Dose Dependent Effects of Atropine on Static and Dynamic Pupil and Accommodation Metrics in Young Adults

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Purpose. The purpose of this study was to investigate short-term effects of a range of low-dose atropine concentrations on static and dynamic pupil and accommodation metrics in young adults.

Methods. This double blinded study tested pupil and accommodation metrics at baseline and 1 hour and 24 hours after topical instillation of a single drop of placebo, 0.01%, 0.025%, 0.05%, and 0.1% atropine in the right eyes of 20 healthy adults (18–35 years). Static pupil diameter was measured under photopic, mesopic, and scotopic illumination, and dynamic responses were recorded as illumination changed from 0.3 to 140 lux using a pupillometer (MYAH). Peak constriction and dilation velocities were extracted. Accommodative lag and maximum accommodation were determined (WAM-5500) and dynamic responses were recorded for targets at 33 cm and 6 m (PowerRef). Dynamic responses were fitted with exponential functions to calculate amplitude, time constant, and peak velocities.

Results. Static pupil diameters under all lighting conditions and dynamic metrics, including constriction amplitude and peak constriction and dilation velocities, showed significant dose-response effects at 1 hour and 24 hours ($P < 0.05$ for all). Maximum accommodation significantly decreased at 1 hour and 24 hours after atropine administration compared to placebo for all concentrations ($P < 0.05$ for all). Accommodative time constant increased and peak velocity decreased over 24 hours after atropine administration ($P < 0.05$). On the other hand, accommodative and disaccommodative amplitudes, disaccommodative time constant, and peak velocity did not significantly change after atropine administration ($P > 0.05$).

Conclusions. A single drop of 0.01%, 0.025%, 0.05%, and 0.1% atropine induced significant changes in static and dynamic pupil and accommodation metrics in a dose-dependent manner in young adults.

Keywords: myopia, atropine, pupil, accommodation

The global prevalence of myopia is currently estimated to be about 34% and is anticipated to reach 50% by 2050.[1] By then, myopia is projected to contribute to 27% to 43% of uncorrectable visual impairment within the US population.[2] Myopia has been recognized as a major public health concern, leading to significant pathological and economic consequences.[3] Various interventions for myopia control in children include pharmacological, optical, and behavioral strategies.[4] Pharmacological interventions are dominated by topical atropine sulfate, a nonselective muscarine acetylcholine receptor antagonist.[4] Atropine has been well recognized for myopia control, with strong support from numerous clinical trials in the last 2 decades.[4,5] The mechanisms of action in myopia control is not fully understood. Traditionally, atropine was believed to control myopia progression by reducing accommodation.[5–7] However, recent animal studies suggest that atropine primarily acts through scleral remodeling and blocking muscarinic receptors in the sclera and retina, reducing biochemical signaling that drives axial elongation.[6,8–10] Although showing effectivity in slowing myopia progression, atropine is associated with side effects due to its nonselective muscarinic effects on the iris sphincter and ciliary muscle, inducing pupil dilation and cycloplegia, thereby resulting in symptoms of glare, photophobia, and blurring of near vision.[4,11]

For myopia control in children, previous studies have used varying concentrations of atropine, ranging from 0.0025% to 1%. Earlier studies, such as Atropine for the Treatment of Myopia (ATOM 1), used 1% atropine, which effectively slowed myopia progression in Asian children, but also resulted in prolonged photophobia and blurring of near vision.[12] More recent studies have utilized lower concentrations of atropine to identify effective doses that minimize adverse side effects. These studies include ATOM 2 (0.01%, 0.5%, and 0.1%),[13] Low-Concentration Atropine for Myopia Progression (LAMP; 0.01%, 0.025%, and 0.05%), Childhood Atropine for Myopia Progression (CHAMP-UK; 0.01%), Western Australia Atropine for the Treatment of Myopia

(WA-ATOM; 0.01%),[14,15] Atropine for Myopia (AIM; 0.01% and 0.02%),[16] and APPLE (0.0025%, 0.005%, and 0.01%).[17,18] However, static and dynamic pupil and accommodation-related side effects of low to moderate concentrations of atropine administration have not been fully investigated.

A recent meta-analysis by Tran et al.,[19] reported the long-term effects of nine concentrations (ranging from 0.01% to 1%) of atropine, including nine randomized control trials conducted in children, that assessed pupil and accommodation effects. The authors' analyses revealed that pupil diameter increased and amplitude of accommodation decreased in a nonlinear dose-dependent manner. However, this meta-analysis was limited by a lack of studies that tested pupil and accommodation dynamics, variations in assessment techniques, and lack of symptomatic outcomes, such as blurred near vision and photophobia. Kaymak et al.,[20] tested the short-term effects of single drop of 0.001%, 0.005%, and 0.01% atropine administration on static pupil diameter and amplitude of accommodation in young adult participants. For 0.005% and 0.01% atropine administration, pupil size under mesopic and photopic conditions significantly increased and amplitude of accommodation significantly decreased, lasting for at least 24 hours. Whereas 0.001% atropine had only minimal effects on pupil size, it should be noted that 0.001%, as well as 0.005%, are not commonly used in myopia control.

Previous studies involving atropine have primarily reported static measurements of the pupil and maximum accommodative amplitude at a single time point.[19,20] Pupil diameter and accommodation are dynamic processes, highlighting the importance of also measuring the dynamic metrics to better understand functional effects of atropine. Dynamic metrics include constriction and dilation velocities of the pupil and velocities and latencies of accommodation and disaccommodation.[21–23] Characterizing pupil and accommodation dynamics after varying doses of atropine will contribute to what is known about biomechanical characteristics of the iris and ciliary muscles and how commonly used concentrations of atropine for myopia control influence these processes.[24] The goal of this study was to investigate short-term effects of 0.01%, 0.025%, 0.05%, and 0.1% atropine administration on static and dynamic pupil and accommodation metrics, as well as examine functional effects in young adults. Although atropine is not typically used for myopia control in adults, and adults and children show differences in accommodative amplitude,[25,26] young adults were chosen for feasibility and accessibility.

Methods

Participants

This randomized, double blinded, repeated measures study enrolled heathy adult participants between the ages of 18 and 35 years. Inclusion criteria were best-corrected vision of 20/25 or better, noncycloplegic autorefraction between +3.00 and –6.00 DS, astigmatism ≤2.00 DC, normal ophthalmological and general health findings, and open anterior chamber angle. Exclusion criteria were narrow angles (Van Herick grade 2 or less), history of adverse reaction with any dilating eye drops, and any myopia control treatment (e.g. atropine and orthokeratology contact lens wearers). Participants were screened through a detailed medical and ocular history, visual acuity assessment, noncycloplegic autorefraction, and accommodation using an open field autorefractometer (WAM 5500, Grand Seiko, Japan), slit-lamp examination, and optical coherence tomography (OCT) fundus examination. Participants accommodating less than 3 diopters (D), as measured objectively with the WAM 5500, were excluded from the study. Iris color was graded using the Iris Classification System ranging from one to five; grade one having the least pigmentation and grade five having the most pigmentation.[27] Spherical equivalent refraction (SER) of the right eye was used to classify participants as myopic (SER ≤ -0.50 D) or non-myopic (SER > -0.50 D).[28] Participants were asked to avoid alcohol, caffeine, tobacco use, and vigorous physical activity on the experimental days, as these have been shown to affect ocular physiology.[29] Written informed consent was obtained after explaining the purpose and risks of the study from all the participants. The study was approved by the Institutional Review Board at the University of Houston and was conducted in accordance with the tenets of the Declaration of Helsinki.

Protocol

Each participant underwent 5 testing conditions, which included 4 atropine concentrations, 0.01%, 0.025%, 0.05%, 0.1%, and a placebo (0.0%). Sterile ophthalmic atropine solutions and placebo were compounded locally (Greenpark Compounding Pharmacy, Houston, TX, USA) and stored at 35 degrees Fahrenheit. The atropine solution was comprised of the appropriate dosage of atropine plus hypromellose, phosphate buffer, ethylene diamine tetra acetic acid, and water for irrigation. The placebo was the same solution, but without atropine. The bottles were coded by one team member (author L.A.O.). The investigator conducting the experiment (author B.L.) and the participants were both masked to the concentration instilled at each session. Concentrations were tested in a randomized order in 5 separate sessions at least 1 week apart. To minimize the influence of diurnal variations across participants, all sessions began between 8:30 and 11:30 AM, and for each individual participant, all repeat sessions began within a 1 hour window.

For each experimental session, measurements were captured at 3 time points, baseline, 1 hour, and 24 hours ($\pm$ 1 hour) after instillation of a single drop of atropine in the right eye. Participants were asked not to wear contact lenses, but to bring their spectacles (where applicable) for the experiment. At baseline and 24 hours, the participants first underwent a 10-minute distance viewing period (watching a television at 6 m) to minimize the impact of prior activities on the measurements. Participants also watched the television after instillation until the 1 hour measurement. At each time point, dynamic and static pupil measurements were recorded with the MYAH (VISIA, San Giovanni Valdarno, Italy; a subsidiary of Topcon Corporation, Japan), static accommodative responses for a range of distances were measured using an open field autorefractometer (WAM-5500; Grand Seiko, Japan) and dynamic accommodative responses were measured with a PowerRefractor (PowerRef 3, PlusOptiX, Germany), in the same order. Measurements were only captured for the right eyes. Additionally, axial length was measured with the MYAH, and OCT and OCT angiography scans (Spectralis, Heidelberg, Germany) were collected at each time point and will be presented elsewhere.

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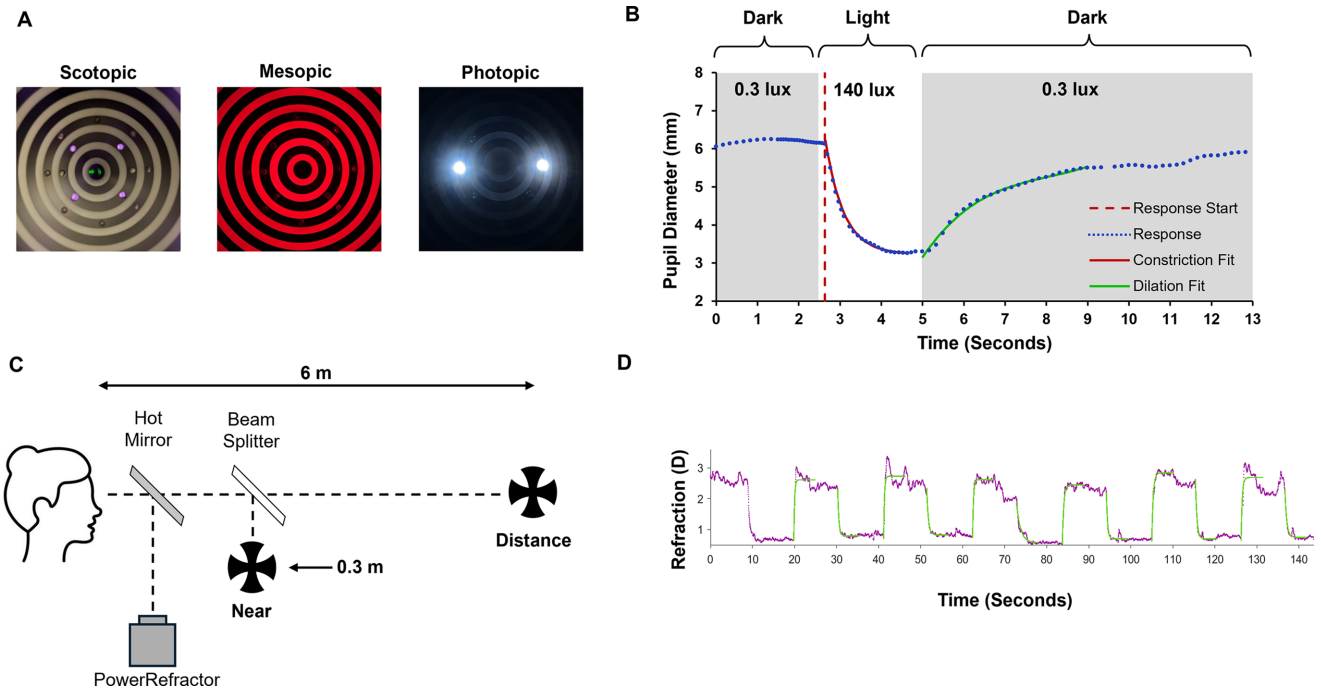

Figure 1. (**A**) Stimuli for scotopic, mesopic, and photopic pupil measurements as presented by the MYAH, (**B**) representative dynamic pupil response to light increment with exponential and polynomial fits for constriction and dilation, (**C**) set up for recording dynamics of accommodation using the PowerRefractor, (**D**) representative dynamic accommodation response (*purple trace*) with exponential fits (*green lines*) for accommodation and disaccommodation.

Pupillometry

Static pupillometry, as well as light-induced dynamic pupillometry, were performed for the right eyes using the MYAH. Additionally, accommodation-induced pupillometry was measured with a PowerRefractor (see below). Pupillometry was performed with the room lights off (0.3 lux) and both eyes open. Participants remained in the darkened room off for approximately 1 to 2 minutes while the test was explained, prior to pupil measurements being taken.

For static measures, pupil diameter was measured over a 7-second period under scotopic illumination (no illumination emitted from the instrument), mesopic illumination (red rings illuminated), and photopic illumination (two LEDS illuminated; Fig. 1A). Using a digital lux meter at the eye level, scotopic, mesopic, and photopic illumination was measured to be approximately 0.3 lux, 2 lux, and 140 lux, respectively (LX1330B; Dr. Meter, Union City, CA, USA). The instrument's software demarcated the pupil margin and provided the average diameter over 7 seconds.

Light-induced dynamic pupil responses were recorded with the MYAH for a 13-second period, with frames captured every 67 to 200 ms (Fig. 1B). This variable frame rate is in-built into the MYAH software using a proprietary algorithm. In dynamic mode, the internal illumination alternates from baseline (approximately 0.3 lux for 2.43 seconds) to photopic (140 lux for 2.57 seconds) and back to baseline (0.3 lux for 8 seconds). The instrument's software demarcated the pupil margin for each frame over the 13-second period. Given the frame period of 67 to 200 ms, the total number of frames for the 13-second period ranged between 90 and 100. To ensure the pupil margin was delineated correctly by the software, the videos for each participant were inspected using i-Map Pro (VISIA, San Giovanni Valdarno, Italy; a subsidiary of Topcon Corporation, Japan). The i-Map pro is an external visualization software that allows remote access to raw pupil data for inspection, export, and analysis. The frames for which the pupil margin was not demarcated correctly or had blink artifacts were excluded from further analysis. The data were then exported and analyzed using a custom MATLAB program (MathWorks, Natick, MA, USA).

To identify the start of light-induced pupil constriction after each stimulus onset, the custom software algorithm searched for a set of three continuously decreasing pupil diameter values (with at least –2 mm/s rate of change) followed by four consecutive values with no more than one sample that was greater than the previous sample.[21] The tolerance for one increasing sample point allowed for noise fluctuations. Baseline pupil diameter was calculated as the average of 2 seconds of pupil diameter prior to stimulus onset. In case the –2 mm/s criterion fails, responses were identified if a sample was below baseline pupil diameter minus 4 standard deviations, and the mean of the next 6 samples stayed below that threshold.

The first of the three continuously decreasing pupil diameter values was considered as the start of light-induced pupil constriction, excluding the data recorded prior to the onset. With the start of the light-induced constriction, pupil response was fitted with exponential functions to a 2-second window, represented by Equation 1.

$$y = y_0 - a\left(1 - e^{-\frac{t}{\tau}}\right) \quad (1)$$

where, y is the pupil diameter, y_0 is the pupil diameter at the start of the constriction response, a is the amplitude of the fit, t is the data point in time, and τ is the time constant.

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The exponential fits provided three dynamic pupil constriction parameters: time constant (i.e. time required for the onset of response), constriction amplitude, and peak constriction velocity. Constriction amplitude (mm) was calculated as the difference between the average of 2 seconds of pupil diameter prior to stimulus onset and the smallest pupil diameter of the exponential fit while the stimulus was on. Peak constriction velocity (mm/s) was identified as the peak value of the first derivative of the exponential functions. Pupil redilation diameters over 2 seconds after stimulus turns off were fitted with fourth order polynomial function, and peak dilation velocity (mm/s) was identified as the peak value of the derivative of the polynomial functions.

Accommodation Measurement

Static accommodation was measured with an open field autorefractor (WAM-5500). Participants wore their habitual distance spectacle correction (where applicable), and the left eye was occluded. Room lights were left on so the targets could be viewed. Right eye refraction was first measured for a distance target at 6 m. Refraction was then measured for stimulus demands of 1 to 9 D in 1 D steps as the participant fixated on a high contrast letter target presented at various distances (1 to 0.11 m). For a 1 D demand, the target was mounted on an external stand at 1 m. For 2 to 6 D demands, the target was mounted on the near-point rod attached to the instrument. For 7 to 9 D demands, targets were mounted in the instrument window using transparent thread. The distance target subtended 0.6 degrees, and the near target at 1 m subtended 1.7 degrees; as the target was moved closer, it increased approximately 1.5 times with each diopter increase in accommodative demand up to 6 D. For 7, 8, and 9 D demands, smaller targets that subtended 2 degrees, 2.3 degrees, and 2.6 degrees, respectively, were used. Measurements were taken on axis by ensuring that the targets were placed within the infrared ring that appears in the instrument's window during measurements. Five measurements were captured for each stimulus, and average spherical power was recorded. Accommodative responses were computed by subtracting the recorded spherical power at each demand from the distance spherical power. The lag of accommodation was calculated by subtracting accommodation response from the demand for each distance. The maximum accommodative response was taken as the accommodative amplitude.

Dynamic accommodation was measured with a PowerRefractor (PowerRef 3, PlusOptiX, Germany) as participants alternately viewed distance and near targets. The PowerRefractor is a dynamic video-based infrared optometer that records both refractive power and pupil diameter at 50 hertz (Hz). The instrument includes 9 infrared LEDs (850 nm) arranged in a trapezoidal pattern, positioned approximately 4 mm above the camera aperture, to measure the eye's refractive power along the vertical meridian. The PowerRefractor was positioned on an optical bench at 1 m (Fig. 1C). A hot mirror was placed along the visual axis of the right eye so that it reflected infrared rays from the power refractor toward the eye, allowing the participant to view the targets, which were high contrast Maltese crosses printed on a white paper. The distance target was placed at 6 m and subtended 0.3 degrees, and the near target was placed at 0.33 m (using a beam splitter) and subtended 2.6 degrees. An accommodative demand of 3 D was selected because it represents a common near working distance for both children and adults.[30,31]

For each of the five sessions for each participant, prior to measuring accommodation, the PowerRefractor was calibrated for the spectacle plane using a previously described method.[22,32] Briefly, the participant placed their chin in a headrest and wore a trial frame with distance correction. An infrared pass filter was placed in front of the right eye, thereby blocking vision, while allowing the instrument to measure refraction. With the room lights off, the left eye fixated on an illuminated distance target as trial lens ranging from +3 to −3 D in 1 D steps were placed in front of the right eye. For each lens, refraction was captured for approximately 5 seconds. Refraction was plotted with lens power, and a linear regression was fitted using a custom-written program in MATLAB, the slope of which provided the calibration factor.[32]

Following calibration, dynamic accommodation responses were recorded as the distance and near targets were alternatively illuminated with ultra-white LEDs for 10 seconds each. The room lights were turned off, ensuring that only one target, either near or far, was visible at any given moment. The participants focused on the targets with their right eye with their habitual correction in a trial frame, while the left eye was occluded. The targets were aligned to the right eye, which was achieved by having the participant view the distant target directly through the beam splitter while simultaneously observing the reflected image of the near target. The participant then rotated the beam splitter until both targets appeared aligned. Participants completed a practice session that included a few near to distance alternations to familiarize themselves with the task, during which no data were collected. Then, a minimum of six accommodation and disaccommodation responses (i.e. 120 seconds) were recorded.

Each accommodation and disaccommodation response was fitted with exponential functions using custom-written programs in MATLAB.[22,33] Given the present study collected refraction data with and without atropine, which affected the ability to accommodate, multiple approaches, described below, were examined to identify the onset of accommodation and disaccommodation response. The performance of these approaches was rigorously inspected for each participant for different conditions to determine the onset of accommodative and disaccommodative responses.

First, the response onset was identified, and the data prior to that was removed. Two normal distributions were fitted to the distance and near responses, and their midpoint was set as the threshold. A sample qualified as an onset candidate if it was the first to exceed the threshold, with at least 75% of samples remaining above it for 1000 ms. Once the onset was identified, the start of the rise of the accommodation response was refined by searching backwards for the first time point, using a previously described method by Bharadwaj et al.[32] The first point where the velocity exceeded 0.5 D/s and continued to do so for the next 100 ms was considered the start of the response. If this method failed in identifying the start of the response, another approach by Kasthurirangan et al. was attempted.[22] This approach was to find three consecutive increases followed by four additional samples with at least three increases with no more than one consecutive decrease. If either of these approaches failed to qualify, then the onset was left at the threshold crossing

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point, which occurred for about 6% of the responses (i.e. 102 of 1800 accommodative responses that were recorded).

To measure disaccommodation, the average refractive response during the first 1000 milliseconds was calculated, and a drop in response was considered to occur if it fell below 90% of this average refractive response. Samples below the drop threshold and within 150% of the stimulus duration were identified as potential starting points for disaccommodation. The initial 100 ms of this interval was line-fitted, and samples with a negative slope (< –0.02 D per sample) were considered further. If at least 75% of the following 1000 ms of responses were below the threshold, the sample was retained and paired with the prior accommodation response.

Exponential functions were the fit from the onset of the accommodation (Equation 2) and disaccommodation responses to half of the stimulus durations (Equation 3), as shown in Figure 1D.[22,33] Responses with poor exponential fits, missing data, or lack of a response were excluded from the analysis.

$$Accommodation\ :\ y = y_0 + a \times \left(1 - e^{-\frac{t}{\tau}}\right) \quad (2)$$

$$Disaccommodation\ :\ y = y_0 - a \times \left(1 - e^{-\frac{t}{\tau}}\right) \quad (3)$$

where y is the accommodation/disaccommodation, y_0 is the accommodation or disaccommodation at the onset, a is the amplitude of accommodation or disaccommodation, t is the time in seconds, and τ is the time constant.

The exponential fits provided accommodation and disaccommodation amplitude in diopters and time constant in seconds. Peak accommodation and disaccommodation velocities were defined as the maximum values of the derivative of the exponential fits in diopters/second.

To assess accommodation-induced pupil responses, pupil diameter (excluding the first 400 ms after the onset of accommodation and disaccommodation and last 2 seconds of the stimulus) was derived from the pupil response captured simultaneously with refraction with the PowerRefractor. This accommodation-induced pupil constriction and disaccommodation dilation were then averaged across the response for comparisons between different time points and concentrations.

Subjective Outcomes

At the 24-hour time point for each concentration tested, participants were asked to rate (1) photophobia and (2) near task related difficulties on a 5-point Likert scale (never, rarely, sometimes, often, and always).

Data Analysis

Statistical analysis was performed using SPSS (version 27; IBM Corp, Armonk, NY, USA) and Excel (version 2404; Microsoft Corporation, Redmond, WA, USA). Descriptive statistics were calculated for all pupil and accommodation parameters and are provided as mean ± standard deviation, unless otherwise noted. Three-way repeated measures analysis of variance (ANOVA) with Bonferroni-adjusted post hoc pairwise comparisons were used to compare pupil and accommodation metrics across time points, concentrations, and refractive groups.

Results

Twenty-one young adults were enrolled. One participant withdrew due to sustained pupil dilation lasting over 1 month following the first dose of atropine; data from this participant are not included. Twenty participants included in the analysis included 10 women and 10 men with a mean age of 25.5 ± 3.4 years (range = 19 to 33 years). Among them, six participants were contact lens users, but also had habitual spectacle distance correction. Mean SER was –1.91 ± 2.24 D (range = –6.62 to +0.75 D), including 12 myopes (mean SER = -3.35 ± 1.72 D) and 8 non-myopes (mean SER = +0.25 ± 0.31 D). There were no refractive group differences in response to atropine in the metrics analyzed below ($P > 0.05$ for all). Therefore, the analyses presented below are for all participants. Across the participants, iris color varied from blue (grade 1, $N = 4$), to light brown (grade 3, $N = 6$), and dark brown (grade 4, $N = 10$).

Static Pupillometry

For one participant, the MYAH was unable to capture scotopic pupil diameter (0.025% and 0.1% at 1 hour; for 1 participant each) and mesopic pupil diameter (placebo at 1 hour; 0.01% at baseline and 1 hour; 0.025%, 0.05%, and 0.1% at 1 hour) due to their large pupil sizes. So, the data presented for these metrics are for 19 participants.

Static pupil diameters measured in scotopic, mesopic, and photopic illumination showed significant changes with concentration and time ($P < 0.001$ for all, Fig. 2). A significant interaction between concentration and time was also noted for all three conditions ($P < 0.001$ for all), indicating a dose-dependent response. Post hoc pairwise comparisons are shown in Table 1. At 1 hour after atropine instillation, there was a significant increase in pupil diameter compared to baseline in all 3 illumination levels for 0.025%, 0.05%, and 0.1% concentrations ($P < 0.001$ for all). Whereas pupil diameter tended to decrease by 24 hours, pupil diameters were still significantly higher than baseline for some concentrations, including 0.01% and 0.05% concentrations in all 3 illumination levels, as well as 0.025% concentration in photopic illumination ($P < 0.001$ for all).

Light-Induced Dynamic Pupillometry

Dynamic pupil metrics are shown in Figure 3, and post hoc comparisons are shown in Table 1. For one participant, the MYAH did not capture pupil diameter for the 0.025% concentration at 1 hour. Additionally, for another participant, the pupil did not react to the light increment for 0.1% concentration at 1 hour. Hence, those metrics include 19 participants. Significant changes with concentrations and time were observed for the light-induced constriction amplitude ($P < 0.001$ for both), peak constriction velocity ($P = 0.008$ for concentration and $P < 0.001$ for time), and peak dilation velocity ($P = 0.02$ for concentration and $P < 0.001$ for time). All the three parameters also showed dose dependent response, that is, significant interactions between concentrations and time ($P < 0.001$ for all).

At 1 hour after instillation, constriction amplitude decreased significantly compared to baseline for all concentrations and did not return to baseline at the 24-hour measurement ($P < 0.05$ for all). Similarly, the peak constriction velocity decreased at 1 hour compared to baseline. Peak constriction velocity remained decreased at 24 hours for

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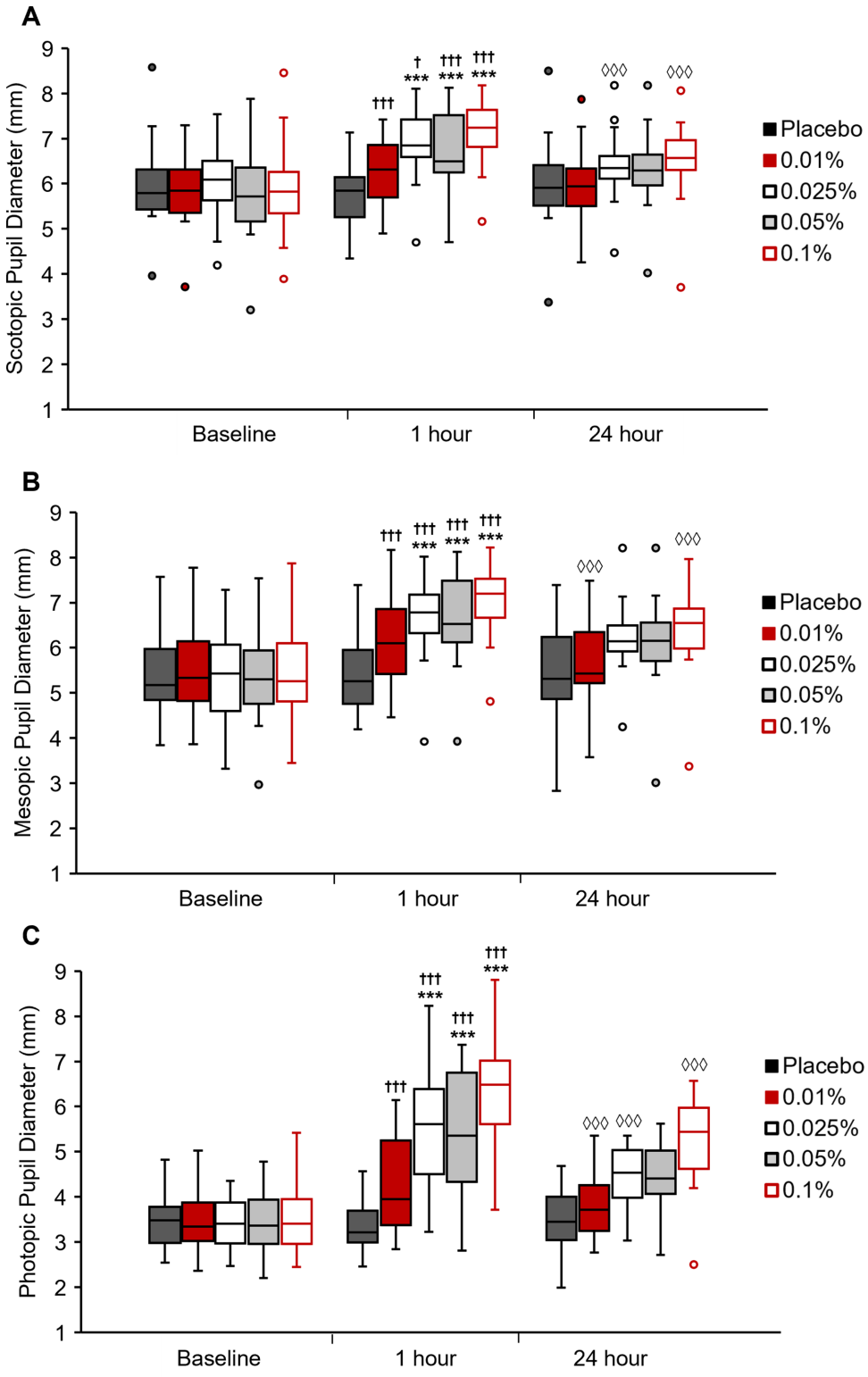

Figure 2. Static pupillometry. (**A**) Scotopic, (**B**) mesopic, and (**C**) photopic pupil diameters at baseline, 1 hour, and 24 hours after each atropine concentration. The *black line* inside the box represents median. Significant Bonferroni-adjusted post hoc pairwise comparisons among * = baseline and 1 hour; ◊ = baseline and 24 hours; and † = 1 hour and 24 hours. Level of significance indicated by ***$P < 0.001$, **$P < 0.01$, and *$P < 0.05$ are the same for ◊ and †.

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Table 1. Significance Values for Bonferroni-Adjusted Pairwise Comparisons for Pupillometry Across Concentrations for Static and Dynamic Metrics

	Time Point	Concentration	0.01%	0.025%	0.05%	0.1%
Scotopic pupil diameter, mm	1 h	Placebo	0.003	<0.001	<0.001	<0.001
		0.01%		0.001	0.001	<0.001
		0.025%			>0.99	0.12
		0.05%				0.009
	24 h	Placebo	>0.99	0.002	<0.001	<0.001
		0.01%		<0.001	0.003	<0.001
		0.025%			>0.99	0.79
		0.05%				0.05
Mesopic pupil diameter, mm	1 h	Placebo	0.003	<0.001	<0.001	<0.001
		0.01%		0.001	<0.001	<0.001
		0.025%			>0.99	0.18
		0.05%				0.009
	24 h	Placebo	0.72	<0.001	0.005	<0.001
		0.01%		<0.001	0.003	0.018
		0.025%			>0.99	0.37
		0.05%				0.005
Photopic pupil diameter, mm	1 h	Placebo	0.002	<0.001	<0.001	<0.001
		0.01%		<0.001	<0.001	<0.001
		0.025%			>0.99	0.02
		0.05%				0.009
	24 h	Placebo	0.91	<0.001	<0.001	<0.001
		0.01%		<0.001	<0.001	<0.001
		0.025%			>0.99	0.004
		0.05%				<0.001
Constriction amplitude, mm	1 h	Placebo	0.02	0.02	<0.001	0.001
		0.01%		0.21	<0.001	0.80
		0.025%			>0.99	0.79
		0.05%				0.005
	24 h	Placebo	0.02	<0.001	>0.99	>0.99
		0.01%		<0.001	<0.001	<0.001
		0.025%			>0.99	0.004
		0.05%				<0.001
Peak constriction velocity, mm/s	1 h	Placebo	0.11	<0.001	<0.001	<0.001
		0.01%		0.04	0.08	0.002
		0.025%			>0.99	0.23
		0.05%				0.64
	24 h	Placebo	>0.99	>0.99	>0.99	0.62
		0.01%		>0.99	>0.99	>0.99
		0.025%			>0.99	0.02
		0.05%				0.39
Peak dilation velocity, mm/s	1 h	Placebo	>0.99	0.99	0.90	<0.001
		0.01%		0.24	0.07	<0.001
		0.025%			0.99	0.05
		0.05%				0.02
	24 h	Placebo	>0.99	>0.99	>0.99	0.38
		0.01%		0.60	0.62	0.03
		0.025%			>0.99	0.57
		0.05%				> 0.99

0.05% and 0.1% concentrations. Similar results were noted for the peak dilation velocity.

Static Accommodation

Maximum amplitude of accommodation exhibited significant time and concentration effects and interactions ($P <$ 0.001 for all; Fig. 4). At baseline, objectively measured amplitude of accommodation was 6.64 ± 1.45 D. At 1 hour after atropine instillation, there was a significant decrease in amplitude for 0.025% (by 0.83 ± 1.10 D), 0.05% (by 1.05 ± 1.32 D), and 0.1% concentrations (by 1.46 ± 1.54 D, $P < 0.01$ for all) compared to baseline. At 24 hours, amplitude was still significantly reduced compared to baseline ($P < 0.01$ for all).

Lag of accommodation at each time point across various concentrations is shown in Figure 5. At 1 hour and 24 hours after atropine instillation, lag increased significantly (main effect $P < 0.001$), with the increase being greatest for higher concentrations and higher stimulus demands. Post hoc comparisons showed that lag of accommodation significantly increased for stimulus demand of 4 D and higher ($P < 0.05$ for all). Significant effects between time points were observed for stimulus demand of 6 D and higher

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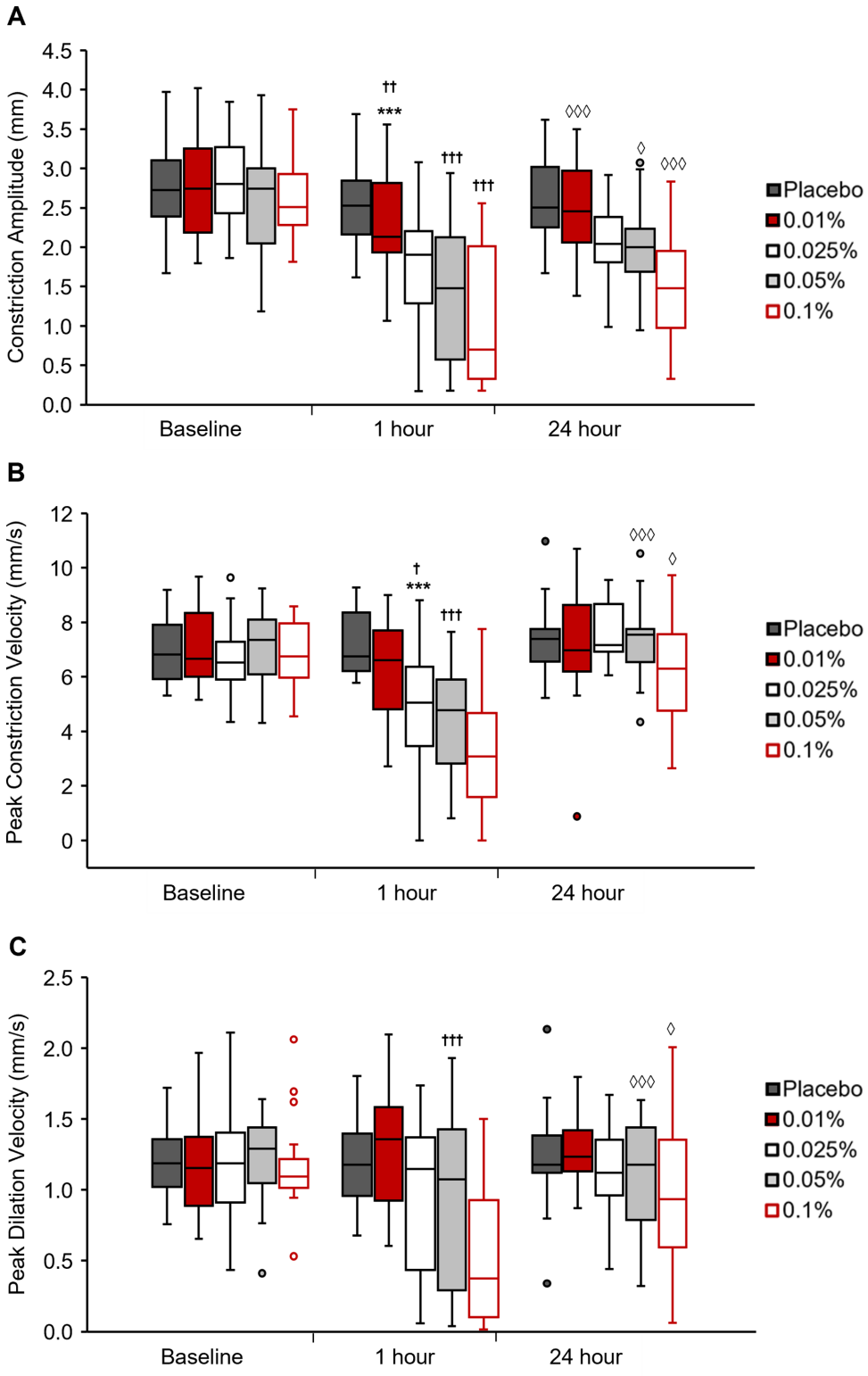

Figure 3. Light-induced dynamic pupillometry. (**A**) Constriction amplitude, (**B**) peak constriction velocity, and (**C**) peak dilation velocity at baseline, 1 hour, and 24 hours after each atropine concentration. The *black line* inside the box represents median. Significant Bonferroni-adjusted post hoc pairwise comparisons among * = baseline and 1 hour; ◊ = baseline and 24 hours; and † = 1 hour and 24 hours. Level of significance indicated by ***$P < 0.001$, **$P < 0.01$, and *$P < 0.05$ are the same for ◊ and †.

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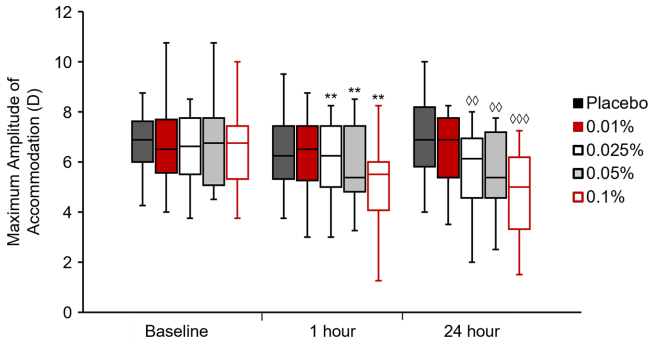

Figure 4. Maximum amplitude of accommodation at baseline, 1 hour, and 24 hours after each atropine concentration. The *black line* inside the box represents median. Significant Bonferroni-adjusted post hoc pairwise comparisons between * = baseline and 1 hour; and ◊ = baseline and 24 hours. Level of significance indicated by ***$P < 0.001$, **$P < 0.01$, and *$P < 0.05$ are the same for ◊ and †.

($P < 0.01$ for all) and between time points and concentrations ($P < 0.01$ for all).

Dynamic Accommodation

Dynamic accommodation and disaccommodation metrics to a 3 D stimulus demand across time and concentrations are shown Table 2. Accommodative time constant and peak velocity demonstrated a significant time effect ($P = 0.03$ and $P = 0.046$), indicating a significantly slower time constant and peak velocity over 24 hours after atropine instillation. However, post hoc comparisons did not show significant pairwise differences (Table 3). Additionally, dynamic accommodative and disaccommodative amplitude, disaccommodative time constant, and disaccommodative peak velocity did not exhibit any significant time points, concentrations, and interactions between them ($P > 0.05$).

Accommodation-induced pupil diameter (fixating at 3 D stimulus) and disaccommodation dilation (fixating at distance), as measured with the PowerRefractor, showed significant changes with concentration and time and interactions between them (see Table 3; $P < 0.001$ for all), indicating less accommodative pupil constriction over 24 hours with increasing concentrations.

Subjective Outcomes

At the 24-hour time point for each atropine concentration, including placebo, participants were asked to rate (1) photophobia and (2) difficulties with near work. Interestingly, despite being blinded to the concentration that was instilled, 100% of participants reported no photophobia or difficulties for near work following placebo administration, whereas all atropine concentrations, including 0.01%, resulted in some side effects. The proportion of participants experiencing photophobia and difficulties during near tasks demonstrated an increase with higher concentrations (Fig. 6).

Discussion

This study investigated 1 hour and 24 hour effects of a single drop of low dose atropine concentrations, those typically used in myopia control for children, on static and dynamic pupil and accommodation metrics using a double blinded, randomized, repeated-measures study design. Results show that a single drop of 0.01%, 0.025%, 0.05%, and 0.1% atropine induce changes in static and dynamic pupil and accommodation metrics in a dose-dependent manner. Most metrics did not return to the baseline at 24 hours, particularly for the higher concentrations.

Topical atropine is one of the most effective modalities for slowing myopia progression.[4] Originally, 1% atropine was found to effectively slow myopia progression and axial elongation compared with eyes that did not receive treatment.[12] However, prolonged side effects, including mydriasis and cycloplegia, limited the clinical use of 1% atropine, spurring interest in low to moderate concentrations of atropine (0.01% to 0.5%). These lower doses were shown to slow myopia progression in a dose dependent manner with comparatively lesser side effects.[18] A study from 2013 suggested that 0.02% atropine was the highest concentration that does not cause significant clinical effects related to accommodation paresis or pupillary dilation.[34] However, atropine as low as 0.01% can result in clinical side effects.[35]

Similar to the current study, previous studies have also investigated accommodation and pupil metrics after a single dose of atropine among adults and children.[20,35–37] However, these studies either investigated only one atropine concentration, randomly assigned different concentrations to different participants, or did not include a dynamic assessment of the responses. In contrast, the present study rigorously investigated the effects of 4 commonly used atropine concentrations over 24 hours in the same participants and included several measures of both static and dynamic physiological changes that may impact on the quality of vision, especially at near vision. Given that the present study was conducted in young adults, caution is advised in translating the study findings in children.

Here, pupil diameter under scotopic, mesopic, and photopic illumination was found to significantly increase in a dose-dependent manner at 1 hour and 24 hourrs after 0.025%, 0.05%, and 0.1% atropine compared to baseline. Findings also showed that pupil diameter was increased 24 hours (but not 1 hour) after 0.01% atropine. A previous study in children administered a single drop of placebo or 0.01%, 0.03%, or 0.05% atropine and reported a significant increase in pupil diameter 1 hour after 0.01% atropine.[36] Similar results for pupil diameter were reported after a single drop of 0.01% atropine in adult eyes,[35] where photopic pupil diameter was significantly increased at 10 hours, 14 hours, and 18 hours. Another study conducted in adults also found significant increase in both scotopic and photopic pupil diameter 12 hours after 0.01% atropine that did not fully return to baseline at 24 hours.[20] Collectively, these studies indicate that even the lowest dose of atropine used in myopia control, 0.01%, has significant mydriatic effects on the pupil measured under different illumination levels that can persist over 24 hours.

Measuring dynamic pupil behavior can provide insight into pharmacological and biomechanical responses of the iris to atropine.[38] Pupil constriction amplitude in response to a light increment demonstrated a dose dependent decrease with increasing atropine concentration, meaning, that with atropine, light-induced pupil constriction was attenuated. These effects lasted for at least 24 hours for all concentrations tested here. Additionally, the pupil did not change as rapidly following atropine. Both peak constriction and dilation velocities showed a dose dependent decrease with increasing concentrations at 1 hour; however, these changes

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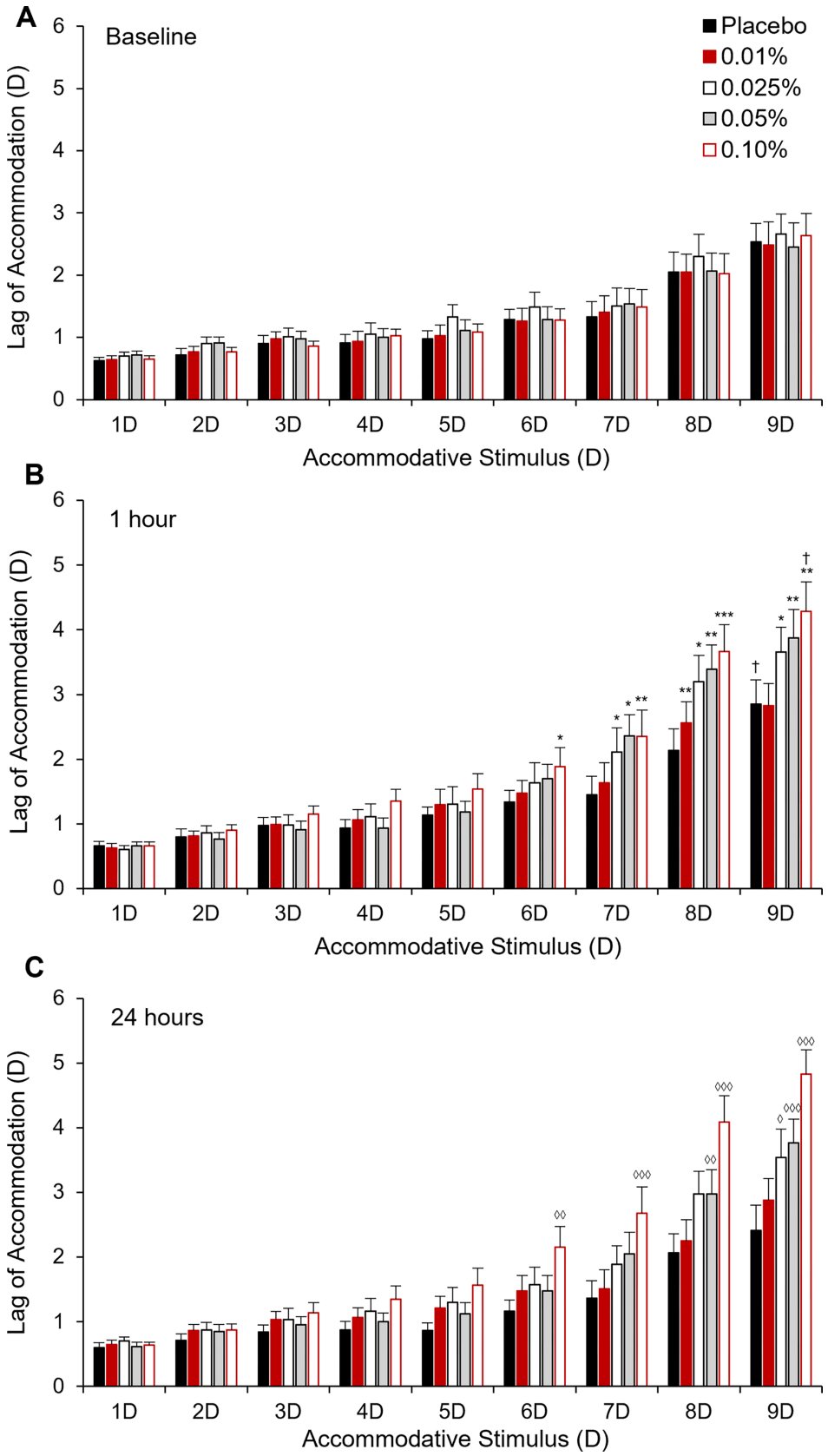

Figure 5. Lag of accommodation (mean ± standard error) of the mean at (**A**) baseline and (**B**) 1 hour and (**C**) 24 hours after each atropine concentration. Significant Bonferroni-adjusted post hoc pairwise comparisons among * = baseline and 1 hour; ◊ = baseline and 24 hours; and † = 1 hour and 24 hours. Level of significance indicated by ***$P < 0.001$, **$P < 0.01$, and *$P < 0.05$ are the same for ◊ and †.

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Table 2. Dynamic Accommodation and Disaccommodation Metrics (Mean ± Standard Deviation) at Baseline, 1 Hour, and 24 Hours After Placebo and Each of Four Atropine Concentrations

		Accommodation				Disaccommodation			
Concentration	Time	Amplitude, D	Time Constant, ms	Peak Velocity, D/s	Pupil Diameter, mm	Amplitude, D	Time Constant, ms	Peak Velocity, D/s	Pupil Diameter, mm
Placebo	Baseline	1.96 ± 0.91	0.39 ± 0.21	7.01 ± 5.98	5.48 ± 0.93	1.75 ± 0.78	0.51 ± 0.47	5.27 ± 3.40	5.83 ± 0.98
	1 h	1.96 ± 0.55	0.42 ± 0.43	7.57 ± 4.29	5.75 ± 1.06	1.78 ± 0.70	0.34 ± 0.14	5.72 ± 2.45	6.03 ± 1.00
	24 h	2.04 ± 0.83	0.41 ± 0.37	7.97 ± 5.87	5.78 ± 1.07	2.04 ± 1.19	0.39 ± 0.16	5.75 ± 3.33	6.08 ± 1.08
0.01%	Baseline	2.20 ± 0.88	0.38 ± 0.24	7.34 ± 4.41	5.56 ± 1.04	2.00 ± 1.00	0.39 ± 0.18	6.17 ± 4.35	5.90 ± 1.02
	1 h	2.15 ± 0.65	0.43 ± 0.29	6.67 ± 3.59	6.38 ± 1.02	2.02 ± 1.16	0.36 ± 0.09	5.86 ± 3.63	6.52 ± 1.04
	24 h	2.23 ± 0.83	0.43 ± 0.36	7.09 ± 3.93	5.99 ± 0.83	1.91 ± 0.86	0.37 ± 0.16	5.75 ± 3.16	6.24 ± 0.88
0.025%	Baseline	1.92 ± 0.68	0.41 ± 0.30	6.53 ± 3.90	5.58 ± 1.15	1.74 ± 1.00	0.35 ± 0.14	6.25 ± 5.32	6.00 ± 1.08
	1 h	1.84 ± 0.80	0.39 ± 0.21	5.72 ± 3.11	6.90 ± 0.83	1.79 ± 1.32	0.36 ± 0.13	5.41 ± 4.35	6.98 ± 0.90
	24 h	1.96 ± 0.63	0.47 ± 0.39	5.92 ± 3.24	6.29 ± 0.89	1.75 ± 0.84	0.40 ± 0.20	5.16 ± 2.57	6.45 ± 0.95
0.05%	Baseline	2.16 ± 0.76	0.38 ± 0.21	7.43 ± 4.14	5.51 ± 1.05	2.01 ± 0.94	0.39 ± 0.31	6.73 ± 4.04	5.91 ± 1.02
	1	1.97 ± 0.63	0.40 ± 0.26	6.51 ± 4.09	6.90 ± 0.74	1.81 ± 0.70	0.39 ± 0.14	5.06 ± 2.54	6.98 ± 0.79
	24 h	2.00 ± 0.72	0.47 ± 0.24	5.29 ± 2.88	6.24 ± 0.80	1.81 ± 0.77	0.36 ± 0.09	5.07 ± 1.95	6.40 ± 0.87
0.1%	Baseline	2.21 ± 0.73	0.38 ± 0.26	7.89 ± 4.70	5.62 ± 1.10	1.96 ± 0.77	0.55 ± 0.63	5.98 ± 4.72	5.99 ± 1.09
	1 h	2.09 ± 0.99	0.43 ± 0.32	6.26 ± 3.48	7.13 ± 0.76	1.79 ± 0.89	0.43 ± 0.29	5.14 ± 3.10	7.18 ± 0.80
	24 h	1.85 ± 0.71	0.48 ± 0.42	5.97 ± 4.12	6.42 ± 0.80	1.76 ± 0.76	0.37 ± 0.16	5.58 ± 3.38	6.52 ± 0.87

were no longer significant at 24 hours for 0.01% and 0.025%. These results demonstrate that a single drop of atropine influences pupil dynamics, including peak pupil constriction and dilation velocities, although the effects may not last a full 24 hours.

Several studies have investigated the effects of atropine on accommodation. Some previous studies reported that a single drop of 0.01%, 0.03%, or 0.05% atropine does not affect amplitude of accommodation over 24 hours in children or adults.[20,36] However, another study in children reported a significant reduction in accommodative amplitude after 3 days treatment of 0.01%, 0.02%, and 0.03%.[39] Here, a significant dose-dependent reduction in objectively measured amplitude of accommodation was observed at 1 hour and 24 hours after 0.025%, 0.05%, and 0.1% atropine administration. No decrease in amplitude of accommodation was observed following 0.01% atropine. Conflicting findings across studies may be attributable to differences in methodology, including subjective measurements of accommodation, different age groups, different iris colors, and different time periods. In line with findings presented here, another study showed that in young adults, 0.01% atropine did not significantly affect accommodative amplitude after 5 days of daily use.[40]

The present study also assessed accommodative lag for increasing stimulus demands before and after atropine. Accommodative lag increased with increasing atropine concentrations and stimulus demands, likely due to effects of atropine on the ciliary muscle. Importantly, concentrations of 0.05% and 0.1% resulted in a significantly increased lag for stimulus demands as low as 4 D, which may cause difficulties with day to day near tasks. However, the extent of the effect on the accommodative lag was greatest for stimulus demand of 6 D and higher. This effect was evident at both 1 hour and 24 hours for concentrations of 0.025%, 0.05%, and 0.1% atropine. Few studies have investigated accommodative lag following atropine for a range of stimulus demands, although some have measured lag for one near demand. Breliant et al.[36] assessed lag for a 3 D stimulus demand over 24 hours following 0.01%, 0.03%, or 0.05% atropine instillation in children ages 6 to 17 years and found no changes from baseline.

Clinical measurements of accommodation traditionally rely on static assessment, capturing a single response as the eye focuses on a near target. However, accommodation is a dynamic process. Dynamic measurements, as used in the current study, allows continuous, high-temporal-resolution assessment as the eye shifts focus between targets at different distances. In addition, accommodation is not stable, but fluctuates over ± 0.50 D.[41] Therefore, dynamic assessment provides a more complete understanding of the accommodative process. Previous studies have reported various dynamic accommodation metrics after instillation of phenylephrine and timolol,[42,43] but not following atropine. Here, accommodation was assessed dynamically using a PowerRefractor as fixation alternated between distance and 0.33 m. Findings show a slower accommodative time constant and peak velocity over 24 hours after atropine administration. Additionally, accommodation-induced pupil constriction decreased following atropine. No disaccommodative parameters were affected by atropine. These results were not entirely unexpected, given that disaccommodative responses are directed towards the resting position of the eye.

The proportion of participants reporting photophobia and difficulties during near tasks increased with atropine concentration, concurring with the objectively measured pupil and accommodation findings. Nearly half of the participants reported some level of photophobia and near task difficulties following one drop of 0.01% atropine, demonstrating that even the lowest atropine concentrations can result in subjective symptoms. Similarly, a previous study among adults reported glare and focusing difficulties after 0.01% atropine over 14 hours.[35] Studies evaluating the tolerability and efficacy of low dose atropine in slowing myopia progression have also reported dose dependent association with photophobia and near vision difficulties.[11,44]

Side effects of atropine, as well as effectiveness for myopia control, may be influenced by iris pigmentation.[45,46] Studies show that atropine binds to melanin, and pigmented irises accumulate more atropine than non-pigmented irises, as demonstrated in pigmented and albino rabbits.[47] Due to the melanin binding and potentially slower release into intraocular tissues, individuals with darker irises may experience a longer duration of atropine's effects, such as pupil dilation. As such, the range of iris colors, and thus, pigmentation, of the participants in the current study may explain the variability in the pupil and accommodation responses. While an attempt was made to enroll participants with a

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Table 3. Significance Values for Repeated Measures ANOVA Comparison Across Concentration and Over Time Along Bonferroni-Adjusted Pairwise Comparison Between Placebo and Four Atropine Concentrations for Static and Dynamic Accommodation Metrics

	Time Points	Concentration	0.01%	0.025%	0.05%	0.1%
Maximum amplitude of accommodation, D	1 h	Placebo	>0.99	0.45	0.31	0.01
		0.01%		>0.99	0.049	0.07
		0.025%			>0.99	0.33
		0.05%				>0.99
	24 h	Placebo	0.64	0.07	0.08	<0.001
		0.01%		>0.99	>0.99	<0.001
		0.025%			>0.99	0.02
		0.05%				0.13
Dynamic accommodative amplitude, D	1 h	Placebo	>0.99	>0.99	>0.99	>0.99
		0.01%		0.72	>0.99	>0.99
		0.025%			>0.99	>0.99
		0.05%				>0.99
	24 h	Placebo	>0.99	>0.99	>0.99	>0.99
		0.01%		>0.99	>0.99	0.69
		0.025%			>0.99	>0.99
		0.05%				>0.99
Dynamic accommodative time constant, s	1 h	Placebo	>0.99	>0.99	>0.99	>0.99
		0.01%		>0.99	>0.99	>0.99
		0.025%			>0.99	>0.99
		0.05%				>0.99
	24 h	Placebo	>0.99	>0.99	>0.99	>0.99
		0.01%		>0.99	>0.99	>0.99
		0.025%			>0.99	>0.99
		0.05%				>0.99
Dynamic accommodative peak velocity, D/s	1 h	Placebo	>0.99	0.28	>0.99	>0.99
		0.01%		0.72	>0.99	>0.99
		0.025%			>0.99	>0.99
		0.05%				>0.99
	24 h	Placebo	>0.99	0.73	0.25	>0.99
		0.01%		>0.99	0.66	>0.99
		0.025%			>0.99	>0.99
		0.05%				>0.99
Dynamic disaccommodative amplitude, D	1 h	Placebo	>0.99	>0.99	>0.99	>0.99
		0.01%		>0.99	>0.99	>0.99
		0.025%			>0.99	>0.99
		0.05%				>0.99
	24 h	Placebo	>0.99	0.88	>0.99	>0.99
		0.01%		>0.99	>0.99	>0.99
		0.025%			>0.99	>0.99
		0.05%				>0.99
Dynamic disaccommodative time constant, s	1 h	Placebo	>0.99	>0.99	>0.99	>0.99
		0.01%		>0.99	>0.99	>0.99
		0.025%			>0.99	>0.99
		0.05%				>0.99
	24 h	Placebo	>0.99	>0.99	>0.99	>0.99
		0.01%		>0.99	>0.99	>0.99
		0.025%			>0.99	>0.99
		0.05%				>0.99
Dynamic disaccommodative peak velocity, D/s	1 h	Placebo	>0.99	>0.99	>0.99	>0.99
		0.01%		>0.99	>0.99	>0.99
		0.025%			>0.99	>0.99
		0.05%				>0.99
	24 h	Placebo	>0.99	>0.99	>0.99	>0.99
		0.01%		>0.99	>0.99	>0.99
		0.025%			>0.99	>0.99
		0.05%				>0.99
Accommodation-induced pupil diameter, mm	1 h	Placebo	<0.001	<0.001	<0.001	<0.001
		0.01%		0.01	0.009	<0.001
		0.025%			>0.99	0.21
		0.05%				0.06
	24 h	Placebo	0.61	0.005	0.005	0.002
		0.01%		0.02	0.15	<0.001
		0.025%			>0.99	>0.99
		0.05%				0.52
Disaccommodated pupil diameter, mm	1 h	Placebo	<0.001	<0.001	<0.001	<0.001
		0.01%		0.011	0.008	<0.001
		0.025%			>0.99	0.24
		0.05%				0.60
	24 h	Placebo	0.48	0.01	0.02	<0.001
		0.01%		0.05	0.24	<0.001
		0.025%			>0.99	>0.99
		0.05%				0.68

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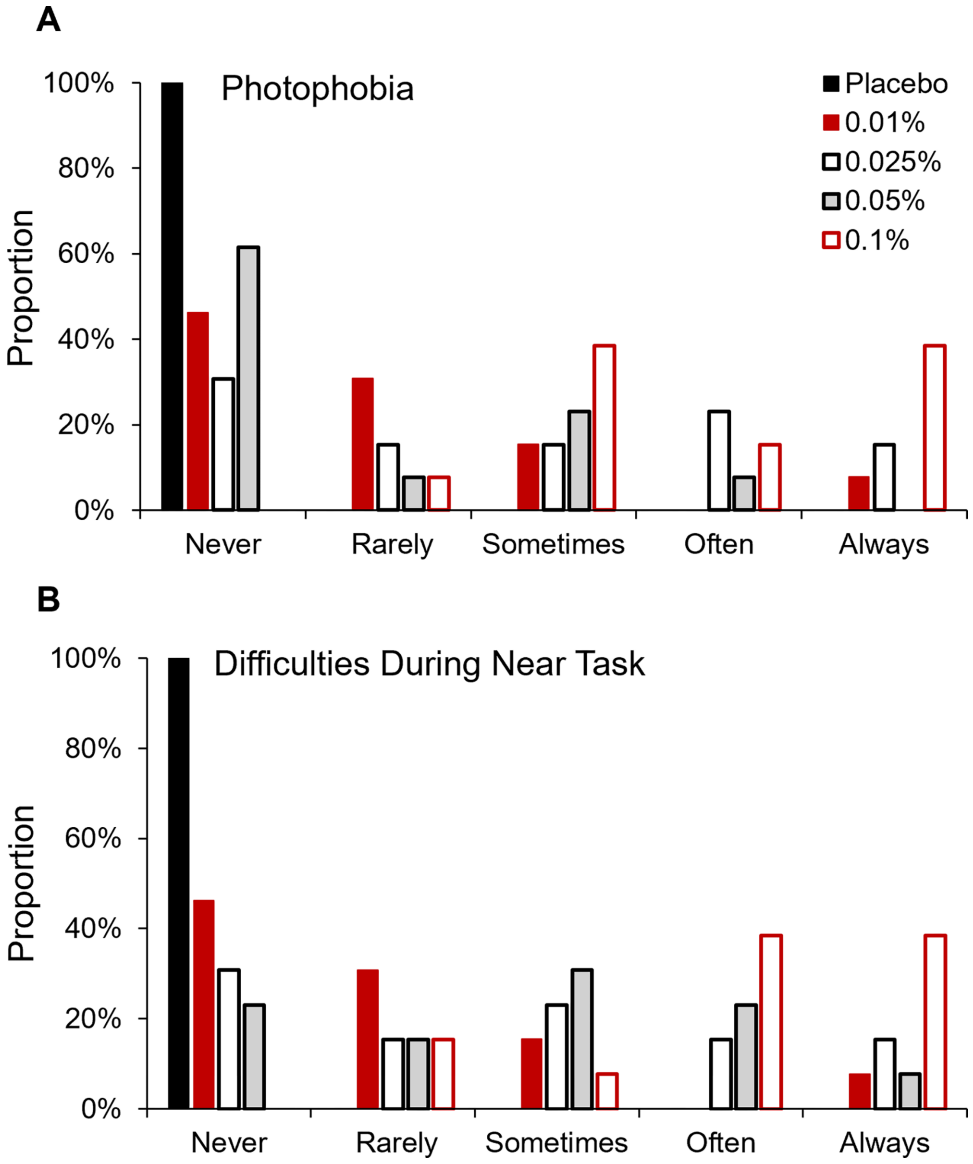

Figure 6. Proportion of participants experiencing (**A**) photophobia and (**B**) difficulties during near task for each concentration.

range of iris colors, the sample size was not large enough to make between group comparisons. Interestingly, one participant with a dark brown iris withdrew following the first dose of atropine due to a significant pupil dilation that took a month to recover. Following unmasking, it was learned that this participant's dose was 0.1% atropine. Atropine binding to iris pigment may have led to a slow release, causing the recovery of the pupil to take longer for this participant. However, this did not occur with any other participants with darker irises, as pupil diameter across experimental sessions had always returned to baseline within the 1 week-minimum between sessions. For another participant with a dark brown iris (grade 4), the pupil did not respond at all to the dynamic light increment for the 0.1% at 1 hour. These events highlight the variable pharmacokinetics across individuals and should be considered in clinical practice. Regarding efficacy of atropine for myopia control, as related to iris color, some studies suggest that iris color may affect treatment outcomes, but the findings are not conclusive.[45,48]

Findings from the present study may also be applicable in amblyopia, where atropine (typically 1%)[49] is used as an alternative to occlusion therapy for amblyopia.[50] Treatment with atropine in the non-amblyopic eye leads to optical defocus by paralyzing the ciliary muscle. It may be of relevance in future studies to consider the effects of atropine on pupil and accommodation dynamics during atropine penalization therapy.

The current study presents the following limitations. Only young adults were included, and only a single atropine instillation was investigated. Differences in accommodation and pupil behavior exist between children and adults: children show a greater accommodative amplitude and lag, larger accommodative fluctuations, and faster accommodative peak velocity and pupil constriction and dilation velocity in compared to young adults.[25,26,51,52] Studies have also noted differences in accommodative-driven pupil responses, with children having a weaker response than adults.[53] Future studies should include children with short-

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term and long-term assessment for better insight into the day-to-day atropine tolerance. Additionally, because atropine for myopia is administered as a nightly eye drop,[5] future studies should consider assessing clinically relevant time points, such as 8, 12, and 18 hours, to better reflect its effects in practice. Another limitation was the use of only one target demand for measuring accommodation dynamics. Investigating additional accommodative demands could unveil more on the extent of atropine effects on accommodative capabilities.

In conclusion, a single drop of low dose atropine ranging between 0.01% and 0.1% produced dose-dependent changes in some static and dynamic pupil and accommodation metrics that can persist up to 24 hours in young adults. These findings suggest that even for the lowest concentrations of atropine used in myopia control, individuals may experience significant pupillary and accommodation side effects.

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