Diurnal Variation of Choroidal Thickness in Normal, Healthy Subjects Measured by Spectral Domain Optical Coherence Tomography

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PURPOSE. To describe the pattern and magnitude of diurnal variation of choroidal thickness (CT), its relation to systemic and ocular factors, and to determine the intervisit reproducibility of diurnal patterns.

METHODS. A prospective study was conducted on 12 healthy volunteers who each underwent sequential ocular imaging on two separate days at five fixed, 2-hour time intervals. Spectral domain optical coherence tomography (OCT) with enhanced depth imaging and image tracking was performed using a standardized protocol. Choroidal and retinal thicknesses were independently assessed by two masked graders. CT diurnal variation was assessed using repeated-measures ANOVA.

RESULTS. A significant diurnal variation in CT was observed, with mean maximum CT of 372.2 μm, minimum of 340.6 μm (P < 0.001), and mean diurnal amplitude of 33.7 μm. Retinal thickness (mean, 235.0 μm) did not exhibit significant diurnal variation (P = 0.621). The amplitude of CT variation was significantly greater for subjects with thicker morning baseline CT compared with those with thin choroids (43.1 vs. 10.5 μm, P < 0.001). There were significant correlations between amplitude of CT and age (P = 0.032), axial length (P < 0.001), and spherical equivalent (P < 0.001). The change in CT also correlated with change in systolic blood pressure (P = 0.031). Comparing CT on two different days, a similar diurnal pattern was observed, with no significant difference between corresponding measurements at the same time points (P = 0.180).

CONCLUSIONS. There is significant diurnal variation of CT, with good intervisit reproducibility of diurnal patterns on two different days. The amplitude of variation varies with morning baseline CT, and is correlated with age, axial length, refractive error, and change in systolic blood pressure. (Invest Ophthalmol Vis Sci. 2012;53:261–266) DOI:10.1167/iovs.11-8782

The choroid is believed to play an important role in the physiology of the eye and in the pathogenesis of a variety of ocular diseases. As a primarily vascular and cavernous structure, certain characteristics of the choroid, in particular its thickness, have been challenging to study histologically. Optimal choroid thickness values have been demonstrated to vary with age and refractive status. Variation in choroidal thickness over time and with time of day, however, has not been studied using SD-OCT. Given its vascular nature, it would not be surprising to find that the choroidal thickness could vary as a result of systemic physiological changes such as hydration and blood pressure. Certain other ocular parameters such as intraocular pressure, anterior chamber depth, and axial length have been found to have a diurnal variation in normal persons. Diurnal variation of choroidal thickness, if of significant magnitude, could be of relevance to both normative data studies as well as studies of longitudinal changes in choroidal thickness. Thus, in this report, we describe the pattern of diurnal variation of foveal choroidal thickness in a cohort of normal, healthy subjects.

METHODS

OCT Acquisition

In this prospective study performed at the Doheny Eye Institute, 12 healthy volunteers with no history of ocular disease underwent spec-

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tal-domain OCT scans of both eyes at five different time points during the course of the day spaced at 2-hour intervals. A repeat series of scans was then performed on this same cohort on a different day (but at the same times of day) at least 3 months later. The study protocol was approved by the Institutional Review Board of the University of Southern California and adhered to the tenets set forth in the Declaration of Helsinki. Written, informed consent was obtained from all participants.

All participants underwent ocular examination by a trained ophthalmologist (SS) to exclude ocular diseases. In addition, color fundus photographs were obtained to further document the absence of ocular diseases.

The OCT scans were performed at five different time points over a single day at 2-hour intervals: 9:00 AM, 11:00 AM, 1:00 PM, 3:00 PM, and 5:00 PM. All OCT scans were performed by the same experienced OCT operator, without pupil dilation, and under standardized mesopic lighting conditions. Participants consumed fluids and food according to their normal dietary practice, and this was not controlled during the course of the study.

A multimodal imaging device (Spectralis OCT; Heidelberg Engineering, Heidelberg, Germany) was used to perform all scans, using a standardized scanning protocol. For each eye, a 31-line raster scan (30° × 25°, 9.2 × 7.6 mm) centered on the fovea (with tracking on) was performed, with 25 frames averaged to improve the image quality. The enhanced depth imaging (EDI) technique, with the zero delay line oriented to the choroidal side, was used to optimize choroidal sensitivity and enhance visualization of the full choroidal thickness. The first (“baseline”) OCT scan (9:00 AM scan on the first day of testing) was set as a reference and all subsequent scans were registered to this to ensure that the same point on the fovea was imaged and graded each time.

The OCT scans were repeated on 11 of 12 subjects over the same time points on a different day, using the same protocol and parameters as before. One of the subjects was not available for the second series of scans.

During each OCT scan, the blood pressure was measured for each subject. Blood pressure was taken using a commercial sphygmomanometer (ADC blood pressure cuff; American Diagnostic Corp., Hauppauge, NY), using the right arm of each subject. Just before obtaining the first OCT scan, axial length was measured (IOL Master 500; Carl Zeiss Meditec, Dublin, CA), and refractive error was measured using an autorefractor (Nidek ARK-700A; Nidek Co. Ltd, Gamagori, Japan).

**Measuring Choroidal Thickness**

Grading of all OCT scans were performed independently by two trained OCT graders from the Doheny Image Reading Center (CT, YO). Measurements from the two graders were compared to assess intergrader reproducibility. However, to facilitate further analyses and comparisons between choroidal thickness over time, graders met face to face in open adjudication to resolve discrepancies and arrive at a single value for each time point for each case. In addition, one grader (CT) repeated all gradings on a different day to determine the intragrader variation.

Choroidal thickness at the fovea was measured using the caliper tools of the proprietary software (Heidelberg Eye Explorer) on the OCT machine. The choroidal thickness was measured using a line drawn perpendicularly from the hyperreflective line believed to represent the retinal pigment epithelium (RPE) to the choroid-scleral junction (Fig. 1). The contrast and brightness of the B-scan were adjusted to allow maximal visualization of the choroid. Graders also viewed B-scans adjacent (superior and inferior) to the central foveal B-scan to aid in confirming the position of the choroid-scleral junction. If the choroid-scleral junction could not be visualized clearly, the case was deemed to be ungradable. A similar technique was used to assess retinal thickness, with the calipers drawn from the internal limiting membrane (ILM) to the RPE. The retinal thickness measurements were intended to serve as a control for the choroidal thickness measurements.

Statistical analysis was performed using commercial analytical software (SPSS for Windows version 16.0; SPSS Inc., Chicago, IL). For inter- and intragrader reliability, intraclass correlation (ICC) was performed. The variation of choroidal thickness was assessed using general linear models with repeated-measures ANOVA. Two within-subject factors (time of day and day of measurement) were used to assess the significant differences in the diurnal change of choroidal thickness within a single day, as well as between two different days.

**RESULTS**

The mean age of the study subjects was 30.0 years (range, 21 to 37 years, SD ± 4.6 years), with 8 males and 4 females. There was a wide range of “baseline” (first day 9:00 AM) choroidal thickness values, with a mean of 372.2 μm (range, 197 to 518 μm, SD ± 100.4 μm). For the purpose of additional subanalyses, eyes were divided into three groups based on “baseline” choroidal thickness: ≤300 μm or “thin” (n = 7), 301–400 μm or “intermediate” (n = 7), and ≥401 μm or “thick” (n = 10). The mean axial lengths and spherical equivalents (SEs) were 23.9 mm (range, 21.9 to 26.3 mm, SD ± 1.3 mm) and −0.46D (range, −4.1 to +2.0, SD ± 1.3 D), respectively.

There was good agreement between graders, with an ICC of 0.994. The mean difference in choroid thickness measurements between graders was 2.0 ± 12.6 μm, with a maximum difference of 24 μm. Intragrader agreement was also excellent with an ICC of 0.998.

A characteristic diurnal pattern was observed in choroid thickness (Fig. 2). The highest mean choroid thickness was 372.2 μm, which occurred at 9:00 AM in the morning. The mean choroid thickness then decreased progressively over the subsequent time points to a low of 340.6 μm at 5:00 PM (Table 1). Using repeated-measures ANOVA with Greenhouse–Geisser correction, the mean choroid thickness differed significantly between time points on a single day (P < 0.0001). Post hoc testing using the Bonferroni correction revealed that the differences were statistically significant between all time points except between the third and fourth time points. Subdividing the eyes into three subgroups based on baseline choroid thickness, a statistically significant overall variation in choroidal thickness was seen for all three subgroups (P = 0.021, P = 0.009, and P < 0.001 for the thin, intermediate, and thick subgroups, respectively).

Over the same period, the retinal thickness (baseline mean, 235.0 μm) measured at five time points did not show significant variation on repeated-measures ANOVA (P = 0.621) (Table 1).

The mean amplitude (difference between maximum and minimum values) in diurnal choroidal thickness change was 33.7 μm (range, 3 to 67 μm, SD ± 21.5 μm) (Table 2).

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**FIGURE 1.** Grading diagram for choroidal and retinal thickness.
Comparing the three subgroups, the mean amplitude was 10.4 μm for the thin group, 41.3 μm for the intermediate group, and 43.1 μm for the thick group. ANOVA with Bonferroni correction revealed that the differences between the thin group and both the intermediate and thick groups were significant (P < 0.001) but the difference between the intermediate and thick groups was not significant (P = 1.00).

Taken as a percentage of the baseline choroidal thickness, the change (from baseline to nadir) in choroidal thickness ranged from 1.0% to 19.3%, with a mean of 8.5 ± 5.2%. Comparing between the three subgroups, the percentage change was 4.2% for the thin group, 11.3% for the intermediate group, and 9.4% for the thick group. The differences were significant between the thin and both the intermediate and thick groups (P = 0.001 and 0.009), respectively.

The mean amplitude for the right and left eyes did not differ significantly (31.5 vs. 31.3 μm, P = 0.943) and there was no significant difference in mean choroidal thickness between right and left eyes at any time point (data not shown). The amplitudes of diurnal change in choroidal thickness between the right and left eyes followed the same pattern over the course of the date and showed significant correlations (P < 0.05) at every time point.

Comparing the diurnal pattern of choroidal thickness variation on different days, a similar diurnal pattern was observed on each day (Fig. 3 and Table 1) for each subject. Performing repeated-measures ANOVA with within-subject factors (time of day and day of measurement), there was no significant variation in choroidal thickness at the same time point between days (P = 0.180).

The amplitude of diurnal variation correlated significantly with age (Pearson correlation, −0.339, P = 0.032), axial length (Pearson correlation, −0.631, P < 0.001), and spherical equivalent (SE) (Pearson correlation 0.626, P < 0.001). Both myopes (defined as SE ≥ −0.5 D) and participants with longer axial length (>23.5 mm) had significantly lower choroidal thickness diurnal variation compared with that of emmetropes and hyperopes (Table 3). Similarly, older individuals had lower diurnal variation in choroidal thickness, although this difference was not statistically significant.

A positive correlation was also observed between the change in choroidal thickness and change in systolic blood pressure (Pearson correlation 0.508, P = 0.031).

**DISCUSSION**

In this study, we observed a significant diurnal variation in choroidal thickness in normal subjects, with a relative peak thickness early in the morning and progressive decrease during the day to a relative nadir at 5:00 PM. Both the overall trend and individual differences between time points were statistically sig-

![Figure 2. Diurnal variation of mean choroidal thickness.](image)

| Table 1: Diurnal Variation of Choroidal and Retinal Thickness on Two Separate Days |
|---------------------------------|------------------|------------------|------------------|------------------|------------------|------------------|
|                                | 9:00 AM          | 11:00 AM         | 1:00 PM          | 3:00 PM          | 5:00 PM          | P Value*         |
| **First Day Measurement (n = 12)** |                  |                  |                  |                  |                  |                  |
| Choroidal thickness, μm (±SD)   | 372.2 (92.7)     | 364.9 (91.8)     | 354.3 (89.9)     | 349.2 (87.1)     | 340.6 (82.9)     | <0.0001          |
| Thin                            | 250.4            | 247.6            | 240.3            | 239.9            | 238.6            | 0.021            |
| Intermediate                    | 571.3            | 559.1            | 550.7            | 540.3            | 533.0            | 0.009            |
| Thick                           | 458.0            | 451.0            | 436.7            | 432.0            | 417.4            | <0.001           |
| Retinal thickness, μm (±SD)     | 235.0 (17.7)     | 235.0 (17.9)     | 235.4 (17.8)     | 235.3 (17.2)     | 234.9 (17.6)     | 0.621            |
| **Second Day Measurement (n = 11)** |                  |                  |                  |                  |                  |                  |
| Choroidal thickness, μm (±SD)   | 379.4 (95.4)     | 369.3 (94.6)     | 360.7 (91.2)     | 355.4 (87.9)     | 344.7 (82.1)     | <0.001           |
| Retinal thickness, μm (±SD)     | 235.8 (19.2)     | 235.9 (19.0)     | 236.4 (19.5)     | 236.3 (19.1)     | 236.1 (18.9)     | 0.167            |

* Repeated-measures ANOVA with Greenhouse-Geisser correction.
significant, except for the difference between the third and fourth time points. Because time points before 9:00 AM and after 5:00 PM were not assessed (due to logistic reasons and lack of availability of the subjects), it is not clear whether these values represent the absolute nadir or peak.

Previous investigators have demonstrated diurnal variation in other ocular parameters such as intraocular pressure\textsuperscript{24–28}, axial length\textsuperscript{24,30,31} and anterior chamber depth\textsuperscript{29,30}. In addition, it has been shown in both chicks\textsuperscript{32,33} and primates\textsuperscript{34} that the choroid thickness varies during the day and it has been suggested that these variations may play some role in the regulation of ocular growth\textsuperscript{33,35–37} and development of myopia. Although two earlier studies have reported variation in choroidal thickness in humans\textsuperscript{30,38}, these studies used partial coherence interferometry instead of OCT. OCT is the most common modality used to assess patients with retinal and choroidal disease in clinical practice and clinical trials. Thus, it is important to describe the diurnal variation in choroidal thickness that is detectable on OCT scanning because this has greater relevance to the interpretation of OCT results described in the literature.

Our results are significant because they indicate that measurement of the choroid thickness should take into account the time of the day that the OCT scan is performed. The mean difference between the thickest and thinnest values was \(33.7 \pm 21.5 \mu m\), with a range of 3 to 67 \(\mu m\). This variation is greater than the variation that can be attributed to measurement error or interobserver variation.\textsuperscript{39} In addition, we have demonstrated that the magnitude of the variation appears to depend on the “baseline” or average choroid thickness. Eyes/individuals with thinner baseline choroids (\(\leq 300 \mu m\)) demonstrated a mean amplitude of 10.5 \(\mu m\) compared with approximately 40 \(\mu m\) for those with thicker baseline choroids. Thus, the time of OCT acquisition may be of particular importance when assessing choroidal thickness in individuals with thicker choroids.

In two earlier studies, both Brown et al.\textsuperscript{38} and Chakraborty et al.\textsuperscript{30} demonstrated significant diurnal variations in choroidal thickness. In the series by Chakraborty and colleagues, the choroidal thickness increased progressively from 12:00 PM through the time points at 3:00 PM and 6:00 PM,\textsuperscript{30} whereas our patients demonstrated a decreasing trend during this time. However, the patterns of diurnal variation differed between the 2 days, and thus the exact pattern of diurnal variation, and whether this is consistent in all individuals, remains uncertain.

The percentage change refers to the change in choroidal thickness as a percentage of the baseline or reference (9:00 AM) choroidal thickness.

\begin{table}[h]
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\begin{tabular}{|c|c|c|c|c|}
\hline
 & Mean Amplitude & Range & Mean Percentage & Range \tabularnewline & (\(\mu m\)) (\(\pm SD\)) & (\(\mu m\)) & Change (\%) (\(\pm SD\)) & (%) \tabularnewline \hline
All eyes & 33.7 (21.5) & 3–67 & 8.5 (5.2) & 1.0–19.3 \tabularnewline Baseline choroid thickness & & & & \tabularnewline \(\leq 300 \mu m\) & 10.5 (7.4) & 3–29 & 4.2 (2.8) & 1.0–11.4 \tabularnewline 301–400 \(\mu m\) & 41.3 (24.1) & 4–67 & 11.3 (6.9) & 1.1–19.3 \tabularnewline \(\geq 400 \mu m\) & 43.1 (13.6) & 16–59 & 9.4 (2.8) & 3.4–13.3 \tabularnewline Laterality & & & & \tabularnewline Right & 33.8 & 3–63 & 8.6 (5.2) & 1.0–19.3 \tabularnewline Left & 33.5 & 4–67 & 8.4 (5.3) & 1.1–18.9 \tabularnewline \hline
\end{tabular}
\caption{Amplitude (Difference between Maximum and Minimum) of Choroid Thickness over 1 Day}
\end{table}

The percentage change refers to the change in choroidal thickness as a percentage of the baseline or reference (9:00 AM) choroidal thickness.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{Figure3}
\caption{Interday variation of choroidal thickness.}
\end{figure}
tion and good agreement between OCT and optical low coherence reflectometry for both retinal and choroidal thickness measurements.22

Earlier studies have reported similar negative correlations between choroidal thickness and age,2,3,5,13,14,16,17,21,23,40 – 43 and axial length,3,21,25,40-44 and a positive correlation with refractive error.5,14

In this study, we found that the amplitude of choroidal thickness diurnal variation correlated significantly with age, axial length, and SE. A previous study using optical low coherence reflectometry reported a negative correlation between change in choroidal thickness and axial length.30 We are unaware of any other studies reporting a correlation between change in choroidal thickness and age or refractive error.

It was reported that arterial blood pressure had no effect on subfoveal choroidal thickness.44 However, in this study, we found that the change in choroidal thickness had positive correlation with the change in systolic blood pressure.

To exclude the possibility of possible small variations in the focal B-scan position between time periods, the retinal thickness was simultaneously assessed at all time points. The retinal thickness in this series was remarkably consistent throughout the study period, with <1 μm of variation between time points. This is not surprising since the tracking and registration functionality of the multimodal imaging device (Spectralis OCT) was used in this study. Importantl, this suggests that the variation detected in choroidal thickness was real and not due to measurement or instrument factors such as magnification or scaling errors or motion artifact. Similarly, a study by Chakraborty et al.30 reported no significant diurnal variation in retinal thickness, with a mean amplitude of 8 ± 2 μm.

In this study, we have demonstrated remarkable congruence in the diurnal pattern of individual eyes on different days (Fig. 3), suggesting that the pattern of change of choroidal thickness may be predictable, and may be regulated by some underlying physiological mechanism.

The strengths of this study include the use of the tracking function on the multimodal imaging device (Spectralis OCT) to ensure that measurement of choroidal thickness at each time point was performed at the same location over the fovea. Since the choroid does not have a uniform thickness along the length of a B scan,25 any horizontal or vertical displacement in the placement of the calipers could affect the accuracy of the results.

This study, however, is not without its limitations. First, the choroidal thickness was assessed during five time points only during daylight hours and did not assess variation during the evening and night. This study included only normal individuals, and had a relatively small cohort. Finally, other systemic and physiologic factors (e.g., state of hydration) were not assessed.

In summary, this study demonstrates a significant and consistent diurnal variation in choroidal thickness in normal individuals. Thus, when assessing choroidal thickness in both clinical practice and clinical trials, it is important to take into account the time of the measurements, and to standardize the time of OCT scan acquisition.

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References


