Diurnal Variations in Axial Length, Choroidal Thickness, Intraocular Pressure, and Ocular Biometrics

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Purpose. To investigate the pattern of diurnal variations in axial length (AL), choroidal thickness, intraocular pressure (IOP), and ocular biometrics over 2 consecutive days.

Methods. Measurements of ocular biometrics and IOP were collected for 30 young adult subjects (15 myopes, 15 emmetropes) at 10 different times over 2 consecutive days. Five sets of measurements were collected each day at approximately 3-hour intervals, with the first measurement taken at ~9 AM and final measurement at ~9 PM.

Results. AL underwent significant diurnal variation ($P < 0.0001$) that was consistently observed across the 2 measurement days. The longest AL was typically observed at the second measurement session (mean time, 12:26) and the shortest AL at the final session of each day (mean time, 21:06). The mean diurnal change in AL was 0.032 ± 0.018 mm. Choroidal thickness underwent significant diurnal variation (mean change, 0.029 ± 0.016 mm; $P < 0.001$) and varied approximately in antiphase to the AL changes. Significant diurnal variations were also found in vitreous chamber depth (VCD; mean change, 0.06 ± 0.029 mm; $P < 0.0001$) and IOP (mean change, 3.54 ± 0.84 mm Hg; $P < 0.0001$). A positive association was found between the variations of AL and IOP ($r^2 = 0.17$, $P < 0.0001$) and AL and VCD ($r^2 = 0.31$, $P < 0.0001$) and a negative association between AL and choroidal thickness ($r^2 = 0.13$, $P < 0.0001$). There were no significant differences in the magnitude and timing of diurnal variations associated with refractive error.

Conclusions. Significant diurnal variations in AL, choroidal thickness, and IOP were consistently observed over 2 consecutive days of testing. (*Invest Ophthalmol Vis Sci.* 2011;52:5121–5129) DOI:10.1167/iovs.11-7364

It is well established that diurnal variations occur in a range of anatomic and physiological parameters of the eye, such as intraocular pressure (IOP),[1–4] corneal thickness,[5–7] corneal topography,[5,8] and anterior chamber biometrics.[4,9] The use of precise noncontact measurement techniques, such as partial coherence interferometry, has led to the finding that significant diurnal variation (range, 25–45 μm) also occurs in the axial length (AL) of the human eye, with the longest AL occurring during the day and the shortest during the night.[3,4,10] However, the relative consistency of diurnal AL rhythms across subjects and between days has been questioned.[3,10] In previous studies in which diurnal measures were performed on 2 separate days, the measurement days were separated by weeks and sometimes months,[3,10] which leaves open the possibility that longer term factors (e.g., seasonal variations) may have influenced some of the reported variability between days.

Although the presence of diurnal rhythms in the AL of human eyes is now well accepted, less is known about the mechanisms and ocular changes underlying these diurnal variations. One factor that is potentially involved in these changes is the eye's IOP. Surgically[11–13] or mechanically[14] induced variations in IOP have been shown to be associated with changes in AL, which leaves open the possibility that the known diurnal variations in IOP[15] play a role in diurnal AL variations. The exact relationship between diurnal AL and IOP changes is unclear, with some recent studies suggesting that there is no relationship between the two variables[3] and others reporting a weak but significant positive association between the variations in AL and IOP.[4]

As AL measurements have typically involved the determination of the distance from the cornea to the retinal pigment epithelium (RPE), diurnal changes in AL could be modulated by variations in the thickness of the choroid. There is evidence from research in chickens[16–18] and marmosets[19] that choroidal thickness (CT) does undergo diurnal variations (increasing during the night and decreasing during the day). However, there have been only very limited investigations of diurnal variations of the choroid in human eyes. Brown et al.[20] in a retrospective analysis of partial coherence interferometry data, recently reported evidence of diurnal variations in CT in human eyes, and a trend for the choroid to fluctuate approximately in antiphase to AL in a small population of subjects.

As AL is the primary biometric determinant of refractive error, diurnal variations in this biometric parameter could be influenced by refractive error. Several animal studies have suggested a relationship between diurnal AL rhythms and refractive error.[16–18,21] Studies of chickens have found that induced form deprivation[16–18] or constant darkness[21] causes significant axial elongation and the development of myopia and also alters the normal diurnal rhythms of both AL and CT. Animal studies also suggest that phase differences between AL and choroidal rhythms may be an important factor in the regulation of ocular growth.[18,21] However, no previous study has prospectively investigated the potential differences in the magnitude and timing of diurnal AL rhythms between myopic and emmetropic human subjects.

In this study we sought to further investigate the underlying origins, relative consistency, and influence of refractive error on the diurnal variation of AL in human eyes, through measurements of ocular biometry (using an instrument capable of determining a comprehensive range of ocular biometric measures including CT) and IOP performed over 2 consecutive

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days, in populations of young adult emmetropic and myopic subjects.

METHODS

Subjects and Procedure

Thirty young adult subjects aged between 18 and 30 years (mean ± SD, 25.16 ± 3.32) were recruited for the study. Seventeen of the subjects were male. None of the participants had any history of significant ocular or systemic disease, ocular injury, or surgery. Before the study, each subject underwent an initial ophthalmic screening to ensure good ocular health and to determine their refractive status. The subjects were classified according to their spherical equivalent refraction (SER) as either emmetropes (SER, +0.75 to −0.75 DS, n = 15; mean, −0.15 ± 0.31 DS) or myopes (SER, ≥ −1.00 DS, n = 15; mean, −3.95 ± 1.41 DS). No subject exhibited anisometropia greater than 1.00 DS or cylindrical refraction greater than 1.25 DC. All subjects had normal logMAR visual acuity of 0.00 or better.

Among the myopes, three subjects wore soft contact lenses. These subjects discontinued wearing the lenses for 1 week before the study and abstained from wearing them for the duration of the study. No wearers of rigid gas-permeable (RGP) contact lenses were included. As the intake of alcohol[22] and caffeine[23] have been found to influence IOP, all participants were asked to abstain from consuming alcoholic and caffeinated beverages from the evening before and for the 2-day duration of the study. Subjects were instructed to maintain consistent sleep/wake cycles for 7 days before commencement of the study, which was confirmed through completion of a modified version of the Pittsburgh Sleep Quality Index (PSQI) questionnaire by each participant.[24] All subjects had an average sleep duration of >5 hours and average sleep efficiency >65%. Approval from the university human research ethics committee was obtained, and written informed consent was obtained from all subjects. All subjects were treated in accordance with the Declaration of Helsinki.

To investigate ocular diurnal variations in each subject, we took a series of measurements of ocular biometrics and IOP over 2 consecutive days. On each day, five measurement sessions were conducted at 2.5- to 3-hour intervals, with the first measurement taken at approximately 9 AM (~1–2 hours after the subjects had awakened) and the final measurement at approximately 9 PM. One emmetropic subject was unable to attend session 3 only on the first day of measurements. Each session took approximately 10 to 15 minutes to complete all the measurements and undertook their regular daily activities between measurement sessions.

Collecting the first measurement 1 to 2 hours after waking avoided the potential confounding of the results by the large changes in anterior eye parameters that are typically observed immediately after waking (as observed in central corneal thickness, [CCT] and anterior chamber depth [ACD]).[4] Noncontact techniques were used for all measurements, to avoid any corneal epithelial disruption as a result of instruments that contact the eye or the use of any anesthetic eye drops.[25] The order of clinical measurements was randomized at each measurement session for each subject, to minimize the risk of systematic bias.

A noncontact optical biometer (Lenstar LS 900; Haag-Streit AG, Köniz, Switzerland) was used to obtain the measurements of AL and other ocular biometric parameters. This instrument works on the principle of optical low-coherence reflectometry (OLCR) and recent studies have found that it provides highly repeatable results (reported intra- and inter-session repeatability for AL is 0.016 and 0.006 mm, respectively) that are comparable with other validated instruments.[26,27] The following ocular biometric measures were collected: CCT (the distance from the anterior to posterior corneal surface), ACD (the distance from the posterior corneal surface to the anterior crystalline lens surface), lens thickness ([LT], the distance from the anterior to posterior lens surface), vitreous chamber depth ([VCD], the distance from the posterior lens surface to the inner limiting membrane), and AL (the distance from the anterior corneal surface to the RPE). Seven measurements were obtained for each biometric parameter at each measurement session and were later averaged.

In addition to these automatically derived ocular measurements, manual analysis of the biometer data was performed to determine retinal thickness ([RT], distance from inner limiting membrane to RPE) and CT (distance from RPE to choroid–sclera interface). Previous studies using instruments based on similar principles have shown that the A-scan data originating from the posterior eye typically contain a series of peaks corresponding to retinal and choroidal structures,[20,28] with the anterior peak (P1) thought to originate from the inner limiting membrane of the retina, the central peak (P3) from the RPE, and the posterior peak (P4) from the choroid–sclera interface. Manually determining interpeak distances from the posterior portion of the A-scan by adjusting the retinal cursors with the biometer's software allows the determination of RT (distance from P1 to P3) and CT (distance from P3 to P4) from each subject's biometry data (method detailed elsewhere).[29–31] RT and CT derived from the biometer (Lenstar; Haag-Streit) have been shown to correlate closely with RT and CT measured with spectral domain OCT.[31] An independent, masked observer performed the manual analysis to determine RT and CT for each subject.

A noncontact tonometer (Ocular Response Analyzer [ORA]; Reichert, Depew, NY) was used to measure IOP at each session. This tonometer[32] has been found to provide IOP measures that agree closely with Goldmann tonometry.[33,34] The instrument provides two estimates of IOP: IOPg, which is a Goldmann-correlated IOP measurement, and IOPcc, which takes corneal biomechanical properties into account and has been reported to be less affected by corneal properties than other tonometric techniques.[32,33] Given that corneal parameters such as CCT are known to vary diurnally,[4] we assumed that using IOPcc should provide a more reliable assessment of diurnal IOP changes. The mean IOPcc was calculated for each subject at each measurement session from a total of four readings at each session.

Data Analysis

After data collection, the average of all biometric parameters and IOP measures for each subject at each measurement session were calculated. The amplitude of change (the difference between the maximum and minimum) for days 1 and 2 and the average amplitude were also calculated for each variable. A repeated-measures analysis of variance (ANOVA) with two within-subject factors (time of day and day of measurement) and one between-subjects factor (refractive error) was performed to determine the significant diurnal changes in each of the parameters and to identify any significant differences between the refractive error groups. This analysis assumes that time of day and day of measurement are categorical variables. Although it was not logistically possible to collect all measurements at each session for each subject at the exact same time, any intersubject variability in measurement time was small compared with the period between each measurement session (as highlighted by the horizontal error bars in Figs. 1–3). To investigate any significant association between the changes in AL and changes in the other measured parameters, an analysis of covariance (ANCOVA) was performed for the analysis of repeated measures.[35] To provide an assessment of the within-session measurement variability of each of the measured ocular parameters, we analyzed the data from each subject to calculate the within-subject SD, range, and coefficient of variation of the repeated measurements of each variable collected at each session (SPSS for Windows; ver. 17.0; Chicago, IL).

Previous studies of ocular diurnal variations have used sine or cosine curve fitting to quantify the timing and amplitude of 24-hour diurnal ocular rhythms.[4,15,36] However, our protocol involved measurements between approximately 9 AM and 9 PM, which meant that no measurements were collected in the 12 hours (including sleep time) between the final measurement on day 1, and the first measurement on day 2. The reliability of any sine curve fitting to this data is therefore likely to be reduced, given that the fitting routines typically

TABLE 1. Overview of the Mean within-Subject Variability for the Repeated Measures Collected at Each Measurement Session for Each of the Measured Ocular Variables

Variables	Mean within-Session Standard Deviation	Mean within-Session Range	Mean Coefficient of Variation (%)
CCT, mm	0.002	0.007	0.46
ACD, mm	0.016	0.042	0.51
LT, mm	0.021	0.055	0.59
VCD, mm	0.021	0.058	0.12
AL, mm	0.009	0.023	0.04
RT, mm	0.006	0.015	2.84
CT, mm	0.015	0.038	5.69
IOP, mm Hg	0.70	1.53	5.21

assume a 24-hour period and that no measurements were performed for 12 of the 24 hours. However, to allow comparison with previous research and to illustrate the average amplitude and timing of the diurnal rhythms of our primary variables of interest (ACD, VCD, AL, CT, and IOP), sine curve fitting was applied, using least-squares fitting to the pooled data for all subjects at all measurement sessions over the 2 days of testing. To allow all subjects' data to be considered together, we normalized the data from each subject to the mean (i.e., expressed as the change from the mean of all measurements) at each time point before performing the sine curve fitting. This fitting determined the group mean amplitude (peak to trough difference) of change, the time at which the peak in each of the measured parameters occurred (the acrophase), as well as the 95% confidence interval (CI) of these parameters and root mean square (RMS) fit error. The following sine curve equation was used to fit the diurnal data:

$$y = \frac{a}{2}\sin\left(2\pi\frac{\text{time}}{24} + c\right)$$

where a is the amplitude (peak-trough difference) and c is the time phase of the sine curve with a fixed period of 24 hours.

RESULTS

Within-Session Repeatability

Table 1 illustrates the within-subject SD, range, and coefficient of variation for the repeated measures of each of the ocular variables collected at each session. It is evident from these data that the within-session variability was small for both the biometric (within-session SD ranges, 0.002–0.021 mm) and IOP (within-session SD, 0.70 mm Hg) measurements.

Diurnal Variation

Repeated-measures ANOVA revealed that a range of ocular biometric parameters (CCT, ACD, AL, VCD, and CT) and IOP measures (Table 2) underwent significant diurnal variation ($P < 0.05$, within-subject effects of time) over the 2 days of testing. The magnitude of diurnal variation in these parameters is typically substantially larger than the within-session measurement variability reported in Table 1. None of the ocular biometrics or IOP measures exhibited a significant time–refractive error interaction ($P > 0.05$), indicating similar magnitude and pattern of diurnal variations for each of the measured variables between the myopic and emmetropic groups. In addition, repeated-measures ANOVA revealed a significant effect of day of measurement ($P < 0.05$) for AL and VCD. There was no significant time–day interaction for any variable.

Axial Length

The mean AL for all subjects was 24.28 ± 1.22 mm. The mean AL in myopic subjects (25.24 ± 1.05 mm) was significantly greater than the mean AL in emmetropic subjects (23.52 ± 0.62 mm; $P < 0.0001$). Significant diurnal variations in AL ($P < 0.0001$) were observed over the 2 days of testing (Fig. 1). Seventy-seven percent of subjects exhibited the longest AL at the second measurement session (mean time of measurement, 12:26) and the shortest at the final session (mean time of measurement, 21:06) on both days of testing. The mean amplitude of change in AL (peak to trough difference) was 0.032 ± 0.018 mm (range, 0.112–0.009), and this measurement remained consistent across days 1 (0.033 ± 0.023 mm) and 2 (0.030 ± 0.017 mm) of testing. The changes in AL did not exhibit a significant time–refractive error interaction ($P > 0.05$, repeated-measures ANOVA), indicating similar timing and amplitude of diurnal AL changes in the myopic (mean amplitude, 0.035 ± 0.023 mm) and emmetropic (mean amplitude, 0.028 ± 0.009 mm) subjects over the 2 days of testing. Repeated-measures ANOVA revealed a significant effect of measurement day for AL, as the mean AL on day 2 was 0.004 ± 0.006 mm shorter than the mean AL on day 1 ($P = 0.011$). There was no significant time–day interaction, which indicates that the pattern of diurnal change in AL was consistent across the 2 days of measurements.

Intraocular Pressure

Diurnal variations in IOP were also significant (Fig. 1, repeated-measures ANOVA; $P < 0.0001$). IOP was typically found to be highest in the morning (session 1, mean time, 09:17) and gradually decreased throughout the day, with the minimum IOP typically observed at the final measurement session (session 5; mean time, 21:06) (Fig. 1). The group mean IOP was

TABLE 2. Diurnal Variation of the Ocular Biometric Parameters

Variables	Group Mean ± SD	Mean Amplitude of Change ± SD	*P* (Time)	*P* (Day)	*P* (Time-day)	*P* (Time-Refractive Error)	*P* (Refractive Error)
CCT, mm	0.53 ± 0.028	0.006 ± 0.003	**<0.0001**	0.575	0.541	0.864	0.590
ACD, mm	3.06 ± 0.27	0.05 ± 0.02	**<0.0001**	0.969	0.183	0.365	**0.022**
LT, mm	3.59 ± 0.21	0.05 ± 0.02	0.369	0.520	0.623	0.099	0.092
AL, mm	24.28 ± 1.22	0.032 ± 0.01	**<0.0001**	**0.011**	0.669	0.339	**<0.0001**
VCD, mm	17.19 ± 1.10	0.06 ± 0.029	**<0.0001**	**0.042**	0.097	0.786	**<0.0001**
RT, mm	0.195 ± 0.015	0.008 ± 0.002	0.162	0.392	0.624	0.423	0.243
CT, mm	0.256 ± 0.049	0.029 ± 0.016	0.011	0.862	0.489	0.231	0.201
IOP, mm Hg	13.35 ± 2.33	3.54 ± 0.83	**<0.0001**	0.312	0.061	0.058	0.385

Data are the summary of group means, amplitude of change over 2 days, and *P* values from repeated-measures ANOVA investigating the within-subjects effects of time and day and the time–day interaction, the between-subjects effect of refractive error, and the time–refractive error interaction for the ocular biometric parameters. Significant *P* values ($P < 0.05$) are highlighted in bold.

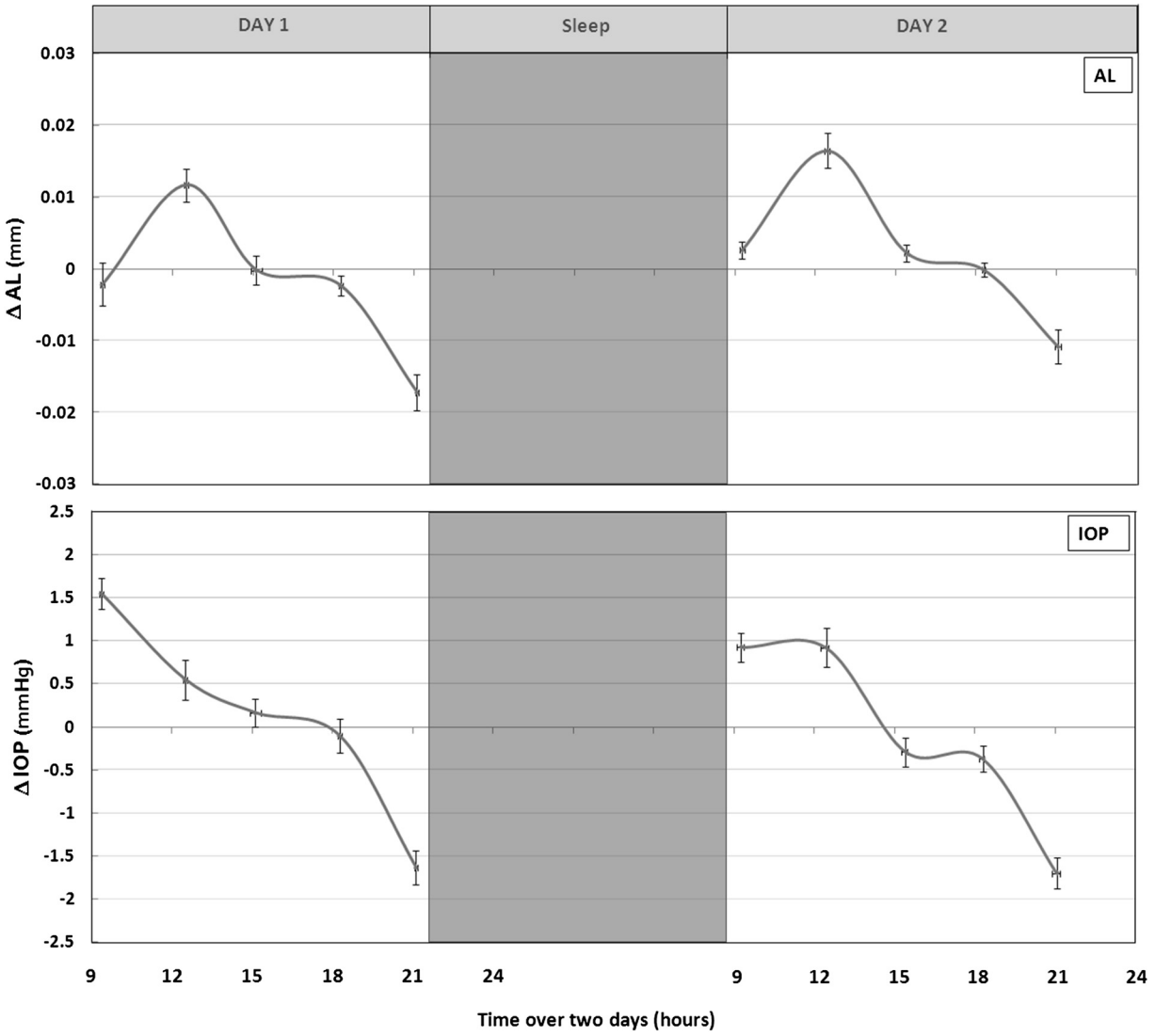

Figure 1. Mean change in AL (*top*) and IOP (*bottom*) for 10 sessions over 2 consecutive days. To highlight the diurnal variations, all values are expressed as the difference from the mean of 10 sessions across 2 measurement days (i.e., all values are normalized to the mean). Repeated-measures ANOVA revealed significant diurnal variations in both AL and IOP ($P < 0.0001$). Vertical error bars, SEM; horizontal error bars, standard error in the mean time that the measurement was taken at each session (in hours).

13.35 ± 2.33 mm Hg, which was not significantly different between the myopes and the emmetropes ($P = 0.385$). The mean amplitude of change in IOP over 2 days of measurements was 3.54 ± 0.83 mm Hg (range, 5.45–1.82), which was not significantly different between myopes (3.57 ± 0.77 mm Hg) and emmetropes (3.50 ± 0.91 mm Hg; $P = 0.058$).

Anterior Eye Biometrics

Figure 2 illustrates the pattern of diurnal variation in the measured anterior eye biometrics (CCT, ACD, and LT). CCT underwent significant diurnal variation ($P < 0.0001$). The cornea was typically found to be thicker in the morning (session 1) and gradually became thinner throughout the day, with the minimum corneal thickness typically observed at the measurement sessions toward the end of each day (mean amplitude of change 0.006 ± 0.003 mm). Significant diurnal variations ($P < 0.0001$) in ACD were also observed, with ACD typically found to be shallowest in the morning and deepest toward the end of the day. The group mean ACD was 3.06 ± 0.27 mm, which was significantly deeper in the myopic subjects (mean ACD, 3.18 ± 0.27 mm) than in the emmetropic subjects (mean ACD, 2.95 ± 0.24 mm, $P = 0.022$). The mean amplitude of change in ACD was 0.05 ± 0.02 mm (range, 0.12–0.01). The diurnal variation in LT was assessed in 27 subjects (14 emmetropes and 13 myopes), as LT measures could not be determined consistently in 3 subjects because of a poor signal from the posterior lens surface in their A-scan data. Repeated-measures ANOVA revealed no significant diurnal variation in LT ($P = 0.369$) over the 2 days of measurement.

Posterior Eye Biometrics

Figure 3 illustrates the diurnal variation in posterior eye biometrics (VCD, RT, and CT). Significant diurnal variations ($P < 0.0001$) were found in VCD. Analogous to the AL results, the longest VCD was typically observed at the second session (mean time, 12:26) and the shortest during the night at the final session (mean time 21:06) on both the days of testing in the 27 subjects with available LT data. The repeated-measures ANOVA (between-subjects effect of refractive error) revealed that the group mean VCD of 17.19 ± 1.19 mm was significantly

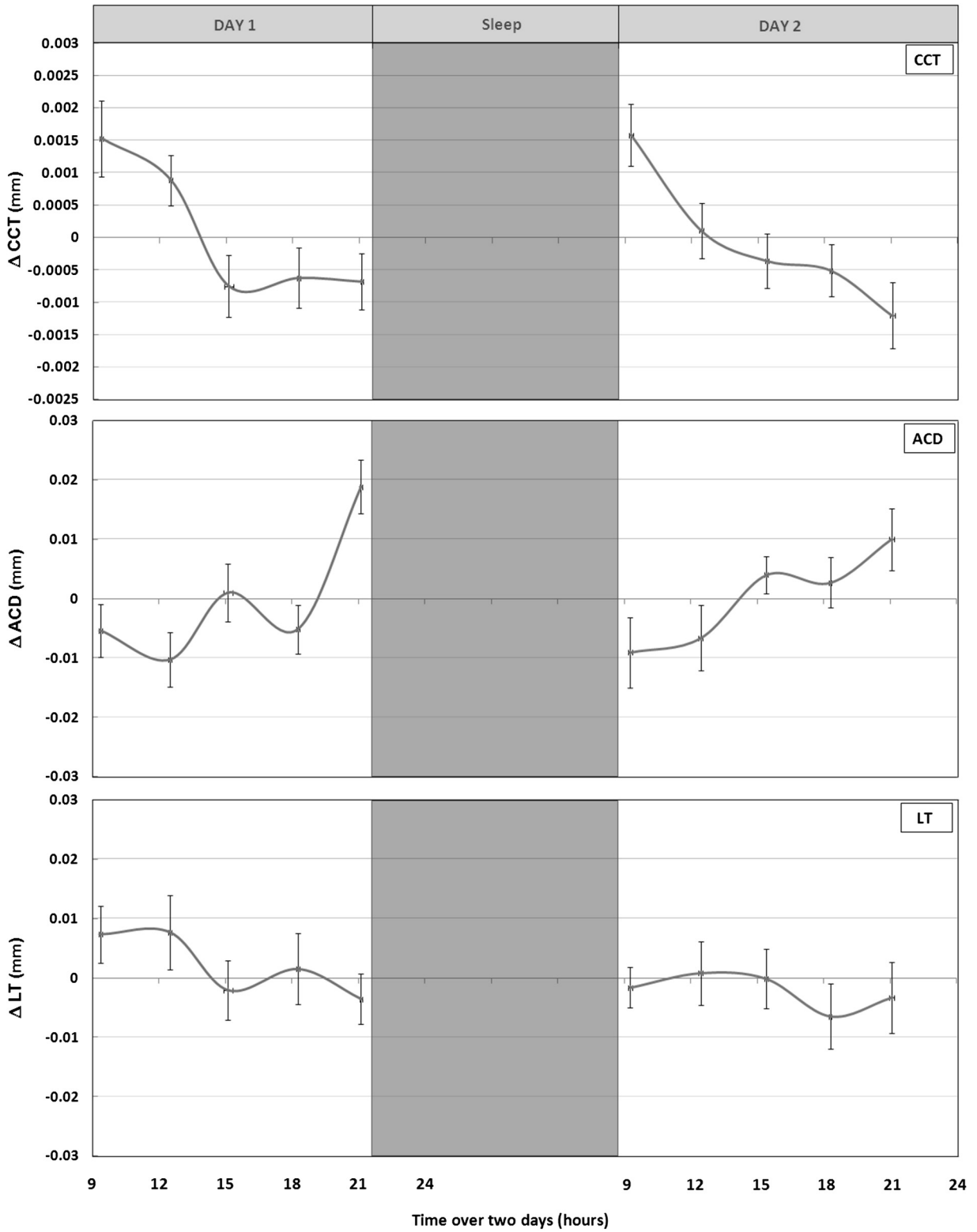

Figure 2. Mean change in anterior eye biometrics: CCT (*top*), ACD (*middle*), and LT (*bottom*) for 10 sessions over 2 consecutive days. All values expressed as the difference from the mean of the 10 sessions across two measurement days. Repeated-measures ANOVA revealed significant diurnal variations in both CCT and ACD ($P < 0.0001$). Vertical error bars, SEM; horizontal error bars, standard error in the mean time that the measurement was taken at each session (in hours).

deeper in the myopic subjects (18.10 ± 0.97 mm) than in the emmetropic subjects (16.35 ± 0.62 mm; $P < 0.0001$). The mean amplitude of change in VCD was 0.06 ± 0.029 mm (range, 0.15–0.02 mm), which was not significantly different between the refractive error groups (0.066 ± 0.033 and 0.073 ± 0.024 mm for the emmetropes and the myopes, respectively; $P = 0.786$). The change in VCD also exhibited a significant effect of day of measurement ($P = 0.042$; repeated-measures ANOVA).

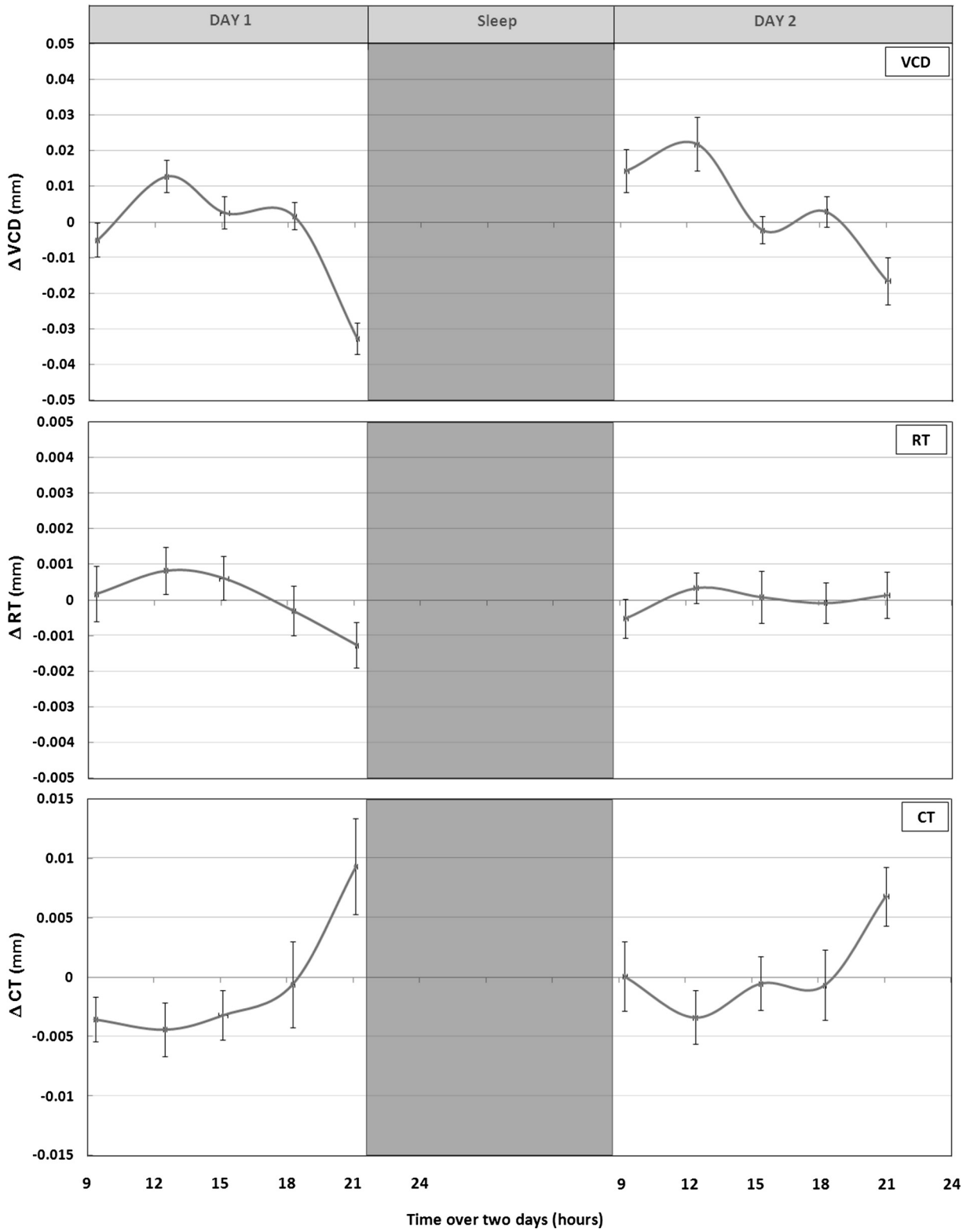

Figure 3. Mean change in posterior eye biometrics: VCD (*top*), RT (*middle*), and CT (*bottom*) for 10 sessions over 2 consecutive days. All values expressed as the difference from the mean of 10 sessions across 2 measurement days. Repeated-measures ANOVA revealed significant diurnal variations in both VCD ($P < 0.0001$) and CT ($P = 0.011$). Vertical error bars, SEM; horizontal error bars, standard error in the mean time that the measurement was taken at each session (in hours).

Consistent P4 choroidal peaks could not be detected by the independent observer in all measurements for six subjects (three from each refractive error group), therefore the CT analysis represents data from 24 subjects. The group mean CT of 0.256 ± 0.049 mm was not significantly different between myopes (mean, 0.242 ± 0.020 mm) and emmetropes (mean,

TABLE 3. Summary of Amplitude, Acrophase and RMS Fit Error from the Sine Curve Modeling to the Pooled Data (Normalized to the Mean) for the Changes in Ocular Parameters

Variables	Mean Amplitude (Peak–Trough Difference) (95% CI)	Mean Acrophase (Peak Time) (h:min) (95% CI)	RMS Fit Error
ACD, mm	0.018 (0.01–0.026)	22:28 (20:36–00:19)	0.026
VCD, mm	0.031 (0.022–0.041)	11:17 (10:04–12:31)	0.028
AL, mm	0.019 (0.016–0.023)	11:22 (10:34–12:09)	0.012
CT, mm	0.010 (0.005–0.014)	23:26 (21:31–01:23)	0.013
IOP, mm Hg	2.4 (2.1–2.7)	09:47 (09:12–10:23)	1.1

0.269 ± 0.065 mm; $P = 0.201$). Significant diurnal variations ($P = 0.011$) were observed in CT. The choroid was typically found to be thicker at night and thinnest in the morning on both measurement days. The mean amplitude of change in CT was 0.029 ± 0.016 mm (range, 0.079 – 0.011 mm), which was not significantly different between the myopic (mean, 0.029 ± 0.013 mm) and the emmetropic (mean, 0.030 ± 0.019 mm) subjects ($P = 0.231$). Repeated-measures ANOVA revealed no significant diurnal variation in RT for 30 subjects ($P = 0.162$) over the 2 days of the experiment.

Sine Curve Modeling

Table 3 displays the estimates of the mean amplitude (peak-trough difference), acrophase (peak timing of rhythm), and 95% CI from the sine curve modeling for the changes in ACD, VCD, AL, CT, and IOP. It should be noted that the amplitude of change from the sine curve modeling (Table 3) is typically of smaller magnitude than the amplitude of change based on the raw data (Table 2) for these parameters, which is most likely a reflection of the limitations of the sine curve model to optimally fit the data when there were no measurements performed for 12 hours of the 24-hour period. It is evident from this modeling that the diurnal variations in AL and CT are in approximate antiphase to each other, with the average timing of the shortest AL coinciding with the thickest choroid. The changes in VCD appear approximately in phase with the changes in AL, and the changes in ACD are in approximate antiphase with AL and VCD. Figure 4 illustrates the sine curve modeling of the pooled data for the three main parameters of interest AL, CT, and IOP.

Association between Variables

Repeated-measures ANCOVA revealed that the changes in AL were significantly associated with changes in a range of other biometry and tonometry parameters. A moderate positive correlation was found between the changes in AL and VCD (slope = 0.266; $r^2 = 0.31$, $P < 0.0001$) and a significant negative correlation between AL and ACD (slope = −0.184; $r^2 = 0.12$, $P < 0.001$). ANCOVA also revealed a significant negative association (slope = −0.31; $r^2 = 0.13$, $P < 0.0001$) between the changes in AL and CT. A significant positive association was observed between the changes in AL and IOP (slope = 0.004; $r^2 = 0.17$, $P < 0.0001$).

DISCUSSION

This study confirms and extends previous research investigating the diurnal rhythms in AL of the human eye. We have shown in this population of young adult myopes and emmetropes that AL undergoes significant diurnal variation, and the amplitude and timing of these AL rhythms were consistent with previous studies.[3,4,10] Furthermore, we have shown that these diurnal rhythms are consistently observed in terms of both their timing and amplitude across 2 consecutive days. Previous studies investigating diurnal AL rhythms have involved measurements over a single 24-hour period[4] or if data from 2 days were collected, the 2 days were separated by weeks or months,[3,10] and some inconsistencies have been noted between days of measurement. In our present study, the collection of data over 2 consecutive days revealed a more

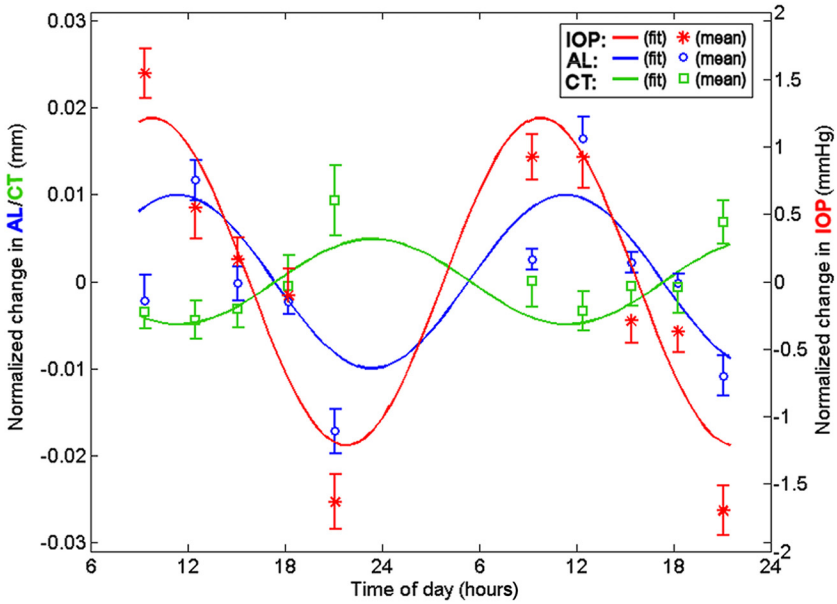

FIGURE 4. Sine curve modeling of the mean changes in AL, CT, and IOP. *Solid lines*: the best-fitting sine curve to the pooled data; *symbols*: the group mean change of each parameter at each measurement time (normalized to the mean). *Left side y*-axis: the normalized change in ocular biometric parameters (AL or CT); *right side y*-axis: the normalized change in IOP. Note the approximately antiphase relationship of diurnal variations in CT and AL rhythms, and in-phase relationship between the diurnal changes in AL and IOP. Error bars represent the SEM.

consistent pattern of variation between days, which suggests that longer term factors may have influenced the observed consistency of diurnal variations in previous studies.[3,10]

The mean amplitude of change in AL of 0.032 ± 0.018 mm in the present study equates to ~0.083-D change in refraction of the eye.[37] However, given that the depth of focus of the human eye is ±0.28 to 0.43 D,[38] the diurnal changes in AL would not be expected to substantially influence vision throughout the day or to affect clinical measures of refraction. However, research applications requiring highly precise measures of AL should be aware of these diurnal changes and take these variations into account when interpreting measured changes in AL.

Investigations of diurnal rhythms of human AL typically defined this biometric parameter as the distance from the anterior corneal surface to the RPE.[3,4,10] Therefore, the variations in AL could be modulated by changes in the anterior segment, changes in the posterior segment, or a combination of the two. We used instrumentation that allowed a more comprehensive range of ocular biometric measures to be assessed than has been possible, which allowed us to provide the first evidence in human subjects that VCD undergoes significant diurnal variations. The moderate association between the changes in AL and VCD ($r^2 = 0.31$, $P < 0.0001$) suggests that the diurnal AL fluctuations occur largely because of changes in the posterior segment of the globe. Furthermore, the fact that the ACD variations (which are potentially related to movement in the crystalline lens) were out of phase with the changes in the VCD (mean phase difference, 11 hours 11 minutes) suggests that changes in the posterior segment and not an outward movement of the cornea underlie the changes in AL that we observed.

Another important finding of this study is the significant diurnal variation observed in CT. Our population's mean CT of 0.256 ± 0.049 mm is within the range of previously reported normative values of the average human foveal CT, measured using a variety of different methods.[20,39–41] Brown et al.[20] provided some evidence of diurnal fluctuations in human CT in a retrospective analysis of partial coherence interferometry data. However, they noted some inconsistencies in the pattern of choroidal change between subjects and between days of testing in their small population of subjects. In our larger population of subjects, we found a consistent pattern of diurnal variation, with the choroid typically being thickest at night and thinnest during the day. These results correspond closely with previous research in other species, such as birds[16–18,21]and marmosets,[19] where the choroid was also typically found to be thicker at night and thinner during the morning.

We found a significant negative association between the variations in AL and CT. The average timing of the shortest AL tended to coincide with the thickest choroid with a phase difference of 11 hours 56 minutes (i.e., nearly exact antiphase). As the present study defined AL from the anterior corneal surface to the RPE and as the choroid is adjacent to the retina, it can be reasonably postulated that expansion and contraction of the choroid could lead to anterior and posterior movements of the RPE and thus be directly involved in the diurnal variations of AL. The mean amplitude of change in CT was of similar magnitude to the changes in AL. However, only approximately 13% ($r^2 = 0.13$) of the diurnal variations in AL can be explained by variations in CT in the present study. It should be noted that the within-subject variability in the determination of CT was slightly higher (involving subjective estimation of peak locations in the A-scan data) than the AL data (Table 1), which may have reduced the strength of the association between these variables.

Diurnal variations in IOP have been extensively studied.[1,3,15,42,43] Our results are consistent with those in several previous studies in terms of both the timing and magnitude of diurnal IOP rhythms in healthy adult eyes.[2,15,42,44,45] Our results also confirm a prior observation of a significant, but relatively weak, association ($r^2 = 0.17$) between the diurnal changes in AL and IOP.[4] There was not a strong trend for those subjects with larger amplitudes of change in IOP to show larger amplitudes of change in AL, which suggests that changes in the two variables may not be causally related and that the association between AL and IOP may be largely due to the similar timing of the two rhythms. Previous animal research[17] has noted small phase differences between the rhythms of AL and IOP, which suggests that the two rhythms may not have a direct causal relationship. Although both IOP and CT were significantly associated with variations in AL, the strength of these associations were relatively weak, which suggests there may be other factors involved in the diurnal AL rhythms.

Previous research in animal models indicates that diurnal variations in AL and CT may be altered during refractive error development.[16–19] In contrast to these findings, the diurnal variations in AL and CT were not significantly different between our populations of young adult myopes and emmetropes. However, previous animal research has shown that diurnal rhythms in AL are different in older adolescent eyes than in younger juvenile eyes[16,19] and that the magnitude of phase difference between AL and CT is associated with the ocular growth rate,[18] which may explain the lack of refractive error effects observed in the AL and choroidal rhythms of our young adult population (mean age, 24.4 ± 2.94 years), whose myopia was only progressing relatively slowly (mean progression rate calculated from previous clinical data −0.20 ± 0.15 D/year). Further research is needed to determine whether younger, faster progressing myopic human eyes exhibit differences in their diurnal AL and CT rhythms.

Previous studies have also reported a significant diurnal variation in CCT, with trends similar to those in our present study.[5–7] However, the magnitude of CCT change in our study is substantially smaller than previous studies that have typically measured corneal thickness immediately after eye opening in the morning when corneal swelling is associated with the relatively hypoxic closed eye environment.[5,7,46] We have also shown that human ACD undergoes significant diurnal variation. The nocturnal enlargement of ACD is consistent with previous observations in humans[4] and animal models.[17,47] The fact that LT did not change significantly and that VCD changed in approximate antiphase to ACD suggests that the changes in ACD most likely reflect a posterior movement of the crystalline lens. We found that the deepest ACD tended to coincide with the lowest IOP, which is consistent with previous studies that have noted that decreases in IOP are associated with a deepening of the ACD[14,48] and suggests that these findings may reflect diurnal changes in aqueous humor fluid dynamics within the eye that would be expected to influence both ACD and IOP.[14,49]

Conclusion

In summary, AL, CT, anterior chamber biometrics, and IOP all underwent significant diurnal variations that were consistently observed over 2 consecutive days of testing. AL was typically longest during the day and shortest during the night, and the changes appear to be largely due to variations in the posterior segment of the eye. CT changed by similar measured amplitude and was approximately in antiphase to the AL variations.

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