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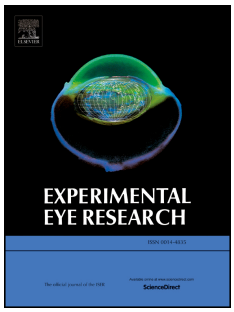

Influence of the time of day on axial length and choroidal thickness changes to hyperopic and myopic defocus in human eyes

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Abstract

Research in animal models have shown that exposing the eye to positive or negative spectacle lenses can lead to predictable changes in eye growth. Recent research indicates that brief periods (1 – 2 hours) of monocular defocus results in small, but significant changes in axial length and choroidal thickness of human subjects. However, the effects of the time of day on these ocular changes with defocus are not known. In this study, we examined the effects of monocular myopic and hyperopic defocus on axial length and choroidal thickness when applied in the morning (change between 10 am and 12 pm) vs the evening (change between 5 and 7 pm) in young adult human participants (mean age, 23.44 ± 4.52 years). A series of axial length (using an IOL Master) and choroidal thickness (using an optical coherence tomographer) measurements were obtained over three consecutive days in both eyes. Day 1 (no defocus) examined the baseline ocular measurements in the morning (10 am and 12 pm) and in the evening (5 and 7 pm), day 2 investigated the effects of hyperopic and myopic defocus on ocular parameters in the morning (subjects wore a spectacle lens with +3 or -3 DS over the right eye and a plano lens over the left eye between 10 am and 12 pm), and day 3 examined the effects of defocus in the evening (+3 or -3 DS spectacle lens over the right eye between 5 and 7 pm). Exposure to myopic defocus caused a significant reduction in axial length and thickening of the subfoveal choroid at both times; but, compared to baseline data from day 1, the relative change in axial length (-0.021 ± 0.009 vs +0.004 ± 0.003 mm, p=0.009) and choroidal thickness (+0.027 ± 0.006 vs +0.007 ± 0.006 mm, p=0.011) with defocus were significantly greater for evening exposure to defocus than for the morning session. On the contrary, introduction of hyperopic defocus resulted in a significant increase in axial length when given in the morning (+0.026 ± 0.006 mm), but not in the evening (+0.001 ± 0.003 mm) (p=0.047). Furthermore, hyperopic defocus resulted in a significant thinning of the choroid (p=0.005), but there was no significant influence of the time of day on choroidal changes associated with hyperopic defocus (p=0.672). Exposure to hyperopic and myopic defocus at different times of the day was also associated with changes in the parafoveal regions of the choroid (measured across 1.5 mm nasal and temporal choroidal regions on either side of the fovea). Our results show that ocular response to optical defocus varies significantly depending on the time of day in human subjects. These findings represent a potential interaction between the signal associated with the eye's natural diurnal rhythm and the visual signal associated with the optical defocus, making the eye perhaps more responsive to hyperopic defocus (or 'go' signal) in the morning, and to myopic defocus (or 'stop' signal) in the latter half of the day.

1. Introduction

During the postnatal period, an active regulatory process harmonises the expansion of axial length with the corneal and crystalline lens powers so that distant images focus at the retinal plane (Smith, 1998; Wallman and Winawer, 2004). This process is known as emmetropization. Any disruption to this coordinated mechanism of ocular growth results in the development of refractive errors; eyes being either too short (hyperopia) or too long (myopia) with respect to the optical power of the eye (Wallman and Winawer, 2004).

Myopia is the most common refractive disorder (Morgan et al., 2012; Saw et al., 1996), and represents the highest incidence of all refractive errors globally (Morgan et al., 2012). In some regions of Asia, its prevalence reaches 70-80% (Au Eong et al., 1993; Edwards and Lam, 2004; Lam et al., 2004; Lin et al., 2001; Morgan et al., 2012) to as high as 96% (Jung et al., 2012) of young adults. Myopia represents a "complex" disorder with both environmental (such as near work and outdoor light exposure) and genetic origins (Farbrother et al., 2004; Jones et al., 2007; Morgan et al., 2017; Rose et al., 2008; ZADNIK, 1997). However, despite extensive research, the exact mechanism underlying the development and progression of myopia remains elusive.

A large body of work on different animal models have shown that the visual environment (or image quality) regulates the refractive development of the eye (Wallman, 1993; Wallman and Winawer, 2004). Previous studies have frequently used two experimental approaches: 1) form-deprivation myopia, where image degradation through a diffuser induces myopia (Howlett and McFadden, 2006; Siegwart Jr and Norton, 1998; Smith et al., 2002; Wallman et al., 1978; Wallman et al., 1995); and 2) wearing spectacle lenses to shift the image plane in front or behind the retina, inducing compensatory changes in eye growth to reposition the retina at the defocused image plane (Howlett and McFadden, 2009; Hung et al., 1995; Irving et al., 1991; Schaeffel et al., 1988; Smith and Hung, 1999; Wallman et al., 1995). Myopic defocus with plus lenses leads to a thickening of the choroid (pushing the retina forward) and a reduction in the overall growth of the eye, thus, causing a hyperopic refractive error. Conversely, hyperopic defocus with minus lenses leads to a thinning of the choroid (moving the retina backward) and an increase in ocular growth, resulting in myopia.

In chicks, the ocular response to lens defocus depends on the frequency of exposure and the duration of each exposure, and not just the "total duration" per day (Winawer and Wallman, 2002; Zhu, 2013; Zhu et al., 2005; Zhu and Wallman, 2009). In addition, recent chick studies have shown the influence of the time of day on the eye's response to lens defocus (Nickla et al., 2017a; Nickla et al., 2017b). A brief 2-hour exposure to hyperopic defocus in the morning results in a small but significant increase in eye growth of chicks, compared to mid-day exposure to defocus (Nickla et al., 2017a). Conversely, the same duration of myopic defocus stimulates less inhibitory effect on ocular growth when imposed in the morning, compared to the evening or mid-day (Nickla et al., 2017b).

Recent human studies have documented small, transient changes in axial length (measured from corneal apex to the retinal pigment epithelium or RPE) and choroidal thickness in response to 1 to 2 hours of myopic and hyperopic defocus in young adult subjects (Chiang et al., 2015; Read et al., 2010a; Wang et al., 2016). This suggests that the human visual system is able to detect the presence and sign of imposed defocus and make compensatory changes in axial length. However, these

studies have employed optical defocus at only one specific time of the day (i.e. either in the morning or in the evening) (Chiang et al., 2015; Read et al., 2010a; Wang et al., 2016) or for a continuous period of 12 hours (Chakraborty et al., 2012, 2013). Furthermore, there were significant differences in the timing of imposed defocus between studies, ranging from 9 am to 12 pm (Read et al., 2010a; Wang et al., 2016) and 12 pm to 5 pm (Chiang et al., 2015). Given that human axial length and choroidal thickness undergo significant diurnal variations throughout the day (Chakraborty et al., 2011; Read et al., 2008; Stone et al., 2004; Tan et al., 2012), the ocular response to defocus may significantly vary depending on the time of day it is imposed. For instance, the increase in axial length with hyperopic defocus may vary when given in the morning (when the eyes are naturally longer) vs when given in the evening (when the eyes are naturally shorter) (Chakraborty et al., 2011; Stone et al., 2004).

In this study, we examined whether the ocular response to myopic and hyperopic defocus differ at different times of the day. More specifically, we investigated the effects of myopic and hyperopic defocus on axial length and choroidal thickness when imposed in the morning (between 10 am and 12 pm) and when imposed in the evening (5 to 7 pm) in young adult human participants.

2. Materials and methods

2.1 Participants and procedures

Young adult, near-emmetropic participants between the ages of 19 and 30 years (mean ± SD, 23.44 ± 4.52) were recruited to examine the effects of myopic (n=12, male=6; female=6) and hyperopic (n=13, male=6, female=7) defocus at different times of the day. A total of ten subjects participated in both hyperopic and myopic defocus experiments. Prior to participation, all subjects underwent a comprehensive ophthalmic examination to assess their refractive status and ocular health. The mean spherical equivalent refraction (SER) was -0.03 ± 0.29 and -0.04 ± 0.28 DS for participants in myopic and hyperopic defocus experiments respectively. All subjects had normal logMAR visual acuity of 0.00 or better, and astigmatic refractive error of ≤ 0.75 DC. Approval from the Southern Adelaide Local Health Network (SALHN, ID:156.17) was obtained, and all subjects gave written informed consent. All subjects were treated in accordance with the Declaration of Helsinki.

To investigate the effects of myopic and hyperopic defocus at different times of day, measurements of axial length and choroidal thickness were obtained from both eyes over three consecutive days (Figure 1). On day 1 (baseline day, no defocus), normal ocular biometric measurements in the absence of defocus were collected at 10 am, 12 pm, 5 pm and 7 pm. On day 2 (morning defocus day), following the first measurement session at 10 am, participants wore spectacle lenses that induced either a myopic or hyperopic defocus in their right eye only (+3.00 or - 3.00 DS right eye, plano left eye) for a period of two hours. The influence of optical defocus on ocular parameters was measured 2 hours after the introduction of defocus (i.e. 12 pm). Day 3 (evening defocus day) examined the effects of defocus in the evening with measurements taken at 5 pm, and 2 hours after exposing the eye to defocus at 7 pm (+3.00 or - 3.00 DS in the right eye). It has been shown that imposing hyperopic or myopic defocus may change the magnitude and/or phase of axial length and choroidal thickness rhythms in human participants (Chakraborty et al., 2012, 2013).

Therefore, to avoid the confounding effects of altered diurnal variations on ocular changes with optical defocus, morning and evening experiments were performed on separate days. For individuals that participated in both hyperopic and myopic defocus experiments, a single baseline measurement (day 1) was used for both defocus conditions if the two experiments were completed within a period of 1 week (n=4). For participants where myopic and hyperopic defocus experiments were separated by >1 week (n=6), two separate baseline sessions (i.e. three consecutive days each for myopic and hyperopic defocus) were carried out for optimal comparison.

On the day of experiment, each measurement took approximately 12 – 15 minutes, and subjects were instructed to carry out their normal daily activities between the measurement sessions. Given that factors such as accommodation (Mallen et al., 2006; Read et al., 2010b; Woodman et al., 2012) and exercise (Read and Collins, 2011) can cause short-term changes in axial length and choroidal thickness, a period of 10 minutes of binocular distance viewing (sitting and viewing an object at 6 m) in dim light (10 lux) was observed to wash out any residual effects of previous visual activities on measurements, as described elsewhere (Chakraborty et al., 2012). Spectacles were removed immediately prior to the measurements.

Axial length (measured from the anterior corneal surface to the RPE) measurements from each eye were obtained using the IOLMaster 500 (IOLMaster 500, Carl Zeiss, Jena, Germany). Three measurements of axial length (i.e. an average of 15 readings) for each subject at each measurement session were averaged for further analysis. The instrument has an excellent test-retest repeatability of ≤ 0.01 mm for axial length measurements (Carkeet et al., 2004; Sheng et al., 2004). The Cirrus HD spectral domain optical coherence tomographer (OCT) was used for choroidal thickness measurements (Cirrus HD-OCT 5000, Carl Zeiss Meditec Inc, Dublin, CA, USA). Three horizontal 6 mm, HD 5-line raster scans were taken from each eye at each measurement session using the enhanced depth imaging (EDI) mode. The spacing between the lines was kept at 0 mm to allow for multiple B-scans to be collected from the same retinal location. The instrument uses an 840 nm superluminescent diode and a scanning speed of 27,000 to 68,000 A-scans/second to capture high definition images of the posterior eye with axial resolution of 5 µm and transverse resolution of 15 µm in the tissue.

2.2. Data analysis

Following data collection, the OCT scans were exported from the instrument for detailed analysis using custom written software. The software allowed for automatic segmentation of chorio-retinal images for the calculation choroidal thickness (Alonso-Caneiro et al., 2013). Automatic segmentations were checked for accuracy, and if required, were manually adjusted for any segmentation errors.

Choroidal thickness (the distance from the RPE to the inner edge of the choroid/scleral interface) could not be calculated for one participant in each defocus condition due to poor visibility of the choroid/scleral interface. As illustrated in Figure 2, we calculated the thickness of the choroid at the fovea (or subfoveal choroidal thickness, SFCT) and across 1.5 mm nasal and temporal parafoveal choroidal regions on either side of the fovea (PFCT) (data combined for a series of 0.5 mm width

zones located at 0.5, 1.0 and 1.5 mm on either side of the fovea as described elsewhere) (Chakraborty et al., 2012).

The average of all biometric parameters for each subject at each measurement session was calculated. The data from the right and the left eye were analysed separately in order to examine the effects of defocus in the right eye, and any potential cross-over effects of defocus in the non-defocused left eye. From the average of ocular measurements at each measurement session, the mean change in the morning (difference between the 12 pm and 10 am measurement), and the mean change in the evening (difference between the 7 and 5 pm measurement) were calculated for each measured variable. Similar to our study, previous human studies have commonly presented the effect of optical defocus on ocular parameters as absolute change with reference to the baseline (or beginning of the defocus treatment) measured over a given period of time (Chiang et al., 2015; Read et al., 2010a; Wang et al., 2016). There were no significant differences in ocular biometrics at morning 10 am on day 1 and day 2 or evening 5 pm on day 1 and day 3 for either defocus experiment (Student's t-test, $p>0.05$, data not shown). Therefore, "change in the morning" and "change in the evening" measurements allowed for optimal comparison of ocular changes with defocus at different times of the day (days 2 and 3) in relation to the baseline ocular measurements on day 1 (no defocus). The net effect of defocus on each ocular parameter in the morning (i.e. change in the morning with defocus on day 2 - change in the morning without defocus on baseline day 1) and evening (i.e. change in the evening with defocus on day 3 - change in the evening without defocus on baseline day 1) were also calculated for both right and left eyes (presented in Table 2 and Supplementary data).

Statistical analyses were performed using commercial software (SigmaStat 3.5, Aspire Software International, Ashburn, VA). For each eye, changes in axial length and choroidal thickness (both SFCT and PFCT) with defocus at different times of day were analysed with repeated-measures two-way analysis of variance (ANOVA) and Holm-Sidak post-hoc tests for statistical significance, using two within-subjects factors ("time of day" and "defocus"). In case of a significant cross-over interaction between the two main variables, while one or both main effects are not significant, SigmaStat automatically runs the post hoc analysis on the data to identify the cause of the interaction. This is done to study the effect or impact of one variable in terms of the impact on the second variable (and vice versa) (Marascuilo and Levin, 1970). To examine the baseline differences between the nasal and temporal regions of the parafoveal choroid, the repeated-measures ANOVA was used with "region" and "time of day" as within-subjects factors. To investigate the association between the changes in axial length and choroidal thickness, a regression analysis was performed using the least-squares approach. To provide an assessment of the within-session variability for each of the measured parameters, the average within-session range and within-session standard deviation (Bland and Altman, 1999), and the mean coefficient of variation for each variable were also calculated. A p-value of less than 0.05 was considered to be statistically significant. All data are expressed as mean ± standard error of mean (SEM).

3. Results

3.1 With-in session measurement repeatability

The with-session variability (i.e. within-subject SD and coefficient of variation) was small for axial length (mean coefficient of variation of 0.04% for both defocus conditions), and subfoveal choroidal thickness (mean coefficient of variation, 3.72% and 3.94% for myopic and hyperopic defocus respectively), but slightly greater for parafoveal choroidal thickness (mean coefficient of variation, 4.71% and 4.88% for myopic and hyperopic defocus conditions) (Table 1). Overall, the measured with-in session repeatability for choroidal measurements was found to be slightly higher than previously published research (average coefficient of variation, 1.82% and 2.50% for subfoveal and parafoveal measures) (Chakraborty et al., 2012), which may be due to different segmentation methods applied in these studies (manual segmentation vs automated segmentation with minimal manual adjustment in the current study).

3.2 Myopic defocus

3.2.1 Axial length

The raw mean axial length at each measurement session across each of the three days, the mean change in axial length with myopic defocus in the morning and in the evening, and the net effect of defocus on axial length at different times of the day for the right and left eyes are presented in Table 2. In figures 3 and 4, blue bars represent normal diurnal changes in ocular parameters without defocus measured at different times of the day, whereas red bars represent ocular changes with morning and evening exposure to defocus. For the right eye (that was exposed to defocus on days 2 and 3), the effects of myopic defocus were significantly different depending on the time of day (two-way repeated measures ANOVA time of day by defocus interaction $F(1,47) = 9.896$, $p=0.009$, Figure 3A). On the baseline day (no defocus), there was a significant increase in axial length in the morning (mean change between 12 pm and 10 am: +0.016 ± 0.006 mm), but no major change in axial length the evening (mean change between 7 and 5 pm, +0.002 ± 0.002 mm, Holm-Sidak multiple comparisons, $p<0.05$). Introduction of myopic defocus in the morning (day 2) as well as in the evening (day 3) led to a significant reduction in axial length compared to the baseline sessions on day 1; however, the change in axial length was found to be significantly larger for defocus in the evening (mean change, -0.021 ± 0.009 mm) than that resulting from defocus in the morning (+0.004 ± 0.003 mm, Holm-Sidak multiple comparisons, $p<0.05$), indicating differential effects of the time of day on ocular response to myopic defocus.

There were small changes in the left eye (that was not exposed to defocus) associated with myopic defocus in the right eye (two-way repeated measures ANOVA time of day by defocus interaction $F(1,47) = 7.367$, $p=0.020$, Figure 3B). In comparison with day 1, myopic defocus in the evening on day 3 caused a small, but significant increase in axial length of the left eye (change on day 3, +0.007 ± 0.003 mm; change on day 1, -0.002 ± 0.003 mm, Holm-Sidak multiple comparisons, $p<0.05$); however, morning exposure to defocus on day 2 had no significant effect on left eye's axial length (change on day 2, +0.007 ± 0.002 mm; change on day 1, +0.012 ± 0.002 mm, $p>0.05$).

3.2.2 Subfoveal choroidal thickness

Consistent with axial length changes in the right eye, subfoveal choroid exhibited significant differences in its thickness depending on the time of day of myopic defocus (two-way repeated measures ANOVA time of day by defocus interaction $F(1,42) = 9.474$, $p=0.011$, Table 2 and Figure 4A). On baseline day 1, the change in SFCT in the morning (change between 10 am and 12 pm: -0.013 ± 0.007 mm) was not significantly different from the change in SFCT observed in the evening (change between 5 and 7 pm: -0.005 ± 0.006 mm, Holm-Sidak multiple comparisons, $p>0.05$). Importantly, exposure to myopic defocus on days 2 and 3 resulted in significant thickening of the choroid, but the change in SFCT was significantly greater for the evening defocus (day 3, +0.027 ± 0.006 mm) than for the morning defocus (day 2, +0.007 ± 0.006 mm, Holm-Sidak multiple comparisons, $p<0.05$, Figure 4A).

Myopic defocus in the right eye caused smaller changes in SFCT of the left eye (two-way repeated measures ANOVA time of day by defocus interaction $F(1,41) = 8.743$, $p=0.018$, Table 2 and Figure 4B). With reference to day 1, defocus in the morning between 10 am and 12 pm led to a greater thickening of the choroid (change on day 2, +0.005 ± 0.005 mm; change on day 1, -0.012 ± 0.004 mm, Holm-Sidak multiple comparisons, $p<0.05$) compared to when given in the evening between 5 and 7 pm (change on day 3, +0.008 ± 0.003 mm; change on day 1, +0.006 ± 0.002 mm, $p>0.05$).

3.2.3 Parafoveal choroidal thickness

Supplementary data shows the raw mean PFCT, changes in the parafoveal choroid (both nasal and temporal regions) with myopic and hyperopic defocus imposed at different times of the day, as well as the net effect of defocus on PFCT on each eye. In the right eye, there were significant differences in the PFCT between the nasal and temporal regions (average thickness of all four measurement sessions on day 1: nasal choroid, 0.370 ± 0.011 mm; temporal choroid, 0.385 ± 0.009 mm, two-way repeated measures ANOVA main effect of the region of the choroid $F(1, 263) = 22.004$, $p<0.001$). Similar to the right eye, temporal choroid was found to be significantly thicker than the nasal choroid in the left eye as well (nasal choroid, 0.367 ± 0.011 mm; temporal choroid, 0.393 ± 0.011 mm, two-way repeated measures ANOVA main effect of the region of the choroid $F(1, 263) = 70.552$, $p<0.001$).

Upon examining the changes in the parafoveal choroid of the right eye on day 1, both nasal and temporal choroid became slightly thinner between 10 am and 12 pm, but thicker in the evening between 5 and 7 pm (nasal choroid: change in the morning, -0.004 ± 0.004 mm; change in the evening, +0.006 ± 0.002 mm; temporal choroid: change in the morning, -0.004 ± 0.004 mm; change in the evening, +0.006 ± 0.003 mm, two-way repeated measures ANOVA time of day by defocus interaction, $p<0.05$, Supplementary data). Furthermore, myopic defocus in the evening on day 3 led to a greater thickening of the parafoveal choroid than morning exposure to defocus on day 2 (nasal choroid: change on day 2, +0.003 ± 0.003 mm; change on day 3, +0.016 ± 0.003 mm; temporal choroid: change on day 2, +0.004 ± 0.003 mm; change on day 3, +0.018 ± 0.003 mm) (two-way repeated measures ANOVA, Holm-Sidak multiple comparisons, $p<0.05$).

Myopic defocus in the right eye caused significant changes in both the nasal and temporal choroidal regions of the left eye (two-way repeated measures ANOVA time of day by defocus interaction, $p<0.05$, Supplementary data). In comparison with day 1, exposure to defocus in the morning caused a significant thickening of the parafoveal choroid (nasal choroid: change on day 2, +0.007 ± 0.003 mm; change on day 1, -0.013 ± 0.002 mm; temporal choroid: change on day 2, +0.008 ± 0.003 mm; change on day 1, -0.009 ± 0.002 mm, Holm-Sidak multiple comparisons, $p<0.05$); however, the effect of defocus was small and insignificant in the evening.

3.3 Hyperopic defocus

3.3.1 Axial length

Table 2 illustrates the raw mean axial length, the change in axial length with hyperopic defocus imposed in the morning and evening hours, and the net effect of defocus on axial length at different times of the day in both right and left eyes. In figures 5 and 6, blue bars represent normal diurnal changes in ocular parameters without defocus measured at different times of the day, whereas green bars represent ocular changes with morning and evening exposure to defocus. In the right defocused eye, the impact of hyperopic defocus on axial length was significantly different at different times of the day (two-way repeated measures ANOVA time of day by defocus interaction $F(1,50) = 4.908$, $p=0.047$, Figure 5A). On day 1, the change in axial length in the morning (+0.010 ± 0.003 mm) was not significantly different from the change in axial length observed in the evening (+0.001 ± 0.004 mm, Holm-Sidak multiple comparisons, $p>0.05$). Compared to the baseline measurement on day 1, exposure to hyperopic defocus in the morning on day 2 led to a significant increase in axial length of +0.026 ± 0.006 mm (Holm-Sidak multiple comparisons, $p<0.05$). However, hyperopic defocus in the evening on day 3 did not induce any significant ocular change (+0.001 ± 0.003 mm) in comparison with the baseline evening session on day 1 (+0.001 ± 0.004 mm, $p>0.05$), suggesting differences in the sensitivity of the eye to hyperopic defocus at different times of the day.

In the left eye, the average change in axial length was significantly different between the morning (mean change of day 1 and day 2 morning sessions, +0.009 ± 0.003 mm) and evening measurement sessions (mean change of day 1 and day 3 evening sessions, -0.009 ± 0.004 mm, two-way repeated measures ANOVA main effect of the time of day $F(1,50) = 17.233$, $p=0.001$, Figure 5B). However, there was no significant effect of defocus or significant interaction between the time of day and defocus on axial length changes in the left eye (two-way repeated measures ANOVA, $p>0.05$).

3.3.2 Subfoveal choroidal thickness

In the right eye, there was a significant thinning in the subfoveal choroid with hyperopic defocus on days 2 and 3 (mean change with morning and evening defocus, -0.013 ± 0.006 mm) compared to day 1 with no defocus (mean change without defocus, -0.002 ± 0.004 mm, two-way repeated measures ANOVA main effect of defocus $F(1,41) = 12.401$, $p=0.005$, Figure 6A). However, there was no significant interaction between the time of day and defocus treatment on SFCT (two-way repeated measures ANOVA time of day by defocus interaction $F(1,41) = 0.193$, $p=0.672$), suggesting that hyperopic defocus at different times of the day had similar effect on subfoveal choroidal changes.

Importantly, the net effect of hyperopic defocus on SFCT was similar between the morning and evening defocus sessions (both ~ -0.010 mm, Table 2).

Exposure to hyperopic defocus was found to have a small, but significant effect on SFCT of the left eye (mean change with morning and evening defocus on days 2 and 3, +0.001 ± 0.006 mm; mean change without defocus on day 1, -0.009 ± 0.005 mm, two-way repeated measures ANOVA main effect of defocus $F(1,44) = 5.261$, $p=0.039$, Figure 6B). However, hyperopic defocus at different times of the day had a similar effect on left eye's SFCT (two-way repeated measures ANOVA time of day by defocus interaction $F(1,44) = 0.91$, $p=0.368$).

3.3.3 Parafoveal choroidal thickness

On average, the temporal choroid was found to be significantly thicker than the nasal choroid in both right (nasal choroid, 0.386 ± 0.010 mm; temporal choroid, 0.395 ± 0.010 mm) and left (nasal choroid, 0.389 ± 0.011 mm; temporal choroid, 0.401 ± 0.011 mm) eyes (two-way repeated measures ANOVA main effect of the region of the choroid, $p<0.001$, Supplementary data).

In the right eye, hyperopic defocus on days 2 and 3 induced significant thinning only in the nasal choroid (mean change with morning and evening defocus, -0.010 ± 0.003 mm; mean change without defocus, -0.001 ± 0.004 mm, two-way repeated measures ANOVA main effect of defocus, $F(1,129) = 7.115$, $p=0.011$, Supplementary data). The temporal choroid did not exhibit any significant change with hyperopic defocus (two-way repeated measures ANOVA, $p=0.173$). There was no significant interaction between the time of day and defocus for either parafoveal region (two-way repeated measures ANOVA, $p>0.05$).

Defocus in the right eye did not induce any significant change in the PFCT of the left non-defocused eye (two-way repeated measures ANOVA, $p>0.05$).

4. Discussion

Various animal models have shown that ocular growth and choroidal thickness can be influenced by optical defocus (Graham and Judge, 1999; Hung et al., 1995; Irving et al., 1992; Nathan et al., 1984; Schaeffel et al., 1988; Smith and Hung, 1999; Wallman et al., 1995; Wildsoet and Wallman, 1995). This study adds to the evidence that human eyes are also able to respond to the presence of defocus, and make small, predictable changes in axial length and choroidal thickness, depending on the sign of imposed defocus.

The novel findings in this study were that the effects of monocular myopic and hyperopic defocus on axial length significantly varied depending on the time of day in young adult human subjects. Myopic defocus caused a significantly greater reduction in axial length when imposed in the evening than in the morning. However, hyperopic defocus led to a significant increase in axial length when imposed in the morning, but had no effect on axial length when imposed in the evening. Research in young adult humans (Read et al., 2010a) and children (Wang et al., 2016) have found that 1 - 2 hours of exposure to monocular hyperopic and myopic defocus during the day (between 9 am to 12 pm) led to small but significant changes in axial length. In our study, the mean change in axial length with 2 hours of hyperopic and myopic defocus in the morning was in close agreement

with previously reported changes of 0.010 mm in human subjects (Read et al., 2010a; Wang et al., 2016).

Interestingly, two recent studies in chicks have found hyperopic (Nickla et al., 2017a) and myopic (Nickla et al., 2017b) defocus to exhibit different effects on axial length and ocular growth depending on the time of exposure to defocus. Similar to our results, Nickla et al found that a brief 2-hour exposure to myopic defocus was more effective in inhibiting axial elongation and slowing ocular growth in chicks when given in the evening or mid-day than in the morning, while 2 hours of hyperopic defocus in the morning caused a significantly greater increase in axial length and corresponding ocular growth compared to the same duration of defocus given at mid-day. It should be noted that both studies (Nickla et al., 2017a; Nickla et al., 2017b) measured axial length from the anterior corneal surface to the inner surface of the sclera. Using the scleral interface for measuring axial length provides a "true" change in the length of the globe, and discerns the changes in choroidal thickness from the total change in the length of the eye (or the globe). As the present study defined axial length from corneal apex to the RPE and as the choroid is adjacent to the retinal photoreceptor-RPE complex, we conjectured that axial length changes measured in this study were associated with changes in the thickness of the choroid (discussed below). Because commonly used optical biometers (including the IOL Master) measure axial length only up to the RPE, it is difficult to determine the actual contribution of choroidal thickness to the changes in axial length in human participants. It is prudent to keep these differences in mind when interpreting the axial length changes across different studies, and drawing parallels with human studies.

We found that hyperopic and myopic defocus at different times of the day had varying effects on the thickness of the subfoveal choroid. Myopic defocus in the evening induced a significantly greater thickening of the subfoveal choroid compared to the same duration of defocus in the morning. Hyperopic defocus resulted in significant thinning of the choroid, and although there was a trend for a greater change in SFCT with hyperopic defocus in the morning ($p=0.672$), the net effect of defocus on choroidal thickness was found to be similar for the morning and evening sessions of defocus. The changes in choroidal thickness were generally consistent with previous studies that have reported a change of approximately 0.010 to 0.020 mm in SFCT with similar periods of hyperopic and myopic defocus in young human eyes (Chakraborty et al., 2013; Chiang et al., 2015; Read et al., 2010a; Wang et al., 2016). It is noteworthy that some differences between the two defocus conditions may be due to slightly lower precision of the SFCT estimates in the hyperopic defocus experiment (mean coefficient of variation of 4.13% compared to 3.58% with myopic defocus). Furthermore, myopic defocus has been shown to have a stronger influence on ocular parameters than hyperopic defocus in animal studies (Winawer and Wallman, 2002; Zhu, 2013).

Similar to chicks (Nickla, 2006; Nickla et al., 1998), primates (Nickla et al., 2002) and mammals (Liu and Farid, 1998), axial length and choroidal thickness undergo significant diurnal variations in human eyes (Brown et al., 2009; Chakraborty et al., 2011; Read et al., 2008; Stone et al., 2004). The axial length of the human eye is typically longest at mid-day and shortest at night, while the choroid is thickest during the night and thinnest during the day (i.e. antiphase to one another). These diurnal fluctuations may differentially influence the ocular response to hyperopic and myopic

defocus, depending on the time of day. In this study, we found a significant increase in axial length with hyperopic defocus in the morning when the eyes were naturally longer; whereas, the decrease in axial length with myopic defocus was significantly greater in the evening when the eyes were naturally shorter. These results suggest a potential interaction between eye's natural diurnal rhythms and visual signals associated with the optical defocus, making the eye perhaps more responsive to hyperopic defocus (or 'go' signal) in the morning, and to myopic defocus (or 'stop' signal) in the latter half of the day (Chakraborty et al., 2018). This may be due to diurnal variations in retinal defocus 'integrator', molecular/cellular structure of the choroid, and/or scleral growth factors that are perhaps most susceptible to hyperopic defocus in the morning, and myopic defocus in the evening (Nickla et al., 2017a). However, this study measured the ocular effects of defocus only at a single time point (i.e. 2 hours after the introduction of defocus), and not for an extended period of time. Therefore, future studies with longer periods of defocus and/or more frequent measurements over the course of the day may provide detailed insights into the effects of time of day on ocular response to optical defocus in human subjects.

In both right and left eyes, the choroid was thickest in the subfoveal region, and thinner in the parafoveal region on either side of the fovea. In addition, the temporal choroid was found to be thicker than the nasal choroid (Ikuno et al., 2010; Manjunath et al., 2010; Margolis and Spaide, 2009). Of interest, we found that choroidal regions peripheral to the fovea were significantly influenced by optical defocus. Although the effect of the time of day on defocus induced PFCT changes were generally similar to the changes in SFCT, the changes were not as marked as the subfoveal choroid, particularly for the hyperopic defocus condition. This may be due to the greater within-session variability in the PFCT data (mean coefficient of variation, 4.79%). Consistent with our results, previous human studies have also found both central and peripheral regions of the choroid to be sensitive to both defocus conditions (Chakraborty et al., 2012, 2013; Hoseini-Yazdi et al., 2018). In the defocused eye, the nasal choroid was found to be sensitive to both hyperopic and myopic defocus at different times of the day, whereas the temporal choroid was only responsive to myopic defocus and not hyperopic defocus. Interestingly, a recent study has also reported increased sensitivity of the nasal choroid to both hyperopic and myopic defocus stimuli in young human subjects (Hoseini-Yazdi et al., 2018). Overall, these results suggest potential regional differences in the sensitivity of the human choroid to short-term optical defocus, which may be due to regional variations in the autonomic innervation to the choroid, distribution of non-vascular smooth muscles and/or choroidal blood flow (Nickla and Wallman, 2010).

The regression analyses revealed a significant, but weak association between the changes in axial length and choroidal thickness across the three measurement days for either defocus condition (myopic defocus: slope = -0.412, r^2 = 0.13, $p<0.001$; hyperopic defocus: slope = -0.378; r^2 = 0.10, $p<0.001$). Previous human studies have also reported moderate to weak association between the axial length and choroidal thickness changes in response to optical defocus (Chakraborty et al., 2013; Read et al., 2010a). The weak association between the two variables suggest that there may be other factors (such as corneal thickness, lens thickness or vitreous chamber depth) involved in defocus induced axial length changes in human eyes that were not measured in this study. Further

investigation of the influence of defocus at different times of day using a more comprehensive instrument (e.g., the Lenstar) therefore seems warranted.

Defocus in the right eye had relatively small effect on axial length and choroidal thickness of the fellow left (non-defocused) eye. On average, the net changes were generally small on the order of <0.010 mm (except for modest choroidal changes in the morning with myopic defocus and in the evening with hyperopic defocus). Previous human studies have documented small changes in axial length and choroidal thickness in response to accommodation due to mechanical effects of ciliary muscle contraction (Drexler et al., 1998; Mallen et al., 2006; Woodman et al., 2012). In the current study, accommodation was not strictly controlled (i.e. no cycloplegia was used) or measured during the defocus treatment. As a result, the exact state of accommodation in each eye, while exposed to defocus, was not known. Although previous human studies have reported only minimal changes in accommodation with similar periods of myopic and hyperopic defocus in young human eyes (Chakraborty et al., 2012, 2013; Read et al., 2010a), it is possible that subtle changes in accommodation during the period of defocus may have contributed to the small contralateral changes observed in the left eye.

These findings may have some practical clinical applications to myopia prevention in children. For instance, it has been hypothesised that hyperopic defocus induced by accommodative lag during prolonged near work could stimulate myopic eye growth in children (Gwiazda et al., 1995; Ip et al., 2008). If the eye has a greater sensitivity to hyperopic defocus in the morning, as suggested by this study, scheduling intensive reading activities in the evening, along with frequent breaks for distance viewing (i.e. exposing the eye to myopic defocus) may prevent myopia development. In addition, optical treatments employing myopic defocus for myopia prevention (such as multifocal soft contact lenses) may be more effective when worn later in the day. Future research will answer whether these results are clinically translatable for myopia prevention.

5. Conclusions

In conclusion, we found that the ocular response to short-term monocular hyperopic and myopic defocus varies significantly depending on the time of exposure in young adult human subjects. Myopic defocus led to a significantly greater reduction in axial length and thickening of the choroid when imposed in the evening rather than in the morning. Hyperopic defocus caused a significant increase in axial length only in the morning, with no major effect in the evening. Furthermore, hyperopic defocus resulted in a significant thinning of the choroid, but there was no significant influence of the time of day on choroidal changes associated with hyperopic defocus. These results may represent a potential interaction between the signal associated with the eye's natural diurnal rhythm and the visual signal associated with the optical defocus that may lead to a greater axial growth in the morning (due to increased sensitivity to hyperopic defocus) and a reduced eye growth in the latter half of the day (due to increased sensitivity to myopic defocus).

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Table 1: Overview of the mean within-subject variability for the repeated measures collected at each measurement session across the three measurement days in the right (defocused) and left (non-defocused) eyes for axial length, subfoveal choroidal thickness (SFCT) and parafoveal choroidal thickness (PFCT, averaged across the nasal and temporal regions) for myopic and hyperopic defocus experiments.

Type of defocus	Measured variables	Right eye (defocused eye)			Left eye (non-defocused eye)		
		Mean within-session standard deviation (mm)	Mean within-session range (mm)	Mean coefficient of variation	Mean within-session standard deviation (mm)	Mean within-session range (mm)	Mean coefficient of variation
Myopic defocus	Axial length	0.011	0.022	0.04%	0.010	0.022	0.04%
	SFCT	0.013	0.024	3.58%	0.014	0.027	3.86%
	PFCT	0.015	0.031	4.77%	0.017	0.032	4.64%
Hyperopic defocus	Axial length	0.010	0.021	0.04%	0.009	0.019	0.03%
	SFCT	0.015	0.030	4.13%	0.013	0.026	3.74%
	PFCT	0.018	0.032	4.74%	0.019	0.035	5.01%

Table 2: Summary of raw group means and mean change in axial length and subfoveal choroidal thickness (SFCT) with hyperopic and myopic defocus at each measurement session for each of the three measurement days (day 1 baseline day, day 2 morning defocus day and day 3 evening defocus day) in the right (defocused) and left (non-defocused) eyes, along with p-values from repeated measures ANOVA illustrating the mean change in the morning and evening as effects of time of day, defocus and time of day by defocus interaction. The net effect of defocus in the morning and evening (i.e. mean change with defocus on day 2 or 3 - mean change with no defocus on baseline day 1) are shown in boxes adjacent to the mean change. Group means of axial length have been rounded to 2 decimal places, whereas group means of SFCT and mean change for all parameters have been rounded to 3 decimal places. Significant p values ($p<0.05$) are highlighted in bold.

Defocus	Eye	Measured variables	Measurement time	Measurement day	Group mean ± SEM (mm)	Mean change ± SEM (mm)		*p* value (time of day)	*p* value (defocus)	*p* value (defocus by time of day interaction)
Myopic Defocus	Right eye (defocused eye)	Axial length	Morning	Day 1, 10 am (baseline)	23.66 ± 0.35	+0.016 ± 0.006	-0.012 ± 0.005	**0.01**	0.556	**0.009**
				Day 1, 12 pm (baseline)	23.68 ± 0.35					
				Day 2, 10 pm (defocus)	23.67 ± 0.35	+0.004 ± 0.003				
				Day 2, 12 pm (defocus)	23.67 ± 0.35					
			Evening	Day 1, 5 pm (baseline)	23.67 ± 0.35	+0.002 ± 0.002	-0.023 ± 0.006			
				Day 1, 7 pm (baseline)	23.67 ± 0.35					
				Day 3, 5 pm (defocus)	23.67 ± 0.35	-0.021 ± 0.009				
				Day 3, 7 pm (defocus)	23.65 ± 0.35					
		SFCT	Morning	Day 1, 10 am (baseline)	0.397 ± 0.018	-0.013 ± 0.007	+0.020 ± 0.007	**0.001**	0.821	**0.011**
				Day 1, 12 pm (baseline)	0.384 ± 0.018					
				Day 2, 10 am (defocus)	0.400 ± 0.018	+0.007 ± 0.006				
				Day 2, 12 pm (defocus)	0.407 ± 0.018					
			Evening	Day 1, 5 pm (baseline)	0.405 ± 0.019	-0.005 ± 0.006	+0.032 ± 0.006			
				Day 1, 7 pm (baseline)	0.400 ± 0.021					
				Day 3, 5 pm (defocus)	0.403 ± 0.019	+0.027 ± 0.006				
				Day 3, 7 pm (defocus)	0.430 ± 0.021					
	Left eye (non-defocused eye)	Axial length	Morning	Day 1, 10 am (baseline)	23.70 ± 0.35	+0.012 ± 0.002	-0.005 ± 0.002	0.488	**0.045**	**0.02**
				Day 1, 12 pm (baseline)	23.71 ± 0.35					
				Day 2, 10 am (defocus)	23.70 ± 0.35	+0.007 ± 0.002				
				Day 2, 12 pm (defocus)	23.71 ± 0.35					
			Evening	Day 1, 5 pm (baseline)	23.71 ± 0.35	-0.002 ± 0.003	+0.009 ± 0.003			
				Day 1, 7 pm (baseline)	23.70 ± 0.35					
				Day 3, 5 pm (defocus)	23.70 ± 0.35	+0.007 ± 0.003				
				Day 3, 7 pm (defocus)	23.71 ± 0.35					
		SFCT	Morning	Day 1, 10 am (baseline)	0.415 ± 0.017	-0.012 ± 0.004	+0.017 ± 0.005	0.076	**0.007**	**0.018**
				Day 1, 12 pm (baseline)	0.403 ± 0.019					
				Day 2, 10 am (defocus)	0.405 ± 0.019	+0.005 ± 0.005				
				Day 2, 12 pm (defocus)	0.410 ± 0.021					
			Evening	Day 1, 5 pm (baseline)	0.402 ± 0.021	+0.006 ± 0.002	+0.002 ± 0.003			
				Day 1, 7 pm (baseline)	0.408 ± 0.021					
				Day 3, 5 pm (defocus)	0.399 ± 0.020	+0.008 ± 0.003				
				Day 3, 7 pm (defocus)	0.406 ± 0.021					

Hyperopic Defocus	Right eye (defocused eye)	Axial length	Morning	Day 1, 10 am (baseline)	23.27 ± 0.31	+0.010 ± 0.003	+0.016 ± 0.005	**0.001**	0.072	**0.047**
				Day 1, 12 pm (baseline)	23.28 ± 0.31					
				Day 2, 10 am (defocus)	23.36 ± 0.30	+0.026 ± 0.006				
				Day 2, 12 pm (defocus)	23.39 ± 0.30					
			Evening	Day 1, 5 pm (baseline)	23.36 ± 0.30	+0.001 ± 0.004	0.000 ± 0.004			
				Day 1, 7 pm (baseline)	23.36 ± 0.30					
				Day 3, 5 pm (defocus)	23.37 ± 0.30	+0.001 ± 0.003				
				Day 3, 7 pm (defocus)	23.37 ± 0.30					
		SFCT	Morning	Day 1, 10 am (baseline)	0.431 ± 0.016	-0.011 ± 0.004	-0.010 ± 0.005	**0.009**	**0.005**	0.672
				Day 1, 12 pm (baseline)	0.420 ± 0.016					
				Day 2, 10 am (defocus)	0.426 ± 0.021	-0.021 ± 0.005				
				Day 2, 12 pm (defocus)	0.405 ± 0.018					
			Evening	Day 1, 5 pm (baseline)	0.410 ± 0.018	+0.006 ± 0.004	-0.010 ± 0.006			
				Day 1, 7 pm (baseline)	0.417 ± 0.017					
				Day 3, 5 pm (defocus)	0.414 ± 0.022	-0.004 ± 0.007				
				Day 3, 7 pm (defocus)	0.410 ± 0.022					
	Left eye (non-defocused eye)	Axial length	Morning	Day 1, 10 am (baseline)	23.33 ± 0.31	+0.008 ± 0.002	+0.001 ± 0.003	**0.001**	0.428	0.213
				Day 1, 12 pm (baseline)	23.34 ± 0.31					
				Day 2, 10 am (defocus)	23.43 ± 0.30	+0.009 ± 0.003				
				Day 2, 12 pm (defocus)	23.43 ± 0.30					
			Evening	Day 1, 5 pm (baseline)	23.43 ± 0.30	-0.006 ± 0.005	-0.007 ± 0.004			
				Day 1, 7 pm (baseline)	23.43 ± 0.30					
				Day 3, 5 pm (defocus)	23.44 ± 0.30	-0.013 ± 0.003				
				Day 3, 7 pm (defocus)	23.43 ± 0.30					
		SFCT	Morning	Day 1, 10 am (baseline)	0.432 ± 0.018	-0.011 ± 0.004	+0.005 ± 0.005	0.103	**0.039**	0.368
				Day 1, 12 pm (baseline)	0.420 ± 0.017					
				Day 2, 10 am (defocus)	0.407 ± 0.022	-0.006 ± 0.006				
				Day 2, 12 pm (defocus)	0.401 ± 0.021					
			Evening	Day 1, 5 pm (baseline)	0.419 ± 0.017	-0.006 ± 0.005	+0.015 ± 0.005			
				Day 1, 7 pm (baseline)	0.413 ± 0.019					
				Day 3, 5 pm (defocus)	0.401 ± 0.019	+0.009 ± 0.005				
				Day 3, 7 pm (defocus)	0.410 ± 0.022					

Figure captions

Figure 1. A timeline of experimental paradigm and measurements collected over three consecutive days. On day 1 (no defocus, baseline day), normal ocular biometric measurements in the absence of defocus were collected at 10 am, 12 pm, 5 pm and 7 pm. On day 2 (morning defocus day), the effects of myopic and hyperopic defocus in the morning were examined with measurements taken at 10 am, and 2 hours after exposing the eye to defocus at 12 pm (+3.00 or - 3.00 DS in the right eye, plano left eye). On day 3 (evening defocus day), the effects of myopic and hyperopic defocus in the evening were examined with measurements taken at 5 pm, and 2 hours after exposing the eye to defocus at 7 pm (+3.00 or - 3.00 DS in the right eye, plano left eye). Measurements of axial length (using IOL Master) and choroidal thickness (using spectral-domain optical coherence tomography) were obtained from both right (defocused) and left (non-defocused) eyes.

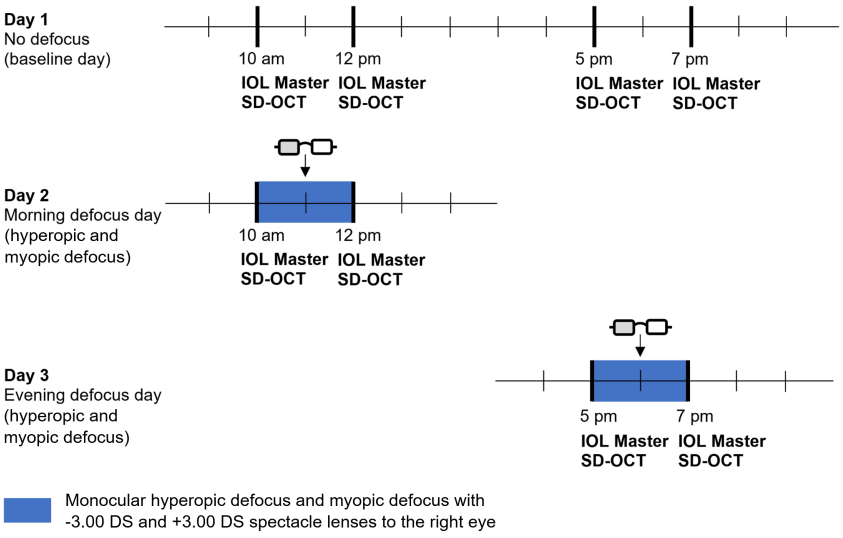
Figure 2. Example of an OCT image from one measurement session, showing subfoveal choroidal thickness (the distance from the RPE to the inner edge of the choroidal/scleral interface at the centre of the fovea) and parafoveal choroidal thickness (3 mm diameter) across 1.5 mm nasal and temporal parafoveal choroidal regions on either side of the fovea.

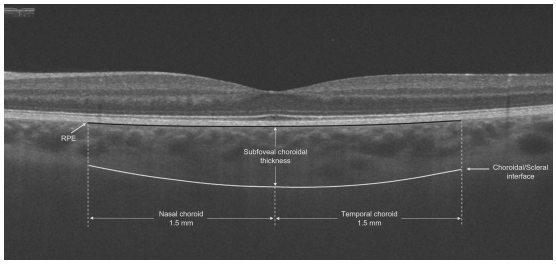
Figure 3: Mean change in axial length with myopic defocus in the morning (between 10 am and 12 pm) and in the evening (5 pm to 7 pm) for the right defocused (A) and left non-defocused (B) eyes. Morning and evening changes in axial length were calculated for the baseline day (day 1, no defocus, shown in blue bar), and following 2 hours of exposure to defocus in the morning (day 2) and evening (day 3) on two separate days (shown in red bar). (A) In the right (defocused) eye, the effects of myopic defocus were significantly different between the morning and evening sessions (two-way repeated measures ANOVA time of day by defocus interaction $F(1,47) = 9.896$, $p=0.009$). (B) Left (non-defocused) eye showed small changes in axial length associated with defocus in the right eye (two-way repeated measures ANOVA time of day by defocus interaction $F(1,47) = 7.367$, $p=0.020$). Significant interactions from post-hoc tests are indicated by asterisks. Error bars represent standard error of mean.

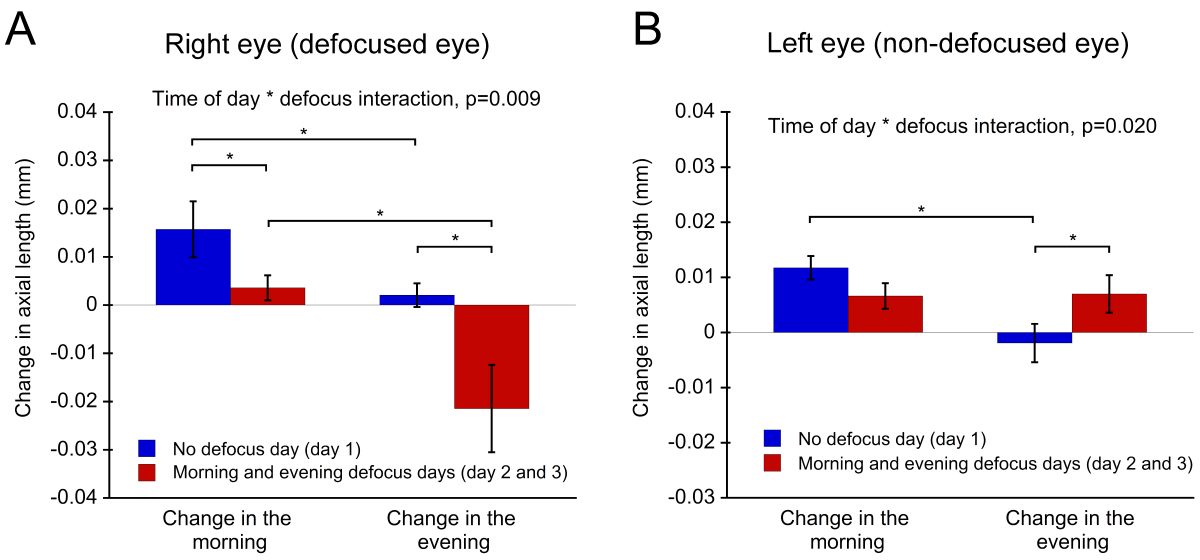
Figure 4: Mean change in subfoveal choroidal thickness (SFCT) with myopic defocus in the morning (between 10 am and 12 pm) and in the evening (5 pm to 7 pm) for the right defocused (A) and left non-defocused (B) eyes. Morning and evening changes in SFCT were calculated for the baseline day (day 1, no defocus, shown in blue bar), and following 2 hours of exposure to defocus in the morning (day 2) and evening (day 3) on two separate days (shown in red bar). (A) In the right (defocused) eye, SFCT exhibited significant differences in its thickness depending on the time of day of myopic defocus (two-way repeated measures ANOVA time of day by defocus interaction $F(1,42) = 9.474$, $p=0.011$). (B) Left (no-defocused) eye showed small changes in SFCT with defocus in the right eye (two-way repeated measures ANOVA time of day by defocus interaction $F(1,41) = 8.743$, $p=0.018$). Significant interactions from post-hoc tests are indicated by asterisks. Error bars represent standard error of mean.

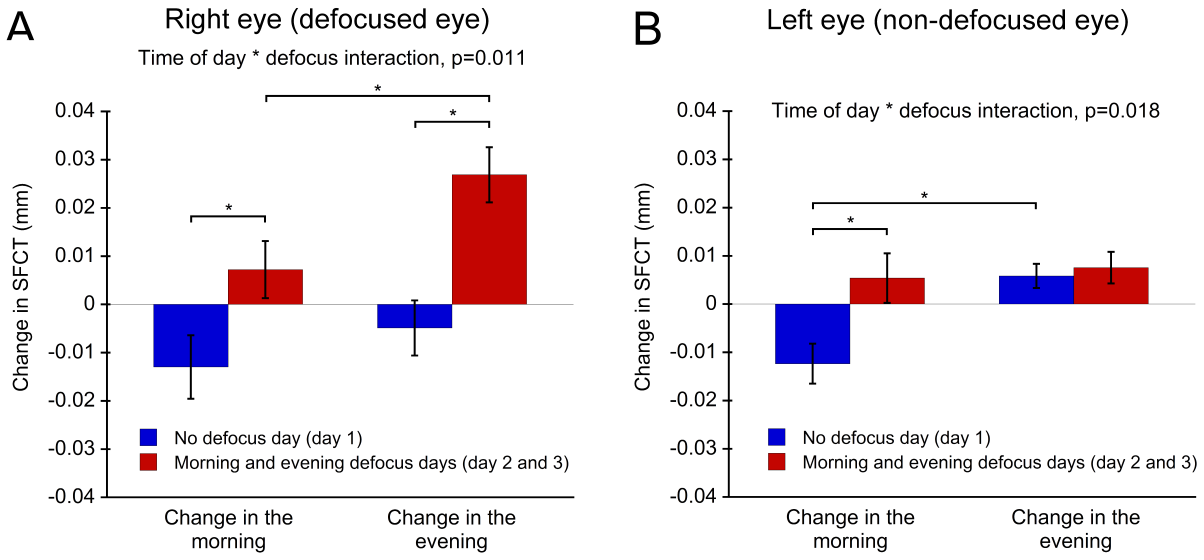
Figure 5: Mean change in axial length with hyperopic defocus in the morning (between 10 am and 12 pm) and in the evening (5 pm to 7 pm) for the right defocused (A) and left non-defocused (B) eyes. Morning and evening changes in axial length were calculated for the baseline day (day 1, no defocus, shown in blue bar), and following 2 hours of exposure to defocus in the morning (day 2) and evening (day 3) on two separate days (shown in green bar). (A) In the right (defocused) eye, hyperopic defocus resulted in a significant increase in axial length when given in the morning, but not in the evening (two-way repeated measures ANOVA time of day by defocus interaction $F(1,50) = 4.908$, $p=0.047$). (B) Hyperopic defocus in the right eye had no significant effect on the axial length of the left (non-defocused) eye (two-way repeated measures ANOVA main effect of defocus $F(1,50) = 0.668$, $p=0.428$). Significant interactions from post-hoc tests are indicated by asterisks. Error bars represent standard error of mean.

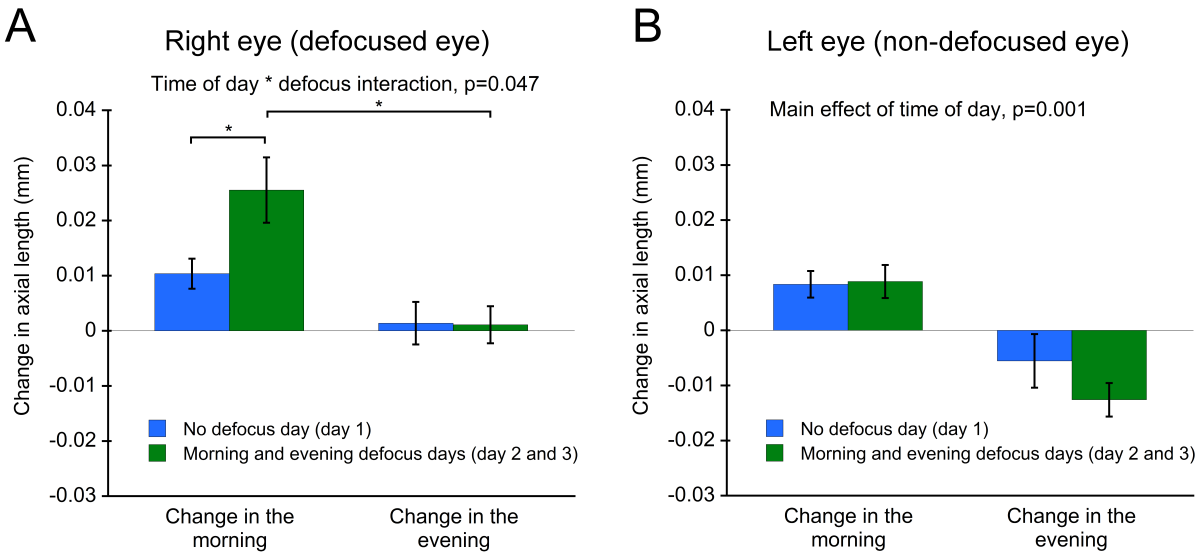
Figure 6: Mean change in sub-foveal choroidal thickness (SFCT) with hyperopic defocus in the morning (between 10 am and 12 pm) and in the evening (5 pm to 7 pm) for the right defocused (A) and left non-defocused (B) eyes. Morning and evening changes in SFCT were calculated for the baseline day (day 1, no defocus, shown in blue bar), and following 2 hours of exposure to defocus in the morning (day 2) and evening (day 3) on two separate days (shown in green bar). (A) In the right eye (defocused) eye, hyperopic defocus caused significant thinning of the choroid on both defocus days (two-way repeated measures ANOVA main effect of defocus $F(1,41) = 12.401$, $p=0.005$). There was no significant interaction between the time of day and defocus treatment (two-way repeated measures ANOVA time of day by defocus interaction $F(1,41) = 0.193$, $p=0.672$). (B) Defocus in the right eye was associated with small changes in the subfoveal choroid of the left (non-defocused) eye (two-way repeated measures ANOVA main effect of defocus $F(1,44) = 5.261$, $p=0.039$). Significant interactions from post-hoc tests are indicated by asterisks. Error bars represent standard error of mean.

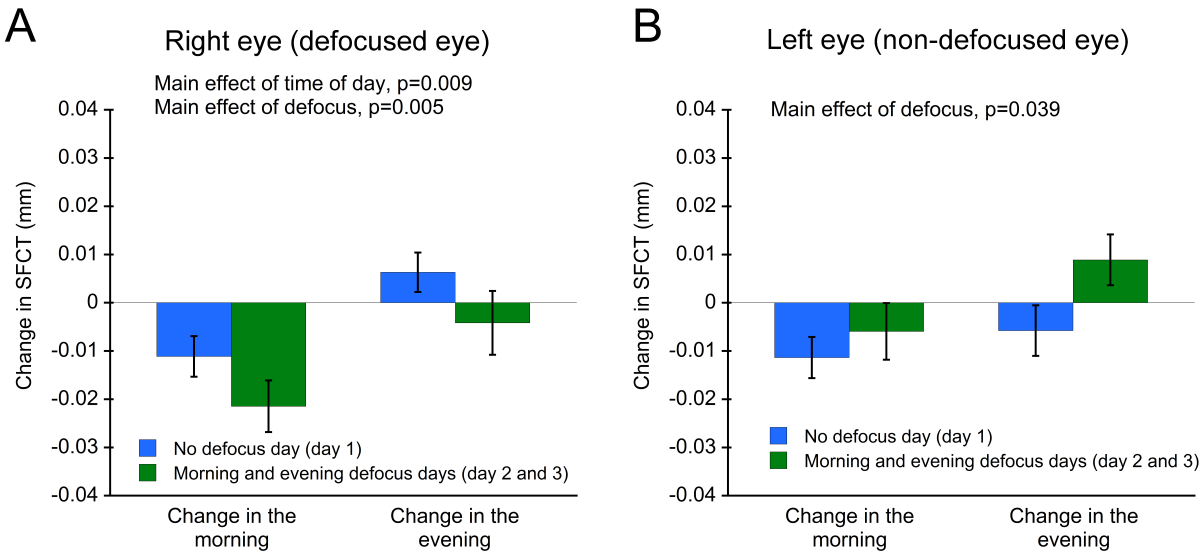












Highlights

- Ocular response to defocus varies depending on the time of day in human subjects
- Evening exposure to myopic defocus causes a larger reduction in axial length
- Only morning exposure to hyperopic defocus causes an increase in axial length
- Axial length changes are associated with opposite changes in choroidal thickness
- Ocular diurnal rhythms may influence the eye's response to defocus in humans