Vision Research 214 (2024) 108341

Contents lists available at ScienceDirect

Vision Research

journal homepage: www.elsevier.com/locate/visres

The effect of topical 1 % atropine on ocular dimensions and diurnal rhythms of the human eye

Nickolai G. Nilsen [a], Stuart J. Gilson [a], Hilde R. Pedersen [a], Lene A. Hagen [a], Christine F. Wildsoet [b], Rigmor C. Baraas [a,*]

[a] National Centre for Optics, Vision and Eye Care, Faculty of Health and Social Sciences, University of South-Eastern Norway, Kongsberg, Norway
[b] Herbert Wertheim School of Optometry & Vision Science, University of California Berkeley, USA

A B S T R A C T

The effect of topical 1 % atropine on the diurnal rhythms of the human eye was investigated. Participants wore an activity monitor on Days 1–7. A set of measures (epochs) encompassing intraocular pressure (IOP), ocular biometry, and retinal imaging were obtained on Day 7 (baseline), followed by eight epochs on Day 8, and one on Day 9 from both eyes of healthy participants (n = 22, 19–25 years). The sleep time of participants (collected via actigraphy) was used as a reference in scheduling epochs. Topical 1 % atropine was instilled in the dominant eye on Day 8, 2 h after habitual wake time, using the fellow eye as control (paired-eye design). Sinusoids with a 24-h period were fitted to the data, and a non-linear mixed-effects model was used to estimate rhythmic statistics. There were no interocular differences in any of the measured parameters at baseline. Comparing pre- versus post-atropine in treated eyes revealed lower IOP, deeper anterior chamber (ACD), decreased crystalline lens thickness and shorter axial length (AL). The same trends were observed when comparing atropine-treated versus fellow control eyes, except for IOP and AL (no differences). Both atropine-treated and fellow control eyes showed significant diurnal variations in all ocular parameters, with atropine-treated eyes revealing larger AL and retinal thickness amplitudes, smaller vitreous chamber depth (VCD) amplitudes, and a significant phase advancement for ACD and VCD. There were no interocular differences in choroidal thickness rhythms. In conclusion, while ocular diurnal rhythms persisted after instillation of 1 % atropine, many rhythmic parameters were altered.

1. Introduction

Atropine, a non-selective anti-muscarinic agent, is now commonly used as a myopia control therapy in children and adolescents, in a low concentration, topical formulation. However, the mechanism of action by which atropine slows myopia progression (Chia et al., 2012; Upadhyay and Beuerman, 2020) remains poorly understood. While muscarinic receptors are present in both the human ocular iris sphincter and ciliary muscles (i.e., mediating pupil constriction and ocular accommodation), atropine's anti-myopia action appears to be unrelated to its cycloplegic effects, as supported by findings from myopia-related studies involving chicks whose accommodation is unaffected by atropine (McBrien et al., 1993; Wildsoet, 2003; Schaeffel et al., 1990). Other potential sites of action for atropine, based on the premise that its action is mediated by interactions with muscarinic receptors, include the retina, retinal pigment epithelium (RPE), choroid and sclera, as all contain muscarinic receptors.

There is mounting evidence tying short-term choroidal thickness (ChT) changes to axial length changes (Ostrin et al., 2023). The choroid also represents a plausible site of action for topical atropine. For example, subfoveal choroidal thickening and axial length (AL) shortening have been reported in humans 60 min after instillation of either 0.01 % atropine (Sander et al., 2019), or of its close relative, homatropine, in a 2 % concentration (Sander et al., 2014; Sander et al., 2018). Atropine has also been reported to inhibit the choroidal thinning induced by imposed hyperopic defocus in similar short-term studies (Chiang & Phillips, 2018; Chiang, Turnbull, & Phillips, 2020). In a longer term (2-year) myopia control clinical trial of topical atropine, concentration-dependent thickening of the choroid was reported (Yam et al., 2022). These observations are consistent with earlier studies in animal models linking the choroid with ocular growth modulation and emmetropization (Marzani and Wallman, 1997), first in the chick (Nickla et al., 2001) and more recently in other animal models (Nickla and Wallman, 2010).

Topical atropine, when used as a myopia control therapy in humans, is typically given just prior to bedtime, to allow partial recovery from its secondary inhibitory effects on accommodation and the pupil's light response, and so minimize its impact on daytime visual function (Chua et al., 2006; Azuara-Blanco et al., 2020; Larkin et al., 2019). This practice raises the question of whether this therapy may affect one or

* Corresponding author.
E-mail address: rigmor.baraas@usn.no (R.C. Baraas).

https://doi.org/10.1016/j.visres.2023.108341
Received 10 July 2023; Received in revised form 10 November 2023; Accepted 15 November 2023
Available online 8 December 2023

more of the ocular diurnal rhythms, some of which are known to be differentially altered during myopia development (Ostrin et al., 2023). For example, significant diurnal variations have been reported in intraocular pressure (IOP) and several ocular dimensions, including AL and ChT, in both humans and experimental animals (Burfield, Patel, & Ostrin, 2018; Chakraborty, Read, & Collins, 2011; Ostrin, Jnawali, Carkeet, & Patel, 2019; Read, Collins, & Iskander, 2008; Stone, Quinn, & Francis, 2004; Troilo, Smith, & Nickla, 2019). In human eyes, the acrophase (peak) for AL, IOP and retinal thickness (RT) rhythms is around mid-day (from 11:30 to 16:00), while for anterior chamber depth (ACD), crystalline lens thickness (LT), and ChT, it is around midnight (from 21:30–04:00) (Burfield, Carkeet, & Ostrin, 2019; Burfield, Patel, & Ostrin, 2018; Nilsen et al., 2022; Ostrin, Jnawali, Carkeet, & Patel, 2019). Furthermore, an early study involving myopia induction and recovery from the same in chicks, revealed changes in the phase relationship of AL and ChT rhythms from being nearly anti-phase during normal growth (≈9h), to become fully anti-phase during accelerated ("myopic") growth (≈12 h), and fully in-phase during the recovery period (≈0h) (Nickla et al., 1998). The possibility that the phase relationship between AL and ChT rhythms is a biomarker of the rate of eye elongation, as suggested by such animal model studies (Nickla, 2013), raises the question of whether the anti-myopia action of topical atropine may be tied to changes in one or more ocular diurnal rhythms. For example, might atropine mimic the changes observed during decelerated AL elongation in chicks (Nickla et al., 1998) and marmosets (Nickla et al., 2002) — a phase-advance in the ChT rhythm (c.f. Fig. 12 in Ref. (Nickla et al., 1998), with the AL and ChT rhythms shifting from anti-phase to becoming more in-phase after the instillation of atropine.

To date, there have been no investigations in humans, into the effect of topical atropine on ocular diurnal rhythms, and potential effects on the diurnal phase relationship between AL and ChT rhythms. The primary aim of this study was to correct this deficiency by investigating the short-term effects of topical 1 % atropine on ocular diurnal rhythms, building on our previous work on untreated eyes (Nilsen et al., 2022). The secondary aim was to investigate whether our previous observation of crystalline lens thickening in the evening (Nilsen et al., 2022) remains in the absence of any accommodative demand, by way of understanding its origin. Specifically, in our earlier study, the diurnal rhythms of ACD and LT were observed to be in phase, increasing in depth and thickness throughout the evening (Nilsen et al., 2022). This behaviour is different from that seen during accommodation, where increases in LT leads to a shallowing of ACD (anti-phase behaviour) (Kaufman et al., 2011; Xiang et al., 2021). To this end, the short-term effects of topical 1 % atropine on ocular diurnal rhythms over a 26-hour period in healthy young adults were investigated, utilizing the same methodologies as in our previous study, which also served as a source of additional control (reference) data (Nilsen et al., 2022).

2. Methods

Twenty-two young healthy adults (19 females, 3 males, 19–25 years of age) participated in this study. While there were no exclusion criteria related to refractive errors, all participants were required to have best corrected visual acuity of $\leq$ 0.00 logMAR (TestChart 2000, Thomson Software Solutions, London, UK), stereo acuity of $\leq$ 120 s of arc (TNO Stereotest, Laméris Ootech, WC Ede, Netherlands), and make no errors in either of two colour vision tests (Ishihara, 24-plate edition, Kanehara Trading Inc., Tokyo, Japan; Hardy–Rand–Rittler Pseudoisochromatic Plate Test, 4th edition, Richmond Products, Albuquerque, NM, USA). Other exclusion criteria included current or previous myopia control therapy, systemic and/or ocular disease, sleep disorders and/or mental disorders known to affect sleep, and the use of melatonin supplements. The study was approved by the Regional Committee for Medical and Health Research Ethics (Southern Norway Regional Health Authority) and was carried out in accordance with the tenets of the Declaration of Helsinki. Written informed consent was obtained from the participants prior to their participation.

2.1. Study design and data collection protocols

This study extends our previous study investigating seasonal variations in diurnal rhythms of the eye (Nilsen et al., 2022), using a similar methodology, with data collection at similar time-of-year, at the same geographical location, and involving participants of a similar age-group, to allow inter-study comparisons as appropriate. The current study spanned 9 days, with ocular data collection limited to the last 3 days of this period, when the dominant eyes of all participants underwent topical 1 % atropine treatment (minims single dose; Bausch + Lomb, Bridgewater, NJ 08807). Fig. 1 shows the sequence and timing of data collection. On Day 1, participants were given an Actigraph GT3X wrist-mounted activity monitor (Actigraph Corp, Pensacola, USA) to wear for the following seven days and nights to objectively measure their sleep-onset times. For each participant, weekday data were averaged to determine their habitual sleep time (HST). Self-reported habitual wake time (HWT) data (based on estimates by participants of their morning wake-up time) were gathered for the preceding month, and the average used in combination with HST to schedule the various sets of measurements (epochs).

At each epoch, ocular biometry (IOLMaster 700, Carl Zeiss Meditec AG, Jena, Germany), tonometry (iCare, Tiolat Oy, Helsinki, Finland) and posterior segment SD-OCT imaging (Spectralis OCT2-EDI, Heidelberg Engineering, Heidelberg, Germany) were undertaken and diurnal ocular rhythms were derived from collected data. During each epoch, participants remained seated on a wheeled chair and were pushed between instruments, to avoid the potential influence of postural changes on measurements (Lockley, 2020; De Bernardo et al., 2019). From ocular biometric measurements, pupil size, central corneal thickness (CCT), corneal curvature (CR), anterior chamber depth (ACD), crystalline lens thickness (LT), retinal thickness (RT) and axial length (AL) data were extracted, with vitreous chamber depth (VCD) derived as AL − (ACD + LT + RT). IOP data represents the average of three sets of readings, where each set represents six single measurements. Refractions were measured for both far and near fixation distances (6 m and Maltese cross at 30 cm; Nvision-K 5001 Open-field autorefractor, Shin-Nippon, Tokyo, Japan), and used to derive refractive errors and accomm odation amplitudes.

The first set of ocular measurements (first epoch, baseline data) was made on Day 7, approximately 4 h after the self-reported habitual wake time (HWT+4). On Day 8, atropine was instilled in the dominant eye, 2 h after HWT (HWT+2), with additional epochs scheduled 1 and 4 h after HWT (HWT+1 and HWT+4), and at 9, 4, 3, 2, 1 and 0 h before the participant's habitual sleep time (HST: HST-9, HST-4, HST-3, HST-2, HST-1, HST +0). A final measurement session was carried out on Day 9 (two days after the collection of baseline data) at HWT+4. Thus, for each participant, the timing of the epochs was aligned with their respective chronotype. This measurement schedule was chosen to capture the sinusoidal characteristics of observed parameters, including the descriptive melatonin onset (DLMO) from which rhythm statistics were estimated (Lockley, 2020). Atropine dosing was adjusted based on iris colour (Mackey et al., 2011), with one drop of atropine instilled in those with lightly pigmented (blue to green) irides and two drops instilled in those with more heavily pigmented (green-with-brown-iris-ring-to-brown) irides (Salazar et al., 1976). To relax accommodation in untreated (fellow control) eyes prior to the start of each measurement session, participants watched a movie (binocularly) at 5 m for 15 min through their habitual distance correction as necessary (Chakraborty et al., 2013). On all occasions, right eyes were measured before left eyes, irrespective of ocular dominance.

Radial OCT scans (6 orientations with 100 B-scans averaged at each orientation, all with enhanced-depth information enabled) were collected at each epoch, using the baseline scan as the reference image for the instrument's retinal tracking system. The method for correction

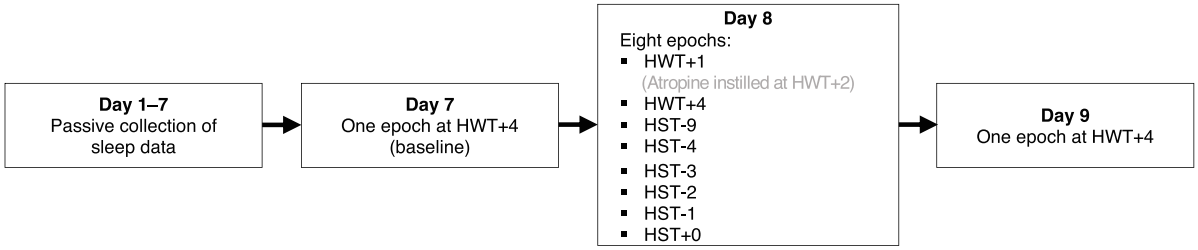

Fig. 1. Data collection schedule showing timing of measurements (epochs) from Day 1 to Day 9. Habitual wake time (HWT) represents individual self-reported average time for the past month; habitual sleep time (HST) was determined for each individual from actigraphy data collected on Day 1–7.

for lateral magnification and segmentation was the same as that described previously (Nilsen et al., 2022). Only horizontal and vertical scans were analysed, with mean thicknesses derived for the central 1 mm of retina and underlying choroid (Fig. 2).

Circulating melatonin (MEL) levels were evaluated from collected saliva samples (Salivette Saliva examination kit, Sarstedt, Nümbrecht, Germany). The first sample was self-collected at wake time before participants got out of bed (HWT+0), and thereafter samples were collected during the last 5 min of the accommodation washout period, at HWT+1, HST-4, HST-3, HST-2, HST-1 and HST+0. Upon collection, saliva samples were immediately centrifuged at −4°C, 4400 RPM for 5 min (Centrifuge 5702R, Eppendorf SE, Germany), and stored in a −25 °C freezer. The samples were analysed by VITAS Analytical services (Oslo, Norway), using appropriate ELISA kits (Bühlmann Laboratories, Schönenbuch, Switzerland).

During all measurements, independent of time of day, ambient light levels were kept below 20 lx to minimize the risk of suppressing evening melatonin secretion, including levels emanating from the TV that participants watched prior to measurements (Crowley, Suh, & Molina, 2017). Ambient light levels remained below 20 lx between evening measurements (HST-4 to HST + 0), when the participants were confined to the laboratory. In accordance with published recommendations for similar studies (Benloucif, Burgess, & Klerman, 2008; Crowley, Suh, & Molina, 2017), participants were also instructed to avoid consuming NSAIDs, nicotine, bananas, and chocolate for 36 h prior to and during data collection to minimize their potentially confounding effects on saliva melatonin levels. For similar reasons, participants were also asked to avoid alcohol and drinks containing caffeine or artificial food colorants for 24 h prior to and during the data collection. While measurement schedules did not require participants to sleep in the laboratory at any time during the study, all were encouraged to maintain their habitual wake and sleep schedules over the study period. Also, none reported travelling across more than 2 time zones within the month preceding the study, ruling out such influences on diurnal rhythms.

2.2. Data analysis

Statistical analyses made use of R statistical software, version 3.6.3, with the NLME package (R Core Team, 2016). Parametric tests were used where the data were confirmed to be normally distributed; otherwise non-parametric alternative tests were used. Statistical significance was set at α=0.05. Participants were characterized by the refractive error of their atropine-treated eye based on the autorefractor data from the Day 8 HWT+4 measurement of their atropine-treated eye. Spherical equivalent refractive errors (SER) were calculated as sphere + ½ cylinder, with myopia being defined as SER $\leq$ −0.50 diopters (D), emmetropia as −0.50 D < SER < +0.50 D, and hyperopia as SER $\geq$ +0.50 D. Accommodation responses were calculated as differences between autorefractor data (expressed as SER), captured under distant and near viewing conditions. Inter-rater reliability for segmentation of the choroid has been reported to be high (intraclass correlation = 0.95, 95 % confidence interval = 0.929–0.965) (Nilsen et al., 2022). To provide a between-session repeatability for OCT derived choroidal thickness for the central 1 mm, the images obtained from the fellow control eye on Day 7 HWT+4 and Day 8 HWT+4 were compared. The coefficient of variation and the limits of agreement were determined with Bland-Altman analysis (Supplementary Fig. S1) (Bland and Altman, 1986). Data processing and analyses of Actigraph data have been described elsewhere (Nilsen et al., 2022).

A non-linear mixed-effects model was used to estimate rhythm statistics (Midline Estimating Statistic of Rhythm, MESOR), amplitude and phase, and dim-light melatonin onset (DLMO), as described previously (Nilsen et al., 2022). To better capture the floor effect typically observed with daytime melatonin measurements, a sinusoid was fitted to the log of the measured melatonin levels (Kennaway, 2019). One-way repeated measures ANOVAs, with the epoch as the within-subject factor and refractive error group as the between-subjects factor, were used to identify significant diurnal variations in saliva melatonin levels and/or one of the measured ocular parameters (Burfield, Patel, & Ostrin, 2018; Ostrin, Jnawali, Carkeet, & Patel, 2019; Read, Collins, & Iskander, 2008). One-way ANOVAs were also used to examine differences between refractive error groups and the effect of topical atropine on

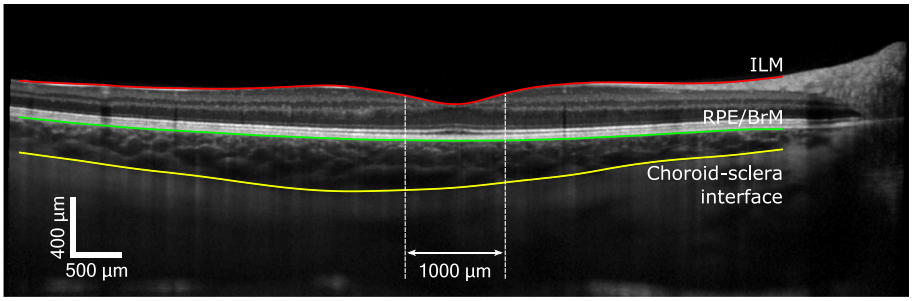

Fig. 2. A horizontal OCT B-scan annotated with segmented retinal and choroidal layers. Retinal and choroidal thicknesses were derived as the distances between the inner limiting membrane (ILM) and retinal pigment epithelium (RPE), and between the RPE and choroid-scleral interface respectively, averaged over the central 1 mm, with reported values representing averages derived from both horizontal and vertical B-scans.

rhythm statistics for a given ocular parameter by calculating the difference between the atropine-treated eyes with the fellow-control eyes. For these comparisons, effect size η^2 (eta-squared) was calculated to be ≥ 0.40, indicating a large effect size (Cohen and Cohen, 1983). Diurnal analyses made use of data collected during the 7 epochs on Day 8 after atropine instillation (HWT+4 until HST+0) in all cases except the case of choroidal thickness, for which the HWT+4 data were uncharacteristically noisy (possibly due to variable patterns of atropine uptake between participants), which prevented convergence of the NLME model.

3. Results

Of the 22 participants, nine were myopic, five were emmetropic, and eight were hyperopic. Table 1 summarizes baseline refractive errors and ocular dimensions of both of their eyes as measured on Day 7, HWT+4, and the corresponding data collected on Day 8, HWT+4 (2 h after instillation of atropine in the dominant eyes). The refractive errors of participants (SER) ranged between −4.13 and +1.82 D (mean ± SD: −0.26 ± 1.60 D), with no anisometropia ≥ 0.75 D. All but one participant had astigmatism ≤ 1.25 D, the one exception being a myope with astigmatism of −2.25 D in both eyes.

3.1. Acute ocular effects of topical atropine, 2 h after atropine instillation

While there were no significant interocular differences in any ocular parameters at baseline on Day 7 ($p > 0.05$), the monocular instillation of atropine introduced significant interocular differences in SER, ACD and LT after just 2 h, reflecting the changes in atropine-treated eyes ($p < 0.001$, Table 1, pairwise statistics provided in Supplementary Table S1–S2). Specifically, treated eyes showed a more positive SER, deeper ACD and reduced LT compared with their fellow control eyes (Day 8 HWT+4; $p < 0.001$). Similar trends are evident in the changes from baseline profiles in treated eyes (Day 7 HWT+4 versus Day 8 HWT+4; all $p < 0.001$); in addition, the instillation of atropine resulted in decreases from baseline in IOP, AL and VCD (all $p \leq 0.005$). While there was no statistically significant atropine-induced change in ChT at this 2 h time point (interocular differences, Day 7 HWT+4 versus Day 8 HWT+4), there were notable individual variations, unrelated to the refractive error groups to which participants belonged (Supplementary Table S3). Based on independent t-tests, there were no differences between the group receiving 1 atropine drop ($n = 14$) versus the group receiving 2 drops ($n = 8$) for any ocular parameters (IOP, CCT, ACD, LT, VCD, AL, RT, ChT, and accommodation amplitude), at day 8 HWT + 4 ($p \geq 0.061$), and likewise, no group-related difference between day 7 HWT+4 and day 8 HWT+4 ($p \geq 0.178$).

3.2. Time-course of changes induced by topical atropine

3.2.1. Effects on ocular biometric parameters

For all ocular biometric parameters (ACD, LT, VCD, AL, RT, and ChT), instillation of atropine resulted in persistent, measurable changes in atropine-treated eyes (Fig. 3). ACD initially deepened, while LT, VCD and AL all decreased. Additionally, ChT showed a small, measurable increase (HWT+1 to HWT+4) at the time-point where a diurnal decrease was expected, based on the change in fellow control eyes (Nilsen et al., 2022). In all cases, the opposite behaviour, i.e., barely-noticeable change, was observed of these parameters in fellow control eyes. On the other hand, increases in RT were observed in both treated and fellow control eyes, albeit larger in the former. For fellow control eyes, the patterns of change across the day for all parameters were generally consistent with those recorded from untreated right and left eyes in our previous study, which involved a different study population (Nilsen et al., 2022), with the one exception being ChT and the HST-2 evening epoch, when a decrease was observed in fellow control eyes, as well as treated eyes.

3.2.2. Enduring (26 h post instillation) effects of atropine

Accommodation and pupillary responses were assessed to confirm the expected ocular effects of atropine. As expected, accommodation responses to a target at 30 cm were significantly reduced by atropine ($t(20) = 9.15$, $p < 0.001$), with this effect persisting over the 26-hour monitoring period, with only a slight increase from 0.09 ± 0.19 D at 2 h to 0.24 ± 0.19 D across subsequent epochs ($R^2 = 0.11$, $p = 0.001$, Supplementary Fig. S2). Accommodative responses in fellow control eyes remained relatively stable and unaffected over the same period ($R^2 < 0.01$, $p = 0.75$), i.e., +2.12 ± 0.84 D and +2.15 ± 0.81 D, respectively. The pupils of treated eyes became dilated with the instillation of atropine, with minimal recovery over the 26-hour monitoring period, as reflected in the lack of any significant differences in pupil size across epochs ($R^2 < 0.01$, $p > 0.20$ for both treated and fellow untreated eyes).

On Day 9 (HWT+4), most of the atropine-induced changes, i.e., as reflected in pre- (Day 7) versus post-atropine treatment, remained; ACD was deeper, LT smaller, VCD shorter and SER more positive (Supplementary Table S4). Also of these parameters, only changes in ACD showed refractive error-related differences at this last time point ($F(2, 17) = 6$, $p = 0.009$).

3.3. Diurnal rhythms and the effects of topical atropine

For each of the measured ocular parameters, data were fitted with sine waves, from which estimates of MESORs, amplitudes and

Table 1
Mean ocular parameters for fellow control and atropine-treated eyes, measured pre- and post-instillation of 1 % atropine (i.e., Day 7 HWT + 4 and 2 h after instillation Day 8 HWT+4). Significance was calculated by pairwise comparisons of Day 7 versus Day 8 for fellow control eyes and atropine eyes (*), respectively, and for atropine versus fellow control eyes on Day 8 (†). Differences between Day 8 HWT+4 and Day 7 HWT+4 data are also shown for both control and atropine-treated eyes.

	Fellow (control) eyes					Atropine treated eyes				
	Day 7 HWT+4		Day 8 HWT+4		Difference	Day 7 HWT+4		Day 8 HWT+4		Difference
Parameter	Mean	SD	Mean	SD	Mean	Mean	SD	Mean	SD	Mean
SER [D]	−0.56	1.57	−0.62	1.54	−0.06	−0.60	1.48	−0.26*	1.56	0.34†
IOP [mm Hg]	14.52	3.70	13.36	4.23	−1.16	15.09	3.61	13.56	4.70	−1.53†
CCT [μm]	548	25	548	25	0	547	25	548	25	1
ACD [mm]	3.588	0.249	3.589	0.240	0.001	3.590	0.262	3.714*	0.244	0.124†
LT [mm]	3.665	0.267	3.673	0.270	0.008	3.661	0.283	3.595*	0.262	−0.066†
VCD [mm]	16.115	0.876	16.106	0.880	−0.009	16.118	0.838	16.050	0.847	−0.068†
AL [mm]	23.593	0.798	23.591	0.801	−0.002	23.592	0.754	23.583	0.758	−0.009†
RT‡ [μm]	254	19	253	19	−1	253	21	254	21	1
ChT‡ [μm]	332	78	330	78	−2	335	81	335	81	0

* Significant pairwise difference between atropine-treated and fellow control eyes at Day 8 HWT+4; $p < 0.05$.
† Significant pairwise difference between values measured pre- and post- atropine instillation in treated eyes; $p < 0.05$.
‡ Averaged over the central 1 mm.

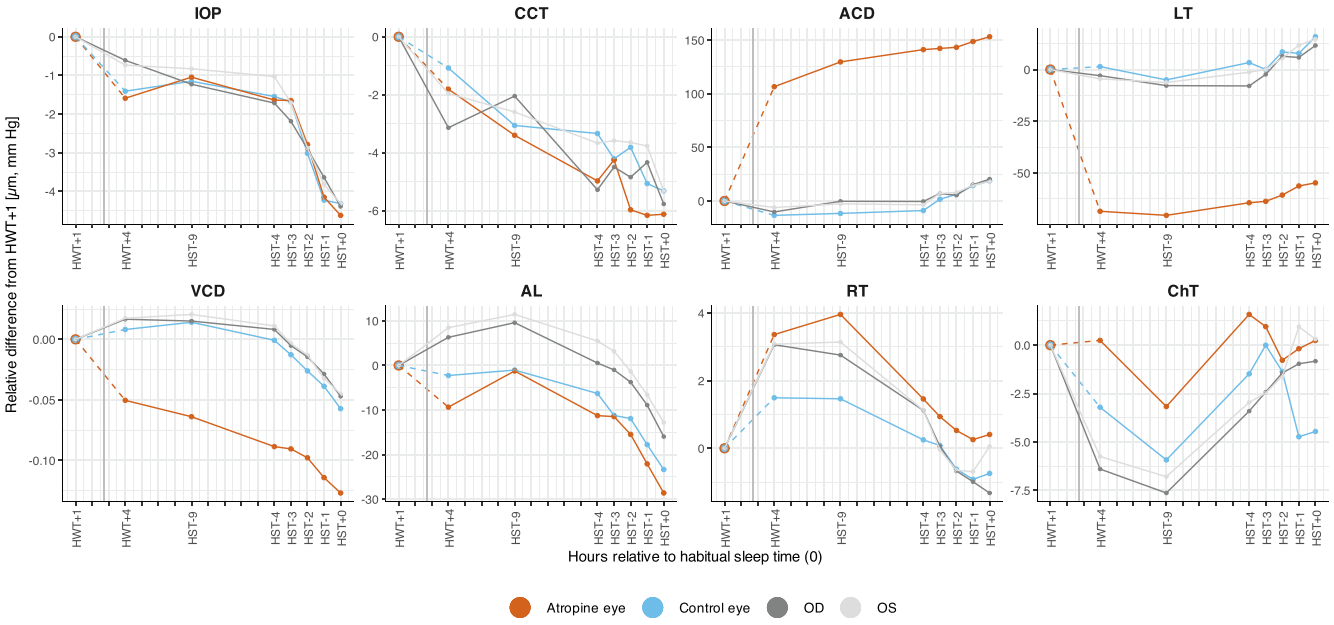

Fig. 3. The time-course of changes in ocular parameters in atropine-treated (orange) and fellow control eyes (blue), pre- and post-instillation of 1 % atropine; also included for comparison are data from a previous study for which both eyes remained untreated (grey, OD, OS; n = 35) (Nilsen et al., 2022) for intraocular pressure (IOP), central corneal thickness (CCT), anterior chamber depth (ACD), crystalline lens thickness (LT), vitreous chamber depth (VCD), axial length (AL), retinal thickness (RT) and choroidal thickness (ChT, central 1 mm for RT and ChT). Change is calculated as the relative difference from HWT+1 (left-most point in each graph), i.e., measurements taken 1–2 h prior to atropine instillation. The average time of atropine instillation (between HWT+2 and HWT+3) is indicated by solid vertical lines and the dashed orange/blue lines link the pre- and first post-instillation measurements. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

acrophases were obtained. These derived data are summarized in Table 2 andTable 3.

3.3.1. *Effects of atropine on ocular rhythms*

Both atropine-treated and fellow control eyes exhibited significant diurnal variations in the following parameters: IOP, CCT, ACD, LT, VCD, AL, RT and ChT ($p < 0.05$, Supplementary Table S5). Compared to the rhythms in fellow control eyes, treated eyes had a smaller VCD amplitude, but larger AL and RT amplitudes ($p < 0.049$ for all), as well as phase advances in their ACD and VCD rhythms ($p < 0.001$ for all) (Fig. 4 A–D). Nonetheless, for both eyes, ACD and LT rhythms were in phase (<1h versus ≈5 h phase-difference in the fellow control and atropine-treated eye, respectively), and in the case of both AL and ChT rhythms, they were nearly in-phase (≈5–6 h phase-difference, Table 2). Also, during the evening, the crystalline lens thickened and the ACD increased, despite the absence of any accommodative demand (mean raw time course data in Fig. 3 and sinusoidal model fits in Fig. 4). Overall, MESORs showed similar trends to values recorded 2 h after the instillation of atropine, with treated eyes having significantly increased ACDs on average, but smaller LTs and VCDs compared to their fellow control eyes (Table 2, statistics provided in Supplementary Table S6). These trends are also reflected in the interocular differences in MESORs ($p < 0.038$ for all).

3.3.2. *Melatonin*

Saliva melatonin levels (MEL) also showed significant diurnal variation (MESOR: 1.23 log pg/mL, amplitude 3.17 log pg/mL, acrophase 3.34 h; 95 % CI: 1.01–1.45; 2.82–3.52, 3.02–3.64, respectively).

3.4. *Differences between refractive error groups*

3.4.1. *Differences in rhythm characteristics between refractive error groups*

When, for a given ocular parameter, there was significant individual variation in MESORs, amplitudes and/or acrophases in the NLME model, a random component representing them was retained in the model. This procedure yielded individual estimates, which were used to assess differences between SER groups (Fig. 5 A–D). Such cases included MESORs for all parameters, amplitudes for ACD, LT, VCD, AL and RT, and acrophases for MEL, ACD, LT and VCD. In the case of fellow control eyes, hyperopes exhibited an earlier acrophase for ACD compared to emmetropes and myopes ($F(2, 19) = 6.54$, $p = 0.007$, Tukey's honestly significant difference (HSD) test $p \leq 0.036$). Differences between atropine-treated and fellow control eyes for MESOR and acrophase were calculated by way of assessing the effects of atropine and differences between the refractive error groups. In the case of MESORs and the effect of atropine, hyperopes also showed greater deepening of ACD ($F(2, 19) = 18.5$, $p < 0.001$, Tukey's HSD test $p < 0.001$) and reduction in LT ($F(2, 18) = 6.85$, $p = 0.006$, Tukey's HSD test $p \leq 0.023$) compared to emmetropes and myopes. All three refractive error groups exhibited atropine-induced phase advances in their ACD rhythms, with hyperopes exhibiting a significantly larger phase-advance compared to the other two groups ($F(2, 19) = 11.6$, $p < 0.001$, Tukey's HSD test $p \leq 0.003$). No other derived parameter showed difference across the refractive error groups.

3.4.2. *Accommodation and pupillary responses*

Myopes showed smaller mean accommodation responses compared to emmetropes and hyperopes, as reflected in significant intergroup differences in results for fellow control eyes, as well as in interocular difference between atropine and fellow control eyes (i.e., $F(2, 19) = 7.83$, $p = 0.003$, Tukey's HSD test $p < 0.0443$, $F(2, 19) = 6.22$, $p = 0.008$, Tukey's HSD test $p = 0.010$, respectively). On the other hand, no refractive error-related differences in either the pupil size of fellow control eyes or atropine-induced pupil size changes were observed.

3.5. *Inter-study comparisons*

Table 4 and Fig. 6 show data collected in the current study (E–F) and in our previous study [where both eyes were untreated, for winter (C) and summer (D)] (Nilsen et al., 2022). Comparison of Fig. 6 E and C

Table 2
Population estimates (Est.) of MESORs, amplitudes and acrophases derived from sine wave fits to data from fellow control and atropine-treated eyes, calculated from the NLME model. Acrophase is estimated relative to habitual sleep time. 95% confidence intervals (95% CI) are included in the table.

	Fellow control eye						Atropine eye					
	MESOR		Amplitude		Acrophase (hour)		MESOR		Amplitude		Acrophase (hour)	
Parameter	Est.	95 % CI	Est.	95 % CI	Est.	95 % CI	Est.	95 % CI	Est.	95 % CI	Est.	95 % CI
IOP [mm Hg]	12.13	10.57–13.69	2.19	1.72–2.77	−8.77	−9.46—8.06	12.37	10.69–14.05	2.45	1.97–3.05	−8.49	−9.07—7.89
CCT [μm]	546	536–556	2	1–2	−12.90	−15.26—9.33	546	536–556	2	1–3	−12.47	−14.49—9.76
ACD [mm]	3.603	3.501–3.706	0.023	0.018–0.03	3.190	2.12–3.99	3.736*	3.636–3.835	0.021	0.018–0.025	−1.820*	−3.07—0.67
LT [mm]	3.678	3.563–3.794	0.014	0.010–0.021	3.54	2.46–4.48	3.600*	3.488–3.712	0.009	0.006–0.014	3.05	1.41–4.43
VCD [mm]	16.079	15.722–16.436	0.045	0.038–0.053	−8.78	−9.25—8.32	16.017*	15.671–16.362	0.034*	0.028–0.041	−11.08*	−11.71—10.4
AL [mm]	23.585	23.242–23.928	0.012	0.009–0.017	−9.45	−9.97—8.91	23.571	23.245–23.896	0.016*	0.012–0.020	−8.81	−9.23—8.38
RT† [μm]	252	245–260	1	1–2	−10.73	−11.94—9.38	252	244–261	2*	2–2	−11.32	−12.3—10.25
ChT† [μm]	325	292–357	7	5–12	−4.37	−5.14—3.60	331	298–365	4	2–9	−3.29	−5.23—1.34

* Significant effect of atropine on MESOR, amplitude or acrophase set at $p < 0.05$.
† Averaged over the central 1 mm.

reveals a notable phase difference in ChT, consistent with a more in-phase relationship between AL and ChT in atropine-treated eyes (E).

4. Discussion

This study investigated the short-term effects of a one-time instillation of topical 1 % atropine on various diurnal ocular rhythms, including IOP, with specific interest in whether some or all persisted after its instillation and to what extent their amplitudes and/or their phase relationships are affected. In all cases, the various diurnal ocular rhythms were found to persist in atropine-treated eyes (see below), although significant differences from those of untreated fellow control eyes were found, in terms of MESORs [ACD (deeper), LT (thinner), VCD (shallower)], amplitudes [AL (larger), RT (larger), VCD (smaller)], and acrophases [≈5h and 2 h 20 min phase-advance for ACD and VCD, respectively]. In the case of both AL and ChT rhythms, treated and fellow-untreated eyes showed approximately the same acrophases, which nonetheless, become more in-phase in comparison with our previous study (Table 4) (Nilsen et al., 2022). In the case of ACD and LT rhythms, they were fully in phase for fellow-untreated eyes, and near in phase for treated eyes, with the anterior chamber deepening, and the crystalline lens thickening over the course of the evening (Figs. 3–4).

Overall, eyes treated with topical 1 % atropine did not show any change in choroidal thickness after instillation of atropine, contrasting with reports of thickening in children undergoing myopia-control treatment (Yam et al., 2022; Xu et al., 2023). However, perhaps noteworthy, the choroids of treated eyes did not thin when diurnal thinning was expected (Fig. 3, c.f. HWT+1 pre-instillation of atropine with HWT + 4). This finding is also consistent with a report of choroidal thickening 30 and 60 min after day-time instillation of 0.01 % atropine, i.e., within a time window of 09:00–14:00 (Sander et al., 2019). In the current study, this "relative choroidal thickening" effect of atropine also altered the phase relationship between choroidal thickness and axial length rhythms, to become more in-phase, in atropine-treated eyes, and curiously also in fellow control eyes (Fig. 4 and 6E–F). That the effect is similar in both eyes is consistent with a cross-over effect of atropine, as evident in accommodation data (discussed further below), and highlights the importance of having available for comparison, data from other studies involving healthy, untreated eyes (Burfield, Carkeet, & Ostrin, 2019; Burfield, Patel, & Ostrin, 2018; Chakraborty, Read, & Collins, 2011; Nilsen et al., 2022). That the changes induced by atropine brought the AL and ChT rhythms more in phase, here by advancing the ChT rhythm by approximately 6 h compared to our previous study (c.f. Fig. 6 C and E) (Nilsen et al., 2022), whilst leaving the AL rhythm unchanged, has a parallel in observations from another human study involving imposed myopic defocus and in which the choroid was found to thicken and also undergo phase-advance in its rhythm, by 9 h, accompanied by a phase-delay in the AL rhythm by 6 h—resulting in AL and ChT rhythms also becoming more in-phase (Chakraborty et al., 2012). Both optical and atropine treatment, when applied longer term, have been linked to slowed myopia progression (Yam et al., 2022; Xu et al., 2023). Interestingly, in our previous study using the same methodologies to track seasonal changes in ocular diurnal rhythms in untreated eyes (Fig. 6 C–D), an anti-phase relationship between AL and ChT rhythms was observed in winter (~12 h phase-difference, Fig. 6C), with these rhythms becoming more in-phase in summer (1–3 h phase-shift, Fig. 6D), but only in eyes with coordinated or decelerated growth. Thus, when comparing these two studies we can see that the AL and ChT phase relationship was related to a change in the phase of ChT only, with the phase of AL remaining the same as for the same time of year and also reported in other studies (Burfield, Patel, & Ostrin, 2018; Chakraborty, Read, & Collins, 2011). Importantly, in related studies involving experimental animal models, AL and ChT rhythms are typically found to be fully in anti-phase (≈12 h phase-difference) during periods of accelerated growth (Fig. 6A), and more in-phase during periods of normal growth (≈9h phase-difference, Fig. 6B) (Nickla et al.,

Table 3
Estimates of relative acrophase for listed ocular parameters and both fellow control and atropine-treated eyes (Table 2) converted to standard clock time (ST). Interocular differences in acrophase are listed in the right most column. Phase advance is indicated with a – sign, and phase delay is indicated with a + sign.

	Fellow control eye		Atropine eye		
Parameter	**Acrophase (ST)**	**95 % CI**	**Acrophase (ST)**	**95 % CI**	**Acrophase difference**
IOP	16:01	15:19–16:43	16:18	15:43–16:54	+0:17
CCT	11:53	09:31–15:27	12:19	10:18–15:01	+0:26
ACD	03:58	02:54–04:46	22:58	21:43–00:07	-5:01*
LT	04:19	03:15–05:16	03:50	02:12–05:13	-0:29
VCD	16:00	15:32–16:28	13:42	13:04–14:23	-2:18*
AL	15:20	14:49–15:52	15:58	15:33–16:24	+0:38
RT†	14:03	12:51–15:24	13:28	12:29–14:32	-0:35
ChT†	20:25	19:39–21:11	21:30	19:33–23:27	+1:05

* Significant effect of atropine on acrophase set at $p < 0.05$.
† Averaged over the central 1 mm.

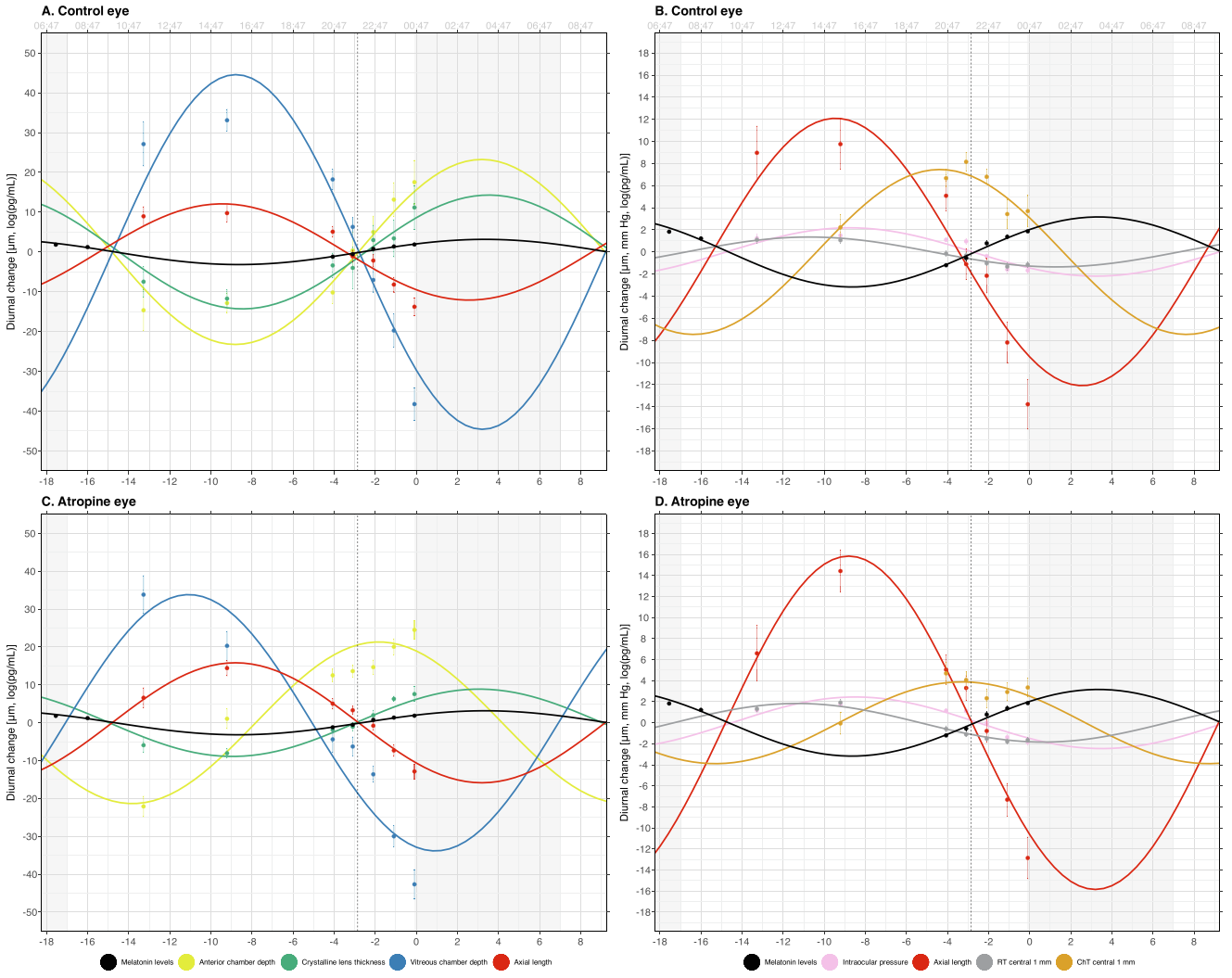

Fig. 4. A-D. Group means (±SE error bars) for measured ocular parameters and saliva melatonin level (MEL), normalized to each individual's MESOR. The curves represent sinusoidal model fits from the population estimate based on the fixed effect estimates from the non-linear mixed effects model. The grey areas represent the averaged estimated sleep period. The x-axis is relative to habitual sleep time (HST) where 0 is HST (the upper x-axis shows standard clock time for easier comparison with other studies) (A, C). Fitted models for melatonin (MEL), anterior chamber depth (ACD), crystalline lens thickness (LT), vitreous chamber depth (VCD) and axial length (AL) for fellow control eyes (A) and atropine-treated eyes (C) Fitted models for MEL, intraocular pressure (IOP), AL, retinal thickness (RT) and choroidal thickness (ChT) (central 1 mm for RT and ChT), with a rescaled y-axis for clarity for fellow control eyes (B) and atropine-treated eyes (D). The dashed vertical lines indicate the timings of dim light melatonin onset (DLMO) relative to HST. The first MEL measurement taken when awakening at Day 8 differed in timing from the self-reported habitual wake time.

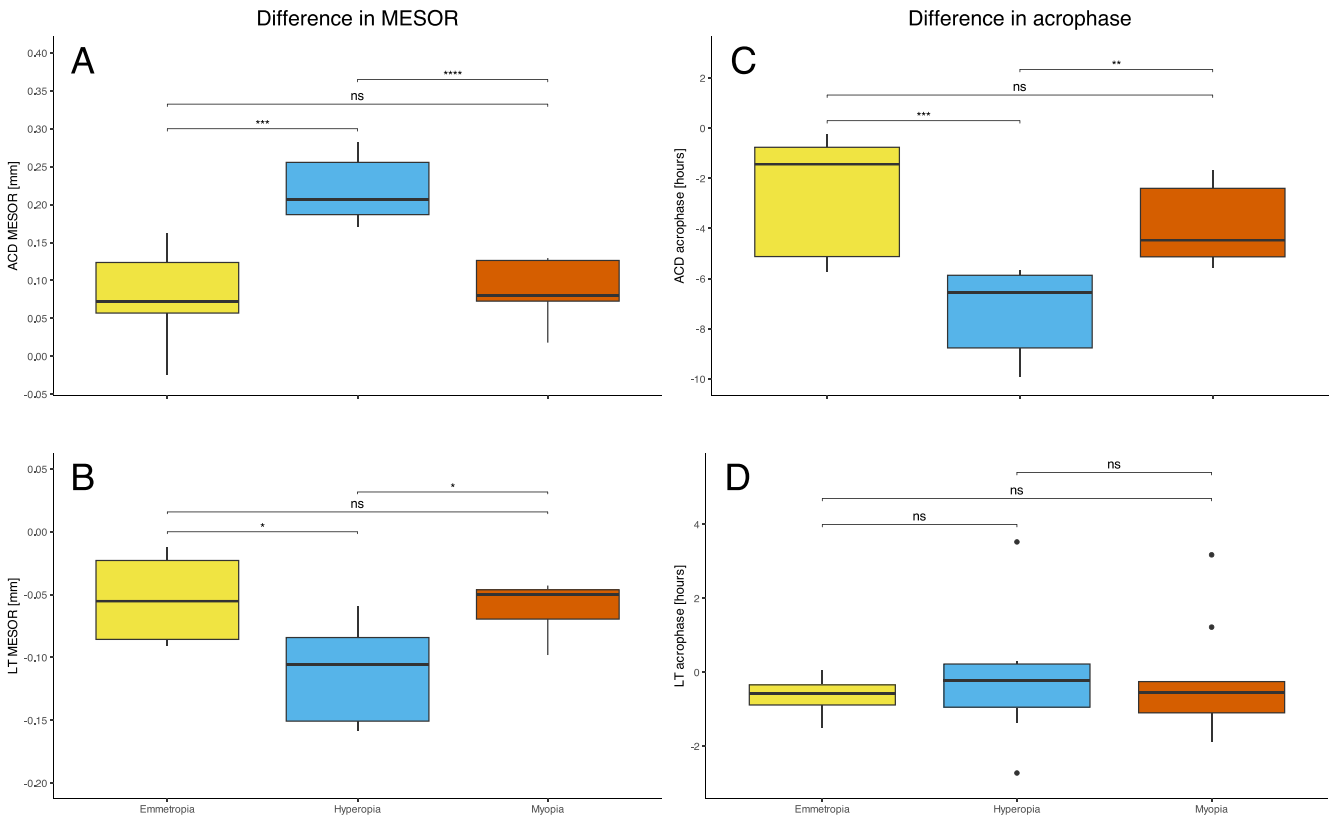

Fig. 5. A–D. Differences in MESOR (A–B) and acrophase (C–D) between atropine-treated and fellow control eyes for anterior chamber depth (ACD, top row) and crystalline lens thickness (LT, bottom row) for emmetropes, hyperopes and myopes. Statistical significance for all panels estimated using one-way ANOVAs, p-values shown in figure are Tukey HSD adjusted: *p < 0.05, **p < 0.01, ***p < 0.001).

Table 4
Comparisons between untreated eyes (previous study) (Nilsen et al., 2022) with atropine-treated eyes for data collected in November (winter), but not in the same year. Time is shown in standard clock time.

	Untreated eyes	Atropine treated eyes	Differences
AL acrophase	15:20	15:58	38 mins
ChT acrophase	03:14	21:30	-5 h 44 mins
AL and ChT phase relationship	11 h 54 mins	5 h 32 mins	6 h 22 mins

1998). On the other hand, such changes in acrophase were not observed in a study involving chicks treated with atropine, although in this case, delivered by intravitreal injection and limited to eyes undergoing myopia induction using negative lenses (Nickla et al., 2019). Together, these observations lend support to the notion that retinal signals arising from behavioural, optical and/or pharmacological interventions have potential to interact with, and perturb, the eye's natural diurnal rhythms to alter eye growth (Nickla, 2013; Chakraborty et al., 2018).

4.1. Accommodation and cross-over effects

The observed differences between the atropine-treated eyes and their fellow eyes (deeper anterior chambers, thinner lenses, and shallower vitreous chambers) are consistent with an inhibitory effect of atropine on accommodation and on the order of magnitude of that expected, based on the difference in residual accommodation between the two eyes (≈2 D). Nonetheless, that AL shows the same trend for both eyes and that atropine-treated and fellow eyes show similar ChT rhythm in the current study, yet the findings for the latter untreated eyes differ from results in our previous study where both eyes were untreated, were unexpected and warrant explanation.

The possibility of a cross-over (contralateral) effect warrants consideration in this context. Such effects have been documented with tropicamide and phenylephrine in humans (Kara et al., 2014; Patsiopoulos et al., 2003), as well as with topical atropine in a study involving rabbits (Wang et al., 2019). Another alternative explanation for similar effects on AL and ChT rhythm in untreated eyes is interocular yoking, which describes interactions between the two eyes that are presumed to be mediated by central neuronal pathways (Zhu, McBrien, & Smith E.L., Troilo D., Wallman J., 2013). Although this has been reported in several paired-eye animal studies, this would seem, however, to be the least plausible explanation for our findings, given the many other influences on the choroid and its very high blood flow (Wang et al., 2019; Nickla and Schroedl, 2019). Among other possibilities that cannot be ruled out as contributing factors leading to the observed altered phase relationship, is that it is a by-product of the changes occurring secondary to the monocular reduction in accommodation and/or decreases in cholinergic stimulation of non-vascular smooth muscle in the choroid (Meriney and Pilar, 1987; Poukens et al., 1998; Flügel-Koch et al., 1996). That choroidal thickness is affected by accommodation has been reported in several studies (Ghosh, Collins, Read, Davis, & Chatterjee, 2014; Kaphle, Schmid, Suheimat, Read, & Atchison, 2023; Woodman, Read, & Collins, 2012; Woodman-Pieterse, Read, Collins, & Alonso-Caneiro, 2015), with attribution to changes in the tone of the choroidal nonvascular smooth

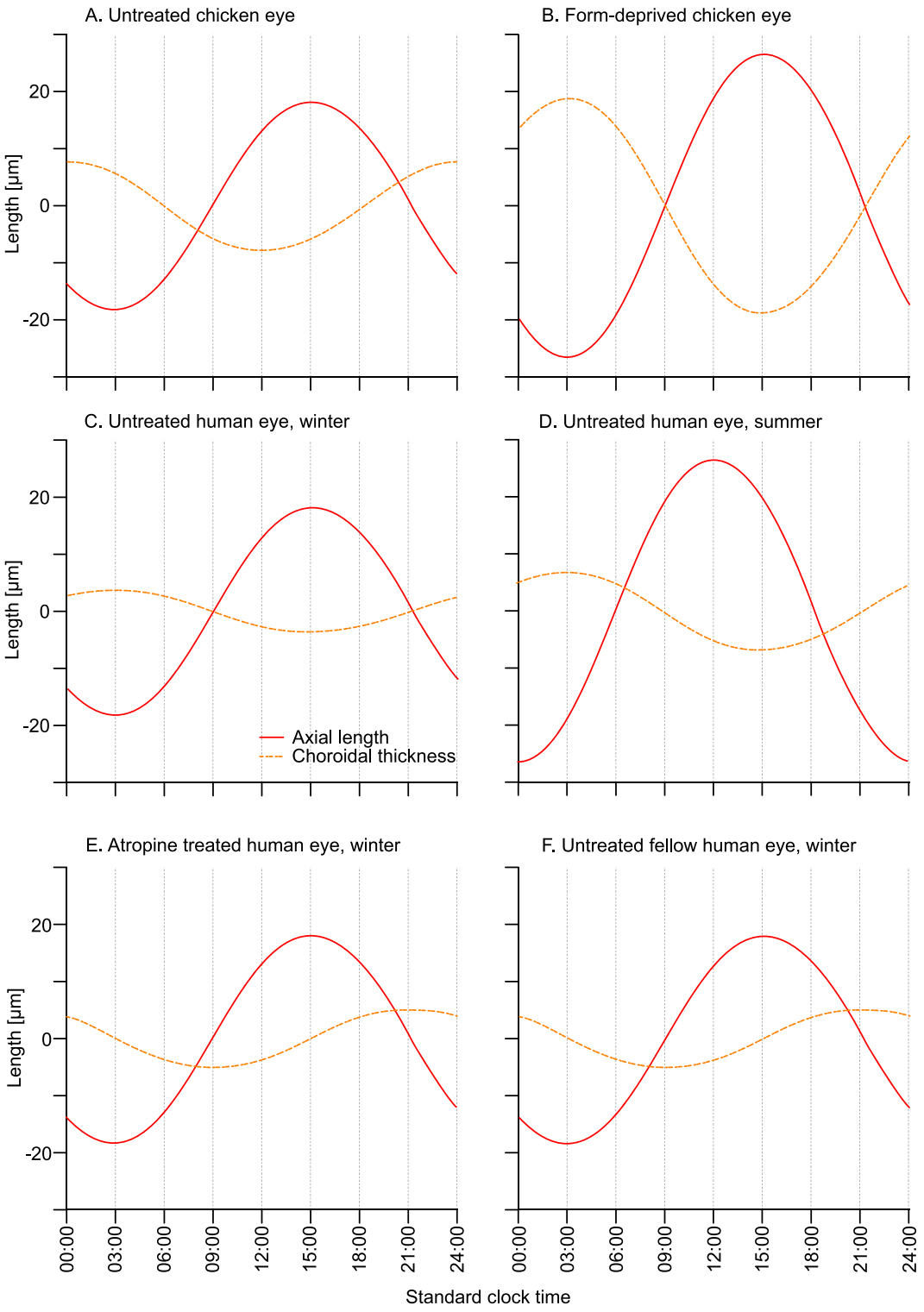

Fig. 6. A–F. Phase relationships between axial length and choroidal thickness rhythms as a function of standard clock time. In chicks, the two rhythms were found to be approximately 9 h out-of-phase in normal (untreated) eyes (A: top left) while the choroid rhythm shifted this to 12 h out-of-phase in form-deprived eyes (B: top right). A and B are adapted from Figure 12 in Ref. (Nickla et al., 1998). In our previous study on humans (C, D), where both eyes remained untreated (Nilsen et al., 2022), the two rhythms were found to be approximately 12 h out-of-phase in winter (C: middle left), with AL advancing to become a few hours more in-phase in summer (9 h out-of-phase), for those who had experienced coordinated or decelerated growth (D: middle right). In this study, the two rhythms were found to be even more in-phase (6 h out-of-phase) both in the atropine (E: bottom left) and control (F: bottom right) eyes. The difference was related to a 6 h phase advance of choroidal thickening, while AL phase remained the same when comparing the atropine treated eye with the untreated human eye in winter (E versus C) and untreated chicken eyes (E versus A).

muscle and/or choroidal blood flow (Woodman-Pieterse et al., 2015).

4.2. Diurnal changes in crystalline lens thickness

The diurnal rhythm in crystalline lens thickening persisted, despite accommodation being paralyzed by atropine, and atropine-treated eyes maintained the same acrophase as fellow control eyes (Table 2). This finding parallels our previous observation of evening thickening of the crystalline lens that was mechanistically different from accommodation-related thickness changes (Nilsen et al., 2022). That the rhythms of ACD and LT were also in-phase implies that the changes were largely confined to the posterior part of the lens (Chakraborty et al., 2011). This interpretation is also corroborated by the observed antiphase relationship of VCD and LT rhythms, and in contrast to accommodation-related changes in the curvature of the anterior lens (and, to a lesser extent, the posterior lens), which lead to shorter ACD and VCD (Kaufman et al., 2011).

The mechanism (or mechanisms) underlying the diurnal changes of crystalline lens thickness is not known, although it is tempting to speculate that changes in ciliary muscle tonus and independent metabolic changes within the crystalline lens may be involved (Burfield, Patel, & Ostrin, 2018). That the diurnal changes persist in atropine-treated eyes tend to rule out the former, as tonic accommodation is minimized in these eyes yet the LT acrophase persists (Choi and Cho, 1963), whilst also the anterior lens surface flattens and LT is reduced (Bartlett & Jaanus, 2008; Hashemi, Asharlous, & Khabazkhoob, 2020) Furthermore, melatonin appears to be synthesized by the crystalline lens (Alkozi et al., 2017), in addition to the pineal gland and retina (Ostrin, 2019), with levels of melatonin reported to increase in the evening in the rabbit lens (Abe et al., 1999). There is a noteworthy similarity in the timing of evening thickening in the human crystalline lens and increases in melatonin levels (Fig. 4 A–D).

ACD and VCD exhibited significant phase differences between the fellow control and atropine-treated eyes (Table 2). Since these ocular parameters are interdependent and most likely reflect the combined effects of the crystalline lens moving and changing its thickness (no interocular differences in neither CCT nor RT, Table 2), the phase differences could be explained by increased noise, as reflected in the less optimal curve fitting (c.f. ACD and VCD in Fig. 4 A and C).

4.3. Differences between refractive error groups

The between-individual intra-ocular variation in ChT for the effect of atropine 1 % was independent of refractive error (Supplementary Table S3). There were no differences between the refractive error groups in terms of the effects of 1 % atropine on IOP, AL, RT and ChT, consistent with previous findings in young adults with 2 % homatropine, another non-selective antimuscarinic drug (Sander et al., 2018). Atropine did, however, induce greater deepening of ACDs and reductions in LTs on average (i.e., MESORs) in the hyperopic group compared with emmetropic and myopic groups. That ciliary muscle tone is likely to be highest in hyperopes and lowest in myopes offers a plausible explanation for differences between these two refractive error groups in the effect of atropine (Zadnik et al., 1999). These findings are also in line with results from a study conducted on children (6–12 years old) involving topical 1 % cyclopentolate (Hashemi et al., 2020).

4.4. Strengths and limitations

Key strengths of this study include the measurement schedule, which was designed to respect individual chronotypes, as well as the nature of the data used to derive them, as described previously (Nilsen et al., 2022). Importantly, wake-up and sleep-onset times of participants were measured objectively (via actigraphy) over the week preceding the lab-based data collection, and were used along with self-reported wake times to create a customized measurement schedules for each individual. Respecting habitual sleep times in this manner allowed diurnal ocular rhythms to be assessed in relation to each individual's chronotype. Melatonin rhythms, derived from assayed saliva samples, were used to further validate derived chronotypes; where the sinusoid was fitted to the logarithm of the measurement to account for the sharp drop and rise in secretion in the morning and evening, respectively (equivalently, a floor effect during the day) (Kennaway, 2019). The participants were confined to the lab for the evening measurements, during which time ambient light levels were kept below 20 lx to avoid artificially suppressing melatonin secretion, as there can be large between-individual variations in sensitivity in suppressing melatonin (Phillips et al., 2019).

When modelling periodic behaviour, it is desirable to sample at regular intervals (say, hourly for a circadian rhythm) for the duration of at least one cycle. There are, however, significant logistical and practical problems with disturbing the sleep patterns of volunteers during their working week and, consequently, we did not attempt any measures after their habitual bedtimes. Further, it has been shown that sleep-disturbing measurements are of questionable value due to the release of stress hormones affecting cardiovascular function, and changes of body posture influencing ocular biometry, intraocular pressure (Liu et al., 1998), and choroidal thickness (Anderson et al., 1985). Instead, we aimed to densely sample parameters around the expected time of dim-light melatonin onset (DLMO), which increased the likelihood of capturing this useful measure of an individual's melatonin cycle (Kennaway, 2019), while also allowing rich sampling in the region of maximum rate-of-change in the other biometric parameters (which is most evident in the early evening measures in Fig. 3). The dense sampling at such an influential part of the rhythm greatly restricted the search space of the sinusoid fitting algorithm, giving confidence to the parameters estimated to assess rhythmic behaviour.

We did not include any measures after habitual bedtime, and while it has been shown that sleep-disturbing measurements are of questionable value in a study like this (Kennaway, 2019), we cannot rule out such influences on our results in the absence of such data.

In the current study, we did not collect a full (day long) set of baseline data before initiating the monocular 1 % atropine treatment, choosing instead to make use as "reference baseline" data, equivalent data from the previous diurnal study, which was conducted at the same time of year the year before. Although these data were collected from different participants, the age range and time of year for these two studies are comparable.

As described, the instillation of atropine affected the MESOR, amplitude and acrophase of various ocular diurnal rhythms, as modelled with a sinusoid with a 24-hour period. Nonetheless, it is plausible that our atropine intervention may have altered the periodicity of the choroid rhythm (Nickla and Schroedl, 2019), which appears to be the more dynamic of choroidal and scleral structures (Read, Fuss, Vincent, Collins, & Alonso-Caneiro, 2019; Zhu, Goto, Singh, Torres, & Wildsoet, 2022). We cannot rule out the possibility that changes (or the absence thereof) in MESOR, amplitude and acrophase of ChT reflect a change in periodicity. Distinguishing between these two possibilities would require significantly more epochal measurements over several days (both pre- and post-atropine-instillation), which would introduce additional questions and challenges, including decisions in relation to atropine dosing.

The potential cross-over and yoking effects are limitations to the paired-eye design used in this study. One approach to circumvent this potentially confounding effect would be to use a "2-day" design, with baseline data collected from both eyes on day 1, followed by repeated measurements on day 2 after instillation of atropine in one (or both) eyes.

The use of different atropine doses, according to iris colour, did not appear to have influenced study outcomes. This outcome is in line with the expected greater inactivation of atropine through binding to melanin in eyes with darker irides, despite their exposure to a higher dose of

atropine (2 drops) (Salazar et al., 1976; Bahrpeyma et al., 2022).

A high concentration (1 %) of atropine was administered once in the morning, in order to give a large – but short-term – measurable effect on rhythmic statistics. While the timing of atropine treatment was found not to influence outcomes in one study in chicks (Nickla et al., 2019), a longer term study is warranted to assess the effects on the various ocular rhythms of repeated daily (evening) atropine treatment with lower concentrations, as currently used for myopia control. Questions of interest in undertaking such a study include whether the observed alteration in the phase-relationship between AL and ChT rhythms persists with long term therapy, and thus its relationship to changes in the rate of eye elongation, with slowed growth being the expected outcome with topical atropine in myopic children. Finally, with drug-eluting contact lenses likely to become available soon, understanding the effect of the timing of atropine instillation may be an especially important factor to investigate in the context of treatment efficacy.

5. Conclusion

During a 24 h period post instillation of topical 1 % atropine, the diurnal rhythms of ocular parameters persisted, although MESORs, amplitudes and/or acrophases were altered in many cases. The rhythmicity of choroidal thickness changes was also affected in both atropine-treated and fellow control eyes, with changes in the phase-relationship between axial length and choroidal thickness rhythms akin to that observed under conditions favouring slowed eye growth. Our results add support to the choroid being a key site for atropine's myopia control effect, while they do not address the question of whether it is the primary site of action as opposed to the down-stream target of an atropine-initiated anti-myopia signal. Additionally, we report for the first time that the crystalline lens undergoes diurnal variation in its thickness even when accommodation is minimized by topical atropine. The significance of this observation for ocular growth regulation and control of myopia progression, along with the effects on all rhythms of more extended use of topical atropine and different concentrations of atropine, warrant further investigation, given increasingly popular use of "low dose" atropine for control of myopia in children.

Disclosure

N.G. Nilsen, **None**; S.J. Gilson, **None**; H.R. Pedersen, **None**; L.A. Hagen, **None**; C.F. Wildsoet, **None**; R.C. Baraas, **None**.

Funding

NGN holds a PhD position funded by the Norwegian Ministry of Education and Research, Norway.

CRediT authorship contribution statement

Nickolai G. Nilsen: Conceptualization, Methodology, Investigation, Formal analysis, Writing – original draft. **Stuart J. Gilson:** Methodology, Software, Investigation, Data curation, Formal analysis, Writing – original draft. **Hilde R. Pedersen:** Investigation, Validation, Writing – review & editing. **Lene A. Hagen:** Investigation, Writing – review & editing. **Christine F. Wildsoet:** Conceptualization, Methodology, Writing – review & editing. **Rigmor C. Baraas:** Conceptualization, Methodology, Investigation, Validation, Writing – original draft, Funding acquisition, Supervision, Project administration.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be shared on Figshare upon publication if manuscript is accepted

Acknowledgments

The authors thank Grethe Thorvaldsen for her help with collecting data and Rodenstock Norge AS for providing iCare probes.

Preliminary data from this study have been published previously as an ARVO abstract: Nilsen et al., IOVS 2020; 61:1922-1922; and at the 2022 International Myopia Conference Meeting (P118).

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.visres.2023.108341.

References

Abe, M., Itoh, M. T., Miyata, M., Ishikawa, S., & Sumi, Y. (1999). Detection of Melatonin, Its Precursors and Related Enzyme Activities in Rabbit Lens. *Experimental Eye Research, 68*, 255–262.

Alkozi, H. A., Wang, X., Perez de Lara, M. J., & Pintor, J. (2017). Presence of melanopsin in human crystalline lens epithelial cells and its role in melatonin synthesis. *Experimental Eye Research, 154*, 168–176.

Anderson, A. P., Babu, G., Swan, J. G., et al. (1985). Ocular changes over 60 min in supine and prone postures. *Journal of Applied Physiology, 2017*(123), 415–423.

Azuara-Blanco, A., Logan, N., Strang, N., et al. (2020). Low-dose (0.01%) atropine eye-drops to reduce progression of myopia in children: A multicentre placebo-controlled randomised trial in the UK (CHAMP-UK)-study protocol. *The British Journal of Ophthalmology, 104*, 950–955.

Bahrpeyma, S., Reinisalo, M., Hellinen, L., Auriola, S., Del Amo, E. M., & Urtti, A. (2022). Mechanisms of cellular retention of melanin bound drugs: Experiments and computational modeling. *Journal of Controlled Release, 348*, 760–770.

Zhu, X., McBrien, N. A., Smith, E. L., Troilo, D., & Wallman, J. (2013). Eyes in various species can shorten to compensate for myopic defocus. *Investigative Ophthalmology & Visual Science, 54*, 2634–2644.

Bartlett, J. D., & Jaanus, S. D. (2008). *Clinical Ocular Pharmacology* (5th ed.). St. Louis, MO: Butterworth-Heinemann.

Benloucif, S., Burgess, H. J., Klerman, E. B., et al. (2008). Measuring Melatonin in Humans. *Journal of Clinical Sleep Medicine: JCSM : official publication of the American Academy of Sleep Medicine, 4*, 66–69.

Bland, J. M., & Altman, D. G. (1986). Statistical methods for assessing agreement between two methods of clinical measurement. *Lancet, 1*, 307–310.

Chakraborty, R., Read, S. A., & Collins, M. J. (2011). Diurnal variations in axial length, choroidal thickness, intraocular pressure, and ocular biometrics. *Investigative Ophthalmology & Visual Science, 52*, 5121.

Chakraborty, R., Read, S. A., & Collins, M. J. (2012). Monocular myopic defocus and daily changes in axial length and choroidal thickness of human eyes. *Experimental Eye Research, 103*, 47–54.

Burfield, H. J., Patel, N. B., & Ostrin, L. A. (2018). Ocular Biometric Diurnal Rhythms in Emmetropic and Myopic Adults. *Investigative Ophthalmology & Visual Science, 59*(12), 5176–5187.

Burfield, H. J., Carkeet, A., & Ostrin, L. A. (2019). Ocular and Systemic Diurnal Rhythms in Emmetropic and Myopic Adults. *Investigative Ophthalmology & Visual Science, 60*(6), 2237–2247.

Chakraborty, R., Ostrin, L. A., Nickla, D. L., Iuvone, P. M., Pardue, M. T., & Stone, R. A. (2018). Circadian rhythms, refractive development, and myopia. *Ophthalmic and Physiological Optics, 38*, 217–245.

Chakraborty, R., Read, S. A., & Collins, M. J. (2013). Hyperopic defocus and diurnal changes in Human Choroid and Axial Length. *Optometry and Vision Science, 90*, 1187–1198.

Chia, A., Chua, W.-H., Cheung, Y.-B., et al. (2012). Atropine for the treatment of childhood myopia: Safety and efficacy of 0.5%, 0.1%, and 0.01% doses (Atropine for the Treatment of Myopia 2). *Ophthalmology, 119*, 347.

Chiang, S. T. H., & Phillips, J. R. (2018). Effect of Atropine Eye Drops on Choroidal Thinning Induced by Hyperopic Retinal Defocus. *Journal of Ophthalmology, 2018*, 8528315.

Chiang, S. T. H., Turnbull, P. R. K., & Phillips, J. R. (2020). Additive effect of atropine eye drops and short-term retinal defocus on choroidal thickness in children with myopia. *Scientific Reports, 10*, 18310.

Choi, O., & Cho, S. C. (1963). The studies on the residual accomodation of Koreans. I. The residual accomodation under 1 percent atropine and 5 percent homatropine cycloplegia. *Yonsei Medical Journal, 4*, 73–76.

Chua, W.-H., Balakrishnan, V., Chan, Y.-H., et al. (2006). Atropine for the Treatment of Childhood Myopia. *Ophthalmology, 113*, 2285–2291.

Cohen, J., & Cohen, P. (1983). *Applied multiple regression-correlation analysis for the behavioral sciences*. Hillsdale, NJ: L. Erlbaum Associates.

Crowley, S. J., Suh, C., Molina, T. A., et al. (2017). Estimating the dim light melatonin onset of adolescents within a 6-h sampling window: the impact of sampling rate and threshold method. *Sleep medicine, 20*, 59–66.
De Bernardo, M., Borrelli, M., Cembalo, G., & Rosa, N. (2019). Intraocular Pressure Measurements in Standing Position with a Rebound Tonometer. *Medicina (Kaunas, Lithuania), 55*, 701.
Flügel-Koch, C., May, C. A., & Lütjen-Drecoll, E. (1996). Presence of a contractile cell network in the human choroid. *Ophthalmologica, 210*, 296–302.
Ghosh, A., Collins, M. J., Read, S. A., Davis, B. A., & Chatterjee, P. (2014). Axial Elongation Associated with Biomechanical Factors during Near Work. *Optometry and Vision Science, 91*, 322–329.
Hashemi, H., Asharlous, A., Khabazkhoob, M., et al. (2020). The Effect of Cyclopentolate on Ocular Biometric Components. *Optometry and Vision Science, 97*, 440–447.
Kaphle, D., Schmid, K. L., Suheimat, M., Read, S. A., & Atchison, D. A. (2023). Central and peripheral choroidal thickness and eye length changes during accommodation. *Ophthalmic & Physiological Optics, 43*, 311–318.
Kara, N., Demircan, A., Karatas, G., et al. (2014). Effects of two commonly used mydriatics on choroidal thickness: Direct and crossover effects. *Journal of Ocular Pharmacology and Therapeutics, 30*, 366–370.
Kaufman, P. L., Alm, A., Levin, L. A., & Adler, F. H. (2011). *Adler's physiology of the eye* (11th ed.). Edinburgh: Saunders Elsevier.
Kennaway, D. J. (2019). A critical review of melatonin assays: Past and present. *Journal of Pineal Research, 67*, e12572.
Larkin, G. L., Tahir, A., Epley, K. D., Beauchamp, C. L., Tong, J. T., & Clark, R. A. (2019). Atropine 0.01% Eye Drops for Myopia Control in American Children: A Multiethnic Sample Across Three US Sites. *Ophthalmology and Therapy, 8*, 589–598.
Liu, J. H., Kripke, D. F., Hoffman, R. E., et al. (1998). Nocturnal elevation of intraocular pressure in young adults. *Investigative Ophthalmology & Visual Science, 39*, 2707–2712.
Lockley, S. W. (2020). Journal of Pineal Research guideline for authors: Measuring melatonin in humans. *Journal of Pineal Research, 69*(2), Article e12664.
Mackey, D. A., Wilkinson, C. H., Kearns, L. S., & Hewitt, A. W. (2011). Classification of iris colour: Review and refinement of a classification schema. *Clinical & Experimental Ophthalmology, 39*, 462–471.
Marzani, D., & Wallman, J. (1997). Growth of the two layers of the chick sclera is modulated reciprocally by visual conditions. *Investigative Ophthalmology & Visual Science, 38*, 1726–1739.
McBrien, N. A., Moghaddam, H. O., & Reeder, A. P. (1993). Atropine reduces experimental myopia and eye enlargement via a nonaccommodative mechanism. *Investigative Ophthalmology & Visual Science, 34*, 205–215.
Meriney, S. D., & Pilar, G. (1987). Cholinergic innervation of the smooth muscle cells in the choroid coat of the chick eye and its development. *The Journal of Neuroscience, 7*, 3827–3839.
Nickla, D. L. (2013). Ocular diurnal rhythms and eye growth regulation: Where we are 50 years after Lauber. *Experimental Eye Research, 114*, 25.
Nickla, D., & Schroedl, F. (2019). Effects of autonomic denervations on the rhythms in axial length and choroidal thickness in chicks. *Neuroethology, Sensory, Neural, and Behavioral Physiology, 205*, 139–149.
Nickla, D. L., & Wallman, J. (2010). The multifunctional choroid. *Progress in Retinal and Eye Research, 29*, 144–168.
Nickla, D. L., Wildsoet, C., & Wallman, J. (1998). Visual Influences on Diurnal Rhythms in Ocular Length and Choroidal Thickness in Chick Eyes. *Experimental Eye Research, 66*, 163–181.
Nickla, D. L., Wildsoet, C. F., & Troilo, D. (2001). Endogenous Rhythms in Axial Length and Choroidal Thickness in Chicks: Implications for Ocular Growth Regulation. *Investigative Ophthalmology & Visual Science, 42*, 584–588.
Nickla, D. L., Wildsoet, C. F., & Troilo, D. (2002). Diurnal Rhythms in Intraocular Pressure, Axial Length, and Choroidal Thickness in a Primate Model of Eye Growth, the Common Marmoset. *Investigative Ophthalmology & Visual Science, 43*, 2519–2528.
Nickla, D. L., Jordan, K., Yang, J., & Singh, P. (2019). Effects of time-of-day on inhibition of lens-induced myopia by quinpirole, pirenzepine and atropine in chicks. *Experimental Eye Research, 181*, 5–14.
Nilsen, N. G., Gilson, S. J., Pedersen, H. R., Hagen, L. A., Knoblauch, K., & Baraas, R. C. (2022). Seasonal Variation in Diurnal Rhythms of the Human Eye: Implications for Continuing Ocular Growth in Adolescents and Young Adults. *Investigative Ophthalmology & Visual Science, 63*, 20.
Ostrin, L. A. (2019). Ocular and systemic melatonin and the influence of light exposure. *Clinical and Experimental Optometry, 102*, 99.
Ostrin, L. A., Jnawali, A., Carkeet, A., & Patel, N. B. (2019). Twenty-four hour ocular and systemic diurnal rhythms in children. *Ophthalmic & Physiological Optics: The Journal of the British College of Ophthalmic Opticians (Optometrists), 39*, 358–369.
Ostrin, L. A., Harb, E., Nickla, D. L., et al. (2023). IMI—The Dynamic Choroid: New Insights, Challenges, and Potential Significance for Human Myopia. *Investigative Ophthalmology & Visual Science, 64*, 4.
Patsiopoulos, G., Lam, V., Lake, S., & Koevary, S. B. (2003). Insulin and tropicamide accumulate in the contralateral, untreated eye of rats following ipsilateral topical administration by a mechanism that does not involve systemic uptake. *Optometry (St Louis, Mo), 74*, 226–232.
Phillips, A. J. K., Vidafar, P., Burns, A. C., et al. (2019). High sensitivity and interindividual variability in the response of the human circadian system to evening light. *Proceedings of the National Academy of Sciences, 116*, 12019–12024.
Poukens, V., Glasgow, B. J., & Demer, J. L. (1998). Nonvascular contractile cells in sclera and choroid of humans and monkeys. *Investigative Ophthalmology & Visual Science, 39*, 1765–1774.
R Core Team. R: A. (2016). *Language and Environment for Statistical Computing*. Vienna, Austria: R Foundation for Statistical Computing.
Read, S., Fuss, J. A., Vincent, S. J., Collins, M. J., & Alonso-Caneiro, D. (2019). Choroidal changes in human myopia: insights from optical coherence tomography imaging. *Clinical and Experimental Optometry, 102*(3), 270–285.
Read, S. A., Collins, M. J., & Iskander, D. R. (2008). Diurnal Variation of Axial Length, Intraocular Pressure, and Anterior Eye Biometrics. *Investigative Ophthalmology & Visual Science, 49*, 2911–2918.
Salazar, M., Shimada, K., & Patil, P. N. (1976). Iris pigmentation and atropine mydriasis. *The Journal of Pharmacology and Experimental Therapeutics, 197*, 79–88.
Sander, B. P., Collins, M. J., & Read, S. A. (2014). The effect of topical adrenergic and anticholinergic agents on the choroidal thickness of young healthy adults. *Experimental Eye Research, 128*, 181–189.
Sander, B. P., Collins, M. J., & Read, S. A. (2018). The interaction between homatropine and optical blur on choroidal thickness. *Ophthalmic & Physiological Optics, 38*, 257–265.
Sander, B. P., Collins, M. J., & Read, S. A. (2019). Short-Term Effect of Low-Dose Atropine and Hyperopic Defocus on Choroidal Thickness and Axial Length in Young Myopic Adults. *Journal of ophthalmology, 2019*, 4782536.
Schaeffel, F., Troilo, D., Wallman, J., & Howland, H. C. (1990). Developing eyes that lack accommodation grow to compensate for imposed defocus. *Visual Neuroscience, 4*, 177–183.
Stone, R. A., Quinn, G. E., Francis, E. L., et al. (2004). Diurnal Axial Length Fluctuations in Human Eyes. *Investigative Ophthalmology & Visual Science, 45*, 63–70.
Troilo, D., Smith, E. L., Nickla, D. L., et al. (2019). IMI - Report on Experimental Models of Emmetropization and Myopia. *Investigative Ophthalmology & Visual Science, 60*, M31–M88.
Upadhyay, A., & Beuerman, R. W. (2020). Biological Mechanisms of Atropine Control of Myopia. *Eye & Contact Lens, 46*, 129–135.
Wang, L. Z., Syn, N., Li, S., et al. (2019). The penetration and distribution of topical atropine in animal ocular tissues. *Acta Ophthalmologica, 97*. e238–e247.
Wildsoet, C. F. (2003). Neural pathways subserving negative lens-induced emmetropization in chicks - Insights from selective lesions of the optic nerve and ciliary nerve. *Current Eye Research, 27*, 371–385.
Woodman, E. C., Read, S. A., & Collins, M. J. (2012). Axial length and choroidal thickness changes accompanying prolonged accommodation in myopes and emmetropes. *Vision Research, 72*, 34–41.
Woodman-Pieterse, E. C., Read, S. A., Collins, M., & Alonso-Caneiro, D. (2015). Regional Changes in Choroidal Thickness Associated With Accommodation. *Investigative Ophthalmology & Visual Science, 56*, 6414–6422.
Xiang, Y., Fu, T., Xu, Q., et al. (2021). Quantitative analysis of internal components of the human crystalline lens during accommodation in adults. *Scientific Reports, 11*, 6688.
Xu, H., Ye, L., Peng, Y., et al. (2023). Potential Choroidal Mechanisms Underlying Atropine's Antimyopic and Rebound Effects: A Mediation Analysis in a Randomized Clinical Trial. *Investigative Ophthalmology & Visual Science, 64*, 13.
Yam, J. C., Jiang, Y., Lee, J., et al. (2022). The Association of Choroidal Thickening by Atropine With Treatment Effects for Myopia: Two-Year Clinical Trial of the Low-concentration Atropine for Myopia Progression (LAMP) Study. *American Journal of Ophthalmology, 237*, 130–138.
Zadnik, K., Mutti, D. O., Kim, H. S., Jones, L. A., Qiu, P. H., & Moeschberger, M. L. (1999). Tonic accommodation, age, and refractive error in children. *Investigative Ophthalmology & Visual Science, 40*, 1050–1060.
Zhu, Q., Goto, S., Singh, S., Torres, J. A., & Wildsoet, C. F. (2022). Daily or Less Frequent Topical 1% Atropine Slows Defocus-Induced Myopia Progression in Contact Lens-Wearing Guinea Pigs. *Translational Vision Science & Technology, 11*, 26.