Diurnal Axial Length Fluctuations in Human Eyes

Richard A. Stone,[1,2] *Graham E. Quinn,*[1,2] *Ellie L. Francis,*[1] *Gui-shuang Ying,*[2] *D. Ian Flitcroft,*[3] *Parag Parekh,*[1] *Jamin Brown,*[1] *Joshua Orlow,*[1] *and Gregor Schmid*[4]

Purpose. This study sought diurnal variations of eye length in human subjects, analogous to those reported in laboratory animals.

Methods. Seventeen subjects, ages 7 to 53 (median 16) years and mean spherical equivalent refractive error −0.68 D (range, −3.00 to +1.00 D), underwent axial length measurements at multiple times during the day between 7 AM and 1 AM the following day, using partial coherence interferometry (PCI), a highly precise, noncontact method. Diurnal axial length measurements were obtained on two or more days in 10 of these subjects.

Results. During at least 1 day, 15 subjects showed a statistically significant (ANOVA, $P < 0.05$) diurnal fluctuation of axial length, with a magnitude generally between 15 and 40 μm. From the diurnal tracings that fit a sine curve using statistical criteria, the mean period of fluctuation was 21.6 ± 4.33 hours (SD), the mean amplitude was 27.1 ± 11.9 μm (SD; range, 12.8–41.4 μm), and the maximum axial length tended to occur at midday. Each of the subjects with multiple daily measurements showed axial length fluctuations on at least 1 day, but there were day-to-day differences in the diurnal variations: most notably, four subjects showed axial length fluctuations on each day; in others, the fluctuations were not observed on each testing day.

Conclusions. The human eye undergoes diurnal fluctuations in axial length, with a pattern suggesting maximum axial length at midday. Based on repeated measurements, these daily fluctuations may not appear regularly in all subjects, suggesting the possibility of physiologic influences that must be defined. (*Invest Ophthalmol Vis Sci.* 2004;45:63–70) DOI:10.1167/iovs.03-0294

Numerous daily rhythms occur in the eye, including variations in visual sensitivity,[1] melatonin production,[1] photoreceptor disc shedding,[1] intraocular pressure (IOP),[2] pupil size,[3] and corneal epithelial thickness.[4,5] Some, though not all, of these diurnal rhythms are circadian; that is, they are endogenous and free-running under constant darkness. First identified in the eyes of young chicks,[6] the anatomic dimensions of the eye vary in definable patterns during the day. So far, diurnal fluctuations of axial length and of vitreous chamber and choroidal thickness have been identified in young chicks, the species most extensively evaluated.[6–9] Daily fluctuations in axial length occur in rabbits,[10] and fluctuations in axial length and choroidal thickness have been found in marmosets.[11] It has been suggested that, in chick and rabbit, these rhythms in ocular dimensions are endogenous, perhaps circadian.[10,12]

We sought in the present study to learn whether the dimensions of the human eye fluctuate during the day. We conducted this investigation using partial coherence interferometry (PCI), a technique that provides highly precise axial eye measurements.[13] In previously validating the PCI instrument that we used, we assessed the SE of the measurement ($SE_{measurement}$), a conservative estimate of precision from which the 95% confidence interval can be determined.[14,15] We found an $SE_{measurement}$ of 8 μm (95% confidence interval, 16 μm) for a single measurement series in individual subjects, aged 3 to 12 years.[14] The precision of the axial length measurement can be increased further by making multiple measurement series. The resultant precision is thus represented by the $SE_{measurement}$ divided by $\sqrt{n}$, where n is the number of measurement series. For example, if the results from five axial length measurement series are averaged, the $SE_{measurement}$ is reduced to 8.0 μm $\sqrt{5}$, or 3.6 μm. Besides high precision, PCI is a noncontact technique and thus is well suited to clinical application, not only in adults, but also in children.

Methods

Subjects

Subjects were 17 volunteers aged between 7 and 53 years, with best-corrected acuity of 20/20 or better in the eye measured. The right eye was measured, except in one subject whose left eye was measured because of decreased vision in the right eye. Because visual acuity was excellent in all subjects, we either recorded the refractive correction from the subjects' glasses if spectacles were worn or measured the refraction using a autorefractor (Retinomax; Nikon, Tokyo, Japan) without cycloplegia in subjects not wearing spectacles. The spherical equivalent refraction of the subjects at the first measurement day ranged between −2.875 and +1.00 D. The protocol was reviewed and approved by the Institutional Review Board of the Children's Hospital of Philadelphia and was in accord with the tenets of the Declaration of Helsinki.

Procedures

Subjects underwent axial length measurement with the PCI, without cycloplegia, using procedures described previously.[14] In brief, subjects, stabilized by a head- and chin-rest and gently held in position by a hand supporting the back of the head, fixated on the instrument's alignment beam. The eye was aligned in the apparatus with the aid of a video monitor. Measurements, using a measurement beam coaxial with the alignment beam, lasted 0.8 second. At each time of measurement during the day, three to five measurement series were obtained, each series comprising 16 individual PCI tracings. For the initial stud-

From the [1]Division of Pediatric Ophthalmology, The Children's Hospital of Philadelphia, Philadelphia, Pennsylvania; the [2]Department of Ophthalmology, Scheie Eye Institute-University of Pennsylvania, Philadelphia, Pennsylvania; the [3]The Children's Hospital, Dublin, Ireland; and the [4]Pennsylvania College of Optometry, Philadelphia, Pennsylvania.

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Corresponding author: Graham E. Quinn, Division of Pediatric Ophthalmology, The Children's Hospital of Philadelphia, One Children's Center, Philadelphia, PA 19104; quinn@email.chop.edu.

ies, measures were taken at four or five different measurement intervals between 7 AM to just after 12 AM.

To examine the consistency of intraday fluctuations, we requested that 10 subjects return for repeat measurements at intervals from 5 days to 8 months after the first measurement day. For these repeat daily measurements, subjects were measured at five to eight different times between 7 AM and 1 AM the following day. Eight of these 10 subjects were measured on one additional day; one subject had two repeat measurements, and another had three repeat measurements.

Data Analysis

A semiautomated algorithm[14] was used to determine the axial length, defined as the distance from the corneal surface to an interference peak corresponding to RPE/Bruch's membrane.[13,14,16] This axial length definition is analogous to ultrasonography, which measures axial length from the corneal surface to the inner retinal surface.[17] An average daily axial length was calculated for each day for each subject, using the mean axial length of all measurement series taken on that day for that subject.

Maximum measured axial length fluctuation during the day for each subject was calculated as the difference between the mean axial length at the time of longest axial length and that of the shortest axial length. A one-way analysis of variance (ANOVA) with replicate measures using a generalized linear model (SAS 8.2; SAS Institute, Inc., Cary, NC) was fit to each individual's data from the study day to determine whether the axial length measured at any of the time points differed significantly from the others. We used a criterion of $P < 0.05$ from the ANOVA to identify subjects that showed significant intraday fluctuation in measured axial length.

For those diurnal axial length readings showing a statistically significant measured fluctuation (Tables 1 and 2), an adjusted axial length was calculated by subtracting the average daily length from the measured axial lengths, and sine curve functions were fit to the adjusted length versus time of day data (SAS 8.2; SAS Institute, Inc.). The following model was used to curve-fit the axial length data:

$$y = (a/2) \cdot \sin(2\pi \cdot \text{time}/b + c)$$

where y represents the adjusted axial length, a represents the peak-to-trough difference, b represents period, and c represents the phase of the sine wave. The period was constrained in the model to be 24 ± 12 hours. The model yielded estimates with 95% confidence intervals for the amplitude of the peak-to-trough difference, the period and phase for each individual and, as indicators of goodness-of-fit of the model, the correlation coefficient (R^2) and the probability (P) of the model fit. In addition, the time of maximum axial length was estimated by solving the equation $\sin(2\pi \cdot \text{time}/\hat{b} + \hat{c}) = 1$ for time, with the constraint of time between 0 and 24 hours and where $\hat{b}$ and $\hat{c}$ were the estimated period and phase, respectively, from the sin-curve fitting.

The principal descriptive analyses of axial length fluctuations are based on the amplitude of fluctuation measured directly from the PCI readings and on estimates of periodicity and time of maximum axial length from acceptable sine curve fits to data with measured daily fluctuations.

Results

Measured Intraday Fluctuation

Table 1 provides data on demographics, refraction, and average daily axial length on the first day of assessment for each of the 17 subjects. Based on ANOVA, the direct axial length measurements showed a statistically significant variation over the day in 12 of these 17 subjects during their first measurement day (Table 1). The mean axial length fluctuation measured in these 12 subjects was 25.0 ± 7.1 μm (SD; range, 15.8–36.8).

Repeat studies were performed on a different day in 10 subjects (Table 2), 7 of whom had shown statistically significant measured intraday fluctuations at the initial study (subjects A, B, C, E, G, H, and J) and 3 of whom had not (subjects N, P, and Q). The repeat studies were performed at time intervals from 5 days to 8 months, and several repeats were obtained in two subjects. The repeat measurements showed that, for a given individual, axial length fluctuations were not manifest on every examination day. Measured intraday axial length fluctuations were present in 9 of the 10 subjects at their second measurement day. In the five subjects with significant measured intraday axial length fluctuations at the first session who had only one additional session (subjects A, B, C, G, and H), four (all but subject A) had statistically significant measured fluctuations on the second testing day (Table 2). Of the two subjects with more than two measurement sessions, one (subject E) showed significant axial length fluctuations in one of two additional sessions; the other (subject J), in two of three additional sessions. All three subjects who had not demonstrated a statistically significant fluctuation on the initial study day (N, P, and Q) showed significant measured fluctuations on repeat testing. The mean measured amplitude of daily axial length fluctuations for all studies that had significant measured axial length fluctuation was 27.3 ± 11.2 μm (SD; range, 14.2–64.2). When the significantly fluctuating axial length data were stratified by subject age, the mean fluctuation amplitude was 35.4 ± 13.9 μm (range, 14.2–64.2) for subjects 12 years of age or less ($n = 8$ studies). The mean fluctuation amplitude was 23.5 ± 6.6 μm (range, 15.8–35.3) for subjects more than 20 years of age ($n = 10$ studies). Despite the small sample size, this age difference reached statistical significance ($P = 0.02$, using the general equation estimate with correlation adjusted for repeated measurements).

Sine Curve Fitting

To provide a descriptive model to the pattern of intraday fluctuations, the data on all daily readings showing a statistically significant measured fluctuation were fit with a sine function (see the Methods section), constraining the period to 24 ± 12 hours. By using such a broad time constraint in the model, periodicity could be estimated from the available data. Examples of these fits are provided for subjects B (Fig. 1A) and C (Fig. 1B). We used $P < 0.05$ as our main criterion for acceptable modeling by a sine curve. Sine fits for 13 studies in 12 subjects met this criterion with $P < 0.05$; for these fits, R^2 ranged from 0.41 to 0.91 (Tables 1 and 2). Thus, only approximately 60% of daily axial length readings with statistically significant measured intraday fluctuation could be modeled appropriately with a sine curve. An example of a waveform from a subject with significant measured intraday fluctuation that was not suitably modeled by a sine curve is shown for subject H (Fig. 1C).

Based on the modeling of the 13 studies that showed statistically significant fits, the sine curves estimated the mean magnitude of the diurnal axial length fluctuation at 27.1 ± 8.6 μm (range, 12.8–41.4). This amplitude of diurnal fluctuation estimated by the sine fit (peak–trough difference) corresponded well to the mean amplitude of 27.0 μm of the axial length fluctuations actually measured in these 13 studies and also with the 27.3-μm amplitude from all 22 studies with significant intraday fluctuation. The mean period for the 13 studies was 21.6 ± 4.33 hours (SD), and the maximum axial length was calculated to occur at an average time of 12:55 PM (SD 2 hours 17 mintes).

In the subjects who returned for repeat measurement series at subsequent dates, the average daily axial lengths (Table 2) between the first and last measurement days were normalized

TABLE 1. Demographics, Axial Length and Intraday Fluctuation of Eye Length in All Subjects

Subject*	Age	Sex	Spherical Equiv.	Average Axial Length (mm)	Maximum Measured Axial Length Fluctuation (Highest − Lowest Mean Reading) (μm)	*P* (Measured Intraday Fluctuation†)	Sine Curve Modeling‡					
							a (Peak-Trough Difference; μm)	95% CI for *a* (μm)	*b* (Period in hours)§	Time of Maximum Axial Length (h:min)	R^2 (Sine Fit)	*P* (Sine Fit)
A	12	M	−2.875	24.458	36.8	<0.0001	30.8	7.2–54.4	21.8	13:45	0.52	0.07
B	7	F	−0.25	23.235	35.4	0.0002	36.8	28.0–45.6	20.7	12:39	0.89	<0.001
C	11	M	−0.50	23.882	30.2	0.004	34.4	21.8–47.2	17.3	9:54	0.73	0.001
D	12	M	0.00	24.002	29.3	0.004	33.8	19.8–48.0	25.3	12:00	0.79	0.002
E	25	M	−2.125	25.206	25.9	<0.05	20.8	3.4–38.0	24.9	13:01	0.15	0.15
F	22	F	−1.375	23.589	25.4	0.003	23.0	15.0–31.0	22.4	12:46	0.77	0.0004
G	20	F	−0.125	22.520	24.2	0.003	30.4	17.8–43.0	22.2	16:39	0.80	0.002
H	18	M	0.00	24.730	23.8	0.006	20.2	4.0–36.4	19.1	17:57	0.39	0.11
I	17	M	−2.75	25.121	19.9	0.02	21.0	13.0–28.8	32.6	9:41	0.91	0.01
J	53	M	0.00	23.712	17.2	0.0001	23.0	17.2–29.0	21.7	16:08	0.91	<0.001
K	26	M	0.00	23.670	15.8	0.02	19.4	7.8–31.0	21.1	15:05	0.67	0.02
L	16	F	+0.25	22.963	15.8	0.005	22.0	11.2–32.6	21.8	15:45	0.74	0.005
M	15	M	+0.75	23.541	44.4	0.71	—	—	—	—	—	—
N	12	M	0.00	24.545	20.9	0.19	—	—	—	—	—	—
O	12	M	−2.00	24.270	12.4	0.27	—	—	—	—	—	—
P	10	M	0.00	23.654	12.0	0.92	—	—	—	—	—	—
Q	44	F	+1.00	23.423	4.4	0.49	—	—	—	—	—	—

— Model not provided, as maximum measured axial length fluctuation not significant by ANOVA.

* Initial diurnal axial length in all subjects. The initial readings from subjects measured on more than one day are reproduced in Table 2, for comparison purposes.

† One-way ANOVA.

‡ $y = (a/2) \cdot \sin(2\pi \cdot \text{time}/b + c)$.

§ Constrained to 24 ± 12 hours.

Table 2. Repeat Studies of Intraday Fluctuation of Axial Length

Subject*	Date of Study	Average Axial Length (mm)	Maximum Measured Axial Length Fluctuation (Highest − Lowest Mean Reading; μm)	*P* (Measured Intraday Fluctuation†)	Sine Curve Modeling‡					
					a (Peak–Trough Difference; μm)	95% CI for *a* (μm)	*b* (Period in Hours)§	Time of Maximum Axial Length (h:min)	R^2 (Sine Fit)	*P* (Sine Fit)
A	08/12/01	24.458	36.8	<0.0001	30.8	7.2–54.4	21.8	13:45	0.52	0.07
A-2	03/20/02	24.875	32.6	0.27	—	—	—	—	—	—
B	08/12/01	23.235	35.4	0.0002	36.8	28.0–45.6	20.7	12:39	0.89	<0.001
B-2	03/20/02	23.403	14.2	0.001	10.5	2.3–19.6	19.1	12:48	0.10	0.12
C	08/12/01	23.882	30.2	0.004	34.4	21.8–47.2	17.3	9:54	0.73	0.001
C-2	03/20/02	24.054	36.5	<0.001	34.8	26.0–43.6	18.7	12:02	0.77	<0.001
E	08/12/01	25.206	25.9	<0.05	20.8	3.4–38.0	24.9	13:01	0.15	0.15
E-2	08/17/01	25.255	7.2	0.83	—	—	—	—	—	—
E-3	03/20/02	25.269	35.3	0.008	16.2	0.3–32.0	33.6	19:27	0.61	0.11
G	07/20/01	22.520	24.2	0.003	30.4	17.8–43.0	22.2	16:39	0.80	0.002
G-2	11/17/01	22.603	32.5	0.02	24.2	4.2–44.4	21.6	16:28	0.52	0.11
H	08/12/01	24.730	23.8	0.006	20.2	4.0–36.4	19.1	17:57	0.39	0.11
H-2	03/20/02	24.712	23.3	0.007	10.8	−1.0–22.8	14.2	13:16	0.17	0.32
J	07/20/01	23.712	17.2	0.0001	23.0	17.6–29.0	21.7	16:08	0.91	<0.001
J-2	08/12/01	23.712	21.5	0.02	16.6	4.8–28.0	21.7	14:06	0.14	0.16
J-3	08/17/01	23.718	27.7	0.10	—	—	—	—	—	—
J-4	03/20/02	23.828	21.1	<0.001	19.6	14.0–25.0	18.4	12:36	0.73	<0.001
N	11/17/01	24.545	20.9	0.19	—	—	—	—	—	—
N-2	03/20/02	24.567	64.2	0.001	41.4	20.8–62.0	23.5	11:18	0.44	0.001
P	11/17/01	23.654	12.0	0.92	—	—	—	—	—	—
P-2	03/20/02	23.718	36.5	0.003	20.6	−7.8–59.0	12.6	10:24	0.13	0.45
Q	11/17/01	23.423	4.4	0.49	—	—	—	—	—	—
Q-2	03/20/02	23.428	16.2	0.03	12.8	5.2–20.4	14.7	11:34	0.41	0.01

— Model not provided, as maximum measured axial length fluctuation not significant by ANOVA.

* Subject identifications without a numerical qualifier identify rows with data from the first testing day, transferred from Table 1 to facilitate comparisons. The rows with the numerical qualifiers −2, −3, and −4 provide data from the second, third or fourth measurement days, respectively for a particular subject.

† One-way ANOVA.

‡ $y = (a/2) \cdot \sin(2\pi \cdot \text{time}/b + c)$.

§ Constrained to 24 ± 12 hrs.

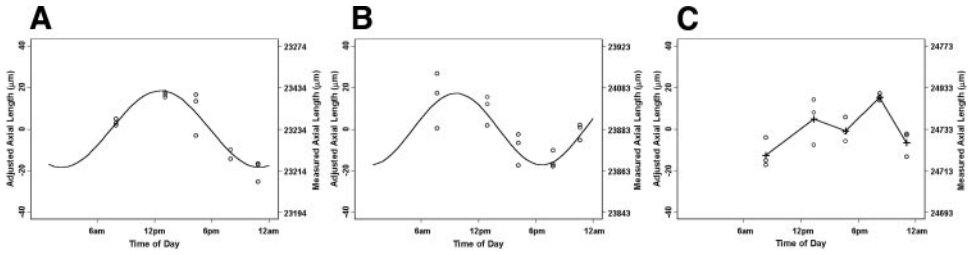

FIGURE 1. Examples of daily axial length fluctuations for three subjects. Panel (**A**) (subject B, Table 1; $P < 0.001$, $R^2 = 0.89$) and panel (**B**) (subject C, Table 1; $P = 0.001$, $R^2 = 0.73$) showed intraday fluctuations that were significantly modeled by the sine curve: $y = (a/2) \cdot \sin(2\pi \cdot \text{time}/b + c)$. Panel (**C**) (subject H, Table 1; $P = 0.11$, $R^2 = 0.39$) shows a tracing that was not suitably modeled by a sine curve. The *y*-axis on the *left* of each tracing shows the diurnal changes from the adjusted axial length and, on the *right*, the actual axial length measurements.

to the initial series to illustrate eye growth during the study (Fig. 2). Of the 10 subjects with at least two series, 9 showed axial lengthening: 4 by at least 100 μm (subjects A, B, C, and J), and 5 by < 100 μm (subjects E, G, N, P, and Q). In one subject, the last measurement was shorter than the first by 18 μm (subject H).

DISCUSSION

These results establish that the human eye undergoes daily axial length fluctuations, generally in the range of 15 to 40 μm. Fluctuations of this small magnitude cannot be identified by conventional A-scan ultrasonography, which lacks the needed precision, but instead require a method with greater resolution, such as PCI. The PCI signal analyzed in this study derives from an interference peak generated from Bruch's membrane/RPE, just beneath the photoreceptors, and the fluctuations in axial length measured by PCI could result from fluctuations in the anatomic length of the eye (i.e., the cornea-to-sclera distance), from fluctuations in choroidal thickness, or from a combination of the two.

Assuming that the optical power of the eye remains constant and that a 1-mm shift in the distance from the cornea to Bruch's membrane corresponds to 2.7 D of optical defocus,[18]

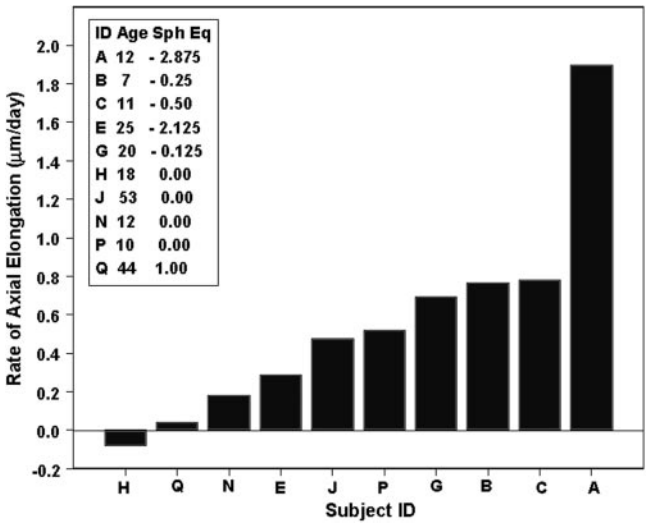

FIGURE 2. Mean rate of axial elongation (micrometers/day) between the first and last measurement session in the 10 subjects with at least two measurement sessions (Table 2). The key provides the subject identification (ID), age, and spherical equivalent refractive error (Sph Eq) at the first measurement day.

the mean daily axial length fluctuation, 27 μm, would correspond to a diurnal shift in the photoreceptor position of some $\pm$ 0.073 D in relation to the eye's image plane. While highly dependent on pupil size, illumination, contrast, and criteria for defocus, the focal depth for the human eye approximates 0.3 D.[18] Thus, diurnal axial length fluctuations are probably too small to be detectable subjectively as shifting image clarity.

Daily axial length fluctuations were measured on most subjects but not on every day. For the first set of measurements, 12 of 17 eyes showed a diurnal axial length change (Table 1). In the repeat diurnal measurements, obtained on 10 of these 17 subjects, fluctuating axial lengths were measured in three subjects (subjects N, P, and Q) who did not previously demonstrate them (Table 2). Among the subjects with fluctuations on the initial day, all but one (subject A) showed fluctuation at another visit (Table 2). Of the subjects measured on more than 2 days (subject E: 3 days; subject J: 4 days), all demonstrated axial length fluctuations on all days but one (Table 2).

Explaining why subjects do not necessarily show a diurnal axial length fluctuation on every measurement day necessitates further research. Although we controlled for time of day, other potential influences were not controlled before or during the day of measurement. These could include sleep/wake times, diurnal lighting exposure, visual activity and diet, among the many factors that may influence a diurnal cycle. Designed as a pilot, the study included subjects of both sexes, with a range of ages and with varied refractions, and the sample size was too small to make definitive comparisons in relation to these conventional demographic variables.

The peak-to-trough amplitude of the daily axial length fluctuations in humans conforms broadly, though not precisely, to results in laboratory animals. In young marmosets, the peak-to-trough amplitude measures some 25 μm, increasing to some 40 to 60 μm in adolescent animals.[11] In chicks, the peak-to-trough amplitude of axial length fluctuations approximates some 40 μm,[8,9] perhaps double that,[6] after correcting eye measurements for the particularly rapid growth in these eyes. The anterior chamber of chicks also undergoes a diurnal change in depth of approximately 20 μm, out-of-phase with the axial length fluctuations.[9] Year-old chickens correspond developmentally to human adolescents. The eyes of year-old chickens do not undergo statistically significant differences in length between the beginning and end of the light phase of a 12-hour light–dark cycle, but a diurnal fluctuation could have been missed by inadvertently sampling at times when the axial lengths may have been similar.[8] In young adult rabbits,[10] a considerably higher peak-to-trough amplitude, some 160 μm, has been measured. In rabbit, some 80% of the axial length fluctuation can be explained by in-phase fluctuations in ante-

rior chamber depth[10,19]; that is, structures different from the anterior chamber depth generate only some 30 μm of the diurnal axial length fluctuation.

Available data in marmosets[11] and chicks[8] suggest that daily axial length fluctuations vary with age. Further, the amplitude of daily axial length fluctuations in chicks may even be larger in faster-growing form-deprived eyes compared to eyes with intact visual input, though these comparisons did not reach statistical significance.[9] Despite our small sample size, daily axial length fluctuations in human eyes seem to be larger in children, suggesting a dependency on perhaps age or ocular growth rate, but more research is needed both to substantiate and to clarify this result.

Because animal research has described daily eye length fluctuations as a diurnal rhythm, we assumed that the human data would also reveal a diurnal rhythm. Traditionally, physiological rhythms are fit with sine (or cosine) curves,[20] and this model has been adopted for the eye length fluctuations of chicks,[7,9,12] marmosets,[11] and rabbits.[10] We used this modeling strategy to learn whether the eye length fluctuations in humans conform to reports in laboratory animals, and then to estimate both the period of fluctuations and the time of maximum axial length. Many, but not all, of the measured fluctuations were reasonably well approximated by a sine curve (Tables 1 and 2). Determination of why sine curves do not consistently model daily axial length fluctuations requires further study. For example, uncontrolled parameters, such as sleep/wake times, lighting exposure, visual activity, and diet, could be shifting a true diurnal axial length rhythm within a day and could account for poor sine fits. Alternatively, the length fluctuations could actually be induced by physiologic parameters that need to be defined. One potentially confounding parameter can be eliminated in humans, as PCI measurements in humans can be obtained without anesthesia, but the investigations in laboratory animals have required general or local anesthesia for ocular measurements.

The acceptable sine fits estimated a period of some 22 hours for axial length fluctuations. Circadian rhythms typically have endogenous periods on the order of 24 hours, ranging from 19 to 28 hours.[21] Our estimate of the period of axial length fluctuations in humans not only conforms to these established periods for other physiologic rhythms, but it is close to the 20-hour period of axial length fluctuations estimated for chicks reared under constant darkness to reveal the endogenous rhythm.[12] The period for axial length fluctuations in humans cannot be readily compared with other available data in animal eyes. Some animal studies[6,8] only obtained readings twice daily and therefore cannot provide an estimate of periodicity. The other reports in chick, marmoset, and probably rabbit that fit data to a sine or cosine curve set the period at 24 hours,[9–11] rather than using the data to determine the period.

The maximum axial length in our subjects occurred at midday or early afternoon. Only those animal studies with more than two measurements within a day permit any estimate of the time of maximum axial length. The approximate time of maximum axial length was found to be late in the light phase in young marmosets,[11] near the onset of the light phase in adolescent marmosets,[11] at the end of the dark phase in rabbits,[10] and in the afternoon in chicks.[9] Within the qualifications that the number of sampling times was limited and that general anesthesia was used in most of these animal studies, the time of maximum axial length in young marmosets and chicks seems to conform most closely to that observed in the present study in humans.

Diurnal axial length fluctuations are a recently described ocular rhythm in humans and several other species, and the physiologic control mechanism of this phenomenon has not yet been elucidated. One possibility is that the eye wall may passively stretch in response to diurnal changes in intraocular pressure (IOP). We did not measure IOP in the current study, as we were uncertain whether IOP measurement would impact on the accuracy of PCI measurements by disrupting the corneal epithelium either by the direct mechanical impact of applanation or by the pharmacological effects of local anesthetics. However, several observations suggest that IOP has a minor role, if any, in generating axial length fluctuations. The time of maximum axial length corresponds to the peak IOP in chicks[7,8] and adolescent marmosets,[11] but the timing of the maximum axial length is out of phase with the peak IOP in rabbits[10] and younger marmosets.[11] With twice daily measurements in chicks at the onset of light and just before the onset of dark, sympathectomy abolishes the daily IOP changes without altering daily axial length changes, thus dissociating the two rhythms.[22] In rabbit, transection of the preganglionic input to the superior cervical ganglion markedly diminishes the dark-phase increase in IOP[23] but has comparatively little effect on increasing axial length occurring during this time,[10] similarly dissociating the diurnal IOP and axial length fluctuations. On balance, available results thus do not now support a major etiologic role for diurnal IOP fluctuations in generating daily axial length fluctuations.

Corneal thickness also undergoes daily changes, presumably from altered hydration.[4,5,24,25] Even though PCI measures from the corneal surface and the magnitude of the daily variation in corneal thickness can approach that of the daily fluctuations in axial length, corneal thickness changes do not seem to explain the axial length fluctuations measured in the current study because of different time courses. Typically, the cornea is most hydrated and thickest on awakening, and it then thins rapidly over the first hour or two after eyelid opening.[5,25] Because our subjects awoke at home and traveled to our facility, the PCI measurements began each day after the initial phases of corneal thinning on eyelid opening. In addition, the longest axial length occurred at midday or later, not at early morning when the cornea is believed to be thickest. As currently understood, changing corneal thickness is thus unlikely to be a primary determinant of the axial length fluctuations measured here. Importantly though, interactions of a diurnal cycle in axial length with other time-varying parameters such as corneal thickness or IOP may have contributed to some of the variability observed in the axial length measurements.

Differences in anterior chamber depth, comparing measurements at 7 AM to 7 PM, have been described in humans using a photographic method of comparatively low precision.[26] The mean anterior chamber was found to be some 60 μm greater in the morning than later in the day,[26] a larger amplitude than the mean axial length fluctuation measured in the present study. Because the stated precision of the photographic technique was only some ±100 μm and the data are not reported in a format that reveals the fluctuation amplitude in individual subjects,[26] it is difficult to resolve the extent to which fluctuations in anterior chamber depth might contribute to the axial length fluctuations found in our study. Nonetheless, these results indicate a need to study diurnal fluctuations in anterior chamber depth with high-resolution methods and raise the possibility that, like rabbits, daily oscillations in anterior chamber depth could contribute significantly to the axial length fluctuations in humans.

The results presented herein address only the distance from the anterior corneal surface to RPE/Bruch's membrane. Other techniques are needed to address fluctuations of anterior and vitreous chamber depths and of choroidal thickness in human subjects— other ocular parameters that remain to be investigated in humans.[7–11,19,26] Our PCI measurements would reflect changes in these parameters but are not able to isolate the relative contributions from the anterior segment, vitreous

chamber, or choroid. Because the PCI signal deep to RPE/Bruch's membrane in humans is broadened with multiple peaks, assessing choroidal thickness by PCI requires methodological refinements we are presently investigating. Certainly, refining high-resolution techniques to assess simultaneously the conventional ocular components such as anterior and vitreous chamber depths as well as choroidal thickness is justified to define fully the anatomic basis for the length fluctuations measured in this study.

Regarding potential implications, animal studies suggest that daily axial length fluctuations may relate to eye growth control mechanisms. A neurotransmitter implicated in myopia and emmetropization, retinal dopamine undergoes diurnal fluctuations in its storage levels and release; physiologically, retinal dopamine fluctuations modulate retinal mechanisms involved in light and dark adaptation.[27] In experimental myopia, the daytime rise in retinal dopamine is attenuated, and a variety of dopamine-related drugs reduce the progression of experimental myopia.[28] Stimulated by the implication of these findings that some aspect of the light–dark cycle might influence refractive development, Weiss and Schaeffel[6] obtained axial length measurements in chicks every 12 hours, finding that normally growing eyes lengthen during the day and shrink slightly during the night. They also found that this intraday growth pattern changes in eyes that are becoming myopic so that the eyes lengthened during both the day and night.[6] Others have obtained analogous results in chicks.[8,9,12] Twice daily measurements do not permit full characterization of a diurnal cycle. With more frequent sampling, a phase shift of the diurnal axial length rhythms appears to account for the altered day–night patterns of myopic eye growth.[9,12] Altered patterns of daily axial length fluctuations in eyes developing ametropia have not yet been described in other species. Determining whether and how daily axial length fluctuations might be linked mechanistically to emmetropization mechanisms in humans requires further research. Nonetheless, because larger amplitude fluctuations seemed to occur in subjects 12 years old or less, an interaction of daily axial length fluctuations and refractive development in children might be a productive area to explore.

As the daily fluctuations in the distance between the cornea and Bruch's membrane/RPE approximates the 25-μm length of photoreceptor outer segments,[29] considerable dynamic shifting of the outer retinal position relative to the cornea seemingly occurs each day. Age-related changes in the biochemistry and histology of Bruch's membrane have long been recognized, and hypothesized biomechanical mechanisms related to such parameters as elasticity, permeability, have been suggested for a variety of outer retinal abnormalities, including lacquer cracks, choroidal neovascularization, and macular degeneration.[30,31] Studying eye length fluctuations also may provide a novel approach for investigating biomechanical mechanisms in outer retinal diseases.

In conclusion, we have demonstrated with the PCI technique that axial length of the human eye fluctuates during the day. Because PCI as performed herein provides the distance from cornea to RPE/Bruch's membrane, further research is needed to learn the extent to which these fluctuations result from changes in the anatomic length of the eye (i.e., cornea-to-sclera distance), fluctuations in choroidal thickness, or both. Further research is also needed to learn the relative contributions of changes in anterior chamber and vitreous chamber depth to the altered lengths measured in our study. Nonetheless, daily fluctuations of the eye's dimensions are a newly recognized physiologic parameter. Axial length studies using high-resolution technologies such as PCI may need to account for the time of day. Further, daily fluctuations in ocular dimensions may be a mechanistically informative parameter to include in future studies of ocular disorders, such as refractive development and outer retinal degenerations.

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