Efficacy and Safety of Low Dose Atropine and Caffeine Eye Drops for Myopia Control

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EFFICACY AND SAFETY OF LOW DOSE ATROPINE AND CAFFEINE EYE DROPS FOR MYOPIA CONTROL

By

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A thesis submitted in fulfilment of the requirements for the degree of

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Presented to

Brien Holden Vision Institute & School of Optometry and Vision Science

Faculty of Medicine, The University of New South Wales, Sydney, Australia

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Thesis/Dissertation Sheet

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Abstract

Aim: To determine the role of topical Caffeine, a Xanthine derivative in slowing myopia either as a single-drug or in combination with Atropine.

Methods: A systematic review and meta-analysis for Atropine in myopia control was followed by a short-term dispensing trial to select a single Atropine concentration to use in combination with Caffeine. In a prospective, randomized, dispensing trial, children with myopia were assigned to daily use of either Caffeine-2%, Atropine-0.02% with Caffeine-2% or Atropine-0.02%. A parallel non-randomised group of spectacle lens wearers were controls. The six-month change in spherical equivalent, axial length, pupillary diameter, and accommodative amplitude were compared between groups. Finally, a validation trial monitored pupillary and accommodative amplitude changes over 24 hours with various concentrations of Atropine and Caffeine, either single or combined. Comparison between groups were performed using repeated measures Analysis of Variance with significance set at 5%. Post-hoc multiple comparisons conducted using Bonferroni correction.

Results: Meta-analysis confirmed dose-dependent efficacy and side effects for all concentrations of Atropine excepting 0.01%. Similarly, short-term trial demonstrated no pupillary diameter/accommodative change with Atropine-0.01% in approximately 30% of eyes. Atropine-0.02% was selected to be used in combination with Caffeine and at six months, change in spherical equivalent/axial length was -0.20±0.34D/0.08±0.11mm, -0.20±0.30D/0.11±0.11mm, -0.39±0.38D/0.19±0.15mm and -0.33±0.29D/0.18±0.11mm with Atropine-0.02%, Atropine-0.02% with Caffeine-2%, Caffeine-2%, and single vision spectacles respectively. The pupillary diameter increase/reduction in accommodative amplitude was 1.20±0.85mm/-3.14±4.08D, 0.76±0.58mm/-2.84±4.35D, -0.07±0.47mm/-0.78±3.43D and -0.10±0.32mm/-0.22±3.81D respectively. Temporal observations of pupil diameter indicated, a) no significant variation with Caffeine, b) Post instillation to 60 minutes – Caffeine-2% combined with 0.05% and 0.1%-Atropine resulted in significantly fewer eyes reaching higher pupillary diameter compared to monotherapy with 0.05% and 0.1%-Atropine. There were no significant changes for accommodative amplitude.

Conclusion: Caffeine-2% did not slow myopia when used either individually or in combination with Atropine. However, Caffeine in combination with Atropine significantly minimised the increase in pupillary diameter that occurs with use of Atropine.

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Publication Details #1

Full Title:	A Meta-Analysis Assessing Change in Pupillary Diameter, Accommodative Amplitude, and Efficacy of Atropine for Myopia Control
Authors:	Tran, Huy D.M. MD, MSc; Sankaridurg, Padmaja BOptom, PhD; Naduvilath, Thomas PhD; Ha, Thao T.X. MD; Tran, Tuan D. MD, PhD; Jong, Monica OD, PhD; Coroneo, Minas MD, MS; Tran, Yen H. MD, PhD
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The Candidate's Contribution to the Work:	- Huy Tran and Padmaja Sankaridurg developed the idea of conducting a meta-analysis of atropine to assess the side-effects over concentrations as well as myopia control efficacy. - Huy Tran and Padmaja Sankaridurg collected the data and related research following the proposed protocol. - Huy Tran wrote the manuscript and sought input from co-authors and managed submission and wrote responses to reviewer comments. - Thomas Naduvilath and Thao Ha significantly contributed to the statistical analysis and the responses to reviewer's comments, - Sankaridurg, P. Coroneo, M., Ha, T.T.X., Naduvilath, T., Jong, M., Tran, Y.H. and Tran, T.D. reviewed the manuscript. - In overall, Huy Tran contributed about 65% of the work
Location of the work in the thesis and/or how the work is incorporated in the thesis:	- Chapter 2 mainly involves the information, the research protocol and the data analysis of the paper, - Multiple information of the paper above were incorporated in the thesis.

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I confirm that where I have used a publication in lieu of a chapter, the listed publication(s) above meet(s) the requirements to be included in the thesis. I also declare that I have complied with the Thesis Examination Procedure.

DEDICATION

This dissertation work is dedicated to my warm and generous parents Du Thi-Thu Cuc and Tran Dinh Dung, my dear brother Tran Dinh-Minh Tu, my loving wife Tang Kha Tu and our two lovely sons (Duy and Tan). I am forever beholden to my parents for their tireless care, nurture, and support over the past thirty years and, hopefully for many more to come. Over this journey, my wife and our two sons continue to be my biggest spiritual base: listening and sharing all the difficulties of life.

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THESIS ABSTRACT

Background: There exists a significant risk of visual impairment and/or blindness with higher levels of myopia and therefore, slowing myopic growth is an imperative. Atropine, a non-selective muscarinic receptor antagonist is effective in slowing myopia, however, its use is associated with an increase in pupillary diameter and reduction in accommodative amplitude. Adenosine receptor antagonists such as 7-Methylxanthine may play a role in slowing myopia. We aimed to determine the role of topical Caffeine, a Xanthine derivate in slowing myopia either alone or in combination with Atropine.

Methods: A systematic review and meta-analysis of published literature (1980 to 2020) was conducted to evaluate the efficacy and side effects of various concentrations of Atropine. Thereafter, in a two-week, dispensing trial, 58 children with myopia were randomised to either 0.01%, 0.02% and 0.03% Atropine and changes in pupillary diameter and accommodative amplitude were studied. A single concentration of Atropine was selected for use in a prospective, randomized dispensing trial, where 96 children with myopia were randomised to nightly use of either Caffeine-2%, Atropine plus Caffeine-2% and Atropine. At 6 months, change in spherical equivalent and axial length, pupillary diameter and accommodative amplitude were compared between groups and to a single vision spectacle control group (n=86). Finally, a validation trial was conducted in young adults (n=30) to observe over 24 hrs, temporal changes in pupillary diameter and accommodative amplitude with 2% Caffeine, 0.05% Atropine, 0.05% Atropine with 2% Caffeine, 0.10% Atropine and 0.10% Atropine with 2% Caffeine. Comparison between groups was performed using repeated measures Analysis of Variance (ANOVA) with the level of significance set at 5% and post-hoc multiple comparisons were conducted using Bonferroni correction.

Results: The meta-analysis confirmed that all concentrations except for 0.01% Atropine slowed myopia. The increase in pupillary diameter and reduction in accommodative amplitude was non-linear with a steep rise for <0.1% Atropine and a more gradual slope for ≥0.1% Atropine. Change

in pupillary diameter/reduction in accommodative amplitude was <1mm/ <2.0D vs 3.2mm/10.7D for <0.1% vs ≥0.1% Atropine. The 2-week trial found Atropine-0.03% to significantly increase pupillary diameter by >3mm compared to 0.02% and 0.01% (23.5% vs 10.5% vs 4.8%, respectively), whereas Atropine-0.01% demonstrated no change in pupillary diameter or accommodative amplitude in 29% to 33% of eyes. Atropine-0.02% was selected for use in the dispensing trial. At six months, change in spherical equivalent/axial length was -0.20±0.34D/0.08±0.11mm, -0.20±0.30D/0.11±0.11mm, -0.39 ± 0.38D/0.19±0.15mm and -0.33±0.29D/0.18±0.11mm with 0.02% Atropine, 0.02% Atropine with 2% Caffeine, 2% Caffeine and single vision spectacles, respectively. The pupillary diameter increase/reduction in accommodative amplitude at 6-month visit was 1.20±0.85mm/-3.14±4.08D, 0.76±0.58mm/-2.84±4.35D, -0.07±0.47mm/-0.78±3.43D and -0.10±0.32mm/-0.22±3.81D, respectively. Temporal observations over 24 hrs for change in pupil diameter indicated, a) no significant variation with Caffeine, b) 0.05% Atropine and 0.1% Atropine significantly increased pupillary diameter and c) 2% Caffeine in combination with Atropine - 0.05% and 0.1% resulted in less variation of the pupillary response and fewer eyes reaching higher pupillary diameter. These observations were significant for Day 0 when pupils were in the dilation phase. There were no significant changes for accommodative amplitude.

Conclusion: Caffeine at a concentration of 2% did not slow myopia but when used in combination with Atropine minimised the increase in pupillary diameter. The results indicate potential avenues for the use of Caffeine in combination with Atropine to improve efficacy whilst minimising the risk of side effects.

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LIST OF ABBREVIATIONS

D: Dioptre

MMD: myopic macular degeneration

SE or Sph-Eqv: spherical equivalent

AL: axial length

ACh: acetylcholine

mAChRs: muscarinic acetylcholine receptors

ADORs: adenosine receptors

logMAR: logarithm of the Minimum Angle of Resolution

RCT: randomized clinical trial

AA or AoA: amplitude of accommodation

PD: pupillary diameter

SD: standard deviation

CI: confidence of interval

Atr: Atropine

Caff: Caffeine

SV specs: Single vision spectacles

VA: Visual acuity

vs: versus

IOP: intraocular pressure

ANOVA: Analysis of Variance

Chapter 1. Introduction

1.1 Definition of myopia

Myopia, also known as near-sightedness, is the most common cause of distance vision impairment. It is characterized by an incongruity or mismatch between the optical components and the length of the eye causing the light rays from distant objects to be focused in front of the retina and therefore, resulting in blurred vision for distance (Flitcroft et al. 2019).

1.2 Trends in prevalence of myopia, high myopia, and associated burden

Myopia has reached epidemic proportions with high and rising prevalence in many parts of the world. For example, myopia prevalence in the United States nearly doubled from 25% in 1972 to 41.6% in 2004 (Vitale et al. 2009). A more recent report from US found 42.2% of myopia prevalence in 10 to 15 years old, and 72.4% in adults aged 20 to 25 years (Hrynchak et al. 2013). This prevalence was nearly 4-times higher compared to the myopia prevalence of 17% reported in 10,153 young Americans in 1931 (Tassman 1932). In Taiwan, from 1983 to 2017, myopia prevalence increased from approximately 5.4% to 25.4% in 7-year old's and from 30.7% to 76.7% in 12-year old's. (Tsai et al. 2021).

The rate of myopia prevalence in many Asian countries is staggering. Taiwan had the highest prevalence with >80% of children myopic by 16-18 years of age (Lin et al. 2004). In China, the prevalence varied between urban and rural regions; prevalence was high at 73.1% by 15 years of age in urban areas (He et al. 2004; He et al. 2019). Although data from Vietnam is sparse with only two cross-sectional studies reporting prevalence from rural provinces, myopia prevalence was already moderate at 14.2% and 20.4% in schoolchildren aged 12 to 16 years respectively (Paudel et al. 2014; Hung et al. 2020). Furthermore, both studies demonstrated an increasing prevalence with age. In Korea, a nationwide survey reported an overall myopia prevalence of 53.7% whereas prevalence was 50% for children aged 5 to 11 years and increased to 78.8% for

those aged 12 to18 years (Yoon et al. 2011). In urban areas, the prevalence of myopia was staggeringly high at 96.5% in a sample of 23,616 male participants aged 19 years (Jung et al. 2012). In Hong Kong, myopia prevalence increased with age, from 17% at below 7 years old to 53.1% at 11 years old and above (Fan et al. 2004).

Holden et al reported that the worldwide prevalence will continue to rise with nearly 50% of the world's population myopic by the year 2050; estimates for the many regions of the world varies from 22.7% to up to 66.4% (Holden et al. 2016). As a consequence of the rising prevalence of myopia, high myopia is also set to rise and estimated to affect 10% of the world population or nearly 1 billion people by the year 2050 (Holden et al. 2016). Presently, the prevalence of high myopia ranges between 2% to 5.5% in different countries (Katz et al. 1997; Sawada et al. 2008). The rising prevalence of high myopia is a concern as it not only imposes a greater level of financial burden (Naidoo et al. 2019), but is also associated with visual impairment and other significant sight-threatening complications such as myopic macular degeneration (MMD) (Haarman et al. 2020), glaucoma (Xu et al. 2007; Jonas et al. 2017), and post-cataract extraction retinal detachment (Neuhann et al. 2008). MMD was already identified as one of the leading causes of vision impairment worldwide, in Copenhagen (Buch et al. 2004), and in Rotterdam (Klaver et al. 1998), and was the primary cause of blindness in Japan (Iwase et al. 2006). Furthermore, a study from Netherlands reported that permanent macular damage was the most common cause of blindness in high myopia (worse than -6.0D) (Verhoeven et al. 2015). High myopia (at the level of worse than -10.0D) was said to have 22 times higher risk of visual impairment compared to emmetropic eyes (Flitcroft 2012). Additionally, highly myopic eyes may be associated with the reduction of visual quality due to either retinal stretching and/or poorer performance resulting from high-prescription spectacles (Strang et al. 1998; Atchison et al. 2006; Jong et al. 2018). Given the significant loss of productivity which was estimated to be up to US$244 billion for the year 2015 alone (Naidoo et al. 2019) and decrements in quality of life due to visual impairment

associated with myopia (Klein et al. 1995; Sankaridurg et al. 2021), controlling or reducing the risk of myopia progression is an urgent priority.

1.3 Myopia progression

Each dioptre of myopia that can be slowed significantly reduces the risk of myopic complications later in life (Hsu et al. 2004; Iwase et al. 2006; Bullimore and Brennan 2019). Myopia commonly progresses in childhood and teenage years. Although the condition is said to stabilize in late teens to early adulthood, there is significant individual variation and thus it is difficult to determine when an individual eye will stop progressing. Progression of myopia is commonly monitored using change in axial length (AL) and/or change in spherical equivalent (SE) (Cooper et al. 2012). Data on annual axial elongation in myopic eyes is limited but may vary from 0.1 mm to 0.45 mm depending on ethnicity and age, (Saw, Chua, et al. 2005; Fledelius et al. 2014; Diez et al. 2019) but there is more data available for annual change in spherical equivalent. In general, it is observed that children with myopia tend to progress about -0.15 to -0.25 D/year faster at younger ages, i.e. 6 to 9 years old compared to older children, i.e. aged 10 years and above (Sankaridurg and Holden 2014). In Asian eyes, mean annual spherical equivalent progression was about 1.1 D/year at 7 years and significantly decreased to about 0.5 D/year at 12 years (Donovan et al. 2012). Similar findings with reduced annual spherical equivalent progression with age were reported from other independent studies (Chua et al. 2016; Baird et al. 2020; Grzybowski et al. 2020). Additionally, annual rate of progression was greater in females than in males (Zhao et al. 2002; Donovan et al. 2012), in Asian ethnicities than Caucasians (Donovan et al. 2012; French, Morgan, Burlutsky, et al. 2013; Zhou et al. 2016), and in urban residents than those from rural area (Zhao et al. 2002; Chua et al. 2016; Zhou et al. 2016). In United States, the mean annual spherical equivalent progression in children was reported to be between -0.38 and -0.5 D/year (Gwiazda et al. 2003; Mutti et al. 2011). In Hong Kong, in 7,560 children with myopia aged 5 to 16 years old, the mean annual spherical equivalent progression was at -0.63 D/year (Fan et al. 2004). In Singapore, the

annual rate of spherical equivalent progression was reported at -0.8 D/year at 7 years of age, -0.66 D/year at 8 years, -0.57 D/year at 9 years old (Saw, Tong, et al. 2005).

1.4 Risk factors

The mechanisms underlining myopia development and progression remain unclear but are considered to result from a complex multifactorial interplay involving genetic and environmental factors. Although not entirely clear, it is generally regarded that the factors underlying onset and progression are similar (McBrien et al. 1993; Flitcroft 2012; Pan et al. 2012; McBrien et al. 2013; Stambolian 2013).

1.4.1 *Genetic factors*

The role of genetics/heredity in the pathogenesis of myopia is supported by studies demonstrating family aggregation and myopia (Yap et al. 1993; Wojciechowski et al. 2005; Ip et al. 2007; Pan et al. 2012; Tedja et al. 2019). Although the estimates varied, heritability between twins was found to be as high as 98% (Teikari et al. 1988; Lyhne et al. 2001; Tsai et al. 2009; Sanfilippo et al. 2010). Hammond and associates (Hammond et al. 2001) analysed data from 506 twin pairs (280 fraternal and 226 identical pairs) and found a higher interclass correlation for spherical equivalent refractive error in the monozygotic compared to the dizygotic group. Other studies also supported the finding of strong correspondence in monozygotic twins (identical genetic complex) and the mapped loci were found responsible for scleral remodelling and axial elongation (Lyhne et al. 2001; Weng et al. 2006; Hornbeak and Young 2009; Wojciechowski 2011; Verhoeven et al. 2013) Heritability was also demonstrated in those with family history of myopia; there was an association with the level of myopia and risk of progression in those with a family history of parental myopia (Ip et al. 2007). Additionally, the heritability correlation with myopia onset was slightly but significantly higher in women than in men (Dirani et al. 2008).

Over the past 30 years, many linkage studies were conducted and identified nearly 200 independent loci that correlated with myopia (Tedja et al. 2019) such as MYP 1 to 20 and others (Young et al. 1998; Naiglin et al. 2002; Paluru et al. 2003; Teare and Barrett 2005; Zhang et al. 2005; Nallasamy et al. 2007; Lam et al. 2008; Hornbeak and Young 2009; Baird et al. 2010; Jacobi and Pusch 2010; Wojciechowski 2011).

A more recent meta-analysis of GWAS (genome-wide association studies) involving 542,934 European participants identified more than 336 novel loci but found refractive error to be genetically heterogeneous. Interestingly, they found genetic factors associated with circadian rhythm and pigmentation to be involved in the development of refractive error including myopia (Hysi et al. 2020). Despite these promising findings, at the present time, there are no approaches to predict and treat myopia using genetic factors.

1.4.2 *Environmental factors*

There is much evidence from animal models and human data that supports remodelling/adjustment of eye growth in response to form deprivation and optical defocus and indicates that visual feedback plays a significant role in eye growth.

1.4.2.1 Form-deprivation myopia

Form-deprivation myopia has been demonstrated in a number of species including monkeys (Smith III et al. 1999), guinea pigs (Li et al. 2010), tree shrews (Siegwart and Norton 2001), fishes (Shen et al. 2005), and the mice (Tejedor and de la Villa 2003). An association between myopia and severe unilateral ptosis was reported in humans indicating that form-deprivation occurs in human eyes (Huo et al. 2012). Similarly, Gusek-Schneider et al. reported a greater association with myopia in ptotic eyes than in contralateral normal eyes (Gusek-Schneider and Martus 2001). Although the mechanism underlying form-deprivation and optical defocus myopia remains unknown, it is thought that visual feedback plays an integral role with an unclear/blurred image

on the retina being suspected to provoke or stimulate myopia progression (Duncan and Collison 2003).

1.4.2.2 Optical defocus

Similar to results obtained with form-deprivation, optical defocus using plus or minus lens was observed to modulate eye growth (Schaeffel et al. 1988). In general, relative hyperopic defocus accelerated the progression of eye growth whereas relative myopic defocus slowed/halted the axial elongation (Smith III et al. 2014). Experiments on chick eyes suggested that the retina could detect the sign of optical defocus in order to compensate for the induced defocus (Schaeffel et al. 1990; Diether and Schaeffel 1997). Modulation of eye growth in response to optical defocus was also replicated in many species including monkeys (Hung et al. 1995; Smith III and Hung 1999; Whatham and Judge 2001), guinea pigs (Howlett and McFadden 2009), or mice (Pardue et al. 2013; Jiang et al. 2018).

Recent human studies indicated that the central retina is capable of detecting and correcting optical defocus to result in a clear retinal image; the resulting changes in eye length were bidirectional and implied that the system was capable of detecting the sign of defocus (Read et al. 2010). Indeed, similar results were observed in axial length and sub-foveal choroidal thickness of myopic children (8 to 16 years of age) with either hyperopic or myopic defocus compared to control (Wang et al. 2016). Furthermore, it appears that the peripheral retina also plays an important role in regulating eye growth in response to environmental factors such as defocus. The evidence from key experiments in primates showed that despite a non-functional/damaged fovea, the peripheral retina alone could guide emmetropisation and furthermore, modulation of defocus at the peripheral retina predictably altered eye growth (Smith III et al. 2007; Huang et al. 2015). Further evidence for the role of the central and/or peripheral retina was from clinical trials involving modulation of the peripheral retinal refractive status using specially designed spectacles

and contact lenses including orthokeratology lenses (Sankaridurg et al. 2010; Cho and Cheung 2012; Chamberlain et al. 2019; Cho and Tan 2019; Lam et al. 2020).

1.4.2.3 Near activities, outdoor, education and others

Some of the environmental factors considered to influence the onset and progression of myopia include near work or time spent on near activities, outdoor time, rural-urban location, socio-economic status, and education (Sankaridurg and Holden 2014; Rudnicka et al. 2016; He et al. 2019; Ho et al. 2019). Studies have indicated an association between myopia progression and intelligence (Rosner and Belkin 1987; Wensor et al. 1999; Wong et al. 2000; Verma and Verma 2015), and level of education (Wu et al. 2001; Mountjoy et al. 2018). In an Orthodox Jewish study of 870 teenagers (both sexes), males with fewer breaks from study were more likely to become myopic (Zylbermann et al. 1993) and data from the Sydney Myopia Study (Ip et al. 2008; Rose et al. 2008) indicated that myopia onset was affected by the continuous work at near distances. Questionnaire data from 2,353 Australian school-children found continuous reading of over 30 minutes to increase the risk of myopia (Ip et al. 2008), and higher level of near work was associated with less hyperopia (Rose et al. 2008). In 2015, Huang and associates reviewed data from 27 studies published from 1989 to 2014, covering 25,025 participants aged 6 to 18 years old. The reported that the risk of myopia increases 2% for every additional hour of near work (Huang et al. 2015). An association between myopia development and increased time on near-work activities and reading was also reported from other studies (Khader et al. 2006; Yingyong 2010).

A much stronger association was reported for myopia and less time outdoors (French, Morgan, Mitchell, et al. 2013). Data from both an older (mean baseline age of 12 years) and younger cohort (mean baseline age of 6 years) indicated a significant higher risk for myopia with high levels of time indoors and reading (OR between 5.1 (95% CI, 1.91 – 13.45) and 15.9 (95% CI, 3.45 – 73.40)) (French, Morgan, Mitchell, et al. 2013). In 2017, data of 34,420 participants from 25

relevant studies were pooled for a meta-analysis on the role of time outdoors on myopia onset and progression, and found that greater time-outdoors was protective for myopia onset but not for progression (Xiong et al. 2017). A more recent meta-analysis indicated that more than 120 minutes per day of outdoor exposure can prevent both development and progression of myopia (Ho et al. 2019). A high level of time-spent outdoors was significantly effective in preventing myopia onset and reducing myopic shift (He et al. 2015; Wu et al. 2018; Yao et al. 2019).

A greater incidence of myopia is also observed for children living in urban compared to rural areas and demonstrated across various countries such as China (Saw et al. 2001; He et al. 2009), Iran (Hashemi et al. 2004; Fotouhi et al. 2007), and Thailand (Yingyong 2010). In 2016, a meta-analysis of pooled data from more than 370,000 subjects, aged between 1 and 18 years from 42 countries and 143 relevant articles, found urban living to significantly increase the risk of myopia development compared to rural living and this was demonstrated across many ethnicities, OR 2.87 (95% CI 1.53 to 4.66) in East Asian, 4.21 (95% CI 1.69 to 11.9) in South Asian, and 6.69 (95% CI 2.69 to 18.3) in Southeast Asian countries (Rudnicka et al. 2016).

1.5 Strategies to control myopia progression

Given the rising burden associated with higher levels of myopia, there is a need to slow progression of myopia. Presently, there are two main strategies utilizing either optical and/or pharmaceutical approaches to control progression of myopia. In 2016, Huang and associates conducted a systematic review of 30 randomized clinical trials covering 16 myopia control interventions compared to either single vision spectacles or lenses or placebo (Huang et al. 2016). The authors then classified the effectiveness of the intervention as marked, moderate or minimal based on the change in spherical equivalent and axial length with intervention compared to control. Of the various interventions assessed, the highest efficacy for slowing myopia was achieved with Atropine eye drops, followed by pirenzepine whereas orthokeratology and

peripheral defocus-modifying contact lenses showed moderate effects, and myopia-control spectacles were found to exert only minimal effects in controlling myopic growth.

1.5.1 *Optical strategies for myopia control*

In general, optical interventions for slowing myopia are based on two main mechanisms: a) reduction of hyperopic blur/defocus at the central retina due to accommodative dysfunction and/or b) reduction of peripheral retinal hyperopic defocus. Undercorrection, in earlier trials, was attempted as a measure to reduce accommodative lag associated with myopia. However, in two randomized clinical trials (Chung et al. 2002; Adler and Millodot 2006) undercorrection (approximately 0.5D and 0.75D) had no effect or was even found to exacerbate myopia progression. Other spectacle-lens based myopia control treatments include (1) progressive additional spectacles (PAL) (Leung and Brown 1999; Gwiazda et al. 2003; COMET 2011; Berntsen et al. 2013; Hasebe et al. 2014), (2) positively aspherized progressive additional spectacles (PA-PAL) (Hasebe et al. 2014), (3) peripheral defocus spectacles (Peri-def) (Sankaridurg et al. 2010), (4) executive bifocal with or without prisms (exec bifocal) (Cheng et al. 2014) and more recently, (5) defocus incorporated multiple segments (DIMS) (Lam et al. 2020) or(6) multiple aspherical lenslets (Bao et al. 2021). Although many spectacle-lens based approaches found limited myopia control effect, i.e., about 20% or less, compared to other interventions (Huang et al. 2016), there are some notable exceptions. A study using executive bifocals, both with and without prisms, reported myopia control of up to 54% with higher efficacy in a sub-group with low lag of accommodation (Cheng et al. 2014). More recent data from a study that utilised multiple lens segments in the mid-periphery of the lens (DIMS spectacle) found a significant treatment efficacy of up to 60% over two years (Lam et al. 2020). Additionally, after one year of lens wear, highly aspherical lenslet spectacles slowed myopia by 67% and 64%with regards to spherical equivalent refractive error and axial elongation respectively; in comparison,

slightly aspherical lenslet spectacles were able to slow myopia by 41% and 31%, respectively (Bao et al. 2021).

Similar to spectacles, contact-lens based interventions for myopia control were developed with the objective of a) reducing the relative hyperopic defocus at the peripheral retina and/or, b) inducing myopic defocus at central retina (Anstice and Phillips 2011; Walline et al. 2013; Fujikado et al. 2014; Lam et al. 2014; Aller et al. 2016; Sankaridurg et al. 2017; Ruiz-Pomeda et al. 2018; Chamberlain et al. 2019). Additionally, orthokeratology (Ortho-K) lenses were also found to be effective in slowing progression of myopia; the post orthokeratology corneal profile resulting in less hyperopic defocus at the peripheral retina was thought to play a role (Cho et al. 2005; Walline et al. 2009; Kakita et al. 2011; Cho and Cheung 2012; Hiraoka et al. 2012; Santodomingo-Rubido et al. 2012; Chen et al. 2013). The mean axial length reduction in groups using orthokeratology varied from approximately 30% to slightly more than 50% compared to the control group.

Although the earlier spectacle-lens based approaches including those based on reduction of peripheral defocus were not highly effective, contact-lens based approaches consistently and significantly slowed myopia with an efficacy of up to 72% observed in myopic children with eso fixation disparities (Aller et al. 2016). In a 3-year clinical study, MiSight soft contact-lenses significantly slowed myopia with up to 59% slowing of spherical equivalent progression and 52% reducing of axial elongation as compared to a control group (Chamberlain et al. 2019). The MiSight lens has been approved by the FDA for myopia control use in young children.

Table 1.1 lists the efficacy of various optical interventions for myopia control treatment.

Table 1.1: Annual change of myopia progression with different optical strategies

Authors and study	Method of treatment	Sample [age range]	Design [myopia range, SER]	Control group	Cycloplegic refraction (Y/N) [Eye drops]	Myopia control effects (%)	
						SE	**AL**
Chung et al. (Chung et al. 2002)	UC +0.75D	94 [9-14]	Nonrandomized [-0.50 and worse]	SVL	N	Worse 29.9	N/A
Adler et al. (Adler and Millodot 2006)	UC +0.50D	48 [6-15]	Randomized [-1.00 to -5.00]	SVL	N	Worse 20.0	N/A
Koomson et al. (Koomson et al. 2016)	UC +0.50D	150 [10-15]	Randomized [-1.25 to -4.50]	SVL	Y (N/A)	7.4	12.5
Spectacles-based interventions							
Leung and Brown (Leung and Brown 1999)	PAL +1.50 D	80 [9-12]	Nonrandomized [-1.00 to -5.00]	SVL	N	38.2	33.8
Leung and Brown (Leung and Brown 1999)	PAL +2.00 D	94 [9-14]	Nonrandomized [-1.00 to -5.00]	SVL	N	46.3	44.6
Edwards et al. (Edwards et al. 2002)	PAL +1.50 D	298 [7-10.5]	Randomized [-1.25 to -4.50]	SVL	Y (1% cyclopentolate)	11.1	3.2
Gwiazda et al. (Gwiazda et al. 2003)	PAL +2.00 D	469 [6-11]	Randomized [-1.25 to -4.50]	SVL	Y (1% tropicamide)	13.5	N/A
Yang et al. (Yang et al. 2009)	PAL	149 [7-13]	Randomized [-0.50 to -3.00]	SVL	Y (0.5% tropicamide + 0.5% phenylephrine)	17.3	N/A
COMET 2 (COMET 2011)	PAL +2.00 D	180 [8-12]	Randomized [-0.75 to -2.50]	SVL	Y (1% tropicamide)	24.3	N/A
Berntsen et al. (Berntsen et al. 2013)	PAL +2.00 D	85 [6-11]	Randomized [-0.75 to -4.00]	SVL	Y (1% tropicamide)	32.7	26.7
Cheng et al. (Cheng et al. 2014)	Bif	135 [8-13]	Randomized [-1.00 or worse]	SVL	Y (1% cyclopentolate)	39.8	33.3
Cheng et al. (Cheng et al. 2014)	Bif + Pr	135 [8-13]	Randomized [-1.00 or worse]	SVL	Y (1% cyclopentolate)	53.8	43.6

Sankaridurg et al. (Sankaridurg et al. 2010)	Peri-def 1	210 [6-16]	Randomized [-0.75 to -3.75]	SVL	Y (1% tropicamide)	5.6	0.0
Sankaridurg et al. (Sankaridurg et al. 2010)	Peri-def 2	210 [6-16]	Randomized [-0.75 to -3.75]	SVL	Y (1% tropicamide)	Worse 2.2	2.8
Sankaridurg et al. (Sankaridurg et al. 2010)	Peri-def 3	210 [6-16]	Randomized [-0.75 to -3.75]	SVL	Y (1% tropicamide)	16.7	13.9
Hasebe et al. (Hasebe et al. 2014)	PAL (Phase1)	197 [6-12]	Randomized [-1.25 to -6.00]	SVL	Y (0.5% tropicamide + 0.5% phenylephrine)	25.8	N/A
Hasebe et al. (Hasebe et al. 2014)	PA-PAL +1.00 D	197 [6-12]	Randomized [-0.50 to -4.50]	SVL	Y (0.5% tropicamide + 0.5% phenylephrine)	13.7	7.3
Hasebe et al. (Hasebe et al. 2014)	PA-PAL +1.50 D	197 [6-12]	Randomized [-0.50 to -4.50]	SVL	Y (0.5% tropicamide + 0.5% phenylephrine)	20.0	11.7
Lam et al. (Lam et al. 2020)	DIMS	160 [8-13]	Randomized [-1.00 to -5.00]	SVL	Y (1% cyclopentolate)	51.8	61.8
Contact lens-based interventions							
Anstice et al. (Anstice and Phillips 2011)	DF CLs	40 [11 – 14]	Randomized cross-over [-1.25 to -4.50]	Paired-eye SVCLs	Y (1% tropicamide)	36..2	50.0
Sankaridurg et al. (Sankaridurg et al. 2011)	Novel CLs	85 [7 – 14]	Randomized [-0.75 to -3.50]	SVL	Y (1% tropicamide)	33.7	32.5
Walline et al. (Walline et al. 2013)	Multifocal CLs	54 [8 – 11]	Historical control [-1.00 to -6.00]	SVCLs	Y (1% tropicamide)	50.5	29.3
Fujikado et al. (Fujikado et al. 2014)	PA CLs	24 [10 – 16]	Randomized [-0.75 to -3.50]	SVCLs	Y (1% tropicamide)	26.2	25.0
Lam et al. (Lam et al. 2014)	DIMS CLs	128 [8 – 13]	Randomized [-1.00 to -5.00]	SVCLs	Y (1% cyclopentolate)	25.0	27.8
Aller et al. (Aller et al. 2016)	Bif CLs	79 [8 – 18]	Randomized [-0.50 to -6.00]	SVCLs	Y (1% tropicamide)	72.2	79.2

Cheng et al. (Cheng et al. 2016)	+SA CLs	109 [8-11]	Randomized [-0.75 to -4.00]	SVL	Y (1% tropicamide + 1% cyclopentolate)	20.2	38.6
Sankaridurg et al. (Sankaridurg et al. 2017)	PDM CLs 1	508 [7-13]	Randomized [-0.75 to -4.25]	SVCLs	Y (1% tropicamide)	28.4	41.2
Sankaridurg et al. (Sankaridurg et al. 2017)	PDM CLs 2	508 [7-13]	Randomized [-0.75 to -4.25]	SVCLs	Y (1% tropicamide)	20.9	32.4
Sankaridurg et al. (Sankaridurg et al. 2017)	EDOF CLs 1	508 [7-13]	Randomized [-0.75 to -4.25]	SVCLs	Y (1% tropicamide)	28.4	32.4
Sankaridurg et al. (Sankaridurg et al. 2017)	EDOF CLs 2	508 [7-13]	Randomized [-0.75 to -4.25]	SVCLs	Y (1% tropicamide)	23.9	35.3
Ruiz-Pomeda et al. (Ruiz-Pomeda et al. 2018)	MiSight (DF CLs)	89 [8-13]	Randomized [-0.75 to -4.00]	SVCLs	Y (1% cyclopentolate)	48.8	50.0
Chamberlain et al. (Chamberlain et al. 2019)	MiSight (DF CLs)	144 [8-12]	Randomized [-0.75 to -4.00]	SVCLs	Y (1% tropicamide)	69.0	62.5
Orthokerotology interventions							
Cho et al. (Cho et al. 2005)	Ortho-K	35 [7-12]	Historical control [-0.25 to -4.50]		N		46.3
Walline et al. (Walline et al. 2009)	Ortho-K	28 [8-11]	Historical control [-0.75 to -4.00]		N		56.1
Kakita et al. (Kakita et al. 2011)	Ortho-K	101 [8-16]	Nonrandomized [-0.50 to -10.00]	SVL	N		36.1
Hiraoka et al. (Hiraoka et al. 2012)	Ortho-K	59 [8-12]	Nonrandomized [-0.50 to -5.00]		N		29.8
Santodomingo-Rubido et al. (Santodomingo-Rubido et al. 2012)	Ortho-K	61 [6-12]	Nonrandomized [-0.75 to -4.00]		N		31.9
Cho and Cheung (Cho and Cheung 2012)	Ortho-K	78 [6-10]	Randomized [-0.50 to -4.00]	SVL	N		42.9
Chen et al. (Chen et al. 2013)	Ortho-K	58 [6-12]	Nonrandomized [-0.50 to -5.00]		N		51.6

Notes: UC: Undercorrection; DF: Dual-Focus; CLs: Contact lens; Bif: Bifocals; Bif+Pr: Bifocals with prism; PAL: Progressive Addition Lens; PA-PAL: Positively Aspherized Progressive Addition Lens; MFs: multifocal spectacles; SVL: single vision spectacles; SVCLs: single vision soft contact lens; DIMS: defocus incorporated multiple segments; MFCLs: multifocal contact lenses;+SA: positive spherical aberration; Peri-def: Peripheral defocus; PDM CLs: Peripheral defocus-modifying contact lenses; Ortho-K: Orthokeratology

1.5.2 *Pharmaceutical strategies for myopia control*

So far, two main groups of pharmaceutical agents have been considered for myopia control: a) muscarinic receptor antagonists including Atropine and Pirenzepine and, b) adenosine receptor antagonists such as 7-Methylxanthine. Other compounds such as Timolol and Tropicamide were trialled early on, however, there have been no recent investigations conducted using these compounds (Sankaridurg et al. 2018).

1.5.2.1 Muscarinic receptor antagonists

Of the various muscarinic receptor antagonists, Atropine remains the only pharmaceutical agent that has been widely adopted for myopia control (Fang et al. 2013; Leo 2017; Walline et al. 2020). Despite the promising data from clinical trials wherein Pirenzepine was demonstrated to slow myopia progression by up to 50% with fewer side effects than high dose Atropine (Tan et al. 2005; Siatkowski et al. 2008), this compound is no longer pursued in further investigations with unknown reasons and is also not commercially available. On the other hand, Atropine is rising in popularity, has been investigated in a number of research studies around the world (Yen et al. 1989; Shih et al. 1999; Chua et al. 2006; Fan et al. 2007; Chia et al. 2012; Clark and Clark 2015; Polling et al. 2016) and is broadly adopted by many practitioners worldwide to manage myopia (Lee et al. 2006; Yi et al. 2015; Lee et al. 2016; Wang et al. 2017; Joachimsen et al. 2019; Sacchi et al. 2019). A more thorough discussion of Atropine is provided in section 1.6, Chapter 1.

1.5.2.2 Adenosine receptor antagonists

The second class of pharmaceutical agents for myopia are based on Xanthine compounds. Trier et al. (Trier et al. 2008) randomised 68 children with myopia (mean age 11.3 years) to either oral 7-Methylxanthine or a placebo for one year; oral 7-Methylxanthine for all participants in the second year and a washout phase in the third year. At the end of 2 treatment years, axial length

progression was slowed but not spherical equivalent (Trier et al. 2008). In primates and rabbits, 7-Methylxanthine proved effective in reducing axial myopia induced by hyperopic defocus (Hung et al. 2018). Furthermore, topically instilled Caffeine, another Xanthine compound was found to perform similarly to 7-Methylxanthine with a reduced likelihood of primate eyes responding to hyperopic defocus (Arumugam et al. 2017). However, in recent studies conducted in chicks, 7-Methylxanthine had no effect on form-deprivation myopia or on the retinal dopamine system (Liu et al. 2020).

In the following paragraphs, the various properties of Atropine and Caffeine and their possible role in myopia control are provided.

1.5.3 *Combination strategies for myopia management (the following section was published in part on the website of Review of Myopia Management in April 2021, https://reviewofmm.com/combination-strategies-for-myopia-management/)*

A combination therapy is a logical and methodical approach to improve either the wearer experience and/or efficacy obtained with monotherapy alone. Indeed, the approach of improving wearer experience is already well-known and applied frequently; for example, children prescribed with higher concentrations of atropine are fitted with bi-focal or multifocal to restore vision for both distance and near (Chou et al. 1997; Syniuta and Isenberg 2001; Polling et al. 2016). However, of greater interest is the aim of improving efficacy over that realized with monotherapy alone. Many precedents exist for this approach in management of health and disease where combination therapies are frequently used to boost efficacy. They are particularly useful where multiple pathways are involved in the condition as it allows targeting via more than a single pathway. It is probable that some practitioners already adopt this route using the available evidence on efficacy and risks versus benefits to maximize efficacy for their myopic patient – an example would be recommending improved outdoor time in combination with an optical

intervention or combination between an optical strategy and a pharmaceutical compound or more than one pharmaceutical composition. In fact, combination therapy showed superior efficacy compared to monotherapy in many reports in the world (Shih et al. 2001; Kinoshita et al. 2018; Wan et al. 2018; Kinoshita et al. 2020).

Furthermore, if combination therapy is instituted as first line treatment prior to monotherapy and does not produce the intended benefits it may be difficult to know as to which of the two systems is at fault. On the other hand, a combination therapy provides a greater chance of efficacy, may be a valuable option especially in children with fast risk of progression where the risk of side effects and rebound of atropine precludes use of higher doses of Atropine.

1.6 Atropine for myopia control (the following section was submitted in part and published as (Tran et al. 2018) in Journal of Ocular Pharmacology and Therapeutics, 34(5), 2018 and on the website of Review of Myopia Management, in October 2020; https://reviewofmm.com/mechanism-of-action-of-Atropine-in-controlling-myopia-progression/)

1.6.1 *Biochemistry of Atropine*

Atropine Sulfate, Figure 1.1, is the sulfate salt of Atropine, an alkaloid that can be derived from plants such as *Datura stramonium*, *Atropa belladonna*, *Hyoscyamus niger* and *Mandragora officinarum*. Atropine consists of an organic base (tropine) and an aromatic (tropic) acid to complete the structure of an organic ester. Atropine is a non-selective anti-muscarinic receptor agent with a high affinity to all five subtypes of muscarinic acetylcholine M1 to M5 receptors (North and Kelly 1987). The tropine base and tropic acid itself do not possess anti-muscarinic activity while the ester is considered to be responsible for the activity (North and Kelly 1987).

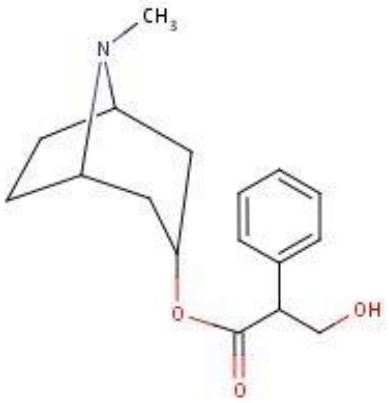

Figure 1.1: Chemical structure of Atropine

In the eye, Atropine was used topically at up to 4% concentration (North and Kelly 1987). The two main effects of topical application of 1% Atropine are mydriasis (dilation of pupil) and cycloplegia (paralysis of ciliary muscle resulting in loss of accommodation). While the mydriatic effect commences with full mydriasis at 30 minutes after the instillation and full recovery is in seven to ten days, the full cycloplegic effect is observed at approximately 40-minute post instillation and its full recovery is ranging from ten days to two weeks. In the clinical settings, other than its extensive application for cycloplegic refraction, Atropine has been used for amblyopia treatment (Chen and Cotter 2016), accommodative spasm (Chatzistefanou and Mills 2000; Laria et al. 2015) and as mentioned previously for myopia control.

1.6.2 *Mechanisms underlying myopia control with Atropine*

The various hypotheses that have been considered so far on the proposed mechanism of action of Atropine for myopia control are detailed below.

1.6.2.1 Via the accommodative pathway

Atropine was first used for myopia control based on the hypothesis that excessive accommodative effort caused myopia. Earlier studies considered that Atropine exerted the efficacy by its

cycloplegic action on the smooth ciliary muscle and blocking the accommodative function of the eye (McKanna and Casagrande 1981; Raviola and Wiesel 1985). However, studies in animal models demonstrated that sectioning of the optic nerve (Troilo et al. 1987) or lesioning of the Edinger–Westphal nucleus (Schaeffel et al. 1990) did not inhibit the development or recovery of experimental myopia and therefore indicated that local visual stimuli but not accommodative mechanism of Atropine might be involved in the complex interplay of myopia development and progression. Furthermore, there was some indication that Atropine may be exerting its myopia control effect *via* a non-accommodative pathway, as both Atropine and pirenzepine (another muscarinic antagonist), inhibited myopia in chicks, which lack muscarinic receptors (McBrien et al. 1993; Leech et al. 1995). This led to a shift away from considering accommodative pathways. Since in most mammalian species, the muscarinic receptors (M1 to M5) are also present across the retina, choroid, and sclera they have been explored as sites of action for Atropine.

1.6.2.2 Via muscarinic receptor pathways in the Retina

In chicks, increased ocular length and myopia progression was found in the presence of damaged retinal ganglion cells, photoreceptors and amacrine cells (Fischer et al. 1999); thus, it was possible that Atropine may be exerting its effect by altering retinal neurotransmission. However, contrary to expectations, ablation of the amacrine cells did not prevent Atropine from inhibiting axial elongation (Fischer et al. 1998). It was then concluded that Atropine may be exerting its growth-suppressing influence by acting on extraretinal muscarinic receptors, possibly in retinal pigment epithelium, the choroid, or the sclera.

Muscarinic receptors are also found in the retinal pigment epithelium which is involved in transferring the signalling cascade toward the target tissue—i.e., the choroid and/or the sclera (Seko et al. 1997; Lind et al. 1998). Atropine was found to increase the release of dopamine but reduced the electroretinogram (ERG) *b* and *d* waves and damped oscillations of retinal pigment

epithelium potentials by which Atropine was suggested to boost dopamine release from cellular stores and then to control eye growth (Schwahn et al. 2000). Indeed, in many experimental animal studies, the use of either dopamine or nonselective dopamine receptor agonists was found to inhibit the development of myopia (Schaeffel et al. 1995; Dong et al. 2011).

However, a retinal site of action cannot yet be ruled out as a study in chicks involving selective M4 and M1 receptors found strong support for muscarinic antagonists exerting myopia inhibition *via* M4 receptors that are likely located in the retina (McBrien et al. 2011). These pathways need to be explored further.

1.6.2.3 Via muscarinic receptor pathways in the Choroid

The choroid is a vascular structure that plays an active role in emmetropisation by changing thickness and moving the retinal image plane in response to optical defocus. Use of muscarinic antagonists, including Atropine, resulted in rapid and transient choroidal thickening and therefore altered eye growth as it was suggested that the two responses, i.e. choroidal thickening and eye growth might be linked (Nickla et al. 2013). In more recent studies, administration of 1% and even 0.01% Atropine resulted in increased sub and para-foveal choroidal thickness and was considered to be related to either due to blockage of accommodation, or due to loss of excitatory input to the choroidal non-vascular smooth muscle resulting in less contraction, or possibly due to the cross-reactivity of Atropine on other receptors resulting in blocking of receptors such as α-adrenoreceptors (Sander et al. 2014; Zhang et al. 2016). Furthermore, Atropine was found to inhibit choroidal thinning induced by hyperopic defocus (Nickla et al. 2013; Chiang and Phillips 2018). The mechanism leading to increased choroidal thickness is not clear, as use of Atropine did not result in alteration of choroidal blood flow in response to light/dark transitions (Fuchsjäger-Mayrl et al. 2003) or during isometric exercise (Polak et al. 2003).

1.6.2.4 Via muscarinic receptor pathways in the Sclera

On the other hand, there is a view that the sclera is the target site of Atropine for myopia inhibition. Cultured human scleral fibroblasts demonstrate mRNA expression of the five muscarinic receptors indicating that the sclera may be a potential site of action (Qu et al. 2006). The synthesis of glycosaminoglycan which is known as scleral extracellular matrix was inhibited by Atropine in isolated scleral tissue from chicks (Lind et al. 1998). Although at higher concentrations, Atropine was toxic to the scleral chondrocytes, this opened up another pathway *via* which Atropine may be exerting its effects. In addition, real-time PCR showed upregulation of the mRNA levels of M1, M3 and M4 receptors in the sclera after subconjunctival Atropine treatment in addition to lens-induced myopia in mice, compared to no changes found in the group of mice having lens-induced myopia without Atropine and the normal group of mice (Barathi and Beuerman 2011).

1.6.2.5 Via other receptors

Another consideration is that Atropine slows myopia progression *via* receptors other than muscarinic acetylcholine receptors (mAChRs). Firstly, only certain muscarinic antagonists inhibit myopia while the majority fail to do so. Other than Atropine and Pirenzepine, only Oxphenonium prevented form-deprivation myopia (FDM) in chicks. Other compounds resulted in partial rescue (Propantheline, Scopolamine, Tropicamide, Dexetimide) or had no effects (Mepenzolate, Procyclidine, Methoctramine) (Lind et al. 1998). Secondly, Atropine is not only a competitive antagonist that works by blocking the action of agonist – acetylcholine (Ach) but also has effects on glycosaminoglycan synthesis of isolated chicken scleral tissues in the absence of Ach (Lind et al. 1998). Hence, mAChRs may not be the only target of Atropine. In this regard, it was found that there was cross-reactivity of some muscarinic antagonists with adrenergic receptors, for example, MT3 binds not only to mAChR M4 but also α_{1A}-, α_{1D}-, α_{2A}-adrenoceptors (Näreoja et

al. 2011). In addition, α_2-adrenergic receptors found in human retina (Woldemussie et al. 2007) may play a role in regulating dopamine synthesis, which affects ocular elongation (Iuvone and Rauch 1983; Schaeffel et al. 1995).

In summary, the mechanism underlying Atropine's efficacy in slowing myopic growth remains unclear, but it is possible that Atropine may be exerting its effect *via* many sites of action rather than one.

1.6.3 *Efficacy data for myopia control with Atropine in human eyes*

Atropine was first investigated for myopia control in the 1970s (Gimbel 1973; Bedrossian 1979). Despite the controversies with respect to the site of action and the lack of clarity on the exact mechanism underlying myopia control effect of Atropine, the effectiveness of Atropine drops with various concentrations and in various ethnic groups has been consistently proven.

Table 1.2 lists the various studies that have published data on the myopia control efficacy of Atropine in human eyes; other pharmaceutical strategies for myopia are also reported.

Table 1.2: Annual change of myopia progression with Atropine and other pharmaceutical strategies

Authors and study	Method of treatment	Sample [age range]	Design [myopia range, SER]	Control group	Cycloplegic refraction (Y/N) [Eye drops]	Myopia control effects (%)	
						SE	**AL**
Atropine treatments							
Yen et al. (Yen et al. 1989)	1% Atrop	96 [6-14]	Randomized [-0.50 to -4.00]	Placebo	Y (1% Tropicamide + 1% Cyclopentolate)	75.8	N/A
Shih et al. (Shih et al. 1999)	0.1% Atrop	186 [6-13]	Randomized [-0.50 to -6.75]	0.5% Tropicamide	Y (1% Tropicamide)	55.7	N/A
Shih et al. (Shih et al. 1999)	0.25% Atrop	186 [6-13]	Randomized [-0.50 to -6.75]	0.5% Tropicamide	Y (1% Tropicamide)	57.5	N/A
Shih et al. (Shih et al. 1999)	0.5% Atrop	186 [6-13]	Randomized [-0.50 to -6.75]	0.5% Tropicamide	Y (1% Tropicamide)	96.2	N/A
Shih et al. (Shih et al. 2001)	0.5% Atrop	227 [6-13]	Randomized [N/A]	SVL	Y (1% Tropicamide)	70.0	62.7
Lee et al. (Lee et al. 2006)	0.05% Atrop	57 [6-12]	Retrospective case-control [-0.50 to -5.50]	SVL	Y (1% Tropicamide + 1% Cyclopentolate)	62.7	N/A
Chua et al. (Chua et al. 2006)	1% Atrop	400 [6-12]	Randomized [-1.00 to -6.00]	Placebo	Y (1% Cyclopentolate)	103.9	170.0
Fan et al. (Fan et al. 2007)	1% Atrop	46 [5-10]	Prospective case-control [-3.00 and worse]	SVL	Y (1% Cyclopentolate)	105.0	87.1
Chia et al. (Chia et al. 2012)	0.01% Atrop	400 [6-12]	Randomized [-2.00 and worse]	Historical controls	Y (1% Cyclopentolate)	43.4	Worse 20.0
Chia et al. (Chia et al. 2012)	0.1% Atrop	400 [6-12]	Randomized [-2.00 and worse]	Historical controls	Y (1% Cyclopentolate)	59.2	35.0

Chia et al. (Chia et al. 2012)	0.5% Atrop	400 [6-12]	Randomized [-2.00 and worse]	Historical controls	Y (1% Cyclopentolate)	77.6	45.0
Clark et al (Clark and Clark 2015)	0.01% Atrop	60 [6-15]	Retrospective case-control [-0.25 to -8.00]	SVL	Y (N/A)	83.3	N/A
Yi et al. (Yi et al. 2015)	1% Atrop	132 [7-12]	Randomized [-0.50 to -2.00]	SVL	Y (1% Cyclopentolate)	137.6	109.4
Lee et al. (Lee et al. 2016)	0.125% Atrop	56 [6-12]	Randomized [-0.50 to -3.00]	SVL	Y (N/A)	95.2	N/A
Lee et al. (Lee et al. 2016)	0.25% Atrop	56 [6-12]	Randomized [-0.50 to -3.00]	SVL	Y (N/A)	100	N/A
Polling et al. (Polling et al. 2016)	0.5% Atrop	77 [6-16]	Cohort [-3.00 and worse]	SVL	Y (1% Cyclopentolate)	90.0	
Yam et al. (Yam et al. 2019)	0.01% Atrop	327 [4-12]	Randomized [-1.00 and worse]	Placebo	Y (1% Cyclopentolate)	27.2	12.2
Yam et al. (Yam et al. 2019)	0.025% Atrop	327 [4-12]	Randomized [-1.00 and worse]	Placebo	Y (1% Cyclopentolate)	43.2	29.3
Yam et al. (Yam et al. 2019)	0.05% Atrop	327 [4-12]	Randomized [-1.00 and worse]	Placebo	Y (1% Cyclopentolate)	66.7	51.2
Joachimsen et al. (Joachimsen et al. 2019)	0.01% Atrop	56 [6-17]	Cohort [N/A]	Historical controls	N	61.9	
Larkin et al. (Larkin et al. 2019)	0.01% Atrop	198 [6-15]	Retrospective case-control [-1.00 to -6.00]	SVL	N	66.7	
Sacchi et al. (Sacchi et al. 2019)	0.01% Atrop	102 [5-16]	Retrospective case-control [N/A]	SVL	N	50.5	
Fu et al. (Fu et al. 2020)	0.01% Atrop	400 [6-14]	Randomized [-1.25 to -6.00]	SVL	Y (0.5% Tropicamide + 0.5% Phenylephrine)	32.9	19.6

Fu et al. (Fu et al. 2020)	0.02% Atrop	400 [6-14]	Randomized [-1.25 to -6.00]	SVL	Y (0.5% Tropicamide + 0.5% Phenylephrine)	45.7	34.8
Selective muscarinic receptor antagonist							
Siatkowski et al. (Siatkowski et al. 2004)	2% Piren	174 [8-12]	Randomized [-0.75 to -4.00]	Placebo	Y (1% Tropicamide + 1% Cyclopentolate)	50.9	17.4
Tan et al. (Tan et al. 2005)	2% Piren	400 [6-14]	Randomized [-1.25 to -6.00]	Placebo	Y (1% Tropicamide + 1% Cyclopentolate)	44.0	39.4
Xanthine treatments							
Trier et al. (Trier et al. 2008) – moderate growth rate	7-MX	77 [8-14]	Randomized [-0.75 and worse]	Placebo tablets	Y (1% Cyclopentolate)	20	24
Trier et al. (Trier et al. 2008) – high growth rate	7-MX	77 [8-14]	Randomized [-0.75 and worse]	Placebo tablets	Y (1% Cyclopentolate)	11.8	7.9
Other pharmaceutical compounds							
Jensen et al. (Jensen 1988)	Timolol	51 [9-12]	Randomized [-1.25 and worse]	SVL	Y (1% Cyclopentolate)	8.5	N/A
Abraham (Abraham 1966)	1% Tropicamide	150 [6-20]	Non-randomized [-0.25 and worse]	SVL	N/A	52.2 – Males 42.8 - Females	N/A
Yen et al. (Yen et al. 1989)	1% Cyl	96 [6-14]	Randomized [-0.50 to -4.00]	Placebo	Y (1% Tropicamide + 1% Cyclopentolate)	36.3	N/A

SE: Spherical equivalent; AL: axial length; Ade-R: Adenosine receptor antagonists; 7-MX: 7 – methylxanthine; Musca-R: Muscarinic receptor antagonists; Atrop: Atropine eye drops; Piren: pirenzepine; Cyl: Cyclopentolate; N/A: not available;

In late 1980s, a trial from Taiwan reported 1% Atropine to be more effective for myopia control than 1% Cyclopentolate or control (saline) over a one-year period (Yen et al. 1989). Compared to saline (control), 1% Atropine slowed myopia by up to 75.8% (Yen et al. 1989). This was followed by two randomized clinical trials (RCT) of 2 years duration that evaluated the effectiveness of different concentrations of Atropine (Shih et al. 1999; Shih et al. 2001); the first RCT evaluated the effectiveness of various concentrations of Atropine (0.5%, 0.25% and 0.1%) compared to placebo (0.5% Tropicamide) (Shih et al. 1999) and the latter RCT compared efficacy between groups of single vision lens (SVL) spectacles, multifocal lens (MFL) spectacles and combination of MFL with 0.5% Atropine drops (Shih et al. 2001). The change in the mean spherical equivalent with 0.5%, 0.25%, 0.1% Atropine were 0.04D/year, 0.45D/year and 0.47D/year, respectively compared to the 1.06D/year in the control group. A total of 61%, 49% and 42% of participants did not progress in myopia (the mean change being less than 0.25D) over two years compared to 8% in control group. Despite it being a randomized trial, there was no true control as 0.5% Tropicamide had a cycloplegic effect which is considered to play a role in the complex multifactorial interplay of myopia control and furthermore, 0.5% Atropine group used bifocal glasses. In the second study, over 18 months of follow-up, the mean refractive changes in the group using 0.5% Atropine plus MFL spectacles was significantly lower than MFL spectacles and SVL spectacles group, with -0.41D, -1.19D, -1.40D, respectively, and the percentage of eyes showing nil progression were significantly higher with Atropine combined treatment compared to MFL spectacles and SVL spectacle groups, at 57.6%, 9.8%, and 4.9% respectively. Although there were some differences between MFL and SVL groups, there was no convincing impact of MFL on myopia. Despite these limitations, the trials validated the use of Atropine (Yen et al. 1989; Shih et al. 1999; Shih et al. 2001) and consequently, Atropine is now routinely prescribed to prevent myopia progression in Taiwanese schoolchildren, with Atropine use by ophthalmologists increasing from 36.9% in 2000 to 49.5% in 2007 (Fang et al. 2013).

Although Atropine eye drops was widely adopted especially in Asia where the rate of myopia was high, concerns due to common side effects including photophobia and difficulties in doing near tasks (Chua et al. 2006; McBrien et al. 2013; Pineles et al. 2017) limited its uptake. The various concentrations investigated to date can be divided into three main categories: high-dose (0.5% and 1%), moderate dose (from 0.1% to under 0.5%) and low-dose (less than 0.1%) (Huang et al. 2016). Due to concerns related to side effects, low dose Atropine became increasingly as the choice for myopia control.

In 2006, Lee and associates (Lee et al. 2006) investigated the efficacy of 0.05% Atropine in a retrospective study using an untreated group as control. Despite the weakness of a retrospective study and an untreated control group rather than a placebo control group, the study indicated a significant difference between groups with mean change in spherical equivalent at -0.28D in 0.05% Atropine group and -0.75D in untreated control group. However, myopia was found to progress in an uncontrolled manner in 16.7% of participants in the group using 0.05% Atropine.

In 2010, Fang and associates recruited either premyopes or emmetropes (from +1.0D to -1,0D) who were assigned either 0.025% Atropine once nightly or categorized as control (Fang et al. 2010). The retrospective data showed that 0.025% Atropine was able to significantly reduce the onset of myopia. There was only 8% of subjects in the Atropine group having a fast myopic shift compared to 58% in the control group. Moreover, myopia onset happened in 21% versus 54% of participants, respectively (Fang et al. 2010).

Between 2006 and 2016, the ATOM (Atropine for the Treatment of Childhood Myopia) group published a number of studies that investigated different concentrations of Atropine for myopia control in schoolchildren in Singapore. The studies were randomized, double-masked clinical trials. In the first study (Chua et al. 2006; Chia et al. 2009; Tong et al. 2009), 400 subjects were randomly allocated to use topical 1% Atropine in one eye and placebo/vehicle eye drops in the contralateral eye. At the end of two years, there was a 13.5% of drop-out rate overall, which may

have been related to the use of eye drops in one eye only and the need for additional photochromic lenses during the treatment period. The mean changes of spherical equivalent (SE) and axial length (AL) in the Atropine and control eyes were significantly different, -0.28D versus -1.20D, and -0.02mm versus +0.38mm respectively. Atropine treatment had no effect on changing astigmatic power (Chia et al. 2009). In the year following discontinuation of Atropine, mean change in SE was significantly greater in the eyes that previously received Atropine compared to the control eyes at -1.14D and -0.38D respectively and indicating a significant rebound in Atropine eyes (Tong et al. 2009). The rebound effect was greater in the first 6–month period compared to the second 6-month period. Furthermore, in spite of the rebound, at the end of three years (i.e. two years in Atropine and one year of follow-up post Atropine), the overall myopic progression in the 1% Atropine group was significantly less than in the placebo group (Tong et al. 2009). The data also indicated that side effects such as reduced near vision were reversible upon discontinuation of Atropine. In fact, there was no significant difference in the near visual acuity and the amplitude of accommodation at 30 months between groups, and the data were comparable to those from the initial visit indicating that effects related to 1% Atropine resolved within six months after cessation (Tong et al. 2009). The second ATOM study (Chia et al. 2012; Chia et al. 2014; Chia et al. 2016) investigated the effectiveness of concentrations <1% Atropine; 400 participants were recruited into a double-masked randomized clinical trial with groups randomized to use of 0.5%, 0.1% and 0.01% Atropine once nightly in both eyes and photochromic progressive additional lenses as needed. Over two years, all three concentrations slowed the progression; 75%, 70% and 60% with 0.5%, 0.1% and 0.01% respectively for spherical equivalent compared to the historical control group from ATOM1 study. However, although 0.5% and 0.1% Atropine significantly controlled axial elongation by 28.9% and 26.3%, respectively, the change with 0.01% Atropine as compared to historical control was insignificant (-7.9%). After cessation of Atropine, over the following year, rebound of myopia in a dose-dependent manner was

observed in all groups. with a mean change in spherical equivalent -1.15D, 1.04D, and -0.72D with 0.5%, 0.1% and 0.01% Atropine. The mean change in axial length with 0.5%, 0.1% and 0.01% was 0.61mm, 0.60mm, and 0.58mm respectively with no differences between the groups. In the final third phase of the ATOM2, participants that progressed by 0.5D or worse in the washout phase (68%, 59%, and 24% of 0.5%, 0.1% and 0.01% Atropine respectively) were retreated with 0.01% Atropine once nightly for two years. At the end of five years, of all three phases, mean spherical equivalent progression was -1.38D with 0.01% Atropine and significantly lower than -1.98D and -1.83D with 0.5% and 0.1% respectively. Thus, it was concluded that 0.01% Atropine offers a reasonable myopia control efficacy with an appropriate risk-benefit ratio with regards to side effects over the long-term use. However, this trial indicated that the efficacy t of 0.01% Atropine in slowing axial elongation remains in doubt.

A more recent randomized, double-blinded, placebo-controlled trial of Atropine eye drops for myopia control treatment in Hong Kong (Yam et al. 2019; Yam et al. 2020) enrolled 438 children (4-12 years of age, myopia with spherical equivalent of -1.0D or worse) and randomly allocated them to four groups of 0.05%, 0.025%, 0.01% topical Atropine and placebo eye drops. After one year of use, compared to control group, all concentrations significantly controlled the progression of spherical equivalent (66.7%, 43.2% and 27.2% with 0.05%, 0.025% and 0.01% respectively), whereas the control efficacy of Atropine on progression of axial length was significant with 0.05% and 0.025% at 51.2% and 29.3% respectively, but was not statistically significant with 0.01% at 12.2%(Yam et al. 2019). In addition to the significantly better myopia control with 0.05% and 0.025% Atropine, there was no difference in photophobia between the three groups at 7.8%, 6.6% and 2.1%, respectively, and therefore indicated that it may be feasible to use higher concentrations than 0.01% Atropine without an increase in side effects.

Although much of the efficacy data of Atropine was from Asian eyes, its efficacy in other ethnic groups is supported by data from Clark and associates (Clark and Clark 2015). In a retrospective

study (60 participants, 6 to 15 years of age with varying ethnicity), after 1.1 years of follow-up, only spherical equivalent refractive error was reported and the mean spherical equivalent change in treated (0.01% Atropine) versus control group was -0.1D/year versus -0.6D/year respectively. Another recent cross-sectional one-year report involving 56 German children, aged between 6 and 17 years, indicated that 0.01% Atropine was safe and efficient for myopia control with efficacy of 61.9% in slowing myopia progression with respect to spherical equivalent compared to one-year period preceding the treatment (Joachimsen et al. 2019). Other studies reported better efficacy in the older group (12 to 17 years old) and dark irides compared to younger group (6 to 11 years old) and light irides (Joachimsen et al. 2019).

1.6.4 *Safety of topical Atropine*

The common side effects associated with topical Atropine include a) an increase in pupillary diameter due to mydriatic action of Atropine on the iris, and b) a decrease in the accommodative amplitude due to paralysis of the ciliary body. Although as much as 4% Atropine was reported to have been used (North and Kelly 1987), such use appeared to be rare with 1% Atropine being the highest concentration that was frequently used (Walline et al. 2020). The most common adverse events reported with high concentration Atropine, i.e. 0.5% or 1% include photophobia, loss of amplitude of accommodation and ocular allergies (Yen et al. 1989; Shih et al. 1999; Chua et al. 2006; Yi et al. 2015; Huang et al. 2016; Polling et al. 2016; Wang et al. 2017). Indeed, Yen and associates reported that all the myopic children using 1% Atropine had experienced significant photophobia that prevented participation in outdoor activities (Yen et al. 1989). At 1% concentration, Atropine reduced the mean accommodative amplitude from 13.76 D to 2.81D indicating a high risk of near visual disturbance (Tong et al. 2009). In European children, 0.5% Atropine resulted in 72% of the participants experiencing photophobia and 38% having issues while reading (Polling et al. 2016). Allergic reactions were also reported by about 5% of

participants or less (Chua et al. 2006; Fan et al. 2007; Chia et al. 2012). To date, there have been no other serious adverse events reported even with long-term follow-up of more than 10 years (Kennedy et al. 2000; Chiang et al. 2001; Syniuta and Isenberg 2001; Tong et al. 2009). Figure 1.2 is a summary of the data from studies that reported loss of accommodative amplitude (blue line) and dilation of pupil diameter (orange line) with the various concentrations of Atropine.

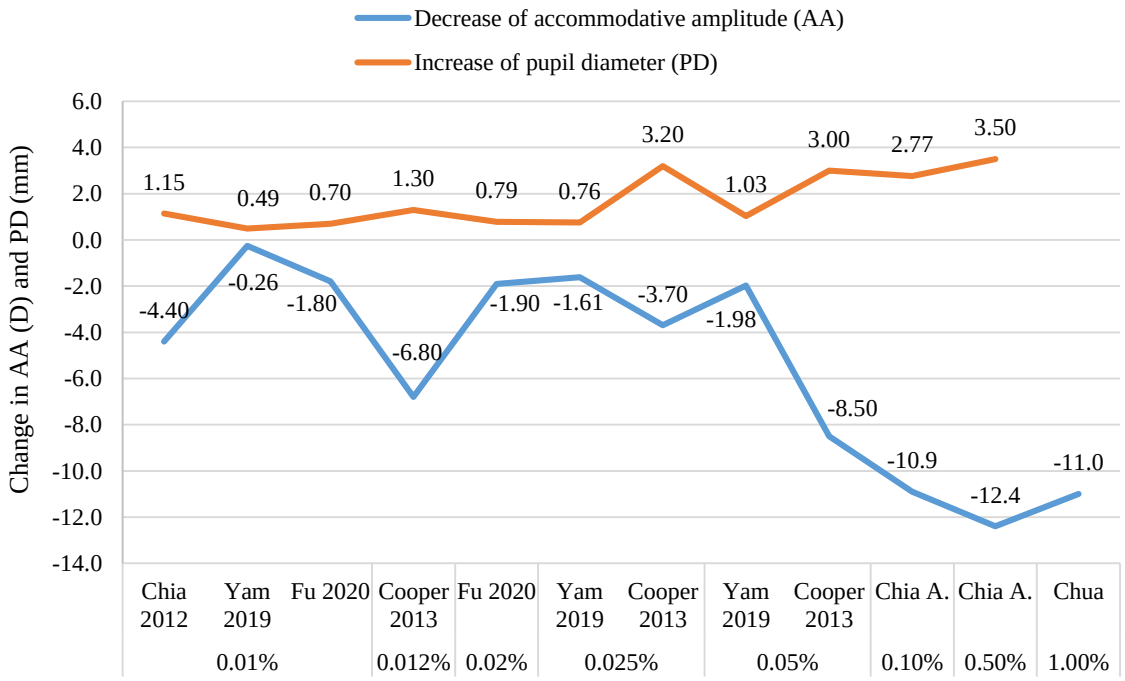

Figure 1.2: Published data on loss of amplitude of accommodation (D) and increase in pupillary diameter (mm) with various concentrations

The reported frequency of symptoms was reduced with lower concentrations of Atropine (Shih et al. 1999; Chia et al. 2012). After 4 weeks of use, photophobia was reported in 22% , 7% and none with 0.5% Atropine ,0.25% and 0.1% Atropine, respectively (Shih et al. 1999). Only less than 10% of participants receiving 0.01% Atropine had requested photochromatic lenses (Chia et al. 2012). A more recent randomized placebo-controlled trial showed no difference between the treatment groups, i.e. 0.05%, 0.025% and 0.01% Atropine, and placebo group with respect to the

participants requesting photochromatic glasses or progressive additional spectacles (Yam et al. 2019).

Although there was a single report on transient increase of intraocular pressure due to the application of ophthalmic Atropine (Spaeth 1985), other studies did not report such an association (Wu et al. 2012). Moreover, the results of electroretinograms of those using Atropine eye-daily for 2 years showed no harmful effects (Luu et al. 2005). Interestingly, the safety of long-term daily usage of ophthalmic Atropine on the endothelial cells was considered and found Atropine to be toxic in a dose- and time-dependent manner and an Atropine concentration of less than 0.038% being considered to be safe for the endothelium (Wen et al. 2016). Cooper et al. suggested that the use of Atropine would remain tolerable if the residual accommodative amplitude remained greater than five dioptres and the change in pupillary diameters was not greater than three millimetres (Cooper et al. 2013).

1.7 Caffeine for ocular usage: mechanism of action, efficacy, and safety

The general population is widely exposed to caffeine, largely related to consumption of coffee, tea, soft drinks, energy drinks, chocolate, and foods. Daily intake ranges from <1mg up to 1000 mg/day. Caffeine occurs naturally in foods such as coffee, tea, and chocolate, and has a long history of use by man-kind as a mild stimulant, added to many foods, medicines and cosmetics (Australia and New Zealand 2015). For example, Caffeine is an ingredient in over-the-counter fat burning products, is used as a supplement for migraine medication (e.g., Panadol extra) and as a cosmetic cream (e.g., cream to enhance the tone of the skin). One cup of coffee (150mL) contains between 60 and 150 mg of Caffeine, a cup of tea 60 mg, a cup of cocoa 5 mg and a glass of Coca-Cola 20 mg (U.S.Food_and_Drug_Administration 2015). Furthermore, Caffeine is a central nervous system stimulant that the U.S.FDA classifies as both a safe drug and a safe food additive (U.S.Food_and_Drug_Administration 2015). A recent report showed that nearly 90% of healthy

adults in U.S.A regularly consume Caffeine with an average intake of over 190 mg/day (Frary et al. 2005). Caffeine is a product approved for use in many foods and beverages for human use and including as a preservative in eye drops (U.S.Food_and_Drug_Administration 2015).

1.7.1 *Biochemistry of Caffeine*

Caffeine's chemical name is 1,3,7-Trimethylxanthine. It is a Xanthine compound with three methyl (CH3) groups attached at the 1,3, and 7 carbons on the Xanthine backbone (Figure 1.3).

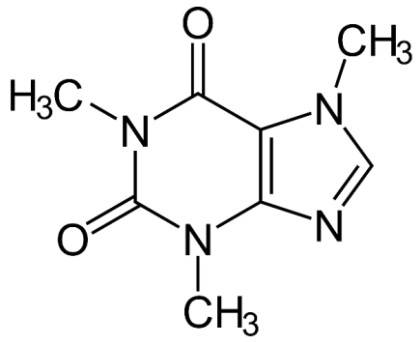

Figure 1.3: Molecular structure of Caffeine

Caffeine is a bitter, white crystalline powder, extracted from the coffee bean which is the seed of the Coffea plant and has a structure that consists of adenine and guanine bases. Caffeine is a class of Xanthines classified as a nootropic as it sensitizes neurons, provides mental stimulation and exerts its effect by competitively and non-selectively binding *via* the adenosine receptors (ADORs) (Nehlig et al. 1992). While adenosine causes sedation and relaxation (Sheth et al. 2014), Caffeine prevents this by antagonizing adenosine receptors located in the brain, hence causing alertness and wakefulness (Holtzman et al. 1991). This inhibition of adenosine can influence the release of other central and systemic neurotransmitters including the dopamine, serotonin, acetylcholine, and adrenaline systems which therefore may impact the autonomic nervous system (Benowitx 1990).

In 2003, Nawrot and associates conducted a review of the literature and concluded that for normal adults, moderate daily Caffeine intake at doses up to 400mg/day (equivalent to 6mg/kg body weight) is not associated with adverse effects on cardiovascular health, general toxicity, bone status and calcium balance, cancer, and fertility. In children, an upper exposure of 2.5mg/kg body weight/day was suggested as a cautious upper limit (Nawrot et al. 2003).

The solubility of Caffeine in water is 2.0%; it is soluble in ethyl acetate and chloroform; moderately soluble in alcohol and acetone; slightly soluble in ether and benzene (Benowitx 1990).

1.7.2 *Pharmacology of Caffeine*

Caffeine is easily dissolved either in water or lipids (Fredholm et al. 2011). Furthermore, Caffeine has been proven to penetrate through the blood-brain barrier and the placenta (Benowitx 1990; Ferré 2008) and therefore it is assumed that it can pass through the blood-retinal barrier when applied topically in the eye. Topical Caffeine appeared to permeate into the ocular tissues (Varma et al. 2011; Kronschläger et al. 2013). In animals, topical Caffeine reached the lens and anterior chamber at about one hour after instillation by using high-performance liquid chromatography analysis (Kronschläger 2014), whereas low concentration of Caffeine in blood indicates minimal absorption of topical Caffeine into the systemic circulation (Varma 2016).

Caffeine is a non-selective adenosine receptor antagonist that has varying affinity to all subtypes of adenosine receptors including A_1, A_{2A}, A_{2B} and A_3, with the affinity (K_D) ranging between 2 µM and up to 80 µM (Froestl et al. 2012). In the absence of Caffeine or its metabolites, adenosine accumulates over time in the central nervous system during wakefulness and therefore, somnolence is cumulatively gained (Nehlig et al. 1992; Cunha 2001). Following the competitive binding effects to the adenosine receptors as an antagonist over the A_{2A} receptor, Caffeine consumption promotes wakefulness by temporarily relieving drowsiness (Ferré 2008).

In addition to the direct competitive action on the adenosine receptor, Caffeine can indirectly enhance the concentration of intracellular cAMP (Essayan 2001), synthesis of leukotriene (Peters-Golden et al. 2005), increase of acetylcholine, a neurotransmitter (Pohanka and Dobes 2013), *via* the role of the inhibitor of the phosphodiesterase enzymes especially acetylcholinesterase. Moreover, by binding to adenosine receptor in the complex of adenosine-dopamine receptor, Caffeine also promotes the effects of dopamine (Ferré 2008; Ferré et al. 2016).

1.7.3 *Pharmacokinetics of Caffeine*

Caffeine is absorbed in the intestine 30 to 60 minutes after oral ingestion whereas the absorption *via* intramuscular administration is slower. It is then metabolised in the liver by the microsomal enzymes, cytochrome P450 oxidase enzyme system, where it undergoes N-demethylation and phase 2 conjugation (Liguori et al. 1997). The volume of distribution is 0.61 l/kg and the distribution is equally rapid throughout the entire water pool of the body (Blanchard and Sawers 1983). In consuming Caffeine, it is nearly all absorbed within the first hour and the peak plasma concentration occurs 30 minutes to 2 hours following Caffeine ingestion (Blanchard and Sawers 1983). Caffeine is proven to have the plasma half-life of about 3 to 4 hours in adults but is found to be prolonged by up to 2 to 3 times in the 3rd trimester of pregnancy. (Nehlig et al. 1992; Fredholm et al. 2011). The plasma protein binding is approximately 17% (Fredholm et al. 2011). Effects of Caffeine appeared to be dose-dependent (Cheng et al. 1990; Ramakrishnan et al. 2014). Caffeine and its metabolites are excreted *via* bile, urine, and faeces. Approximately 10% of a given dose of Caffeine is excreted unchanged in the urine (Wikoff et al. 2017).

1.7.4 *Ocular use of Caffeine use and myopia control*

In addition to being a US FDA approved drug and food additive, Caffeine has been proven to be safe and effective and has a long history of intravenous use in premature babies for preventing

blindness from retinopathy of prematurity (Aranda et al. 2010; Beharry et al. 2016; Abu-Shaweesh and Martin 2017; Chen et al. 2017; Zhang et al. 2017).

1.7.4.1 In animals

In animal models, topical Caffeine used at high concentrations, between 30 to 100 mg/kg, significantly increased the production of aqueous humour which was then reversed by the application of topical beta-blockers (Kurata et al. 1997; Kurata et al. 1998). Most recently, 72mM of Caffeine eye drops (in sterile 0.3% hydroxyl-propyl methylcellulose) was assessed for UV-cataract prevention in rat eyes and there were no changes to the ocular surface and lens (Kronschlager et al. 2013; Kronschlager et al. 2014). The concentration in the blood reached a peak at 120 minutes and no changes in the appearance of the eyelids, the cornea or the conjunctiva were observed at 120 minutes after application of Caffeine eye drops. The rat blood concentrations achieved were far below the equivalent threshold dose of FDA recommended daily dose for humans. In a more recent study, oral Caffeine was able to partially decrease IOP in ocular hypertensive adult rats (Madeira et al. 2016).

1.7.4.2 In humans

Caffeine was originally investigated for its use in glaucoma (Leydhecker 1955; Peczon and Grant 1964). More recently, it was evaluated for cataract prevention. While the lens capsule was found to be target tissue of Caffeine consumption (Kronschläger et al. 2018), topical Caffeine appeared to protect the formation of lens opacity (Varma 2016). There is data that indicates that oral Caffeine may induce thinning of sub-foveal choroid but no changes were found for IOP and ocular pulse amplitude (Vural et al. 2014; Dervişoğulları et al. 2016). Furthermore, 1% Caffeine eye drops was trialled for treatment of glaucoma in humans with multiple instillations over a single day- although found to be safe, it had no effect on intraocular pressures (Chandra et al. 2011).

Similarly, use of Caffeine 3 times a day over a week did not influence intraocular pressures or blood pressures (Chandra et al. 2011).

1.7.4.3 Current evidence for use in myopia

At present, the action of Caffeine, a non-selective ADOR antagonist has been considered in a limited manner in animal models for use in myopia but has not yet been explored in humans. Caffeine may have an influence on myopia *via* antagonism on ADOR subtypes (Cui et al. 2010; Cui et al. 2011), but may also exert an effect *via* non-ADOR pathway (Sanderson et al. 2014). ADORs and the subtypes have been identified to be present in the key ocular components involved in the signalling cascade of the emmetropisation including retina, retinal pigment epithelium, choroid, and sclera (Braas et al. 1987; Cui et al. 2008; Beach et al. 2018; Smith III et al. 2021). Table 1.3 illustrates the ocular components along with the suggested mechanism of action on adenosine receptors *via* which Caffeine may influence emmetropisation.

Adenosine receptors are said to influence the release of neurotransmitters in the retina such as dopamine and acetylcholine (Cunha 2001; Fredholm et al. 2011; Pohanka and Dobes 2013; Sodhi and Hartwick 2014; Ferré et al. 2016), also involved with intrinsically photosensitive retinal ganglion cells and thus may be involved in emmetropization (Dong et al. 2007). Caffeine was considered to alter choroidal thickness in animal models and thought to support reduction of axial elongation (Smith III et al. 2021). Adenosine receptors may also be involved in collagen synthesis of the sclera by fibroblasts (Trier et al. 1999; Cui et al. 2008; Nie et al. 2012) and influence the signals of metabolic transportation across the retinal pigment epithelium (Kawahara et al. 2005; Mitchell and Reigada 2008; Zhang and Wildsoet 2015). Interestingly, genetic deletion of A2A receptors was found to produce myopia in mice (Zhou et al. 2010).

Table 1.3: Role of adenosine receptors related to the emmetropisation

Ocular component	Mechanism	Authors and Year
Neurotransmitter	Alter the release of the neurotransmitters of which their roles were proved in the ocular development (e.g. dopamine, acetylcholine)	(Cunha 2001; Fredholm et al. 2011; Pohanka and Dobes 2013; Sodhi and Hartwick 2014; Ferré et al. 2016)
Choroidal thickness, blood flow and ocular perfusion pressure	Alter the choroidal blood flow	(Portellos et al. 1995; Polska et al. 2003; Schmidl et al. 2011; Smith III et al. 2021)
Retinal pigment epithelium	Influence the signals of metabolic transportation across the retinal pigment epithelium	(Kawahara et al. 2005; Mitchell and Reigada 2008; Zhang and Wildsoet 2015)
Sclera	Alter the changes of scleral strength	(Trier et al. 1999; Cui et al. 2008; Nie et al. 2012)

Additionally, Caffeine was thought to be more potent than the current other available Xanthines, 7-Methylxanthine (7-MX) (Daly et al. 1983). Oral 7-MX, a metabolite of Caffeine, was found effective in myopia control in children with myopia in Denmark (Trier et al. 2008) and it was considered that topical Caffeine may exert similar effects and be more advantageous with use compared to other Xanthines (Smith III et al. 2021). Additionally, evidence supported the permeability of topical Caffeine to the anterior chamber (Varma et al. 2011; Kronschlager et al. 2013; Varma 2016) prior to directly inhibiting the ADOR on the potential targets including retinal pigment epithelium, choroid or sclera (Johnson et al. 2017). While 7-MX, does not show the ability to cross the blood-brain barrier (Smith III et al. 2021), Caffeine may conceivably be the only Xanthine that may cross the retinal barrier and thus the only ADOR antagonist that can be topically used.

Recently, 1.4% Caffeine (in sterile 0.3% hydroxyl-propyl methylcellulose) eye drops were investigated for efficacy in slowing myopia in primates and found to slow myopia in some eyes while preventing myopia in others. There were no ocular or systemic side effects (Arumugam et al. 2017).

1.7.5 *Safety of Caffeine eye drops*

In-vitro cytotoxicity evaluations of 1.4% and 2% Caffeine found the formulation to be completely safe with no damaging effect on human corneal epithelial cells (UNSW Cytotoxicity report file dated 8th December 2017). In vivo, Caffeine has been trialled in primate eyes at 1.4% in a base of 0.3% hydroxyl-propyl-methyl cellulose and proven effective for myopia control (Arumugam et al. 2017). Topical Caffeine was also investigated in human trials (Chandra et al. 2011; Kronschläger et al. 2013; Bardak et al. 2016). Additionally, Caffeine used alone and in combination with Atropine was found to be safe in a clinical trial involving adult human participants (Bellberry application number 2018-01-036; ANZCTR registration no 12618000196246, BHVI, Sydney, Australia). In this cross over clinical study conducted at the Brien Holden Vision Institute Limited, Sydney, Australia, 22 healthy adult participants cross-over used and being followed with topical 0.02% Atropine, 1.4% Caffeine, 2.0% Caffeine and 0.02 % Atropine plus 1.4% Caffeine over 7 days for each compound (ANZCTR registration no 12618000196246, BHVI database). No adverse events such as ocular redness, staining etc. were observed with the use of eye drops. In addition, in an ongoing clinical study conducted at Brien Holden Vision Institute Limited, Sydney, Australia, 30 healthy adult participants are enrolled for an assessment of ocular response (change in pupillary diameter, accommodative response) to various concentrations of Atropine, Caffeine and combination (ANZCTR registration no 12620000433909) over a 24-hour period.

1.8 Rationale of the study

Although Atropine is effective in slowing myopia, higher concentrations of Atropine induce significant side effects whereas lower concentrations such as 0.01% Atropine may not be effective in slowing axial elongation. Thus, there is a need to improve myopia control efficacy with lower

concentrations of Atropine and/or reduce the risk of side effects associated with higher concentrations of Atropine.

In this regard, it may be useful to determine if Caffeine has a role to play in myopia control. Therefore, we aim to investigate the effectiveness in of topical Caffeine (alone and in combination with Atropine) in myopia control.

1.9 Hypothesis

Topical Caffeine either alone or in combination with Atropine has no role in myopia control.

1.10 Objectives

The objectives of this thesis are:

- To conduct a meta-analysis to determine the efficacy and side effects of various concentrations of Atropine used for slowing myopia.
- To determine the effect of various low concentrations of Atropine on pupil size and accommodative amplitude in children with myopia.
- To determine using a randomized, longitudinal, dispensing trial the rate of progression of myopia and side effects with use of Caffeine, low concentration Atropine, and a combination of low concentration Atropine and Caffeine.
- To compare the rate of progression of myopia with use of Caffeine, low concentration Atropine and a combination of low concentration Atropine and Caffeine in comparison to a matched group of children wearing single vision spectacles.

Interim summary:

With an estimated 50% of the world population to be myopic by the year 2050, the burden of myopia is substantial. Higher levels of myopia are associated with significant risk of vision impairment. Atropine, a non-selective muscarinic receptor antagonist can slow myopia in a dose-dependent manner. However, its use is associated with side effects that appear to be dose-dependent. Adenosine receptor antagonists, Methylxanthines are metabolites of Caffeine and evidence suggests that they may play a role in myopia control. This thesis aims to determine if Caffeine either alone or in combination with Atropine has a role to play in myopia control.

1.11 Outline of the chapters

Chapter 1 provides an overview of myopia, prevalence of myopia and high myopia, progression of myopia and related risk factors, strategies for controlling myopia progression, structure of Atropine and Caffeine, their safety profile and use if any for myopia control. The rationale, hypotheses and aims of the thesis are detailed.

Chapter 2 is a comprehensive literature review and meta-analyses of literature from 1980 to 2020 for use of Atropine in myopia control. The purpose was to evaluate and understand the evidence for efficacy of various concentrations of Atropine in controlling myopia as well as side effects relating to its use.

Chapter 3 reports the results of a short-term two-week randomized clinical trial investigating the effect of 0.01%, 0.02% or 0.03% Atropine on pupillary diameter, accommodative amplitude, and subjective acceptability in children with myopia.

Chapter 4 reports and examines the efficacy of 0.02% Atropine, 0.02% Atropine combined with 2% Caffeine and 2% Caffeine in slowing myopia compared to a control group wearing single vision spectacles. The progression of myopia, both in terms of spherical equivalent and axial length, as well as changes in pupillary diameter and accommodative amplitude, were evaluated

in a prospective, randomised, dispensing clinical trial. Results for 6-months in the trial are presented.

Chapter 5 evaluates the temporal effect of a single drop of 0.05% Atropine, 0.1% Atropine, 0.05% Atropine plus 2% Caffeine, 0.1% Atropine plus 2% Caffeine and 2% Caffeine on the pupillary diameter and accommodative amplitude in a prospective, randomized, cross-over trial.

Chapter 6 summarises the major findings of the thesis. Future implications in the field of pharmaceutical myopia control treatment in addition to the recommendation for further research are also provided.

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Chapter 2. Effects of Atropine on pupil size and accommodative amplitude

The following chapter was published "A meta-analysis assessing change in pupillary diameter, accommodative amplitude, and efficacy of Atropine for myopia control" in the Asia-Pacific Journal of Ophthalmology (doi: 10.1097/APO.0000000000000414. Epub ahead of print. PMID: 34456234) and is reproduced in this chapter.

2.1 Introduction

Considered to be the most common cause of distance vision impairment (Flaxman et al. 2017), myopia is estimated to affect half of the population worldwide by 2050 (Holden et al. 2016). In addition to resulting in a significant economic burden (Smith et al. 2009), high myopia (-5.0D or worse) may also induce pathologic changes that result in vision impairment and/or blindness. Data from many countries, especially from East Asia, show that myopic macular degeneration is one of the leading causes of blindness (Hsu et al. 2004; Iwase et al. 2006). Additionally, any level of myopia is associated with the risk of visual impairment (Flitcroft 2012), which is significantly higher with high myopia (Verhoeven et al. 2015). Given the rising burden, there has been an increasing interest in solutions to better manage and control myopia.

Presently, of all the known strategies to slow or reduce myopia progression, Atropine, a nonselective anti-muscarinic agent, is considered superior and widely used despite the lack of understanding relating to its mode of action as well as lack of clarity with respect to the efficacy of 0.01% Atropine (Huang et al. 2016). Although other pharmaceutical agents such as Pirenzepine, a selective anti-muscarinic agent and 7-methylxanthine showed promise in early clinical trials (Siatkowski et al. 2008; Trier et al. 2008), presently Atropine is the only pharmaceutical agent that is broadly adopted for myopia control. It was reported that, in Taiwan, up to 50% of ophthalmologists nationwide prescribe Atropine for children with myopia (Fang et al. 2013). However, the use of Atropine is not without issues. Despite no reports of serious side effects to date, photophobia and difficulty with near-work remain the major concerns for the

application of Atropine at higher concentrations. Another significant concern is the "rebound" of myopia that occurs on ceasing treatment (Tong et al. 2009; Chia et al. 2014). There are other concerns related to the unknown effects of long-term use on pupils, macula, and/or retina, although some data shows that the accommodative amplitude and near vision recovered to pretreatment within months after cessation of Atropine (Tong et al. 2009).

Although previous meta-analyses (Li et al. 2014; Huang et al. 2016; Gong et al. 2017) have considered the effect of various concentrations of Atropine on axial elongation and refractive error, its effect on pupillary diameter (PD) and amplitude of accommodation (AA) has not been considered so far. In this meta-analysis, we considered the effect of various concentrations of Atropine on pupillary dilation and accommodative amplitude as well as for efficacy in myopia control (change in spherical equivalent and axial length).

2.2 Methods

2.2.1 *Literature search from multiple resources*

We searched the electronic medical databases, including PUBMED, EMBASE, Scopus, ProQuest, and Cochrane library. A combination of keywords and Boolean operators using search terms "myopia", "myopia progression", "myopia control", "Atropine", "Atropine eye drops", along with the main Boolean operators "AND", "OR" were used. Databases were searched for articles from 1980 until June 2020 following the PRISMA guidelines (Table 2.1 and Figure 2.1).

Table 2.1: Number of records from multiple resources

	Duplicates removed	Remaining data	Summary	Notes (Language, period of time)
PUBMED	174	90	264	English, January 1980 - June 2020
EMBASE	238	544	782	English, January 1980 - June 2020
SCOPUS	469	516	985	English, Range of time January 1980 - June 2020, books and chapters excluded
ProQuest	208	166	374	English, Range of time January 1980 - June 2020, books and chapters excluded
Cochrane	3	1	4	English, January 1980 - June 2020
Total	1092	1317	2409	

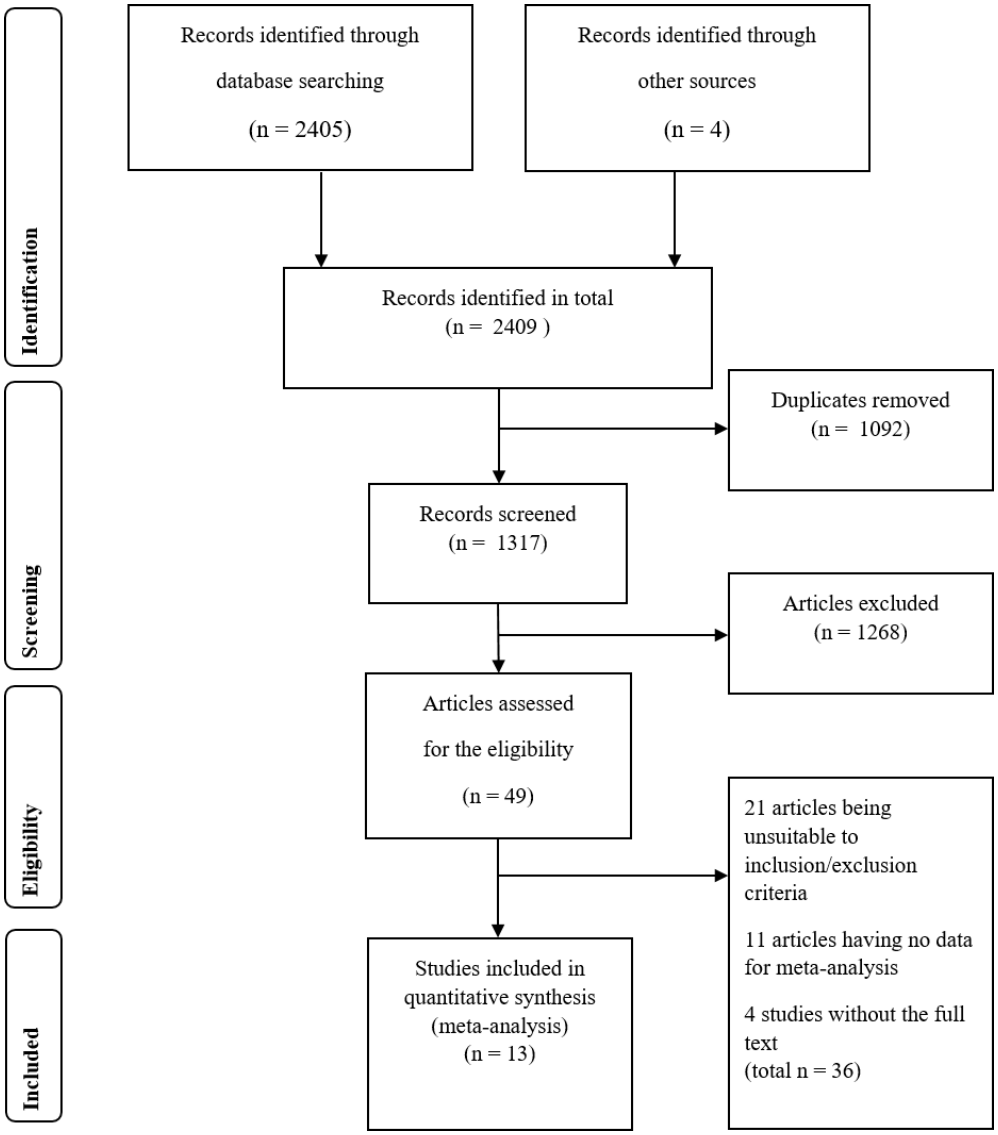

Figure 2.1: Process of search and inclusion/exclusion of eligible articles into the final meta-analysis

2.2.2 *Inclusion/Exclusion criteria*

To be included in the meta-analysis, the articles had to meet the following criteria:

a) participants were myopic;

b) aged 16 years or younger;

c) the data was from a randomised clinical trial (RCT) or an observational study;
d) a control group or a historical control group was included in the trial or study;
e) at entry, participants had myopia with spherical equivalent of -0.50 Dioptre (D) or worse;
f) participants were followed for at least 12 months; and
g) the full-text article was published in English language.

Articles were excluded if:

a) at baseline, participants had spherical equivalent refractive error of -0.25D or better;
b) were 17 years old or older;
c) each of the participants was on multiple concentrations of Atropine during the observation period;
d) participants were treated with a combination of Atropine and other myopia control treatment methods.

In our review, we considered control groups as those that were treated with either single-vision spectacles, 0.50% tropicamide or placebo eye drops.

2.2.3 *Data collection and extraction*

Two of the authors (HTDM and PS) individually reviewed the extracted data using the inclusion/exclusion criteria. Initially, the titles and abstracts were reviewed, and ineligible articles and duplicated data were removed. All selected articles were assessed using the 'Newcastle-Ottawa' scale for the observational studies and the "Cochrane" risk of bias evaluation tool for randomised clinical trials (Higgins et al. 2019). The following data were extracted from selected articles: authors, year of publication, country of research, study design, age of participants, baseline spherical equivalent and axial length, and prescribed concentration of Atropine. Additionally, we also collected pupillary diameter, accommodative amplitude, annual change in spherical equivalent, and annual change in axial length. If there were multiple articles reporting

data of a single study, we extracted in priority the data from the earliest published publication that provided the annual change.

2.2.4 *Statistical analysis*

The primary outcomes were the change in pupillary diameter and accommodative amplitude with various concentrations of Atropine compared to control. The secondary outcomes included the annual mean change in spherical equivalent refractive error and axial elongation with Atropine compared to control. Statistical analysis was conducted using Review Manager (version 5.3 by Cochrane Collaboration), GraphPad Prism 8 (version 8.4.3 by GraphPad Software, LLC), and Microsoft Excel (version 2016 Microsoft Office by Microsoft Co.).

To calculate the effect size, the difference in accommodative amplitude and pupillary diameter with various concentrations of Atropine compared to control was pooled. Similarly, the mean annual change in myopia progression, i.e., spherical equivalent and axial length with use of Atropine compared to control, was assessed. Cohen's d formula along with the random effect model was applied. The I^2 statistical test was used to quantify the heterogeneity and the risk of bias evaluation for all the included studies and trials. A positive effect size for spherical equivalent and pupil dilation, as well as the mean difference, represented that the Atropine was superior to the control, whereas it was the converse for axial length and accommodative amplitude (Fritz et al. 2012). For the analysis related to side effects, i.e., change in pupillary diameter and the accommodative amplitude, baseline data prior to instillation of Atropine was used as the control with the exception of the randomised clinical trial conducted by Chia et al. 2012 where a historical control group was used (Chia et al. 2012). Further analysis was conducted to determine the relation between the change in pupillary diameter and accommodative amplitude to the various concentrations of Atropine. The relation between weighted data from the meta-analysis versus different concentrations of Atropine was determined using linear, logarithmic, inverse, quadratic, and cubic fits; R^2 and standardised residual errors were used to determine the model that best fits

the data. Cubic, quadratic, and logarithmic fits had high R^2 values. However, both quadratic and cubic fits were found to either estimate values beyond the range of the observed data or have significant standardised residual errors, and therefore, a dose-response curve was generated using only the logarithmic fit.

2.3 Results

2.3.1 *Characteristics of studies*

The search yielded a total of 2405 scientific items. After removing duplicates and completing eligibility assessment, a total of 13 eligible articles (6 RCTs and 7 observational studies) remained (Figure 2.1). These comprised data from 2907 participants aged 4 to 16 years old and with at least one year of follow-up. A total of 9 different concentrations of Atropine were used in these trials. Table 2.2 presents the basic characteristics of the trials/studies included in the meta-analysis. The trials were from various countries and/or regions across the world. Moreover, the quality of the trials/studies were assessed using several features which are presented in Figure 2.2 for the randomized clinical trials and Table 2.3 for observational studies. Of the 13 trials/studies that were eligible, the study of Chia and associates in 2012 lacked a control group (Chia et al. 2012), however, was a well-conducted clinical trial using a historical control group, and therefore included in the analysis (Chua et al. 2006).

Table 2.2: Characteristics of the eligible randomized clinical trials included in the meta-analysis

Author and Year	Country	Age	Follow-up	Concentration investigated	Participants in the treatment arms	Type of study	Spherical equivalent	Axial length	Pupil size	Amplitude of accommodation
Randomized clinical trials (6)										
Yam 2019 (Yam et al. 2019)	Hong-Kong	4-12	1 year	0.05% 0.025% 0.01%	109 108 110	RCTs	x	x	x	x
Yi 2015 (Yi et al. 2015)	China	7-12	1 year	1%	68	RCTs	x	x		
Chia 2012 (Chia et al. 2012)	Singapore	6-12	2 years	0.5% 0.1% 0.01%	161 155 84	RCTs	x	x	x	x
Chua 2006 (Chua et al. 2006)	Singapore	6-12	2 years	1%	200	RCTs	x	x		x
Shih 1999 (Shih et al. 1999)	Taiwan	6-13	1.5 year	0.5% 0.25% 0.1%	41 47 49	RCTs	x			
Yen 1989 (Yen et al. 1989)	Taiwan	6-14	1 year	1%	32	RCTs	x			
Observational studies (7)										
Fu 2020 (Fu et al. 2020)	China	6-14	1 year	0.02% 0.01%	117 119	Observational study	x	x	x	x
Sacchi 2019 (Sacchi et al. 2019)	Italia	5-16	1 year	0.01%	52	Observational study	x			
Larkin 2019 (Larkin et al. 2019)	USA	6-15	2 years	0.01%	100	Observational study	x			
Lee CY 2016 (Lee et al. 2016)	Taiwan	6-12	1 year	0.25% 0.125%	12 32	Observational study	x			
Clark 2015 (Clark and Clark 2015)	USA	6-15	1 year	0.01%	32	Observational study	x			
Fan 2007 (Fan et al. 2007)	Taiwan	5-10	1 year	1%	23	Observational study	x	x		
Lee JJ 2006 (Lee et al. 2006)	Taiwan	6-12	2 years	0.05%	21	Observational study	x			

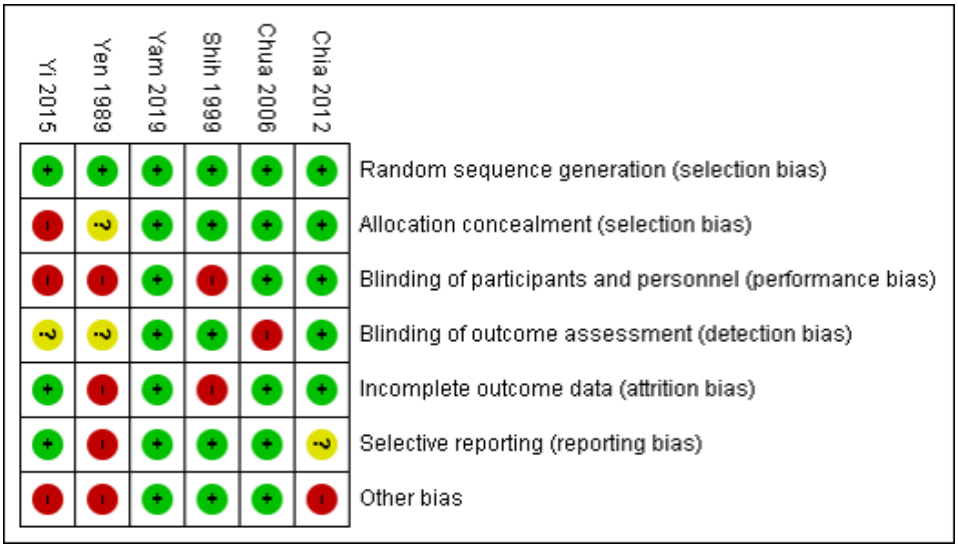

Figure 2.2: Quality assessment of the randomized clinical trials

Table 2.3: Quality assessment of the observational studies using the Newcastle-Ottawa Scale

	Representativeness	Control definition	Ascertainment of exposure	Outcome not present at start	Comparability	Assessment of outcome	Follow-up length	Adequacy of follow-up
Fu 2020	**	**	*	*	**	**	*	*
Sacchi 2019	*	*	*	*	**	**	*	*
Larkin 2019	*	*	*	*	**	*	*	*
Lee CY 2016	*	*	*	*	*	*	*	*
Clark 2015	*	*	*	*	**	*	*	*
Fan 2007	*	*	*	*	*	*	*	*
Lee 2006	*	*	*	*	*	*	*	*

* represents score, each individual feature can be labelled a maximum of one star, except the comparability can be given up to two stars.

2.3.2 *Effect of Atropine on pupillary diameter and amplitude of accommodation*

Only three of the 13 studies included data on pupil diameter and accommodative amplitude. A further single study, Chua et al. 2006 reported only the change in accommodative amplitude (Chua et al. 2006). The effect of seven concentrations of Atropine was extracted from these studies. All concentrations resulted in a reduction in accommodative amplitude (mean change -4.67 D, 95% CI, -7.44 to -1.89D, p<0.001; I^2 = 99%) and an increase in pupillary diameter (mean increase 1.33 mm, 95% CI, 0.57 to 2.09 mm, p<0.001; I^2 = 99%, Figure 2.3 and Figure 2.4).

Figure 2.5 presents the relation between the weighted data for change in amplitude of accommodation and increase in pupillary diameter to various concentrations of Atropine using a logarithmic equation and is given by "Effect size for change in pupillary diameter = 8.151 + 0.849 x LN (Atropine concentration)" and "Effect size for amplitude of accommodation = -23.131 – 2.389 x LN (Atropine concentration) where LN is the natural log of Atropine. The curve indicates a non-linear dose response; the curve was steep for lower concentrations and appears to slow/plateau for points at ≥0.10% Atropine. Therefore, we categorised the effect sizes for concentrations <0.10% and ≥0.10% Atropine and the data is presented in Table 2.4.

At concentrations of <0.10% Atropine, the mean change in pupillary diameter and accommodative amplitude was 0.7mm (95% CI, 0.1 to 1.4) and -1.6D (95% CI, -3.9 to 0.7) whereas for ≥0.1% Atropine concentrations it increased to 3.2 mm (95% CI, 2.8 to 3.5) and -10.7 D (95% CI, -12.2 to -9.2) respectively. These differences for change in pupillary diameter and accommodative amplitude between the two groups, i.e. <0.10% and ≥0.10%, were significant (Table 2.4).

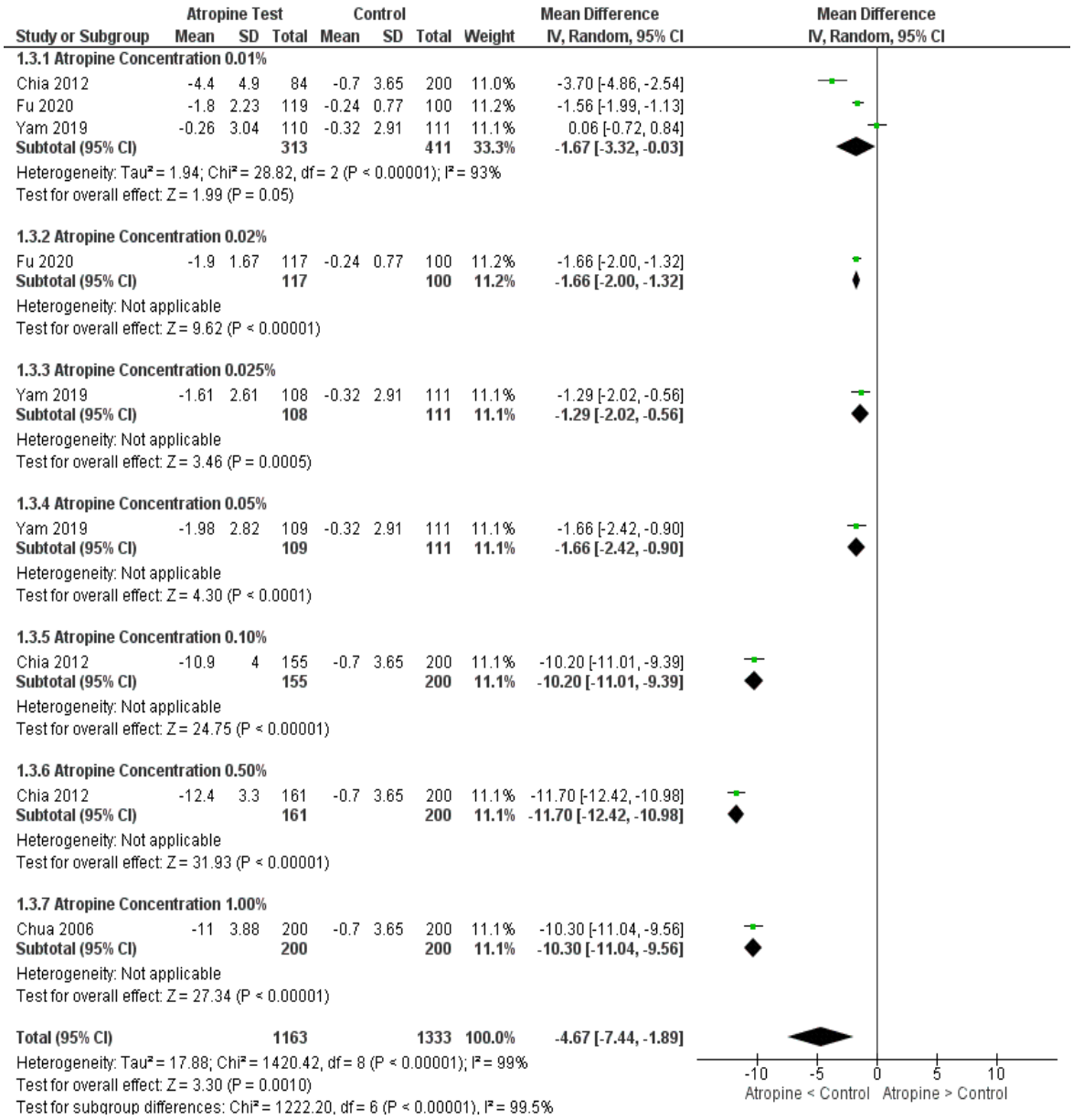

Figure 2.3: The effect of Atropine on accommodative amplitude

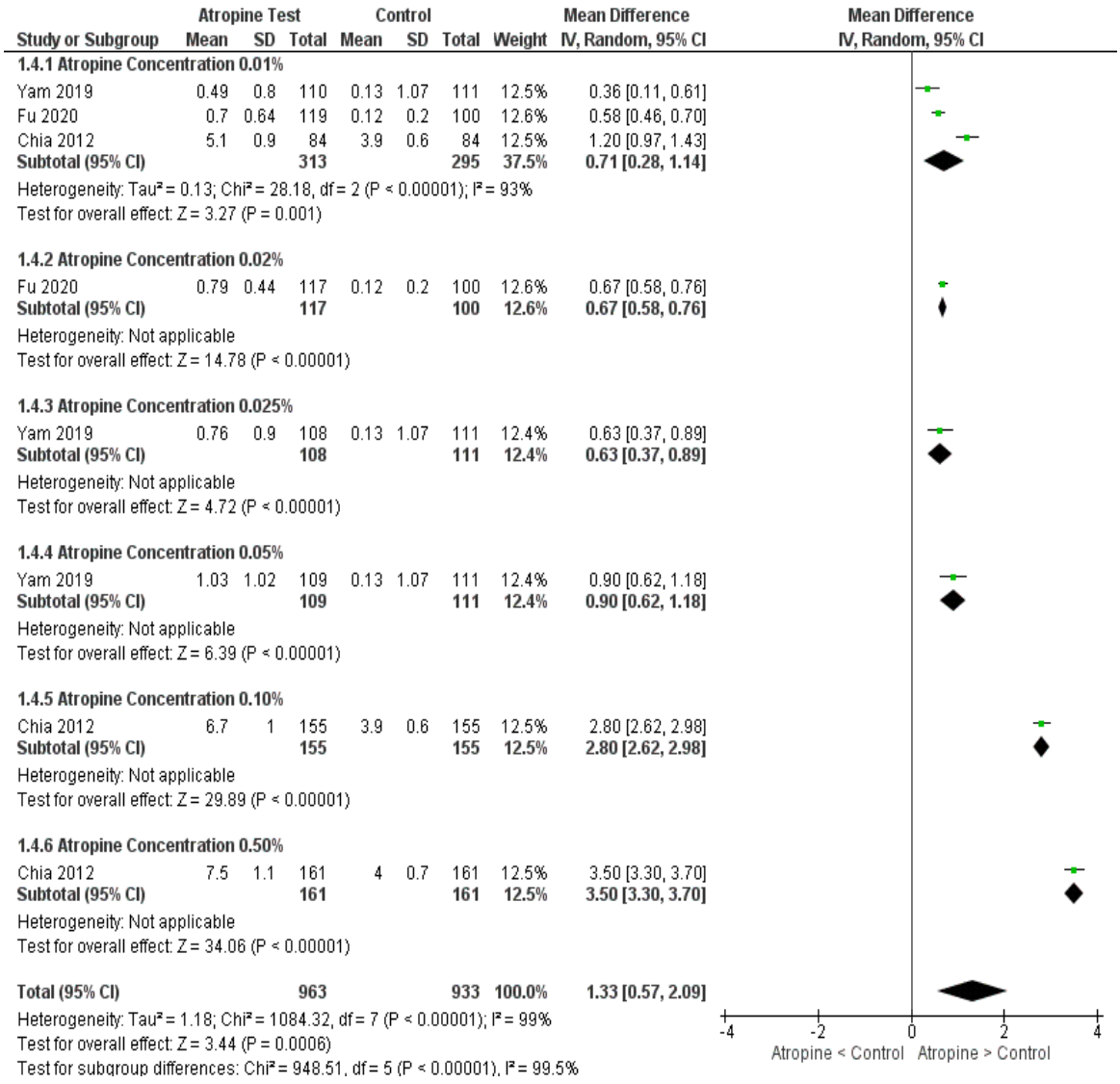

Figure 2.4: The effect of Atropine on pupillary diameter

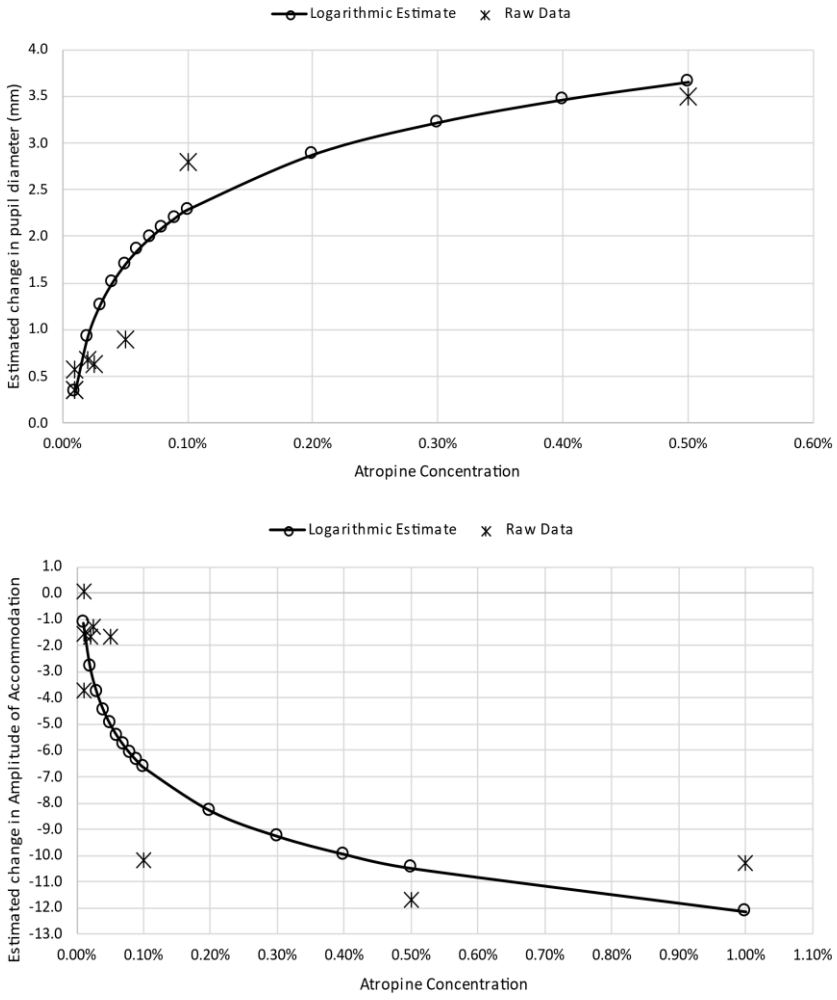

Figure 2.5: Relation between Atropine concentration and change in pupillary diameter and accommodative amplitude

Table 2.4: Effects size of change in pupillary diameter and accommodative amplitude with various concentrations of Atropine"

Atropine Concentration	Effect Estimate	Standard error	CI Start	CI End	Weight	Subgroup weight	Weighted mean	Weighted standard error	Subgroup Mean	Subgroup standard error	95% Lower	95% Upper	p-value
Amplitude of accommodation [Dioptre]													
Overall	-4.67		-7.44	-1.89	100.00								
0.01%	-1.67	1.65	-3.32	-0.03	33.30	0.50	-0.83	0.82	-1.60	1.13	-3.86	0.65	
0.02%	-1.66	0.34	-2.00	-1.32	11.20	0.17	-0.28	0.06					
0.025%	-1.29	0.73	-2.02	-0.56	11.10	0.17	-0.21	0.12					
0.05%	-1.66	0.76	-2.42	-0.90	11.10	0.17	-0.28	0.13					
0.10%	-10.20	0.81	-11.01	-9.39	11.10	0.33	-3.40	0.27	-10.73	0.75	-12.24	-9.22	
0.50%	-11.70	0.72	-12.42	-10.98	11.10	0.33	-3.90	0.24					
1.00%	-10.30	0.74	-11.04	-9.56	11.10	0.33	-3.43	0.25					
							Mean Difference		**-9.13**	**0.94**	**9.71**	**0.000**	***p = 0.000***
Pupil size [mm]													
Overall	1.33		0.57	2.09	100.00								
0.01%	0.71	0.43	0.28	1.14	37.50	0.50	0.36	0.22	0.72	0.32	0.08	1.36	
0.02%	0.67	0.09	0.58	0.76	12.60	0.17	0.11	0.02					
0.025%	0.63	0.26	0.37	0.89	12.40	0.17	0.10	0.04					
0.05%	0.90	0.28	0.62	1.18	12.40	0.17	0.15	0.05					
0.10%	2.80	0.18	2.62	2.98	12.50	0.50	1.40	0.09	3.15	0.19	2.77	3.53	
0.50%	3.50	0.20	3.30	3.70	12.50	0.50	1.75	0.10					
							Mean Difference		**2.43**	**0.25**	**9.53**	**0.000**	***p = 0.000***

2.3.3 *Efficacy of Atropine for myopia control*

A total of nine concentrations of Atropine were assessed in 21 treatment arms and were as follows: 0.01% (6 treatment arms), 0.02% (1), 0.025% (1), 0.05% (2), 0.10% (2), 0.125% (1), 0.25% (2), 0.50% (2) and 1.00% (4). The pooled estimates suggest that, compared to the control, reduction in annual progression of spherical equivalent with 0.01% Atropine was 0.34D (95% CI, 0.25 to 0.44), 0.02% was 0.32D (95% CI, 0.19 to 0.45), 0.025% was 0.35D (95% CI, 0.22 to 0.48), 0.05% was 0.51D (95% CI, 0.40 to 0.62), 0.10% was 0.46D (95% CI, 0.37 to 0.56), 0.125% was 1.00D (95% CI, 0.20 to 1.80), 0.25% was 0.64D (95% CI, 0.41 to 0.86), 0.50% was 0.79D (95% CI, 0.37 to 1.21), 1.00% was 0.92D (95% CI, 0.63 to 1.21) and overall was 0.57D (95% CI, 0.43 to 0.71) (Figure 2.6). The differences between groups were significant ($p < 0.001$). The data was highly heterogeneous (I^2 =93%, $p<0.001$). The reduction in spherical equivalent with Atropine compared to controls for concentrations <0.10 and ≥0.10% was 0.37D (95% CI, 0.16 to 0.58) versus 0.75D (95% CI, 0.18 to 1.33) and not statistically significant between the two groups ($p=0.055$); however, the mean difference was 0.38 D/year and considered to be clinically relevant (Table 2.5).

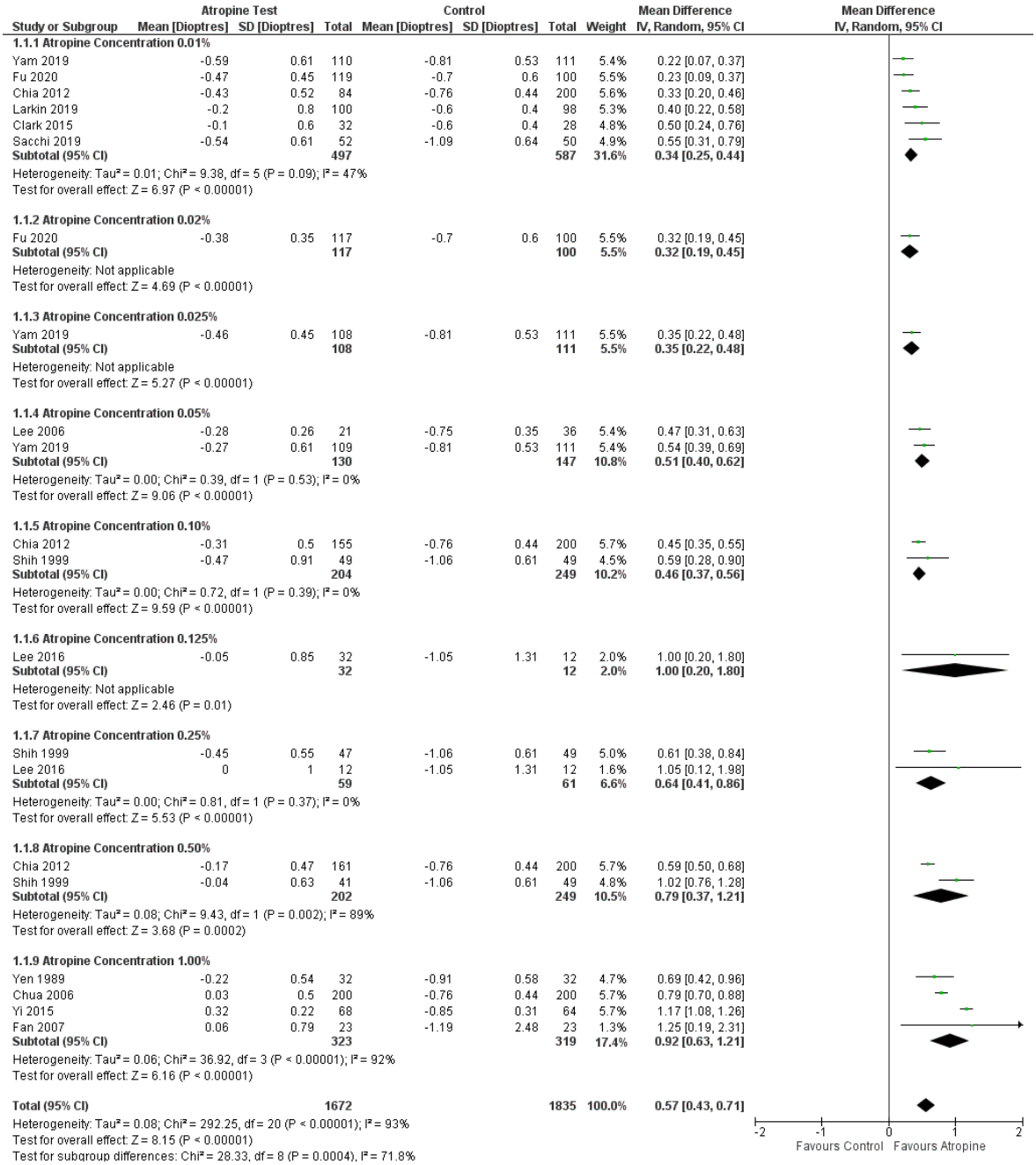

Figure 2.6: Annual change in spherical equivalent refractive error with various concentrations of Atropine as compared to Control

Table 2.5: Effect estimate for various concentrations of Atropine on annual change in refractive error and axial length

Atropine Concentration	Effect Estimate	Standard error	CI Start	CI End	Weight	Subgroup weight	Weighted mean	Weighted standard error	Subgroup Mean	Subgroup standard error	95% Lower	95% Upper	p-value
Spherical equivalent progression [Dioptre]													
Overall	0.57		0.43	0.71	100.00								
0.01%	0.34	0.10	0.25	0.44	31.60	0.59	0.20	0.06	0.37	0.11	0.16	0.58	
0.02%	0.32	0.13	0.19	0.45	5.50	0.10	0.03	0.01					
0.025%	0.35	0.13	0.22	0.48	5.50	0.10	0.04	0.01					
0.05%	0.51	0.11	0.40	0.62	10.80	0.20	0.10	0.02					
0.10%	0.46	0.10	0.37	0.56	10.20	0.22	0.10	0.02	0.75	0.29	0.17	1.33	
0.125%	1.00	0.80	0.20	1.80	2.00	0.04	0.04	0.03					
0.25%	0.64	0.23	0.41	0.86	6.60	0.14	0.09	0.03					
0.50%	0.79	0.42	0.37	1.21	10.50	0.22	0.18	0.09					
1.00%	0.92	0.29	0.63	1.21	17.40	0.37	0.34	0.11					
							Mean Difference		0.38	0.20	1.92	0.055	**p = 0.055**
Axial elongation [mm]													
Overall	-0.17		-0.25	-0.08	100.00								
0.01%	-0.03	0.08	-0.11	0.05	28.10	0.50	-0.01	0.04	-0.10	0.07	-0.24	0.05	
0.02%	-0.16	0.08	-0.24	-0.08	9.20	0.16	-0.03	0.01					
0.025%	-0.12	0.06	-0.18	-0.06	9.60	0.17	-0.02	0.01					
0.05%	-0.21	0.06	-0.27	-0.15	9.50	0.17	-0.04	0.01					
0.10%	-0.07	0.05	-0.12	-0.02	9.60	0.22	-0.02	0.01	-0.23	0.05	-0.34	-0.13	
0.50%	-0.09	0.05	-0.14	-0.04	9.70	0.22	-0.02	0.01					
1.00%	-0.36	0.05	-0.41	-0.30	24.40	0.56	-0.20	0.03					
							Mean Difference		-0.14	0.06	2.18	0.029	***p = 0.029***

Similarly, Atropine was statistically superior to the controls in slowing axial elongation with a pooled difference in annual mean of -0.17mm (95% CI -0.25 to -0.08, p<0.001) (Figure 2.7). The data was highly heterogeneous (I^2 =95%, p<0.001) and the efficacy varied across concentrations with 0.01% Atropine statistically insignificant compared to the control (mean difference of -0.03D, 95% CI, -0.11 to 0.05, p=0.45). The reduction in axial elongation for concentrations of <0.10 and ≥ 0.10% % Atropine compared to controls was -0.10mm (95% CI, -0.24 to 0.05) and -0.23mm (95% CI, -0.34 to -0.13) respectively (p=0.029) (Table 2.5).

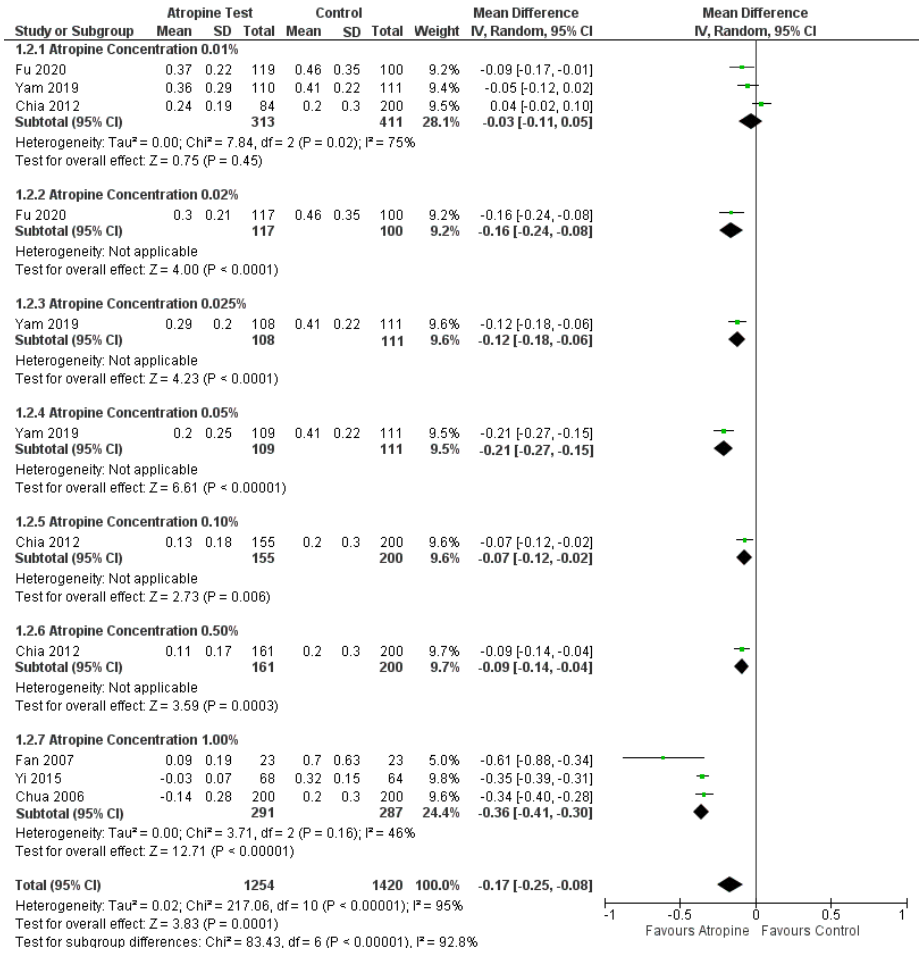

Figure 2.7: Annual change in axial length with various concentrations of Atropine as compared to Control

2.4 Discussion

None of the previous meta-analyses had systematically reviewed the data for the two common side effects related to use of Atropine, i.e. an increase in pupillary diameter and a reduction in accommodative amplitude (Li et al. 2014; Gong et al. 2017). Since these two factors appear to be mostly responsible for the initial choice of concentration of Atropine in myopia management (Lee et al. 2006; Fang et al. 2010; Chia et al. 2012; Clark and Clark 2015; Polling et al. 2016), they were analysed and reported as the primary outcomes in the current meta-analysis. Since data on pupillary diameter and accommodative amplitude with use of Atropine was not always reported in efficacy trials, the data is somewhat limited. Furthermore, where information was available on the effect of Atropine on pupillary size and accommodative amplitude, there was no information on efficacy (Loughman and Flitcroft 2016; Tran et al. 2019). Notwithstanding the above, a quantification of the effect size for an increase in pupillary diameter and a reduction in accommodative amplitude indicates a greater effect or an increased magnitude of these side effects at higher concentrations (Figure 2.3 and Figure 2.4). This relationship was examined further (Figure 2.5) and the data highlights a non-linear dose response relationship characterised by a) a steep increase in the effect versus dose at concentrations <0.10% Atropine and b) a higher effect but a slower rise or some plateauing of the curve for concentrations ≥0.10% Atropine and c) the trend is similar for both the side effects. Our findings align with published reports wherein most of the reports of photophobia or near work problems were with the use of 0.50% Atropine and infrequent in those using ≤0.10% Atropine (Shih et al. 1999; Shih et al. 2001; Chia et al. 2012). However, whilst the relationship indicates a steep increase for concentrations <0.10% Atropine, previous reports also found no significant difference in the rate of side effects with the use of 0.05% and 0.025% Atropine (Fang et al. 2010; Yam et al. 2019). More data is needed to better understand and confirm this relationship. In addition to the lack of sufficient data, other factors that may be confounding include a possible variability in the stability of Atropine as well as the time of measurement versus instillation. Atropine is mostly available as a compounded

product that is diluted for use; thus, ensuring that the stability and therefore strength is appropriate is important especially for the lower concentrations of Atropine. In this respect, although there are some reports of Atropine being unstable at certain conditions, others have not found an issue (Saito et al. 2019; Berton et al. 2020; Puri et al. 2020). Furthermore, the side effects maybe time related and may peak for example, within certain hours after instillation of drop and may reduce in intensity thereafter; therefore, the time at which the measurements were collected post-instillation might also influence the results.

Although there have been several meta-analyses reporting on the efficacy of Atropine in slowing myopia (Song et al. 2011; Li et al. 2014; Gong et al. 2017), since the last reported analysis in 2017, there have been a further four studies that have assessed the efficacy of 0.01% Atropine. Therefore, we also considered the efficacy of Atropine in controlling progression of myopia. In comparison to controls, all concentrations were observed to slow myopia when spherical equivalent refractive error was considered as the outcome measurements; similarly, all except 0.01% Atropine had a significant effect in slowing axial length. Utilising the cut-off criteria of <0.10% and ≥0.10% Atropine, compared to controls, the reduction in progression was 0.37D (95% CI, 0.16 to 0.58) versus 0.75D (95% CI, 0.18 to 1.33) for spherical equivalent refractive error and -0.10mm (95% CI, -0.24 to 0.05) versus -0.23mm (95% CI, -0.34 to -0.13) for axial length. Although there was lesser control of myopia at the lower concentrations, the overlap of CI indicates that the groups were not significantly different. Evidence indicates that the younger the age of onset, the faster the progression of myopia, and thus interventions should be considered as early as possible (Sankaridurg and Holden 2014). Furthermore, the second-year data of LAMP study indicated that the progression of myopia in those that were not treated in the first year (controls) and treated using 0.05% Atropine in the second year was clinically and statistically equivalent to the group using 0.01% Atropine over two years suggesting a more aggressive approach early on may be a useful strategy.

Additionally, although our analysis found some differences between the various concentrations for reduction in spherical equivalent refractive error and/or axial length, two of the previous meta-analyses did not find significance between the various doses/concentrations (Huang et al. 2016; Gong et al. 2017). The differences may be related to variation between the studies with respect to the articles considered for the meta-analysis as well as the categorisation. For example, Gong et al 2017 (Gong et al. 2017) considered the results of a 3-year follow-up study of ATOM 1 study for 1% Atropine (Chua et al. 2006; Tong et al. 2009), whereas we had considered only the original ATOM 1 study (Chua et al. 2006). With respect to categorisation, Huang et al. 2016 had combined both 1% and 0.5% into high dose, 0.1% as moderate and 0.01% as low dose and had considered only 5 articles (Huang et al. 2016). Furthermore, whilst the previous articles had considered the efficacy over the study period (for example, 24 months), we have provided the annualised difference in progression to ensure uniformity across the studies.

In addition to concerns related to dilation of pupil and significant decrease of accommodative amplitude, there are other concerns related to long term use of Atropine. Use of Atropine with preservatives has been reported to result in red and irritated eyes (Tan et al. 2016). Importantly, rebound of myopia that occurs on discontinuation of Atropine and is said to occur especially with high dose Atropine (Tong et al. 2009; Chia et al. 2014). However, the data is limited and was not explored further. Other concerns such as retinal damage due to long term exposure to ultraviolet light. In this regard, there have been some trials that have followed participants using Atropine for ten years or more with no adverse events (Brodstein et al. 1984; Kennedy et al. 2000; Syniuta and Isenberg 2001), and recent evidence indicates no damage to the retinal layers (Luu et al. 2005; Chia et al. 2013). However, the available information is inadequate to analyse this data systematically. Although the data support the role of Atropine, including low dose Atropine, in myopia management, the effect of the lowest dose i.e., 0.01% Atropine remains inconclusive as its effect on axial length was not significantly different (Chia et al. 2012).

There are several limitations associated with this meta-analysis. Although there were enough studies, most of these studies used low concentrations of Atropine, and only a few had reported on pupil size and accommodative amplitude. Therefore, there was limited information on the use of higher concentrations of Atropine where there was a greater risk of side effects. Additionally, there is variation in the techniques used to measure accommodative amplitude and pupillary diameter between studies. However, rather than absolute values, we reported change in pupillary diameter and accommodative amplitude comparing it to the control used in those studies. Therefore, the effects of such variation are likely minimised. Furthermore, whilst the change in pupil size and accommodative amplitude was considered, the functional outcomes of these structural variations, such as loss of vision or glare or photophobia was not explored as these were not always available or easily quantifiable due to the variation in subjective questionnaires between studies (Shih et al. 1999; Chia et al. 2012; Yam et al. 2019). Except for LAMP study and the ATOM2, subjective symptoms were not evaluated in a systematic manner (Chia et al. 2012; Yam et al. 2019). Despite a robust study design and conduct of the trial, the use of a historical control group is a source of bias (Chia et al. 2012). Nonetheless, it was considered reliable as both studies were conducted in a similar population and with comparable designs. Finally, all the studies that reported on pupil size and accommodation were from Asian eyes; the effects of mydriatics and cycloplegics on pigmented versus non-pigmented or lesser pigmented eyes are well known (Polling et al. 2016). Therefore, although the current meta-analysis found little variation in pupillary size and accommodative amplitude for concentrations of Atropine <0.10%, it is possible that there may be a greater variation in eyes with lesser pigmentation.

In summary, the data indicate that at concentrations of <0.10%, the effect on pupillary size and accommodative amplitude was small with insignificant variation between the various concentrations. On average, the change in pupil size was < 1mm, and the change in accommodative amplitude was <2.0D. Despite some discomfort due to the increase in pupil size, the loss of a small amount of accommodative amplitude is unlikely to have resulted in any

significant symptoms as children have large reserves of accommodation (Chia et al. 2012; León et al. 2016; Yam et al. 2019). Similarly, the ability to slow myopia progression was lower for concentrations <0.10%. Although higher concentrations of Atropine (≥ 0.10%) can significantly slow ocular elongation, they are accompanied by large increases in pupillary diameter and accommodative amplitude that limit their use. Further work needs to be conducted to determine the concentration of Atropine that achieves the maximal efficacy without a significant increase in pupillary diameter and accommodative amplitude.

Interim summary:

Nine concentrations of Atropine (0.01% to 1%) were considered in 13 trials and based on the concentration, the pooled estimates for reduction in annual progression of spherical equivalent varied from 0.34D to 0.92D. A corresponding slowing of axial elongation was observed for all concentrations excepting 0.01% Atropine (mean diff: -0.03mm compared to control). The effect on pupillary diameter and accommodative amplitude was reported for seven concentrations; all increased pupillary diameter and reduced accommodative amplitude. The relation between the concentration and the observed change in accommodative amplitude/pupillary diameter was non-linear; at <0.1%, the slope of relation was rising and steep but the overall change in accommodative amplitude/pupillary diameter was small at <2D/<1mm. At >0.1% Atropine, the effect was significant, but the slope was flatter.

Chapter 3. Effects of low concentration Atropine on pupillary diameter and accommodative amplitude in children

3.1 Introduction

As detailed in Chapter 1, with the exception of 0.01% Atropine, other higher concentrations (>0.01% to 1.0%) of Atropine, have consistently demonstrated a reduction in progression of myopia in human eyes of different ethnicities (Brodstein et al. 1984; Yen et al. 1989; Kennedy et al. 2000; Syniuta and Isenberg 2001; Fan et al. 2007; Yi et al. 2015; Chia et al. 2016; Wang et al. 2017; Moon and Shin 2018; Yam et al. 2019; Fu et al. 2020; Polling et al. 2020). However, the underlying mechanisms remain unknown. Furthermore, a meta-analysis of published data (Chapter 2) indicates that there is a) limited data on the side effects related to Atropine, b) at higher concentrations, use of Atropine results in a significant proportion of the users experiencing unwanted effects such as photophobia and blurred near vision due to dilation of pupils and loss of accommodation which may consequently limit compliance and lead to discontinuing the treatment and, c) cessation of Atropine especially at higher concentrations results in significant rebound of myopia (Tong et al. 2009; Chia et al. 2016; Tran et al. 2018). With respect to 0.01% Atropine, although a 5-year study reported that it was effective in slowing myopia, efficacy was demonstrated only with respect to change in spherical equivalent but not with change in axial length (Chia et al. 2016). Similar result was observed in a more recent, prospective randomized clinical trial where there was no significant difference for change in eye length with 0.01% Atropine compared to the control group (Yam et al. 2019).

Thus, it is not clear from the evidence that 0.01% Atropine is the optimal concentration that combines efficacy with the least risk of side effects. Cooper and co-workers (Cooper et al. 2013) evaluated various strengths of Atropine (0.012%, 0.025% and 0.05%) to determine the concentration that provides least side effects (defined as pupillary difference no greater than 3

millimetres and residual accommodation greater than five dioptres) and found the highest clinically tolerable concentration of Atropine was approximately 0.02% with anything beyond resulting in clinical loss of accommodation and significant pupillary dilation. However, the sample size of the study was considerably small at just 12 adult participants enrolled into a 3x 3 phase I clinical trial paradigm that limited the number of participants assessed per dose (Cooper et al. 2013). Additionally, in other independent studies, concentrations above 0.03% Atropine were cytotoxic to the corneal endothelium in a concentration-dependent and time-dependent manner (Wen et al. 2016). These results suggest that although better efficacy may be achieved at higher concentrations, the concentration that achieves good efficacy with minimal side effects may be in the region of ≥0.01% and ≤0.03% Atropine.

Prior to undertaking large scale, dispensing trials, we aimed to determine and select a greater concentration than 0.01% Atropine to ensure that the chosen concentration provides efficacy in our future clinical trials whilst also lowering the risk of side effects. This would serve as a benchmark against which we would compare the efficacy of other compounds such as Caffeine and the combination of Atropine and Caffeine. Although 0.01% Atropine was not effective in controlling myopic growth, it was identified as the concentration with the least side effects and therefore included in the study to evaluate the effects of other concentrations against 0.01% Atropine. We therefore compared the effects of 0.01%, 0.02 and 0.03% Atropine on pupillary diameter, accommodative amplitude, and subjective symptoms in a short term, dispensing clinical trial assessment in a group of Vietnamese children with myopia.

3.2 Aims

- To compare the effect of 0.01%, 0.02 and 0.03% Atropine on pupillary diameter and accommodative amplitude in a two-week, randomised dispensing clinical trial.

- To determine the subjective acceptability and symptoms associated with 0.01%, 0.02% and 0.03% Atropine in children with myopia in a two-week, randomised, dispensing clinical trial.

3.3 Methods

In a prospective, two-week, double-masked clinical trial conducted at An-Sinh Hospital and Hai Yen Eye Center in Ho Chi Minh City, Vietnam, 58 children with myopia were successfully enrolled. The study was conducted from March to May 2018. All children were aged between 6 and 12 years, had myopia worse than -0.50D with ≤ -2.00 D of astigmatism, vision correctable to at least 20/25 or better in each eye with single-vision spectacles (SV Specs), did not wear contact lenses in the 3 months prior to trial and had normal ocular findings. In addition, their parents/legal guardians indicated they were willing to apply the eye drops once every night for fourteen days, follow the visit schedule as advised by the clinicians, and were willing to sign the informed consent.

Children were excluded from participation if they had:

a) any pre-existing ocular injury or condition or systemic condition that may affect ocular health, b) any current use of systemic or topical medication that may affect eye health
b) history of eye surgery
c) history of interventions for myopia control in the past 12 weeks
d) contraindications or known allergic reactions to Atropine or any other medications used in the trial
e) current involvement in another clinical trial.

The study protocol was approved by the Institutional Research Ethics Committee of An-Sinh Hospital (No CS/AS/18/07), Human Research Ethics Committee of University of New South Wales (No HC200725) and adhered to the Declaration of Helsinki for experimentation on human subjects.

3.3.1 *Sample size determination*

A minimum of 20 participants in each group, were required to determine a paired difference of 0.5 ± 0.6 mm in change of pupillary dilation (Chia et al. 2012). The power was set at 80% at the 5% level of significance. The sample size was also adjusted for a 15% of drop-out rate.

3.3.2 *Visits and measurements*

On enrolment, children were randomised to one of three groups (A, B or C coded for 0.01%, 0.02% or 0.03% Atropine, CustomCare Compounding Pharmacy, Dural, NSW, Australia respectively) at the allocation ratio 1:1:1. Investigators, participants and their carers remained masked to the identity of the drops dispensed. The children were advised to continue to use their corrective device, i.e. single vision spectacles, during the trial and to apply the drop once nightly at bedtime in both eyes. Children and their parents or carers were encouraged to report any health issues or side effects related to the usage of eye drops over the trial period.

At baseline, assessments were conducted pre- and post-cycloplegia. Visual acuity was measured at distance and near with best-corrected prescription. Distance visual acuity was measured using Snellen chart at 5 meters. Near visual acuity was measured using the ETDRS (Early Treatment of Diabetic Retinopathy Study) chart at 40 centimetres. Data related to the distance and near visual acuity were recorded in logMAR scale. Following visual acuity measurement, pupillary diameter was measured using OCULUS Park 1 (OCULUS Optikgerate GmBH, Wetzlar, Germany) under photopic and mesopic conditions. Photopic state was defined as use of direct light of 300 lux at the eye level and mesopic condition was ambient room light (30 lux) (Figure 3.1). Pupil diameter (photopic and mesopic), amplitude of accommodation and intraocular pressure were measured by three repeats followed by an average for each participant. A Royal Air-Force Near Point Ruler was used to measure the monocular accommodative amplitude with the best-corrected distance spectacle correction in place (Figure 3.1). Protocol was repeated for both eyes of each participant. Briefly, a push-up method was used wherein the child was instructed to focus on the smallest line

on the target drum, equivalent to N5 (N-scale) print. The assessor then slowly moved the drum towards the testing eye until the child reported the blur of that line. Intraocular pressure was measured using Tonopen (Reichert Technologies, Reichert Inc., Depew, USA) at each visit.

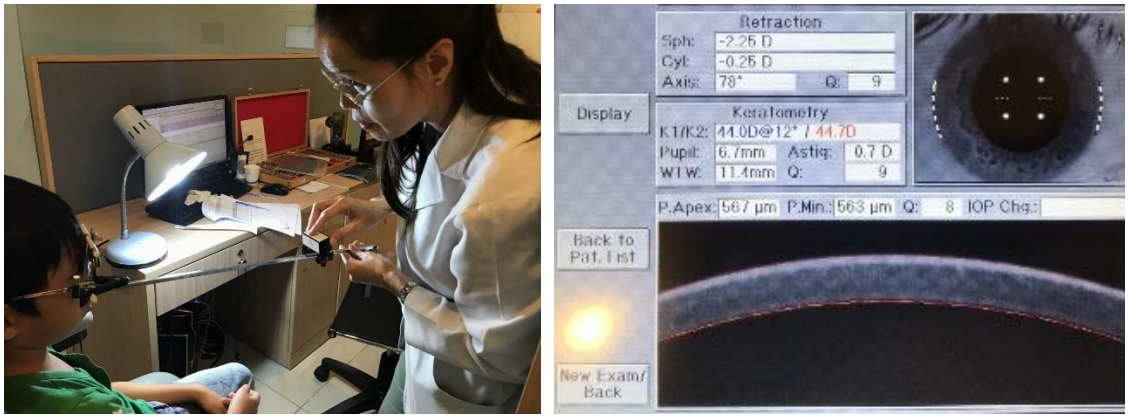

Figure 3.1: Measurement of accommodative amplitude with the Royal Air-Force Near Point Ruler and pupil size assessment with the OCULUS Park1.

Thereafter, eyes were cyclopleged using three drops of 1% cyclopentolate (Cyclogyl, Alcon-Convreur, Rijksweg, Belgium) instilled three times at five-minute intervals. After approximately thirty minutes, eyes were checked for dilation using a pen torch (size and response of pupil assessed). Cyclopleged eyes underwent axial length measurement with the IOL Master 500 (Carl Zeiss Meditec, Germany), and refractive error assessment with Oculus Park 1 (OCULUS Optikgerate GmBH, Wetzlar, Germany)

A short safety check was conducted on day 3. On day 14, a comprehensive examination that included measurements of pupillary diameter under photopic and mesopic conditions, accommodative amplitude, intraocular pressure, visual acuity (distance and near) and slit-lamp biomicroscopy was conducted. Thereafter, participants were discharged from the trial.

3.3.3 *Statistical analysis*

Demographic and ocular characteristics including age, baseline spherical equivalent, baseline axial length, intraocular pressure, best-corrected distance visual acuity, near visual acuity using single vision spectacles, photopic and scotopic pupillary diameter, and accommodative amplitude were summarised as means ± standard deviations for continuous measurements. Data from both eyes were considered. The level of significance was set at 5%. Comparison between study groups over time was performed using repeated measures Analysis of Variance (ANOVA). The threshold or cut-off criteria for an increase in pupillary diameter that was considered tolerable was set at <3 millimetres and for residual amplitude of accommodation was set at 5 dioptres (Cooper et al. 2013). Post-hoc multiple comparisons were corrected using Bonferroni correction. Level of significance was set at 5%. Statistical analysis was conducted using the IBM SPSS Statistics software, version 25.0.

3.4 Results

3.4.1 *Baseline Data*

Fifty-eight children were enrolled and randomised to one of the three groups and Table 3.1 provides the biometric data and demographic characteristics. The mean age of the participants was 9.3 ± 1.7 years, the mean cycloplegic spherical equivalent was -3.53 ± 1.79D and there were no differences between the groups for any of the baseline characteristics. Figure 3.2 presents the participant flow through the study. One child assigned to 0.03% Atropine was lost to follow-up. Fifty-seven children completed the 2-week trial. None of the parents reported missing application of Atropine at more than 1 day/week, and therefore all data were considered.

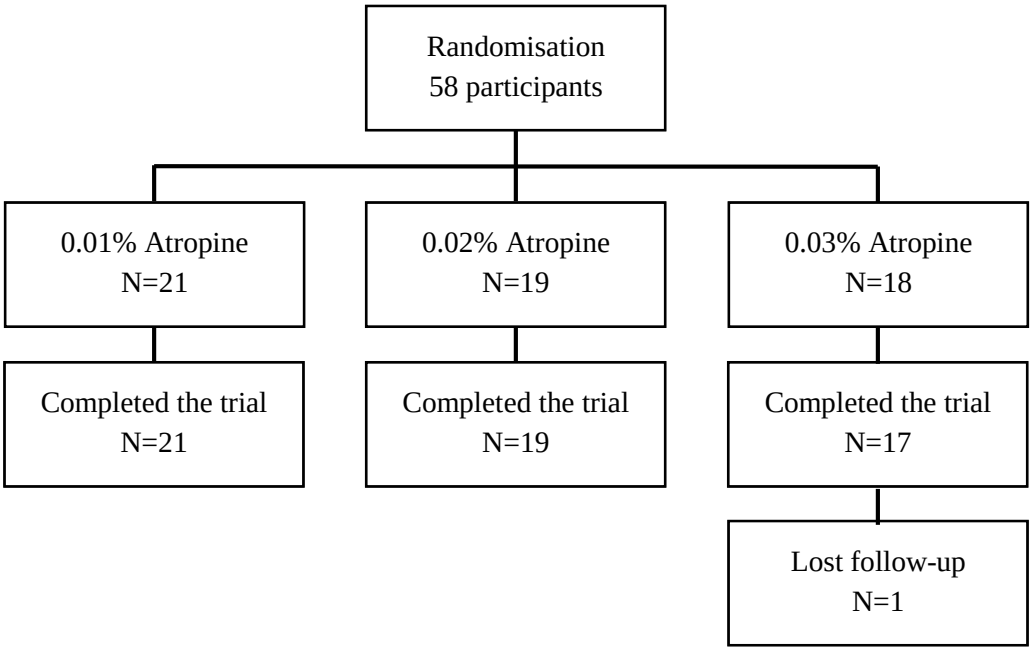

Figure 3.2: Flow of participants

Table 3.1: Baseline demographic and ocular characteristics of participants enrolled

Characteristic	0.01% Atropine	0.02% Atropine	0.03% Atropine	Overall	p-value
Subjects	21	19	17	57	
Age (years old)	9.2 ± 1.9	9.1 ± 1.7	9.5 ± 1.3	9.3 ± 1.7	0.764
CI 95%	[8.3;10.1]	[8.3;9.9]	[8.8;10.2]	[8.8;9.7]	
Spherical equivalent (D)	-3.66 ± 1.95	-3.22 ± 1.45	-3.73 ± 1.93	-3.53 ± 1.79	0.417
CI 95%	[-4.26;-3.05]	[-3.69;-2.74]	[-4.40;-3.05]	[-3.86;-3.20]	
Axial length (mm)	25.00 ± 1.07	24.77 ± 0.79	24.79 ± 0.96	24.86 ± 0.95	0.738
CI 95%	[24.67;25.33]	[24.51;25.03]	[24.45;25.12]	[24.68;25.03]	
IOP (mmHg)	16.77 ± 2.08	16.98 ± 3.25	17.20 ± 2.67	16.97 ± 2.67	0.819
CI 95%	[16.12;17.42]	[15.91;18.05]	[16.26;18.12]	[16.47;17.46]	
BCVA (logMAR)	0.03 ± 0.05	0.03 ± 0.03	0.03 ± 0.03	0.03 ± 0.04	0.875
CI 95%	[0.02;0.05]	[0.02;0.04]	[0.02;0.04]	[0.02;0.04]	
NearVA (logMAR)	0.08 ± 0.06	0.09 ± 0.06	0.06 ± 0.04	0.08 ± 0.05	0.159
CI 95%	[0.06;0.10]	[0.07;0.10]	[0.05;0.08]	[0.07;0.09]	
Accommodative amplitude (D)	19.40 ± 1.72	19.75 ± 0.93	19.27 ± 1.74	19.48 ± 1.50	0.570
CI 95%	[18.86;19.93]	[19.44;20.05]	[18.67;19.88]	[19.20;19.76]	
Photopic pupil (mm)	3.91 ± 0.63	4.30 ± 0.79	4.18 ± 0.84	4.12 ± 0.76	0.069
CI 95%	[3.72;4.11]	[4.04;4.56]	[3.89;4.47]	[3.97;4.26]	
Mesopic pupil (mm)	5.68 ± 0.73	6.23 ± 0.81	5.75 ± 1.06	5.88 ± 0.90	0.059
CI 95%	[5.45;5.91]	[5.97;6.50]	[5.38;6.12]	[5.72.;6.05]	

IOP: intraocular pressure; BCVA: best-corrected distance visual acuity; logMAR: logarithm of the Minimum Angle of Resolution; Near VA: near visual acuity on best-corrected distance spectacles; CI: confidence of interval

3.4.2 *Baseline to two weeks*

Over the two weeks in trial, no serious or significant side effects were reported by any of the participants.

3.4.2.1 Accommodative amplitude and change over time

Table 3.2, Figure 3.3 and Figure 3.4 present a) the mean amplitude of accommodation at each of the three visits and b) the change in accommodative amplitude from baseline for each of the follow-up visits. At baseline, mean accommodative amplitude was 19.40 ± 1.72 D, 19.75 ± 0.93 D, and 19.27 ± 1.74 D for participants assigned to 0.01%, 0.02%, and 0.03% Atropine, respectively and there was no significant difference between groups.

Compared to baseline, at both the 3-day and two-week visits, mean accommodative amplitude was significantly lower across all three groups. The drop in accommodative amplitude from baseline to three-day visit was significant; thereafter there was little change from the three-day visit to 2-week visit. At two weeks, there was a greater decrease in accommodative amplitude with 0.03% followed by 0.02% and 0.01%. However, the difference in the mean accommodative amplitude between 0.02% and 0.03% Atropine was within ≤1D (14.17 ± 4.40 D, 10.47 ± 3.16 D, and 9.95 ± 3.04 D at 2 weeks with 0.01%, 0.02% and 0.03% Atropine, $p < 0.001$, Table 3.2).

Compared to baseline, the mean reduction in accommodative amplitude at 2 weeks was 5.23 ± 4.11D, 9.28 ± 3.26D and 9.32 ± 2.83D with 0.01, 0.02 and 0.03% Atropine respectively. The percent of eyes wherein the mean residual accommodative amplitude was <10D was 31% with 0.01% and significantly higher at 60.5% and 58.8% for eyes 0.02% and 0.03% Atropine respectively. Furthermore, a total of 5.3% and 5.9% of the 0.02% and 0.03% Atropine eyes had a mean residual accommodative amplitude of <5D (Figure 3.4).

Table 3.2: Changes in accommodative amplitude over time

Time	Concentration	Combined		p-value each time	Posthoc		
		N	Mean ± SD [CI 95%]		0.01% Atropine	0.02% Atropine	0.03% Atropine
Pre-treatment	0.01% Atropine	42	19.40 ± 1.72 [18.86;19.93]	0.578			
	0.02% Atropine	38	19.75 ± 0.93 [19.44;20.05]				
	0.03% Atropine	34	19.27 ± 1.74 [18.67;19.88]				
3-day	0.01% Atropine	42	15.83 ± 3.98 [14.59;17.07]	***0.004***		0.519	***0.003***
	0.02% Atropine	38	13.99 ± 4.41 [12.54;15.44]		0.519		0.112
	0.03% Atropine	34	10.99 ± 4.71 [9.35;12.64]		***0.003***	0.112	
2-week	0.01% Atropine	42	14.17 ± 4.40 [12.80;15.54]	***0.001***		***0.005***	***0.002***
	0.02% Atropine	38	10.47 ± 3.16 [9.43;11.51]		***0.005***		1.000
	0.03% Atropine	34	9.95 ± 3.04 [8.89;11.01]		***0.002***	1.000	
3-day vs Pre-treatment	0.01% Atropine	42	-3.57 ± 3.84 [-4.76;-2.37]	***0.000***		0.078	***0.000***
	0.02% Atropine	38	-5.76 ± 4.42 [-7.21;-4.30]		0.078		***0.045***
	0.03% Atropine	34	-8.28 ± 4.79 [-9.95;-6.61]		***0.000***	***0.045***	
2-week vs Pre-treatment	0.01% Atropine	42	-5.23 ± 4.11 [-6.51;-3.95]	***0.000***		***0.000***	***0.000***
	0.02% Atropine	38	-9.28 ± 3.26 [-10.35;-8.20]		***0.000***		1.000
	0.03% Atropine	34	-9.32 ± 2.83 [-10.31;-8.33]		***0.000***	1.000	

Note: N: number of subjects; SD: standard deviations; Posthoc: post-hoc analysis between groups; CI: confidence of interval

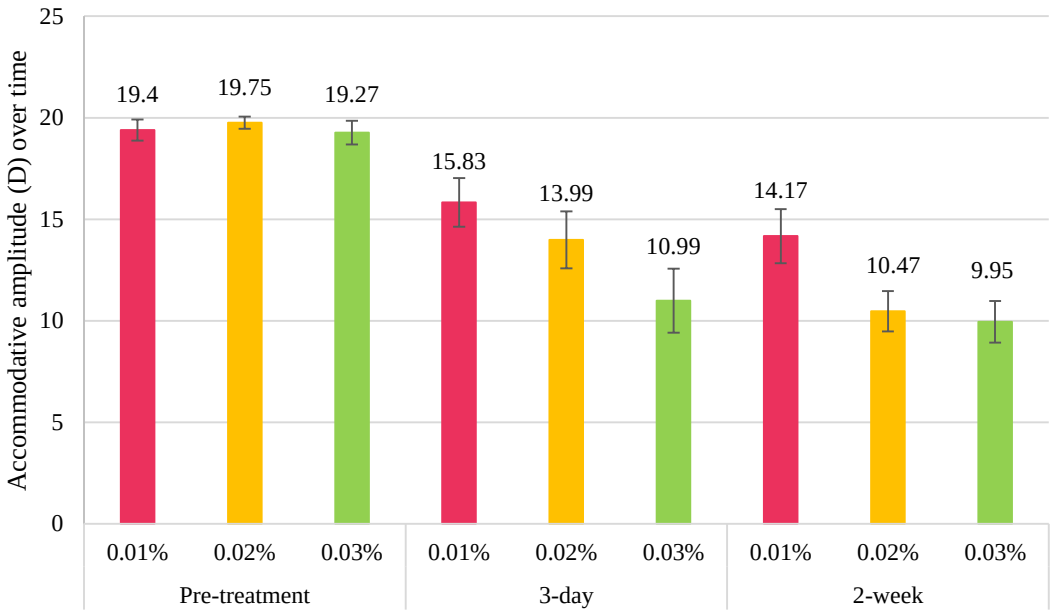

Figure 3.3: Accommodative amplitude at baseline and follow up visits

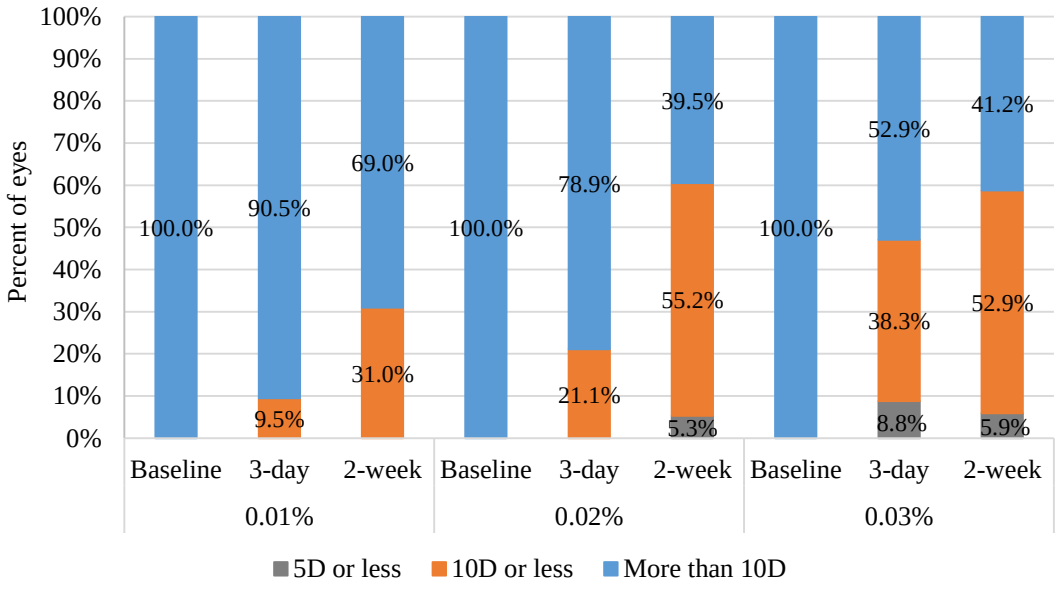

Figure 3.4: Percent of eyes with varying levels of residual accommodative amplitude

3.4.2.2 Pupillary diameter and change over time

Table 3.3, and Figure 3.6 present a) the mean photopic and mesopic pupillary diameter at baseline and 2-week visit and, b) the change in photopic and mesopic pupillary diameter from baseline to

two weeks. At baseline, the mesopic pupil diameter was 5.68 ± 0.73 mm, 6.23 ± 0.81 mm, and 5.75 ± 1.06 mm and, the photopic pupil diameter was 3.91 ± 0.63 mm, 4.30 ± 0.79 mm, and 4.18 ± 0.84 mm for eyes assigned to 0.01%, 0.02% and 0.03% Atropine, respectively. There were no differences between the groups for both the photopic and mesopic pupillary diameter ($p = 0.096$, 0.218, respectively).

At two weeks, mean mesopic and photopic pupil diameter had increased in size across all groups The mean mesopic pupillary diameter was 6.23 ± 0.61 mm, 6.98 ± 0.79 mm, and 7.03 ± 0.51 mm and photopic pupillary diameter was 4.87 ± 1.19 mm, 5.95 ± 1.04 mm and 6.34 ± 0.56 mm for eyes assigned to 0.01%, 0.02% and 0.03% Atropine groups, respectively (Table 3.3).

The difference in the mean photopic pupillary diameter (2-week visit versus baseline visit) was 0.98 ± 1.00 mm, 1.65 ± 0.93 mm, and 2.16 ± 0.88 mm with 0.01%, 0.02% and 0.03% Atropine ($p < 0.001$). Furthermore, a significantly higher number of eyes assigned to 0.03% Atropine had an increase in pupillary diameter of both ≥2mm and ≥3mm or compared to eyes with 0.02% and 0.01% Atropine. The difference between groups was statistically significant ($p = 0.043$ at 3mm of dilation and $p < 0.001$ at 2mm of dilation) (Figure 3.6).

Table 3.3: Changes in mesopic and photopic pupil size over time

Time	Concentration	Pupillary diameter		p-value each time	Posthoc		
		N	Mean ± SD [CI 95%]		0.01% Atropine	0.02% Atropine	0.03% Atropine
Mesopic pupil size (measured in dim light at 30 lux)							
Pre-treatment	0.01% Atropine	42	5.68 ± 0.73 [5.45;5.91]	0.096			
	0.02% Atropine	38	6.23 ± 0.81 [5.97;6.50]				
	0.03% Atropine	34	5.75 ± 1.06 [5.38;6.12]				
2-week	0.01% Atropine	42	6.23 ± 0.61 [6.04;6.42]	***0.000***		***0.001***	***0.001***
	0.02% Atropine	38	6.98 ± 0.79 [6.72;7.24]		***0.001***		1.000
	0.03% Atropine	34	7.04 ± 0.51 [6.86;7.21]		***0.001***	1.000	
2-week vs Pre-treatment	0.01% Atropine	42	0.55 ± 0.73 [0.33;0.78]	***0.000***		0.738	***0.000***
	0.02% Atropine	38	0.75 ± 0.79 [0.49;1.01]		0.738		***0.012***
	0.03% Atropine	34	1.27 ± 0.74 [1.01;1.53]		***0.000***	***0.012***	
Photopic pupil size (measuring bright light at 300 lux)							
Pre-treatment	0.01% Atropine	42	3.91 ± 0.63 [3.72;4.11]	0.218			
	0.02% Atropine	38	4.30 ± 0.79 [4.04;4.56]				
	0.03% Atropine	34	4.18 ± 0.84 [3.89;4.47]				
2-week	0.01% Atropine	42	4.87 ± 1.19 [4.50;5.24]	***0.000***		***0.002***	***0.000***
	0.02% Atropine	38	5.95 ± 1.00 [5.62;6.28]		***0.002***		0.634
	0.03% Atropine	34	6.34 ± 0.56 [6.15;6.54]		***0.000***	0.634	
2-week vs Pre-treatment	0.01% Atropine	42	0.98 ± 1.00 [0.67;1.29]	***0.000***		***0.006***	***0.000***
	0.02% Atropine	38	1.65 ± 0.93 [1.34;1.95]		***0.006***		0.067
	0.03% Atropine	34	2.16 ± 0.88 [1.86;2.47]		***0.000***	0.067	

Note: N: number of subjects; SD: standard deviations; Posthoc: post-hoc analysis between groups

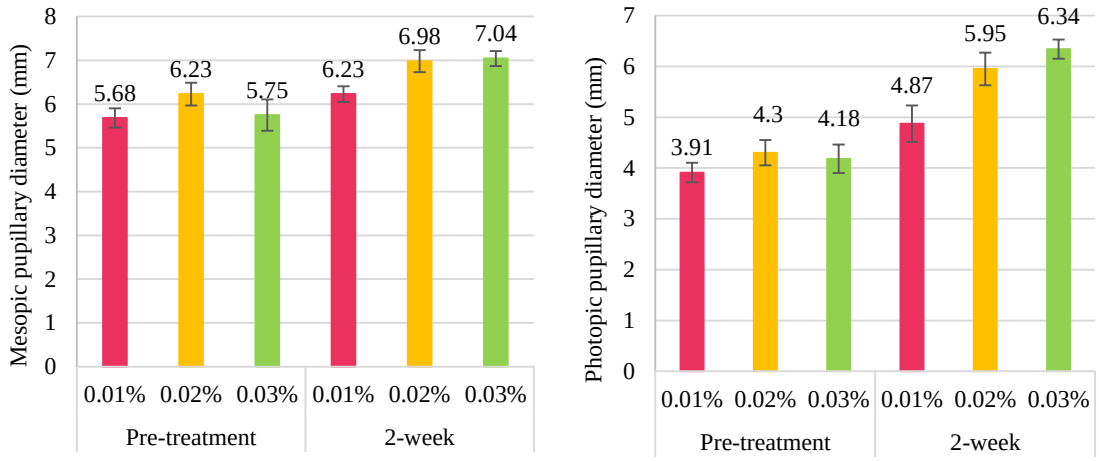

Figure 3.5: Mesopic and photopic pupillary diameter at baseline and 2-week visit.

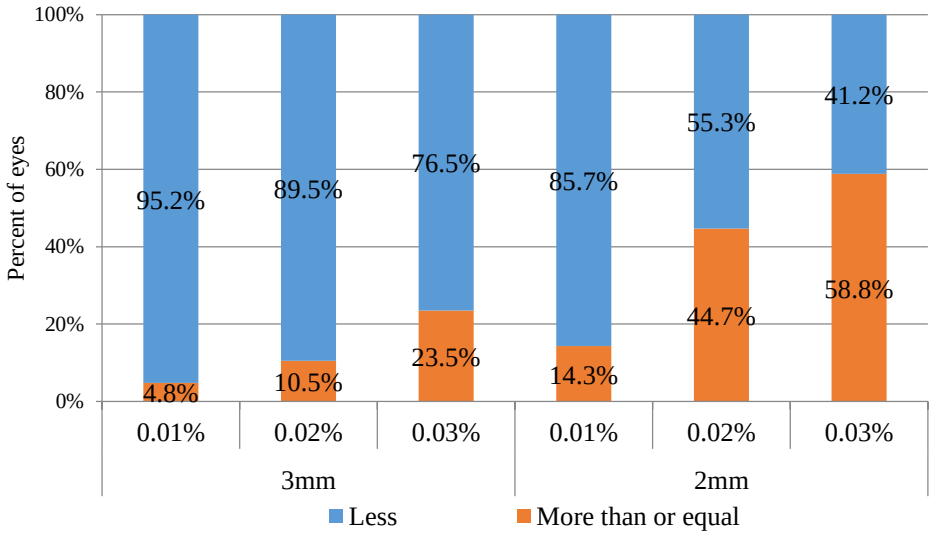

Figure 3.6: Percent of eyes with photopic pupillary diameter increase of ≥2mm and ≥3mm

3.4.2.3 Relation between change in accommodative amplitude and in pupillary diameter

The relation if any between change in accommodative amplitude to change in mesopic and photopic pupillary diameter was explored (Figure 3.7). Overall, the correlation between change in accommodative amplitude to pupillary diameter (either photopic or mesopic) was low to moderate.

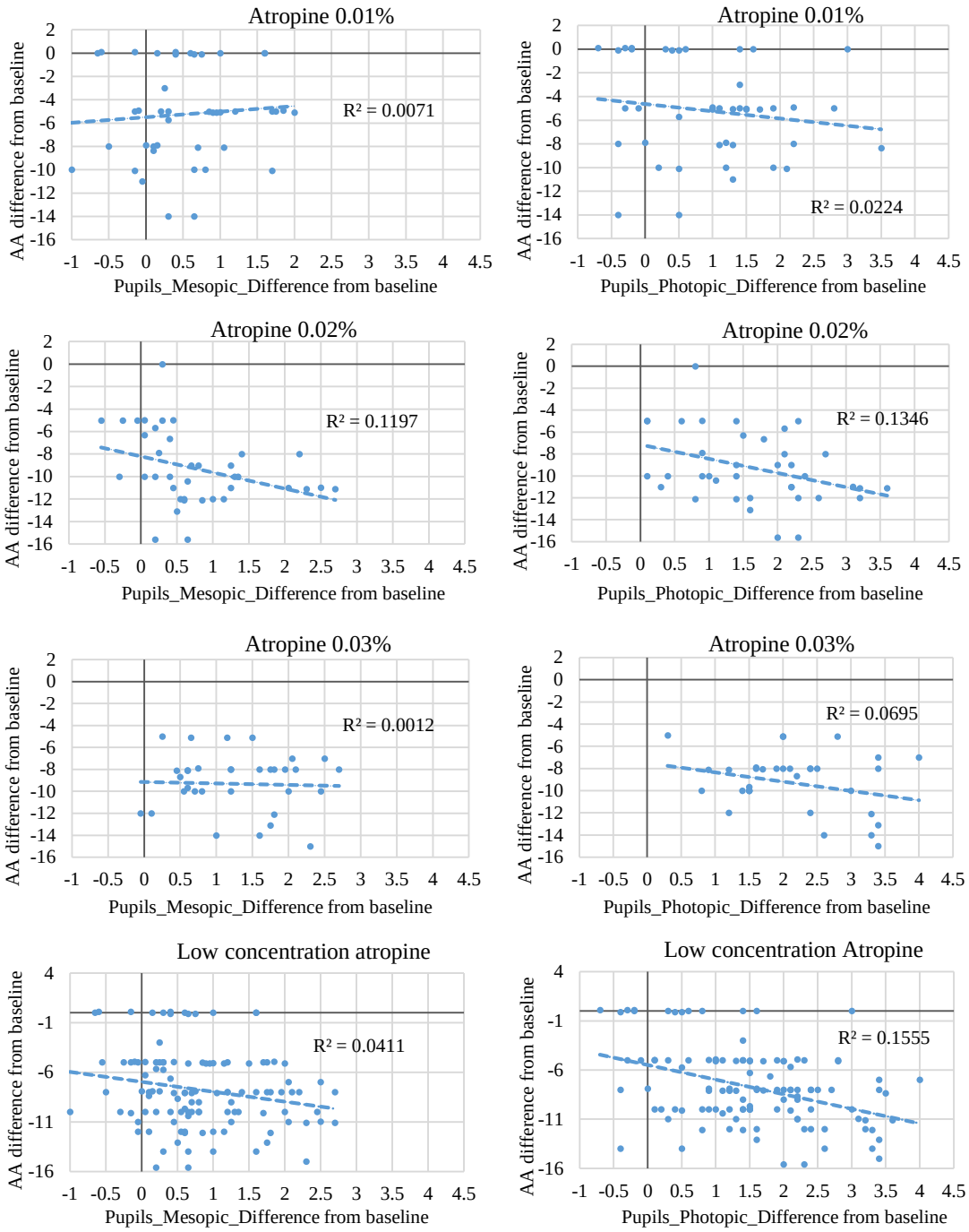

Figure 3.7: Correlation of changes in accommodative amplitude versus photopic and mesopic pupil diameter

Interestingly, with 0.01% Atropine, approximately 29 to 33% of eyes did not demonstrate any change in either pupillary diameter or change in accommodative amplitude (Figure 3.8). In

comparison, only 3 to 18% of eyes did not demonstrate any change in either accommodative amplitude or pupillary diameter with 0.02% Atropine. With 0.03% Atropine, a small percent (5.9% for mesopic and 2.9% for photopic) had no change in pupil size (Figure 3.8).

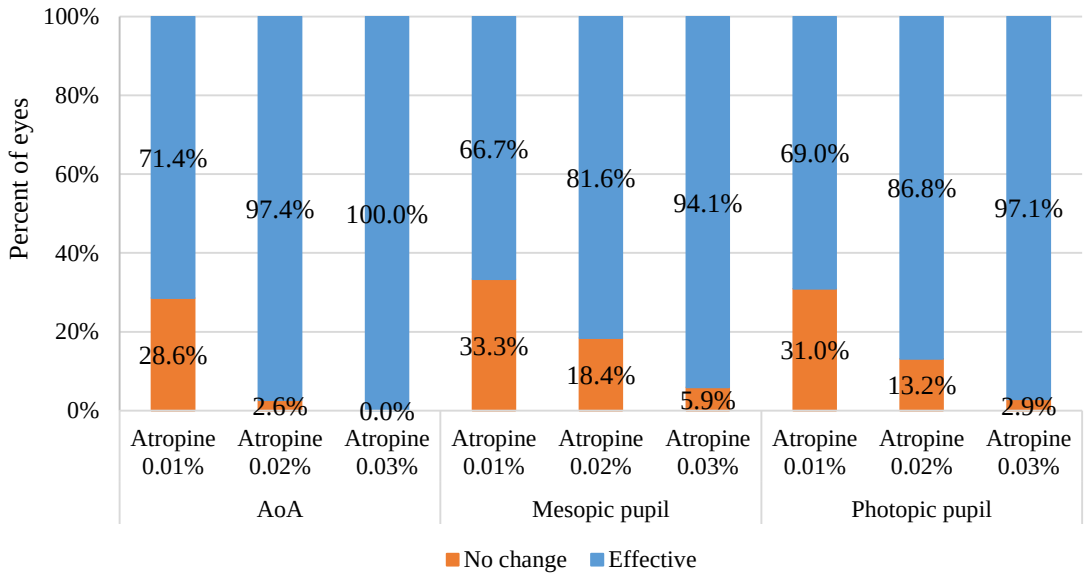

Figure 3.8: Percent of eyes showing no change in amplitude of accommodation, mesopic and photopic pupillary diameter at 2-week visit

3.4.2.4 *Subjective responses with 0.01%, 0.02% and 0.03% Atropine*

After two weeks of Atropine use, whilst only a small percent (<10%) reported blur at distance (board work in classroom), a greater percent reported blur at near (homework: 18.9% to 23% depending on concentration). In most eyes, blur was observed only sometimes (sometimes blurry) except for a single participant each assigned to 0.02% (5.3%) and 0.03% Atropine (5.9%) who reported their near vision to be mostly blurred (Figure 3.9). The blur of participants with 0.02% correlated with objective measurements; residual accommodative amplitude in both eyes was 8.0D, change or reduction in accommodative amplitude from baseline was -12.0D and near visual acuity decreased by 0.06 (logMAR) from baseline. In comparison, the objective measurements and the subjective reports for the participant reporting blur with 0.03% group were at odds; the

residual accommodative amplitude was 15.0D, the change in accommodative amplitude from baseline was only 5.1D in furthermore there was no change of near visual acuity compared to pre-instillation data.

Although a significant percentage of children reported blur while looking at the whiteboard or doing homework, there was no significant difference between groups ($p > 0.05$, Figure 3.9).

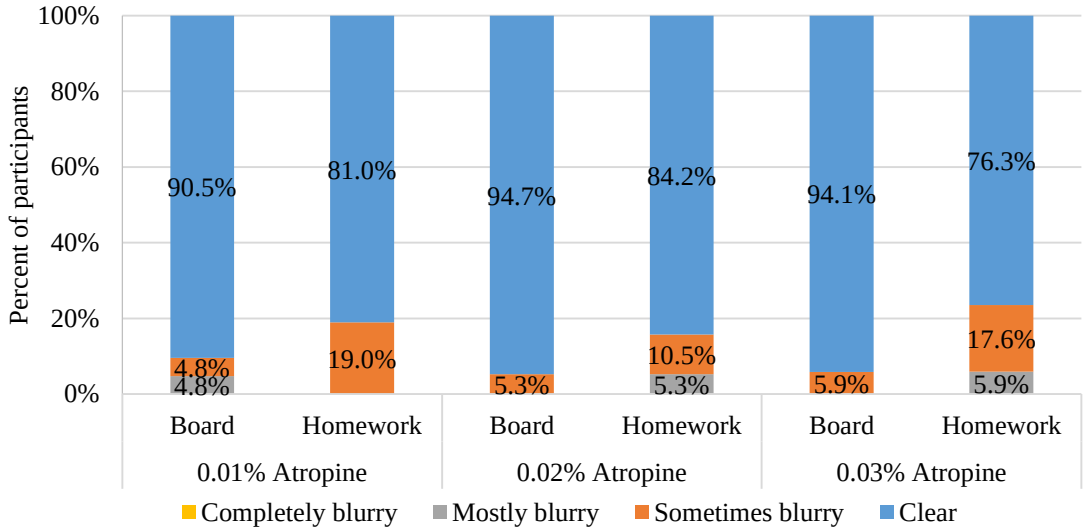

Figure 3.9: Frequency of children reporting blurriness in classroom or during homework

Light sensitivity was reported by >20% of all eyes with 28.6%, 21.1% and 29.4% of 0.01%, 0.02% and 0.03% Atropine users, respectively (Figure 3.10) and was reported to be slight to moderate. Differences between groups were not significant ($p > 0.05$). Comparing between the participants reporting presence or absence of light sensitivity, the mean change in pupillary diameter of the group reporting light sensitivity with 0.01%, 0.02%, and 0.03% was 0.73 ± 0.79 mm, 2.53 ± 0.77 mm, 2.46 ± 0.70 mm, whereas the mean change in pupillary diameter of those with no light sensitivity was 1.08 ± 1.07 mm, 1.41 ± 0.83 mm, 2.04 ± 0.94 mm respectively.

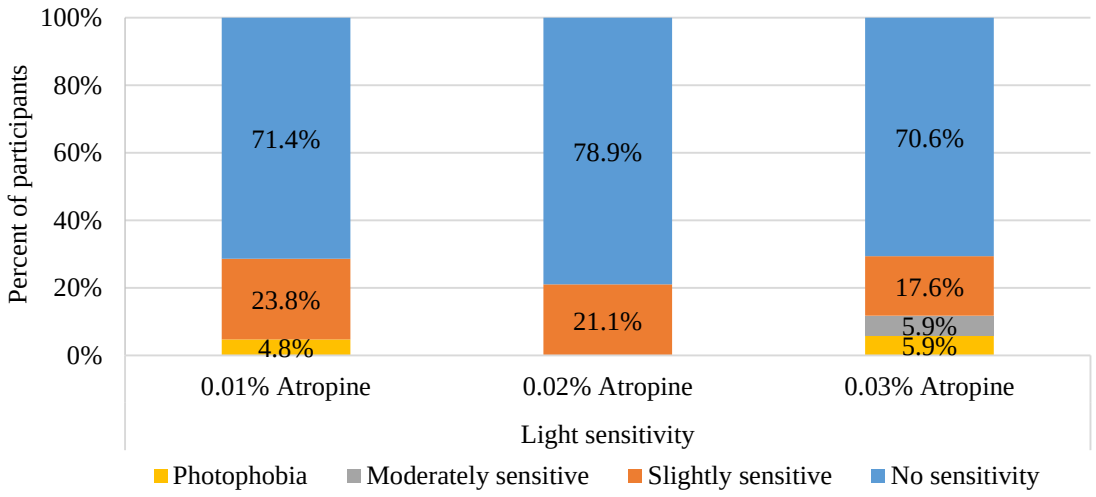

Figure 3.10: Frequency of children reporting light sensitivity with outdoor activities

3.5 Discussion

We aimed to investigate the effect of various low concentrations of Atropine on pupillary diameter, accommodative effort and subjective symptoms. After two weeks of use, all the concentrations assessed, i.e. 0.01%, 0.02% and 0.03%, Atropine resulted in an increase in pupillary diameter and a reduction in accommodative amplitude with a greater decrement in ocular functions observed with use of higher concentrations.

3.5.1 *Change in accommodative amplitude and pupillary diameter*

3.5.1.1 Accommodative amplitude

Our observations indicate a rapid decline in accommodative amplitude with use of Atropine; at 3 days, the decrease was approximately -3.57D, -5.76D and -8.28D with 0.01%, 0.02% and 0.03% Atropine and at two weeks it had further decreased to -5.23D, -9.28D and -9.32D. These results are similar to those reported previously with an initial and rapid decline in accommodative response soon after instillation of Atropine (Chia et al. 2012; Kaymak et al. 2018). Although there was some decrease in accommodative amplitude after 3 days, much of the decrease occurred from baseline from 3 days, thereafter, the change from 3 days to 2 weeks was minimal. Interestingly,

with 0.03% Atropine, there was less than 1D difference between 3-day and 2-week visits whereas with 0.01% and 0.02% Atropine, there was a further decrease of about -1.5D and -3.5D between 3-day and 2-week visits. This suggests that accommodative paralysis is achieved much faster and earlier with higher concentrations.

Our meta-analysis (Figure 2.3, Chapter 2) indicated that at concentrations <0.10% Atropine, the mean change in accommodative amplitude was -1.6D (95% CI, -3.9 to 0.7) whereas with ≥0.1%, the reduction in accommodative amplitude was -10.7D (95%CI, 12.2 to 9.2D). In comparison, the decrease in accommodative amplitude of 5.23D with 0.01% Atropine in this study was much higher. Whilst it was similar to the reduction of 4.90 D reported by Chia et al during 0.01% Atropine use over two weeks (Chia et al. 2012) and of 6.8D reported by Cooper et al with 0.012% (Cooper et al. 2013), it was much higher than that of the reduction reported by Yam et al. (Yam et al. 2019) and Fu et al. (Fu et al. 2020). Similarly, a greater reduction in accommodative amplitude was observed with higher concentrations; the reduction at -9.28D and -9.32D with 0.02% and 0.03% Atropine respectively was slightly less to the reduction in accommodative response observed by Cooper et al. with 0.025% and 0.05% Atropine at between 3.7D and 8.5D (Cooper et al. 2013) but was significantly less than that reported by Yam et al and Fu et al (Yam et al. 2019; Fu et al. 2020). The reasons for this discrepancy with Yam et al and Fu et al (Yam et al. 2019; Fu et al. 2020) is unclear and may be related to the methodology adopted between studies (monocular versus binocular measurement, subjective nature of assessment from our conventional push-up method, bias from investigators etc). Previous reports have indicated the lack of agreement between measurements of accommodation collected using different methods on using the same method but in different populations. (Sterner et al. 2004; Ovenseri-Ogbomo et al. 2012; Abu et al. 2018). For example, although both Chia et al. (Chia et al. 2012) and Yam et al. (Yam et al. 2019) reported using a similar protocol for measurements of amplitude of accommodation, the difference of the mean change in accommodative amplitude between two 0.01% Atropine groups was -3.76D. The difference in results of accommodative measurements

varied up to 4.6D between different methods (Abu et al. 2018). However, it is possible that we have seen the maximal or peak effect of 0.01% Atropine at 2 weeks with no further effect on accommodative amplitude even if Atropine continued to be instilled daily beyond 2 weeks. Interestingly, with 0.01% Atropine, nearly 29% (or approximately 3 of 10 eyes) had no demonstratable change in accommodative amplitude; although the current mechanisms of action for Atropine mostly indicate non-accommodative pathways, if a reduction in accommodative amplitude and efficacy were to be related, then this data indicates that 0.01% Atropine may not be effective across 1/3rd of the population.

At the end of 2 weeks, approximately 31%, 60.5% and 58.8% of 0.01%, 0.02% and 0.03% Atropine eyes had <10D of accommodative amplitude and a small percent of eyes with 0.03% had <5D of residual accommodative amplitude. These data suggest that approximately one-third to a half of users of Atropine would have issues with very close near work. However, in spite of a large number of eyes having a significant drop in accommodative amplitude, not many children reported near vision problems. This data is similar to results from other studies (Fang et al. 2010; Yam et al. 2019; Fu et al. 2020). The subjective responses are discussed in detail further below but may indicate that that the residual accommodative amplitude was sufficient for near work; furthermore, the accommodative amplitude was measured monocularly in the current study. It is possible that binocularly, there would have been an improvement in near functions and may have accounted for the lack of near vision problems with the subjective questionnaire.

3.5.1.2 Pupillary diameter

With respect to pupillary diameter, it was anticipated that with Atropine use, a greater change would be evident with photopic than mesopic pupillary diameter; the mesopic pupillary diameter was already substantially large at baseline and thus there would be minimal to little observable change with Atropine. Indeed, at 2 weeks, the mean increase in the photopic pupillary diameter was 0.95 ± 1.05 mm, 1.65 ± 0.93 mm and 2.16 ± 0.88 mm and the increase in mesopic pupillary

diameter e was 0.55 ± 0.73 mm, 0.75 ± 0.79 mm, and 1.27 ± 0.74 mm with 0.01%, 0.02% and 0.03% Atropine groups, respectively. The increase in the pupillary diameter demonstrated a dose-dependency which is supported by other evidence. However, as with change in accommodative amplitude, a slightly greater change in pupillary diameter was observed in the current study compared to the results of the meta-analysis (Figure 2.4) where at concentrations of <0.10% Atropine, the mean change in pupillary diameter was 0.7mm (95% CI, 0.1 to 1.4) whereas for ≥0.1% Atropine concentrations it increased to 3.2 mm (95% CI, 2.8 to 3.5) respectively. Whilst they were also higher than those reported by Yam et al. (Yam et al. 2019) and Fu et al. (Fu et al. 2020), they are comparable to those reported by Chia et al. 2012 (Chia et al. 2012). We used a standardized procedure with fixed lighting conditions for our measurements; it is not clear that the previous studies had adopted such measurements. Furthermore, if the measurements for the studies by Fu et al and Yam et al were taken at ambient room illumination, then it is possible that there would be less change (similar to the results observed with mesopic condition in the current study).

In this group of children with darker pigmented irides, the baseline photopic pupil diameter ranged from about 3.9 to 4.3mm and was about 5.7 to 6.2mm in dim room illumination. It is expected that the baseline pupillary diameters may be larger in those with less pigmented irides; indeed in European eyes, mean photopic pupil diameter at baseline ranged between 5.51 to 5.81mm (Loughman and Flitcroft 2016).

The relation between an increased pupillary diameter and symptoms of photophobia is well known; although it is well known that large pupils are associated with photophobia, it is not clear that there is a threshold/cut-off criteria for a pupillary diameter that correlates with photophobia. The pupil diameter at which photophobia occurs may vary from individual to individual and may be related to other factors such as clarity of optical media, environmental exposure (bright sunlight versus indoors) etc. In the current trial, the mean photopic pupil diameter at 2-week visit with 0.03% Atropine was larger than the mean mesopic pupillary diameter at baseline ($p < 0.001$); this

indicates that the mean pupillary diameter had exceeded the threshold at which the eye operates in dim and bright light and therefore the eye is at great risk of photophobia and other effects related to increased light exposure. Approximately 24% of eyes on 0.03% Atropine had an increase in the photopic pupil diameter of ≥3mm compared to 11% and 5% of eyes on 0.02% and 0.01% Atropine respectively. In this study, we found that 21% to 29% of the eyes reported light sensitivity and is likely related to the increase in pupil diameter; although a greater change in pupillary diameter was observed with 0.03% Atropine, the increase in reported light sensitivity with 0.03% versus 0.01% was not significant indicating that other factors may be at play; these may include individual variations in sensitivity, light exposure, age, and other factors.

3.5.2 *Relation between change in accommodative amplitude to change in pupil diameter and subjective responses*

Difficulties with near-work and photophobia remain some of the major side effects associated with Atropine and limiting its adoption and use to manage myopia progression (Tran et al. 2018). Atropine is a non-selective muscarinic antagonist, and in the eye, the ciliary body and iris express all five (M1-M5) receptor subtypes (Gupta et al. 1994; Zhang et al. 1995; Gil et al. 1997). Our results show a concentration-dependent decrease in accommodative amplitude and pupillary response with the use of Atropine. The effects of Atropine on pupillary size and accommodation are well characterised and found to be mediated by the non-selectively blocking of the muscarinic receptors at the iris sphincter and ciliary body (McDougal and Gamlin 2015; McLendon and Preuss 2020). As both the mechanisms underlying regulation of pupillary diameter and accommodative amplitude share the same parasympathetic pathway (Miller et al. 2005), it is reasonable to hypothesise that with use of Atropine, there would be a correlation between the change in pupillary diameter to the decrease in accommodative amplitude. However, our data indicate that although higher doses are associated with a greater change in pupillary diameter and accommodative amplitude, the relation between the two parameters is low. It indicates that for a

given pupil diameter there is a range of accommodative responses and vice versa. The reasons are not well understood and beyond the scope of this thesis.

An increase in the pupillary diameter increases aberrations of the eyes that may result in a decrement in vision performance; the reduction in vision performance is likely more noticeable at near as normally near tasks are associated with pupillary constriction (Miller et al. 2005). If accommodative amplitude with Atropine falls to a certain level and remains stable, any further variations or increases in the pupillary diameter may affect the depth of field and thus visual performance.

Any blurred vision, both distance and near, reported by participants with Atropine may have resulted either from an increased pupil size or due to a reduction in accommodative response. Although one participant each from the groups using concentrations of 0.02% (1/19, 5.3%) and 0.03% (1/17, 5.9%) reported blurred vision during near activities, the majority of children completed the trial without impact on their daily activities. As seen in Figure 3.9 and Figure 3.10, a slightly greater percentage of participants in the group of 0.03% Atropine compared to reported "blurriness" for near tasks and higher light sensitivity while doing outdoor activities compared to other groups. In comparison, it was reported that use of 0.05% Atropine resulted in 9.5% of participants reporting side effects such as blurred near vision (Lee et al. 2006). The mean near visual acuity at 40 centimetres with best-corrected spectacles ranged between 0.11 and 0.14 LogMAR in all three groups (equivalent to 20/30 to 20/25) and indicated that children were able to accommodate to see or carry out tasks at near distances.

Interestingly, we found that 0.01% atropine had no change in either the accommodative amplitude or the mesopic and photopic pupillary diameter in approximately 28% to 33% of the eyes (Figure 2.8). If this is indicative of atropine having no effect on the eye, then this aligns with the reported that nearly 30% of participants on 0.01% atropine in the LAMP trial had a progression of >1.0D/year (Chia et al. 2012; Yam et al. 2019).. It has been said that the mean progression of SE of an 8-year old children was about 0.75-1.0 D/year (Sankaridurg and Holden 2014). In

comparison, less than 20% of eyes on 0.02% atropine and less than 6% of eyes on 0.03% atropine had no effect on accommodative amplitude/pupillary diameter.

3.5.3 *Strengths and limitations of the study*

One of the key strengths of the study was the randomized nature of the study with myopic children enrolled in the study. The study groups were not different at baseline which ensured that differences between groups could be attributed to the assigned treatment. Furthermore, the duration of the trial, i.e., 2 weeks, ensured that the effects of Atropine could be well ascertained. However, the trial suffered from some limitations. More frequent visits especially during the initial few days could have determined the time-to-effect response more closely. However, the nature of the trial involving children meant that multiple visits were not suitable for this population. Furthermore, accommodative amplitude was measured at three days and two weeks using a push-up method for determining amplitude of accommodation. The push-up method for determining accommodative amplitude is considered to result in a higher mean accommodative compared to minus lens method. It is considered that the push-up method may overestimate the accommodative ability of the individual due to the increased angular sub-tense and therefore perceived magnification resulting from an approaching target (Burns et al. 2014). In addition, with the moving target, the delay from the time the individual calls out "blur" to the time the observer records the distance may also result in a higher accommodative response. Additionally, we used a compounding agency to provide the Atropine and were unable to conduct quality assessments to determine the final concentration of the Atropine that was received. All Atropine was received in unit dose vials (opaque plastic vials) and was stored per the manufacturing instructions out of light and in a fridge (4 degrees Celsius), however we could not ascertain if these conditions were met when children were provided with the product. Further, compliance could not be ascertained beyond asking the parents. Additionally, subjective responses were

gathered with a questionnaire, and it is well known that these questionnaires are subject to various biases such as recall bias, order bias and a courteous bias.

3.5.4 *Summary*

All the concentrations investigated in the study, i.e. 0.01%, 0.02% and 0.03% Atropine had an effect on pupillary and accommodative mechanisms. A greater reduction of accommodative amplitude and an increase in pupillary diameter was observed with 0.03% and 0.02%. Use of 0.03% atropine resulted in 23% of eyes having an increase in photopic pupil diameter at ≥3mm as compared to 10.5% and 4.8% with 0.02% and 0.01% Atropine. Similarly, a large number of eyes on 0.03% atropine had accommodative amplitude of ≤10D (58.8% vs 47.1% vs 0% with 0.03%, 0.02% and 0.01% Atropine). On the other hand, use of 0.01% atropine did not result in any change in either accommodative amplitude or pupillary diameter in 29% to 33% of eyes. Based on these results, 0.02% atropine appears to be the concentration that has an effect on a large number of eyes without a substantial change in accommodative amplitude/pupillary diameter.

Interim summary:

In myopic children aged 6 to 12 years, use of 0.01%, 0.02%, and 0.03% Atropine for two weeks resulted in an increase in pupillary diameter and reduction of accommodative amplitude in a dose-dependent manner. Reduction in accommodative amplitude was 5.23 ± 4.11 D, 9.28 ± 3.26 D and 9.32 ± 2.83 D and the increase in photopic pupillary diameter was 0.95 ± 1.05 mm, 1.65 ± 0.93 mm and. 2.16 ± 0.88 mm with 0.01%, 0.02% and 0.03% Atropine respectively. Use of 0.03% Atropine resulted in a significant number of eyes having a photopic pupillary diameter increase of >3mm (23.5% vs 10.5% vs 4.8% with 0.03%, 0.02% and 0.01% Atropine) and residual accommodative amplitude of ≤10D (58.8% vs 47.1% vs 0%). On the contrary,

nearly 29% to 33% of eyes had no change of accommodative amplitude or pupillary diameter with 0.01% Atropine.

Chapter 4. Myopia control with novel eye drops

4.1 Introduction

As discussed in Chapter 1, topical Caffeine, 1,3,7-Trimethylxanthine, an adenosine receptor agent, was found to increase choroidal thickness in primates (Arumugam et al. 2017). In previously published trials, oral use of 7-methylxanthine, a natural metabolite of Caffeine, was reported to be effective in slowing axial length in human clinical trials (Trier et al. 2008), and was also demonstrated to exert myopia control effects on sclera in animal models (Trier et al. 1999; Cui et al. 2011; Nie et al. 2012; Hung et al. 2018). Furthermore, Caffeine, as a topical composition, has been previously investigated as eye drops in other studies and proven its permeability into anterior chamber tissues, e.g., chamber and anterior lens capsules (Chandra et al. 2011; Bardak et al. 2016; Varma 2016; Smith III et al. 2021).

Evidence in section 1.5.3 supported that a combination therapy appears to provide a logical approach either reducing side-effects and/or improving efficacy compared to monotherapy alone. Therefore, we aimed to determine if a combination of Atropine and Caffeine may be used to slow the progression of myopia and determine if there were any benefits to the combination therapy over monotherapy. In the previous chapter, we reported the effect of low concentration Atropine (0.01%, 0.02% and 0.03%) over a two-week period on pupillary diameter, accommodative amplitude, and subjective responses. The photopic pupil diameter increased by ≥3mm in 4.8%, 10.5% and 23.5% and accommodative amplitude decreased by an average of 5.23D, 9.28D and 9.32D with 0.01%, 0.02% and 0.03% Atropine respectively. Since the efficacy of 0.01% Atropine in controlling eye growth was found to be negligible, we chose the next higher concentration that was likely to induce minimal side effects, i.e., 0.02% Atropine to be used in the combination therapy. With respect to Caffeine, we chose the highest or maximum soluble concentration of Caffeine in H_2O which was 2%. (Yalkowsky H.S. 2010). In a cross over clinical study conducted at Brien Holden Vision Institute Limited, Sydney, Australia, 22 healthy adult participants used

topical Atropine 0.02%, Caffeine 1.4%, Caffeine 2.0% and 0.02 % Atropine plus Caffeine 1.4% (ANZCTR registration no 12618000196246, BHVI data on file). No adverse events such as ocular redness, staining etc. were observed with the use of eye drops. To determine if the combination therapy of Atropine and Caffeine has any benefits over monotherapy, we instituted a long-term randomized, dispensing study in children with myopia. Although the trial is of two-year duration, with a minimum of one year in treatment mode, only results up to six months in the trial are presented as the progress in the trial is beyond the timelines of this thesis. In previously published trials, differences between test and control strategies were apparent at 6 months (Shih et al. 1999; Polling et al. 2016; Joachimsen et al. 2019; Lam et al. 2020) and therefore, we believe that the first six months will determine the trend for the duration of the trial.

In the dispensing trial, we randomized children with myopia to 0.02% Atropine, 0.02% Atropine with 2% Caffeine and 2% Caffeine. We also instituted a parallel, matched group study of children wearing single vision spectacles at the same time. Although it would have been ideal to randomize children into a group wearing single vision spectacles in the main study, we anticipated a high dropout rate in children randomized to single vision spectacles as they may have preferred the treatment and therefore instituted a parallel group.

4.2 Aims

- To determine the rate of progression of myopia as determined by change in spherical equivalent and axial length over a six-month period with 0.02% Atropine, 2% Caffeine, and 0.02% Atropine combined with 2% Caffeine in a prospective, randomised clinical trial.
- To determine the rate of progression of myopia as determined by change in spherical equivalent and axial length over a six-month period with 0.02% Atropine, 2% Caffeine, and 0.02% Atropine combined with 2% Caffeine compared to a non-randomised, matched group of children using single vision spectacles.

4.3 Methods

In a prospective, dispensing trial conducted at An-Sinh Hospital and Hai Yen Eye Center in Ho Chi Minh City, Vietnam, children aged six to 13 years with myopia ranging from -0.50D to -6.00D were enrolled and randomized at an allocation ratio of 1:1:1 to 3 groups to either 0.02% Atropine, 2% Caffeine, or 0.02% Atropine with 2% Caffeine to be administered daily. Additionally, a matched, non-randomized control group of children with myopia were recruited with similar inclusion and exclusion criteria as in the randomized group. The study protocol was approved by the Institutional Research Ethics Committee of An-Sinh Hospital (No CS/AS/18/12), Institutional Research Ethics Committee of Ministry of Health of Vietnam (No 84/CN-HDDD), Human Research Ethics Committee of University of New South Wales (No HC200725), was registered at ClinicalTrials.gov (NCT04301323) and adhered to the Declaration of Helsinki for experimentation on human subjects. The trial is intended to run for a duration of two years and results for up to six months are presented in this thesis.

4.3.1 *Sample size determination*

Prior studies indicated that the annual progression of refractive error in Asian children using single vision spectacles was -0.70 ± 0.45D (Donovan et al. 2012). Therefore, a minimum of 35 successfully enrolled subjects were required in each study group to demonstrate a statistically significant 50% difference in annual myopia progression (0.35 ± 0.45D) between test and control groups at the 5% level of significance with 80% power, using a 2-tailed distribution and assuming 20% dropouts. The detectable difference of 0.35±0.45 equates to an effect size of 0.78. G*power was used to compute the sample (Bartlett 2019). It was estimated that a minimum of 60 participants was to be enrolled in the control group wearing single vision spectacles. The lack of randomization to the control group could result in differences in baseline demographics and consequently a higher combined variance of the outcome variable. Furthermore, a higher drop-

out may occur in the control group. This sample size for controls would ensure that a minimum power of 80% was maintained even if the combined standard deviation resulted in a 20% increase (0.45 to 0.55D) due to lack of randomization and accounts for a higher dropout rate (25%).

4.3.2 *Participant selection and enrolment*

All participants were screened for general suitability using the inclusion/exclusion criteria as detailed below.

Participants were enrolled in the trial if they:

- were accompanied by a parent or guardian who was able to read and comprehend Vietnamese/English and gave informed consent as demonstrated by signing a record of informed consent.
- at baseline, were within the age range of 6 to 13 years old inclusive.
- were diagnosed as myopic with spherical equivalent refractive error between -0.50 and-6.00 D.
- were willing to comply with instilling the eye drops once nightly at bedtime and follow the clinical trial visit schedule as directed by the Investigator.
- had ocular findings deemed to be normal; and
- had vision correctable to at least 20/25 or better in each eye with spectacles.

Additionally, they were excluded from the trial if they

- had any pre-existing ocular irritation, allergic conjunctivitis, injury, or condition, including infection or disease.
- had any systemic disease that would have had an adverse effect on ocular health.
- used or had a need for concurrent category S3 and above ocular medication at enrolment and/or during the clinical trial.

- used or had a need for any systemic medication or topical medications that may have affected a participant's ocular health/physiology.

NB: Systemic antihistamines were allowed on an "as needed basis", provided they are not used prophylactically during the trial and at least 24 hours before the clinical trial product is used.

- had history of eye trauma
- had history of use of myopia control interventions such as Orthokeratology or eye surgery.
- had contraindications to Atropine and Caffeine such as pulmonary disease, heart conditions and ADHD
- had known allergy or intolerance to ingredients to Atropine eye drops, Xanthines and other derivatives of anti-muscarinic receptor agents.
- were currently enrolled in another clinical trial.

A participant was considered "successfully enrolled" when the Investigator agreed that they conformed to the inclusion/exclusion criteria and signed the Informed Consent Form. All care was provided free of charge to participants. Also, participants were provided with lenses and frames.

4.3.3 *Masking procedure and maintenance*

On enrolment, eligible children and parents were allowed to decide on either the intervention (treatment) or control group. If they chose the intervention group, they were randomised to one of three groups (0.02% Atropine, 2% Caffeine or 0.02% Atropine plus 2% Caffeine, CustomCare Compounding Pharmacy, Dural, NSW, Australia respectively) at the allocation ratio 1:1:1.

In the intervention groups, investigators, participants and their carers remained masked to the identity of the drops dispensed throughout the study.

4.3.4 *Clinical trial randomisation*

The randomisation plan was generated from http://www.randomization.com/. The plan was used to randomise each study participant from the intervention group into one of 3 test groups using the method of randomly permuted blocks. Randomization plan was for a minimum sample of 105 participants and used 7 blocks with 15 subjects per block. The randomization plan was applied through the Clinic Data Management system installed by the Brien Holden Vision Institute at the Hai Yen Eye Care centre for the purpose of the trial. In addition to the principal investigator, the study was supported by a clinical coordinator and clinicians who were trained and aided in study examinations when needed.

The randomisation code was applied at the baseline visit and clearly documented. The Investigator (Huy Tran) was not allowed to access this randomisation code until six months in the trial were completed and data analysis was performed. The trial responsibilities were then passed over to a practicing ophthalmologist at the site.

4.3.5 *Product information*

4.3.5.1 Product description

0.02% Atropine, 2% Caffeine, 0.02% Atropine with 2% Caffeine in sterile water were manufactured by CustomCare Compounding Pharmacy, Sydney, Australia in preservative-free single-use and disposable vials.

4.3.5.2 Eye-drop instillation and disposal

In the intervention groups, each participant was instructed to use one drop of the allocated eye-drop once nightly each day. A new unit dose was to be used for each instillation and the unit dose applicator was discarded safely after use. Participants were provided with 30-unit doses and resupplied towards the end of the 30-day schedule. All unit dose eye drops were required to be refrigerated and had to be applied once nightly at bedtime in both eyes. Children were also

provided with an up-to-date single vision spectacles for use during the day. Children and their parents or carers were encouraged to report any health issues or side effects related to the usage of eye drops over the trial period.

At the clinic, the study product was kept refrigerated in storage facility with access limited to study personnel. Participants were instructed to return any unused eye drops at the end of each visit that were then disposed safely.

For the control group assigned to wear single vision spectacles, the participants were not prescribed any drops or solutions but were provided with single vision spectacles fitted in a frame of the participant's choosing. Participants in the single vision group had similar follow-up visits and examination process as the intervention groups.

4.3.6 *Visits and measurements*

Prior to the initial assessment, participants were given the opportunity to read the Participant Information/Informed Consent Form and to choose either the intervention or the control group. For the intervention groups, visits in the trial included the baseline visit, 2-week (quick health check only) and three-monthly visits in the first year and 6 monthly visits in the second year. For the control group, the study consisted of an initial baseline visit followed by six monthly visits (Figure 4.1).

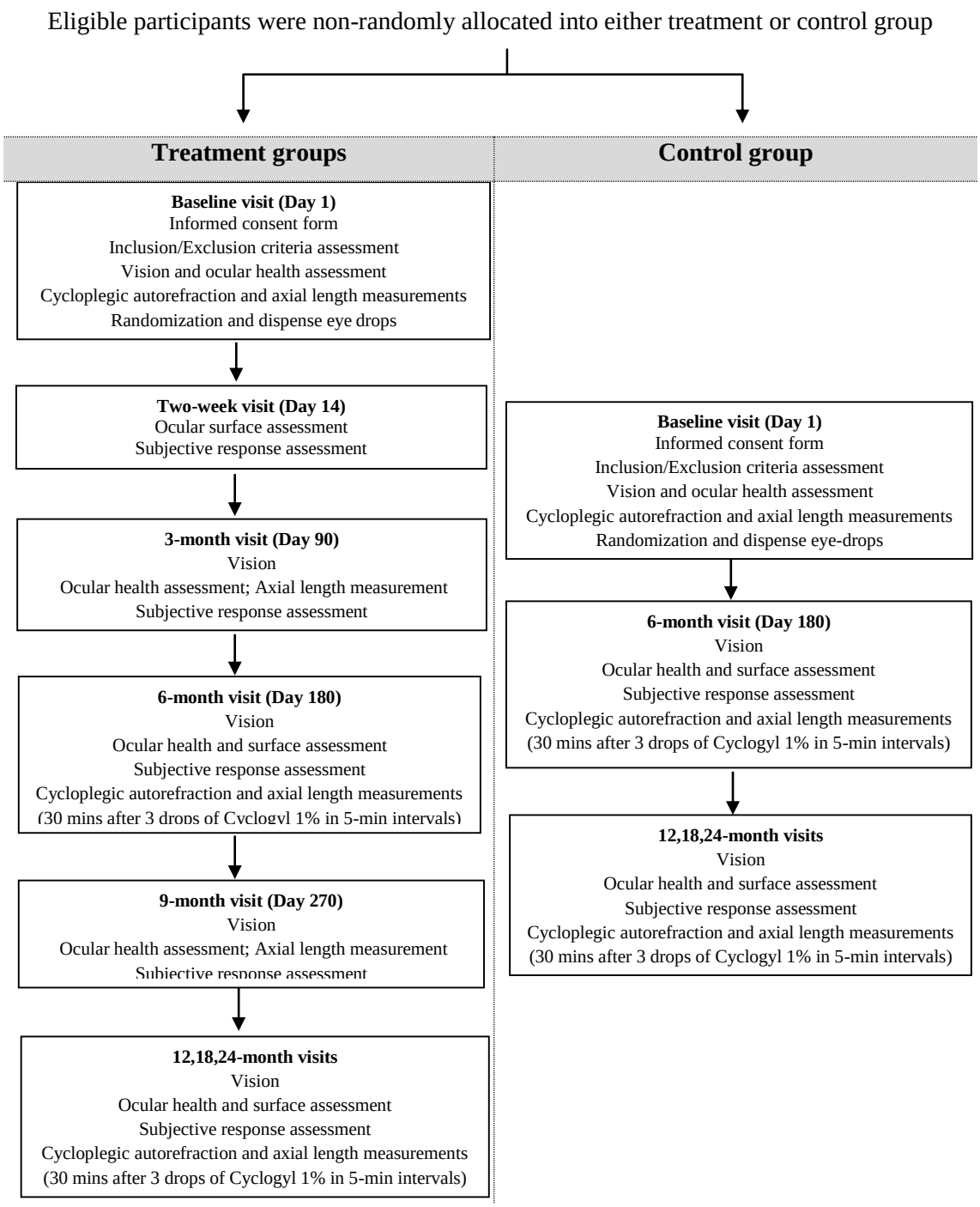

Figure 4.1: Flow-chart of study visits

At baseline, assessments were conducted pre- and post-cycloplegia. Visual acuity was measured at both distance and near. Distance visual acuity was assessed using Snellen chart at 6 meters. Near visual acuity was measured using the ETDRS (Early Treatment of Diabetic Retinopathy

Study) chart at 40 centimetres. Data related to the distance and near visual acuity were recorded on a logMAR scale. Following visual acuity measurement, pupil size was measured using Oculus Park 1 (OCULUS Optikgerate GmBH, Wetzlar, Germany). Photopic conditions were defined as the use of direct light maintained at (300 lux) at the eye level and mesopic conditions were ambient room light (30 lux). Pupil diameter, under both photopic and mesopic conditions, amplitude of accommodation and intraocular pressure were measured three times per participants (Figure 4.2). Pupil area, the circular opening of the pupil, were interpreted by using formula of (pupil diameter/2)^2 * 3.14.

Figure 4.2: Set-up of Oculus Park 1

A Royal Near Point Ruler was used to measure the monocular accommodative amplitude with the best-corrected distance spectacle correction in place (Figure 4.3). Briefly, a push-up method with a minus -4.0D lens was used wherein the child was instructed to focus on the smallest line on the target drum, equivalent to N5 (N-scale) print. The assessor then slowly moved the drum towards the testing eye until the child reported the blur of that line.

Figure 4.3: Royal Air Force Ruler for accommodative amplitude measurements

Intraocular pressure was measured using Tonopen (Reichert Technologies, Reichert Inc., Depew, USA) at each visit. Slit-lamp examination was also performed by certified clinicians. Thereafter, eyes were cyclopleged using three drops of 1% cyclopentolate (Cyclogyl, Alcon-Convreur, Rijksweg, Belgium) instilled three times at five-minute intervals. After approximately thirty minutes, eyes were checked for dilation using a pen torch (size and response of pupil assessed) and thereafter, axial length was measured with Lenstar 900 (Haag-Streit, Switzerland) (Figure 4.4) and refractive error of the eye was measured using open-field auto-refractometer NKVision 5001 (Shin-Nippon by Rexxam, Japan) (Figure 4.5).

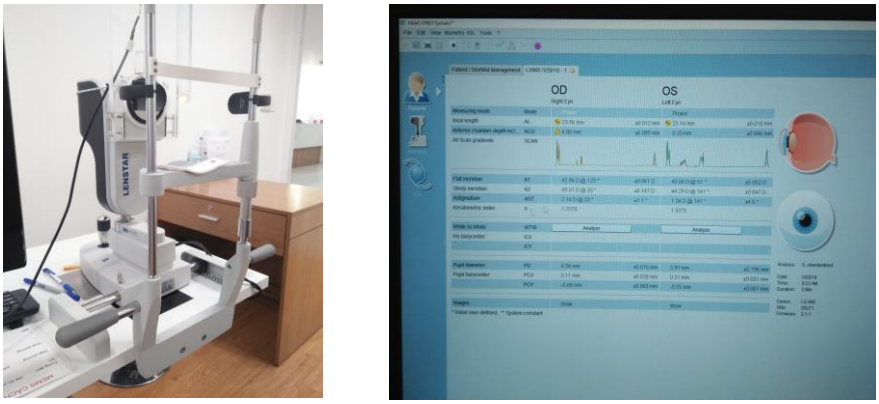

Figure 4.4: Axial length measurements with Lenstar 900

Figure 4.5: Open-field auto-refractor with NKVision 5001

Follow-up visits were conducted as detailed in the flow diagram and assessments conducted at each visit are presented in Table 4.1 below.

Table 4.1: Assessment of variables and window period for each visit

Procedures / Data	Baseline visit	3-month follow-up visit (on Day 90)	6-month follow-up visit (on Day 180)
Visit window	N/A	10 days	10 days + with cycloplegia
Informed consent	✓	-	-
Meet inclusion / exclusion criteria	✓	-	-
History	✓	✓	✓
Visual acuity (VA) at distance and near (habitual and BCVA)	✓	✓	✓
Accommodation	✓	✓	✓
Pupil diameter	✓	✓	✓
Distance and Near Phoria	✓	✓	✓
Slit-lamp biomicroscopy - Ocular assessment	✓	✓	✓
Non cycloplegic subjective refraction	✓	✓*	✓
Cycloplegia	✓	-	✓
Cycloplegic autorefraction	✓	*	✓
Axial length measurement	✓	✓	✓
Cycloplegic subjective refraction	✓	-	✓
Intraocular pressures	✓	✓	✓
Questionnaire	✓	✓	✓
Compliance	-	✓	✓
All the participants in the control group will be collected data similar to baseline measurements before and after cycloplegia for every six months over two years			
Note: "✓" required information; "-" not required; "*" at investigator's discretion			

4.3.7 *Statistical analysis*

Demographic and ocular characteristics including age, baseline spherical equivalent, baseline axial length, intraocular pressure, best-corrected visual acuity, near visual acuity using single vision spectacles, photopic and mesopic pupillary diameter, and accommodative amplitude were summarised as means ± standard deviations for continuous measurements. Data from both eyes were considered. The level of significance was set at 5%. Comparison between trial groups over time was performed using repeated measures Analysis of Variance (ANOVA).

The threshold or cut-off criteria for an increase in pupillary diameter that was considered tolerable was set at <3 millimetres and for residual amplitude of accommodation was set at 5 dioptres (Cooper et al. 2013). In the model, treatment group was factored as between-subject factor, while

visits and eyes were factored as within-subject factors. Interactions were tested. If there was a significant treatment x visit interaction, the data were analysed for each visit. Post-hoc multiple comparisons were corrected using Bonferroni correction. Level of significance was set at 5%. Statistical analysis was conducted using the IBM SPSS Statistics software, version 25.0, Microsoft Excel 2016.

4.4 Results

4.4.1 *Enrolment and discontinuation*

A total of 200 children were enrolled in the trial with 112 children opting to participate in the intervention group and 88 children in the control group. Following an assessment of the inclusion/exclusion criteria, 96 of the 112 participants were randomized to the intervention groups and 86 participants to the control group.

The study flow for the participants for the first six months is presented in Figure 4.6. After successful enrolment and randomization, and prior to completion of baseline visit, a further five participants from the intervention group and three participants from the control group were permanently discontinued. After successful completion of the baseline visit, there were only few discontinuations until the 6-month period (a single participant from 0.02% Atropine (0.02% Atr), two participants from combination of 0.02% Atropine plus 2% Caffeine (0.02% Atr+2% Caff) and one participant from 2% Caffeine (2% Caff)). In comparison, five participants discontinued from the single vision group.

The total discontinuation rate in the intervention and control groups was 9.4% (9/96) and 9.3% (8/86) respectively. Reporting group-wise, 5.9% (2/34), 13.5% (4/30), 9.4% (3/32), and 9.3% (8/86) discontinued from 0.02% Atropine, 0.02% Atropine plus 2% Caffeine, 2% Caffeine, and Single Vision spectacles control groups, respectively and the difference between the groups was not significant (p=0.65).

Table 4.2 details the reasons for discontinuations and these were: loss to follow-up (3.8%), time conflicts (3.3%), worried about side effects/cycloplegia (1.1%), and withdrawal of consent (1.1%).

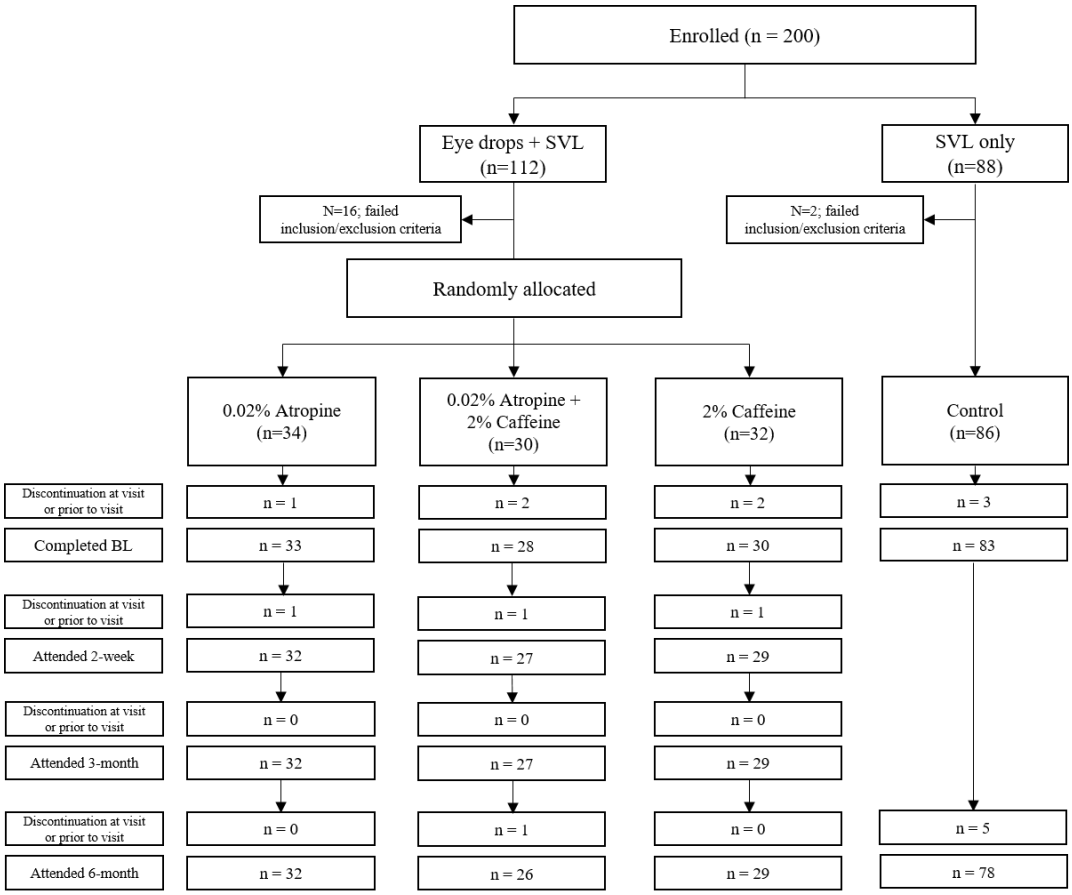

Figure 4.6: Flow of participants

Table 4.2: Reasons of discontinuations over the follow-up

		Lost to follow up	Worry of the side effects	Time conflicts	Withdrawal of consent	Total	p-value
0.02% Atr	n	0	1	0	1	34	0.648
	%	0.0	2.9	0.0	2.9	100.0	
0.02% Atr + 2% Caff	n	2	1	1	0	30	
	%	6.7	3.3	3.3	0.0	100.0	
2% Caff	n	1	0	2	0	32	
	%	3.1	0.0	6.3	0.0	100.0	
Control	n	4	0	3	1	86	
	%	4.7	0.0	3.5	1.2	100.0	
Total	n	7	2	6	2	182	
	%	3.8	1.1	3.3	1.1	100.0	

4.4.2 *Baseline data*

Table 4.3 presents the demographic data of all participants who successfully completed the baseline visits; there were no significant differences between the study groups ($p > 0.05$). The study cohort was aged between 6 and 13 years with an average of 10.2 ± 2.0 years. There were 95 (54.6%) females and 79 (45.4%) males. A total of 40.2% of participants had no parental myopia whereas 42.5% and 17.2% had one and two parents myopic, respectively.

Table 4.3: Demographic data of participants who completed baseline

Variables	n	0.02% Atr	n	0.02% Atr + 2% Caff	n	2% Caff	p-Value	n	Interven-tion	n	Control	p-Value
Age Mean±SD	33	10.1±2.0	28	10.6±1.9	30	10.3±2.1	0.591	91	10.3±2.0	83	10.1±2.1	0.657
Gender (%) Female:Male	33	72.7:27.3	28	46.4:53.6	30	53.3:46.7	0.093	91	58.2:41.8	83	51.8:48.2	0.145
Parental Myopia None: 1 Parent: 2 Parents	33	14:10:9	28	9:15:4	30	15:9:6	0.268	91	15:9:6	83	32:40:11	0.220
Computer Use (hours) Mean±SD	33	1.67±1.64	28	1.63±1.73	30	1.25±1.04	0.479	91	1.52±1.50	83	1.75±2.18	0.937
Outdoors/ in sunlight (hours) Mean±SD	33	1.37±0.96	28	0.99±0.79	30	1.28±0.91	0.235	91	1.23±0.90	83	1.14±1.06	0.253
Homework (hours) Mean±SD	33	1.64±1.12	28	1.48±0.88	30	1.56±0.90	0.828	91	1.56±0.97	83	1.43±1.10	0.208
Reading (hours) Mean±SD	33	0.60±0.48	27	0.55±0.47	30	0.54±0.52	0.844	90	0.56±0.48	83	0.64±0.60	0.675

Notes: 0.02%Atr – 0.02% Atropine; 0.02% Atr+2% Caff - combination eye drops including 0.02% Atropine plus 2% Caffeine; 2% Caff – 2% Caffeine. Data of reading (hours) was not available for one participant in group 0.02% Atr + 2% Caff.

Details of the baseline refractive error and axial length for the intervention and control groups are presented in Table 4.4. Significant differences were found between the groups for cycloplegic spherical equivalent and subjective refractive error and post-hoc comparisons (Table 4.4) indicated that the control group had less myopia in comparison to the test groups. No differences

were seen for any of the other baseline variables including axial length, flat and steep radius of curvature, amplitude of accommodation, photopic and mesopic pupil diameter (Figure 4.7 and Table 4.4).

Table 4.4: Baseline clinical data of participants (completed the baseline visits)

Variables Mean±SD (95% CI)	eyes	**0.02% Atr**	eyes	**0.02% Atr + 2% Caff**	eyes	**2% Caff**	eyes	**SV Specs**	p-Value
Cyclo Sph Eq (D)	66	-4.06±1.32 (-4.39;-3.74)	56	-4.39±1.40 (-4.76;-4.01)	60	-3.95±1.17 (-4.25;-3.64)	164	-3.21±1.35 (-3.42;-3.00)	***0.000***
Subjective Sph eqv (D)	66	-3.42±1.32 (-3.75;-3.11)	56	-3.84±1.37 (-4.21;-3.48)	60	-3.34±1.22 (-3.66;-3.03)	166	-2.69±1.31 (-2.89;-2.49)	***0.000***
Axial Length (mm)	58	24.85±0.81 (24.64;25.07)	54	25.14±0.74 (24.93;25.34)	60	25.09±0.90 (24.86;25.33)	166	24.79±0.82 (24.67;24.92)	***0.013***
K Flat Radius (mm)	58	7.92±0.27 (7.85;7.99)	54	7.88±0.24 (7.81;7.94)	60	7.98±0.24 (7.91;8.04)	166	7.93±0.23 (7.89;7.97)	0.470
K Steep Radius (mm)	58	7.67±0.28 (7.60;7.74)	54	7.60±0.23 (7.53;7.66)	60	7.70±0.26 (7.63;7.77)	166	7.68±0.25 (7.64;7.72)	0.443
Intraocular Pressure (mmHg)	66	16.27±2.06 (15.76;16.77)	56	16.40±1.75 (15.93;16.87)	60	16.04±1.96 (15.53;16.55)	166	16.47±2.07 (16.15;16.79)	0.773
AA (D)	66	16.46±2.93 (15.74;17.18)	56	15.58±3.11 (14.75;16.42)	60	16.16±2.72 (15.46;16.86)	166	15.95±3.24 (15.45;16.44)	0.719
Mesopic pupil size (mm)	64	6.14±0.75 (5.95;6.33)	56	6.08±0.58 (5.92;6.23)	60	5.80±0.90 (5.57;6.03)	166	5.91±0.66 (5.81;6.01)	0.055
Mesopic pupil size (mm^2)	64	30.03±7.25 (28.22;31.84)	56	29.26±5.55 (27.77;30.75)	60	27.04±8.31 (24.89;29.19)	166	27.73±6.07 (26.80;28.66)	0.054
Photopic pupil size (mm)	64	3.04±0.57 (2.89;3.18)	56	2.97±0.38 (2.87;3.08)	60	3.03±0.34 (2.94;3.12)	166	2.99±0.45 (2.92;3.06)	0.805
Photopic pupil size (mm^2)	64	7.49±3.35 (6.65;8.33)	56	7.06±1.78 (6.58;7.53)	60	7.29±1.65 (6.87;7.72)	166	7.17±2.31 (6.82;7.52)	0.749

Notes: 0.02%Atr – 0.02% Atropine; 0.02%Atr+2%Caff - 0.02% Atropine plus 2% Caffeine; 2%Caff – 2% Caffeine; Cyclo Sph Eqv: cycloplegic auto-refraction spherical equivalent; CI: confidence of interval; D: dioptre; mm: millimetre. Data were not available: - in group 0.02% Atr, 8 eyes for axial length, flat and steep radius of curvature, 2 eyes for mesopic and photopic pupil size; - in group of 0.02% Atr + 2% Caff, 2 eyes for axial length, flat and steep radius of curvature; - in control group, 2 eyes for cycloplegic Sph Eqv.

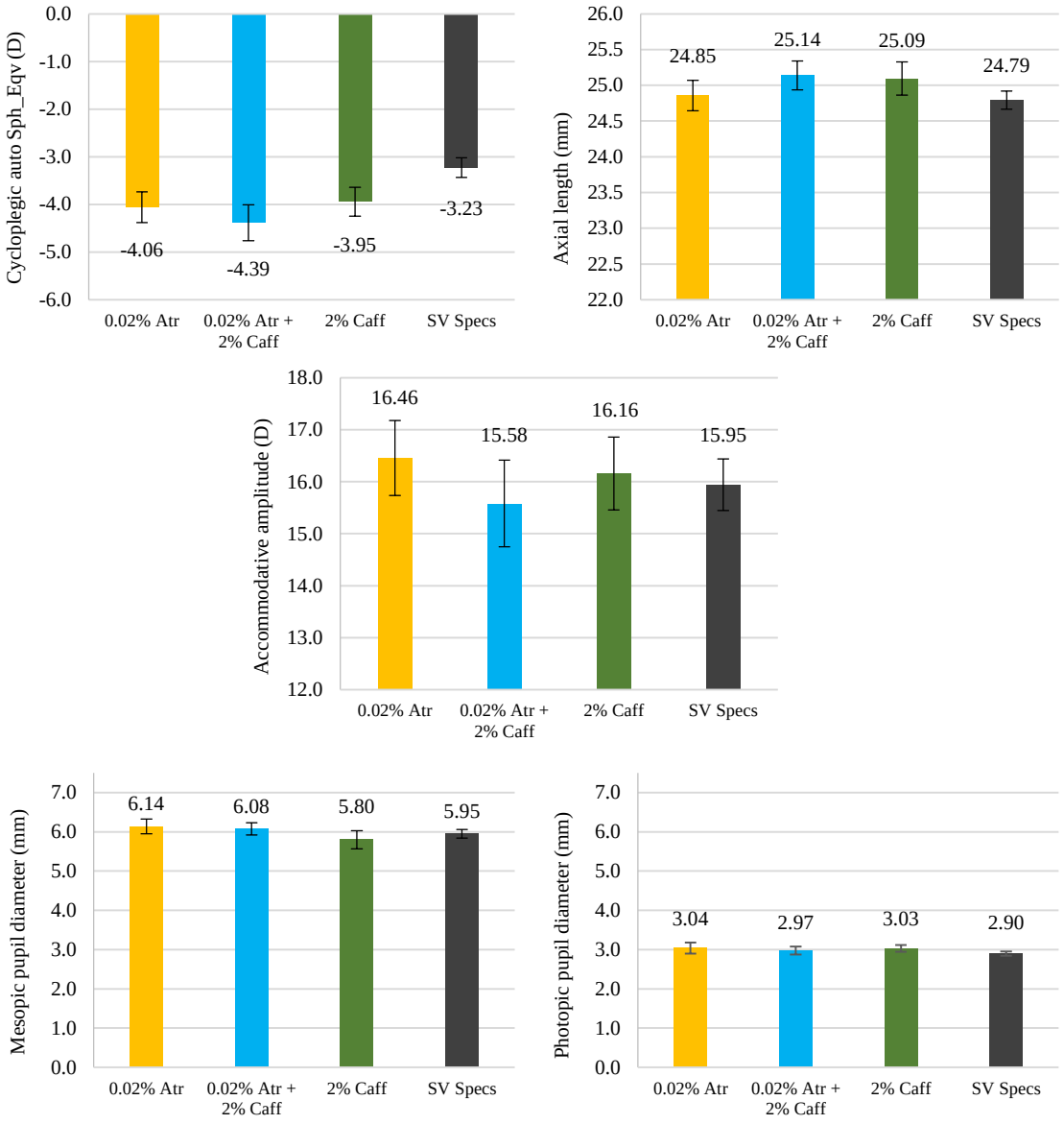

Figure 4.7: Baseline cycloplegic spherical equivalent refractive error, axial length, accommodative amplitude, and pupil diameter between study groups

4.4.3 *Myopia progression*

4.4.3.1 Change in refractive error from baseline to six months

Table 4.5 presents the mean spherical equivalent refractive error at baseline and 6-month visits as well as the change in spherical equivalent refractive error for each of the groups. As previously

indicated, the single vision control group had less myopia at baseline. All groups progressed in myopia over the six months of the study.

The mean change in spherical equivalent (SE) refractive error in the single vision (SV) control group was -0.33 ± 0.29D and of the intervention groups, the mean change in spherical equivalent in the Caffeine 2% group was similar at -0.39 ± 0.38D. In comparison, both 0.02% Atropine and 0.02% Atropine plus 2% Caffeine showed a reduction in spherical equivalent at -0.20 ± 0.34 and -0.20 ± 0.30D respectively. The change over time was mostly related to the spherical component with no change observed for astigmatism. Post-hoc comparisons demonstrated that change in spherical equivalent with 0.02% Atropine was significantly different to change in spherical equivalent with 2% Caffeine and single vision spectacles. The change in spherical equivalent between the two Atropine groups was not significant.

Figure 4.8 illustrates the change in refractive error in groups over time and demonstrate that 0.02% Atropine and 0.02% Atropine plus 2% Caffeine slowed myopia compared to 2% Caffeine and single vision control groups.

Table 4.5: Change in cycloplegic refractive error over time

	Visit	Group	Eyes	Mean ± SD (D)	p-value	Post-hoc			
						0.02% Atr	0.02% Atr + 2% Caff	2% Caff	SV Specs
Spherical equivalent (D)	Baseline	0.02% Atr	66	-4.06 ± 1.32	***0.000***		1.000	1.000	***0.000***
		0.02% Atr + 2% Caff	56	-4.39 ± 1.40		1.000		0.451	***0.000***
		2% Caff	60	-3.95 ± 1.17		1.000	0.451		***0.002***
		SV Specs	164	-3.21 ± 1.35		***0.000***	***0.000***	***0.002***	
	6-Month	0.02% Atr	64	-4.19 ± 1.35	***0.000***		0.605	1.000	***0.025***
		0.02% Atr + 2% Caff	52	-4.60 ± 1.53		0.605		1.000	***0.000***
		2% Caff	58	-4.35 ± 1.23		1.000	1.000		***0.002***
		SV Specs	152	-3.60 ± 1.35		***0.025***	***0.000***	***0.002***	
	Changes from Baseline	0.02% Atr	64	-0.20 ± 0.34	***0.001***		1.000	***0.006***	***0.044***
		0.02% Atr + 2% Caff	52	-0.20 ± 0.30		1.000		***0.011***	0.074
		2% Caff	58	-0.39 ± 0.38		***0.006***	***0.011***		1.000
		SV Specs	150	-0.33 ± 0.30		***0.044***	0.074	1.000	
Sphere (D)	Changes from Baseline	0.02% Atr	64	-0.15 ± 0.40	***0.003***		1.000	***0.021***	***0.015***
		0.02% Atr + 2% Caff	52	-0.20 ± 0.35		1.000		0.187	0.223
		2% Caff	58	-0.38 ± 0.39		***0.021***	0.187		1.000
		SV Specs	150	-0.35 ± 0.37		***0.015***	0.223	1.000	
Cylinder (D)	Changes from Baseline	0.02% Atr	64	-0.10 ± 0.41	0.204				
		0.02% Atr + 2% Caff	52	0.00 ± 0.38					
		2% Caff	58	-0.02 ± 0.38					
		SV Specs	150	0.04 ± 0.47					

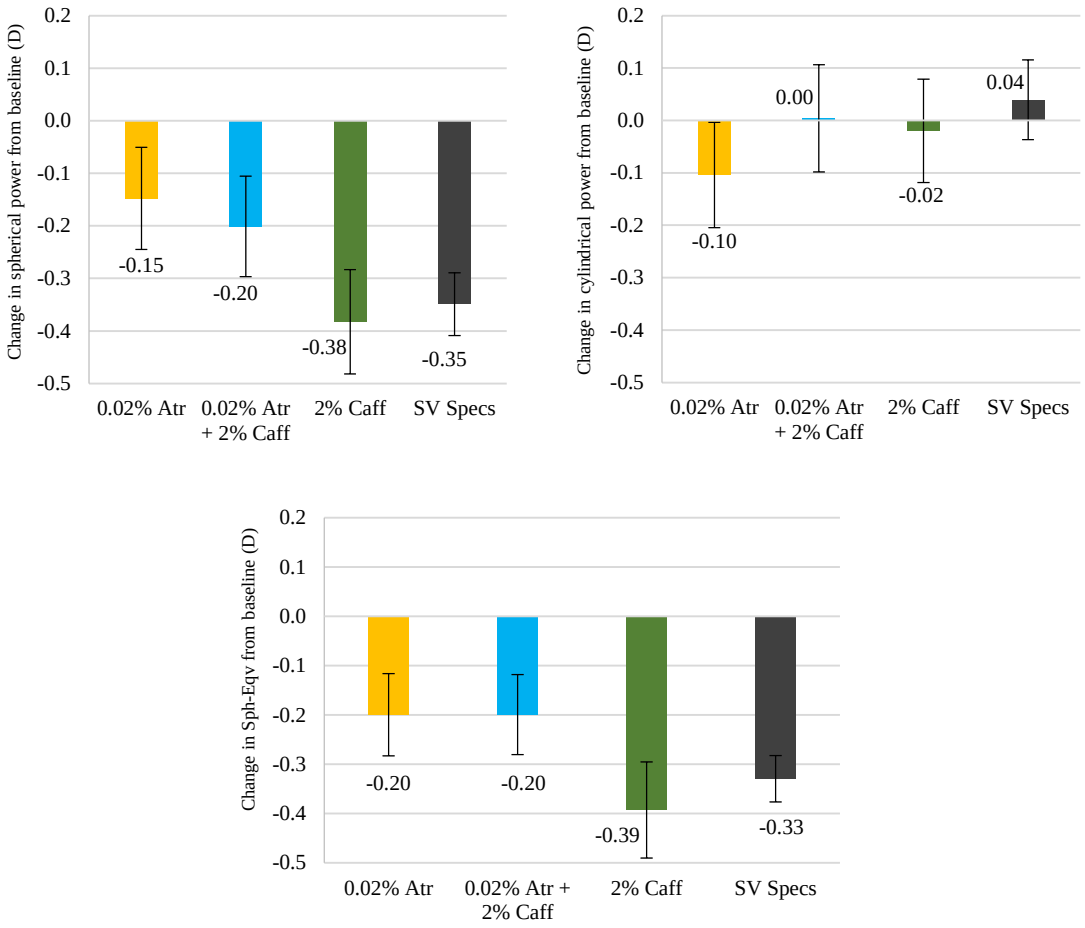

Figure 4.8: Change in refractive error over time

4.4.3.2 Progression ≥0.5D over 6-month follow-up

Significantly fewer eyes from the two Atropine groups progressed by ≥0.5D in comparison to the Caffeine 2% and control groups. Over the 6 months, progression of ≥0.5D was found in 18.8%, and 13.5% in the two Atropine groups (0.02% Atropine and 0.02% Atropine plus 2% Caffeine, respectively) compared to 36.2% of the eyes in the Caffeine 2% and 30% in the control group (Figure 4.9).

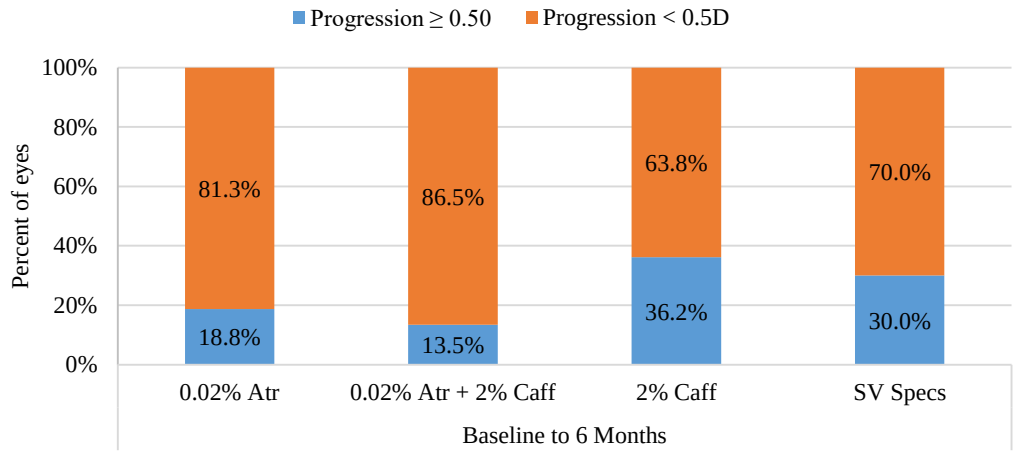

Figure 4.9: Percent of eyes with spherical equivalent progression of ≥0.5D versus <0.5D over 6 months

4.4.3.3 *Changes of axial length over time*

The mean axial length at baseline, and the change in axial length at the 3-month and 6-month visits from baseline are presented in Table 4.6. At baseline, the mean axial length was 24.85 ± 0.81 mm, 25.14 ± 0.74 mm, 25.09 ± 0.90 mm in 0.02% Atropine, 0.02% Atropine plus 2% Caffeine and 2% Caffeine groups with no significant difference between the groups. Similarly, mean baseline axial length was 24.79 ± 0.82 mm in the single vision control group with no differences compared to other groups (Table 4.6). Axial length increased with time in all groups. At 3 months, axial length was measured only in the intervention groups, and the change from baseline was significantly different between the groups ($p < 0.001$, Table 4.6). The least change in axial length was in the 0.02% Atropine group and significantly different compared to 2% Caffeine group. Post-hoc comparisons showed that the difference between 0.02% Atropine and 2% Caffeine was significant ($p < 0.001$).

Similarly, the change in axial length from baseline to 6 months was significantly different between the groups. The least change in axial length was observed in the 0.02% Atropine and 0.02% Atropine plus 2% Caffeine group compared to 2% Caffeine and single vision control groups.

Figure 4.10 illustrates the change in axial elongation of groups over time. Over 6 months, significantly less axial elongation was observed with the use of 0.02% Atropine or 0.02% Atropine plus 2% Caffeine compared to 2% Caffeine or single vision spectacles.

Table 4.6: Changes in axial length over time

Visit	Group	Eyes	Mean ± SD (mm)	p-value	Posthoc			
					0.02% Atr	0.02% Atr + 2% Caff	2% Caff	SV Specs
Baseline	0.02% Atr	58	24.85 ± 0.81	***0.013***		0.413	0.686	1.000
	0.02% Atr + 2% Caff	54	25.14 ± 0.74		0.413		1.000	***0.045***
	2% Caff	60	25.09 ± 0.90		0.686	1.000		0.091
	SV Specs	168	24.79 ± 0.82		1.000	***0.045***	0.091	
Changes of 3-month from BL	0.02% Atr	54	0.03 ± 0.07	***0.000***		0.133	***0.000***	
	0.02% Atr + 2% Caff	54	0.06 ± 0.09		0.133		0.087	
	2% Caff	58	0.09 ± 0.07		***0.000***	0.087		
Changes of 6-month from BL	0.02% Atr	56	0.08 ± 0.11	***0.000***		1.000	***0.000***	***0.000***
	0.02% Atr + 2% Caff	52	0.11 ± 0.11		1.000		***0.002***	***0.001***
	2% Caff	58	0.19 ± 0.15		***0.000***	***0.002***		1.000
	SV Specs	156	0.18 ± 0.11		***0.000***	***0.001***	1.000	

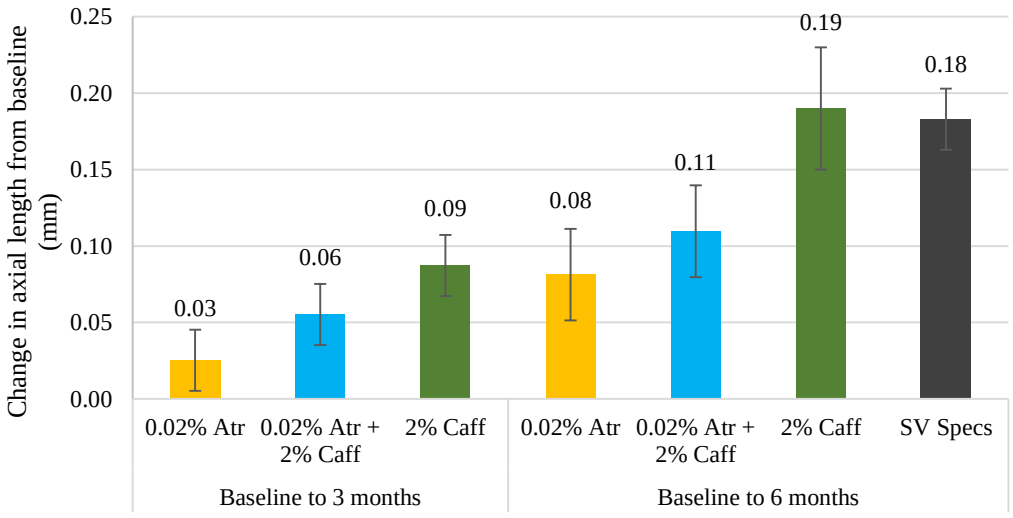

Figure 4.10: Change in mean axial elongation between groups (from baseline visit)

4.4.3.4 *Progression of axial length over 6-month follow-up*

Fewer eyes from Atropine groups had axial length change of ≥0.25mm. Over the six-month period, axial elongation of 0.25mm or more was found in 7.1%, 13.5%, 32.8% and 29.5% of 0.02% Atropine, 0.02% Atropine plus 2% Caffeine, 2% Caffeine and single vision spectacles control groups respectively (Figure 4.11).

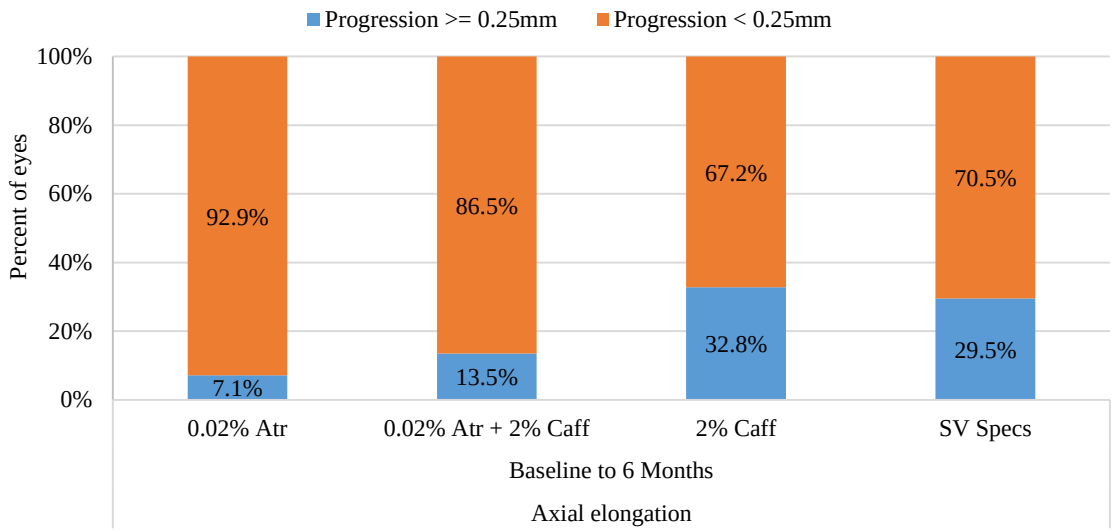

Figure 4.11: Percent of eyes with axial elongation of ≥0.25mm versus <0.25mm over 6 months

4.4.4 *Correlation of the progression of spherical equivalent and axial length in groups*

Across all groups, the correlation between change in spherical equivalent to change in axial length was high indicating that myopic progression was due to change in axial length. Figure 4.12 presents the correlation between change in spherical equivalent to change in axial length.

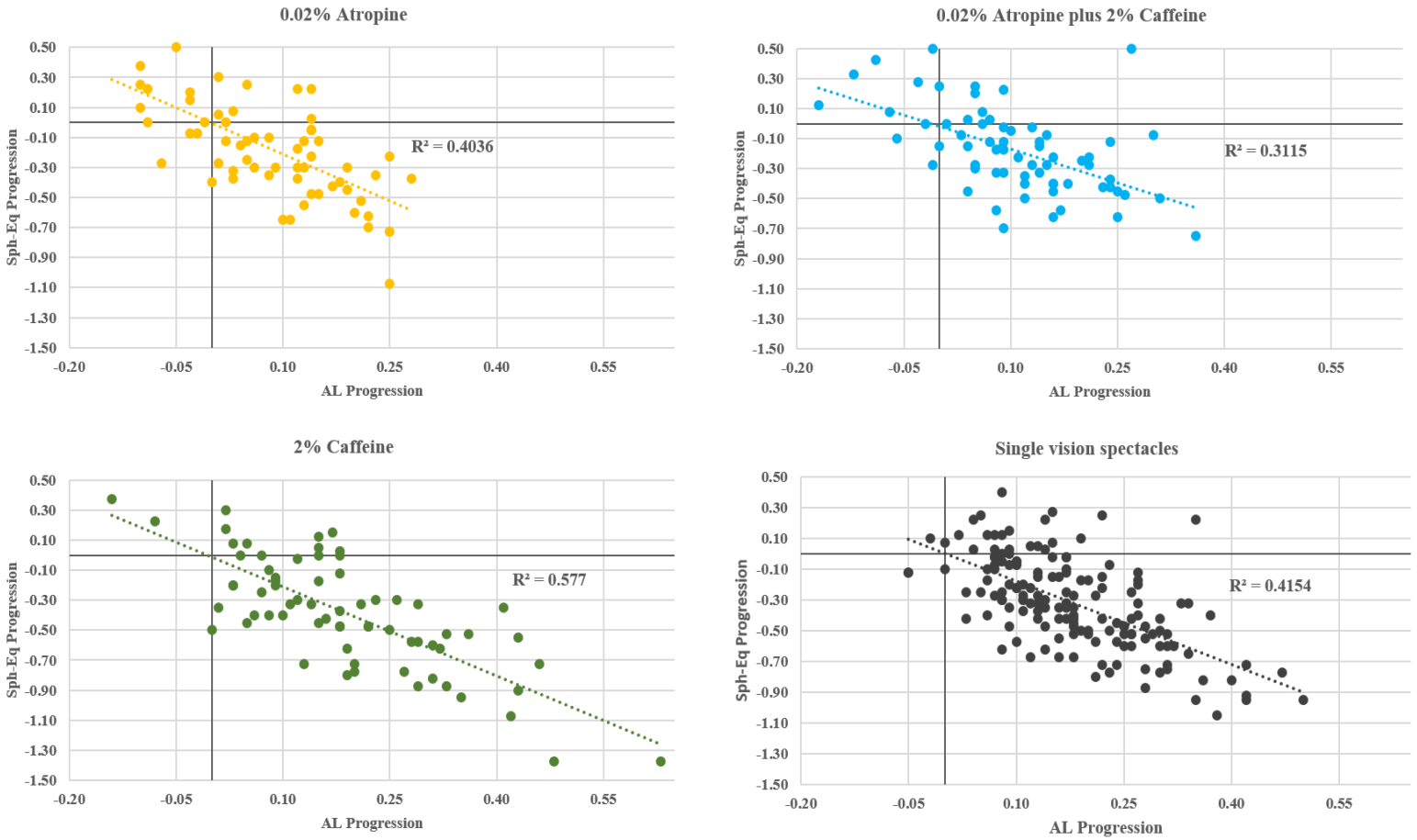

Figure 4.12: Correlation of the change in spherical equivalent refractive and axial length in the study groups

4.4.5 *Change in accommodative amplitude with time: Between groups*

At baseline, mean accommodative amplitude was 16.46 ± 2.93 D, 15.58 ± 3.11 D, 16.16 ± 2.72 D, and 15.95 ± 3.24 D for participants assigned to 0.02% Atropine, 0.02% Atropine plus 2% Caffeine, 2% Caffeine and single vision spectacles groups respectively. There was no significant difference between the groups ($p = 0.443$).

Table 4.7 shows the change in accommodative amplitude from baseline to three and six-month visits for each of the groups. At the three-month visit, a significant decrease in accommodative amplitude was observed between the intervention groups with the maximal decrease observed in the 0.02% Atropine group. The mean decrease in accommodative amplitude was 3.77D, 2.28D and 0.68D with 0.02% Atropine, 0.02% Atropine plus 2% Caffeine and 2% Caffeine respectively (Figure 4.13). There was approximately a 1.5D difference between the two Atropine groups although the difference was not significant. The difference in accommodative amplitude for both the Atropine groups was significantly different to the 2% Caffeine group.

Similarly, at the six-month visit, the greatest decline in accommodative amplitude was observed with 0.02% Atropine with a mean decrease of 3.14D. The decline in the 0.02% Atropine plus 2% Caffeine was 2.84D and not significantly different to Atropine. In comparison, change in accommodative amplitude was minimal with 2% Caffeine and single vision control group at 0.78D and 0.22D respectively.

Figure 4.14 details the percent eyes with >15D, 15D to >10D, 10D to >5D and ≤5D of AA at each visit. At baseline, all eyes had accommodative amplitude higher than 10D (between 32.1% and 54.5% of eyes in groups had more than 15D). At 3 months of the eyes receiving 0.02% Atropine, only 11.3% had AA >15D. Importantly, 25.8% of eyes had AA of 10D or less and a further 3.2% of eyes had ≤5D. In comparison, there were fewer eyes with reduced amplitude with Atropine 0.02% plus Caffeine 2%, with approximately 17% of eyes with AA ≤10D. No such changes were observed with Caffeine 2%. Similar changes were observed at 6 months; however, at this visit, the percent of eyes with ≤10D was similar between both the Atropine groups (Figure 4.14).

Table 4.7: Change in accommodative amplitude over time

Visit	Group	Eyes	Mean ± SD (D)	p-value	Posthoc			
					0.02% Atr	0.02% Atr + 2% Caff	2% Caff	SV Specs
Baseline	0.02% Atr	66	16.46±2.93	0.443				
	0.02% Atr + 2% Caff	56	15.58±3.11					
	2% Caff	60	16.16±2.72					
	SV Specs	166	15.95±3.24					
From baseline to 3-month	0.02% Atr	62	-3.77±4.44	***0.000***		0.073	***0.000***	
	0.02% Atr + 2% Caff	54	-2.28±2.94		0.073		***0.049***	
	2% Caff	58	-0.68±2.80		***0.000***	***0.049***		
From baseline to 6-month	0.02% Atr	64	-3.14±4.08	***0.000***		1.000	***0.005***	***0.000***
	0.02% Atr + 2% Caff	52	-2.84±4.35		1.000		***0.035***	***0.000***
	2% Caff	58	-0.78±3.43		***0.005***	***0.035***		1.000
	SV Specs	156	-0.22±3.81		***0.000***	***0.000***	1.000	

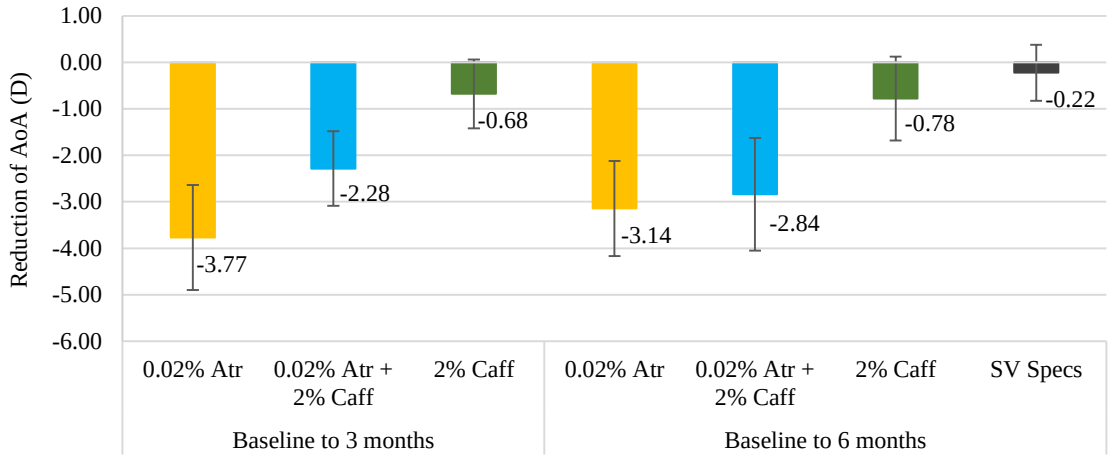

Figure 4.13: Change in amplitude of accommodation between the study groups

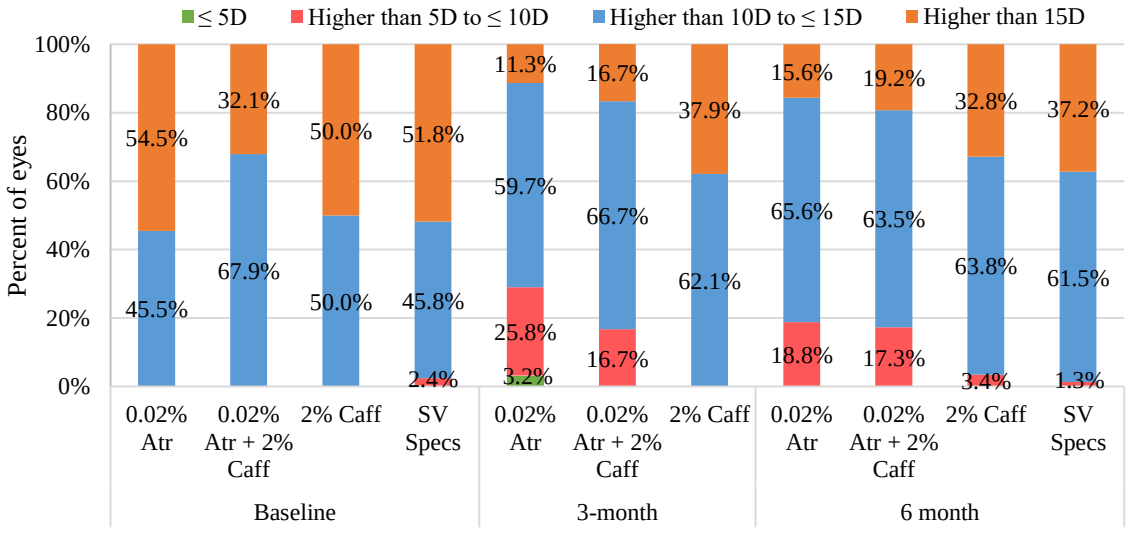

Figure 4.14: Percent of eyes with varying levels of residual accommodative amplitude

4.4.6 *Change in pupil diameter: differences between groups*

At baseline, the photopic pupil diameter was 3.04 ± 0.57 mm, 2.97 ± 0.38 mm, 3.03 ± 0.34 mm and 2.90 ± 0.33 mm and the mesopic pupil diameter was 6.14 ± 0.75 mm, 6.08 ± 0.58 mm, 5.80 ± 0.90 mm and 5.91 ± 0.66 mm in eyes assigned to 0.02% Atropine, 0.02% Atropine plus 2% Caffeine, 2% Caffeine and control single vision spectacles groups, respectively. There were no differences between the groups for either the photopic or mesopic pupillary diameter (p = 0.805, 0.055, respectively).

Figure 4.15, Figure 4.16, and Table 4.8 illustrate the mean pupil diameter for both photopic and mesopic conditions as well as the mean change in pupillary diameter from baseline to three and six months visit for each of the groups.

At three months, there was a significant increase in photopic pupil diameter in eyes with 0.02% Atropine with a difference from baseline of 1.10 ± 0.94 mm. In comparison, the increase of the photopic pupil diameter in eyes with 0.02% Atropine plus 2% Caffeine was significantly less at 0.59 ± 0.61 mm (mean pupillary diameter 3.56 ± 0.47 mm). The decrease in pupillary diameter with the combination eye drops in comparison to Atropine alone was 46.7%. The change in

photopic pupil diameter was least in eyes with 2% Caffeine at -0.07 ± 0.50 mm and was similar to baseline diameter.

Similarly, at the 6-month visit, the change in photopic pupillary diameter from baseline was most with 0.02% Atropine at 1.20 ± 0.85mm (mean photopic pupil diameter of 4.25 ± 0.72 mm). Although there was an increase in the photopic pupil diameter in eyes with 0.02% Atropine plus 2% Caffeine, it was significantly less at 0.76 ± 0.58 mm (mean pupil diameter 3.72 ± 0.46 mm). The decrease in pupillary diameter with the combination in comparison to Atropine was 37.0%. There appeared to be no change in photopic pupillary diameter in the 2% Caffeine and the single vision spectacles control groups with a mean change of -0.07 ± 0.47 and -0.10 ± 0.32 mm respectively.

Similar changes were observed for mesopic conditions. At three months, the largest increase in mesopic pupil diameter was observed in eyes receiving 0.02% Atropine with a difference from baseline of 0.42 ± 0.77 mm (mean mesopic pupil diameter was 6.53 ± 0.59 mm). In comparison, the mean dilation of mesopic pupil diameter in eyes using 0.02% Atropine plus 2% Caffeine was significantly less 0.24 ± 0.63mm and was 42.3% less compared to 0.02% Atropine alone. The least change was observed in 2% Caffeine group at 0.12 ± 0.57 mm, respectively.

At six months, the mean change in mesopic pupillary diameter with 0.02% Atropine was 0.59±0.80mm and in comparison, the decrease was 20.2% less with 0.02% Atropine plus 2% Caffeine at 0.47±0.62mm (mean mesopic pupil size was 6.69 ± 0.59 and 6.50 ± 0.51 mm with 0.02% Atropine and 0.02% Atropine plus 2% Caffeine respectively). In comparison, the change in mesopic pupillary diameter with 2% Caffeine and the single vision group was 0.37 ± 0.61 and 0.22 ± 0.43 (mean mesopic pupil diameter was 6.17 ± 0.80 mm and 6.18 ± 0.64 mm respectively).

Figure 4.17 illustrates the percentage of eyes with > 2mm and ≤ 2mm change in photopic pupillary diameter. At three and six months, 15% and 13% of eyes on 0.02% Atropine had >2mm increase in pupillary diameter. In comparison, only 4% of the eyes on the combination eye drops had >2mm

increase at 6 months and the other groups did not demonstrate any increase in pupillary diameter with time (p <0.001, Figure 4.17).

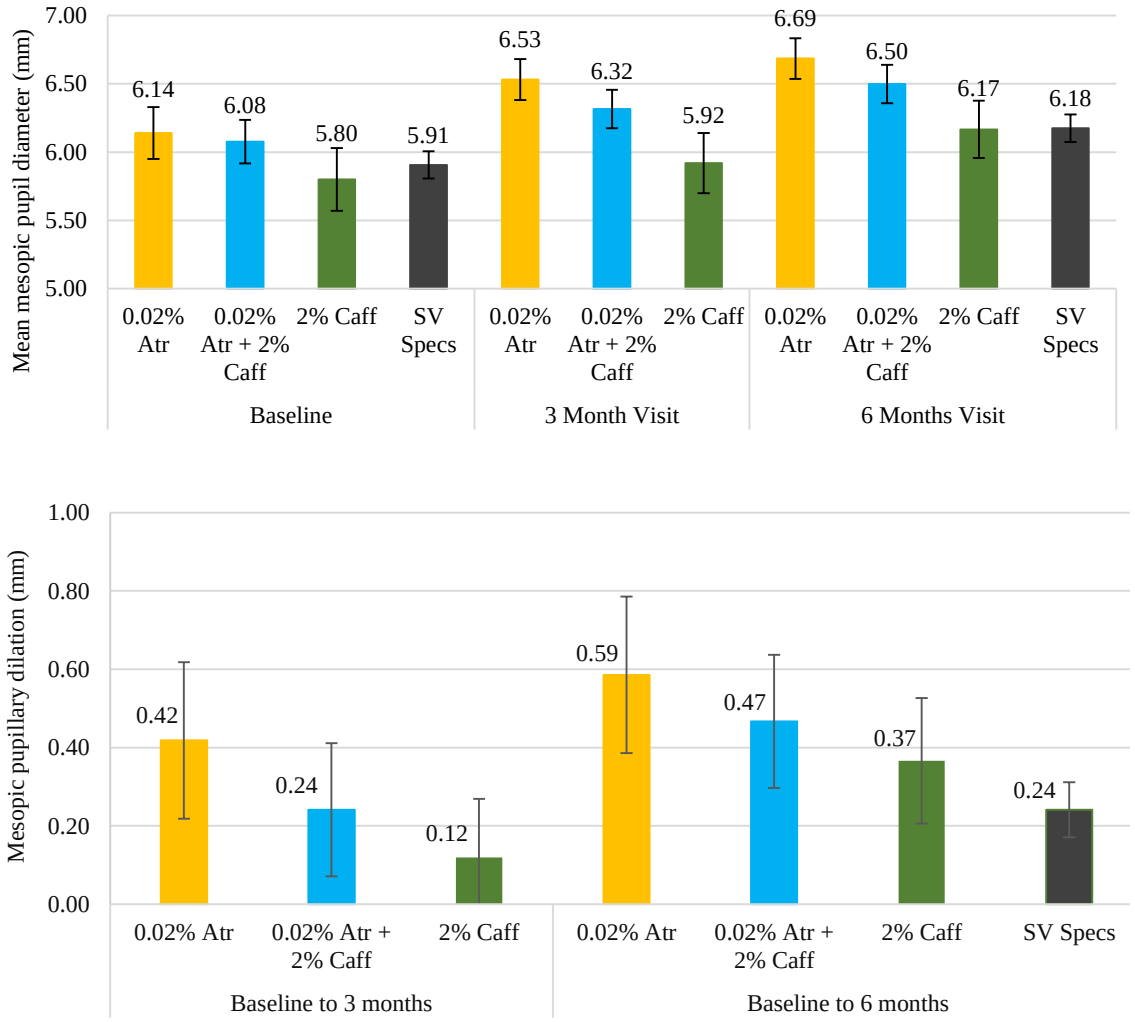

Figure 4.15: Mesopic pupillary diameter between groups over time

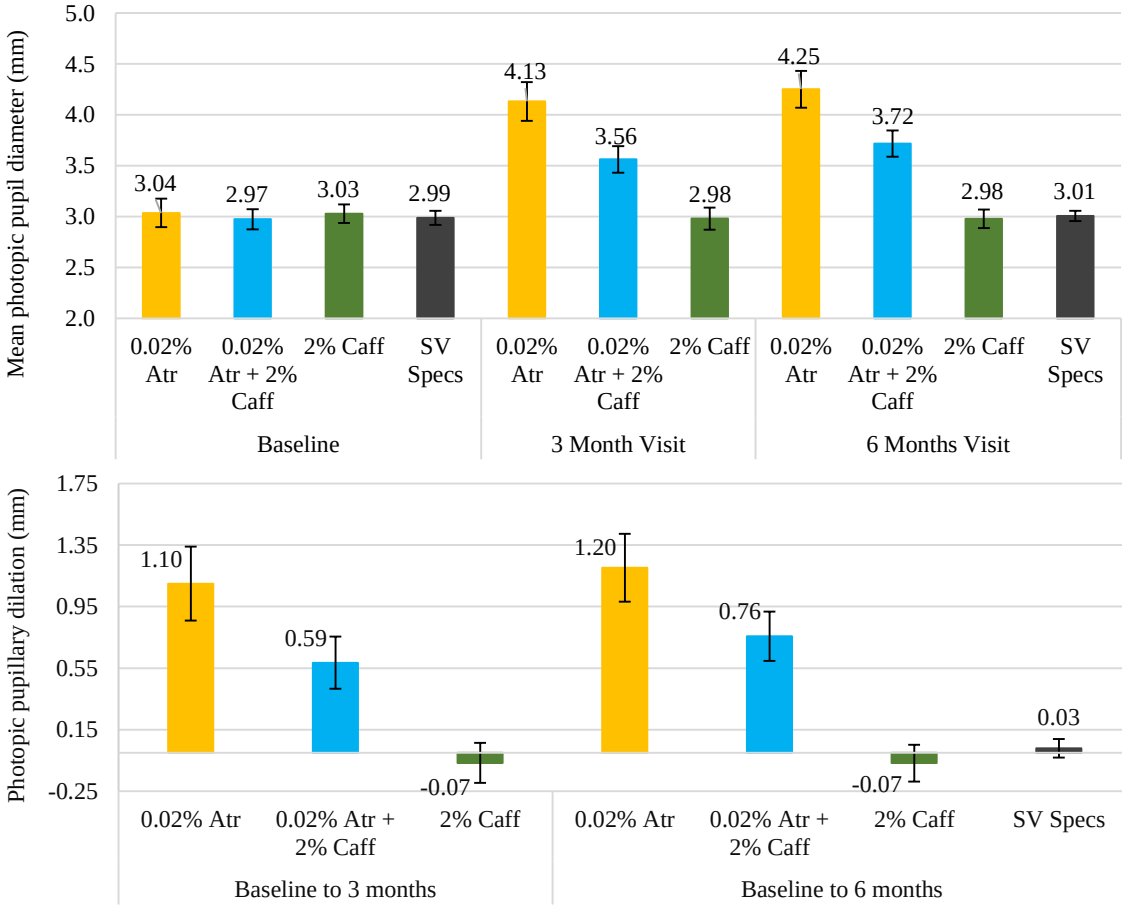

Figure 4.16: Photopic pupillary diameter between groups over time

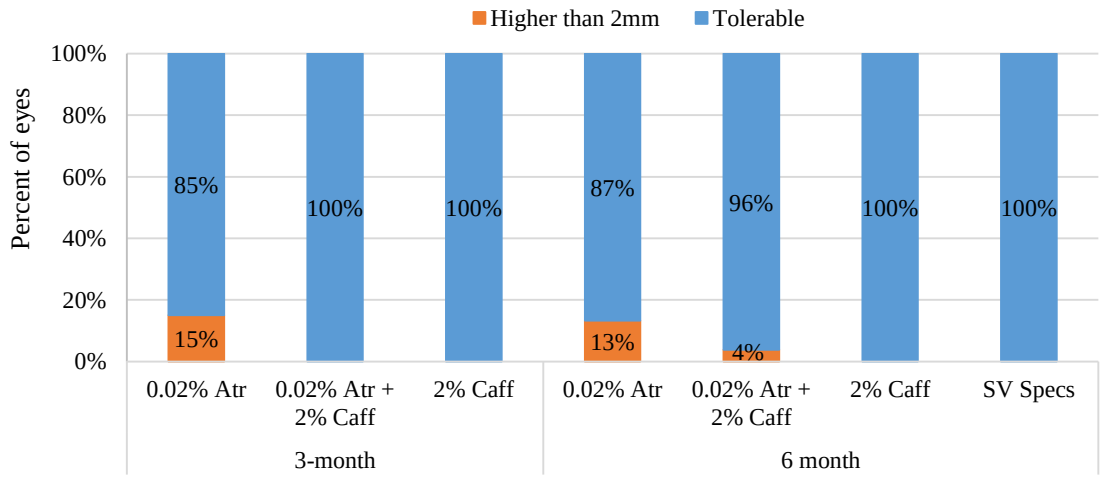

Figure 4.17: Percent eyes with photopic pupillary diameter increase of >2mm and ≤2mm

Table 4.8: Change in pupillary diameter over time

Visit	Group	Eyes	Mean ± SD	p-value	Posthoc			
					0.02% Atr	0.02% Atr + 2% Caff	2% Caff	SV Specs
Photopic pupil size								
Baseline	0.02% Atr	66	3.04±0.57	0.805				
	0.02% Atr + 2% Caff	56	2.97±0.38					
	2% Caff	60	3.03±0.34					
	SV Specs	166	2.90±0.33					
From baseline to 3-month	0.02% Atr	60	1.10±0.94	***0.000***		***0.000***	***0.000***	
	0.02% Atr + 2% Caff	54	0.59±0.61		***0.000***		***0.000***	
	2% Caff	58	-0.07±0.50		***0.000***	***0.000***		
From baseline to 6-month	0.02% Atr	64	1.20±0.85	***0.000***		***0.000***	***0.000***	***0.000***
	0.02% Atr + 2% Caff	52	0.76±0.58		***0.000***		***0.000***	***0.000***
	2% Caff	58	-0.07±0.47		***0.000***	***0.000***		1.000
	SV Specs	156	-0.10±0.32		***0.000***	***0.000***	1.000	
Mesopic pupil size								
Baseline	0.02% Atr	64	6.14±0.75	0.055				
	0.02% Atr + 2% Caff	56	6.08±0.58					
	2% Caff	60	5.80±0.90					
	SV Specs	166	5.91±0.66					
From baseline to 3-month	0.02% Atr	60	0.42±0.77	***0.038***		0.467	***0.033***	
	0.02% Atr + 2% Caff	54	0.24±0.63		0.467		0.827	
	2% Caff	58	0.12±0.57		***0.033***	0.827		
From baseline to 6-month	0.02% Atr	62	0.59±0.80	***0.001***		1.000	0.245	***0.001***
	0.02% Atr + 2% Caff	52	0.47±0.62		1.000		1.000	0.098
	2% Caff	58	0.37±0.61		0.245	1.000		0.990
	SV Specs	156	0.22±0.43		***0.001***	0.098	0.990	

4.4.7 *Subjective responses*

4.4.7.1 Visual acuity between the groups

Visual acuity at each of the visits is presented in Table 4.9 and shows no difference between the groups at any of the visits. Visual acuity was generally high across all groups. At baseline, while the mean binocular and monocular best-corrected visual acuity (BCVA) in high contrast condition were not significantly different between groups (p>0.05, Table 4.9), the mean of near visual acuity

was statistically different between groups ($p<0.05$, Table 4.9). However, the difference of near visual acuity between groups was clinically minimal.

Table 4.9: Change of high contrast visual acuity over time

Visit	Group	N	Mean ± SD (logMAR)	Min	Max	p-value
Monocular Best Corrected Visual Acuity in high contrast condition						
Baseline	0.02% Atr	66	0.05 ± 0.03	0.00	0.12	0.872
	0.02% Atr + 2% Caff	56	0.05 ± 0.03	0.02	0.14	
	2% Caff	60	0.05 ± 0.04	0.00	0.16	
	SV Specs	166	0.05 ± 0.03	0.00	0.18	
3-month	0.02% Atr	62	0.05 ± 0.03	0.00	0.12	0.964
	0.02% Atr + 2% Caff	54	0.05 ± 0.03	0.00	0.12	
	2% Caff	58	0.05 ± 0.04	0.00	0.16	
6-month	0.02% Atr	64	0.05 ± 0.03	0.02	0.16	0.303
	0.02% Atr + 2% Caff	52	0.05 ± 0.03	0.00	0.12	
	2% Caff	58	0.04 ± 0.02	0.00	0.10	
	SV Specs	156	0.06 ± 0.04	0.00	0.18	
Binocular Best Corrected Visual Acuity in high contrast condition						
Baseline	0.02% Atr	33	0.04 ± 0.03	0.00	0.10	0.622
	0.02% Atr + 2% Caff	28	0.04 ± 0.03	0.00	0.12	
	2% Caff	30	0.04 ± 0.03	0.00	0.14	
	SV Specs	83	0.03 ± 0.04	-0.10	0.14	
3-month	0.02% Atr	31	0.03 ± 0.02	0.00	0.10	0.781
	0.02% Atr + 2% Caff	27	0.04 ± 0.03	0.00	0.10	
	2% Caff	29	0.04 ± 0.04	0.00	0.12	
6-month	0.02% Atr	32	0.04 ± 0.03	0.00	0.12	0.452
	0.02% Atr + 2% Caff	26	0.03 ± 0.02	0.00	0.10	
	2% Caff	29	0.03 ± 0.02	0.00	0.08	
	SV Specs	78	0.04 ± 0.03	0.00	0.14	
Near Visual Acuity						
Baseline	0.02% Atr	66	0.03 ± 0.03	0.00	0.10	***0.017***
	0.02% Atr + 2% Caff	56	0.04 ± 0.03	0.00	0.12	
	2% Caff	60	0.02 ± 0.03	0.00	0.10	
	SV Specs	166	0.02 ± 0.03	0.00	0.10	
3-month	0.02% Atr	62	0.03 ± 0.02	0.00	0.10	0.535
	0.02% Atr + 2% Caff	54	0.02 ± 0.03	0.00	0.10	
	2% Caff	58	0.03 ± 0.03	0.00	0.10	
6-month	0.02% Atr	64	0.02 ± 0.02	0.00	0.06	0.408
	0.02% Atr + 2% Caff	52	0.02 ± 0.03	0.00	0.10	
	2% Caff	58	0.02 ± 0.03	0.00	0.10	
	SV Specs	156	0.02 ± 0.02	0.00	0.10	

4.4.7.2 Subjective ratings

Figure 4.18 and Table 4.10 present the mean subjective ratings for overall comfort, distance vision, haloes at night vision, outdoor activities with sunlight and near readings. Subjective ratings were on a scale of 1-10 where 1 was poor and 10 was excellent. Generally, the mean ratings for overall comfort were high for all groups with no significant differences between the groups.

With regards to distance vision, there were no differences between the groups at the 3-month visit. At six months, the single vision control group reported poorer distance vision compared to other groups; it is possible that this rating was reflective of the poorer distance vision. Greater myopia progression was observed in the single vision group from baseline to six months and it is likely that children suffer poorer vision over time and gave an overall worst score.

Interestingly, despite the significant dilation of pupils in both mesopic (in night light) and photopic (in day light) conditions with the Atropine groups, the mean subjective ratings for night vision haloes, outdoor performance and near reading was not different between the groups.

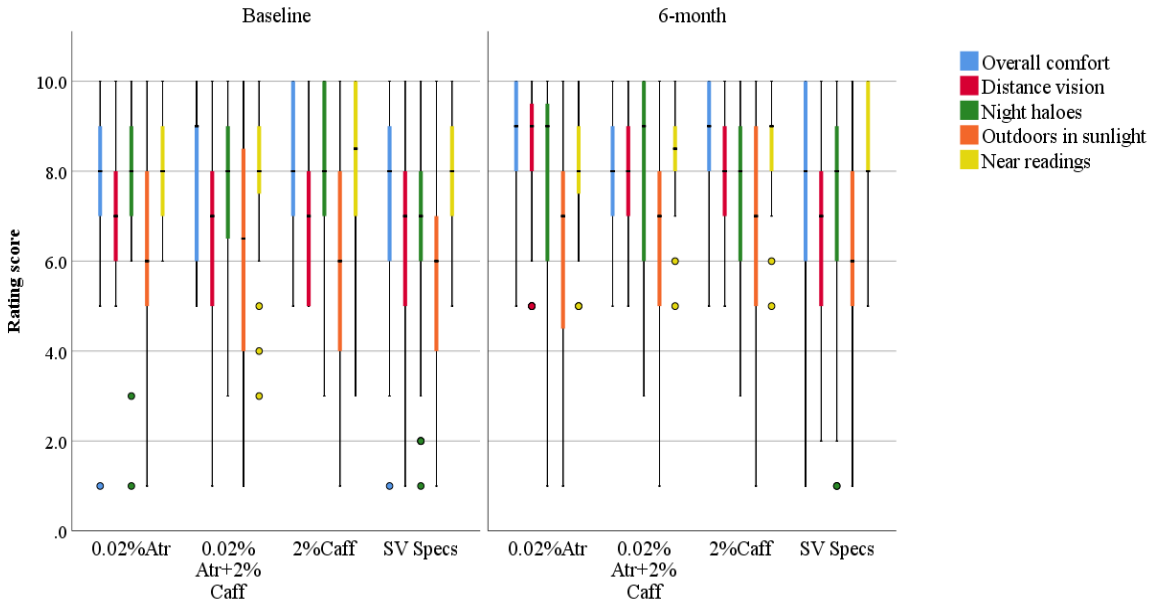

Figure 4.18: Subjective ratings between groups at baseline and 6-month visits

Table 4.10: Differences in subjective ratings between groups at baseline and 6 month visits

Visit	Group	N	Mean ± SD	Min	Max	p-value	Post-hoc			
							0.02% Atr	0.02% Atr + 2% Caff	2% Caff	SV Specs
Subjective Ratings Comfort Overall										
Baseline	0.02% Atr	32	7.88 ± 1.88	1.00	10.00	0.298				
	0.02% Atr + 2% Caff	28	7.93 ± 1.61	5.00	10.00					
	2% Caff	30	8.20 ± 1.67	5.00	10.00					
	SV Specs	83	7.49 ± 2.00	1.00	10.00					
6-month	0.02% Atr	32	8.63 ± 1.56	5.00	10.00	0.078				
	0.02% Atr + 2% Caff	26	8.08 ± 1.49	5.00	10.00					
	2% Caff	29	8.59 ± 1.62	5.00	10.00					
	SV Specs	78	7.77 ± 2.14	1.00	10.00					
Subjective Ratings Distance Vision										
Baseline	0.02% Atr	32	7.33 ± 1.43	5.00	10.00	0.377				
	0.02% Atr + 2% Caff	28	6.64 ± 2.25	1.00	10.00					
	2% Caff	30	7.00 ± 1.60	5.00	10.00					
	SV Specs	83	6.77 ± 1.76	1.00	10.00					
6-month	0.02% Atr	32	8.28 ± 1.61	5.00	10.00	***0.000***		1.000	0.551	***0.000***
	0.02% Atr + 2% Caff	26	8.04 ± 1.54	5.00	10.00		1.000		1.000	***0.009***
	2% Caff	29	7.52 ± 1.62	5.00	10.00		0.551	1.000		0.289
	SV Specs	78	6.76 ± 1.92	2.00	10.00		***0.000***	***0.009***	0.289	

Subjective Ratings Night Vision Haloes						
Baseline	0.02% Atr	32	7.67 ± 2.03	1.00	10.00	0.095
	0.02% Atr + 2% Caff	28	7.68 ± 1.87	3.00	10.00	
	2% Caff	30	7.83 ± 1.90	3.00	10.00	
	SV Specs	83	6.95 ± 2.11	1.00	10.00	
6-month	0.02% Atr	32	7.66 ± 2.51	1.00	10.00	0.457
	0.02% Atr + 2% Caff	26	7.92 ± 2.13	3.00	10.00	
	2% Caff	29	7.45 ± 1.74	3.00	10.00	
	SV Specs	78	7.19 ± 2.18	1.00	10.00	
Subjective Ratings Outdoors/ in sunlight						
Baseline	0.02% Atr	32	6.18 ± 2.28	1.00	10.00	0.444
	0.02% Atr + 2% Caff	28	6.29 ± 2.68	1.00	10.00	
	2% Caff	30	5.70 ± 2.38	1.00	10.00	
	SV Specs	83	5.58 ± 2.38	1.00	10.00	
6-month	0.02% Atr	32	6.44 ± 2.46	1.00	10.00	0.430
	0.02% Atr + 2% Caff	26	6.27 ± 2.74	1.00	10.00	
	2% Caff	29	6.86 ± 2.20	1.00	10.00	
	SV Specs	78	6.01 ± 2.36	1.00	10.00	
Subjective Ratings Near Reading Chart						
Baseline	0.02% Atr	32	8.18 ± 1.18	6.00	10.00	0.881
	0.02% Atr + 2% Caff	28	7.93 ± 1.82	3.00	10.00	
	2% Caff	30	8.20 ± 1.75	3.00	10.00	
	SV Specs	83	8.04 ± 1.47	5.00	10.00	
6-month	0.02% Atr	32	8.19 ± 1.45	5.00	10.00	0.822
	0.02% Atr + 2% Caff	26	8.35 ± 1.29	5.00	10.00	
	2% Caff	29	8.52 ± 1.30	5.00	10.00	
	SV Specs	78	8.38 ± 1.39	5.00	10.00	

4.4.7.3 Relation between near reading ratings with the decrease of accommodative amplitude and the increase of pupillary area

Despite the significant decrease in accommodative amplitude and an increase of pupillary diameter in the Atropine groups, the near reading ratings were high and showed no difference between study groups (Table 4.10). Furthermore, the correlation between decrease in accommodative amplitude and pupil area versus near vision was low across all groups (Figure 4.19).

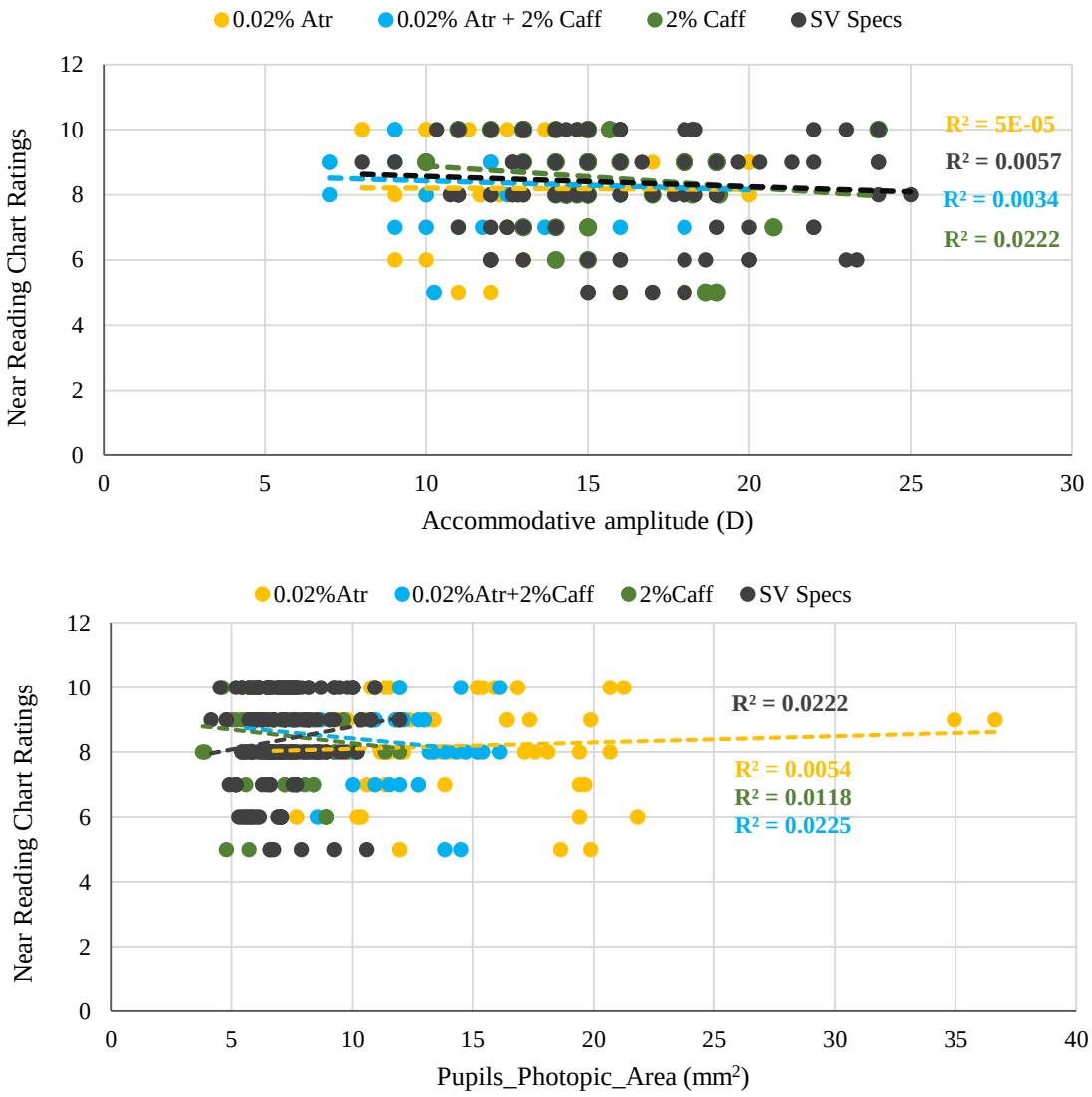

Figure 4.19: Correlation between subjective near vision to mean accommodative amplitude and mean pupil area

4.4.8 *Intraocular pressure*

Table 4.11 presents the intraocular pressure measurements at baseline and the changes at 3- and 6-month visits compared to baseline between groups. There was no statistically significant difference between groups over 3- or 6-month use of either 0.02% Atropine vs 0.02% Atropine+2% Caffeine vs 2% Caffeine vs SV Specs ($p > 0.05$, Table 4.11).

Table 4.11: Change in intraocular pressure over time

Visit	Group	Eyes	Mean ± SD (mm)	p-value	Posthoc			
					0.02% Atr	0.02% Atr + 2% Caff	2% Caff	SV Specs
Baseline	0.02% Atr	66	16.27 ± 2.06	0.773				
	0.02% Atr + 2% Caff	56	16.40 ± 1.75					
	2% Caff	60	16.04 ± 1.96					
	SV Specs	166	16.47 ± 2.07					
Changes of 3-month from BL	0.02% Atr	62	-0.33 ± 1.91	0.470				
	0.02% Atr + 2% Caff	54	-0.51 ± 2.25					
	2% Caff	58	-0.02 ± 2.24					
Changes of 6-month from BL	0.02% Atr	62	-0.21 ± 1.66	0.266				
	0.02% Atr + 2% Caff	52	-0.68 ± 1.64					
	2% Caff	58	-0.20 ± 2.38					
	SV Specs	154	-0.07 ± 1.88					

4.5 Discussion

4.5.1 *Myopia control*

Over six months, a significant decrease in progression of myopia was found with use of Atropine 0.02% and combination (0.02% Atropine plus 2% Caffeine) as compared to use of Caffeine 2% or the single vision control group. These results support previous reports that indicate the efficacy of Atropine in slowing myopia (Clark and Clark 2015; Chia et al. 2016; Joachimsen et al. 2019; Sacchi et al. 2019; Yam et al. 2019; Fu et al. 2020),

For the first time, we assessed the efficacy of 2% Caffeine for myopia control in human eyes. Previous trials indicated that 7-Methylxanthine, a metabolite of Caffeine had a role to play in myopia (Trier et al. 2008), but the results were equivocal. When used orally (400mg) in myopic children for 24 months, 7-Methylxanthine slowed axial length but not spherical equivalent; furthermore, a shorter period of use (12 months) did not appear to confer any benefit (Trier et al. 2008). However, support was found for its use in animal models (primates, guinea pigs and rabbits) where 7-Methylxanthine reduced axial myopia induced by hyperopic defocus (Cui et al. 2011; Nie et al. 2012; Hung et al. 2018). Furthermore, topically instilled Caffeine was found to reduce the

likelihood of primate eyes responding to hyperopic defocus by having an effect on choroidal thickness (Arumugam et al. 2017; Smith III et al. 2021). More recently, studies conducted in chicks with 7-methylxanthine found no effect either for form deprivation myopia or retinal dopamine.

Our data indicate that when used as a primary agent, 2% Caffeine had no effect on the progression of myopia. The change in spherical equivalent and axial length observed with 2% Caffeine over the 6-month period was similar to that observed with the use of single vision spectacles. Furthermore, the addition of 2% Caffeine to 0.02% Atropine did not alter the efficacy of Atropine as the progression in both the Atropine groups was similar (Figure 4.8). Figure 4.20 illustrates the slope in the mean cycloplegic spherical equivalent and axial length for the four groups from baseline to 6 months and demonstrates a much steeper and identical slope with Caffeine 2% and the single vision spectacle group as compared to the Atropine 0.02% and the combination group. The steeper slope was indicative of greater progression with the Caffeine 2% and the single vision spectacle group.

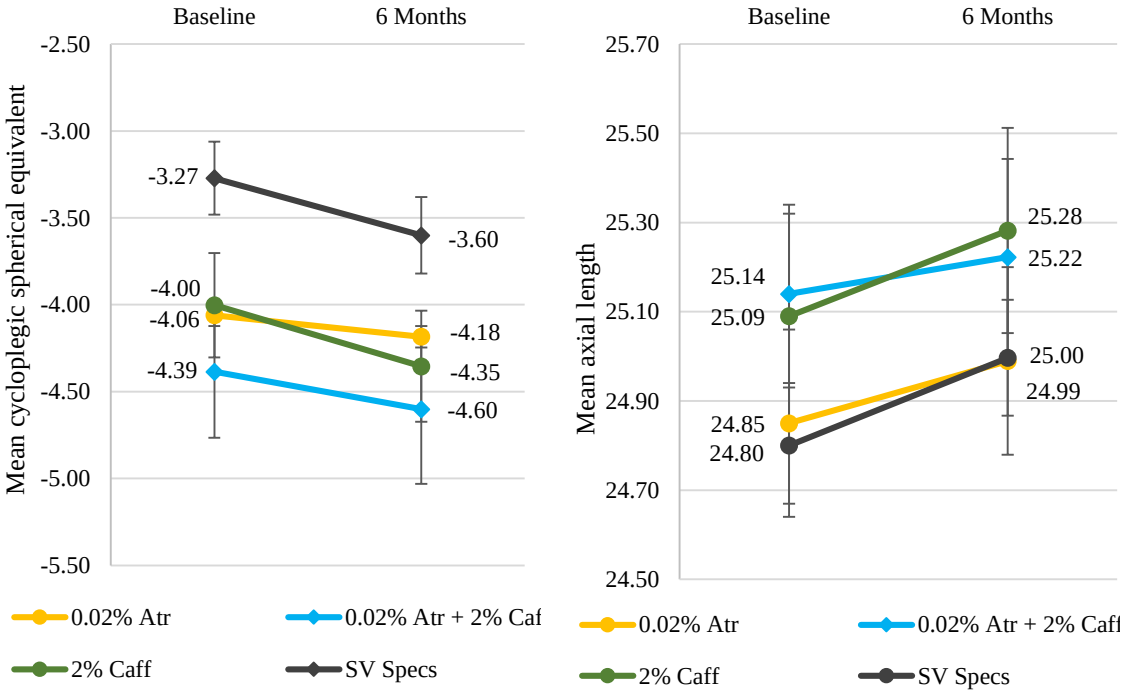

Figure 4.20: Cycloplegic spherical equivalent refractive error and axial length of the study groups at baseline and 6-month.

Caffeine is a non-selective adenosine receptor antagonist, and it was considered that adenosine receptors were involved *via* multiple pathways in the regulation of eye growth, for example, *via* release of neurotransmitters such as dopamine, or *via* collagen synthesis by scleral fibroblasts or *via* choroidal blood flow. Further evidence was found from studies conducted in mice, where genetic deletion of adenosine receptor ($A_{2A}R$) was found to promote the development of myopia with an increased axial length and an altered scleral collagen structure (Zhou et al. 2010). Given the association with eye growth in animal models, the lack of efficacy with topical 2% Caffeine was unexpected and perplexing. If Caffeine did impact eye growth *via* adenosine receptor pathways or other pathways, we would have seen either a) altered progression in eyes that received Caffeine alone or b) improved or greater or altered efficacy with the combination rather than Atropine monotherapy due to a synergistic mechanism involving both the muscarinic receptor and adenosine receptor pathways. It is possible that the concentration of Caffeine as used in the current trial at 2% may not have been sufficient to influence eye growth. The maximum solubility of Caffeine in water is 2.17 g/100ml and Caffeine was found to be stable at 12 weeks in studies conducted by BHVI (data on file). Although higher concentrations of Caffeine are soluble with the addition of, for example citrate, they were not pursued in the current trial as such solutions affect the pH and composition, may cause stinging and we wanted the same composition as a primary agent and in combination with Atropine. Interestingly, studies conducted by Smith and associates in 2021 used a much lower concentration of Caffeine at 1.4%; it may be that the concentration was sufficient to impact on the smaller eye/volume of the infant monkey (13 to 15 mm in infant monkey) but not that of the eye of a young child (Smith III et al. 2021). However, given the effects of Caffeine on pupillary diameter, we have evidence that the drug was absorbed into the eye and has an effect on the ocular tissues. It also may be possible that Caffeine is excreted faster before it had an impact on the eye. Published evidence suggests that many subtypes of ADOR are present mainly in the posterior eye, i.e. at the retina, retinal pigment epithelium and sclera (Cui et al. 2010; Beach et al. 2018). Therefore, although 2% Caffeine was able to reach the anterior segment (Varma

et al. 2011; Kronschläger et al. 2013), it may be possible that given its rapid excretion from the eye it may not have reached in sufficient quantity to affect the receptors located in or more layers of the posterior eye (Johnson et al. 2017). Since we had not evaluated choroidal thickness, we were unable to determine if Caffeine reached the posterior structures. Additionally, since there was no impact on the efficacy in combination with Atropine it appears that Caffeine has no influence on the pathways *via* which Atropine is exerting its ocular growth effects.

There has been a single article reporting the efficacy of 0.02% Atropine in slowing myopia. (Fu et al. 2020). Although the current results are limited to six months, the data is indicative of a decrease in SE and axial elongation similar to the study of Fu et al. (Table 4.12). Approximately 43- 46% of reduction in spherical equivalent progression and a slightly lesser axial length change of 29% to 35% was reported with concentrations of 0.02% and 0.025%. Our results are comparable for change in spherical equivalent but found a higher reduction in axial length change; however, it should be noted that the previous trials had reported data of one to two years duration (Yam et al. 2019; Fu et al. 2020).

Table 4.12: Myopia progression from current study compared to published data

	Single vision spectacles	0.02% Atropine (Fu et al. 2020)	0.025% Atropine (Yam et al. 2019)
Mean difference in SE progression (95% CI) / **%**		Mean (95% CI) / %	
0.02% Atropine	0.13 (0.00;0.26) / **39.4%**	0.32 (0.19; 0.45) / **45.7%**	0.35 (0.22; 0.48) / **43.2%**
0.02% Atropine plus 2% Caffeine	0.13 (0.00;0.27) / **39.6%**		
Mean difference in AL progression (mm / **%**)		Mean (95% CI) / %	
0.02% Atropine	-0.10 (-0.15;-0.05) / -**55.6%**	-0.16 (-0.24; -0.08) / -**34.8%**	-0.12 (-0.18; -0.06) / -**29.3%**
0.02% Atropine plus 2% Caffeine	-0.07 (-0.12;-0.02) / -**40.1%**		

4.5.2 *Change of pupil size and accommodative amplitude*

As summarized in the meta-analysis, although Atropine at higher concentrations was more effective in slowing myopia, it was associated with a greater risk of significant side effects such as

blurred near vision, glare, and photophobia due to an increase in pupillary diameter and reduction in accommodative amplitude. Although the intensity of side effects is reduced with lower concentrations, they continue to be seen. As demonstrated from the current results, use of 0.02% Atropine resulted in a significant change in pupil diameter – a mean 1 to 1.2mm increase in photopic pupil diameter was observed at the 3- and 6-month visits; furthermore, approximately 15% of 0.02% Atropine users had a pupil diameter of >2mm. An increase in mesopic pupil diameter was also observed. Similarly, 0.02% Atropine also reduced accommodative amplitude; there was approximately 3 to 4D decrease in accommodative amplitude with use of 0.02% Atropine.

Interestingly, our results indicate a benefit for use of Caffeine in combination with Atropine. The data indicates that when Caffeine is used in combination with Atropine, the change in pupillary diameter and accommodative amplitude is minimised. The data for minimization of pupillary diameter increase was consistent and observed at both the 3-month and 6-month visits. This minimization of side effects was significant; with respect to photopic pupil diameter, Caffeine in combination with Atropine controlled the increase in pupillary diameter by approximately 47% and 37% (3 and 6 months respectively); the mean increase in pupil diameter with of the combination was approximately 0.6 to 0.75mm compared to 1.1 to 1.20mm change observed with Atropine monotherapy. With the mesopic pupil diameter, this benefit was approximately 42% and 20% (3 and 6 months respectively). Similarly, eyes on Atropine 0.02% plus Caffeine 2% had lesser reduction in accommodative amplitude compared to those on 0.02% Atropine alone. This difference was much greater at the 3-month visit (approximately 0.5D) compared to the 6-month visit (approximately 0.3D). Interestingly, there was a trend to slight decrease in photopic pupillary diameter with Caffeine 2% alone at both the 3-and 6-month visits; however, no such changes were observed with mesopic pupillary diameter (it had increased slightly from baseline) or accommodative amplitude. These results for minimisation of pupillary diameter are exciting and suggest a pathway for reduction of side effects associated with use of Atropine whilst maintaining

efficacy. Although it was anticipated that adenosine receptor antagonists such as Caffeine may have an impact on the side effects (Coroneo et al. 2020), they have not been reported previously.

In the eye, the iris sphincter and ciliary body are rich with m3 muscarinic receptors (approximately 60 to 75% receptors are thought to be m3 subtype) (McDougal and Gamlin 2015). Atropine is thought to block the action of the muscarinic receptors to acetylcholine resulting in mydriasis of the circular muscle or the iris sphincter, and resulting in passive dilation and paralysis of the ciliary muscle (Tran et al. 2018). It appears that Caffeine may have no additional benefit in normal eyes when the parasympathetic system is functioning normally. However, when combined with Atropine, it may be that Caffeine works on the muscarinic receptors by competing with Atropine with muscarinic receptors and therefore Atropine is less effective in blocking the receptors. Alternatively, Caffeine may act on the neurons to increase acetylcholine that compete with Atropine for the muscarinic receptors at the iris sphincter leading to a reduced pupillary dilation. Caffeine inhibits the adenosine by binding the ADOR and is said to result in the rise of acetylcholine (Kardon 2005). Indeed it has been suggested that oral Caffeine consumption may enhance the accommodative amplitude with the peak within the first hour after use by modulating the acetylcholine *via* blocking ADOR and therefore stimulating the ciliary body in addition to accommodative amplitude (Abokyi et al. 2017).

Caffeine is considered to have a half-life of 5 hours with peak effects within 30 to 60 minutes. In comparison, Atropine has a slower onset with more prolonged effect with a half-life of about 4 hours and maximum mydriatic effect at about 30mins and cycloplegic effect at about 4 hours. Given the short half-life of Caffeine it is possible that it has an effect only on pupil size and not accommodation. Despite there being only approximately 0.5mm difference between the dilation of photopic pupil between the 0.02% Atropine and 0.02% Atropine plus 2% Caffeine group at 6-month visit (Figure 4.16), the difference in the photopic pupillary area is reduced by about 4 to 5x and therefore significantly reduces the amount of light entering the eye and its associated risks.

4.5.3 *Strengths and Limitations*

There are several strengths and limitations to this trial. The double-masked randomized nature of the trial where children were randomized to one of three groups is a significant strength and ensures that the outcomes were not influenced by bias between the sample groups. However, the lack of randomization of the single vision spectacle group was a limitation. We expected that if randomized, the single vision group would have experienced a higher-than-normal rate of discontinuation. Parents of children who expected to benefit from participation in the trial would have discontinued. However, not randomizing the children to the control group had the effect of parents with children who had lower baseline refractive error preferring the single vision group; it is possible that this group may not have been as concerned about myopia progression compared to those with higher baseline refractive error.

It is possible that the trial population is biased as those parents that seek myopia control strategy for their children have enrolled in the trial; this was also evident from the fact that the myopia of the randomization group was higher than that of the single vision group.

Additionally, we had not assessed other factors such as choroidal thickness as part of this longitudinal study. The primate studies demonstrated the effect of Caffeine on choroidal thickness and assessment of choroidal thickness would have provided data on whether Caffeine influences choroidal thickness in humans. However, due to the nature of the clinical trial and the need to ensure that the visit schedule is kept to a minimum we had elected to perform choroidal thickness measurements in this trial. Since changes in choroidal thickness appeared to be rapid, appearing within minutes of exposure to stimuli and required a highly controlled environment, we felt that inclusion of thickness in the trial may not provide results that are accurate. Therefore, it was felt that this was best addressed in a short-term experiment. Furthermore, although there is some data that suggests that choroidal thickness responds to myopic and hyperopic defocus in a consistent manner with thickening to myopic defocus and thinning with hyperopic defocus, there is other data that suggests that the response is not always predictable. We had also not trialled other

concentrations of Caffeine to determine if for example, higher concentrations were likely to result in a greater benefit. To improve the solubility of Caffeine past the 2% solubility, it would have required modification with for example, use of citrate salts etc and such alterations and their effects on efficacy, use in human eyes etc was beyond the timelines and scope of this thesis.

The use of a subject push-up test to measure amplitude of accommodation is also a significant limitation. The subjective nature of the test with variability in call-out times and responder times makes this a difficult assessment; furthermore, there is bias introduced due to the nature of the pupil diameter which would have been visible to the examiner. We had not also assessed other biomarkers such as blood, serum assessments to evaluate the bioavailability of the drug; such assessments and measurements were beyond the scope of the trial. Additionally, the questionnaires to address visual symptoms were confounded by progression; these were likely best addressed over short-term. When children returned for 3- and 6-month visits, the nature of the assessment -for example, rate your distance vision on a scale of 1-10 appeared to be weighted more towards an assessment of poor vision due to progression rather than an assessment due to the side effects of Atropine.

Although the treatment phase of the study was expected to last for a year, we are able to present only up to 6 months. Due to unforeseen circumstances, lengthy ethics, and health authority approvals as well as COVID, there have been delays and therefore, we are unable to present these results in this thesis. However, based on a vast body of evidence, the observed progression and efficacy of myopia control options at 6 months reflect long-term efficacy.

4.5.4 *Summary*

Caffeine either alone or in combination with Atropine had no effect on myopia progression. However, there was an unexpected and beneficial effect of a reduced change in pupillary diameter when Caffeine was combined with Atropine. Although the underlying mechanisms for this finding are not entirely understood, it would be of interest to determine if Caffeine is effective at higher

concentrations of Atropine. This would enable delivery of higher concentrations of Atropine that maximise efficacy with reduced risk of side effects.

Interim summary:

A prospective, randomised clinical trial in myopic children involving 3 intervention groups of 2% Caffeine, 0.02% Atropine (monotherapy), or 0.02% Atropine plus 2% Caffeine (combination) and a single vision (SV) spectacle lens wearing group. At 6 months, all groups progressed in myopia. The mean change in spherical equivalent/axial length was -0.33±0.29D / 0.18±0.11mm and -0.39 ± 0.38D / 0.19±0.15mm with SV and Caffeine 2%. In comparison, change was slower at -0.20±0.34D / 0.08±0.11mm and -0.20±0.30D / 0.11±0.11mm with Atropine monotherapy and combination, respectively. Caffeine 2% performed similarly to SV spectacle group and had no effect on spherical equivalent or axial length. Use of 0.02% Atropine resulted in a significant increase in photopic pupil diameter, i.e., >1mm at 3 and 6 months. This increase was reduced by 46.7% and 37.0% with the combination therapy at 3 and 6 months respectively. Similar changes were observed with the change in mesopic pupillary diameter. Although not significant, Caffeine when used with Atropine minimised the impact on accommodative amplitude as compared to Atropine monotherapy.

Chapter 5. Effects of low concentration Atropine and Caffeine eye drops either alone or in combination on accommodative response and pupil size

5.1 Introduction

Topical application of Caffeine, a metabolite of Methylxanthine was found effective in increasing choroidal thickness and slowing eye growth in primates. Importantly, there were no systemic side effects reported (Arumugam et al. 2017; Smith III et al. 2021). Additionally, oral use of Methylxanthine was also effective in slowing myopia in humans (Trier et al. 2008). In human eyes, Caffeine was previously investigated for treatment of glaucoma (Chandra et al. 2011), cataract prevention (Varma 2016), and treatment of dry eye (Osei et al. 2014). Caffeine was also commonly used as an inactive ingredient in commercially available eye-drop preparations (Australia and New Zealand 2015; Kaiserman et al. 2017). Topical Caffeine used either alone or in combination with Atropine was found to be safe in a short term, human clinical trial conducted in Sydney, Australia (Bellberry application number 2018-01-036; ANZCTR registration no 12618000196246, BHVI database). In that cross-over study conducted at Brien Holden Vision Institute Limited, Sydney, Australia, twenty-two healthy adults were monitored for use of topical 0.02% Atropine, 1.4% Caffeine, 2.0% Caffeine and 0.02 % Atropine plus 1.4% Caffeine. No adverse events such as ocular redness, staining etc. were observed with the use of Caffeine. In a long-term dispensing study, we evaluated the efficacy of 0.02% Atropine, 0.02% Atropine with 2% Caffeine and, 2% Caffeine compared to single vision spectacles for slowing of myopia progression. Results of our recent trial (Chapter 4) indicated that Caffeine did not slow myopia; however, when used in combination with Atropine, it had a protective effect in reducing the risk of an increase in pupillary diameter resulting from the use of Atropine. These results were unexpected and require to be validated. Since changes in pupillary diameter and accommodative amplitude occur rapidly after instillation of Atropine, we elected to conduct a short-term, 24-hour

investigation to determine if Caffeine 2% eye drops when used in combination with higher concentrations of Atropine, i.e., 0.05% and Atropine 0.1% influenced the increase in pupillary diameter observed with Atropine.

5.2 Aims

- To determine the change in pupillary diameter over a 24-hour period with 0.05% Atropine, 0.1% Atropine, 2% Caffeine, 0.05% Atropine plus 2% Caffeine and 0.1% Atropine plus 2% Caffeine.
- To determine the change in accommodative amplitude over a 24-hour period with 0.05% Atropine, 0.1% Atropine, 2% Caffeine, 0.05% Atropine plus 2% Caffeine and 0.1% Atropine plus 2% Caffeine.

5.3 Methods

A prospective, randomized, double-masked, cross-over clinical trial was conducted at Hai Yen Eye Center in Ho Chi Minh City, Vietnam involving adult human participants. The study consisted of two arms with 15 participants, aged between 18 and 35 years, randomly allocated into each intervention arm. One treatment arm evaluated each of the compositions comprising 0.05% Atropine, 0.05% Atropine plus 2% Caffeine and 2% Caffeine; similarly, participants randomized to the other arm evaluated each of the compositions comprising 0.1% Atropine, 0.1% Atropine plus 2% Caffeine, and 2% Caffeine. The order of the compositions in each arm was further randomised. Ocular assessments for each composition were over a 24-hour period followed by a minimum of 4-night washout period prior to evaluation of the next composition.

The study protocol was approved by the Institutional Research Ethics Committee of An-Sinh Hospital (No 511-21/AS-QD), Human Research Ethics Committee of University of New South Wales (No HC200993), and adhered to the Declaration of Helsinki for experimentation on human subjects.

5.3.1 *Sample size determination*

Sample size was based on change in pupil size which was the primary outcome variable. Previous publication (Yam et al. 2019) and the estimated data from our meta-analysis (section 2.3.2) on the change in pupil size with 0.05% Atropine indicated that the SD of the change in pupil size was 1.7±1 mm. In both photopic and mesopic light conditions, there was a minimum of 1mm difference to the control non-treated eye. A minimum of fifteen participants was required in each arm to determine a paired difference of 1±1 mm change in pupil size with 80% power at the 5% level of significance, using G*power to compute sample size (Bartlett 2019).

5.3.2 *Participant selection and enrolment*

All participants were screened for general suitability using the inclusion/exclusion criteria as detailed below.

Participants were enrolled in the trial if they:

- were able to read and comprehend Vietnamese and give informed consent as demonstrated by signing a record of informed consent.
- at baseline, were between 18 to 35 years of age, male or female.
- were willing to comply with instilling the eye drops and clinical trial visit schedule as directed by the Investigator.
- had ocular health findings considered to be “normal”.

Additionally, they were excluded from the trial if they:

- had any pre-existing ocular irritation, injury, or condition (including infection or disease).
- had any systemic disease that adversely affects ocular health e.g. Graves disease, and auto-immune diseases such as multiple sclerosis, Sjögrens syndrome and systemic lupus erythematosus.

- used or had a need for concurrent category S3 and above ocular medication at enrolment and/or during the clinical trial.
- used or had a need for any systemic medication or topical medications that may have affected a participant's ocular health / physiology.
- had undergone eye surgery within 12 weeks immediately prior to enrolment for this trial.
- had previous corneal refractive surgery.
- had known allergy or intolerance to ingredients in any of the clinical trial products (Atropine and Caffeine).
- were currently enrolled in another clinical trial.
- had participated in a clinical trial within the previous 2 weeks for dispensing studies and 48 hours between in-house studies.
- reported to be pregnant or were breastfeeding*.

A participant was considered "successfully enrolled" when the Investigator agreed that they conformed to the inclusion/exclusion criteria and signed the Informed Consent Form.

5.3.3 *Masking procedure and maintenance*

The study was double-masked. Both the enrolled participants and the investigator remained masked to the type of eye drops for the duration of the clinical trial. All eye drops were identically packaged and dispensed by an unmasked study coordinator.

5.3.4 *Clinical trial randomisation*

After enrolment into the study, participants were randomly allocated to either of the two intervention arms at an allocation ratio of 1:1. The randomisation plan was generated from http://www.randomization.com/. The website's second random generator was used to create a random permutation for each participant for all three eye drops within each study arm. The

randomization plan was based on balanced block design. A randomisation list was generated by the biostatistician and applied through the Clinic Data Management system installed by Brien Holden Vision Institute at the Hai Yen Eye Center for the purpose of the trial. In addition to the principal investigator, the study was supported by a clinical coordinator and clinicians who were trained and aided in study examinations.

The randomisation code for eye drops was applied at baseline visit by a biostatistician and clearly documented. The Investigator (Huy Tran) was not allowed to access this randomisation code until the trial was completed and final data analysis was performed.

5.3.5 *Product information*

5.3.5.1 Product description

All trial solutions (Table 5.1) were manufactured by CustomCare Compounding Pharmacy, Sydney, Australia and provided in preservative-free single-use and disposable vials.

Table 5.1: Description of eye drops used in this clinical trial

Eye Drops	Manufacturer	Active Ingredients	Artificial Tear Solution
Arm 1			
Test 1.1	Custom Compounding Pharmacy	2.0% Caffeine	0.3% Hydroxyl-propyl methyl cellulose, 0.1% EDTA, sterile water
Test 1.2		0.05% Atropine	
Test 1.3		0.05% Atropine plus 2.0% Caffeine	
Arm 2			
Test 2.1	Custom Compounding Pharmacy	2.0% Caffeine	0.3% Hydroxyl-propyl methyl cellulose, 0.1% EDTA, sterile water
Test 2.2		0.1% Atropine	
Test 2.3		0.1% Atropine plus 2.0% Caffeine	

5.3.5.2 Eye-drop instillation and disposal

In this clinical trial, the study investigator instilled a single drop of the allocated composition just once in the right eye of the participant. All eye drops were stored refrigerated in the investigational product storeroom. Participants were advised to not use any other eye drops during the trial.

5.3.6 *Visits and measurements*

Those participants wearing a corrective device were advised to continue to use their corrective device, as needed for the duration of the trial. They were encouraged to report any health issues or side effects related to the use of eye drops over the trial period.

Figure 5.1 presents the flow-chart for visits over the course of the trial. Prior to the initial assessment visit, participants were given the opportunity to read the Participant Information/Informed Consent Form. At the baseline visit (approximately 1-3 pm), one of the 3 allocated eye drops was chosen at random to be instilled in the right eye of the participant. An assessment of the pupillary and accommodative response was conducted soon after the instillation of the drop and repeated every 20 minutes for the first 60 minutes. On the following day (Day 2), a follow-up assessment took place at approximately 9 (±2 hours) am and then repeated every 3 hours until 3 pm. This schedule of instillation and assessment visits was repeated for all 3 eye drops per group in a random order with a minimum of four-night washout period before assessment of the next drop. Although the eye drops were instilled only in right eyes, both eyes were measured.

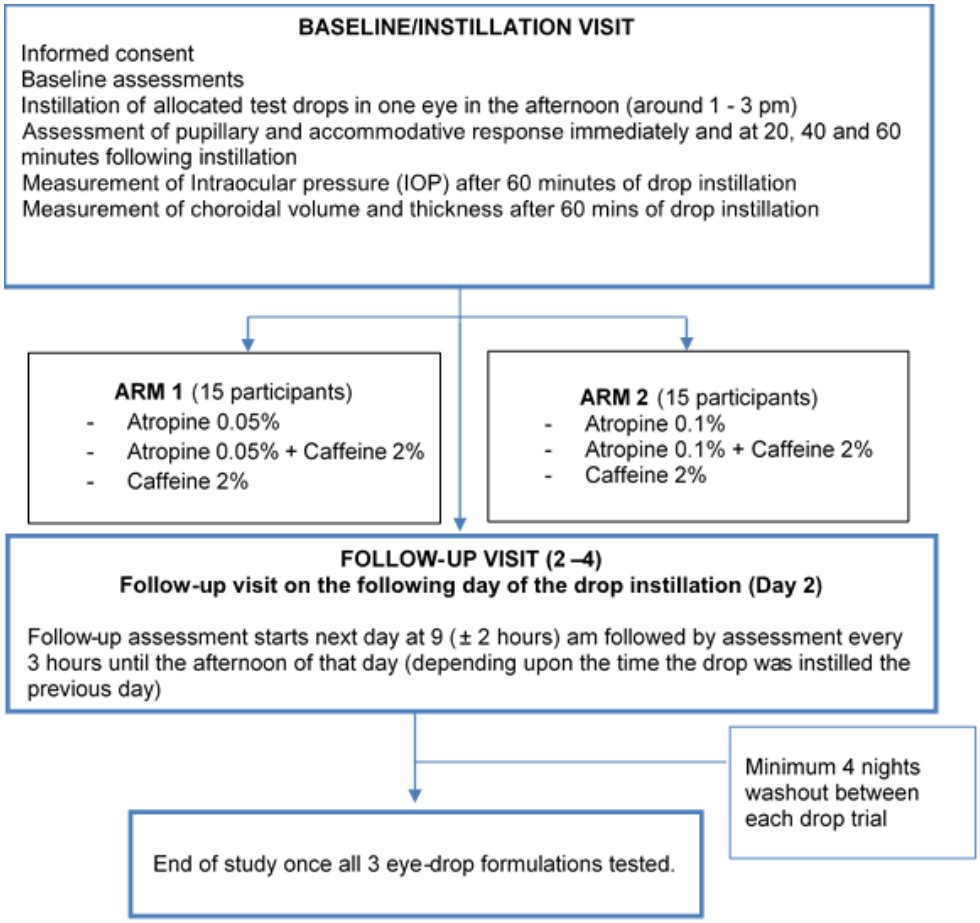

Figure 5.1: Flow chart of study visits

At baseline, assessments were conducted without cycloplegia. Visual acuity was measured at distance and near. Distance visual acuity was measured using Snellen chart at 6 meters. Near visual acuity was measured using the ETDRS (Early Treatment of Diabetic Retinopathy Study) chart at 40 centimetres. Data related to the distance and near visual acuity were recorded on logMAR scale. Following visual acuity measurement, pupil size was measured using Oculus Park 1 (OCULUS Optikgerate GmBH, Wetzlar, Germany, Figure 4.2). Photopic condition was defined as use of direct light maintained at (300 lux) at the eye level and mesopic condition was ambient room light (30 lux). Pupil diameter, in both photopic and mesopic conditions, amplitude of accommodation and introcular pressure were measured by three repeats for each participants. A Royal Near Point Ruler (Figure 4.3) was used to measure the monocular accommodative

amplitude with the best-corrected distance spectacle correction in place. Briefly, a push-up method with the –4.0D spherical lens over the best-corrected refraction was used wherein the participant was instructed to focus on the smallest line on the target drum, equivalent to N5 (N-scale) print. The assessor then slowly moved the drum towards the testing eye until the participant reported the blur of that line (Rosenfield and Cohen 1996). Intraocular pressure was measured using Tonopen (Reichert Technologies, Reichert Inc., Depew, USA) at each visit. Axial length and keratometry were measured with Lenstar 900 (Haag-Streit, Switzerland, Figure 4.4) and refractive error of the eye was measured using open-field auto-refractometer NKVision 5001 (Shin-Nippon by Rexxam, Japan, Figure 4.5) with five repeats each.

A detailed outline of the tests performed for each arm at the baseline visit, instillation and assessment visit are listed in Table 5.2.

Table 5.2: Assessment of variables and window period for each visit

Procedures / Data	**BL**	**Drop Instillation Visit between 1 and 3 pm (for 2nd and 3rd drops only)**	**Visit 2 (Day 2) At 9 (± 2 hrs) am**	**Visit 3 (Day 2) At 12 (± 2 hrs) pm**	**Visit 4 (Day 2) At 3 (± 2 hrs) pm**	**Unscheduled/ Adverse Events**
Visit window	N/A	At least 4 nights after previous drop assessment	≤ 20 hours from BL/Drop instillation visit	≤ 4 hours from visit 2	≤ 4 hours from visit 3	
Informed consent	✓	-	-	-	-	-
Meet inclusion / exclusion criteria	✓	-	-	-	-	-
Demographics	✓	-	-	-	-	-
Presenting VA	✓	✓	✓	✓	✓	✓
History at baseline	✓	-	-	-	-	-
History since last visit, including updated medical problems / previous events / treatment	-	✓	✓	✓	✓	✓
Auto-refraction + keratometry Subjective refraction + VA	✓	*	*	*	✓	*
Slit-lamp biomicroscopy - Ocular assessment	✓	*	*	*	✓	✓
Pupil diameter and response (photopic and mesopic)	✓	✓	✓	✓	✓	-
Accommodative amplitude	✓	✓	✓	✓	✓	-
Intra Ocular Pressure (IOP)	✓	✓	✓	✓	✓	-
Choroidal volume and thickness	✓	✓	-	-	✓	-
Photos / video	*	*	*	*	*	*
Lid and conjunctiva swabs	*	*	*	*	*	*
Adverse event assessment	✓	✓	✓	✓	✓	✓
Visit summary	✓	✓	✓	✓	✓	✓
All the participants in the control group will be collected data similar to baseline measurements before and after cycloplegia for every six months over two years						
Note: "✓" required information; "-" not required; "*" at investigator's discretion						

5.3.7 *Statistical analysis*

Demographic and ocular characteristics including age, baseline spherical equivalent, baseline axial length, intraocular pressure, best-corrected visual acuity, near visual acuity using single vision spectacles, photopic and mesopic pupillary diameter, and accommodative amplitude were

summarised as means ± standard deviations for continuous measurements. Data from both eyes were considered. The level of significance was set at 5%.

5.3.7.1 Pupillary response and accommodative response

Data were recorded in millimetres for pupil size and dioptres for accommodative amplitude on an interval scale. Data were summarized as means ± standard deviations. No transformation was required. The change in pupillary diameter and accommodative amplitude from baseline were computed for each eye. Within each study arm, changes in pupillary diameters and accommodative response if any were compared between Atropine, Caffeine and combination eye drops at each study visit. Data were analysed using repeated measures ANOVA or linear mixed model with subject random intercepts. Factors in the model included eye drops, visit and interaction of visit x eye drops. If the interaction of visit x eye drops is significant, the difference between eye drops was determined at each visit. Between study arm analysis was also be conducted using linear mixed models.

A tolerable increase in pupillary diameter was set at <3 millimetres change for baseline and for residual amplitude of accommodation it was set at 5 dioptres (Cooper et al. 2013).

5.3.7.2 Other variables

Data was summarised as means ± standard deviations for variables measured on an interval scale and median ± interquartile range for ordinal variables and percentages for those that are categorical. Linear mixed model and logistic regression was used to determine significant differences between trial groups and between visits. Other commonly used tests of significance at each visit included paired t-tests and group t-test for parametric data and Wilcoxon signed-rank test and rank sum test for non-parametric data. Test of other variables also included Analysis of Variance (ANOVA) and repeated measures ANOVA to test for between-participant and within-

participant factors. Test of other categorical variables involved McNemar's, Fisher's Exact and Chi-Square tests for within- and between-participant factors.

Post-hoc multiple comparisons were corrected using Bonferroni correction. Level of significance was set at 5%. Statistical analysis was conducted using the IBM SPSS Statistics software, version 25.0, Microsoft Excel 2016.

5.4 Results

5.4.1 *Enrolment and discontinuation*

Overall, 30 adult participants, aged between 18 and 35 years old, were successfully allocated to the two intervention arms at an allocation ratio of 1:1. Table 5.3 shows the participants successfully enrolled at baseline and the patient flow for each stage of the trial. None of the participants reported any serious side effects, allergic reactions, or other reactions to the eye drops. No discontinuation was reported during the trial, but one participant in group 1 missed the third composition. Since there was complete data for first two investigated compositions, the participant was included in the final analysis.

Table 5.3: Participants involved at each phase of the trial

Trial Indicators / Visits	Recruitment	BL	Stage 1					Stage 2						Stage 3				
			V1.0	V1.1	V1.2	V1.3	V1.4	V2.0	V2.1	V2.2	V2.3	V2.4	V3.0	V3.1	V3.2	V3.3	V3.4
Eligible Pxs	33																	
Px excluded	3																	
Px allocated to groups	30																	
Group 1																		
Px completed		15	15	15	15	15	15	15	15	15	15	15	14	14	14	14	14	
Missed visit		0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1	
Permanent discontinuations		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
Cumulative discontinuations		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
Group 2																		
Px completed		15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	
Missed visit		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
Permanent discontinuations		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
Cumulative discontinuations		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	

5.4.2 *Baseline data*

Table 5.4 and Table 5.5 detail the biometric data for the enrolled participants. There were no differences between the participants enrolled in the two arms ($p > 0.05$). The photopic and mesopic pupil diameter of the population was 3.05 ± 0.55mm and 5.22± 0.95mm respectively and the accommodative amplitude was 9.91± 1.50D.

Table 5.4: Age and gender distribution

Variables	n	Group 1	n	Group 2	n	Overall	Min	Max	p-Value
Age Mean±SD	15	20.4 ± 2.4	15	21.1 ± 2.6	30	24.4 ± 2.4	20.4	30.7	0.710
Gender (%) Female:Male	15	60.0:40.0	15	73.3:26.7		66.7:33.3	-	-	0.439

Table 5.5: Baseline biometry data of all participants

Biometry measurements	N	Mean ± Std. Deviation	Minimum	Maximum
Auto Refraction Cylinder Non-cycloplegic (D)	60	-0.74 ± 0.80	-4.25	0.00
Auto Refraction Sphere Non-cycloplegic (D)	60	-3.39 ± 2.34	-8.50	0.75
Keratometry Flat-Power R1 (D)	60	42.35 ± 1.41	39.00	46.75
Keratometry Steep-Power R2 (D)	60	43.75 ± 1.43	40.50	47.75
Subjective Refraction Cylinder (D)	60	-0.70 ± 0.77	-4.00	0.00
Subjective Refraction Sphere (D)	60	-2.64 ± 2.40	-7.75	0.25
Intraocular pressure (mmHg)	60	15.18 ± 1.83	12.33	20.00
Accommodative amplitude (D)	60	9.91 ± 1.50	7.50	15.00
Mesopic pupil size (mm)	60	5.22 ± 0.95	3.30	6.87
Photopic pupil size (mm)	60	3.05 ± 0.55	2.23	5.97
Near visual acuity at 40cm (logMAR)	60	0.01 ± 0.02	0.00	0.16
High contrast BCVA at 6m (logMAR)	60	0.02 ± 0.02	0.00	0.08

At the baseline visit, pupillary diameters and accommodative amplitude were measured both prior to (pre-instillation) and soon after the instillation of the allocated composition (Post_Day0 or Post instillation). All follow-up measurements were compared to the post-instillation data.

Table 5.6 describes and compares the measurements between treated (right) eyes versus non-treated (left) eyes immediately prior to and after instillation of drops. There were no differences between the eyes at both visits, for accommodative amplitude, mesopic and photopic pupil sizes ($p > 0.05$).

Table 5.6: Pupil diameter and accommodative amplitude between eyes at baseline – pre- and post-instillation

		Eye	N	Mean ± Std. Deviation	Minimum	Maximum	p-value
AA (D)	Pre-Instil	Left	30	9.96 ± 1.65	7.50	15.00	0.91
		Right	30	9.86 ± 1.36	8.00	13.00	
	Post-Instil(0)	Left	30	9.81 ± 1.61	7.00	13.00	0.69
		Right	30	9.65 ± 1.50	7.00	12.50	
	Change	Left	30	-0.16 ± 1.19	-3.17	2.75	0.47
		Right	30	-0.21 ± 1.20	-2.00	3.00	
Mesopic pupil diameter (mm)	Pre-Instil	Left	30	5.15 ± 0.96	3.30	6.77	0.49
		Right	30	5.28 ± 0.94	3.43	6.87	
	Post-Instil(0)	Left	30	5.25 ± 0.84	3.60	6.53	0.25
		Right	30	5.50 ± 0.82	4.20	6.73	
	Change	Left	30	0.09 ± 0.42	-0.94	0.93	0.25
		Right	30	0.21 ± 0.36	-0.77	0.90	
Photopic pupil diameter (mm)	Pre-Instil	Left	30	2.96 ± 0.38	2.30	3.93	0.17
		Right	30	3.15 ± 0.67	2.23	5.97	
	Post-Instil(0)	Left	30	2.92 ± 0.30	2.30	3.57	0.41
		Right	30	2.99 ± 0.37	2.23	3.97	
	Change	Left	30	-0.04 ± 0.21	-0.63	0.37	0.24
		Right	30	-0.15 ± 0.51	-2.70	0.23	

5.4.3 *Temporal variation in pupillary diameter*

5.4.3.1 Pupil diameter - At baseline between groups

Table 5.7 presents the mean pupillary diameter with each of the compositions immediately following instillation of drop and compares between the right (treatment eye) versus left (control) eyes. There were no differences between treatment versus control eyes except for photopic pupil

size with 0.1% Atropine and 2% Caffeine, wherein the right eyes had a slightly greater photopic pupil size. However, the inter-ocular difference in pupillary diameter was smaller than 0.5mm and not considered to be clinically relevant.

Table 5.7: Mean pupillary diameter immediately post-instillation (Day0-Post) in groups

		N	Mean ± SD (mm)	95% CI		p-value
				Lower CI	Upper CI	
Mesopic pupil size						
0.05% Atropine	Left	14	5.36 ± 1.02	4.78	5.95	0.30
	Right	14	5.67 ± 0.99	5.09	6.24	
0.05% Atropine plus 2% Caffeine	Left	15	5.42 ± 0.95	4.89	5.95	0.33
	Right	15	5.72 ± 0.68	5.35	6.10	
0.1% Atropine	Left	15	5.18 ± 0.81	4.73	5.63	0.20
	Right	15	5.56 ± 0.73	5.15	5.96	
0.1% Atropine plus 2% Caffeine	Left	15	5.09 ± 0.88	4.60	5.57	0.28
	Right	15	5.43 ± 0.82	4.98	5.88	
2% Caffeine	Left	30	5.32 ± 0.97	4.96	5.69	0.23
	Right	30	5.59 ± 0.74	5.32	5.87	
Photopic pupil size						
0.05% Atropine	Left	14	3.22 ± 0.81	2.75	3.69	0.14
	Right	14	3.43 ± 0.87	2.93	3.93	
0.05% Atropine plus 2% Caffeine	Left	15	3.02 ± 0.28	2.87	3.18	0.49
	Right	15	3.09 ± 0.26	2.95	3.24	
0.1% Atropine	Left	15	2.88 ± 0.27	2.73	3.03	***0.02***
	Right	15	3.34 ± 0.66	2.97	3.70	
0.1% Atropine plus 2% Caffeine	Left	15	2.93 ± 0.35	2.73	3.12	0.13
	Right	15	3.19 ± 0.55	2.88	3.49	
2% Caffeine	Left	30	2.92 ± 0.33	2.80	3.05	***0.04***
	Right	30	3.13 ± 0.41	2.98	3.28	

The change in pupillary diameter, both in photopic and mesopic conditions, were monitored at 20, 40 and 60-minute post instillation (Day 0), on the following day (Day 1) at approximately 9 AM (9Hrs), 12 PM (12Hrs) and 3 PM (15Hrs).

5.4.3.2 Change in photopic pupil diameter with 0.05% Atropine, 0.05% Atropine + 2% Caffeine and 2% Caffeine

Figure 5.2 and Supplement E.E.2 summarise the effect of 0.05% Atropine, 0.05% Atropine + 2% Caffeine and 2% Caffeine from post-instillation to 60 minutes and at 9Hrs, 12Hrs and 15Hrs on Day 1.

As seen from the figure, there was no noticeable variation in the pupillary diameter with 2% Caffeine, whereas pupillary diameter increased with both the Atropine groups. Importantly, a greater change and variability was observed with 0.05% Atropine alone. At 20-minutes post-instillation, the 25^{th}/ median/75^{th} percentiles were 0.36/1.10/1.75mm versus 0.23/0.96/1.53mm with 0.05% Atropine versus 0.05% Atropine + 2% Caffeine, respectively. Similar but greater difference was observed at other visits on Day0 after the instillation and on Day1.

The mean change in photopic pupillary diameter over the observation period (Supplement E.E.3a) indicates a greater change from baseline in eyes receiving 0.05% Atropine alone at all-time points (Supplement E.E.3b). However, the differences between groups (0.05% Atropine versus 0.05% Atropine plus 2% Caffeine) were not significant (Supplement E.E.9).

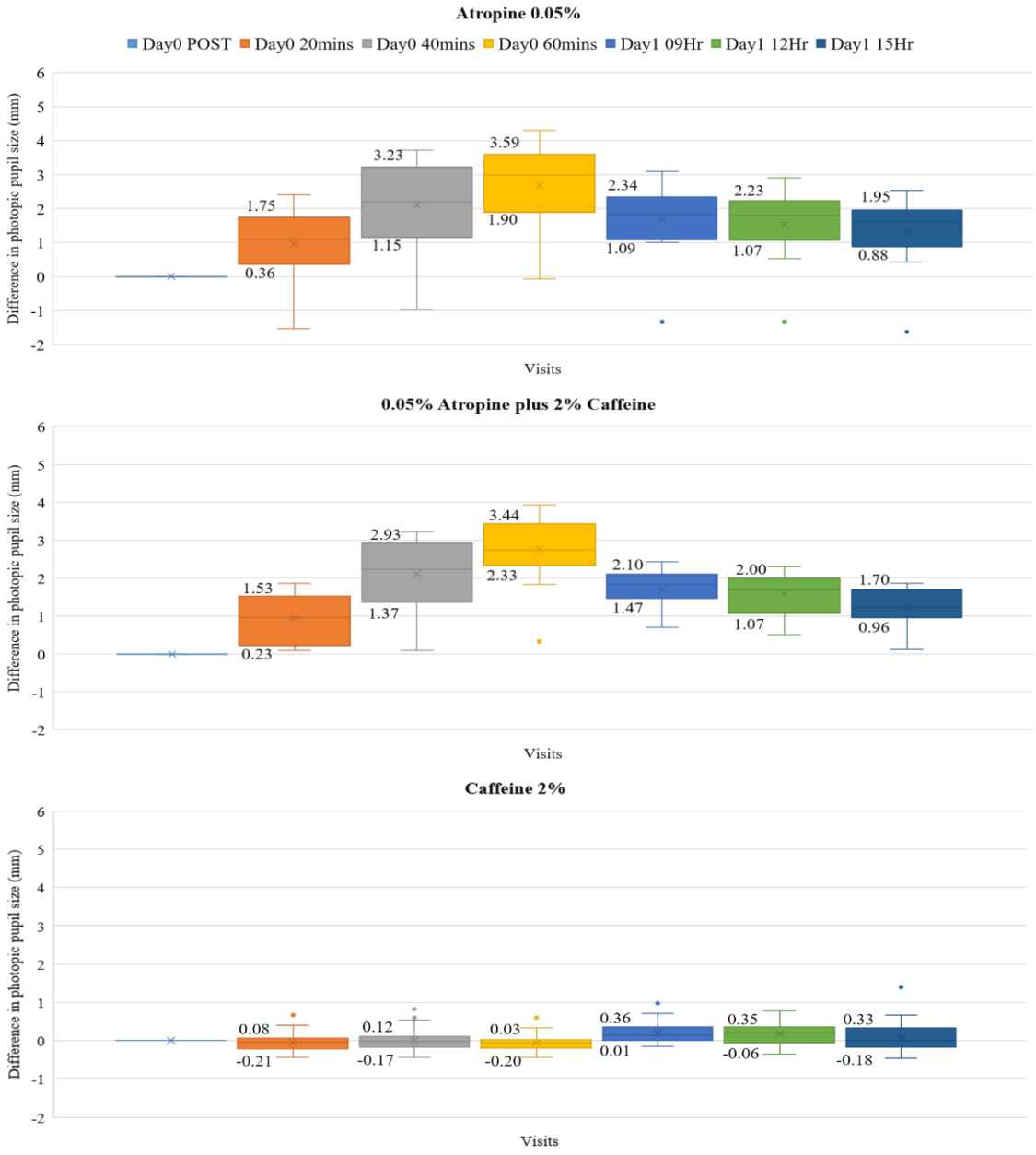

Figure 5.2: Temporal variation in photopic pupillary diameter with 0.05% Atropine, 0.05% Atropine+2% Caffeine and 2% Caffeine

Furthermore, the frequency of eyes demonstrating change in 1 mm steps at each of the time points was considered (Figure 5.3). Soon after instillation, none of the eyes exhibited any change of ±1mm. Similarly, at 20-, 40- and 60-minutes post instillation, none of the eyes that received

Caffeine exhibited any change of ±1mm steps. In comparison, a greater percent of eyes on Atropine 0.05% exhibited a greater shift/increase in pupillary diameter of +1mm steps compared to eyes that received 0.05% Atropine + 2% Caffeine. At 20 minutes, 14.3% vs 0% of 0.05% Atropine versus 0.05% Atropine + 2% Caffeine had an increase in pupil diameter of 2 mms. At 40 minutes, 35.7% vs 20% of 0.05% Atropine vs 0.05% Atropine + 2% Caffeine eyes had increased in pupil diameter by 3 mms. At 60 minutes, 14.3% vs 0% of 0.05% Atropine vs 0.05% Atropine plus 2% Caffeine increased in pupil diameter by 4 mms.

Results for Day 1 (post-instillation, Figure 5.4) indicate that eyes on 0.05% Atropine and combination eye drop were in recovery phase with a smaller increase in pupil diameter as compared to 60 minutes after instillation. At 9 hours on day 1, 7.1% vs 0% of eyes on Atropine 0.05% versus combination had a pupillary increase of 3mm. At approximately 12 hours on day1, 42.9% vs 26.7% of eyes on 0.05% Atropine vs 0.05% Atropine plus 2% Caffeine, respectively had a 2 mm increase in pupil diameter from baseline. At 15 hours on day 1, 21.4% vs 0% of eyes on 0.05% Atropine vs 0.05% Atropine plus 2% Caffeine had a pupillary increase of 2 mm from baseline. Although an equal number of both the Atropine groups demonstrated a change in photopic pupil diameter, a greater proportion of the 0.05% eyes had a faster increase in pupil diameter with a larger change compared to those on the combination.

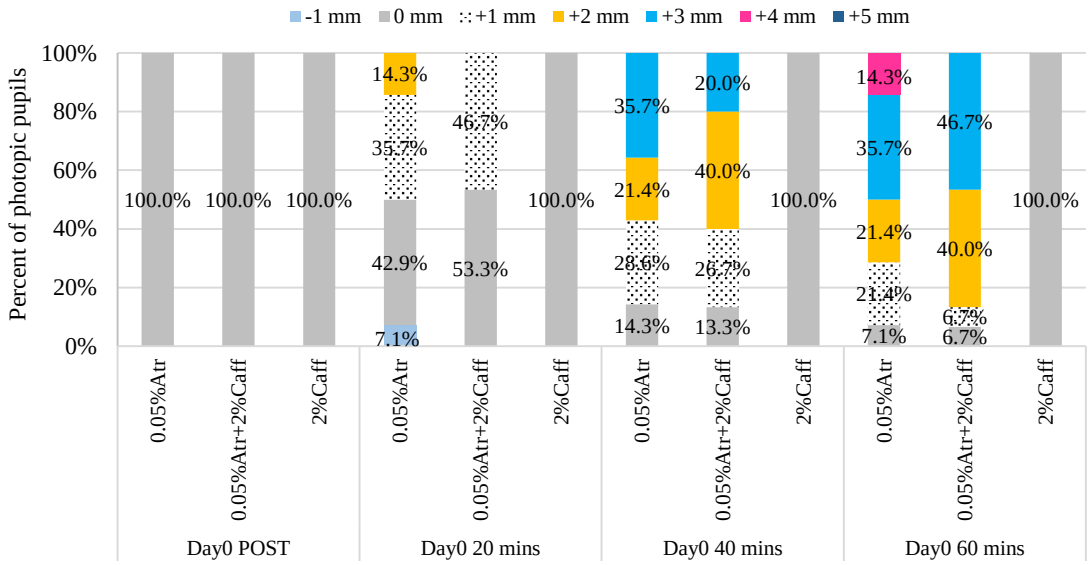

Figure 5.3: Temporal variation in photopic pupillary diameter with 0.05% Atropine: post-instillation to 60 minutes

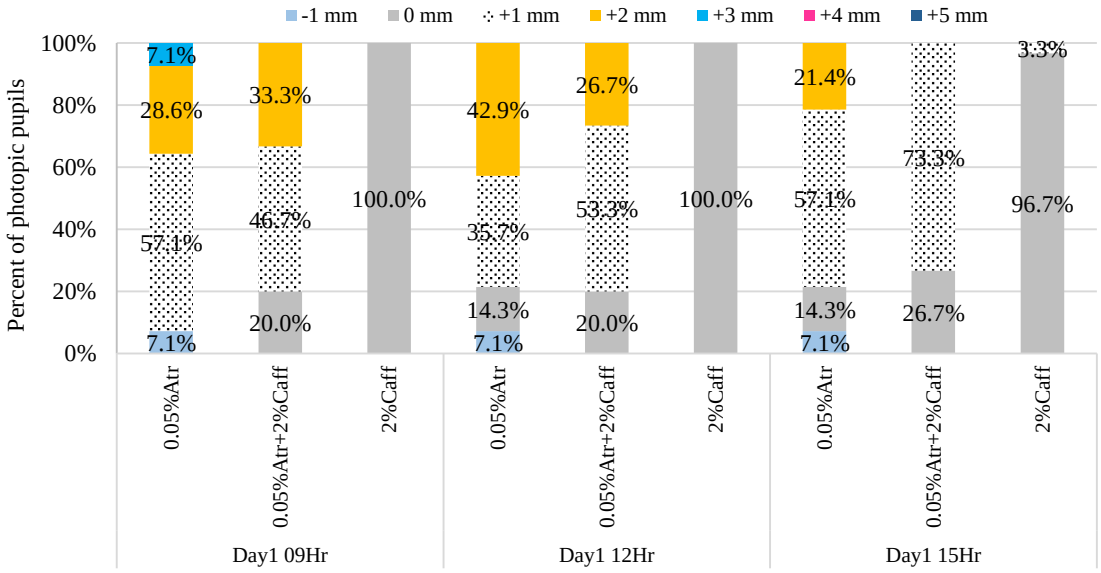

Figure 5.4: Temporal variation in photopic pupillary diameter with 0.05% Atropine: Day 1

5.4.3.3 Change in mesopic pupil diameter with 0.05% Atropine, 0.05% Atropine + 2% Caffeine and 2% Caffeine

Over the observation period, similar to changes seen with the photopic pupil diameter, a greater change and more variability was observed with 0.05% Atropine alone (Figure 5.5 and Supplement E.E.2). At 60 minutes after the instillation, the 25th/median/75th percentiles were 0.86/1.15/1.95mm vs 0.84/1.30/1.83mm with 0.05% Atropine vs 0.05% Atropine+2%Caffeine, respectively. Similar but greater difference was observed at other visits on Day0 after the instillation and on Day1. The mean mesopic pupillary diameter and change over time is presented in Supplement E.E.4. However, the differences between groups (0.05% Atropine versus 0.05% Atropine plus 2% Caffeine) were not significant (Supplement E.E.9).

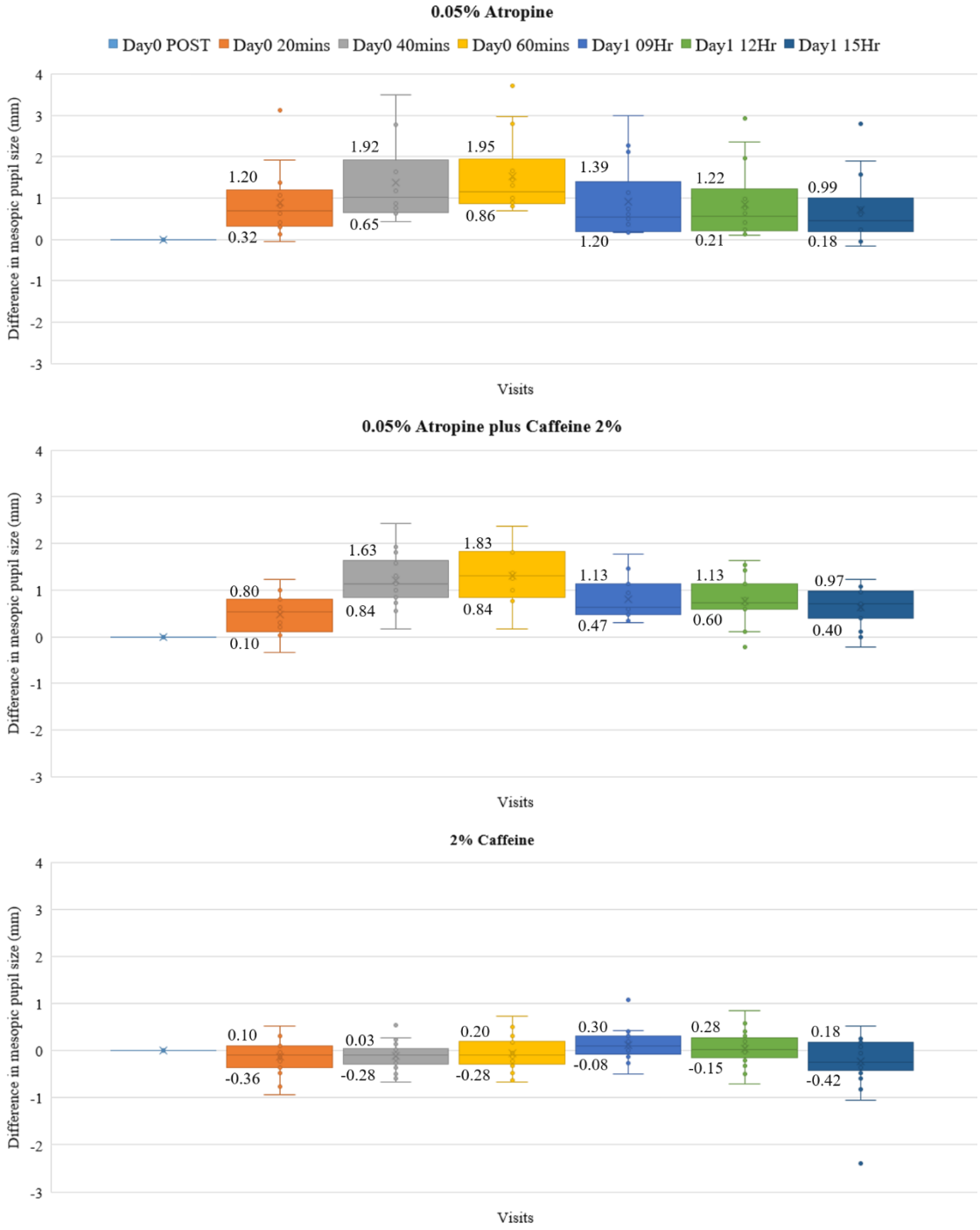

Figure 5.5: Temporal variation in mesopic pupillary diameter with 0.05% Atropine, 0.05% Atropine+2% Caffeine and 2% Caffeine

Furthermore, observations of change in pupillary diameter recorded in 1 mm steps (Figure 5.6 and Figure 5.7) for each of the time points demonstrate that a greater no of Atropine 0.05% eyes had a greater increase at each of the time points. At 20-, 40- and 60-minutes post instillation, none of the eyes that received Caffeine exhibited any pupil diameter changes of ±1mm steps. At 20, 40 and 60 minutes, 7.1% versus 0% of 0.05% Atropine vs 0.05% Atropine plus 2% Caffeine had a change in pupil diameter by 3 mms (Figure 5.6). On the following day (post-instillation Day 1, Figure 5.7), results indicate that a greater percent of eyes on 0.05% Atropine alone had a larger increase in mesopic pupillary diameter at all-time points.

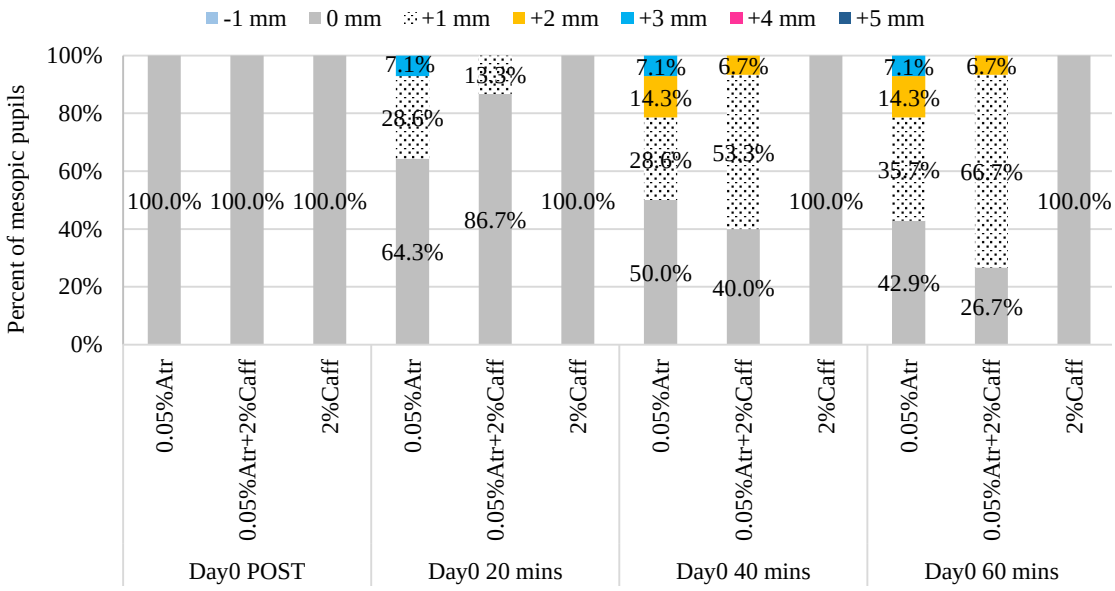

Figure 5.6: Temporal variation in mesopic pupillary diameter with 0.05% Atropine: post-instillation to 60 minutes

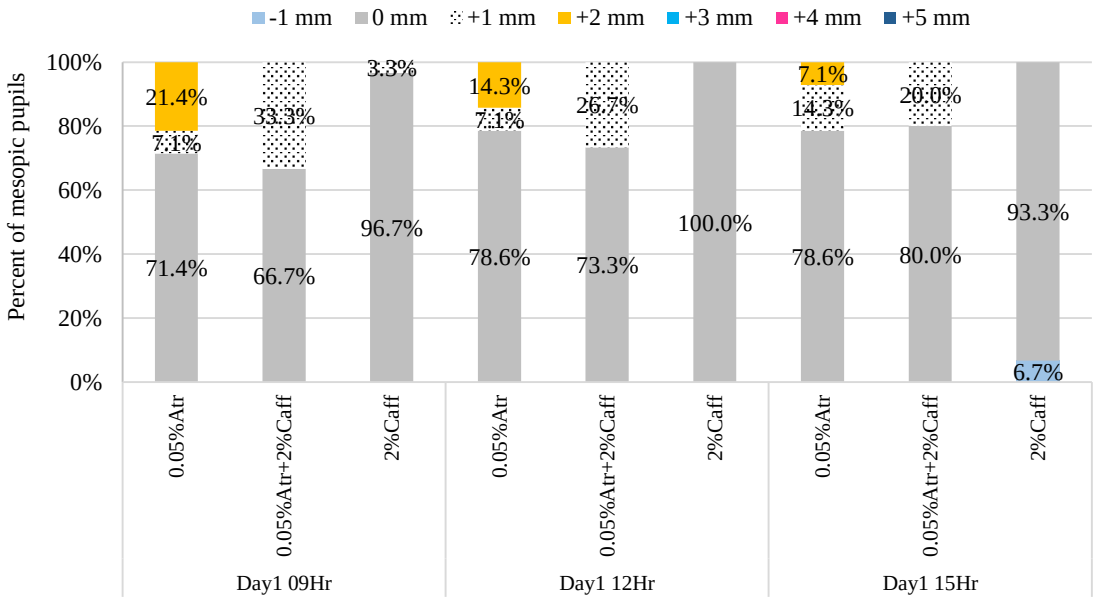

Figure 5.7: Temporal variation in mesopic pupillary diameter with 0.05% Atropine: Day 1

5.4.3.4 Change in photopic pupil diameter with 0.1% Atropine, 0.1% Atropine + 2% Caffeine and 2% Caffeine

Figure 5.8 shows that there was no variation in the pupil diameter with 2% Caffeine. In comparison, a greater change in pupil diameter and more variability was observed with 0.1% Atropine at each of the time points (Figure 5.8 and Supplement E.E.2). At 20 minutes after the instillation, the 25th /median/75th percentiles were 0.77/1.44/2.57mm vs 0.86/1.40/1.86mm with 0.1% Atropine vs 0.1% Atropine+2% Caffeine, respectively. The mean differences between groups (0.1% Atropine versus 0.1% Atropine plus 2% Caffeine) were not significant (Supplements E.E.5 and E.E.9).

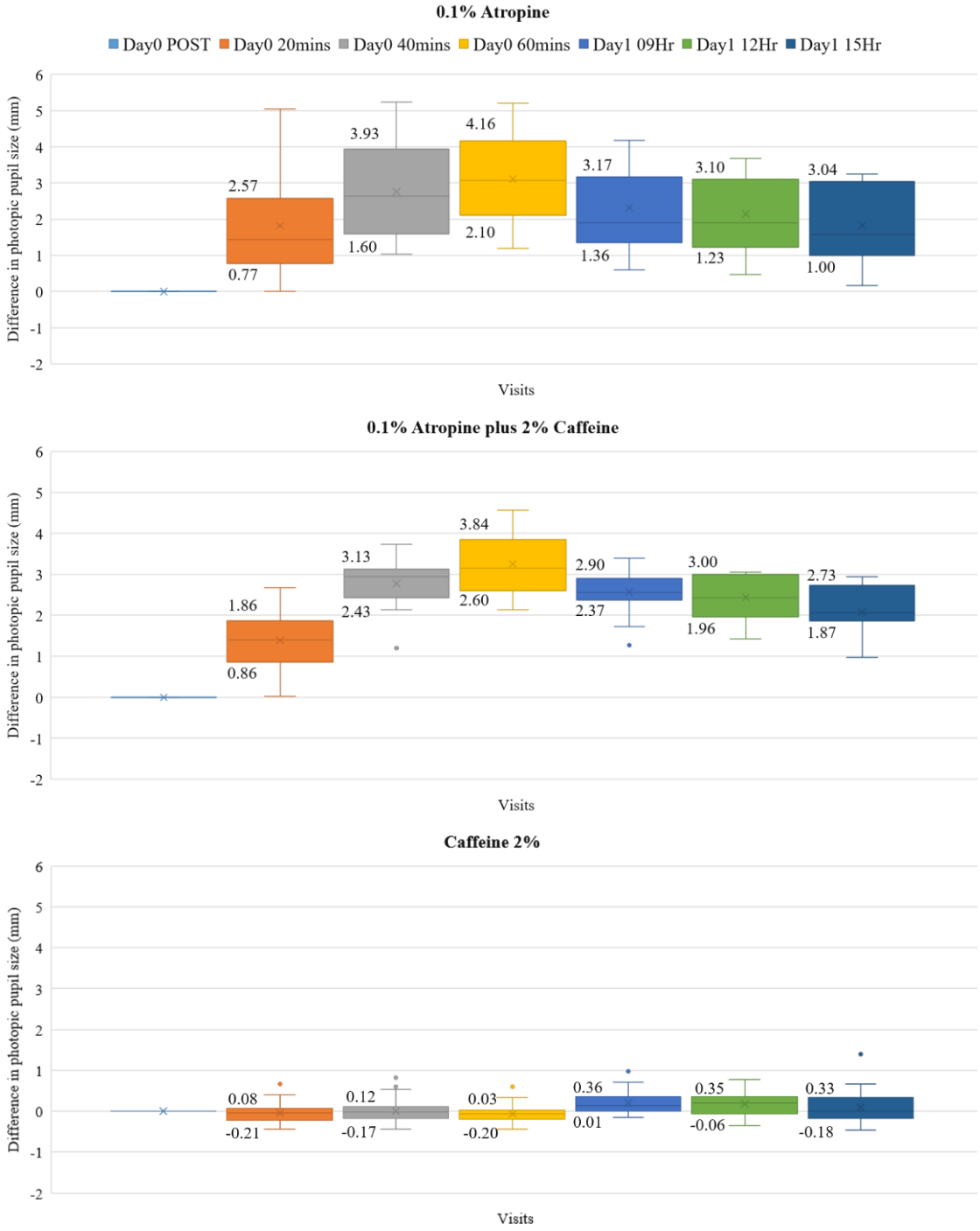

Figure 5.8: Temporal variation in photopic pupillary diameter with 0.1% Atropine, 0.1% Atropine+2% Caffeine and 2% Caffeine

Furthermore, observations of change in photopic pupillary diameter recorded in 1 mm steps (Figure 5.9 and Figure 5.10) for each of the time points demonstrate that a greater no of 0.1%

Atropine eyes had a greater increase at each of the time points. At 20 minutes, 13.4% vs 0% of 0.1% Atropine vs 0.1% Atropine plus Caffeine 2% had a change in pupil diameter of 4mms and more. Similarly, at 40 minutes, 20% vs 0% of eyes on 0.1% Atropine vs 0.1% Atropine plus 2% Caffeine had increased in pupil diameter by 4 mms or more. At 60 minutes, 33.4% vs 13.3% of eyes on 0.1% Atropine and 0.1% Atropine plus Caffeine 2% had increased in pupil diameter by 4 mms. The difference in groups was significant ($p<0.001$, Figure 5.9).

The day 1 (post instillation, Figure 5.10) results indicate that at 9 Hrs on day 1, 6.7% vs 0% of eyes on 0.1% Atropine versus 0.1% Atropine plus 2% Caffeine had a pupillary increase of 4mm. At approximately 12 Hrs on day 1, there was no difference between 0.1% Atropine groups but at 15Hrs, 26.79% vs 0% of eyes had a 3 mm increase in pupil diameter from baseline with 0.1% Atropine vs 0.1% Atropine plus 2% Caffeine, respectively.

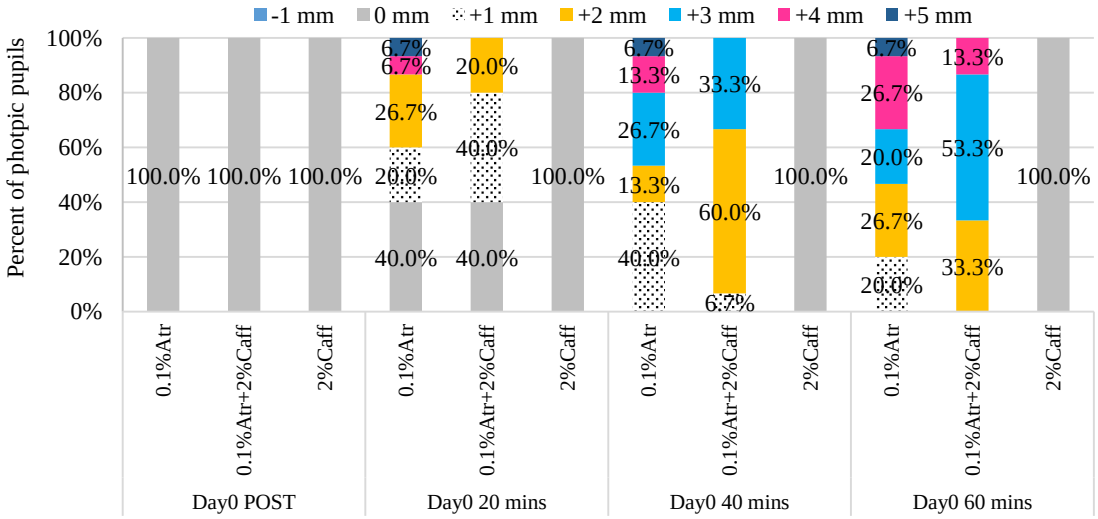

Figure 5.9: Temporal variation in photopic pupillary diameter with 0.1% Atropine: post-instillation to 60 minutes

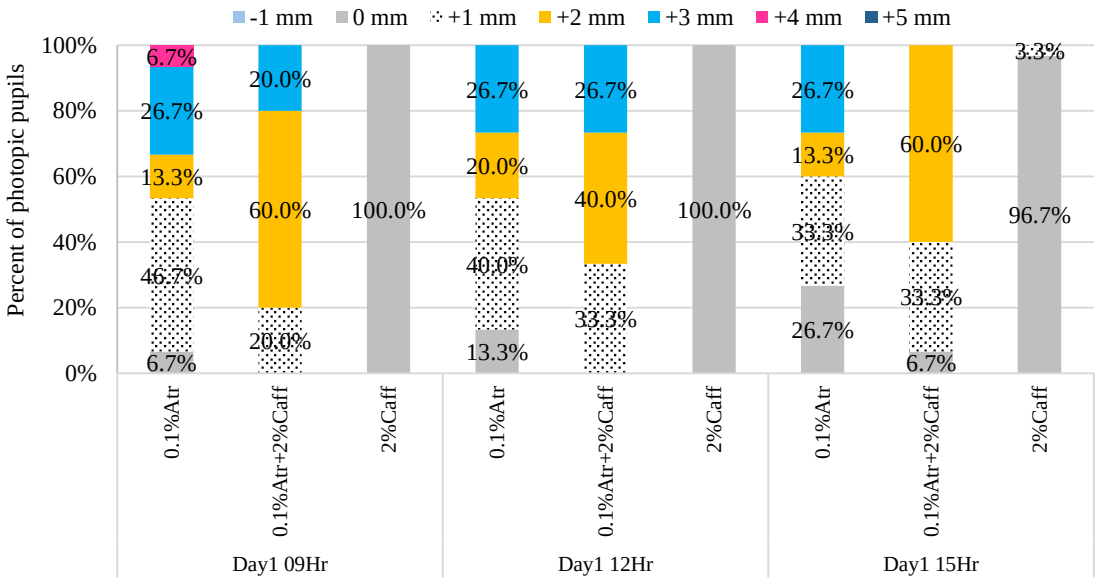

Figure 5.10: Temporal variation in photopic pupillary diameter with 0.1% Atropine: Day 1

5.4.3.5 Change in mesopic pupil diameter with 0.1% Atropine, 0.1% Atropine + 2% Caffeine and 2% Caffeine

Figure 5.11 shows that there was no variation in the pupil diameter with 2% Caffeine. In comparison, a greater change in pupil diameter and more variability was observed with 0.10% Atropine at each of the time points (Figure 5.11 and Supplement E.E.2). At 60 minutes after the instillation, the 25th /median/75th percentiles were 0.90/1.50/2.34 mm vs 1.13/1.43/2.63 mm with 0.1% Atropine vs 0.1% Atropine+2%Caffeine, respectively. There was more controlled response with 0.1% Atropine+2% Caffeine but the mean responses between groups were not significant (Supplements E.E.6 and E.E.9).

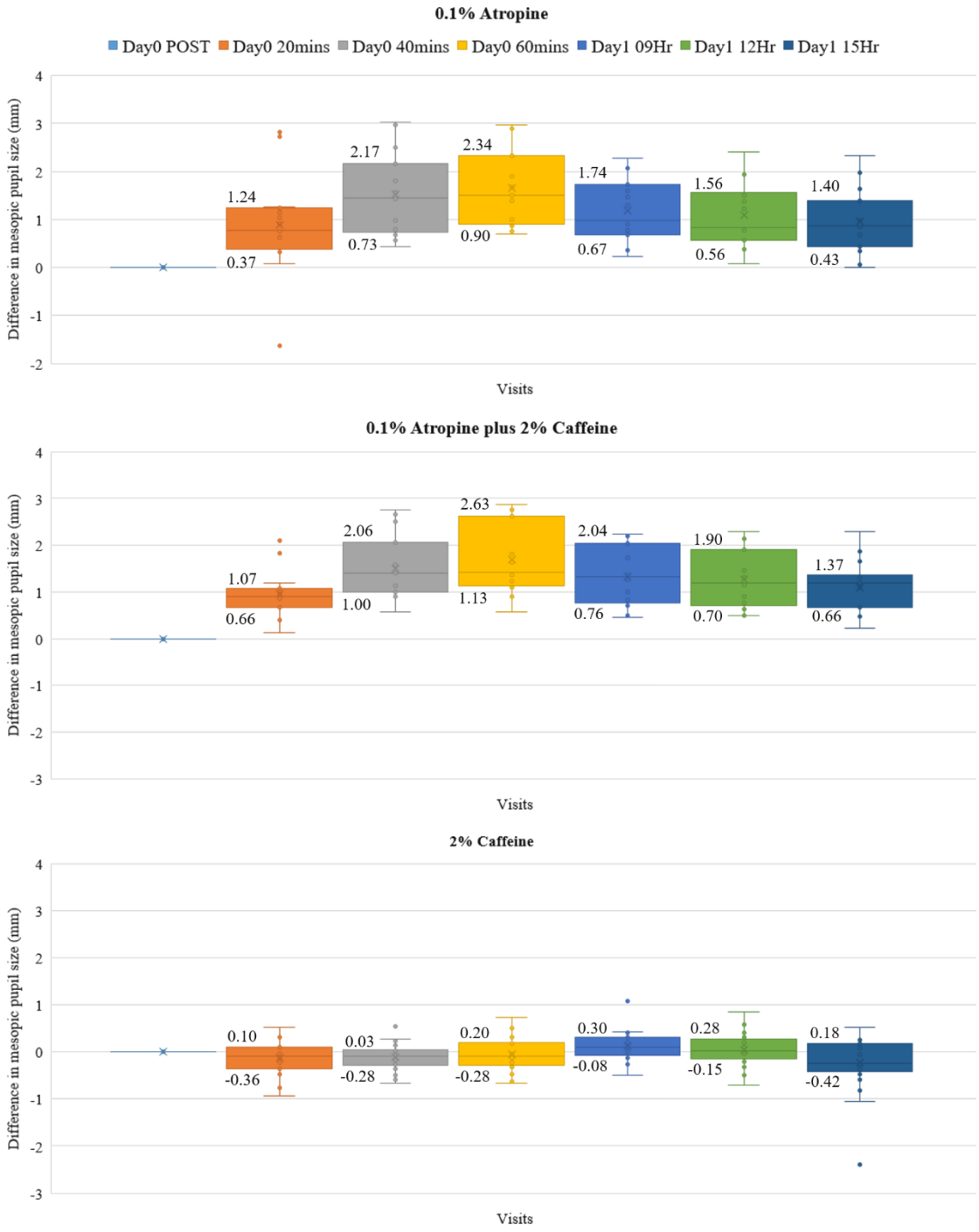

Figure 5.11: Temporal variation of mesopic pupillary diameter with 0.1% Atropine, 0.1% Atropine+2% Caffeine and 2% Caffeine

Figure 5.12 presents the percentage of eyes exhibiting the increase in mesopic pupillary diameter from baseline to 60 minutes post instillation (dilation phase, change recorded in 1 mm steps).

None of the eyes exhibited any change of ±1mm immediately post instillation. Similarly, at 20-, 40- and 60-minutes post instillation, none of the eyes that received 2% Caffeine exhibited any pupil diameter changes of ±1mm steps. In comparison, at 20 minutes, a greater percent of eyes on Atropine 0.1% alone exhibited a greater shift/increase in pupillary diameter of +2mm steps compared to eyes that received 0.1% Atropine plus 2% Caffeine, with 13.3% vs 6.7%, respectively. At 40 minutes, 6.7% versus 0% of 0.1% Atropine versus 0.1% Atropine plus 2% Caffeine had a change in pupil diameter of +3 mms. On the following day 1 (post instillation, Figure 5.13), results indicate that a greater, but insignificantly different, percent of eyes on 0.1% Atropine plus 2% Caffeine had a larger increase in mesopic pupillary diameter of +2mms at all-time points on day 1. There was none of eyes in all groups having increase of mesopic diameter (Figure 5.13).

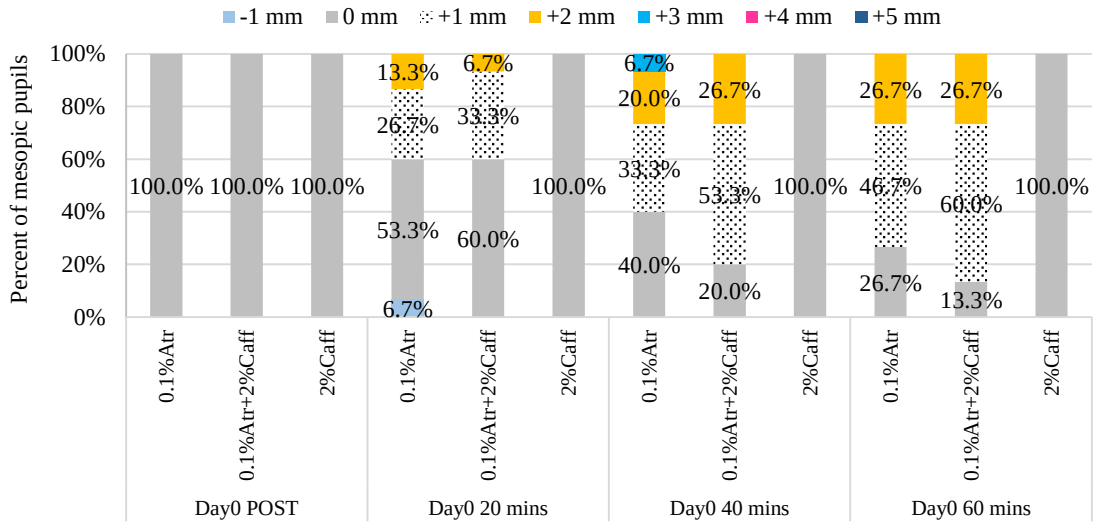

Figure 5.12: Temporal variation in mesopic pupillary diameter with 0.1% Atropine: post instillation to 60 minutes

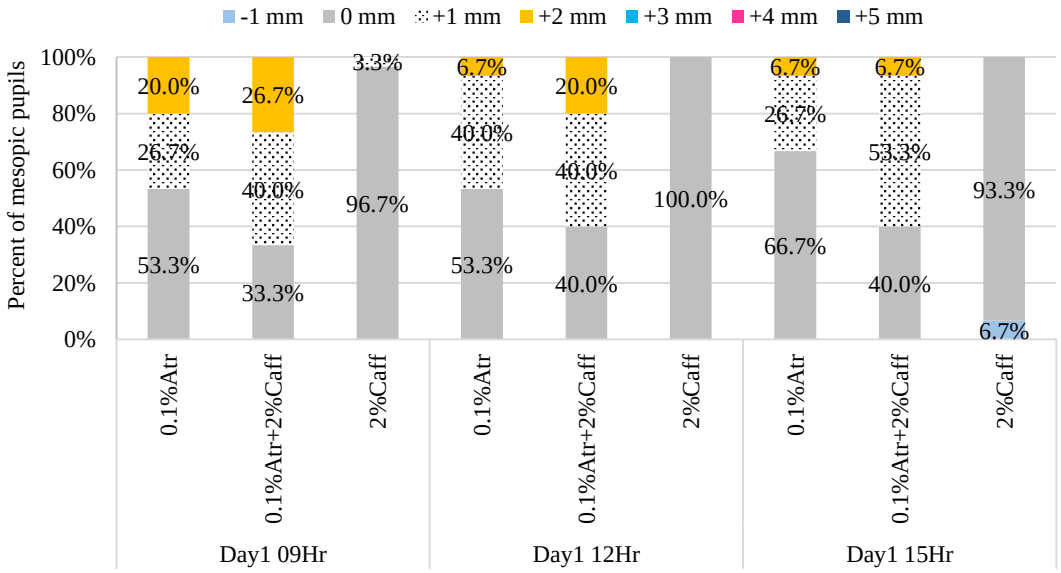

Figure 5.13: Temporal variation in mesopic pupillary diameter with 0.1% Atropine: Day 1

5.4.3.6 Change in pupil diameter - Monotherapy versus combination therapy

The change in pupillary diameter with the monotherapy versus combination therapy for each time-point, for photopic versus mesopic conditions and between the concentrations, was considered

and cut off criteria determined for a significant increase in pupil diameter. The cut-off limit varied for each time point as the pupil diameter increased with time on Day 0 and reduced on Day 1 (Table 5.8). Thereafter, for each time point, the frequency of eyes that had a pupillary diameter increase ≥ the pupil limit and <pupil limit was determined, and a logistic regression analysis was conducted for time points on Day 0 and Day 1.

Table 5.8: Cut-off criteria for change in pupil diameter under mesopic and photopic condition

Pupils Diameter (mm)	Day0 20min	Day0 40min	Day0 60min	Day1 09Hr	Day1 12Hr	Day1 15Hr
Photopic condition	2	3	4	3	3	3
Mesopic condition	2	2	2	2	2	2

Table 5.9 presents the logistic regression model which indicates a significant difference between monotherapy versus combination therapy on Day 0 and for 40 minutes versus 20 minutes. The odds of the pupil crossing the limit was greater with combination therapy compared to monotherapy. The differences on Day 1 were not significant (Table 5.9).

Table 5.9: Logistic regression model

Visit	Factors	p-value	Odds Ratio	95% CI.	
				Lower	Upper
Day 0	Atropine (Mono vs. Combo)	***0.023***	2.39	1.13	5.08
	Study_Arm (0.05% vs 0.1%)	0.201	0.51	0.18	1.44
	Light (Mesopic vs. Photopic)	0.192	0.63	0.31	1.26
	Visit 40 vs 20	***0.004***	2.66	1.37	5.18
	Visit 60 vs 20	0.279	1.51	0.72	3.16
Day 1	Atropine (Mono vs. Combo)	0.347	1.87	0.51	6.87
	Study_Arm (0.05% vs 0.1%)	***0.022***	0.21	0.06	0.80
	Light (Mesopic vs. Photopic)	0.731	0.88	0.44	1.78
	Visit 12 vs 9Hr	0.058	0.68	0.46	1.01
	Visit 15 vs 9Hr	***0.000***	0.31	0.17	0.56

5.4.4 *Temporal variation in accommodative amplitude with the various compositions*

Immediately post instillation (Day0), the mean accommodative amplitude of the treated right eyes with 0.05% Atropine, 0.05% Atropine plus 2% Caffeine, 0.1% Atropine, 0.1% Atropine plus 2% Caffeine, and 2% Caffeine was 9.41 ± 1.63 D, 9.72 ± 1.32 D, 9.29 ± 1.28 D, 9.39 ± 1.78 D, 9.44 ± 1.44 D, respectively and was not different compared to the accommodative amplitude in the non-treated left eyes ($p > 0.05$, Table 5.10).

Table 5.10: Mean accommodative amplitude post-instillation (Day0_Post)

Accommodative amplitude		Eyes	Mean ± SD	95% CI		p-value
				Lower CI	Upper CI	
0.05% Atropine	Left	14	10.96 ± 1.85	8.98	11.11	0.24
	Right	14	9.41 ± 1.63	8.47	10.35	
0.05% Atropine plus 2% Caffeine	Left	15	9.99 ± 1.49	9.16	10.82	0.60
	Right	15	9.72 ± 1.32	8.98	10.45	
0.1% Atropine	Left	15	9.48 ± 1.22	8.81	10.16	0.62
	Right	15	9.29 ± 1.28	8.59	10.00	
0.1% Atropine plus 2% Caffeine	Left	15	9.57 ± 1.86	8.54	10.60	0.79
	Right	15	9.39 ± 1.78	8.40	10.38	
2% Caffeine	Left	30	10.09 ± 1.57	9.50	10.67	0.10
	Right	30	9.44 ± 1.44	8.91	9.98	

5.4.4.1 Change in accommodative amplitude with 0.05% Atropine, 0.05% Atropine + 2% Caffeine and 2% Caffeine

Table 5.11, Figure 5.14 and Figure 5.15 present the frequency of eyes with accommodative amplitude categorized into groups with ≤5D, more than 5D to 10D, more than 10D to 15D and >15D for each of the time points considered in the study. A single eye with 0.05% Atropine was recorded at <5D of accommodative amplitude from 60-minute post instillation until Day 1.

Table 5.11: Percent of treatment eyes with 0.05% Atropine at different levels of accommodative amplitude at each visit

		Count (Row %)					p-value
		AA ≤5D	5D<AA ≤10D	10D<AA ≤15D	AA >15D	Total	
Day0 POST	0.05% Atr	0 (0.0%)	10 (71.4%)	4 (28.6%)	0 (0.0%)	14 (100,0%)	0.479
	0.05% Atr + 2% Caff	0 (0.0%)	8 (53.3%)	7 (46.7%)	0 (0.0%)	15 (100,0%)	
	2% Caff	0 (0.0%)	21 (70.0%)	9 (30.0%)	0 (0.0%)	30 (100,0%)	
Day0 20 mins	0.05% Atr	0 (0.0%)	10 (71.4%)	4 (28.6%)	0 (0.0%)	14 (100,0%)	0.316
	0.05% Atr + 2% Caff	0 (0.0%)	11 (73.3%)	4 (26.7%)	0 (0.0%)	15 (100,0%)	
	2% Caff	0 (0.0%)	16 (53.3%)	14 (46.7%)	0 (0.0%)	30 (100,0%)	
Day0 40 mins	0.05% Atr	0 (0.0%)	11 (78.6%)	3 (21.4%)	0 (0.0%)	14 (100,0%)	0.432
	0.05% Atr + 2% Caff	0 (0.0%)	12 (80.0%)	3 (20.0%)	1 (6.7%)	15 (100,0%)	
	2% Caff	0 (0.0%)	22 (73.3%)	8 (26.7%)	0 (0.0%)	30 (100,0%)	
Day0 60 mins	0.05% Atr	1 (7.1%)	11 (78.6%)	2 (14.3%)	0 (0.0%)	14 (100,0%)	0.091
	0.05% Atr + 2% Caff	0 (0.0%)	14 (93.3%)	0 (0.0%)	1 (6.7%)	15 (100,0%)	
	2% Caff	0 (0.0%)	22 (73.3%)	8 (26.7%)	0 (0.0%)	30 (100,0%)	
Day1 09Hr	0.05% Atr	1 (7.1%)	12 (85.7%)	1 (7.1%)	0 (0.0%)	14 (100,0%)	0.327
	0.05% Atr + 2% Caff	0 (0.0%)	13 (86.7%)	2 (13.3%)	0 (0.0%)	15 (100,0%)	
	2% Caff	0 (0.0%)	21 (70.0%)	8 (26.7%)	1 (3.3%)	30 (100,0%)	
Day1 12Hr	0.05% Atr	1 (7.1%)	12 (85.7%)	1 (7.1%)	0 (0.0%)	14 (100,0%)	0.053
	0.05% Atr + 2% Caff	0 (0.0%)	14 (93.3%)	1 (6.7%)	0 (0.0%)	15 (100,0%)	
	2% Caff	0 (0.0%)	20 (66.7%)	10 (33.3%)	0 (0.0%)	30 (100,0%)	
Day1 15Hr	0.05% Atr	1 (7.1%)	12 (85.7%)	1 (7.1%)	0 (0.0%)	14 (100,0%)	***0.032***
	0.05% Atr + 2% Caff	0 (0.0%)	14 (93.3%)	1 (6.7%)	0 (0.0%)	15 (100,0%)	
	2% Caff	0 (0.0%)	19 (63.3%)	11 (36.7%)	0 (0.0%)	30 (100,0%)	

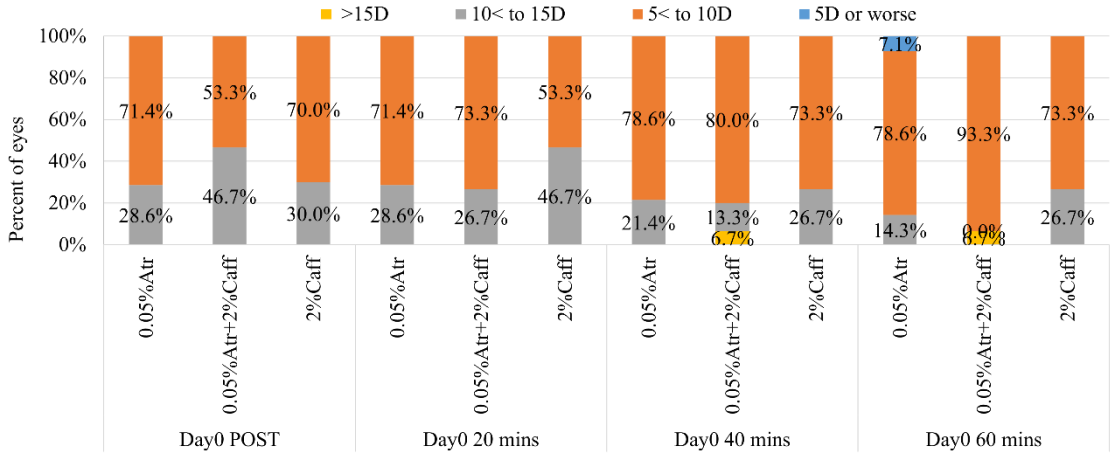

Figure 5.14: Temporal variation in accommodative amplitude with 0.05% Atropine: post-instillation to 60 minutes

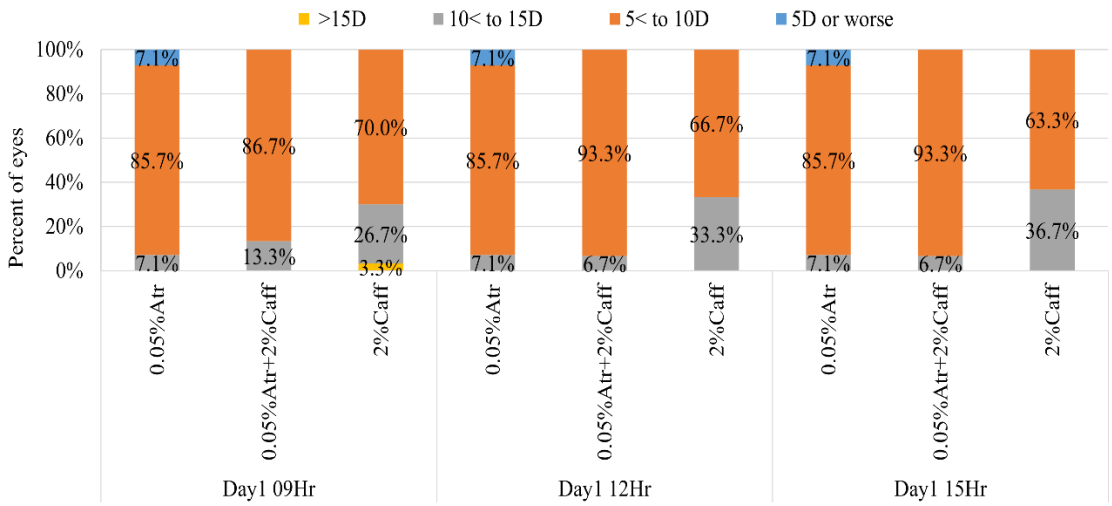

Figure 5.15: Temporal variation in accommodative amplitude with 0.05% Atropine: Day 1

Figure 5.16 and Supplement E.E.2 illustrate the change in accommodative amplitude over the observation period. The temporal variation in the accommodative amplitude is detailed in Supplement E.E.7. In the control left eyes, there were no differences between the groups at any of the visits ($p>0.05$, Supplement E.E.7a and Supplement E.E.9).

In the treatment right eyes, a) there was no significant effect on accommodative amplitude in eyes that received 2% Caffeine; b) both the Atropine groups (0.05% Atropine and 0.05% Atropine plus 2% Caffeine) demonstrated a significant reduction in accommodative amplitude that reached a peak at about 60 minutes and continued until 12 hrs. on Day 1 ($p < 0.05$, Supplement E.E.9), c) reduction in accommodative amplitude ranged from 0.26D to 1.48D with 0.05% Atropine and 0.40 to 1.65D with 0.05% Atropine plus 2% Caffeine and the differences between the groups were not significant. ($p>0.05$, Supplement E.E.7b and Supplement E.E.9) and d) difference in accommodative amplitude with the two 0.05% Atropine groups compared to non-treated eyes and 2% Caffeine groups was statistically significant at all visits (Supplement E.E.7b and Supplement E.E.9).

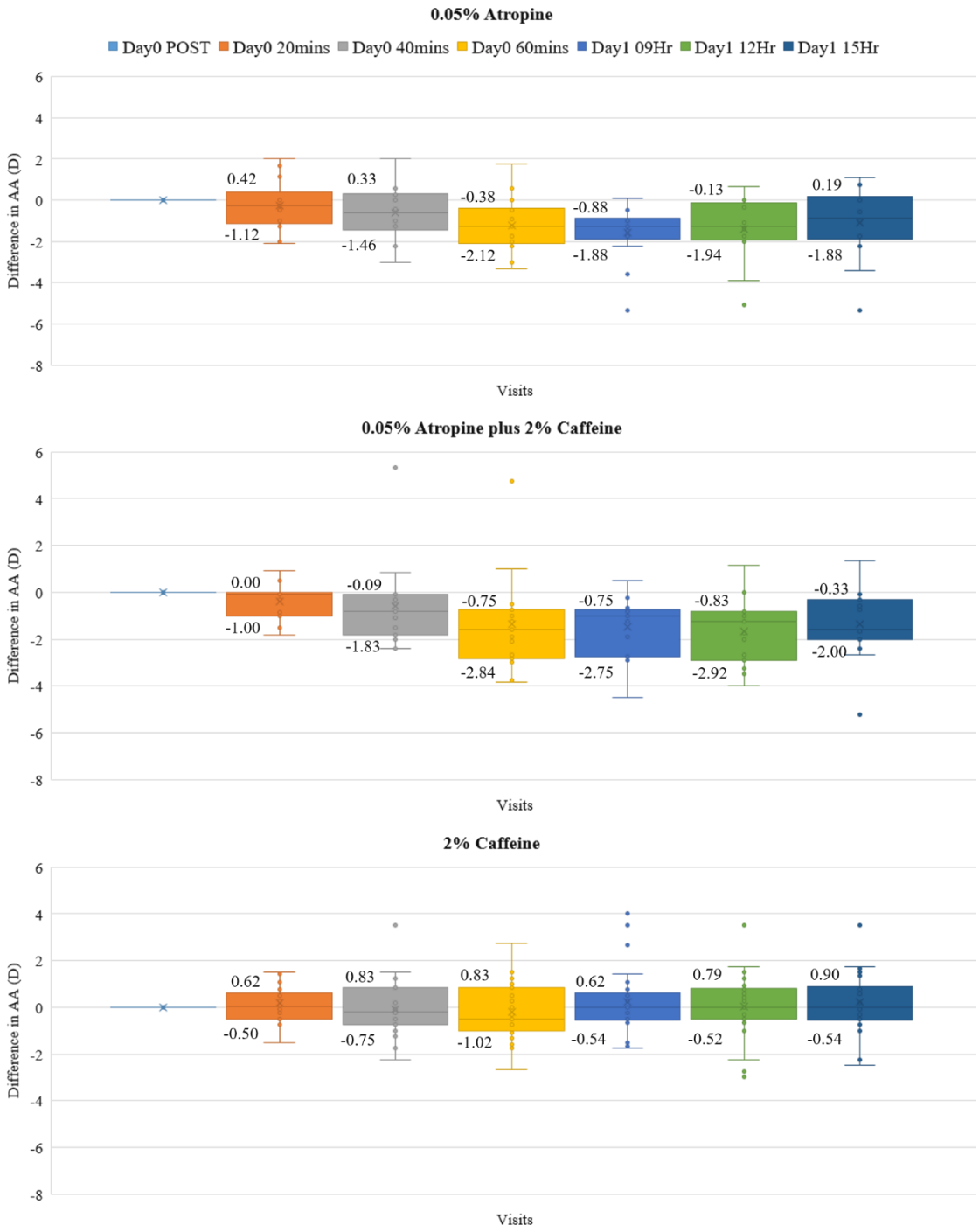

Figure 5.16: Temporal variation in accommodative amplitude with 0.05% Atropine, 0.05% Atropine+2% Caffeine and 2% Caffeine

5.4.4.2 Change in accommodative amplitude with 0.1% Atropine, 0.1% Atropine + 2% Caffeine and 2% Caffeine

Table 5.12, Figure 5.17 and Figure 5.18 present the frequency of eyes with accommodative amplitude categorized into groups with ≤5D, more than 5D to 10D, more than 10D to 15D and > 15D for each of the time points considered in the study.

At 20 and 40 minutes after the instillation, a greater percent of 0.1% Atropine had lower accommodative amplitude, whereas at 60-minute post instillation and on Day 1 time points, greater percent of eyes on 0.1% Atropine+2% Caffeine had accommodative amplitude ≤5D (Table 5.12).

Table 5.12: Percent of treatment eyes with 0.10% Atropine at different levels of accommodative amplitude at each visit

		Count (Row %)					p-value
		AA ≤5D	5D<AA ≤10D	10D<AA ≤15D	AA >15D	Total	
Day0 POST	0.1% Atr	0 (0.0%)	11 (73.3%)	4 (26.7%)	0 (0.0%)	15 (100,0%)	0.774
	0.1% Atr + 2% Caff	0 (0.0%)	12 (80.0%)	3 (20.0%)	0 (0.0%)	15(100,0%)	
	2% Caff	0 (0.0%)	21 (70.0%)	9 (30.0%)	0 (0.0%)	30 (100,0%)	
Day0 20 mins	0.1% Atr	0 (0.0%)	13 (86.7%)	2 (13.3%)	0 (0.0%)	15 (100,0%)	***0.006***
	0.1% Atr + 2% Caff	0 (0.0%)	14 (93.3%)	1 (6.7%)	0 (0.0%)	15(100,0%)	
	2% Caff	0 (0.0%)	16 (53.3%)	14 (46.7%)	0 (0.0%)	30 (100,0%)	
Day0 40 mins	0.1% Atr	0 (0.0%)	15 (100.0%)	0 (0.0%)	0 (0.0%)	15 (100,0%)	***0.036***
	0.1% Atr + 2% Caff	0 (0.0%)	14 (93.3%)	1 (6.7%)	0 (0.0%)	15(100,0%)	
	2% Caff	0 (0.0%)	22 (73.3%)	8 (26.7%)	0 (0.0%)	30 (100,0%)	
Day0 60 mins	0.1% Atr	0 (0.0%)	15 (100.0%)	0 (0.0%)	0 (0.0%)	15 (100,0%)	***0.017***
	0.1% Atr + 2% Caff	1 (6.7%)	14 (93.3%)	0 (0.0%)	0 (0.0%)	15(100,0%)	
	2% Caff	0 (0.0%)	22 (73.3%)	8 (26.7%)	0 (0.0%)	30 (100,0%)	
Day1 09Hr	0.1% Atr	0 (0.0%)	15 (100.0%)	0 (0.0%)	0 (0.0%)	15 (100,0%)	***0.004***
	0.1% Atr + 2% Caff	3 (20.0%)	12 (80.0%)	0 (0.0%)	0 (0.0%)	15(100,0%)	
	2% Caff	0 (0.0%)	21 (70.0%)	8 (26.7%)	1 (3.3%)	30 (100,0%)	
Day1 12Hr	0.1% Atr	0 (0.0%)	15 (100.0%)	0 (0.0%)	0 (0.0%)	15 (100,0%)	***0.005***
	0.1% Atr + 2% Caff	1 (6.7%)	14 (93.3%)	0 (0.0%)	0 (0.0%)	15(100,0%)	
	2% Caff	0 (0.0%)	20 (66.7%)	10 (33.3%)	0 (0.0%)	30 (100,0%)	
Day1 15Hr	0.1% Atr	0 (0.0%)	15 (100.0%)	0 (0.0%)	0 (0.0%)	15 (100,0%)	***0.010***
	0.1% Atr + 2% Caff	1 (6.7%)	12 (86.7%)	1 (6.7%)	0 (0.0%)	15(100,0%)	
	2% Caff	0 (0.0%)	19 (63.3%)	11 (36.7%)	0 (0.0%)	30 (100,0%)	

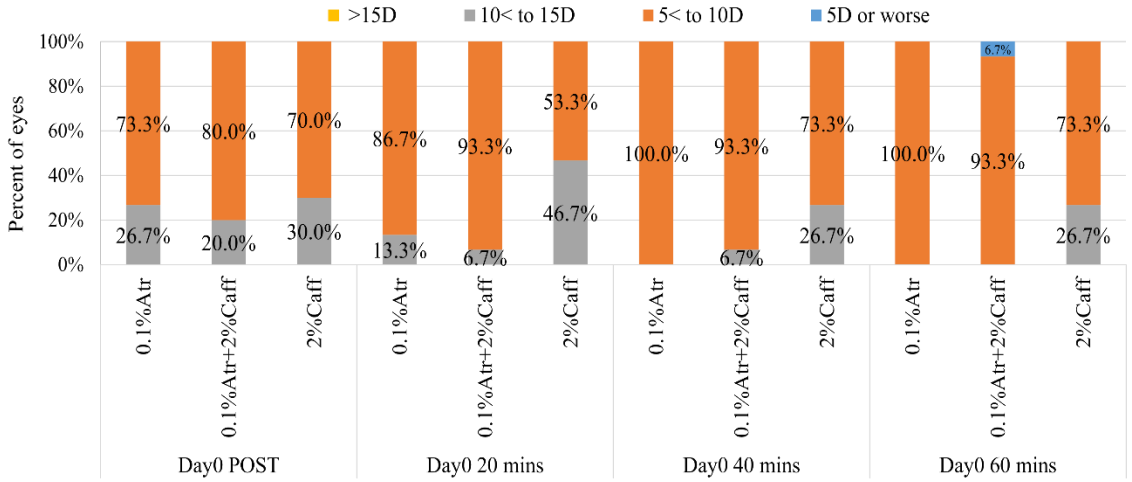

Figure 5.17: Temporal variation in accommodative amplitude with 0.1% Atropine: post-instillation to 60 minutes

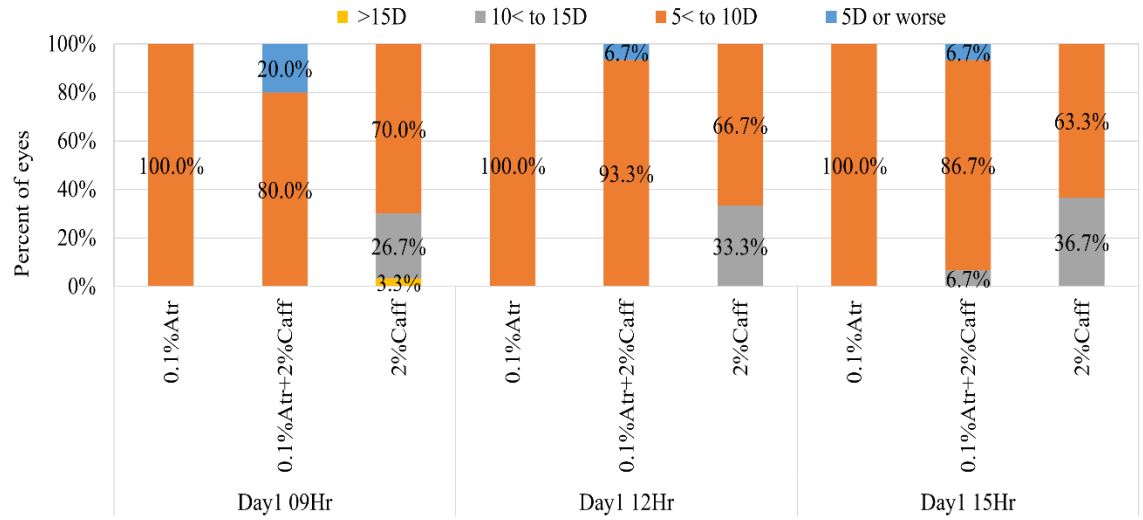

Figure 5.18: Temporal variation in accommodative amplitude with 0.1% Atropine: Day 1

Figure 5.19 and Supplement E.E.2 illustrate the peak effect of reducing the accommodative amplitude of both 0.1% Atropine groups at 9 AM on Day1 and the variation of change in AA over time with 0.1% Atropine versus 0.1% Atropine plus 2% Caffeine. Data of 2% Caffeine were also provided as vehicle composition as there was no change with the use of 2% Caffeine. A greater

range of variation was found with 0.1% Atropine plus 2% Caffeine than with 0.05% Atropine at all times (Supplements E.E.8 and E.E.9).

The temporal variation in the accommodative amplitude is additionally described in Supplements E.E.8. In the non-treated left eyes, there were no differences between the groups at any of the visits ($p>0.05$, Supplements E.E.8 and E.E.9).

In the treatment eyes, a) there was no significant effect on accommodative amplitude in eyes that received 2% Caffeine (Supplement E.E.8a); b) both the Atropine groups (0.1% Atropine and 0.1% Atropine plus 2% Caffeine) demonstrated a significant reduction in accommodative amplitude that reached a peak at about 60 minutes and continued until 12 hrs. on Day 1 ($p < 0.05$), c) reduction in accommodative amplitude ranged from 0.74D to 1.85D with 0.1% Atropine and 0.66 to 2.65D with 0.1% Atropine plus 2% Caffeine and the differences between the groups were not significant. ($p>0.05$, Supplement E.E.9) and d) difference in accommodative amplitude with the two 0.10% Atropine groups compared to control eyes and 2% Caffeine groups was statistically significant at all visits (Supplement E.E.8b and Supplement E.E.9).

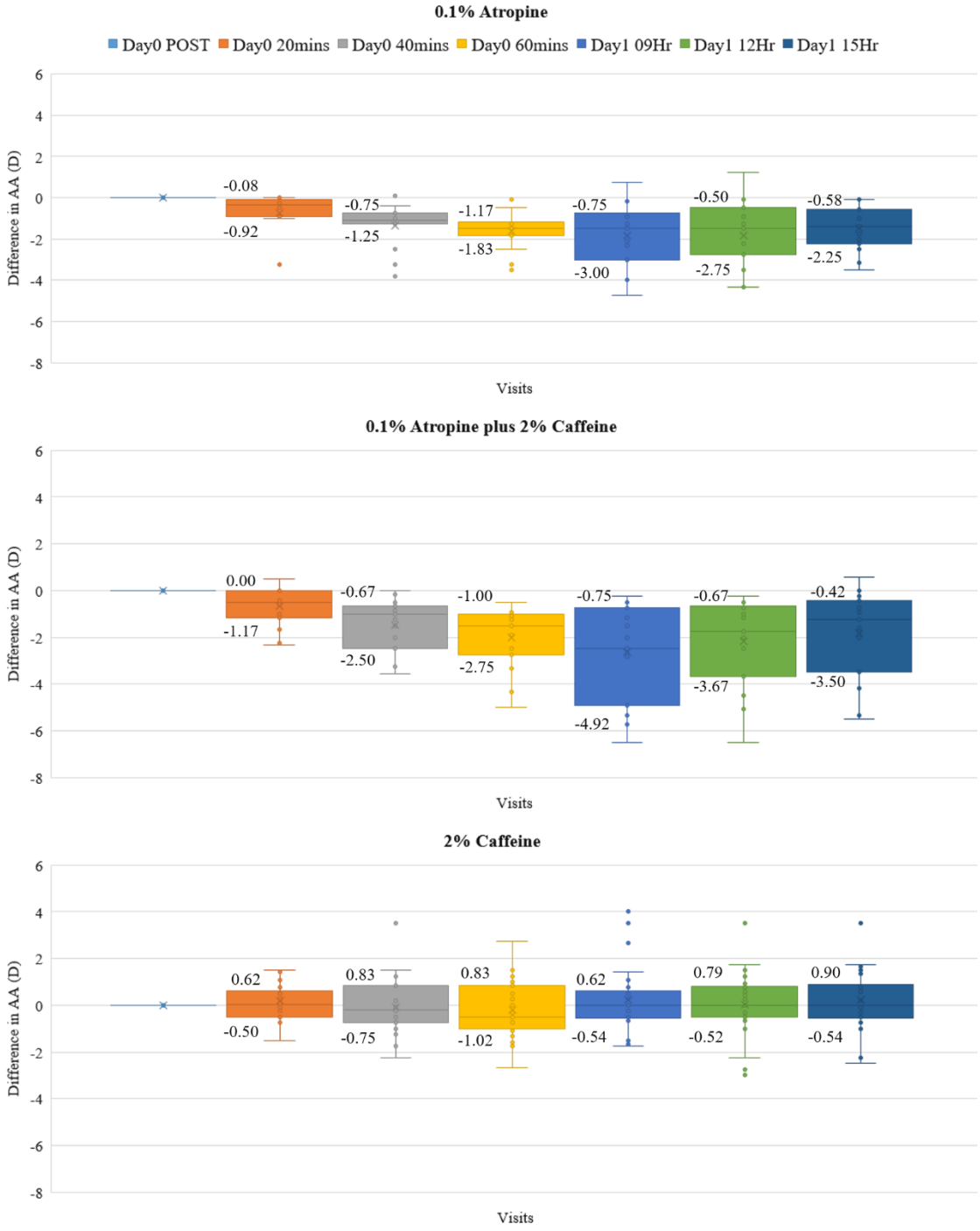

Figure 5.19: Boxplots of temporal variation accommodative amplitude with 0.1% Atropine, 0.1% Atropine+2% Caffeine and 2% Caffeine

5.4.5 *Intraocular pressure*

Table 5.13 describes the intraocular pressure measurements at baseline, 60 minutes after instillation, on day1 at 9 AM, 12 PM and 3 PM and compares between treated eyes and non-treated eyes. There was no statistically significant difference between eyes at any visits with any compounds ($p > 0.05$, Table 5.13).

Table 5.13: Intraocular pressure

Intraocular pressure	eyes		Pre-instillation		60 mins after instillation		On day1 at 9Hr		On day1 at 12Hr		On day1 at 15Hr	
			Mean ± SD	p-value	Mean ± SD	p-value	Mean ± SD	p-value	Mean ± SD	p-value	Mean ± SD	p-value
0.05% Atropine	Left	14	13.52 ± 1.44	0.42	13.57 ± 2.10	1.00	13.29 ± 2.40	0.64	12.93 ± 1.94	1.00	13.07 ± 1.98	0.68
	Right	14	13.05 ± 1.62		13.57 ± 2.17		12.86 ± 2.32		12.93 ± 1.98		12.71 ± 2.49	
0.05% Atropine plus 2% Caffeine	Left	15	14.80 ± 2.55	0.76	13.93 ± 2.31	0.94	14.27 ± 2.58	0.77	13.60 ± 2.75	0.78	13.73 ± 3.28	0.64
	Right	15	14.51 ± 2.51		14.00 ± 2.24		14.00 ± 2.27		13.33 ± 2.53		13.20 ± 2.88	
0.1% Atropine	Left	15	13.74 ± 2.12	0.26	13.60 ± 2.06	0.88	14.47 ± 1.46	1.00	14.07 ± 1.83	0.71	13.93 ± 1.44	0.52
	Right	15	14.64 ± 2.15		13.73 ± 2.55		14.47 ± 1.96		14.33 ± 2.09		14.33 ± 1.91	
0.1% Atropine plus 2% Caffeine	Left	15	14.09 ± 1.91	0.51	14.20 ± 2.04	0.64	14.20 ± 1.57	0.32	14.13 ± 1.68	0.26	13.40 ± 1.72	0.43
	Right	15	13.69 ± 1.37		13.87 ± 1.77		13.67 ± 1.29		13.53 ± 1.13		12.93 ± 1.44	
2% Caffeine	Left	30	13.94 ± 2.07	0.61	13.50 ± 2.18	0.72	13.90 ± 2.28	0.78	13.73 ± 2.08	0.34	13.37 ± 2.11	1.00
	Right	30	14.25 ± 2.67		13.70 ± 2.15		13.73 ± 2.23		13.20 ± 2.22		13.37 ± 2.17	

5.5 Discussion

The current trial demonstrates that eyes receiving combination therapy, i.e., Atropine and Caffeine had a lesser increase in pupillary diameter compared to those receiving Atropine alone. Utilising cut-off criteria for pupil diameter at each time point, the differences were found to be significant for Day 0 with eyes on atropine alone showing a greater increase in pupil diameter. This finding was consistent for both the Atropine concentrations studied, i.e., 0.05% and 0.1%. This finding is useful as it appears that combining Caffeine with Atropine may aid in reducing the risk of adverse side effects associated with long term Atropine monotherapy for myopia without impacting efficacy. The impact of the combination therapy on accommodative amplitude appears to be insignificant.

5.5.1 *Change of pupil size and accommodative amplitude*

5.5.1.1 Temporal change of pupillary diameter

Overall, 2% Caffeine did not result in any significant variation in the pupil diameter over the observation period; furthermore, the pupillary diameter in eyes receiving 2% Caffeine was similar to the pupillary diameter in the placebo left eyes. (Figure 4.15 and Figure 4.16). In comparison, both monotherapy (Atropine) and dual therapy (Atropine+Caffeine) resulted in an increase in pupillary diameter. For both the compositions (mono and combination), the pupil diameter had enlarged substantially at 20-minute post instillation and continued to increase in diameter until 60-minutes post instillation. On Day 1, the pupils appeared to be in the recovery mode, but were still larger compared to baseline and had not reached the baseline diameter at approximately 15 hrs (3 PM). Although at all time points there were fewer eyes with larger change in pupillary diameter and lesser variability in response when Atropine was combined with Caffeine, the differences were significant for Day 0 when the pupils were increasing in diameter. This change was consistent for both photopic and mesopic conditions. The combination therapy was well tolerated and there were no adverse effects. The improvement of this key measure relating to side

effect of Atropine indicates that Caffeine should be further explored as an option for limiting the side effect of Atropine.

We compared the results for mean increase in pupillary diameter with Atropine monotherapy to other published data (Table 5.14). Whilst there was some variation between studies, the differences were not vastly apart. For example, whilst the Atropine mean change in photopic and pupil diameter with 0.05% Atropine in the current is higher than that reported by Yam and co-workers (Table 5.14, (Yam et al. 2019)), it was comparable to Cooper (Cooper et al. 2013). Some of these variations may have resulted from study methodology or from the drug not having achieved the ceiling effect with a single dose. For example, in the current study, pupillary responses were tracked at frequent intervals post instillation and data from 60-minute post instillation were used for comparison as the pupillary dilation was maximal at this time point. In comparison, it appears that Yam et al, may be reporting Day 1 results as in their study Atropine instillation was nightly with follow-up examinations conducted during the day (Yam et al. 2019). Indeed, the change in mean photopic and mesopic pupillary diameter with 0.05% Atropine at the end of Day1 in our study were 1.31 ± 1.04 mm and 0.62 ± 0.42 mm respectively and align with the measurements of 1.03 ± 1.02 mm and 0.58 ± 0.63 mm reported by Yam (Yam et al. 2019). Similarly, we compared the change in pupillary diameter with 0.1% from the current trial to that reported by Chia and associates in 2012. Whilst the change in photopic pupil diameter was comparable, we observed a slightly lesser change in the mesopic pupil diameter (Table 5.14). The data for change in photopic pupillary diameter was comparable but a slightly greater difference in mesopic pupillary diameter was observed with Chia et al. 2012. Atropine(Chia et al. 2012). Differences may also result if the use of the drug does not result in the maximal or ceiling effect – this may be more likely with lower concentrations, but it appears that it might be unlikely with the concentrations considered in the current study.

Table 5.14: Comparison of pupil diameter and accommodative amplitude

Change at peak effects		Accommodative amplitude (D) - on Day1 9Hr	Mesopic pupillary dilation (mm) - on Day0 60mins	Photopic pupillary dilation (mm) - on Day0 60mins
0.05% Atropine	Our study	-1.58 ± 1.39	1.53 ± 0.95	2.68 ± 1.24
	Yam et al. 2019	-1.98 ± 2.82	0.58 ± 0.63	1.03 ± 1.02
	Cooper et al. 2013	-8.50	N/A	3.00
0.1% Atropine	Our study	-1.85 ± 1.59	1.65 ± 0.82	3.10 ± 1.25
	Chia et al. 2012	-10.9 ± 4.0	2.42 ± 0.91	2.77 ± 1.03

5.5.1.2 Temporal change of accommodative amplitude over 24-hour

Both monotherapy and combination therapy resulted in a decrease in accommodative amplitude on Day-0 with subsequent recovery on Day-1, however, there was more variability in the accommodative response with combination eye drops in comparison to Atropine alone. The reasons for this discrepancy with the accommodative response is not entirely clear but may also be related to the study procedure. We used a modified push-up technique with a -4D minus lens to evaluate accommodative amplitude. Table 5.14 provides a comparison of the reduction in accommodative amplitude on Day1-9Hr, with other evidence. Our data were similar to the decrease in AA reported by Yam et al. (Yam et al. 2019) but significantly smaller than those reported by Cooper et al with the use of 0.05% Atropine (Cooper et al. 2013) and Chia et al. for the use of 0.1% Atropine (Chia et al. 2012). Others had not used the minus lens (Cooper et al. 2013) for measurement of accommodative amplitude or had used push-up plus push-down method (Chia et al. 2012; Yam et al. 2019) which may have resulted in the discrepancy. It is well known that measurement of accommodative amplitude is influenced by many confounding factors, including methodology, age of individuals, ocular characteristics, clinical settings for the measurement (Rosenfield and Cohen 1996), individual psychological effects (Sterner et al. 2004; Burns et al. 2014) and diurnal change (Park et al. 2019). Majumder and Afnan indicated that varying the technique can result in significant variation in the accommodative amplitude (Abu et al. 2018; Majumder and Afnan 2020)

Table 5.15 indicates that the accommodative amplitude measurements may vary significantly depending on the type of measurement that is adopted. In young adults, use of modified push-up (as in the current study) indicates significantly less accommodative amplitude compared to for example a conventional push-up technique (see Abu et al. 2018 and Majumdar and Afnan 2020). The mean amplitude of 9.91 ± 1.50D from the current study is less than the 11.38 ± 2.03D reported from an Asian population (Majumder 2020). It is therefore not clear that given the lower accommodative amplitudes, the technique was discriminative to pick up variations in accommodative amplitude between the monotherapy versus dual therapy.

Table 5.15: Accommodative amplitude with age

Author – Year	Age group	Method of measurement	Mean ± SD	Notes and ethnicity
Abu 2018 (Abu et al. 2018)	10-19	Push-up	14.25 ± 3.33	- African
	20-29	Push-up	12.62 ± 1.81	
Mordi 1998 (Mordi and Ciuffreda 1998)	20-29	Push-up	9 ± 2	- Caucasian
Duane 1912 (Duane 1912)	20-29	Push-up	10 ± 3.5	- Caucasian
Yekta 2016 (Yekta et al. 2016)	11	Push-up	15.33	- West-Asian
	17	Push-up	10.40	
Park 2019 (Park et al. 2019)	10-19	Push-up	14.67 ± 3.29	- Asian - Diurnal change at between 1.1 and 1.8D (20-29 years old)
	20-29	Push-up	11.13 ± 2.74	
	40-49	Push-up	5.53 ± 1.10	
Majumder 2020 (Majumder and Afnan 2020)	21-27	Push-up	11.38 ± 2.03	- Asian
		Pull-away	10.35 ± 1.64	
		Modified push-up	8.26 ± 1.44	
Rosenfield 1995 (Rosenfield and Cohen 1995)	23-29	Push-up	10.11 ± 0.73	- Caucasian
Our study in Chapter 5	20-30	Modified push-up	9.91 ± 1.50	- Asian

5.5.2 *Safety data of the combination compounds with higher concentrations of Atropine*

Over the observation period, there were no incidents of raised intraocular pressure or significant reduction in near visual acuity. In comparison to the control eyes, there was no difference in

intraocular pressure with any of the compositions considered in the present study. Moreover, no serious adverse events were reported over the use of eye drops. Furthermore, other evidence reported that the use of Atropine and Caffeine did not affect the change in IOP (Chandra et al. 2011; Chia et al. 2012; Lee et al. 2016; Yam et al. 2019), thus in together, supported the safety aspects of the investigating compounds.

5.5.3 *Mechanisms underlying the difference between the change in accommodative amplitude and pupillary diameter with Atropine alone versus combination compounds*

The data suggest that the effects of the combination on pupillary diameter and accommodative amplitude were greater at the lower of the two concentrations of Atropine; a greater effect of Caffeine was demonstrated at 0.05% compared to 0.1% Atropine. Importantly, the data indicate a faster recovery with the combination compared to Atropine alone.

Atropine is a non-selective muscarinic receptor antagonist that binds to muscarinic receptors and blocks the release of acetylcholine. Muscarinic receptors are present at the iris sphincter and ciliary muscle. When Atropine is instilled in the eye, Atropine blocks the contraction of the iris sphincter thus causing an increase in pupillary diameter or mydriasis. The underlying mechanisms for the smaller pupillary increase with the combination are unclear. It is possible that when Atropine is combined with Caffeine, Caffeine may be selectively competing with Atropine for the muscarinic receptors at the iris and thus reducing the influence of Atropine on the iris. Or alternatively, Caffeine is thought to inhibit the acetylcholinesterase and therefore influence the concentration of acetylcholine (Ribeiro and Sebastião 2010; Pohanka and Dobes 2013) which may competitively reduce the antagonism effects of Atropine. Other possible mechanisms are that when Atropine is combined with Caffeine, the permeability of Atropine through the tissues is reduced or delayed; however, our data from the longitudinal study indicates that such a pathway did not alter the efficacy of Atropine on eye growth.

Greater variability of the accommodative amplitude was observed with Atropine 0.1% when combined with Caffeine. It is possible that Caffeine at the concentration used in the current study is not effective with higher doses of Atropine. Caffeine is considered to induce the release and enhancing the binding effect of dopamine at its receptor *via* the receptor complex, heterotetramer A_{2A}-D_2 (Volkow et al. 2015; Ferré et al. 2016).

5.5.4 *Strengths and limitations of the study*

The strength of the study was the longitudinal cross-over design wherein each participant evaluated all three compositions. We were able to demonstrate at an individual level that Atropine when used in the combination had an effect of reduced pupillary diameter in most participants. We also used a consistent approach to measure the pupil diameter- the positioning of the patient on the chin rest and the distance from the eye and alignment ensured that parallax errors associated with pupillary diameter measurement were mostly eliminated. The trial suffered from some limitations. We had expected to see differences in the average or mean response between the groups and the sample size was powered to observe such differences. However, we found variation with eyes achieving higher or lower increases in pupillary diameter and our sample size was not adequate to explain these differences. Therefore, further explorations may be needed with higher sample size. Furthermore, the observation period in the study was limited to 24 hours; this was partly driven by the need to minimize the number of follow-up visits that each individual had to attend; each individual had to evaluate all three compositions and was required to attend 7 visits per composition. However, the pupil diameter and accommodative amplitude had not returned to baseline at the end of this observation period, and we had not tracked the eye until it returned to normal baseline levels. Furthermore, although it appears that the pupils returned to normal after the washout period (a minimum of 4 days), we are unsure if there were any lingering effects from the prior dose. The trial enrolled young Vietnamese adults with pigmented eyes; although the pupillary responses and accommodative amplitude in young adults are robust, we are unsure if

there might be differences in responses compared to children who have a higher amplitude of accommodation and larger pupil. More importantly, the subjective nature of the accommodative amplitude assessment may have resulted in the variability observed in the study (Sun et al. 1988; Lockhart and Shi 2010; Park et al. 2019).

5.5.5 *Summary*

In summary, data from the current trial indicates that dual therapy (Atropine plus Caffeine) appears to reduce the risk for a larger pupillary increase as compared to monotherapy alone and additionally, the response was less variable. There appeared to be no differences in accommodative amplitude. These findings support the results from Chapter 4 where a smaller pupillary increase was found with a lower concentration of Atropine 0.02% when combined with Caffeine 2%.

Interim summary:

In a 24-hour prospective, randomised, cross-over trial, as compared to Atropine monotherapy, combination therapy, Atropine+Caffeine, resulted in lesser variability in the pupil response and fewer eyes reaching a larger pupil diameter at each time point. This was observed for both mesopic and photopic conditions. No such changes were evident for accommodative amplitude.

Chapter 6. Discussion and conclusions

This thesis aimed to investigate the role of Caffeine in management of myopia. Specifically, the thesis aimed to determine whether Caffeine when used either as a single-drug or in combination with Atropine had any role in improving the efficacy and/or reducing the side effects of Atropine.

6.1 Key findings and discussion

6.1.1 *Chapter 1: Introduction*

With respect to pharmaceutical strategies for slowing myopia, Atropine remains the only known effective agent. Atropine is an anticholinergic non-selective muscarinic receptor antagonist but its mechanism of action underlying myopia control remains unclear. It was previously considered that Atropine blocked the excessive accommodative effort resulting in myopia, however, recent evidence suggests that it may be influencing eye growth *via* non-accommodative mechanisms. As outlined in Chapter 1, whether it acts to slow axial elongation *via* muscarinic receptors present in the retina, choroid, or sclera, or *via* other non-muscarinic receptors or pathways, or *via* direct action on ocular tissues remains unclear. Importantly, although Atropine has been widely adopted to control myopia, its use is associated with an increase in pupillary diameter and loss of accommodative amplitude that result in light sensitivity, glare, and difficulty at near-work for the individual. There appears to be a dose-dependent relation between the concentration and the side effects, with significant issues at higher concentrations, and therefore, in recent years there has been a shift to using lower concentrations of Atropine in myopia management (Lee et al. 2006; Fang et al. 2010; Chia et al. 2012; Clark and Clark 2015; Polling et al. 2016). Presently 0.01% Atropine remains the most widely used concentration for myopia control. However, evidence indicates that 0.01% Atropine may not be effective in slowing myopia. Therefore, there is a need for other effective compounds to slow myopia and/or minimise the risk of side effects during use. In this regard, 7-Methylxanthine, an adenosine receptor antagonist was explored for its ability to slow myopia, but in a limited manner. 7-Methylxanthine was thought to influence the scleral

collagen and thus axial elongation. However, the data on 7-Methylxanthine for slowing myopia remains equivocal. In this regard, Caffeine, a Xanthine derivative is of interest due to its widespread use in human health and well-being and can be applied topically. Topical Caffeine was found to alter choroidal thickness in animal models and thought to play a role in modulating axial elongation. It may be possible that a combination of Caffeine and low concentration Atropine may have more potent action on efficacy. Therefore, the objective of this thesis was to investigate the effectiveness of topical Caffeine both alone and in combination with Atropine eye drops for its role in myopia control treatment.

6.1.2 *Chapter 2: Meta-analysis of Atropine for assessing the change of accommodative amplitude and pupillary dilation as well as the myopia control efficacy*

A systematic review and meta-analysis of data (1980 to 2020) for use of Atropine in the treatment of myopia considered data from 13 independent trials that included 6 randomised clinical trials and 7 observational studies. The aim of the meta-analysis was to systematically study the evidence for efficacy and side effects and to determine the Atropine concentration that would be most suitable for use in combination with Caffeine. The 13 published trials had considered nine different concentrations of Atropine ranging from 0.01% to 1.0%., i.e., 0.01%, 0.02%, 0.025%, 0.05%, 0.1%, 0.125%, 0.25%, 0.5% and 1%. Atropine slowed myopia and the pooled estimates indicated a reduction in annual progression of spherical equivalent that varied from 0.34D (95% CI, 0.25 to 0.44) with the lowest concentration of 0.01% to 0.92D (95% CI, 0.63 to 1.21) with the highest concentration of 1.0%. However, with respect to slowing axial elongation, the data was more heterogeneous (I^2 =95%, p<0.001) and the efficacy with 0.01% Atropine statistically insignificant compared to the control (mean difference of -0.03mm, 95% CI, -0.11 to 0.05, p=0.45).

The mydriatic and cycloplegic effects of Atropine with an increase in pupillary diameter and reduction in accommodative amplitude are commonly understood (McDougal and Gamlin 2015)

and indeed, were the basis for the fundamental shift towards use of lower concentrations of Atropine for myopia control. However, surprisingly there is limited literature that systematically considered the effects of various concentrations of Atropine on pupillary diameter and accommodative amplitude. Only few studies reported changes in pupillary diameter and accommodative amplitude that occurred with Atropine. Three studies reported a total of seven concentrations (Chia et al. 2012; Yam et al. 2019; Fu et al. 2020), and all concentrations excepting for 0.01% Atropine increased the pupillary diameter and reduced the accommodative amplitude. Interestingly, the relation for change in amplitude of accommodation and increase in pupillary diameter with varying Atropine concentrations was non-linear. There was a steep and a rising curve from 0.01% to 0.10% Atropine indicating a significant increase with increasing concentrations, but the curve appeared to slow/plateau for points ≥0.10% Atropine and was suggestive that a peak/ceiling effect on pupillary diameters/accommodative amplitude may have been reached at concentrations at ≥0.10% with only minimal to small change in effects on pupillary responses and accommodative efforts for concentrations ≥0.10%. At <0.1% Atropine, although the slope was rising the change in PD (+0.7mm; 95% CI: +0.1 to +1.4) and AA (-1.6D; 95% CI: -3.9 to +0.7) was small whereas at ≥0.10% Atropine, the change in PD (+3.2mm, 95% CI: +2.8 to +3.5) and AA (-10.7D; 95% CI: -12.2 to -9.2) was high. Reduction in myopia progression with Atropine at < 0.10% and ≥ 0.10% as compared to controls was 0.37D (95% CI: 0.16 to 0.58) versus 0.75D (95% CI: 0.17 to 1.33) for spherical equivalent and -0.10mm (95% CI: -0.24 to 0.05) versus -0.23mm (95% CI: -0.34 to -0.13) for axial length.

Although the meta-analysis indicated minimal effect on pupillary diameter and accommodative amplitude with <0.1% Atropine (<1mm pupillary change and <2D decrease in accommodative amplitude), there were some limitations. The limited data points across the various concentrations as well as the variations in techniques used to measure the accommodative amplitude and the pupillary diameter indicated a need for further data. Therefore, the meta-analysis highlighted a

need to determine the highest concentration of Atropine that demonstrates efficacy with the lest risk of side effects.

6.1.3 *Chapter 3: Short-term trial assessing the effects of low-concentration Atropine on pupillary dilation and decrease of accommodative amplitude*

Although the meta-analysis had identified that concentrations <0.1% Atropine resulted in small changes in accommodative amplitude and pupillary diameter (<2D reduction in accommodative amplitude/<1mm change in pupillary diameter), other short-term clinical studies/trials reported concentrations beyond 0.02% Atropine to result in a significant loss of accommodation and an increased pupillary diameter (Cooper et al. 2013). Furthermore, concentrations higher than 0.03% were found to be cytotoxic to the corneal endothelium (Wen et al. 2016). Therefore, we assessed the effects of 0.01%, 0.02% and 0.03% Atropine in a short-term, prospective, randomized trial where 57 myopic participants, 6 and 12 years of age were enrolled and randomized to use one of the three concentrations. The mean age of the participants was 9.26 ± 1.66 years, the mean spherical equivalent refractive error of -3.53 ± 1.79 D and a mean axial length of 24.86 ± 0.95mm. Pupillary diameter was measured under photopic (300 lux) and mesopic (30lux) using standard procedures. A push-up method was used to measure monocular accommodative amplitude.

Interestingly, the results of this short term clinical trial indicated a greater effect on pupillary diameter and accommodative amplitude with all the three concentrations as compared to the results from the meta-analysis. The reduction in accommodative amplitude from baseline to 2 weeks was at 5.23 D, 9.28D and 9.32D and the increase in photopic pupillary diameter was 0.95 ± 1.05 mm,1.65 ± 0.93 mm and. 2.16 ± 0.88 mm with 0.01%, 0.02% and 0.03% Atropine respectively. There was a significant change from baseline to the follow-up visit that was conducted on Day 3. Beyond that, there was minimal change indicating that the ceiling or maximal effect of the concentration may have been reached. Approximately 23.5% of eyes had a pupillary diameter increase of ≥3mm with 0.03% as compared to 10.5% and 4.8% with 0.02%

and 0.01% Atropine. Similarly, more of the 0.03% Atropine eyes had residual accommodative amplitude of 10D or less (58.8% versus 47.1% versus 0% with 0.03%, 0.02% and 0.01% Atropine). AtropineAtropine This indicated approximately 1 of 4 eyes on 0.03% Atropine had a change in accommodative amplitude/pupillary diameter that was likely to affect day-to-day vision. At the other end, approximately 29% to 33% of the eyes demonstrated no change with 0.01% Atropine for either accommodative amplitude or pupillary diameter. This data for 0.01% Atropine was aligned with the data from the meta-analysis that indicated that 0.01% had no significant effect on accommodative amplitude or axial length elongation and therefore it appeared that 0.01% may not be an effective agent to use in future clinical trials.

6.1.4 *Chapter 4: Longitudinal dispensing randomized clinical trial investigating the role of 2% Caffeine either alone or in combination in controlling myopia progression*

Based on the results from the 2-week trial of 0.01%, 0.02% and 0.03% Atropine, 0.02% Atropine was chosen as the concentration of choice to use in a longitudinal randomized clinical trial that investigated the efficacy of once nightly use of either 0.02% Atropine, 2% Caffeine or 0.02% Atropine plus 2% Caffeine on progression of myopia. The aim of the trial was to determine if Caffeine used in combination with Atropine increased the potency/efficacy of the drug in slowing myopia either via a synergistic or additive mechanism. The concentration of Caffeine was set at 2% as it was the maximum concentration soluble in water (Benowitx 1990; Australia and New Zealand 2015). The data from the randomized groups were compared to a parallel, non-randomized control group of single vision spectacle lens wearers. In this prospective, randomized clinical trial that was conducted at An Sinh Hospital, Hai Yen Eye Care, Vietnam a total of 96 children aged between 6 and 13 years, myopic refractive error ranging from -0.50 to -6.00D were randomized to one of the three groups of 0.02% Atropine, 2% Caffeine or 0.02% Atropine plus 2% Caffeine. A total of 86 participants were enrolled in the parallel group to wear single vision spectacles. The six-month data of this 2-year trial was presented in this thesis.

All groups progressed in myopia over the six months in the study.

At six months, the mean change in spherical equivalent refractive error/axial length in the single vision (SV) control group was -0.33 ± 0.29D/0.18±0.11mm and from the intervention groups, the mean change in spherical equivalent/axial length in the 2% Caffeine group was similar at -0.39 ± 0.38D/0.19±0.15mm. In comparison, use of both Atropine groups (monotherapy and combination therapy) demonstrated a reduction in myopia progression with a change of -0.20 ± 0.34/0.08±0.11mm and -0.20 ± 0.30D/0.11±0.11mm with 0.02% Atropine and 0.02% Atropine plus 2% Caffeine respectively. These 6-month results indicated that 2% Caffeine performed similarly to single vision spectacle group and had no effect on axial length or refractive error change. Similarly, combining Caffeine with Atropine 0.02% did not alter/reduce/improve the efficacy of Atropine.

Contrary to expectations, we found that Caffeine alone had no effect on myopia progression. However, surprisingly, combining Caffeine with Atropine resulted in minimisation of the pupillary diameter increase and possibly to a lesser extent also minimised the change in accommodative amplitude. At three- and six-month visits, there was a significant increase in photopic pupil diameter in eyes with 0.02% Atropine with a difference from baseline of 1.10 ± 0.94 mm and 1.20 ± 0.85mm respectively. In comparison, the increase of the photopic pupil diameter in eyes with 0.02% Atropine plus 2% Caffeine was significantly less at 0.59 ± 0.61 mm and 0.76 ± 0.58mm respectively. There was a 46.7% and 37.0% reduction in change in photopic pupillary diameter with the combination therapy as compared to monotherapy at 3 and 6 months respectively. Similar changes were observed with the change in mesopic pupillary diameter.

At 3 months, accommodative amplitude was reduced by 3.77D, 2.28D and 0.68D with 0.02% Atropine, 0.02% Atropine plus 2% Caffeine and 2% Caffeine, respectively. Similarly, at the six-month visit, accommodative amplitude was reduced by 3.14D, 2.84D and 0.78D, respectively. Although the combination composition resulted in a slightly lower reduction in accommodative amplitude, the difference between the two Atropine groups was not significant.

These results support previously published results that indicate that low concentration Atropine is effective in slowing myopia but also found that 2% Caffeine had no effect on the progression of myopia. The reasons for lack of efficacy with Caffeine are not understood. If Caffeine did have a role influencing eye growth *via* adenosine receptor pathways, an effect on eye growth would have been observed with either Caffeine alone or in combination with Atropine. It may be possible that the concentration of Caffeine as used in the current trial at 2% may not have been sufficient to influence eye growth or the half-life of Caffeine may not have been sufficient (Johnson et al. 2017) or alternatively, Caffeine does not have a role to play in slowing myopia.

Although Caffeine did not impact ocular growth, when combined with Atropine, there was a significant minimisation of the increase in pupillary diameter seen with monotherapy of Atropine. Additionally, it also appeared to have an impact on the change in accommodative amplitude. The data for minimization of pupillary diameter increase and possibly reduction of accommodative amplitude was consistent and was observed at both the 3 month and 6-month visits.

These results are exciting and possibly indicate a role for Caffeine in minimizing side effects related to the use of Atropine. There are many limitations to this trial and the foremost was that Caffeine was not optimized in concentration. Furthermore, it needs to be studied if the effects are sustained over long term use of the combination drug. Additionally, it needs to be studied if Caffeine can induce similar responses with other concentrations of Atropine. Despite these limitations, if the results were valid and if Caffeine does have a role to play in minimizing pupillary diameter change, especially with higher doses of Atropine where the impact on pupillary diameter is significant, then it paves the way for a new generation of treatment strategies that can be used to minimize the impact of side effects associated with Atropine use for myopia control.

6.1.5 *Chapter 5: 24-hour effects of single-use 2% Caffeine either alone or in combination with 0.05% or 0.1% Atropine on pupil size and accommodative amplitude*

Methylxanthines, when used orally (7-Methylxanthine) and topically (Caffeine) were found to influenced ocular growth patterns in both human and animal studies. It should be noted that although oral use of Methylxanthine was effective in slowing myopia in children with myopia (Trier et al. 2008), its effect on spherical equivalent remained uncertain. In a prospective, randomised clinical trial that evaluated the use of 2% Caffeine either alone or in combination with 0.02% Atropine, there was no effect of Caffeine on progression of myopia. However, when used in combination with 0.02% Atropine, the increase in pupillary diameter that results from use of Atropine was reduced by 37% or more. To further explore and determine if Caffeine had a similar effect on reducing pupillary diameter increase with higher concentrations of Atropine, we conducted a 24-hour prospective, randomized, cross-over, observational study to monitor the effects of 0.05% and 0.1% Atropine both alone (monotherapy) and in combination with Caffeine 2% (combination) on pupillary diameter and accommodative amplitude. The composition was instilled in the right eye and both eyes were examined 20-, 40- and 60-minute post instillation and then were examined at 09:00, 12:00 and 15:00 hrs on the following day.

Although the mean differences were not significant, the results indicated that at any given time point, the upper limit to the change in pupillary diameter was smaller in eyes on combination therapy and there were fewer eyes with larger increase in pupillary diameter. Furthermore, the change in pupillary diameter was less variable with the combination therapy compared to monotherapy. This was consistent for both photopic and mesopic pupillary diameter. This was more evident for when Atropine was used at concentration of 0.05% compared to 0.1%. No such changes were evidence for accommodative amplitude. The reasons for the lack of response with accommodative amplitude is unclear; both the long-term dispensing trial and the 24-hour observational trial used a -4D minus lens push-up method and this resulted in significantly less accommodative amplitude at baseline and a surprising number of eyes on Atropine did not

demonstrate any change over time even with higher concentrations of Atropine. Thus, it is not clear, if the results with accommodative amplitude are related to methodology or inherent with the use of the compositions. Additionally, we had not optimized or studied other concentrations of Caffeine. In summary, this study indicated that Caffeine has a role in modulating the pupillary change observed with use of Atropine.

6.2 Implications of the research and future directions

The burden of myopia and high myopia is increasing and there is a growing need for more effective strategies to reduce the risk of the eye reaching higher levels of myopia. Although the underlying mechanism of action remains unclear, the use of Atropine for slowing myopia is widespread (Chou et al. 1997; Shih et al. 1999; Syniuta and Isenberg 2001; Lee et al. 2006; Chia et al. 2012; Clark and Clark 2015; Yi et al. 2015; Lee et al. 2016; Moon and Shin 2018; Joachimsen et al. 2019; Larkin et al. 2019; Sacchi et al. 2019; Yam et al. 2019; Fu et al. 2020; Polling et al. 2020). More importantly, there is increasing adoption of 0.01% Atropine although its role in slowing myopia has not been substantiated in well-conducted clinical trials (Chia et al. 2012; Yam et al. 2019; Fu et al. 2020) and is driven by the need to minimize the side effects associated with higher concentrations of Atropine (Chua et al. 2006; Chia et al. 2012).

This thesis explored the use of Caffeine, a non-selective adenosine receptor antagonist for its role ins slowing myopia. To our knowledge, our trials were the first reports for the human use of topical Caffeine for myopia control treatment. Although our results indicate that Caffeine, at 2% concentration, does not appear to have a role in slowing myopia, its use resulted in an unexpected benefit. The results showed that use of Caffeine in combination with Atropine resulted in a smaller variation in pupillary diameter. This result is significant as it paves the way for possible avenues to minimize the risk of side effects with Atropine whilst maintaining the efficacy. Further work needs to be conducted to understand if higher concentrations of Caffeine either alone or in combination have a role to play in myopia control. Similarly, further work needs to be conducted

to determine the strength and nature of Caffeine that provides the optimal results with Atropine. Additionally, it needs to be determined if these results vary between pigmented versus non-pigmented eyes. It also needs to be determined if there is a role for Caffeine on accommodation with more robust techniques evaluating various aspects of accommodation. Furthermore, due to the nature of the clinical studies, we had not addressed issues related to chemical compositions, stability, pharmacokinetics etc.

Despite these limitations and the need for further investigations, this thesis indicates that there is a role for Caffeine in myopia control when used in conjunction with Atropine. Our experiments have discovered a novel use for Caffeine in regulating pupillary responses.

6.3 Conclusion

Based on the results from the trials, we accept the null hypothesis that topical Caffeine alone has no role in myopia control. However, Caffeine when used in combination with Atropine has a role to play as it has the potential to minimize one of the significant side effects of Atropine. This potentially enables avenues where higher levels of Atropine could be used with a lowered risk of pupillary diameter increase and therefore, we reject the premise that Caffeine in combination with Atropine has no role in myopia control.

APPENDICES

Appendix 1: Supplemental documents and data for chapters

A. For Chapter 1

UNSW SYDNEY | Australia's Global University

SIGN-OFF APPROVAL SHEET

Surname/Family Name	:	Tran
Given Name/s	:	Huy Dinh Minh
Abbreviation for degree as give in the University calendar	:	PhD
Faculty	:	Medicine
School	:	School of Optometry and Vision Science
Publication	:	Mechanism of action of atropine in controlling myopia progression.
Authors	:	Tran, H. D. M., Ha, T. T. X.
Journal	:	Review of myopia management. Online on 1st October 2020. https://reviewofmm.com/mechanism-of-action-of-atropine-in-controlling-myopia-progression/.

CO-AUTHOR AUTHORISATION APPROVAL:

Signing off on this document signifies that the approving author, Tran,H.D.M, is allowed to incorporate the data and information of the paper above as a part or in lieu of a Chapter of his PhD dissertation. The co-authors acknowledge satisfaction and approval for the authorisation.

Name of author	Signature	Approved by
1. Ha, Thao T.X. MD.		19-July-2021

B. For Chapter 2

UNSW SYDNEY | Australia's Global University

SIGN-OFF APPROVAL SHEET

Surname/Family Name	:	Tran
Given Name/s	:	Huy Dinh Minh
Abbreviation for degree as give in the University calendar	:	PhD
Faculty	:	Medicine
School	:	School of Optometry and Vision Science
Publication	:	A meta-analysis assessing change in pupillary diameter, accommodative amplitude, and efficacy of Atropine for myopia control.
Authors	:	Tran, H. D. M., Sankaridurg, P., Naduvilath, T., Ha, T. T. X., Tran, T. D., Jong, M., Coroneo, M., Tran, Y. H.
Journal	:	Asia-Pacific Journal of Ophthalmology (accepted for publication)

CO-AUTHOR AUTHORISATION APPROVAL:

Signing off on this document signifies that the approving author, Tran,H.D.M, is allowed to incorporate the data and information of the paper above in lieu of a Chapter of his PhD dissertation. The co-authors acknowledge satisfaction and approval for the authorisation.

Name of author	Signature	Approved by
1. Prof. Padmaja Sankaridurg		23 June, 2021
2. A/Prof. Thomas Naduvilath		23 June, 2021 Thomas Naduvilath
3. Dr. Thao T.X. Ha		23 June, 2021
4. Prof. Tuan D. Tran		23 June, 2021
5. A/Prof. Monica Jong		23 June, 2021
6. Prof. Minas Coroneo		23 June, 2021
7. A/Prof. Yen H. Tran		22 June, 2021

C. For Chapter 3

Informed Consent Form (the first page is attached below)

PARTICIPANT INFORMATION AND CONSENT

"EFFECTS OF SHORT-TERM USE OF 0.01%, 0.02% AND 0.03% ATROPINE EYE DROPS"

This consent form may contain words that you do not understand. Please ask the doctor or the research staffs to explain any words or information that you concern. You may take home an unsigned copy of this consent form to think about or discuss with family before making your decision.

1. **What is the research study about?**

You are invited to consent for your child to participate in a short-term research study, being collaborated between four organisations:

- University of New South Wales.
- Brien Holden Vision Institute.
- An Sinh Hospital, Ho Chi Minh City, Vietnam.
- Hai Yen Eye Center, Hai Yen Eye Care, Ho Chi Minh City, Vietnam.

This study aims to examine the short term effects on your children's eyes by using 0.01%, 0.02%, 0.03% atropine as eye drops. The eye drops will be used once nightly before bedtime, one drop to each eye for both eyes.

Atropine has been used since long time ago for many treatments on the eyes. There are many concentrations commercially available prescribed by the ophthalmologists such as 1%, 0.5%, 0.1% and 0.01% in various countries, e.g. Singapore, Taiwan, Australia, India. Regarding the myopia control treatment, atropine has been investigated in the United States, Netherlands, Taiwan, Singapore, India, China with different concentrations varied from 1% to the lowest reported at 0.01% and consistently shown the strong efficacy in slowing the progression of myopia without any serious adverse events reported (even in the long-term cohort study with up to 11 years of follow-up).

2. **Who is conducting this research?**

Role	Name	Organisation
Scientific and Clinical supervisor	Assoc.Prof. Tran Hai Yen.MD. PhD	Hai Yen Eye Care Pham Ngoc Thach Medical University, Ho Chi Minh City, Vietnam
Chief Investigator	Tran Dinh Minh Huy. MD. MSc	Hai Yen Eye Care University of Medicine and Pharmacy at Ho Chi Minh City, Vietnam Brien Holden Vision Institute

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D. For Chapter 4

- *Informed Consent Form for Treatment Groups (the first page is attached below)*

AN SINH HAIYEN EYE CARE

Participant information and consent
"Myopia control with novel eye drops"

This consent form may contain words that you do not understand. Please ask the doctor or the study staffs to explain any words or information that you concern. You may take home an unsigned copy of this consent form to think about or discuss with family befor make your decision.

1. What is the research study about?

You are invited to consent for your child to participate in a short-term research study to examine the response of either 0.02% atropine or 2% caffeine or 0.02% atropine plus 2% caffeine as eye-drops. The drops will be used once every day at bedtime, one drop to each eye for both eyes.

Atropine has long been used in the eye for many conditions and is available in various concentrations and currently low concentration atropine (0.01%, 0.025%) is being used in countries for slowing myopia. Similarly, a caffeine metabolite taken orally was effective in controlling myopia progression in children in Denmark. And use of low concentration caffeine 2% in eye drops was found to be safe for use in eyes in a short term assessment conducted at Sydney.

2. Who is conducting this research?

The study is being carried out by the following researchers:		
Role	**Name**	**Organisation**
Scientific supervisor – Principal investigator	Assoc. Prof. Tran Hai Yen MD. PhD.	An Sinh Hospital, Ho Chi Minh City, Vietnam Hai Yen Eye Care (belongs to Hai Yen Anh Tran Co.Ltd, Ho Chi Minh City, Vietnam)
Co-Principal Investigator	Tran Dinh Minh Huy MD. MSc.	Ho Chi Minh City Medical and Pharmacy University Hai Yen Eye Care (belongs to Hai Yen Anh Tran Co.Ltd, Ho Chi Minh City, Vietnam)
Co-Investigator	Pham Ngoc Dan Thanh MD.	Hai Yen Eye Care (belongs to Hai Yen Anh Tran Co.Ltd, Ho Chi Minh City, Vietnam)
Research funder	This research is being funded by Hai Yen Anh Tran Co. Ltd	

3. Inclusion/Exclusion Criteria

Your child is eligible to participate in the study if they are between 6 and 13 years old, have myopia between -0.50 diopter and -6.00 diopter, have normal eye findings and vision and willing to follow the visit schedule in 2 years.

They are not eligible if they have any ocular or general health condition, or are enrolled in another trial or known to have allergy to the drops being used in the study.

Version dated 17/07/2019
TREATMENT GROUP

- *Informed Consent Form for Control Group (the first page is attached below)*

AN SINH | HAIYEN EYE CARE

Participant information and consent
"Myopia control with novel eye drops"

This consent form may contain words that you do not understand. Please ask the doctor or the study staffs to explain any words or information that you concern. You may take home an unsigned copy of this consent form to think about or discuss with family befor make your decision.

1. **What is the research study about?**

You are invited to consent for your child to participate in a short-term research study to examine the response of either 0.02% atropine or 2% caffeine or 0.02% atropine plus 2% caffeine as eye-drops. The drops will be used once every day at bedtime, one drop to each eye for both eyes. However, your child will take part in the control group and will be followed up with the standard procedures for myopia evaluation in children.

Atropine has long been used in the eye for many conditions and is available in various concentrations and currently low concentration atropine (0.01%, 0.025%) is being used in countries for slowing myopia. Similarly, a caffeine metabolite taken orally was effective in controlling myopia progression in children in Denmark. And use of low concentration caffeine 2% in eye drops was found to be safe for use in eyes in a short term assessment conducted at Sydney.

2. **Who is conducting this research?**

The study is being carried out by the following researchers:		
Role	**Name**	**Organisation**
Scientific supervisor – Principal investigator	Assoc. Prof. Tran Hai Yen MD. PhD.	An Sinh Hospital, Ho Chi Minh City, Vietnam Hai Yen Eye Care (belongs to Hai Yen Anh Tran Co.Ltd, Ho Chi Minh City, Vietnam)
Co-Principal Investigator	Tran Dinh Minh Huy MD. MSc.	Ho Chi Minh City Medical and Pharmacy University Hai Yen Eye Care (belongs to Hai Yen Anh Tran Co.Ltd, Ho Chi Minh City, Vietnam)
Co-Investigator	Pham Ngoc Dan Thanh MD.	Hai Yen Eye Care (belongs to Hai Yen Anh Tran Co.Ltd, Ho Chi Minh City, Vietnam)
Research funder	This research is being funded by Hai Yen Anh Tran Co. Ltd	

3. **Inclusion/Exclusion Criteria**
Your child is eligible to participate in the study if they are between 6 and 13 years old, have myopia between -0.50 diopter and -6.00 diopter, have normal eye findings and vision and willing to follow the visit schedule in 2 years.

Version dated 17/07/2019
CONTROL GROUP

E. For Chapter 5

E.1. Informed Consent Form (*the first page is attached below)*

Hai Yen Eye Care, Hai Yen Anh Tran Co.
Brien Holden Vision Institute Limited

PARTICIPANT INFORMATION STATEMENT AND CONSENT FORM

Effect of a single dose of low dose atropine eye drops, caffeine eye drops and caffeine plus atropine eye drops on accommodative response and pupil size

Chief Investigator: Prof. Padmaja Sankaridurg B.Optom PhD MIP
Principal Investigator: Huy Dinh Minh Tran MD. MSc.
Co-Investigator: A/Prof. Yen Hai Tran MD. PhD.; Thao Ha MD.

1. **What is the research study about?**
   You are invited to take part in this research study.
   This study aims to determine the response of the eye to six different eye-drop formulations given below (Table 1).

Table 1: Description of eye drops used in this clinical trial

Eye-Drops	Manufacturer	Active Ingredients	Artificial Tear Solution
Arm 1			
Test 1.1	Custom Compounding Pharmacy	2.0% caffeine	0.3% Hydroxyl-propyl methyl cellulose, 0.1% EDTA, sterile water
Test 1.2		0.05% atropine	
Test 1.3		2.0% caffeine/0.05% atropine	
Arm 2			
Test 2.1	Custom Compounding Pharmacy	2.0% caffeine	0.3% Hydroxyl-propyl methyl cellulose, 0.1% EDTA, sterile water
Test 2.2		0.1% atropine	
Test 2.3		2.0% caffeine/0.1% atropine	

   The effect of these drops on human eyes has been studied previously and no significant discomfort has been reported.
   In this study, we wish to assess your eye's focussing ability (accommodation) and pupil size and response after instilling these test eye drops. A minimum of 30 people will take part in this study, each arm will have at least 15 participants. Each participant will assess 3 eye drops.

2. **Who is conducting this research?**
   The study is being carried out by the following researchers:
   - Professor Padmaja Sankaridurg, B.Optom, PhD, MIP
     - Brien Holden Vision Institute Limited
     - School of Optometry and Vision Science, University of New South Wales
   - Huy Dinh Minh Tran, MD. MSc.
     - PhD Student of School of Optometry and Vision Science, University of New South Wales
     - Brien Holden Vision Institute Limited
     - Hai Yen Eye Care
   - A/Prof Yen H. Tran, MD. PhD.
     - Founder
     - Clinical Researcher of Hai Yen Eye Care, Ho Chi Minh City, Vietnam
   - Thao T.X. Ha, MD.
     - Ophthalmologist
     - Clinical Researcher of Hai Yen Eye Care, Ho Chi Minh City, Vietnam

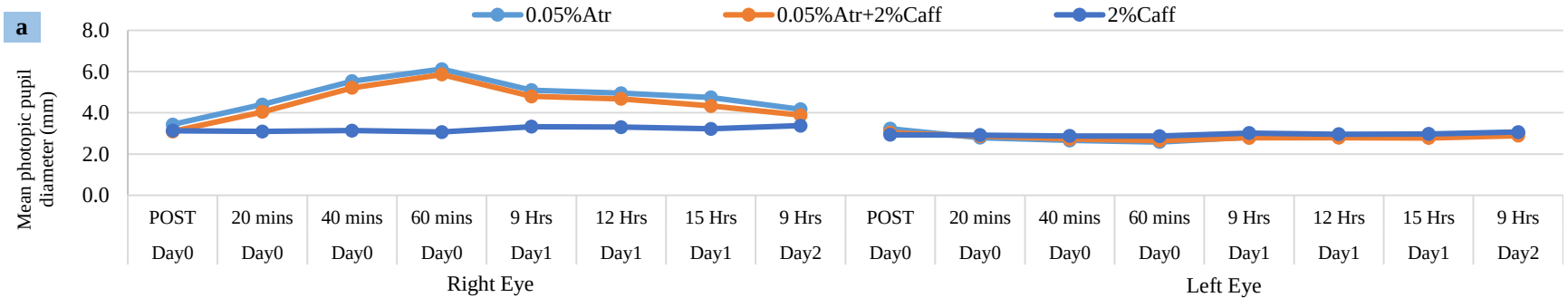
   Research Funder: This research is being funded by Brien Holden Vision Institute Ltd., Sydney, Australia and Hai Yen Eye Care, an entity of Hai Yen Anh Tran Co., Ho Chi Minh City, Vietnam

3. **Inclusion/Exclusion Criteria**

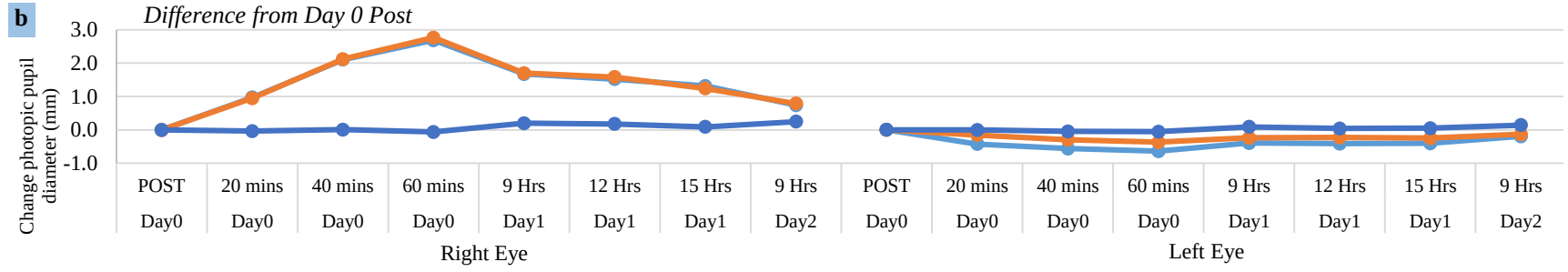
E.2. Clinical measurements of groups over the visits

	Amplitude_of_Accommodation						Pupils_Size_Mesopic						Pupils_Size_Photopic					
	Day0 20mins	Day0 40mins	Day0 60mins	Day1 09Hrs	Day1 12Hrs	Day1 15Hrs	Day0 20mins	Day0 40mins	Day0 60mins	Day1 09Hrs	Day1 12Hrs	Day1 15Hrs	Day0 20mins	Day0 40mins	Day0 60mins	Day1 09Hrs	Day1 12Hrs	Day1 15Hrs
Eyes (N)	14								0.05% Atropine									
Mean	-0.26	-0.62	-1.23	-1.58	-1.42	-1.10	0.90	1.37	1.53	0.91	0.86	0.71	0.97	2.11	2.68	1.67	1.52	1.31
Median	-0.25	-0.63	-1.25	-1.25	-1.25	-0.88	0.69	1.02	1.15	0.55	0.56	0.45	1.10	2.20	2.98	1.83	1.80	1.60
Min	-2.08	-3.00	-3.33	-5.33	-5.08	-5.33	-0.04	0.44	0.70	0.16	0.10	-0.16	-1.53	-0.97	-0.07	-1.33	-1.33	-1.63
Max	2.00	2.00	1.75	0.08	0.67	1.08	3.13	3.50	3.73	3.00	2.93	2.80	2.40	3.73	4.30	3.10	2.90	2.54
Percent 25	-1.12	-1.46	-2.12	-1.88	-1.94	-1.88	0.32	0.65	0.86	0.20	0.21	0.18	0.36	1.15	1.90	1.09	1.07	0.88
Percent 75	0.42	0.33	-0.38	-0.88	-0.13	0.19	1.20	1.92	1.95	1.39	1.22	0.99	1.75	3.23	3.59	2.34	2.23	1.95
Eyes (N)	15								0.05% Atropine + 2% Caffeine									
Mean	-0.40	-0.58	-1.36	-1.48	-1.65	-1.36	0.47	1.20	1.31	0.80	0.76	0.62	0.95	2.12	2.76	1.70	1.58	1.24
Median	-0.08	-0.83	-1.58	-1.00	-1.25	-1.58	0.53	1.13	1.30	0.63	0.73	0.70	0.96	2.23	2.74	1.83	1.70	1.23
Min	-1.83	-2.42	-3.83	-4.50	-4.00	-5.25	-0.33	0.17	0.17	0.30	-0.23	-0.23	0.10	0.10	0.33	0.70	0.50	0.13
Max	0.92	5.33	4.75	0.50	1.17	1.33	1.23	2.43	2.37	1.77	1.63	1.23	1.87	3.23	3.93	2.43	2.30	1.87
Percent 25	-1.00	-1.83	-2.84	-2.75	-2.92	-2.00	0.10	0.84	0.84	0.47	0.60	0.40	0.23	1.37	2.33	1.47	1.07	0.96
Percent 75	0.00	-0.09	-0.75	-0.75	-0.83	-0.33	0.80	1.63	1.83	1.13	1.13	0.97	1.53	2.93	3.44	2.10	2.00	1.70
Eyes (N)	15								0.1% Atropine									
Mean	-0.74	-1.34	-1.62	-1.85	-1.82	-1.51	0.88	1.53	1.65	1.18	1.09	0.97	1.82	2.76	3.10	2.31	2.14	1.83
Median	-0.33	-1.08	-1.50	-1.50	-1.50	-1.42	0.77	1.44	1.50	0.97	0.83	0.86	1.44	2.64	3.07	1.90	1.90	1.57
Min	-3.25	-3.83	-3.50	-4.75	-4.33	-3.50	-1.64	0.43	0.70	0.23	0.07	0.00	0.00	1.03	1.20	0.60	0.47	0.17
Max	0.00	0.09	-0.08	0.75	1.25	-0.08	2.83	3.03	2.97	2.27	2.40	2.33	5.04	5.24	5.20	4.17	3.67	3.24
Percent 25	-0.92	-1.25	-1.83	-3.00	-2.75	-2.25	0.37	0.73	0.90	0.67	0.56	0.43	0.77	1.60	2.10	1.36	1.23	1.00
Percent 75	-0.08	-0.75	-1.17	-0.75	-0.50	-0.58	1.24	2.17	2.34	1.74	1.56	1.40	2.57	3.93	4.16	3.17	3.10	3.04
Eyes (N)	15								0.1% Atropine + 2% Caffeine									
Mean	-0.66	-1.47	-2.02	-2.65	-2.19	-1.83	0.95	1.51	1.69	1.32	1.26	1.10	1.39	2.78	3.26	2.56	2.44	2.08
Median	-0.50	-1.00	-1.50	-2.50	-1.75	-1.25	0.90	1.40	1.43	1.33	1.20	1.20	1.40	2.94	3.16	2.56	2.43	2.06
Min	-2.33	-3.58	-5.00	-6.50	-6.50	-5.50	0.13	0.57	0.57	0.46	0.50	0.23	0.03	1.20	2.13	1.27	1.43	0.97
Max	0.50	0.00	-0.50	-0.25	-0.25	0.58	2.10	2.76	2.87	2.23	2.30	2.30	2.67	3.74	4.57	3.40	3.06	2.94
Percent 25	-1.17	-2.50	-2.75	-4.92	-3.67	-3.50	0.66	1.00	1.13	0.76	0.70	0.66	0.86	2.43	2.60	2.37	1.96	1.87
Percent 75	0.00	-0.67	-1.00	-0.75	-0.67	-0.42	1.07	2.06	2.63	2.04	1.90	1.37	1.86	3.13	3.84	2.90	3.00	2.73
Eyes (N)	30								2% Caffeine									
Mean	0.18	-0.08	-0.18	0.22	0.05	0.24	-0.13	-0.12	-0.06	0.12	0.04	-0.24	-0.04	0.01	-0.06	0.20	0.18	0.09
Median	0.04	-0.21	-0.50	0.00	0.00	0.00	-0.09	-0.10	-0.10	0.10	0.02	-0.25	-0.05	-0.03	-0.07	0.13	0.20	0.00
Min	-1.50	-2.25	-2.67	-1.75	-3.00	-2.50	-0.93	-0.67	-0.67	-0.50	-0.70	-2.40	-0.44	-0.43	-0.43	-0.16	-0.36	-0.47
Max	1.50	3.50	2.75	4.00	3.50	3.50	0.53	0.54	0.73	1.07	0.84	0.53	0.67	0.83	0.60	0.97	0.77	1.40
Percent 25	-0.50	-0.75	-1.02	-0.54	-0.52	-0.54	-0.36	-0.28	-0.28	-0.08	-0.15	-0.42	-0.21	-0.17	-0.20	0.01	-0.06	-0.18
Percent 75	0.62	0.83	0.83	0.62	0.79	0.90	0.10	0.03	0.20	0.30	0.28	0.18	0.08	0.12	0.03	0.36	0.35	0.33

E.3. Photopic pupillary diameter in groups with 0.05% Atropine over time

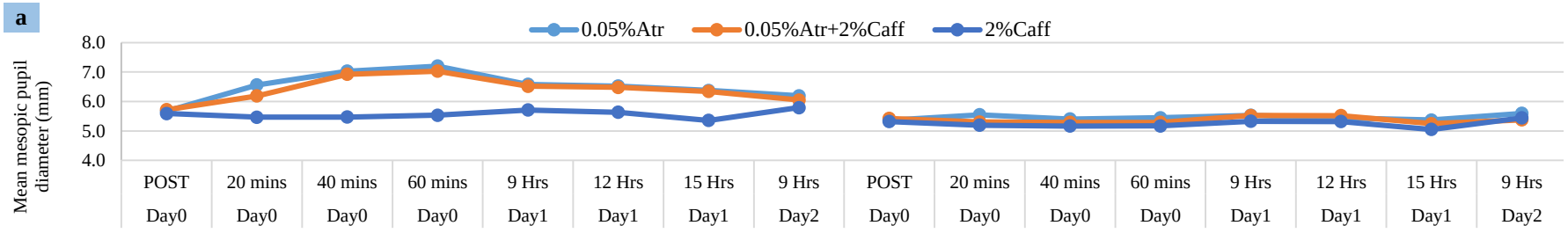


	Right Eye								Left Eye							
	POST Day0	20 mins Day0	40 mins Day0	60 mins Day0	9 Hrs Day1	12 Hrs Day1	15 Hrs Day1	9 Hrs Day2	POST Day0	20 mins Day0	40 mins Day0	60 mins Day0	9 Hrs Day1	12 Hrs Day1	15 Hrs Day1	9 Hrs Day2
0.05%Atr Mean±SD	3.43 ±0.87	4.40 ±0.82	5.53 ±0.97	6.11 ±0.92	5.10 ±0.60	4.95 ±0.66	4.74 ±0.60	4.17 ±0.55	3.22 ±0.33	2.79 ±0.30	2.66 ±0.81	2.58 ±0.32	2.82 ±0.38	2.81 ±0.31	2.82 ±0.40	3.03 ±0.31
0.05%Atr + 2%Caff	3.09 ±0.26	4.04 ±0.79	5.21 ±0.98	5.86 ±0.99	4.79 ±0.55	4.68 ±0.56	4.33 ±0.53	3.88 ±0.47	3.02 ±0.31	2.87 ±0.31	2.73 ±0.28	2.65 ±0.35	2.79 ±0.32	2.80 ±0.29	2.78 ±0.28	2.89 ±0.25
2%Caff	3.13 ±0.41	3.09 ±0.43	3.14 ±0.49	3.06 ±0.41	3.33 ±0.42	3.30 ±0.43	3.22 ±0.48	3.38 ±0.49	2.92 ±0.27	2.92 ±0.24	2.88 ±0.33	2.87 ±0.31	3.01 ±0.33	2.96 ±0.31	2.98 ±0.37	3.07 ±0.35

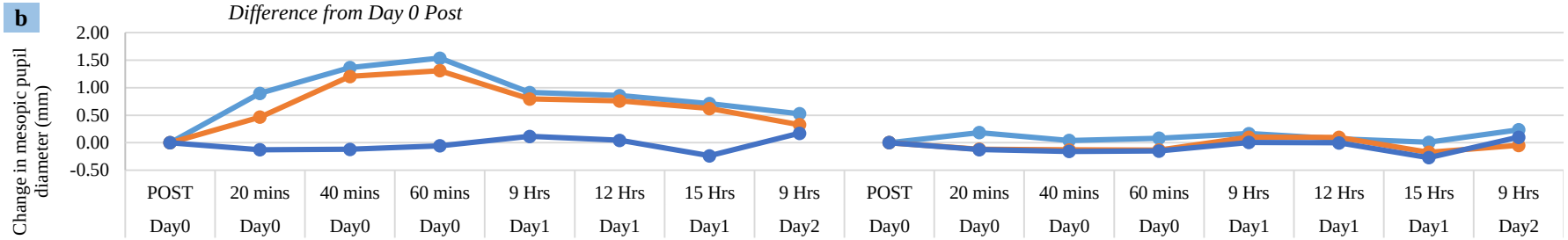


	Right Eye								Left Eye							
	POST Day0	20 mins Day0	40 mins Day0	60 mins Day0	9 Hrs Day1	12 Hrs Day1	15 Hrs Day1	9 Hrs Day2	POST Day0	20 mins Day0	40 mins Day0	60 mins Day0	9 Hrs Day1	12 Hrs Day1	15 Hrs Day1	9 Hrs Day2
0.05%Atr Mean±SD	0.00 ±0.00	0.97 ±1.04	2.11 ±1.33	2.68 ±1.24	1.67 ±1.08	1.52 ±1.06	1.31 ±1.04	0.74 ±0.96	0.00 ±0.00	-0.43 ±0.66	-0.56 ±0.67	-0.64 ±0.63	-0.40 ±0.67	-0.41 ±0.68	-0.40 ±0.65	-0.19 ±0.63
0.05%Atr + 2%Caff	0.00 ±0.00	0.95 ±0.64	2.12 ±0.92	2.76 ±0.90	1.70 ±0.55	1.58 ±0.54	1.24 ±0.50	0.79 ±0.42	0.00 ±0.00	-0.15 ±0.26	-0.29 ±0.29	-0.37 ±0.23	-0.24 ±0.19	-0.23 ±0.20	-0.25 ±0.24	-0.13 ±0.23
2%Caff	0.00 ±0.00	-0.04 ±0.24	0.01 ±0.29	-0.06 ±0.22	0.20 ±0.27	0.18 ±0.26	0.09 ±0.38	0.25 ±0.28	0.00 ±0.00	0.00 ±0.14	-0.04 ±0.15	-0.05 ±0.15	0.09 ±0.17	0.04 ±0.17	0.05 ±0.35	0.14 ±0.14

E.4. Mesopic pupillary diameter in groups with 0.05% Atropine over time

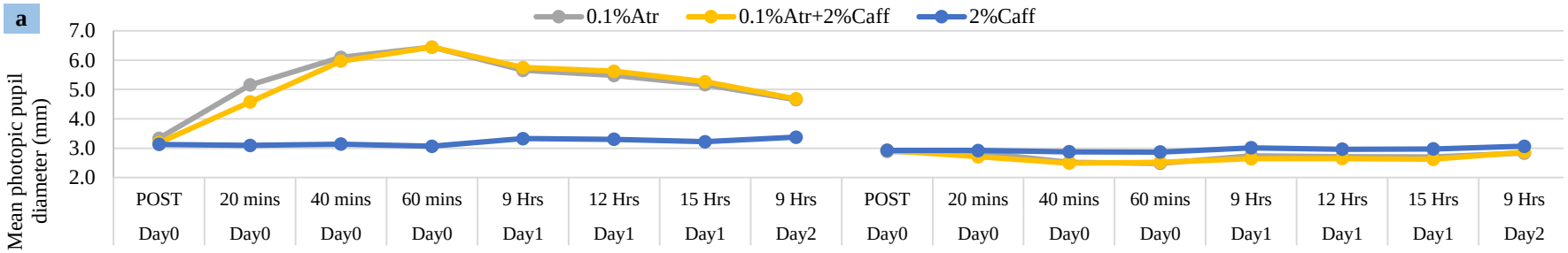


	Right Eye								Left Eye							
0.05%Atr Mean±SD	5.67 ±0.99	6.56 ±0.55	7.03 ±0.42	7.20 ±0.41	6.58 ±0.29	6.52 ±0.31	6.38 ±0.36	6.19 ±0.41	5.36 ±1.02	5.55 ±0.99	5.41 ±0.96	5.45 ±0.93	5.53 ±1.03	5.44 ±1.00	5.37 ±0.98	5.60 ±0.85
0.05%Atr + 2%Caff	5.72 ±0.68	6.19 ±0.63	6.93 ±0.43	7.03 ±0.48	6.52 ±0.32	6.48 ±0.38	6.34 ±0.43	6.05 ±0.48	5.42 ±0.95	5.31 ±1.00	5.29 ±0.92	5.29 ±0.99	5.52 ±0.97	5.52 ±0.97	5.25 ±0.99	5.38 ±1.14
2%Caff	5.59 ±0.74	5.47 ±0.71	5.47 ±0.74	5.54 ±0.83	5.71 ±0.77	5.64 ±0.78	5.36 ±0.98	5.79 ±0.80	5.32 ±0.97	5.20 ±0.98	5.17 ±0.97	5.17 ±0.98	5.33 ±1.04	5.32 ±0.99	5.05 ±1.02	5.44 ±1.08

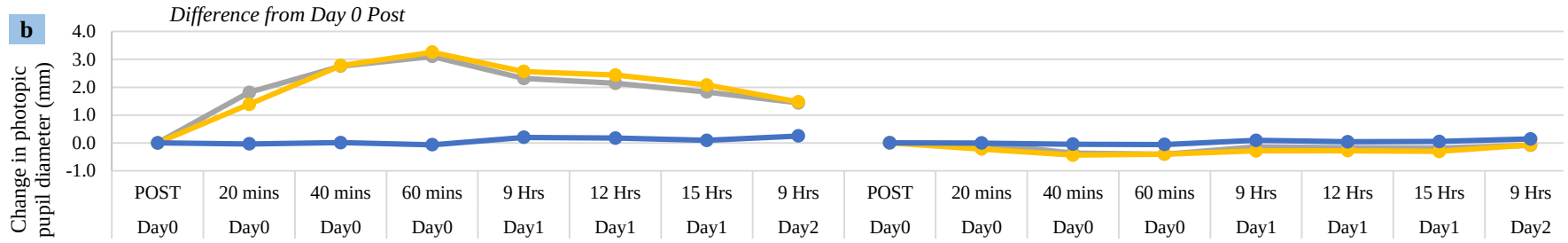


	Right Eye								Left Eye							
0.05%Atr Mean±SD	0.00 ±0.00	0.90 ±0.83	1.37 ±0.96	1.53 ±0.95	0.91 ±0.90	0.86 ±0.91	0.71 ±0.84	0.53 ±0.90	0.00 ±0.00	0.19 ±0.81	0.04 ±0.86	0.08 ±0.84	0.17 ±0.82	0.07 ±0.84	0.01 ±0.80	0.24 ±1.01
0.05%Atr + 2%Caff	0.00 ±0.00	0.47 ±0.42	1.20 ±0.59	1.31 ±0.57	0.80 ±0.43	0.76 ±0.52	0.62 ±0.42	0.33 ±0.38	0.00 ±0.00	-0.12 ±0.22	-0.13 ±0.27	-0.13 ±0.33	0.10 ±0.42	0.10 ±0.38	-0.17 ±0.35	-0.05 ±0.56
2%Caff	0.00 ±0.00	-0.13 ±0.32	-0.12 ±0.27	-0.06 ±0.35	0.12 ±0.29	0.04 ±0.35	-0.24 ±0.55	0.17 ±0.38	0.00 ±0.00	-0.12 ±0.23	-0.16 ±0.30	-0.15 ±0.30	0.01 ±0.27	0.00 ±0.28	-0.27 ±0.37	0.10 ±0.32

E.5. Photopic pupillary diameter in groups with 0.1% Atropine over time

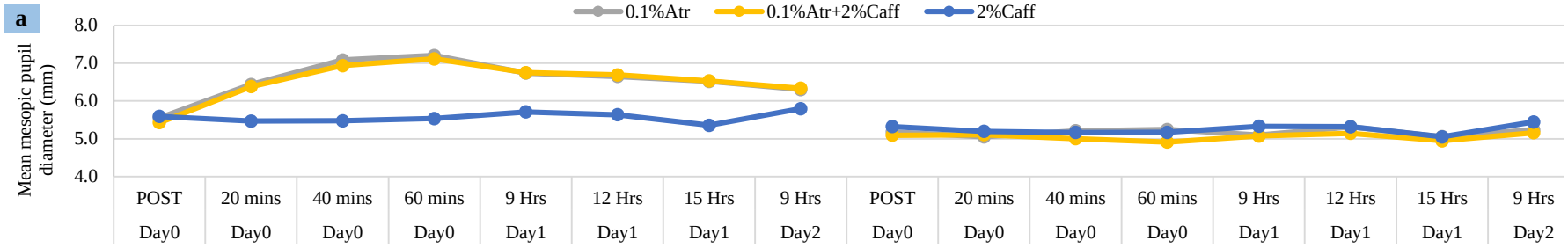


	Right Eye								Left Eye							
0.1%Atr Mean±SD	3.34 ±0.66	5.15 ±1.44	6.10 ±1.37	6.44 ±1.24	5.65 ±1.00	5.48 ±1.01	5.17 ±0.99	4.65 ±0.77	2.88 ±0.27	2.86 ±0.66	2.52 ±0.21	2.48 ±0.24	2.74 ±0.44	2.72 ±0.34	2.71 ±0.36	2.83 ±0.31
0.1%Atr + 2%Caff	3.19 ±0.55	4.58 ±1.10	5.96 ±0.85	6.44 ±0.75	5.75 ±0.66	5.62 ±0.58	5.26 ±0.64	4.68 ±0.44	2.93 ±0.35	2.71 ±0.31	2.49 ±0.22	2.52 ±0.26	2.64 ±0.31	2.65 ±0.35	2.62 ±0.37	2.87 ±0.40
2%Caff	3.13 ±0.41	3.09 ±0.43	3.14 ±0.49	3.06 ±0.41	3.33 ±0.42	3.30 ±0.43	3.22 ±0.48	3.38 ±0.49	2.92 ±0.27	2.92 ±0.24	2.88 ±0.33	2.87 ±0.31	3.01 ±0.33	2.96 ±0.31	2.98 ±0.37	3.07 ±0.35

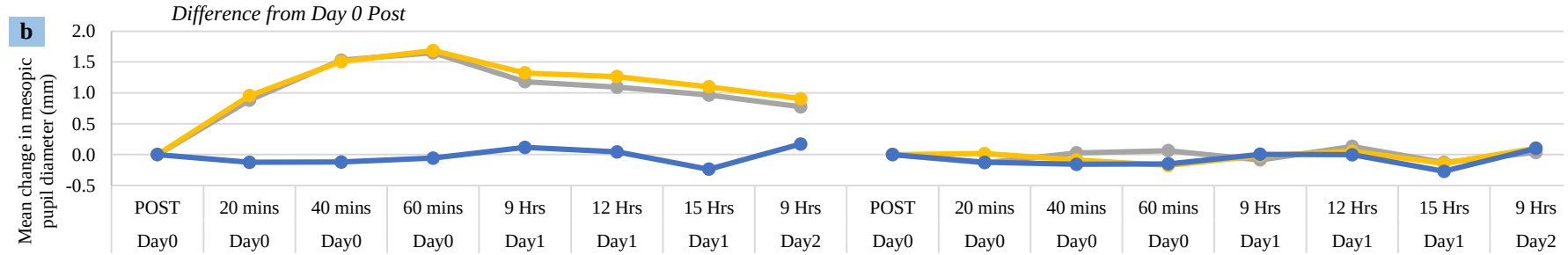


	Right Eye								Left Eye							
0.1%Atr Mean±SD	0.00 ±0.00	1.82 ±1.41	2.76 ±1.40	3.10 ±1.25	2.31 ±1.09	2.14 ±1.06	1.83 ±1.04	1.44 ±0.73	0.00 ±0.00	-0.02 ±0.59	-0.36 ±0.20	-0.40 ±0.18	-0.14 ±0.40	-0.17 ±0.26	-0.18 ±0.30	-0.09 ±0.19
0.1%Atr + 2%Caff	0.00 ±0.00	1.39 ±0.73	2.78 ±0.59	3.26 ±0.68	2.56 ±0.60	2.44 ±0.56	2.08 ±0.57	1.47 ±0.44	0.00 ±0.00	-0.22 ±0.18	-0.44 ±0.26	-0.40 ±0.16	-0.29 ±0.17	-0.28 ±0.15	-0.30 ±0.21	-0.07 0.19
2%Caff	0.00 ±0.00	-0.13 ±0.32	-0.12 ±0.27	-0.06 ±0.35	0.12 ±0.29	0.04 ±0.35	-0.24 ±0.55	0.17 ±0.38	0.00 ±0.00	-0.12 ±0.23	-0.16 ±0.30	-0.15 ±0.30	0.01 ±0.27	0.00 ±0.28	-0.27 ±0.37	0.10 ±0.32

E.6. Mesopic pupillary diameter in groups with 0.1% Atropine over time

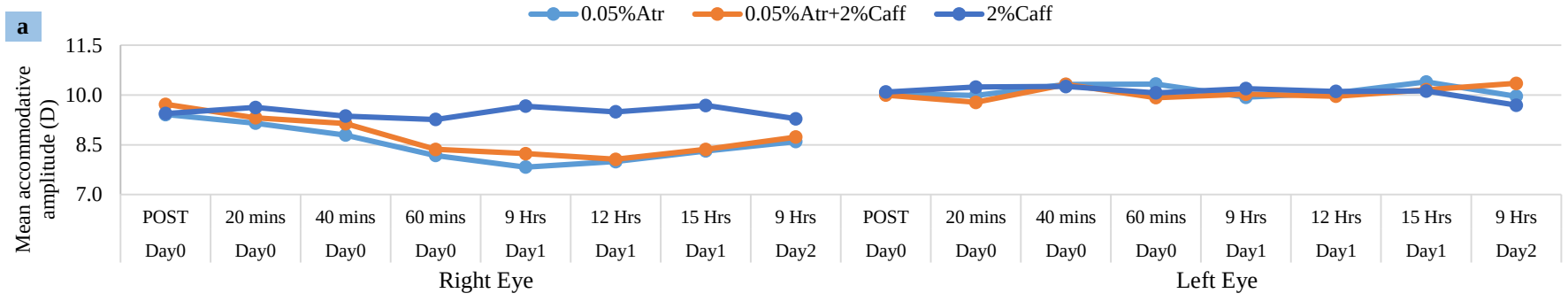


	Right Eye								Left Eye							
0.1%Atr Mean±SD	5.56 ±0.73	6.44 ±1.13	7.08 ±0.77	7.20 ±0.75	6.74 ±0.64	6.65 ±0.67	6.52 ±0.71	6.30 ±0.61	5.18 ±0.81	5.05 ±1.02	5.21 ±0.97	5.25 ±0.98	5.10 ±1.27	5.32 ±1.01	5.06 ±0.99	5.24 ±1.04
0.1%Atr + 2%Caff	5.43 ±0.82	6.38 ±0.74	6.94 ±0.65	7.11 ±0.64	6.75 ±0.52	6.69 ±0.53	6.53 ±0.53	6.34 ±0.53	5.09 ±0.88	5.11 ±0.92	5.00 ±0.87	4.91 ±0.92	5.07 ±0.92	5.14 ±0.91	4.95 ±0.98	5.16 ±1.02
2%Caff	5.59 ±0.74	5.47 ±0.71	5.47 ±0.74	5.54 ±0.83	5.71 ±0.77	5.64 ±0.78	5.36 ±0.98	5.79 ±0.80	5.32 ±0.97	5.20 ±0.98	5.17 ±0.97	5.17 ±0.98	5.33 ±1.04	5.32 ±0.99	5.05 ±1.02	5.44 ±1.08

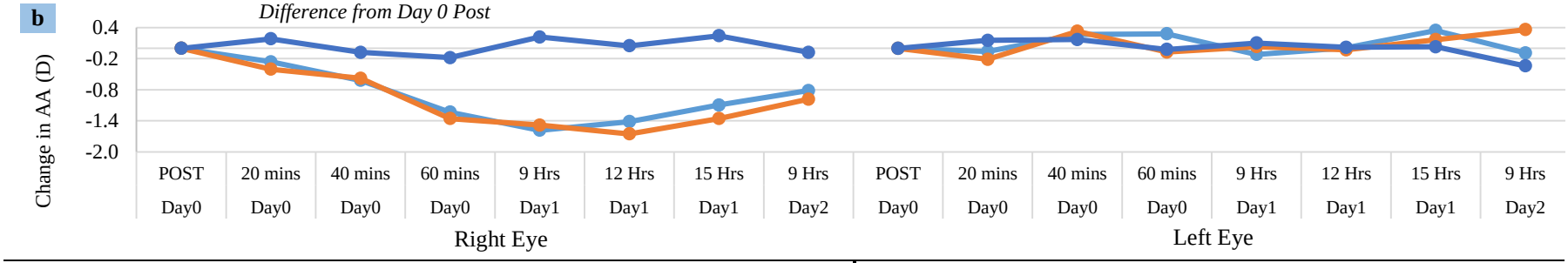


	Right Eye								Left Eye							
0.1%Atr	0.00 ±0.00	0.88 ±1.04	1.53 ±0.86	1.65 ±0.82	1.18 ±0.67	1.09 ±0.68	0.97 ±0.67	0.77 ±0.58	0.00 ±0.00	-0.13 ±0.60	0.03 ±0.41	0.06 ±0.48	-0.09 ±0.62	0.13 ±0.45	-0.13 ±0.44	0.04 ±0.41
0.1%Atr + 2%Caff	0.00 ±0.00	0.95 ±0.50	1.51 ±0.68	1.69 ±0.74	1.32 ±0.61	1.26 ±0.62	1.10 ±0.57	0.91 ±0.50	0.00 ±0.00	0.02 ±0.30	-0.09 ±0.40	-0.18 ±0.48	-0.02 ±0.37	0.05 ±0.43	-0.14 ±0.38	0.11 ±0.36
2%Caff	0.00 ±0.00	-0.13 ±0.32	-0.12 ±0.27	-0.06 ±0.35	0.12 ±0.29	0.04 ±0.35	-0.24 ±0.55	0.17 ±0.38	0.00 ±0.00	-0.12 ±0.23	-0.16 ±0.30	-0.15 ±0.30	0.01 ±0.27	0.00 ±0.28	-0.27 ±0.37	0.10 ±0.32

E.7. Accommodative amplitude in groups with 0.05% Atropine over time

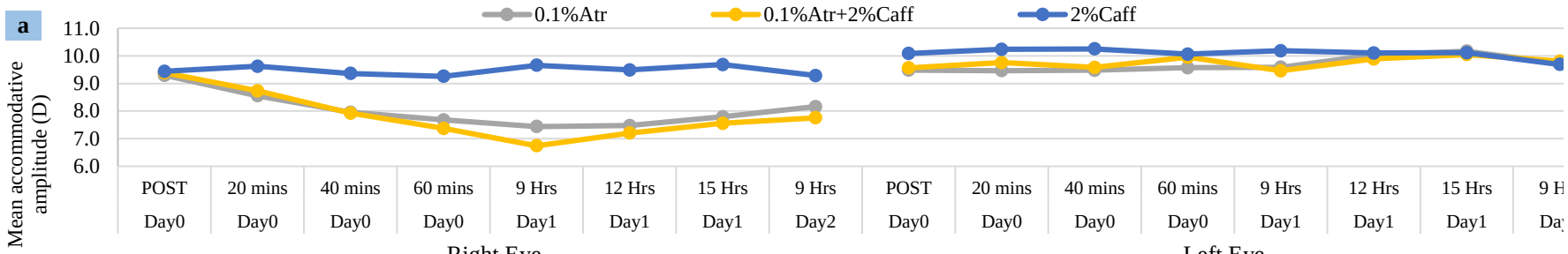


0.05%Atr Mean±SD	9.41 ±1.63	9.15 ±1.50	8.79 ±1.36	8.18 ±1.74	7.83 ±1.47	7.99 ±1.43	8.32 ±1.61	8.60 ±1.74	10.05 ±1.85	9.99 ±1.86	10.32 ±1.76	10.33 ±2.04	9.93 ±1.72	10.06 ±1.87	10.39 ±2.20	9.96 ±1.88
0.05%Atr + 2%Caff	9.72 ±1.32	9.31 ±1.42	9.14 ±2.44	8.36 ±2.53	8.23 ±1.34	8.06 ±1.06	8.36 ±1.09	8.73 ±1.20	9.99 ±1.49	9.78 ±1.45	10.32 ±2.50	9.92 ±1.56	10.02 ±1.95	9.96 ±1.48	10.16 ±1.43	10.35 ±1.73
2%Caff	9.44 ±1.44	9.62 ±1.56	9.36 ±1.61	9.26 ±1.40	9.66 ±1.95	9.49 ±1.37	9.68 ±1.62	9.28 ±1.53	10.09 ±1.57	10.24 ±1.68	10.26 ±1.96	10.06 ±1.81	10.19 ±1.75	10.11 ±1.65	10.12 ±1.75	9.69 ±1.57

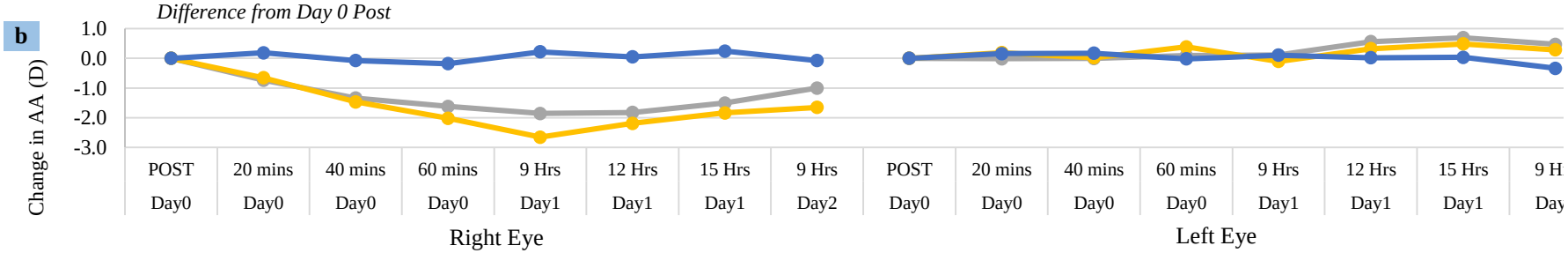


0.05%Atr Mean±SD	0.00 ±0.00	-0.26 ±1.23	-0.62 ±1.33	-1.23 ±1.37	-1.58 ±1.39	-1.42 ±1.56	-1.10 ±1.74	-0.82 ±1.61	0.00 ±0.00	-0.06 ±1.43	0.27 ±1.63	0.28 ±1.55	-0.12 ±1.48	0.01 ±1.94	0.35 ±2.16	-0.09 ±1.71
0.05%Atr + 2%Caff	0.00 ±0.00	-0.40 ±0.77	-0.58 ±1.88	-1.36 ±2.13	-1.48 ±1.27	-1.65 ±1.41	-1.36 ±1.51	-0.98 ±1.79	0.00 ±0.00	-0.21 ±0.69	0.33 ±1.53	-0.07 ±0.89	0.03 ±1.37	-0.03 ±1.09	0.17 ±1.68	0.36 ±1.52
2%Caff	0.00 ±0.00	0.18 ±0.77	-0.08 ±1.16	-0.18 ±1.18	0.22 ±1.36	0.05 ±1.33	0.24 ±1.35	-0.08 ±1.40	0.00 ±0.00	0.15 ±0.91	0.17 ±1.06	-0.02 ±1.07	0.10 ±1.27	0.02 ±1.15	0.03 ±1.37	-0.34 ±1.37

E.8. Accommodative amplitude in groups with 0.1% Atropine over time



0.1%Atr Mean±SD	9.29 ±1.28	8.56 ±0.96	7.96 ±0.76	7.68 ±0.76	7.44 ±1.18	7.47 ±1.29	7.79 ±0.88	8.16 ±1.25	9.48 ±1.22	9.47 ±1.13	9.48 ±1.17	9.57 ±0.97	9.59 ±0.95	10.04 ±1.58	10.17 ±1.82	9.73 ±1.39
0.1%Atr + 2%Caff	9.39 ±1.78	8.73 ±1.26	7.92 ±1.18	7.37 ±1.39	6.74 ±1.36	7.20 ±1.39	7.56 ±1.58	7.76 ±1.49	9.57 ±1.86	9.76 ±1.57	9.58 ±1.60	9.95 ±1.81	9.46 ±1.18	9.89 ±1.51	10.05 ±1.34	9.81 ±1.39
2%Caff	9.44 ±1.44	9.62 ±1.56	9.36 ±1.61	9.26 ±1.40	9.66 ±1.95	9.49 ±1.37	9.68 ±1.62	9.28 ±1.53	10.09 ±1.57	10.24 ±1.68	10.26 ±1.96	10.06 ±1.81	10.19 ±1.75	10.11 ±1.65	10.12 ±1.75	9.69 ±1.57



0.1%Atr Mean±SD	0.00 ±0.00	-0.74 ±1.03	-1.34 ±1.05	-1.62 ±0.91	-1.85 ±1.59	-1.82 ±1.58	-1.51 ±1.04	-1.00 ±1.19	0.00 ±0.00	-0.02 ±0.96	0.00 ±0.78	0.09 ±0.87	0.11 ±1.02	0.56 ±1.45	0.69 ±1.65	0.47 ±1.30
0.1%Atr + 2%Caff	0.00 ±0.00	-0.66 ±0.87	-1.47 ±1.17	-2.02 ±1.33	-2.65 ±2.06	-2.19 ±1.91	-1.83 ±1.92	-1.65 ±1.82	0.00 ±0.00	0.19 ±0.88	0.02 ±0.76	0.38 ±1.00	-0.11 ±1.31	0.32 ±0.60	0.48 ±1.48	0.29 ±2.01
2%Caff	0.00 ±0.00	0.18 ±0.77	-0.08 ±1.16	-0.18 ±1.18	0.22 ±1.36	0.05 ±1.33	0.24 ±1.35	-0.08 ±1.40	0.00 ±0.00	0.15 ±0.91	0.17 ±1.06	-0.02 ±1.07	0.10 ±1.27	0.02 ±1.15	0.03 ±1.37	-0.34 ±1.37

E.9. Pairwise comparison of mean at visits within groups and of difference from post-instillation (Day0_Post) within groups

Pairwise Comparisons for Data at each visit		Right eye (treated eyes)														
		Day0 Post														
		POST 20 mins					POST 40 mins					POST 60 mins				
		0.05% Atr	0.05% Atr + 2% Caff	0.1% Atr	0.1% Atr + 2% Caff	2% Caff	0.05% Atr	0.05% Atr + 2% Caff	0.1% Atr	0.1% Atr + 2% Caff	2% Caff	0.05% Atr	0.05% Atr + 2% Caff	0.1% Atr	0.1% Atr + 2% Caff	2% Caff
Amplitude of Accommodation	0.05% Atr		1.000	1.000	1.000	1.000		1.000	0.983	0.869	1.000		1.000	1.000	0.825	0.247
	0.05% Atr + 2%	1.000		1.000	1.000	1.000	1.000		0.161	0.137	1.000	1.000		1.000	0.296	0.742
	0.1% Atr	1.000	1.000		1.000	***0.045***	0.983	0.161		1.000	***0.012***	1.000	1.000		1.000	***0.002***
	0.1% Atr + 2%	1.000	1.000	1.000		0.173	0.869	0.137	1.000		***0.009***	0.825	0.296	1.000		***0.000***
	2% Caff	1.000	1.000	***0.045***	0.173		1.000	1.000	***0.012***	***0.009***		0.247	0.742	***0.002***	***0.000***	
Pupils Size Mesopic	0.05% Atr		0.849	1.000	1.000	***0.000***		1.000	0.563	1.000	***0.000***		1.000	1.000	1.000	***0.000***
	0.05% Atr + 2%	0.849		0.498	0.829	***0.006***	1.000		0.215	1.000	***0.000***	1.000		0.468	1.000	***0.000***
	0.1% Atr	1.000	0.498		1.000	***0.000***	0.563	0.215		1.000	***0.000***	1.000	0.468		1.000	***0.000***
	0.1% Atr + 2%	1.000	0.829	1.000		***0.000***	1.000	1.000	1.000		***0.000***	1.000	1.000	1.000		***0.000***
	2% Caff	***0.000***	***0.006***	***0.000***	***0.000***		***0.000***	***0.000***	***0.000***	***0.000***		***0.000***	***0.000***	***0.000***	***0.000***	
Pupils Size Photopic	0.05% Atr		1.000	***0.034***	1.000	***0.000***		1.000	0.308	0.831	***0.000***		1.000	1.000	1.000	***0.000***
	0.05% Atr + 2%	1.000		***0.001***	0.310	***0.002***	1.000		***0.012***	***0.047***	***0.000***	1.000		0.251	0.249	***0.000***
	0.1% Atr	***0.034***	***0.001***		0.204	***0.000***	0.308	***0.012***		1.000	***0.000***	1.000	0.251		1.000	***0.000***
	0.1% Atr + 2%	1.000	0.310	0.204		***0.000***	0.831	***0.047***	1.000		***0.000***	1.000	0.249	1.000		***0.000***
	2% Caff	***0.000***	***0.002***	***0.000***	***0.000***		***0.000***	***0.000***	***0.000***	***0.000***		***0.000***	***0.000***	***0.000***	***0.000***	
		Day1 09Hr					Day1 12Hr					Day1 15Hr				
Amplitude of Accommodation	0.05% Atr		1.000	1.000	0.802	***0.001***		1.000	1.000	0.443	***0.000***		1.000	1.000	1.000	***0.001***
	0.05% Atr + 2%	1.000		1.000	0.105	***0.022***	1.000		0.789	0.177	***0.001***	1.000		1.000	1.000	***0.003***
	0.1% Atr	1.000	1.000		1.000	***0.000***	1.000	0.789		1.000	***0.000***	1.000	1.000		1.000	***0.000***
	0.1% Atr + 2%	0.802	0.105	1.000		***0.000***	0.443	0.177	1.000		***0.000***	1.000	1.000	1.000		***0.000***
	2% Caff	***0.001***	***0.022***	***0.000***	***0.000***		***0.000***	***0.001***	***0.000***	***0.000***		***0.001***	***0.003***	***0.000***	***0.000***	
Pupils Size Mesopic	0.05% Atr		1.000	***0.047***	***0.039***	***0.000***		1.000	0.473	0.268	***0.000***		1.000	0.552	0.509	***0.000***
	0.05% Atr + 2%	1.000		***0.027***	***0.022***	***0.000***	1.000		0.431	0.241	***0.000***	1.000		0.629	0.581	***0.000***
	0.1% Atr	***0.047***	***0.027***		1.000	***0.000***	0.473	0.431		1.000	***0.000***	0.552	0.629		1.000	***0.000***
	0.1% Atr + 2%	***0.039***	***0.022***	1.000		***0.000***	0.268	0.241	1.000		***0.000***	0.509	0.581	1.000		***0.000***
	2% Caff	***0.000***	***0.000***	***0.000***	***0.000***		***0.000***	***0.000***	***0.000***	***0.000***		***0.000***	***0.000***	***0.000***	***0.000***	
Pupils Size Photopic	0.05% Atr		1.000	0.145	***0.043***	***0.000***		1.000	0.081	***0.011***	***0.000***		0.517	0.152	***0.046***	***0.000***
	0.05% Atr + 2%	1.000		***0.002***	***0.000***	***0.000***	1.000		***0.002***	***0.000***	***0.000***	0.517		***0.000***	***0.000***	***0.000***
	0.1% Atr	0.145	***0.002***		1.000	***0.000***	0.081	***0.002***		1.000	***0.000***	0.152	***0.000***		1.000	***0.000***
	0.1% Atr + 2%	***0.043***	***0.000***	1.000		***0.000***	***0.011***	***0.000***	1.000		***0.000***	***0.046***	***0.000***	1.000		***0.000***
	2% Caff	***0.000***	***0.000***	***0.000***	***0.000***		***0.000***	***0.000***	***0.000***	***0.000***		***0.000***	***0.000***	***0.000***	***0.000***	

Pairwise Comparisons for Data at each visit		Left eye (non-treated eyes)														
		Day0 Post														
		POST 20 mins					POST 40 mins					POST 60 mins				
		0.05% Atr	0.05% Atr + 2% Caff	0.1% Atr	0.1% Atr + 2% Caff	2% Caff	0.05% Atr	0.05% Atr + 2% Caff	0.1% Atr	0.1% Atr + 2% Caff	2% Caff	0.05% Atr	0.05% Atr + 2% Caff	0.1% Atr	0.1% Atr + 2% Caff	2% Caff
Amplitude of Accommodation	0.05% Atr		1.000	1.000	1.000	1.000		1.000	1.000	1.000	1.000		1.000	1.000	1.000	1.000
	0.05% Atr + 2%	1.000		1.000	1.000	1.000	1.000		0.561	0.829	1.000	1.000		1.000	1.000	1.000
	0.1% Atr	1.000	1.000		1.000	0.138	1.000	0.561		1.000	0.391	1.000	1.000		1.000	1.000
	0.1% Atr + 2%	1.000	1.000	1.000		0.903	1.000	0.829	1.000		0.667	1.000	1.000	1.000		1.000
	2% Caff	1.000	1.000	0.138	0.903		1.000	1.000	0.391	0.667		1.000	1.000	1.000	1.000	
Pupils Size Mesopic	0.05% Atr		1.000	1.000	1.000	1.000		1.000	1.000	1.000	1.000		1.000	1.000	1.000	1.000
	0.05% Atr + 2%	1.000		1.000	1.000	1.000	1.000		1.000	1.000	1.000	1.000		0.584	1.000	1.000
	0.1% Atr	1.000	1.000		1.000	1.000	1.000	1.000		0.482	0.996	1.000	0.584		***0.008***	0.171
	0.1% Atr + 2%	1.000	1.000	1.000		1.000	1.000	1.000	0.482		1.000	1.000	1.000	***0.008***		1.000
	2% Caff	1.000	1.000	1.000	1.000		1.000	1.000	0.996	1.000		1.000	1.000	0.171	1.000	
Pupils Size Photopic	0.05% Atr		1.000	1.000	1.000	1.000		1.000	1.000	1.000	***0.001***		1.000	1.000	1.000	***0.000***
	0.05% Atr + 2%	1.000		1.000	1.000	1.000	1.000		1.000	1.000	***0.023***	1.000		1.000	1.000	***0.001***
	0.1% Atr	1.000	1.000		1.000	1.000	1.000	1.000		1.000	***0.000***	1.000	1.000		1.000	***0.000***
	0.1% Atr + 2%	1.000	1.000	1.000		0.544	1.000	1.000	1.000		***0.000***	1.000	1.000	1.000		***0.000***
	2% Caff	1.000	1.000	1.000	0.544		***0.001***	***0.023***	***0.000***	***0.000***		***0.000***	***0.001***	***0.000***	***0.000***	
		Day1 09Hr					Day1 12Hr					Day1 15Hr				
Amplitude of Accommodation	0.05% Atr		1.000	1.000	1.000	1.000		1.000	1.000	1.000	1.000		1.000	1.000	1.000	1.000
	0.05% Atr + 2%	1.000		1.000	1.000	1.000	1.000		1.000	1.000	1.000	1.000		1.000	1.000	1.000
	0.1% Atr	1.000	1.000		1.000	1.000	1.000	1.000		1.000	1.000	1.000	1.000		1.000	1.000
	0.1% Atr + 2%	1.000	1.000	1.000		1.000	1.000	1.000	1.000		1.000	1.000	1.000	1.000		1.000
	2% Caff	1.000	1.000	1.000	1.000		1.000	1.000	1.000	1.000		1.000	1.000	1.000	1.000	
Pupils Size Mesopic	0.05% Atr		1.000	1.000	1.000	1.000		0.977	1.000	1.000	1.000		1.000	1.000	1.000	1.000
	0.05% Atr + 2%	1.000		1.000	1.000	1.000	0.977		1.000	1.000	1.000	1.000		1.000	1.000	1.000
	0.1% Atr	1.000	1.000		1.000	1.000	1.000	1.000		0.797	1.000	1.000	1.000		1.000	1.000
	0.1% Atr + 2%	1.000	1.000	1.000		1.000	1.000	1.000	0.797		1.000	1.000	1.000	1.000		1.000
	2% Caff	1.000	1.000	1.000	1.000		1.000	1.000	1.000	1.000		1.000	1.000	1.000	1.000	
Pupils Size Photopic	0.05% Atr		1.000	1.000	1.000	***0.016***		1.000	1.000	1.000	***0.006***		1.000	1.000	1.000	***0.019***
	0.05% Atr + 2%	1.000		1.000	1.000	***0.004***	1.000		1.000	1.000	***0.003***	1.000		1.000	1.000	***0.004***
	0.1% Atr	1.000	1.000		1.000	***0.012***	1.000	1.000		1.000	***0.004***	1.000	1.000		1.000	0.155
	0.1% Atr + 2%	1.000	1.000	1.000		***0.000***	1.000	1.000	1.000		***0.000***	1.000	1.000	1.000		***0.007***
	2% Caff	***0.016***	***0.004***	***0.012***	***0.000***		***0.006***	***0.003***	***0.004***	***0.000***		***0.019***	***0.004***	0.155	***0.007***	

Pairwise Comparisons for Difference from Day0		Right eye (treated eyes)														
		Day0 Post POST 20					POST 40					POST 60				
		0.05% Atr	0.05% Atr + 2% Caff	0.1% Atr	0.1% Atr + 2% Caff	2% Caff	0.05% Atr	0.05% Atr + 2% Caff	0.1% Atr	0.1% Atr + 2% Caff	2% Caff	0.05% Atr	0.05% Atr + 2% Caff	0.1% Atr	0.1% Atr + 2% Caff	2% Caff
Amplitude of Accommodation	0.05% Atr		1.000	1.000	1.000	1.000		1.000	1.000	0.874	1.000		1.000	1.000	1.000	0.227
	0.05% Atr + 2%	1.000		1.000	1.000	0.465	1.000		1.000	0.684	1.000	1.000		1.000	1.000	0.095
	0.1% Atr	1.000	1.000		1.000	***0.021***	1.000	1.000		1.000	***0.033***	1.000	1.000		1.000	***0.017***
	0.1% Atr + 2%	1.000	1.000	1.000		***0.049***	0.874	0.684	1.000		***0.013***	1.000	1.000	1.000		***0.001***
	2% Caff	1.000	0.465	***0.021***	***0.049***		1.000	1.000	***0.033***	***0.013***		0.227	0.095	***0.017***	***0.001***	
Pupils Size Mesopic	0.05% Atr		0.545	1.000	1.000	***0.000***		1.000	1.000	1.000	***0.000***		1.000	1.000	1.000	***0.000***
	0.05% Atr + 2%	0.545		0.787	0.385	***0.026***	1.000		1.000	1.000	***0.000***	1.000		1.000	1.000	***0.000***
	0.1% Atr	1.000	0.787		1.000	***0.000***	1.000	1.000		1.000	***0.000***	1.000	1.000		1.000	***0.000***
	0.1% Atr + 2%	1.000	0.385	1.000		***0.000***	1.000	1.000	1.000		***0.000***	1.000	1.000	1.000		***0.000***
	2% Caff	***0.000***	***0.026***	***0.000***	***0.000***		***0.000***	***0.000***	***0.000***	***0.000***		***0.000***	***0.000***	***0.000***	***0.000***	
Pupils Size Photopic	0.05% Atr		1.000	***0.043***	1.000	***0.001***		1.000	0.581	0.510	***0.000***		1.000	1.000	0.789	***0.000***
	0.05% Atr + 2%	1.000		***0.032***	1.000	***0.001***	1.000		0.521	0.456	***0.000***	1.000		1.000	1.000	***0.000***
	0.1% Atr	***0.043***	***0.032***		1.000	***0.000***	0.581	0.521		1.000	***0.000***	1.000	1.000		1.000	***0.000***
	0.1% Atr + 2%	1.000	1.000	1.000		***0.000***	0.510	0.456	1.000		***0.000***	0.789	1.000	1.000		***0.000***
	2% Caff	***0.001***	***0.001***	***0.000***	***0.000***		***0.000***	***0.000***	***0.000***	***0.000***		***0.000***	***0.000***	***0.000***	***0.000***	
		Day1 09Hr					Day1 12Hr					Day1 15Hr				
Amplitude of Accommodation	0.05% Atr		1.000	1.000	0.657	***0.001***		1.000	1.000	1.000	***0.025***		1.000	1.000	1.000	***0.022***
	0.05% Atr + 2%	1.000		1.000	0.425	***0.002***	1.000		1.000	1.000	***0.003***	1.000		1.000	1.000	***0.002***
	0.1% Atr	1.000	1.000		1.000	***0.000***	1.000	1.000		1.000	***0.001***	1.000	1.000		1.000	***0.001***
	0.1% Atr + 2%	0.657	0.425	1.000		***0.000***	1.000	1.000	1.000		***0.000***	1.000	1.000	1.000		***0.000***
	2% Caff	***0.001***	***0.002***	***0.000***	***0.000***		***0.025***	***0.003***	***0.001***	***0.000***		***0.022***	***0.002***	***0.001***	***0.000***	
Pupils Size Mesopic	0.05% Atr		1.000	1.000	0.594	***0.000***		1.000	1.000	0.876	***0.000***		1.000	1.000	0.888	***0.000***
	0.05% Atr + 2%	1.000		0.647	0.128	***0.002***	1.000		1.000	0.271	***0.001***	1.000		1.000	0.342	***0.000***
	0.1% Atr	1.000	0.647		1.000	***0.000***	1.000	1.000		1.000	***0.000***	1.000	1.000		1.000	***0.000***
	0.1% Atr + 2%	0.594	0.128	1.000		***0.000***	0.876	0.271	1.000		***0.000***	0.888	0.342	1.000		***0.000***
	2% Caff	***0.000***	***0.002***	***0.000***	***0.000***		***0.000***	***0.001***	***0.000***	***0.000***		***0.000***	***0.000***	***0.000***	***0.000***	
Pupils Size Photopic	0.05% Atr		1.000	0.179	***0.012***	***0.000***		1.000	0.188	***0.006***	***0.000***		1.000	0.518	***0.045***	***0.000***
	0.05% Atr + 2%	1.000		0.213	***0.014***	***0.000***	1.000		0.315	***0.012***	***0.000***	1.000		0.241	***0.016***	***0.000***
	0.1% Atr	0.179	0.213		1.000	***0.000***	0.188	0.315		1.000	***0.000***	0.518	0.241		1.000	***0.000***
	0.1% Atr + 2%	***0.012***	***0.014***	1.000		***0.000***	***0.006***	***0.012***	1.000		***0.000***	***0.045***	***0.016***	1.000		***0.000***
	2% Caff	***0.000***	***0.000***	***0.000***	***0.000***		***0.000***	***0.000***	***0.000***	***0.000***		***0.000***	***0.000***	***0.000***	***0.000***	

Pairwise Comparisons for Difference from Day0		Left eye (non-treated eyes)														
		Day0 Post														
		POST 20					POST 40					POST 60				
		0.05% Atr	0.05% Atr + 2% Caff	0.1% Atr	0.1% Atr + 2% Caff	2% Caff	0.05% Atr	0.05% Atr + 2% Caff	0.1% Atr	0.1% Atr + 2% Caff	2% Caff	0.05% Atr	0.05% Atr + 2% Caff	0.1% Atr	0.1% Atr + 2% Caff	2% Caff
Amplitude of Accommodation	0.05% Atr		1.000	1.000	1.000	1.000		1.000	1.000	1.000	1.000		1.000	1.000	1.000	1.000
	0.05% Atr + 2%	1.000		1.000	1.000	1.000	1.000		1.000	1.000	1.000	1.000		1.000	1.000	1.000
	0.1% Atr	1.000	1.000		1.000	1.000	1.000	1.000		1.000	1.000	1.000	1.000		1.000	1.000
	0.1% Atr + 2%	1.000	1.000	1.000		1.000	1.000	1.000	1.000		1.000	1.000	1.000	1.000		1.000
	2% Caff	1.000	1.000	1.000	1.000		1.000	1.000	1.000	1.000		1.000	1.000	1.000	1.000	
Pupils Size Mesopic	0.05% Atr		0.597	0.467	1.000	0.248		1.000	1.000	1.000	1.000		1.000	1.000	1.000	1.000
	0.05% Atr + 2%	0.597		1.000	1.000	1.000	1.000		1.000	1.000	1.000	1.000		1.000	1.000	1.000
	0.1% Atr	0.467	1.000		1.000	1.000	1.000	1.000		1.000	1.000	1.000	1.000		1.000	1.000
	0.1% Atr + 2%	1.000	1.000	1.000		1.000	1.000	1.000	1.000		1.000	1.000	1.000	1.000		1.000
	2% Caff	0.248	1.000	1.000	1.000		1.000	1.000	1.000	1.000		1.000	1.000	1.000	1.000	
Pupils Size Photopic	0.05% Atr		0.611	0.062	1.000	***0.011***		0.288	1.000	1.000	***0.000***		0.170	0.359	0.380	***0.000***
	0.05% Atr + 2%	0.611		1.000	1.000	1.000	0.288		1.000	1.000	0.161	0.170		1.000	1.000	***0.012***
	0.1% Atr	0.062	1.000		1.000	1.000	1.000	1.000		1.000	***0.027***	0.359	1.000		1.000	***0.004***
	0.1% Atr + 2%	1.000	1.000	1.000		0.808	1.000	1.000	1.000		***0.003***	0.380	1.000	1.000		***0.003***
	2% Caff	***0.011***	1.000	1.000	0.808		***0.000***	0.161	***0.027***	***0.003***		***0.000***	***0.012***	***0.004***	***0.003***	
		Day1 09Hr					Day1 12Hr					Day1 15Hr				
Amplitude of Accommodation	0.05% Atr		1.000	1.000	1.000	1.000		1.000	1.000	1.000	1.000		1.000	1.000	1.000	1.000
	0.05% Atr + 2%	1.000		1.000	1.000	1.000	1.000		1.000	1.000	1.000	1.000		1.000	1.000	1.000
	0.1% Atr	1.000	1.000		1.000	1.000	1.000	1.000		1.000	1.000	1.000	1.000		1.000	1.000
	0.1% Atr + 2%	1.000	1.000	1.000		1.000	1.000	1.000	1.000		1.000	1.000	1.000	1.000		1.000
	2% Caff	1.000	1.000	1.000	1.000		1.000	1.000	1.000	1.000		1.000	1.000	1.000	1.000	
Pupils Size Mesopic	0.05% Atr		1.000	1.000	1.000	1.000		1.000	1.000	1.000	1.000		1.000	1.000	1.000	0.650
	0.05% Atr + 2%	1.000		1.000	1.000	1.000	1.000		1.000	1.000	1.000	1.000		1.000	1.000	1.000
	0.1% Atr	1.000	1.000		1.000	1.000	1.000	1.000		1.000	1.000	1.000	1.000		1.000	1.000
	0.1% Atr + 2%	1.000	1.000	1.000		1.000	1.000	1.000	1.000		1.000	1.000	1.000	1.000		1.000
	2% Caff	1.000	1.000	1.000	1.000		1.000	1.000	1.000	1.000		0.650	1.000	1.000	1.000	
Pupils Size Photopic	0.05% Atr		1.000	0.448	1.000	***0.000***		1.000	0.409	1.000	***0.000***		1.000	0.879	1.000	***0.002***
	0.05% Atr + 2%	1.000		1.000	1.000	***0.024***	1.000		1.000	1.000	0.090	1.000		1.000	1.000	0.096
	0.1% Atr	0.448	1.000		1.000	0.301	0.409	1.000		1.000	0.427	0.879	1.000		1.000	0.588
	0.1% Atr + 2%	1.000	1.000	1.000		***0.006***	1.000	1.000	1.000		***0.022***	1.000	1.000	1.000		***0.034***
	2% Caff	***0.000***	***0.024***	0.301	***0.006***		***0.000***	0.090	0.427	***0.022***		***0.002***	0.096	0.588	***0.034***	

Appendix 2: Publications and Presentations

A. Publications related to the thesis

1. **Tran, H. D.**, Tran, Y. H., Tran, T. D., Jong, M., Coroneo, M., & Sankaridurg, P. (2018). A review of myopia control with Atropine. *Journal of Ocular Pharmacology and Therapeutics*, *34*(5), 374-379.
2. Sankaridurg, P., Conrad, F., **Tran, H.**, & Zhu, J. (2018). Controlling progression of myopia: optical and pharmaceutical strategies. *The Asia-Pacific Journal of Ophthalmology*, *7*(6), 405-414.
3. **Tran, H. D. M.**, Tran, Y. H., Coroneo, M., Tran, T. D., Pham, T., Naduvilath, T., & Sankaridurg, P. (2019). Effects of low concentration Atropine on pupillary size and accommodative amplitude in children with myopia. *Investigative Ophthalmology & Visual Science*, *60*(9), 4333-4333.
4. Sankaridurg, P., & **Tran, H. D.** (2019). The lowdown on low-concentration Atropine for myopia progression. *Ophthalmology*, *126*(1), 125-126.
5. **Tran, H. D. M.**, Sankaridurg, P., Naduvilath, T., Ha, T. T. X., Tran, T. D., Jong, M., Coroneo, M., Tran, Y. H. (2021). A Meta-Analysis Assessing Change in Pupillary Diameter, Accommodative Amplitude, and Efficacy of Atropine for Myopia Control. Asia Pac J Ophthalmol (Phila). 2021 Aug 27. doi: 10.1097/APO.0000000000000414. Epub ahead of print. PMID: 34456234.

Tran, H. D., Tran, Y. H., Tran, T. D., Jong, M., Coroneo, M., & Sankaridurg, P. (2018). A review of myopia control with Atropine. *Journal of Ocular Pharmacology and Therapeutics*, *34*(5), 374-379.

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INVITED REVIEW

A Review of Myopia Control with Atropine

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Abstract

Myopia is a global public health issue with a worldwide prevalence of ~30% and is estimated to rise to 50% by 2050. In addition to the burden associated with routine management of the condition, high myopia predisposes the eye to sight-threatening complications such as myopic maculopathy and glaucoma in adult life. Controlling onset and progression of myopia at a young age can reduce the risk of morbidity associated with high myopia. Progression of myopia can be slowed with various optical, environmental, and pharmaceutical strategies, of which atropine has proven to be the most effective. High-dose atropine (0.5%–1%) is the most effective, but it has significant trade-offs with respect to rebound of myopia on discontinuation and side effects such as photophobia and difficulty with near work (decreased accommodation). Low doses of atropine have been trialed and show a dose-dependent efficacy. However, its mode of action on the ocular tissues leading to slowing eye growth remains unclear and multiple mechanisms and sites in the eye have been postulated to play a role. This review summarizes the role of atropine in controlling myopia and the mechanisms studied to date.

Keywords: myopia, myopia control, atropine, muscarinic antagonists

Background

In the United States, the prevalence of myopia (nearsightedness) has nearly doubled in just over 30 years from 25% in 1972 to 41.6% in 2004.[1] Even more startling is its prevalence in many East Asian countries such as China, Taiwan, Hong Kong, Singapore, and Korea where ~42.4% to 96.5% of children aged between 6 and 19 years are affected by the condition.[2–5] Importantly, the prevalence of high myopia (worse than –5.00D) is also high with 2.8% of the world's population being affected in 2010.[6] Considered to be one of the leading public health issues of our times, evidence shows a rising prevalence of myopia and high myopia, with estimates indicating that myopia will affect between 23% and 66% of the global population by 2050.[6] That puts an estimated 5 billion people at risk of developing myopia, of which 1 billion (10%) people are at risk of developing high myopia.[6]

Myopia, a progressive condition, is a leading cause of uncorrected refractive error, and the annual global cost relating to the decrease of productivity associated with untreated refractive error was more than US$200 billion over 5 years.[7,8] Beside the fact that uncorrected myopia is one of the leading causes of visual impairment,[9] pathological changes associated with axial elongation such as chorioretinal degeneration or myopic choroidal neovascularization also significantly increase the risk of visual impairment and/or blindness. Indeed, studies from Copenhagen[10] and Rotterdam[11] report that myopic degeneration is among the leading causes of visual impairment in the population, while the primary cause of blindness in Japan is myopic macular degeneration.[12] In addition, high myopia also increases the risk of vision impairment and other serious conditions such as glaucoma[13,14] and retinal detachment.[15,16] Slowing myopic progression can prevent the risk of complications and the future burden of visual impairment and blindness.

Myopia commonly results from axial elongation of the eye. The annual progression of eye length in children varies between 0.1 and 0.45 mm and is influenced by age, family history, and ethnicity.[17,18] In terms of spherical equivalent, mean myopia progression in children aged from 6 to 15 years of age is estimated to range between 0.4D/year[19] and 1.0D/year or more[20–22] for Caucasian and Asian eyes, respectively. The exact mechanism underlying progression of myopia is

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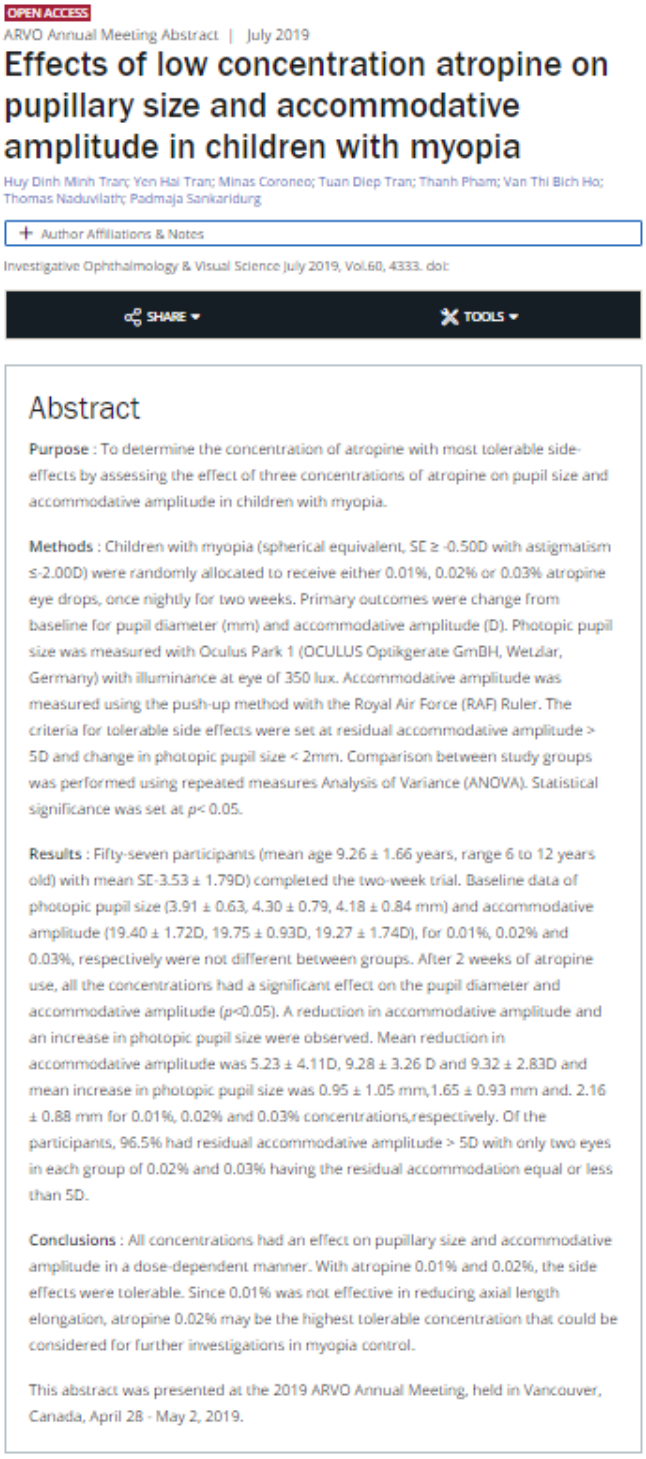
Tran, H. D. M., Tran, Y. H., Coroneo, M., Tran, T. D., Pham, T., Naduvilath, T., & Sankaridurg, P. (2019). Effects of low concentration Atropine on pupillary size and accommodative amplitude in children with myopia. *Investigative Ophthalmology & Visual Science*, *60*(9), 4333-4333.



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Effects of low concentration atropine on pupillary size and accommodative amplitude in children with myopia

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Abstract

Purpose : To determine the concentration of atropine with most tolerable side-effects by assessing the effect of three concentrations of atropine on pupil size and accommodative amplitude in children with myopia.

Methods : Children with myopia (spherical equivalent, SE ≥ -0.50D with astigmatism ≤-2.00D) were randomly allocated to receive either 0.01%, 0.02% or 0.03% atropine eye drops, once nightly for two weeks. Primary outcomes were change from baseline for pupil diameter (mm) and accommodative amplitude (D). Photopic pupil size was measured with Oculus Park 1 (OCULUS Optikgerate GmBH, Wetzlar, Germany) with illuminance at eye of 350 lux. Accommodative amplitude was measured using the push-up method with the Royal Air Force (RAF) Ruler. The criteria for tolerable side effects were set at residual accommodative amplitude > 5D and change in photopic pupil size < 2mm. Comparison between study groups was performed using repeated measures Analysis of Variance (ANOVA). Statistical significance was set at $p < 0.05$.

Results : Fifty-seven participants (mean age 9.26 ± 1.66 years, range 6 to 12 years old) with mean SE -3.53 ± 1.79D) completed the two-week trial. Baseline data of photopic pupil size (3.91 ± 0.63, 4.30 ± 0.79, 4.18 ± 0.84 mm) and accommodative amplitude (19.40 ± 1.72D, 19.75 ± 0.93D, 19.27 ± 1.74D), for 0.01%, 0.02% and 0.03%, respectively were not different between groups. After 2 weeks of atropine use, all the concentrations had a significant effect on the pupil diameter and accommodative amplitude ($p<0.05$). A reduction in accommodative amplitude and an increase in photopic pupil size were observed. Mean reduction in accommodative amplitude was 5.23 ± 4.11D, 9.28 ± 3.26 D and 9.32 ± 2.83D and mean increase in photopic pupil size was 0.95 ± 1.05 mm,1.65 ± 0.93 mm and. 2.16 ± 0.88 mm for 0.01%, 0.02% and 0.03% concentrations,respectively. Of the participants, 96.5% had residual accommodative amplitude > 5D with only two eyes in each group of 0.02% and 0.03% having the residual accommodation equal or less than 5D.

Conclusions : All concentrations had an effect on pupillary size and accommodative amplitude in a dose-dependent manner. With atropine 0.01% and 0.02%, the side effects were tolerable. Since 0.01% was not effective in reducing axial length elongation, atropine 0.02% may be the highest tolerable concentration that could be considered for further investigations in myopia control.

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REVIEW ARTICLE

OPEN

A Meta-Analysis Assessing Change in Pupillary Diameter, Accommodative Amplitude, and Efficacy of Atropine for Myopia Control

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Purpose: To determine the effect of atropine on pupillary diameter, accommodative amplitude as well as myopia progression.

Methods: Medical databases and Cochrane Library were systematically searched for studies from 1980 until June 2020. The primary and secondary outcomes were: a) change in pupillary diameter (PD) and accommodative amplitude (AA) and b) annualized mean change in spherical equivalent and axial length with various concentrations of atropine compared to control.

Results: Thirteen trials (6 RCTs, 7 observational studies) that studied 9 atropine concentrations (0.01–1.0%) were included. The relation between atropine and change in PD and AA was nonlinear; at < 0.10% atropine, the slope of the curve was steep but the change in PD (+0.7 mm; 95% CI: +0.1 to +1.4) and AA (−1.6D; 95% CI: −3.9 to +0.7) was smaller whereas at ≥0.10% atropine, the slope plateaued but change in PD (+3.2 mm, 95% CI: +2.8 to +3.5) and AA (−10.7D; 95% CI: −12.2 to −9.2) was high.

Reduction in myopia progression with atropine at <0.10% and ≥0.10% as compared to controls was 0.37D (95% CI: 0.16 to 0.58) versus 0.75D (95% CI: 0.17 to 1.33) for spherical equivalent and −0.10 mm (95% CI: −0.24 to 0.05) versus −0.23 mm (95% CI: −0.34 to −0.13) for axial length.

Conclusions: A nonlinear dose-response relationship exists between atropine and PD and AA. Further work is warranted to determine the concentration that provides maximal efficacy with tolerable side effects.

Key Words: accommodative amplitude, atropine, muscarinic antagonists, myopia control, pupillary diameter

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The authors have no conflicts of interest to declare.

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Myopia

Considered to be the most common cause of distance vision impairment,[1] myopia is estimated to affect half of the population worldwide by 2050.[2] In addition to resulting in a significant economic burden,[3] high myopia (−5.0D or worse) may also induce pathologic changes that result in vision impairment and/or blindness. Data from many countries, especially from East Asia, show that myopic macular degeneration is one of the leading causes of blindness.[4,5] Additionally, any level of myopia is associated with the risk of visual impairment[6], which is significantly higher with high myopia.[7] Given the rising burden, there has been an increasing interest in solutions to better manage and control myopia.

Atropine

Presently, of all the known strategies to slow or reduce myopia progression, atropine, a nonselective antimuscarinic agent, is considered superior and widely used despite the lack of understanding relating to its mode of action as well as lack of clarity with respect to the efficacy of 0.01% atropine.[8] Although other pharmaceutical agents, such as Pirenzepine, a selective antimuscarinic agent, and 7-methylxanthine, showed promise in early clinical trials,[9,10] presently atropine is the only pharmaceutical agent that is broadly adopted for myopia control. It was reported that, in Taiwan, up to 50% of ophthalmologists nationwide prescribe atropine for children with myopia.[11] However, the use of atropine is not without issues. Despite no reports of serious side effects to date, photophobia and difficulty with near-work remain the major concerns for the application of atropine at higher concentrations. Another significant concern is the "rebound" of myopia that occurs on ceasing treatment.[12] There are other concerns related to the unknown effects of long-term use on pupils, macula, and/or retina, although some data shows that the accommodative amplitude (AA) and near vision recovered to pretreatment within months after cessation of atropine.[13]

Although previous meta-analyses[8,14,15] have considered the effect of various concentrations of atropine on axial elongation and refractive error, its effect on pupillary diameter (PD) and amplitude of accommodation has not been considered so far. In this meta-analysis, we considered the effect of various concentrations of atropine on pupillary dilation and AA as well as for efficacy in myopia control (change in spherical equivalent and axial length).[16–19]

METHODS

Design

We searched the electronic medical databases, including PubMed, EMBASE, Scopus, ProQuest, and Cochrane library.

B. Presentations related to the thesis

1. **Tran, H. D. M**., Sankaridurg, P. (2018). Pharmaceutical strategies for myopia control. Vietnam Ophthalmological Society Congress, Ho Chi Minh City, Vietnam.
2. **Tran, H. D. M.** (2019). Low concentration Atropine for myopia control treatment. Ho Chi Minh City Ophthalmology Annual Meeting, Ho Chi Minh City, Vietnam.
3. **Tran, H. D. M**, Tran., Y. H., Jong, M., Sankaridurg, P. (2020). Updates of topical Atropine for myopia control treatment. Myopia Seminar - Hanoi Ophthalmological Society, Hanoi, Vietnam.
4. **Tran, H. D. M.**, Sankaridurg, P. (2021). Pharmaceutical strategies for myopia control. Thai Optometry Conference, Bangkok, Thailand
5. **Tran, H. D. M.**, Sankaridurg, P. (2021). Atropine for myopia control treatment, a meta-analysis. Myopia Masterclass. Shroff Eye Hospital, New Delhi, India.
6. **Tran, H. D. M.**, Sankaridurg, P. (2021). Pharmaceutical strategies for myopia control. Myopia Symposium – Mero Eye Center, Kathmandu, Nepal.

Appendix 3: Awards and Scholarships

2021	Higher Degree Research UNSW Completion Scholarship
2019	UNSW PRSS (Postgraduate Research Support Scheme) scholarship supporting the attendance at the international conference
2017 - 2021	Brien Holden Vision Institute Scholarship worth 26,282 AUD per year as living allowance
2017 - 2021	UNSW TFS (Tuition Fee Scholarship) for the candidature of Doctor of Philosophy

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