Circadian rhythms are biological or physiological variations that follow an approximate 24-hour cycle. Circadian rhythms are driven intrinsically by endogenous clocks and regulated extrinsically by environmental cues. Light exposure is the most potent environmental cue that regulates circadian rhythms. Circadian rhythms persist under constant conditions, such as constant darkness. A diurnal rhythm is a biological rhythm that is synchronized to the day/night cycle and may or may not be circadian in nature. Diurnal rhythms exist in multiple ocular parameters, including retinal thickness, intraocular pressure, axial length, and choroidal thickness. Diurnal rhythms in axial length and choroidal thickness are altered by light exposure. However, only a limited number of studies have investigated the effects of light exposure on choroidal thickness in the human eye. In humans, increased daily light exposure in the morning for one week resulted in an increase in choroidal thickness throughout the day. In contrast, exposure to 1000 lux for four hours before sleep for five days resulted in a decrease in choroidal thickness in humans. These studies demonstrate that light exposure and ocular diurnal rhythms have been implicated in the regulation of eye growth and refractive error development. It has been shown that rearing chicks under constant light causes excessive eye growth and corneal flattening, resulting in the development of refractive errors. It is hypothesized that the absence of a regular light/dark cycle alters ocular diurnal rhythms, resulting in abnormal eye growth. A previous study showed that a minimum of four hours of continuous darkness provided in an increase in choroidal thickness throughout the day. In contrast, exposure to 1000 lux for four hours before sleep for five days resulted in a decrease in choroidal thickness in humans. These studies demonstrate that light exposure and ocular diurnal rhythms have been implicated in the regulation of eye growth and refractive error development.

PURPOSE. To determine the effects of monocular light deprivation on diurnal rhythms in retinal and choroidal thickness.

METHODS. Twenty participants, ages 22 to 45 years, underwent spectral domain optical coherence tomography imaging every three hours, from 8 AM to 8 PM, on two consecutive days. Participants wore an eye patch over the left eye starting at bedtime of day 1 until the end of the last measurement on day 2. Choroidal, total retinal, photoreceptor outer segment + retinal pigment epithelium (RPE), and photoreceptor inner segment thicknesses were determined.

RESULTS. For both eyes, significant diurnal variations were observed in choroidal, total retinal, outer segment + RPE, and inner segment thickness (P < 0.001). For light-deprived eyes, choroid diurnal variation persisted, although the choroid was significantly thinner at 8 AM and 11 AM (P < 0.01) on day 2 compared to day 1. On the other hand, diurnal variations in retinal thickness were eliminated in the light-deprived eye on day 2 when the eye was patched (P > 0.05). Total retinal and inner segment thicknesses significantly decreased (P < 0.001) and outer segment + RPE thickness significantly increased (P < 0.05) on day 2 compared to day 1.

CONCLUSIONS. Blocking light exposure in one eye abolished the rhythms in retinal thickness, but not in choroidal thickness, of the deprived eye. Findings suggest that the rhythms in retinal thickness are, at least in part, driven by light exposure, whereas the rhythm in choroidal thickness is not impacted by short-term light deprivation.

Keywords: diurnal rhythms, retinal thickness, choroidal thickness, light exposure, light deprivation.
exposure impacts choroidal thickness, although the influence of light on the rhythm in choroidal thickness remains unclear. Diurnal rhythms in retinal thickness have also been demonstrated in humans.\textsuperscript{3,4} Burfield et al.\textsuperscript{4} showed that total retinal thickness and photoreceptor inner segment thickness are thickest during the day and thinnest at night, whereas photoreceptor outer segment + retinal pigment epithelium (RPE) thickness is thinnest during the day and thickest at night. The photoreceptors located in the outer retina are responsible for detection of light entering the eye and are sensitive to changes in light exposure. Photoreceptor outer segment equivalent length has been shown to shorten after 10 to 20 minutes of light adaptation,\textsuperscript{25} which has been suggested to be due to photoreceptor disc shedding, changes in the subretinal space, or RPE variations. However, the effects of light on the diurnal rhythms in retinal thickness have not been extensively investigated.

Given the importance of light exposure in regulating diurnal rhythms and the potential role of light exposure in eye growth and myopia development, the effects of light deprivation on ocular rhythms are of interest. The amount of time spent in different lighting levels has been shown to differ between emmetropic and myopic children.\textsuperscript{26} There is speculation that altered light exposure patterns affect ocular diurnal rhythms and that this may underlie the development of refractive errors.\textsuperscript{15} The purpose of this study was to examine the role of light deprivation in mediating the rhythms in retinal and choroidal thickness in humans.

**METHODS**

**Participants**

Twenty participants, ages 22 to 45 years, were enrolled in this study. The study was approved by the institutional review board at the University of Houston and procedures followed the tenets of the Declaration of Helsinki. Informed consent was obtained after the purpose and risks of the study were explained.

Visual acuity was measured with the participant’s habitual correction. All participants had a best corrected visual acuity of 20/25 or better. Non-cycloplegic autorefraction was measured for both eyes (WAM-5000; Grand Seiko, Tokyo, Japan). Axial length and central corneal thickness were measured using a non-contact low-coherence optical biometer (LenStar; Haag-Streit, Kôniz, Switzerland). Five measurements were collected and averaged for each eye. Exclusion criteria included any ocular pathology, use of prescription or over-the-counter medications known to affect sleep or circadian rhythms, use of sleep aids such as melatonin, and shift work or travel across more than two time zones during the month prior to the study visit. No participants had any systemic disease with ocular manifestations, such as diabetes or hypertension.

**Experimental Protocol**

The study timeline is depicted in Figure 1. Experimental sessions occurred over the course of two consecutive days. Participants wore an actigraphy device (Actiwatch Spectrum Plus; Philips Respironics, Murrysville, PA, USA) to measure light exposure throughout the two days of the study to assess whether light exposure and sleep patterns were similar for the two experimental days. The Actiwatch is a wrist-worn actigraphy device that records ambient illuminance (lux) and activity (counts per minute) at 32 Hz. The Actiwatch was configured to record in one-minute epochs continuously for the two experimental days. Participants were instructed to put on the Actiwatch the day before the first experimental day, and the device was worn continuously until the end of the last measurement on day 2.

**Figure 1.** Study timeline. The experimental sessions occurred over the course of two consecutive days. Participants wore an Actiwatch (indicated in purple) starting the day before experimental day 1 until the end of the final measurement on day 2. SD-OCT imaging was conducted at 8:00 AM, 11:00 AM, 2:00 PM, 5:00 PM, and 8:00 PM on day 1 and day 2. Participants wore an eye patch over the left eye (indicated in gray) starting at their habitual bedtime on day 1 until the end of the final measurement on day 2.
On day 1, ocular imaging was conducted every three hours over a 12-hour period, at 8:00 AM, 11:00 AM, 2:00 PM, 5:00 PM, and 8:00 PM, for a total of five time points. After the last measurement session on day 1, participants were given two eye patches to wear over the left eye, (1) an adhesive eye patch (Nexcare Opticlude; 3M, Maplewood, MN, USA), and (2) a black cloth patch. Participants were instructed to put on both eye patches over the left eye before their habitual bedtime on day 1 and to keep the eye patches on until the end of the final measurement (8:00 PM) on day 2. Ocular imaging was repeated on day 2, with measurements conducted every three hours at the same time points as day 1, from 8:00 AM to 8:00 PM. Eye patches were worn over the left eye throughout the entire experimental day on day 2. Participants were only allowed to remove the eye patches when instructed to do so at each measurement session after the room lights were turned off. Participants went about their daily activities in between measurement sessions on both days. On both measurement days, participants were asked to abstain from caffeinated and alcoholic beverages, which have been shown to affect choroidal thickness.\textsuperscript{27–30}

**Ocular Imaging**

Before each ocular imaging session, participants underwent a 10-minute distance viewing period to minimize effects of prior ocular accommodation and physical activity. During this time, participants sat in the lab and viewed a television 4 m away. Illumination in the laboratory was 350 lux, measured at eye level in the vertical plane (LX1330B; Dr. Meter, Union City, CA, USA). Then, ocular imaging was conducted with spectral domain optical coherence tomography (SD-OCT; Spectralis, Heidelberg, Germany). Two high-quality images (signal strength > 35 dB) of the back of each eye were collected. On day 1, images for both eyes were collected under typical laboratory illumination (350 lux). On day 2, images for the right eye were collected under typical laboratory illumination (350 lux), and images for the left eye (light-deprived eye) were collected in the dark with the computer monitor at the dimmest setting. Eye patches were removed immediately prior to SD-OCT imaging in the dark and put back on immediately after the imaging was complete. The scan protocol included a six-line 30° radial scan centered at the fovea (Fig. 2A). Images were acquired in enhanced depth imaging mode and B-scan averaging was set at 30 frames. For each participant, the first image at the first measurement session on day 1 was set as the reference for subsequent imaging for that particular eye.

**Data Analysis**

SD-OCT images were exported and analyzed with a custom MATLAB program (MathWorks, Natick, MA, USA). A three-surface schematic eye was constructed for each individual as described by Bennett et al.\textsuperscript{31,32} Lateral magnification was calculated based on each individual’s axial length and corneal curvature data.\textsuperscript{33} Images were preprocessed to enhance the contrast of the boundary between the choroid and sclera.\textsuperscript{34} Bruch’s membrane, the inner segment/outer segment junction, and the external and internal limiting membranes were segmented and manually corrected for any segmentation errors (Fig. 2B). The choroid/sclera interface was manually segmented and axial thickness was determined for 1536 points along each of the six scan lines. Total retinal, photoreceptor outer segment + RPE, photoreceptor inner segment, and choroidal thickness were determined from the segmentations. A retinal thickness map was generated from the segmentations and the center of the fovea was manually located for each image based on the thinnest region in the center of the retinal thickness map (Fig. 2C). Although the full scan was 30°, the average thickness in a 1-mm diameter centered at the fovea was calculated for each parameter. It has been shown that the choroid follows a similar diurnal amplitude of change in the central 3-mm and 6-mm regions surrounding the fovea.\textsuperscript{4} Similarly, Read et al.\textsuperscript{23} also reported that the 2-mm regions adjacent to the 1-mm foveal region exhibited similar changes in choroidal thickness after light therapy. Bland-Altman analysis\textsuperscript{55} was used to assess within-session repeatability for retinal and choroidal thickness measurements in the central 1-mm region based on the first measurement session on each day for the right eye. The mean difference and 95% limits of agreement between the two images collected at the same time point were determined.

Actiwatch data were downloaded and analyzed using the Actiware software (Actiware Version 6.0.8; Philips Respironics). Wake-up time and bedtime were determined, and average hourly light exposure in lux was calculated for each day.

**Statistical Analysis**

Statistical analysis was performed with IBM SPSS Statistics version 28 (IBM, Armonk, NY, USA). Data are expressed...
as mean ± standard deviation unless otherwise stated. For retinal and choroidal thickness, the change in thickness at each time point was calculated relative to the first measurement on day 1. Two-way repeated measures ANOVA with within-subjects factors, measurement day and time of day, was performed. Post hoc paired t-tests were corrected for multiple comparisons using the Benjamini-Hochberg post hoc correction. Right and left eyes were analyzed separately. Normality was assessed with the Shapiro-Wilk test. Paired t-tests, or Wilcoxon signed-rank tests for nonparametric data, were used to compare the amplitudes of change in retinal and choroidal thickness, measured as the difference between the maximum and minimum thickness measurements, between the two days of measurement. Light exposure data were not normally distributed (Shapiro-Wilk test, P < 0.05). Data were log transformed and two-way repeated measures ANOVA was used to compare hourly light exposure between the two days of measurement. P < 0.05 was considered statistically significant.

**RESULTS**

Mean age of all participants was 28.5 ± 6.8 years. Mean spherical equivalent refraction of the right and left eyes was −2.19 ± 2.54 D and −2.07 ± 2.55 D, respectively (P = 0.34). Mean axial length of the right and left eyes was 24.72 ± 1.45 mm and 24.66 ± 1.42 mm, respectively (P = 0.17).

Average wake-up time, determined from the Actiwatch, for the two experimental days was 6:48 AM (±35 minutes), and bedtime was 11:49 PM (±92 minutes). Average hourly light exposure for day 1 and day 2 is shown in Figure 3. There was no significant difference in average hourly light exposure from 6:00 AM to 8:00 PM between day 1 (427 ± 200 lux) and day 2 (355 ± 126 lux; P = 0.42), indicating that the control eye, which was exposed to the normal light/dark cycle, experienced similar light exposure on both days.

**Bland-Altman analysis** was used to assess within-session repeatability of the retinal and choroidal thickness measurements (Fig. 4). For choroidal thickness, the mean difference between the two images collected at the same time point was −0.73 ± 4.50 μm (95% limits of agreement from 8.08 to −9.55 μm). The mean difference between the two images collected at the same time point was 0.29 ± 1.12 μm (95% limits of agreement from 2.48 to −1.90 μm) for total retinal thickness, 0.21 ± 0.86 μm (95% limits of agreement from 1.90 to −1.48 μm) for photoreceptor outer segment + RPE thickness, and 0.11 ± 0.78 μm (95% limits of agreement from 1.63 to −1.41 μm) for photoreceptor inner segment thickness.

**Choroidal Thickness**

Choroidal thickness for both eyes and for each day is shown in Figure 5A. For control eyes exposed to the normal light/dark cycle on both days, repeated measures ANOVA revealed a significant main effect of time of day (P < 0.001), but not for day of measurement (P = 0.13). The interaction between time of day and day of measurement was not significant (P = 0.43; Table 1). Therefore, mean choroidal thickness was not significantly different between day 1 (339.3 ± 105.1 μm) and day 2 (337.2 ± 101.9 μm), but significant diurnal variation was observed on both days. The choroid was thinnest at 2:00 PM and thickest at 8:00 AM and 8:00 PM. The amplitude of change in choroidal thickness (i.e., the difference between the maximum and minimum measurements) in control eyes was not significantly different between day 1 and day 2 (P = 0.48; Table 2).

For light-deprived eyes, which were light deprived only on day 2, repeated measures ANOVA revealed a significant main effect of time of day (P < 0.001) and day of measurement (P = 0.01; Table 1). Mean choroidal thickness was 354.9 ± 105.9 μm and 351.5 ± 103.9 μm on day 1 and day 2.
FIGURE 4. Bland-Altman analysis of the difference between scan 2 and scan 1 collected at the same time point in the right eye for (A) choroidal thickness, (B) total retinal thickness, (C) photoreceptor outer segment + RPE thickness, and (D) photoreceptor inner segment thickness to assess within-session repeatability. The dashed and dotted lines represent the mean difference and 95% limits of agreement, respectively. Shaded areas represent the 95% confidence intervals for the mean difference and 95% limits of agreement.

respectively. Similar to control eyes, the choroid was thinnest at 2:00 PM and thickest at 8:00 AM and 8:00 PM on both days. A significant interaction effect between time of day and day of measurement was also observed ($P = 0.02$). The choroid was significantly thinner on day 2, when eyes were light deprived, compared to day 1 at 8:00 AM and 11:00 AM ($P < 0.001$ and $P = 0.005$, respectively). However, the amplitude of change in choroidal thickness in light-deprived eyes was not significantly different between day 1 and day 2 ($P = 0.06$; Table 2).

**Total Retinal Thickness**

Total retinal thickness for both eyes and for each day is shown in Figure 5B. For control eyes, total retinal thickness exhibited significant diurnal variation on both days ($P < 0.001$). Total retinal thickness was thickest at 2:00 PM and thinnest at 8:00 AM and 8:00 PM. Mean total retinal thickness was not significantly different between day 1 ($272.1 \pm 16.5 \mu m$) and day 2 ($272.0 \pm 16.2 \mu m$; $P = 0.67$). The interaction effect between time of day and day of measurement was not significant ($P = 0.26$; Table 1). The amplitude of change in total retinal thickness in control eyes was not significantly different between day 1 and day 2 ($P = 0.22$; Table 2).

For light-deprived eyes, mean total retinal thickness was significantly thinner on day 2 ($268.2 \pm 15.9 \mu m$), when eyes were light deprived, compared to day 1 ($270.7 \pm 16.2 \mu m$; $P < 0.001$). There was a significant interaction effect between time of day and day of measurement ($P = 0.01$; Table 1). Post hoc pairwise comparisons showed that the photoreceptor outer segment + RPE was significantly thicker on day 2 compared to day 1 at all time points ($P < 0.001$ for all), except 8:00 AM ($P = 0.05$). Total retinal thickness exhibited significant diurnal variation on day 1. However, no diurnal variation was observed on day 2 when eyes were light deprived. The amplitude of change in total retinal thickness in light-deprived eyes was significantly reduced on day 2 compared to day 1 ($P = 0.006$; Table 2).

**Photoreceptor Outer Segment + RPE Thickness**

Photoreceptor outer segment + RPE thickness for both eyes and for each day is shown in Figure 5C. For control eyes, significant diurnal variation was observed in photoreceptor outer segment + RPE thickness on both days ($P < 0.001$). The photoreceptor outer segment + RPE was thickest at 8:00 AM and significantly thinned throughout the day. Mean photoreceptor outer segment + RPE thickness was $60.0 \pm 2.0 \mu m$ and $60.0 \pm 2.2 \mu m$ on day 1 and day 2, respectively ($P = 0.97$). The interaction effect between time of day and day of measurement was not significant ($P = 0.82$; Table 1). The amplitude of change in photoreceptor outer segment + RPE thickness in control eyes was not significantly different between day 1 and day 2 ($P = 0.32$; Table 2).

For light-deprived eyes, mean photoreceptor outer segment + RPE thickness was significantly thicker on day 2 ($61.4 \pm 2.3 \mu m$), when eyes were light deprived, compared to day 1 ($59.9 \pm 2.0 \mu m$; $P < 0.001$). A significant interaction effect between time of day and day of measurement was observed ($P = 0.004$; Table 1). Post hoc pairwise comparisons showed that the photoreceptor outer segment + RPE was significantly thicker on day 2 compared to day 1 at all time points ($P < 0.05$ for all). Significant diurnal variation in photoreceptor outer segment + RPE thickness was observed on day 1, but no diurnal variation was observed on day 2 when eyes were light deprived. The amplitude of change in photoreceptor outer segment + RPE thickness in
light-deprived eyes was significantly reduced on day 2 compared to day 1 ($P = 0.002$; Table 2).

**Photoreceptor Inner Segment Thickness**

Photoreceptor inner segment thickness for both eyes and for each day is shown in Figure 5D. For control eyes, photoreceptor inner segment thickness exhibited significant diurnal variation on both days ($P < 0.001$). The photoreceptor inner segment was thinnest at 8:00 AM and significantly thickened throughout the day. Mean photoreceptor inner segment thickness was $30.4 \pm 1.7 \mu m$ and $30.2 \pm 1.6 \mu m$ on day 1 and day 2, respectively ($P = 0.32$). The interaction effect between time of day and day of measurement was not significant.

**Table 1.** Summary of Results From Two-Way Repeated Measures ANOVA for Factors Day of Measurement and Time of Day and the Interaction Between Day of Measurement and Time of Day

|                      | Eye                | Day of Measurement | Day of Measurement × Time of Day |
|----------------------|--------------------|--------------------|----------------------------------|
|                      |                    | Time of Day        |                                  |
| Choroidal thickness  | Control Eye        | 0.13               | 0.43                             |
|                      | Deprived Eye       | 0.01               | 0.02                             |
| Total retinal thickness | Control Eye   | 0.67               | 0.26                             |
|                      | Deprived Eye       | $<0.001^*$         | 0.07                             |
| Photoreceptor outer segment + RPE thickness | Control Eye | 0.97               | 0.82                             |
|                      | Deprived Eye       | $<0.001^*$         | 0.004                             |
| Photoreceptor inner segment thickness | Control Eye | 0.32               | 0.31                             |
|                      | Deprived Eye       | $<0.001^*$         | $<0.001^*$                        |

Control eyes were exposed to the normal light/dark cycle on day 1 and day 2, whereas experimental eyes underwent monocular light deprivation on day 2. * Indicates significance at $P < 0.05$. 

*Asterisk indicates significant difference between day 1 and day 2 in the light-deprived eye (red lines). Error bars represent standard error of the mean. Control eyes were exposed to the normal light/dark cycle on day 1 and day 2, whereas experimental eyes underwent light deprivation on day 2.*
(P = 0.31; Table 1). The amplitude of change in photoreceptor inner segment thickness in control eyes was not significantly different between day 1 and day 2 (P = 0.10; Table 2).

For light-deprived eyes, mean photoreceptor inner segment thickness was significantly thinner on day 2 (28.6 ± 1.5 μm), when the eye was light deprived, compared to day 1 (30.1 ± 1.5 μm; P < 0.001). There was a significant interaction effect between time of day and day of measurement (P < 0.001; Table 1). Post hoc pairwise comparisons showed that the photoreceptor inner segment was significantly thinner on day 2 compared to day 1 at all time points (P < 0.001 for all). Significant diurnal variation in photoreceptor inner segment thickness was observed on day 1, similar to the amplitude observed in control eyes. The amplitude of change in photoreceptor inner segment thickness in light-deprived eyes was significantly reduced on day 2 compared to day 1 (P = 0.01; Table 2).

**DISCUSSION**

This study demonstrates that short-term monocular light deprivation alters diurnal rhythms in retinal thickness, but not in choroidal thickness. Additionally, light deprivation had a significant effect on the overall thickness of both the retina and the choroid. The diurnal rhythms in retinal thickness were eliminated when the eye was light deprived, indicated by the lack of a significant variation in thickness throughout the day. Consistent with previous studies, choroidal thickness thinned in the morning and thickened from afternoon to evening. This pattern of diurnal variation in choroidal thickness persisted even when one eye was light deprived during the day, with a small decrease in thickness in the light-deprived eye.

Recent studies in humans examining the effects of light exposure on choroidal thickness have reported changes in the thickness of the choroid with increased light exposure. Read et al. examined the effects of 30 minutes of morning light therapy for seven days on choroidal thickness and reported a statistically significant increase in choroidal thickness after one week. Alun et al. examined the effects of four hours of high level of illumination (1000 lux) before sleep for five consecutive nights and showed that the choroid significantly thinned after nighttime light exposure. Both studies measured the diurnal variation in choroidal thickness and found no significant changes in the phase or timing of the diurnal variation, despite an overall change in thickness. In this study, we found that monocular light deprivation resulted in a decrease in the thickness of the choroid in the eye that was light deprived only in the morning, with no effect on the choroid of the fellow control eye that was exposed to the normal light/dark cycle. The magnitude of the decrease in choroidal thickness in the morning was approximately 5 to 6 μm, similar to the magnitude of change in choroidal thickness reported by Read et al.25

The effect of constant darkness on the rhythm in choroidal thickness has been previously examined in chicks. The rhythm in choroidal thickness was shown to persist when chicks were reared in constant darkness for four days, defining it as an endogenous circadian rhythm. The authors also showed that the choroid thinned by approximately 7 μm/d in constant darkness. Previous studies in humans have reported changes in choroidal thickness with short-term dark exposure. In one study, subfoveal choroidal thickness was shown to increase after 30 minutes of dark adaptation in the evening. In contrast, one hour of dark exposure in the morning resulted in choroidal thinning. Overall, results of these previous studies suggest that the timing and duration of light or dark exposure may have differential effects on choroidal thickness. While we observed that the choroid was thinner in the morning hours when the eye was light deprived, whether the decrease in choroidal thickness was a result of an overall thinning of the choroid or a change in the amplitude or phase of the rhythm in choroidal thickness requires further investigation. Future studies measuring the change in choroidal thickness over a 24-hour period and over several days of light deprivation would better characterize the influence of light on the phase and amplitude of the rhythm in choroidal thickness.

It is speculated that the rhythm in choroidal thickness may play a role in the regulation of eye growth. The rhythm in choroidal thickness is altered in chicks during the development of experimental myopia or hyperopia. In addition, increased exposure to light at night was shown to disrupt the rhythm in choroidal thickness in chicks, which may be associated with increased eye growth. However, disruptions in the rhythm in choroidal thickness were not consistently associated with changes in eye growth when chicks were exposed to brief periods of bright light at different times of day. Thus the relationship between light exposure, choroidal rhythms, and refractive error development is not fully understood. Given that light exposure is important for synchronizing diurnal rhythms, understanding how light influences the rhythm in choroidal thickness may help to elucidate its role in myopia development.

The mechanisms underlying the diurnal variation in choroidal thickness are also not fully understood. There is speculation that changes in choroidal blood flow play a role in the changes in choroidal thickness. In guinea pigs, changes in choroidal blood perfusion were positively correlated with changes in choroidal thickness during
experimentally induced myopia. However, whether changes in choroidal blood perfusion are responsible for changes in choroidal thickness or vice versa, or whether there is another common factor involved, is unknown. Moreover, it has been shown that the diurnal changes in choroidal thickness are primarily due to changes in the luminal area (i.e., the vascular area) and not the stromal area of the choroid. In humans, subfoveal choroidal blood flow was also shown to be affected by light exposure, decreasing during dark adaptation, then increasing after subsequent light adaptation. Other potential mechanisms underlying the changes in choroidal thickness that have been proposed include changes in the synthesis of osmotically active proteoglycans to regulate water content, modulation of fluid transport between the retina and choroid, or changes in the tenuis of the nonvascular smooth muscle of the choroid (for a review see Nickla and Wallman, 2010). Further studies are required to determine whether these processes are involved in the mechanisms underlying the diurnal changes in choroidal thickness and changes with light exposure.

In addition to choroidal thickness, we examined the changes in the diurnal variation in retinal thickness with monocular light deprivation. The effects of light exposure on outer retinal thickness have been examined previously in humans using SD-OCT. Abràmoff et al. examined changes in photoreceptor outer segment equivalent length, measured from the inner segment/outer segment junction to the inner surface of the RPE, after 10 to 20 minutes of light adaptation, and reported a significant decrease in outer segment equivalent length of approximately 2 μm. Alagöz et al. measured photoreceptor layer thickness, defined as the overall thickness of the myoid zone, ellipsoid zone, and outer segments of the photoreceptors, under dark and light adaptation and found no significant changes in photoreceptor layer thickness between adaptation states. Ahn et al. examined the effects of high level of illumination before sleep on subfoveal photoreceptor layer thickness, measured from the inner surface of the ellipsoid zone to the outer surface of the RPE, and reported no changes in subfoveal photoreceptor layer thickness after exposure to high level of illumination. Differences in the results from previous studies could be attributed to the differences in the definition of the borders of the photoreceptor layer or outer segment or other potential methodological differences.

In the current study, we measured both photoreceptor outer segment + RPE thickness, defined as the distance from the inner segment/outer segment junction to Bruch’s membrane, and photoreceptor inner segment thickness, defined as the distance from the external limiting membrane to the inner segment/outer segment junction. We have previously shown in our laboratory that it is possible to measure diurnal changes in total retinal, photoreceptor outer segment + RPE, and photoreceptor inner segment thickness with high repeatability. Here, we observed that the rhythms in retinal thickness were abolished when the eye was light deprived during the day. Light deprivation caused opposite changes in the photoreceptor outer segment + RPE and photoreceptor inner segment thicknesses. When the eye was light deprived, the photoreceptor outer segment + RPE was thicker, whereas the photoreceptor inner segment was thinner, compared to day 1 when the eye was exposed to the normal light/dark cycle. The rhythms in the fellow eye, which was not light deprived, were maintained. Given that these rhythms do not persist under light deprivation (i.e., constant darkness), the findings suggest that these retinal rhythms are not endogenous. Although the magnitude of the changes in outer retinal thickness was small, the changes observed were consistent between individuals (standard deviation <2 μm) and similar in magnitude to previously reported diurnal changes, as well as changes under light adaptation. A more complete quantification of the changes in the retina with light deprivation would require measuring the inner retinal layers, which was outside the scope of this study.

The circadian clock in the retina has been shown to drive several processes, including gene expression, melatonin release, dopamine synthesis, and rod photoreceptor disc shedding. Both rod and cone outer segments undergo daily disc shedding and renewal. The pattern of photoreceptor disc shedding exhibits a diurnal rhythm and is influenced by the light/dark cycle, which has been demonstrated in several species ex vivo. In rodents, rod outer segment disc shedding was shown to persist in constant darkness, defining it as a circadian rhythm, and evidence suggests that circadian control of the rod disc shedding rhythm occurs locally within the eye. Whether photoreceptor disc shedding in humans is also controlled by an endogenous retinal clock is unknown. However, it is speculated that photoreceptor disc shedding may contribute to the changes observed in outer retinal thickness measured with OCT.

Cone photoreceptor disc shedding has been measured in humans in vivo with adaptive optics OCT and reported to be highest in the morning after light onset and lowest in the evening. The authors reported that the cone photoreceptor outer segment decreased by 2.1 μm on average per shedding event, which is similar in magnitude to the changes observed here in photoreceptor outer segment + RPE thickness. The diurnal rhythm in cone photoreceptor disc shedding was also shown to be affected by the timing of light onset (Zhang F, et al. IOVS 2018;59:ARVO E-Abstract 730). In addition to the photoreceptor outer segments, changes may also occur in the subretinal space and RPE in response to light exposure. It has been demonstrated in animals ex vivo that light exposure leads to an expansion of the subretinal space volume, and the RPE is believed to be involved in the light-dependent changes in subretinal space volume. In humans, a decrease in optical path length between the inner segment/outer segment junction and RPE, measured in vivo using OCT, was reported after light stimulation in the peripheral retina. This change was attributed to a decrease in the volume of the subretinal space and not a change in the length of the photoreceptor outer segments. Further studies are warranted to characterize the mechanisms underlying the light-dependent changes in outer retinal thickness observed with OCT.

In the current study, participant ages ranged from 22 to 45 years, and refractive errors ranged from −6.3 to +1.5 D. We did not observe variations in ocular rhythms based on age in this study. A previous study in our laboratory examined ocular rhythms in children ages 5 to 14, including retinal and choroidal thickness rhythms, and found that the amplitude and phase of the rhythms were similar to ocular rhythms reported in adults ages 18 to 30 and across 25 to 41. Studies in humans have reported that there are no significant differences in diurnal rhythms in choroidal thickness between emmetropic and myopic adults. Therefore it is unlikely that the range of ages and refractive errors of the participants in the current study influenced the observed findings.
During the experiment, we did not control for participants’ bedtimes and wakeup times. However, we tracked the times objectively using a wrist-worn actigraphy device. On average, there were no significant differences in bedtime and wakeup time between day 1 and day 2. In addition, the rhythms in retinal and choroidal thickness of the control eye were similar on day 1 and day 2. As such, we do not believe that variable bedtimes and wakeup times between participants had a significant effect on the overall results. Participants were asked to avoid caffeine and alcohol during the experiment, and otherwise went about their daily routine between measurements. Therefore there may have been other variables in behavior that could influence ocular measurements. To minimize the effects of prior near work and physical activity and to normalize light exposure conditions, all participants underwent 10 minutes of distance viewing under standardized light conditions in the laboratory before each measurement session.

In conclusion, these findings contribute to a better understanding of the role of light exposure in regulating ocular diurnal rhythms. Given the growing evidence that light exposure and circadian rhythms are involved in eye growth, these findings may provide further insight into their involvement in refractive error development. We found that monocular light deprivation altered the rhythms in retinal thickness, but not in choroidal thickness. The diurnal rhythms in retinal thickness were eliminated in the eye that was light deprived, with no effect on the fellow eye. In contrast, the rhythm in choroidal thickness was maintained in both eyes, albeit with a small amount of choroidal thinning in the morning. Findings suggest that the rhythms in outer retinal thickness are influenced by the light/dark cycle, rather than being driven solely by an endogenous clock, whereas the diurnal rhythm in choroidal thickness is robust to alterations in the light exposure pattern.

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