to the retina during distance vision: both would be expected to reduce axial eye elongation, and thus myopia progression. However, it has been shown that undercorrection is not an effective therapy (Adler and Millodot 2006), and Chung et al. even concluded that undercorrecting myopia in children may potentially enhance their myopia progression (Chung, Mohidin et al. 2002). However, a study by Phillips (Phillips 2005), evaluated the effectivity of a monovision spectacle correction on myopia progression. The study concluded that monovision was not effective in reducing accommodation in children but that myopia progression was significantly reduced in the near-corrected eye (Phillips 2005), as would be consistent with the results of animal studies (Schaeffel, Glasser et al. 1988). It has been proposed (Phillips 2005) that the reason for the different effects of binocular versus monocular undercorrection is because undercorrecting one eye has quite different optical consequences than bilateral undercorrection. Bilateral undercorrection results in myopic defocus at distance, but clear retinal images at near in both eyes. In contrast, unilateral undercorrection results in continuous myopic defocus in the

undercorrected eye at both distance and near. This is because accommodation at near is driven by the fully corrected eye, rather than the undercorrected eye in children wearing monovision. However, monovision would have very limited applicability in clinical myopia control because it only affects myopia progression in the undercorrected eye and potentially disrupts binocular vision and stereopsis.

### 2.2.2 Bifocal & Progressive addition lenses (PALs)

Several studies have been conducted to investigate the effectiveness of bifocal and PAL spectacle corrections for controlling myopia. Bifocal and PAL (Hyman, Gwiazda et al. 2001, Yang, Lan et al. 2009, Berntsen, Sinnott et al. 2012, Berntsen, Barr et al. 2013) corrections aim to reduce accommodative demand during near tasks and therefore reduce accommodative lag and the hyperopic retinal defocus that potentially promotes eye elongation. The advantage over undercorrection is that distance vision is not compromised. The PALs are usually more widely acceptable than bifocals both cosmetically and visually, as there is no obvious reading addition segment and PALs provide a range of effective additions, rather than just one. The Correction of Myopia Evaluation Trial (COMET) was a large, multicentre, randomised, double-masked trial (Hyman, Gwiazda et al. 2001, Gwiazda, Hyman et al. 2003, Hyman, Gwiazda et al. 2005) which evaluated the effectiveness of PALs versus a single vision correction in reducing myopia progression. The original COMET study found that children (aged 6 – 11 years) assigned to wear PALs had less myopia progression compared to those wearing single vision lenses by a small amount (0.20 ± 0.08 D after 3 years, 0.18 D after 1 year), and it was concluded that this was of minor clinical significance (Gwiazda, Hyman et al. 2003, Hyman, Gwiazda et al. 2005). More recent studies by Bernsten et al. (Berntsen, Sinnott et al. 2012, Berntsen, Barr et al. 2013) provide comparative data in children of the same age group, but over a shorter period. In the

first study (Study of Theories About Myopia Progression – STAMP) of 85 children aged 6 – 11 years, 42 in PALs, with high accommodative lag and low myopia of -2.25D spherical equivalent or less) progression was reduced by 0.18 D after 1 year – a very similar result to the COMET study. The second study by Bernsten (Berntsen, Barr et al. 2013) examined the effect of PALs versus SVLs on progression and peripheral defocus in myopic children. That study reported a difference of 0.27 D in progression between groups wearing PALs and SVLs after one year, which would appear to suggest a greater effect than observed in their previous study and the COMET study (0.18 D after one year). It is not clear what might account for the different efficacies between studies, but the COMET study (469 children with 235 in PALs) included many more children than the Bernsten studies. In the COMET study, PALs were more effective in some subgroups of children than in others. The observed differences between studies may simply be the result of variability in participant characteristics. The hypothesised explanations for the reduced progression with PALs was different for the COMET study and the Bernsten studies. The hypothesis associated with the COMET study was that PALs slowed progression by reducing accommodation and thus accommodative lag and in turn hyperopic defocus during near work. The Bernsten study suggested that SVLs caused a hyperopic shift in peripheral defocus at all locations, and that PALs caused a myopic shift in peripheral defocus in three of four locations measured. The authors concluded that superior retinal myopic defocus was associated with less on-axis myopia progression (Berntsen, Barr et al. 2013).

A three-year randomized study of effects of bifocal and bifocal with base-in prism spectacles, examined a total of 135 Chinese-Canadian children (aged 8-13 years) with myopia progression rates ≥ 0.5 D in the preceding year. Over the three years of

the study, children wearing bifocals progressed by −1.25 D, those wearing prismatic bifocals progressed by −1.01 D, and those wearing single vision spectacles progressed by −2.06 D (Cheng, Woo et al. 2014).

### 2.2.3 Orthokeratology

Orthokeratology (Ortho-K) lenses are worn overnight to reshape the central cornea into a flatter profile in order to correct refractive error. The lenses are removed during the day to allow unaided vision. The Ortho-K lens changes anterior corneal curvature by thinning the central epithelial layer and thickening the epithelium in the mid-periphery (Wang, Fonn et al. 2003). Over time the epithelial cells return to their original condition and therefore Ortho-K lenses must be worn regularly (typically every night) to maintain their effect. Several clinical trials report that Ortho-K reduces the rate of axial elongation of the eye and hence controls myopia progression (See *Table 1*). Meta analyses of changes in the rate of eye elongation indicate that Ortho-K reduces progression by ~45% (Sun, Xu et al. 2015), or ~0.27mm slower in axial elongation over 2 years (Li, Kang et al. 2016). It has been proposed that Ortho-K slows myopia progression because although the central cornea is moulded to a flatter shape, the mid-peripheral cornea is steepened, which results in myopic defocus in the peripheral retina (Kang and Swarbrick 2011) and hence reduced eye elongation. The effect of pupil diameter on axial elongation was investigated in 52 Chinese children aged between 9 to 14 years in which 25 children wore Ortho-K lenses over 2 year period (Chen, Niu et al. 2012). Their results showed that axial growth was significantly slower in subjects with above-average pupil sizes than those with below average pupil sizes in the Ortho-K wearing group. The authors proposed that the effect of pupil diameter on axial growth was because of enhancement of the myopic shift in the peripheral retina.

| Study | Age (yrs) | n | Control | Drop-out | Reduction in axial length (Study length) |
|---|---|---|---|---|---|
| Cho et al. 2005 | 7–12 | 43 | Specs | 19% | 46% (2yrs) |
| Walline et al. 2009 | 8–11 | 40 | SCLs | 30% | 56% (2yrs) |
| Kakita et al. 2011 | 8–16 | 45 | Specs | 7% | 36% (2yrs) |
| Cho, Cheung 2012 | 7–10 | 51 | Specs | 27% | 43% (2yrs) |
| Santodomingo-Rubido et al. 2012 | 6–12 | 31 | Specs | 7% | 32% (2yrs) |
| Hiraoka et al. 2012 | 8–12 | 29 | Specs | 24% | 30% (5yrs) |
| Charm, Cho 2013 | 8–12 | 52 | Specs | 46% | 63% (2yrs) |
| Chen et al. 2013 | 6 - 12 | 43 | Specs | 19% | 52% (2yrs) |
| Li et al. 2016 | Meta-analysis | | | | 43% (2yrs) |
| Sun et al. 2015 | Meta-analysis | | | | 45% (2yrs) |

***Table 1.*** *Summary of results of studies of myopia control using Orthokeratology.*

### 2.2.4 Dual focus / Multifocal contact lenses

A variety of soft contact lens designs have been tested for their ability to slow myopia progression, e.g. (Anstice and Phillips 2011, Lam, Tang et al. 2014, Aller, Liu et al. 2016, Cheng, Xu et al. 2016). For example, the Dual-Focus (DF) lens is a soft contact lens with a central correction zone surrounded by a series of treatment and correction zones designed for use with the large pupils of children (Anstice and Phillips 2011). The central correction zone is designed to provide full correction, while the concentric treatment zones create 2.00D of simultaneous myopic defocus to the retina during distance and near viewing. The DF lens is based on the idea that simultaneous myopic retinal defocus might reduce axial elongation of the eye and hence control myopia progression, even when a clear retinal image is also present. A human paired-eye comparison clinical trial showed significant reductions in eye elongation

(49%) and progression of myopia (37%) in eyes wearing DF lenses compared to eyes wearing single vision contact lenses (Anstice and Phillips 2011). Conventional soft multifocal contact lenses have also been tested as possible treatments for myopia control. The results of a study in children wearing soft multifocal contact lenses with a +2.00 D add (Walline, Greiner et al. 2013) suggested that multifocal contact lens wear resulted in a 50% reduction in the progression of myopia (-0.51D for the multifocal contact lens wearers versus -1.03D for single-vision contact lens wearers), with a 27% reduction in eye elongation over 2 years. However, the control group used for comparison was taken from another study (i.e. was a historical control group).

A recent study investigated the effect of bifocal contact lenses on myopia progression in children with eso fixation disparities at near (Aller, Liu et al. 2016). The results showed that bifocal contact lenses significantly slowed myopia progression, with statistically significant differences between the treatment groups. After 12 months of study, the single vision contact lenses group had progressed by −0.79 ± 0.43 D, compared with −0.22 ± 0.34 D in the bifocal contact lenses group. The studies described above indicate that myopic defocus presented to the retina in conjunction with a clear retinal image can slow myopia progression. However, the majority of the studies are of short duration. Recently the preliminary results of a multicentre clinical trial of the Dual Focus lens indicates that the efficacy is maintained over three years (Chamberlain 2017).

## 2.3 Pharmaceutical control of myopia progression

### 2.3.1 Clinical studies

Recent reviews have concluded that the most effective current method for slowing myopia progression, at least in the short term, is the use of the non-selective muscarinic antagonist, atropine e.g. (Walline, Lindsley et al. 2011, Huang, Wen et al. 2016). Atropine eye drops appear to be tolerated by children and act by inhibiting the abnormal elongation of the eye, thus slowing myopia progression e.g. (Chia, Chua et al. 2012, Chia, Lu et al. 2016). Atropine is widely used in Taiwan, and the prescribing of atropine to slow childhood myopia progression increased significantly from 37% of children diagnosed with myopia by Ophthalmologists in 2000, to 50% in 2007 (Fang, Chou et al. 2013).

A large paired-eye, double-masked study (Chua, Balakrishnan et al. 2006) recruited 400 Asian children aged between 6 and 12 years, to receive either 1% atropine for one eye or a placebo vehicle for one eye nightly for two years. A total of 346 children completed the two-year study, and their results showed that topical atropine was well tolerated and effective in slowing low and moderate myopia (mean sphere change: -0.28 ± 0.92 D; change in axial eye length (AL): −0.02 ± 0.35 mm in atropine-treated eyes compared with changes in placebo-treated eyes (mean sphere change: −1.20 ± 0.69 D; change in AL: 0.38 ± 0.38 mm). However, after cessation of atropine drops, previously-treated eyes appeared to show faster myopia progression than non-treated eyes, although the overall increase in myopia was still less than that in placebo-treated eyes 1 year after cessation (Tong, Huang et al. 2009).

Although 1% atropine treatment appeared to be effective in slowing myopia progression, administering 1% atropine topically induces unwanted side-effects including pupil dilation, causing glare, and paralysis of accommodation, causing

difficulties with near work. In attempts to reduce the severity of these side-effects, the efficacy of lower concentrations of atropine has been investigated e.g. (Chia, Chua et al. 2012). Four-hundred children aged 6 to 12 years were randomly assigned to receive 0.5%, 0.1% or 0.01% atropine, in both eyes nightly for 2 years. The study concluded that 0.01% atropine has minimal side-effects compared to those on 0.1% and 0.5% atropine, while retaining comparable efficacy in controlling myopia progression (Chia, Chua et al. 2012). The effects of ceasing administration of lower concentrations of atropine on myopia progression and ocular parameters have also been investigated (Chia, Chua et al. 2014). Three hundred and fifty-six children were recruited into this one-year washout phase of the study. The results showed that myopia progression during the washout period was greater in eyes that had received 2 years of 0.5% atropine treatment (−0.87 ± 0.52 D), compared with the other groups (0.1%: −0.68 ± 0.45 D and 0.01%: −0.28 ± 0.33 D). Eye elongation was also greater in the 0.5% and 0.1% treated eyes, compared to the 0.01% treated eyes. Thus, the study showed that there was a rebound effect (increase in progression) after ceasing atropine treatment, and the rebound effect was greater in children who had received 0.5% and 0.1% atropine compared to those who had received 0.01% atropine (Chia, Chua et al. 2014). Chia and colleagues (Chia, Lu et al. 2016) have also investigated the safety and efficacy of lower concentrations of atropine in controlling myopia progression for up to 5 years. Their findings indicate that in the first 24 months of atropine treatment, myopia progression was dose-related with a greater effect for higher doses (concentrations). However, in the following 12-month washout period, there was an inverse dose-related increase in myopia progression (i.e. the higher the dose, the greater the progression after stopping), which resulted in atropine 0.01% being the most effective in reducing myopia progression at 3 years (i.e. at the end of the washout period). At this point, children who had progressed by 0.50 D or more in

the washout period (regardless of the concentration of their original treatment) were restarted on atropine 0.01% for a further 2 years. At the end of the five-year study period, the authors concluded that 0.01% atropine was more effective in slowing myopia progression (approximately 50% of reduction in myopia progression), with far fewer side-effects than higher doses (Chia, Lu et al. 2016).

Wu et al. (Wu, Yang et al. 2011) have also evaluated the long-term efficacy of low-concentration atropine eye drops. They followed school-children for at least 3 years who were initially prescribed 0.05% atropine. If the myopia progression was greater than −0.50 D during the first 6 months (45% of children), then the concentration was changed to 0.1% atropine. Their results indicated that myopia progression in the atropine treatment groups (−0.23 D per year) was significantly lower than those in their control group that was not on atropine (−0.86 D per year).

The highest possible concentration of atropine that does not result in significant symptoms from the side-effects of mydriasis and cycloplegia is reportedly 0.02% atropine (Cooper, Eisenberg et al. 2013).

### 2.3.2 Potential site and mode of action of atropine

The mechanism by which atropine inhibits myopia progression is debated. Originally it was thought that the reduction in progression was due to the cycloplegic action of atropine blocking accommodation, which was believed to cause myopia progression when driven excessively with large amounts of near-work. However, following the demonstration that atropine also inhibits the development of experimental myopia in the chick animal model of myopia, which has nicotinic receptors on the ciliary body which are not blocked by atropine (McBrien, Moghaddam et al. 1993), it became clear that atropine inhibits myopia via a non-accommodative mechanism, maybe acting via the retina, choroid or sclera, as different subtypes of muscarinic Acetylcholine

receptors (mAChRs) predominate in the retina, choroid and sclera (Fischer, McKinnon et al. 1998, Qu, Zhou et al. 2006).

It has been proposed that the sclera may be the main target site of atropine based on the finding that muscarinic antagonists applied to isolated cultures of sclera inhibited chick scleral chondrocytes (Lind, Chew et al. 1998). However, the following arguments suggest that the sclera is unlikely to be the main target site. The doses applied to the cultures were so high that it most likely produced a toxic effect on the cells, hence inhibiting glycosaminoglycan synthesis required for scleral growth (McBrien, Stell et al. 2013). Moreover, doses of atropine as dilute as 0.01% are nearly as effective as 1% in slowing myopia progression in children (Chia, Chua et al. 2012). It would be expected that relatively high doses of atropine would be necessary to cross the tissue barriers of the choroid and scleral boundaries if muscarinic antagonist control of myopia was initiated at the sclera. Highly-selective muscarinic antagonists MT3 (M4 receptor antagonist) and MT7 (M1 receptor antagonist) are effective in preventing experimentally-induced myopia at low nanomolar concentrations close to the receptor affinity constants, which would support the view that the retina, rather than the sclera is the important site of action of atropine (McBrien, Stell et al. 2013). Furthermore, the low concentration of the injected intravitreal doses would likely have been in the picomolar range at the choroid and sclera due to ocular barriers, and thus below functional thresholds of muscarinic receptors in the choroid or sclera (Cottriall, McBrien et al. 1999). However, some evidence argues against the retina being atropine's primary site of action. A study by Fischer et al. (Fischer, Miethke et al. 1998) found that atropine prevented form deprivation myopia in chicks whose retinas had been depleted of muscarinic receptors and cholinergic amacrine cells by injected toxins. The results suggested that if cholinergic mechanisms were involved at all,

cholinergic pathways in the retina were unlikely to have participated in the atropine-mediated suppression of form deprivation myopia.

It has been proposed by some researchers that atropine may in fact reduce myopia progression via a non-muscarinic receptor mechanism. This view is supported by the Fischer et al. study above and by the fact that many other mAChR antagonists (e.g. methoctramine, dicyclomine, mepenzolate) fail to produce the same inhibitory effects on myopia as atropine (Stone, Lin et al. 1991, Luft, Ming et al. 2003).

Other than atropine, a large number of other pharmacological agents involved in eye growth control have been tested in animals. These studies have been reviewed elsewhere, e.g. (Ganesan and Wildsoet 2010).

#### *2.3.2.1 Nitric oxide*

The gaseous neurotransmitter nitric oxide (NO) is a potent vasodilator (Ignarro, Lippton et al. 1981, Yamamoto, Bredt et al. 1993, Alderton, Cooper et al. 2001) synthesized by the enzyme nitric oxide synthase (NOS). Unlike other neurotransmitters, NO is only synthesized immediately before its release. There are three isoforms of NOS: neuronal NOS (nNOS) and endothelial NOS (eNOS) require calcium for activation, while inducible NOS (iNOS) is transcriptionally regulated. NOS is present in the RPE (Goureau, Hicks et al. 1994, Goldstein, Ostwald et al. 1996), retinal neurons (Fischer and Stell 1999), intrinsic choroidal neurons (Flugel, Tamm et al. 1994), ciliary parasympathetic terminals (Nilsson 1996) and pterygopalatine ganglia of vertebrate eyes (Yamamoto, Bredt et al. 1993). Since axon terminals containing NOS are found on the non-vascular smooth muscle cells of the choroid and these cells are densely arranged in the subfoveal region (Flügel-Koch, May et al. 1996). It has been proposed that NO plays an important role in regulating choroidal

thickness and volume by varying the degree of vascular and non-vascular smooth muscle contraction. The contraction may directly thin the choroid, and also oppose the lacunae gaining fluid via osmotic pressure (Nickla, Zhu et al. 2013).

Nickla and colleagues (Nickla and Wildsoet 2004) have examined the potential role of NO on the choroidal compensatory response to myopic defocus in chick eyes. Their results showed that intravitreal injections of a non-specific NOS inhibitor (L-NAME) transiently inhibited the choroidal thickening response to myopic defocus induced by either prior form deprivation or by positive spectacle lenses (+15 D). This choroidal inhibition was also associated with a dis-inhibition of ocular growth rate (i.e. myopic defocus failed to slow growth). This effect was dose-dependent and the authors concluded that NO plays a role in modulating choroidal thickness. A study by Nickla et al. (Nickla, Damyanova et al. 2009) further investigated various specific NOS inhibitors: nNOS, eNOS and iNOS for their effects on the choroid and on eye-growth. They concluded that the choroidal compensatory response is influenced by nNOS, possibly released from the intrinsic choroidal neurons.

Recently, the essential role of NO in atropine's ability to inhibit myopia development in animals has been demonstrated (Carr and Stell 2016). Atropine and NO-sources (L-arginine or sodium nitroprusside) inhibited form deprivation myopia in a dose-dependent manner. In addition, NOS inhibitors blocked the atropine-mediated inhibition of myopia. Therefore, the authors concluded that prevention of myopia by atropine requires the production of NO. They also proposed two potential mechanisms (direct and indirect) for induction of NO synthesis by atropine. In the direct pathway, atropine could cause excitation of a target cell and an increase in intracellular calcium, thus activation of nNOS or eNOS, leading to NO synthesis and release, and consequently prevention of myopia. In an indirect pathway, atropine

could inhibit an inhibitory cell (disinhibition) finally leading to an induction of NO synthesis and release, and prevention of myopia.

#### *2.3.2.2 Dopamine*

It has been proposed that dopamine, a neuromodulator synthesized by interplexiform and amacrine cells, plays an important role in a signal pathway mediating emmetropization (Nickla and Wallman 2010). It was previously shown that dopamine was decreased in retinas of form-deprived (Stone, Lin et al. 1989) and negative lens-wearing chick eyes (Guo, Sivak et al. 1995). In comparison, dopamine was increased in retinas that were recovering from form-deprivation myopia (Pendrak, Nguyen et al. 1997). In addition, Ashby et al. (Ashby and Schaeffel 2010) have shown that the protective effect of light against myopia development (see section 2.4) can be abolished by dopamine antagonists such as Spiperone, in the chick model of myopia. It has also been shown that intravitreal injections of atropine massively increase (+209 ± 70%) the release of dopamine (Schwahn, Kaymak et al. 2000). A study investigating the effects of dopamine agonists and antagonists (Nickla, Totonelly et al. 2010) showed that agonists (apomorphine and quinpirole) inhibited myopia development and caused transient choroidal thickening in negative lens-wearing eyes. Therefore, the authors proposed that the release of dopamine from the retina triggers the release of NO from either the retina or choroid, leading to choroidal thickening and inhibition of ocular growth. The order in which this occurs has subsequently been confirmed by experiments in the chick by Nickla and colleagues (Nickla, Lee et al. 2013).

A number of studies have investigated the relationship between light and the release of dopamine. Among them, Cohen et al. (Cohen, Peleg et al. 2012) showed that dopamine release by the retina is linearly related to luminance between about 50 to

10,000 lux and that refractive development is associated with luminance-dependent release of dopamine, i.e. myopia associated with low luminance results from low retinal dopamine levels. In addition, Sekeran et al (Sekaran, Cunningham et al. 2005) investigated the relationship between nitric oxide release and dopamine during illumination. The authors found that dopamine stimulates the release of retinal nitric oxide (NO) in response to flickering light, which is consistent with the later findings of Nickla et al. (Nickla, Lee et al. 2013) showing the link between dopamine and nitric oxide.

#### *2.3.2.3 Acetylcholine*

Muscarinic acetylcholine antagonists such as atropine prevent form-deprivation myopia in chicks (Stone, Lin et al. 1991, McBrien, Moghaddam et al. 1993, Luft, Ming et al. 2003), monkeys (Young 1965, Tigges, Iuvone et al. 1999), and also inhibit myopia progression in humans (Bedrossian 1979, Shih, Chen et al. 1999, Kennedy, Dyer et al. 2000), suggesting that acetylcholine is an important component in the development of myopia. Animal studies using a mammalian model, the tree shrew, found M1 and M4 muscarinic receptor specific antagonists to be effective in reducing form deprivation myopia. The authors concluded that the inhibition of form deprivation myopia by muscarinic antagonists involves both M1 and M4 muscarinic receptor signalling pathways (Arumugam and McBrien 2012). Nickla et al 2013 (Nickla, Zhu et al. 2013) examined the effects of several muscarinic agents (Agonists: oxotremorine, pilocarpine, carbachol and arecaidine; Antagonists: Atropine, pirenzepine, oxyphenonium and dicyclomine) on chick choroids *in vitro* and *in vivo*. Their results showed that 24 hours after a single intravitreal injection of muscarinic agents, all agonists caused choroidal thinning *in vitro*, and all except pilocarpine, thinned choroids *in vivo*. In contrast, antagonists, atropine, pirenzepine and oxyphenonium

caused rapid, transient choroidal thickening and inhibited the development of myopia in negative lens-wearing eyes (Nickla, Zhu et al. 2013). These findings suggest that acetylcholine plays a major role in the development of myopia.

## 2.4 Other methods of controlling myopia

Several studies have explored the association between environmental factors and onset and progression of myopia (Guo, Liu et al. 2013, Ramamurthy, Lin Chua et al. 2015, Read 2016, Rose, French et al. 2016). Sherwin et al. (Sherwin, Reacher et al. 2012) published a meta-analysis of seven cross-sectional studies on the relationship between time outdoors and myopia development in children. Their results showed that a one-hour increase per week in time spent outdoors could reduce the odds of myopia development by 2%.A study by Read et al. (Read, Collins et al. 2015) reported a significant association between greater average daily light exposure and slower axial eye growth in children. A study in Taiwan (Wu, Tsai et al. 2013) investigated the effect of increasing time outdoors during class recess, on myopia onset and progression in children. The authors concluded that time outdoors had a significant effect on myopia onset and refractive change in non-myopic children, but no effect on myopia progression in myopic children. Similarly, a two-year study in nearly 2000 Chinese children (Li, Li et al. 2015) concluded that greater time spent outdoors was associated with slower axial elongation in non-myopic children, but not in pre-existing myopic children. Jones-Jordan et al. also reported that outdoor/sports activity had no beneficial effect on myopia progression in myopic children (Jones-Jordan, Sinnott et al. 2012). Guo et al. (Guo, Liu et al. 2013) investigated the potential factors associated with myopia in urban and rural school children in Beijing. Their study showed that myopia is significantly associated with less time spent in

outdoor activities and more time spent indoors (e.g. study and watching television). A recent meta-analysis by Xiong et al. (Xiong, Sankaridurg et al. 2017) further confirmed that more time outdoor is protective for myopia development. They concluded that an increase of approximately 76 minutes per day compared to controls would reduce the incident of myopia by 50%. Their analysis also showed a stronger protective effect of outdoor activities in children aged 6 years compared with 11 to 12 year-olds. This may be due to the more rapid growth of the ocular components in younger children. Their analysis also showed that myopic children on average spent less time outdoors than non-myopic children. Time outdoors was also shown to have a protective effect against the myopic shift in refractive error that precedes myopia development. Interestingly, this protective effect from time outdoors seemed to be greater in non-myopic than myopic children. The meta-analysis also showed that there was no significant association between outdoor time and myopia progression in myopic children.

Rather than increasing time outdoors, other researchers have investigated the effects of increasing light level indoors on myopia development. Hua et al. (Hua, Jin et al. 2015) showed that elevating light levels in the classroom can effectively reduce the onset of myopia: there was a 4% onset of myopia in the intervention group compared to 10% for the non-intervention (control) group. They also observed a smaller myopic shift among non-myopic children. Therefore the authors concluded that elevating light levels in classrooms could have a significant effect on myopia onset.

Vitamin D level in the blood is a biomarker for sun exposure: low levels have been linked with less time outdoors and consequently an increased risk of myopia development. However, a recent review by Pan et al. (Pan, Qian et al. 2017) suggests that the evidence linking vitamin D levels and myopia is weak and the mechanisms

are unclear. Therefore the vitamin D level in blood may only serve as a biomarker of outdoor exposure, which is the real protective factor for myopia.

Seasonal effects on eye growth and myopia progression in children have been reported, with a faster progression rate in winter months and a slower rate in summer (Fulk, Cyert et al. 2002, Donovan, Sankaridurg et al. 2012, Gwiazda, Deng et al. 2014). It has been suggested that this seasonal effect may be light-based, with children spending more time outdoors during the summer, or the associated effect of summer vacation and less near-work.

## 2.5 Anatomy of the ocular choroid

The choroid (*Figure 1*) is a thin, pigmented, highly vascular layer situated between the retina and sclera of the eye. It is part of the uveal tract and is derived from the mesenchyme surrounding the optic vesicle during eye development. The choroid has the highest blood flow rate per unit-tissue-weight in the body to fulfil the metabolic demands of the outer layers of the retina (Alm and Bill 1973). The choroid forms a ring around the optic nerve at the posterior part of the eye and extends forwards to join the ciliary body anteriorly. Blood is supplied to the choroid primarily from the posterior ciliary arteries and some recurrent branches of anterior ciliary arteries (Hayreh and Baines 1972, Lieberman, Maumenee et al. 1976, Hayreh 2004).

The choroid is usually thickest at the posterior pole of the eye, gradually becoming thinner towards the anterior of the eye (Margolis and Spaide 2009, Manjunath, Taha et al. 2010). *In vivo* studies with Optical Coherence Tomography (OCT) report that choroidal thickness at the posterior pole is around 366 µm in the first decade of life, decreasing by 15 µm for each decade of life thereafter (Margolis and Spaide 2009).

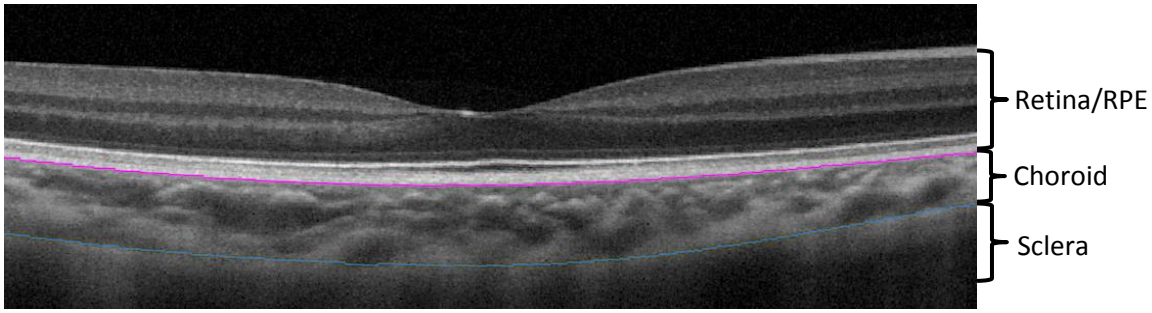


***Figure 1.*** *Image of the retina and choroid of a myopic participant taken with Optical Coherence Tomography (OCT). The choroid lies between the pink and blue borders, below the retina and the retinal pigment epithelium (RPE) and above the sclera.*

The anatomy of the choroid has been described in detail elsewhere (Hogan, Alvarado et al. 1971, Nickla and Wallman 2010). Briefly, the choroid has five main layers including Bruch's membrane, the choroiocapillaris, two vascular layers (Sattler's and Haller's layers) and the suprachoroidea adjoining the sclera. The choriocapillaris contains numerous capillaries, which are supplied with blood from the arterioles of the vascular layers and which drain into the veins of the vascular (Sattler's and Haller's) layers. Sattler's layer consists of medium and small arteries and arterioles that feed the capillary network, whereas Haller's layer consists of larger blood vessels. The extravascular tissue of the vascular layers contains loosely arranged connective tissue and is rich in melanocytes, non-vascular smooth muscle cells, elastic fibres and fibroblasts. The suprachoroidea, the transition zone between choroid and sclera, consists of collagen fibres, melanocytes and fibroblasts. Previous studies in avian species (De Stefano and Mugnaini 1997) showed that the choroid contains an extensive system of thin-walled, endothelial-lined spaces known as lacunae. Lacunae, which are similar to lymphatic vessels, eventually merge to form larger lacunae in the suprachoroidea layer and empty into veins (Krebs and Krebs 1988, De Stefano and

Mugnaini 1997). These thin-walled lacunae are also found in primates (Krebs and Krebs 1988) as well as chicks (Junghans, Crewther et al. 1998).

### 2.5.1 Innervation of the choroid

The smooth muscles of the choroidal vessel walls are innervated by both the parasympathetic and sympathetic divisions of the autonomic nervous system. The parasympathetic input mainly originates from the pterygopalatine ganglion via the facial nerve and mediates vasodilation (Ruskell 1970, Ruskell 1971, Stone 1986). The parasympathetic fibres are largely cholinergic and immunoreactive for nitric oxide synthase (NOS) and vasoactive intestinal peptide (VIP) (Yamamoto, Bredt et al. 1993, Flugel, Tamm et al. 1994). The sympathetic innervation originates from the superior cervical ganglion and mediates vasoconstriction (Kirby, Diab et al. 1978): the neuron terminals form a dense perivascular nerve plexus and axon terminals can also be found throughout the stroma (Sattler's layer) terminating on Intrinsic Choroidal Neurons (ICNs, i.e. choroidal ganglion cells) and non-vascular smooth muscle cells. In all species studied, ICNs typically stain positive for neuronal NOS and/or nicotinamide-adenine dinucleotide phosphate (NADPH)-diaphorase (Flugel, Tamm et al. 1994, Bergua, Mayer et al. 1996) which suggests the use of nitric oxide as a transmitter. Studies comparing different mammalian eyes have found that significant numbers of ICNs are only present in eyes with a well-developed fovea like the eyes of humans and higher primates (Flügel-Koch, Kaufman et al. 1994, Flugel, Tamm et al. 1994). Human choroids have approximately 2000 ICNs per eye, with more than fifty percent concentrated in the central and temporal quadrant and the rest unevenly distributed in all other quadrants (Flugel, Tamm et al. 1994). Monkey choroids contain approximately 500 ICNs in each eye, but they are more evenly distributed throughout the entire choroid (Flügel-Koch, Kaufman et al. 1994). The non-vascular smooth

muscle cells in primate choroids are found in two arrangements: in a layer that runs parallel and underneath Bruch's membrane, and in a reticulum of flattened lamellae that run oblique to the choroid and lie between lacunae (Poukens, Glasgow et al. 1998). Although the non-vascular smooth muscle cells are distributed throughout the choroid, they are most densely arranged in the posterior part of the suprachoroidea of the submacular region (Flügel-Koch, May et al. 1996), and it has been suggest that they play a role in the change of choroidal thickness in response to retinal defocus (Wallman, Wildsoet et al. 1995). This topic is further discussed in section 2.7.6.

### 2.5.2 Functions of the choroid

A variety of functions have been associated with the choroid in addition to the supply of blood and associated nutrients and oxygen to the outer retina. These functions have been investigated in animal studies (Parver, Auker et al. 1982, Parver, Auker et al. 1982) and include thermoregulation (i.e. by dissipating heat), and light absorption. The very high choroidal blood flow might protect the retina from damage resulting from increased temperature, (e.g. from heat generated during exposure to bright lights (Parver, Auker et al. 1982, Parver, Auker et al. 1982) or by exposure of the eye to extreme increases in environmental temperature) by acting as a heat sink. Similarly, if the eye was exposed to an extremely cold environment, choroidal blood flow could act maintain the temperature of the retina near the core temperature.

## 2.6 Methods of imaging the choroid

The choroid is situated between the RPE and the opaque sclera and consequently visualizing the *in vivo* choroid with standard methods (e.g. Biomicroscopy) is not possible. As different imaging techniques have been developed, observing the *in vivo* choroid in health and disease has become possible. Prior to the widespread availability of OCT, assessment of choroidal integrity was typically assessed using techniques such as Fluorescein angiography with fundus photography (Novotny and Alvis 1961). More recently Fluorescein angiography has been replaced by Indocyanine Green (ICG) angiography to acquire an angiogram of the choroid (Yannuzzi, Slakter et al. 1992, Yannuzzi, Slakter et al. 2012). The procedure of ICG angiography is similar to fluorescein angiography, but uses Indocyanine Green dye that fluoresces in infra-red light. Infrared wavelengths have the ability to penetrate the retinal layers making the circulation in deeper layers visible when photographed with an infra-red sensitive camera. Although the two angiography methods provide information about blood vessel damage, leakage sites, vascular filling etc, it does not provide information about choroidal thickness or give a cross-sectional view of the choroid. Ocular biometry with ultrasound has been used to provide information regarding the axial dimensions of the ocular components (Connors Iii, Boseman Iii et al. 2002, Chen, Hirnschall et al. 2011) including the choroid, but the low resolution of ultrasound and the reflectivity of the retina and RPE generally makes the retina difficult to distinguish from the sclera, making choroidal thickness measurement difficult with ultrasound. More recently, optical low coherence reflectometry, as employed in the LenStar LS 900 biometer (LenStar LS 900; Haag-Streit AG, Koeniz, Switzerland) has come to replace ultrasound biometry. The LenStar LS 900 can measure keratometry readings, pupil size, corneal diameter, central corneal thickness, anterior chamber depth, crystalline lens thickness, axial length, retinal thickness and

choroidal thickness (Cruysberg, Doors et al. 2010). However, it can only provide numerical measurements of ocular components along a single axis.

### 2.6.1 Optical Coherence Tomography (OCT)

Optical coherence tomography (OCT) is a non-invasive optical imaging method that provides a real-time cross-sectional image of the ocular tissue of interest. The measuring principle of OCT is based on the interferometric technique that uses relatively long wavelength light to penetrate the biological tissues. It detects the path length distribution of low coherence light that is reflected back from the interfaces between the tissues being sampled (Huang, Swanson et al. 1991). OCT can provide images of ocular tissues in three-dimensions with resolution of a few micrometres. The types of OCT devices that are currently available can roughly be divided into three categories: time-domain (TD-OCT), spectral-domain (SD-OCT), and swept source (SS-OCT).

The time-domain OCT uses an optical beam splitter to split a super-luminescent diode light source into two perpendicular beams (i.e. a reference beam and a sample beam); one is directed to a known reference arm while the sample beam enters the patient's eye. Backscatter from the layers of the ocular tissues (e.g. retina and superficial choroid) is co-mingled with those from the reference arm. In TD-OCT, a moving mirror is required for data collection. Since it can only sample a very small area at a time, it requires a reasonable amount of time and number of samples to obtain a reproducible image. Further, the long sampling time increases light exposure, which is restricted by safety standards, limiting the number of samples and resolution of the scan (Podoleanu 2012).

In contrast, spectral-domain OCT (SD-OCT) uses a broadband light source which is split in a similar manner to TD-OCT, but no moving mirror is used in data capture. Instead, SD-OCT employs a spectrometer to analyse light frequency changes that occur from reference beam and reflected ocular beam interactions and the information from the full depth scan can be acquired and recorded within a single exposure. It has the ability to perform up to 50 times faster and with higher resolution than TD-OCT (Wojtkowski, Srinivasan et al. 2005). SD-OCT typically employs wavelengths centred around 800 - 870 nm (Adhi, Liu et al. 2014), but SD-OCTs employing longer wavelengths (around 1050 - 1060 nm) have also been developed and used to analyse the choroid. These SD-OCTs have the advantage of reduced light scattering and deeper penetration into the tissue, thus improving visualization of the choroid (Považay, Bizheva et al. 2003, Esmaeelpour, Povazay et al. 2010). The high signal-to-noise ratio and high-speed performance make acquisition of a three-dimensional image possible as there are less movement artefacts, and the axial resolution can be as good as 2-3 μm (Drexler, Sattmann et al. 2003, Wojtkowski, Srinivasan et al. 2005). Since SD-OCT can provide high-resolution images in a relatively short period of time, it is widely used in clinical settings such as measuring macular oedema (Pelosini, Hull et al. 2011, Ricci, Airaghi et al. 2016) and the extent of drusen in age-related macular degeneration (Gregori, Wang et al. 2011, Ko, Cao et al. 2013). However, despite the improvement in sampling time and resolution compared to TD-OCT, the ability of SD-OCT to penetrate tissue and to image the choroid and deeper tissues is still limited by the highly absorbing and scattering pigments of the RPE. This problem has been partially addressed by Enhanced Depth Imaging (EDI OCT) introduced by Spaide et al. (Spaide, Koizumi et al. 2008) to provide better imaging quality of the choroid. This technique involves positioning the OCT instrument closer to the eye, which makes the OCT focus at deeper tissue layers,

i.e. the choroid and slightly further, with an inverted mirror image being formed and captured. Spaide et al. concluded that this technique provided a more detailed and measurable image of the choroid compared with the standard method (Spaide, Koizumi et al. 2008). The EDI technique has also been used to investigate choroidal thickness maps in highly myopic eyes by Ohsugi et al. (Ohsugi, Ikuno et al. 2013) who concluded that the EDI technique provided a sufficiently clear image of the RPE and the choroid-scleral interface, that precise measurement of the choroidal thickness was possible.

Swept source OCT (SS-OCT), also known as Time Encoded Frequency Domain OCT (TEFD-OCT) works on a different principle to the instruments mentioned above. It encodes the spectral components in time instead of spatial separations. The SS-OCT has a higher acquisition rate (up to 100-fold (Waldstein, Faatz et al. 2015) than SD_OCT (e.g. Topcon Atlantis): 100,000 A-scans per second with a wavelength-sweeping laser centred at 1050 nm). Since the light source has longer wavelengths, SS-OCT provides deeper penetration of tissue with higher sampling speed than typical SD-OCT. SS-OCT also exhibits less sensitivity roll-off with image depth compared to the SD-OCT method, which allows a wider and more detailed scanning of deeper structures (Waldstein, Faatz et al. 2015). These improvements make three-dimensional choroidal thickness maps and choroidal volume calculation possible (Ellabban, Tsujikawa et al. 2012, Ellabban, Tsujikawa et al. 2012). The SS-OCT can obtain high-quality images at ultra-high speed, while at the same time increasing penetration into the deeper tissues such as the choroid and choroid-sclera boundary (Yasuno, Hong et al. 2007, Hirata, Tsujikawa et al. 2011). A study comparing the abilities of SD-OCT (with the reference position set at the choroid, similar to Enhanced Depth Imaging) and SS-OCT to measure choroidal thickness

(Copete, Flores-Moreno et al. 2014) concluded that SD-OCT was only able to reproducibly measure choroidal thickness in 74% of eyes versus 100% with SS-OCT, suggesting that SS-OCT is the better option for measurement of choroidal thickness.

The axial resolution claimed for the various types of OCT varies between models. However, the literature suggests that typical TD-OCT has an axial resolution of approximately 8 – 10 µm, SD-OCT around 4 – 7 µm and SS-OCT around 8 – 9 µm (Hirata, Tsujikawa et al. 2011, Adhi and Duker 2013, Copete, Flores-Moreno et al. 2014, Novais, Adhi et al. 2016).

These new OCT imaging technologies have led to better visualization of *in-vivo* eye anatomy and made clinical examinations more precise. In particular, the development of SS-OCT and the EDI technique have significantly improved visualization of the choroid and enabled rapid and accurate measures of choroidal thickness and choroidal volume to be made. Such measures help to better understand the role of the choroid in healthy and diseased eyes.

## 2.7 Choroidal thickness in health and disease

Many factors are known to affect the choroidal thickness in health and disease; these factors have been the subject of a recent review (Tan, Gupta et al. 2016). Topics relevant to this thesis are discussed below.

### 2.7.1 Choroidal thickness: relationship with age and degree of myopia

In healthy adult eyes SFCT is inversely proportional to age (Ramrattan, van der Schaft et al. 1994, Fujiwara, Imamura et al. 2009, Margolis and Spaide 2009, Manjunath, Taha et al. 2010) decreasing with age by about 15 µm per decade, from approximately 366 µm in the first decade of life (Margolis and Spaide 2009). However, in children, the choroidal thickness increases from early childhood to adolescence (Read, Collins et al. 2013). Read and colleagues found that the choroidal thickness was significantly thinner in 4 to 6 year-old children (312 ± 62 µm), compared to 7 to 9 year-olds (337 ± 65 µm).

It is well known that the choroid is significantly thinned in myopic eyes (Leila El Matri, Bouladi et al. 2012, Flores-Moreno, Lugo et al. 2013, Ho, Liu et al. 2013, Ohsugi, Ikuno et al. 2013, Read, Collins et al. 2013, Vincent, Collins et al. 2013, Chiang, Phillips et al. 2015). The thickness of the choroid is highly correlated with the degree of myopia, although different studies suggest very different relationships: i.e. a decrease in SFCT of 6 µm (Ho, Liu et al. 2013), 7 µm (Leila El Matri, Bouladi et al. 2012), 19 µm (Read, Collins et al. 2013) or up to 39 µm (Chiang, Phillips et al. 2015) per dioptre of myopia. Furthermore, the overall thickness profile of the choroid also seems to vary depending on the refractive status. The choroid in highly myopic eyes tends to be thickest temporal to the fovea and thinnest nasally (Fujiwara, Imamura et al. 2009), whereas in normal eyes, it tends to be thickest at the fovea and gradually

thinner towards both nasal and temporal sides (Margolis and Spaide 2009). The relevance of choroidal thickness in relation to myopia and sight threatening diseases is discussed in Section 2.7.7.1.

A recent study investigated the longitudinal changes in choroidal thickness and its relationship with eye growth in 10 - 15 year-old children over an 18-month period. Their results showed a significant increase in average SFCT over 18 months in both myopic and non-myopic children. However, those children undergoing faster axial eye growth and myopia progression exhibited less thickening or a thinning of the choroid (Read, Alonso-Caneiro et al. 2015).

### 2.7.2 Choroidal thickness and accommodation

Near work and accommodation have long been implicated in the development of myopia (Hepsen, Evereklioglu et al. 2001, Gwiazda, Hyman et al. 2004) and most myopia develops due to axial elongation of the eye. Several studies have investigated the short-term relationship between accommodation and eye length (Drexler, Findl et al. 1998, Woodman, Read et al. 2011).

Woodman et al., 2011 demonstrated that the axial length (i.e. the distance from anterior corneal surface to RPE) of the eye slightly increased during prolonged near tasks (Woodman, Read et al. 2011). In 2012, the same group further showed that there was significant axial elongation immediately following the commencement of an accommodation task (Woodman, Read et al. 2012). This elongation was sustained for the duration of the near task, and returned to the baseline levels 10 minutes after accommodation ceased. During this axial elongation, they also observed that a small amount of choroidal thinning occurred (Woodman, Read et al. 2012). This choroidal thickness change during accommodation could potentially account for a portion of the increased axial length during accommodation. However, it was unclear why the

choroidal thickness changed during accommodation. One possible explanation put forward was that since the ciliary body inserts into the anterior choroid, the contraction of ciliary muscle during accommodation might be transmitted to the choroid to stretch it and thus reduce its thickness (Tamm, Lutjen-Drecoll et al. 1991). However, this did not directly explain why, in their study (Woodman, Read et al. 2012), the crystalline lens thickness returned to baseline immediately following the near task, but the choroidal thickness only returned to baseline ten minutes after the accommodation task ceased. Since the ciliary muscle receives both parasympathetic and sympathetic innervation, another possibility put forward was that during accommodation, the associated changes in the autonomic innervation potentially also affected the blood flow and/or the non-vascular smooth muscle of the choroid, and hence led to the choroidal thickness change. Recently, Woodman et al. further investigated the regional changes in choroidal thickness across the posterior pole associated with accommodation (Woodman-Pieterse, Read et al. 2015). Their results showed choroidal thinning during accommodation to a 6 D stimulus in both subfoveal and parafoveal regions, and the regional variation in the parafoveal thinning appeared to correspond to the distribution of the nonvascular smooth muscle within the uvea. This result suggests that these cells play a role in the mechanism for choroidal thinning during accommodation (Woodman-Pieterse, Read et al. 2015).

Several other studies have investigated the choroid in myopia from different perspectives. Nishida et al. (Nishida, Fujiwara et al. 2012) investigated the relationship between choroidal thickness, visual acuity and refractive errors and concluded that the SFCT is a significant predictor of visual acuity in high myopia, and the choroidal thickness is inversely related to the degree of refractive error (Nishida, Fujiwara et al. 2012).

### 2.7.3 Choroidal thickness undergoes diurnal fluctuation

It is well known that intraocular pressure (IOP) undergoes diurnal fluctuation (Bengtsson and Heijl 2005) and studies in animals and humans have demonstrated that axial eye length and choroidal thickness also vary throughout the day. In chick and also marmoset monkey, the choroid becomes thicker during the night and thinner during the day in approximate anti-phase to the diurnal rhythms of eye length (Nickla, Wildsoet et al. 1998, Nickla, Wildsoet et al. 2002). Axial eye length also fluctuates in humans, typically reaching a maximum during the day and minimum at night (Stone, Quinn et al. 2004, Read, Collins et al. 2008). Human choroid also has a consistent pattern of diurnal variation: again approximately in anti-phase to the axial length change. Thus, the choroid is thinnest during the day (around midday) and thickest around midnight, and so the timing of shortest axial length tends to coincide with the timing of the thickest choroid (Brown, Flitcroft et al. 2009, Chakraborty, Read et al. 2011, Chakraborty, Read et al. 2012). However, two other studies based on OCT measures of SFCT report that the choroid is thinnest in the late afternoon (i.e. 5 – 6 pm) and thickest in the morning (e.g 3.00am (Usui, Ikuno et al. 2012) and 9.00am (Tan, Ouyang et al. 2012)). However, Tan et al. (Tan, Ouyang et al. 2012) only made measures between 9.00am and 5.00pm, and so was unable to confirm measures outside that period.

### 2.7.4 Other factors affecting the choroid in healthy individuals

Choroidal thickness also appears to depend on gender: Li et al. (Li, Larsen et al. 2011) reported that SFCT was 18% higher in men than in women when adjusted for age and axial length. Choroidal thickness is also increased after the water-drinking test (WDT – a stress test, in which one litre of water is consumed within 5 minutes) used in glaucoma patients to evaluate aqueous drainage from the eye (Hatanaka, Alencar et al. 2013). The WDT reportedly increases peripapillary and macular choroidal

thickness by 4 to 6% (Mansouri, Medeiros et al. 2013). Alwassia et al. examined choroidal thickness changes in 15 patients who underwent cardiac exercise stress testing. However, despite a significant increase in systolic blood pressure after stress testing, the mean choroidal thickness measurements showed no significant difference before and immediately after the exercise test (Alwassia, Adhi et al. 2013). The effect of smoking on choroidal thickness has been investigated by Sızmaz et al. (Sizmaz, Kucukerdonmez et al. 2013) who examined 17 otherwise healthy smokers (and 17 non-smokers as control, who did not smoke in the experiment). Choroidal thickness was measured at baseline, and at 1 and 3 hours following smoking of one cigarette by the smokers group. The results showed that the mean SFCT decreased significantly from 301 ± 63 μm at baseline to 284 ± 57 μm at 1 hour and 271 ± 80 μm at 3 h after smoking. There were no significant change in SFCT in controls (who did not smoke).. Ulaş et al. (Ulas, Celik et al. 2014) investigated the short-term (within 60 minutes of smoking) and longer-term (smoking histories over 10 years) effects of smoking on choroidal thickness in young males. Their results showed an acute and significant increase in choroidal thickness within 5 minutes of smoking, that returned to baseline levels after 1 hour. Baseline choroidal thickness, did not differ significantly between the healthy young smokers (who had smoked for over 10 years) and non-smokers. Based on the two studies above, smoking caused acute thickening of the choroid which returned to baseline in 1 hour, and progressive thinning until three hours after smoking. But there was no clear evidence that long term cigarette smoking affects choroidal thickness.

Previous studies have also investigated the effect of caffeine on choroidal thickness. Caffeine causes a significant decrease in choroidal thickness for at least 3 to 4 hours (Vural, Kara et al. 2014, Zengin, Cinar et al. 2015) following oral intake in young

healthy adults. The authors speculated that the decrease in choroidal thickness might be a result of reduced ocular blood flow due to the vasoconstrictive effect of caffeine (Zengin, Cinar et al. 2015).

### 2.7.5 Retinal image defocus alters choroidal thickness

#### *2.7.5.1 Animal studies*

During the lens-rearing experiments investigating the effects of imposed retinal defocus on refractive development in animals, described in Section 2.1.1, it was observed that imposed retinal defocus also affected the thickness of the choroid (Wallman, Wildsoet et al. 1995, Hung, Wallman et al. 2000). In particular, it was found that myopic defocus led to a thickening of the choroid, therefore moving the retina forward towards the displaced image plane, and hyperopic defocus caused thinning of the choroid, moving the retina back, again towards the displaced image plane. Moreover, the mechanism of choroidal response to defocus appeared to act entirely within the eye (Wallman, Wildsoet et al. 1995) and at least in the chick, it only required as little as 10 minutes of myopic defocus to initiate the thickening of the choroid (Zhu, Park et al. 2005). The choroid required a longer time to respond to hyperopic defocus and then only responded to a lesser extent compared to its response to myopic defocus (Zhu, Park et al. 2005). Similar results have also been found in monkeys and guinea pigs (Hung, Wallman et al. 2000, Howlett and McFadden 2009). Troilo et al., 2000 showed that in marmosets with induced refractive errors, eyes that were shorter and more hyperopic had thicker choroids than control eyes, and in eyes that were longer and more myopic than control eyes the choroids were thinner (Troilo, Nickla et al. 2000). In summary, in experimental animals including chicks, guinea pigs and monkeys, the sign of defocus causes predictable changes in choroidal thickness: in

each case the choroid changes thickness such that the retina is moved towards the displaced image plane.

#### *2.7.5.2 Human studies*

Read et al. (Read, Collins et al. 2010) investigated ocular axial length and choroidal thickness changes when eyes were exposed to four different types of monocular defocus (no defocus; myopic defocus with +3.00 DS; hyperopic defocus with -3.00 DS; and 0.2 density Bangerter filter) while watching television at 6 meters for 60 minutes. The mean change in axial eye length after 60 minutes in each condition was: 0 µm with no defocus (Control), a 13 µm decrease in axial length with +3.00 DS myopic defocus; an 8 µm increase with -3.00DS hyperopic defocus, and a 6 µm increase in diffuse defocus conditions (Bangerter filter). The mean choroidal thickness increased by 5 µm with no defocus; increased 12 µm with +3.00 DS myopic defocus; decreased 3 µm with -3.00DS hyperopic defocus and decreased 6 µm with Bangerter filter. These results suggested that a short period of defocus in the human eye could lead to small, bi-directional changes in eye length and choroidal thickness. Myopic defocus led to a thickening of the choroid and a reduced axial eye length, whereas hyperopic defocus resulted in thinning of the choroid and an increase in axial eye length as found in animal studies. However, the magnitude of axial length decrease after exposing the retina to myopic defocus seemed to be larger than the increase with hyperopic defocus (Read, Collins et al. 2010). Chiang et al. (Chiang, Phillips et al. 2015) investigated the effect of 2.00 D of hyperopic or myopic retinal defocus induced with contact lenses in 12 East Asian subjects. The results showed that myopic defocus caused a rapid increase in SFCT (statistical significant by 10 min and increased to approximately 20 µm within 60 minutes) and hyperopic defocus led to a decrease in SFCT (significant by 20 – 35 minutes and decreased by approximately 20

μm within 60 minutes). The results may imply that the human eye is more sensitive to myopic defocus, or the choroid is more capable of expanding rather than thinning when exposed to defocus. Wang et al. (Wang, Chun et al. 2016) also compared the effect of defocus on choroidal thickness in 51 Chinese children. While they saw choroidal thinning in response to hyperopic defocus (~4 μm), the response to myopic defocus was more modest. This may be because they used different subjects for each defocus group (17 subjects in each), and each participant received three drops of cyclopentolate 1% prior to the measurement of choroidal thickness, which may have confounded their results.

As discussed earlier in Section 2.7.3, the axial eye length and the choroidal thickness undergo diurnal changes, and the choroidal changes tend to be in anti-phase with the axial eye length changes. Chakraborty et al. further investigated the possible impacts of myopic (Chakraborty, Read et al. 2012) and hyperopic (Chakraborty, Read et al. 2013) defocus on daily rhythms of choroid thickness and eye length. They concluded that the normal daily rhythms of eye length and choroidal thickness in both subfoveal and parafoveal locations could be significantly disrupted when exposed to either myopic or hyperopic defocus (Chakraborty, Read et al. 2012, Chakraborty, Read et al. 2013).

### 2.7.6 Potential mechanisms that alter choroidal thickness

Based on the research described above, the human choroid seems able to change its thickness (both thicken and thin) relatively rapidly when the retina is exposed to defocus. Several theories have been proposed to explain how the choroid might change its thickness in such a short time.

#### 2.7.6.1 Contraction and relaxation of non-vascular smooth muscle

The human choroid and inner sclera contain a network of non-vascular smooth muscle α-actin positive cells (Flügel-Koch, May et al. 1996, Poukens, Glasgow et al. 1998). As discussed in the basic anatomy section (2.5), there are two arrangements of non-vascular smooth muscle cells found in primate choroid: a layer that runs parallel and underneath Bruch's membrane, and a reticulum of flattened lamellae that are oblique to the choroid and lie between lacunae (Poukens, Glasgow et al. 1998). Although the non-vascular smooth muscle cells are distributed throughout the entire choroid, a previous study (Flügel-Koch, May et al. 1996) also showed that they are most densely arranged in the posterior part of the suprachoroidea of the submacular region. The innervation of choroidal non-vascular smooth muscle cells remains debated, but most evidence suggests that non-vascular smooth muscle cells are in close contact with nerve terminals and it is thought that they are innervated by both parasympathetic and sympathetic inputs (Poukens, Glasgow et al. 1998, Schrödl, Tines et al. 2001, May, Neuhuber et al. 2004). Since the choroid contains abundant non-vascular smooth muscle cells which also run between the lacunae spaces, changes in the tonus of these smooth muscle cells could potentially play a role in changing choroidal thickness.

#### 2.7.6.2 Osmotic changes and fluid redistribution

It has been suggested that the choroid could potentially change its thickness and volume via osmotic changes and redistribution of fluid by a variety of mechanisms (Wallman, Wildsoet et al. 1995). The choroid is part of the uveal tract and is the posterior section of the uveoscleral outflow pathway. Animal studies have shown that horseradish peroxidase injected into the anterior chamber can be found in the lacunae of the suprachoroidea after 4 hours (Wallman, Wildsoet et al. 1995). Therefore, changes in the amount of aqueous humour drained through the uvea might account

for the increase in size of lacunae and hence the thickness of the choroid. Another potential way in which the thickness of the choroid may change is by changing the vascular permeability of the blood vessels within the choroid. With increased vascular permeability, plasma proteins would move into the extracellular matrix which in turn would result in an influx of fluid into the extracellular spaces and an increase in thickness of the choroid (Pendrak, Papastergiou et al. 2000). A further potential way in which the thickness and volume of the choroid might be altered is by changing the tonicity of the extracellular fluid via synthesis of osmotically active elements such as proteoglycans molecules. Wallman et al. (Wallman, Wildsoet et al. 1995) found that the choroid in chicks with form deprivation myopia were thickened, with increased amounts of highly charged proteoglycans in the choroidal extracellular matrix which could have caused fluid to enter the choroid and increase its volume and thickness. A similar hypothesis is supported by the work of Nickla et al. (Nickla, Wildsoet et al. 1997) who found that chicks wearing plus-powered (+15D) lenses to create myopic defocus, synthesized greater amounts of proteoglycan molecules than those wearing plano lenses, and significantly less proteoglycans were synthesized in those with hyperopic defocus (wearing -15D lenses).

#### *2.7.6.3 Changes in choroidal blood flow*

The highly vascular nature of the choroid raises the possibility that choroidal thickness changes might be based on changes in blood volume or blood flow. Laser Doppler flowmetry (LDF) has been used for the investigation of choroidal blood flow (ChBF) in chicks (Shih, Fitzgerald et al. 1993) and in humans (Longo, Geiser et al. 2000, Fuchsjäger-Mayrl, Polska et al. 2001). It is a non-invasive technique which investigates the local choroidal blood flow (velocity) in the foveal region, as this region is free of retinal vessels. The patient fixates directly on the laser light: the Doppler

signal is believed to originate predominantly from the moving red blood cells of the choriocapillaris (Riva, Cranstoun et al. 1994). Increased choroidal thickness is reportedly correlated with increased choroidal blood flow (Berenberg, Metelitsina et al. 2012, Kim, Silverman et al. 2013, Ko, Cao et al. 2013) although this relationship has not always been found (Sogawa, Nagaoka et al. 2012).

### 2.7.7 The choroid in common ocular diseases

Recent research has clarified the association between many common eye diseases and the condition of the choroid. McCourt et al. 2010 (McCourt, Cadena et al. 2010) studied 194 eyes with ocular disease and reported that the SFCT in patients with age-related macular degeneration (both wet and dry AMD), diabetic retinopathy, and glaucoma was significantly less than in healthy eyes. However, once the confounding variable of age had been accounted for (choroidal thickness decreases with age), they found no significant difference in choroidal thickness with disease. Nevertheless, many other studies have reported changes in choroidal thickness and choroidal blood flow with disease, as described below.

#### *2.7.7.1 Myopia and risks of sight-threatening diseases*

Myopia is a serious concern in many parts of East Asia (Ding, Shih et al. 2017). Lim et al. (Lim, Gazzard et al. 2009) reported that the mean annual direct costs of myopia for each school child in Singapore (aged 7 to 9 years) was approximately US$148. The National Health and Nutrition Examination Survey in the United States reported that correcting distance refractive error cost between US$3.9 to US$7.2 Billion dollars (Vitale, Cotch et al. 2006). As discussed in Section 2.7.1, myopic eyes are known to have significant thinner choroids, and the choroidal thickness is highly correlated with the degree of myopia. In addition, as myopia is associated with abnormal elongation and structural changes to the eye, myopia dramatically increases the risk for

developing sight-threatening diseases such as myopic maculopathy, retinal detachment, cataract and glaucoma (Saw, Gazzard et al. 2005, Flitcroft 2012).

#### 2.7.7.1.1 Myopic maculopathy

Myopic maculopathy is a common complication of high myopia (Flitcroft 2012). This progressive sight-threatening condition is one of the most common causes of visual impairment in the UK (Evans, Fletcher et al. 2004). Common features of myopic maculopathy include lacquer cracks, choroidal neovascularization, tessellated fundus (corresponding to RPE atrophy), diffuse and patchy chorioretinal atrophy, and macular atrophy (Hayashi, Ohno-Matsui et al. 2010). Vongphanit et al. (Vongphanit, Mitchell et al. 2002) reported a highly non-linear relationship between the prevalence of myopic maculopathy and myopic refractive error. Refractive errors greater than 5 D were 60-fold more likely to develop myopic maculopathy than normal. The prevalence of myopic maculopathy increased to over 50% for myopia greater than 9 D.

#### 2.7.7.1.2 Cataract and Glaucoma

Myopic eyes are structurally different from emmetropic eyes as they have longer axial length and vitreous chamber depths (Scott and Grosvenor 1993). Longer eyes appear to have larger discs, higher cup–disc ratios, increased optic nerve fibre layer defects and possibly a weaker lamina cribrosa, which may result in a higher susceptibility to glaucoma (Fong, Epstein et al. 1990). A meta-analysis of 11 studies of myopia as a risk factor for glaucoma concluded that for myopia up to −3 D, the odds ratio was 1.65 and for higher levels of myopia the odds ratio was 2.46 (Marcus, de Vries et al. 2011).

The Blue Mountain Eye Study (Lim, Mitchell et al. 1999) showed that the odds ratio for the development of Posterior Subcapsular Cataract (PSC) increased from 2.1 for low myopia to 3.1 for moderate myopia and 5.5 for high myopia and that hyperopia appeared protective against PSC (OR 0.6). The Salisbury Eye Evaluation Study

(Chang, Congdon et al. 2005) found weaker associations with nuclear cataract and no association between myopia and cortical cataract. The Tanjong Pagar survey also reported significant associations between nuclear cataract and posterior subcapsular cataract but not cortical cataract (Wong, Foster et al. 2003). The mechanism linking myopia to cataract appears to be unknown although it has been suggested that damage to the rod outer segments in myopia may result in release of cataractogenic substances (Zigler, Bodaness et al. 1983).

#### *2.7.7.2 The choroid in age-related macular degeneration*

Age-related macular degeneration (AMD) is one of the most common causes of blindness in developed countries (Mitchell, Smith et al. 1995, Klein, Klein et al. 1999, Kawasaki, Yasuda et al. 2010). Patients with AMD experience a slow but progressive loss of central vision: vision loss can occur within months, or over many years, depending on the type and severity of AMD. The pathophysiology of AMD is not fully understood, although smoking, diet, genetic factors and UV light are believed to play a role in this condition (Age-Related Eye Disease Study Research 2000, McCarty, Mukesh et al. 2001, Hyman and Neborsky 2002). Some studies have suggested that ischemia may play a major role in the pathogenesis of AMD (Berenberg, Metelitsina et al. 2012, Coleman, Silverman et al. 2013). Specifically, it has been suggested that the lipoid deposition in the choroid and Bruch's membrane leads to increased resistance to blood flow (Friedman 1997) and thus decreased choroidal perfusion. Berenberg & Metelitsina et al. (2012) studied 239 eyes to investigate the association between the extent of drusen and foveolar choroidal blood flow in AMD. They suggested that increased extent of drusen may lead to a decrease in choroidal blood volume and flow and an increased risk of developing advanced disease (Berenberg, Metelitsina et al. 2012). One recent study has also suggested a strong correlation between extent of

drusen, progression of AMD and reduction in choroidal thickness (Ko, Cao et al. 2013). These studies support the idea that ischemia may play a significant role in the pathogenesis of AMD. Coleman et al. (2013) further suggested that AMD should be treated using pharmacological agents to increase the choroidal perfusion to reduce ischemia and thus reduce disease progression (Coleman, Silverman et al. 2013).

#### *2.7.7.3 The choroid in diabetic retinopathy*

Diabetes mellitus is a serious medical issue worldwide. Untreated diabetes ultimately leads to a variety of secondary complications, such as neuropathy, heart disease, kidney failure and retinopathy. In the United States, among adults aged between 20-74 years, diabetic retinopathy (DR) is reportedly the leading cause of new cases of blindness (Rabb, Gagliano et al. 1990, Harris 1998). A study by Fong and colleagues found that the prevalence of retinopathy was about 80% after 15 years of diabetes having been diagnosed (Fong, Aiello et al. 2004).

The clinical signs of diabetic retinopathy within the retinal circulation, include microaneurysms, haemorrhages, intraretinal microvascular abnormalities and neovascularization (Wilkinson, Ferris et al. 2003). In addition to the clinical signs in the retina that can be visualised via ophthalmoscopy, evidence also suggests that choroidal angiopathy may coexist along with retinal vascular damage (Fukushima, McLeod et al. 1997, Adhi, Regatieri et al. 2013).

Several recent studies have shown that patients with diabetic retinopathy have thinner choroids than age-matched normals (Esmaeelpour, Povazay et al. 2011, Esmaeelpour, Brunner et al. 2012, Adhi, Regatieri et al. 2013). Adhi et al. analysed the morphology of the choroid in diabetic retinopathy; their results suggested that the SFCT, and the thickness of the medium choroidal vessel (Sattler's) layer and choriocapillaris layer were significantly reduced in patients with proliferative diabetic

retinopathy (with and without macular oedema) (Adhi, Regatieri et al. 2013). Studies of three-dimensional choroidal OCT maps have also found that the central and inferior choroids are thinner in diabetic eyes when compared to normal. In addition, the thickness of the choroid is inversely proportional to the severity of diabetic retinopathy (Esmaeelpour, Povazay et al. 2011, Esmaeelpour, Brunner et al. 2012), suggesting that choroidal thinning could be one of the predictors for progression of diabetic retinopathy.

#### *2.7.7.4 The choroid in glaucoma*

Glaucoma is the second leading cause of blindness worldwide (Quigley and Broman 2006). It refers to a group of ocular disorders with multifactorial etiology, which often cause an asymptomatic gradual loss of vision until the disease becomes advanced. The exact underlying mechanisms of glaucoma remain largely unknown. In the ischemic theory, ganglion cell death is thought to be a result of ischemia (Osborne, Ugarte et al. 1999, Sun, Pang et al. 2010). An OCT study (Banitt 2013) investigated choroidal thickness in relation to glaucoma: the results indicated that the peripapillary choroid is thinner in some subsets of glaucoma compared to normal. A recent study by Bayhan et al. evaluated the choroidal thickness in pseudoexfoliative glaucoma. Their results concluded that pseudoexfoliative glaucoma causes significant thinning in the nasal choroid (Bayhan, Bayhan et al. 2016).

#### *2.7.7.5 The choroid in retinitis pigmentosa*

Retinitis pigmentosa is an inherited degenerative eye disease which is characterized by progressive loss of peripheral vision and night vision, which ultimately leads to severe visual impairment and loss of central vision. Adhi et al. (Adhi, Regatieri et al. 2013) evaluated the morphology and vascular layers of the choroid in retinitis pigmentosa and concluded that there was thinning of the large choroidal vessel

(Haller's) layer in retinitis pigmentosa (Adhi, Regatieri et al. 2013). Investigations using magnetic resonance imaging (MRI) have also demonstrated that retinal-choroidal blood flow is reduced in retinitis pigmentosa patients (Zhang, Harrison et al. 2013).

In summary, recent research has demonstrated that many of the most common eye diseases are characterized by changes in the morphology of the choroid and thinning of the choroid. Although the causal relationships between the choroidal changes and the diseases (and disease progression) are yet to be established, it seems likely that choroidal changes play an important role in the pathogenesis of several of the most prevalent and sight-threatening ocular conditions.

## 2.8 Effects of pharmaceutical agents on choroidal thickness

With the advancement of OCT imaging, researchers are now able to image the choroid *in vivo,* and in a fast, safe and almost real-time manner. A number of recent studies have examined the effect of various topical agents on human choroidal thickness, and a summary of the effect of mydriatic pharmaceutical agents on human choroidal thickness is given in *Table 2*.

### 2.8.1 Muscarinic cholinergic antagonists

Mwanza et al. (Mwanza, Sayyad et al. 2013) compared choroidal thickness before and after pupil dilation with 1% tropicamide in healthy individuals and in patients with glaucoma. They found no statistically significant differences between pre- and post-dilation choroidal thickness in both normal and glaucomatous eyes. Similar findings were reported by Oner et al. (Oner, Bulut et al. 2016), who investigated the effects of 1% tropicamide on 37 healthy adults, with the topical eye drops applied three times at 10-minute intervals, and choroidal thickness measures made at

baseline and 40 minutes after drop instillation. However, some studies have reported opposite results. Kara et al. (Kara, Demircan et al. 2014) investigated the effect of 1% tropicamide instilled monocularly 3 times at 5-minute intervals in healthy Participants, with SFCT measured before and 45 minutes after drop administration. They found that SFCT significantly decreased in both the dilated eye and contralateral eye after tropicamide instillation. A similar result was also found by Yuvaci et al. (Yuvaci, Pangal et al. 2015), where administration of 1% tropicamide caused thinning of the choroid.

In the same study, Yuvaci et al. also investigated the effects of cyclopentolate 1%, another muscarinic antagonist, on choroidal thickness. Again, their results showed that cyclopentolate also caused a decrease in choroidal thickness (Yuvaci, Pangal et al. 2015). In contrast, Oner et al. reported that instillation of 1% cyclopentolate induced significant choroidal thickening (Oner, Bulut et al. 2016).

The effect of homatropine hydrobromide on human choroid has also been examined. Homatropine is a non-selective anticholinergic agent that is closely related to atropine, but has shorter-lasting mydriatic and cycloplegic effects. Topical 2% homatropine hydrobromide resulted in a small but significant increase in subfoveal and parafoveal choroidal thickness 60 minutes after eye drop instillation (Sander, Collins et al. 2014).

Recently, Sander et al. have published an Abstract further investigating the effect of the interaction between retinal image blur and homatropine 2% on the choroidal thickness of young healthy adults (Sander, Collins et al. 2016). Their findings showed a significant interaction between homatropine and optical blur. The subfoveal choroid underwent a small but significant thinning with hyperopic blur and placebo (mean change of -11.3 ± 2.9 μm at 60 minutes), hyperopic blur and homatropine treatment led to small amounts of subfoveal choroidal thickening (mean change of +2.9 ± 2.4

μm at 60 minutes). The effects of myopic blur and placebo, myopic blur and homatropine, and homatropine alone, all had similar effects in increasing SFCT. Therefore, the authors concluded that homatropine 2% appeared to block the thinning effect of hyperopic blur on choroidal thickness, but did not enhance the thickening effect of myopic blur.

The short-term effect of atropine eye drops on human choroidal thickness has also been investigated (Zhang, Zhou et al. 2016). Choroidal thickness in the eyes of 30 healthy Chinese children was measured before and after the use of 1% atropine gel twice daily for a week. Their results showed that 1% atropine gel can significantly increase choroidal thickness by ~15 μm (Zhang, Zhou et al. 2016).

### 2.8.2 Adrenergic receptor agonists

Phenylephrine is a selective α1-adrenergic receptor agonist commonly used in ophthalmic practice to dilate the pupil. Two studies (Kara, Demircan et al. 2014, Yuvaci, Pangal et al. 2015) report decreased choroidal thickness with phenylephrine 2.5% eye drops, although a similar study by Sander (Sander, Collins et al. 2014) found no significant change in choroidal thickness with 2.5% phenylephrine. Instillation of Mydrin-P (Tropicamide 5 mg/mL and phenylephrine hydrochloride 5 mg/mL; Santen Pharmaceuticals, Japan) administered three times at 5-minute intervals, was found to have no significant influence on choroidal thickness (Kim, Kwon et al. 2012).

### 2.8.3 Summary table of the effect of various mydriatic pharmaceutical agents on human choroidal thickness

| Mydriatic | Authors | Protocol | Effect on ChT |
|---|---|---|---|
| **Atropine 1%** | Zhang et al., 2016 | Gel given 2 times daily for 1 week, OCT scan before and after 1 week | Thicker |
| **Cyclopentolate 1%** | Oner et al., 2016 | Drops given 3 times at 10-min intervals, OCT scan before and at 40-min after drop instillation | Thicker |
| | Yuvaci et al., 2015 | Drops given 3 times at 5-min intervals, OCT scan before and at 50-min after drop instillation | Thinner |
| **Homatropine 2%** | Sander et al., 2014 | Drop given once, OCT scan before and 30 and 60-min after drop instillation. | Thicker |
| | Sander et al., 2016 | Drop given once, OCT scan before and 30 and 60-min after drop instillation | Thicker |
| **Mydrin-P*** | Kim et al., 2012 | Drops given 3 times at 5-min intervals, OCT scan before and after pupil size >6 mm | No significant effect |
| **Phenylephrine 2.5%** | Kara et al., 2014 | Drops given 3 times at 5-min intervals, OCT scan before and at 45-min after drop instillation | Thinner |
| | Sander et al., 2014 | Drop given once, OCT scan before and 30 and 60-min after drop instillation. | No significant effect |
| | Yuvaci et al., 2015 | Drops given 3 times at 5-min intervals, OCT scan before and at 50-min after drop instillation | Thinner |
| **Tropicamide 1%** | Kara et al., 2014 | Drops given 3 times at 5-min intervals, OCT scan before and at 45-min after drop instillation | Thinner |
| | Mwanza et al., 2013 | Drop given once, OCT scan before and 20–30-min after drop instillation, with the option of a second drop if the first failed to dilate the pupil enough. | No significant effect |
| | Oner et al., 2016 | Drops given 3 times at 10-min intervals, OCT scan before and at 40-min after drop instillation | No significant effect |
| | Yuvaci et al., 2015 | Drops given 3 times at 5-min intervals, OCT scan before and at 50-min after drop instillation | Thinner |

*Tropicamide 5 mg/mL and phenylephrine 5 mg/mL; Santen Pharmaceuticals, Japan

***Table 2.*** *Summary of the effect of various mydriatic pharmaceutical agents on human choroidal thickness.*

## 2.9 Aims and rationale of this study

The Literature reviewed in the previous sections shows that myopia prevalence has reached high levels throughout the developed world, and that myopia is a significant risk factor for a number of sight threatening diseases including myopic maculopathy, glaucoma etc. The methods available for preventing the development of myopia in children are in their early stages and have yet to receive widespread acceptance. However, methods for slowing progression in children who have developed myopia are available, but are limited in efficacy. Currently, atropine can slow progression by ~50% over the long term and the efficacy of optical methods is similar (~45%). Despite decades of animal research the mechanisms by which optical defocus and also atropine inhibit eye elongation remain unknown and it is therefore not possible to predict how they might interact if both were used together to attempt to slow progression more effectively than either method alone. However, the results of animal studies indicate that imposing hyperopic defocus on the retina with lenses causes rapid thinning of the choroid, followed later by eye elongation and the development of myopia (Wallman, Wildsoet et al. 1995). Conversely, creating myopic defocus causes rapid thickening of the choroid, followed by a slowing of eye growth and the development of hyperopia (Wallman, Wildsoet et al. 1995). Because of the predictable order in which these events occur, it is thought that the choroid is an intermediary between the retina and sclera, with changes in choroidal thickness reflecting changes in the eye-growth signalling pathway between retina and sclera (Wallman and Winawer 2004, Nickla, Wilken et al. 2006, Nickla, Damyanova et al. 2009). Thus, an observed increase in choroidal thickness would indicate signals for reduced eye growth, whereas a decrease in thickness would indicate signals for accelerated growth and myopia development. Similar changes in the thickness of the

human choroid have also been reported following short-term imposition of retinal defocus (Read, Collins et al. 2010, Chiang, Phillips et al. 2015, Wang, Chun et al. 2016) and it has been proposed that these rapid changes in choroidal thickness may serve as an indicator of pending changes to refractive status (Troilo, Nickla et al. 2000, Fitzgerald, Wildsoet et al. 2002, Read 2016, Wang, Chun et al. 2016).

Both atropine and retinal defocus modify myopia progression in humans and the choroid has been implicated in eye growth and myopia development. By taking advantage of the relatively rapid and predictable change in human choroidal thickness in response to hyperopic and myopic retinal defocus, this study investigated the interaction and additive effect of atropine and imposed retinal defocus at the level of the choroid in humans.

**Preliminary study of atropine in young adults:**

The preliminary study served a number of purposes. It allowed the protocol for combining atropine with optical defocus to be tested and refined before conducting the main study in children. It was conducted in young adults (University students) because these were easier to recruit than children. Only one sign of defocus was investigated in the preliminary study in order to reduce the number of visits required from 4 (2 (±) defocus before atropine and 2 (±) defocus after atropine) to 2 (1 defocus before and 1 defocus after atropine). Also, it was assumed that an ethics application to conduct such a study in children would be strengthened with preliminary data in adults. The concentration of atropine used in the preliminary study was chosen as 0.5%, that is midway between the concentration readily available in New Zealand (1.0%) and the very low concentrations that are now commonly used for clinical myopia control (0.01%). The reason for not using very low concentrations (e.g. 0.01%)

was because clinical trials of 0,01% atropine have shown that the effect on myopia progression is not evident for some months (Chia, Chua et al. 2014, Chia, Lu et al. 2016). A concentration of 1% would have produced very long-lasting effects which would have been detrimental to the participants.

The aim of the preliminary study was to test the effect of 0.5% atropine on choroidal thickness changes induced by hyperopic retinal defocus in young adults.

**Main study of atropine in children:**

The main study in children aimed to address the following questions

The study was conducted over 6 months. The primary reason was to check that he short-term effects of atropine on choroidal responses did not diminish over time (tachyphylaxsis). The primary intent was not to assess the efficacy of atropine in slowing progression and that is the reason for not including a control group and not following the participants for longer.

The main study was conducted in children because myopia develops and progresses fastest in children and atropine is generally prescribed to children.

The study was conducted in Taiwan because the prevalence of myopia is high and atropine is regularly prescribed to control myopia in Taiwan and the opportunity was available to conduct the study in Taiwan rather than New Zealand.

The summation of atropine with both myopic and hyperopic defocus was investigated to give a complete picture of the interaction of atropine with the retinal image of the environment, which could contain elements of both hyperopic and myopic defocus.

Specifically, the following questions were asked:

- Does atropine affect choroidal thickness in children prescribed 0.3% atropine for myopia control?
- Does atropine affect choroidal thickness changes resulting from hyperopic or myopic retinal defocus in children prescribed 0.3% atropine for myopia control?
- Do the effects of atropine and optical defocus summate (as a basis for predicting efficacy of future combined optical and pharmaceutical therapy)?
- Do the effects of atropine on choroidal thickness and on choroidal response to induced retinal defocus change after children have taken atropine for 3 and 6 months?

# 3 Preliminary study – Atropine in young Asian adults

The aim of this preliminary study was to test the potential effect of 0.5% atropine on choroidal thickness changes induced by hyperopic retinal defocus in young adults, prior to conducting a more extensive study in children.

## 3.1 Participants

Ten adult East Asian participants aged between 18 and 24 years (mean ± SD, 20.90 ± 1.79 years) participated in this study, which adhered to the tenets of the Declaration of Helsinki. Ethics approval was obtained from the University of Auckland Human Participants Ethics Committee (Reference 010617) and informed consent was obtained from all participants in writing. The inclusion criteria for this study were: age 18 to 25 years, with Spherical Equivalent Refraction (SER) with spectacle prescription between -1.00 to -5.00 D (mean ± SD, -2.63 ± 1.28 D), with little astigmatism (≤ 1.00 D) or anisometropia (≤ 1.00 D). Also, individuals who had undergone any myopia control treatment (including Orthokeratology) were excluded as were individuals with amblyopia, ocular pathology or other ocular anomalies (e.g. surgery, trauma) that might have influenced the measurements. Prior to enrolment, all participants underwent a comprehensive eye examination to confirm their refractive status and to ensure the absence of binocular or pathological abnormalities or history of significant ocular surgery or trauma. All 10 participants had visual acuity of logMAR 0.00 or better and all had previous experience of wearing contact lenses.

## 3.2 SS-OCT system and scan protocols

The choroidal thickness measurements were obtained using a Swept-Source Optical Coherence Tomography (SS-OCT) Topcon DRI OCT-1 Atlantis (Topcon Corp., Tokyo,

Japan; http://www.topcon.co.jp/), with an axial scan rate of 100,000 Hz operated at the 1 μm wavelength region. The wavelength-sweeping laser had a tuning range of approximately 100nm centred at 1,050nm, allowing a high axial resolution of 8μm to be obtained (http://www.topcon.co.jp/). Compared to spectral-domain OCT centred at 800μm, the longer wavelengths enable deeper penetration of ocular tissues and allowed a three-dimensional (3D) high contrast image of the choroid to be obtained. To enhance the signalling and imaging of the choroid, the "chorioretinal" scanning mode of the instrument was selected.

A 3D imaging data set covering an area of 6 x 6 $mm^2$ centred over the macula was obtained from each participant by using a scan protocol of 512 (horizontal) x 128 (vertical) A-scans per data set. The scanning was carried out by an invisible scanning laser so that eye movements were minimised during the scan, while the participant viewed the fixation target. The choroidal thickness was measured by the automatic detection of the outer-border (RPE) and the chorioscleral border. After the choroidal thickness map was obtained from 3D imaging, a grid (*Figure 2*) used previously in Early Treatment Diabetic Retinopathy Study (ETDRS) (Diabetic Retinopathy Study Research 1985) was applied to the map to give automated averaged measures of choroidal thickness within the various segmentations. The ETDRS grid was automatically aligned with the participants fixation, which generally also aligned with the fovea. In situations where there was misalignment with the fovea, the ETDRS grid was manually realigned.

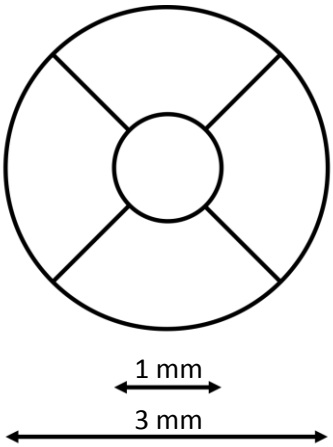


***Figure 2.*** *ETDRS grid overlay centred at the fovea was used to generate automated mean choroidal thickness measures. Central 1 mm diameter circle represents SFCT. Thickness within the 4 segment annulus (1 – 3 mm) diameter represents PFCT.*

The measure provided for the central 1 mm diameter circle was used as the measure of SFCT. The mean of the values for the 4 segments within the 3mm diameter annulus were used as the measure of Parafoveal Choroidal Thickness (PFCT). The variation in axial lengths of the eye across the different participants will have resulted in somewhat different magnification of the OCT image between participants. This magnification effect mainly relates to the transverse directions, rather than the axial (i.e. depth) direction (Parthasarathy and Bhende 2015). Thus, the magnification effect has the potential to slightly alter the position of the ETDRS grid lines relative to the transverse position on the retina between subjects. Since the main comparisons were made within subjects (i.e. before and after atropine) rather than between subjects, the effect of magnification was not corrected for in this study.

## 3.3 Experiment protocol

**Summary:** Participants attended two sessions that allowed choroidal measures to be made at the same time on two consecutive afternoons. At the first visit, 2.00D of hyperopic retinal defocus was applied to the Experimental (non-dominant) eye for 60 minutes, while the fellow (Control, dominant) eye was fully corrected. Eye dominance was determined using a simple pointing task (Coren and Kaplan 1973). OCT measures of SFCT were made in both eyes before applying defocus and at 20-minute intervals during defocus. At the end of the hour of defocus on Day 1, all Experimental eyes were treated with one drop of 0.5% atropine. On Day 2 (i.e. 22 hours after instillation of atropine), the Experimental eye again received 2.00D of hyperopic retinal defocus for 60 minutes, and the fellow (Control) eye was fully corrected. OCT measures of SFCT were made as for Day 1.

**Stabilisation period:** It has been reported that accommodation (Mallen, Kashyap et al. 2006), exercise (Read and Collins 2011) and diurnal fluctuations (Read, Collins et al. 2008, Chakraborty, Read et al. 2011) can all cause short-term changes in axial length and choroidal thickness. Therefore, measurements were made at the same time of day for each participant (between 12 noon and 5 pm). In addition, prior to each session of measurements and before applying defocus, participants viewed a video movie for 20 minutes (binocular viewing, seated at 6 m from the screen with full distance correction for both eyes) to reduce the influence of previous visual and non-visual tasks on choroidal thickness.

**Monocular defocus during the measurement period:** Following the stabilisation period, five consecutive non-cycloplegic autorefraction measures were made with a autorefractor (Shin-Nippon NVision-K 5001; http://www.shin-nippon.jp/) to confirm the refractive status of each eye. To induce and maintain the desired level of retinal

defocus (2.00D of monocular defocus), participants wore single vision disposable contact lenses (Johnson & Johnson ACUVUE Oasys; http://www.jnj.com/). The desired level of defocus was combined with the participant's refractive correction into one single contact lens power (e.g. if a participant's distance prescription was -3.00 DS, then to achieve 2.00 D of hyperopic defocus at the fovea in that eye, a -5.00 DS contact lens was given). After contact lenses had settled on the eye (3 – 4 minutes), five consecutive autorefractor measures were again made in each eye to confirm that the desired refractive status had been achieved, before the measurement period commenced.

Participants then viewed a video movie binocularly at 6m for 60 minutes while remaining seated and as still as possible. OCT measures were made at 20-minute intervals in both eyes during the 60-minute viewing time. To ensure reasonably large pupil diameters which facilitate rapid OCT measures, the ambient lighting was maintained at about 10 lux (as adopted in previous similar studies (Read, Collins et al. 2010, Chiang, Phillips et al. 2015)) measured with a Digital Light Meter (TES-1335; http://www.tes.com.tw/).

At the end of the first session, trial contact lenses were discarded, and one drop of 0.5% atropine was administered to the lower conjunctival fornix of the participant's Experimental (non-dominant) eye. The second-day session also consisted of a 20-minute stabilisation and 60-minute measurement period with the same amount of defocus induced in the Experimental eye, to investigate the influence of atropine on the response to hyperopic defocus. Since the participant's Experimental eye was cyclopleged with atropine on the second day, the autorefractor was again used to measure the participant's refractive error so as to combine the desired level of defocus into one contact lens power.

## 3.4 Statistical analysis

Statistical analyses were performed using IBM® SPSS® Statistics 20. The statistical model employed was repeated measures ANOVA with General Linear Model (GLM), with 3 within-subject factors: time (0, 20, 40, 60 minutes), Experimental versus Control eye, and Day 1 versus Day 2. Bonferroni-corrected pairwise comparisons were performed for any variables with significant within-subject effect and interactions. A p-value ≤ 0.05 was considered statistically significant.

## 3.5 Results

The results in this section are all given as mean ± 1SEM, unless otherwise specified. The presenting SFCT & PFCT in Control eyes were very similar between Day 1 and Day 2 (Day 1 SFCT = 260 ± 37 µm versus Day 2 SFCT = 260 ± 38 µm, p = 0.91. Day 1 PFCT = 261 ± 33 µm versus Day 2 PFCT = 260 ± 33 µm, p = 0.44).

Similarly, the presenting SFCT and PFCT in Experimental eyes were also similar between Day 1 and Day 2, i.e. before and 22 hours after instilling atropine into the Experimental eye. (Day 1 SFCT = 253 ± 32 µm vs Day 2 SFCT = 249 ± 31 µm, p = 0.16. Day 1 PFCT = 252 ± 30 µm vs Day 2 PFCT = 249 ± 30 µm, p = 0.09).

On Day 1, Experimental eyes exposed to 2.00 D hyperopic retinal defocus developed progressively thinner choroids (Maximum change: SFCT = 10 ± 2 µm at 40 mins (p = 0.004); PFCT = 7 ± 2 µm at 60 mins (p = 0.05). Changes in both SFCT and PFCT in Control eyes did not reach statistical significance (SFCT: minimum p = 0.17; PFCT: minimum p = 0.26).

However, unlike for Day 1, 60 minutes exposure to 2.00 D hyperopic defocus failed to thin the choroid. The maximum change in SFCT occurred at 60 minutes with a

non-significant thickening of 2 ± 2 µm (p = 0.36). For PFCT the maximum thinning of 2 ± 1 µm occurred at 20 minutes but did not reach significance (p = 0.19). *Figure 3* shows the change in mean SFCT for Control and Experimental eyes on Day 1 and Day 2 over the 60-minute testing sessions. A summary of SFCT and PFCT and key statistics are shown in *Table 3* below. The mean changes in PFCT of Control and Experimental eyes on Day 1 and Day 2 over the 60-minute testing sessions are shown in *Figure 4*.

| **(a) SFCT** | | Control Eye (µm) | | Exp Eye (µm) | |
|---|---|---|---|---|---|
| Baseline SFCT (mean ± SEM) | | Day 1 (260 ± 37) | Day 2 (260 ± 38) | Day 1 (253 ± 32) | Day 2 (249 ± 31) |
| SFCT change over time | 0 | 0 | 0 | 0 | 0 |
| | 20 | 2 ± 1 | 0 ± 1 | -6 ± 2 | 0 ± 1* |
| | 40 | 2 ± 1 | -1 ± 2 | **-10 ± 2** | 2 ± 1* |
| | 60 | 0 ± 2 | 2 ± 2 | -9 ± 3 | 2 ± 2* |
| **(b) PFCT** | | Control Eye (µm) | | Exp Eye (µm) | |
| Baseline PFCT (mean ± SEM) | | Day 1 (261 ± 33) | Day 2 (260 ± 33) | Day 1 (252 ± 30) | Day 2 (249 ± 30) |
| PFCT change over time | 0 | 0 | 0 | 0 | 0 |
| | 20 | 1 ± 1 | -2 ± 2 | -6 ± 3 | -1 ± 1 |
| | 40 | -2 ± 3 | 0 ± 1 | **-6 ± 1** | -1 ± 1* |
| | 60 | -2 ± 2 | 0 ± 1 | **-7 ± 2** | 1 ± 1* |

***Table 3.*** *Summary table for mean baseline choroidal thickness in Control eyes, Experimental eyes, and the change in choroidal thickness over 60 minutes of optical defocus for (a) subfoveal choroidal thickness and (b) parafoveal choroidal thickness. Bonferroni corrected P-values were calculated with reference to baseline and values ≤ 0.05 are in bold. Asterisks (*) indicate a significant change in choroidal thickness between Day 2 and Day 1.*

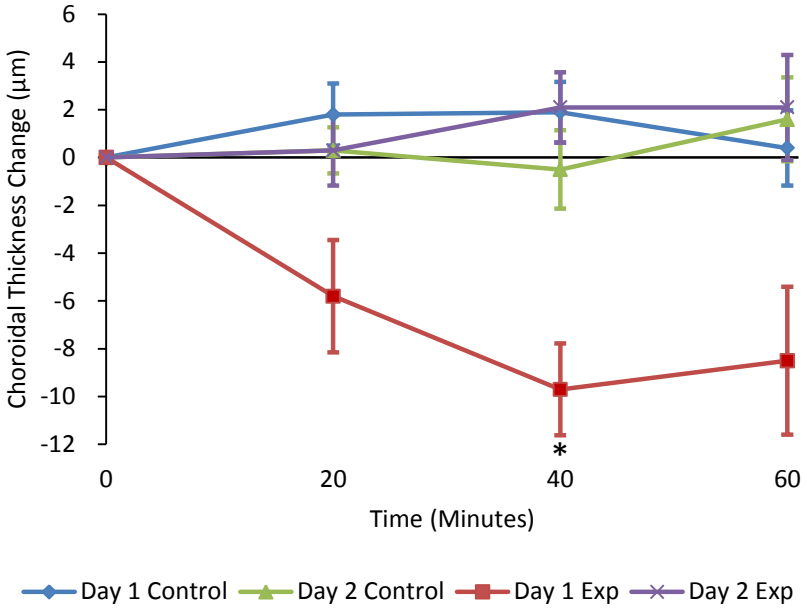


***Figure 3.*** *Mean changes in subfoveal choroidal thickness (SFCT) for Control and Experimental eyes on Day 1 and Day 2 over the 60-minute testing session. Error bars represent standard error of mean. The asterisk (*) indicates a significant change in choroidal thickness from baseline (Bonferroni-corrected pairwise comparison $p \leq 0.05$).*

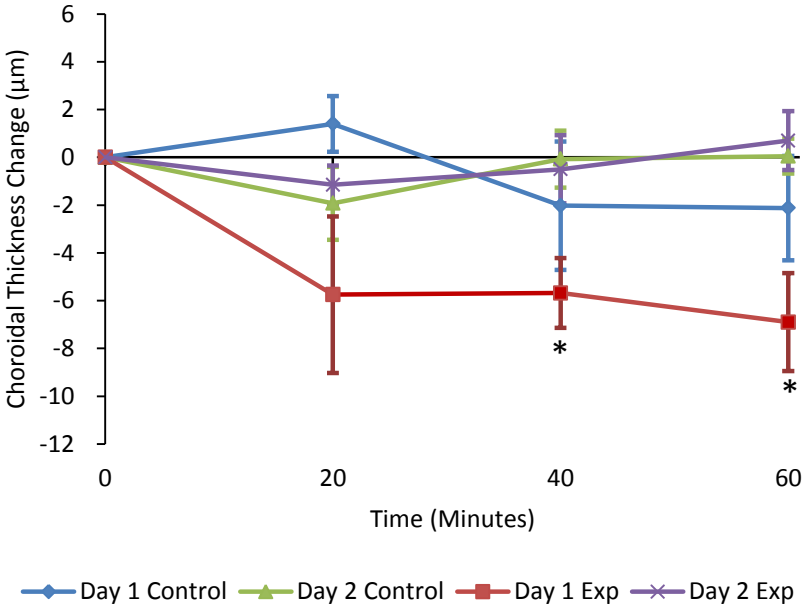


***Figure 4.*** *Mean changes in parafoveal choroidal thickness (PFCT) for Control and Experimental eyes on Day 1 and Day 2 over the 60-minute testing session. Error bars represent standard error of mean. The asterisks (*) indicate a significant change in choroidal thickness from baseline (Bonferroni-corrected pairwise comparison $p \leq 0.05$).*

## 3.6 Discussion

Previous studies have demonstrated that hyperopic defocus applied to the retina of animals (Wallman, Wildsoet et al. 1995, Zhu, Park et al. 2005) and humans (Read, Collins et al. 2010, Chiang, Phillips et al. 2015, Wang, Chun et al. 2016) causes thinning of the choroid. Our results show that in humans, choroidal thinning in response to hyperopic defocus is abolished by one drop of 0.5% atropine, when measured 22 hours after instillation. This concentration is well within the range of concentrations (0.01 – 1%) that have been used for myopia control (Huang, Wen et al. 2016). Our results also show that the resting (baseline) thickness of the choroid is unaffected 22 hours after instillation of 0.5% atropine, which appears contrary to previous reports showing that atropine causes an increase in choroidal thickness. Nickla and colleagues (Nickla, Zhu et al. 2013) showed that in chick, intraocular injections of muscarinic antagonists including atropine, induced thickening of the choroid 3 hours after injection, even in eyes wearing -10 D negative lenses. Zhang and colleagues (Zhang, Zhou et al. 2016) showed that administration of 1% atropine gel twice daily for one week in children resulted in an increase in resting subfoveal choroidal thickness of ~15 µm. There are some potential reasons why the choroidal thickening effect of atropine was not observed in our study. It is possible that 1 drop of 0.5% atropine in the Experimental eye was not potent enough to induce an overall increase in choroidal thickness, or that we had simply not waited long enough to see an effect. However, the fact that the choroidal thinning response to hyperopic defocus was abolished, confirms that atropine had reached receptors or other target sites that were able to either directly or indirectly influence the choroid. The finding that the two actions of atropine, abolition of choroidal thinning and increase in baseline choroidal thickness can occur independently is consistent with the idea that choroidal thinning and thickening are mediated by different mechanisms as suggested by Nickla et al.

(Nickla, Zhu et al. 2013) on the basis of work conducted in the chick model of myopia. Nickla and colleagues proposed that choroidal thinning is likely mediated via contraction of choroidal, non-vascular smooth muscle by acetylcholine, whereas thickening is via a dopaminergic or nitrergic pathway.

Thus, although previous studies have demonstrated that one week of atropine use causes thickening of the human choroid, the results of this study show that one drop of 0.5% atropine does not cause an increase in choroidal thickness when measured 22 hours after instillation. However, atropine does abolish the normal thinning of the choroid caused by hyperopic retinal defocus. This may indicate that the previously reported choroidal thickening effect of atropine is mediated by a different mechanism than that responsible for its inhibitory effect on choroidal thinning induced by hyperopic defocus. This inhibitory effect on choroidal thinning suggests that atropine may act to block the myopiagenic effects of hyperopic retinal defocus present with accommodative lag during near work.

# 4 Study in children

The results of the preliminary study indicate that in young adults atropine can have a significant effect in abolishing the normal thinning response of the choroid to imposed hyperopic retinal defocus. However, as discussed earlier, it is important that the effects of defocus and atropine and their interaction, be examined in children because optical and pharmaceutical methods of myopia control are primarily directed at children. In addition, the preliminary study did not investigate the effect of atropine on the choroidal response to myopic defocus. The possibility remains that atropine may also abolish the choroidal thickening effect of myopic defocus.

This chapter describes the methods used in the main study of atropine in children. Chapter 5 presents the Results for the main study in children and Chapters 6 and 7 provide the Discussion and Conclusions relating to all the results obtained in this thesis.

## 4.1 Participants

Children were recruited primarily through the Department of Ophthalmology, China Medical University Hospital, Taichung, Taiwan. Human Ethics approval was obtained from the Research Ethics Committee of China Medical University & Hospital (Protocol No. / CMUHREC No: CMUH104-REC3-069) and participants were treated in accordance with the Declaration of Helsinki. Before entering the study, all participants were given a written Participant Information Sheet and the opportunity to have their questions answered. Informed consent was obtained from parents in writing. Informed assent was obtained from children verbally, as specified in the ethics approval.

## 4.2 Inclusion and exclusion criteria for participation

### 4.2.1 Inclusion criteria

To participate, children had to have been assessed by an Ophthalmologist and be ready to start on 0.3% atropine for at least 6 months as treatment for myopia control. In addition, the parent/guardian of the participant had to have agreed to be responsible for instilling atropine eye drops at night. Atropine eye drops in a variety of concentrations from 1.0% to 0.01% have been and are currently used to control myopia in children. Over the last few years in Taiwan, there has been a shift from prescribing high concentrations (1 – 0.5%) to prescribing lower concentrations (0.3 – 0.1%) (Fang, Chou et al. 2013) and now 0.05% (Wu, Yang et al. 2011). To maintain consistency with the preliminary study as far as possible, children prescribed 0.3% atropine were the subject of this study. Atropine (0.3%) is currently the highest concentration used routinely at China Medical Hospital from where the children were recruited.

**Age:** Participants were required to be between 6 and 15 years at the time of enrolment. A previous study suggested that myopia progresses most rapidly between the ages of 6 to 15 years (Zadnik 1997), tending to slow after 16 years of age (Goss and Winkler 1983). Thus, parents would be most likely to take their children to see an eye care provider for myopia correction and control between the ages of 6 and 15 years, and this would be the most appropriate time to start a myopia control treatment with atropine.

**Refractive error:** All participants underwent a comprehensive eye examination by a qualified Optometrist to confirm their refractive status. Refractive error was required to be between −0.75 D and −4.50 D spherical equivalent (sphere + ½ cylinder), when prescribing the maximum plus correction that provided the best VA possible. In this

study, cycloplegics were not used because they can potentially affect the choroid (see *Table 2* in Section 2.8.3). A minimum refractive error of −0.75 D was chosen because in typical clinical practice myopia control would likely not be considered for myopia less than -0.75 D. A maximum refractive error of -4.50 D was chosen because myopia progression is more likely to slow after this point (Thorn, Gwiazda et al. 2005).

**Visual acuity:** Best-corrected visual acuity was required to be 6/6 Snellen (0.0 logMAR) or better in both eyes. Good visual acuity was a requirement for this study so as to exclude amblyopia and other unexplained anomalies.

**Informed consent / assent:** All children and their parents had to be able to give informed consent (parent/guardian) in writing and informed assent (children).

### 4.2.2 Exclusion criteria

**Previous myopia control:** Participants who had used any myopia control methods including anti-muscarinic eye drops, orthokeratology, progressive addition lenses etc within the past 6 months were excluded to ensure no carry-over effects from the previous treatments. Four of the participants had used previous myopia control treatments, but more than one year previously. One had used PALs, two had used Tropicamide and one had used unknown eye drops.

**Ocular pathology:** Children with any known ocular pathology were excluded as it may potentially have affected the response of the choroid or other ocular tissue to optical defocus.

**Astigmatism and anisometropia**: Children with astigmatism in either eye greater than -1.00 DC or anisometropia greater than 1.00 D were excluded from the study. A limit on astigmatism was included as it could potentially complicate the accurate presentation of optical defocus. Only a small amount of initial anisometropia was

acceptable as experimentally induced optical defocus was randomly assigned the dominant or non-dominant eye and anisometropia is associated with differences in choroidal thickness between the eyes (Vincent, Collins et al. 2013). These are the same criteria used in previous study (Chiang, Phillips et al. 2015).

**Strabismus and amblyopia:** Participants with amblyopia or strabismus were excluded from the study because of the unknown effects of retinal defocus in these conditions.

**General health:** Children with systemic conditions likely to affect the eye were excluded from the study (e.g. Diabetes could cause fluctuations in refractive error and diabetic eyes tend to have a thinner choroid than normal, see Section 2.7.7.3 ).

## 4.3 Recruitment and retention

In this study, participants were required to attend a total of 8 visits over a period of 6 months (approximately two hours each visit, on two consecutive days at all 4 stages). The time and inconvenience involved for participants and their parents/guardians made recruitment challenging. Participants were recruited between September 2015 and early January 2016, through Ophthalmologists. However, a very large number of potential participants did not volunteer to take part in the study. In addition, although atropine is widely used in Taiwan, many children either were already on atropine or their parents had already decided that they did not want their children to use atropine. Therefore once recruited, every effort was made to ensure that participants were not lost to follow up during the study. When a potential participant who fitted the study inclusion criteria was enrolled, a clear appointment scheduling plan was discussed, written down, and agreed with the participant and their parents/guardians. To ensure

that the dates for the appointment were not forgotten, reminder phone calls were made to the parents/guardians at 1 week and 1 day prior to the appointment. Vouchers were given to cover expenses as approved by the ethics committee.

## 4.4 Administration of atropine eye drops

Prior to enrolment, participants had been assessed by an Ophthalmologist and were about to commence myopia control using 1 drop per day of 0.3% atropine (atropine sulphate 3mg/mL: Sinphar Pharmaceutical Co Ltd. Taiwan). The parent/guardian had agreed to be responsible for instilling the drops at night. Prior to instilling the drops, they were advised to shake the bottle. They were instructed to instil another drop if the first drop did not fall in the eye. While on atropine, participants were advised to wear sunglasses and a hat when outside in sunlight. They had also been warned of a reduction in the ability to accommodate and hence the potential need for reading spectacles for near work. The investigator's cellphone number was given to parents/guardians in case unscheduled appointments were required for any adverse events such as an allergic reaction or other emergency. Parents/guardians were asked to report instances in which eye drops were not instilled for two or more consecutive days.

## 4.5 Sample size calculation

The sample size was calculated using the web-based sample size calculator 'Statistical considerations for clinical trials and scientific experiments,' supported by the Massachusetts General Hospital and National Institutes of Health, available at http://hedwig.mgh.harvard.edu/sample_size/size.html. The original intent was to use data from the pilot study described in Chapter 3, to compute the sample size for the

main study in children. However, the pilot study employed SS-OCT with automated segmentation of choroidal boundaries, but SS-OCT was not available for the study of children in Taiwan because of Taiwan FDA restrictions. The SD-OCT that was available was the same model as that used in our previous published study (Chiang, Phillips et al. 2015), and so data from that study was used to compute sample size. The within-subject variance calculated from the choroidal thickness measurements from emmetropic participants while watching the DVD without defocus in the Chiang et al study had a Standard Deviation (SD) = 7 µm. Sample size calculations showed that to obtain a power of 90% at a p-value of 0.01, 18 participants would be required to detect a choroidal thickness change of 15 µm. Assuming a drop-out-rate of 20%, we therefore aimed to recruit 22 participants.

## 4.6 Randomisation

The Experimental eye of each participant was pseudo-randomly assigned per their ocular dominance by using a permuted-block design with a block size of four. The ocular dominance of each participant had previously been determined by means of a simple pointing task. The words ‘Dominant’ or ‘Non-Dominant’ were printed on pieces of paper which were individually placed inside a series of opaque sealed envelopes arranged in pseudo-random order. The investigators did not have access to the randomisation schedule throughout the study. Once a participant had been enrolled in the study, the investigator selected the next envelope and opened it to determine the identity of the Experimental eye for that participant. The assignment of the Experimental eye for that participant remained the same throughout the study, with the contralateral eye acting as control.

## 4.7 Protocol for participant testing

The study required each participant to attend 8 visits in total. Visits were divided into 4 stages with each stage consisting of two visits on consecutive days (i.e. two at baseline (Stage 1), two at 1 week (Stage 2), two at 3 months (Stage 3) and two at 6 months (Stage 4)). Enrolment was included in the first visit. Visits were scheduled at the same time of day for each participant (between 1pm to 6pm) to minimise the effects of diurnal variations in choroidal thickness, axial length etc. (Read, Collins et al. 2008, Tan, Ouyang et al. 2012). The detailed testing protocols for each visit are described below and summarised in *Table 4* and *Figure 5*. Details of the equipment used to make outcome measures (OCT, ocular biometry, autorefraction etc. are given in Sections 4.7 and 4.8)

At the beginning of each stage, refractive error was checked by autorefraction and refined by subjective refraction. This was to ensure the correct power of ophthalmic lens was used to create the required degree of myopic defocus and to ensure that the Control eye was fully corrected. Then, pupil diameters were measured with a pupil rule. Monocular and binocular amplitudes of accommodation then measured (by the push-up method) to assess accommodative status. In addition for stages 1, 3 and 4, ocular biometry was then performed using the Lenstar 900 as described in Sections 4.7 and 4.8.

**Stage 1:**

***Day 1:***

- The participant was seated to view a video (binocularly) at a viewing distance of 6 metres, wearing their full distance prescription, for a 20-minute stabilisation period.

- The participant then viewed a video at 6 meters viewing distance for 60 minutes with 2.00D hyperopic or myopic retinal defocus applied with an ophthalmic lens to the Experimental eye. Whether myopic or hyperopic defocus was selected on Day 1 (with defocus of the opposite sign being used on the following day) was determined randomly. The contralateral Control eye was fully corrected with an ophthalmic lens. OCT measures of choroidal thickness were taken in both eyes at 0, 20, 40 and 60 minutes.

***Day 2:***

- Again, the participant was seated to view a video (binocularly) at a viewing distance of 6 metres, wearing their full distance prescription, for a 20-minute stabilisation period.
- The participant then viewed a video at 6 meters viewing distance for 60 minutes with the opposite sign of retinal defocus from Day 1 applied to the Experiment eye, while the contralateral Control eye was fully corrected with an ophthalmic lens. OCT measures of choroidal thickness were taken in both eyes at 0, 20, 40 and 60 minutes.
- Nightly instillation of 0.3% atropine to both eyes (prescribed by an Ophthalmologist in the initial visit) was initiated in the evening following the Day 2 measures and continued every night for 6 months.

***Day 3 to Day 7:***

- Home administration (by parent/guardian) of 0.3% atropine, 1 drop to each eye at night.

**Stage 2 (after 1 week on atropine)**

***Day 8:***

- Participants returned to the clinic and the Day 1 study procedures were repeated.

***Day 9****:*

- Participants returned to the clinic and the Day 2 study procedures were repeated.

***Day 9 to 3-month visit:***

- Home administration (by parent/guardian) of 0.3% atropine, 1 drop to each eye before sleep.

**Stage 3: (after 3 months on atropine)**

***Day 1:***

- Participants returned to the clinic and Stage 1 Day 1 study procedures were repeated.

***Day 2:***

- Participants returned to the clinic and the Stage 1 Day 2 study procedures were repeated.

**Stage 4: (after 6 months on atropine)**

***Day 1:***

- Participants returned to the clinic and Stage 1 Day 1 study procedures were repeated.

***Day 2:***

- Participants returned to the clinic and the Stage 1 Day 2 study procedures were repeated.

At the end of 6-month follow up, children were discharged from the study and continued under their Ophthalmologist's care for myopia control.

| | Stage 1 | | Stage 2 | | Stage 3 | | Stage 4 | |
|---|---|---|---|---|---|---|---|---|
| | Defocus Day1 Stage 1 | Defocus Day2 | Defocus Day1 1 week Atrop | Defocus Day2 1 week Atrop | Defocus Day1 3 mo Atrop | Defocus Day2 3 mo Atrop | Defocus Day1 6 mo Atrop | Defocus Day2 6 mo Atrop |
| Autorefraction | ● | ● | ● | ● | ● | ● | ● | ● |
| Accommodation measurement | ● | | ● | | ● | | ● | |
| Pupil diameter | ● | | ● | | ● | | ● | |
| LenStar LS 900 measurement | ● | | | | ● | | ● | |
| Drops compliance confirmation | | | ● | ● | ● | ● | ● | ● |
| History taking | ● | | ● | | ● | | ● | |
| Full subjective refraction | ● | | ● | | ● | | ● | |
| Binocular vision assessment | ● | | | | | | | |
| Ocular health assessment | ● | | | | | | | |

***Table 4.*** *Summary of tests performed at each visit at each stage.*

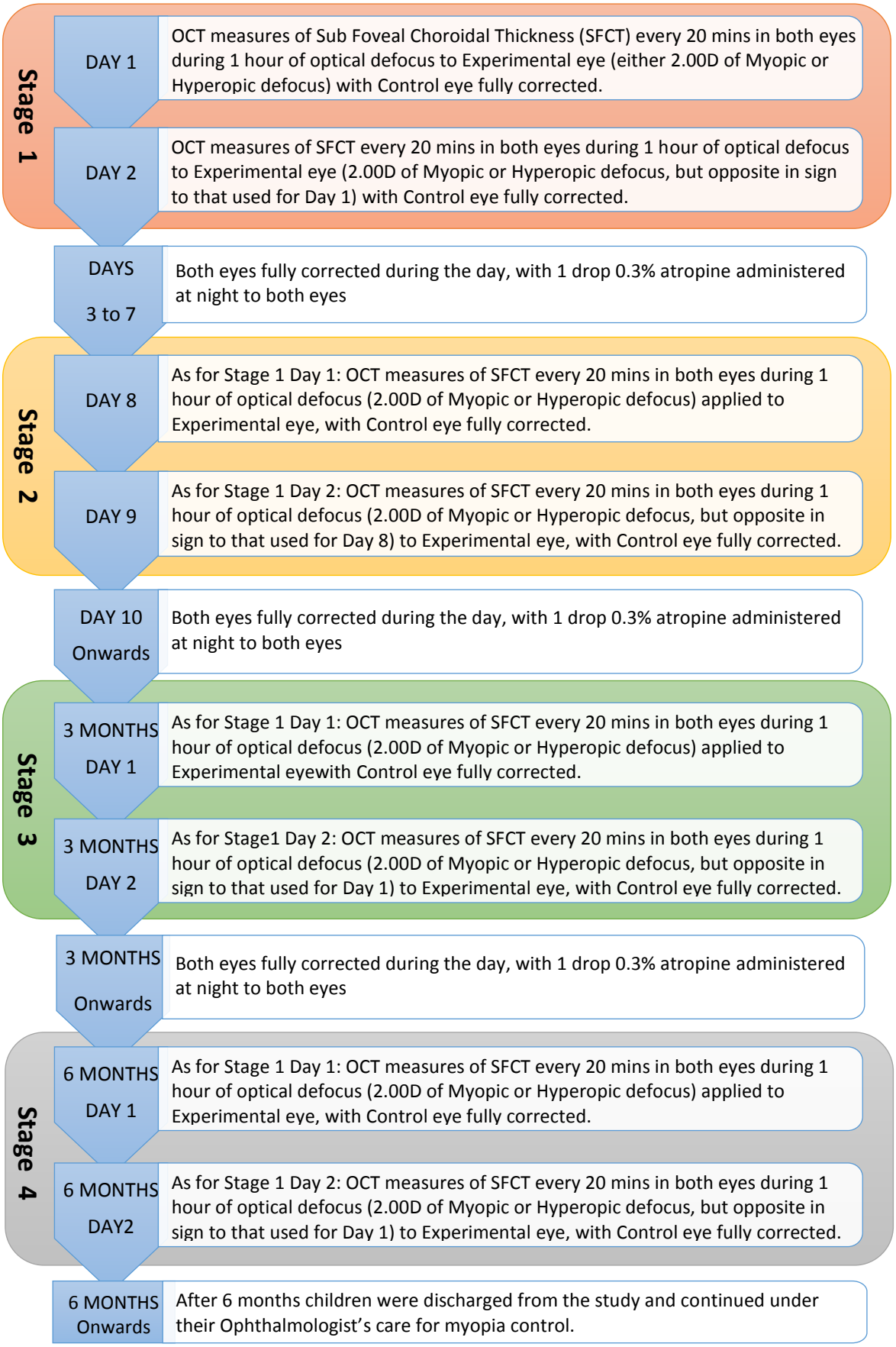


***Figure 5.*** *Flow diagram showing the basic measures made at each stage of the study.*

## 4.8 Primary outcome measure

The primary outcome measure used in this study was change in sub foveal choroidal thickness (SFCT), measured from Optical Coherence Tomography images.

### 4.8.1 OCT equipment

Spectral Domain Optical Coherence Tomography (SD-OCT) Nidek RS-3000 RetinaScan Advance (Nidek Co., Ltd. Aichi, Japan) was used to obtain cross-sectional chorio-retinal images of both eyes from each participant. Images from the OCT were used to measure and analyse SFCT. Initially, the aim was to use the Swept-Source Optical Coherence Tomography (SS-OCT) DRI OCT-1 Atlantis (Topcon Corp., Tokyo, Japan, http://www.topcon.co.jp/) that was used in the preliminary study, for the main study also, as the Atlantis SS-OCT provided automatic segmentation of choroidal boundaries. However, this SS-OCT was not available in Taiwan as it was still awaiting Taiwan FDA approval. Therefore, we used the same model of SD-OCT that was used in our previous published study (Chiang, Phillips et al. 2015) with manual measurement of the choroid. The Nidek RS-3000 RetinaScan Advance is a high-speed Spectral Domain OCT, which uses an 880nm light source and performs up to 53,000 A-scans per second. The advanced speckle-noise-reduction system averages images and reportedly provides 4 microns (depth (Z) direction) digital resolution OCT images (Nidek RS-3000 OCT RetinaScan Specification Sheet, Nidek CO., LTD). In this study, we followed the OCT protocols used in our previous study (Chiang, Phillips et al. 2015) with the 6 mm “Macula Line” function (single-line scan, consisting of 30 overlaid consecutive scans) centred on the fovea and ‘Choroidal’ scanning mode was used to obtain the cross-sectional chorio-retinal images. The participant was asked to look at the fixation target during the scanning. The OCT included an eye-tracer function that allowed the scanned line

to automatically track small movements of the participant's eye. Scanning automatically stopped and then restarted when the participant's eye movement exceeded the tracer limit. No other methods of registering the locations of the scans obtained within and between the different stages were employed.

### 4.8.2 OCT image processing

The Nidek RS-3000 RetinaScan Advance OCT used in-house database software NAVIS-EX (Nidek Advance Vision Information System – Extra) to capture and display data. Although it had advanced imaging analysis functions such as automated 'layer' recognition and 'measure' functions, these could not be used to measure choroidal thickness automatically, because there was no automated function available to detect the choroid-sclera interface (i.e. the outer border of the choroid). Therefore, manual definition of the outer choroidal border by an investigator was still required to measure choroidal thickness. However, the need for manual measurement increased the chance that investigator bias might influence the measures. The approach taken was therefore to export the image files from the NAVIS software and store them in bitmap image file format so that they could be de-identified before measurements were made by masked observers.

### 4.8.3 Masking

Once the OCT image files for each participant had been exported and stored, a research associate who was not involved in analysing the images, generated random codes to replace the file names. The order of the image files for each participant was shuffled, and then passed to the investigator (author) and two other independent trained Optometrists for measurement of choroidal thickness. Once the measures for an individual participant were completed, the original order of the measures (e.g. baseline, measure 1 etc.) was then reconstructed for statistical analysis.

### 4.8.4 Scaling method

Twenty-five raw OCT images were randomly selected from different participants, and an ‘arrow bar’ of random length was added to each image with the built-in ‘measure’ function of the NAVIS software: this function automatically displays the numerical measurement in microns of the length of the inserted arrow bar. The images (each of which included an ‘arrow bar’ and the numerical measurement of its length in microns) were then exported as a bitmap file for analysis with ImageJ software. ImageJ provides distance measurements based on pixels, therefore, the number of pixels associated with the ‘arrow bar’ were then divided by the displayed numerical measure in microns associated with the ‘arrow bar’, to calculate the average scaling factor in microns per pixel. The averaged scaling factor obtained was 1 pixel = 3.01 ± 0.01 µm, and this was applied to all image calculations to determine choroidal thickness.

### 4.8.5 Choroidal thickness measurement

Only the SFCT was measured and analysed in this study. A previous study indicated that the influence of monocular defocus on parafoveal choroidal thickness was similar to the influence observed on SFCT (Chakraborty, Read et al. 2012). In addition, the effect of topical homatropine (a non-specific muscarinic antagonist similar to atropine with shorter lasting mydriatic and cycloplegic effect) reportedly produces a similar increase in choroidal thickness in the parafoveal and subfoveal regions ($p < 0.001$) (Sander, Collins et al. 2014). Therefore, only the SFCT was measured and analysed in this study.

Open-source ImageJ software (Rasband, W.S., ImageJ, U. S. National Institutes of Health, Bethesda, Maryland, USA, http://imagej.nih.gov/ij/, 1997-2014.) was employed to make the measures of choroidal thickness from the coded (de-identified) bitmap image files. ImageJ is widely used internationally in biology, including

Ophthalmology for retinal image analysis. (Abràmoff, Magalhães et al. 2004, Staal, Abramoff et al. 2004, Papadopulos, Spinelli et al. 2007).

The Nidek RS-3000 OCT provides an output of relatively clear and reasonably high-resolution images sufficient to make manual segmentation of the inner and outer borders of the choroid possible. After the cross-sectional chorio-retinal OCT images had been exported and opened with ImageJ, the inner and outer choroidal borders were independently identified and manually measured by the three masked investigators, using the measure function in ImageJ. The inner border of the choroid is generally very well defined, as it is usually a relatively straight and smooth line. The outer border is generally well defined and visible with the OCT. However, in some cases it has irregular and bumpy borders, and a line of best fit had to be applied for manual segmentation. (see *Figure 6* below).

The following procedure was used to measure choroidal thickness. First, in ImageJ the scaling of the image was set, based on the scaling factor specified above (1 pixel = 3.01 ± 0.01 µm). Second, the masked investigator independently placed a line to measure the distance between the inner and outer borders of the choroid. If the outer border was bumpy, a line of best fit (using the ImageJ draw function) was drawn prior to placing the measurement line. The measure function then gave the length of the drawn line (choroidal thickness) directly in microns.

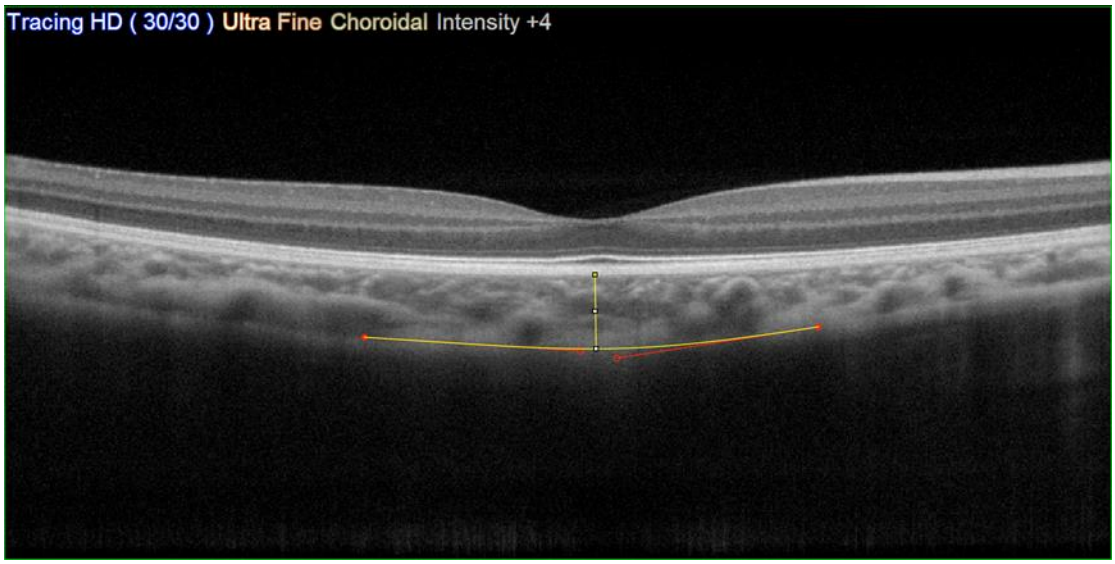


***Figure 6.*** *Example of SFCT manually segmented with the measurement line placed manually with ImageJ by an independent investigator.*

## 4.9 Inter-observer agreement

Since the inner and outer boundaries of the choroid were determined manually in this study, it was of interest to evaluate the repeatability between observers in determining choroidal thickness. Repeatability of the measures between observers was assessed by Bland-Altman analysis (Bland and Altman 1999) and Intra-class correlation coefficient (ICC).

For the Bland-Altman analysis, the mean inter-observer difference and 95% limits of agreement were as follows: A vs B: -1 ± 5 μm (95% CI -11 to +8 μm); A vs C: -1 ± 5 μm (95% CI -10 to +9 μm); B vs C: 0 ± 5 μm (95% CI -9 to +10 μm), see *Figure 7* below for the Bland-Altman plot analyses for the three comparisons. The ICC analysis showed a very high correlation coefficient of 0.99.

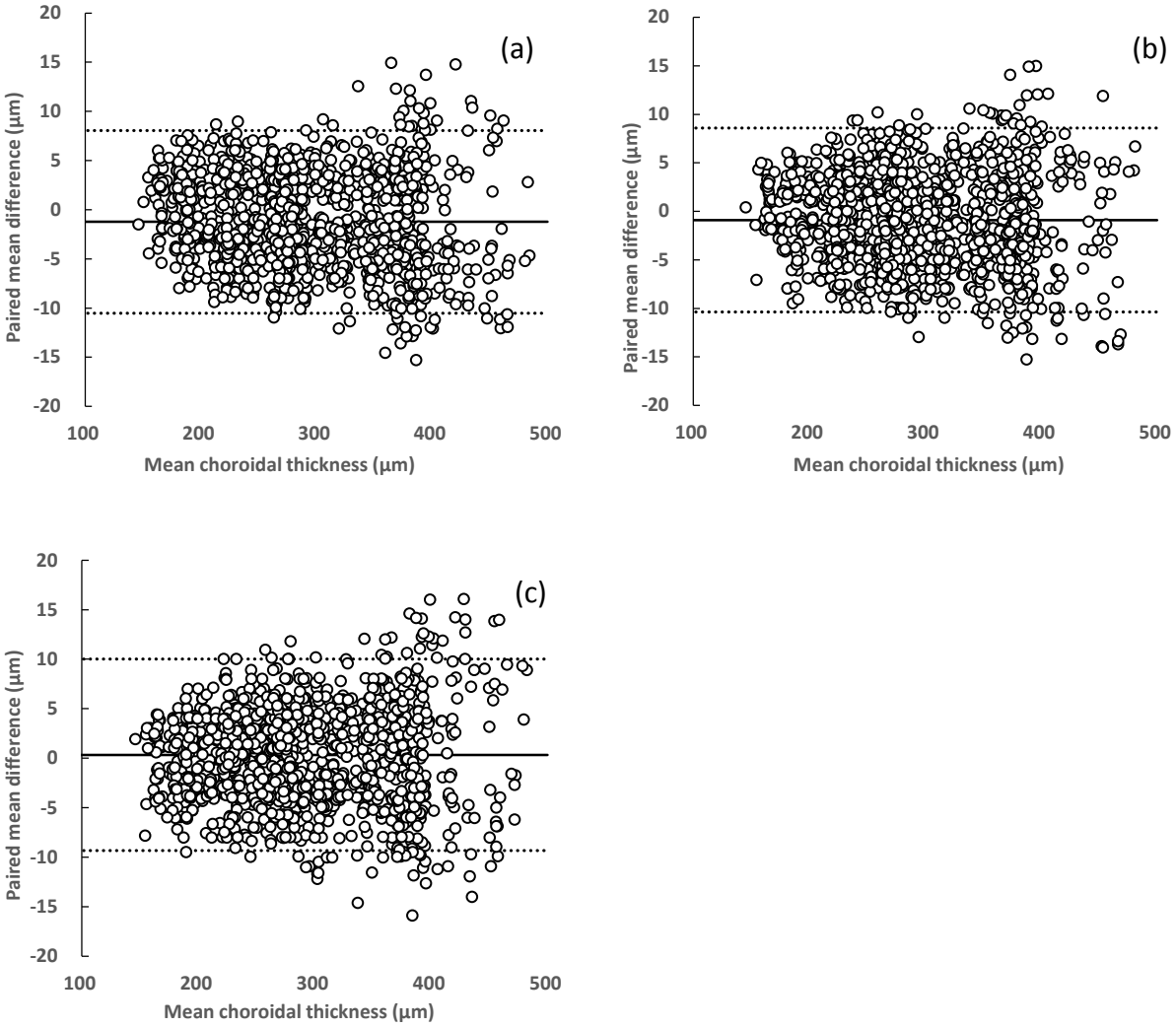


***Figure 7.*** *Bland-Altman plots of inter-observer agreement on subfoveal choroidal thickness measurements between observers. (a) Observer A vs B. (b) Observer A vs C. (c) Observer B vs C.*

## 4.10 Secondary outcome measures

The secondary outcome measures of the study were: Ocular biometry of axial eye dimensions measured using the LenStar reflectometer (LenStar LS 900, Haag Streit AG, Koeniz, Switzerland); Refractive error (mean of 5 readings) measured using an open field autorefractor (Shin-Nippon Auto Refkeratometer NVision-K 5001 (http://www.shin-nippon.jp/); measures of accommodation made using the push-up method and measures of pupil diameter, made with a pupil rule.

The Shin Nippon NVision-K 5001 autorefractor that was usied for this study has previously been validated in young adults (non-cycloplegic refractions in 99 subjects, aged 23.2 +/- 7.4 years) (Davies, Mallen et al. 2003) and in children 8-13 years (cycloplegic refractions in 101 subjects, 10.9 +/- 1.42 years) (Tang, Tang et al. 2014). Davies et al. showed that refractive error measured by the NVision-K was similar ($p = 0.67$) to subjective refraction (difference, 0.14 +/- 0.35 D), and was accurate and repeatable over a wide range of prescription (-8.25 to +7.25 D). The authors concluded that this autorefractor is a valuable addition to the instrumentation currently available to researchers.

Measures of axial eye dimensions (LenStar) were made to compare potential changes caused by administration of atropine eye drops. Ocular biometry measures were obtained at the initial visit (i.e. before atropine) and after 3 and 6 months of atropine administration. At each visit, three consecutive LenStar measures were made in each eye and averaged.

According to the LenStar user manual, the LenStar is able to measure axial length (AL) in the range 12 – 32 mm with a resolution of 0.01 mm and an *in vivo* repeatability (1 SD) of 0.035 mm. There have been several independent studies confirming the

accuracy and repeatability of the LenStar reflectometer (Holzer, Mamusa et al. 2009, Hoffer, Shammas et al. 2010). The LenStar automatically measures Central Corneal Thickness (CCT), Anterior Chamber Depth (AD), Thickness of the Crystalline Lens (LT), Axial length (AL, the distance from the anterior cornea to Retinal Pigment Epithelium), reporting the mean thickness of each ocular structure using their in-house software EyeSuite (Haag Streit, Koeniz, Switzerland). Once these components had been measured, Vitreous Chamber Depth (VCD) was computed by using VCD = AL – (CCT + AD + LT).

## 4.11 Statistical data analysis

After data collection, statistical analyses were performed using IBM® SPSS® Statistics 20 (IBM, Armonk, USA). The choroidal thickness changes were measured as absolute changes in microns with reference to baseline at time zero (start of the relevant 60-minute observation period). Since choroidal thickness changes were measured in the same participants and at regular 20-minute intervals over the 60-minute observation period, repeated measures adjustments were required. The Shapiro–Wilk test was used to verify normality (all $p>0.05$), then the data were analysed using repeated measures ANOVA with General Linear Model (GLM), with 3 within-subject factors: time (minutes), Experimental versus Control eye, as well as before atropine versus 1 week, 3 months and 6 months on atropine. If significant differences were identified, post-hoc pair-wise comparisons with Bonferroni correction were made. This test provides a very conservative measure of the significance of differences. A p-value $\leq 0.05$ was considered statistically significant. Data are presented as Mean and Standard Deviation throughout, unless otherwise specified. Standard deviation (SD) has been used to illustrate variation in values (e.g. age of participants) whereas the standard error of the mean (SEM) has been used to give an indication of reliability in the estimate of the mean, which has typically been used in figures.

# 5 Results

## 5.1 Participants

Twenty-two Taiwanese children (11 Males and 11 Females) were enrolled in the study. Two participants (who were brothers) withdrew in the first week of study participation before the use of atropine eye drops, at the request of their grandparents. Therefore, all their outcome measures were excluded from the following analysis.

Twenty participants (9 Males and 11 Females) aged 6 to 14 (mean ± SD, 8.95 ± 2.31 years) participated throughout the study; there were no missing appointments or further loss to follow-up during the 6-month study period. All 20 children had normal best-corrected visual acuity of logMAR 0.00 or better. The baseline data of participants is summarised in *Table 5* below.

| | | | | |
|---|---|---|---|---|
| **Gender** | Male | 9 | 45% | |
| | Female | 11 | 55% | |
| **Ethnicity** | Taiwanese | 20 | 100% | |
| **Allocation to** | Dominant eye | 10 | 50% | |
| **Exp eye** | Non-dominant eye | 10 | 50% | |
| **Age** | Male | 9.89 ± 2.62 | | Whole group |
| **(Years)** | Female | 8.18 ± 1.78 | | 8.95 ± 2.31 |
| **Mean Sphere** | Control eye | -1.64± 0.95 | p = 0.76 | Whole group |
| **(SER, Dioptres)** | Experimental eye | - 1.61 ± 0.90 | | −1.63 ± 0.90 |
| **Accommodation** | Control eye | 17.55± 1.47 | p = 0.87 | Whole group |
| **(AoA, Dioptres)** | Experimental eye | 17.50± 1.47 | | 17.53 ± 1.30 |
| **Pupil Size** | Control eye | 4.08± 0.54 | p = 0.99 | Whole group |
| **(mm)** | Experimental eye | 4.08± 0.47 | | 4.08 ± 0.47 |

***Table 5.*** *Baseline data of the 20 included participants (9 Males and 11 Females). Gender, ethnicity, eye allocation, age, spherical equivalent refraction (SER, D), amplitude of accommodation (AoA, D) and pupil size (mm). P-values are paired t-test.*

## 5.2 Seasonal variation in Stage 1 Participant visits

Previous studies have shown that eye growth and ocular parameters vary between seasons. Typical seasons in Taiwan are normally classified into spring (March–May), summer (June–August), autumn (September–November), and winter (December–February) (Chen and Chen 2003, Lin, Wang et al. 2005). In this study, the participants were enrolled over a reasonably short period (i.e. over the 4 months from September 2015 to first week of January 2016, which falls in autumn to mid-winter) rather than spread throughout the year. One participant was enrolled in September 2015, thirteen in October 2015, one in November 2015 and five in the first week of January 2016. The Histogram below (see *Figure 8*) shows the enrolment dates (month) of all participants in the study. Enrolment date corresponds to the first day of Stage 1 measures. The within-subject inter-seasonal effect on axial elongation during the 6-months study, and the relationship between axial elongation and season of enrolment (autumn vs winter) is reported in Section 5.9.

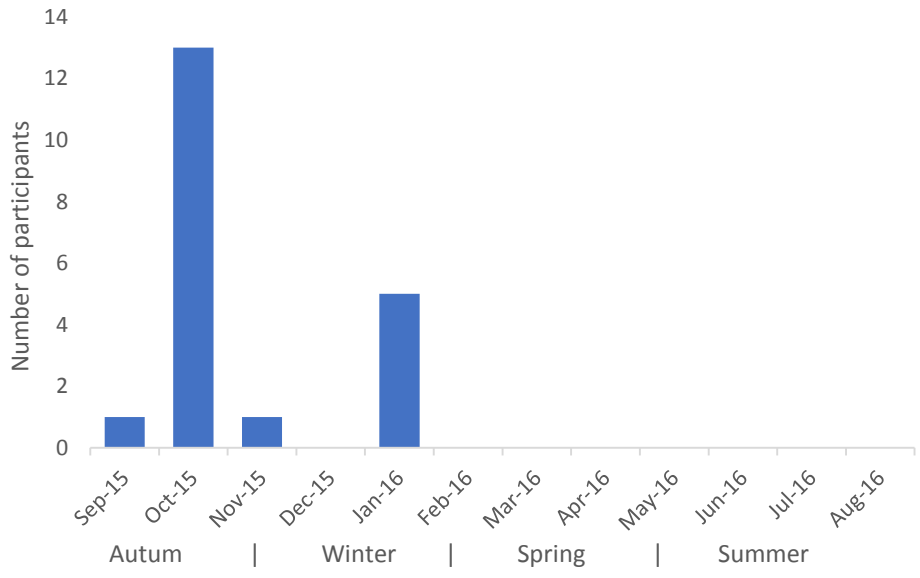


***Figure 8.*** *Histogram showing the enrolment dates (month) of all participants in this study. Enrolment date corresponds to the first day of Stage 1 measures. All participants were enrolled within the autumn / mid-winter period.*

## 5.3 Mean subfoveal choroidal thickness: Control vs Experimental eyes

All Choroidal thickness data presented in the results is derived from the average of the measures made on OCT images by the three masked observers, as described in Section 4.8. At baseline, the mean SFCT in Control eyes was 278 ± 73 µm and the mean SFCT in Experimental eyes was 272 ± 72 µm, with no statistical difference between the two (p = 0.50, paired t-test).

## 5.4 Subfoveal choroidal thickness vs. Degree of myopia

As discussed earlier in section 2.7.1, it has been reported that SFCT in healthy adult eyes is inversely proportional to the degree of myopia (Fujiwara, Imamura et al. 2009, Ikuno and Tano 2009, Li, Larsen et al. 2011, Nishida, Fujiwara et al. 2012, Chiang, Phillips et al. 2015). Also, myopic children have significantly thinner choroids compared to non-myopic children of the same age, especially in the central foveal region (Read, Collins et al. 2013).

The relationship between baseline SFCT (mean of both eyes, prior to any defocus) versus refractive error (mean of both eyes) for all 20 participants in this study is shown in *Figure 9,* which confirms these earlier findings. The regression analysis suggests that refractive error exhibits a positive association (slope, 39 µm / D) with SFCT (i.e. the higher the myopia the thinner the choroid). The correlation is moderate, with $R^2$ = 0.253, p = 0.02.

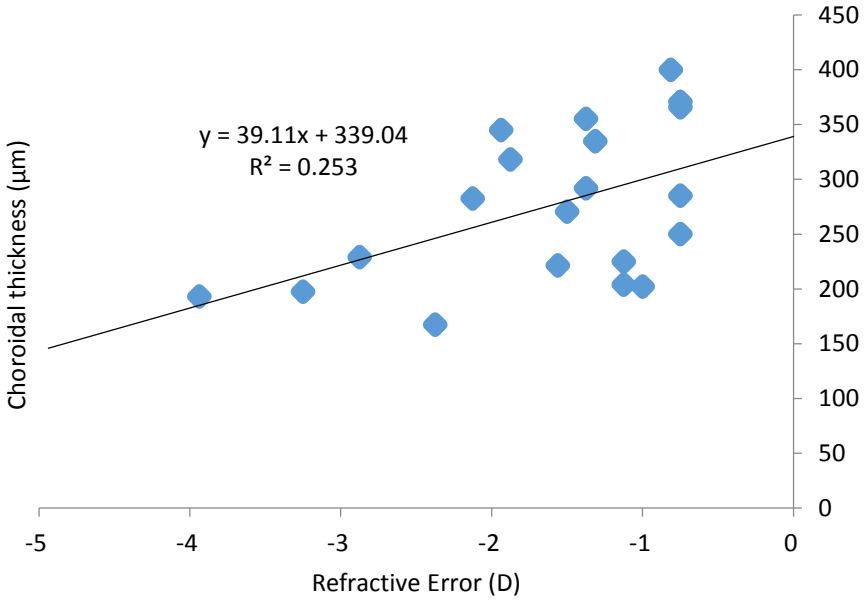


***Figure 9.*** *Baseline subfoveal choroidal thickness vs refractive error before any defocus. The results suggest that choroidal thickness exhibits a positive association (slope, 39 µm/D) with refractive error.* $R^2 = 0.253$, $p = 0.02$.

## 5.5 Choroidal thickness changes in response to retinal image defocus

Participants attended study visits in 4 stages (i.e. Stage 1: before atropine; Stage 2: after 1 week of atropine treatment; Stage 3: after 3 months of atropine treatment and Stage 4: after 6 months of atropine treatment). Each stage consisted of two visits on consecutive days to view a video at 6 metres viewing distance for 60 minutes with either hyperopic or myopic retinal defocus (pseudo-randomly selected) applied with an ophthalmic lens to the Experimental eye, while the contralateral Control eye was fully corrected with an ophthalmic lens. OCT measures of choroidal thickness were taken in both eyes at 0, 20, 40 and 60 minutes.

### 5.5.1 Stage 1 monocular defocus - Before atropine (Day 1 & 2)

The absolute choroidal thickness changes in microns in response to the two signs of monocular defocus are shown in *Figure 10*. The results show that the 2.00D of

monocular hyperopic defocus causes a decrease in choroidal thickness in the Experimental eye over the 60-minute period. The maximum degree of thinning was 12 ± 2 µm (mean ± SEM) at 60 minutes. In contrast, eyes exposed to 2.00D of myopic defocus, thickened over the 60-minute defocus period by 12 ± 2 µm (mean ± SEM) at 40 and 60 minutes. Repeated measures ANOVA showed that the choroidal thickness changes associated with both hyperopic and myopic retinal image defocus were significantly different from baseline ($p < 0.001$) at 20, 40 and 60 minutes. With regards to the contralateral Control eyes, the choroidal thickness appeared to be very slightly increased (~1 ± 1 µm, mean ± SEM) over the 60-minute time course when either sign of defocus was applied to the Experimental eye, but these minor changes did not reach statistical significance ($p > 0.05$) when compared to the initial baseline measure.

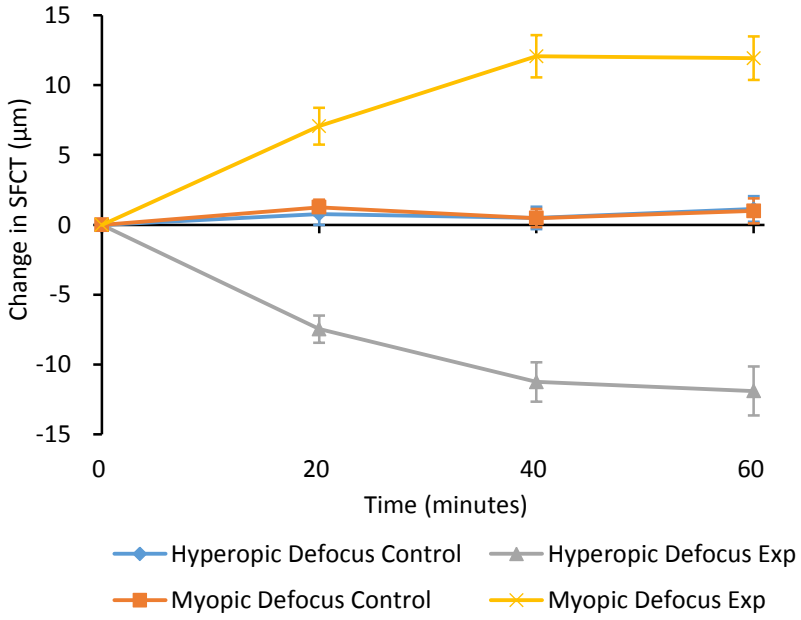


***Figure 10.*** *Time course of changes in choroidal thickness with monocular hyperopic and myopic defocus. The data shows that 2.00D of monocular hyperopic defocus causes a decrease in choroidal thickness in the Experimental eye over the 60-minute period. In contrast, 2.00D of monocular myopic defocus causes an increase in choroidal thickness in the Experimental eye over the 60-minute period. Error bars show ± 1 SEM.*

### 5.5.2 Stage 2 monocular defocus – after 1 week on atropine (Day 8 & Day 9)

After the Stage 1 measures, participants started instilling one drop of 0.3% atropine to each eye every night. *Figure 11* summarises the mean choroidal thickness changes in respond to 2.00D of monocular hyperopic and myopic defocus at Day 8 and Day 9 (after 6 to 7 days of administering atropine eye drops). As for Day 1, myopic defocus caused a steady thickening of the choroid which reached its maximum thickness (mean ± SEM, 13 ± 1 µm) at 60 minutes. Repeated measures ANOVA showed a statistically significant ($p < 0.001$) increase in choroidal thickness with myopic defocus over the 60-minute defocus period. In contrast, hyperopic defocus applied to the Experimental eye failed to thin the choroid throughout the 60 minutes of monocular defocus (mean ± SEM, 2 ± 1 µm, maximum change at 60 minutes). Experimental eyes exposed to hyperopic defocus responded in a similar way to the fully corrected contralateral Control eyes, with minimal changes from baseline ($p > 0.05$), indicating that atropine inhibited the choroidal thinning in response to hyperopic retinal image defocus, as was observed in the preliminary study in adults.

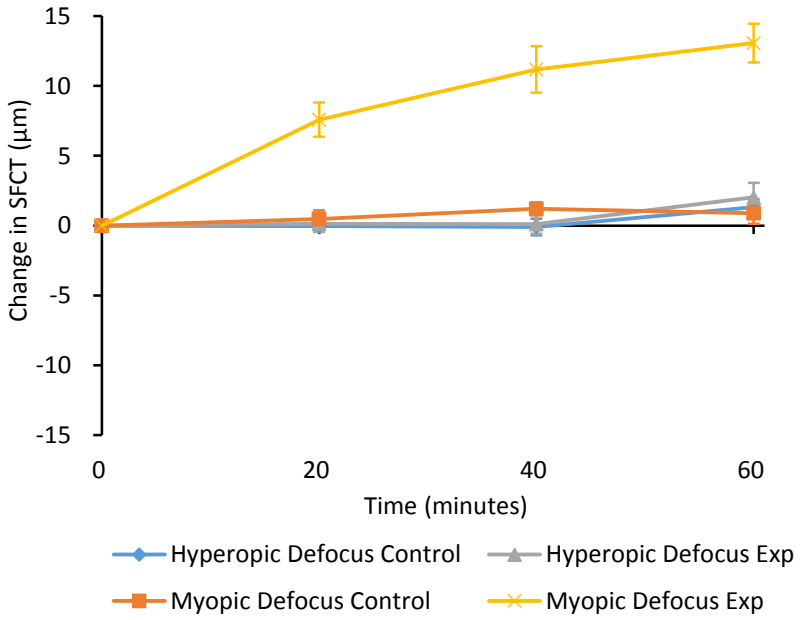


***Figure 11.*** *Time course of changes in choroidal thickness with monocular hyperopic and myopic retinal image defocus in children at stage 2 (i.e. after one week on atropine). The data shows that 2.00D of monocular myopic defocus causes an increase in choroidal thickness in the Experimental eye. However, 2.00D of monocular hyperopic defocus failed to thin the choroid in the Experimental eye over the 60-minute period. Choroidal thickness in contralateral Control eyes remained steady over the 60-minute period. Error bars show ± 1 SEM.*

### 5.5.3 Stage 3 monocular defocus – after 3 months of atropine use

Once participants completed their Stage 2 visits (Day 8 & Day 9), they continued instilling one drop of 0.3% atropine into each eye at night until the 3-month follow-up visits. At the Stage 3 visits, children's Experimental eyes were again exposed to myopic and hyperopic retinal image defocus on two consecutive days and the choroidal responses are shown in *Figure 12*. Again, myopic defocus caused a statistically significant thickening of the choroid ($p < 0.001$), which reached its maximum increase (mean ± SEM, 10 ± 1 µm) at 60 minutes. Again, the choroidal thinning that is expected in response to hyperopic defocus in the absence of atropine, appeared to be abolished in the Experimental eye (mean ± SEM, 1 ± 0.5 µm at 60 minutes). Repeated measures ANOVA for the Stage 3 data was consistent with that

of the Stage 2 data: a statistically significant increase ($p < 0.001$) in choroidal thickness with myopic defocus over the 60 minutes, but failure of hyperopic defocus to induce choroidal thinning ($p = 0.34$). The contralateral Control eyes again showed no statistically significant changes in choroidal thickness ($p > 0.05$). Thus, the effect of atropine in abolishing choroidal thinning induced by hyperopic defocus while leaving the thickening response to myopic defocus unaffected, remained after 3 months of atropine use.

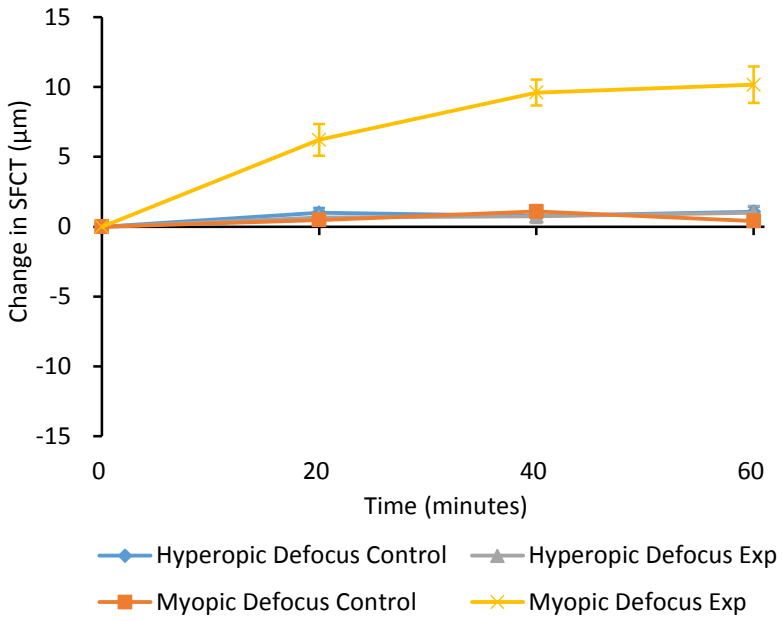


***Figure 12.*** *Time course of changes in choroidal thickness with monocular hyperopic and myopic retinal image defocus in children after 3 months of atropine administration. The data shows that 2.00D of monocular myopic defocus causes an increase in choroidal thickness in the Experimental eye, but 2.00D of monocular hyperopic defocus fails to thin the choroidal in the Experimental eye. Choroidal thickness of contralateral Control eyes remained steady over the 60-minute period. Error bars show ± 1 SEM.*

### 5.5.4 Stage 4 monocular defocus – after 6 months of atropine use

Children continued with their 0.3% atropine administration regime of 1 drop each eye at night before sleep for myopia control after the Stage 3 follow-up visit. All participants returned for the 6-month follow-up (Stage 4) and received monocular

retinal image defocus for 60 minutes on two more consecutive days. *Figure 13* summarises the choroidal responses to different types of defocus at the 6-month visit. The results were consistent with the 3-month visit, when 2.00 D of hyperopic defocus failed to thin the choroid during the 60-minute period (maximum change, mean ± SEM, 1 ± 0.5 µm). The 2.00 D of myopic defocus continued to cause a gradual increase in choroidal thickness (maximum change, mean ± SEM, 12 ± 1 µm). Repeated measures ANOVA (GLM) showed that short-term monocular myopic defocus caused small but significant changes in choroidal thickness ($p < 0.001$) but hyperopic retinal image defocus still failed to induce thinning ($p > 0.05$).

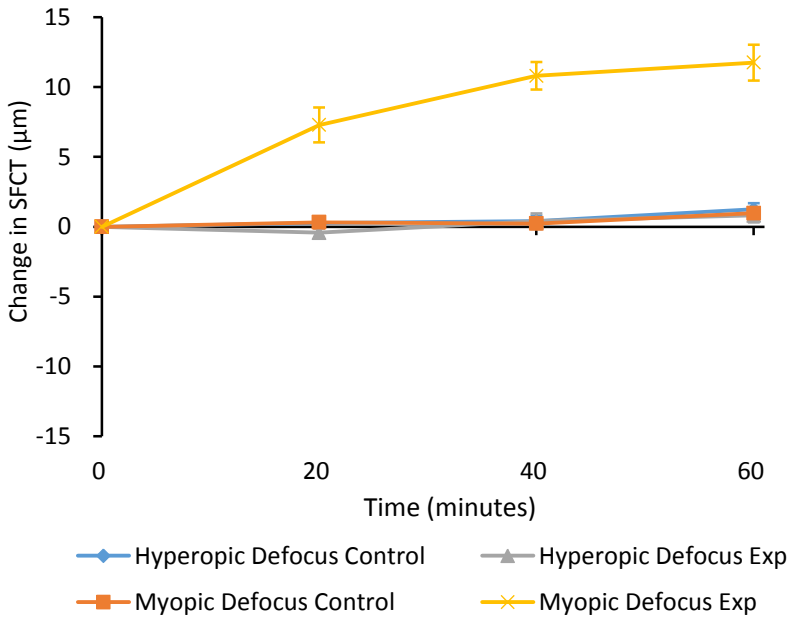


***Figure 13.*** *Time course of changes in choroidal thickness with monocular hyperopic and myopic retinal image defocus in children after 6 months of atropine administration. The data shows that 2.00D of monocular myopic defocus causes an increase in choroidal thickness in the Experimental eye, but 2.00D of monocular hyperopic defocus fails to thin the choroidal in the Experimental eye. Choroidal thickness of contralateral Control eyes remained steady over the 60-minute period. The data at 6 months is essentially the same as at 1 week and 3 months of atropine use. Error bars show ± 1 SEM.*

### 5.5.5 Summary of effects of monocular defocus during the 6-month study

The effects of 60 minutes of 2.00 D of hyperopic and myopic retinal defocus applied to the Experimental eye at all four stages of the study are summarised in *Figure 14* below.

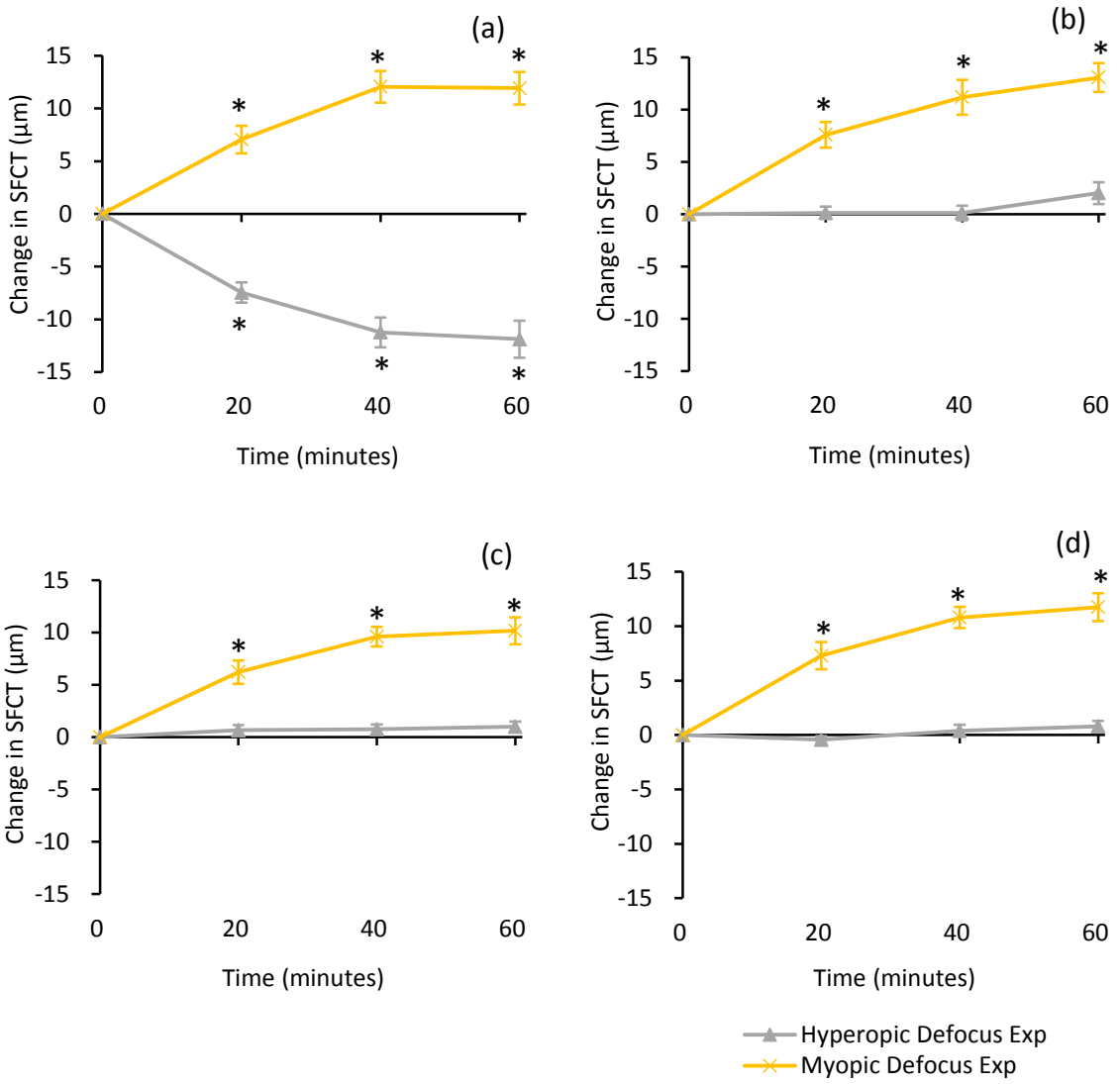


***Figure 14.*** *Summary of the effects of 2.00D hyperopic and myopic defocus on choroidal thickness in the Experimental eyes (Control eye data not shown) at each stage. (a) Stage 1 (before atropine). (b) Stage 2 (after 1 week on atropine). (c) Stage 3 (after 3 months on atropine) (d) Stage 4 (after 6 months on atropine). Error bars show ± 1 SEM. The asterisks (*) indicates a significant change in choroidal thickness within each Stage from baseline (Bonferroni-corrected pairwise comparison $p \leq 0.05$).*

At Stage 1, before atropine administration, children's choroids were thinned when 60 minutes of 2.00 D of hyperopic defocus was applied to the retina ($p < 0.001$), and thickened when 2.00 D of myopic defocus was applied ($p < 0.001$). However, at Stage 2, after 1 week of nightly instillation of 0.3% atropine to both eyes, the choroidal thinning that previously had been induced by hyperopic defocus (as shown in Stage 1) was abolished, while the thickening induced by myopic defocus remained. This inhibition of choroidal thinning by atropine was still present at Stage 3 and Stage 4 (after 3 and 6 months of atropine respectively).

Between stages, the choroidal thickness change induced by hyperopic defocus showed a significant change ($p < 0.001$) before and after atropine administration (i.e. Stage 2, 3, 4, vs Stage 1). However, the choroidal thickness change induced by myopic defocus showed no significant change between all four stages (i.e. the magnitude of choroidal thickening induced by myopic defocus was not significantly different before vs after atropine).

## 5.6 Baseline choroidal thickness before and after atropine

Choroidal thickness measures were made four times per eye during the 60 minutes of defocus (i.e. at 0 (before defocus), 20, 40 and 60 minutes). By comparing the presenting choroidal thickness without retinal image defocus (Time = 0 minutes) before atropine (Stage 1) with the equivalent presenting choroidal thickness after 1 week on atropine (Stage 2) indicates the effect that 1 week of 0.3% atropine alone (without defocus) had on choroidal thickness.

Pairwise comparison analysis showed that the mean presenting choroidal thickness values (at Time 0) for Stage 1 (Time 0 value x 2 defocus conditions x 20 participants) were significantly thicker following 1 week of atropine as follows: Control eyes before vs after atropine (278 ± 73 µm vs 297 ± 74 µm: $p < 0.001$); Experimental eyes before vs after atropine (272 ± 72 µm vs 293 ± 76 µm: $p = 0.002$). The mean of both eyes before vs after atropine (275 ± 70 µm vs 295 ± 72 µm: $p < 0.001$) See *Table 6*. Thus, the mean presenting choroidal thickness was increased by approximately 20 µm after 1 week of 0.3% atropine instillation alone. The raw data for all eyes before atropine and after 1 week of atropine are shown in *Figure 15* below. There are 80 data points in *Figure 15*; 20 participants x 2 eyes x 2 conditions (Time 0 for Hyperopic and Time 0 for Myopic defocus).

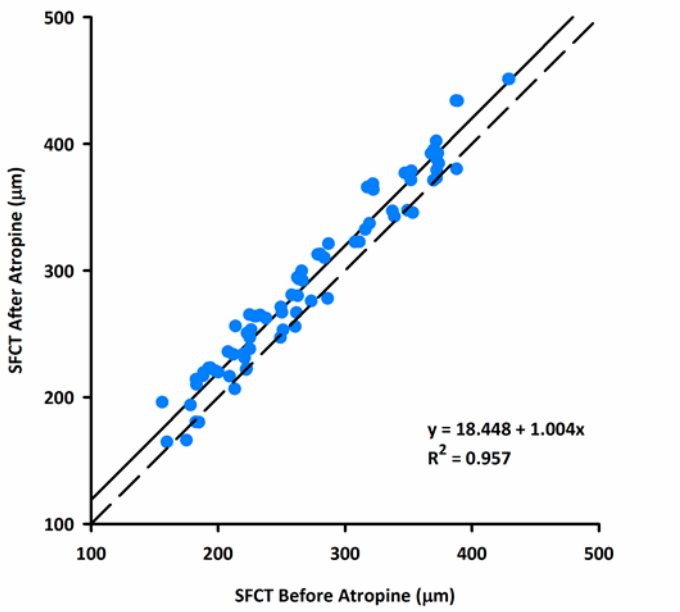


***Figure 15.*** *Baseline choroidal thickness values (Time 0, i.e. before retinal image defocus) before vs. after 1 week of atropine instillation. The data indicates that nightly instillation of 1 drop of 0.3% atropine to both eyes increased choroidal thickness by ~20µm.* $R^2$ *= 0.957, p <0.01. The dashed line corresponds to equality between ordinate and abscissa values.*

The choroidal thickening effect of atropine administration was maintained for the 6 months that the children were on atropine. At Stage 3 (3 months), the mean presenting choroidal thickness values were: Control eyes: 302 ± 70 µm; Experimental eyes (300 ± 71 µm). At Stage 4 (6 months) the mean presenting choroidal thickness values were: Control eyes: 297 ± 71 µm; Experimental eyes: 295 ± 72 µm.

| | Control Eye (µm) | Exp Eye (µm) | Mean (µm) | *Increase from Stage 1* (µm) |
|---|---|---|---|---|
| Stage 1 | 278 ± 73 | 272 ± 72 | 275 ± 70 | - |
| Stage 2 | 297 ± 74 | 293 ± 76 | 295 ± 72 | ~20 * |
| Stage 3 | 302 ± 70 | 300 ± 71 | 301 ± 67 | ~26 * |
| Stage 4 | 297 ± 71 | 295 ± 72 | 296 ± 68 | ~21 * |

***Table 6.*** *Summary table for mean baseline choroidal thickness in Control eyes, Experimental eyes, mean of both eyes, and the increase in baseline choroidal thickness at various stages vs Stage 1. The asterisks (*) indicate a significant change in choroidal thickness at various stages versus thickness at Stage 1 (Bonferroni-corrected pairwise comparison* $p \leq 0.05$*).*

## 5.7 Ocular biometry by reflectometry

Ocular biometry was performed using the LenStar reflectometer on three occasions during the study: at the initial visit (Stage 1) before atropine, and at follow ups during atropine treatment at 3 months (Stage 3) and at 6 months (Stage 4). Values and changes in individual axial components of the eye, including Central Corneal Thickness (CCT), Anterior Chamber Depth (AD), Crystalline Lens Thickness (LT), Vitreous Chamber Depth (VCD) and Axial length (AL) were analysed below and a summary table of the data is given in Section 5.7.6.

### 5.7.1 Central corneal thickness (CCT)

Changes in central corneal thickness (CCT) during the 6 months of the study are summarised in *Figure 16* below. The presenting CCT was very similar for Control and Experimental eyes (552 ± 23 µm and 551 ± 24 µm), and the mean CCT was 552 ± 23 µm. The CCT remained steady over the 6-month study period (3 months: 553 ± 24 µm and 554 ± 24 µm at 6 months). Pairwise comparison showed no significant change at the 3-month and 6-month visits from the baseline ($p = 0.22$ and 0.10).

### 5.7.2 Anterior chamber depth (AD)

Changes in Anterior Chamber Depth (AD: the distance between the central corneal endothelium and the anterior lens surface) during the 6 months of the study are summarised in *Figure 17*. The mean AD (both eyes) was 3.15 ± 0.34 mm at baseline (Control eyes: 3.16 ± 0.34 mm and Experimental eyes: 3.15 ± 0.33 mm). Pairwise comparison showed that the mean AD increased significantly from the baseline value before atropine compared to the 3 and 6-month values obtained during atropine use

(AD at 3 months: 3.26 ± 0.32 mm; AD at 6 months: 3.25 ± 0.32 mm, both $p < 0.001$)

see *Figure 17*.

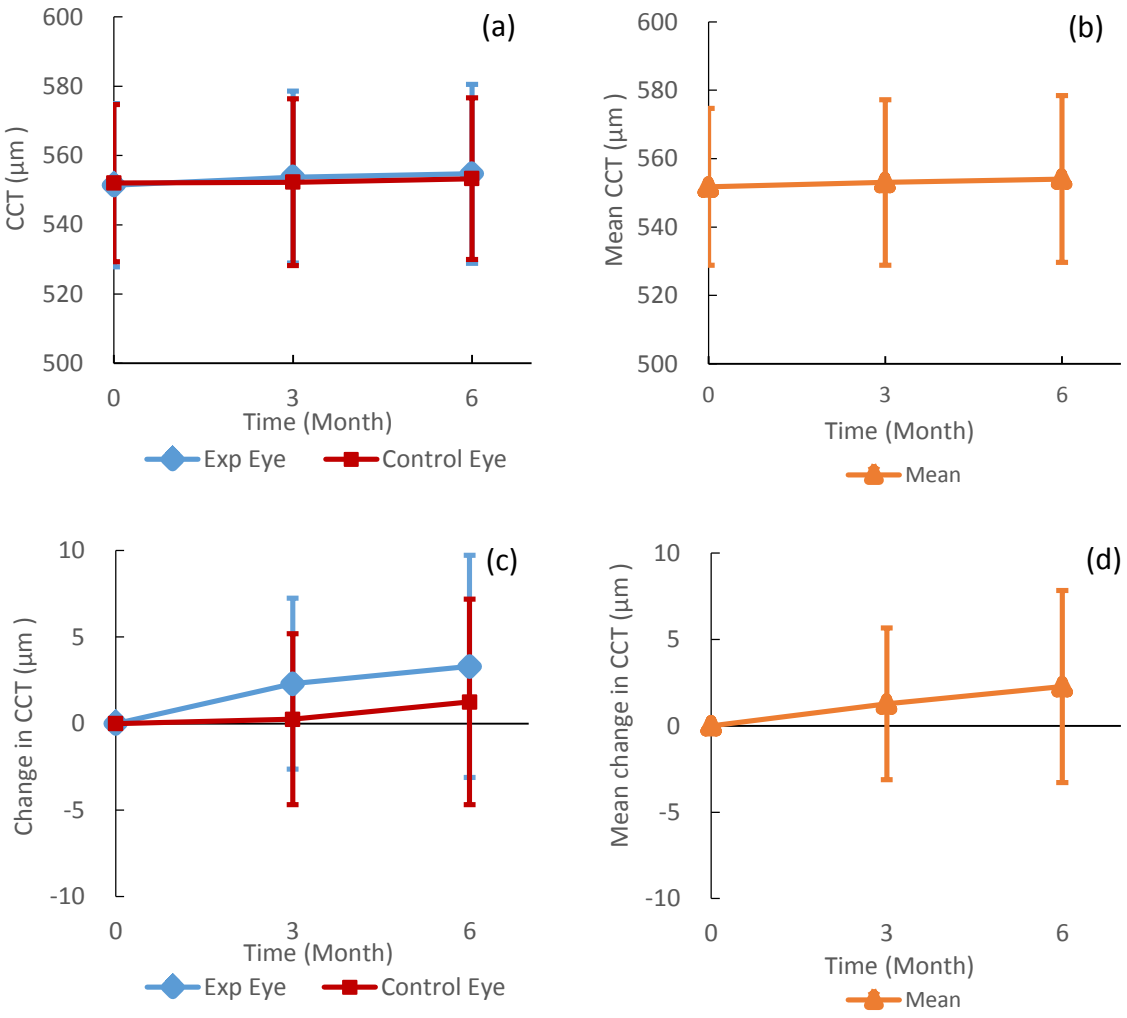


***Figure 16.*** *The central corneal thickness (CCT) was measured at the first visit, and at 3-month and 6-month follow ups. (a) Actual CCT of Control and Experimental eyes. (b) Actual CCT mean. (c) Absolute change in CCT in Control and Experimental eyes. (d) Mean change in CCT. The presenting CCTs were very similar between Control and Experimental eyes and remained steady over the 6-month period. Error bars show ± 1 SD.*

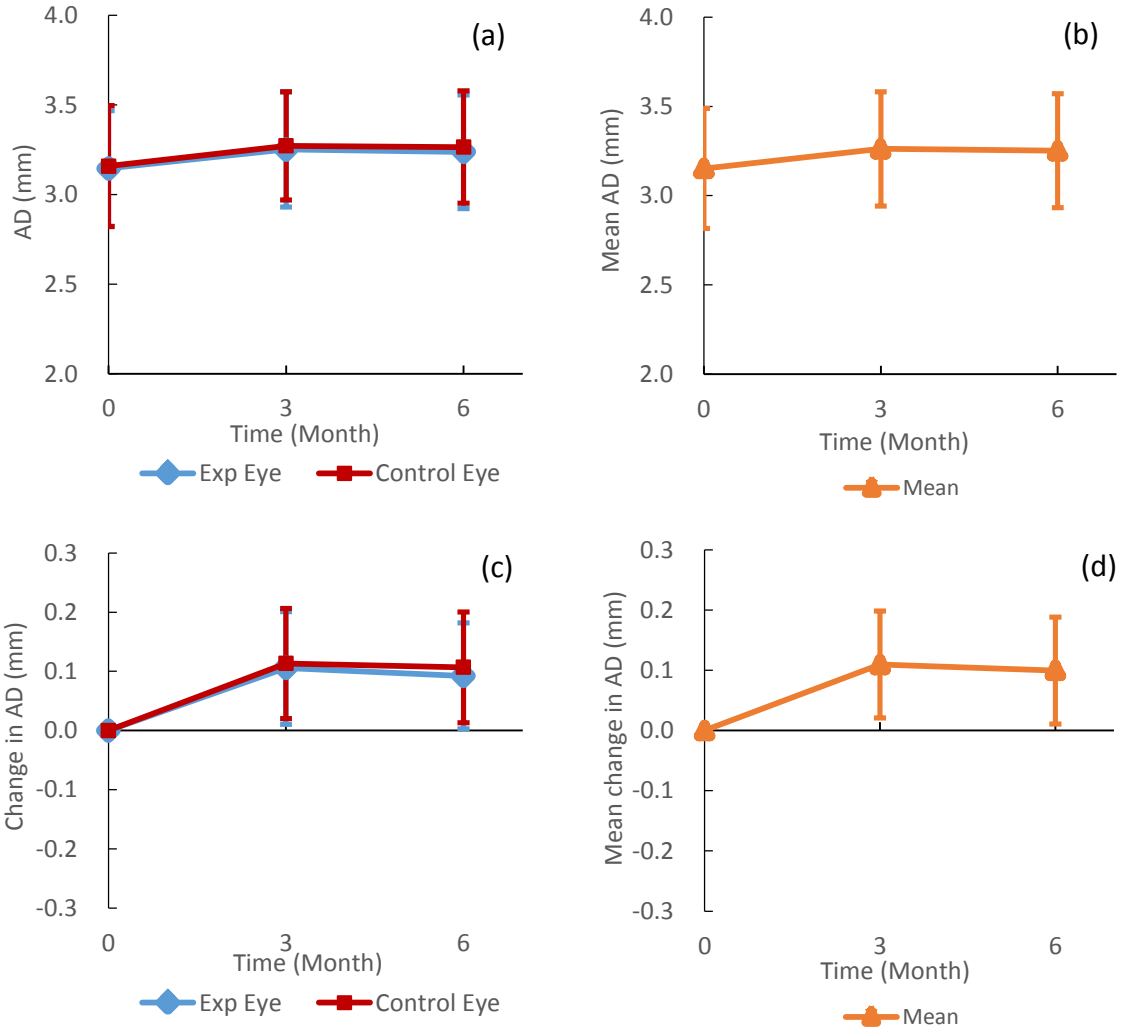


***Figure 17.*** *Summary of anterior chamber depth (AD) over the 6 months study period. (a) Actual AD of Control and Experimental eyes. (b) Actual AD mean. (c) Absolute change in AD in Control and Experimental eyes. (d) Mean change in AD. Error bars show ± 1 SD.*

### 5.7.3 Crystalline lens Thickness (LT)

Changes in crystalline lens thickness (LT) during the 6 months of the study are summarised in *Figure 18* below. Lens thickness data for two participants (PT 4 and PT 18) is missing because of missing posterior lens boundary peaks in the LenStar waveforms and therefore the results below were the mean for 18 participants. The presenting LT of Control and Experimental eyes were similar (3.43 ± 0.19 mm and 3.43 ± 0.17 mm, respectively; p = 0.67, paired t-test), with a mean for both eyes of 3.43 ± 0.18 mm. Pairwise comparison showed a significant thinning of LT from the

baseline value before atropine to the 3 and 6-month values obtained during atropine use (LT at 3 months: 3.37 ± 0.15 mm, p = 0.029; LT at 6 months 3.37 ± 0.16 mm, p = 0.036).

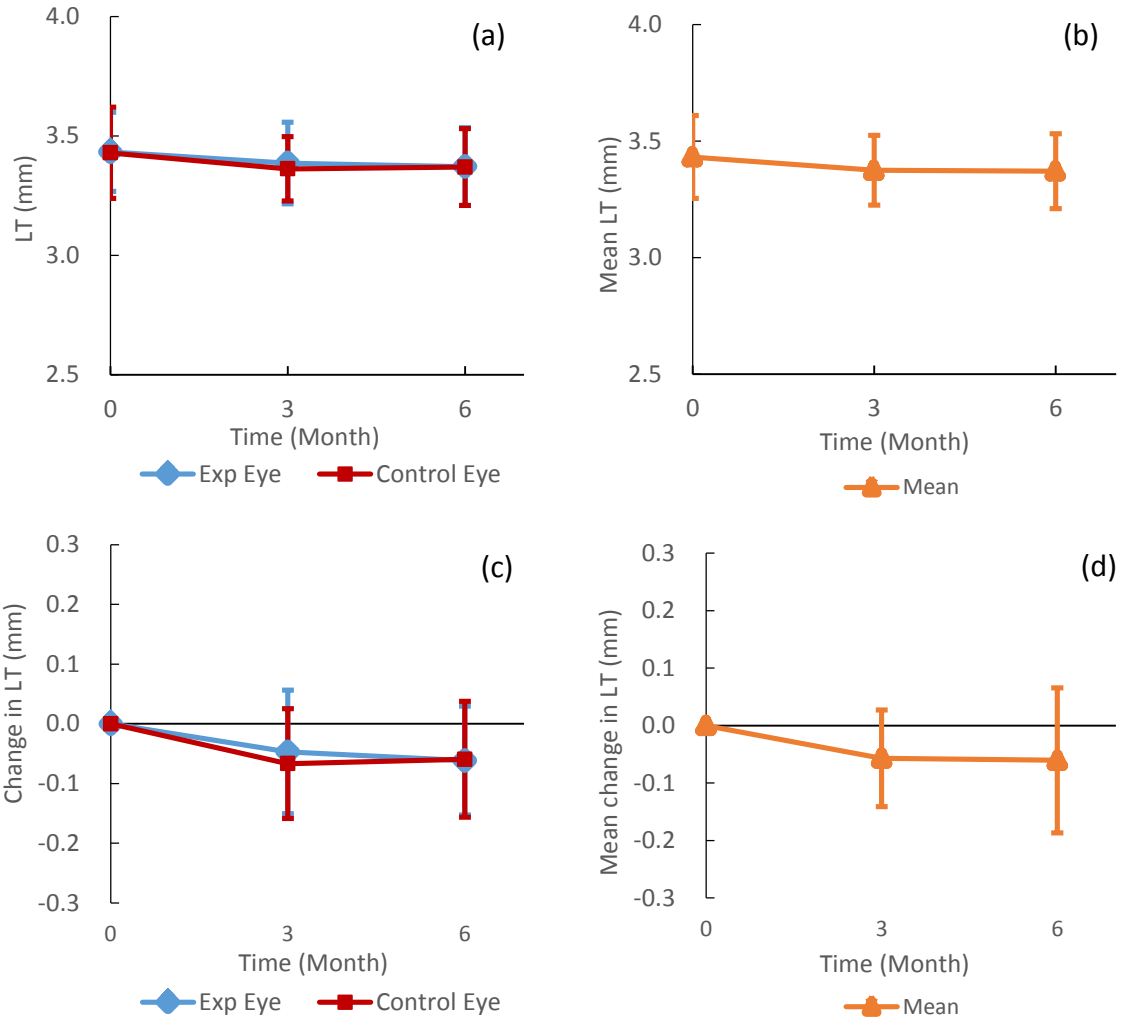


***Figure 18.*** *Summary of crystalline lens thickness (LT) over the 6-month period. (a) Actual LT of Control and Experimental eyes. (b) Actual LT mean. (c) Absolute change in CCT in Control and Experimental eyes. (d) Mean change in LT. Error bars show ± 1 SD; n = 18 (2 children's results were excluded due to missing data)*

### 5.7.4 Vitreous chamber depth (VCD)

Changes in Vitreous Chamber Depth (VCD) during the 6-month study are summarised in *Figure 19*. The VCD was calculated as VCD = AL – (CCT + AD + LT). The VCD derived from LenStar measures corresponds to the distance from the

posterior lens surface to the Retinal Pigment Epithelium. Since participants PT 4 & PT 18 had missing LT results, VCD values were only available for 18 participants (n = 18). The presenting VCD for the Control eyes was 17.07 ± 1.09 mm and for Experimental eyes was 17.11 ± 1.05 mm. The mean VCD was 17.09 ± 1.05 mm at baseline which appeared to shortened at 3 months to 17.04 ± 1.09 mm, (p = 0.026). VCD then elongated at 6 months to be similar to the presenting VCD: 17.12 ± 1.03 mm (p = 0.06).

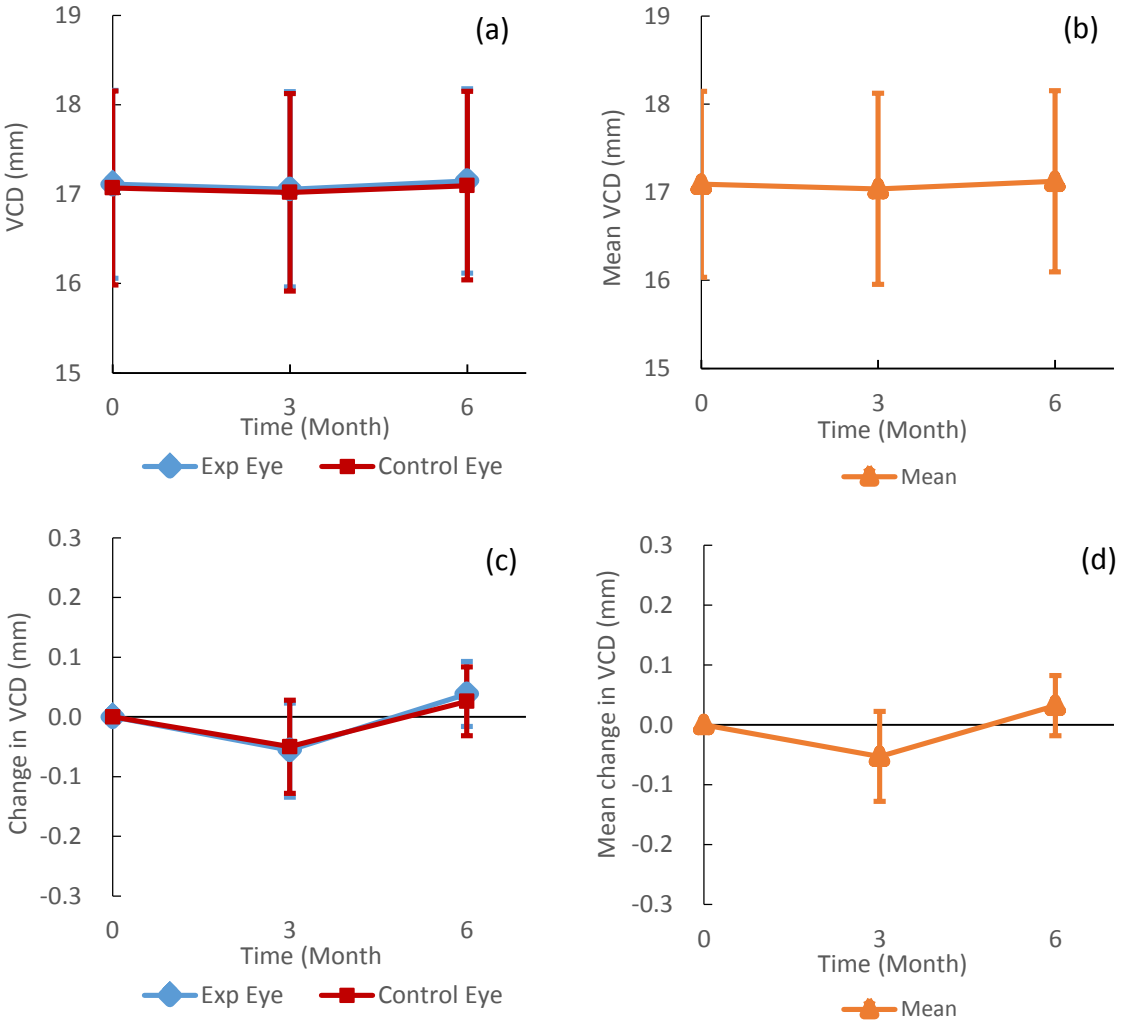


***Figure 19****. Vitreous chamber depth (VCD) over the 6-month study period. (a) Actual VCD of Control and Experimental eyes. (b) Actual VCD mean. (c) Absolute change in VCD in Control and Experimental eyes. (d) Mean change in VCD. Error bars show ± 1 SD. Two children were excluded, n = 18.*

### 5.7.5 Axial length (AL)

Axial length (AL) is an important indicator of eye size and has often been used for monitoring myopia progression. Changes in AL (distance from anterior cornea to Retinal Pigment Epithelium) during the 6 months of the study are summarised in *Figure 20* below.

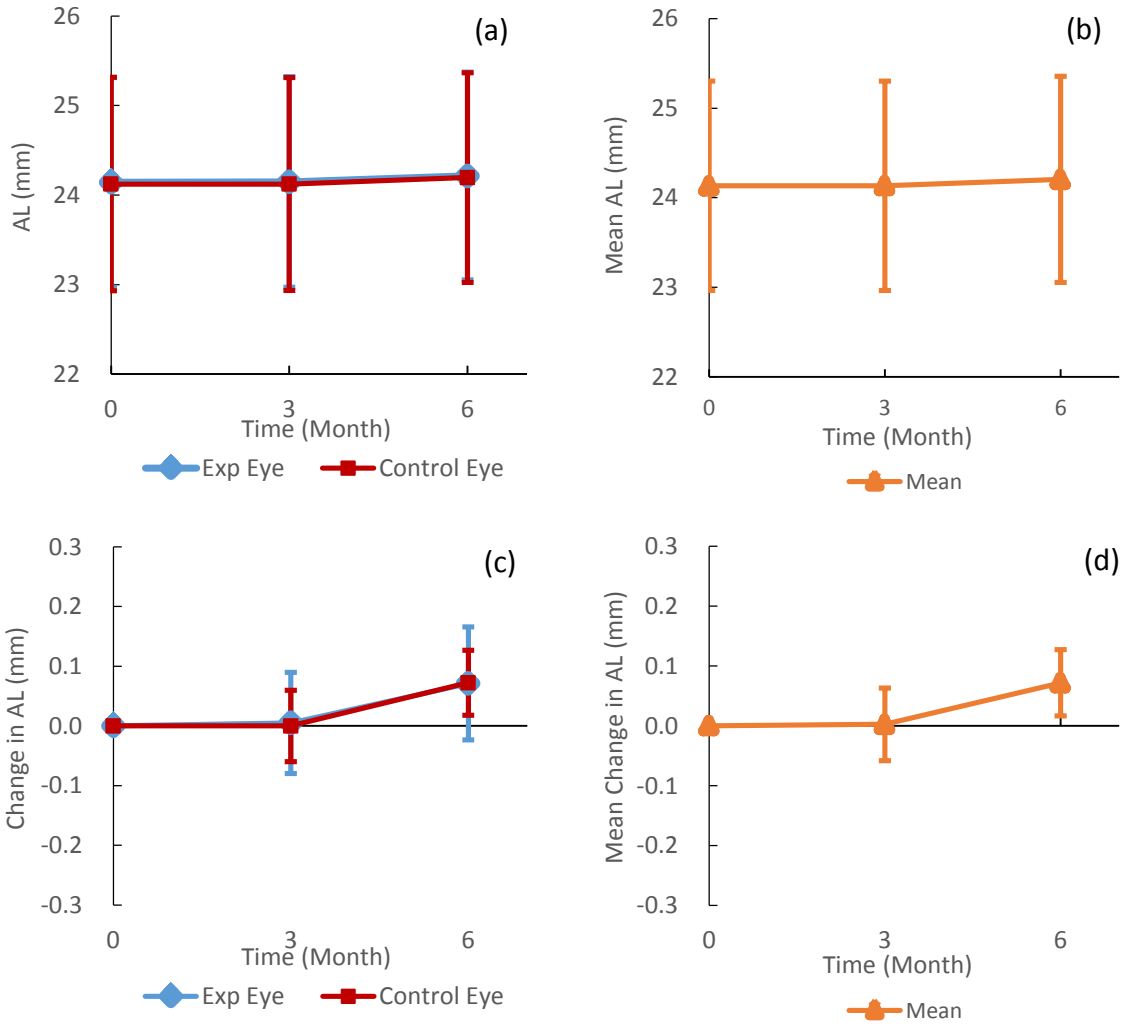


***Figure 20.*** *Summary of Axial length (AL) changes over the 6-month study period. (a) Actual AL of Control and Experimental eyes. (b) Actual AL mean. (c) Absolute change in AL in Control and Experimental eyes. (d) Mean change in AL. Error bars show ± 1 SD, n = 20.*

The mean presenting AL for the Control eyes was 24.12 ± 1.19 mm, and for Experimental eyes was 24.14 ± 1.17 mm with no statistically significant difference between the eyes (p = 0.81, paired t-test, n = 20). The mean presenting AL of both

eyes was 24.13 ± 1.17 mm. The AL remained steady at the 3-month visit (24.13 ± 1.17 mm, p = 0.86), but then showed a statistically significant elongation at 6 months of 0.07 ± 0.06 mm (p < 0.001) from baseline to reach 24.20 ± 1.15 mm.

### 5.7.6 Summary table of ocular biometry

The *Table 7* below summarises the values of each component of ocular biometry obtained with the LenStar at the 3 measurement visits: the initial visit before atropine, and after 3 and 6 months of atropine treatment. The results include Central Corneal Thickness (CCT), Anterior Chamber Depth (AD), Thickness of the Crystalline Lens (LT), Vitreous Chamber Depth (VCD) and Axial length (AL).

| | | Control Eye | | Experimental Eye | | Mean | | Absolute Mean Change | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Month | Mean | SD | Mean | SD | Mean | SD | Mean | SD | P |
| CCT (µm) | 0 | 552.05 | 22.70 | 551.45 | 23.58 | 551.75 | 22.96 | 0.00 | 0.00 | |
| | 3 | 552.30 | 24.05 | 553.75 | 24.82 | 553.03 | 24.23 | 1.28 | 4.39 | 0.22 |
| | 6 | 553.30 | 23.33 | 554.75 | 25.83 | 554.03 | 24.38 | 2.28 | 5.56 | 0.10 |
| AD (mm) | 0 | 3.16 | 0.34 | 3.15 | 0.33 | 3.15 | 0.34 | 0.00 | 0.00 | |
| | 3 | 3.27 | 0.31 | 3.25 | 0.33 | 3.26 | 0.32 | 0.11 | 0.09 | **<0.001** |
| | 6 | 3.27 | 0.32 | 3.24 | 0.32 | 3.25 | 0.32 | 0.10 | 0.09 | **<0.001** |
| LT (mm) | 0 | 3.43 | 0.19 | 3.43 | 0.17 | 3.43 | 0.18 | 0.00 | 0.00 | |
| | 3 | 3.36 | 0.14 | 3.39 | 0.17 | 3.37 | 0.15 | -0.06 | 0.08 | **0.029** |
| | 6 | 3.37 | 0.16 | 3.37 | 0.16 | 3.37 | 0.16 | -0.06 | 0.13 | **0.036** |
| VCD (mm) | 0 | 17.07 | 1.09 | 17.11 | 1.05 | 17.09 | 1.05 | 0.00 | 0.00 | |
| | 3 | 17.02 | 1.11 | 17.06 | 1.09 | 17.04 | 1.09 | -0.05 | 0.08 | **0.026** |
| | 6 | 17.10 | 1.06 | 17.15 | 1.03 | 17.12 | 1.03 | 0.03 | 0.05 | 0.06 |
| AL (mm) | 0 | 24.12 | 1.19 | 24.14 | 1.17 | 24.13 | 1.17 | 0.00 | 0.00 | |
| | 3 | 24.12 | 1.19 | 24.15 | 1.17 | 24.13 | 1.17 | 0.00 | 0.06 | 0.86 |
| | 6 | 24.20 | 1.17 | 24.21 | 1.16 | 24.20 | 1.15 | 0.07 | 0.06 | **<0.001** |

***Table 7.*** *Summary table for ocular biometry components made by LenStar reflectometer measurement. Central Corneal Thickness (CCT), Anterior Chamber Depth (AD), Thickness of the Crystalline Lens (LT), Vitreous Chamber Depth (VCD) and Axial length (AL). P-values were calculated with reference to baseline and Bonferroni corrected values ≤ 0.05 are in bold.*

## 5.8 Change in total eye length over the study period

The ocular biometry parameter AL measures from corneal apex to RPE, and does not give accurate information regarding the size of the scleral coat (Total eye length - TEL), particularly in this study in which atropine markedly altered the choroidal thickness. In order to estimate expansion of the scleral coat itself over time the choroidal thickness measures were added to the AL values to give an indication of change in TEL over time. *Figure 21b* shows that TEL (mean of both eyes) increased in the first three months (from Stage 1 to Stage 3) by 0.02 ± 0.06 mm, p = 0.22. Over the period from 3 months to 6 months (Stage 3 to Stage 4) mean TEL increased by 0.07 ± 0.06 mm, p < 0.01. The changes in each eye were very similar, as shown in *Figure 21a* and *Table 8*. The result confirms that small but significant eye elongation occurred over the 6 months study.

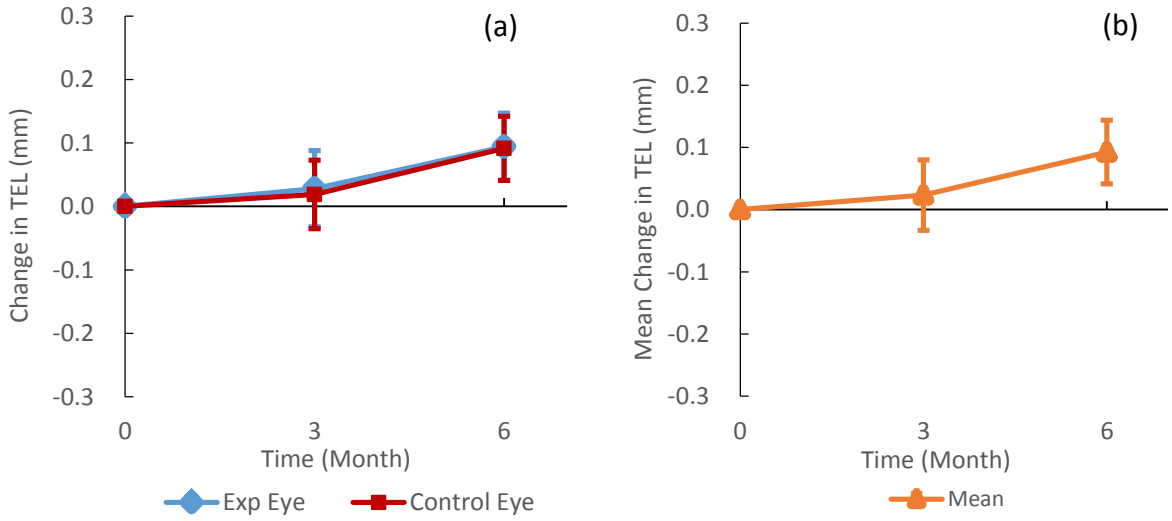


***Figure 21.*** *Summary of Total Eye Length (TEL) changes over the 6-month study period. (a) Absolute change in TEL in Control and Experimental eyes. (b) Mean change in TEL. Error bars show ± 1 SD.*

| | Control Eye (mm) (Mean ± SD) | Exp Eye (mm) (Mean ± SD) | Mean (mm) (Mean ± SD) | *Increase from Stage 1* (mm) |
|---|---|---|---|---|
| Stage 1 | 24.40 ± 1.16 | 24.41 ± 1.14 | 24.41 ± 1.13 | - |
| Stage 3 | 24.42 ± 1.15 | 24.44 ± 1.14 | 24.43 ± 1.13 | 0.02 ± 0.06 |
| Stage 4 | 24.49 ± 1.13 | 24.51 ± 1.13 | 24.50 ± 1.11 | **0.09 ± 0.05** |

***Table 8.*** *Summary table for Total Eye Length (TEL) in Control eyes, Experimental eyes, mean of both eyes, and the increase of TEL at various stages vs TEL at Stage 1. P-values were calculated with reference to baseline and Bonferroni corrected values ≤ 0.05 are in bold.*

## 5.9 Effect of season on axial elongation during the 6 month study

Previous studies have reported seasonal variation in axial eye growth and myopia progression, with slower eye growth in summer and faster eye growth in winter. As reported earlier in Section 5.2, all the children were enrolled over the 4-month period September 2015 to January 2016. Fifteen participants were enrolled in September to November 2015 (the Autumn group), and five participants were enrolled in January 2016 (the Winter group). The mean age for the Autumn group was 9.03 ± 2.30 years and the Winter group 9.00 ± 1.56 years (p = 0.98). The baseline TELs were similar between the two groups (Autumn group 24.42 ± 1.31 mm vs Winter group 24.37 ± 0.19 mm; p = 0.91). The axial elongation (TEL) of the two groups over 6 months were: Autumn group 0.10 ± 0.05 mm vs Winter group 0.07 ± 0.03 mm (p = 0.09, Unpaired t-test), see *Figure 22*. Summary of actual TEL and change in TEL of the Autumn (n=15) and Winter (n=5) groups over the 6 months is shown in *Table 9*. The change in TEL between 0 – 3 months (Between Stage 1 & Stage 3) versus 3 – 6 months (Between Stage 3 & Stage 4) are summarise in *Table 10*.

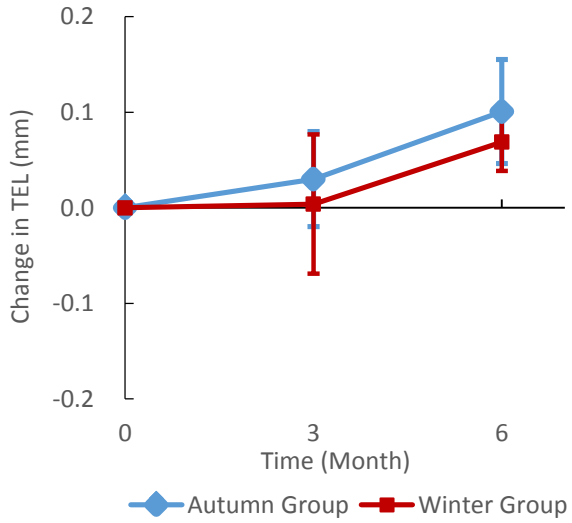


***Figure 22.*** *Absolute change of Total Eye Length (TEL) over 6 months for the Autumn and Winter groups.*

| | (Units: mm) | Control Eye | Exp Eye | Mean | Mean Change in TEL vs Stage 1 | Mean Change in TEL Stage 3 vs Stage 4 |
|---|---|---|---|---|---|---|
| Autumn (n=15) | Stage 1 | 24.42 ± 1.34 | 24.42 ± 1.32 | 24.42 ± 1.31 | - | - |
| | Stage 3 | 24.44 ± 1.33 | 24.45 ± 1.32 | 24.45 ± 1.31 | **0.03 ± 0.05** | - |
| | Stage 4 | 24.52 ± 1.31 | 24.52 ± 1.31 | 24.52 ± 1.29 | **0.10 ± 0.05** | **0.07 ± 0.06** |
| Winter (n=5) | Stage 1 | 24.35 ± 0.18 | 24.39 ± 0.22 | 24.37 ± 0.19 | - | - |
| | Stage 3 | 24.35 ± 0.22 | 24.40 ± 0.21 | 24.37 ± 0.20 | 0.00 ± 0.07 | - |
| | Stage 4 | 24.42 ± 0.17 | 24.46 ± 0.20 | 24.44 ± 0.18 | **0.07 ± 0.03** | 0.07 ± 0.07 |

***Table 9.*** *Summary of actual Total Eye Length (TEL) and change in TEL of the Autumn (n=15) and Winter (n=5) groups over 6 months. Bonferroni corrected values ≤ 0.05 are in bold*

| | 0 – 3 months (Between Stage 1 & Stage 3) | 3 – 6 months (Between Stage 3 & Stage 4) | 0 – 3 vs 3 – 6 |
|---|---|---|---|
| Autumn Group (n=15) | 0.03 ± 0.05 mm | 0.07 ± 0.06 mm | **p = 0.02** |
| Winter Group (n=5) | 0.00 ± 0.07 mm | 0.07 ± 0.07 mm | p = 0.21 |

***Table 10.*** *Summary of change in Total Eye Length (TEL) between 0 – 3 months (Between Stage 1 & Stage 3) versus 3 – 6 months (Between Stage 3 & Stage 4). P-values were calculated between 0 – 3 months vs 3 – 6 months and paired t-test values ≤ 0.05 are in bold.*

## 5.10 Prediction of change in eye length over 6 months from change in choroidal thickness over 1 week on atropine

In this section the change in TEL over 6 months is compared with the change in SFCT induced by 1 week of atropine use, to investigate whether short term changes in the choroid could be used to reliably predict longer term changes in eye length. *Figure 23* shows for each participant the change in TEL over 6 months plotted versus change in SFCT induced by 1 week of atropine use (Experimental eye). Regression analysis shows that there is a weak correlation ($R^2$ = 0.15, p = 0.09) between SFCT and change in TEL at 6 months.

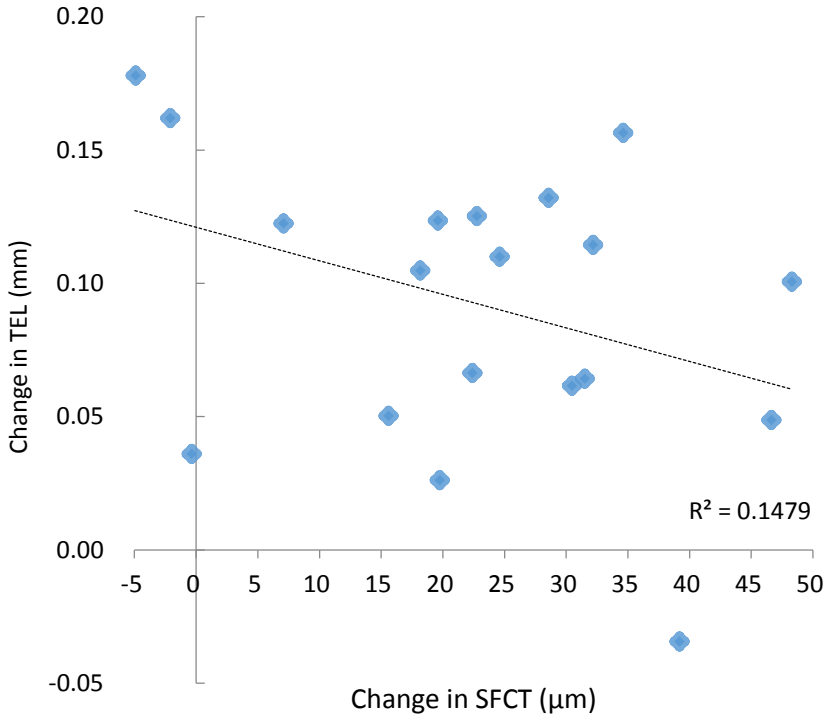


***Figure 23.*** *Change in Total Eye Length (TEL) over 6 months plotted versus change in SFCT induced by 1 week of atropine use in the Experimental eye.*

## 5.11 Prediction of change in eye length over 6 months from change in choroidal thickness after 40 minutes of optical defocus

In this section the change in TEL over 6 months is compared with the change in SFCT induced by imposed myopic and also hyperopic defocus, to investigate whether short term changes in the choroid induced by defocus might be used to predict longer term changes in eye length. *Figure 24a* and *b* show for each participant the change in TEL over 6 months plotted versus change in SFCT induced at 40-minutes of hyperopic and myopic defocus respectively, measured prior to atropine use in the Experimental eye. Low $R^2$ values associated with TEL and hyperopic defocus ($R^2$ = 0.14, p = 0.10) and myopic defocus ($R^2$ = 0.08, p = 0.24) suggest that measures of choroidal thickening induced by both myopic and hyperopic defocus are poor predictors of change in TEL over 6 months. The changes in TEL were small and limited by the

short duration of the study. Moreover, the results would only be applicable to children who are on atropine.

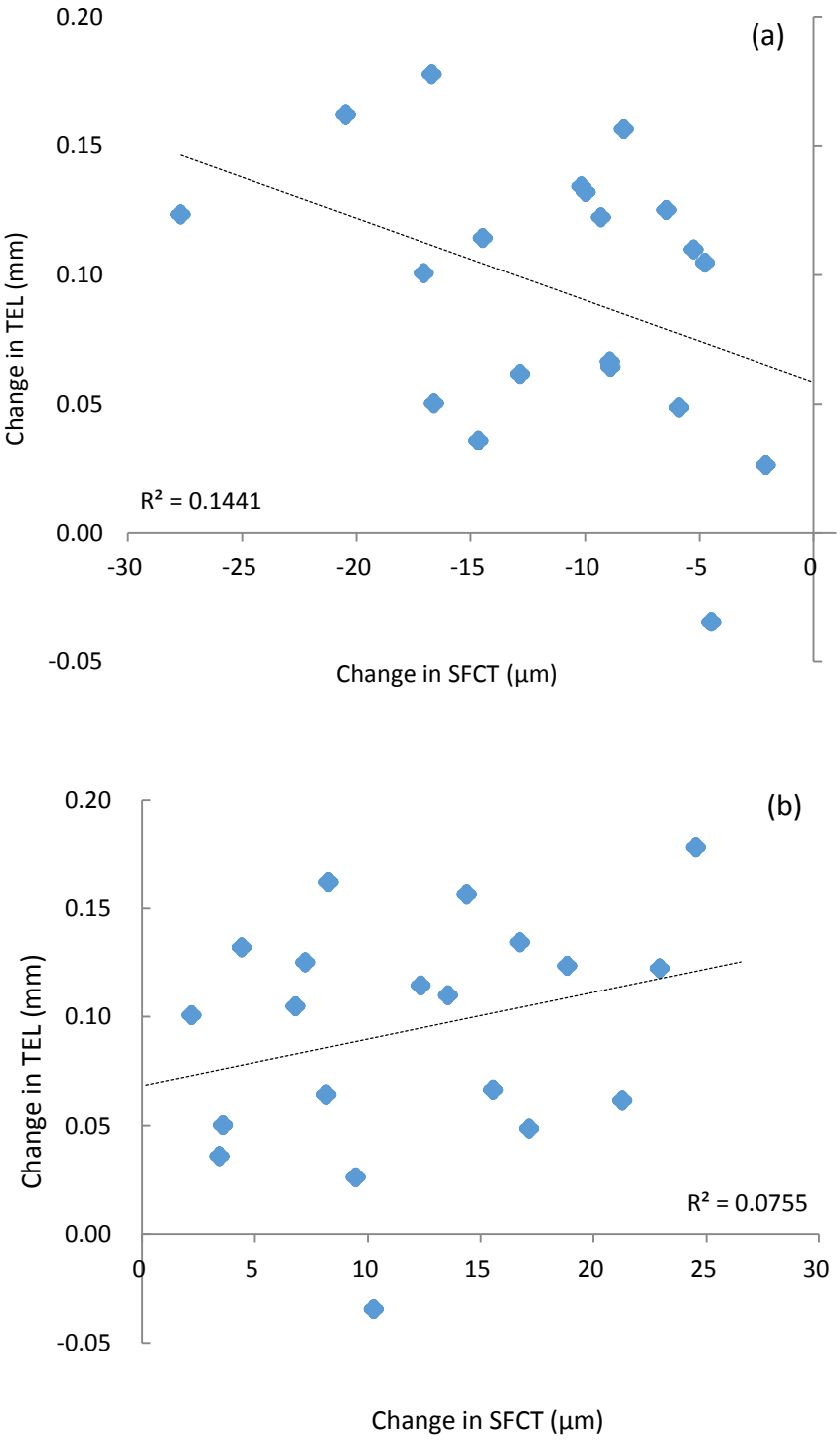


***Figure 24.*** *Change in Total Eye Length (TEL) over 6 months plotted versus change in SFCT induced at 40 minutes of (a) hyperopic defocus and (b) myopic defocus in the Experimental eye.*

## 5.12 Refractive error, amplitude of accommodation and pupil size

Refractive error, amplitude of accommodation and pupil size of both eyes were measured at baseline and at 1 week, 3 months and 6 months of the study to assess potential changes in each factor caused by administration of atropine.

### 5.12.1 Refractive error during the 6-month study

Children's refractive error was monitored over the 6-month study period using the open-field autorefractor results refined by subjective refraction. Baseline refractive error was measured without cycloplegia to avoid unwanted residual effects on choroidal thickness and response to optical defocus.

*Figure 25* summarises the refractive error of all participants during the 6-month study period. The mean refractive error was -1.63 ± 0.90 D at baseline, which slightly reduced after 1 week of atropine administration to -1.43 ± 0.90 D. The refractive error then slowly progressed at 3 and 6 months of atropine administration (to -1.48 ± 0.92 D and -1.63 ± 0.90 D respectively) back to the baseline refractive error. Pairwise comparison showed a significant difference from baseline at 1 week and at 3 months ($p < 0.001$ and $p = 0.002$) but no difference at 6 months ($p = 0.95$).

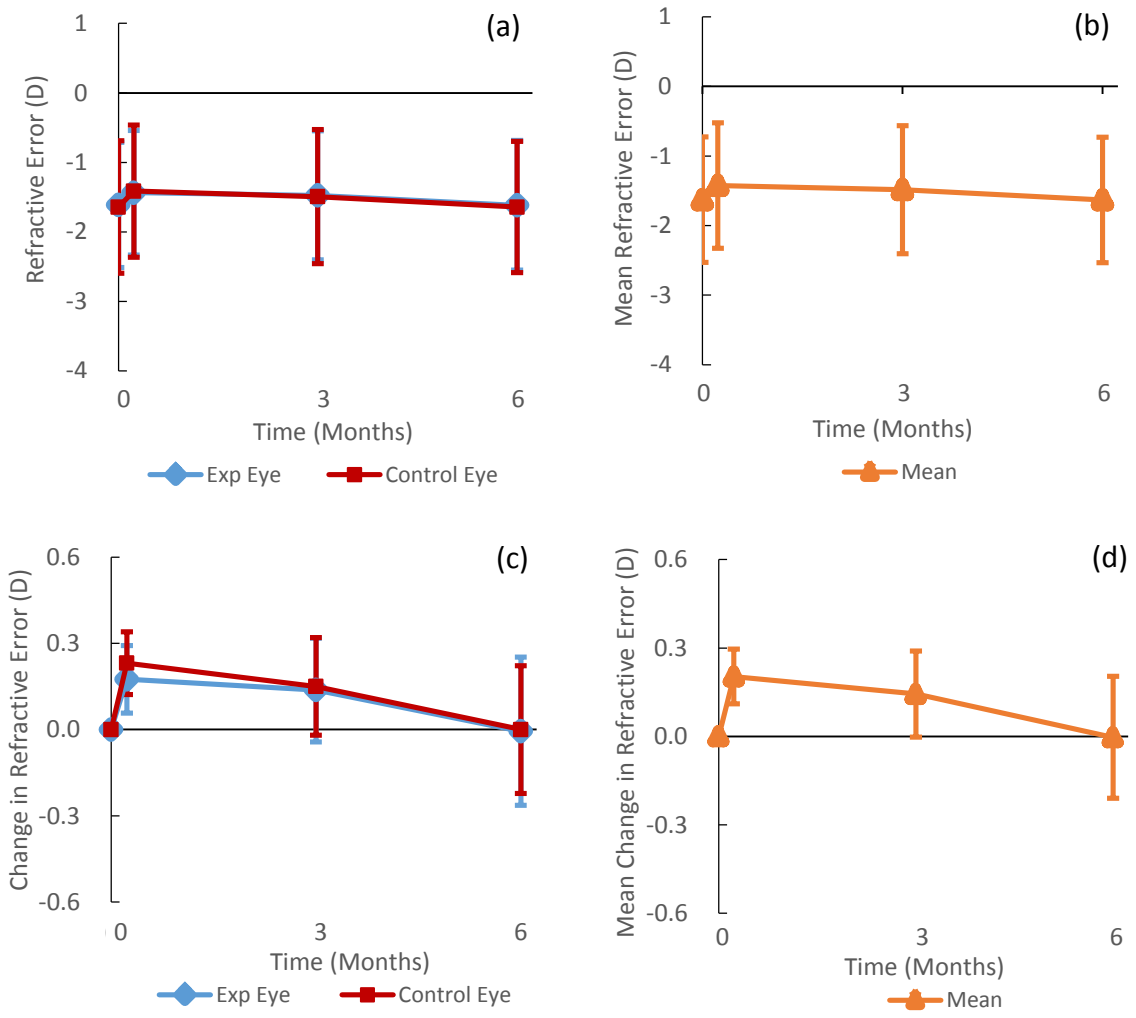


***Figure 25.*** *Summary of children's refractive error over the 6-month study period. (a) Mean refractive error (Rx) for Control and Experimental eyes. (b) Mean refractive error. (c) Mean change in Control and Experimental eye refractive error. (d) Mean change in refractive error. Error bars show ± 1 SD.*

### 5.12.2 Correlation between refractive error and ocular biometry

In progressing myopia, a change in refraction over time is expected to result from changes in ocular biometry, in particular abnormal axial growth of the eye. *Figure 26* illustrates the correlation between change in refractive error versus change in axial length in the Experimental eye over the 6-month study. The result shows a weak correlation ($R^2$ = 0.06, p = 0.29). However, the initial measures of axial length were made prior to atropine use whereas the changes in refraction were all computed from cycloplegic measures. Therefore the axial length measures used for *Figure 26* were

corrected by subtracting the increase in choroidal thickness that resulted from 1 week of atropine use from the AL measures made without cycloplegia. inspection of the graph shows that the changes in refraction of individual participants was typically very small (the mean change in refraction in the Experimental eyes was -0.18 ± 0.23 D, and in Control eyes was -0.23 ± 0.18 D. The mean change in both eyes was -0.21 ± 0.21 D), which is below the smallest step (0,25D) used to determine refraction. (Note that the refractions used were the autorefraction results refined by subjective refraction and therefore to the nearest 0.25D).

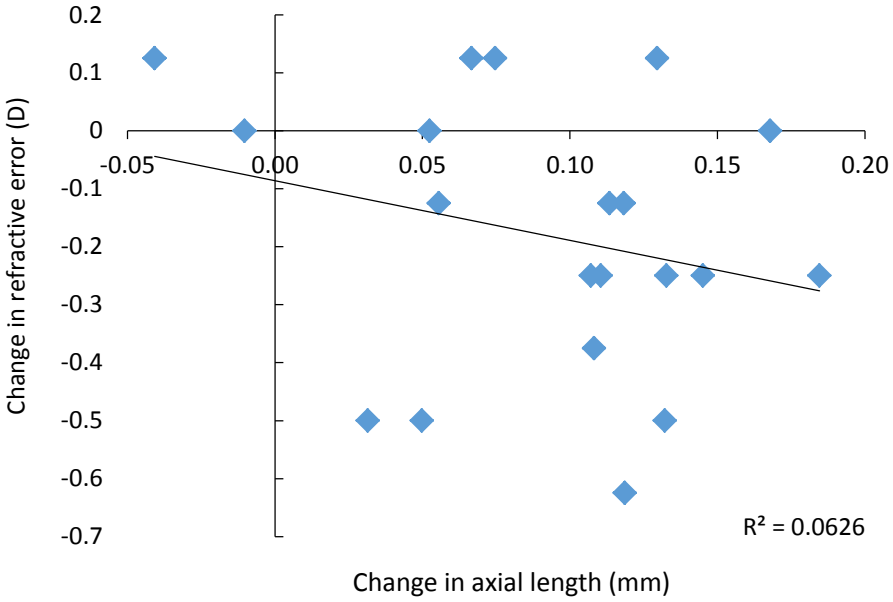


***Figure 26.*** *Correlation between change in refractive error versus change in axial length over the 6-month study.*

### 5.12.3 Amplitude of accommodation (AoA) during the 6-month study

The Amplitude of Accommodation (AoA) of all participants was measured and recorded at each stage of the study, to monitor the potential effect of atropine on AoA. The summary results for AoA are shown in *Figure 27* below. The mean AoA at

baseline (both eyes) was 17.53 ± 1.30 D, which reduced significantly (as expected) after 1 week of atropine administration to 1.37 ± 0.20 D. The AoA remained minimal throughout the 6 months use of atropine (AoA at 3 months: 1.36 ± 0.20 D and at 6 months: 1.36 ± 0.23 D). Pairwise comparison showed a significant change from baseline to 1 week, 3 months and 6 months ($p < 0.001$) of atropine administration.

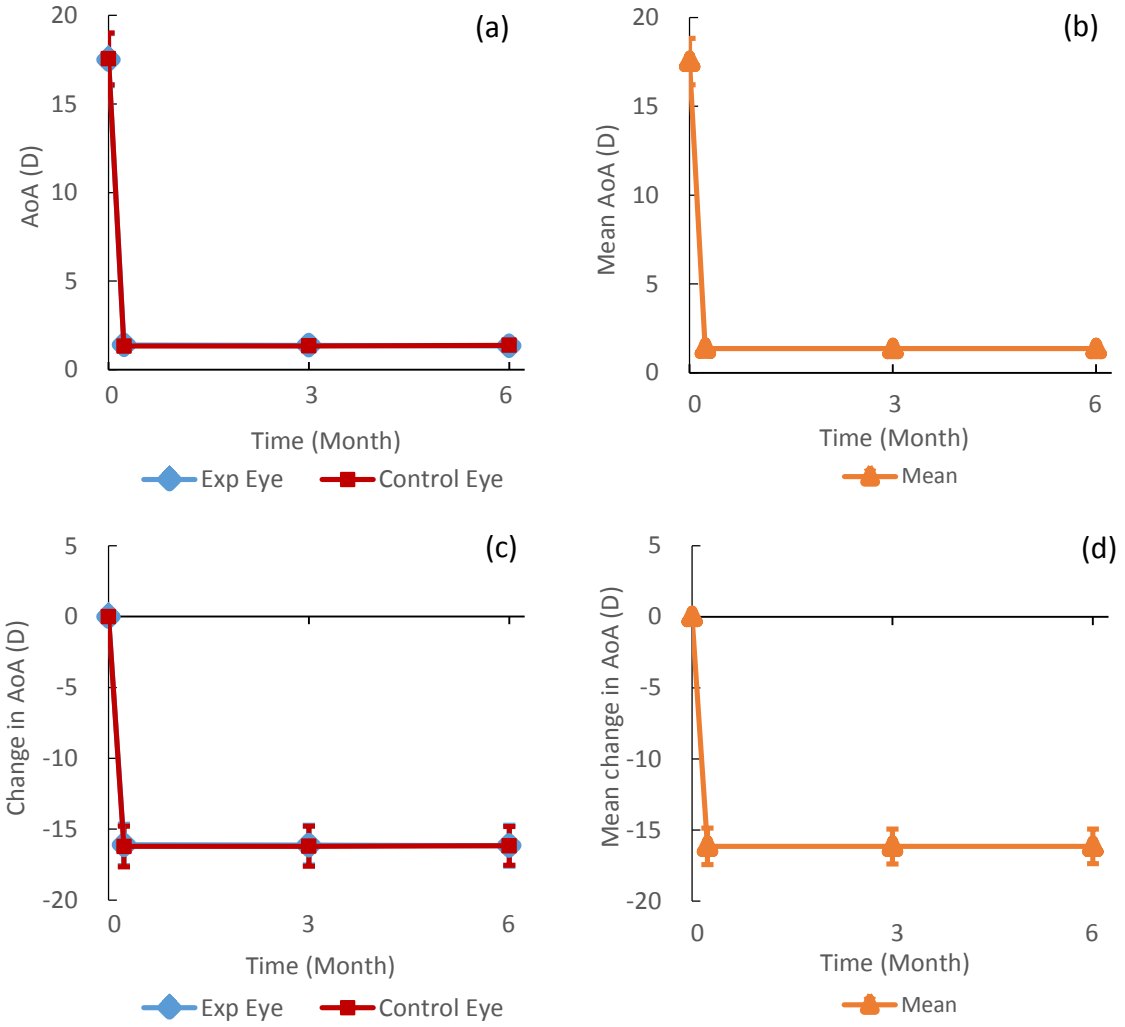


***Figure 27.*** *Summary of amplitude of accommodation (AoA, D) over the 6-month study period. (a) AoA of Control and Experimental eyes. (b) Mean AoA. (c) Change of AoA in Control and Experimental eyes. (d) Mean change in AoA. Error bars show ± 1 SD.*

### 5.12.4 Pupil size during the 6-month study

Pupil size of all participants was measured and recorded at each stage of the study to monitor the effect of atropine on the pupil. A summary of pupil size over the 6-month

study is shown in *Figure 28* below. The mean pupil size was 4.08 ± 0.47 mm at baseline (Control eyes: 4.08 ± 0.54 mm; Experimental eyes 4.08 ± 0.47 mm). Mean pupil size became much larger after 1 week of atropine administration (7.44 ± 0.38 mm) and pupil size remained large throughout the 6-month use of atropine (At 3 months: 7.53 ± 0.37 mm and at 6 months: 7.55 ± 0.34 mm). Pairwise comparison showed a significant change from baseline at 1 week, 3 months and 6 months ($p < 0.001$) of atropine use.

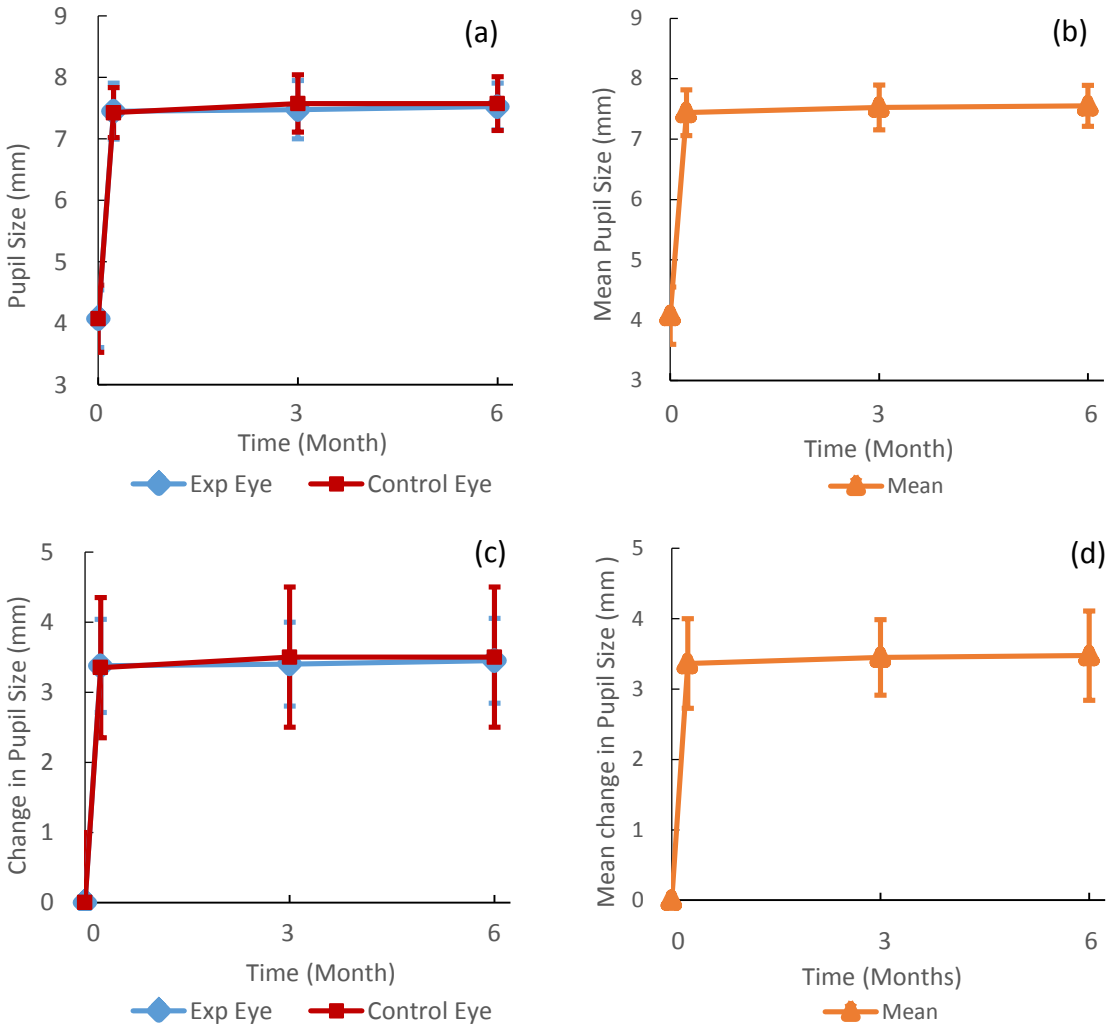


***Figure 28.*** *Summary of pupil size (mm) over the 6-month study period. (a) Pupil size of Control and Experimental eyes. (b) Mean pupil size. (c) Mean change in pupil size in Control and Experimental eyes. (d) Mean change in pupil size. Error bars show ± 1 SD.*

### 5.12.5 Summary of refractive error, amplitude of accommodation and pupil size

A summary of refractive error (refraction), amplitude of accommodation and pupil size of the Control and Experimental eyes, shown as mean, absolute mean change, standard deviation and p-value for secondary outcome measures are shown in *Table 11* below. The absolute mean change at each visit was calculated by subtracting the initial value at baseline (0-week: 0W) from the value at each visit. The p-value shows significance of the comparison between that specific visit and at baseline (0W).

| | | Control Eye | | Experimental Eye | | Mean | | Absolute Mean Change | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Visit | Mean | SD | Mean | SD | Mean | SD | Mean | SD | P |
| Refraction (D) | 0W | -1.64 | 0.95 | -1.61 | 0.90 | -1.63 | 0.90 | | | |
| | 1W | -1.41 | 0.95 | -1.44 | 0.89 | -1.43 | 0.90 | 0.20 | 0.09 | **<0.001** |
| | 3M | -1.49 | 0.97 | -1.48 | 0.93 | -1.48 | 0.92 | 0.14 | 0.15 | **0.002** |
| | 6M | -1.64 | 0.95 | -1.62 | 0.93 | -1.63 | 0.90 | 0.00 | 0.21 | 0.95 |
| AoA (D) | 0W | 17.55 | 1.47 | 17.50 | 1.47 | 17.53 | 1.30 | | | |
| | 1W | 1.33 | 0.16 | 1.39 | 0.27 | 1.37 | 0.20 | -16.16 | 1.28 | **<0.001** |
| | 3M | 1.34 | 0.21 | 1.38 | 0.22 | 1.36 | 0.20 | -16.17 | 1.23 | **<0.001** |
| | 6M | 1.37 | 0.23 | 1.34 | 0.31 | 1.36 | 0.23 | -16.16 | 1.22 | **<0.001** |
| Pupil (mm) | 0W | 4.08 | 0.54 | 4.08 | 0.47 | 4.08 | 0.47 | | | |
| | 1W | 7.43 | 0.41 | 7.45 | 0.46 | 7.44 | 0.38 | 3.36 | 0.64 | **<0.001** |
| | 3M | 7.58 | 0.47 | 7.48 | 0.47 | 7.53 | 0.37 | 3.45 | 0.54 | **<0.001** |
| | 6M | 7.58 | 0.44 | 7.53 | 0.38 | 7.55 | 0.34 | 3.48 | 0.63 | **<0.001** |

***Table 11.*** *Summary of the refraction, amplitude of accommodation and pupil size of the Control and Experimental eyes and their mean, at each stage of the study. 0W= 0-week (Baseline), 1W = 1 week, 3M = 3 months and 6M = 6 months. P-values were calculated with reference to baseline and Bonferroni corrected values ≤ 0.05 are in bold.*

## 5.13 Managing the side-effects of atropine

During the 6-month study period, none of the children experienced any significant clinical adverse event or requested an unscheduled appointment. Medically, there was no report of allergic conjunctivitis, allergic dermatitis of the eyelid, ocular irritation or other severe adverse event during the 6-month use of atropine.

The common and well-known side-effects of atropine administration were reported by some participants. These included photophobia due to pupil dilation, and the need for reading glasses for near work, due to paralysis of accommodation. These common side-effects were explained to participants and their parent/guardians before enrolment.

Five participants reported sensitivity to light with atropine use: these were managed with sunglasses for outdoor activities and they were used as needed.

Nine participants requested a reading correction for near work; this was expected for children with smaller amounts of myopia as their myopic refractive error when they removed their distance correction (if they wore one) did not adequately compensate for the near demand of reading. Children requesting reading spectacles were managed for near work by provision of single vision spectacles (typically +1.50D) for use after removal of the single vision distance correction. No bifocal or progressive addition lenses were prescribed during this short study as participants managed successfully with single vision spectacles.

# 6 Discussion

## 6.1 Defocus-induced changes in choroidal thickness

Both the results from the Preliminary study and those from Stage 1 of the main part of this study extend previous findings from animal studies (e.g. (Wallman, Wildsoet et al. 1995)) and corroborate findings in human adults (Read, Collins et al. 2010, Chakraborty, Read et al. 2011, Chakraborty, Read et al. 2013, Chiang, Phillips et al. 2015) and children (Wang, Chun et al. 2016) to show that hyperopic and myopic defocus induce rapid choroidal thinning and thickening in young Taiwanese children.

The preliminary study found that 60 minutes of 2.00 D hyperopic defocus (prior to atropine) induced significant thinning of SFCT of 10 ± 2 µm (mean ± SEM). In Stage 1 of the main study (before atropine), 60 minutes of 2.00 D hyperopic defocus also induced significant thinning of SFCT of 12 ± 2 µm (mean ± SEM). In contrast, 2.00 D of monocular myopic defocus caused significant thickening of SFCT (12 ± 2 µm: mean ± SEM, see *Figure 10*). The degree of choroidal thinning and thickening in response to defocus found in the present study lie between the values reported by Read et al. 2010 (Read, Collins et al. 2010) of ~8 µm of thinning and ~12 µm of thickening and those reported previously by the author (Chiang, Phillips et al. 2015) of ~20 µm thinning and ~20 µm of thickening. The degree of choroidal thickening and thinning found in the present study would appear to be greater than reported by Wang et al. (Wang, Chun et al. 2016) of approximately 3 µm thickening and 0.5 µm thinning after 1 hour of defocus and 5 µm thickening and 2 µm thinning after 2 hours of defocus . There are several factors that could account for the different results found in these studies. All participants in this study were children whereas the Read et al., 2010 and Chiang et al., 2015 studies investigated young adults. Moreover, children in the present study were all Taiwanese, whereas Read et al.'s participants included

Caucasians, East Asians, and Indians, while Chiang et al.'s participants were all East Asians. Differences in ocular biometry and myopia prevalence have been reported in different ethnicities (Rudnicka, Owen et al. 2010, Rudnicka, Kapetanakis et al. 2016), and it is possible that such variations extend to the choroidal response to defocus. Comparisons with the Wang et al. (Wang, Chun et al. 2016)study are difficult because prior to their study, 3 drops of 1% cyclopentolate were instilled into both eyes of all participant groups, including the control group. Previous studies (Section 2.8.3) indicate that cyclopentolate can affect choroidal thickness and so it may also have affected the responses to optical defocus. One of their findings was that the choroid progressively thinned in the control group over the study period. This made their responses to defocus difficult to interpret. In addition, measures were made under brighter lighting conditions (~430 lux) compared to the other studies (~10 lux).

In addition, Read et al. and Wang el al. used ± 3.00 D of defocus, whereas Chiang et al., 2015 and the present study used ± 2.00 D defocus. The optimal level of defocus for eliciting a choroidal response is not known, and may not be linear.

## 6.2 Effect of atropine on defocus-induced changes in choroidal thickness

This is the first study to our knowledge that has investigated the effect of atropine on choroidal response to hyperopic and myopic retinal image defocus in humans. As discussed in section 6.1, hyperopic retinal defocus induces rapid choroidal thinning and myopic retinal defocus causes rapid thickening of the choroid. The results of this study indicate that atropine abolishes choroidal thinning induced by hyperopic defocus but does not interfere with choroidal thickening caused by myopic defocus.

This differential effect of atropine on the choroidal response to hyperopic and myopic defocus suggests that choroidal thinning and thickening are mediated by different mechanisms as previously suggested by Nickla et al. (Nickla, Zhu et al. 2013). Nickla and colleagues have proposed that choroidal thinning is mediated via contraction of non-vascular smooth muscle by acetylcholine, whereas thickening is via a dopaminergic or nitrergic pathway.

The inhibitory effect of atropine on choroidal response to hyperopic retinal defocus was observed in our preliminary study of young East Asian adults described in Chapter 3. In the preliminary study, prior to instilling atropine, 2.00 D of hyperopic retinal defocus caused subfoveal choroidal thinning of 10 ± 2 µm (mean ± SEM) after 40 minutes of retinal image defocus to the Experimental eye. This choroidal thinning induced by hyperopic defocus was abolished when hyperopic defocus was applied on the following day to the Experimental eye which had been treated with 1 drop of 0.5% atropine 22 hours earlier. However, in the preliminary study the effect of atropine on choroidal thickening induced by myopic defocus was not investigated.

In the main study in children, at Stage 1 (before atropine, see *Figure 10*), 2.00 D of hyperopic defocus induced significant choroidal thinning (~12 µm) and 2.00 D of myopic defocus caused significant choroidal thickening (~12 µm). After 1 week of nightly instillation of 0.3% atropine to both eyes, the choroidal thinning that was normally induced by hyperopic defocus was abolished by atropine (2 ± 1 µm; mean ± SEM, of choroidal thickening was recorded at 60 minutes): this was consistent with the finding in the Preliminary Study that atropine abolished thinning induced by hyperopic defocus. However, after 1 week on atropine the thickening effect of myopic defocus was unaffected (13 ± 1 µm; mean ± SEM, see *Figure 11*). This ability of atropine to abolish choroidal thinning in the presence of hyperopic defocus was

consistent and was present in children on atropine at 3 months (1 ± 0.5 µm; mean ± SEM, see *Figure 12*) and 6 months (1 ± 0.5 µm; mean ± SEM, see *Figure 13*). The choroidal thickening induced by 2.00 D myopic defocus remained unaffected by atropine use at 3 months (10 ± 1 µm at 60 minutes; mean ± SEM) and at 6 months (12 ± 1 µm at 60 minutes; mean ± SEM).

The mechanism by which atropine abolishes choroidal thinning induced by hyperopic defocus is unknown. A related animal study conducted by Nickla and colleagues (Nickla, Zhu et al. 2013) examined the effect of muscarinic antagonists in the chick eye. They showed that muscarinic antagonists: atropine, oxyphenonium (non-selective) and pirenzepine (M1, M4 selective antagonist) thickened the choroidal 3 hours after injection, even in eyes wearing -10 D negative lenses, which were previously shown to thin the choroid in chick. The three antagonists were also effective at inhibiting the development of myopia in the chick (Nickla, Zhu et al. 2013). These results provide further evidence of a potential link between thickening of the choroid and reduced myopia development, at least in animal models. To the extent that comparisons can be made, the results of the present study agree with those of the animal study by Nickla et al. (Nickla, Zhu et al. 2013). In both studies atropine treatment resulted in choroidal thickening, although this was far greater in the chick (42 µm) than was observed in humans (~20 µm). Moreover atropine inhibited choroidal thinning induced by negative lenses in both the animal and human studies.

## 6.3 Atropine eye drops alone thicken the choroid

The main study in children showed that 1 drop of 0.3% atropine instilled at night in both eyes for a week is capable of increasing choroidal thickness by approximately 20

μm without any applied optical defocus (see *Figure 15* and *Table 6*). The mean presenting choroidal thickness of participants at Stage 1 (before atropine) was 275 ± 70 μm (mean ± SD). The mean presenting choroidal thickness had increased significantly ($p < 0.001$) to 295 ± 72 μm (mean ± SD) after 1 week of 0.3% atropine instillation binocularly (Stage 2). This thickening effect remained during the 6-month study period while children were on atropine eye drops. At 3 months (Stage 3), the mean presenting choroidal thickness was 301 ± 67 μm, and at 6 months was 296 ± 68 μm.

Interestingly, this thickening effect of atropine on the choroid was not apparent in our preliminary study on young adults, which employed the protocol of 1 drop of 0.5% atropine in total and applied to the Experimental eye only. The normal time-course of the effects of 1% atropine sulphate eye drops on the pupil and ciliary body are that the peak effect occurs at around 30 minutes to 3 hours, with a progressive reduction in effect over the following 1 to 10 days (Wolf and Hodge 1946), However, the time course of atropine's effects on the choroid do not appear to have been investigated in humans. Nonetheless, it is surprising that no effect of atropine alone on baseline choroidal was seen in the preliminary study. The baseline choroidal thickness (both SFCT and PFCT) were not significantly different on Day 1 and Day 2 (Day 1 SFCT = 253 ± 32 μm versus Day 2 SFCT = 249 ± 31 μm (mean ± SD); $p = 0.16$). However, the choroidal response to hyperopic defocus (thinning) was abolished by this dose of atropine. There are some possible reasons why the choroidal thickening effect of atropine was not observed in the preliminary study. It is possible that 1 drop of 0.5% atropine in the Experimental eye was not potent enough to induce an overall increase in choroidal thickness, or that the effect had occurred and then disappeared, or that we had not waited long enough to see an effect, or that a significant effect was not

seen because of the small number of participants (10) studied. However, the fact that the choroidal thinning response to hyperopic defocus was abolished, confirms that atropine had reached receptors or other target sites that were able to either directly or indirectly influence the choroid. This may imply that the two actions of atropine, abolition of choroidal thinning and increase in baseline choroidal thickness, operate through different mechanisms.

A recent study investigated the effect of 1% atropine twice daily for a week on choroidal thickness in 30 children (Zhang, Zhou et al. 2016). The results showed a significant increase in the SFCT (~15 μm) after 1 week of 1% atropine gel. Our findings are consistent with their results showing atropine alone thickens the choroid. However, our study only used 0.3% atropine eye drops nightly for a week and yet still induced approximately 20 μm of choroidal thickening. This suggests that lower concentrations of atropine may be as effective as higher concentrations in thickening the choroid.

The ability of muscarinic antagonists (e.g. atropine) to cause thickening of the choroid has previously been demonstrated in animal and human studies. An animal study by Nickla et al. (Nickla, Zhu et al. 2013) found that when atropine is injected into the vitreous of chicks wearing -10 D lenses, a choroidal thickening of 42 μm ($p = 0.075$) compared to the saline control was observed 3 hours after the $4^{th}$ day injection. Similar effects were also shown with other muscarinic antagonist (i.e. pirenzepine and oxyphenonium) (Nickla, Zhu et al. 2013).

In human studies, Sander et al., showed that 2% homatropine, a non-selective muscarinic antagonist similar to atropine, caused a small but significant increase in subfoveal and parafoveal choroidal thickness (~14 μm at 60 minutes) and ~12 μm at 60 minutes, 1.5 mm from the fovea) in young adults (Sander, Collins et al. 2014). A

recent study by Oner et al., 2016 (Oner, Bulut et al. 2016) investigated the short-term effects of the two antimuscarinic agents (i.e. tropicamide 1% and cyclopentolate 1%) on SFCT in 74 healthy adults aged between 16 and 64 years. They instilled topical medication three times in a 10-minute interval and performed choroidal thickness measures by OCT at baseline and 40 minutes after the last eye drops. They reported that cyclopentolate caused significant choroidal thickening, whereas tropicamide had no significant effect on choroidal thickness. In contrast, Yuvaci et al., assessed the short-term effects of 1% tropicamide and 1% cyclopentolate on choroidal thickness. Their protocol involved instilling eye drops 3 times at 5-minute intervals, and making OCT measures of choroidal thickness before and 50 minutes after eye drops instillation. They concluded that cyclopentolate and tropicamide both caused a decrease in choroidal thickness (Yuvaci, Pangal et al. 2015).

## 6.4 Additive effects of atropine and myopic defocus

Section 6.2 discussed the finding that atropine abolished the choroidal thinning typically induced by hyperopic retinal defocus, without affecting the choroidal thickening typically induced by myopic retinal defocus. Section 6.3 discussed the finding that 1 week of nightly instillation of atropine alone also induced choroidal thickening. This section discusses the way these two choroidal thickening effects summate.

Section 5.6 showed that 1 drop of 0.3% atropine instilled nightly thickened the choroidal by approximately 20 µm in a week. Furthermore, 2.00 D of myopic defocus could further thicken the choroid by ~13 µm (Section 5.5.2: *Figure 11*). The mean presenting choroidal thickness and absolute changes in choroidal thickness with defocus in the Experimental eyes at each stage are summarised in *Table 12* below.

| | Presenting SFCT (µm) | Max SFCT with MD (µm) | Increase due to MD (µm) |
|---|---|---|---|
| Stage 1 | 271 ± 72 | 283 ± 75 | 12 ± 7 |
| Stage 2 | 293 ± 75 | 306 ± 73 | 13 ± 6 |
| Stage 3 | 300 ± 70 | 310 ± 71 | 10 ± 6 |
| Stage 4 | 295 ± 72 | 306 ± 74 | 12 ± 6 |

***Table 12****. Summary of the mean presenting subfoveal choroidal thickness (SFCT) and absolute change in SFCT with myopic defocus (MD) in Experimental eyes only at each stage. Presenting SFCT = actual SFCT of Experimental eye at 0 minutes. Max SFCT with MD = maximum mean SFCT during the 60 minutes of defocus. Increase due to MD = (Max SFCT – Presenting SFCT). Table shows mean ± 1SD.*

The graphical illustration of increases in choroidal thickness at the 4 Stages is shown in *Figure 29* below. The results show that thickening due to atropine administration and thickening due to myopic defocus simply summate (add). The summation was consistent over the 6 months of the study.

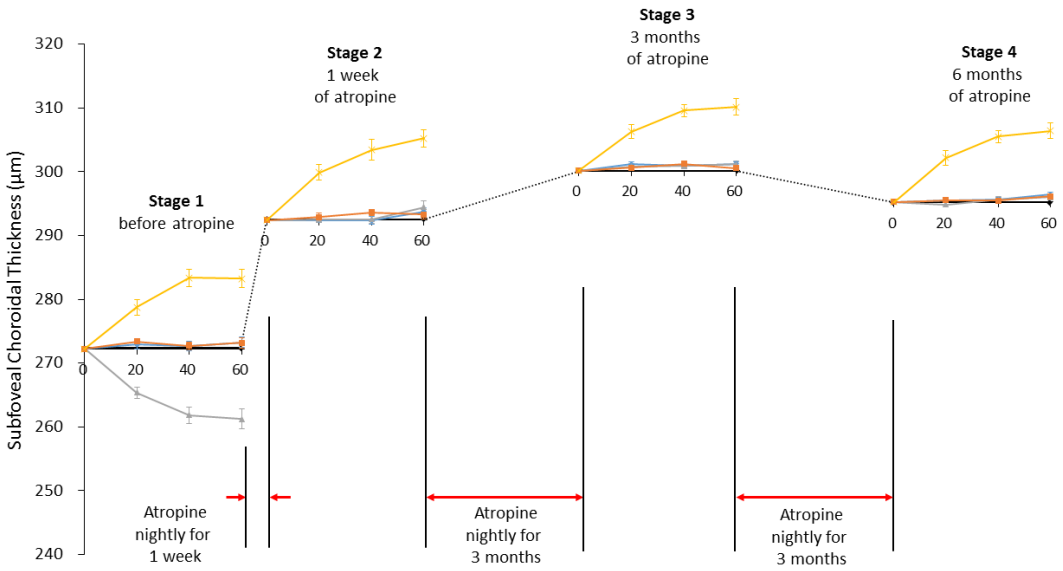


***Figure 29.*** *Graphical illustration of changes in SFCT during the 6 months of the study. The results show that thickening due to atropine administration and thickening due to myopic defocus simply summate (add). The summation was consistent over the 6 months of the study.*

In an ARVO abstract, Sander et al. (Sander, Collins et al. 2016) studied the interactive effects of 2% homatropine and optical blur on choroidal thickness over 60 minutes in young healthy adults. In their study, homatropine alone caused an increase in choroidal thickness. In the adult study described in this thesis, choroidal thickness remained unaltered 22 hours after one drop of 0.5% atropine, suggesting a different time course for choroidal thickening with atropine and homatropine alone. In the main study in children the choroidal thickness had increased after 1 week of atropine treatment as has recently been reported in children by Zhang and colleagues (Zhang, Zhou et al. 2016). Homatropine also abolished choroidal thinning associated with hyperopic defocus in the presence of homatropine-induced choroidal thickening. This finding is consistent with the results from the main study of atropine in children, but different from the result of atropine in adults, in which choroidal thinning was abolished after 22 hours of atropine, even though the baseline choroidal thickness had not been increased with atropine. There are a number of possible explanations for this difference, in addition to the essential differences between atropine and homatropine. For example, the shorter-term effects of atropine on resting choroidal thickness is unknown and it may be that atropine caused choroidal thickening in the adult study in the early stages, which had disappeared after 22 hours. Alternatively, the abolition of choroidal thinning by atropine (and possibly homatropine) may be a separate process from that of their thickening effect on resting choroidal thickness. A further alternative is that inhibition of choroidal thinning in response to optical defocus may require a lower cumulative dose of these agents than is required to cause baseline choroidal thickening.

The effects of homatropine and atropine on the choroidal response to myopic defocus may be different. In the main part of this study the thickening of the choroid in

response to myopic defocus appeared to be similar before and after atropine (at 1 week and 3 and 6 months) despite the fact that the thickness of the choroid was increased by atropine. Thus, myopic retinal defocus further thickened the choroid that had already been thickened by atropine. In contrast Sander et al report that in the short term homatropine appeared not to enhance the thickening effect of myopic defocus and the authors speculated that an upper limit of choroidal thickening had been reached. Possible reasons for these differences include that the choroids in children may be more prone to change than those in adults, or that 0.3% atropine alone may not have increased choroidal thickness to the full amount, thus allowing defocus to still exert further thickening. Another possibility relates to the different time courses of the two studies: over the longer term homatropine may be found to have similar effects to atropine.

## 6.5 Effects of atropine on ocular biometry

Ocular biometry measures via LenStar were made at Stage 1 (baseline), Stage 3 (3 months) and Stage 4 (6 months) of the study. Individual components, i.e. Central Corneal Thickness (CCT), Anterior Chamber Depth (AD), Crystalline Lens Thickness (LT), Vitreous Chamber Depth (VCD) and Axial length (AL) were recorded as secondary outcome measures to monitor potential changes over the 6-month study. The results were summarised in *Table 7* in Section 5.7.6. Overall, the results were as expected and generally consistent with previous studies (e.g. thinning of the lens (Celebi and Aykan 1999), initial shortening in VCD (Kumaran, Htoon et al. 2015). There was no change in CCT over the study period. There was a significant increase in AD of 0.11 mm following atropine administration. This was expected and was partly

accounted for by the fact that lens thickness (LT) was reduced by 0.06 mm after atropine. The mean presenting VCD was 17.09 ± 1.05 mm at baseline, which then shortened by approximately 0.05 mm at 3 months to 17.04 ± 1.09 mm; p = 0.026) but then elongated at 6 months (17.12 ± 1.03 mm) to be similar to the baseline value (p = 0.06). Initial shortening of the VCD (0.06 mm) has previously been reported in a study of multifocal orthokeratology (MOK) lens wear (Lörtscher 2013). In that study, the VCD shortening was believed to be due (at least partly) to the increase in choroidal thickness that was also observed in that study. In the current study the shortening in VCD may also be partly due to the increase in choroidal thickness induced by atropine. The mean presenting choroidal thickness of Control and Experimental eyes combined at Stage 1 (before atropine) was 275 ± 70 µm, which increased to 301 ± 67 µm at 3 months (i.e. approximately a 26 µm or 0.026 mm increase, see *Table 6*). Thus in this case, choroidal thickening could account for approximately 50% of the observed VCD shortening.

The mean presenting AL was 24.13 ± 1.17 mm, which remained steady at 3 months (24.13 ± 1.17 mm, p = 0.86) but had increased by 0.07 ± 0.06 mm at 6 months (24.20 ± 1.15 mm, p < 0.001). This would correspond to a projected annual elongation rate of approximately 0.14 mm per year, which is consistent with previous similar studies showing elongation of 0.13 ± 0.18 mm after one year of 0.1% atropine (Chia, Chua et al. 2012) and 0.11 ± 0.17 mm after one year of 0.5% atropine (Chia, Chua et al. 2012). However, previous studies have shown a seasonal variation on eye growth e.g. (Donovan, Sankaridurg et al. 2012), in which the axial elongation typically increases faster in winter than summer. Children in this study were recruited during autumn and winter, and all were discharged before their summer holidays. Therefore it is likely that we monitored their myopia progression during the fastest time of the year, and simple

extrapolation to predict a yearly progression of 0.14 mm per year may be inappropriate. Annual eye elongation might be expected in this study for two reasons. Participants were children aged between 6 and 14 years of age when natural eye growth is still occurring (Zadnik 1997) and therefore their eye length would be expected to be increasing. Also, all participants had myopia, and although on atropine, their myopia might be expected to be progressing to some degree.

## 6.6 Potential seasonal variations over the six months study

Children in this study were enrolled over a relatively short period of time (over the 4 months from September 2015 to first week of January 2016, which falls in typical autumn to mid-winter time in Taiwan, rather than spread throughout the year. Previous studies (e.g. (Donovan, Sankaridurg et al. 2012, Gwiazda, Deng et al. 2014) showed that eye growth and refractive error progression in childhood tend to be slower in summer and faster in winter. If such a seasonal effect was present in this study, we would have expected to see faster eye growth in the first 3 months and slower in the second 3 months (first 3 months is autumn and winter to early spring, and second 3 months is spring to early summer). However, children in both the Autumn and Winter groups seemed to exhibit a slower eye growth in the first 3 months and a faster growth in the second 3 months (although there was no statistical significance for the Winter group), which is the opposite to the seasonal effect reported in previous studies. A potential explanation for this difference is that all children started on atropine at the beginning of the study so the efficacy may have been higher in the first 3 months than in the second 3 months.

## 6.7 Short-term measures of choroidal thickness and longer-term change in eye length

In clinical practice, a reliable predictor of a child's future myopia progression would be very useful. Read (Read 2016) suggested that measures of choroidal thickness are an important biomarker and potentially a novel predictor of eye growth. Read (Read, Alonso-Caneiro et al. 2015) showed that children with a slower axial eye growth tended to show greater thickening of the choroid over time, and children showing faster axial eye growth displayed less thickening and in many cases a thinning of the choroid over time. However, as shown in Section 5.10 and 5.11, the current study did not find a good correlation between short-term changes in choroidal thickness induced by atropine or defocus and myopia progression over 6 months. However, the current study was limited by the small number of participants and a short duration (6 months) with small changes in axial length (mean change in AL by 0.07 ± 0.06 mm, mean change in TEL by 0.09 ± 0.05 mm) and refractive error (mean change 0.21 ± 0.21 D). In addition, the predictions were limited to children who were using atropine.

## 6.8 New insights from this study regarding the action of atropine on the choroid and also in slowing myopia progression.

### 6.8.1 Action of atropine on the choroid

The results from the main study in children showed that 1 drop of 0.3% atropine instilled nightly in both eyes for a week, induced choroidal thickening by approximately 20 µm which corroborates the finding of Zhang et al. (Zhang, Zhou et al. 2016) who showed choroidal thickening in children after 1 week of 1% atropine use. The current study extends this finding by showing that the effect is undiminished after 6 months of 0.3% atropine use in children.

In addition, the results of the preliminary study show that atropine abolishes choroidal thinning induced by hyperopic retinal defocus even though the baseline choroidal thickness had not been increased with atropine. This finding indicates that the two mechanisms, the thinning caused by defocus and the thickening induced by atropine, can be separated. This supports the proposal by Nickla (Nickla, Zhu et al. 2013) that choroidal thickening and thinning are mediated by different mechanisms. Nickla has proposed that thinning occurs via contraction of non-vascular smooth muscle via acetylcholine and thickening possibly by a dopaminergic or nitrergic mechanism. The present study indicates that myopic defocus can further thicken the choroid that has already been thickened by atropine. This may suggest that there are two mechanisms that can cause choroidal thickening, one driven by atropine alone and the other driven by myopic defocus. Because of the possibility that atropine may exert its effect on the choroid indirectly by acting in the retina as well as by acting directly on the choroid, there are several potential combinations and mechanisms.

### 6.8.2 Atropine's action in slowing myopia progression

We speculate that atropine acts on the retina-choroid-sclera pathway to slow myopia progression by two mechanisms. Atropine inhibits signals associated with choroidal thinning and increased ocular expansion and it also enhances signals associated with choroidal thickening and inhibition of ocular expansion.

However, the main insight of this study in terms of myopia control is that the choroidal thickening associated with atropine can be further thickened by myopic defocus. This suggests that signals associated with slowing of eye expansion mediated by atropine can be added to signals associated with slowing of eye expansion mediated by myopic defocus. This in turn suggests that a dual therapy employing both optical and pharmaceutical approaches would be more efficacious than either therapy alone.

## 6.9 Limitations of the study

Inevitably, there were a number of limitations to this study. The more important ones are discussed below.

### 6.9.1 Participant numbers, ethnicity and age groups

The main part of this study was conducted in Taiwan and involved only Taiwanese children between the ages of 6 and 14 years. Thus, the results are not necessarily generalizable to other ages and ethnic groups. However, these restrictions did remove ethnicity as a potential confounding factor and additional source of variance in the data, which was a potential advantage in the main part of this study which involved a relatively small number of participants (n = 20). With regards to the preliminary study described in Chapter 3, this was also conducted in East Asian participants (n = 10), but they were young adults (18 to 24 years of age) living in New Zealand. Both studies showed that atropine blocked choroidal thinning induced by imposed hyperopic retinal defocus, indicating that this effect is present in children and young adults. Whether it is also present in older age groups and in ocular disease, remains to be investigated.

### 6.9.2 Unknown accommodative status during 60 minutes of retinal image defocus

The refractive status of both eyes of all participants was measured by autorefraction and refined by a qualified optometrist with subjective refraction before starting each stage of the study. The aim was to ensure that when the 2.00 D of myopic or hyperopic defocus was added to the Experimental eye, there was a true difference of 2.00D in the position of the focal planes relative to the retina in the two eyes (within the limits of the 0.25 D ophthalmic lens power increments). Because accommodation in the two eyes is yoked, this relative difference would remain constant, regardless of the accommodative status. However, although participants in both the preliminary and

main studies were asked to focus only on the DVD and nothing else during the 60-minute defocus period, the actual accommodative status of the eyes (when not treated with atropine) was unknown, though hopefully constant, during this period. Moreover, OCT scans, which were made every 20 minutes, inevitably interrupted vision and accommodative status. While taking an OCT scan, participants briefly stopped watching the DVD and shifted their gaze to the internal target in the OCT. Thus, their accommodative status was not the same during the OCT measures as it was while they were watching the DVD. The Nidek RS-3000 Advance OCT machine usually took around 10 to 15 seconds to complete a scan per eye, so distance viewing of the DVD was interrupted for at least 30 seconds each time OCT scans were performed. In the main study the children often found it hard to maintain steady fixation during OCT scanning as they tended to get distracted easily by their surroundings and certainly the fixation target of the OCT machine was not as attractive to them as the DVD that they were watching. Therefore, OCT scans often took longer than 15 seconds; in fact, some could take up to 60 seconds per eye.

This issue of uncontrolled and unknown accommodation was a limitation throughout the preliminary study and in the main study before instillation of atropine eye drops at end of Stage 1 measures. Thereafter, in the main study, measures were made under cycloplegia induced by administration of the atropine eye drops. This ensured that the level of accommodation remained relatively constant and at a low level. However, this did not ensure that the level of retinal defocus was always well controlled. For example, when children on atropine fixated the cross-target of the OCT, their retina would likely have experienced some hyperopic defocus as a result of accommodative lag.

### 6.9.3 Potential effect of DVD viewing on choroidal thickness

To make the DVD interesting and to attract attention while watching, the movies utilised sound, lighting and special effects to tell the story. However, these factors could have led to emotional and other responses which may affect the autonomic nervous system and hence cause potential changes to the choroid via changes in blood pressure, heart rate, blood flow within the choroid, contraction and relaxation of the non-vascular smooth muscle tone within the choroid, etc., and those factors might have resulted in fluctuations in choroidal thickness.

Previous studies have suggested that diffuse luminance flicker can induce an increase in retinal and optic nerve head blood flow in animals (Riva, Harino et al. 1991) and humans (Scheiner, Riva et al. 1994, Formaz, Riva et al. 1997). In this study, we kept the lighting level at around 10 lux throughout the entire process to facilitate OCT image capture. This level of luminance was also adopted by some previous choroidal thickness studies (Read, Collins et al. 2010, Chiang, Phillips et al. 2015). However, while the DVD was playing, the screen caused diffuse flickering light and brightness changes in the room. A previous study (Garhüfer, Huemer et al. 2002) suggested that flicker stimulation increases optic nerve head blood flow, although the same authors concluded that it did not significantly alter choroidal blood flow.

### 6.9.4 Other limitations

Daily spectacle wear during the 6 months was not monitored. This potentially could have affected the baseline choroidal thickness measures at all stages (for example, myopic children not wearing a correction might have been habitually experiencing myopic defocus. This situation was partly dealt with by having a 20 minute stabilisation period with full correction prior to measures of baseline choroidal thickness and responses to defocus.

Although an open field autorefractor was used to measure refractions, the autorefractor results were refined by subjective refraction. The reason for refining the autorefractor measures was because the initial measures were made uncyclopleged and it was believed that subjective refraction would better deal with uncontrolled accommodation. However, the use of subjective refraction lead to rounding of refractive error to 0.25D steps. In retrospect it might have been better to have used autorefractor results alone throughout the study.

The accuracy of the results was limited by the resolution of the OCT machine: this particular OCT (Nidek RS-3000 Advance) reportedly provides about 4 µm of image resolution by averaging images. This is a relatively poor resolution considering that most of the changes in choroidal thickness which were observed were only about ±12 µm.

The fact that the choroidal thickness was observed to change over time in the Experimental eye as a result of the imposed 2.00 D defocus, implies that the relative refraction between the eyes likely changed as a result of the movement of the retina in the Experimental eye and this would have altered the degree of imposed defocus. This change however was likely very small. Assuming that a 1000 µm change in axial length corresponds to about a 2.70 D change in refraction (Bennett and Rabbetts 1989), then the ~15 µm change observed in one eye would correspond to approximately a 0.04 D difference in refraction between the eyes. It seems unlikely that this small change would have had any functional significance in altering the response of the choroid to defocus. Compared to the precision with which refractive error is typically corrected with lenses (i.e. to the nearest 0.25 D), changes of the order of 0.04 D are likely insignificant.

## 6.10 Potential future directions

This study demonstrated that one drop of 0.3% atropine instilled binocularly at night for a week can thicken the choroid by around 20 µm in children. Moreover, 60 minutes of 2.00 D monocular myopic retinal defocus further thickened the choroid by about 12 µm. This suggests that the pharmaceutical and optically induced changes in choroidal thickness may be superimposed. This is turn leads to the hypothesis that the myopia inhibiting effects of atropine and optical defocus can also be superimposed to enhance efficacy.

A future study investigating the efficacy of atropine combined with peripheral defocus associated with orthokeratology or combined with lenses with anti-myopia optics such as 'dual-focus' contact lenses which impose simultaneous myopic defocus to the retina (Anstice and Phillips 2011) should be conducted. The aim would be to test the hypothesis that the effect of a combined therapy would be more effective in slowing myopia progression in clinical practice than either therapy alone.

As discussed in section 2.7.7, currently incurable sight-threatening ischemic retinal diseases such as AMD and Diabetic Retinopathy are known have reduced choroidal thickness and blood flow. Thus, future studies investigating the effects of atropine and optical defocus on the choroid of patients with ischemic eye disease, would appear to be a potentially important and a beneficial direction for future research.

The present study only investigated 0.3% atropine and ± 2.00 D of monocular retinal defocus. Future studies should investigate the most effective concentrations of atropine, and find the optimal amount of defocus in order to maximise the effect. The effects of atropine and optical defocus on choroidal thickness in children of different ethnic groups to make the findings more generalizable, should also be conducted.

The present study investigated the ability of short term measures of the effects of atropine and defocus on choroidal thickness to predict longer term changes in eye growth and myopia progression. Reliable predictors of future myopia progression would be very useful in clinical practice for selecting patients who would most benefit from myopia control therapies. Therefore a future study investigating the ability of short term measures of choroidal thickness to predict longer term changes should be carried out. The ability of the current study to test this potential prediction method was limited by the short duration and consequent small changes in axial length and refractive error and the small number of participants. In addition, the predictions were limited to children who were using atropine.

# 7 Summary and Conclusions

In summary, this study extends previous findings (Read, Collins et al. 2010, Chiang, Phillips et al. 2015) regarding the small but significant choroidal thickness changes that occur when human eyes are exposed to retinal defocus by investigating the effect of atropine on these responses. The results show that:

Choroidal thickness changes resulting from imposed monocular defocus previously reported in young adults also occur in children. In children, 60 minutes of 2.00 D of myopic retinal defocus causes thickening of the choroid by about 12 µm, whereas 2.00 D of hyperopic retinal defocus causes a thinning of the choroid by about 12 µm.

The results confirm that choroidal thickness is inversely correlated with the degree of myopia in children, with choroidal thickness reducing by 39 microns per dioptre of myopia ($R^2 = 0.25$, $p = 0.02$).

One drop of 0.3% atropine instilled into both eyes before sleep for 1 week can increase choroidal thickness by approximately 20 µm ($p < 0.001$). If this result also applies in older patients, this may have a significant beneficial clinical application in currently incurable retinal ischaemic diseases like AMD.

Atropine abolishes the choroidal thinning effect that is generally induced by hyperopic retinal defocus, but it does not interfere with the choroidal thickening induced by myopic defocus. This suggests that atropine may specifically inhibit myopiagenic signals (e.g. hyperopic defocus associated with a lag of accommodation) without interfering with signals that retard eye growth (e.g. myopic defocus).

In terms of mechanism, this study adds to our understanding of the action of atropine in slowing myopia progression. The results may suggest that atropine acts at an early

stage in the retina-choroid-sclera pathway controlling eye growth, to selectively block transmission of signals for scleral expansion.

Myopic retinal defocus can further thicken the choroid that has already been thickened by 0.3% atropine eye drops. This additive effect suggests that combining optical and pharmaceutical therapies into dual therapies is likely to provide more effective myopia control than either therapy alone.

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# 9 Appendices

## 9.1 Individual results for amplitude of accommodation & pupil size

### 9.1.1 Individual results for mean sphere (SER, D)

| | Stage 1 (Before atropine) | | | Stage 2 (after 1 week on atropine) | | | Stage 3 (after 3 months on atropine) | | | Stage 4 (after 6 months on atropine) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Ctrl | Exp | Mean | Ctrl | Exp | Mean | Ctrl | Exp | Mean | Ctrl | Exp | Mean |
| PT 1 | -2.25 | -2.00 | -2.13 | -1.75 | -1.75 | -1.75 | -1.75 | -1.75 | -1.75 | -1.75 | -2.00 | -1.88 |
| PT 2 | -1.50 | -0.75 | -1.13 | -1.25 | -0.63 | -0.94 | -1.25 | -0.75 | -1.00 | -1.50 | -0.50 | -1.00 |
| PT 3 | -0.75 | -0.75 | -0.75 | -0.50 | -0.75 | -0.63 | -0.50 | -0.75 | -0.63 | -0.75 | -0.75 | -0.75 |
| PT 4 | -0.75 | -0.75 | -0.75 | -0.50 | -0.63 | -0.56 | -0.50 | -0.50 | -0.50 | -0.75 | -0.50 | -0.63 |
| PT 5 | -2.38 | -1.50 | -1.94 | -2.13 | -1.50 | -1.81 | -2.50 | -1.75 | -2.13 | -2.75 | -2.00 | -2.38 |
| PT 6 | -2.00 | -2.75 | -2.38 | -1.75 | -2.38 | -2.06 | -2.00 | -2.50 | -2.25 | -2.00 | -2.50 | -2.25 |
| PT 7 | -1.50 | -2.25 | -1.88 | -1.25 | -2.00 | -1.63 | -1.25 | -2.25 | -1.75 | -1.25 | -2.50 | -1.88 |
| PT 8 | -0.75 | -0.75 | -0.75 | -0.50 | -0.50 | -0.50 | -0.50 | -0.50 | -0.50 | -0.75 | -0.75 | -0.75 |
| PT 9 | -4.00 | -3.88 | -3.94 | -3.88 | -3.75 | -3.81 | -3.75 | -3.88 | -3.81 | -4.00 | -4.00 | -4.00 |
| PT 10 | -3.50 | -3.00 | -3.25 | -3.25 | -2.75 | -3.00 | -3.50 | -2.75 | -3.13 | -3.50 | -2.88 | -3.19 |
| PT 11 | -1.50 | -1.50 | -1.50 | -1.50 | -1.50 | -1.50 | -1.50 | -1.50 | -1.50 | -1.75 | -1.75 | -1.75 |
| PT 12 | -1.25 | -0.75 | -1.00 | -1.00 | -0.50 | -0.75 | -1.13 | -0.50 | -0.81 | -1.38 | -1.13 | -1.25 |
| PT 13 | -1.38 | -1.38 | -1.38 | -1.25 | -1.38 | -1.31 | -1.50 | -1.50 | -1.50 | -1.75 | -1.75 | -1.75 |
| PT 14 | -1.25 | -1.38 | -1.31 | -1.00 | -1.25 | -1.13 | -1.00 | -1.00 | -1.00 | -1.50 | -1.38 | -1.44 |
| PT 15 | -1.25 | -1.50 | -1.38 | -1.00 | -1.38 | -1.19 | -1.25 | -1.25 | -1.25 | -1.25 | -1.25 | -1.25 |
| PT 16 | -3.00 | -2.75 | -2.88 | -2.75 | -2.63 | -2.69 | -2.75 | -2.50 | -2.63 | -2.75 | -2.50 | -2.63 |
| PT 17 | -0.75 | -1.50 | -1.13 | -0.75 | -1.25 | -1.00 | -0.75 | -1.63 | -1.19 | -0.75 | -1.75 | -1.25 |
| PT 18 | -0.75 | -0.75 | -0.75 | -0.50 | -0.50 | -0.50 | -0.50 | -0.50 | -0.50 | -0.75 | -0.75 | -0.75 |
| PT 19 | -0.75 | -0.88 | -0.81 | -0.50 | -0.50 | -0.50 | -0.75 | -0.50 | -0.63 | -0.75 | -0.50 | -0.63 |
| PT 20 | -1.63 | -1.50 | -1.56 | -1.25 | -1.25 | -1.25 | -1.25 | -1.25 | -1.25 | -1.25 | -1.25 | -1.25 |
| **Mean** | **-1.64** | **-1.61** | **-1.63** | **-1.41** | **-1.44** | **-1.43** | **-1.49** | **-1.48** | **-1.48** | **-1.64** | **-1.62** | **-1.63** |
| SD | 0.95 | 0.90 | 0.90 | 0.95 | 0.89 | 0.90 | 0.97 | 0.93 | 0.92 | 0.95 | 0.93 | 0.90 |

Units: Dioptres (D)

### 9.1.2 Individual results for amplitude of accommodation (AoA, D)

| | Stage 1 (Before atropine) | | | Stage 2 (after 1 week on atropine) | | | Stage 3 (after 3 months on atropine) | | | Stage 4 (after 6 months on atropine) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Ctrl | Exp | Mean | Ctrl | Exp | Mean | Ctrl | Exp | Mean | Ctrl | Exp | Mean |
| PT 1 | 17.00 | 17.00 | 17.00 | 1.43 | 1.43 | 1.43 | 1.43 | 1.25 | 1.34 | 1.43 | 1.25 | 1.34 |
| PT 2 | 18.00 | 18.00 | 18.00 | 1.25 | 1.25 | 1.25 | 1.43 | 1.43 | 1.43 | 1.25 | 1.11 | 1.18 |
| PT 3 | 18.00 | 18.00 | 18.00 | 1.25 | 1.25 | 1.25 | 1.11 | 1.11 | 1.11 | 1.25 | 1.43 | 1.34 |
| PT 4 | 14.00 | 14.00 | 14.00 | 1.11 | 1.11 | 1.11 | 1.11 | 1.00 | 1.06 | 1.11 | 1.00 | 1.06 |
| PT 5 | 18.00 | 17.00 | 17.50 | 1.43 | 1.43 | 1.43 | 1.43 | 1.67 | 1.55 | 1.67 | 1.67 | 1.67 |
| PT 6 | 20.00 | 20.00 | 20.00 | 1.25 | 1.43 | 1.34 | 1.25 | 1.25 | 1.25 | 1.43 | 1.25 | 1.34 |
| PT 7 | 18.00 | 18.00 | 18.00 | 1.25 | 1.25 | 1.25 | 1.43 | 1.67 | 1.55 | 1.25 | 1.25 | 1.25 |
| PT 8 | 18.00 | 18.00 | 18.00 | 1.43 | 1.43 | 1.43 | 1.25 | 1.43 | 1.34 | 1.67 | 1.67 | 1.67 |
| PT 9 | 20.00 | 20.00 | 20.00 | 1.67 | 1.67 | 1.67 | 2.00 | 1.67 | 1.84 | 2.00 | 1.67 | 1.84 |
| PT 10 | 17.00 | 17.00 | 17.00 | 1.43 | 1.43 | 1.43 | 1.25 | 1.43 | 1.34 | 1.25 | 2.00 | 1.63 |
| PT 11 | 17.00 | 17.00 | 17.00 | 1.43 | 1.25 | 1.34 | 1.25 | 1.11 | 1.18 | 1.67 | 1.25 | 1.46 |
| PT 12 | 17.00 | 18.00 | 17.50 | 1.25 | 1.25 | 1.25 | 1.25 | 1.11 | 1.18 | 1.25 | 1.11 | 1.18 |
| PT 13 | 18.00 | 14.00 | 16.00 | 1.25 | 1.67 | 1.46 | 1.25 | 1.25 | 1.25 | 1.11 | 1.43 | 1.27 |
| PT 14 | 14.00 | 18.00 | 16.00 | 1.43 | 2.00 | 1.72 | 1.43 | 1.67 | 1.55 | 1.25 | 1.43 | 1.34 |
| PT 15 | 18.00 | 18.00 | 18.00 | 1.67 | 2.00 | 1.84 | 1.67 | 1.67 | 1.67 | 1.43 | 1.43 | 1.43 |
| PT 16 | 18.00 | 18.00 | 18.00 | 1.25 | 1.25 | 1.25 | 1.43 | 1.43 | 1.43 | 1.43 | 1.25 | 1.34 |
| PT 17 | 18.00 | 18.00 | 18.00 | 1.43 | 1.43 | 1.43 | 1.25 | 1.43 | 1.34 | 1.25 | 1.43 | 1.34 |
| PT 18 | 17.00 | 17.00 | 17.00 | 1.11 | 1.10 | 1.11 | 1.25 | 1.43 | 1.34 | 1.11 | 0.50 | 0.81 |
| PT 19 | 18.00 | 18.00 | 18.00 | 1.11 | 1.00 | 1.06 | 1.25 | 1.25 | 1.25 | 1.25 | 1.43 | 1.34 |
| PT 20 | 18.00 | 17.00 | 17.50 | 1.25 | 1.25 | 1.25 | 1.11 | 1.25 | 1.18 | 1.43 | 1.25 | 1.34 |
| **Mean** | **17.55** | **17.50** | **17.53** | **1.33** | **1.39** | **1.37** | **1.34** | **1.38** | **1.36** | **1.37** | **1.34** | **1.36** |
| SD | 1.47 | 1.47 | 1.30 | 0.16 | 0.27 | 0.20 | 0.21 | 0.22 | 0.20 | 0.23 | 0.31 | 0.23 |

Units: Dioptres (D)

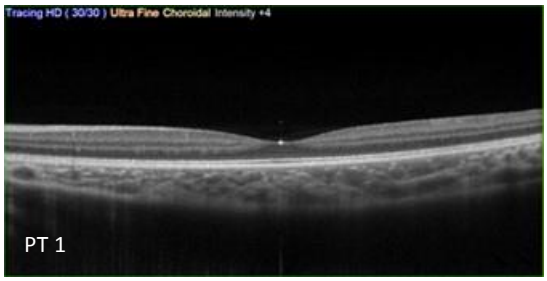
### 9.1.3 Individual results for pupil size (mm)

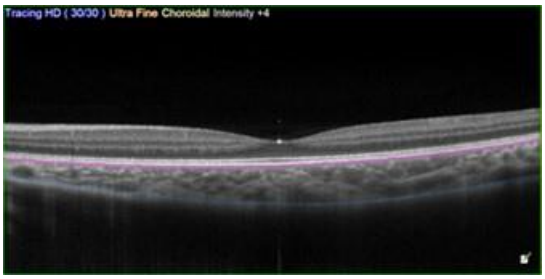
| | Stage 1 (Before atropine) | | | Stage 2 (after 1 week on atropine) | | | Stage 3 (after 3 months on atropine) | | | Stage 4 (after 6 months on atropine) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Ctrl | Exp | Mean | Ctrl | Exp | Mean | Ctrl | Exp | Mean | Ctrl | Exp | Mean |
| PT 1 | 4.00 | 4.00 | 4.00 | 7.00 | 6.50 | 6.75 | 7.00 | 7.00 | 7.00 | 7.50 | 7.50 | 7.50 |
| PT 2 | 3.50 | 4.00 | 3.75 | 7.50 | 7.50 | 7.50 | 7.50 | 7.00 | 7.25 | 7.50 | 8.00 | 7.75 |
| PT 3 | 5.00 | 4.50 | 4.75 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 8.00 | 7.50 | 7.75 |
| PT 4 | 3.50 | 3.50 | 3.50 | 6.50 | 7.00 | 6.75 | 7.50 | 7.00 | 7.25 | 7.50 | 7.00 | 7.25 |
| PT 5 | 4.00 | 4.00 | 4.00 | 7.00 | 7.00 | 7.00 | 7.50 | 8.00 | 7.75 | 7.50 | 7.50 | 7.50 |
| PT 6 | 4.00 | 4.00 | 4.00 | 7.00 | 7.00 | 7.00 | 7.50 | 7.00 | 7.25 | 7.50 | 7.50 | 7.50 |
| PT 7 | 3.50 | 3.50 | 3.50 | 7.50 | 7.00 | 7.25 | 8.00 | 8.00 | 8.00 | 8.00 | 8.00 | 8.00 |
| PT 8 | 5.00 | 5.00 | 5.00 | 7.50 | 7.50 | 7.50 | 8.50 | 8.00 | 8.25 | 7.50 | 7.50 | 7.50 |
| PT 9 | 5.00 | 5.00 | 5.00 | 7.50 | 7.00 | 7.25 | 8.00 | 7.50 | 7.75 | 7.00 | 7.00 | 7.00 |
| PT 10 | 4.50 | 4.00 | 4.25 | 7.00 | 8.00 | 7.50 | 7.00 | 8.00 | 7.50 | 7.00 | 7.50 | 7.25 |
| PT 11 | 4.00 | 4.00 | 4.00 | 8.00 | 7.50 | 7.75 | 7.50 | 8.50 | 8.00 | 8.00 | 8.00 | 8.00 |
| PT 12 | 4.50 | 4.00 | 4.25 | 7.50 | 7.50 | 7.50 | 8.00 | 7.00 | 7.50 | 8.00 | 7.00 | 7.50 |
| PT 13 | 4.00 | 5.00 | 4.50 | 7.50 | 8.00 | 7.75 | 7.50 | 8.00 | 7.75 | 7.00 | 8.00 | 7.50 |
| PT 14 | 4.50 | 4.00 | 4.25 | 8.00 | 7.50 | 7.75 | 8.50 | 7.50 | 8.00 | 8.50 | 8.00 | 8.25 |
| PT 15 | 4.00 | 4.00 | 4.00 | 7.50 | 7.50 | 7.50 | 7.50 | 7.50 | 7.50 | 8.00 | 8.00 | 8.00 |
| PT 16 | 4.00 | 4.00 | 4.00 | 8.00 | 8.00 | 8.00 | 7.50 | 7.50 | 7.50 | 7.50 | 7.00 | 7.25 |
| PT 17 | 3.50 | 3.50 | 3.50 | 7.50 | 8.00 | 7.75 | 7.00 | 7.50 | 7.25 | 8.00 | 7.50 | 7.75 |
| PT 18 | 4.00 | 4.00 | 4.00 | 7.50 | 8.00 | 7.75 | 7.50 | 7.00 | 7.25 | 7.00 | 7.50 | 7.25 |
| PT 19 | 4.00 | 4.00 | 4.00 | 7.50 | 7.50 | 7.50 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 |
| PT 20 | 3.00 | 3.50 | 3.25 | 8.00 | 8.00 | 8.00 | 8.00 | 7.50 | 7.75 | 7.50 | 7.50 | 7.50 |
| **Mean** | **4.08** | **4.08** | **4.08** | **7.43** | **7.45** | **7.44** | **7.58** | **7.48** | **7.53** | **7.58** | **7.53** | **7.55** |
| SD | 0.54 | 0.47 | 0.47 | 0.41 | 0.46 | 0.38 | 0.47 | 0.47 | 0.37 | 0.44 | 0.38 | 0.34 |

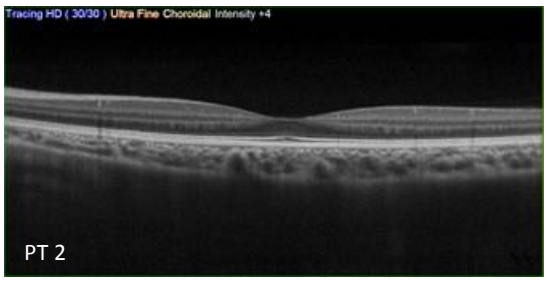
Units: Millimetres (mm)

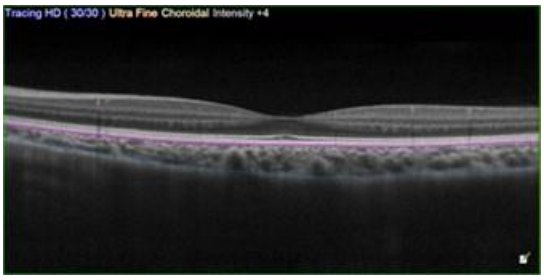
## 9.2 OCT images raw (left) vs with border defined manually (right)

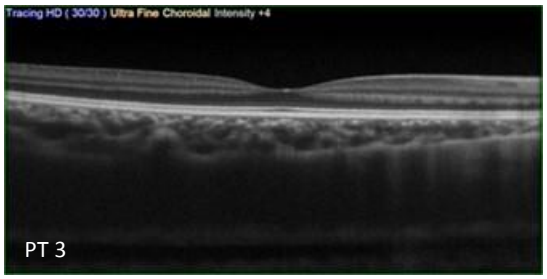
### 9.2.1 Control eyes OCT images

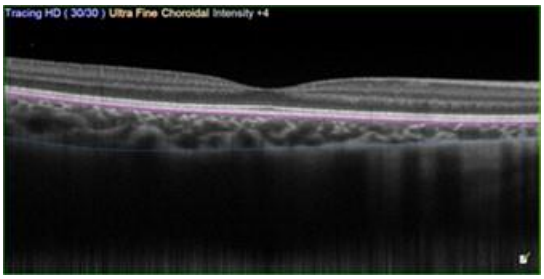


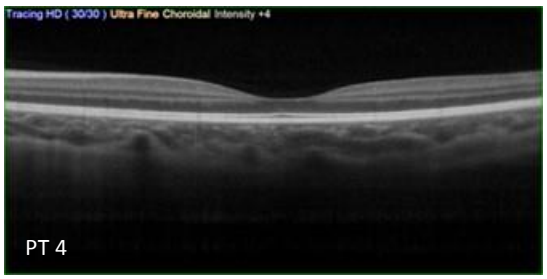



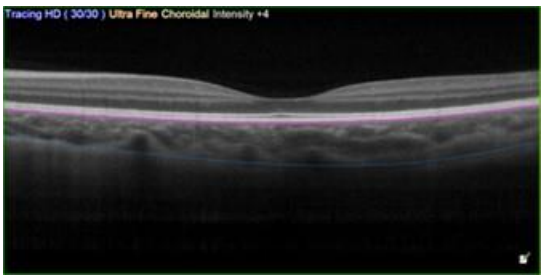



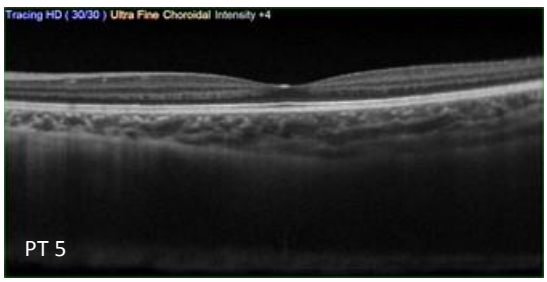



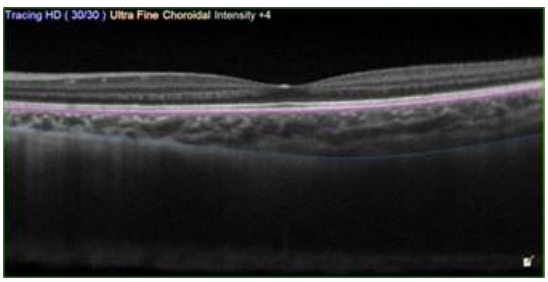



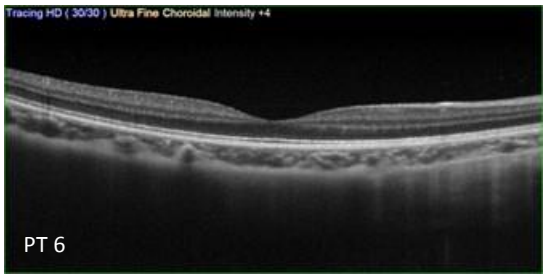
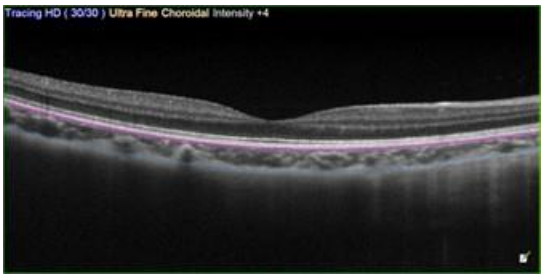
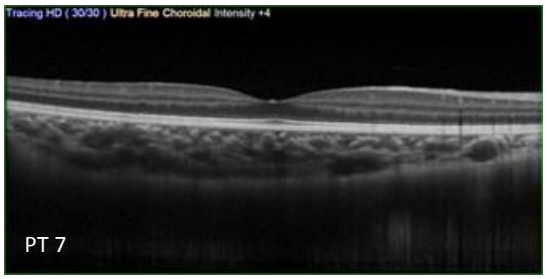
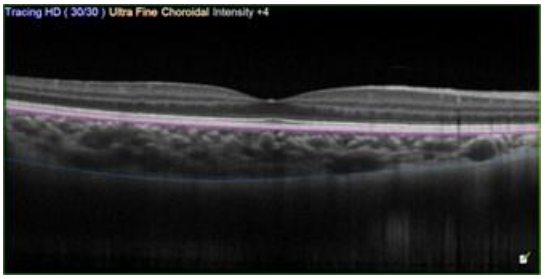
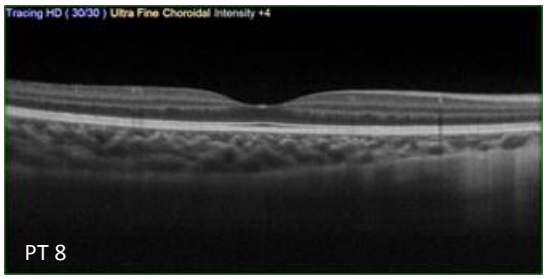
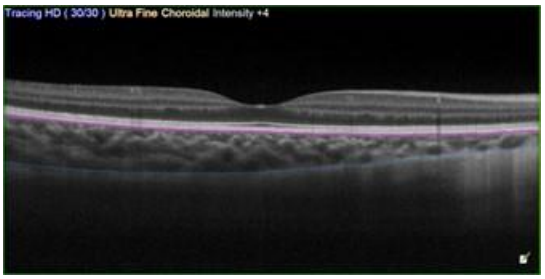
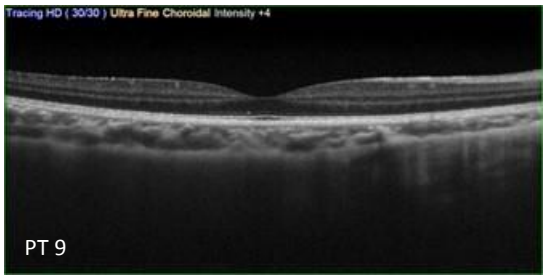
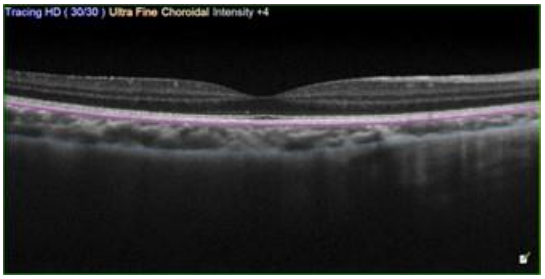
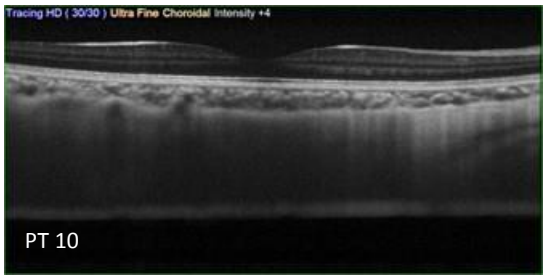
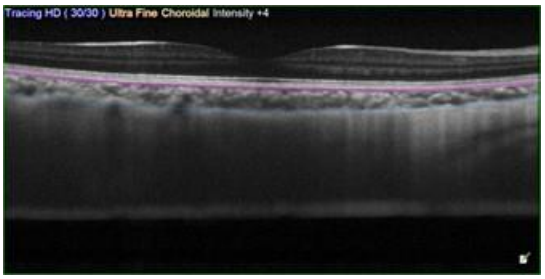



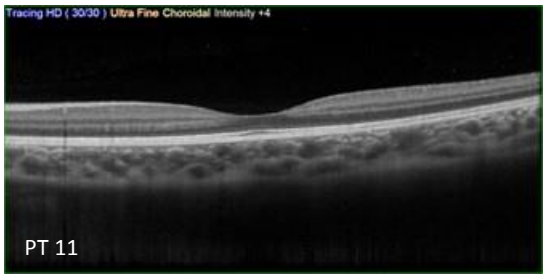
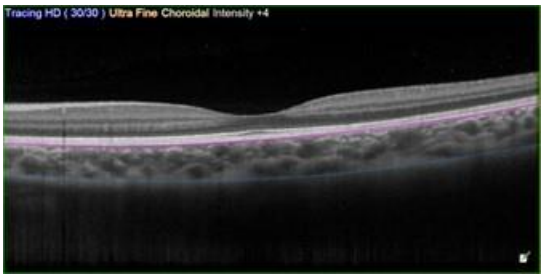
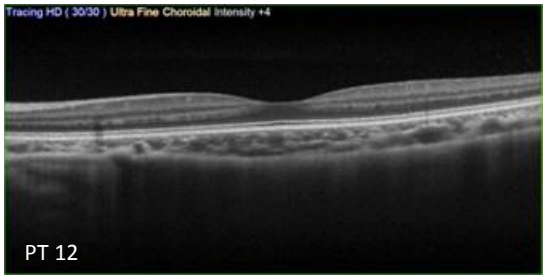
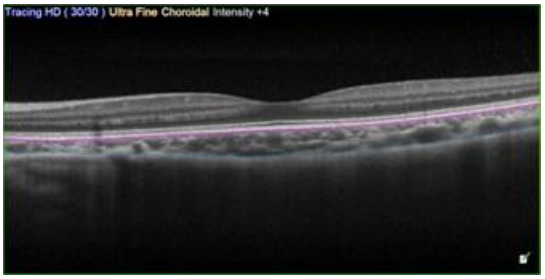
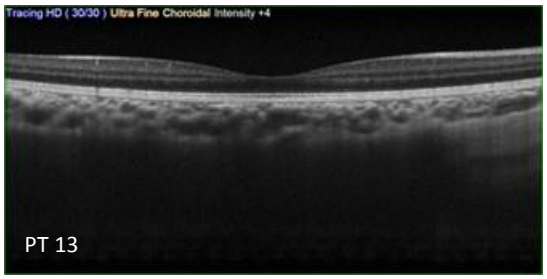
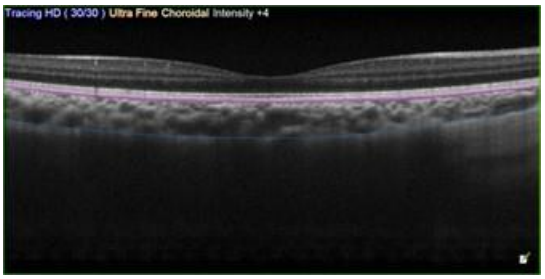
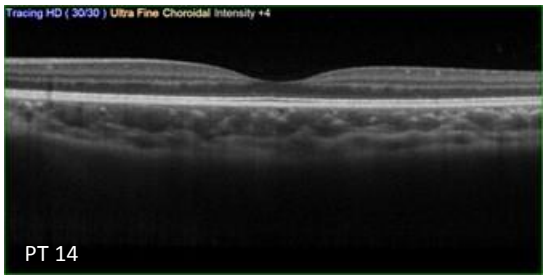
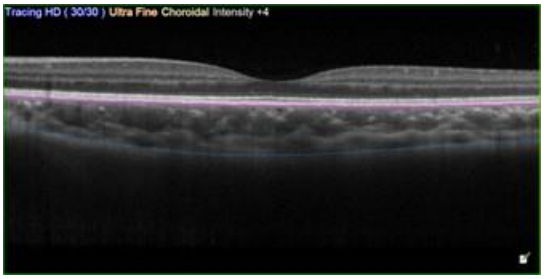
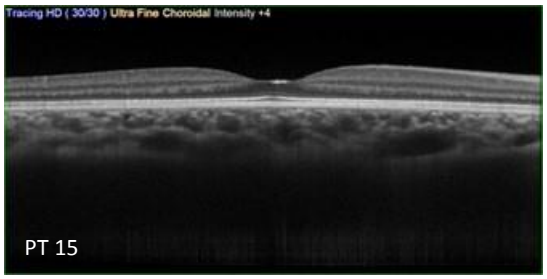
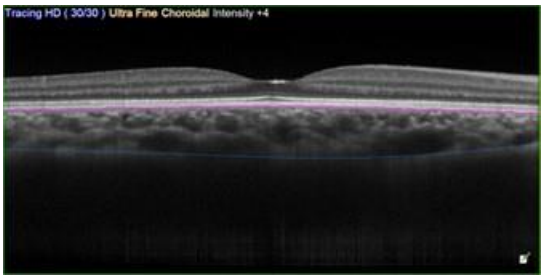



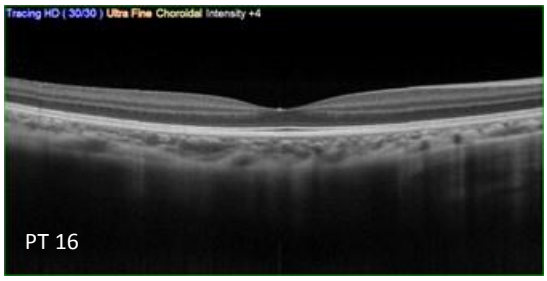
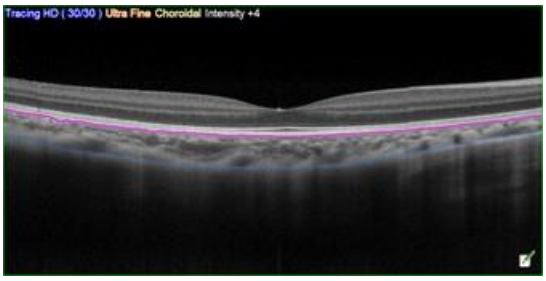
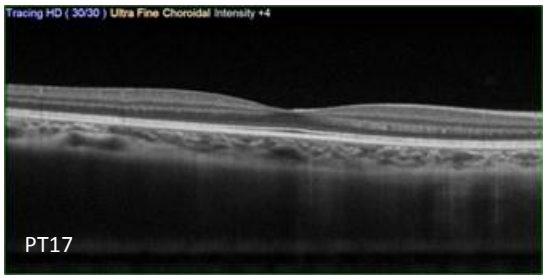
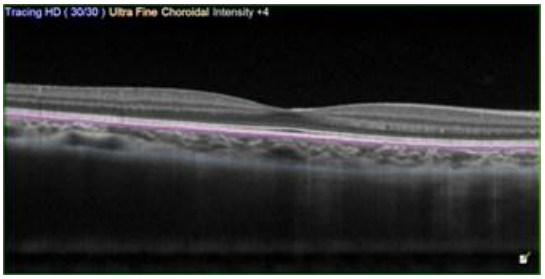
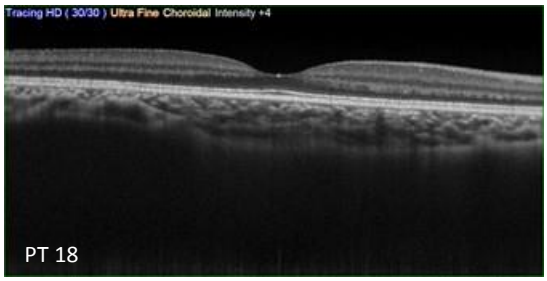
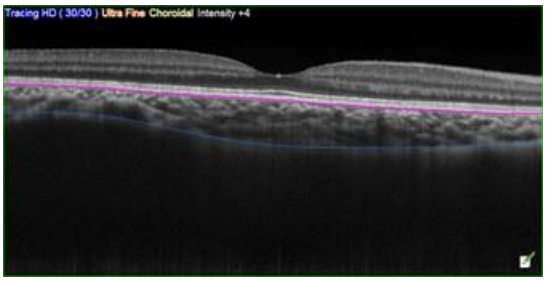
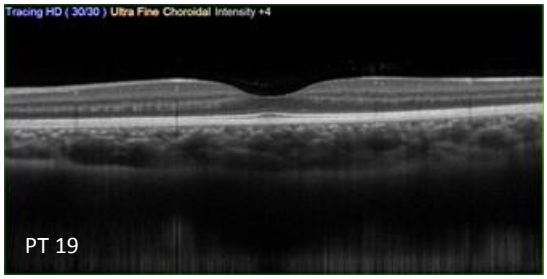
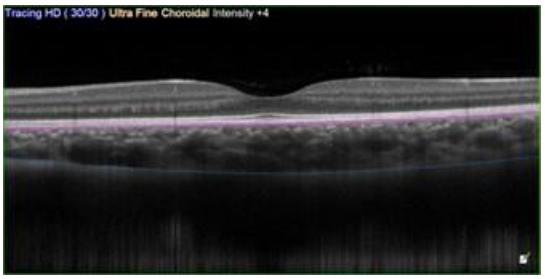
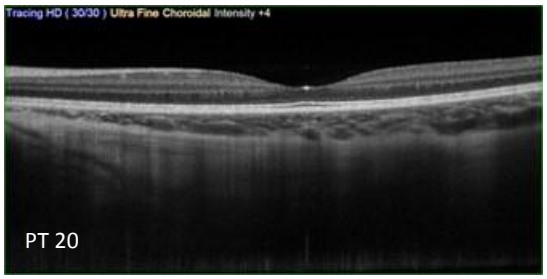
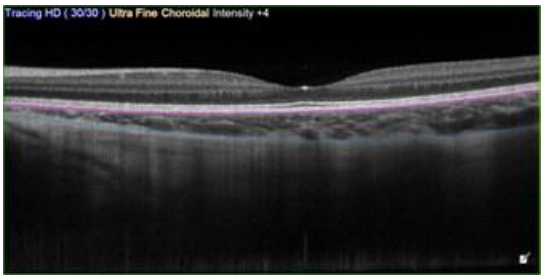



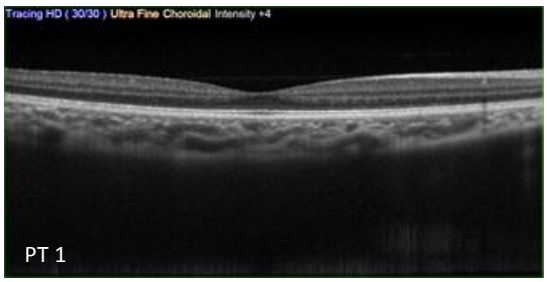



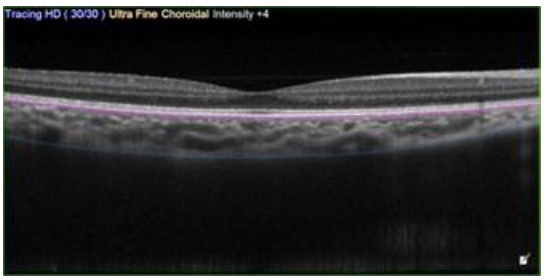
| | |
|---|---|
|  Tracing HD ( 30/30 ) Ultra Fine Choroidal Intensity +4<br>PT 6 |  Tracing HD ( 30/30 ) Ultra Fine Choroidal Intensity +4 |
|  Tracing HD ( 30/30 ) Ultra Fine Choroidal Intensity +4<br>PT 7 |  Tracing HD ( 30/30 ) Ultra Fine Choroidal Intensity +4 |
|  Tracing HD ( 30/30 ) Ultra Fine Choroidal Intensity +4<br>PT 8 |  Tracing HD ( 30/30 ) Ultra Fine Choroidal Intensity +4 |
|  Tracing HD ( 30/30 ) Ultra Fine Choroidal Intensity +4<br>PT 9 |  Tracing HD ( 30/30 ) Ultra Fine Choroidal Intensity +4 |
|  Tracing HD ( 30/30 ) Ultra Fine Choroidal Intensity +4<br>PT 10 |  Tracing HD ( 30/30 ) Ultra Fine Choroidal Intensity +4 |

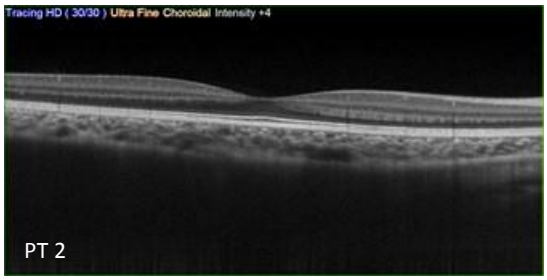
| | |
|---|---|
|  Tracing HD ( 30/30 ) Ultra Fine Choroidal Intensity +4<br>PT 11 |  Tracing HD ( 30/30 ) Ultra Fine Choroidal Intensity +4 |
|  Tracing HD ( 30/30 ) Ultra Fine Choroidal Intensity +4<br>PT 12 |  Tracing HD ( 30/30 ) Ultra Fine Choroidal Intensity +4 |
|  Tracing HD ( 30/30 ) Ultra Fine Choroidal Intensity +4<br>PT 13 |  Tracing HD ( 30/30 ) Ultra Fine Choroidal Intensity +4 |
|  Tracing HD ( 30/30 ) Ultra Fine Choroidal Intensity +4<br>PT 14 |  Tracing HD ( 30/30 ) Ultra Fine Choroidal Intensity +4 |
|  Tracing HD ( 30/30 ) Ultra Fine Choroidal Intensity +4<br>PT 15 |  Tracing HD ( 30/30 ) Ultra Fine Choroidal Intensity +4 |

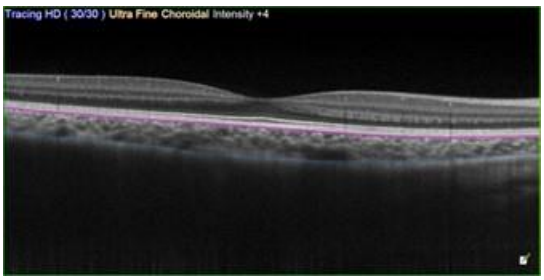
| | |
|---|---|
|  Tracing HD ( 30/30 ) Ultra Fine Choroidal Intensity +4<br>PT 16 |  Tracing HD ( 30/30 ) Ultra Fine Choroidal Intensity +4 |
|  Tracing HD ( 30/30 ) Ultra Fine Choroidal Intensity +4<br>PT17 |  Tracing HD ( 30/30 ) Ultra Fine Choroidal Intensity +4 |
|  Tracing HD ( 30/30 ) Ultra Fine Choroidal Intensity +4<br>PT 18 |  Tracing HD ( 30/30 ) Ultra Fine Choroidal Intensity +4 |
|  Tracing HD ( 30/30 ) Ultra Fine Choroidal Intensity +4<br>PT 19 |  Tracing HD ( 30/30 ) Ultra Fine Choroidal Intensity +4 |
|  Tracing HD ( 30/30 ) Ultra Fine Choroidal Intensity +4<br>PT 20 |  Tracing HD ( 30/30 ) Ultra Fine Choroidal Intensity +4 |

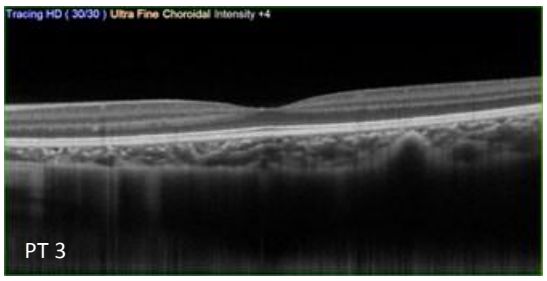
### 9.2.2 Experimental eyes OCT images

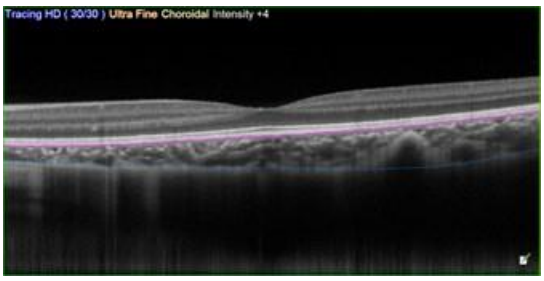


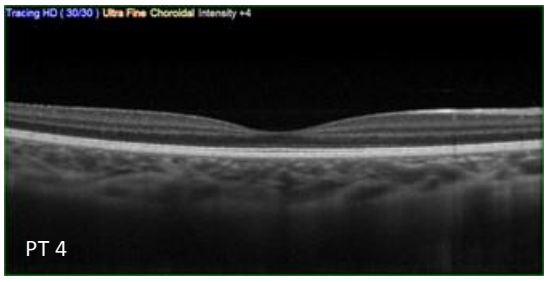



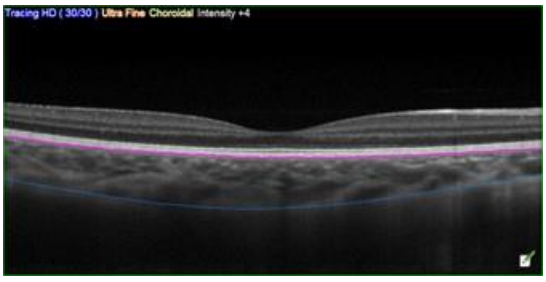



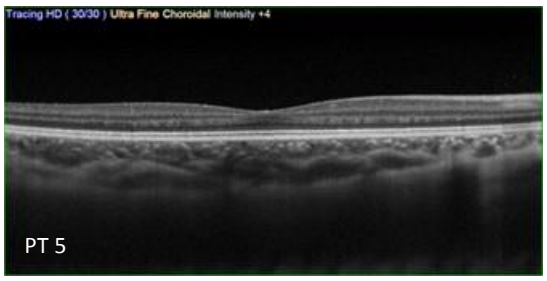



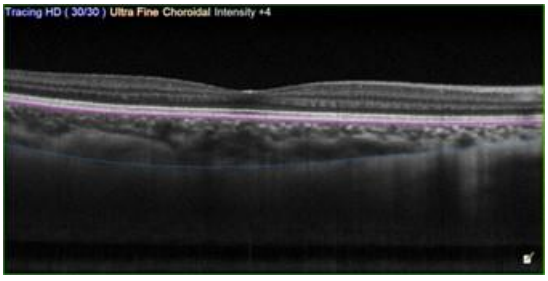



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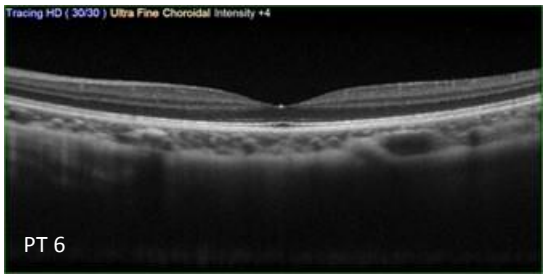
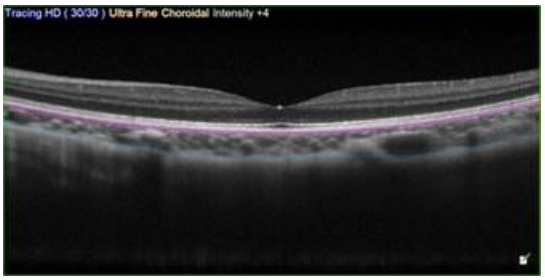
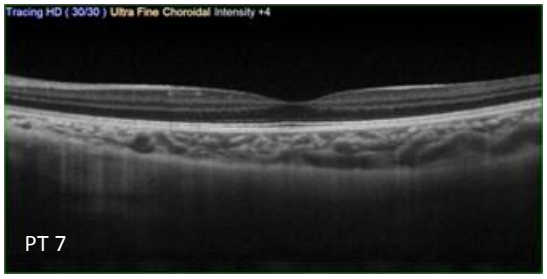
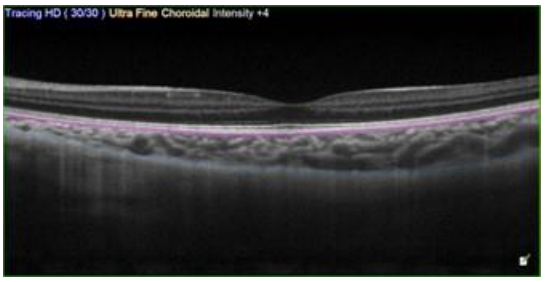
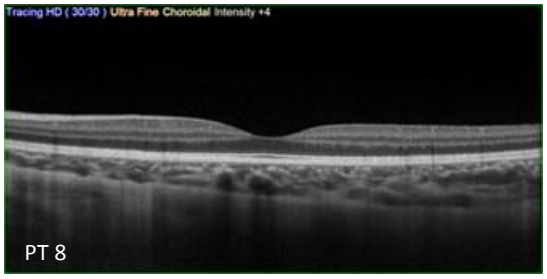
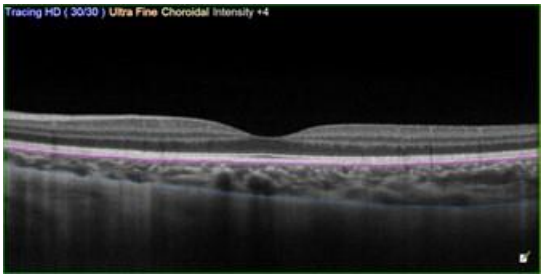
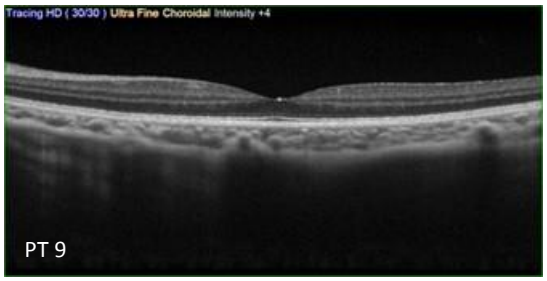
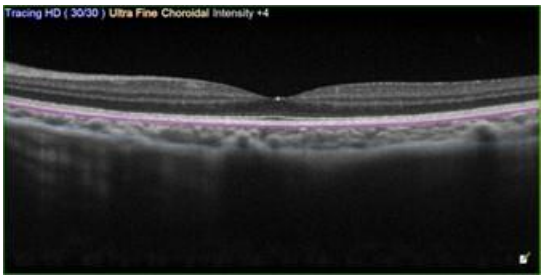
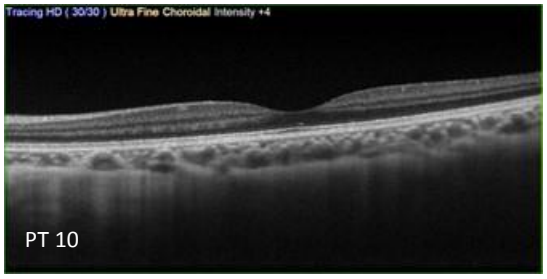
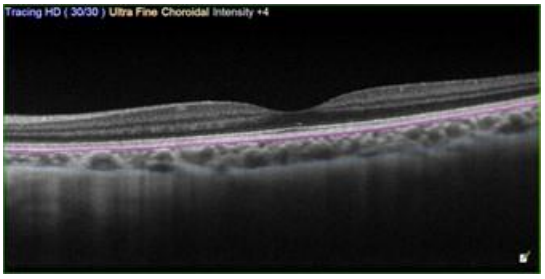
|---|---|
|  Tracing HD ( 30/30 ) Ultra Fine Choroidal Intensity +4<br>PT 6 |  Tracing HD ( 30/30 ) Ultra Fine Choroidal Intensity +4 |
|  Tracing HD ( 30/30 ) Ultra Fine Choroidal Intensity +4<br>PT 7 |  Tracing HD ( 30/30 ) Ultra Fine Choroidal Intensity +4 |
|  Tracing HD ( 30/30 ) Ultra Fine Choroidal Intensity +4<br>PT 8 |  Tracing HD ( 30/30 ) Ultra Fine Choroidal Intensity +4 |
|  Tracing HD ( 30/30 ) Ultra Fine Choroidal Intensity +4<br>PT 9 |  Tracing HD ( 30/30 ) Ultra Fine Choroidal Intensity +4 |
|  Tracing HD ( 30/30 ) Ultra Fine Choroidal Intensity +4<br>PT 10 |  Tracing HD ( 30/30 ) Ultra Fine Choroidal Intensity +4 |

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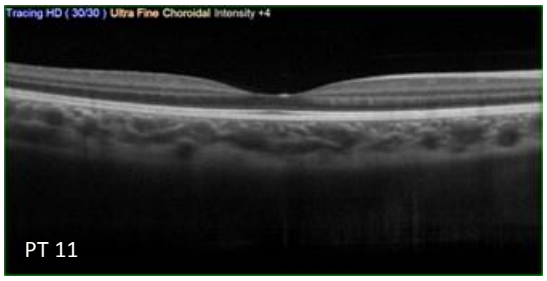
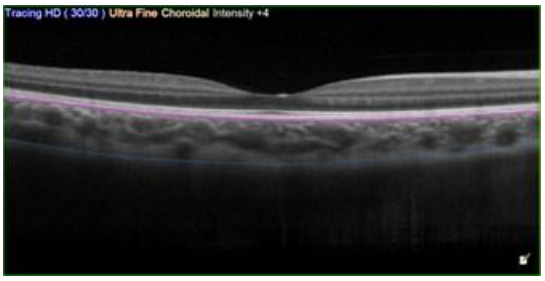
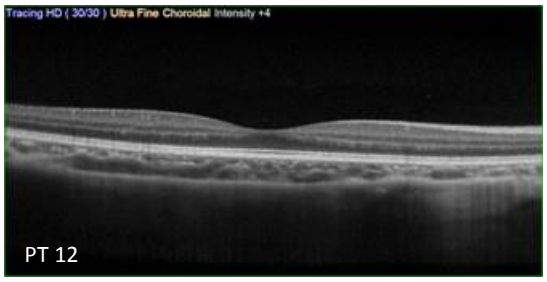
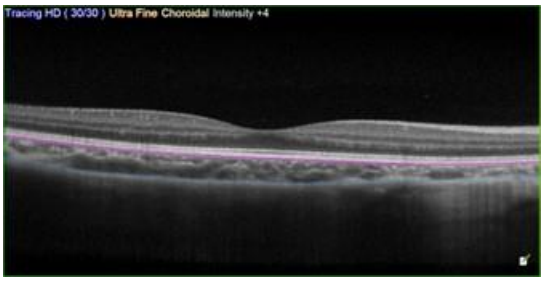
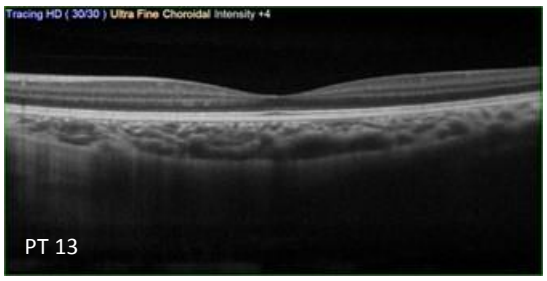
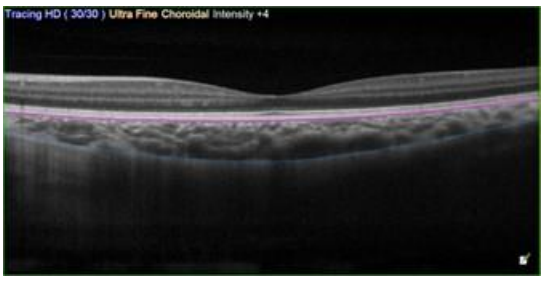
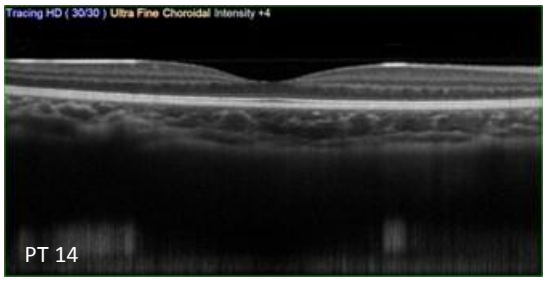
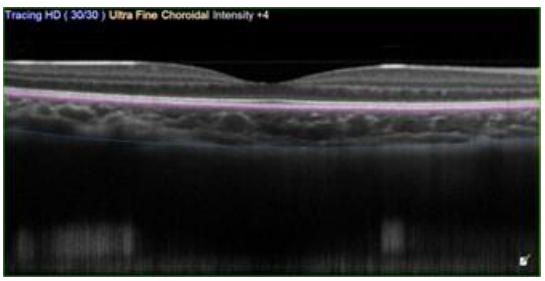
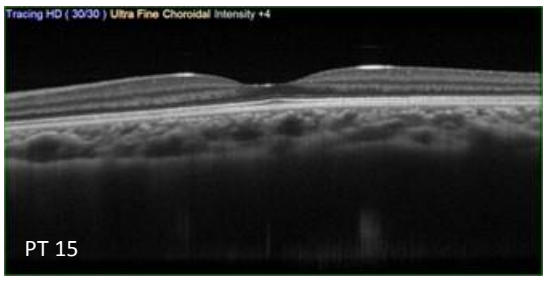
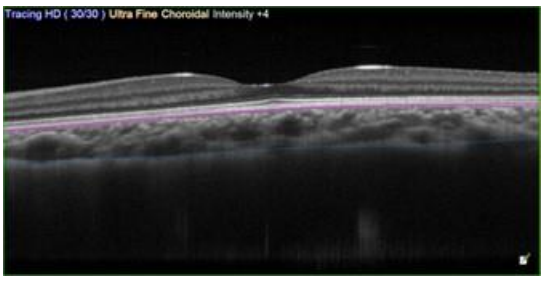
|---|---|
|  Tracing HD ( 30/30 ) Ultra Fine Choroidal Intensity +4<br>PT 11 |  Tracing HD ( 30/30 ) Ultra Fine Choroidal Intensity +4 |
|  Tracing HD ( 30/30 ) Ultra Fine Choroidal Intensity +4<br>PT 12 |  Tracing HD ( 30/30 ) Ultra Fine Choroidal Intensity +4 |
|  Tracing HD ( 30/30 ) Ultra Fine Choroidal Intensity +4<br>PT 13 |  Tracing HD ( 30/30 ) Ultra Fine Choroidal Intensity +4 |
|  Tracing HD ( 30/30 ) Ultra Fine Choroidal Intensity +4<br>PT 14 |  Tracing HD ( 30/30 ) Ultra Fine Choroidal Intensity +4 |
|  Tracing HD ( 30/30 ) Ultra Fine Choroidal Intensity +4<br>PT 15 |  Tracing HD ( 30/30 ) Ultra Fine Choroidal Intensity +4 |

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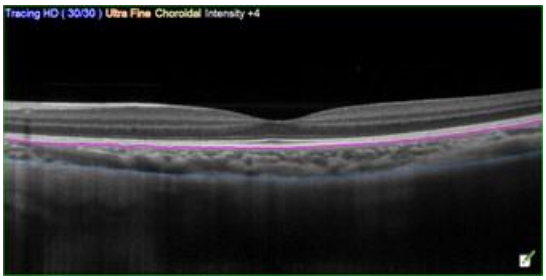
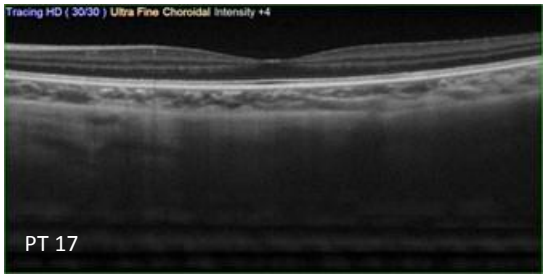
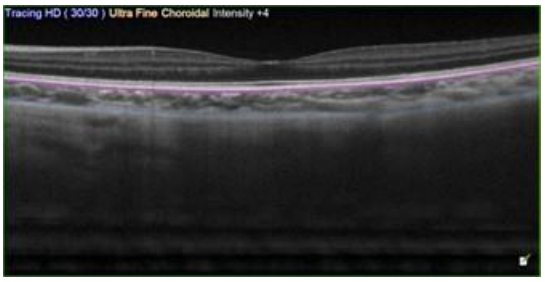
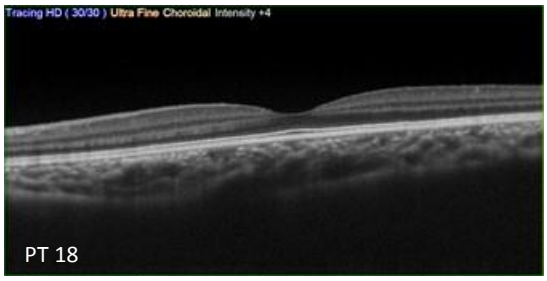
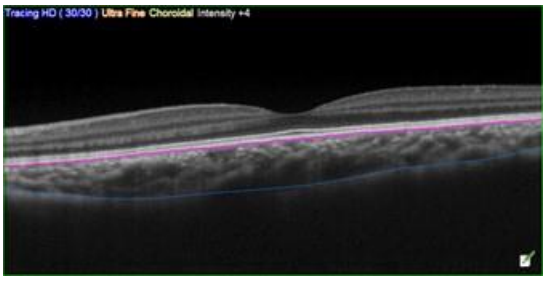
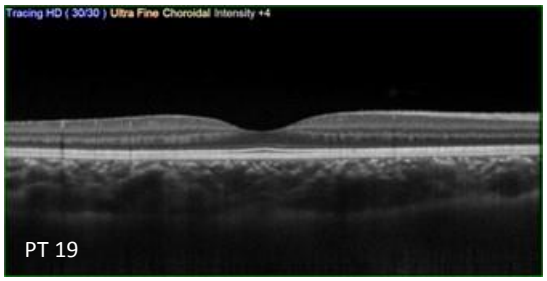
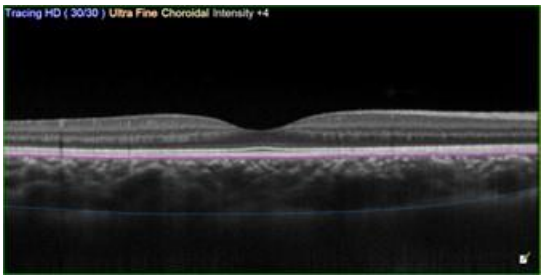
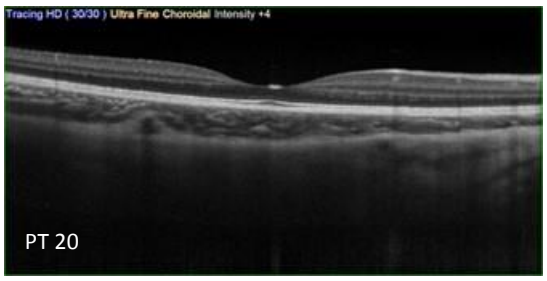
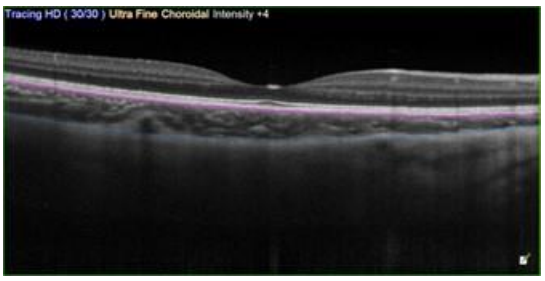
|---|---|
|  Tracing HD ( 30/30 ) Ultra Fine Choroidal Intensity +4<br>PT 16 |  Tracing HD ( 30/30 ) Ultra Fine Choroidal Intensity +4 |
|  Tracing HD ( 30/30 ) Ultra Fine Choroidal Intensity +4<br>PT 17 |  Tracing HD ( 30/30 ) Ultra Fine Choroidal Intensity +4 |
|  Tracing HD ( 30/30 ) Ultra Fine Choroidal Intensity +4<br>PT 18 |  Tracing HD ( 30/30 ) Ultra Fine Choroidal Intensity +4 |
|  Tracing HD ( 30/30 ) Ultra Fine Choroidal Intensity +4<br>PT 19 |  Tracing HD ( 30/30 ) Ultra Fine Choroidal Intensity +4 |
|  Tracing HD ( 30/30 ) Ultra Fine Choroidal Intensity +4<br>PT 20 |  Tracing HD ( 30/30 ) Ultra Fine Choroidal Intensity +4 |

## 9.3 Individual choroidal thickness: raw data for all 20 participants

### 9.3.1 Actual choroidal thickness – Stage 1 (Before atropine)

| | | | PT 1 | PT 2 | PT 3 | PT 4 | PT 5 | PT 6 | PT 7 | PT 8 | PT 9 | PT 10 | PT 11 | PT 12 | PT 13 | PT 14 | PT 15 | PT 16 | PT 17 | PT 18 | PT 19 | PT 20 | Mean | SD | SEM |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Hyperopic Defocus | Control Eye | 0 | 283.65 | 213.69 | 250.17 | 367.78 | 337.27 | 155.93 | 373.71 | 311.17 | 188.46 | 182.50 | 273.40 | 207.78 | 266.46 | 352.21 | 388.75 | 228.57 | 224.90 | 353.24 | 371.99 | 220.89 | **277.63** | 74.23 | 16.60 |
| | | 20 | 284.23 | 224.17 | 248.64 | 370.89 | 335.44 | 156.43 | 371.32 | 316.74 | 189.49 | 179.14 | 270.55 | 209.10 | 262.48 | 352.83 | 390.57 | 229.06 | 223.91 | 352.84 | 375.13 | 224.74 | **278.38** | 74.28 | 16.61 |
| | | 40 | 287.03 | 225.76 | 247.13 | 365.85 | 334.76 | 155.17 | 376.08 | 312.75 | 183.09 | 180.50 | 274.04 | 206.76 | 269.57 | 352.04 | 388.25 | 229.93 | 226.09 | 356.27 | 369.59 | 221.71 | **278.12** | 74.20 | 16.59 |
| | | 60 | 287.05 | 226.27 | 259.01 | 372.28 | 334.99 | 153.31 | 373.04 | 311.13 | 190.30 | 178.67 | 275.08 | 208.90 | 262.39 | 355.82 | 388.02 | 227.83 | 225.46 | 352.42 | 370.51 | 222.74 | **278.76** | 74.02 | 16.55 |
| | Experimental Eye | 0 | 280.40 | 237.66 | 249.09 | 370.05 | 351.81 | 175.07 | 263.50 | 262.67 | 197.57 | 209.05 | 261.68 | 200.14 | 315.87 | 321.81 | 322.19 | 229.98 | 182.96 | 387.97 | 429.29 | 222.45 | **273.56** | 72.29 | 16.17 |
| | | 20 | 269.76 | 237.21 | 231.83 | 358.92 | 344.13 | 163.66 | 256.32 | 259.12 | 188.69 | 205.87 | 255.97 | 195.29 | 304.02 | 312.15 | 315.90 | 220.89 | 179.57 | 375.20 | 427.72 | 219.80 | **266.10** | 71.76 | 16.05 |
| | | 40 | 272.11 | 232.40 | 228.62 | 342.36 | 349.72 | 158.47 | 249.04 | 256.25 | 184.73 | 198.88 | 244.98 | 190.17 | 311.11 | 304.76 | 316.31 | 225.50 | 174.08 | 378.68 | 420.38 | 207.80 | **262.32** | 72.71 | 16.26 |
| | | 60 | 266.85 | 218.54 | 238.57 | 345.20 | 328.07 | 145.99 | 252.47 | 261.36 | 181.17 | 204.01 | 255.10 | 190.32 | 300.66 | 310.83 | 320.19 | 219.98 | 183.26 | 380.33 | 417.62 | 212.87 | **261.67** | 71.91 | 16.08 |
| Myopic Defocus | Control Eye | 0 | 286.93 | 222.79 | 249.39 | 371.76 | 338.80 | 159.64 | 372.05 | 307.78 | 194.03 | 184.88 | 286.21 | 211.83 | 265.77 | 347.03 | 387.53 | 233.15 | 224.98 | 349.24 | 369.68 | 219.78 | **279.16** | 72.15 | 16.13 |
| | | 20 | 288.55 | 223.39 | 253.49 | 368.71 | 338.93 | 163.15 | 370.26 | 308.77 | 190.40 | 190.16 | 289.27 | 216.41 | 269.83 | 347.73 | 387.20 | 233.95 | 226.45 | 350.80 | 371.76 | 219.11 | **280.42** | 71.26 | 15.93 |
| | | 40 | 288.52 | 223.44 | 255.25 | 372.50 | 338.60 | 163.29 | 376.90 | 301.15 | 192.15 | 180.54 | 285.23 | 210.14 | 265.05 | 349.24 | 389.66 | 235.83 | 225.79 | 349.75 | 369.09 | 220.81 | **279.65** | 72.58 | 16.23 |
| | | 60 | 286.30 | 236.49 | 244.38 | 376.02 | 339.04 | 165.06 | 375.60 | 306.39 | 196.55 | 183.63 | 282.38 | 212.86 | 263.39 | 346.09 | 390.39 | 233.85 | 224.07 | 350.86 | 369.05 | 220.75 | **280.16** | 71.92 | 16.08 |
| | Experimental Eye | 0 | 278.47 | 225.94 | 251.13 | 373.08 | 351.83 | 178.17 | 262.45 | 258.04 | 192.54 | 213.04 | 260.67 | 188.17 | 319.14 | 317.51 | 321.85 | 224.65 | 182.58 | 372.15 | 428.78 | 222.13 | **271.12** | 72.30 | 16.17 |
| | | 20 | 289.03 | 231.06 | 251.55 | 384.31 | 350.93 | 187.84 | 263.41 | 259.26 | 194.86 | 220.52 | 281.88 | 199.20 | 327.14 | 321.13 | 324.01 | 232.18 | 191.00 | 381.70 | 447.43 | 225.05 | **278.17** | 73.67 | 16.47 |
| | | 40 | 292.85 | 239.51 | 259.38 | 391.91 | 361.29 | 181.75 | 274.80 | 265.28 | 213.83 | 229.77 | 285.20 | 192.59 | 325.94 | 319.69 | 339.00 | 234.91 | 190.73 | 395.10 | 444.34 | 225.54 | **283.17** | 74.70 | 16.70 |
| | | 60 | 284.78 | 239.92 | 264.75 | 381.98 | 366.63 | 187.55 | 281.81 | 273.54 | 193.53 | 216.31 | 291.30 | 205.47 | 329.11 | 320.31 | 333.29 | 233.13 | 197.90 | 392.69 | 436.49 | 230.26 | **283.04** | 72.86 | 16.29 |

Units: Microns (µm)

### 9.3.2 Actual choroidal thickness – Stage 2 (after 1 week on atropine)

| | | PT 1 | PT 2 | PT 3 | PT 4 | PT 5 | PT 6 | PT 7 | PT 8 | PT 9 | PT 10 | PT 11 | PT 12 | PT 13 | PT 14 | PT 15 | PT 16 | PT 17 | PT 18 | PT 19 | PT 20 | Mean | SD | SEM |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Hyperopic Defocus | Control Eye | 0 | 310.12 | 256.06 | 266.87 | 392.51 | 347.07 | 196.01 | 384.72 | 322.73 | 219.35 | 180.44 | 275.80 | 235.83 | 292.17 | 378.71 | 434.06 | 263.88 | 247.03 | 345.68 | 373.17 | 230.98 | **297.66** | 71.95 | 16.09 |
| | | 20 | 309.21 | 257.10 | 265.03 | 390.92 | 347.41 | 195.66 | 383.02 | 325.95 | 215.46 | 181.42 | 274.98 | 233.41 | 293.59 | 378.95 | 435.01 | 262.16 | 247.95 | 345.49 | 375.71 | 234.00 | **297.62** | 72.21 | 16.15 |
| | | 40 | 313.74 | 253.11 | 271.61 | 391.00 | 344.35 | 195.09 | 384.45 | 327.57 | 215.75 | 178.45 | 278.49 | 234.42 | 292.32 | 377.71 | 434.86 | 267.42 | 245.92 | 342.07 | 372.09 | 230.80 | **297.56** | 72.14 | 16.13 |
| | | 60 | 314.95 | 258.38 | 269.50 | 393.56 | 344.10 | 200.11 | 389.29 | 327.24 | 218.40 | 181.40 | 278.95 | 237.75 | 292.31 | 378.54 | 429.01 | 266.31 | 249.93 | 344.67 | 373.44 | 231.86 | **298.98** | 71.02 | 15.88 |
| | Experimental Eye | 0 | 313.10 | 262.26 | 247.00 | 395.56 | 371.52 | 166.02 | 293.30 | 280.15 | 220.91 | 216.43 | 266.73 | 219.71 | 332.43 | 366.25 | 363.92 | 263.84 | 210.02 | 380.22 | 451.12 | 222.66 | **292.16** | 76.36 | 17.07 |
| | | 20 | 312.67 | 262.10 | 244.03 | 394.60 | 369.67 | 165.34 | 293.01 | 276.56 | 217.72 | 218.79 | 265.02 | 217.70 | 333.94 | 367.83 | 365.60 | 262.41 | 213.59 | 387.33 | 452.78 | 225.13 | **292.29** | 77.10 | 17.24 |
| | | 40 | 309.83 | 259.41 | 244.10 | 400.13 | 371.87 | 164.57 | 294.41 | 279.10 | 221.75 | 218.29 | 262.35 | 218.46 | 334.82 | 366.50 | 359.51 | 260.81 | 212.86 | 383.58 | 458.20 | 224.79 | **292.27** | 77.64 | 17.36 |
| | | 60 | 311.38 | 260.42 | 249.59 | 402.43 | 367.01 | 168.41 | 297.91 | 284.11 | 226.88 | 215.11 | 267.39 | 225.52 | 326.27 | 367.17 | 360.16 | 265.03 | 211.18 | 391.64 | 461.86 | 224.18 | **294.18** | 77.31 | 17.29 |
| Myopic Defocus | Control Eye | 0 | 321.23 | 250.59 | 271.12 | 402.32 | 342.68 | 164.66 | 388.43 | 322.64 | 223.09 | 180.25 | 277.82 | 233.70 | 299.77 | 377.00 | 434.12 | 264.76 | 238.04 | 347.50 | 371.19 | 233.91 | **297.24** | 75.51 | 16.89 |
| | | 20 | 324.91 | 250.36 | 266.69 | 407.54 | 342.09 | 163.66 | 386.13 | 325.58 | 224.80 | 180.83 | 274.99 | 234.59 | 300.78 | 374.98 | 434.03 | 266.18 | 240.23 | 346.06 | 377.29 | 232.79 | **297.73** | 76.01 | 17.00 |
| | | 40 | 323.75 | 250.48 | 273.45 | 407.83 | 345.98 | 162.71 | 389.85 | 320.06 | 222.74 | 181.75 | 281.63 | 236.83 | 301.37 | 378.65 | 434.54 | 266.53 | 237.91 | 347.68 | 373.19 | 231.89 | **298.44** | 76.28 | 17.06 |
| | | 60 | 323.89 | 249.31 | 273.75 | 410.53 | 347.93 | 170.00 | 386.14 | 327.96 | 220.82 | 181.23 | 278.02 | 227.25 | 297.40 | 375.92 | 434.10 | 264.53 | 240.77 | 348.52 | 368.96 | 235.66 | **298.13** | 75.84 | 16.96 |
| | Experimental Eye | 0 | 312.74 | 253.31 | 253.07 | 392.67 | 371.59 | 193.77 | 294.63 | 280.80 | 223.00 | 206.48 | 255.78 | 216.76 | 337.31 | 365.82 | 368.49 | 265.11 | 214.09 | 379.24 | 451.18 | 221.78 | **292.88** | 74.96 | 16.76 |
| | | 20 | 317.45 | 255.78 | 278.47 | 398.18 | 381.31 | 195.68 | 297.33 | 284.31 | 224.63 | 211.04 | 265.82 | 226.56 | 347.20 | 377.83 | 379.09 | 269.91 | 226.11 | 389.54 | 457.39 | 225.53 | **300.46** | 75.92 | 16.98 |
| | | 40 | 325.64 | 262.09 | 282.24 | 421.13 | 379.33 | 198.77 | 306.41 | 283.52 | 233.17 | 211.16 | 258.02 | 229.50 | 354.19 | 377.66 | 381.87 | 271.42 | 232.08 | 387.60 | 456.21 | 229.23 | **304.06** | 76.39 | 17.08 |
| | | 60 | 323.14 | 270.73 | 280.71 | 412.80 | 383.02 | 206.25 | 310.08 | 289.17 | 241.86 | 214.34 | 262.30 | 237.95 | 345.15 | 383.31 | 375.98 | 274.27 | 232.62 | 384.18 | 456.95 | 234.09 | **305.94** | 73.16 | 16.36 |

Units: Microns (µm)

### 9.3.3 Actual choroidal thickness – Stage 3 (after 3 months on atropine)

| | | | PT 1 | PT 2 | PT 3 | PT 4 | PT 5 | PT 6 | PT 7 | PT 8 | PT 9 | PT 10 | PT 11 | PT 12 | PT 13 | PT 14 | PT 15 | PT 16 | PT 17 | PT 18 | PT 19 | PT 20 | Mean | SD | SEM |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Hyperopic Defocus | Control Eye | 0 | 323.99 | 242.37 | 274.85 | 393.65 | 346.15 | 166.28 | 383.82 | 322.53 | 200.66 | 190.57 | 307.75 | 260.12 | 301.42 | 377.86 | 416.23 | 269.24 | 282.10 | 345.75 | 370.86 | 276.52 | **302.64** | 69.85 | 15.62 |
| | | 20 | 325.66 | 242.47 | 275.27 | 396.06 | 346.90 | 166.36 | 385.04 | 321.48 | 200.55 | 191.82 | 310.42 | 261.77 | 300.50 | 382.01 | 418.21 | 268.24 | 280.31 | 348.48 | 372.65 | 278.38 | **303.63** | 70.58 | 15.78 |
| | | 40 | 323.18 | 246.23 | 275.20 | 396.20 | 344.42 | 166.01 | 384.23 | 321.58 | 202.58 | 189.85 | 307.42 | 260.01 | 303.62 | 380.92 | 420.25 | 270.23 | 280.67 | 347.74 | 370.89 | 277.02 | **303.41** | 70.32 | 15.72 |
| | | 60 | 324.19 | 243.93 | 275.15 | 394.84 | 344.81 | 169.34 | 385.00 | 320.51 | 202.62 | 188.53 | 309.91 | 262.87 | 302.14 | 378.92 | 419.39 | 269.20 | 282.25 | 348.71 | 373.91 | 277.78 | **303.70** | 70.06 | 15.67 |
| | Experimental Eye | 0 | 311.43 | 288.30 | 261.56 | 393.04 | 368.93 | 190.73 | 289.93 | 273.03 | 175.52 | 228.08 | 291.85 | 250.98 | 347.12 | 363.83 | 353.97 | 267.40 | 242.64 | 376.93 | 453.84 | 275.46 | **300.23** | 70.64 | 15.80 |
| | | 20 | 313.22 | 290.70 | 262.64 | 391.62 | 366.20 | 191.48 | 290.22 | 276.01 | 178.40 | 230.73 | 290.44 | 250.98 | 345.82 | 366.83 | 359.55 | 266.38 | 243.72 | 375.08 | 452.95 | 274.93 | **300.89** | 69.97 | 15.65 |
| | | 40 | 314.01 | 290.26 | 260.51 | 390.42 | 366.21 | 189.47 | 290.24 | 272.28 | 178.45 | 230.47 | 294.34 | 253.73 | 346.67 | 361.82 | 358.38 | 268.28 | 245.40 | 379.05 | 452.51 | 277.06 | **300.98** | 69.84 | 15.62 |
| | | 60 | 312.03 | 290.27 | 259.15 | 392.08 | 371.88 | 187.50 | 290.53 | 270.35 | 177.40 | 231.12 | 291.91 | 253.97 | 348.03 | 369.11 | 356.09 | 269.27 | 245.91 | 378.26 | 452.84 | 276.78 | **301.22** | 70.80 | 15.83 |
| Myopic Defocus | Control Eye | 0 | 323.72 | 246.33 | 278.36 | 390.62 | 344.18 | 163.68 | 382.57 | 324.26 | 202.37 | 191.51 | 304.45 | 263.11 | 300.50 | 379.97 | 413.21 | 272.38 | 269.77 | 344.35 | 370.96 | 277.46 | **302.19** | 69.22 | 15.48 |
| | | 20 | 325.92 | 244.04 | 282.05 | 390.03 | 342.17 | 165.57 | 381.93 | 323.86 | 204.64 | 192.82 | 303.58 | 261.52 | 301.25 | 381.69 | 412.51 | 271.79 | 272.12 | 345.81 | 370.89 | 279.14 | **302.67** | 68.73 | 15.37 |
| | | 40 | 324.79 | 241.68 | 278.22 | 393.17 | 341.67 | 164.38 | 384.36 | 326.62 | 205.70 | 193.61 | 305.45 | 260.71 | 303.82 | 381.95 | 415.21 | 274.40 | 270.95 | 346.39 | 372.16 | 280.17 | **303.27** | 69.57 | 15.56 |
| | | 60 | 323.63 | 244.39 | 278.18 | 392.19 | 342.65 | 163.35 | 380.96 | 326.62 | 205.67 | 193.22 | 304.04 | 259.49 | 302.49 | 380.08 | 413.37 | 274.13 | 270.61 | 343.33 | 373.19 | 280.30 | **302.59** | 69.07 | 15.44 |
| | Experimental Eye | 0 | 311.65 | 287.92 | 262.83 | 391.36 | 363.40 | 190.49 | 292.44 | 271.23 | 175.50 | 225.73 | 289.43 | 247.05 | 348.23 | 360.59 | 358.50 | 270.17 | 246.41 | 380.88 | 451.58 | 275.15 | **300.03** | 70.39 | 15.74 |
| | | 20 | 314.72 | 299.97 | 265.57 | 398.47 | 367.49 | 201.45 | 293.25 | 278.29 | 178.71 | 231.58 | 303.42 | 250.46 | 346.18 | 367.69 | 360.34 | 278.99 | 265.87 | 388.44 | 453.69 | 280.17 | **306.24** | 69.11 | 15.45 |
| | | 40 | 319.59 | 309.88 | 270.32 | 400.53 | 374.99 | 198.83 | 302.47 | 278.22 | 181.86 | 230.65 | 301.46 | 255.21 | 354.82 | 374.83 | 366.21 | 280.27 | 262.79 | 391.29 | 458.64 | 279.61 | **309.62** | 70.86 | 15.85 |
| | | 60 | 314.64 | 305.44 | 274.27 | 400.48 | 374.92 | 197.04 | 303.08 | 275.44 | 183.42 | 234.40 | 307.71 | 254.07 | 353.95 | 383.94 | 365.97 | 284.63 | 265.54 | 391.72 | 454.89 | 278.19 | **310.19** | 70.50 | 15.76 |

Units: Microns (µm)

### 9.3.4 Actual choroidal thickness – Stage 4 (after 6 months on atropine)

| | | | PT 1 | PT 2 | PT 3 | PT 4 | PT 5 | PT 6 | PT 7 | PT 8 | PT 9 | PT 10 | PT 11 | PT 12 | PT 13 | PT 14 | PT 15 | PT 16 | PT 17 | PT 18 | PT 19 | PT 20 | Mean | SD | SEM |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Hyperopic Defocus | Control Eye | 0 | 317.40 | 251.44 | 246.09 | 389.91 | 323.57 | 163.64 | 374.54 | 319.00 | 202.67 | 189.48 | 298.99 | 223.80 | 283.20 | 368.99 | 415.66 | 272.12 | 287.30 | 357.45 | 384.33 | 274.76 | **297.22** | 71.25 | 15.93 |
| | | 20 | 316.88 | 249.66 | 246.00 | 388.53 | 323.50 | 164.82 | 373.73 | 318.48 | 202.00 | 190.53 | 301.78 | 224.19 | 282.28 | 367.26 | 417.19 | 273.69 | 288.36 | 360.79 | 383.58 | 276.35 | **297.48** | 71.09 | 15.90 |
| | | 40 | 315.79 | 254.46 | 244.40 | 387.90 | 322.92 | 167.48 | 376.32 | 320.71 | 203.60 | 192.15 | 300.91 | 224.85 | 284.56 | 366.52 | 415.20 | 273.16 | 285.95 | 356.62 | 383.62 | 275.16 | **297.62** | 70.19 | 15.70 |
| | | 60 | 316.53 | 254.01 | 250.07 | 387.48 | 321.88 | 166.40 | 377.93 | 320.48 | 202.85 | 190.59 | 302.83 | 223.93 | 285.71 | 366.26 | 417.58 | 274.50 | 290.25 | 357.94 | 386.94 | 275.03 | **298.46** | 70.79 | 15.83 |
| | Experimental Eye | 0 | 287.09 | 300.51 | 241.28 | 385.01 | 347.56 | 185.84 | 276.78 | 273.79 | 178.49 | 229.92 | 286.75 | 230.40 | 335.97 | 346.08 | 350.00 | 273.17 | 250.57 | 396.86 | 468.76 | 267.90 | **295.64** | 72.42 | 16.19 |
| | | 20 | 287.45 | 297.36 | 240.56 | 384.01 | 347.90 | 186.60 | 278.40 | 274.43 | 177.42 | 228.33 | 287.55 | 229.48 | 334.08 | 345.12 | 347.78 | 272.16 | 253.24 | 395.15 | 468.34 | 268.80 | **295.21** | 72.06 | 16.11 |
| | | 40 | 288.02 | 301.71 | 243.53 | 385.00 | 351.67 | 187.05 | 278.16 | 276.70 | 178.44 | 230.59 | 289.43 | 226.77 | 333.27 | 342.24 | 352.08 | 276.35 | 252.92 | 392.68 | 467.95 | 266.07 | **296.03** | 71.85 | 16.07 |
| | | 60 | 288.82 | 302.33 | 242.51 | 382.49 | 347.75 | 186.81 | 278.27 | 277.15 | 181.55 | 231.49 | 288.22 | 230.80 | 339.46 | 342.78 | 351.33 | 276.20 | 253.83 | 394.61 | 467.05 | 265.37 | **296.44** | 71.27 | 15.94 |
| Myopic Defocus | Control Eye | 0 | 313.31 | 253.49 | 244.82 | 391.12 | 328.52 | 165.57 | 372.77 | 318.35 | 203.60 | 188.66 | 303.31 | 223.40 | 283.21 | 365.89 | 419.15 | 272.13 | 290.25 | 357.69 | 382.62 | 272.40 | **297.51** | 71.18 | 15.92 |
| | | 20 | 312.49 | 252.09 | 244.23 | 392.85 | 326.67 | 167.33 | 372.87 | 320.86 | 203.83 | 189.77 | 302.99 | 225.89 | 284.22 | 366.96 | 415.47 | 274.22 | 287.53 | 356.25 | 385.64 | 274.53 | **297.83** | 70.74 | 15.82 |
| | | 40 | 314.03 | 252.23 | 247.09 | 390.76 | 331.96 | 164.69 | 370.92 | 317.60 | 203.72 | 188.22 | 304.04 | 223.83 | 282.94 | 367.19 | 420.47 | 270.58 | 288.36 | 359.69 | 383.36 | 273.18 | **297.74** | 71.53 | 15.99 |
| | | 60 | 312.91 | 250.10 | 246.62 | 392.69 | 330.28 | 166.82 | 375.96 | 317.43 | 205.61 | 189.46 | 306.36 | 225.19 | 283.63 | 362.89 | 421.47 | 274.12 | 291.73 | 358.74 | 386.49 | 271.16 | **298.48** | 71.46 | 15.98 |
| | Experimental Eye | 0 | 284.87 | 297.58 | 241.04 | 386.58 | 347.92 | 188.47 | 276.84 | 273.19 | 174.06 | 227.34 | 288.58 | 230.24 | 333.88 | 348.12 | 350.57 | 270.17 | 246.78 | 394.51 | 465.13 | 268.04 | **294.70** | 72.45 | 16.20 |
| | | 20 | 292.13 | 303.64 | 247.42 | 400.62 | 359.44 | 189.10 | 285.26 | 280.00 | 184.19 | 235.09 | 298.16 | 236.94 | 328.51 | 345.65 | 351.25 | 276.68 | 261.02 | 406.85 | 482.32 | 275.31 | **301.98** | 73.91 | 16.53 |
| | | 40 | 295.39 | 304.99 | 249.71 | 398.21 | 357.82 | 199.00 | 284.20 | 286.44 | 186.28 | 240.58 | 306.97 | 232.83 | 344.00 | 349.11 | 366.17 | 279.50 | 264.10 | 410.02 | 478.32 | 276.17 | **305.49** | 73.10 | 16.35 |
| | | 60 | 291.55 | 313.43 | 250.60 | 406.88 | 361.61 | 202.07 | 281.23 | 286.76 | 184.44 | 236.19 | 307.22 | 244.63 | 338.29 | 348.25 | 364.35 | 279.82 | 262.89 | 415.12 | 481.31 | 272.24 | **306.44** | 74.11 | 16.57 |

Units: Microns (µm)

### 9.3.5 Absolute change in choroidal thickness – Stage 1 (Before atropine)

| | | PT 1 | PT 2 | PT 3 | PT 4 | PT 5 | PT 6 | PT 7 | PT 8 | PT 9 | PT 10 | PT 11 | PT 12 | PT 13 | PT 14 | PT 15 | PT 16 | PT 17 | PT 18 | PT 19 | PT 20 | Mean | SD | SEM |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Hyperopic Defocus | Control Eye | 0 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | **0.00** | 0.00 | 0.00 |
| | | 20 | 0.58 | 10.49 | -1.52 | 3.11 | -1.83 | 0.50 | -2.38 | 5.57 | 1.03 | -3.36 | -2.85 | 1.32 | -3.98 | 0.62 | 1.82 | 0.49 | -0.99 | -0.40 | 3.13 | 3.86 | **0.76** | 3.39 | 0.76 |
| | | 40 | 3.38 | 12.07 | -3.04 | -1.93 | -2.51 | -0.76 | 2.37 | 1.58 | -5.37 | -2.01 | 0.64 | -1.02 | 3.11 | -0.17 | -0.50 | 1.36 | 1.19 | 3.03 | -2.41 | 0.82 | **0.49** | 3.58 | 0.80 |
| | | 60 | 3.40 | 12.58 | 8.84 | 4.50 | -2.29 | -2.62 | -0.67 | -0.04 | 1.85 | -3.83 | 1.68 | 1.11 | -4.07 | 3.61 | -0.74 | -0.73 | 0.56 | -0.82 | -1.48 | 1.86 | **1.14** | 4.07 | 0.91 |
| | Experimental Eye | 0 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | **0.00** | 0.00 | 0.00 |
| | | 20 | -10.64 | -0.45 | -17.26 | -11.14 | -7.69 | -11.41 | -7.17 | -3.56 | -8.88 | -3.17 | -5.71 | -4.85 | -11.85 | -9.65 | -6.29 | -9.09 | -3.39 | -12.77 | -1.57 | -2.65 | **-7.46** | 4.36 | 0.97 |
| | | 40 | -8.29 | -5.26 | -20.47 | -27.69 | -2.09 | -16.60 | -14.46 | -6.43 | -12.85 | -10.16 | -16.70 | -9.97 | -4.76 | -17.05 | -5.88 | -4.48 | -8.88 | -9.29 | -8.91 | -14.65 | **-11.24** | 6.32 | 1.41 |
| | | 60 | -13.55 | -19.13 | -10.52 | -24.85 | -23.74 | -29.08 | -11.03 | -1.32 | -16.40 | -5.04 | -6.58 | -9.82 | -15.21 | -10.97 | -2.00 | -10.00 | 0.30 | -7.64 | -11.66 | -9.58 | **-11.89** | 7.81 | 1.75 |
| Myopic Defocus | Control Eye | 0 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | **0.00** | 0.00 | 0.00 |
| | | 20 | 1.62 | 0.60 | 4.10 | -3.04 | 0.12 | 3.51 | -1.79 | 0.99 | -3.63 | 5.28 | 3.07 | 4.58 | 4.05 | 0.70 | -0.33 | 0.80 | 1.47 | 1.56 | 2.08 | -0.67 | **1.25** | 2.44 | 0.54 |
| | | 40 | 1.59 | 0.65 | 5.85 | 0.74 | -0.20 | 3.65 | 4.85 | -6.63 | -1.88 | -4.34 | -0.98 | -1.69 | -0.72 | 2.21 | 2.13 | 2.67 | 0.81 | 0.52 | -0.59 | 1.03 | **0.48** | 2.90 | 0.65 |
| | | 60 | -0.63 | 13.70 | -5.01 | 4.27 | 0.23 | 5.42 | 3.56 | -1.39 | 2.52 | -1.25 | -3.83 | 1.03 | -2.38 | -0.94 | 2.86 | 0.70 | -0.91 | 1.62 | -0.62 | 0.97 | **1.00** | 3.97 | 0.89 |
| | Experimental Eye | 0 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | **0.00** | 0.00 | 0.00 |
| | | 20 | 10.56 | 5.12 | 0.42 | 11.24 | -0.90 | 9.67 | 0.96 | 1.22 | 2.31 | 7.48 | 21.21 | 11.02 | 8.01 | 3.62 | 2.16 | 7.53 | 8.42 | 9.55 | 18.66 | 2.92 | **7.06** | 5.87 | 1.31 |
| | | 40 | 14.39 | 13.57 | 8.26 | 18.83 | 9.46 | 3.58 | 12.35 | 7.24 | 21.29 | 16.73 | 24.53 | 4.41 | 6.81 | 2.17 | 17.14 | 10.27 | 8.15 | 22.95 | 15.57 | 3.42 | **12.06** | 6.75 | 1.51 |
| | | 60 | 6.31 | 13.98 | 13.63 | 8.90 | 14.80 | 9.37 | 19.36 | 15.50 | 0.99 | 3.27 | 30.62 | 17.30 | 9.98 | 2.79 | 11.44 | 8.49 | 15.32 | 20.54 | 7.71 | 8.14 | **11.92** | 6.96 | 1.56 |

Units: Microns (µm)

### 9.3.6 Absolute change in choroidal thickness – Stage 2 (after 1 wk on atropine)

| | | PT 1 | PT 2 | PT 3 | PT 4 | PT 5 | PT 6 | PT 7 | PT 8 | PT 9 | PT 10 | PT 11 | PT 12 | PT 13 | PT 14 | PT 15 | PT 16 | PT 17 | PT 18 | PT 19 | PT 20 | Mean | SD | SEM |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Hyperopic Defocus | Control Eye | 0 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | **0.00** | 0.00 | 0.00 |
| | | 20 | -0.91 | 1.05 | -1.84 | -1.59 | 0.34 | -0.35 | -1.69 | 3.22 | -3.89 | 0.98 | -0.82 | -2.42 | 1.41 | -1.00 | 0.24 | -5.06 | -1.72 | 0.92 | -0.19 | 3.01 | **-0.04** | 1.87 | 0.42 |
| | | 40 | 3.61 | -2.95 | 4.74 | -1.51 | -2.72 | -0.92 | -0.27 | 4.84 | -3.61 | -1.99 | 2.69 | -1.41 | 0.15 | -1.00 | 0.80 | 3.54 | -1.11 | -3.61 | -1.08 | -0.19 | **-0.10** | 2.65 | 0.59 |
| | | 60 | 4.83 | 2.32 | 2.63 | 1.05 | -2.97 | 4.10 | 4.57 | 4.51 | -0.95 | 0.96 | 3.15 | 1.92 | 0.13 | -0.17 | -5.06 | 2.43 | 2.91 | -1.01 | 0.27 | 0.87 | **1.33** | 2.57 | 0.58 |
| | Experimental Eye | 0 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | **0.00** | 0.00 | 0.00 |
| | | 20 | -0.43 | -0.16 | -2.97 | -0.96 | -1.85 | -0.68 | -0.30 | -3.59 | -3.19 | 2.36 | -1.71 | -2.01 | 1.51 | 1.58 | 1.68 | -1.43 | 3.57 | 7.10 | 1.66 | 2.46 | **0.13** | 2.62 | 0.59 |
| | | 40 | -3.28 | -2.86 | -2.90 | 4.57 | 0.35 | -1.45 | 1.11 | -1.04 | 0.84 | 1.86 | -4.39 | -1.25 | 2.39 | 0.25 | -4.40 | -3.03 | 2.83 | 3.36 | 7.08 | 2.13 | **0.11** | 3.13 | 0.70 |
| | | 60 | -1.72 | -1.84 | 2.59 | 6.86 | -4.51 | 2.39 | 4.61 | 3.96 | 5.97 | -1.33 | 0.65 | 5.81 | -6.16 | 0.91 | -3.76 | 1.19 | 1.16 | 11.42 | 10.74 | 1.52 | **2.02** | 4.68 | 1.05 |
| Myopic Defocus | Control Eye | 0 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | **0.00** | 0.00 | 0.00 |
| | | 20 | 3.68 | -0.23 | -4.43 | 5.23 | -0.58 | -1.00 | -2.30 | 2.94 | 1.71 | 0.57 | -2.83 | 0.88 | 1.01 | -2.01 | -0.10 | 1.42 | 2.19 | -1.45 | 6.10 | -1.12 | **0.48** | 2.67 | 0.60 |
| | | 40 | 2.52 | -0.11 | 2.33 | 5.51 | 3.31 | -1.95 | 1.42 | -2.58 | -0.35 | 1.49 | 3.81 | 3.13 | 1.60 | 1.65 | 0.42 | 1.77 | -0.13 | 0.18 | 2.00 | -2.02 | **1.20** | 2.05 | 0.46 |
| | | 60 | 2.66 | -1.28 | 2.63 | 8.21 | 5.25 | 5.34 | -2.29 | 5.33 | -2.28 | 0.97 | 0.20 | -6.45 | -2.37 | -1.07 | -0.03 | -0.23 | 2.73 | 1.02 | -2.23 | 1.75 | **0.89** | 3.46 | 0.77 |
| | Experimental Eye | 0 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | **0.00** | 0.00 | 0.00 |
| | | 20 | 4.70 | 2.47 | 25.40 | 5.50 | 9.72 | 1.91 | 2.70 | 3.51 | 1.63 | 4.57 | 10.05 | 9.80 | 9.88 | 12.01 | 10.60 | 4.80 | 12.03 | 10.31 | 6.21 | 3.75 | **7.58** | 5.50 | 1.23 |
| | | 40 | 12.90 | 8.78 | 29.17 | 28.45 | 7.74 | 5.00 | 11.79 | 2.72 | 10.17 | 4.68 | 2.24 | 12.74 | 16.88 | 11.84 | 13.38 | 6.30 | 17.99 | 8.36 | 5.04 | 7.45 | **11.18** | 7.42 | 1.66 |
| | | 60 | 10.39 | 17.42 | 27.64 | 20.13 | 11.43 | 12.49 | 15.45 | 8.38 | 18.86 | 7.87 | 6.53 | 21.19 | 7.84 | 17.49 | 7.49 | 9.15 | 18.53 | 4.94 | 5.77 | 12.31 | **13.06** | 6.20 | 1.39 |

Units: Microns (µm)

### 9.3.7 Absolute change in choroidal thickness – Stage 3 (after 3 mo on atropine)

| | | | PT 1 | PT 2 | PT 3 | PT 4 | PT 5 | PT 6 | PT 7 | PT 8 | PT 9 | PT 10 | PT 11 | PT 12 | PT 13 | PT 14 | PT 15 | PT 16 | PT 17 | PT 18 | PT 19 | PT 20 | Mean | SD | SEM |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Hyperopic Defocus | Control Eye | 0 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | **0.00** | 0.00 | 0.00 |
| | | 20 | 1.67 | 0.10 | 0.41 | 2.41 | 0.75 | 0.08 | 1.22 | -1.04 | -0.11 | 1.25 | 2.67 | 1.65 | -0.92 | 4.15 | 1.98 | -1.00 | -1.79 | 2.73 | 1.79 | 1.86 | **0.99** | 1.52 | 0.34 |
| | | 40 | -0.81 | 3.86 | 0.34 | 2.56 | -1.73 | -0.27 | 0.41 | -0.95 | 1.93 | -0.72 | -0.33 | -0.11 | 2.20 | 3.06 | 4.02 | 0.98 | -1.43 | 1.99 | 0.03 | 0.50 | **0.78** | 1.72 | 0.38 |
| | | 60 | 0.20 | 1.56 | 0.29 | 1.19 | -1.35 | 3.06 | 1.18 | -2.01 | 1.96 | -2.04 | 2.16 | 2.75 | 0.72 | 1.06 | 3.16 | -0.04 | 0.15 | 2.96 | 3.04 | 1.26 | **1.06** | 1.61 | 0.36 |
| | Experimental Eye | 0 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | **0.00** | 0.00 | 0.00 |
| | | 20 | 1.79 | 2.40 | 1.08 | -1.43 | -2.73 | 0.74 | 0.29 | 2.98 | 2.88 | 2.64 | -1.41 | 0.00 | -1.30 | 3.00 | 5.59 | -1.02 | 1.09 | -1.85 | -0.89 | -0.54 | **0.67** | 2.13 | 0.48 |
| | | 40 | 2.58 | 1.96 | -1.05 | -2.62 | -2.71 | -1.27 | 0.32 | -0.75 | 2.93 | 2.39 | 2.48 | 2.74 | -0.45 | -2.01 | 4.42 | 0.88 | 2.76 | 2.11 | -1.33 | 1.59 | **0.75** | 2.13 | 0.48 |
| | | 60 | 0.61 | 1.97 | -2.41 | -0.97 | 2.95 | -3.24 | 0.60 | -2.69 | 1.88 | 3.03 | 0.06 | 2.98 | 0.91 | 5.28 | 2.12 | 1.87 | 3.28 | 1.33 | -1.00 | 1.32 | **0.99** | 2.19 | 0.49 |
| Myopic Defocus | Control Eye | 0 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | **0.00** | 0.00 | 0.00 |
| | | 20 | 2.19 | -2.29 | 3.70 | -0.59 | -2.00 | 1.88 | -0.64 | -0.39 | 2.27 | 1.31 | -0.87 | -1.59 | 0.75 | 1.72 | -0.70 | -0.58 | 2.35 | 1.46 | -0.07 | 1.68 | **0.48** | 1.66 | 0.37 |
| | | 40 | 1.07 | -4.65 | -0.14 | 2.55 | -2.50 | 0.70 | 1.79 | 2.36 | 3.32 | 2.10 | 1.00 | -2.39 | 3.32 | 1.98 | 2.00 | 2.03 | 1.18 | 2.04 | 1.20 | 2.70 | **1.08** | 2.06 | 0.46 |
| | | 60 | -0.10 | -1.94 | -0.17 | 1.57 | -1.53 | -0.33 | -1.61 | 2.36 | 3.30 | 1.70 | -0.41 | -3.62 | 1.99 | 0.11 | 0.16 | 1.76 | 0.83 | -1.02 | 2.23 | 2.83 | **0.41** | 1.81 | 0.41 |
| | Experimental Eye | 0 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | **0.00** | 0.00 | 0.00 |
| | | 20 | 3.07 | 12.05 | 2.75 | 7.11 | 4.09 | 10.96 | 0.82 | 7.05 | 3.21 | 5.84 | 13.98 | 3.41 | -2.05 | 7.10 | 1.84 | 8.82 | 19.46 | 7.57 | 2.11 | 5.02 | **6.21** | 5.03 | 1.13 |
| | | 40 | 7.94 | 21.96 | 7.50 | 9.17 | 11.59 | 8.34 | 10.03 | 6.98 | 6.35 | 4.92 | 12.02 | 8.16 | 6.59 | 14.24 | 7.70 | 10.10 | 16.38 | 10.42 | 7.07 | 4.46 | **9.60** | 4.15 | 0.93 |
| | | 60 | 2.99 | 17.52 | 11.45 | 9.12 | 11.52 | 6.55 | 10.65 | 4.20 | 7.91 | 8.67 | 18.28 | 7.02 | 5.72 | 23.35 | 7.47 | 14.47 | 19.13 | 10.84 | 3.31 | 3.04 | **10.16** | 5.80 | 1.30 |

Units: Microns (µm)

### 9.3.8 Absolute change in choroidal thickness – Stage 4 (after 6 mo on atropine)

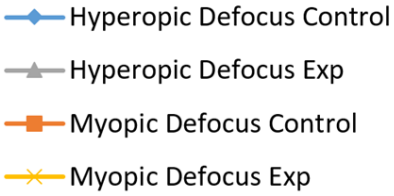
| | | | PT 1 | PT 2 | PT 3 | PT 4 | PT 5 | PT 6 | PT 7 | PT 8 | PT 9 | PT 10 | PT 11 | PT 12 | PT 13 | PT 14 | PT 15 | PT 16 | PT 17 | PT 18 | PT 19 | PT 20 | Mean | SD | SEM |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Hyperopic Defocus | Control Eye | 0 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | **0.00** | 0.00 | 0.00 |
| | | 20 | -0.52 | -1.78 | -0.09 | -1.38 | -0.07 | 1.18 | -0.80 | -0.52 | -0.67 | 1.05 | 2.79 | 0.39 | -0.92 | -1.73 | 1.53 | 1.57 | 1.06 | 3.34 | -0.75 | 1.60 | **0.26** | 1.45 | 0.32 |
| | | 40 | -1.61 | 3.02 | -1.69 | -2.01 | -0.65 | 3.85 | 1.79 | 1.71 | 0.93 | 2.67 | 1.93 | 1.06 | 1.36 | -2.46 | -0.47 | 1.04 | -1.35 | -0.83 | -0.71 | 0.40 | **0.40** | 1.81 | 0.40 |
| | | 60 | -0.86 | 2.56 | 3.98 | -2.43 | -1.69 | 2.76 | 3.39 | 1.48 | 0.18 | 1.12 | 3.85 | 0.14 | 2.51 | -2.73 | 1.92 | 2.39 | 2.95 | 0.49 | 2.61 | 0.27 | **1.24** | 2.02 | 0.45 |
| | Experimental Eye | 0 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | **0.00** | 0.00 | 0.00 |
| | | 20 | 0.36 | -3.15 | -0.72 | -1.00 | 0.34 | 0.76 | 1.62 | 0.64 | -1.08 | -1.59 | 0.80 | -0.92 | -1.89 | -0.96 | -2.22 | -1.00 | 2.67 | -1.71 | -0.42 | 0.89 | **-0.43** | 1.42 | 0.32 |
| | | 40 | 0.93 | 1.20 | 2.25 | -0.01 | 4.11 | 1.22 | 1.38 | 2.91 | -0.06 | 0.68 | 2.68 | -3.63 | -2.70 | -3.84 | 2.08 | 3.19 | 2.35 | -4.18 | -0.81 | -1.83 | **0.40** | 2.48 | 0.56 |
| | | 60 | 1.74 | 1.82 | 1.24 | -2.52 | 0.19 | 0.97 | 1.49 | 3.36 | 3.06 | 1.58 | 1.47 | 0.40 | 3.49 | -3.30 | 1.33 | 3.03 | 3.26 | -2.25 | -1.71 | -2.53 | **0.80** | 2.16 | 0.48 |
| Myopic Defocus | Control Eye | 0 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | **0.00** | 0.00 | 0.00 |
| | | 20 | -0.82 | -1.39 | -0.59 | 1.72 | -1.85 | 1.76 | 0.10 | 2.52 | 0.23 | 1.11 | -0.33 | 2.50 | 1.01 | 1.07 | -3.69 | 2.09 | -2.72 | -1.44 | 3.01 | 2.12 | **0.32** | 1.89 | 0.42 |
| | | 40 | 0.72 | -1.25 | 2.27 | -0.37 | 3.44 | -0.88 | -1.85 | -0.75 | 0.12 | -0.43 | 0.72 | 0.43 | -0.27 | 1.30 | 1.32 | -1.55 | -1.89 | 2.00 | 0.74 | 0.77 | **0.23** | 1.42 | 0.32 |
| | | 60 | -0.40 | -3.38 | 1.79 | 1.57 | 1.76 | 1.25 | 3.19 | -0.92 | 2.01 | 0.80 | 3.04 | 1.79 | 0.42 | -3.00 | 2.32 | 1.99 | 1.48 | 1.05 | 3.87 | -1.24 | **0.97** | 1.92 | 0.43 |
| | Experimental Eye | 0 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | **0.00** | 0.00 | 0.00 |
| | | 20 | 7.26 | 6.06 | 6.38 | 14.04 | 11.52 | 0.63 | 8.42 | 6.82 | 10.13 | 7.76 | 9.58 | 6.70 | -5.37 | -2.47 | 0.68 | 6.51 | 14.24 | 12.34 | 17.19 | 7.28 | **7.28** | 5.61 | 1.25 |
| | | 40 | 10.51 | 7.41 | 8.67 | 11.63 | 9.90 | 10.53 | 7.36 | 13.25 | 12.22 | 13.24 | 18.39 | 2.59 | 10.12 | 0.99 | 15.60 | 9.32 | 17.31 | 15.51 | 13.19 | 8.13 | **10.79** | 4.40 | 0.98 |
| | | 60 | 6.68 | 15.85 | 9.56 | 20.31 | 13.69 | 13.60 | 4.39 | 13.58 | 10.38 | 8.86 | 18.64 | 14.39 | 4.41 | 0.13 | 13.78 | 9.65 | 16.11 | 20.61 | 16.18 | 4.20 | **11.75** | 5.72 | 1.28 |

Units: Microns (µm)

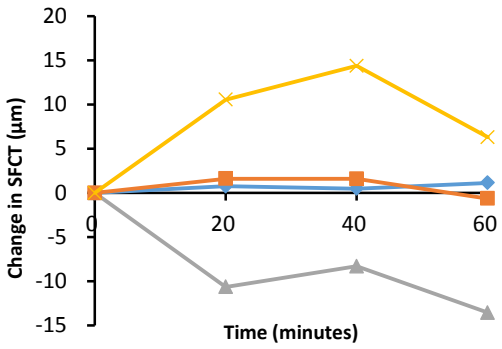
## 9.4 Individual absolute choroidal thickness change in response to retinal image defocus over the 6 months period for all 20 participants

All twenty participants received two different types of retinal image defocus at each stage of the study (i.e. Stage 1 (before atropine); Stage 2 (after 1 week on atropine); Stage 3 (after 3 months on atropine) and Stage 4 (after 6 months on atropine). The individual data is shown below.

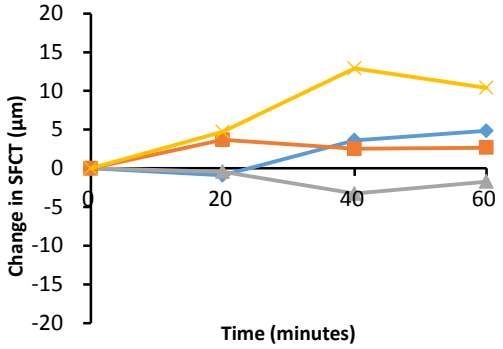
Legend:



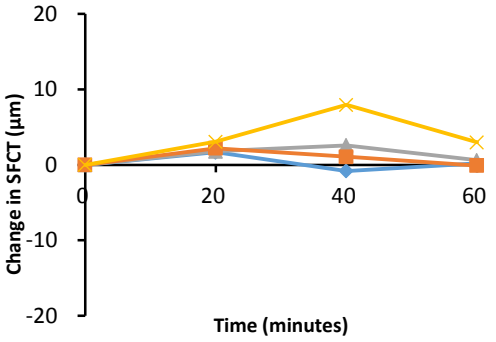
## Participant 1



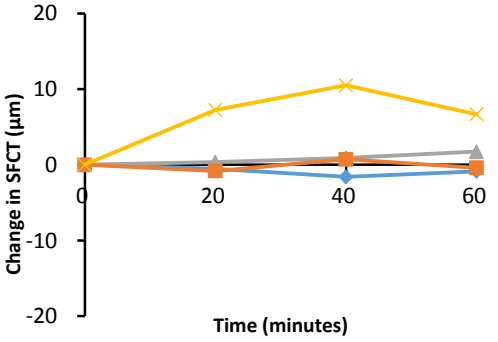

**Stage 1** (Before atropine)




**Stage 2** (1 week after atropine)

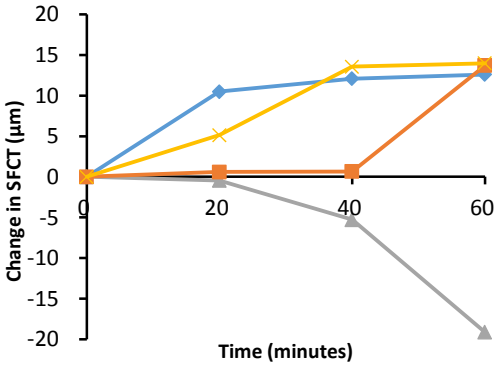



**Stage 3** (3 months after atropine)

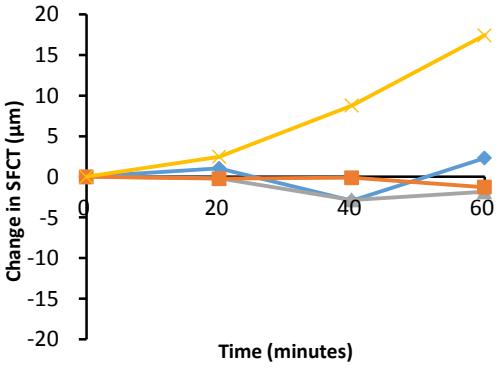



**Stage 4** (6 months after atropine)

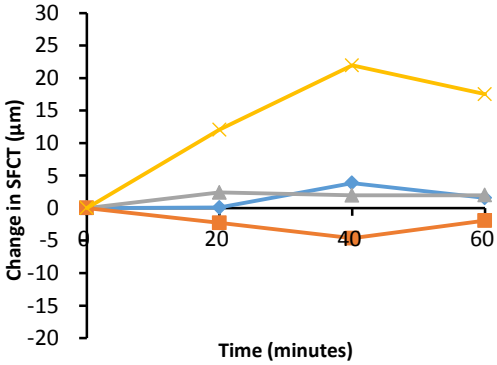
## Participant 2



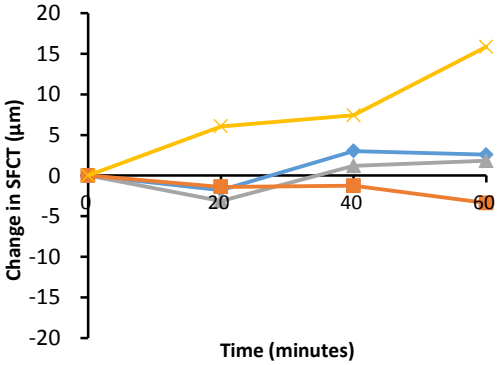

**Stage 1** (Before atropine)




**Stage 2** (1 week after atropine)

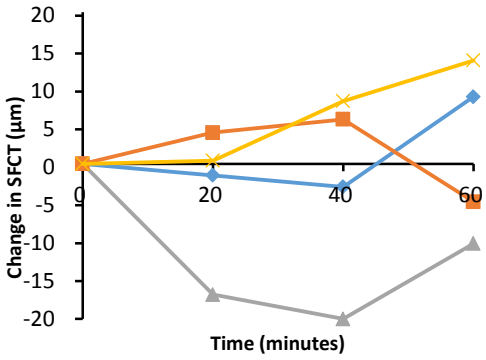



**Stage 3** (3 months after atropine)

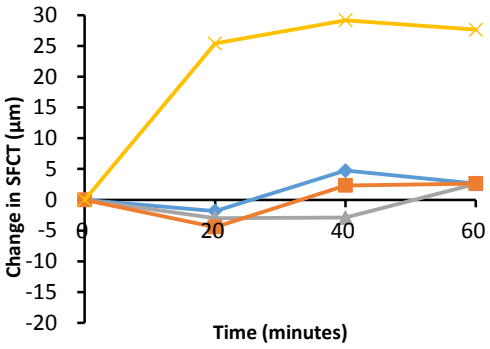



**Stage 4** (6 months after atropine)

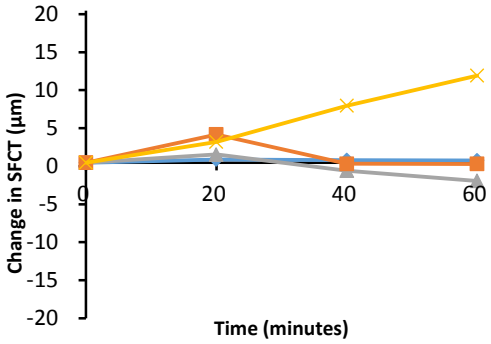
## Participant 3



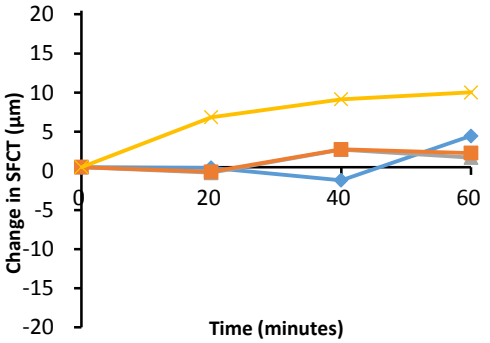

**Stage 1** (Before atropine)




**Stage 2** (1 week after atropine)

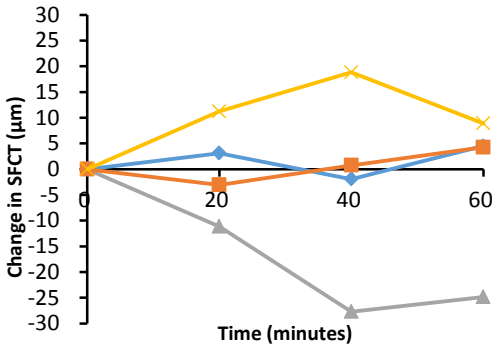



**Stage 3** (3 months after atropine)

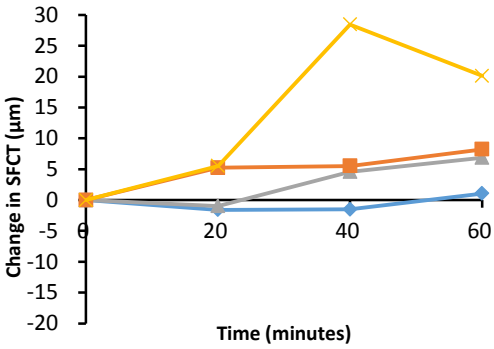



**Stage 4** (6 months after atropine)

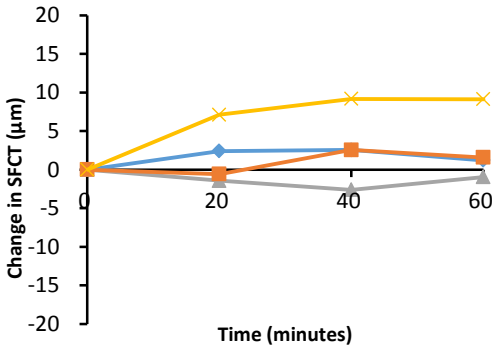
## Participant 4



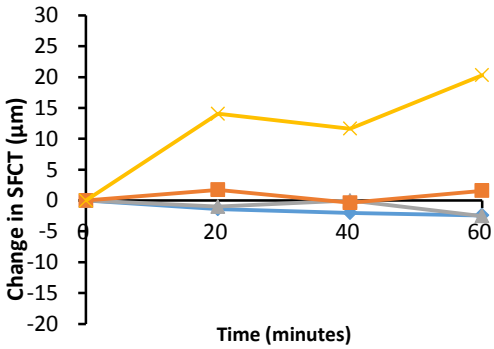

**Stage 1** (Before atropine)




**Stage 2** (1 week after atropine)

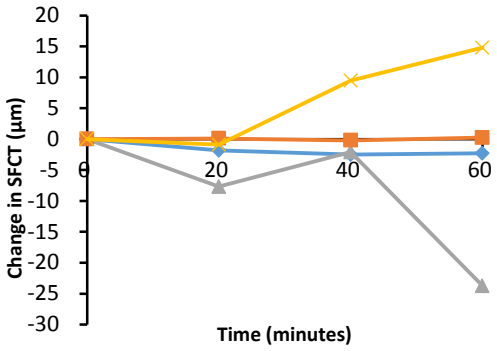



**Stage 3** (3 months after atropine)

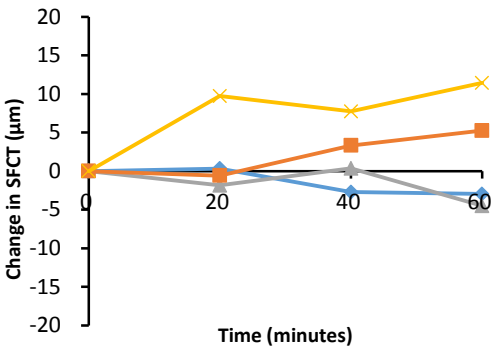



**Stage 4** (6 months after atropine)

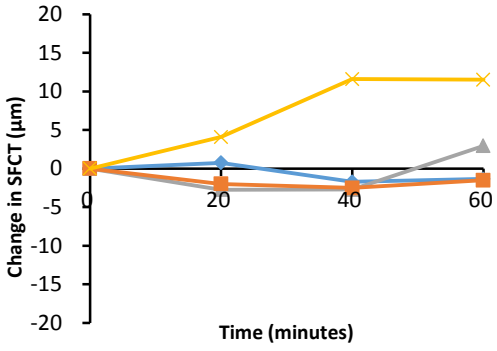
## Participant 5



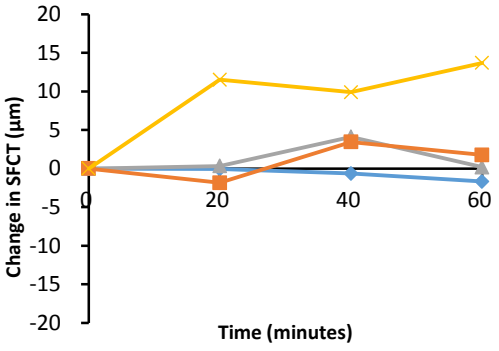

**Stage 1** (Before atropine)




**Stage 2** (1 week after atropine)

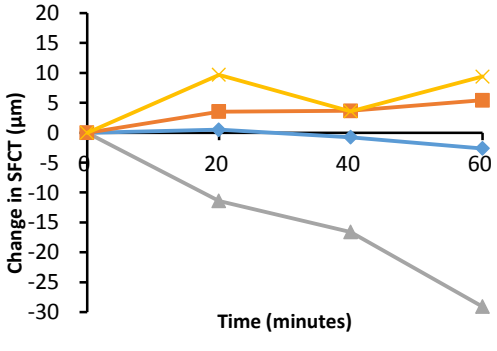



**Stage 3** (3 months after atropine)

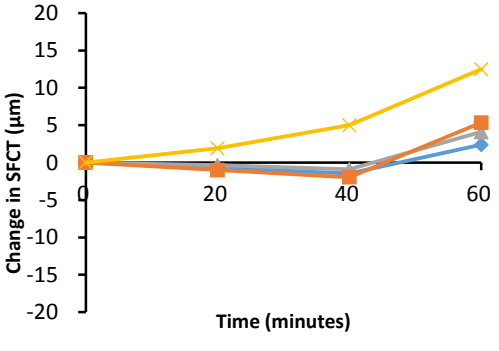



**Stage 4** (6 months after atropine)

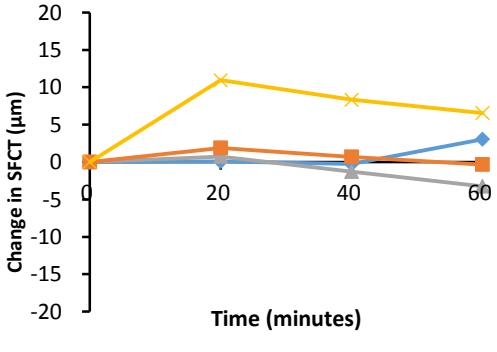
## Participant 6



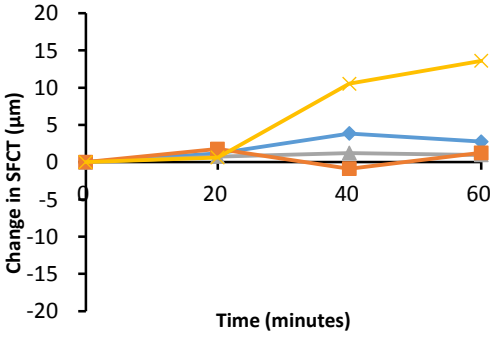

**Stage 1** (Before atropine)




**Stage 2** (1 week after atropine)

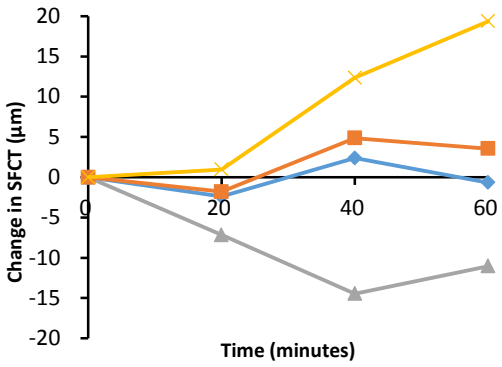



**Stage 3** (3 months after atropine)

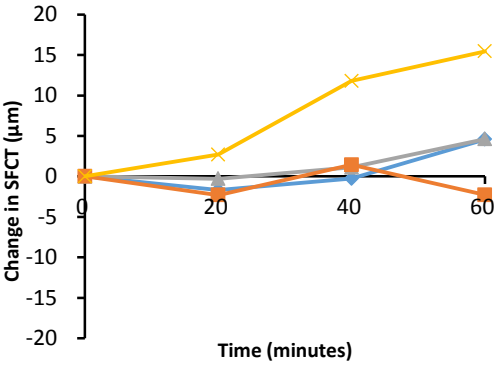



**Stage 4** (6 months after atropine)

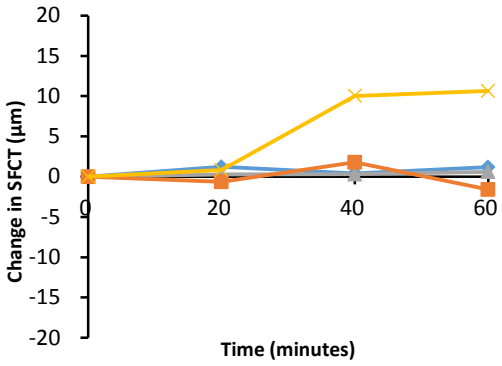
## Participant 7



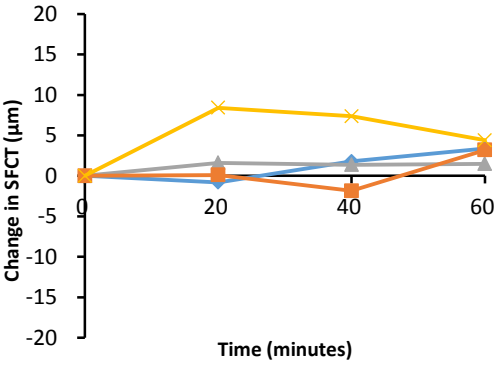

**Stage 1** (Before atropine)




**Stage 2** (1 week after atropine)

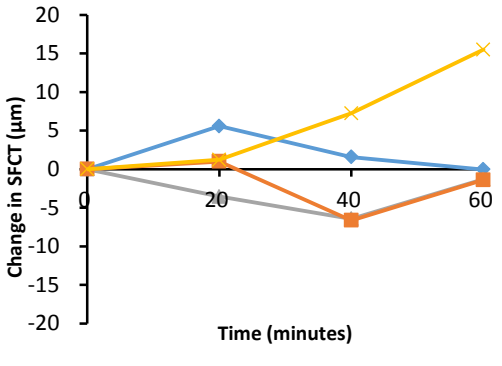



**Stage 3** (3 months after atropine)

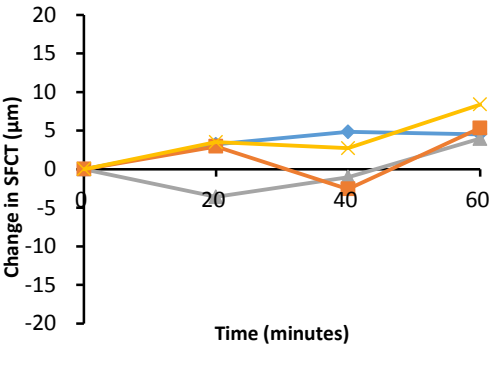



**Stage 4** (6 months after atropine)

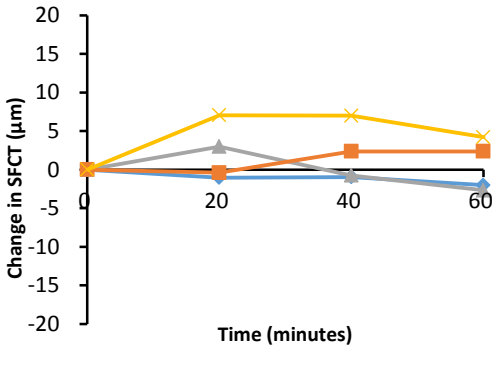
## Participant 8



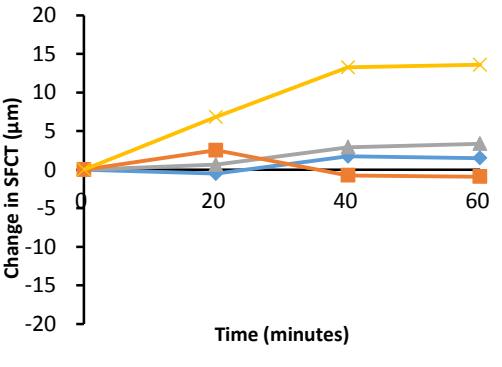

**Stage 1** (Before atropine)




**Stage 2** (1 week after atropine)

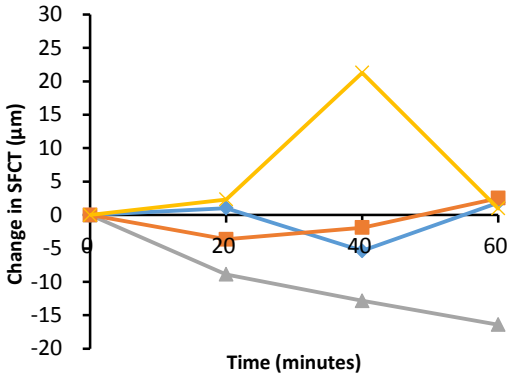



**Stage 3** (3 months after atropine)

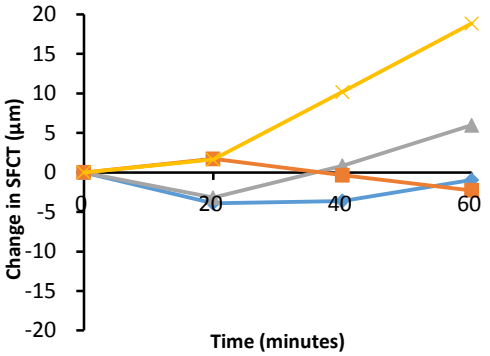



**Stage 4** (6 months after atropine)

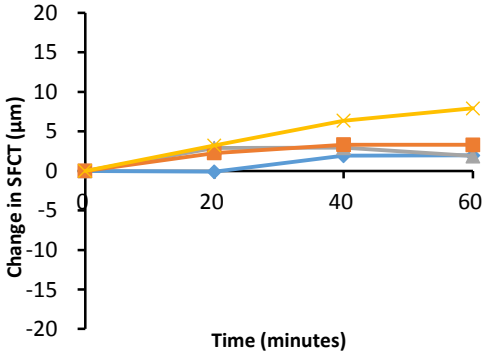
## Participant 9



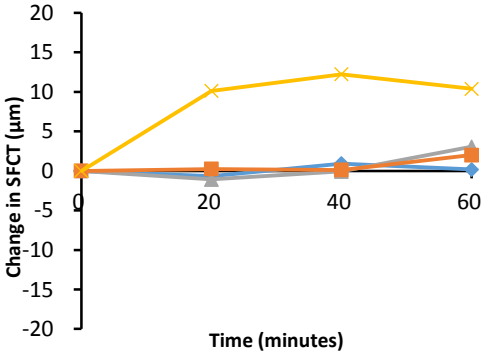

**Stage 1** (Before atropine)




**Stage 2** (1 week after atropine)

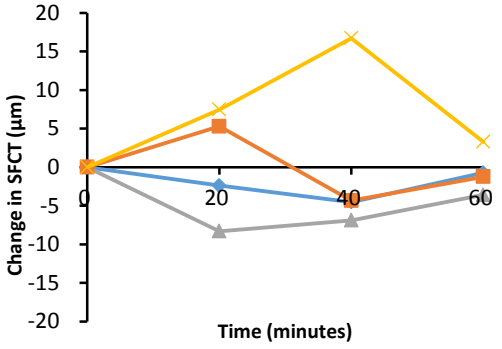



**Stage 3** (3 months after atropine)

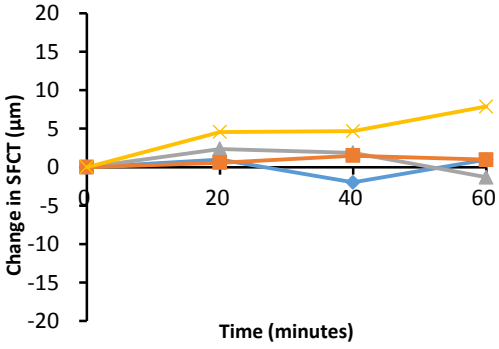



**Stage 4** (6 months after atropine)

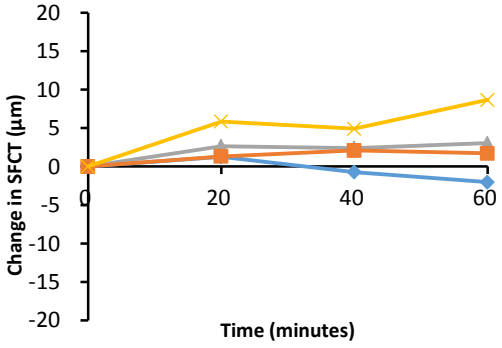
## Participant 10



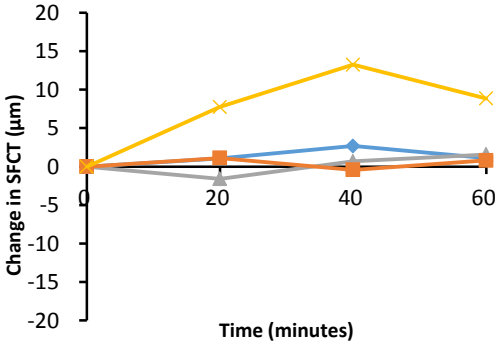

**Stage 1** (Before atropine)




**Stage 2** (1 week after atropine)

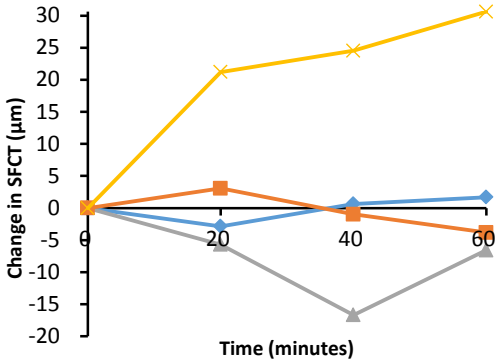



**Stage 3** (3 months after atropine)

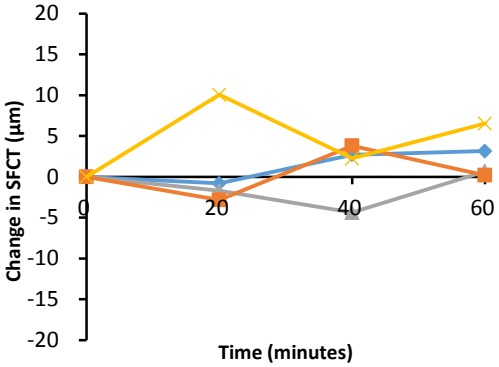



**Stage 4** (6 months after atropine)

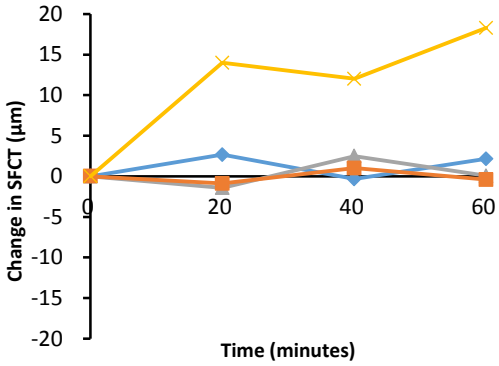
## Participant 11



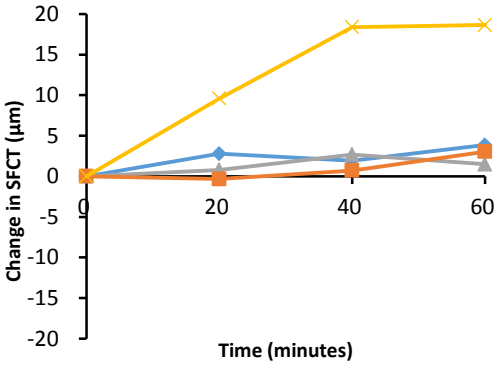

**Stage 1** (Before atropine)




**Stage 2** (1 week after atropine)

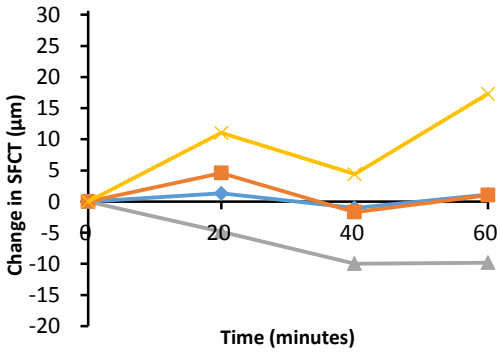



**Stage 3** (3 months after atropine)

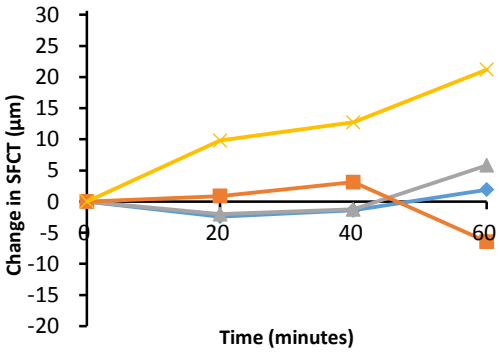



**Stage 4** (6 months after atropine)

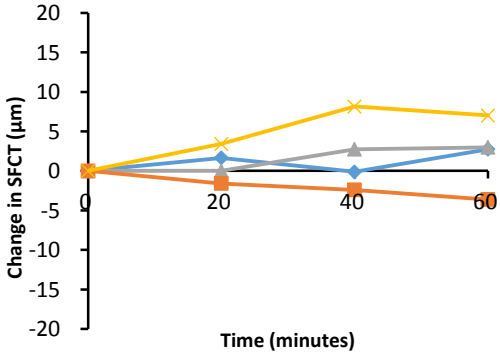
## Participant 12



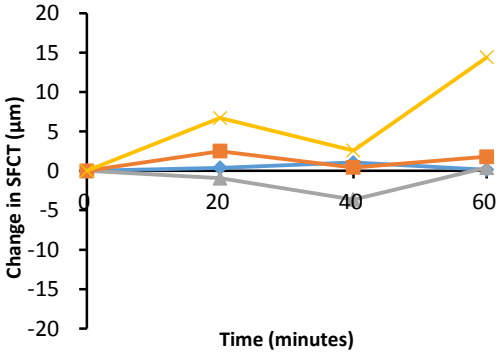

**Stage 1** (Before atropine)




**Stage 2** (1 week after atropine)

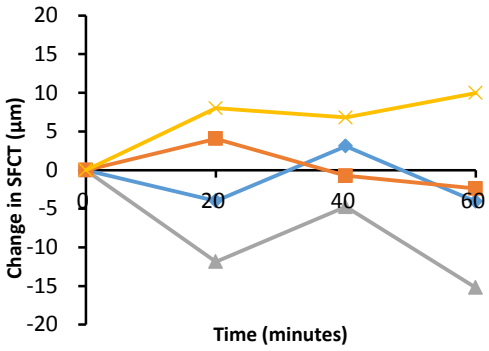



**Stage 3** (3 months after atropine)

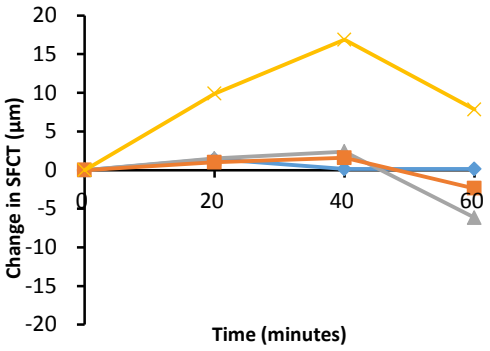



**Stage 4** (6 months after atropine)

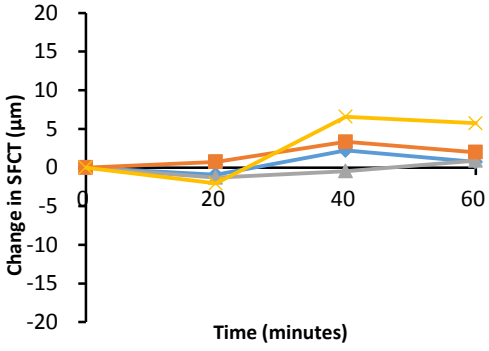
## Participant 13



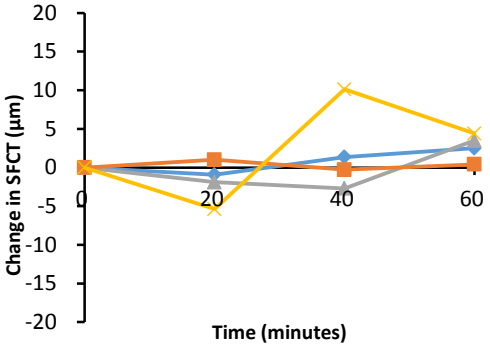

**Stage 1** (Before atropine)




**Stage 2** (1 week after atropine)

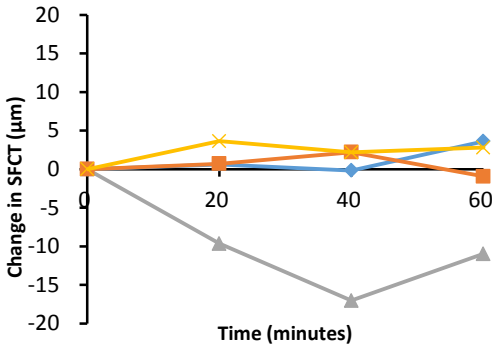



**Stage 3** (3 months after atropine)

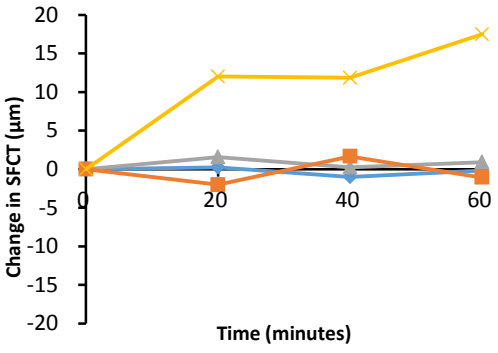



**Stage 4** (6 months after atropine)

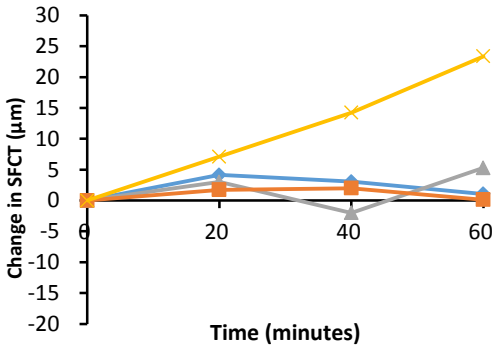
## Participant 14



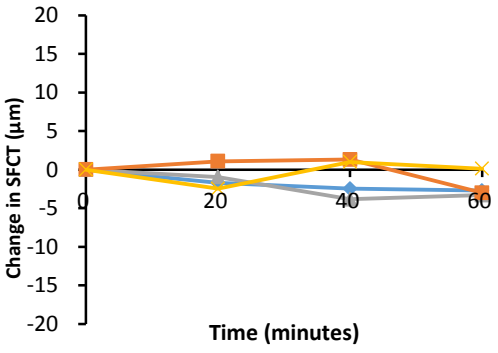

**Stage 1** (Before atropine)




**Stage 2** (1 week after atropine)

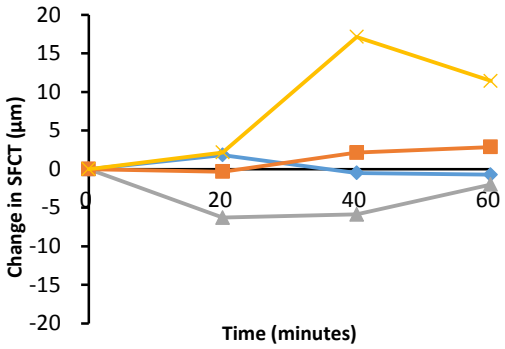



**Stage 3** (3 months after atropine)

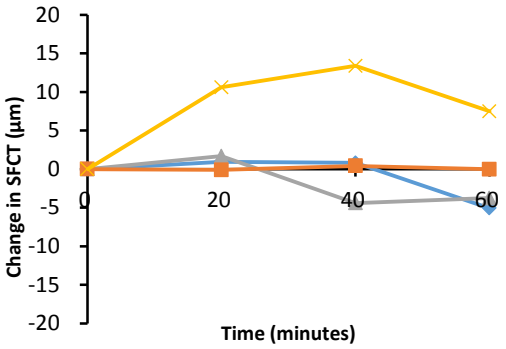



**Stage 4** (6 months after atropine)

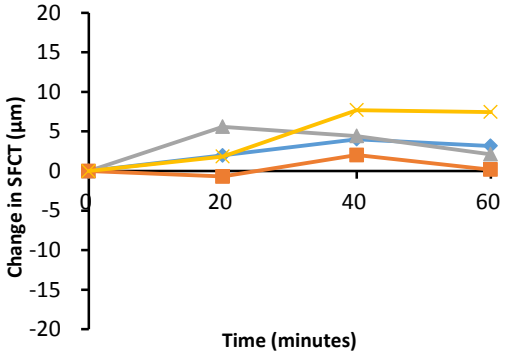
## Participant 15



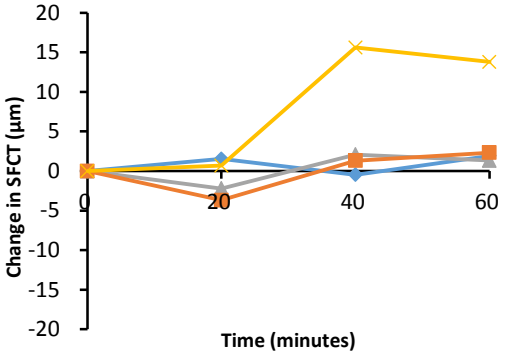

**Stage 1** (Before atropine)




**Stage 2** (1 week after atropine)

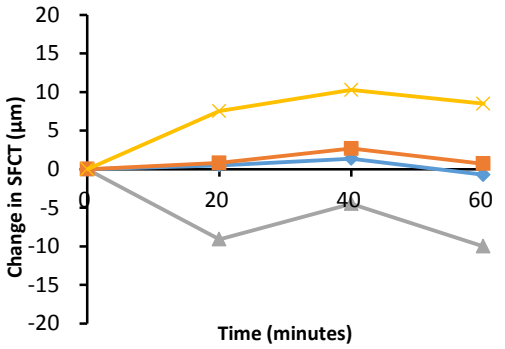



**Stage 3** (3 months after atropine)

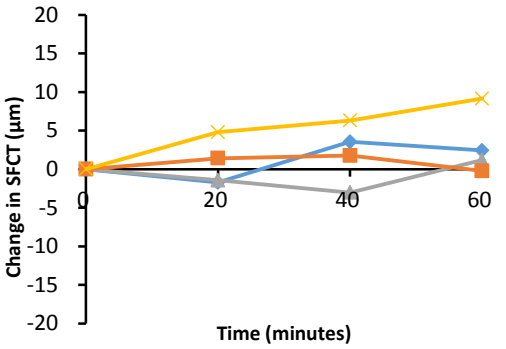



**Stage 4** (6 months after atropine)

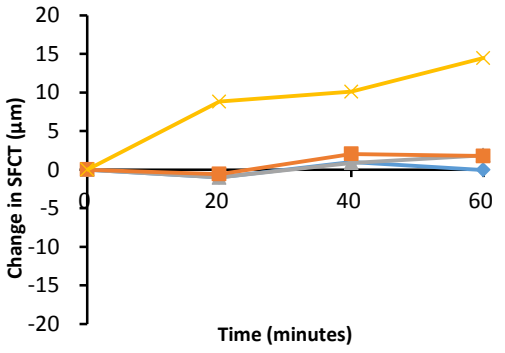
## Participant 16



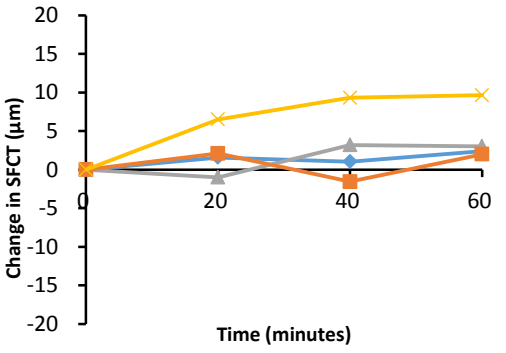

**Stage 1** (Before atropine)




**Stage 2** (1 week after atropine)

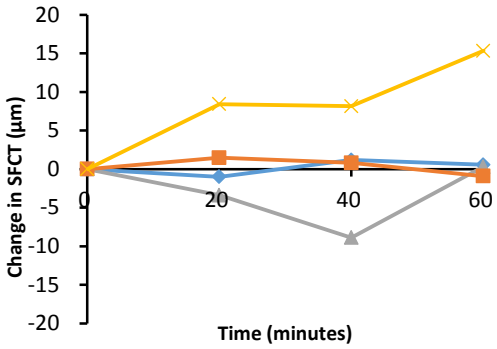



**Stage 3** (3 months after atropine)

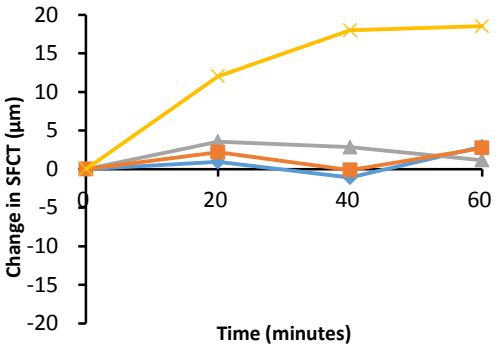



**Stage 4** (6 months after atropine)

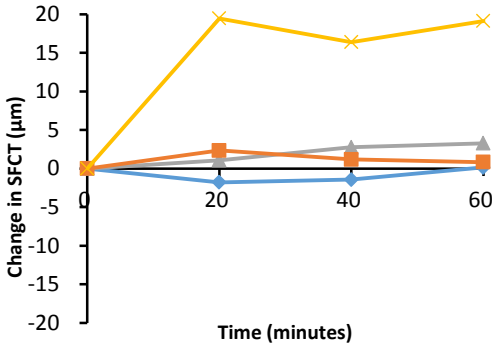
## Participant 17



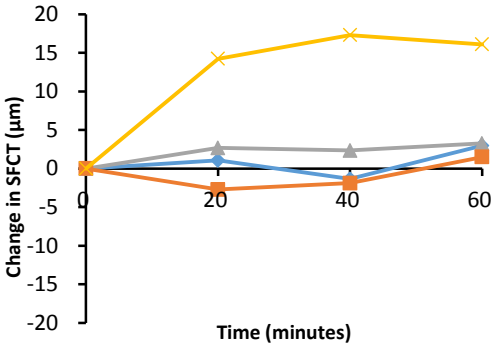

**Stage 1** (Before atropine)




**Stage 2** (1 week after atropine)

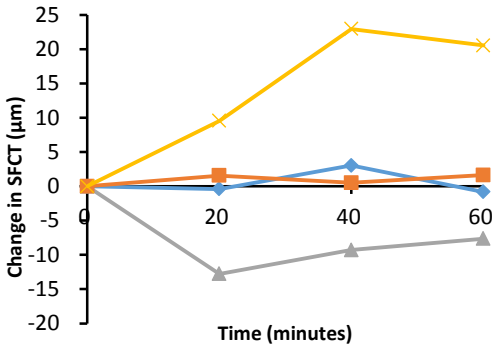



**Stage 3** (3 months after atropine)

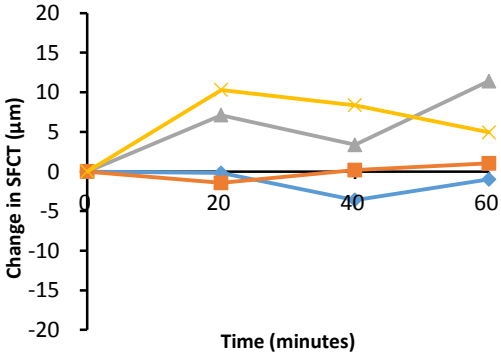



**Stage 4** (6 months after atropine)

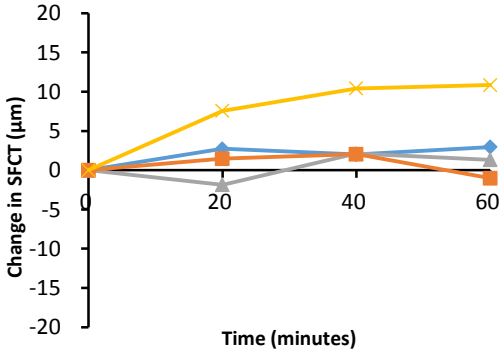
## Participant 18



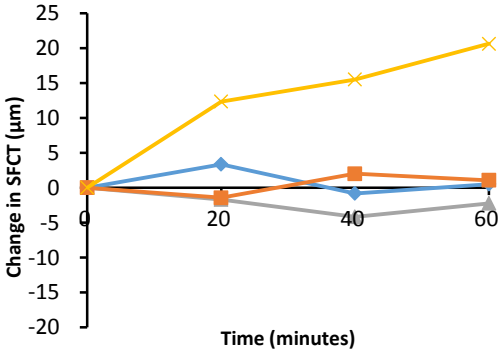

**Stage 1** (Before atropine)




**Stage 2** (1 week after atropine)

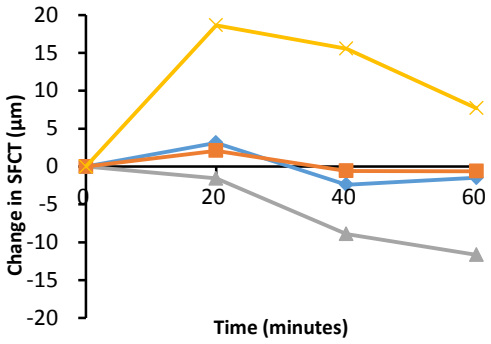



**Stage 3** (3 months after atropine)

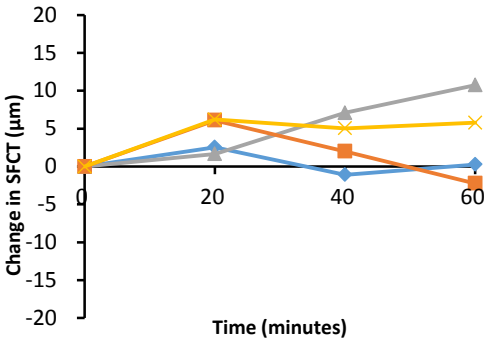



**Stage 4** (6 months after atropine)

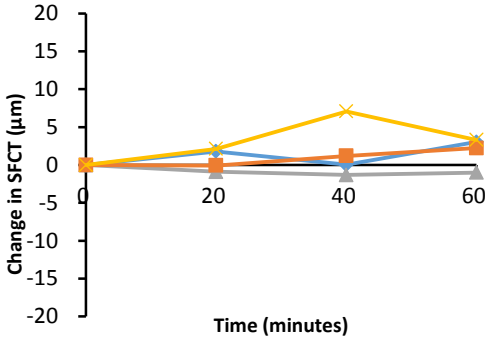
## Participant 19



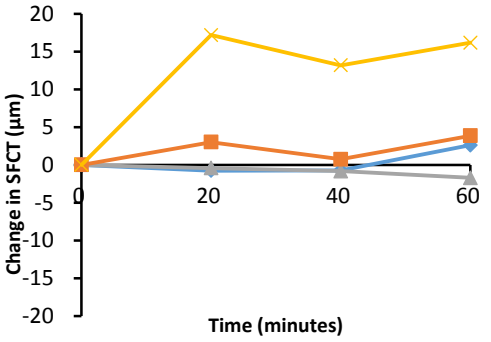

**Stage 1** (Before atropine)




**Stage 2** (1 week after atropine)

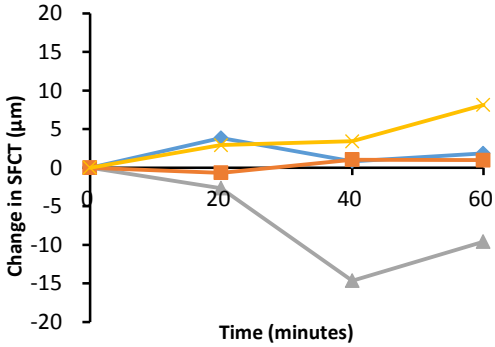



**Stage 3** (3 months after atropine)

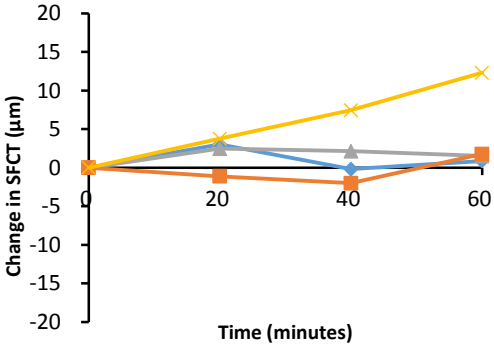



**Stage 4** (6 months after atropine)

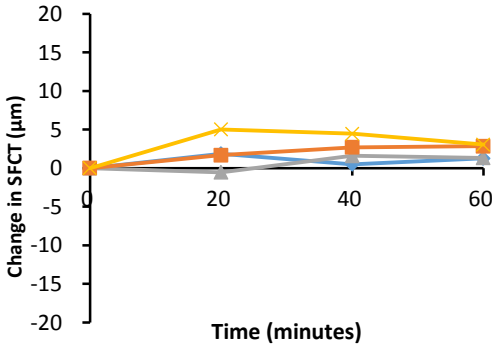
## Participant 20



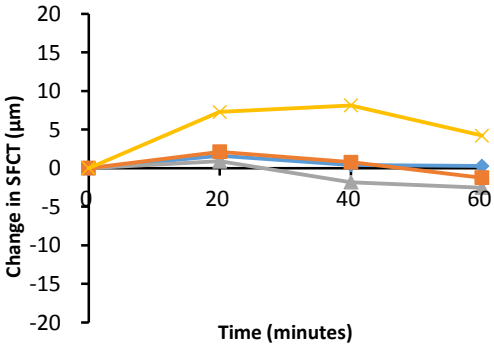

**Stage 1** (Before atropine)




**Stage 2** (1 week after atropine)




**Stage 3** (3 months after atropine)




**Stage 4** (6 months after atropine)

## 9.5 Ocular biometry raw data

### 9.5.1 Central corneal thickness (CCT)

| | Stage 1 (Before atropine) | | | Stage 3 (after 3 months on atropine) | | | Stage 4 (after 6 months on atropine) | | |
|---|---|---|---|---|---|---|---|---|---|
| | Ctrl | Exp | Mean | Ctrl | Exp | Mean | Ctrl | Exp | Mean |
| PT 1 | 557 | 556 | 557 | 550 | 561 | 556 | 556 | 559 | 558 |
| PT 2 | 537 | 525 | 531 | 536 | 530 | 533 | 534 | 527 | 531 |
| PT 3 | 560 | 562 | 561 | 568 | 562 | 565 | 566 | 565 | 566 |
| PT 4 | 547 | 544 | 546 | 543 | 542 | 543 | 539 | 537 | 538 |
| PT 5 | 542 | 545 | 544 | 541 | 532 | 537 | 539 | 539 | 539 |
| PT 6 | 606 | 609 | 608 | 612 | 620 | 616 | 612 | 626 | 619 |
| PT 7 | 533 | 537 | 535 | 532 | 541 | 537 | 537 | 543 | 540 |
| PT 8 | 570 | 572 | 571 | 565 | 569 | 567 | 563 | 565 | 564 |
| PT 9 | 562 | 557 | 560 | 568 | 561 | 565 | 574 | 557 | 566 |
| PT 10 | 561 | 559 | 560 | 560 | 560 | 560 | 566 | 572 | 569 |
| PT 11 | 566 | 557 | 562 | 557 | 556 | 557 | 560 | 560 | 560 |
| PT 12 | 543 | 532 | 538 | 541 | 537 | 539 | 540 | 535 | 538 |
| PT 13 | 513 | 512 | 513 | 516 | 520 | 518 | 518 | 521 | 520 |
| PT 14 | 552 | 559 | 556 | 555 | 564 | 560 | 563 | 571 | 567 |
| PT 15 | 549 | 562 | 556 | 550 | 560 | 555 | 549 | 560 | 555 |
| PT 16 | 547 | 548 | 548 | 541 | 544 | 543 | 543 | 549 | 546 |
| PT 17 | 589 | 587 | 588 | 598 | 593 | 596 | 593 | 593 | 593 |
| PT 18 | 568 | 566 | 567 | 569 | 576 | 573 | 564 | 570 | 567 |
| PT 19 | 520 | 518 | 519 | 522 | 523 | 523 | 528 | 525 | 527 |
| PT 20 | 519 | 522 | 521 | 522 | 524 | 523 | 522 | 521 | 522 |
| **Mean** | **552.05** | **551.45** | **551.75** | **552.30** | **553.75** | **553.03** | **553.30** | **554.75** | **554.03** |
| SD | 22.70 | 23.58 | 22.96 | 24.05 | 24.82 | 24.23 | 23.33 | 25.83 | 24.38 |

Units: Microns (μm)

Ctrl = Control Eye

Exp = Experimental Eye

Mean = (Control Eye + Experimental Eye) /2 of each participant

### 9.5.2 Anterior chamber depth (AD)

| | Stage 1 (Before atropine) | | | Stage 3 (after 3 months on atropine) | | | Stage 4 (after 6 months on atropine) | | |
|---|---|---|---|---|---|---|---|---|---|
| | Ctrl | Exp | Mean | Ctrl | Exp | Mean | Ctrl | Exp | Mean |
| PT 1 | 3.39 | 3.36 | 3.38 | 3.47 | 3.43 | 3.45 | 3.49 | 3.43 | 3.46 |
| PT 2 | 3.52 | 3.43 | 3.47 | 3.54 | 3.49 | 3.52 | 3.56 | 3.47 | 3.51 |
| PT 3 | 2.71 | 2.74 | 2.72 | 2.83 | 2.66 | 2.75 | 2.81 | 2.79 | 2.80 |
| PT 4 | 2.45 | 2.44 | 2.44 | 2.61 | 2.58 | 2.59 | 2.64 | 2.55 | 2.60 |
| PT 5 | 3.18 | 3.15 | 3.16 | 3.21 | 3.17 | 3.19 | 3.21 | 3.17 | 3.19 |
| PT 6 | 2.95 | 2.95 | 2.95 | 3.02 | 2.99 | 3.00 | 2.96 | 2.96 | 2.96 |
| PT 7 | 3.39 | 3.34 | 3.37 | 3.45 | 3.50 | 3.47 | 3.44 | 3.50 | 3.47 |
| PT 8 | 3.10 | 3.15 | 3.12 | 3.23 | 3.19 | 3.21 | 3.29 | 3.26 | 3.27 |
| PT 9 | 3.47 | 3.42 | 3.45 | 3.49 | 3.44 | 3.47 | 3.50 | 3.44 | 3.47 |
| PT 10 | 3.84 | 3.81 | 3.83 | 3.86 | 3.88 | 3.87 | 3.88 | 3.88 | 3.88 |
| PT 11 | 3.10 | 3.15 | 3.13 | 3.18 | 3.24 | 3.21 | 3.17 | 3.24 | 3.21 |
| PT 12 | 2.96 | 2.95 | 2.95 | 3.18 | 3.12 | 3.15 | 3.16 | 3.11 | 3.13 |
| PT 13 | 3.20 | 3.17 | 3.18 | 3.29 | 3.26 | 3.28 | 3.31 | 3.28 | 3.30 |
| PT 14 | 2.76 | 2.77 | 2.76 | 3.09 | 3.09 | 3.09 | 2.82 | 2.79 | 2.80 |
| PT 15 | 2.95 | 2.98 | 2.96 | 3.29 | 3.26 | 3.28 | 3.34 | 3.33 | 3.34 |
| PT 16 | 3.33 | 3.28 | 3.31 | 3.42 | 3.40 | 3.41 | 3.42 | 3.38 | 3.40 |
| PT 17 | 2.79 | 2.83 | 2.81 | 2.81 | 2.85 | 2.83 | 2.87 | 2.85 | 2.86 |
| PT 18 | 3.11 | 3.08 | 3.10 | 3.21 | 3.22 | 3.22 | 3.31 | 3.25 | 3.28 |
| PT 19 | 3.28 | 3.20 | 3.24 | 3.46 | 3.44 | 3.45 | 3.45 | 3.43 | 3.44 |
| PT 20 | 3.68 | 3.72 | 3.70 | 3.80 | 3.84 | 3.82 | 3.68 | 3.67 | 3.67 |
| **Mean** | **3.16** | **3.15** | **3.15** | **3.27** | **3.25** | **3.26** | **3.27** | **3.24** | **3.25** |
| SD | 0.34 | 0.33 | 0.34 | 0.31 | 0.33 | 0.32 | 0.32 | 0.32 | 0.32 |

Units: Millimetres (mm)

Ctrl = Control Eye

Exp = Experimental Eye

Mean = (Control Eye + Experimental Eye) /2 for each participant

### 9.5.3 Lens thickness (LT)

| | Stage 1 (Before atropine) | | | Stage 3 (after 3 months on atropine) | | | Stage 4 (after 6 months on atropine) | | |
|---|---|---|---|---|---|---|---|---|---|
| | Ctrl | Exp | Mean | Ctrl | Exp | Mean | Ctrl | Exp | Mean |
| PT 1 | 3.46 | 3.45 | 3.46 | 3.38 | 3.40 | 3.39 | 3.37 | 3.41 | 3.39 |
| PT 2 | 3.28 | 3.30 | 3.29 | 3.29 | 3.27 | 3.28 | 3.25 | 3.26 | 3.26 |
| PT 3 | 3.65 | 3.59 | 3.62 | 3.54 | 3.83 | 3.69 | 3.55 | 3.62 | 3.59 |
| PT 4 | 3.79 | M | M | 3.63 | 3.66 | 3.65 | 3.60 | 3.64 | 3.62 |
| PT 5 | 3.20 | 3.21 | 3.21 | 3.21 | 3.20 | 3.21 | 3.22 | 3.21 | 3.22 |
| PT 6 | 3.43 | 3.43 | 3.43 | 3.42 | 3.42 | 3.42 | 3.44 | 3.43 | 3.44 |
| PT 7 | 3.24 | 3.33 | 3.29 | 3.20 | 3.18 | 3.19 | 3.21 | 3.19 | 3.20 |
| PT 8 | 3.41 | 3.36 | 3.39 | 3.32 | 3.40 | 3.36 | 3.31 | 3.32 | 3.32 |
| PT 9 | 3.42 | 3.46 | 3.44 | 3.47 | 3.47 | 3.47 | 3.48 | 3.50 | 3.49 |
| PT 10 | 3.13 | 3.14 | 3.14 | 3.20 | 3.18 | 3.19 | 3.15 | 3.16 | 3.16 |
| PT 11 | 3.44 | 3.45 | 3.45 | 3.42 | 3.43 | 3.43 | 3.44 | 3.43 | 3.44 |
| PT 12 | 3.62 | 3.66 | 3.64 | 3.49 | 3.49 | 3.49 | 3.51 | 3.48 | 3.50 |
| PT 13 | 3.32 | 3.37 | 3.35 | 3.26 | 3.32 | 3.29 | 3.27 | 3.30 | 3.29 |
| PT 14 | 3.83 | 3.79 | 3.81 | 3.63 | 3.63 | 3.63 | 3.77 | 3.73 | 3.75 |
| PT 15 | 3.77 | 3.69 | 3.73 | 3.45 | 3.48 | 3.47 | 3.39 | 3.39 | 3.39 |
| PT 16 | 3.35 | 3.37 | 3.36 | 3.23 | 3.27 | 3.25 | 3.28 | 3.29 | 3.29 |
| PT 17 | 3.46 | 3.46 | 3.46 | 3.42 | 3.40 | 3.41 | 3.36 | 3.40 | 3.38 |
| PT 18 | 3.39 | 3.42 | 3.41 | 3.35 | 3.39 | 3.37 | M | 3.34 | M |
| PT 19 | 3.24 | 3.29 | 3.27 | 3.15 | 3.15 | 3.15 | 3.14 | 3.09 | 3.12 |
| PT 20 | 3.47 | 3.45 | 3.46 | 3.44 | 3.43 | 3.44 | 3.51 | 3.48 | 3.50 |
| **Mean** | **3.43** | **3.43** | **3.43** | **3.36** | **3.39** | **3.37** | **3.37** | **3.37** | **3.37** |
| SD | 0.19 | 0.17 | 0.18 | 0.14 | 0.17 | 0.15 | 0.16 | 0.16 | 0.16 |

Units: Millimetres (mm)

M: Missing Data in LenStar Biometry.

Children **PT4 & PT18** were excluded from analysis of LT due to missing data.

Ctrl = Control Eye

Exp = Experimental Eye

Mean = (Control Eye + Experimental Eye) /2 for each participant

### 9.5.4 Vitreous chamber depth (VCD)

| | Stage 1 (Before atropine) | | | Stage 3 (after 3 months on atropine) | | | Stage 4 (after 6 months on atropine) | | |
|---|---|---|---|---|---|---|---|---|---|
| | Ctrl | Exp | Mean | Ctrl | Exp | Mean | Ctrl | Exp | Mean |
| PT 1 | 17.18 | 17.21 | 17.20 | 17.20 | 17.21 | 17.21 | 17.27 | 17.33 | 17.30 |
| PT 2 | 16.70 | 16.43 | 16.57 | 16.65 | 16.41 | 16.53 | 16.81 | 16.47 | 16.64 |
| PT 3 | 16.03 | 15.85 | 15.94 | 16.03 | 15.81 | 15.92 | 16.18 | 15.94 | 16.06 |
| PT 4 | 15.25 | M | M | 15.36 | 15.29 | 15.33 | 15.41 | 15.37 | 15.39 |
| PT 5 | 18.01 | 17.78 | 17.90 | 17.96 | 17.75 | 17.86 | 17.96 | 17.79 | 17.88 |
| PT 6 | 18.34 | 18.32 | 18.33 | 18.31 | 18.30 | 18.31 | 18.37 | 18.33 | 18.35 |
| PT 7 | 16.51 | 17.73 | 17.12 | 16.57 | 17.82 | 17.20 | 16.55 | 17.81 | 17.18 |
| PT 8 | 17.00 | 16.99 | 17.00 | 17.01 | 16.98 | 17.00 | 17.02 | 17.04 | 17.03 |
| PT 9 | 18.19 | 17.84 | 18.02 | 18.14 | 17.85 | 18.00 | 18.20 | 17.86 | 18.03 |
| PT 10 | 19.29 | 19.30 | 19.30 | 19.22 | 19.26 | 19.24 | 19.27 | 19.32 | 19.30 |
| PT 11 | 16.96 | 16.97 | 16.97 | 16.92 | 16.93 | 16.93 | 16.99 | 17.05 | 17.02 |
| PT 12 | 16.96 | 16.84 | 16.90 | 16.83 | 16.71 | 16.77 | 16.96 | 16.95 | 16.96 |
| PT 13 | 16.67 | 16.66 | 16.67 | 16.55 | 16.60 | 16.58 | 16.73 | 16.70 | 16.72 |
| PT 14 | 14.14 | 14.34 | 14.24 | 14.02 | 14.15 | 14.09 | 14.23 | 14.44 | 14.34 |
| PT 15 | 16.96 | 16.87 | 16.92 | 16.97 | 16.83 | 16.90 | 16.99 | 16.84 | 16.92 |
| PT 16 | 17.82 | 17.79 | 17.81 | 17.83 | 17.70 | 17.77 | 17.77 | 17.69 | 17.73 |
| PT 17 | 17.11 | 17.61 | 17.36 | 17.11 | 17.59 | 17.35 | 17.12 | 17.65 | 17.39 |
| PT 18 | 16.61 | 16.83 | 17.72 | 16.75 | 16.53 | 16.64 | M | 16.62 | M |
| PT 19 | 17.01 | 16.96 | 16.99 | 16.94 | 16.85 | 16.90 | 16.95 | 16.96 | 16.96 |
| PT 20 | 16.38 | 16.51 | 16.45 | 16.10 | 16.25 | 16.18 | 16.36 | 16.52 | 16.44 |
| **Mean** | **17.07** | **17.11** | **17.09** | **17.02** | **17.06** | **17.04** | **17.10** | **17.15** | **17.12** |
| SD | 1.09 | 1.05 | 1.05 | 1.11 | 1.09 | 1.09 | 1.06 | 1.03 | 1.03 |

Units: Millimetres (mm)

M: Missing Data in LenStar Biometry.

Children **PT4 & PT18** were excluded from analysis of VCD due to missing data.

Ctrl = Control Eye

Exp = Experimental Eye

Mean = (Control Eye + Experimental Eye) /2 for each participant

### 9.5.5 Axial length (AL)

| | Stage 1 (Before atropine) | | | Stage 3 (after 3 months on atropine) | | | Stage 4 (after 6 months on atropine) | | |
|---|---|---|---|---|---|---|---|---|---|
| | Ctrl | Exp | Mean | Ctrl | Exp | Mean | Ctrl | Exp | Mean |
| PT 1 | 24.59 | 24.58 | 24.59 | 24.60 | 24.60 | 24.60 | 24.69 | 24.73 | 24.71 |
| PT 2 | 24.04 | 23.68 | 23.86 | 24.02 | 23.70 | 23.86 | 24.15 | 23.73 | 23.94 |
| PT 3 | 22.95 | 22.74 | 22.85 | 22.97 | 22.86 | 22.92 | 23.11 | 22.91 | 23.01 |
| PT 4 | 22.04 | 22.03 | 22.04 | 22.14 | 22.07 | 22.11 | 22.19 | 22.14 | 22.17 |
| PT 5 | 24.93 | 24.68 | 24.81 | 24.92 | 24.65 | 24.79 | 24.93 | 24.71 | 24.82 |
| PT 6 | 25.33 | 25.31 | 25.32 | 25.36 | 25.33 | 25.35 | 25.38 | 25.35 | 25.37 |
| PT 7 | 23.67 | 24.94 | 24.31 | 23.75 | 25.04 | 24.40 | 23.74 | 25.04 | 24.39 |
| PT 8 | 24.08 | 24.07 | 24.08 | 24.12 | 24.14 | 24.13 | 24.18 | 24.18 | 24.18 |
| PT 9 | 25.64 | 25.28 | 25.46 | 25.67 | 25.32 | 25.50 | 25.75 | 25.36 | 25.56 |
| PT 10 | 26.82 | 26.81 | 26.82 | 26.84 | 26.88 | 26.86 | 26.87 | 26.93 | 26.90 |
| PT 11 | 24.07 | 24.13 | 24.10 | 24.08 | 24.16 | 24.12 | 24.16 | 24.28 | 24.22 |
| PT 12 | 24.08 | 23.98 | 24.03 | 24.04 | 23.86 | 23.95 | 24.17 | 24.07 | 24.12 |
| PT 13 | 23.70 | 23.71 | 23.71 | 23.62 | 23.70 | 23.66 | 23.83 | 23.80 | 23.82 |
| PT 14 | 21.28 | 21.46 | 21.37 | 21.29 | 21.43 | 21.36 | 21.38 | 21.53 | 21.46 |
| PT 15 | 24.23 | 24.10 | 24.17 | 24.26 | 24.13 | 24.20 | 24.27 | 24.12 | 24.20 |
| PT 16 | 25.05 | 24.99 | 25.02 | 25.02 | 24.91 | 24.97 | 25.01 | 24.91 | 24.96 |
| PT 17 | 23.95 | 24.49 | 24.22 | 23.94 | 24.43 | 24.19 | 23.94 | 24.49 | 24.22 |
| PT 18 | 23.90 | 23.68 | 23.79 | 23.88 | 23.72 | 23.80 | 24.01 | 23.78 | 23.90 |
| PT 19 | 24.05 | 23.97 | 24.01 | 24.07 | 23.96 | 24.02 | 24.07 | 24.00 | 24.04 |
| PT 20 | 24.05 | 24.20 | 24.13 | 23.86 | 24.04 | 23.95 | 24.07 | 24.19 | 24.13 |
| **Mean** | **24.12** | **24.14** | **24.13** | **24.12** | **24.15** | **24.13** | **24.20** | **24.21** | **24.20** |
| SD | 1.19 | 1.17 | 1.17 | 1.19 | 1.17 | 1.17 | 1.17 | 1.16 | 1.15 |

Units: Millimetres (mm)

Ctrl = Control Eye

Exp = Experimental Eye

Mean = (Control Eye + Experimental Eye) /2 for each participant

### 9.5.6 Summary table of secondary outcome measures

| | | | Refraction (D) | | | | AoA (D) | | | | Pupil size (mm) | | | | CCT (µm) | | | | AD (mm) | | | | LT (mm) | | | | VCD (mm) | | | | AL (mm) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | 0 | 1 | 3 | 6 | 0 | 1 | 3 | 6 | 0 | 1 | 3 | 6 | 0 | 3 | 6 | | 0 | 3 | 6 | | 0 | 3 | 6 | | 0 | 3 | 6 | | 0 | 3 | 6 |
| Raw data | Control Eye | Mean | -1.64 | -1.41 | -1.49 | -1.64 | 17.55 | 1.33 | 1.34 | 1.37 | 4.08 | 7.43 | 7.58 | 7.58 | 552.05 | 552.3 | 553.3 | | 3.16 | 3.27 | 3.27 | | 3.43 | 3.36 | 3.37 | | 17.07 | 17.02 | 17.1 | | 24.12 | 24.12 | 24.2 |
| | | SD | 0.95 | 0.95 | 0.97 | 0.95 | 1.47 | 0.16 | 0.21 | 0.23 | 0.54 | 0.41 | 0.47 | 0.44 | 22.7 | 24.05 | 23.33 | | 0.34 | 0.31 | 0.32 | | 0.19 | 0.14 | 0.16 | | 1.09 | 1.11 | 1.06 | | 1.19 | 1.19 | 1.17 |
| | | P | | <0.001 | 0.005 | 1 | | <0.001 | <0.001 | <0.001 | | <0.001 | <0.001 | <0.001 | | 1 | 1 | | | <0.001 | <0.001 | | | 0.021 | 0.056 | | | 0.043 | 0.214 | | | 1 | <0.001 |
| | Experimental Eye | Mean | -1.61 | -1.44 | -1.48 | -1.62 | 17.5 | 1.39 | 1.38 | 1.34 | 4.08 | 7.45 | 7.48 | 7.53 | 551.45 | 553.75 | 554.75 | | 3.15 | 3.25 | 3.24 | | 3.43 | 3.39 | 3.37 | | 17.11 | 17.06 | 17.15 | | 24.14 | 24.15 | 24.21 |
| | | SD | 0.9 | 0.89 | 0.93 | 0.93 | 1.47 | 0.27 | 0.22 | 0.31 | 0.47 | 0.46 | 0.47 | 0.38 | 23.58 | 24.82 | 25.83 | | 0.33 | 0.33 | 0.32 | | 0.17 | 0.17 | 0.16 | | 1.05 | 1.09 | 1.03 | | 1.17 | 1.17 | 1.16 |
| | | P | | <0.001 | 0.018 | 1 | | <0.001 | <0.001 | <0.001 | | <0.001 | <0.001 | <0.001 | | 0.235 | 0.099 | | | <0.001 | <0.001 | | | 0.209 | 0.032 | | | 0.025 | 0.025 | | | 1 | <0.001 |
| | Mean | Mean | -1.63 | -1.43 | -1.48 | -1.63 | 17.53 | 1.37 | 1.36 | 1.36 | 4.08 | 7.44 | 7.53 | 7.55 | 551.75 | 553.03 | 554.03 | | 3.15 | 3.26 | 3.25 | | 3.43 | 3.37 | 3.37 | | 17.09 | 17.04 | 17.12 | | 24.13 | 24.13 | 24.2 |
| | | SD | 0.9 | 0.9 | 0.92 | 0.9 | 1.3 | 0.2 | 0.2 | 0.23 | 0.47 | 0.38 | 0.37 | 0.34 | 22.96 | 24.23 | 24.38 | | 0.34 | 0.32 | 0.32 | | 0.18 | 0.15 | 0.16 | | 1.05 | 1.09 | 1.03 | | 1.17 | 1.17 | 1.15 |
| | | P | | <0.001 | 0.002 | 1 | | <0.001 | <0.001 | <0.001 | | <0.001 | <0.001 | <0.001 | | 0.661 | 0.288 | | | <0.001 | <0.001 | | | 0.029 | 0.036 | | | 0.026 | 0.057 | | | 1 | <0.001 |
| Difference | Control Eye | Mean | 0 | 0.23 | 0.15 | 0 | 0 | -16.22 | -16.21 | -16.18 | 0 | 3.35 | 3.50 | 3.50 | 0 | 0.25 | 1.25 | | 0 | 0.11 | 0.11 | | 0 | -0.07 | -0.06 | | 0 | -0.05 | 0.03 | | 0 | 0 | 0.07 |
| | | SD | 0 | 0.11 | 0.17 | 0.22 | 0 | 1.44 | 1.41 | 1.37 | 0 | 0.71 | 0.65 | 0.71 | 0 | 4.94 | 5.94 | | 0 | 0.09 | 0.09 | | 0 | 0.09 | 0.1 | | 0 | 0.08 | 0.06 | | 0 | 0.06 | 0.05 |
| | | P | | <0.001 | 0.005 | 1 | | <0.001 | <0.001 | <0.001 | | <0.001 | <0.001 | <0.001 | | 1 | 1 | | | <0.001 | <0.001 | | | 0.021 | 0.056 | | | 0.043 | 0.214 | | | 1 | <0.001 |
| | Experimental Eye | Mean | 0 | 0.18 | 0.14 | -0.01 | 0 | -16.11 | -16.12 | -16.16 | 0 | 3.38 | 3.40 | 3.45 | 0 | 2.3 | 3.3 | | 0 | 0.11 | 0.09 | | 0 | -0.05 | -0.06 | | 0 | -0.06 | 0.04 | | 0 | 0.01 | 0.07 |
| | | SD | 0 | 0.12 | 0.18 | 0.26 | 0 | 1.45 | 1.39 | 1.44 | 0 | 0.67 | 0.6 | 0.6 | 0 | 4.94 | 6.42 | | 0 | 0.10 | 0.09 | | 0 | 0.1 | 0.09 | | 0 | 0.08 | 0.05 | | 0 | 0.08 | 0.09 |
| | | P | | <0.001 | 0.018 | 1 | | <0.001 | <0.001 | <0.001 | | <0.001 | <0.001 | <0.001 | | 0.235 | 0.099 | | | <0.001 | 0.001 | | | 0.209 | 0.032 | | | 0.025 | 0.025 | | | 1 | <0.001 |
| | Mean | Mean | 0 | 0.2 | 0.14 | 0 | 0 | -16.16 | -16.17 | -16.16 | 0 | 3.36 | 3.45 | 3.48 | 0 | 1.28 | 2.28 | | 0 | 0.11 | 0.10 | | 0 | -0.06 | -0.06 | | 0 | -0.05 | 0.03 | | 0 | 0 | 0.07 |
| | | SD | 0 | 0.09 | 0.15 | 0.21 | 0 | 1.28 | 1.23 | 1.22 | 0 | 0.64 | 0.54 | 0.63 | 0 | 4.39 | 5.56 | | 0 | 0.09 | 0.09 | | 0 | 0.08 | 0.13 | | 0 | 0.08 | 0.05 | | 0 | 0.06 | 0.06 |
| | | P | | <0.001 | 0.002 | 1 | | <0.001 | <0.001 | <0.001 | | <0.001 | <0.001 | <0.001 | | 0.661 | 0.288 | | | <0.001 | <0.001 | | | 0.029 | 0.036 | | | 0.026 | 0.057 | | | 1 | <0.001 |

NB: p-values were calculated with repeated measure ANOVA with Bonferroni corrections

## 9.6 Key statistical outputs

### 9.6.1 SPSS Repeated measure ANOVA (GLM) – Within stage

Hyperopic Defocus (Before Atropine)

| n=20 | △Con0 | △Con20 | △Con40 | △Con60 |
|---|---|---|---|---|
| Mean±SD | 0.00±0.00 | 0.76±3.39 | 0.49±3.58 | 1.14±4.07 |
| Mean±SEM | 0.00±0.00 | 0.76±0.76 | 0.49±0.80 | 1.14±0.91 |
| △Con0 | - | p=1.000 | p=1.000 | p=1.000 |
| △Con20 | p=1.000 | - | p=1.000 | p=1.000 |
| △Con40 | p=1.000 | p=1.000 | - | p=1.000 |
| △Con60 | p=1.000 | p=1.000 | p=1.000 | - |

| n=20 | △Trl0 | △Trl20 | △Trl40 | △Trl60 |
|---|---|---|---|---|
| Mean±SD | 0.00±0.00 | (7.46)±4.36 | (11.24)±6.32 | (11.89)±7.81 |
| Mean±SEM | 0.00±0.00 | (7.46)±0.97 | (11.24)±1.41 | (11.89)±1.75 |
| △Trl0 | - | p<0.0001 | p<0.0001 | p<0.0001 |
| △Trl20 | p<0.0001 | - | p=0.078 | p=0.103 |
| △Trl40 | p<0.0001 | p=0.078 | - | p=1.000 |
| △Trl60 | p<0.0001 | p=0.103 | p=1.000 | - |

Myopic Defocus (Before Atropine)

| n=20 | △Con0 | △Con20 | △Con40 | △Con60 |
|---|---|---|---|---|
| Mean±SD | 0.00±0.00 | 1.25±2.44 | 0.48±2.90 | 1.00±3.97 |
| Mean±SEM | 0.00±0.00 | 1.25±0.54 | 0.48±0.65 | 1.00±0.89 |
| △Con0 | - | p=0.197 | p=1.000 | p=1.000 |
| △Con20 | p=0.197 | - | p=1.000 | p=1.000 |
| △Con40 | p=1.000 | p=1.000 | - | p=1.000 |
| △Con60 | p=1.000 | p=1.000 | p=1.000 | - |

| n=20 | △Trl0 | △Trl20 | △Trl40 | △Trl60 |
|---|---|---|---|---|
| Mean±SD | 0.00±0.00 | 7.06±5.87 | 12.06±6.75 | 11.92±6.96 |
| Mean±SEM | 0.00±0.00 | 7.06±1.31 | 12.06±1.51 | 11.92±1.56 |
| △Trl0 | - | p<0.0001 | p<0.0001 | p<0.0001 |
| △Trl20 | p<0.0001 | - | p=0.030 | p=0.069 |
| △Trl40 | p<0.0001 | p=0.030 | - | p=1.000 |
| △Trl60 | p<0.0001 | p=0.069 | p=1.000 | - |

Hyperopic Defocus (After Atropine 1wk)

| n=20 | △Con0 | △Con20 | △Con40 | △Con60 |
|---|---|---|---|---|
| Mean±SD | 0.00±0.00 | (0.04)±1.87 | (0.10)±2.65 | 1.33±2.57 |
| Mean±SEM | 0.00±0.00 | (0.04)±0.42 | (0.10)±0.59 | 1.33±0.58 |
| △Con0 | - | p=1.000 | p=1.000 | p=0.196 |
| △Con20 | p=1.000 | - | p=1.000 | p=0.490 |
| △Con40 | p=1.000 | p=1.000 | - | p=0.172 |
| △Con60 | p=0.196 | p=0.490 | p=0.172 | - |

| n=20 | △Trl0 | △Trl20 | △Trl40 | △Trl60 |
|---|---|---|---|---|
| Mean±SD | 0.00±0.00 | 0.13±2.62 | 0.11±3.13 | 2.02±4.68 |
| Mean±SEM | 0.00±0.00 | 0.13±0.59 | 0.11±0.70 | 2.02±1.05 |
| △Trl0 | - | p=1.000 | p=1.000 | p=0.410 |
| △Trl20 | p=1.000 | - | p=1.000 | p=0.674 |
| △Trl40 | p=1.000 | p=1.000 | - | p=0.317 |
| △Trl60 | p=0.410 | p=0.674 | p=0.317 | - |

Myopic Defocus (After Atropine 1wk)

| n=20 | △Con0 | △Con20 | △Con40 | △Con60 |
|---|---|---|---|---|
| Mean±SD | 0.00±0.00 | 0.48±2.67 | 1.20±2.05 | 0.89±3.46 |
| Mean±SEM | 0.00±0.00 | 0.48±0.60 | 1.20±0.46 | 0.89±0.77 |
| △Con0 | - | p=1.000 | p=0.102 | p=1.000 |
| △Con20 | p=1.000 | - | p=1.000 | p=1.000 |
| △Con40 | p=0.102 | p=1.000 | - | p=1.000 |
| △Con60 | p=1.000 | p=1.000 | p=1.000 | - |

| n=20 | △Trl0 | △Trl20 | △Trl40 | △Trl60 |
|---|---|---|---|---|
| Mean±SD | 0.00±0.00 | 7.58±5.50 | 11.18±7.42 | 13.06±6.20 |
| Mean±SEM | 0.00±0.00 | 7.58±1.23 | 11.18±1.66 | 13.06±1.39 |
| △Trl0 | - | p<0.0001 | p<0.0001 | p<0.0001 |
| △Trl20 | p<0.0001 | - | p=0.110 | p=0.009 |
| △Trl40 | p<0.0001 | p=0.110 | - | p=0.833 |
| △Trl60 | p<0.0001 | p=0.009 | p=0.833 | - |

Hyperopic Defocus (After Atropine 3m)

| n=20 | △Con0 | △Con20 | △Con40 | △Con60 |
|---|---|---|---|---|
| Mean±SD | 0.00±0.00 | 0.99±1.52 | 0.78±1.72 | 1.06±1.61 |
| Mean±SEM | 0.00±0.00 | 0.99±0.34 | 0.78±0.38 | 1.06±0.36 |
| △Con0 | - | p=0.056 | p=0.348 | p=0.055 |
| △Con20 | p=0.056 | - | p=1.000 | p=1.000 |
| △Con40 | p=0.348 | p=1.000 | - | p=1.000 |
| △Con60 | p=0.055 | p=1.000 | p=1.000 | - |

| n=20 | △Trl0 | △Trl20 | △Trl40 | △Trl60 |
|---|---|---|---|---|
| Mean±SD | 0.00±0.00 | 0.67±2.13 | 0.75±2.13 | 0.99±2.19 |
| Mean±SEM | 0.00±0.00 | 0.67±0.48 | 0.75±0.48 | 0.99±0.49 |
| △Trl0 | - | p=1.000 | p=0.790 | p=0.342 |
| △Trl20 | p=1.000 | - | p=1.000 | p=1.000 |
| △Trl40 | p=0.790 | p=1.000 | - | p=1.000 |
| △Trl60 | p=0.342 | p=1.000 | p=1.000 | - |

Myopic Defocus (After Atropine 3m)

| n=20 | △Con0 | △Con20 | △Con40 | △Con60 |
|---|---|---|---|---|
| Mean±SD | 0.00±0.00 | 0.48±1.67 | 1.08±2.06 | 0.41±1.81 |
| Mean±SEM | 0.00±0.00 | 0.48±0.37 | 1.08±0.46 | 0.41±0.41 |
| △Con0 | - | p=1.000 | p=0.181 | p=1.000 |
| △Con20 | p=1.000 | - | p=1.000 | p=1.000 |
| △Con40 | p=0.181 | p=1.000 | - | p=0.260 |
| △Con60 | p=1.000 | p=1.000 | p=0.260 | - |

| n=20 | △Trl0 | △Trl20 | △Trl40 | △Trl60 |
|---|---|---|---|---|
| Mean±SD | 0.00±0.00 | 6.21±5.03 | 9.60±4.15 | 10.16±5.80 |
| Mean±SEM | 0.00±0.00 | 6.21±1.13 | 9.60±0.93 | 10.16±1.30 |
| △Trl0 | - | p<0.0001 | p<0.0001 | p<0.0001 |
| △Trl20 | p<0.0001 | - | p=0.008 | p=0.010 |
| △Trl40 | p<0.0001 | p=0.008 | - | p=1.000 |
| △Trl60 | p<0.0001 | p=0.010 | p=1.000 | - |

Hyperopic Defocus (After Atropine 6m)

| n=20 | △Con0 | △Con20 | △Con40 | △Con60 |
|---|---|---|---|---|
| Mean±SD | 0.00±0.00 | 0.26±1.45 | 0.40±1.81 | 1.24±2.02 |
| Mean±SEM | 0.00±0.00 | 0.26±0.32 | 0.40±0.40 | 1.24±0.45 |
| △Con0 | - | p=1.000 | p=1.000 | p=0.075 |
| △Con20 | p=1.000 | - | p=1.000 | p=0.307 |
| △Con40 | p=1.000 | p=1.000 | - | p=0.396 |
| △Con60 | p=0.075 | p=0.307 | p=0.396 | - |

| n=20 | △Trl0 | △Trl20 | △Trl40 | △Trl60 |
|---|---|---|---|---|
| Mean±SD | 0.00±0.00 | (0.43)±1.42 | 0.40±2.48 | 0.80±2.16 |
| Mean±SEM | 0.00±0.00 | (0.43)±0.32 | 0.40±0.56 | 0.80±0.48 |
| △Trl0 | - | p=1.000 | p=1.000 | p=0.676 |
| △Trl20 | p=1.000 | - | p=0.859 | p=0.228 |
| △Trl40 | p=1.000 | p=0.859 | - | p=1.000 |
| △Trl60 | p=0.676 | p=0.228 | p=1.000 | - |

Myopic Defocus (After Atropine 6m)

| n=20 | △Con0 | △Con20 | △Con40 | △Con60 |
|---|---|---|---|---|
| Mean±SD | 0.00±0.00 | 0.32±1.89 | 0.23±1.42 | 0.97±1.92 |
| Mean±SEM | 0.00±0.00 | 0.32±0.42 | 0.23±0.32 | 0.97±0.43 |
| △Con0 | - | p=1.000 | p=1.000 | p=0.212 |
| △Con20 | p=1.000 | - | p=1.000 | p=1.000 |
| △Con40 | p=1.000 | p=1.000 | - | p=1.000 |
| △Con60 | p=0.212 | p=1.000 | p=1.000 | - |

| n=20 | △Trl0 | △Trl20 | △Trl40 | △Trl60 |
|---|---|---|---|---|
| Mean±SD | 0.00±0.00 | 7.28±5.61 | 10.79±4.40 | 11.75±5.72 |
| Mean±SEM | 0.00±0.00 | 7.28±1.25 | 10.79±0.98 | 11.75±1.28 |
| △Trl0 | - | p<0.0001 | p<0.0001 | p<0.0001 |
| △Trl20 | p<0.0001 | - | p=0.059 | p=0.005 |
| △Trl40 | p<0.0001 | p=0.059 | - | p=1.000 |
| △Trl60 | p<0.0001 | p=0.005 | p=1.000 | - |

Note: in these SPSS output tables, data from the Experimental eye is denoted Trl (Trial Eye). P-values were calculated with repeated measure ANOVA with Bonferroni corrections.

### 9.6.2 SPSS Repeated measure ANOVA (GLM) – between stages

Hyperopic Defocus

| △Con20 | Before | After | After 3m | After 6m |
|---|---|---|---|---|
| Mean±SD | 0.76±3.39 | (0.04)±1.87 | 0.99±1.52 | 0.26±1.45 |
| Mean±SEM | 0.76±0.76 | (0.04)±0.42 | 0.99±0.34 | 0.26±0.32 |
| Before | - | p=1.000 | p=1.000 | p=1.000 |
| After | p=1.000 | - | p=0.478 | p=1.000 |
| After 3m | p=1.000 | p=0.478 | - | p=0.726 |
| After 6m | p=1.000 | p=1.000 | p=0.726 | - |

| △Trl20 | Before | After | After 3m | After 6m |
|---|---|---|---|---|
| Mean±SD | (7.46)±4.36 | 0.13±2.62 | 0.67±2.13 | (0.43)±1.42 |
| Mean±SEM | (7.46)±0.97 | 0.13±0.59 | 0.67±0.48 | (0.43)±0.32 |
| Before | - | p<0.0001 | p<0.0001 | p<0.0001 |
| After | p<0.0001 | - | p=1.000 | p=1.000 |
| After 3m | p<0.0001 | p=1.000 | - | p=0.585 |
| After 6m | p<0.0001 | p=1.000 | p=0.585 | - |

Myopic Defocus

| △Con20 | Before | After | After 3m | After 6m |
|---|---|---|---|---|
| Mean±SD | 1.25±2.44 | 0.48±2.67 | 0.48±1.67 | 0.32±1.89 |
| Mean±SEM | 1.25±0.54 | 0.48±0.60 | 0.48±0.37 | 0.32±0.42 |
| Before | - | p=1.000 | p=1.000 | p=0.972 |
| After | p=1.000 | - | p=1.000 | p=1.000 |
| After 3m | p=1.000 | p=1.000 | - | p=1.000 |
| After 6m | p=0.972 | p=1.000 | p=1.000 | - |

| △Trl20 | Before | After | After 3m | After 6m |
|---|---|---|---|---|
| Mean±SD | 7.06±5.87 | 7.58±5.50 | 6.21±5.03 | 7.28±5.61 |
| Mean±SEM | 7.06±1.31 | 7.58±1.23 | 6.21±1.13 | 7.28±1.25 |
| Before | - | p=1.000 | p=1.000 | p=1.000 |
| After | p=1.000 | - | p=1.000 | p=1.000 |
| After 3m | p=1.000 | p=1.000 | - | p=1.000 |
| After 6m | p=1.000 | p=1.000 | p=1.000 | - |

Hyperopic Defocus

| △Con40 | Before | After | After 3m | After 6m |
|---|---|---|---|---|
| Mean±SD | 0.49±3.58 | (0.10)±2.65 | 0.78±1.72 | 0.40±1.81 |
| Mean±SEM | 0.49±0.80 | (0.10)±0.59 | 0.78±0.38 | 0.40±0.40 |
| Before | - | p=1.000 | p=1.000 | p=1.000 |
| After | p=1.000 | - | p=1.000 | p=1.000 |
| After 3m | p=1.000 | p=1.000 | - | p=1.000 |
| After 6m | p=1.000 | p=1.000 | p=1.000 | - |

| △Trl40 | Before | After | After 3m | After 6m |
|---|---|---|---|---|
| Mean±SD | (11.24)±6.32 | 0.11±3.13 | 0.75±2.13 | 0.40±2.48 |
| Mean±SEM | (11.24)±1.41 | 0.11±0.70 | 0.75±0.48 | 0.40±0.56 |
| Before | - | p<0.0001 | p<0.0001 | p<0.0001 |
| After | p<0.0001 | - | p=1.000 | p=1.000 |
| After 3m | p<0.0001 | p=1.000 | - | p=1.000 |
| After 6m | p<0.0001 | p=1.000 | p=1.000 | - |

Myopic Defocus

| △Con40 | Before | After | After 3m | After 6m |
|---|---|---|---|---|
| Mean±SD | 0.48±2.90 | 1.20±2.05 | 1.08±2.06 | 0.23±1.42 |
| Mean±SEM | 0.48±0.65 | 1.20±0.46 | 1.08±0.46 | 0.23±0.32 |
| Before | - | p=1.000 | p=1.000 | p=1.000 |
| After | p=1.000 | - | p=1.000 | p=0.344 |
| After 3m | p=1.000 | p=1.000 | - | p=1.000 |
| After 6m | p=1.000 | p=0.344 | p=1.000 | - |

| △Trl40 | Before | After | After 3m | After 6m |
|---|---|---|---|---|
| Mean±SD | 12.06±6.75 | 11.18±7.42 | 9.60±4.15 | 10.79±4.40 |
| Mean±SEM | 12.06±1.51 | 11.18±1.66 | 9.60±0.93 | 10.79±0.98 |
| Before | - | p=1.000 | p=1.000 | p=1.000 |
| After | p=1.000 | - | p=1.000 | p=1.000 |
| After 3m | p=1.000 | p=1.000 | - | p=1.000 |
| After 6m | p=1.000 | p=1.000 | p=1.000 | - |

Hyperopic Defocus

| △Con60 | Before | After | After 3m | After 6m |
|---|---|---|---|---|
| Mean±SD | 1.14±4.07 | 1.33±2.57 | 1.06±1.61 | 1.24±2.02 |
| Mean±SEM | 1.14±0.91 | 1.33±0.58 | 1.06±0.36 | 1.24±0.45 |
| Before | - | p=1.000 | p=1.000 | p=1.000 |
| After | p=1.000 | - | p=1.000 | p=1.000 |
| After 3m | p=1.000 | p=1.000 | - | p=1.000 |
| After 6m | p=1.000 | p=1.000 | p=1.000 | - |

| △Trl60 | Before | After | After 3m | After 6m |
|---|---|---|---|---|
| Mean±SD | (11.89)±7.81 | 2.02±4.68 | 0.99±2.19 | 0.80±2.16 |
| Mean±SEM | (11.89)±1.75 | 2.02±1.05 | 0.99±0.49 | 0.80±0.48 |
| Before | - | p<0.0001 | p<0.0001 | p<0.0001 |
| After | p<0.0001 | - | p=1.000 | p=1.000 |
| After 3m | p<0.0001 | p=1.000 | - | p=1.000 |
| After 6m | p<0.0001 | p=1.000 | p=1.000 | - |

Myopic Defocus

| △Con60 | Before | After | After 3m | After 6m |
|---|---|---|---|---|
| Mean±SD | 1.00±3.97 | 0.89±3.46 | 0.41±1.81 | 0.97±1.92 |
| Mean±SEM | 1.00±0.89 | 0.89±0.77 | 0.41±0.41 | 0.97±0.43 |
| Before | - | p=1.000 | p=1.000 | p=1.000 |
| After | p=1.000 | - | p=1.000 | p=1.000 |
| After 3m | p=1.000 | p=1.000 | - | p=1.000 |
| After 6m | p=1.000 | p=1.000 | p=1.000 | - |

| △Trl60 | Before | After | After 3m | After 6m |
|---|---|---|---|---|
| Mean±SD | 11.92±6.96 | 13.06±6.20 | 10.16±5.80 | 11.75±5.72 |
| Mean±SEM | 11.92±1.56 | 13.06±1.39 | 10.16±1.30 | 11.75±1.28 |
| Before | - | p=1.000 | p=1.000 | p=1.000 |
| After | p=1.000 | - | p=0.535 | p=1.000 |
| After 3m | p=1.000 | p=0.535 | - | p=1.000 |
| After 6m | p=1.000 | p=1.000 | p=1.000 | - |

Note: in these SPSS output tables, data from the Experimental eye is denoted Trl (Trial Eye). P-values were calculated with repeated measure ANOVA with Bonferroni corrections.

Values of p = 1.000 appear occasionally in tables in Appendices. This is because SPSS Bonferroni post-hoc comparison adjusts the p-value (rather than the α) by multiplying it by the number of comparison groups, which in some cases can results in a p-value ≥ 1. SPSS then replaces this with p = 1.000.

(see http://imaging.mrc-cbu.cam.ac.uk/statswiki/FAQ/SpssBonferroni).

### 9.6.3 SPSS Test of Normality

**Test of Normality**

| | Kolmogorov-Smirnov | | | Shapiro-Wilk | | |
|---|---|---|---|---|---|---|
| | Statistics | df | Sig. | Statistics | df | Sig. |
| Normality | .146 | 20 | .200* | .929 | 20 | .148 |
| BefAHypC20 | .147 | 20 | .200* | .929 | 20 | .150 |
| BefAHypC40 | .142 | 20 | .200* | .932 | 20 | .172 |
| BefAHypC60 | .154 | 20 | .200* | .933 | 20 | .173 |
| BefAHypT0 | .155 | 20 | .200* | .947 | 20 | .330 |
| BefAHypT20 | .139 | 20 | .200* | .953 | 20 | .423 |
| BefAHypT40 | .133 | 20 | .200* | .954 | 20 | .425 |
| BefAHypT60 | .121 | 20 | .200* | .966 | 20 | .675 |
| BefAMyoC0 | .146 | 20 | .200* | .932 | 20 | .167 |
| BefAMyoC20 | .144 | 20 | .200* | .930 | 20 | .154 |
| BefAMyoC40 | .142 | 20 | .200* | .935 | 20 | .192 |
| BefAMyoC60 | .144 | 20 | .200* | .933 | 20 | .177 |
| BefAMyoT0 | .148 | 20 | .200* | .937 | 20 | .214 |
| BefAMyoT20 | .134 | 20 | .200* | .933 | 20 | .178 |
| BefAMyoT40 | .121 | 20 | .200* | .950 | 20 | .366 |
| BefAMyoT60 | .123 | 20 | .200* | .946 | 20 | .315 |
| AftAHypC0 | .119 | 20 | .200* | .966 | 20 | .666 |
| AftAHypC20 | .124 | 20 | .200* | .963 | 20 | .602 |
| AftAHypC40 | .104 | 20 | .200* | .971 | 20 | .767 |
| AftAHypC60 | .111 | 20 | .200* | .967 | 20 | .684 |
| AftAHypT0 | .130 | 20 | .200* | .955 | 20 | .448 |
| AftAHypT20 | .138 | 20 | .200* | .949 | 20 | .353 |
| AftAHypT40 | .150 | 20 | .200* | .953 | 20 | .412 |
| AftAHypT60 | .136 | 20 | .200* | .959 | 20 | .531 |
| AftAMyoC0 | .101 | 20 | .200* | .974 | 20 | .830 |
| AftAMyoC20 | .118 | 20 | .200* | .971 | 20 | .774 |
| AftAMyoC40 | .087 | 20 | .200* | .975 | 20 | .863 |
| AftAMyoC60 | .105 | 20 | .200* | .971 | 20 | .782 |
| AftAMyoT0 | .144 | 20 | .200* | .928 | 20 | .140 |
| AftAMyoT20 | .146 | 20 | .200* | .929 | 20 | .146 |
| AftAMyoT40 | .156 | 20 | .200* | .933 | 20 | .173 |
| AftAMyoT60 | .141 | 20 | .200* | .936 | 20 | .201 |
| AftA3HypC0 | .086 | 20 | .200* | .971 | 20 | .777 |
| AftA3HypC20 | .086 | 20 | .200* | .971 | 20 | .768 |
| AftA3HypC40 | .081 | 20 | .200* | .973 | 20 | .823 |
| AftA3HypC60 | .092 | 20 | .200* | .971 | 20 | .779 |
| AftA3HypT0 | .147 | 20 | .200* | .976 | 20 | .865 |
| AftA3HypT20 | .158 | 20 | .200* | .972 | 20 | .798 |
| AftA3HypT40 | .138 | 20 | .200* | .974 | 20 | .841 |
| AftA3HypT60 | .152 | 20 | .200* | .971 | 20 | .769 |
| AftA3MyoC0 | .090 | 20 | .200* | .968 | 20 | .707 |
| AftA3MyoC20 | .090 | 20 | .200* | .969 | 20 | .734 |
| AftA3MyoC40 | .089 | 20 | .200* | .972 | 20 | .788 |
| AftA3MyoC60 | .097 | 20 | .200* | .971 | 20 | .769 |
| AftA3MyoT0 | .143 | 20 | .200* | .974 | 20 | .836 |
| AftA3MyoT20 | .116 | 20 | .200* | .981 | 20 | .947 |
| AftA3MyoT40 | .111 | 20 | .200* | .978 | 20 | .912 |
| AftA3MyoT60 | .125 | 20 | .200* | .976 | 20 | .864 |
| AftA6HypC0 | .101 | 20 | .200* | .974 | 20 | .833 |
| AftA6HypC20 | .113 | 20 | .200* | .973 | 20 | .826 |
| AftA6HypC40 | .100 | 20 | .200* | .974 | 20 | .828 |
| AftA6HypC60 | .100 | 20 | .200* | .973 | 20 | .820 |
| AftA6HypT0 | .147 | 20 | .200* | .965 | 20 | .648 |
| AftA6HypT20 | .142 | 20 | .200* | .966 | 20 | .671 |
| AftA6HypT40 | .137 | 20 | .200* | .970 | 20 | .752 |
| AftA6HypT60 | .143 | 20 | .200* | .966 | 20 | .679 |
| AftA6MyoC0 | .101 | 20 | .200* | .978 | 20 | .909 |
| AftA6MyoC20 | .096 | 20 | .200* | .975 | 20 | .864 |
| AftA6MyoC40 | .107 | 20 | .200* | .978 | 20 | .906 |
| AftA6MyoC60 | .100 | 20 | .200* | .979 | 20 | .924 |
| AftA6MyoT0 | .134 | 20 | .200* | .969 | 20 | .726 |
| AftA6MyoT20 | .141 | 20 | .200* | .962 | 20 | .589 |
| AftA6MyoT40 | .142 | 20 | .200* | .971 | 20 | .781 |
| AftA6MyoT60 | .130 | 20 | .200* | .969 | 20 | .739 |

*. This is a lower bound of the true significance

a. Lilliefors Significance Correction