EFFECT OF ATROPINE EYE DROPS ON THE ANTERIOR AND POSTERIOR SEGMENT STRUCTURES AND FUNCTIONS

A thesis presented to the graduate faculty

of New England College of Optometry in partial fulfillment

of the requirements for the degree of Master of Science

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May, 2023

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This manuscript has been read and accepted by the
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Abstract

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Courtney Liang

New England College of Optometry, 2023

Background: Previous studies have investigated the mechanism of action of atropine in myopia management without conclusive results. The series of studies presented here aimed to understand how atropine eye drops affect a number of anterior and posterior segment structures and functions. We hypothesize that low dose atropine drops affect not only the posterior segment of the eye, but also ocular rigidity and other anterior segment structures.

Methods: We conducted two cross-sectional studies using two separate groups of young healthy adults. The first study investigated the repeatability of Icare measurements on the sclera (n = 25, age = 24.28 ± 1.74 years old). For this, we measured intraocular pressure (IOP) in the central cornea, at 4mm inferior, nasal, superior and temporal from the limbus, as well as at 8mm inferior, nasal, superior and temporal from the limbus. A week later, we repeated measurements in the same positions. We used Bland-Altman analyses, Wilcoxon signed rank tests and coefficients of repeatability analyses. The results from this pilot study helped us design the main study and determine the optimal scleral locations to measure IOP.

The main study investigated how the use of 0.05% atropine eye drops affect the following measures: accommodation (open-field autorefractor in static and dynamic modes and a clinical test, MEM), pupil sizes, glare (straylight, Oculus C-quant), subjective visual function (questionnaires), reading speed and comprehension, biometry (axial length - AXL, anterior chamber depth - ACD, lens thickness - LT, keratometry - K, and vitreous chamber depth - VCD), anterior segment OCT (cornea, anterior sclera at the same locations as the IOP measures, ciliary body and crystalline lens), corneal and scleral IOP (at 4mm inferior, 5mm nasal, 4mm superior and 5mm temporal from the limbus), and posterior segment OCT (retinal and choroidal thicknesses). All measures were taken before and after the use of

atropine eye drops during an initial visit, and one time in a second visit, after 7 days of usage of the atropine eye drops. Related-samples Friedman's two-way analysis of variance by ranks were used to assess the changes in these metrics between the three time points. Biometry, anterior segment OCT and IOP measurements were used to calculate ocular rigidity. Subjects with Emmetropia (n = 15, age = 24.65 ± 1.67 years old, RE = 0.27 ± 0.42D) or Myopia (n = 15, age = 24.96 ± 1.66 years old, RE = -1.89 ± 1.86D) were recruited.

Results: The first, pilot, study results showed that measurements of IOP were repeatable in the right eye for all locations ($p > 0.05$) except superior ($p = 0.04$). These results indicated the best locations to test IOP in the main study were 4mm inferior and superior, and 5mm nasal and temporal from the limbus.

The second, main, study resulted in many interesting results. The most novel results are that atropine use caused a significant thickening of the temporal anterior sclera only in Emmetropes ($p < 0.01$). Additionally, ocular rigidity as evaluated with Laplace's formula significantly increased temporally ($p < 0.01$) and nasally ($p = 0.01$) in all subjects. As expected, the use of atropine decreased static and dynamic ($p < 0.01$) accommodation responses, associated with a significant increase in pupil sizes for all subjects with the use of atropine ($p < 0.01$). Lastly, significant regional changes in choroidal thickness were also noted with atropine at 3mm from the fovea in Myopes and was thicker temporally ($p = 0.02$), with a trend towards thicker choroid inferiorly ($p = 0.06$) in Myopes only.

Conclusions: Although atropine has been used as a method of myopia control for many years, its mechanism of action remains unknown. Our results support the hypothesis that atropine affects not only the retina and choroid, but also anterior structures and functions of the eye. While temporal posterior choroidal thickness is, as expected, thickened with atropine use, so are anterior ocular rigidity and ciliary body structure and function. Further work is necessary to understand the contributing role of each of these in the control of myopia. Our results also show the usefulness and limitations of various clinical tests that may be used to monitor the effect of atropine eye drops in myopia control.

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Specific Aims and Hypotheses

The overall objective of my studies is to further the understanding of the mechanism of action of atropine in myopia management. To achieve this, I evaluated the effect of low dose atropine eye drops in the anterior and posterior segment structures and functions in a group of young adults with emmetropia or myopia. I took the measurements described below three times in each subject: twice during an initial (baseline) visit, before and after the use of 0.05% atropine eye drops, and once after one week of daily use of the eye drops.

The specific aims of my project are:

Aim-1: Repeatability of using Icare Tonometry to Evaluate Intraocular Pressure

Intraocular pressure (IOP) was measured at various locations within the globe with an Icare tonometer to evaluate the repeatability of these measurements (Chapter 2).

Hypothesis: Icare measurements will be repeatable, which will allow us to use these to monitor changes with the use of low dose atropine eye drops.

Aim-2: Investigate the Effect of Low Dose Atropine Eye Drops in Accommodation and Glare.

The following measurements were taken: (1) static and dynamic accommodation using a motor-controlled open field autorefractor, (2) near accommodation using MEM retinoscopy, (3) pupil sizes, (4) glare, (5) symptoms with questionnaires delivered in the lab and online each evening the subject was using the drops (Chapter 4).

Hypotheses: (1) Static and dynamic accommodation responses, measured with the open-field autorefractor or MEM, will decrease after the use of atropine short-term (pre- and post-within the initial visit), with no further decrease after using the drops for one week. (2) The decrease in accommodation responses with atropine will be greater in subjects with emmetropia. (3) Pupil sizes will increase under all conditions for all subjects with no differences between refractive groups. (4) As a consequence, glare will increase similarly in

all subjects, and (5) all subjects will become significantly more symptomatic for near blur and light sensitivity.

Aim-3: Investigate the Effect of Low Dose Atropine Eye Drops in Retinal and Choroidal Thickness.

Posterior segment images obtained with a high-resolution wide-field OCT were used to calculate retinal and choroidal thickness at different locations (Chapter 5).

Hypotheses: (1) Subfoveal and overall choroid will thicken after one week of use of atropine drops with no changes short-term, within the same visit, with a larger change in myopes (thinner choroids to start with). (2) Total and peripheral retinal thickness will not change with the use of atropine eye drops in either refractive group.

Aim-4: Investigate the Effect of Low Dose Atropine Eye Drops in Ocular Rigidity.

The following measurements were taken: (1) anterior segment high-resolution wide-field OCT (cornea, anterior sclera, ciliary body, crystalline lens), (2) ocular biometry (axial length, anterior chamber depth, lens thickness, vitreous chamber depth and keratometry), and (3) ocular pressure at the central cornea (with Icare and GAT) and various locations of the conjunctiva (with Icare). Ciliary body and scleral thickness and attenuation coefficients, as well as scleral wall stress and ocular rigidity coefficients were calculated from these measurements (Chapter 6).

Hypotheses: (1) The use of low dose atropine eye drops will increase anterior sclera thickness and as a consequence ocular rigidity, and (2) will decrease ciliary body thickness. (3) Anterior scleral and ciliary body thickness changes and therefore ocular rigidity will be larger in subjects with myopia than in subjects with emmetropia.

1. General Background

Myopia is a refractive disorder in which light entering a non-accommodating eye focuses in front of, rather than on, the retina. Myopia usually occurs because the eye elongates more than it is needed to compensate for the optical components. Myopia is considered an epidemic in certain areas of the world (Chua and YinWong 2016; Dolgin 2015; Grosvenor 2003; Holden et al. 2014; Morgan 2016; Park and Congdon 2004), and it is a major public health concern with rapidly increasing prevalence (Foster and Jiang 2014; Hrynchak et al. 2013; Pärssinen 2012; Recko and Stahl 2015; Vitale, Sperduto, and Ferris 2009; Williams et al. 2015; Wolfram et al. 2014; Xiang et al. 2013; Zhou et al. 2016). Between 1971-72 and 1999-2004, the estimated prevalence of myopia in the US in persons of ages 12 to 54 years increased from 25% to 42% (Vitale, Sperduto, and Ferris 2009). The prevalence of myopia is expected to reach 4,758 million people (49.8% of the world population; 3,620-6,056 million [95% CI, 43.4%-55.7%]) in the year 2050 (Holden, Jong, et al. 2015; Holden, Wilson, et al. 2015). The high and rapidly rising prevalence of myopia, the development of myopia earlier in life and an increasing aging of the world population is causing an increase in higher myopias, with three main effects: (1) increased risk of visual impairment and blindness (Chang et al. 2013; Holden et al. 2014; Jonas and Xu 2014; Li et al. 2014; Ohno-Matsui et al. 2016; Perera 2010; Saw 2006), (2) almost one billion people are expected to suffer from ocular complications, and visual loss related to myopia in the year 2050 (Holden et al. 2016), decreased quality of life for those affected (Rose et al. 2000), and (3) a heavy global economic burden. The World Health Organization (WHO) recognizes that myopia is a major cause of visual impairment and constitutes a significant risk for potentially blinding ocular diseases. It is expected that in the next decades, there will be increased numbers of myopic maculopathy, retinal detachment, cataracts, retinal breaks, and glaucoma events (Sankaridurg et al. 2018). A recent study conducted in Beijing revealed degenerative myopia was the most common cause of visual impairment and blindness in adults between 40-49 years and the second most common cause in adults older than 50 years (Mak et al. 2018). The WHO's recent report on the cost of correcting vision impairment (Fricke et al. 2012; Holden, Wilson et al. 2015; Mak et al. 2018) from uncorrected refractive error claims US $202 billion as the

annual estimated loss in the global gross domestic product (Fricke et al. 2012; Holden, Wilson et al. 2015).

Myopia is caused by an excessive elongation of the eye that causes a mismatch between its optical power and the location of the retina with respect to the focal point (Atchison 2006; Bueno-Gimeno et al. 2014; Gilmartin, Nagra, and Logan 2013; Jiang et al. 2013; Sergienko and Shargorogska 2012; Tekiele and Semes 2002; Tian, Tarrant, and Wildsoet 2011; Vera-Diaz et al 2010). Longer myopic eyes also exhibit weakened sclera (Bryant and McDonnell 1998; Jonas and Xu 2014; Lewis et al. 2014; Norton and Rada 1995). Although it is yet unclear whether histological and structural differences in the myopic sclera are a consequence of myopia or may be involved in its development, there is irrefutable evidence that the development of myopia is associated with changes in scleral properties (Mesquita et al. 2020), and most myopic eyes have a biomechanical weakness in the scleral shell compared to emmetropic and hyperopic eyes (Sergienko and Shargorogska 2012). Myopia is also associated with abnormal scleral constitution and remodeling, there is for example a reduction in scleral collagen fibers, generalized and localized thinning, loss of scleral tissue and scleral expansibility (He et al. 2017; Woodman-Pieterse et al. 2018). Myopia affects scleral structure, extracellular matrix, and biomechanical properties (Niyazmand et al. 2020). For these reasons, the role of intraocular pressure (IOP) in the excessive eye growth that underlies myopia development has been the subject of many investigations (Cho and Tan 2019; Jost B. Jonas et al. 2021; Remón et al. 2020), although the nature and extent of the influence of IOP remains poorly understood. It is likely that factors related to ocular rigidity and other mechanical properties of the ocular globe are important in the development of myopia (Ruiz-Pomeda and Villa-Collar 2020). The pressure exerted on the ocular wall depends on the rigidity of its outer layers. These factors may also be the cause of the controversial results found in measures of IOP in myopia (Mingguang He et al. 2015).

Myopia represents a failure of the natural process of emmetropization (Cho and Tan 2019; Flitcroft 2014; Jonas et al. 2021). Normally, the eye grows to match the changes that happen to its optics, so that it maintains a clear focus on the retina. If the eye does not have

adequate visual signals to emmetropize normally, it grows excessively, and myopia occurs (Flitcroft 2014).

In recent years, several treatments have been proposed to prevent the development of myopia and to reduce the progression of myopia, including lifestyle modifications, pharmacological approaches with low-dose atropine and Pirenzepine eye drops, and optical treatments such as multifocal contact lenses (Mak et al. 2018; Remón et al. 2020), orthokeratology (Cho and Tan 2019; Mak et al. 2018) and spectacles with various peripheral optics designs (Jonas et al. 2021; Mak et al. 2018). An example is MiSight contact lenses, the first in a group of bifocal or dual-focused contact lenses based in a concentric ring design (Ruiz-Pomeda and Villa-Collar 2020). The central zone corrects the myopic refractive error (RE) and the concentric zone of rings has plus power addition to simultaneously deliver peripheral myopic defocus (Ruiz-Pomeda and Villa-Collar 2020). Orthokeratology lenses have shown significant effects in controlling axial elongation by flattening the central cornea overnight (Mak et al. 2018). Lifestyle aspects that may affect myopia include increasing the amount of time outdoors. In a study conducted in Guangzhou, China, an additional 40 minutes of outdoor activity reduced the incidence of myopia over 3 years (He et al. 2015).

1.1. Low Dose Atropine in Myopia Management

Atropine is a non-selective broad muscarinic acetylcholine receptor antagonist that works as a reversible competitive antagonist for an affinity of all five subtypes of acetylcholine, muscarinic receptors that are found in most ocular tissues, including the cornea, iris, ciliary body, ciliary muscle, epithelium of crystalline lens, retina, retinal pigment epithelium, choroid and sclera (Upadhyay and Beuerman 2020). Atropine has also been shown to reduce epidermal growth factor receptor (EGFR) activity in mice (Upadhyay and Beuerman 2020).

Atropine has been found to suppress visually induced myopia in humans and animals. Schwahn et al (2000) studied an in vitro preparation of the retina pigment epithelium-choroid complex in chick eyes with atropine injected into the vitreous in two-day intervals.

Dopamine release from the retina following atropine injection was quantified using HPLC-EC and was found to be increased after injection; retina and vitreous dopamine concentrations were elevated after atropine injection in chicks (Schwahn et al. 2000). Atropine reduced ERG b-wave and d-waves, led to damped oscillations of RPE potentials and reversed the ERG c-wave in chicks (Schwahn et al. 2000).

Atropine has been used for myopia control for decades, first in higher doses, and more recently in low doses (typically 0.01%, 0.025%, or 0.05%) (Upadhyay and Beuerman 2020). In the first large controlled study on the use of atropine for myopia, the ATOM study, Chia et al (2016) concluded that a daily dose of 0.01% atropine is an effective first-line treatment in children aged 6 to 12 years with documented myopia progression of ≥ 0.50 D in the preceding year. Atropine was found to be more successful in the second year than the first, so it was recommended that the treatment be continued for at least 2 years (Chia, Lu, and Tan 2016). In a later study, LAMP, Yam et al (2020) indicated a possible higher efficacy of atropine 0.05% compared to 0.01% over 2 years, although the reports on this study have significant limitations. Both the ATOM and LAMP studies postulated the efficacy of low dose atropine was similar to higher doses of 0.1% and 0.5% atropine over 2 years (Chia, Lu, and Tan 2016; Yam et al. 2020). Many other studies have shown a positive effect of low doses of atropine in reducing myopia progression, although the effect varies in reducing ocular elongation (axial length, AXL, measured in mm) (Yam et al. 2020).

Many clinicians consider the potential side effects of atropine dosage for myopia control. Cyphers et al (2022) tested subjects on 0.01% atropine on changes to vision, accommodation, reading speed or subjective side effects which were noted to be not statically significant (Cyphers, Huang, and Walline 2022). Loughman et al (2016) similarly agreed by reporting 0.01% atropine was tolerated with no adverse effects on visual acuity (distance and near) and reading speed. Joachimsen et al (2021) noted stronger side effects in their 0.05% atropine group compared to their 0.01% atropine group but both groups did not have a significant reduction in near visual acuity (Joachimsen et al. 2021).

It is important to note that there were no significant side effects when using these low doses of atropine (Chia, Lu, and Tan 2016; Upadhyay and Beuerman 2020; Yam et al. 2020). Atropine causes pupil mydriasis that leads to photophobia and blurred near vision from a loss of accommodation. Yam et al (2020) indicated the pupil size increase was atropine concentration dependent and pupil size change remained similar after the first-year result and was well-tolerated. Atropine 0.01% caused minimal pupil dilation, minimal loss of accommodation and no near vision loss compared with higher doses used in the ATOM studies (Chia, Lu, and Tan 2016). The side effects of atropine are not uncommon, but they are rarely serious. The toxic effects of atropine are known as "Belladonna poisoning" which has a severity of symptoms that vary from mild to severe depending on the dose and source (O'Shaughnessy 2012). Atropine is an alkaloid from the deadly nightshade, Atropa belladonna. In general, the effects of atropine are inhibitory, but in large doses, it stimulates the central nervous system.

Table 1.1. Possible side effects of atropine (Kaiti 2022).

Systemic side effects	**Ocular side effects**
• Dry mouth	• Dilated pupil
• Difficulty in swallowing and talking	• Dreadful visual hallucinations
• Dry, flushed and hot skin	• Photophobia
• Difficulty in micturition, decreased bowel sounds	• Accommodation paralysis
• Excitement, ataxia, agitated delirium, acute psychosis	• Local allergic response
• Hypotension, weak and rapid pulse, cardiovascular failure and respiratory depression	
• Convulsions and coma only in events of severe poisoning	

1.2. Low Dose Atropine and Ocular Rigidity

As mentioned before, the precise mechanism of action for atropine as a treatment for myopia control is unknown. Even though it has been hypothesized that atropine affects primarily the retina, where ocular growth regulatory signals pass to the sclera through the choroid (Upadhyay and Beuerman 2020; Chia, Lu, and Tan 2016), some have proposed that it may act directly in the sclera, modulating the stiffness of the eye. Gallego et al (2012) proposed that atropine works by restoration of thickness in the fibrous layer of the sclera, therefore delaying the progression of myopia on the chick model. On the other hand, Cristaldi et al (2020) suggested that a passive elongation evokes a reduced thickness and/or rigidity of the extracellular matrix of the sclera, leading the eye globe to prolapse posteriorly. Atropine would be expected to reinforce the sclera, by increasing its extracellular matrix elements biosynthesis. Myopia results in thinning of the sclera, which becomes more extensible and favors further eye elongation. It would be of interest to understand if the sclera characteristics are different in those children who will develop myopia in the future. The remodeling of the sclera in myopia is associated with decreased amounts of type I collagen, and treatment with atropine has been shown to increase type I collagen in the extracellular matrix (Cristaldi et al. 2020). Zhou et al (2020) noted that atropine also affects the anterior segment structures, as measured by ultrasound biomicroscopy, such as the central anterior chamber depth, the trabecular meshwork-iris angle, the angle opening at a distance of 500μm from the scleral spur, the iris thickness at 750μm and 1500μm from the scleral spur, the trabecular-ciliary angle, the trabecular-ciliary process distance, the sclera-iris angle, and the sclera-ciliary process angle (Zhou et al. 2020).

It is now known why atropine has a larger effect in controlling the progression of myopia (D) than controlling the elongation of the eye (mm). We hypothesize that the ciliary muscle is involved in this process and that long term use of low dose of atropine eye drops may be simply (or mostly) causing further relaxation of the ciliary muscle with the subsequent reduction in power of the eye, but not a true control of myopia (i.e., elongation of the eye). We therefore evaluate the effect of low dose atropine eye drops on ocular anterior and

posterior segment structures and functions in subjects with emmetropia or myopia. We measured the impact of using 0.05% atropine eye drops for one week on the size and thickness of the ciliary body, crystalline lens, anterior chamber, irido-corneal angle, conjunctiva, and anterior sclera. We will also evaluate anterior segment functions, including corneal and scleral IOP, accommodation, the optical quality of the eye, pupillary functions, and posterior segment measurements, including retinal and choroidal thicknesses. In order to use measurements of scleral IOP, we first need to know whether those measurements are reliable, which we do in a pilot study (Chapter 3).

1.3. Low Dose Atropine and Accommodation

Due to its muscarinic antagonistic effect, atropine eye drops, even at low doses, have a significant effect on the accommodation response. Several recent studies have shown this effect in children who use low dose atropine eye drops for myopia management. For example, Fu et al evaluated the effect of 0.01% and 0.02% atropine vs. placebo eye drops on accommodative amplitude in myopic children and found an overall decrease in amplitude of accommodation of 1.50 ± 0.25 D for the 0.01% group and 1.61 ± 0.31 D for the 0.02% group after 12 months, both changes were significantly different compared to the placebo group (Fu et al. 2020). There were no significant differences in the amplitude of accommodation between the 0.01% and 0.02% groups (Fu et al. 2020). A study investigating the short-term effects of low dose (0.01%, 0.005%, and 0.001%) atropine eye drops on accommodation in young adults found reduced accommodation and increased pupil size at all times tested (morning, afternoon, and late afternoon). They noted a minor decline in accommodation amplitude in the morning only in the group of subjects using 0.01% atropine (Kaymak et al. 2018).

A meta-analysis looking at the changes in accommodative amplitude searched for studies that used various concentrations of atropine. Tran et al (2021) concluded that there is a non-linear dose-response relationship between atropine and accommodative amplitude.

Prescribers are advised to consider the maximal efficacy with the most tolerable side effects. A look at the effect size of a reduction in accommodative amplitude shows a greater effect of side effects at higher concentrations (Tran et al. 2021). From a literature review, atropine concentrations from 0.01% to 1.00% all had a significant decrease in accommodative ability, with the most variability in the 0.01% atropine group (Tran et al. 2021).

Clinicians consider potential side effects of atropine dosage when used for myopia control. Cyphers et al (2022) tested subjects on 0.01% atropine on changes to vision, accommodation, reading speed or subjective side effects which were noted to be not statically significant. Loughman et al (2016) similarly agreed by reporting 0.01% atropine was tolerated with no adverse effects on visual acuity (distance and near) and reading speed. Joachimsen et al (2021) noted stronger side effects in their 0.05% atropine group compared to their 0.01% atropine group but both groups did not have a significant reduction in near visual acuity.

1.4. Low Dose Atropine and Retinal and Choroidal Thickness

Although the exact mechanism for atropine to control myopia is unknown, the generally accepted hypothesis is that atropine works directly or indirectly on the retina and/or the sclera, ultimately inhibiting thinning or stretching of the sclera, and thereby inhibiting eye growth (Chia, Lu, and Tan 2016). Atropine is a non-specific muscarinic receptor antagonist and M1 receptors are highly concentrated in the retina (Walline 2016). Most previous work has shown atropine does not change retinal thickness. For example, Wang et al (2022) found no changes in intrafoveal retinal thickness with 0.01% atropine use in myopic children. On the other hand, Liu et al noted a significant total retinal and outer intrafoveal retinal thickness increase in guinea pigs that received atropine injections every 3 days for 2 weeks (Liu et al. 2022). To our knowledge, no previous studies have investigated the effect of atropine eye drops in retinal thickness across the entire retina in humans.

Anti-muscarinic drugs, including atropine, have been shown to significantly increase the subfoveal choroidal thickness (Sander, Collins, and Read 2019; Yam et al. 2022) as well as overall choroidal thickness (Wang et al. 2022; Ye et al. 2020). This transient thickening of the choroid has also been shown also in animal models treated with hyperopic defocus, which otherwise would lead to choroidal thinning. The choroid thickens significantly 30 minutes after use of atropine but returns to normal values 60 minutes after instillation (Sander, Collins, and Read 2019). It appears that the effect of atropine is strong enough to inhibit the effect of hyperopic defocus, even if short-term. Most studies have investigated the effect of atropine on subfoveal choroidal thickness, such as the above mentioned by Sander et al (2019). Others such as Yam et al (2022) reported low concentrations of atropine (0.05%, 0.025% and 0.01%) induced a subfoveal choroidal thickening with a concentration-dependent response in children. Since choroidal thickening was associated with slower progression of myopia and axial elongation in their study, the investigators concluded that choroidal thickness could be used as a measure to assess the effectiveness of myopia control treatments (Yam et al. 2022). A few studies have investigated the effect of atropine on the overall choroid, not just the subfoveal choroid. Ye et al (2020) showed that higher doses of atropine (1%) increased overall choroidal thickness, however, contrary to other studies on subfoveal thickness, they found that 0.01% atropine caused a decrease, not an increase, in choroidal thickness after 6 months use in children. Ye et al (2020) analyzed average choroidal thickness in subfoveal, parafoveal, and perifoveal areas (1mm, 3mm, and 6mm respectively), then further divided these into superior, inferior, temporal and nasal quadrants. Wang et al (2022) also found that low dose atropine (0.01%) did not increase overall choroidal thickness, although the trend in this study was towards thicker choroids. The effect of low dose atropine in choroidal thickness is therefore inconclusive, and more studies evaluating the effect of low dose atropine across the entire choroid long term in children are needed. The main study of my thesis includes measurements of choroidal thickness throughout the central 55 degrees, before and after using low dose atropine eye drops for one week.

In conclusion, my studies aim to evaluate the effect of 0.05% atropine eye drops on anterior and posterior segment structures and functions. Some of the parameters investigated in these studies have previously been examined, but with inconclusive results, and no previous study has comprehensively investigated all these parameters in the same group of subjects, in particular the effect of low dose atropine on ocular rigidity in humans. The overall aim is to investigate how each of these parameters changes with daily use of 0.05% atropine eye drops in young adults with emmetropia or myopia.

2. Pilot Study - Feasibility of Measuring Ocular Pressure through the Sclera

2.1. Introduction

There are many methods to measure IOP clinically, including the Icare rebound tonometer. The Icare IC100 is commonly used in clinical settings to measure IOP quickly and safely. The accuracy and repeatability of the Icare rebound tonometer to measure IOP at the cornea are established to be high (Liu et al. 2022; Walline 2016; Wang et al. 2022). However, studies of Icare or other methods to measure IOP at the sclera are scarce. Badakere et al (2018) evaluated scleral IOP using a Tonopen tonometer and compared the measurements with those from the gold-standard (Goldmann applanation tonometry, GAT) on the central cornea, temporal limbus and sclera. Ruland et al (2019) compared the accuracy of Tonopen and Icare at the central cornea, the limbal cornea and the sclera (at 2mm from the limbus). In this study, Tonopen was more accurate in the central cornea, and no significant difference at the limbus was noted between Tonopen and Icare. Senthil et al (2019) estimated IOP with corneal GAT, Icare, IcarePro, and Schiotz on the cornea and at the temporal sclera (3 to 5 mm from the limbus); it was found that IOP was significantly overestimated by Icare and Icare pro in the sclera. Although there have been several studies that use Icare in the sclera, they have each measured at different points from the limbus and none have clearly explained how they determined where they were measuring on the sclera.

Numerous instruments have been used to obtain IOP measurements in two groups: indentation tonometers and applanation tonometers. One of the first indentation tonometers is the Schiotz tonometer. This tonometer works when the cornea is indented by a plunger loaded with different weights; IOP is based on the depth of indentation (Brusini and Salvetat 2021). The value on a scale between 0 to 20 units in which movement of the plunger of 0.05mm is each unit of measurement; the value needs to be converted in mmHg using a conversion scale. The Schiotz tonometer is no longer used due to several sources of error, including improper positioning on the eye, defective or dirty instruments, and high variability

compared to other devices; this instrument is also more invasive and patients are required to be in a supine position for testing (Brusini and Salvetat 2021).

Applanation tonometers are the most reliable instruments for an accurate IOP. These instruments use the Imbert - Fick law: P = F / S where P is pressure, F is the force needed to flatten a fixed corneal area, and S is the surface of the flattened area (Brusini and Salvetat 2021). In GAT, a truncated cone is illuminated by a blue light, pushed on the center of the anesthetized cornea (Brusini and Salvetat 2021). A doubling prism divides the circular meniscus on the surface of the cornea in two arcs that are aligned to obtain a precise and standardized applanation (Brusini and Salvetat 2021). The force required to flatten the corresponding surface of the cornea is directly proportional to IOP, in mmHg. Since the GAT is calibrated for a cornea 520μm thick, thin corneas cause underestimation of true IOP and vice versa (Brusini and Salvetat 2021).

In the initial pilot study reported here, we used an Icare rebound tonometer to measure the IOP throughout the conjunctiva (anterior sclera) in a pre-experimental study that has shown promising results. The goal of this pilot study was to explore the possibility of evaluating scleral rigidity in our main study (methods described Chapter 3) using Icare tonometry. One of the goals of the main study is to assess whether there are differences in scleral IOP measured at various locations between emmetropic and myopic adult eyes as well as the effect of atropine eye drops in scleral rigidity (Chapter 6). For this, we needed to first determine whether the measurements of IOP in the conjunctiva (anterior sclera) were repeatable. We hypothesized that we would observe intersubject variability, but we would be able to measure pressures across various locations of the globe and will find reliable and repeatable results.

2.2. Methods

2.2.1. Subjects

This was a cross-sectional study that was conducted over two visits in a group of healthy young adults. Recruitment occurred within the New England College of Optometry (NECO) student population. We enrolled a total of n = 27 subjects of whom n = 25 completed the study.

All subjects met the following inclusion criteria: between the ages of 18 and 35 years (an upper limit was used because older eyes may have different scleral rigidity). Subjects with ocular history, including extraocular muscle surgery for strabismus, or ocular disease that may affect the globe rigidity will be excluded from the study.

2.2.2. Experimental Procedures

In two visits, each to last up to 0.5 hours, we conducted the following specific tests:

a) After an explanation of the purposes of the study and the potential subject read the Informed Consent Form (ICF), the PI or Investigator answered any questions the subject had and ensured that the procedures and purposes were understood.

   Once the ICF was signed, a de-identifier form was used to assign a study number to the subject; this study number, with no identifiable information, was used from that point on.

b) Vision screening to determine the subject's visual acuity and RE:

- Screening questionnaire including eye and health history and refraction history.
- Habitual logMAR VA at 4 m and at 40 cm.
- Lensometry to verify spectacle prescription, when applicable.

c) Icare ic100 measurements:

The ocular pressure was measured at seven different positions in each eye (Fig 2.1): central cornea, 4 mm inferior, nasal, superior and temporal from the limbus, and 8 mm temporal from and nasal from the limbus. We obtained three reliable measures for each corneal and conjunctival position from the limbus, except for the 2 mm temporal position, where we obtained six reliable measures. The measures were taken at the distances from the limbus marked by an aseptic surgical marker to help with accuracy. This was to improve the repeatability of the measurements.

2.2.3. Data Analyses

The standardized effect size (SD) (Norton and Rada 1995; Tian, Tarrant, and Wildsoet 2011) using the Icare ic100 in the cornea has been found to be about 10% (13.0 ± 2.8 mmHg) (Bryant and McDonnell 1998), using Icare PRO in the cornea was about 20% (24.5 ± 11.0 mmHg) and using Icare PRO in the sclera was about 15% (45.9 ± 14.9 mmHg) (Lewis et al. 2014). For alpha 0.05% (two-sided), power of 0.80, and effect size of 10% using Icare ic100, the sample size would be n = 17. For this initial study, a total of n = 25 subjects was chosen.

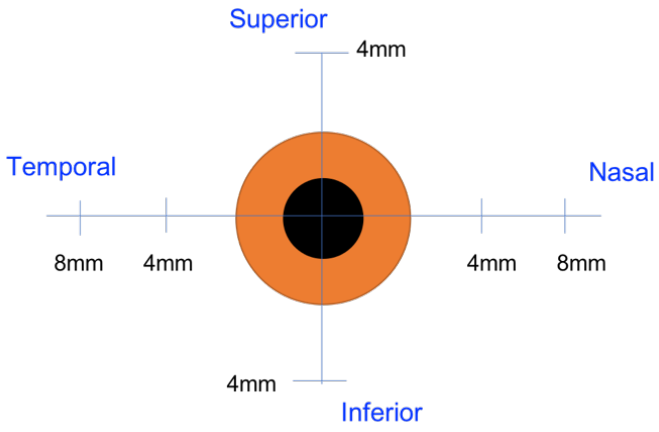


Figure 2.1. Schematic diagram of the areas tested with the Icare tonometer. The black circle represents the pupil and the orange circle the iris. We measured IOP at seven different positions in each eye: central cornea, 4 mm inferior, 4 mm and 8mm nasal, 4 mm superior and 4 mm and 8mm temporal from the limbus.

Test-retest variability was assessed using Bland-Altman analysis and coefficient of repeatability (95% confidence interval of the difference between the compared tests). Scatter plots were used to plot the distribution comparisons between the two tests. Tests for normality showed some measures were normally distributed and others were not based, so we used non-parametric tests: Wilcoxon Signed Rank Test for each pair.

2.3. Results

The 25 participants who completed this pilot study had a mean age of 24.28 ± 1.75 years. The DVA mean (± SD) at visit 1 for OD was 20/18 ± 4 and for OS was 20/18 ± 4. For Visit 2, the DVA mean (± SD) for OD was 20/16 ± 3 and for OS was 20/17 ± 3.5. The Wilcoxon Signed Rank Test indicated significant differences when comparing the results of Visit 1 and Visit 2 in the right eyes' superior and nasal (4 mm) positions and in the left eyes' nasal (8 mm) and inferior positions as shown in Table 2.1.

Table 2.1. Statistical analyses comparing the IOP results between Visit #1 and Visit #2.

	OD		OS	
	Test-statistic	2-side significance	Test-statistic	2-side significance
Central	83	0.06	83	0.06
Superior	57	0.04*	131.5	0.40
Nasal (4mm)	80	0.05	186	0.30
Nasal (8mm)	132.5	0.85	190.5	0.04*
Inferior	139.5	0.96	73.5	0.02*
Temporal (4mm)	130	0.57	161.5	0.48
Temporal (8mm)	137.5	0.44	132.5	0.42

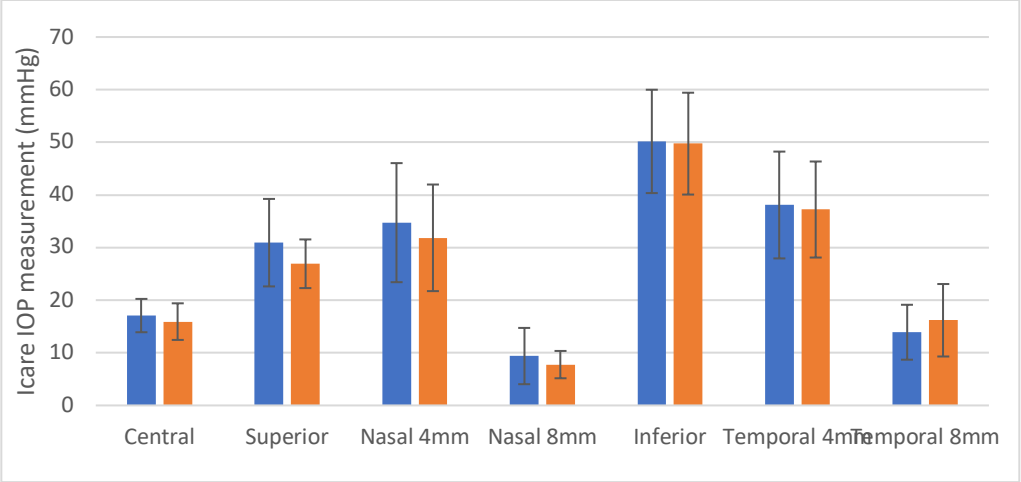


Figure 2.2a. Results of IOP (mmHg) for Visit #1 (blue) and Visit #2 (orange) were measured with the Icare at various points throughout the cornea and conjunctiva for the right eye. Error bars show standard deviation.

Raw data from the 25 participants is shown in figures 2.2a (right eye) and 2.2b (left eye). As seen in these graphs, there is a wide range of IOP measurements throughout the various positions tested. For both eyes, the inferior measurements were highest and nasal measurements were lowest.

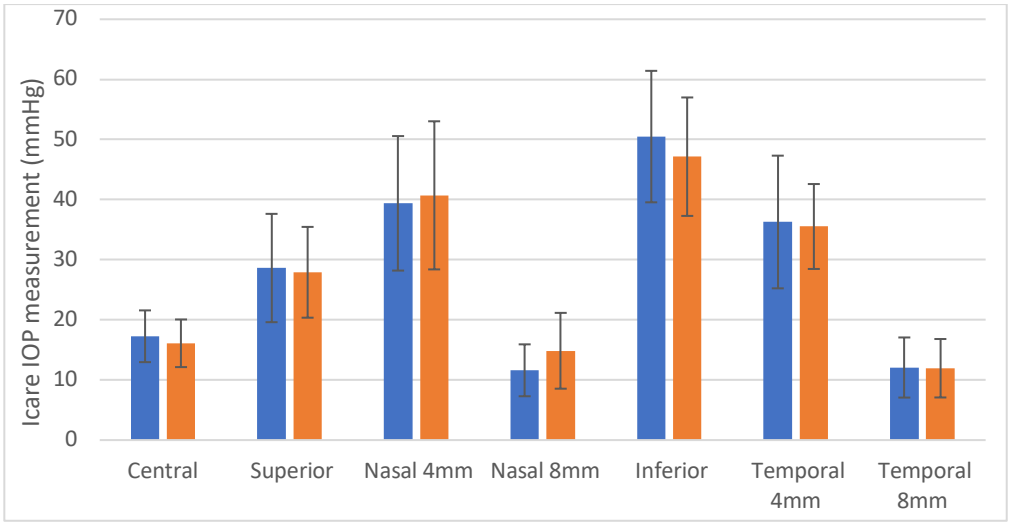


Figure 2.2b. Results of IOP (mmHg) for Visit #1 (blue) and Visit #2 (orange) were measured with the Icare at various points throughout the cornea and conjunctiva for the left eye. Error bars show standard deviation.

2.4. Discussion

This pilot study (Appendix V) examined the feasibility of evaluating scleral rigidity using Icare tonometry, which we intend to use in our main study (Chapter 6). Results from previous studies that measured scleral rigidity using the Icare in different locations indicated very large intersubject variability (Senthil et al. 2019; Sergienko and Shargorogska 2012), although the accuracy and repeatability of the Icare rebound tonometer to measure the cornea is high (Davies et al. 2006; Khanal, Walton, and Davey 2017; Schweier et al. 2013).

The results from our study indicate no significant differences between the measurements taken at the first and second visits (one week apart) for most of the locations tested. Icare can provide repeatable measurements at the cornea as well as most locations at the conjunctiva. The only location for which the measurements were different between the two sessions for the right eye was superiorly ($p = 0.04$), and for the left eye inferiorly ($p = 0.02$) and nasal at 8mm only ($p = 0.04$). We, therefore, did not use these locations in our main study (see Chapters 3 and 6).

3. Main Study - Effect of Atropine Eye Drops on the Anterior and Posterior Segment Structures and Functions - Methods

3.1. Subjects

The main study was a cross-sectional study that was conducted over two visits in a group of healthy young adults. Recruitment occurred within the NECO student population. A total of n = 30 healthy young adult subjects with emmetropia (n = 15) or myopia (n = 15) were enrolled.

All subjects met the following inclusion criteria:

- Within 18 - 30 years of age,
- SE in each eye between +0.75 DS and -5.00 DS, with less than 1.00 DC of astigmatism in each eye and less than 1.00 D of anisometropia,
- Best corrected logMAR VA = 0.0 (20/20 Snellen equivalent) or better in each eye,
- No history of ocular surgery or disease that may have resulted in visual consequences,
- Not using drugs that may affect their vision.

RE grouping was determined using binocular subjective refraction that followed objective refraction. Subjects were classified as having Emmetropia (EMM), defined as having a RE (spherical equivalent, SE) in each eye determined by wavefront autorefractor refraction between +0.75 to -0.25 D; or Myopia (MYOP), with an SE between -0.50 to -5.00 D at the initial visit.

3.2. Procedures

Visit #1:

During an initial visit that lasted up to 3 hours, the following tests were performed in the given order:

a) Vision screening, to determine the subject's eligibility and RE group inclusion:

- Screening questionnaire (Appendix I) for eye and health history, including allergy or reaction to drops and possibility of pregnancy or nursing and refraction history.
- Habitual distance and near visual acuity using a logMAR chart.
- Habitual cover test for distance and near.
- Near point of convergence (NPC) using an accommodative target. Results are presented in Chapter 4.
- Counting fingers confrontation fields (CFF).
- Extraocular muscle (EOMs) testing.
- Pupil evaluation, including measures of pupil sizes under dim, near, and bright conditions. Results are presented in Chapter 4.
- Monocular estimation method (MEM) to measure accommodative accuracy at near. Results are presented in Chapter 4.
- Objective refraction with static retinoscopy.
- Subjective refraction with binocular balance to determine the subject's RE.
- Slit lamp anterior ocular health evaluation of the ocular media health.
- Questionnaire 1 to determine baseline near work and photophobia (Appendix I). Results are presented in Chapter 4.

All data were entered in a data form in hard copy and electronic versions.

If subjects were eligible, they continued with the experimental testing of this visit (*Visit #1 Pre-atropine*) as follows:

b) Reading Speed and Comprehension

Measurements of using reading speed were done using Radner reading charts and the New York Public Library reading test passages (Appendix II). The results of these tests are presented in Chapter 4.

c) Measures of Static and Dynamic Accommodation:

Measurements of the accuracy and stability of static and dynamic accommodation responses were done using a WAM-5500 open-field autorefractor WMT-2 Moving System (Ait, Luneau, Japan). Detailed methods and results for these experiments are presented in Chapter 4.

d) Ocular Biometry:

- OCT Spectralis (Heidelberg Engineering, USA) with widefield imaging module.

We imaged the posterior segment of the right eye using a widefield imaging module with a 55-degree lens attached to the OCT Spectralis. From these images, we segmented and measured the thickness of the retinal and choroidal layers. Detailed methods and results for this test are presented in Chapter 5.

- OCT Spectralis with anterior segment module.

We also imaged the anterior segment of the right eye, including the cornea, the anterior sclera for the area beyond 5mm from the limbus (to compare with the Icare results), the ciliary body, and the crystalline lens. Detailed methods and results for these tests are presented in Chapter 6.

- Lenstar LS900 biometry (Haag-Streit, USA).

Five measurements (within 0.02mm) were taken to obtain: axial length (AXL), anterior chamber depth (ACD), lens thickness (LT), keratometry (K), and vitreous chamber depth (VCD), as well as angle Kappa. The data obtained from the Lenstar were used to:

- Determine the RE correlation with AXL between subjects.
- Evaluate the within subjects' changes in AXL between the three testing times.
- Correct the IOP values measured to a norm due to variations in ocular rigidity. Details on methods and results are presented in Chapter 6.

- Icare ic100 (Icare, Finland).

We used the Icare ic100 and a surgical marker to measure IOP at five different positions in each eye: central cornea, 4mm inferior, 5mm nasal, 4mm superior, and 5mm temporal from the limbus. We obtained five reliable measures for each corneal and conjunctival position.

We also measured central cornea IOP using the standard of care GAT. We used the GAT values in the ocular rigidity calculations (Chapter 6).

e) Glare.

We used the Oculus C-quant machine to measure the amount of straylight in a human eye. Detailed methods and results are presented in Chapter 4.

f) Instillation of low dose atropine eye drops:

- Instill 1 gtt 0.05% atropine OD and OS, and record the time.
- **Wait 30 minutes.** During this time, the subject watched a movie on a large TV, at a 6 m distance.

- **Repeat all tests** described above **(c), (d), (e), (f), and (g)** in the same order, at 30 minutes following the drop instillation (*Visit #1 Post-atropine*).

- Deliver Questionnaire 1 (Appendix I) to determine near work and photophobia short-term after atropine instillation.

- **Give** the subject their prescribed **bottle** (4 ml) of atropine eyedrops with their name. Provide verbal and written usage instructions (Appendix III). Subjects were asked to instill 1gtt 0.05% atropine OD and OS each night before bed, for 7 days, until the next visit. We educated subjects on the importance of adherence to eye drop instructions. The atropine eye drops were obtained from Johnson Compounding and Wellness (Waltham, MA).

- Inform the subject that an electronic questionnaire and compliance check (Questionnaire 2, Appendix IV) will be automatically sent to them via Google Forms each day between the two visits.

- Schedule *Visit #2*, within 1 week (±1 day) of the first visit, and within 2 hours of the daytime of the first visit. Educate the subject on the importance of adherence to the visit schedule.

Visit #2:

Subjects were asked to return for a second visit that lasted up to 2 hours to perform tests **(a)** through **(g)** once (*Visit #2*), as described for Visit #1.

3.3. Power Analyses

Sample size calculations were conducted before the initiation of the main study (Hulley et al. 2013). Whether the null hypothesis is true or false in the population, there are 4 possible

situations listed in Table 3.1. To determine the maximum chance that we would tolerate making a type I (alpha, α, or the level of statistical significance) or type II errors (β). Usually, α is set to 0.05, where there is a 5% maximum chance of incorrectly rejecting the null hypothesis. The value of $|1-\beta|$ indicates the power, which is the probability of correctly rejecting the null hypothesis in the sample. Often $|1-\beta|$ is set to 0.20 where there is a 20% chance of missing an association of the effect size; this is a power of 0.80 where there is an 80% chance of finding an association of that size.

The effect size chosen to calculate sample size is based on the hypothesis that requires the largest sample, accommodation. The accommodation variability effect size (standard deviation) in EMMs compared to MYOPs is about 0.20% (0.21 ± 0.08 D) according to previous literature (Abbott, Schmid, and Strang 1998; Langaas et al. 2008; Vera-Diaz et al. 2004), which gives a sample size of 16 subjects in each group. For this study, a sample size of n = 30 would be reasonable.

Table 3.1. Truth in the population versus results in the study sample: the four possibilities. Adapted from Hulley (2013).

	Truth in the population	
Results in the study sample	Association between predictor and outcome	No association between predictor and outcome
Reject null hypothesis	Correct	Type I error
Fail to reject null hypothesis	Type II error	Correct

The specific data analyses are described under each experimental test in the following Chapters 4 through 7.

Reliable data was obtained from the 30 participants who completed this study for each of the tests described in Chapters 4, 5, and 6. Subjects (n = 22 females) had a mean age of 24.79 ± 1.64 years (EMMs: n = 11 Females, SE: +0.27 ± 0.42 D; MYOPs: n = 11 Females, SE: -1.89 ± 1.86 D).

4. Chapter 4: Low Dose Atropine and Accommodation

4.1. Introduction

Accommodation is the mechanism through which the eye can alter the refractive power of the crystalline lens to maintain a clear image of a near target on the retina. The accommodative reflex consists of accommodation, convergence, and pupillary constriction. The accommodative response also involves bilateral contraction of the ciliary muscle which is comprised of longitudinal, radial, and ciliary fibers. Accommodation is thought to thicken the anterior ciliary muscle and thin posteriorly, causing a forward and inward displacement of the ciliary body. Due to proximity, changes in the choroid are strongly associated with affecting scleral remodeling and metabolism (Prousali et al. 2022). In accommodation, the anterior sclera shows thinning, which is more evident in eyes with myopia (Prousali et al. 2022; Wagner, Zrenner, and Strasser 2019). A thinner, more extensile sclera due to biochemical changes including proteoglycan synthesis, collagen, and/or matrix metalloproteinase activity is hypothesized to cause axial elongation (Prousali et al. 2022).

Many previous studies have hypothesized the relationship between accommodation and myopia progression. When there is a lag in accommodation, the eye attempts to accommodate to eliminate or reduce the hyperopic defocus and blurred retinal image that may lead to axial elongation and eventually myopia development. Accommodative amplitude is the maximum ability of the eye to change its refractive power from the relaxed state by accommodating when fixating on a near target. This is often tested with the push-up and push-down test, minus lens method and retinoscopy. There have been variable results in the assessment of the relationship between myopia and accommodative amplitude. Many describe myopia development as an inadequate accommodative response to a near stimulus leading to axial elongation (Langaas et al. 2008; Prousali et al. 2022). Accommodative accuracy can be tested with monocular estimated method (MEM) retinoscopy, which we did in this study. Accommodative facility is how quickly the eye is able to respond to blur and reflects how adaptable the accommodative response is. There has been reported improvement

in accommodative facility with myopia treatment including single vision spectacles, orthokeratology lenses, and multifocal lenses (Prousali et al. 2022).

Low dose atropine eye drops are the most common treatment used for myopia control. As discussed in the general introduction chapter, it is established that atropine has a significant effect on accommodative amplitude that is dose-dependent (Fu et al. 2020; Kaymak et al. 2018; Tran et al. 2021). Cyphers et al (2022) used 0.01% atropine on subjects and examined the effect on accommodation. They found there was no change in accommodative lag or accommodative facility. Kaymak et al (2018) also used very low dose 0.01%, 0.005%, and 0.001% atropine on young adults and reported a significant effect on accommodative amplitude. There are no previous studies that have examined the effect of low dose atropine eye drops on the dynamics of the accommodation response, which is the goal of this sub-study.

4.2. Methods

4.2.1. Accommodation Responses Tested with an Open-Field Autorefractor

The subject's static and dynamic accommodation responses were tested using a Grand Seiko WAM-5500 binocular accommodation autorefractor with a dynamic WMT-2 moving system. The accommodation measurements were taken in the order of static accommodation response at 6m, dynamic accommodation response, and static accommodation response at 40cm.



Figure 4.1. Target attached to steel apparatus

First, five static measurements were taken for the right and left eyes while the subject looked at a target at 6 m (target: green light) and a target (ovals of various spatial frequencies, Figure 4.1.) at 40 cm. The near static accommodation response was calculated as the difference between the WAM SE measures at 6 m and 40 cm.

Following the static measurements, dynamic measures of accommodation were taken while the subject observed (trying to keep it clear) a target that moved sinusoidally at a rate of 0.2 Hz and amplitude of 3.00 D starting at 2.50 D of accommodation demand (range 1.50 to 4.50 D), during three cycles that lasted 9 seconds each. The raw dynamic accommodation results were fitted to a sinusoidal function (Figure 4.2). From these fitted functions we obtained the maximum accommodation response, the amplitude, and the phase (latency) of the accommodation responses.

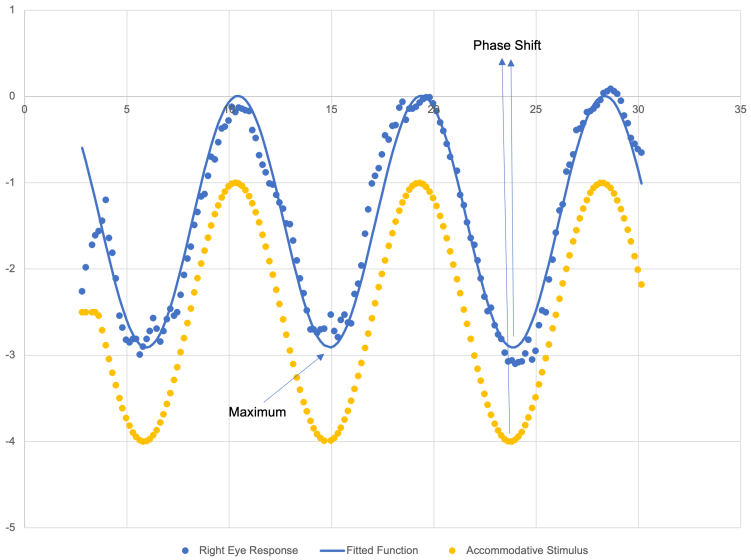


Figure 4.2. Sample of dynamic accommodation measurements and sinusoidal fitting. The parameters obtained from these sinusoidal fittings and evaluated included the maximum accommodation response, the phase shift, and the amplitude.

A general linear model with repeated measures and RE group as the between subjects' factor was used to compare the results across visits. Related samples Friedman's two-way analysis of variance by ranks were also used.

4.2.2. MEM Retinoscopy

During this clinical test, subjects wore their habitual correction while they read words on a target that was placed at the retinoscope level while wearing. Loose lenses (positive or negative powered) were briefly placed in front of the subject's eyes to neutralize the reflex.

4.2.3. Near Point of Convergence

Subjects also used their habitual correction to perform this clinical test. They were asked to look at a small letter in a near stick and to keep clear and single as long as they could, then

report when the letters became double, and then back to single. The examiner held a ruler next to the subject's glasses to determine the break and recovery points based on the subject's reported diplopia, observation of fixation loss of one eye, or determine there is no breakpoint.

4.2.4. Pupil Sizes

Pupils' functions were examined using standard clinical procedures to determine if they were round, centered and responsive to light (direct and consensual responses), and accommodation (looking at a small target at 6 m and back at 40 cm). Possible relative afferent pupillary defects were also assessed.

Pupil sizes were measured with a pupil ruler under three conditions: Dim (without glasses while the subject is fixated on a large distance target, 20/400 optotype), Bright illumination (same conditions but lights as bright as possible), and Near conditions (looking at a small letter at 40 cm under normal illumination).

4.2.5. Glare

Glare was examined using a C-quant device (Oculus, Germany). The C-quant measures the amount of straylight in the eye, which creates glare. It was set to the A range field (lowest setting) as subjects were young and with healthy eyes are more effectively studied. The A range field setting had the smallest difference in intensity between the left and right sides of the target circle compared to the other higher settings. The stimulus field of concentric light and dark circles was presented to the subjects, with a vertical line dividing the circles in half for each eye's view. With the left eye patched and wearing their habitual correction, the subject was instructed to click the left or right button on the C-quant, choosing the side that appeared brighter to them (Figure 4.3).

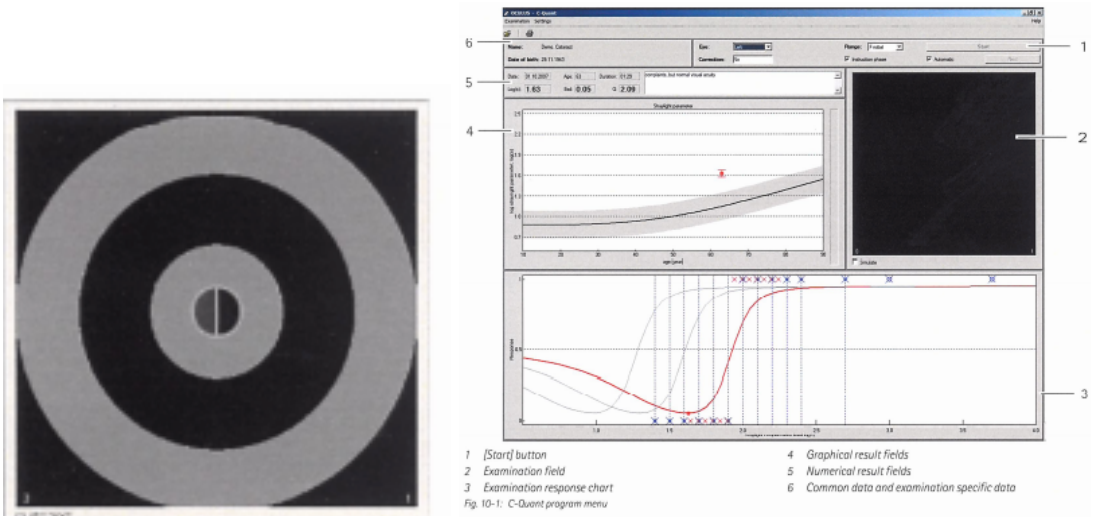


Figure 4.3. Left: Fixation target inside Oculus C-quant. Right: Graphic presentation of straylight compared to age-related normal examination time.

The C-quant presents results as an absolute straylight value in log(s), a graphic display of the straylight compared to an age-related normal examination time, a standard deviation of the individual measuring points (Esd), and a reliability coefficient (Q). The expected standard deviation of the individual measurement value is taken in cases of repeated measurements; the smaller the value indicating the more reliable result. The higher the quality criterion, the more reliable the measurement is. An Esd < 0.08 and Q > 1 result is considered to be good, while an Esd < 0.08 and Q > 0.5 is considered to be acceptable (Figure 4.3).

4.2.6. Reading Tests

Two reading tests were administered (Appendix II), one test that evaluates mainly reading speed, and another that assesses comprehension. The Radner reading charts, designed for clinical and research use, provide different parameters from a single examination. Each reaching chart has standardized “sentence optotypes” that vary in size, that are standardized

by reliability and validity analyses. The subject was instructed to hold the Radner reading chart at a comfortable reading distance while wearing their reading glasses. One sentence on the optotype was uncovered at a time, and the subject was timed on how long they took to read each sentence and how many errors were made during the reading. Subjects continued to read the next sentence in the optotype as the letters become smaller. At the point where the subject indicated that they could no longer read the sentence, the test ended. For each of the three rounds of testing the subject read a different version of the Radner standardized reading chart.

The New York Public Library has paragraph texts that we used as comprehension tests with 3 correlated questions. Note these are no longer available on their website. We timed how long the subject took to read each paragraph and how accurate they were when answering the three comprehension questions that followed. For each of the three rounds of testing the subject read a different paragraph.

4.2.7. Questionnaires

Subjects were presented with a paper questionnaire (Appendix I) at the beginning and end of both visits. The questionnaire includes questions related to difficulty doing near work and increased light sensitivity. Within each question, where the subject reported they did have difficulty with near work or light sensitivity, they were asked to rate the level of difficulty on a scale of 1 to 10.

Each of the 7 evenings that subjects used the low dose atropine eye drops at home, they were also sent and instructed to fill out an online questionnaire delivered using Google Forms (Questionnaire 2, Appendix IV).

4.3. Results

4.3.1. Accommodation Responses with the Open-Field Autorefractor

As expected, static accommodation responses (distance autorefractor SE – near autorefractor SE for the right eyes) decreased with the use of low dose atropine eye drops. General linear models with repeated measures showed an overall effect of visits ($F = 10.57$, $p < 0.001$) and visits with RE group ($F = 4.34$, $p = 0.02$) (Figure 4.4).

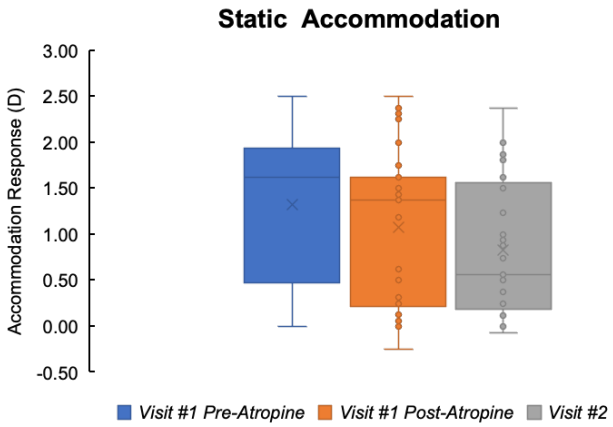


Figure 4.4. Boxplot with static accommodation responses (distance – near WAM measurements) for all subjects at each visit.

The effect of visits was significant between each visit (Mean estimated decrease from *Visit #1 Pre-atropine* until *Visit #1 Post-atropine*: 1.89 D, $p = 0.006$, Mean estimated decrease from *Visit #1 Pre-atropine* until *Visit #2:* 0.46 D, $p < 0.001$, and Mean difference *Visit #1 Post-atropine* with *Visit #2:* 0.27 D, $p = 0.01$). Related-samples Friedman's two-way analysis of variance by ranks also showed an overall effect of visits ($p < 0.001$), pairwise analyses showed differences between *Visit #1 Pre-atropine* and *Visit #1 Post-atropine* ($p = 0.02$) and between *Visit #1 Pre-atropine* and *Visit #2* ($p < 0.001$).

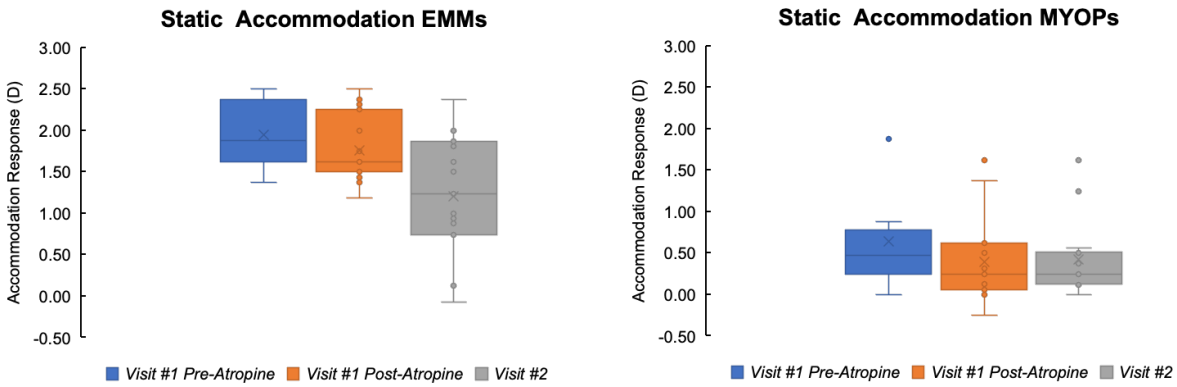


Figure 4.5. Boxplots with static accommodation responses for EMMs (left) and MYOPs (right) at each visit.

Interestingly, when subjects were split into EMMs and MYOPs, we noted that the effect of visits was significant for all visits comparisons in the group with EMMs ($p < 0.04$ for all), but in MYOPs, it was only significantly smaller between *Visit #1 Pre-atropine* and *Visit #1 Post-atropine* (Mean estimated difference 0.19 D, $p = 0.049$), not for the other conditions (Figure 4.5). This was also shown in Friedman's analysis (EMMs: $p < 0.001$, MYOPs: $p = 0.081$). The static accommodation response for EMMs was larger than for MYOPs (Mean estimated difference at baseline: 1.17 D, $p < 0.001$) for all visits ($p < 0.01$ for all).

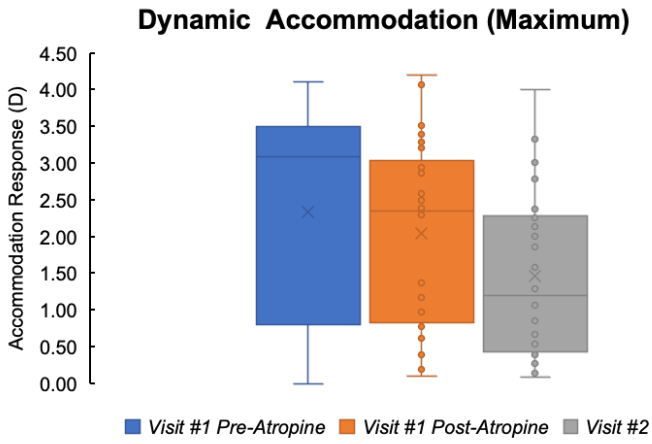


Figure 4.6. Boxplot with dynamic accommodation responses (maximum response) for all subjects.

Similarly, maximum response during the dynamic accommodation responses decreased with the use of low dose atropine eye drops. General linear models with repeated measures showed an overall effect of visits ($F = 22.65$, $p < 0.001$) and visits with RE group ($F = 9.68$, $p < 0.001$) (Figure 4.6). The effect of visits was significant between each visit (Mean estimated difference *Visit #1 Pre-atropine* with *Visit #1 Post-atropine*: 0.30 D, $p < 0.001$, Mean estimated difference *Visit #1 Pre-atropine* with *Visit #2:* 0.88 D, $p < 0.001$, and Mean estimated difference *Visit #1 Post-atropine* with *Visit #2:* 0.58 D, $p < 0.001$). Related-samples Friedman's two-way analysis of variance by ranks also showed an overall effect of visits ($p < 0.001$), with pairwise analyses showing differences between all visits ($p < 0.03$). There was a high correlation between the static and dynamic measurements (Figure 4.7).

When subjects were split into EMMs and MYOPs, linear regression analyses showed that the effect of visits was only significant for all visit comparisons in the group with EMMs ($p < 0.001$ for all), but not in MYOPs (Figure 4.8). In Friedman's analysis, the overall effect of visits was significant for both groups (EMMs: $p < 0.001$, MYOPs: $p = 0.038$). The dynamic accommodation maximum response for EMMs was also larger than for MYOPs (Mean estimated difference at baseline: 1.81 D, $p < 0.001$) for all visits ($p < 0.01$ for all).

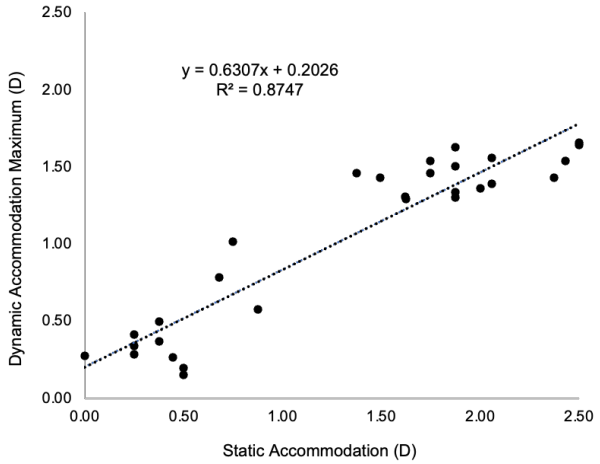


Figure 4.7. Correlation between static accommodation responses and dynamic accommodation maximum responses for all subjects at *Visit #1 Pre-atropine*.

For phase (latency) of the dynamic accommodation response, general linear models with repeated measures showed no significant effects of visits or visits and RE group (F = 4.000, p = 0.14), although there was a trend of shorter phase in *Visit #2* compared to *Visit #1* (F = 0.517, p = 0.05). MYOPs had shorter phases than EMMs for all visits (F = 6.41, p = 0.002). Related-samples Friedman's two-way analysis of variance by ranks showed no overall effects for all subjects (p = 0.06) or when split into RE groups (EMMs p = 0.08, MYOPs p = 0.40).

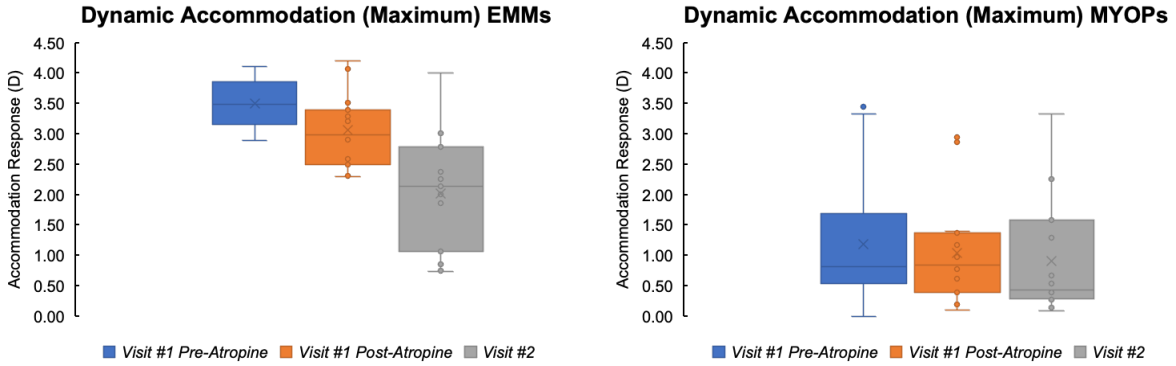


Figure 4.8. Boxplots with dynamic accommodation responses (maximum) for EMMs (left) and MYOPs (right) at each visit.

4.3.2. MEM Retinoscopy

The clinical test MEM retinoscopy, used in standard practice to evaluate lag of accommodation, also showed significant effects of visits in general linear models with repeated measures (F = 11.37, p < 0.001) and related-samples Friedman's two-way analysis of variance by ranks (p < 0.001), but no interaction of visits with RE group (F = 0.29, p = 0.75). The effect was significant between *Visit #1 Pre-atropine* and *Visit #2* (p < 0.001), and between *Visit #1 Post-atropine* and *Visit #2* (p = 0.002), but not between *Visit #1 Pre-atropine* and *Visit #1 Post-atropine* (p = 0.26). No differences were noted on this test between RE groups (Mean estimated difference = 0.06D, p = 0.61) at any visit. When split

into RE groups, both groups showed an effect of visits (EMMs: p = 0.002, MYOPs: p = 0.034).

4.3.3. Near Point of Convergence

The clinical test NPC was to-the-nose for most subjects, which means that no differences were expected within visits. Indeed, no significant effects of visits were found in general linear models with repeated measures (F = 0.24, p = 0.79) or related samples Friedman's two-way analysis of variance by ranks (p = 0.88). No differences were noted when data were split into RE groups either.

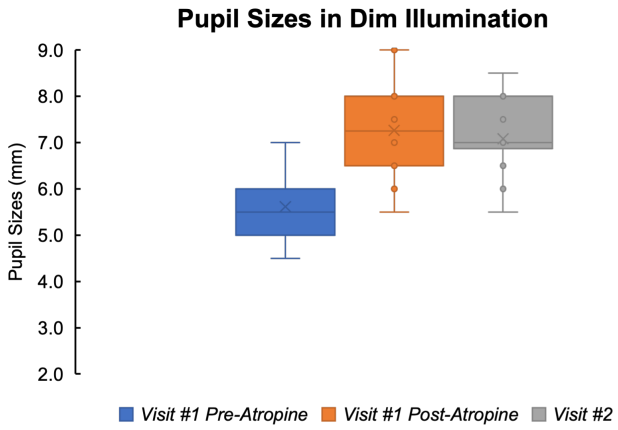


Figure 4.9. Boxplot with pupil sizes under dim conditions for all subjects at each visit.

4.3.4. Pupil Sizes

As expected, general linear models with repeated measures showed a significant effect of visits in Dim illumination pupil sizes (F = 108.34, p < 0.001), but no effects of visits and RE group (F = 1.04, p = 0.32). The effect was also noted when using related samples Friedman's two-way analysis of variance by ranks (p < 0.001), with pupils being an average of 1.63 mm larger after atropine use in *Visit #1* (p < 0.001), but did not increase after one week of use (Mean estimated difference *Visit #1 Post-atropine* compared to *Visit #2* difference = 0.16

mm, p = 0.305) (Figure 4.9). The effect was still significant when subjects were grouped into EMMs and MYOPs for both groups, and no overall significant differences were found in Dim pupil sizes between EMMs and MYOPs (Mean estimated difference = 0.38 mm smaller in myopes, p = 0.12), but pupils were larger for EMMs compared to MYOPs at *Visit #2* (Mean estimated difference = 0.58 mm, p = 0.036).

A similar effect of visits was found for pupil sizes under Bright illumination (F = 123.73, p < 0.001). The effect was still significant for both groups when subjects were grouped into EMMs and MYOPs. Differently from Dim pupil sizes, there was a significant difference between EMMs and MYOPs in Bright pupil sizes, with smaller pupils in MYOPs (Mean estimated difference 0.93 mm, p = 0.014) (Figure 4.10).

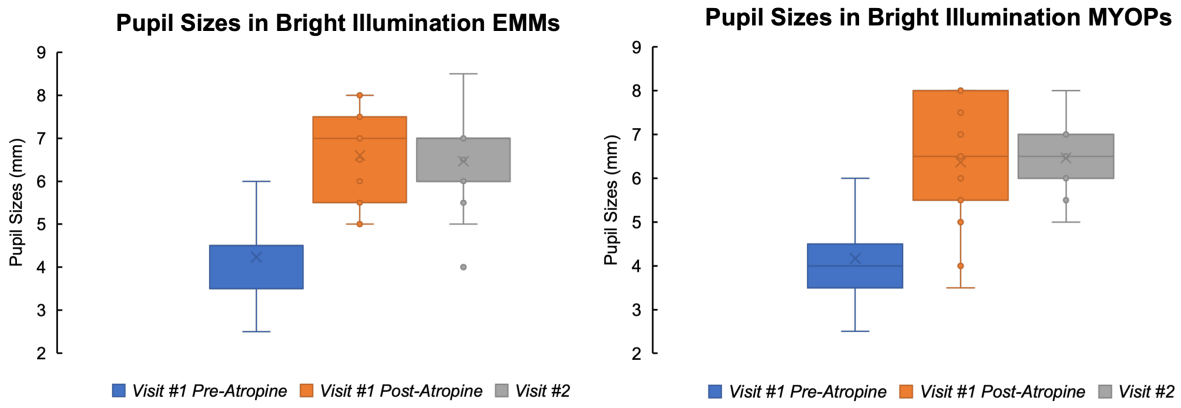


Figure 4.10. Boxplots with pupil sizes under bright illumination conditions for EMMs (left) and MYOPs (right) at each visit.

Lastly, for Near pupil sizes there was also a significant overall effect of visits F = 110.257, p < 0.001), with larger pupils in *Visit #1 Post-atropine* compared to *Visit #1 Pre-atropine* (Mean estimated difference = 2.055 mm, p < 0.001) and *Visit #2* compared to *Visit #1 Post-atropine* (Mean estimated difference = 2.050 mm, p < 0.001), but not *Visit #1 Post-atropine* to *Visit #2* (Mean estimated difference = 0.005 mm, p = 0.98). No correlation was found between pupil sizes at near and static or dynamic accommodation responses (p > 0.50 for all).

4.3.5. Glare

A significant effect of visits on stray light ($F = 14.94$, $p < 0.001$) was noted, with no overall effect of visits and RE group ($F = 3.10$, $p = 0.062$). Stray light increased from *Visit #1 Pre-atropine* to *Post-atropine* (Mean estimated difference 0.10 log, $p = 0.001$), and it was also larger at *Visit #2* compared to *Visit #1 Pre-atropine* (Mean estimated difference 0.14 log, $p < 0.001$), with no differences between *Visit #1 Post-atropine* and *Visit #2* (Mean estimated difference 0.04 log, $p = 0.25$) (Figure 4.11).

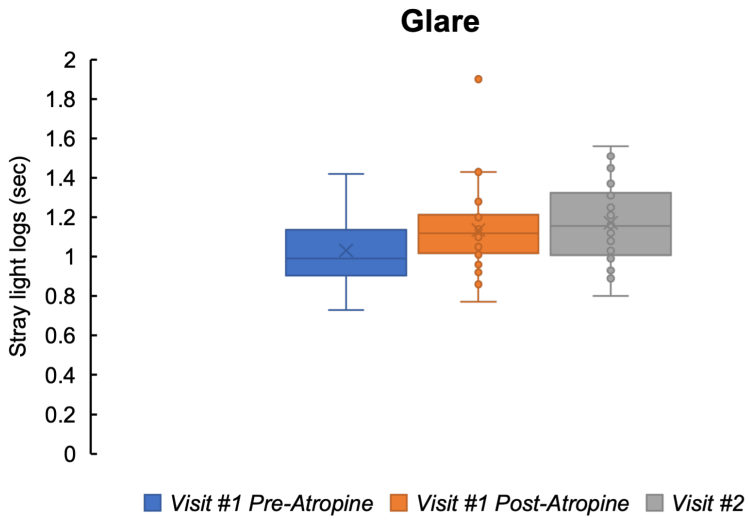


Figure 4.11. Boxplot stray light for all subjects at each visit.

When split into RE groups, the effect was only found in EMMs ($p < 0.001$), not in MYOPs ($p = 0.076$), although there was a trend. The standard deviation of the individual measuring points (Esd) and reliability coefficient (Q) was acceptable (Mean estimated Esd in EMMs = 0.072 and in MYOPs = 0.063).

4.3.6. Reading

General linear models with repeated measures showed a significant effect of visits for Radner reading speed (F = 6.007, p = 0.007), with no effect of visits * RE group (F = 0.800, p = 0.460). Radner speed was an estimated 7.731 secs faster in *Visit #2* compared to *Visit #1 Pre-atropine* (p = 0.002) and 5.760 secs faster in *Visit #2* compared to *Visit #1 Post-atropine* (p = 0.042).

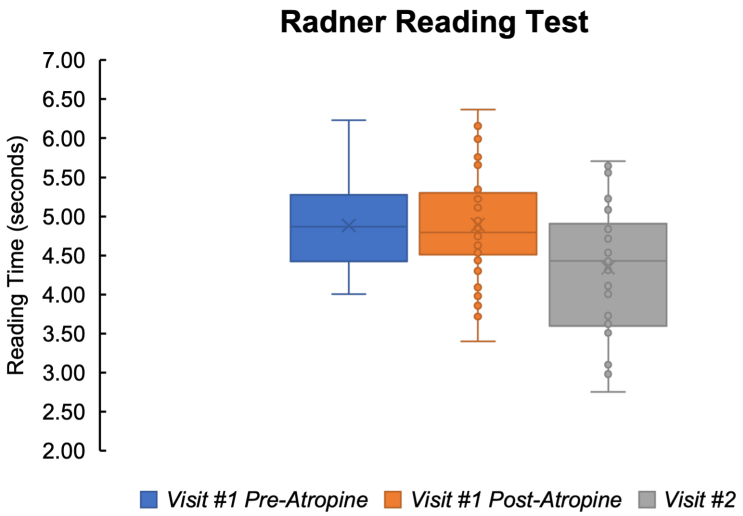


Figure 4.12. Boxplot showing Radner reading times for all subjects at each visit.

When divided into EMMs and MYOPs, the difference is only significant for EMMs (p = 0.011), not for MYOPs (p = 0.265).

Similarly, for the New York Public Library reading test, reading times were shorter after atropine use (F=121.163, P<0.001), likely due to a learning effect and habituation. Pairwise analyses showed estimated mean differences of 35.584 secs faster in *Visit #1 Pre-atropine* compared to *Visit #1 Post-atropine* (p < 0.001), and 37.978 secs faster in *Visit #1 Post-atropine* compared to *Visit #2* (p < 0.001).

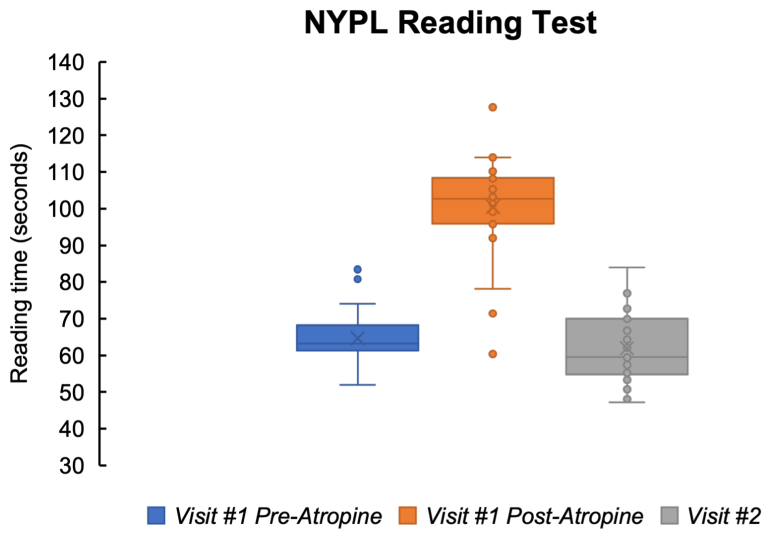


Figure 4.13. Boxplot for NYPL reading times for all subjects at each visit.

When divided into EMMs and MYOPs, the differences were very similar in each group. No significant differences in reading times were noted between EMMs and MYOPs in either test (Radner: $p = 0.276$; New York Library: $p = 0.954$).

Reading test errors were very small for both reading tests (Radner: 1.89 ± 2.20 errors; New York Library: 0.06 ± 2.50 errors) and showed no significant effect of visits.

4.3.7. Questionnaires

Each subject was asked 6 questions on difficulty with near work and 7 questions on symptoms of light sensitivity (Appendices I and II). If the subject answered yes to one of the questions, they were asked to rate their symptoms on a scale of 1-10. Overall, the subjects noted the side effects of using atropine to be greater after using atropine for a week. More of the subjects noted symptoms of light sensitivity than near blur. The subjects were asked "Do you generally have symptoms of light sensitivity such as: bothered sunlight or glare?" with Yes = 1 and No = 0. These worsen from *Visit #1 Pre-atropine* (0.17 ± 0.38) to *Visit #1 Post-atropine* (0.47 ± 0.51) to *Visit #2* (0.47 ± 0.51). The glare symptoms of the subjects who

answered Yes were asked to rate severity from 1-10; there was an increase in symptoms from *Visit #1 Pre-atropine* (0.03±0.18) to *Visit #1 Post-atropine* (0.17±0.38) to *Visit #2* (0.17±0.38).

4.4. Discussion

As expected, static accommodation responses were reduced with the use of low dose atropine eye drops. The effect was significant short-term (*Visit #1 Pre-atropine* to *Visit #1 Post-atropine*) and long-term (*Visit #1 Post-atropine* to *Visit #2*), although the effects were larger after 1 week of use of atropine. MEM retinoscopy, a clinical measure of static accommodation responses at near, showed similar effects but no differences within *Visit #1*, so it may not be as effective as detecting changes in accommodation with the use of atropine in clinic.

We show for the first time that dynamic accommodation is also affected by the use of atropine. The maximum response during the dynamic accommodation test was decreased after atropine, more after 1 week of use. However, the phase of the accommodation response was not affected by the use of atropine eye drops.

Both static and dynamic accommodation responses were significantly more accurate for this group of young adult subjects in those with emmetropia compared to those with myopia. Interestingly, when the analyses were repeated with subjects split into EMMs and MYOPs, the effect of visits on static and dynamic accommodation responses was significant for all visits in EMMs, but not in MYOPs, perhaps because their response was rather small to start with (baseline).

Whether the effect in accommodation caused any changes in binocularity cannot be ascertained from our data. All subjects had normal cover test and NPC values at baseline, and no changes were noted.

With the decrease in static accommodation and maximum dynamic accommodation, we would expect reading speed would also be slower with the use of atropine. However, we show that the average Radner reading speed was significantly faster in *Visit #1 Post-atropine* and *Visit #2* compared to *Visit #1 Pre-atropine*. These results may be due to subjects learning the methods of the Radner reading test and subjects quickly deciding to stop reading right away when they could not see the text. The New York Public Library reading test correlated with increased reading time in *Visit #1 Post-atropine* and Visit #2 compared to *Visit #1 Pre-atropine*, so subjects read slower in this test after the use of atropine, likely due to the decrease in accommodation and increase in pupil diameter. The two reading tests showed very different results, likely due to a variety of reasons. First, when subjects completed the Radner test, they stopped reading as soon as they were unable to see the small print. However, when subjects completed the New York Public Library reading test, they all continued to read until the end regardless of how blurry the near print may have appeared. The Radner test has a regular number of words and letters in each line while the New York Public Library reading test has a different number of sentences, words, and letters in each reading paragraph. Subjects also knew beforehand that there were comprehension questions to be answered after finishing the New York Public Library reading test, so they likely took their time to read the test to ensure they could answer the comprehension questions correctly. In conclusion, it appears that the use of low dose atropine eye drops may affect reading speed, at least when reading comprehension.

With the use of atropine eye drops, even at these low doses, we expected a significant increase in pupil diameter, which we noted under all conditions (dim, bright, and near). Interestingly, the effect was statistically significant short term (*Visit #1 Pre-atropine* to *Visit #1 Post-atropine*), but not long-term (*Visit #1 Post-atropine* to *Visit #2*), which indicates that possible side effects are likely to improve with time, as previously suggested (Yam et al. 2020). The effect of atropine on pupil sizes was not different between EMMs and MYOPs, and were not different their pupil sizes at baseline.

The increase in pupil sizes would be expected to cause an associated increase in glare. Indeed, stray light measurements were significantly increased short term (*Visit #1 Pre-atropine* to *Visit #1 Post-atropine*), but similarly to pupil sizes, not long term (*Visit #1 Post-atropine* to *Visit #2*). These results indicate an improvement in objective measurements of glare after a week of use of low dose atropine eye drops.

On the other hand, the subjective questionnaires delivered to subjects before, after, and during the use of atropine (Appendices I and IV), indicate that symptoms significantly increased during the week that subjects were using the atropine eye drops. (*Visit #1* compared to *Visit #*2), but they noted no differences short term (*Visit #1 Pre-atropine* compared to *Visit #1 Post-atropine*). This may be due to subjects staying in the lab during the entire *Visit #1*, they did not perform routine activities during that time as they did when they took the drops home for one week. Interestingly subjective symptoms were more affected by atropine than objective measures, which should be considered in clinical care and future studies.

5. Chapter 5: Low Dose Atropine and Retinal and Choroidal Thickness

5.1. Introduction

Emmetropization is the process whereby the ocular length adjusts to match the optical power of the eye. This axial elongation is associated with a thinning of the retina, choroid, and sclera that starts at the equator and is most prominent at the posterior pole. However, some studies have noted retinal thickness at the macula is independent of AXL (Jonas, Ohno-Matsui, and Panda-Jonas 2019). Previous studies have also hypothesized the relationship between retinal thickness and myopia progression, although findings differ. No studies have investigated the effect of atropine eye drops across a large area of the retina in humans.

As discussed in the general background chapter, multiple studies have hypothesized choroidal thickness thins with myopia progression and ocular elongation. This thinning of the choroid affects several normal mechanisms, including the choroid vasculature, crucial to nourish the outer retina. Thinning of the choroid causes a loss of vascular tissue, with possible collateral damage to the retina (Yan et al. 2017). For this and other reasons described in the background section, the study of choroidal thickness is important in understanding myopia etiology. Previous research studies on choroidal thickness have focused on subfoveal choroid or the extent of the macula (6 x 6 mm, or 20 x 20 degrees). These studies have noted a significant increase in subfoveal (Sander, Collins, and Read 2019; Yam et al. 2022) and also overall choroidal thickness, up to peripheral 20 degrees, with associated myopia progression control (Wang et al. 2022; Ye et al. 2020). Some have also divided the choroid into quadrants to evaluate changes in different locations (Wang et al. 2022; Ye et al. 2020). No studies have investigated the effect of atropine eye drops across a large area of the choroid in humans.

The choroid of the eye is primarily a vascular structure but also contains non-vascular smooth muscle cells (Nickla and Wallman 2010). The vasculature of the choroid is the major supply of the outer retina (Nickla and Wallman 2010). The thinning in choroidal thickness

noted in myopia and the thickening noted with the use of myopia control treatments move the retina backward and forward, respectively, potentially bringing the photoreceptor into the plane of focus. A function demonstrated by the thinning of the choroid occurs when the focal plane is moved back by the wearing of negative lenses (Nickla and Wallman 2010). Nickla et al. (2010) argued that choroidal thickness changes are also correlated with the changes in the growth of the sclera, and thus the myopic elongation of the eye. The choroid can control the growth of the sclera through 1. Secreting growth factors that stimulate or inhibit scleral growth in response to signals from the retina and RPE, 2. Differences in choroidal thickness may affect the molecular signals reaching the sclera because a thicker choroid may be a greater barrier to signals from the RPE or retina, or a thicker choroid may act as a sponge to facilitate access of molecules to the sclera, 3. Lateral area of choroid may mechanically influence the area of the sclera, and hence size of the globe (Nickla and Wallman 2010). When studying the choroidal thickness differences in myopes and emmetropes, it has been noted in myopes, the choroid progressively thinned in the macular region and beyond the parafovea towards the periphery (Hoseini-Yazdi et al. 2019). Myopic eyes have an excessive elongation of the eyes which can cause biomechanical stretching and thinning of the choroid, retina, and sclera (Teberik, Kuddusi; Kaya 2017).

5.2. Methods

We used a Heidelberg Spectralis Widefield Imaging Module OCT with a wide field (55 degrees) lens to take radial scan fundus images of all 30 subjects (Figure 5.1). Subjects were instructed to place their chin and forehead against the rests, then blink regularly before taking the image to maintain a good tear film for increased OCT image quality.

The large eye size was selected for the OCT to avoid touching the patient's eye. The OCT scan was set to an evenly-illuminated, well-focused fundus image when placed in the middle of the image window and the image was only taken when the signal-to-noise ratio (SNR) bar was blue with a value > 15. The ART function was set to a frame value that would

provide the best quality B-scan image. We waited until the entire ART function bar was filled before accepting an image.

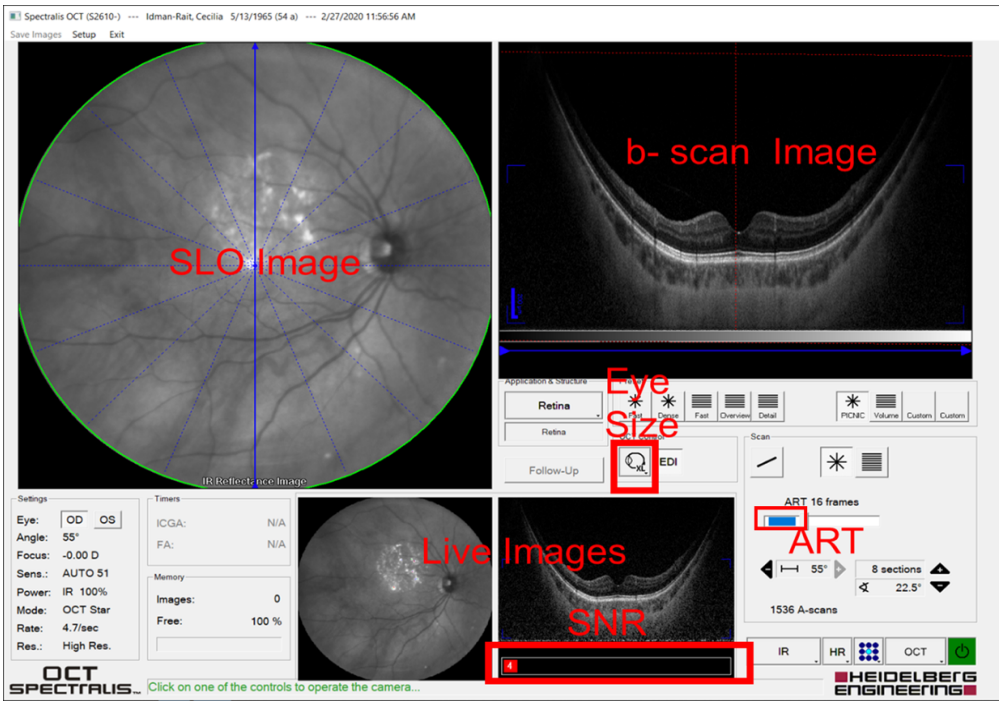


Figure 5.1. Screen capture of retinal and choroidal radial scan with widefield SLO image on Heidelberg Spectralis OCT.

We analyzed the raw images (.vol) obtained from the Heidelberg OCT using custom Matlab codes. First, we pre-process the OCT files by converting the files using a custom Matlab program and then conducted image segmentation with another Matlab custom program. Using a drawing pad and the radial scan segmentation program, we traced the internal limiting membrane (green line in Figure 5.2), photoreceptor integrity line at the RPE/Bruch's membrane boundary (white line in Figure 5.2), and choroid (pink line in Figure 5.2). The program measured the differences between each line to determine retinal and choroidal thicknesses. The thickness for the retina and choroid was calculated using the thickness calculation program. After each OCT image was segmented, a thickness calculation program was used to measure the retinal and choroidal thickness through the

global means thickness in the inferior, nasal, superior, and temporal quadrants (Figure 5.2 bottom) and in five different eccentricities (1mm, 3mm, 5mm, 8mm, and 12mm rings).

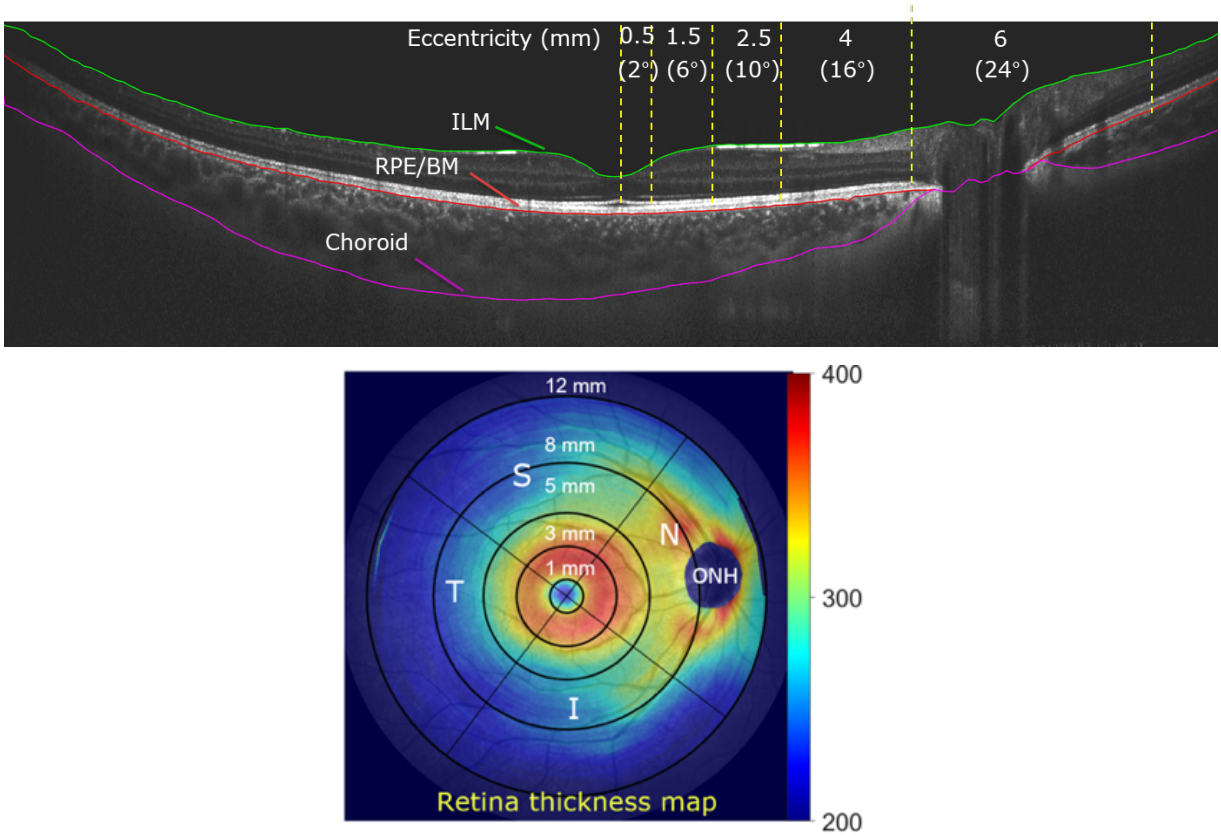


Figure 5.2. Top: sample scan segmentation used to measure the retinal and choroidal thickness (green = internal limiting membrane, red = RPE, pink = choroid). Bottom: sample thickness map of the retina with the regions for which the retinal thickness was calculated for (4 zones and 5 eccentricities).

5.3. Results

Related-samples Friedman's two-way analysis of variance by ranks showed no significant changes in retinal thickness for any of the locations studied (Temporal: $p = 0.648$, Nasal: $p = 0.122$, Inferior: $p = 0.195$, or Superior: $p = 0.792$), although there was a trend of thicker retinae at *Visit #2* compared to *Visit #1 Pre-atropine* in the Temporal ($p = 0.06$), Nasal ($p =$

0.05), and Inferior (p = 0.09) retina (Figure 5.3). When split into RE groups, MYOPs showed a trend towards thinning in the nasal retina with atropine (p = 0.155), whereas EMMs did not (p = 0.420).

We also analyzed changes in retinal thickness at 1mm, 3mm, and 5mm from the fovea. At 1mm, there was an overall effect of visits (p = 0.036) with a significant increase from *Visit #1 Post-atropine* to *Visit #2* (p = 0.010) for all subjects. At 3mm, there was no overall effect of visits (p = 0.174), but approached significance for thicker at *Visit #2* compared to *Visit #1 Post-atropine* (p = 0.082). At 5mm, there was no overall effect of visits (p = 0.185), but approached significance for thicker at Visit #2 compared to Visit #1 Post-atropine (p = 0.066).

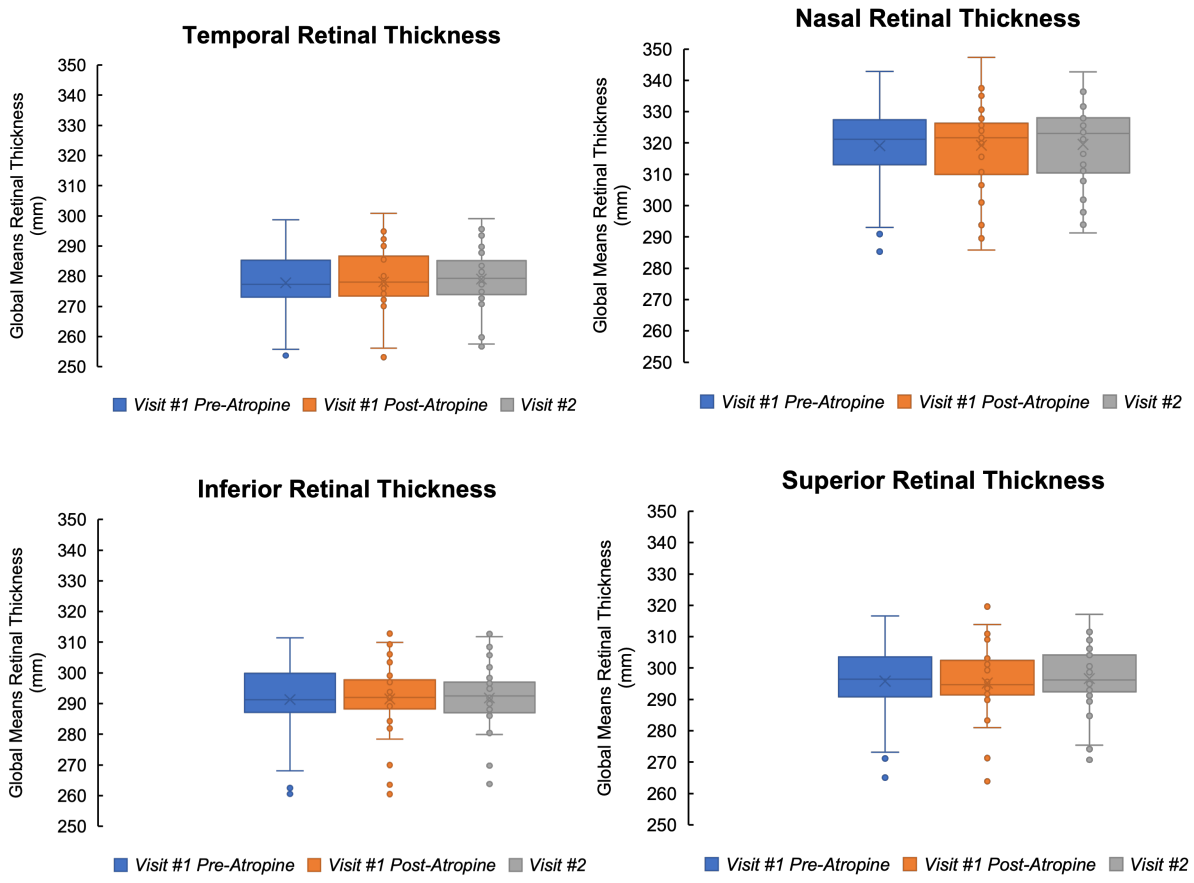


Figure 5.3. Retinal thickness for all subjects in the temporal, nasal, inferior, and superior quadrants.

When split into RE groups, MYOPs approached an overall significant effect of atropine ($p = 0.070$) inducing increased retinal thickness from *Visit #1 Pre-atropine* and *Visit #1 Post-atropine* ($p = 0.028$). There was no overall significance when split into RE groups.

Choroids were also not significantly affected by the use of low dose atropine for any of the four quadrants studied (Temporal: $p = 0.670$, Nasal: $p = 0.875$, Inferior: $p = 0.177$, or Superior: $p = 0.905$), although there was a trend toward thicker choroids at *Visit #2* compared to *Visit #1 Pre-atropine* in the Inferior choroid retina ($p = 0.07$) (Figure 5.4).

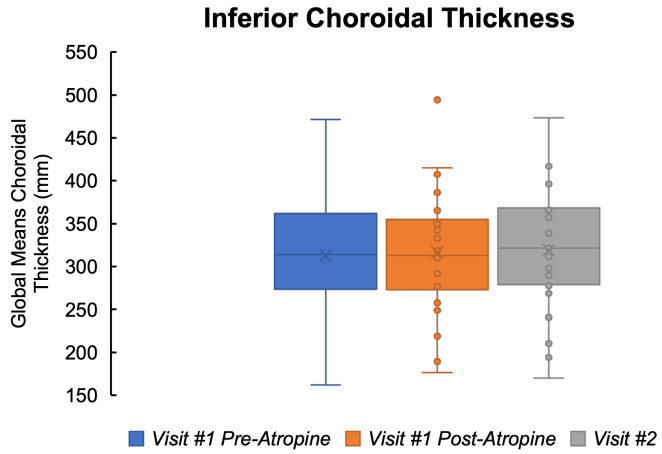


Figure 5.4. Change in choroidal thickness in the inferior quadrant for all subjects.

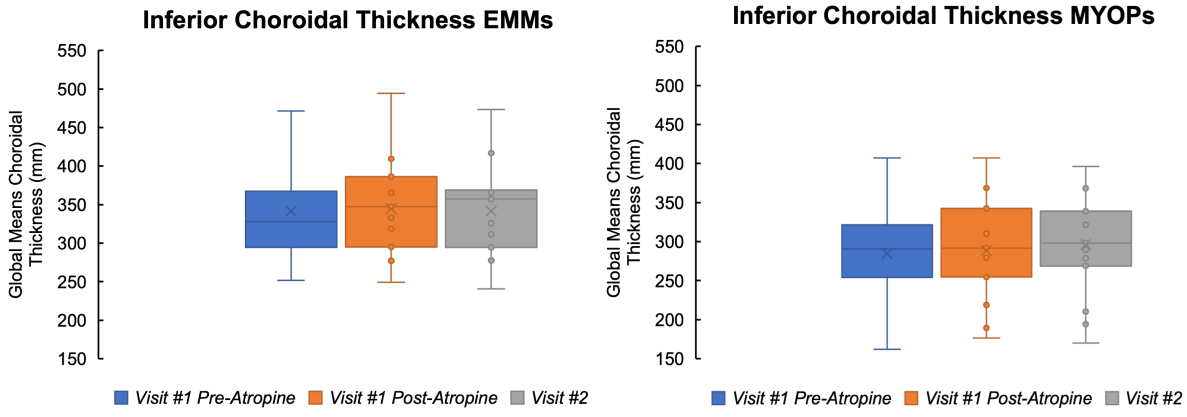


Figure 5.5. Significant thickening in inferior choroidal quadrant in myopes.

When split into refractive groups, it was noted this trend of thickening in the inferior quadrant in the overall subjects was due to the effect of the MYOP group ($F_{15,2} = 5.733$, $p = 0.057$) (Figure 5.5), no trend was noted in EMMs ($F_{15,2} = 1.333$, $p = 0.936$). There was no significant change in Nasal or Superior choroidal thickness in EMMs (Nasal: $F_{15,2} = 0.533$, $p = 0.766$; Superior: $F_{15,2} = 0.400$, $p = 0.819$) or MYOPs (Nasal: $F_{15,2} = 1.600$, $p = 0.449$; Superior: $F_{15,2} = 0.576$, $p = 0.652$). There was also no significant change in Temporal choroidal thickness in EMMs ($F_{15,2} = 3.600$, $p = 0.165$), but there was a significant thickening in MYOPs ($F_{15,2} = 7.600$, $p = 0.022$) (Figure 5.6).

When analyzing changes in choroidal thickness at different eccentricities we noted no effect of atropine at 1mm ($p = 0.875$), 3mm ($p = 0.587$), or 5mm ($p = 0.648$). There was no significance when split into RE groups, but MYOPs approached significance between *Visit #1 Post-atropine* and *Visit #2* at 1mm ($p = 0.068$) and had significantly thicker choroid for the 3mm ring ($p = 0.028$). There was no effect at 5mm ($p = 0.282$).

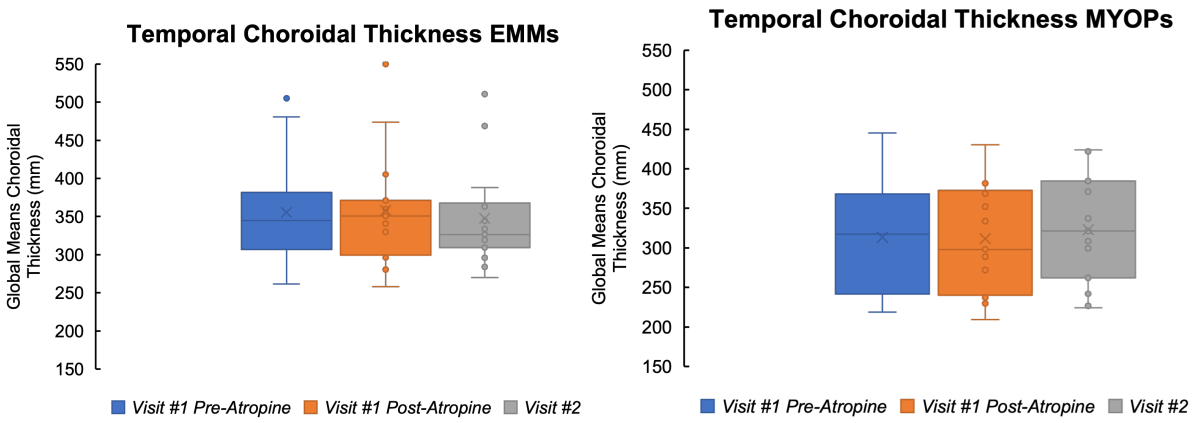


Figure 5.6. Significant thickening in temporal choroidal quadrant in myopes.

5.4. Discussion

There is a generally accepted hypothesis that atropine works directly or indirectly on the posterior choroid, retina and/or sclera, ultimately inhibiting thinning or stretching of the sclera, thereby inhibiting eye growth (Chia, Lu, and Tan 2016). Most previous studies, including the recent one by Wang et al (2022), noted no changes in intrafoveal retinal thickness after young myopic children used 0.01% atropine. On the other hand, others, such as a recent study by Liu et al (2022) who injected 2% atropine in guinea pigs, noted significantly increased total retinal and outer retinal thickness. In our studies in humans, when the entire retina was divided in four quadrants, we found no significant changes in retinal thickness with low dose atropine drops in the central 55 degrees for any quadrants, so no effect in retinal thickness overall. However, when divided into rings of various eccentricities, we did find a significant retinal thickening with the use of low dose atropine eye drops but only in the central area (intrafoveal, 1mm), which may be the main reason for the discrepancy among previous studies, other reasons may be the dose of atropine used.

When subjects were divided into refractive groups, we noted an interesting trend for thicker retinae after one week in the temporal, nasal and inferior retina but only in MYOPs, not in EMMs. Myopic retinae also showed thickening for the 1mm rings. These results are likely a consequence of subjects with myopia having thinner retinas to begin with, subjects with emmetropia do not seem to have more room to thicken.

The effect of atropine on the choroid has been investigated extensively. Nickla et al (2019) first noted that atropine prevents excessive lens-induced ocular elongation in chicks. In another study, Nickla et al (2013) injected 20μL of atropine into a chick eye wearing a -10D lens for 4 days. Their study showed results of choroidal thickening and inhibition of development of myopia in the negative lens-wearing eyes. The effect of myopia is thinning of the choroid. Findings noted in some studies of human subjects using low dose atropine eye drops, which have been shown to significantly increase subfoveal choroidal thickness (Sander, Collins, and Read 2019); Yam et al. 2022). The effect of Atropine is thickening of

the choroid. It was expected that Atropine would thicken the choroid in myopic subjects, which aligns with our findings.

A few studies have also investigated the effect of atropine eye drops in the peripheral choroid, not only subfoveal. For example, Ye et al (2020) noted significant increase in overall average choroidal thickness with higher doses of atropine (1%), which is higher than those doses used in myopia control.

All these studies have only included subjects with myopia, they have not included subjects with emmetropia. We studied subjects with emmetropia and myopia and did not find a significant increase in choroidal thickness overall after using 0.05% atropine eye drops for all subjects, but did note a trend toward thicker choroids at *Visit #2* compared to *Visit #1 Pre-atropine* in the Inferior choroid ($p = 0.07$). No effect was noted in the entire group of subjects when the choroid was divided into rings of various eccentricities.

However, when we investigated the difference between refractive errors, we noted that although EMMs showed no changes in choroidal thickness, MYOPs showed a significant increase in choroidal thickness in the temporal quadrant and a trend towards inferior choroidal thickening. When examining the various distances from the fovea, the significant increase in choroidal thickness was found to be only in the 3mm ring in MYOPs. The differences between refractive groups may be a consequence of MYOPs having baseline thinner choroids, and therefore larger room for thickening. These results indicate a need to study the choroid in different areas than subfoveal and may have implications in the understanding of myopia etiology.

6. Chapter 6: Low Dose Atropine and Ocular Rigidity

6.1. Introduction

As discussed in the general background chapter, the role of IOP in the excessive eye growth that underlies myopia development has been the subject of many investigations (Chan et al. 2016; J. H. K. Liu et al. 2002; Manny et al. 2011), but the nature and extent of its influence remains poorly understood. Likely, factors related to *Ocular Rigidity* (OR) and other mechanical properties of the ocular globe are important in the development of myopia (McBrien and Gentle 2001). The pressure exerted on the ocular wall depends on the rigidity of its outer layers. It is known that OR is affected by RE. These factors may also be the cause of the controversial results found in measures of IOP in myopia (Schmid et al. 2003).

The parameter that we are ultimately interested in this study of myopia is OR. Previous studies have evaluated OR using various metrics, including IOP, age of the patient, AXL and CCT, or certain pathologic conditions of the eye (Agrawal et al. 1991; Drance 1960; Friedenwald 1937; Goldmann and Schmidt 1957; Pallikaris et al. 2005). The basic definition of OR refers to the association between ocular pressure and volume in the eyeball. OR is affected by the elastic properties of the sclera and the cornea, but also the vascular uveal layer. Several *in vivo* studies have examined OR via invasive techniques using conventional manometric pressure-volume methods and non-invasive techniques such as Schiotz tonometry and assessment of pulse amplitude and fundus pulsation (Hommer et al. 2008; Pallikaris et al. 2005; Schmid et al. 2003). He et al (2017) found that eyes with high myopia had a greater deformation amplitude, defined as the corneal apex movement in the anterior-posterior direction, compared to those with emmetropia. Sergienko and Shargorgoska also used external pressure on the eye to evaluate the biomechanical properties of the scleral coat (Sergienko and Shargorogska 2012). OR has also been previously evaluated using GAT, a technique that deforms the corneal apex and displaces volume from the anterior segment (Detorakis, Tsaglioti, and Kymionis 2015). These authors developed an algorithm for the calculation of OR based on differences in IOP and volume between the deformed and non-

deformed eye (Detorakis, Tsaglioti, and Kymionis 2015) using *Friedenwald's Equation* (Friedenwald 1954). This equation has been used to measure OR based on the pressure-volume relationship in the eye (Pallikaris et al. 2005), but it was determined from enucleated eyes, so it cannot be directly applied to living eyes. Several investigators (Eisenlohr, Langham, and Maumenee 1962; Silver, DM; Geyer 2000; Ytteborg 1960) tried to use manometric measurements in living human eyes to obtain an estimate of ocular rigidity, but the sample sizes were small. Nevertheless, it can be used indirectly through the use of both applanation and indentation tonometry (Pallikaris et al. 2005). Using this method, OR has been shown to increase with higher IOP; a larger volume increment for a larger pressure was provided by *Friedenwald's Equation* (Pallikaris et al. 2005; Silver, DM; Geyer 2000). In this study, we measured ocular rigidity, among other methods, using *Friedenwald's Equation* for GAT (applanation) and Icare (indentation) tonometry. For their calculations, Detorakis et al (2015) included the use of biometric measures such as axial length (AXL), corneal curvature (K), central corneal thickness (CCT) as well as measurements of the maximal eyeball diameter (corresponding to the equatorial region) along both transverse (TD) and coronal (CD) planes), with OCT. All these parameters will be investigated in the proposed study.

We also plan to measure IOP at the cornea and different scleral zones and with these measures compute the ocular wall stress. *Scleral Wall Stress*, the pressure exerted on the outer wall of the eye (Schmid et al. 2003), has been proposed as a parameter to evaluate ocular rigidity. Schmid et al (2003) suggested that eye wall stress is not different in children with and without myopia, but there is a significant correlation between the myopic RE shift and *Scleral Wall Stress* in myopic children (Schmid et al. 2003). We will use the same modified Laplace's Equation they used to evaluate the *Scleral Wall Stress* before and after the use of low dose atropine eye drops.

Another very relevant work for our purposes is that of Mesquita et al (2020). They defined the region of interest for the changes associated with myopic elongation as located in depth from the episclera-sclera boundary to the sclera-ciliary muscle boundary, and laterally from the apex of the ciliary muscle to the episclera (Mesquita et al. 2020). They used this

area to calculate scleral and ciliary muscle "attenuation coefficients" that have been shown to correlate with differences in scleral collagen ultrastructure and vasculature (Mesquita 2017). The *Attenuation Coefficient* describes how quickly light is attenuated when passing through the sclera or ciliary body, as analyzed with anterior segment OCT (S. Chang and Bowden 2019), and is derived from the slope of the linear fit of the averaged intensity profile of 100 A-scan lines in a region of interest (Mesquita et al. 2020). It has been suggested REs contribute to the individual variability found in the *Scleral Attenuation Coefficient*; a higher amount of myopia had a higher scleral attenuation coefficient (Mesquita et al. 2020). Thickness of the ciliary muscle may also be affected by RE due to internal equatorial growth restriction in myopia, and poor contractility due to muscular hypertrophy (Jeon et al. 2012). It is known that the ciliary muscle in myopic eyes moves more, but thickens less anteriorly than the emmetropic ciliary muscle (Wagner, Zrenner, and Strasser 2019). While ciliary muscle shape, movement, and thickness differences have been shown with RE, this effect does not seem to translate to accommodation responses (Wagner, Zrenner, and Strasser 2019). In our study, we measured scleral and ciliary body dimensions and calculated *Scleral* and *Ciliary Body Attenuation Coefficients*.

Atropine is thought to reinforce the sclera in myopia by increasing the extracellular matrix biosynthesis (Cristaldi et al. 2020). We investigate OR before and after the use of low dose atropine to determine if this variable provides insights into the etiology of myopia and the mechanism of atropine in myopia control.

6.2. Methods

The same Heidelberg OCT described in Chapter 5 was used to examine the anterior segment, after replacing the 55 degrees wide angle lens with the anterior segment lens attachment. We took various anterior segment OCT measurements on the right eye of each subject. First, we took corneal scans to evaluate CCT (Figure 6.1), we took 5 scans to ensure at least one was centered.

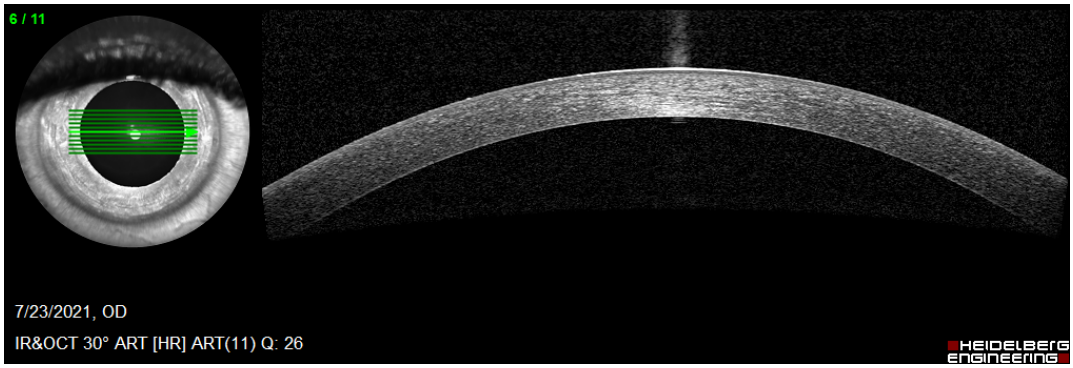


Figure 6.1. Corneal thickness scan on a Heidelberg OCT.

We then took scans (x60 using the AT ANG setting) of the temporal angle, with the OCT segment line centered over the limbus (Figure 6.2). For this, the subject was asked to look at an external fixation light to the left of the instrument.

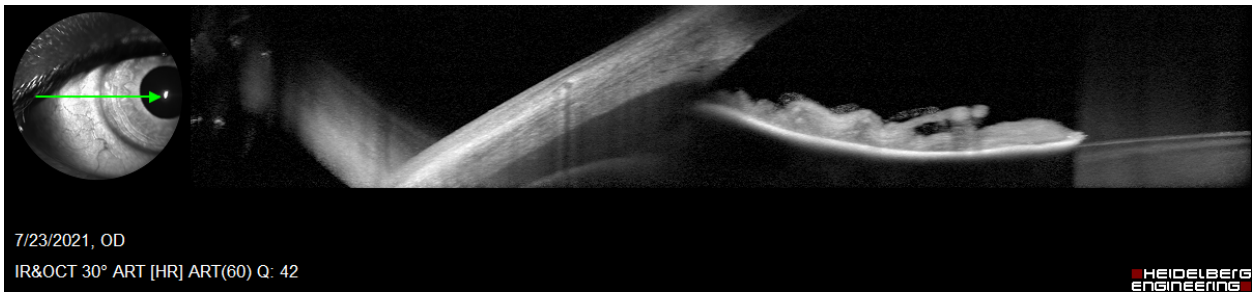


Figure 6.2. Angle scan on Heidelberg OCT.

Lastly, we took scans at various scleral locations that correspond to the locations we measure IOP with the Icare (similarly to the method described in Chapter 2). We started measuring OCT scans (x60, using AT SCL setting) in the temporal sclera, at 5mm from the limbus (Figure 6.3). We then repeated for nasal sclera (5mm from the limbus), superior sclera (4mm from the limbus), and inferior sclera (4mm from the limbus).

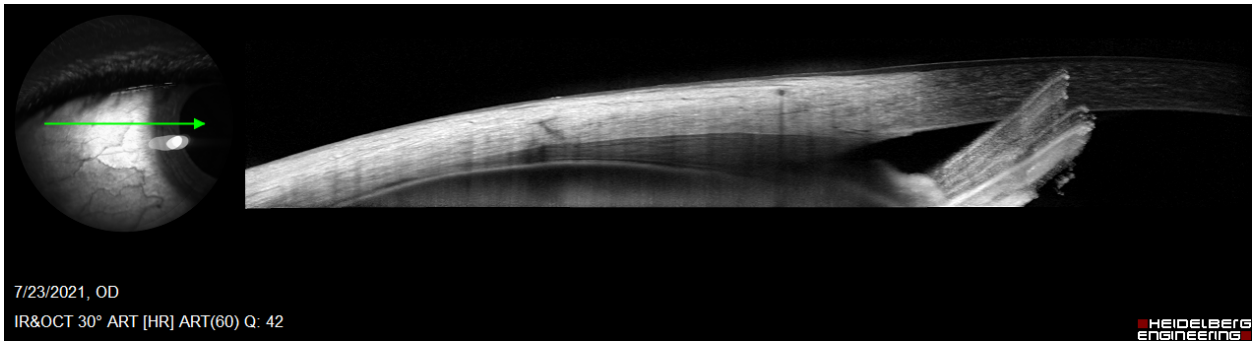


Figure 6.3. Scleral thickness scan on Heidelberg OCT.

To increase the precision of the scleral locations used and ensure that these matched the IOP measurements, we used a sterile surgical marker to mark the exact locations to be tested (measured with a flexible ruler). The marked dots were visible in the image obtained with the OCT, so the examiner was able to center the measurements at that spot.

All scans were assessed for quality and repeated when needed. Immediately after taking the OCT image, the Icare was used to measure IOP at each of the marked points. Raw images were exported as jpg files with measurements in pixels. Since the current OCT Heidelberg program does not give us the anterior segment images in microns, we were only able to get measurements in pixels. We calculated a ratio to determine the scleral thickness in microns by using the OCT cornea thickness images. Each cornea .jpg file (Figure 6.4, left) was uploaded into the Image J program and the number of pixels in the center corneal thickness was measured three times and then averaged. Since we also had the values of the central corneal thickness from the Lenstar in microns, we were able to convert from pixels to microns. The sclera .jpg files were uploaded into the Image J program (Figure 6.4, right) to determine the average of the scleral thickness. From these, the various OR metrics described in the Introduction section (6.1.) were calculated as explained below.

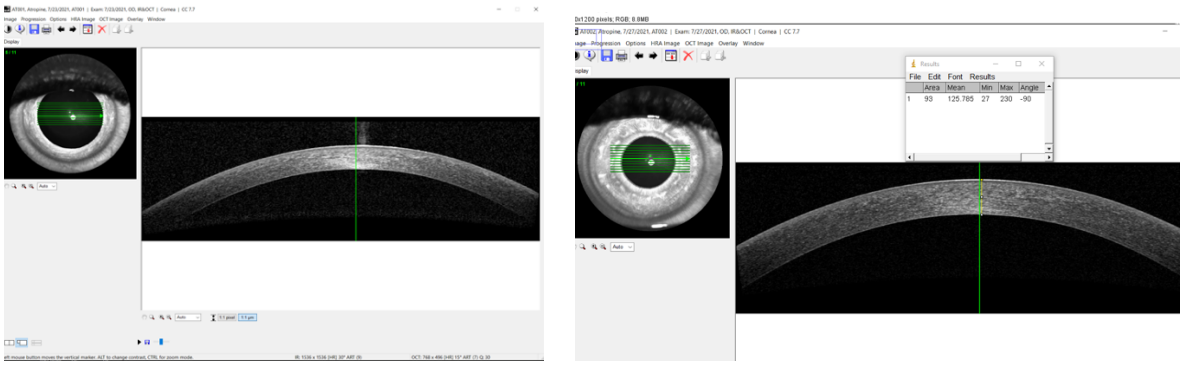


Figure 6.4. OCT Heidelberg Central Cornea Scan (left) and measurement in pixels using Image J (right).

6.2.1. Friedenwald's Equation

OR was computed using *Friedenwald's* transformation algorithms (Friedenwald 1937, 1954; Goldmann and Schmidt 1957; Patel et al. 2011) based on differences in pressure and volume between the deformed and non-deformed eye. We used the formula previously used by Detorakis et al (2015) for GAT, with the GAT values as the "deformed" values and the Icare values as the "non-deformed" values:

$$K = (\log IOP_1 - \log IOP_2) / (V)$$

where IOP_1 refers to the "deformed" values and IOP_2 refers to the "non-deformed" values of intraocular pressure (mmHg), V is the difference in volume expected to be displaced by GAT, which has been calculated to be approximately 0.50 µl, (Detorakis, Tsaglioti, and Kymionis 2015) and K is the calculated OR coefficient.

6.2.2. Scleral Wall Stress

Scleral wall stress is the pressure exerted on the outer wall of the eye and has been proposed as a parameter to evaluate ocular rigidity.

The modified Laplace's equation to evaluate the *Scleral Wall Stress* (Schmid et al. 2003) is:

$$P = IOP * r / 2t,$$

where r is the AXL divided by 2, t is the wall thickness, and P is the resulting *wall stress* coefficient.

6.2.3. Attenuation Coefficients

To calculate the *Attenuation Coefficients*, we loaded the raw angle scan (Figure 6.5, left) into ImageJ. The region of interest within the ciliary muscle was selected around a green line that was automatically located at the limbus when taking the scans (Figure 6.5). The region of interest had a width of 100 units (from 50 each way of the green line) and a height of 50 (Figure 6.5). The image needed to be rotated 90 degrees for the graph to be plotted with the intensity average along the x-axis for the scleral horizontal cross-sections (Figure 6.6). The profile of the image was plotted before adding a line of best fit. The b-value, slope, of the line of best fit, was noted to calculate -1/b, slope for the scleral attenuation coefficient (Figure 6.6). The gray value of the image is the intensity profile of pixels in the region of interest (Figure 6.6).

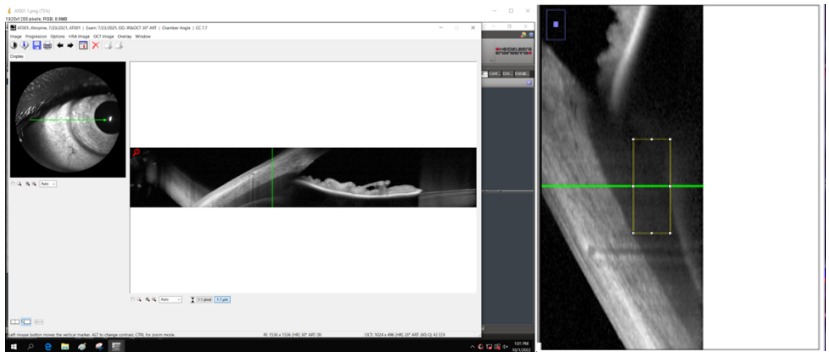


Figure 6.5. OCT Heidelberg Angle Scan (left) and Ciliary Muscle region of interest (right).

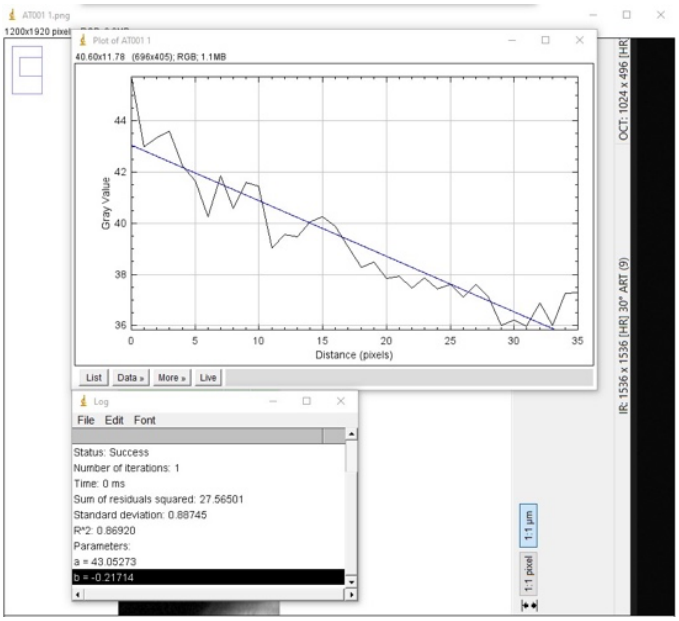


Figure 6.6. Plot of Intensity Profile in Region of Interest on ImageJ

We used the Laplace's equation to calculate *Scleral Attenuation coefficients* (Mesquita et al. 2017; Mesquita et al. 2020).

6.3. Results

The 30 participants who completed the study had complete reliable data for this section of the study.

6.3.1. Cornea

Related-samples Friedman's two-way analysis of variance by ranks with post-hoc analyses showed a **significant thickening of CCT** ($F_{30,2} = 7.60$, $p = 0.022$) in all subjects

overall. When comparing *Visit #1 Pre-atropine* and *Visit #1 Post-atropine*, a trend towards thinner CCT was noted ($p = 0.07$), whereas, between *Visit #1 Pre-atropine* and *Visit #2*, the trend was towards thicker CCT ($p = 0.05$). When divided into refractive groups, the effects were no longer significant for either EMMS ($F_{15,2} = 4.750$, $p = 0.093$) or MYOPs ($F_{15,2} = 4.966$, $p = 0.084$), likely due to smaller group sizes.

6.3.2. Central and Scleral IOP

Related-samples Friedman's two-way analysis of variance by ranks with post-hoc analyses on central IOP measured with GAT showed no overall significant change in ($F_{30,2} = 2.10$, $p = 0.350$). However, when central IOP was measured with Icare, a significant decrease in IOP was noted after atropine use ($F_{30,2} = 8.29$, $p = 0.016$). Post-hoc analysis showed this was due to the difference between *Visit #1 Pre-atropine* and *Visit #1 Post-atropine* ($p = 0.029$), with no difference between *Visit #1 post-atropine* and *Visit #2*. When the analyses were conducted with the refractive groups split, no effect was noted in either group in central IOP measured with GAT (EMMs: $F_{15,2} = 4.625$, $p = 0.099$; MYOPs: $F_{15,2} = 0.040$, $p = 0.980$). There was also no significant change in Icare central IOP in EMMs ($F_{15,2} = 4.360$, $p = 0.113$) or MYOPs ($F_{15,2} = 4.189$, $p = 0.123$).

Analyses of scleral IOP show no overall effect of atropine at any eccentricity ($F_{30,2} = 3.54$, $p = 0.170$), with no significant effect in Nasal ($F_{30,2} = 2.313$, $p = 0.315$), Inferior ($F_{30,2} = 2.99$, $p = 0.224$) or Superior ($F_{30,2} = 4.54$, $p = 0.103$) sclera IOP. When divided into refractive groups, no overall scleral IOP effect of atropine was noted (EMMs: $F_{15,2} = 1.393$, $p = 0.498$; MYOPs $F_{15,2} = 2.582$, $p = 0.282$). Although there was no overall significant change in Nasal scleral IOP, there was significantly higher Nasal IOP from *Visit #1 Post-atropine* and *Visit #2* ($p = 0.036$) in MYOPs.

6.3.3. Sclera thickness

Related-samples Friedman's two-way analysis of variance by ranks with post-hoc analyses on anterior scleral thickness shows a significant thickening of the Temporal sclera ($F_{30,2} = 11.67$, $p = 0.003$) overall, with a larger effect 1 week after atropine use ($p = 0.004$). There were no significant changes in Nasal anterior scleral thickness ($F_{29,2} = 2.14$, $p = 0.343$), Inferior anterior scleral thickness ($F_{30,2} = 0.60$, $p = 0.74$), or Superior anterior scleral thickness ($F_{30,2} = 2.067$, $p = 0.356$), although all changed towards thicker values.

When divided into refractive groups, a significant increase in Temporal anterior scleral thickness with atropine use was noted only in EMMs ($F_{15,2} = 11.200$, $p = 0.004$), not in MYOPs ($F_{15,2} = 2.533$, $p = 0.282$) (Figure 6.7). The largest increase was noted after 1 week of use of atropine, between *Visit #1 Pre-atropine* and *Visit #2* ($p = 0.001$), with some increase in thickness between *Visit #1 Pre-atropine* and *Visit #1 Post-atropine* ($p = 0.085$), always in EMMs only. There was no significant change in Nasal, Inferior, or Superior sclera thickness in either refractive group.

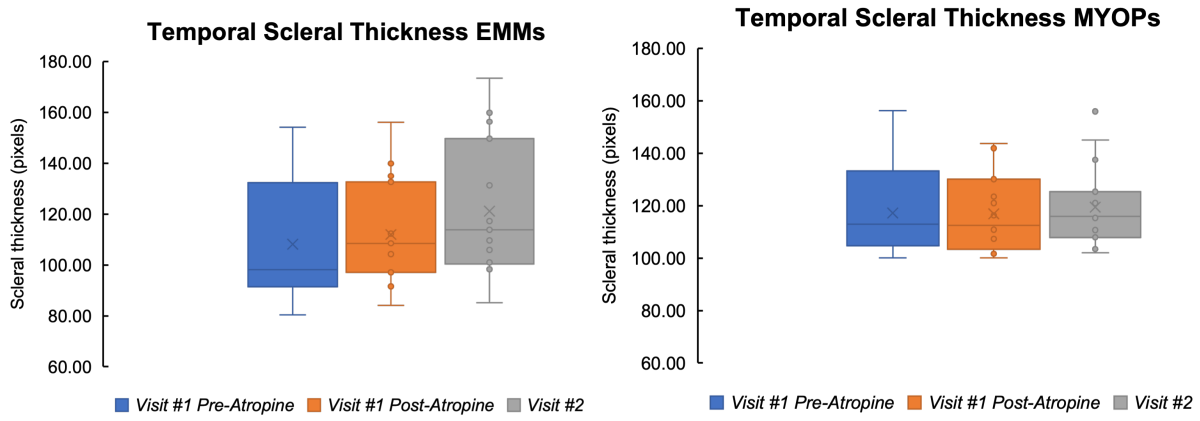


Figure 6.7. Significant thickening in temporal scleral thickness in emmetropes

6.3.4. Friedenwald's Equation

Related-samples Friedman's two-way analysis of variance within subjects' analyses showed no effects of visits overall ($F_{30,2} = 2.512$, $p = 0.101$) (Figure 6.8).

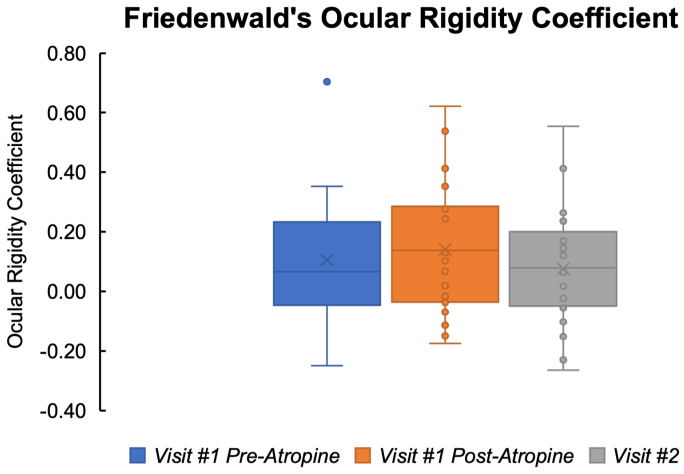


Figure 6.8. OR Coefficient for all subjects at each visit.

When split into refractive groups, there were also no significant differences (Mean estimated difference between groups = 0.141, $p = 0.086$) (Figure 6.9).

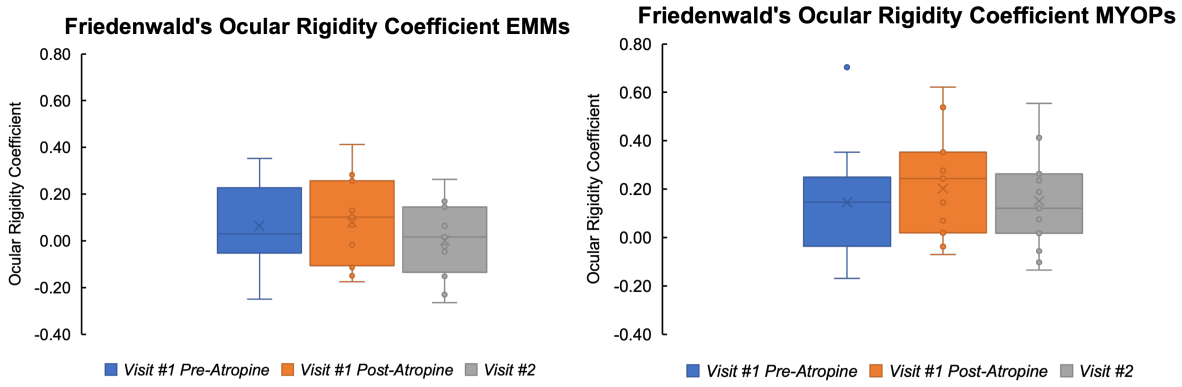


Figure 6.9. OR Coefficient in emmetropic subjects (left) and in myopic subjects (right).

6.3.5. Scleral Wall Stress

Related-samples Friedman's two-way analysis of variance by ranks with post-hoc analyses on scleral wall stress shows no effects of visits ($F_{30,2} = 4.373$, $p = 0.018$). When split into refractive groups, there were also no significant differences overall (Mean estimated difference = 1.425, $p = 0.391$).

Related-samples Friedman's two-way analysis of variance by ranks with post-hoc analyses on Temporal *Scleral Wall Stress* shows a significant effect of visits overall ($F_{30,2} = 6.661$, $p = 0.005$) (Figure 6.10). When split into refractive groups, there were also significant differences (Figure 6.11). In EMMs, the difference was significant between *Visit #1 Pre-atropine* and *Visit #2* ($F_{15,2} = 75.365$, $p = 0.40$), whereas in MYOPs the difference was significant between *Visit #1 Pre-atropine* and *Visit #2* ($F_{15,2} = 76.228$, $p = 0.044$) but also between *Visit #1 Pre-atropine* and *Visit #1 post-atropine* ($F_{15,2} = 76.670$, $p = 0.018$). There were no significant differences in Temporal *Scleral Wall Stress* between EMMs and MYOPs overall.

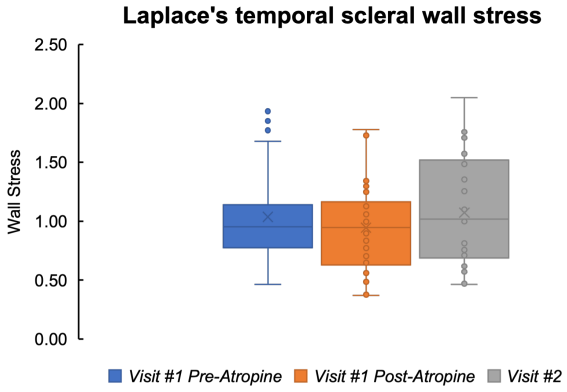


Figure 6.10. Temporal *Scleral Wall Stress* for all subjects.

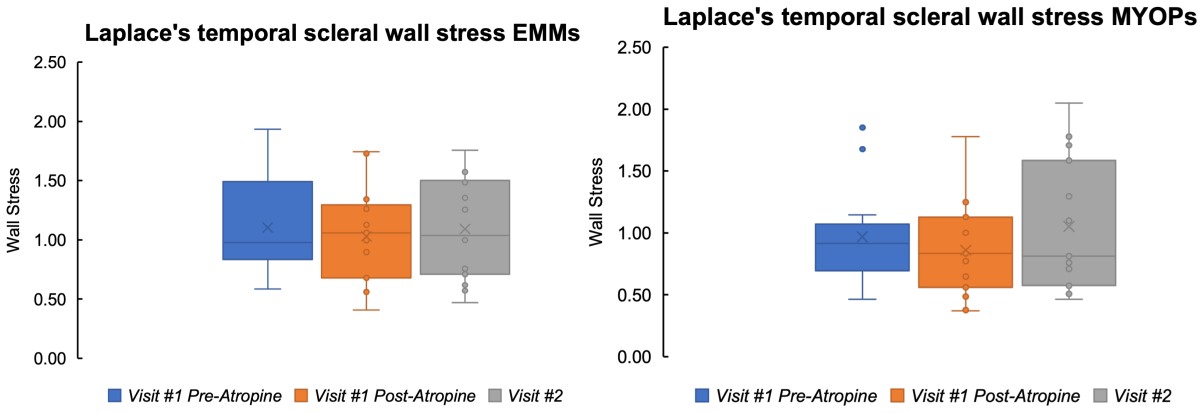


Figure 6.11. Temporal Scleral Wall Stress for EMMs (left) and MYOPs (right).

Analyses of Nasal *Scleral Wall Stress* also showed a significant effect over visits overall ($F_{30,2} = 5.248$, $p = 0.012$) (Figure 6.12). When split into refractive groups, there was a significant difference between *Visit #1 Pre-atropine* and *Visit #1 Post-atropine* ($F = 91.175$, $p = 0.003$) only in MYOPs, with no differences in EMMs (Figure 6.13). There were no overall significant differences in Nasal Temporal *Scleral Wall Stress* between EMMs and MYOPs.

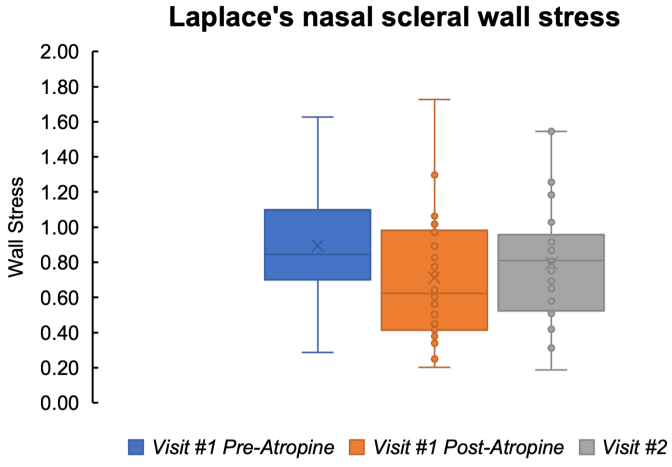


Figure 6.12. Nasal Scleral Wall Stress for all subjects.

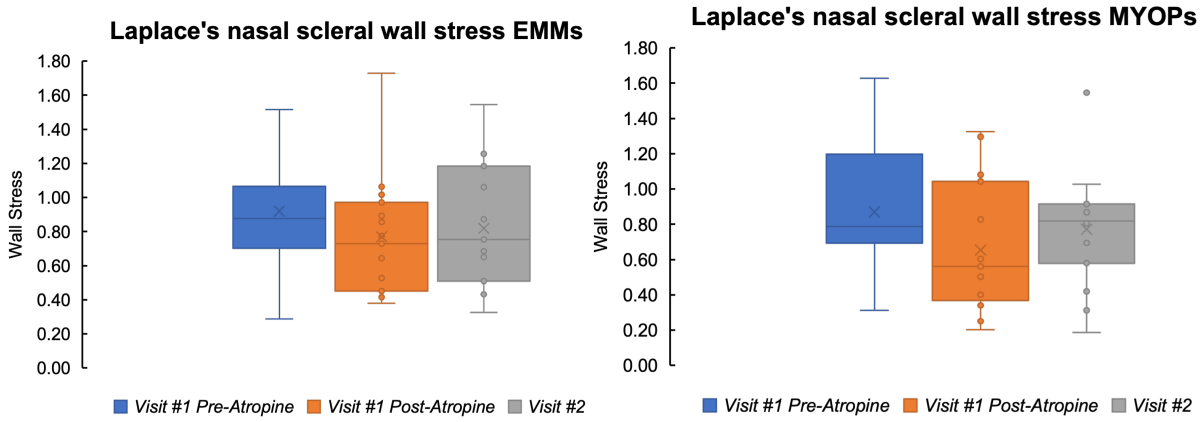


Figure 6.13. Nasal *Scleral Wall Stress* for EMMs (left) and MYOPs (right).

No overall significant effect of atropine was noted on Inferior *Scleral Wall Stress* ($F_{30,2}$ = 1.860, p = 0.175). However, when divided into refractive groups, MYOPs showed a significant increase between *Visit #1 Pre-atropine* and *Visit #1 Post-atropine* ($F_{15,2}$ = 88.893, p = 0.035). The effect was not noted in EMMs. There were no overall significant differences in Inferior *Scleral Wall Stress* between EMMS and MYOPs.

Similarly, no overall significant effect of atropine was noted on Superior *Scleral Wall Stress* ($F_{30,2}$ = 1.534, p = 0.225). When split into refractive groups, we again noted a significant difference in MYOPs between *Visit #1 Pre-atropine* and *Visit #1 Post-atropine* ($F_{15,2}$ = 96.076, p = 0.033), with no effect in EMMs. There were no overall significant differences in Superior *Scleral Wall Stress* between EMMS and MYOPs.

6.4. Discussion

The results from this study showed a significant decrease in IOP with Icare. This effect was short-term between *Visit #1 Pre-atropine* and *Visit #1 Post-atropine* and therefore may

be related to the time of day or activities performed by subjects in between the two measurements rather than the effect of atropine.

The results from this part of the study show significant thickening of the temporal anterior sclera with the use of low dose atropine eye drops in all subjects, but more so in subjects with emmetropia. This result being only significant in the temporal sclera could simply be because this is the most visible part of the sclera and has the most contact with atropine drops when topically applied.

Interestingly, subjects with emmetropia showed more scleral thickening than those with myopia and the group of subjects with myopia did not show significant thickening. This may indicate emmetropic eyes have a different structure of the anterior sclera that may protect them against progression to myopia. It would be of interest to conduct these measurements in children who do not yet have myopia and follow them over time until they develop (or do not develop) myopia.

Friedenwald's transformation algorithms were used to calculate OR in the past using invasive techniques performed on enucleated eyes (Detorakis, Tsaglioti, and Kymionis 2015; Friedenwald 1937). Due to the impracticality of *Friedenwald's* techniques, Detorakis et al altered the original formula to be more easily applied to clinical data (Detorakis, Tsaglioti, and Kymionis 2015). We use the modified formula provided by Detorakis et al in this study, with GAT values as the "deformed" values and the Icare values as the "non-deformed" values. No changes in OR were noted using this method. Given that we did find changes in OR using other methods, as described below, we suggest that this method is not effective in determining OR in living human eyes.

Laplace's formula has also been used to calculate OR indirectly (*Scleral Wall Stress*). This technique may be more applicable to living human eyes and more clinically applicable. Using this method, we noted significant increases in the Temporal and Nasal *Scleral Wall Stress* in all subjects. This is very interesting because this is an indicator of OR changes with atropine, which was our hypothesis. From these findings, the use of low dose atropine eye

drops increases OR of the eye, which may be one of the mechanisms for how myopia progression is controlled. The Nasal and Temporal regions were most significantly affected, perhaps because when atropine drops are applied topically, these areas are more exposed. Interestingly, there were significant changes in the MYOPs group between *Visit #1 Pre-atropine* and *Visit #1 Post-atropine* in the inferior and superior scleral wall stress. This is unlikely due to the effect of atropine and rather the effect of the number of tests conducted on the subjects.

7. General Conclusions

The mechanism of atropine in myopia control has confounded researchers for decades. Recent hypotheses aim to examine how atropine affects several anterior and posterior segment structures and functions, including OR. To study OR, we needed to first evaluate the repeatability of measurements of IOP in the sclera at various positions from the limbus, which we completed with a pilot study. The pilot study showed that Icare measurements can consistently be used in the sclera at least at certain locations, to measure IOP. We hypothesized that results from this study may also indicate that scleral IOP may be a measure that can be used in clinic to assess the effect of atropine eye drops in myopia control, but our results did not support this hypothesis as we noted no significant changes in scleral IOP with 0.05% atropine use for of one week in the main study.

During the main study where subjects used daily use of 0.05% atropine eye drops for one week, we found that static and dynamic accommodation responses measured with an open-field autorefractor were decreased with atropine use, as expected, but not previously reported for dynamic responses (see Appendices VI and VII). We also included a clinical test, MEM, with the hypothesis that it would provide similar results to those found with the open-field autorefractor. However, our results indicate that this measurement is not reliable in determining the effect of atropine or whether patients are applying atropine when prescribed for myopia control. Atropine use had significant negative effects on reading and glare, which is expected but has not consistently been reported in previous studies, perhaps because children are less likely to report symptoms.

When retina and choroidal thickness were evaluated, we noted interesting results. As expected, the peripheral retina thickness measurements were not significantly affected by the use of low dose atropine eye drops. However, thicker intrafoveal retinae were noted after the use of atropine only in MYOPs short-term. The choroid thickness measurements were significantly thicker temporally and trended towards significance inferiorly after the use of atropine only in MYOPs. These measurements may be useful in clinics because OCTs are

commonly available in clinical care and could be used to measure the choroidal thickness effects of myopia control.

The most important result of these studies was the measure ocular rigidity because it has not previously been evaluated after the use of atropine in humans. We used all available techniques for humans, including a derivative of Friedenwald's formula by Detorakis et al (2015), Scleral Attenuation Coefficients (Mesquita et al. 2020), and the Laplace's *Scleral Wall Stress* equation. Friedenwald's formula was determined by enucleated eyes and is not easily used clinically. In our study, we did not note a significant change in OR with Detorakis et al derivative of Friedenwald's formula. Laplace's *Scleral Wall Stress* equation is more likely to be used clinically because the formula requires IOP, which is always measured at each visit, "r", which is the axial length measured easily with Lenstar, and "t" or wall thickness, which can be measured with OCT. With a stronger *Scleral Wall Stress*, the eye is more resistant to axial length growth and could indicate a decrease in myopia progression. In our study, we noted significant increases in wall stress temporally and nasally. This interesting finding could indicate one potential mechanism for atropine in the control of myopia progression. It also must be considered that our subjects have gone through the emmetropization process.

The mechanism of atropine for myopia control has been speculated for a long time. Our study shows anterior segment structures and functions can be used to monitor how atropine affects a patient's eye and can be used to monitor myopia progression. Accommodative responses, retinal and choroidal thicknesses, and ocular rigidity measurements can be taken before and during the treatment with atropine to monitor myopia control.

8. References

Abbott, Meredith L., Katrina L. Schmid, and Niall C. Strang. 1998. “Differences in the Accommodation Stimulus Response Curves of Adult Myopes and Emmetropes.” *Ophthalmic and Physiological Optics*.

Agrawal, K. K., D. P. Sharma, G. Bhargava, and D. K. Sanadhya. 1991. “Scleral Rigidity in Glaucoma, before and during Topical Antiglaucoma Drug Therapy.” *Indian Journal of Ophthalmology*.

Atchison, David A. 2006. “Optical Models for Human Myopic Eyes.” *Vision Research*.

Badakere, Swathi Vallabh et al. 2018. “Comparison of Scleral Tono-Pen Intraocular Pressure Measurements with Goldmann Applanation Tonometry.” *Optometry and Vision Science*.

Brusini, Paolo, and Maria Letizia Salvetat. 2021. “How to Measure Intraocular Pressure : An Updated Review of Various Tonometers.”

Bryant, Michael R., and Peter J. McDonnell. 1998. “Optical Feedback-Controlled Scleral Remodeling as a Mechanism for Myopic Eye Growth.” *Journal of Theoretical Biology*.

Bueno-Gimeno, Inmaculada et al. 2014. “Relationship among Corneal Biomechanics, Refractive Error, and Axial Length.” *Optometry and Vision Science*.

Chan, Michelle P.Y. et al. 2016. “Associations with Intraocular Pressure in a Large Cohort: Results from the UK Biobank.” *Ophthalmology*.

Chang, Lan et al. 2013. “Myopia-Related Fundus Changes in Singapore Adults with High Myopia.” *American Journal of Ophthalmology*.

Chang, Shuang, and Audrey K. Bowden. 2019. “Review of Methods and Applications of Attenuation Coefficient Measurements with Optical Coherence Tomography.” *Journal of Biomedical Optics* 24(09): 1.

Chia, Audrey, Qing Shu Lu, and Donald Tan. 2016. “Five-Year Clinical Trial on Atropine for the Treatment of Myopia 2 Myopia Control with Atropine 0.01% Eyedrops.” *Ophthalmology* 123(2): 391–99.

Cho, Pauline, and Qi Tan. 2019. “Myopia and Orthokeratology for Myopia Control.” *Clinical and Experimental Optometry* 102(4): 364–77.

Chua, Jacqueline, and Tien YinWong. 2016. “Myopia-the Silent Epidemic That Should Not Be Ignored.” *JAMA Ophthalmology*.

Cristaldi, Martina et al. 2020. “Atropine Differentially Modulates ECM Production by Ocular Fibroblasts, and Its Ocular Surface Toxicity Is Blunted by Colostrum.” *Biomedicines* 8(4): 1–11.

Cyphers, Ben, Juan Huang, and Jeffrey J. Walline. 2022. “Symptoms and Ocular Findings Associated

with Administration of 0.01% Atropine in Young Adults." *Clinical and Experimental Optometry* 00(00): 1–11. https://doi.org/10.1080/08164622.2022.2033603.

Davies, Leon N., Hannah Bartlett, Edward A.H. Mallen, and James S. Wolffsohn. 2006. "Clinical Evaluation of Rebound Tonometer." *Acta Ophthalmologica Scandinavica*.

Detorakis, Efstathios T., Emmanuela Tsaglioti, and George Kymionis. 2015. "Non-Invasive Ocular Rigidity Measurement: A Differential Tonometry Approach." *Acta medica (Hradec Králové) / Universitas Carolina, Facultas Medica Hradec Králové*.

Dolgin, Elie. 2015. "The Myopia Boom." *Nature*.

Drance, S. M. 1960. "The Coefficient of Scleral Rigidity in Normal and Glaucomatous Eyes." *A.M.A. Archives of Ophthalmology*.

Eisenlohr, John E., M. E. Langham, and A. E. Maumenee. 1962. "Manometiric Studies of the Pressure-Volume Relationship in Living and Enucleated Eyes of Individual Human Subjects." *British Journal of Ophthalmology* 46(9): 536–48.

Flitcroft, D. I. 2014. "Emmetropisation and the Aetiology of Refractive Errors." *Eye (Basingstoke)* 28(2): 169–79. http://dx.doi.org/10.1038/eye.2013.276.

Foster, P. J., and Y. Jiang. 2014. "Epidemiology of Myopia." *Eye (Basingstoke)*.

Fricke, TR et al. 2012. "Global Cost of Correcting Vision Impairment from Uncorrected Refractive Error." *Bulletin of the World Health Organization*.

Friedenwald, Jonas S. 1937. "Contribution to the Theory and Practice of Tonometry." *American Journal of Ophthalmology*.

———. 1954. *Tonometer Calibration: An Attempt to Remove Discrepancies Found in the 1954 Calibration Scale for Schiotz Tonometers*.

Fu, Aicun et al. 2020. "Effect of Low-Dose Atropine on Myopia Progression, Pupil Diameter and Accommodative Amplitude: Low-Dose Atropine and Myopia Progression." *British Journal of Ophthalmology* 104(11): 1535–41.

Gallego, Patricia et al. 2012. "Scleral Changes Induced by Atropine in Chicks as an Experimental Model of Myopia." *Ophthalmic and Physiological Optics*.

Gilmartin, Bernard, Manbir Nagra, and Nicola S. Logan. 2013. "Shape of the Posterior Vitreous Chamber in Human Emmetropia and Myopia." *Investigative Ophthalmology and Visual Science*.

GOLDMANN, H., and T. SCHMIDT. 1957. "Friedenwald's Rigidity Coefficient." *Ophthalmologica. Journal international d'ophtalmologie. International journal of ophthalmology. Zeitschrift fur Augenheilkunde*.

Grosvenor, Theodore. 2003. "Why Is There an Epidemic of Myopia?" *Clinical and Experimental*

Optometry.

He, Miao, Wei Wang, Hui Ding, and Xingwu Zhong. 2017. "Corneal Biomechanical Properties in High Myopia Measured by Dynamic Scheimpflug Imaging Technology." *Optometry and Vision Science*.

He, Mingguang et al. 2015. "Effect of Time Spent Outdoors at School on the Development of Myopia among Children in China a Randomized Clinical Trial." *JAMA - Journal of the American Medical Association* 314(11): 1142–48.

Holden, B. et al. 2014. "Myopia, an Underrated Global Challenge to Vision: Where the Current Data Takes Us on Myopia Control." *Eye (Basingstoke)*.

Holden, Brien A., David A. Wilson, et al. 2015. "Myopia: A Growing Global Problem with Sight-Threatening Complications." *Community Eye Health Journal*.

Holden, Brien A., Monica Jong, et al. 2015. "Nearly 1 Billion Myopes at Risk of Myopia-Related Sight-Threatening Conditions by 2050 - Time to Act Now." *Clinical and Experimental Optometry*.

Holden, Brien A. et al. 2016. "Global Prevalence of Myopia and High Myopia and Temporal Trends from 2000 through 2050." *Ophthalmology*.

Hommer, Anton et al. 2008. "Estimation of Ocular Rigidity Based on Measurement of Pulse Amplitude Using Pneumotonometry and Fundus Pulse Using Laser Interferometry in Glaucoma." *Investigative Ophthalmology and Visual Science*.

Hoseini-Yazdi, Hosein et al. 2019. "Wide-Field Choroidal Thickness in Myopes and Emmetropes." *Scientific Reports* 9(1): 1–10. http://dx.doi.org/10.1038/s41598-019-39653-w.

Hrynchak, Patricia K. et al. 2013. "Increase in Myopia Prevalence in Clinic-Based Populations across a Century." *Optometry and Vision Science*.

Hulley, Stephen B et al. 2013. Optometry Vision Science *Designing Clinical Research : An Epidemiologic Approach. 4th Ed. Philadelphia, PA: Lippincott Williams & Wilkins*.

Jeon, Sohee, Won Ki Lee, Kook Lee, and Nam Ju Moon. 2012. "Diminished Ciliary Muscle Movement on Accommodation in Myopia." *Experimental Eye Research*.

Jiang, Zipei et al. 2013. "Interocular Evaluation of Axial Length and Retinal Thickness in People with Myopic Anisometropia." *Eye and Contact Lens*.

Joachimsen, Lutz et al. 2021. "Side Effects of Topical Atropine 0.05% Compared to 0.01% for Myopia Control in German School Children: A Pilot Study." *International Ophthalmology* 41(6): 2001–8.

Jonas, J. B., and L. Xu. 2014. "Histological Changes of High Axial Myopia." *Eye (Basingstoke)*.

Jonas, Jost B. et al. 2021. "IMI Prevention of Myopia and Its Progression." *Investigative*

Ophthalmology and Visual Science 62(5): 8–10.

Jonas, Jost B., Kyoko Ohno-Matsui, and Songhomitra Panda-Jonas. 2019. "Myopia: Anatomic Changes and Consequences for Its Etiology." *Asia-Pacific Journal of Ophthalmology* 8(5): 355–59.

Kaiti, Raju. 2022. "Role of Atropine in the Control of Myopia Progression- A Review." *Beyoglu Eye Journal* 7(3): 157–66.

Kaymak, Hakan et al. 2018. "Short-Term Effects of Low-Concentration Atropine Eye Drops on Pupil Size and Accommodation in Young Adult Subjects." *Graefe's Archive for Clinical and Experimental Ophthalmology* 256(11): 2211–17.

Khanal, Safal, Meredith Walton, and Pinakin Gunvant Davey. 2017. "Evaluation of Intraocular Pressure Estimates Obtained Using an ICare Rebound Tonometer." *Clinical and Experimental Optometry*.

Langaas, Trine et al. 2008. "Variability of the Accommodation Response in Early Onset Myopia." *Optometry and Vision Science*.

Lewis, Jacob A., Mariana B. Garcia, Lakshmisahithi Rani, and Christine F. Wildsoet. 2014. "Intact Globe Inflation Testing of Changes in Scleral Mechanics in Myopia and Recovery." *Experimental Eye Research*.

Li, Ying et al. 2014. "Refractive Error and Risk of Early or Late Age-Related Macular Degeneration: A Systematic Review and Meta-Analysis." *PLoS ONE*.

Liu, Hong, Donglong Chen, Zhikuan Yang, and Xiaoning Li. 2022. "Atropine Affects the Outer Retina During Inhibiting Form Deprivation Myopia in Guinea Pigs." *Current Eye Research* 47(4): 614–23. https://doi.org/10.1080/02713683.2021.2009515.

Liu, John H.K. et al. 2002. "Twenty-Four-Hour Pattern of Intraocular Pressure in Young Adults with Moderate to Severe Myopia." *Investigative Ophthalmology and Visual Science*.

Loughman, James; Flitcroft, DI. 2016. "The Acceptability and Visual Impact of 0.01% Atropine in a Caucasian Population." *British Journal of Ophthalmology* (100): 1525–29.

Mak, C. Y. et al. 2018. "Epidemiology of Myopia and Prevention of Myopia Progression in Children in East Asia: A Review." *Hong Kong Medical Journal* 24(6): 602–9.

Manny, Ruth E. et al. 2011. "Intraocular Pressure, Ethnicity, and Refractive Error." *Optometry and Vision Science*.

McBrien, Neville A., and Alex Gentle. 2001. "The Role of Visual Information in the Control of Scleral Matrix Biology in Myopia." *Current Eye Research*.

Mesquita, Gabrielle M.; Chang, Yu-Cherng; Cabot, Florence; Ruggeri, Marco; Yoo, Sonia H; Parel, Jean-Marie A.; Manns, Fabrice. 2017. "In Vivo Measurement of the Attenuation Coefficient of the Sclera and Ciliary Muscle from Transscleral Optical Coherence Tomography Images." In

ARVO Annual Meeting Abstract,.

Mesquita, Gabrielle M.; Patel, Disha M.; Channg, Yu-Cherng; Cabot, Florence; Ruggeri, Marco; Yoo, Sonia; Parel, Jean-Marie; Ho, Arthur; Manns, Fabrice. 2020. "Relation between Refractive Error and Scleral Attenuation Coefficient Obtained from Transceleral OCT Images." In Miami, Florida: ARVO Imaging in the Eye Conference Abstract.

Morgan, Ian G. 2016. "What Public Policies Should Be Developed to Deal with the Epidemic of Myopia?" In *Optometry and Vision Science*,.

Nickla, Debora L.; Wallman, Joseph. 2010. "The Multifunctional Choroid." *Progress in Retinal and Eye Research* 29(2): 144–68.

Nickla, Debora L., Kelsey Jordan, Jane Yang, and Puneet Singh. 2019. "Effects of Time-of-Day on Inhibition of Lens-Induced Myopia by Quinpirole, Pirenzepine and Atropine in Chicks." *Experimental Eye Research* 181(181): 5–14.

Nickla, Debora L., Xiaoying Zhu, and Josh Wallman. 2013. "Effects of Muscarinic Agents on Chick Choroids in Intact Eyes and Eyecups: Evidence for a Muscarinic Mechanism in Choroidal Thinning." *Ophthalmic and Physiological Optics* 33(3): 245–56.

Niyazmand, Hamed, Scott A. Read, David A. Atchison, and Michael J. Collins. 2020. "Effects of Accommodation and Simulated Convergence on Anterior Scleral Shape." *Ophthalmic and Physiological Optics*.

Norton, Thomas T., and Jody A. Rada. 1995. "Reduced Extracellular Matrix in Mammalian Sclera with Induced Myopia." *Vision Research*.

O'Shaughnessy, Kevin. 2012. "Cholinergic and Antimuscarinic (Anticholinergic) Mechanisms and Drugs."

Ohno-Matsui, Kyoko, Timothy Y.Y. Lai, Chi Chun Lai, and Chiu Ming Gemmy Cheung. 2016. "Updates of Pathologic Myopia." *Progress in Retinal and Eye Research*.

Pallikaris, Ioannis G. et al. 2005. "Ocular Rigidity in Living Human Eyes." *Investigative Ophthalmology and Visual Science*.

Park, D. J.J., and Nathan G. Congdon. 2004. "Evidence for an 'Epidemic' of Myopia." *Annals of the Academy of Medicine Singapore*.

Pärssinen, Olavi. 2012. "The Increased Prevalence of Myopia in Finland." *Acta Ophthalmologica*.

Patel, Hetal, Bernard Gilmartin, Robert P. Cubbidge, and Nicola S. Logan. 2011. "In Vivo Measurement of Regional Variation in Anterior Scleral Resistance to Schiotz Indentation." *Ophthalmic and Physiological Optics*.

Perera, Shamira A. 2010. "Refractive Error, Axial Dimensions, and Primary Open-Angle Glaucoma." *Archives of Ophthalmology*.

Prousali, Efthymia et al. 2022. "'The Role of Accommodative Function in Myopic Development: A Review.'" *Seminars in Ophthalmology* 37(4): 455–61. https://doi.org/10.1080/08820538.2021.2006724.

Recko, Matthew, and Erin D.urrie Stahl. 2015. "Childhood Myopia: Epidemiology, Risk Factors, and Prevention." *Missouri medicine*.

Remón, Laura et al. 2020. "Bifocal and Multifocal Contact Lenses for Presbyopia and Myopia Control." *Journal of Ophthalmology* 2020.

Rose, K. et al. 2000. "Quality of Life in Myopia." *British Journal of Ophthalmology*.

Ruiz-Pomeda, Alicia, and César Villa-Collar. 2020. "Slowing the Progression of Myopia in Children with the MiSight Contact Lens: A Narrative Review of the Evidence." *Ophthalmology and Therapy* 9(4): 783–95.

Ruland, Kelly et al. 2019. "Accuracy of Tonopen versus ICare in Human Cadaveric Eyes with Edematous Corneas over a Wide Range of Intraocular Pressures." *Journal of Glaucoma*.

Sander, Beata P., Michael J. Collins, and Scott A. Read. 2019. "Short-Term Effect of Low-Dose Atropine and Hyperopic Defocus on Choroidal Thickness and Axial Length in Young Myopic Adults." *Journal of Ophthalmology* 2019: 1–8.

Sankaridurg, Padmaja, Fabian Conrad, Huy Tran, and Jianfeng Zhu. 2018. "Controlling Progression of Myopia: Optical and Pharmaceutical Strategies." *Asia-Pacific Journal of Ophthalmology* 7(6): 405–14.

Saw, Seang Mei. 2006. "How Blinding Is Pathological Myopia?" *British Journal of Ophthalmology*.

Schmid, Katrina L., Roger W.H. Li, Marion H. Edwards, and John K.F. Lew. 2003. "The Expandability of the Eye in Childhood Myopia." *Current Eye Research*.

Schwahn, Hartmut; Kaymak, Hakan; Schaeffel, Frank. 2000. "Effects of Atropine on Refractive Development, Dopamine Release, and Slow Retinal Potentials in the Chick." *Visual Neuroscience* 17(2): 165–76.

Schweier, Caterina, James V.M. Hanson, Jens Funk, and Marc Töteberg-Harms. 2013. "Repeatability of Intraocular Pressure Measurements with Icare PRO Rebound, Tono-Pen AVIA, and Goldmann Tonometers in Sitting and Reclining Positions." *BMC Ophthalmology*.

Senthil, Sirisha et al. 2019. "Schiotz Scleral Intraocular Pressure Readings Predict Goldmann Applanation Readings Better Than Rebound Tonometry." *Cornea*.

Sergienko, Nikolai M., and Irina Shargorogska. 2012. "The Scleral Rigidity of Eyes with Different Refractions." *Graefe's Archive for Clinical and Experimental Ophthalmology*.

Silver, DM; Geyer, O. 2000. "Pressure-Volume Relation for the Living Human Eye." *Current Eye Research* 20(2): 115–20.

Teberik, Kuddusi; Kaya, Murat. 2017. “Retinal and Choroidal Thickness in Patients with High Myopia without Maculopathy.” *Pak J Med Sci* 33(6): 1438–43.

Tekiele, Bernard C., and Leo Semes. 2002. “The Relationship among Axial Length, Corneal Curvature, and Ocular Fundus Changes at the Posterior Pole and in the Peripheral Retina.” *Optometry*.

Tian, Yibin, Janice Tarrant, and Christine F. Wildsoet. 2011. “Optical and Biometric Characteristics of Anisomyopia in Human Adults.” *Ophthalmic and Physiological Optics*.

Tran, Huy D.M. et al. 2021. “A Meta-Analysis Assessing Change in Pupillary Diameter, Accommodative Amplitude, and Efficacy of Atropine for Myopia Control.” *Asia-Pacific journal of ophthalmology (Philadelphia, Pa.)* 10(5): 450–60.

Upadhyay, Aradhana, and Roger W. Beuerman. 2020. “Biological Mechanisms of Atropine Control of Myopia.” *Eye & contact lens* 46(3): 129–35.

Vera-Diaz, F. A. 2010. “Myopia.” In *Encyclopedia of the Eye*,.

Vera-Diaz, Fuensanta A., Jane Gwiazda, Frank Thorn, and Richard Held. 2004. “Increased Accommodation Following Adaptation to Image Blur in Myopes.” *Journal of Vision*.

Vitale, Susan, Robert D. Sperduto, and Frederick L. Ferris. 2009. “Increased Prevalence of Myopia in the United States between 1971-1972 and 1999-2004.” *Archives of Ophthalmology*.

Wagner, Sandra, Eberhart Zrenner, and Torsten Strasser. 2019. “Emmetropes and Myopes Differ Little in Their Accommodation Dynamics but Strongly in Their Ciliary Muscle Morphology.” *Vision Research* 163(July): 42–51. https://doi.org/10.1016/j.visres.2019.08.002.

Walline, Jeffrey J. 2016. “Myopia Control: A Review.” *Eye and Contact Lens* 42(1): 3–8.

Wang, Yuliang et al. 2022. “Short-Term Effects of Atropine 0.01% on the Structure and Vasculature of the Choroid and Retina in Myopic Chinese Children.” *Ophthalmology and Therapy* 11(2): 833–56. https://doi.org/10.1007/s40123-022-00476-0.

Williams, Katie M. et al. 2015. “Increasing Prevalence of Myopia in Europe and the Impact of Education.” *Ophthalmology*.

Wolfram, Christian et al. 2014. “Prevalence of Refractive Errors in the European Adult Population: The Gutenberg Health Study (GHS).” *British Journal of Ophthalmology*.

Woodman-Pieterse, Emily C., Scott A. Read, Michael J. Collins, and David Alonso-Caneiro. 2018. “Anterior Scleral Thickness Changes with Accommodation in Myopes and Emmetropes.” *Experimental Eye Research*.

Xiang, F. et al. 2013. “Increases in the Prevalence of Reduced Visual Acuity and Myopia in Chinese Children in Guangzhou over the Past 20 Years.” *Eye (Basingstoke)*.

Yam, Jason C. et al. 2020. “Two-Year Clinical Trial of the Low-Concentration Atropine for Myopia

Progression (LAMP) Study: Phase 2 Report." *Ophthalmology* 127(7): 910–19. https://doi.org/10.1016/j.ophtha.2019.12.011.

———. 2022. "The Association of Choroidal Thickening by Atropine With Treatment Effects for Myopia: Two-Year Clinical Trial of the Low-Concentration Atropine for Myopia Progression (LAMP) Study." *American Journal of Ophthalmology* 237: 130–38. https://doi.org/10.1016/j.ajo.2021.12.014.

Yan, Honggai, Jun Li, Jing Zhang, and Liu Yang. 2017. "Retinal and Choroidal Thickness in Patients with Uveitis." *Ocular Immunology and Inflammation* 25(2): 202–9.

Ye, Luyao et al. 2020. "Effects of Atropine Treatment on Choroidal Thickness in Myopic Children." *Investigative Ophthalmology and Visual Science* 61(14): 1–10.

Ytteborg, J. 1960. "Further Investigations of Factors Influencing Size of Rigidity Coefficient." *Acta Ophthalmologica* 38(643–657).

Zhou, Wen Jun et al. 2016. "Five-Year Progression of Refractive Errors and Incidence of Myopia in School-Aged Children in Western China." *Journal of Epidemiology*.

Zhou, Yue et al. 2020. "Comparative Study of the Effects of 1% Atropine on the Anterior Segment." *Journal of Ophthalmology* 2020(1).

Appendix I: Questionnaire 1

Effect of Atropine Eye Drops on the Anterior and Posterior Segment Structures and Functions

Subject ID: _____ Date: ___/___/_____ Examiner(s): _____

1. <u>At the beginning of Visit #1:</u>

Do you *generally* have difficulty doing near work activities such as:

1. reading small print in a book or paper? Yes ☐ No ☐ If Yes, how often?
2. writing or reading? Yes ☐ No ☐ If Yes, how often?
3. reading in your smart phone? Yes ☐ No ☐ If Yes, how often?
4. working on your laptop or tablet? Yes ☐ No ☐ If Yes, how often?
5. maintaining focus for long periods of time? Yes ☐ No ☐ If Yes, how often?
6. seeing up close in poor or dim light? Yes ☐ No ☐ If Yes, how often?

Comments:

Do you *generally* have symptoms of light sensitivity such as:

1. being bothered by indoor bright lights? Yes ☐ No ☐ If Yes, how often?
2. being bothered by sun light? Yes ☐ No ☐ If Yes, how often?
3. often wearing sunglasses outdoors? Yes ☐ No ☐ If Yes, how often?
4. squinting one or both eyes? Yes ☐ No ☐ If Yes, how often?
5. glare? Yes ☐ No ☐ If Yes, how often?
6. pain in the eye? Yes ☐ No ☐ If Yes, how often?
7. frontal headaches? Yes ☐ No ☐ If Yes, how often?

Comments:

2. <u>At the end of Visit #1:</u>

Are you experiencing more difficulty doing near work activities compared to before we started testing?

1. reading small print in a book or paper? Yes ☐No ☐ If Yes, how severe?

2. writing or reading? Yes ☐ No ☐ If Yes, how severe?

3. reading in your smart phone? Yes ☐ No ☐ If Yes, how severe?

4. working on your laptop or tablet? Yes ☐ No ☐ If Yes, how severe?

5. seeing up close in poor or dim light? Yes ☐ No ☐ If Yes, how severe?

Comments:

Are you experiencing more symptoms of light sensitivity compared to before we started testing?

1. being bothered by indoor bright lights? Yes ☐No ☐ If Yes, how severe?

2. being bothered by sun light? Yes ☐ No ☐ If Yes, how severe?

3. squinting one or both eyes? Yes ☐ No ☐ If Yes, how severe?

4. glare? Yes ☐ No ☐ If Yes, how severe?

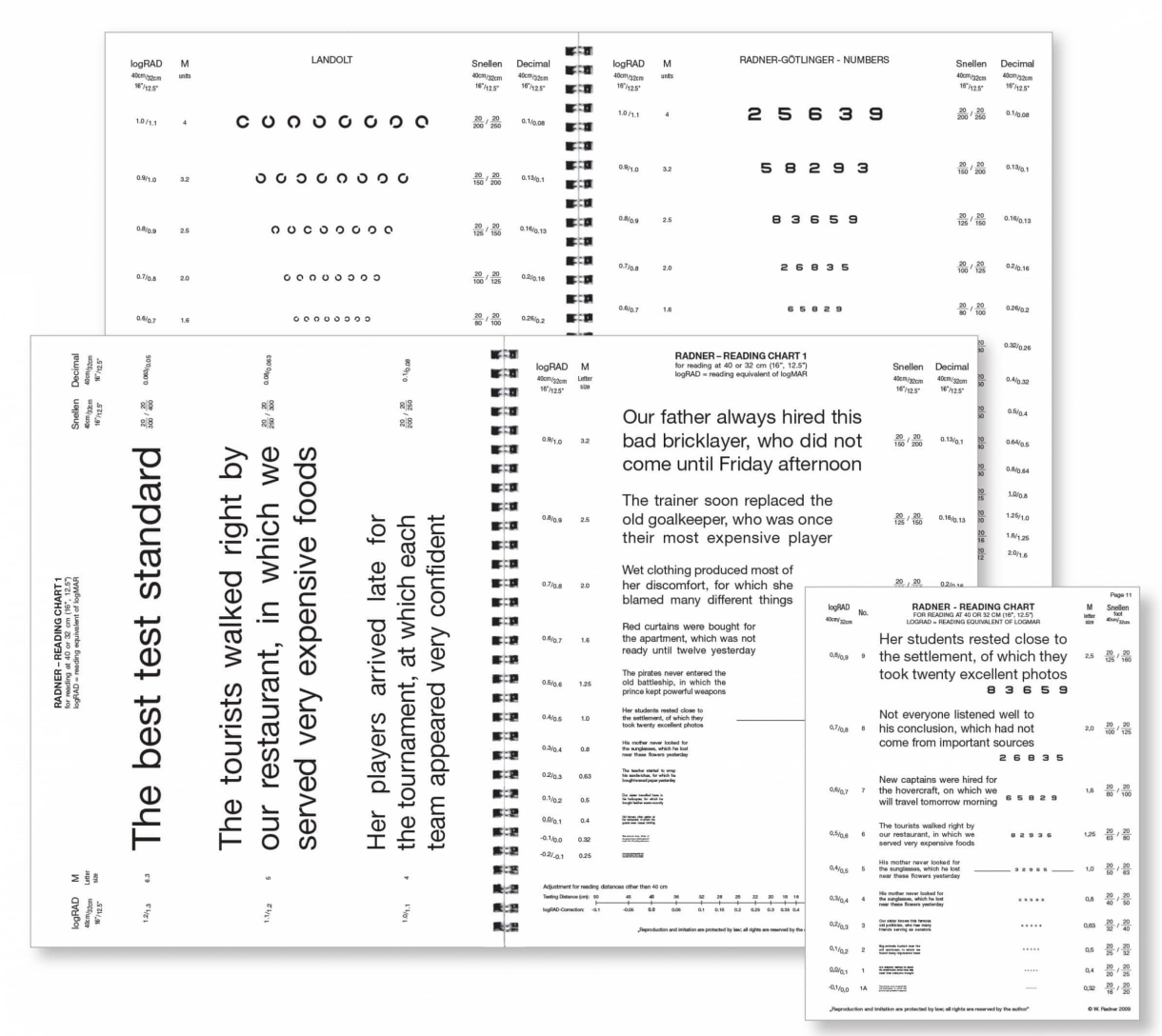
5. pain in the eye? Yes ☐ No ☐ If Yes, how severe?

6. frontal headaches? Yes ☐ No ☐ If Yes, how severe?

Comments:

Appendix II: Reading Tests

Radner Reading Test



New York Public Library Reading Test

The New York Public Library Reading Test we used is no longer available online. There were 3 passages that increased in length. Each passage had 3 reading comprehension questions listed after the passage.

Appendix III: Written tropine usage instructions

Effect of Atropine Eye Drops on the Anterior and Posterior Segment Structures and Functions

Visit Date #1: ___/___/_____ Name: _____

Instill 1 drop of 0.05% atropine OD and OS each eye for 7 days until next visit.

Always wash your hands before handing your eye drops or touching your eyes. Remove the cap of the eye drop medication but do not touch the dropper tip. Tilt your head back slightly and look up. Use one hand to pull your lower lid down, away from the eye. Squeeze the bottle gently and let the eye drop fall into the lower lid.



Day #1: ___/___/_____
☐ Instilled atropine drops Time: _____ ☐ Completed questionnaire

Day #2: ___/___/_____
☐ Instilled atropine drops Time: _____ ☐ Completed questionnaire

Day #3: ___/___/_____
☐ Instilled atropine drops Time: _____ ☐ Completed questionnaire

Day #4: ___/___/_____
☐ Instilled atropine drops Time: _____ ☐ Completed questionnaire

Day #5: ___/___/_____
☐ Instilled atropine drops Time: _____ ☐ Completed questionnaire

Day #6: ___/___/_____
☐ Instilled atropine drops Time: _____ ☐ Completed questionnaire

Day #7: ___/___/_____
☐ Instilled atropine drops Time: _____ ☐ Completed questionnaire

Visit Date #2: ___/___/_____ Time: _____

Appendix IV: Questionnaire 2 (via automated email Google Form each day while using the atropine eye drops)

Effect of Atropine Eye Drops on the Anterior and Posterior Segment Structures and Functions

Do you have *today* difficulty doing near work activities such as:

1. reading small print in a book or paper? Yes ☐ No ☐ If Yes, how often?
2. writing or reading? Yes ☐ No ☐ If Yes, how often?
3. reading in your smart phone? Yes ☐ No ☐ If Yes, how often?
4. working on your laptop or tablet? Yes ☐ No ☐ If Yes, how often?
5. maintaining focus for long periods of time? Yes ☐ No ☐ If Yes, how often?
6. seeing up close in poor or dim light? Yes ☐ No ☐ If Yes, how often?

Comments:

Do you have *today* symptoms of light sensitivity such as:

1. being bothered by indoor bright lights? Yes ☐ No ☐ If Yes, how often?
2. being bothered by sun light? Yes ☐ No ☐ If Yes, how often?
3. often wearing sunglasses outdoors? Yes ☐ No ☐ If Yes, how often?
4. squinting one or both eyes? Yes ☐ No ☐ If Yes, how often?
5. glare? Yes ☐ No ☐ If Yes, how often?
6. pain in the eye? Yes ☐ No ☐ If Yes, how often?
7. frontal headaches? Yes ☐ No ☐ If Yes, how often?

Comments:

How many hours did you spend *today* doing near work (including reading, studying, writing, etc.)? (Rounded to nearest higher hour)

How many hours did you spend *today* using a computer or other electronic device? (Rounded to nearest higher hour)

Appendix V: American Academy of Optometry Poster 2021

NECO | New England College of Optometry

Measuring Scleral Rigidity using iCare – a Pilot Study

Courtney Liang[1], Fuensanta A. Vera-Diaz[1]
[1]New England College of Optometry, Boston, MA

BACKGROUND

- Longer, myopic, eyes exhibit biomechanical thinning and weakness in the scleral shell.[1-4] It is likely that factors related to ocular rigidity and other mechanical properties of the ocular globe are important in the development of myopia.[5]
- Ocular rigidity relates to the association between pressure and volume changes in the eyeball. Rigidity has been examined using invasive techniques such as manometric pressure-volume methods and Schiotz tonometry, as well as non-invasive assessment of fundus pulsation.[6-8]
- iCare tonometer shows good accuracy and repeatability at the cornea, but previous studies show large variability when measuring IOP in the conjunctiva.
- Previous studies have evaluated scleral IOP at 3-5mm from the limbus[9] and up to 8mm away from scleral spur to avoid effect of extraocular muscle insertion.[10]

PURPOSE

- ***To explore the feasibility of obtaining repeatable IOP measures at various locations of the conjunctiva (anterior sclera) using iCare tonometry.***

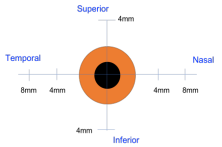
METHODS

Subjects:

- N = 25 young (24.28 ± 1.7) adults with emmetropia or myopia and LogMAR BCVA = 0.00 or better in each eye.
- N was based on preliminary studies of effect size (SD) of about 15% (45.9 ±14.9 mmHg).

Measurements:

- GAT IOP at the central cornea for each eye.
- iCare IOP at 7 positions in each eye (central cornea and 6 conjunctival locations):



- To increase accuracy of the conjunctival locations, the distances from the limbus were marked by an aseptic surgical marker. Three measures within ± 2mmHg were taken at each location.
- Repeated measurements 1 week later (7.08 ± 0.27 days), within 2 hours time window of the first visit.

RESULTS

- No significant differences in IOP at any of the locations measured were found between visit #1 and visit #2 for the right or left eyes (all P<0.01) (Figure 1).

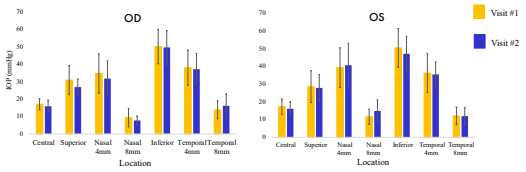


Figure 1. Average IOP at each location. Error bars represent ±1 standard deviation. Outliers were removed when the values were outside two standard deviations of the average.

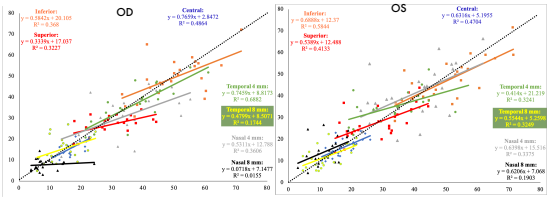


Figure 2. Correlation of IOP measured during visits 1 and 2 in each eye at the marked locations

- Test-retest variability was assessed using calculated Pearson's correlation coefficients (Figure 2), Bland-Altman analysis (Figure 3), and coefficient of repeatability.

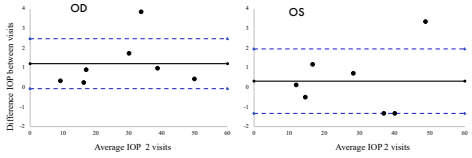


Figure 3. Bland-Altman plots: average IOP between the 2 visits at each location vs. the difference between the 2 visits. Two points outside 2 standard deviations, denoted by dashed lines, are 4mm nasal OD and 4mm inferior OS, indicating these two points were not repeatable between visit 1 and 2.

- The coefficient of repeatability (95% confidence interval of the difference between the compared tests) showed good repeatability for all locations except in the right eye Superiorly (p = 0.04) and at 8 mm Nasally (p = 0.05), and for the left eye Inferiorly (p = 0.02) and at 8 mm Nasally (p = 0.04).

DISCUSSION

- Following this initial pilot study, a technique for repeatedly measuring IOP at various location of the conjunctiva (anterior sclera) has been established.
- We found good repeatability for all locations except in the right eye Superiorly and for the left eye Inferiorly and for both eyes at 8 mm Nasally.
- These differences may be due to difficulties taking measurements in upper and lower positions when it is more difficult for the patient to hold steady gaze.
- Lower repeatability noted at 8mm Nasally may be due to differences in amount of bulbar conjunctiva visible between subjects and more strain holding eye position in this extreme gaze.
- We are using the information from this pilot study in subsequent research. The main study involves evaluation of changes in a number of both anterior and posterior segment ocular structures and visual functions before and after usage of topical Atropine drops. These measurements include biometry, scanning aberrometer, open field autorefractor, anterior and posterior segment ocular coherence tomography, and pattern electroretinograms.
- The findings from this study will be used with other biometry and OCT measures to compute ocular rigidity.

CONCLUSIONS

- No significant differences in IOP between the first and second visits were noted at most locations.
- The iCare can provide repeatable measurements at the conjunctiva.
- This technique is useful to evaluate IOP in peripheral locations of the conjunctiva (anterior sclera).

ACKNOWLEDGEMENTS

- NECO Internal Funding, PI Vera-Diaz
- NIH T35 (EY007149), PI J. Gwiazda

REFERENCES

1. Jonas JB, Xu L. Histological changes of high axial myopia. *Eye*. 2014;28(2):113-117. doi:10.1038/eye.2013.223.
2. Norton TT, Rada JA. Reduced extracellular matrix in mammalian sclera with induced myopia. *Vision Research*. 1995;35(9):1271-1281. doi:10.1016/0042-6989(94)00243-F.
3. Bryant MR, McDonnell PJ. Optical feedback controlled scleral remodeling as a mechanism for myopic eye growth. *Journal of Theoretical Biology*. 1998;193(4):613-622. doi:10.1006/jtbi.1998.0727.
4. Lewis JA, Garcia MB, Rani L, Wildsoet CF. Intact globe inflation testing of changes in scleral mechanics in myopia and recovery. *Experimental Eye Research*. 2014;127:42-48. doi:10.1016/j.exer.2014.07.004.
5. McBrien NA, Gentle A. The role of visual information in the control of scleral matrix biology in myopia. *Current Eye Research*. 2001;23(5):313-319. doi:10.1076/ceyr.23.5.313.5440.
6. Schmid KL, Li RWH, Edwards MH, Lew JKF. The expandability of the eye in childhood myopia. *Current Eye Research*. 2003;26(2):25-71. doi.org/10.1076/ceyr.26.2.65.14513.
7. Pallikaris IG, Kymionis GD, Ginis HS, Kounis GA, Tsilimbaris MK. Ocular rigidity in living human eyes. *Investigative Ophthalmology and Visual Science*. 2005;46(2):409-414. doi.org/10.1167/iovs.04-0162.
8. Hommer A, Fuchsjager-Mayrl G, Resch H, Vass C, Garhofer G, Schmetterer L. Estimation of ocular rigidity based on measurement of pulse amplitude using pneumotonometry and fundus pulse using laser interferometry in glaucoma. *Investigative Ophthalmology and Visual Science*. 2008;49(9):4046-4050. doi: 10.1167/iovs.07-1342.
9. Senthil S, Chary R, Ali MH, et al. Schiotz Scleral Intraocular Pressure Readings Predict Goldmann Applanation Readings Better Than Rebound Tonometry. *Cornea*. 2019. doi:10.1097/ICO.0000000000002033.
10. Dhakal R, Vupparaboina KK, Verkicharla PK. Anterior sclera undergoes thinning with increasing degree of myopia. *Investigative Ophthalmology and Visual Science*. 2020. doi:10.1167/iovs.61.4.6.

Appendix VI: Association for Research in Vision and Ophthalmology Poster 2022

NECO | New England College of Optometry

Effect of Low Dose Atropine Eye Drops on Ocular Structure and Function

Courtney Liang[1], Ashutosh Jnawali[1], Sarah Curtin[1], Fuensanta A. Vera-Diaz[1]
[1]New England College of Optometry, Boston, MA

Background

- Atropine is a non-selective broad muscarinic acetylcholine receptor antagonist that works as a reversible competitive antagonist for an affinity of all five subtypes of acetylcholine, muscarinic receptors found in different ocular tissues including the cornea, iris, ciliary body, ciliary muscle, epithelium of crystalline lens, retina, RPE, choroid and sclera.[1]
- Hypermetropic and emmetropic eyes have stiffer scleras compared to myopic eyes.[2]
- Anterior sclera is also thinner in myopia, with differences among superior and inferior quadrants.[3].

Purpose

Investigate the effect of low dose Atropine eye drops on scleral thickness and pressure to evaluate ocular rigidity and other visual parameters in emmetropes and myopes.

Methods - Subjects

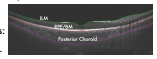
27 young adults (24.53±1.40 yrs) with Emmetropia (n=13, SE=+0.24±0.41D) or Myopia (n=14, SE=-1.89±1.86D).

Methods - Design

- Tested at baseline (Visit #1) and after 7±1 days of using 1gtt Atropine 0.05% QHS OU (Visit #2)
- Measurements taken: twice during Visit #1 (before and 30mins after instillation of Atropine drops), and once during Visit #2 (same time as Visit #1, ±2 hours)

Methods - Tests

- GAT and iCare: central corneal IOP
- iCare at 4 scleral locations (surgical marker): Temporal 5mm, Superior 4mm, Nasal 5mm, Inferior 4mm
  Repeatability assessed in pilot study.
- Spectralis SD-OCT anterior segment module: anterior scleral thickness same locations
- Spectralis SD-OCT wide field posterior module: Retinal and Choroidal Thickness across central 55 degrees: Four quadrants analyzed.



- Lenstar (LS900): axial length (AXL), corneal thickness (CCT), anterior chamber (ACD), lens thickness (LT)
- Other tests: Pupil Sizes (Dim, Bright, and Near), MEM, TBUT, Contrast Sensitivity (CS, Rubin).

Conclusions

Corneal IOP significantly decreased after short and long-term use of 0.05% Atropine. Nasal and temporal scleral IOP showed a tendency to increase after Atropine use, which matched the increased thickness in the temporal sclera. Findings suggest that anterior scleral thickening may be involved in the mechanism of Atropine in myopia control.

As expected, ACD and pupil sizes increased and accommodation decreased with the use of 0.05% Atropine.

References

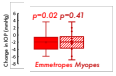
1. Upadhyay & Beuerman. Biological Mechanisms of Atropine Control of Myopia. Eye Contact Lens, 2020.
2. Dhakal, Vupparaboina, &Verkicharla. Anterior sclera undergoes thinning with increasing degree of myopia. IOVS, 2020.
3. Sergienko & Shargorogska. The scleral rigidity of eyes with different refractions. Graefe's Arch Ophthalmol, 2012

Acknowledgements

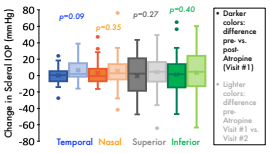
• NIH T35 (EY007149), PI J. Gwiazda
• NECO Internal Funding, PI Vera-Diaz
• Conflicts of interest: None.

Results

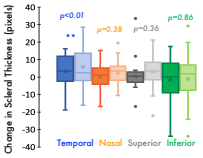
- Corneal IOP: Overall decrease in IOP after Atropine use (iCare for all subjects: F(27,2)=7.23, p=0.03), significant pre- and post-Atropine (Visit #1) in Emms (p=0.01), no change Visit #2. No significant in Myops.

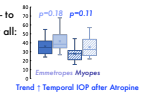


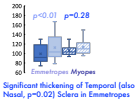
- Scleral IOP with iCare:
  No significant differences among visits, but ***trend to increase with atropine*** for temporal sclera:



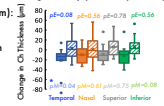
- Scleral Thickness:
  Significantly increased Temporal Sclera Visit #1 pre- to post-Atropine, similar Visit #2. *Trend to increase* for all:







- Choroidal Thickness increased significantly post-Atropine only in Myopes (F(14,2)=6.14, p=0.04), about 9µm, with the same trend for Emmetropes (about 10µm): Similar trends, but not significant, were found in the other quadrants:





- Pupils were significantly larger for Dim, Near and Bright conditions after Atropine use (F(27,2)>41.10, p<0.01). The effect was only significant between pre- and post-Atropine in Visit #1 (p<0.01), no changes after 1 week of use (p>0.71). Larger effects noted for Myopes than Emmetropes (had larger baseline sizes).
- MEM had larger LAGs in Visit #1 compared to Visit #2, about 0.50D (p<0.01), but no differences within Visit #1 measures.
- CS decreased with Atropine in Emmetropes (p=0.03), not in Myopes (p=0.13).
- TBUT became about 2sec shorter after 1 week Atropine use (p<0.01) for all.
- Retinal Thickness showed no significant changes with Atropine for all quadrants (F<5.00, p>0.08).
- AXL did not change with Atropine in Emmetropes (F(13,2)=0.04, p=0.98), but was >0.20mm **longer in Myopes** after Atropine instillation (F(14,2)=7.66, p=0.02), with no difference Visit #1 vs. Visit #2.
- ACD was deeper (Emmetropes: F(13,2)=9.26, p=0.01; Myopes: F(14,2)=12.69, p<0.01) after instillation of Atropine.
- LT did not significantly change, but approached significance for thinning after 1 week of Atropine in Emmetropes only (p=0.09).

Appendix VII: American Academy of Optometry Abstract 2023

Title:
Effect of Low Dose Atropine Eye Drops on Retinal Responses to Clear and Blurred Images

Authors:
Fuensanta A. Vera-Diaz, Courtney Liang, Sarah Curtin, Thanasis Panorgias

Purpose:
To investigate the effect of low dose atropine eye drops on retinal responses to clear and blurred images in a group of young adults with emmetropia or myopia.

Methods:
Dead-leaves pattern electroretinograms (DLPs) were recorded from N=30 young adults (24.7±1.6yrs) with emmetropia (+0.4±0.4D) or myopia (-2.0±1.3D) at two visits. Retinal responses were recorded while subjects viewed naturalistic images under three different conditions: (1) no blur (CLEAR), (2) convoluted to simulate Defocus blur (DEF, 0.5μm), or (3) to simulate Spherical Aberration (SA, 0.365μm) of equivalent perceptual magnitude, across the entire "dead leaves" naturalistic image. Optical biometry and open-field autorefractor were also measured. Subjects instilled 1 drop of 0.05% atropine in each eye every evening for 7 days. All measurements were taken: twice during Visit-1 (before and 30mins after instillation of atropine), and once during Visit-2 (1 week later, at the same time: ±2 hours). The Amplitude (AMP, μV) and Latency (LAT, ms) of the first peak (P1) and second negative trough (N2) were analyzed with repeated measures general linear models with pupil size change as a covariate.

Results:
Low dose atropine increased P1 AMP responses when DLPs were recorded using images blurred with DEF ($F=3.46$, $P=0.04$, mainly short-term: $P<0.01$) and SA ($F=6.22$, $P<0.01$, short- $P<0.01$ and long-term $P=0.02$), but did not affect recordings using CLEAR images ($F=0.51$, $P=0.61$). When refractive groups were considered separately, atropine increased the P1 AMP for DEF images only for MYOPs ($F=11.17$, $P<0.01$), with a trend also for SA also only in MYOPs ($F=2.78$, $P=0.11$). The effect of atropine in P1 AMP was not observed in EMMs. On the other hand, low dose atropine decreased P1 LAT ($F=7.39$, $P<0.01$) with CLEAR images, with no effect for blurred images. There were no effects on N2 AMP or LAT responses.

Conclusions:
Using 0.05% atropine eye drops significantly affected retinal responses. Amplitude responses were not affected for clear images but increased for blurred images in myopes. The results appear to represent retinal effects since the change in pupil size was considered and reduced accommodation caused by atropine eye drops would reduce, not increase amplitudes.