In Vivo Human Choroidal Thickness Measurements: Evidence for Diurnal Fluctuations

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Purpose. The authors applied partial coherence interferometry (PCI) to estimate the thickness of the human choroid in vivo and to learn whether it fluctuates during the day.

Methods. By applying signal processing techniques to existing PCI tracings of human ocular axial length measurements, a signal modeling algorithm was developed and validated to determine the position and variability of a postretinal peak that, by analogy to animal studies, likely corresponds to the choroidal/scleral interface. The algorithm then was applied to diurnal axial eye length datasets.

Results. The postretinal peak was identified in 28% of subjects in the development and validation datasets, with mean subfoveal choroidal thicknesses of 307 and 293 μm, respectively. Twenty-eight of 40 diurnal PCI datasets had at least two time points with identifiable postretinal peaks, yielding a mean choroidal thickness of 426 μm and a mean high-low difference in choroidal thickness of 59.5 $\pm$ 24.2 μm (range, 25.9–103 μm). The diurnal choroidal thickness fluctuation was larger than twice the SE of measurement (24.5 μm) in 16 of these 28 datasets. Axial length and choroidal thickness tended to fluctuate in antiphase.

Conclusions. Signal processing techniques provide choroidal thickness estimates in many, but not all, PCI datasets of axial eye measurements. Based on eyes with identifiable postretinal peaks at more than one time in a day, choroidal thickness varied over the day. Because of the established role of the choroid in retinal function and its possible role in regulating eye growth, further development and refinement of clinical methods to measure its thickness are warranted. (*Invest Ophthalmol Vis Sci.* 2009;50:5–12) DOI:10.1167/iovs.08-1779

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At present, there are no reliable methods to accurately estimate choroidal thickness in human eyes in vivo. Recent animal studies have linked diurnal and longer term variations of choroidal thickness to the regulation of eye growth and refractive development.[1–4] Thus, in addition to the long-appreciated vascular function of the choroid, the thickness of the choroid in humans may be dynamically regulated. The limited in vivo clinical tools for evaluating the choroid in humans restrict our understanding of choroidal contributions to retinal diseases such as age-related macular degeneration and central serous retinopathy, which lack good animal models.

High-frequency ultrasound can identify choroidal thickness in animal eyes[5–7] with reasonable accuracy. However, because of the large size of the human eye, energy dispersion precludes safe application of high-frequency ultrasound to the human posterior segment. In recent years, posterior segment imaging of the human eye has been revolutionized by optical coherence tomography (OCT). To date, OCT has not proven effective for assessing choroidal structure because of signal attenuation posterior to the retinal pigment epithelium (RPE),[8,9] though future modifications of OCT methodology may provide some information about choroidal structure (Unterhuber A, et al. *IOVS* 2006;47:ARVO E-Abstract 3507).

Partial coherence interferometry (PCI) offers the possibility of providing a detailed quantitative measurement tool for posterior ocular structures with simultaneous axial length measurement. Axial PCI measurements provide data analogous to those obtained with A-scan ultrasound but with much greater precision; they have been clinically applied for biometric calculations for cataract surgery (e.g., IOLMaster; Carl Zeiss Meditec, Oberkochen, Germany). In chicks and humans, the PCI reflectance signal from the posterior eye walls consists of multiple peaks[10–12] (Fig. 1). In chick, the likely anatomic sources of these peaks were identified using sequential dissection of the outer layers of an eye, and it was found that the inner scleral surface generates a reliable and identifiable PCI signal.[12] Using both the robust peak from the RPE/Bruch membrane interface and the signal from the inner scleral surface, PCI thus enables precise measurements of choroidal thickness and daily choroidal thickness fluctuations in chick.[13,14]

Similarly, in humans, PCI waveforms from the posterior eye wall may contain a peak 250 to 400 μm behind the robust and reproducible peak at the RPE/Bruch membrane interface. By its location posterior to the RPE/Bruch membrane interface and by analogy with chick studies, this peak likely represents a signal originating at or near the choroidal/scleral interface. Our data cannot distinguish in humans whether P4 originates at the anatomic boundary of the choroid and sclera or from a prominent vascular or other structure in the outer choroid. As shown in Figure 1, we number PCI reflectance signals, by convention, from the inner limiting membrane peak, with P4 identifying this putative choroidal/scleral peak and P3 identi-

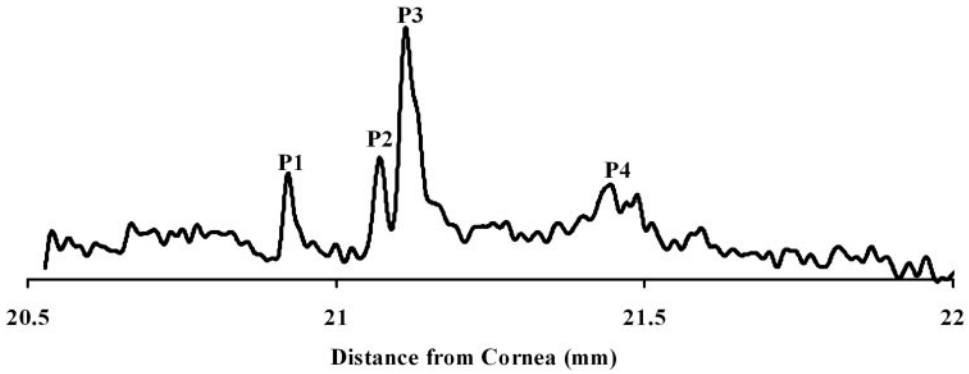

FIGURE 1. PCI axial length data showing optimum visualization of the four PCI peaks at the posterior wall of a representative human eye in a signal-averaged waveform. The likely anatomic correlates of the peaks are P1 (inner limiting membrane), P2 (consistent internal retinal peak, perhaps at the outer limiting membrane), P3 (Bruch membrane/RPE interface), and P4 (choroidal/scleral signal). The ambiguity of the location of P4 in this figure illustrates the difficulty of obtaining a precise measurement for P4 given the low signal-to-noise ratio. The noise at the P4 peak precludes simple identification of P4 as the largest peak deep to the RPE. Here the P4 label is placed over the location of P4, as identified by the algorithm. The same problem complicates the curve fitting (see Fig. 3).

fying the RPE/Bruch membrane interface. An estimate of choroidal thickness can be obtained by determining the distance from P3 to P4. This measurement may represent true choroidal thickness if the P4 signal originates from the choroidal/scleral interface or partial choroidal thickness if the P4 signal originates from a deep choroidal structure. In either case, the methodology would allow us to extract a reliable estimate of choroidal thickness or partial choroidal thickness in humans and to attribute fluctuations in the distance from P3 to P4 to thickness changes within the choroid.

Compared with that of chicks, which have less ocular pigmentation than humans, the P4 PCI signal of the human choroid/scleral region is attenuated and broadened, often with multiple peaks. The presence and morphology of the P4 signal varies widely between human subjects and between measurements within subjects. Because of a low signal-to-noise ratio of the attenuated PCI reflectance signal deep to the RPE, P4 is most evident in signal-averaged data (Fig. 2).

To demonstrate the potential usefulness of in vivo human choroidal thickness measurements, we developed an algorithm that uses a sequence of averaging, filtering, and curve fitting to determine the location of P4. A bootstrap method was then applied to determine the statistical variability for the location of P4. This article describes the algorithm and provides validation of the techniques with a dataset independent of the original data used to develop the approach. To extend our understanding of the choroid in humans, we then applied these methods to the question of diurnal variations in human choroidal thickness using data from subjects who participated in a previous study of diurnal variation in axial length.[15]

METHODS

Subjects

We used existing PCI axial length data from three separate and previously reported subject groups.[15,16] The first group was composed of 64 patients of the Division of Pediatric Ophthalmology at The Children's Hospital of Philadelphia (Philadelphia, PA) and ranged in age from 3 to 12 years.[15] The second group was composed of 39 University of Pennsylvania undergraduates, medical students, and employees (Greenberg KP, et al. *IOVS* 2003;44: ARVO E-Abstract 3612). The third group was composed of 10 University of Pennsylvania undergraduates ranging in age from 18 to 24 years.[16] Recruitment and ocular testing protocols of all three groups were reviewed and approved by the Institutional Review Board of The Children's Hospital of Philadelphia and were in accord with the Declaration of Helsinki.

Procedures

Subjects from each study group underwent PCI axial eye length measurements using our previously described instrument and protocol.[15,16] During each measurement session, the subject's head position and corneal reflex were monitored and adjusted so that the central corneal reflex and alignment beam were coaxial. Subjects were encouraged to maintain fixation on the alignment beam of the PCI for the 0.8 second required for each measurement, and 80 PCI axial length tracings were acquired over a period of 5 to 7 minutes. If the subject changed fixation from the alignment beam, lateral readjustment of the subject's eye was necessary to obtain an axial length measurement. This, together with continuous monitoring of the corneal reflex, ensured that the subject was fixating on the alignment beam. The first two datasets used in this study contained one measurement session on

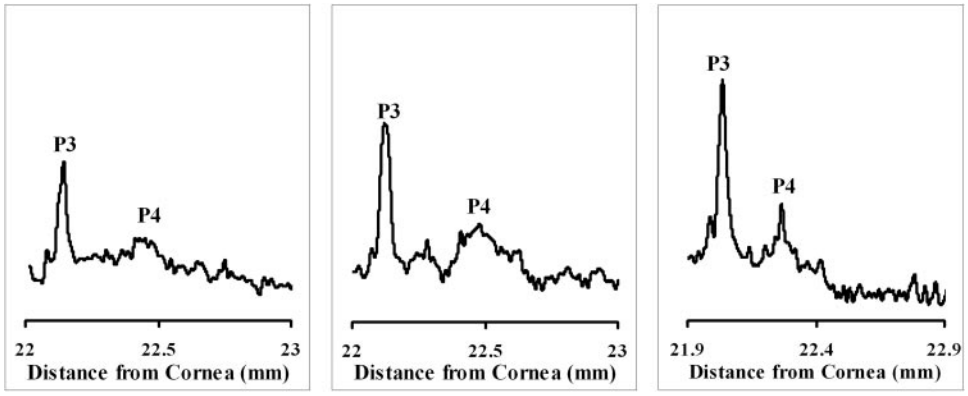

FIGURE 2. Signal-averaged PCI axial length data from three subjects.

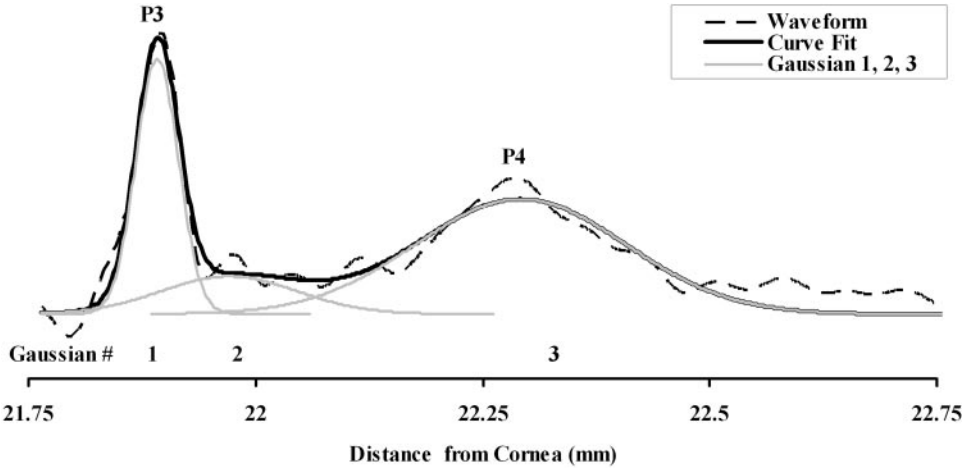

FIGURE 3. Example of a 3-Gaussian curve fit to the signal-averaged and filtered P4-P3 complex. The subject's waveform (*dashed line*) has been fit (*solid line*) with 3 Gaussian curves (*gray lines*). The leftmost Gaussian (no. 3) is fit to the P4 component of the P4-P3 complex.

a single eye of each subject[15] (Greenberg KP, et al. *IOVS* 2003;44: ARVO E-Abstract 3612). The third dataset contained bilateral diurnal axial length measurements on each subject, obtained six times over the course of 1 day at 3-hour intervals between 7 AM and 10 PM and another set of diurnal axial length measurements obtained on another day in the same time frame.[16]

Algorithm for Determination of P4

Using a variation of a previously reported algorithm,[15] we developed an algorithm in MatLab (version 6; The Math Works, Natick, MA) to determine the position and variability of the choroidal/scleral peak (P4) in PCI axial length tracings. Briefly, the algorithm analyzed the raw data consisting of up to 80 PCI tracings from a given subject. Each waveform was examined to determine whether it contained eye movements (characterized by large amplitude signal transients), whether reflections were in a consistent location, and whether there was a high signal-to-noise ratio. Based on these criteria, the algorithm accepted tracings that contained potential peaks and excluded tracings containing only noise or artifact. The accepted tracings with potential peaks (i.e., *n* tracings) were signal averaged and filtered to generate one posterior eye wall waveform that was curve-fit with three Gaussian curves (Fig. 3). The resultant curve-fit waveform was then analyzed for the presence of a P4 signal. In summary, a P4 was present if the P4 Gaussian peak was above the level of the noise and was within 550 μm from P3 (i.e., the RPE). If a subject's curve-fit waveform contained a P4 signal, the variability of the P4 determination was estimated from the original dataset using a bootstrap technique.[17] Novel bootstrap datasets were generated by random sampling with replacement *n* times from the original accepted dataset of *n* tracings.[17] The bootstrap datasets were signal averaged and filtered to generate one bootstrap waveform that was curve-fit and analyzed for the presence of a P4 signal. The process was repeated until 200 bootstrap P4s were extracted, from which the variability was estimated. A more detailed description of the algorithm is available in the Appendix, online at http://www.iovs.org/cgi/content/full/50/1/5/DC1.

Given the limited amount of data available in a single session, the assumptions for the algorithm were conservative because it was automated and user-independent when used to analyze a single measurement session for the presence and variability of a P4 signal. In analyzing the first two datasets containing subjects with only one measurement session, the algorithm sequentially ran through the signal-averaged data from each subject and determined whether there was a P4. If the subject's readings contained a P4, the bootstrap statistical method was applied to calculate the variability for P4.

In contrast, for subjects in the diurnal dataset, there were 12 measurement sessions for each eye (six on each day). The additional measurement sessions in essence increased the signal-to-noise ratio of the P4 signal and the number of subjects whose data had an identifiable P4. Figure 4 illustrates how the availability of multiple measurement

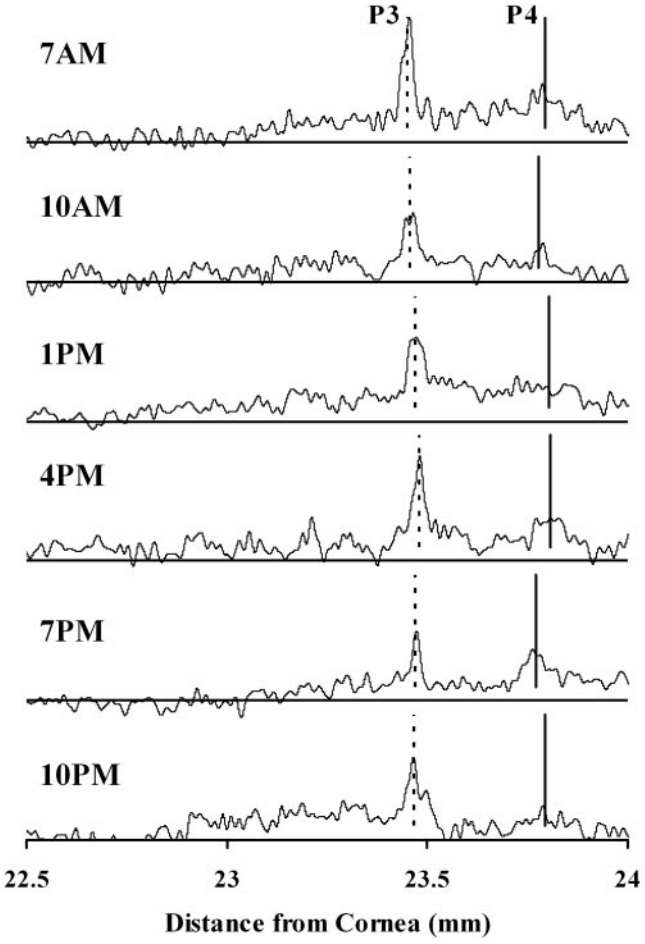

FIGURE 4. Signal-averaged waveform from six time points in a typical diurnal dataset, in this instance for the right eye of subject 2 on testing day 2. The intensity and morphology of P4 varies with each time point. This figure exemplifies how difficult it is to examine the signal-averaged waveform at any single time point and to identify the location of P4. Examining all six time points simultaneously allowed us to more easily identify P4. *Dotted lines* and *solid lines* indicate the positions of P3 and P4, respectively, as identified by the P4 algorithm. Note that the waveform from 1 PM showed no apparent P4 on the signal-averaged waveform, but the P4 algorithm identified a P4 for this time point likely because certain bootstrapped subsets of data contained a P4.

sessions at different time points for a single eye improved the identification of P4. It provided face validity to the approach used for the diurnal dataset, whereby the composite data from each eye were examined by one of the authors (JSB), and the algorithm was applied to each time point when there was visible evidence of a P4.

Data Analysis

Choroidal thickness estimates were defined as the difference between the location of P3 and P4 using bootstrapped estimates from eyes with identifiable P4 peaks. For the two study groups with only one measurement session, the precision of measurement of choroidal thickness was summarized by $SE_{measurement}$.[18] For diurnal data with six measurement sessions in each eye on each day, mean daily choroidal thickness was calculated by using all measurement sessions containing a P4 signal for that eye and day. An estimate for change in choroidal thickness during the day for each subject was calculated as the difference between the largest and the smallest mean choroidal thickness for a single measurement session.[16] For each subject, axial length and choroidal thickness were normalized to their respective daily mean values. Unless otherwise specified, data are shown as mean ± SD.

Results

Initial Development of Algorithm to Detect P4

The algorithm was developed using the data from a single measurement session obtained in 64 subjects, ages 3 to 12 years.[15] As shown in Table 1A for this group, P4 could be identified in 18 subjects (28%); the mean P4-P3 distance was 307 μm (median, 309 μm; range, 204–422 μm; $SE_{measurement}$, 16 μm).

Algorithm Validation

The algorithm was validated using a 39-subject data set from a study that examined the relation of routine ultrasonographic and PCI measurements of axial length with one measurement session per subject (Greenberg KP, et al. *IOVS* 2003;44: ARVO E-Abstract 3612). The algorithm identified P4 in 11 of the 39 subjects (28%; Table 1B). The mean P4-P3 distance for these subjects was 293 μm (median, 296 μm; range, 206–389 μm; $SE_{measurement}$, 19 μm).

Application of the Algorithm to Detect Diurnal Fluctuations in the P4-P3 Distance

To determine whether the P4-P3 distance fluctuated during the day, the algorithm was applied to the datasets from both eyes of 10 subjects who had undergone multiple axial PCI measurements on two different days. These axial PCI data had been accumulated as part of a study to examine the relation of IOP and axial length.[16] In the analysis of these 40 diurnal datasets, a P4 waveform was identified in the data of at least two measurement times in 28 datasets (Table 2). Seven subjects contained a P4 waveform in each eye on each day of testing, and the remaining three contained no P4 waveform in either eye on either testing day.

Among the seven subjects in whom bilateral P4 waveforms could be identified, the mean P4-P3 distance was 426 μm (range, 308–527) with the $SE_{measurement}$ of 24.5 μm. The mean change in P4-P3 over the day from the 28 datasets was 59.4 ± 24.2 μm (median, 56.4; range, 25.9–103 μm; Table 2). The diurnal choroidal thickness fluctuation was larger than twice the $SE_{measurement}$ (24.5 μm) in 16 of the 28 datasets. In 18 of the 28 datasets there was a statistically significant change in axial length over the day. This group of 18 datasets had a mean change in axial length of 38.5 μm (last column of Table 2) and an average P4-P3 distance of 424 μm (median, 431 μm; range, 342–518 μm).

Table 1. Choroidal Thickness Measurements in the Algorithm Development Study and in the Algorithm Validation Study

A. Algorithm Development Cohort

Subject* (*n* = 18)	Choroidal Thickness† (μm)
7	422.3 ± 26.4
9	310.7 ± 7.7
10	270.4 ± 24.4
15	234.1 ± 8.2
24	352.4 ± 13.8
27	400.8 ± 8.9
29	334.9 ± 6.8
30	204.4 ± 6.7
32	204.0 ± 7.2
34	236.6 ± 6.5
52	377.6 ± 46.9
55	332.0 ± 5.8
57	395.7 ± 5.3
58	307.4 ± 10.4
60	261.8 ± 7.6
61	360.1 ± 18.6
65	220.0 ± 27.9
69	300.3 ± 57.2
Average of all subjects	307.0 ± 70.1

B. Algorithm Validation Cohort

Subject (*n* = 11)	Choroidal Thickness† (μm)
1	268.2 ± 9.3
2	368.8 ± 19.3
3	389.4 ± 8.4
4	206.0 ± 42.0
5	264.9 ± 12.4
6	298.9 ± 11.3
7	339.6 ± 10.5
8	311.3 ± 5.8
9	266.8 ± 9.9
10	218.1 ± 19.7
11	295.5 ± 26.4
Average of all subjects	293.4 ± 57.3

* Subject numbers correspond to those in the original report.[15]

† Choroidal thickness is represented as mean P4-P3 ± bootstrap SD.

There was no consistent relation between the diurnal fluctuations in choroidal thickness and axial length in these data. Representative waveforms illustrate the inconsistency of comparing the diurnal fluctuations in the two parameters, in which choroidal thickness and axial length were normalized to their respective daily mean values (Fig. 5). Figure 5A shows a group of two datasets with a statistically significant change in axial length and in which the qualitative change in choroidal thickness was in approximate antiphase with the change in axial length. Figure 5B shows a dataset in which the axial length had no statistically significant diurnal variation and the choroidal thickness also showed little diurnal change. In 5 of the 18 datasets in which there was a significant change in the axial length, the diurnal changes in choroidal thickness were in approximate antiphase with the axial length fluctuations at all but one time point (examples are provided in Fig. 5C). In a few datasets, the relation between axial length and choroidal thickness suggested that they were unrelated (Figs. 5D, 5E). Figure 5D shows one dataset in which there was a statistically significant change in axial length and the choroidal thickness was essentially unchanged. Figure 5E shows two datasets that had no statistically significant change in axial length and in which

TABLE 2. Differences in Daily Occurrences of P4 and P3, Peak P4-P3, High-Low P4-P3, and Axial Length High-Low

Subject/Eye/Day	P4/P3 (d)	Peak P4-P3 (μm)	Maximum and Minimum Values for P4-P3 Difference	Axial Length High-Low Difference (μm)
B/OD/1	5/6	356	42.6	81.8*
B/OS/1	4/6	347	29.1	96.2*
B/OD/2	6/6	342	38.7	30.5*
B/OS/2	4†/5	308	32.7	26.0‡
C/OD/1	2/6	478	39.7	21.8‡
C/OS/1	3/6	518	45.7	22.8*
C/OD/2	5/6	437	45.5	9.8‡
C/OS/2	3/6	527	55.9§	6.0‡
D/OD/1	5/6	510	92.3§	31.4‡
D/OS/1	6/6	441	77.2§	47.8*
D/OD/2	6/6	479	65.1§	45.7*
D/OS/2	6/6	487	82.2§	47.3*
F/OD/1	6/6	375	95.1§	27.0*
F/OS/1	5/6	348	33.7	36.0*
F/OD/2	6/6	438	89.0§	25.3*
F/OS/2	6/6	382	56.8§	25.4*
G/OD/1	3/4	373	51.9§	28.0‡
G/OS/1	5/6	397	25.9	39.5‡
G/OD/2	6/6	379	40.0	58.8‡
G/OS/2	4/6	396	62.7§	18.3‡
H/OD/1	6/6	444	58.2§	14.2*
H/OS/1	6/6	490	103§	7.4‡
H/OD/2	5/6	424	37.5	42.2*
H/OS/2	5/6	467	100§	19.8*
I/OD/1	6/6	516	80.9§	44.5*
I/OS/1	6/6	411	28.3	33.6*
I/OD/2	6/6	457	65.8§	22.8*
I/OS/2	6/6	406	88.0§	29.4*
Mean(SD)		426 (61)	59.4 (24.2)	38.5 (21.1)‖
Median		431	56.4	32.1
Range		308–527	25.9–103	14.2–96.2

Data are from the diurnal study.

* One-way ANOVA ($P < 0.01$).

† One data point was excluded because more than one choroidal peak identified indiscernible from each other.

‡ NS by ANOVA ($P > 0.05$).

§ Greater than 2× the standard error of measurement (24.5 μm).

‖ Mean, SD, median, and range are given only for the 18 subjects with axial length high-low differences ($P < 0.01$; one-way ANOVA).

the mean change during the day in choroidal thickness was more than twice the $SE_{measurement}$.

DISCUSSION

This study demonstrates that signal processing techniques can identify a reasonably reproducible PCI peak in the deep choroid that, based on analogy to work in laboratory animals, we believe originates at or near the choroidal/scleral interface. If so, then comparing the location of this peak to the PCI signal originating at the Bruch membrane/RPE interface permits an in vivo estimate of choroidal thickness or of partial choroidal thickness if the P4 signal originates from a deep choroidal structure. These techniques provide the first evidence of diurnal variation in choroidal thickness or partial choroidal thickness in humans that parallel the diurnal fluctuations in choroidal thickness observed in several species of laboratory animals. A function for choroidal thickness fluctuations in humans and laboratory animals is speculative. Chicks, marmosets, and rhesus monkeys show changes in choroidal thickness in response to the alterations in retinal image quality, and it has been suggested that choroidal thickness changes participate in mechanisms regulating eye growth and refractive development.[1–4] Proof of a similar response in humans would be a crucial link between clinical and animal research in this field, but methods for measuring human choroidal thickness in vivo have heretofore been unavailable.

Although techniques to estimate choroidal thickness require further development to increase the proportion of subjects with recordable choroidal signals, our methods of analyzing PCI axial data demonstrate a structure deep to the Bruch/RPE interface in 28% to 70% of test subjects. When our algorithm was applied to a single measurement session taken at one time point, only 28% of tested subjects across all ages showed evidence of P4 (Table 1). When multiple measurement sessions at different times over a day were analyzed, we used a subjective judgment of the waveforms along with the P4 algorithm to aid in identifying P4 (Fig. 4). Repeat measurements significantly increased the number of identifiable P4 signals by taking advantage of averaging of the inherently low signal-to-noise ratio of the PCI choroidal/scleral signal. Of the 10 subjects in the diurnal study reported here, seven (70%) showed evidence of a P4 signal during at least 2 of 6 measurement sessions for each eye.

Despite the increased percentage of subjects showing evidence of a P4 with more data in the diurnal study, three subjects still did not have evidence of a P4 in either eye. In the diurnal study the presence of a P4 was subject dependent, not eye dependent. Either subjects had a P4 signal in both eyes or the P4 signal was not detected in either eye. The most likely

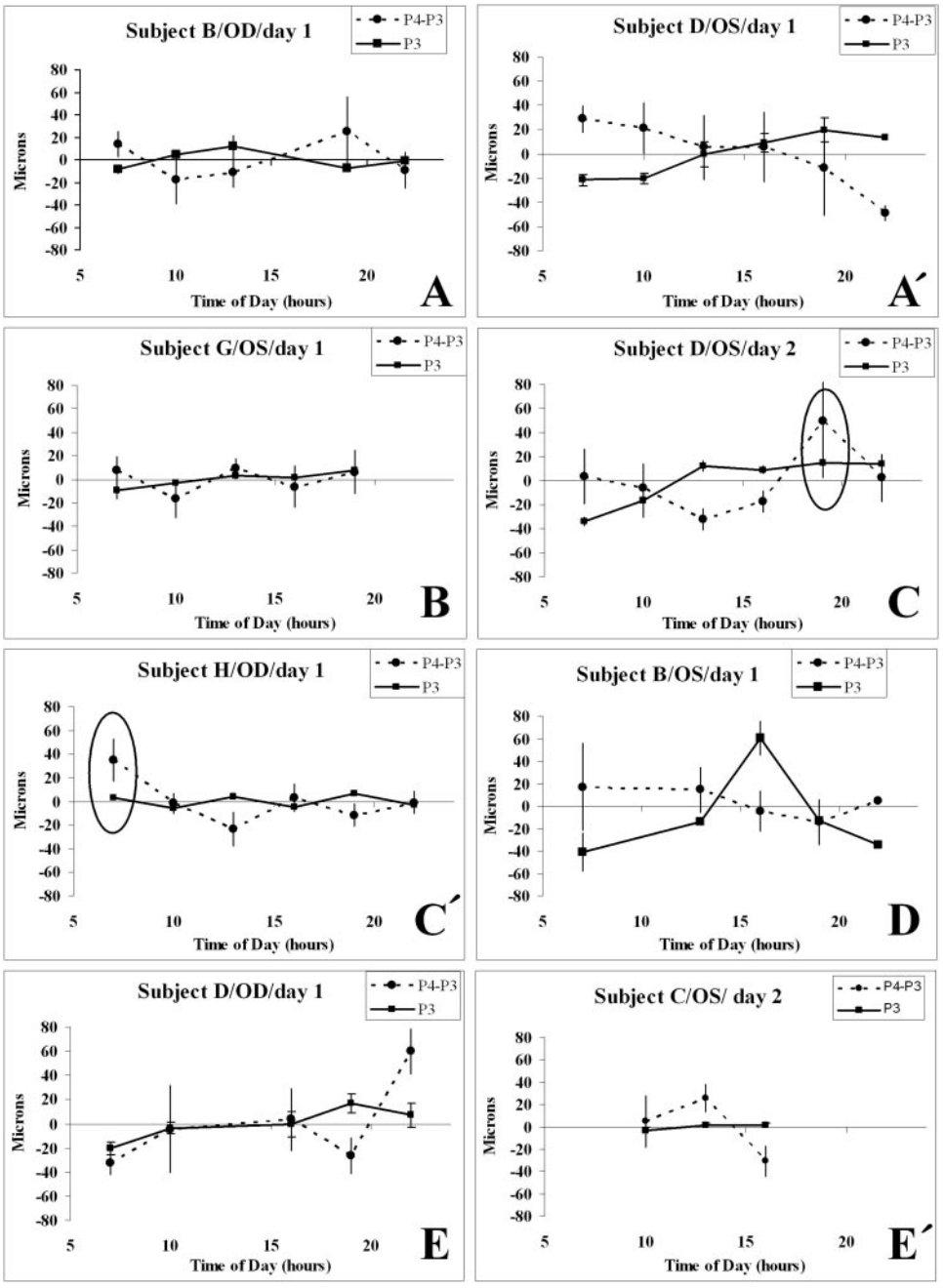

FIGURE 5. (**A**, **A**′) Examples from subjects with significant changes in axial length (P3) in whom choroidal thickness fluctuations (P4-P3) are in antiphase with axial length fluctuations. (**B**) Data from a subject in whom no significant change in axial length was observed and whose choroidal thickness showed little fluctuation over the day. (**C**, **C**′) Sample data from subjects with significant changes in axial length and in whom the choroidal thickness fluctuations are in antiphase with the axial length fluctuations for all but one time point (*circled*). (**D**) This is the only dataset in which the axial length showed significant change but the choroidal thickness changed little, indicating that choroidal thickness did not account for the observed fluctuations in axial length. (**E**, **E**′) Both subjects showed no significant change in axial length, and the choroidal thickness had a mean difference of 2× the $SE_{measurement}$, indicating that it likely changed over the course of the day.

reason for the absence of a P4 signal was decreased signal intensity from absorption because of some anatomic characteristic unique to the subjects, such as thicker choroids, increased choroidal pigmentation, or some property of Bruch membrane. To the extent that race may be a surrogate for choroidal pigmentation, we found no correlation between the presence and absence of a P4 signal based on race in any of the study cohorts.

Choroidal thickness is likely a dynamic parameter influenced by oscillations in IOP or the cardiac cycle. IOP undergoes relatively rapid oscillation associated with the cardiac cycle, and this may translate to a rapid change in choroidal thickness over seconds. However, given that we averaged up to 80 measurements to obtain a single estimate of choroidal thickness, any transient variation on the order of seconds in the choroidal thickness would be expected to average out and probably not influence the mean measurements obtained at each time point.

Other investigators and we[19,20] have previously shown that humans undergo diurnal fluctuation in axial length, as previously demonstrated in chicks,[5,6,13,21] rabbits,[22] and marmosets.[7] The physiologic basis for this variation and its implications are not understood. Results in chicks, rabbits, and humans indicate that the diurnal fluctuations in ocular dimensions are not solely passive expansions and contractions of the globe in response to IOP fluctuations.[5,6,16,19,22] Axial diurnal fluctuations in humans have also been shown to have no correlation to intraday variations in corneal thickness (Glassman RD, et al. *IOVS* 2006;47:ARVO E-Abstract 1798).

The choroids of chicks and marmosets, measured with high-frequency ultrasound, undergo diurnal fluctuations in thickness that are in antiphase with axial length fluctua-

tions.[6,7,13] In contrast, the results of the present study do not establish a consistent phase relationship between diurnal fluctuations of the choroidal thickness and axial eye length, perhaps because of inherent variability in the patterns of humans in contrast to those of laboratory animals.

Comparison with Other Methods of Choroidal Measurement

A major problem in developing methods to measure human choroidal thickness in vivo is the lack of good comparison data from similar techniques. Overall, the mean P4-P3 distance for every measurement session from all subjects described herein with a P4 was 326 μm (range, 204–490). With the increased number of measurement sessions used in the diurnal study, we were able to estimate choroidal thickness in an increased proportion of subjects (70% compared with 28%). The increased number of measurement sessions in the diurnal study also resulted in a greater mean choroidal thickness (426 μm) compared with the one measurement session (307 and 293 μm), whereas the equipment and the measurement technique were otherwise unaltered, possibly because the increased number of measurements allowed the detection of smaller signals in the noise of the waveform. Given that the power of the PCI signal would be expected to decrease as it proceeded through the eye and was scattered at density interfaces, it is logical that reflections further back in the eye would have smaller signal-to-noise ratios and therefore would be identifiable only by increased averaging of data. Thus, we speculate that the improved sensitivity in detecting P4 in the diurnal study was associated with the ability to detect signals that had penetrated further through the choroid. Whether P4 represents the histologic choroidal/scleral junction or some other prominent vascular or other structure in the outer choroid cannot be established from these data. Assuming that P4 represents the same structure for a given eye when detected, this limitation in the method does not invalidate the conclusions of diurnal variation. Improvements in detecting P4 signals beyond the present methods may lead to further increases in estimated mean value for choroidal thickness. Part of the difference in mean choroidal thickness may also be an artifact of the time of day in the studies with a single session.

Historically, accurate histologic measurements of choroidal thickness have been difficult to obtain, at least in part because of postmortem drainage of blood and loss of tone from the choroidal blood vessels.[23] Thickness estimates of 220 μm were reported in 1912 for the posterior choroid based on in vitro injection of choroidal vascular channels,[24] a technique still considered likely to underestimate the true choroidal thickness.[25] This thickness estimate, however, continues to be cited in contemporary texts.[26] An in vivo technique using radiofrequency signals resulted in thicker choroidal measurements. Choroidal thickness was calculated with the use of extrapolated ocular radiofrequency velocities; measurements taken in the macula ranged from 420 to 450 μm and had a reported error of 20 μm.[25] We are unaware of the application of any other methods other than our technique and radiofrequency signal analysis to estimate choroidal thickness in the living human eye.

Current Limitations and Future Directions

Although the PCI instrument used in this study provides highly precise measurements of the retina from the inner limiting membrane to Bruch membrane at the fovea, there are limitations for its use to assess in vivo choroidal thickness in humans. Specifically, the intrasubject variability in the choroidal/scleral P4 peak may limit its generalizability. On the other hand, the intrasubject variability may be a clue to individual differences in function that are related to differences in structure. The relatively small and demographically limited diurnal study population also limits the generalizability of these results. However, the diurnal cohort does provide the first evidence that diurnal variation in choroidal thickness may occur in humans and serves as a rationale for continuing efforts to analyze choroidal thickness.

In addition, the PCI used in this study makes use of a light source with a longer wavelength than that of the only commercially available axial PCI device (IOLMaster; Carl Zeiss Meditec). This difference may enhance the ability of our device to penetrate the RPE and choroid because the absorption coefficient of melanin declines with increasing wavelength.[27] The PCI used in this study provides higher spatial resolution than the commercially available axial PCI device (IOLMaster; Carl Zeiss Meditec) because of its superluminescent diode and shorter coherence length (30 vs. 120 μm). The resultant output eliminates the secondary artifactual peaks that are generated by the multimode laser diode of the commercially available axial PCI device (IOLMaster; Carl Zeiss Meditec). These observations suggest that using still longer wavelengths may facilitate choroidal measurements. Recently, an OCT system using a longer wavelength at 1040 nm was demonstrated to be capable of producing images of the superficial choroid of approximately 200 μm (Unterhuber A, et al. *IOVS* 2006;47: ARVO E-Abstract 3507). Extending the wavelength for axial PCI measurements to use the local minimum in water infrared absorption at 1050 nm while minimizing melanin absorption may improve the signal-to-noise ratio of the P4 signal. Multispectral measurements may further enhance the signal quality from the choroid. Correlation of the PCI measurement with multispectral high-resolution OCT imaging may also give insight into the exact structures measured by the P4 signal. These technical issues merit further evaluation because a device that is better able to penetrate the heavily pigmented human choroid and thus provide a stronger reflectance signal from the choroidal/scleral interface would facilitate exploring the complex interaction between choroidal thickness, emmetropization, eye growth, and other disease processes.

Summary and Conclusions

Choroidal thickness estimates were obtained from 28% of human subjects at a single PCI measurement session and from 70% of subjects who provided multiple PCI measurement sessions as part of diurnal studies. The accuracy of the measurements, as determined by the SE of measurement, was 16 to 24.5 μm. Choroidal thickness tended to fluctuate, with a mean change larger than twice the SE of measurement (24.5 μm) in 57% (16 of 28) of the diurnal datasets. The growing literature on the role of the choroid in controlling eye growth in animal models emphasizes the need to further develop the methods to measure human choroidal thickness in vivo. The ability to determine choroidal thickness reliably in humans will likely improve our understanding of the pathology underlying age-related macular degeneration, central serous chorioretinopathy, and other disorders of the posterior retina and choroid. Techniques such as those described in this article, combined with improved instrumentation, may find broad future application.

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