Diurnal variation of axial length, intraocular pressure and anterior eye biometrics.

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Abstract

Purpose: To investigate the diurnal variation in axial length and anterior eye biometrics, whilst simultaneously measuring intraocular pressure (IOP) with dynamic contour tonometry in human subjects.

Methods: Fifteen young adult near emmetropic subjects had their axial length, anterior eye biometrics (central corneal thickness and anterior chamber dimensions), and IOP measured at 6 different times across a 24 hour measurement period. Repeated measures ANOVA and sine curve fitting was used to analyze the diurnal rhythms in each measured parameter.

Results: Axial length was found to undergo significant diurnal variation ($p = 0.0006$). The mean amplitude of axial length change was 0.046 ± 0.022 mm. The mean peak in axial length was found to occur at 11:13 am. Intraocular pressure and ocular pulse amplitude were also found to undergo significant diurnal change ($p < 0.0001$ and 0.0006 respectively). The variation in axial length exhibited a significant association with the change in IOP ($r = 0.37$, $p = 0.001$). No significant difference was found between the mean peak times of axial length and intraocular pressure. Anterior eye biometric measures of central corneal thickness and anterior chamber depth were also found to undergo significant diurnal changes ($p < 0.0001$ and 0.0368 respectively).

Conclusion: Axial length undergoes significant variation over a 24 hour period. Associations exist between the change in axial length and the change in IOP as measured by dynamic contour tonometry. These results may have significant implications **for the role of ocular diurnal rhythms in emmetropization.**

Key words: Axial length, intraocular pressure, anterior eye biometrics

Introduction

It has been well established that axial length undergoes a significant diurnal variation in both animal[1-10] and human subjects.[11,12] Evidence from animal studies indicates that the amplitude and phase of these daily fluctuations in axial length may play an important role in the control of eye growth and refractive error development.[3,5,9] Whilst the pattern of these daily fluctuations in ocular dimensions has been well documented, the physiological causes and mechanisms underlying these changes are less well understood.

As intraocular pressure (IOP) is also known to undergo diurnal variation in animals[4,7,13,14] and in humans,[15-27] it is conceivable that mechanical expansion and contraction of the globe as a result of changes in IOP may be responsible in part for the daily rhythms observed in axial length. It has also been found that large alterations in IOP (due to mechanical or surgical interventions) can cause significant and predictable changes in axial length consistent with the globe expanding and contracting in response to the IOP.[28-32] However, the exact role of natural IOP variations in the diurnal variation of axial length is still unclear. Phase shifts observed between the diurnal rhythms of axial length and IOP present in chicks suggest a more complex relationship between the two variables than a simple passive stretch of the globe in response to IOP.[3] Autonomic denervation in chicks has also been found to disassociate the rhythms of axial length and IOP.[6] A recent study investigating the diurnal variations of IOP and axial length in human subjects found similar mean peak times for the two

rhythms, but no significant correlation was observed for the amplitude, phase or period of the IOP and axial length rhythms in individual subjects.[12]

Anterior eye dimensions such as corneal thickness[33-37] and anterior chamber depth[38-40] are also known to undergo significant diurnal change. As axial length is typically defined as the distance from the anterior corneal surface to the retina, changes in these anterior eye dimensions may influence the observed changes in axial length. IOP measures with most modern tonometric techniques are also known to be influenced by the thickness of the cornea.[41-46] Given that both axial length and IOP measures may be influenced by the dimensions of the anterior eye, it is conceivable that changes in anterior eye biometrics could potentially confound studies aiming to investigate the relationship between IOP and axial length.

To further investigate the factors influencing the diurnal fluctuation in axial length, we have measured axial length and anterior eye biometrics (central corneal thickness and anterior chamber depth) over a 24 hour period **(but not during the subjects' sleep periods)** in a group of healthy young adult subjects whilst simultaneously assessing IOP using a tonometric technique (Pascal dynamic contour tonometry), that is thought to provide measurements of IOP independent of corneal thickness and biomechanical properties.[47-50]

Methods

Subjects and Procedure

Fifteen young healthy, near emmetropic adult subjects aged between 20 and 27 years (mean age 22 years) were recruited for this study. Subjects were primarily recruited from the students and staff of the QUT School of Optometry. Eight of the subjects were male. All subjects were free of any ocular or systemic disease and had no history of ocular surgery or significant trauma. None of the subjects were contact lens wearers. Prior to the study, each subject underwent an initial ophthalmic examination to ensure good ocular health and to determine their refractive status. All subjects had normal visual acuity of logMAR 0.00 or better. The subjects' mean ± SD best sphere refraction was found to be -0.3 ± 0.4 DS (range +0.25 to -1.125 DS), with a mean ± SD cylinder refraction of -0.2 ± 0.3 DC (range 0.00 to -1.00 DC). No subject exhibited anisometropia of greater than 0.75 DS. Slit-lamp biomicroscopy revealed no evidence of narrow anterior chamber angles in any subject.

Approval from the university human research ethics committee was obtained prior to commencement of the study and subjects gave written informed consent to participate. All subjects were treated in accordance with the tenets of the declaration of Helsinki.

This study took place over 5 separate measurement days, with 2-4 subjects participating on each day. On each measurement day, the axial length, anterior

eye biometrics and IOP of each subject's right eye were measured in the sitting position every 3-7 hours at 6 separate measurement sessions over a 24 hour period. The initial measurement for all subjects took place in the morning, at least two hours after their reported time of awaking on that day. Following the initial measurement, subjects undertook their regular daily activities, and returned to the research laboratory for each measurement session. Over the course of the study, measurement sessions occurred at the following mean times: session 1: 09:40 (range: 08:35-11:00), session 2: 13:00 (12:00-14:10), session 3: 17:30 (17:00-18:30), session 4: 22:30 (22:00-23:20), session 5: 06:00 (05:00-06:40), session 6: 09:20 (08:00-10:20). One subject was unable to attend for one of their scheduled measurement sessions. Following session 4 (mean time 22:30), subjects went to sleep in individual darkened rooms within the research laboratory. To ensure that postural variations in IOP[27,51,52] did not influence the results, the next morning subjects were woken and were instructed to sit for 5 minutes with their eyes closed prior to the beginning of measurement session 5. At each measurement session, the time taken to perform the entire series of measurements on each subject was approximately 20 minutes. To make sure that the axial length and anterior eye biometric measurements were not influenced by any corneal epithelial disruption brought about by contact tonometry or local aesthetic instillation, the tonometry was always the final measurement performed at each session.

Axial length (defined in this case as the distance from the anterior corneal surface to the retinal pigment epithelium) was measured with the IOLMaster (Zeiss Meditec, Jena, Germany). The IOLMaster is a non-contact instrument based upon the principles of partial coherence laser interferometry (PCI)[53] and has been found to provide highly precise measurements of axial length.[54-56] For each subject a total of 5 measurements of axial length were taken at each measurement session and the mean of these readings calculated. Any measurements of axial length from the IOLMaster with a reported signal-to-noise ratio of less than 2.0 were repeated until 5 valid readings were attained.

Anterior eye biometrics were measured using the Pentacam HR system (Oculus Inc, Wetzlar Germany). The Pentacam is a non-contact instrument that utilizes a rotating Scheimpflug camera to measure the anterior segment. Previous studies have shown the Pentacam to have excellent repeatability for measuring both central corneal thickness[57-60] and anterior chamber depth.[60-63] We used the Pentacam's "50 picture 3D Scan" measurement mode for all measures. At each measurement session a total of 5 scans were performed on each subject. Any measurements flagged by the instrument's quality specification as unreliable were repeated until 5 valid measures were obtained. For this study, central corneal thickness (CCT) (centred on the corneal apex), anterior chamber depth (ACD) (the axial distance from the corneal endothelium to the anterior lens surface) and anterior chamber volume (ACV) (calculated for a 12mm diameter around the corneal apex) from each measurement were all recorded, and the

mean of each parameter calculated for each subject at each measurement session.

All IOP measures were carried out using the Pascal Dynamic contour tonometer (DCT) (Ziemer Ophthalmic Systems, Port, Switzerland). The DCT is a contact tonometer that works on the principle of contour matching. The instrument outputs mean IOP and ocular pulse amplitude (OPA) (defined as the difference between the diastolic and systolic IOP over the measurement time) as well as a quality score (where a score of 4 or 5 indicates an unreliable result) for each measurement. The DCT has been found to exhibit good inter- and intra-observer repeatability, comparable to Goldmann applanation tonometry.[47] Furthermore, the DCT has been found to provide IOP measures that are closer to true manometric pressures than Goldmann applannation tonometry[64] and also less influenced by corneal thickness than other tonometric techniques.[47-50] Measurements with the DCT were taken according to manufacturer instructions, following the instillation of a drop of local anesthetic (0.4 % oxybuprocaine hydrochloride). A total of 3 DCT measurements were taken for each subject at each measurement session and the mean IOP and OPA measures from the three measurements calculated. Any measurement displaying a quality score of 4 or 5 was repeated until 3 valid measures were obtained. All DCT measurements were taken by one clinician, experienced in the use of the instrument. For one subject at one measurement session, valid readings with quality better than 4 were unable to be obtained, and therefore no IOP or OPA

measures were recorded for this session for this subject. Following the IOP measurements, a careful slit lamp examination was carried out to ensure that no substantial epithelial disruption occurred as a result of the contact tonometry procedure or anesthetic drops.

Data Analysis

Following data collection, the mean axial length, DCT measures (IOP and OPA), and anterior eye biometric measures (CCT, ACD, ACV) were calculated for each subject for each measurement session**. Based upon the data collected for all subjects across all sessions, the average coefficient of variation was calculated for each of the measurements, and was found to be 0.08% for axial length, 4.40% for IOP, 10.39% for OPA, 0.69 % for CCT, 0.63 % for ACD, and 2.14 % for ACV.**

Each individual subject's data was combined and the group mean for each parameter at each measurement session was then calculated. To investigate the significance of changes occurring in the group mean values of each of the measured variables over the 24 hour measurement period, a repeated measures analysis of variance was carried out with one within-subject factor (time of day). The two subjects who did not have complete sets of data from all 6 measurement sessions (as outlined above) were not included in this ANOVA, since it required all subjects to have 6 complete data sets for all variables. Each individual subject's average daily axial length, IOP, OPA, CCT, ACD and ACV were also

calculated as the mean of all measurements across all sessions for each parameter. The difference from each subject's daily mean for each variable at each measurement session was then calculated. The amplitude of change (the difference between the maximum **change** and minimum **change** from the mean) in each variable for each individual subject over the 24 hour period was also calculated, as well as the group mean amplitude of change in each variable.

To investigate associations between the variations in axial length and the variation occurring in the other measured variables, an analysis of covariance was carried out as described by Bland and Altman[65] for the analysis of repeated observations. This analysis reveals a correlation coefficient (r) and a regression coefficient (slope) to describe the relationship between axial length and each of the other parameters. The significance of the association is determined based upon the F statistic from the analysis of covariance. Similar analyses were also carried out to investigate the relationship between the change in IOP and the change in anterior eye biometrics.

To further investigate the 24 hour rhythms in each of the measured variables with a mathematical model, the change from the daily mean of each variable at each session for each individual subject was modeled with sine curve fitting. The best fitting sine curve was calculated for each subject from the 6 measurements taken of each of the measured variables over the 24 hour measurement period. The following equation was used to fit the data for each variable:

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

The fitted curve therefore had a fixed period of 24 hours, and was defined by terms a (**peak to trough difference**) and c (phase). The parameters a and c were fit to the data using a linear least squares method. For each variable, the acrophase (i.e. the time at which the peak value occurred) was also determined based upon the above model and expressed as the actual clock time of the occurrence of the fitted peak. The group mean **peak to trough difference** and acrophase were also calculated. To investigate whether the distribution of acrophases across our population of subjects for each measured variable was statistically different to a Gaussian distribution, the Rayleigh statistical test was used.[66] **The Wilcoxon signed-rank test was used to determine if there were significant differences between the group mean acrophase for each of the measured variables.**

Results

Significant diurnal variation was found to occur in axial length, DCT measures and anterior eye biometrics in this population of near emmetropic young adult subjects. Repeated measures ANOVA revealed a significant effect of time of day ($p < 0.05$) for all measured variables. Table 1 displays the mean values (as measured for all subjects across all time points), **measured amplitude of**

change, and repeated measures ANOVA results for all of the measured variables. **Pair-wise comparisons (with Bonferroni adjustment for multiple comparisons) revealed no significant difference (p>0.05) between the two morning measurement sessions carried out at similar times (i.e. session1 compared to session 6) for all measured variables, indicating minimal day-to-day variation in the measured parameters.**

The group mean axial length calculated from all measurements from all subjects was found to be 23.77 ± 0.7 mm. Axial length displayed a relatively consistent pattern of change over the measurement period for all subjects, with the measured maximum in mean axial length occurring at measurement session 2 (mean time of measurement 13:00) and the minimum in the evening at measurement session 4 (mean time of measurement 22:30). The mean measured amplitude of change in axial length (maximum to minimum difference) for all subjects was 0.046 ± 0.022 mm (range 0.020 – 0.092 mm). Repeated measures ANOVA revealed the diurnal variation in axial length to be highly significant (p = 0.0006). Figure 1 illustrates the mean change in axial length over the study period.

Consistent diurnal variations in DCT measures **of IOP and OPA** were also observed. The group mean IOP was found to be 14.49 ± 1.7 mmHg and mean OPA was 2.10 ± 0.79 mmHg. Both IOP and OPA measures exhibited a significant change over the 24 hour measurement period (ANOVA p <0.0001 and

0.0006 for IOP and OPA respectively). The mean measured amplitude of change in IOP over the study was 3.12 ± 0.94 mmHg (range 1.97 – 4.97 mmHg) and in OPA was 1.27 ± 0.44 mmHg (range 0.43 – 2.50 mmHg). Both IOP and OPA exhibited their mean maximum values at measurement session 1 (9:40) and mean minimum values at measurement session 4 (22:30) (Figure 1).

Anterior eye biometrics were also found to undergo significant diurnal change. The group mean CCT was 0.532 ± 0.029 mm with a mean amplitude of change of 0.018 ± 0.008 mm (range 0.007-0.03 mm) over the 24 hour study period. The mean maximum CCT was observed to occur at measurement 5 (06:00) immediately after waking, and the mean minimum in CCT occurred at measurement session 4 (22:30) just before subjects went to sleep. Repeated measures ANOVA revealed the change in CCT over the 24 hour study period to be highly significant (**p <0.0001**). The group mean ACD was found to be 3.16 ± 0.27 with a measured mean amplitude of change of 0.073 ± 0.037 mm (range 0.034 - 0.178 mm). The mean peak in ACD (i.e. deepest ACD) occurred at measurement session 4 (22:30) and the mean trough in ACD (i.e. shallowest ACD) occurred at measurement session 5 immediately after waking. The change in anterior chamber depth over the course of the study also just reached statistical significance (repeated measures ANOVA, p = 0.036). Figure 2 illustrates the mean change observed in CCT, ACD and axial length over the course of the 24 hour study period. ACV exhibited a similar pattern of change to that observed in the anterior chamber depth throughout the day. The mean ACV

was found to be 187.1 ± 30.1 mm^3, with a mean amplitude of change of 15.10 ± 6.95 mm^3 (range 5.6 – 27.3 mm^3). The diurnal change in ACV was highly significant (repeated measures ANOVA p <0.0001).

Analysis of covariance revealed a number of significant associations between the changes occurring in axial length and the changes in the other measured variables (Table 2). A highly significant positive correlation was found between the change in axial length and the change in IOP (r = 0.370, p = 0.001). The regression coefficient for these two variables was 0.0059, indicating a change of 5.9 µm in axial length for every 1mmHg change in IOP. Figure 3 illustrates the relationship between the change in IOP and the change in axial length. A relatively weak but significant correlation was also found between the change in axial length and change in OPA (r = 0.300, p = 0.009) and a weak correlation that just reached significance was also found between the change in axial length and the change in ACD (r = -0.232, p = 0.045). The change in IOP was also found to have a strong positive correlation with the change in OPA (r = 0.659, **p <0.0001**) and a weak negative correlation with the change in ACD (r = -0.271, p = 0.02).

Sine curve fitting was performed for each subject to provide a mathematical model for the 24-hour rhythm occurring in axial length, IOP, OPA, CCT, ACD and ACV data. This modeling provided estimates for each individual subject of the mean **peak to trough difference** and acrophase for each variable. Table 3 displays the mean peak to trough difference, and acrophase for each of the

measured variables as well as the results of the Rayleigh statistical test. The Rayleigh test revealed that the timing of the peak (acrophase) was significantly different from a random Gaussian distribution for all variables measured ($p < 0.05$), suggesting that significant synchronized 24-hour rhythms occurred in each of the measured variables across our population of subjects. The Wilcoxon signed-rank test revealed no significant difference ($p > 0.05$) between the mean acrophases of axial length (mean acrophase 11:13) IOP (mean acrophase 10:23) and OPA (mean acrophase 11:30) suggesting that the mean timing of the peaks in the rhythms of these three variables coincided. Ten of the 15 subjects exhibited peak times for axial length occurring within 4 hours of the peak of IOP. Significant differences were found between the mean acrophase of the axial length and CCT (mean acrophase 4:49) ($p = 0.001$) and axial length and ACD (mean acrophase 19:13) ($p = 0.002$) indicating significant phase differences between axial length and these other rhythms.

Discussion

We have shown that significant variation occurs in axial length over a 24-hour period in our population of young adult near emmetropic subjects. Whilst the diurnal rhythms in axial length in animals have been well studied, there have been relatively few studies exploring the diurnal variation of axial length in human subjects.[11,12] The mean amplitude of change (0.046 mm) and timing of the peak axial length (11:13) found in our present study are in relatively close agreement to the results from the two previous studies of the diurnal variation in axial length

in human subjects who also used PCI techniques to measure axial length. Differences in the age of subjects tested, and/or differences in subjects' refractive status may account for some of the small differences between our current study and previous investigations.

We also found that a significant association exists between the variations occurring in axial length and the variations of IOP as measured by DCT. The mean phase timing of the peak of these two rhythms also appeared to be similar. The association observed between IOP and axial length is consistent with the hypothesis of passive expansion and contraction of the globe in response to IOP. Previous studies have found significant associations between IOP and axial length when large changes in IOP are surgically or mechanically induced,[28-32] but to our knowledge, this is the first study to show that associations exist between the natural, physiological changes in IOP and those of axial length in human subjects. The precise measurements of axial length with PCI and the fact that our IOP measures were taken with DCT (and are therefore unlikely to be confounded by concurrent changes in corneal thickness), has revealed the association between these two physiological rhythms in this study.
The association found between the change in axial length and IOP, although highly statistically significant **was not strong** ($r = 0.370$), indicating that **only** 14% of the variation in the change in axial length could be accounted for by the change in IOP. The regression coefficient for these two variables suggests approximately 5.9 microns of change in axial length per 1 mmHg change in IOP.

The mean amplitude of change in IOP was 3.12 mmHg, which based upon this regression analysis would lead to 18 microns of change in axial length. As the measured mean amplitude of change in axial length was 46 microns, the total change observed in axial length cannot be explained completely by the change in IOP. Changes in IOP therefore may be involved in the diurnal variation of axial length in human subjects, but are not the sole reason for the changes observed. Changes in choroidal thickness,[3,7-9] and/or scleral proteoglycan synthesis[67] as noted in previous animal studies may potentially also be involved in the diurnal variation of axial length in human subjects. As the IOLMaster measures from anterior cornea to the retinal pigment epithelium, it cannot differentiate choroidal thickness changes from scleral changes and therefore further research is required to comprehensively characterize the origins of the axial length changes found.

The fact that the diurnal change in IOP and axial length exhibit a significant association may have important implications for eye growth and refractive error development. Liu et al[68] found that young subjects with moderate levels of myopia exhibited differences in both the amplitude and phase of their 24 hour rhythms of IOP compared to age matched emmetropic or mildly myopic subjects. As we have found that associations exist between the change in IOP and the change in axial length in our population of emmetropic subjects, it is plausible that a population of young myopic subjects may exhibit differences in their pattern of diurnal axial length change compared to our study population. Studies

with animals have found that significant differences exist in the phase of axial length rhythms in chicks undergoing myopic eye growth, and it has been suggested that these rhythms may play an important role in the control of eye growth in these animals.[3,5,9] Further research to characterize the diurnal rhythms occurring in axial length and IOP in human myopic subjects may therefore help to clarify the etiological factors involved in the development of myopia and the control of eye growth in humans.

The diurnal variation in IOP has been the focus of numerous investigations. Previous studies investigating the diurnal variation of IOP have used a number of different tonometric techniques including Goldmann applanation tonometry,[18,22] pneumotonometry,[25,27] non contact "air-puff" tonometry[19,20,24] and Tono-pen.[23] All of these tonometric techniques have been found to be influenced to different degrees by corneal thickness.[41-46] Our current study is one of the first to report on the diurnal variation of IOP with DCT, a tonometric technique that is not influenced by corneal thickness. Our results however showed the same general trend as a number of the previous studies in healthy adult subjects using older tonometric techniques, with most subjects exhibiting their peak in IOP in the morning, and their minimum or trough in IOP observed in the afternoon/evening.[15,19,22,24,25,27,69] The magnitude of change that we found in IOP (mean amplitude of 3.12 mmHg) is also consistent with previous studies into the diurnal variation of IOP in similar populations of healthy young adult non-glaucomatous subjects.[25,27,69] Patients with glaucoma have been found to exhibit

differences in their diurnal pattern of IOP change[15,22,70-72] and diurnal variations in IOP may also be an important risk factor in the development and progression of glaucoma.[73-75] As the use of DCT helps to remove some of the variability in IOP measures associated with corneal biometric parameters, investigation of the diurnal variation in IOP with DCT in glaucomatous subjects may help to further the understanding of the role of these IOP variations in glaucoma.

A recent study by Hamilton et al[76] reported highly significant associations between the change in IOP and the change in CCT after waking, suggesting that peaks in IOP measured upon waking may relate to errors in IOP estimates due to the overnight swelling in corneal thickness (due to the associations between IOP and corneal thickness with applanation tonometers). Our current study with the DCT, generally did not find peaks in IOP to occur upon waking (for the majority of our subjects), and also found no significant association between the change in CCT and IOP ($r= 0.14$ $p = 0.23$). As DCT measures are less influenced by corneal thickness measures than other tonometric techniques, our findings are in general agreement with the suggestion of Hamilton et al[76] that peaks in IOP upon waking may relate to errors in IOP estimates due to corneal swelling. It is also possible that any peaks in IOP as a result of sleep may have subsided before our DCT measures were carried out, (the data collection procedures at each measurement session took approximately 20 minutes to complete after subjects awoke), as it has been shown previously that peaks in IOP during sleep return rapidly to normal levels within 15 minutes of waking.[19,20] We did not want to

interrupt our subjects' sleep patterns with the relatively lengthy measurement protocol (i.e. 20 minutes), so we therefore did not take any measurements with the DCT during the subjects' sleep period. However, it may aid in the understanding of the "true" IOP during sleep, to investigate the IOP with DCT during the nocturnal/sleep period.

DCT also provides measures of the OPA, a parameter thought to provide information regarding intraocular blood flow as it represents the dynamic changes occurring in IOP with the cardiac cycle (i.e. the change that occurs in IOP when a bolus of blood enters the ocular circulation with the cardiac pulse). We have demonstrated that significant change occurs in this parameter over a 24 hour period, with the highest levels being present in the morning and lowest in the evening. A previous study utilizing the Langham Ocular Blood Flow System also reported a slight decrease in OPA in the evening compared to daytime measures in normal subjects.[77] Other studies,[78,79] including a recent study using DCT[79] have reported no significant diurnal variation in OPA over their measurement period. However, neither of these two recent studies[78,79] measured OPA over a 24 hour period. By collecting OPA data from our subjects over a 24 hour period we were able to establish that significant diurnal variation does occur in this parameter. The decrease in OPA at night could be indicative of a decrease in ocular blood flow at this time. However, the change in OPA may simply be related to the observed association between OPA and IOP. We found that a significant positive correlation existed between the change in IOP and the

change in OPA. Previous cross sectional studies have also noted a significant positive association between IOP and OPA in normal subjects.[80-82]

We have also observed that significant changes occurred in anterior eye biometrics over the 24 hour study period. The significant swelling of the cornea observed in our subjects on waking is consistent with previous studies into the diurnal variation in CCT.[33-37] Axial length was also observed to be increased upon waking (compared to the previous night time measure), which is consistent with the corneal swelling upon waking contributing to this axial length change. However, no significant association was found between the change in axial length and the change in CCT ($r = 0.07$, $p = 0.56$).

Whilst there have been numerous studies investigating the diurnal variation in CCT, there have been only relatively limited studies investigating the diurnal change in ACD. We found a significant change in ACD over the 24 hour study period, with the maximum ACD observed at night and the minimum observed in the morning. The changes found in ACD were out of phase with the changes in axial length and IOP, with peaks in ACD occurring at a similar time to the troughs in the other two variables. The narrowing of the ACD observed in the morning coincides with the swelling of the cornea also observed on waking. This ACD change is therefore consistent with a swelling of the cornea in the posterior direction, as has been previously reported.[83] The amplitude of ACD change in the morning was larger than the amplitude of change in corneal thickness,

indicating that some anterior movement of the lens may also be present at first waking.

Studies with rabbits[2,40] and chicks[3,10] have reported similar changes in ACD, with increases in ACD also noted at night. Contrary to these studies with animals and to the results from our current study, the two previous studies investigating the diurnal variation of ACD in human subjects (both using relatively low resolution imaging techniques), reported either irregular changes in ACD with lowest values generally occurring at midday[38] or a decrease in ACD at night.[39] The reason for this discrepancy with the previous studies on human subjects may be due to the measurement techniques used, or the data collection protocols utilized in the different studies. In our current study we took 6 measurements over 24 hours, as opposed to two measurements 12 hours apart[39] or serial measurements between 9am and 5 pm[38] in the previous studies.

Clinically, the measurement of ACD is important in a number of applications including planning of cataract surgery and the diagnosis and management of closed angle glaucoma. All of our subjects had wide anterior chamber angles. However it is known that the physiological properties and anatomical characteristics of the anterior chamber are different in patients with angle closure glaucoma.[84] Investigation of the pattern of diurnal variation in anterior chamber parameters with high resolution techniques in populations of subjects with narrow anterior chamber angles may be an area worthy of future research and may lead

to improved understanding of the patho-physiology underlying closed angle glaucoma.

In summary we have found diurnal variation to occur in a range of ocular parameters over the period of 24 hours. We also found that significant associations exist between the change in IOP and the change in axial length. Whilst the associations found are consistent with a passive expansion of the globe in response to IOP, they do not prove that IOP changes are causing the changes in axial length. **These results may have important implications for the role of ocular diurnal variations in emmetropization and ocular growth.**

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FIGURES AND TABLES:

Figure 1: Mean change in axial length (top), IOP (middle) and OPA (bottom) over the 24 hour measurement period. All values expressed as the average difference from the daily mean at each measurement session. Vertical error bars represent the standard error of the mean, whereas the horizontal error bars represent the standard error in the mean time that the measurement was taken at each session (in hours)

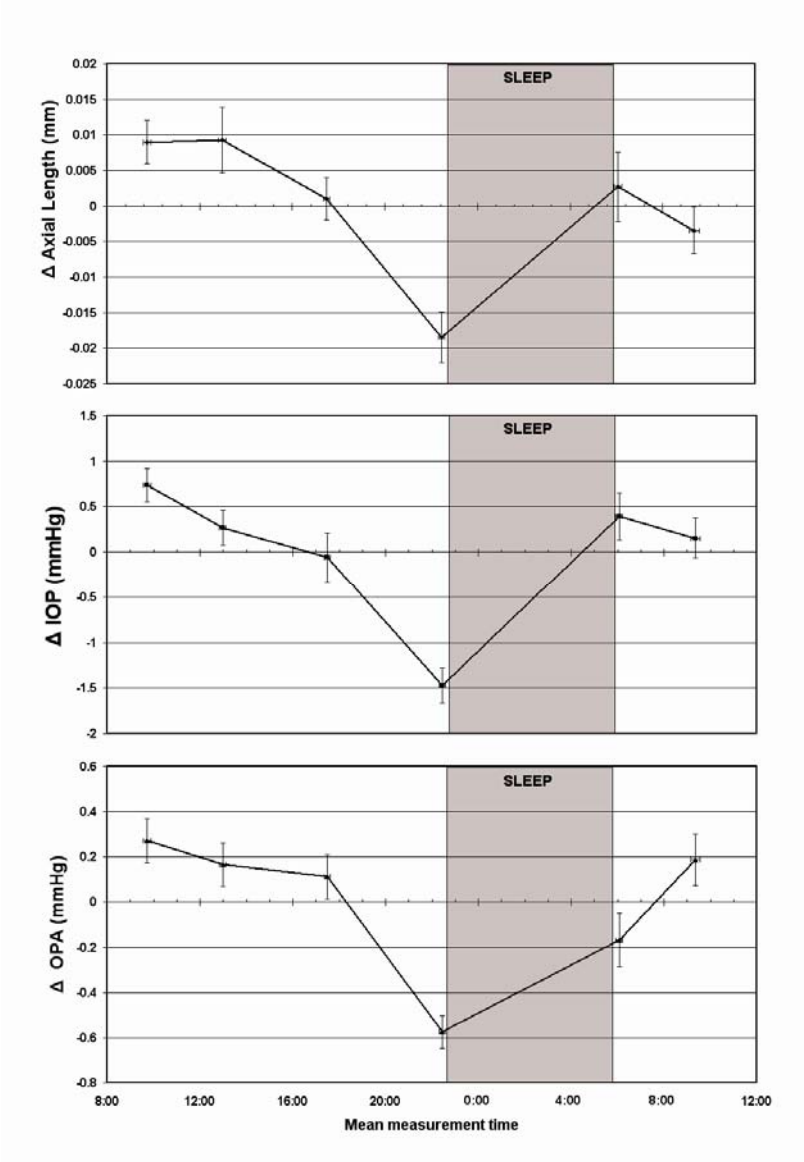

Figure 2: Mean change in CCT, ACD and axial length over the 24 hour study period. All values expressed as the average difference from the daily mean at each measurement session.

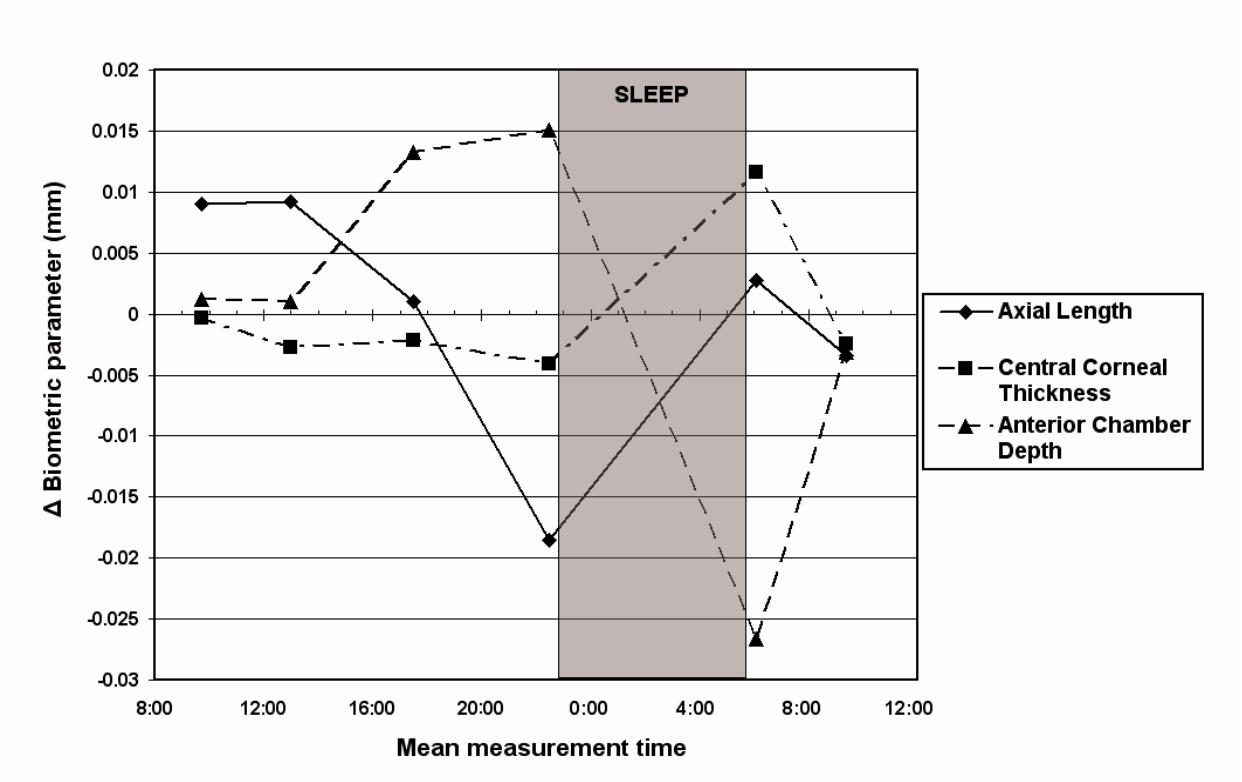

Figure 3: Change in axial length versus change in IOP. Difference in IOP from each individual subject's mean daily IOP at each measurement session plotted against the difference in axial length from each subject's individual mean daily axial length at each measurement session.

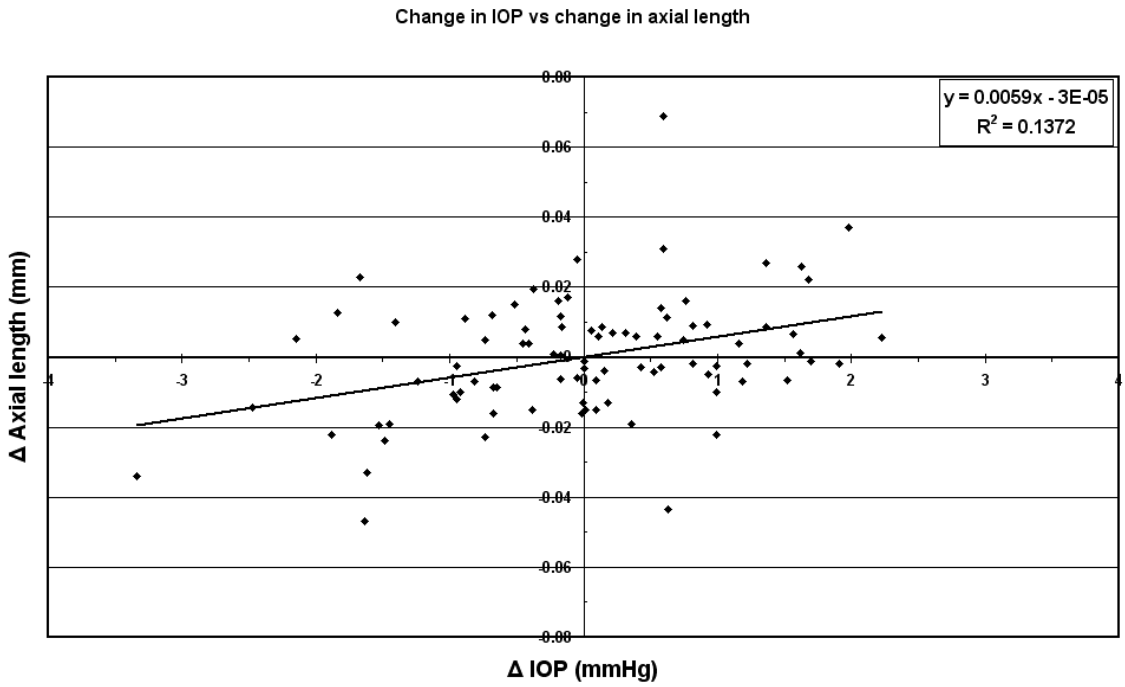

Table 1: Summary of group mean levels and amplitude of change for axial length, DCT measures and anterior eye biometrics. P-values of the F statistic from repeated measures ANOVA are displayed

Variable	Mean ± SD	Mean measured amplitude of change ± SD	p-value
Axial length (mm)	23.77 ± 0.7	0.046 ± 0.022	0.0006
IOP (mmHg)	14.49 ± 1.7	3.12 ± 0.94	<0.0001
OPA (mmHg)	2.10 ± 0.79	1.27 ± 0.44	0.0006
CCT (mm)	0.532 ± 0.029	0.018 ± 0.008	<0.0001
ACD (mm)	3.16 ± 0.27	0.073 ± 0.037	0.0368
ACV (mm^3)	187.1 ± 30.1	15.10 ± 6.95	<0.0001

Table 2: Summary of analysis of covariance investigating the relationship between the variation in axial length and the other measured variables. Note that the p-value represents the significance of the F statistic from the ANCOVA.

VARIABLE	Regression coefficient (slope)	Correlation coefficient (r)	Significance 'p'
Δ IOP	0.0059	0.370	0.001
Δ OPA	0.0109	0.300	0.009
Δ ACD	-0.143	-0.232	0.045
Δ CCT	0.180	0.068	0.563
Δ ACV	-0.0002	-0.056	0.637

Table 3: Summary of sine curve modeling of the 24 hour rhythm in axial length, IOP, OPA, CCT and ACD.

VARIABLE	Sine wave modelling				
	Peak to trough difference		Acrophase (time)		Rayleigh test
	Mean ± SD	Range	Mean ± SD	Range	p-value
Axial Length	0.029 ± 0.015	0.005 – 0.06	11:13 ± 3:27	5:50 – 16:37	0.0015
IOP	2.27 ± 0.89	0.47– 3.64	10:23 ± 3:31	5:13 – 15:36	0.019
OPA	1.04 ± 0.41	0.39 – 1.75	11:30 ± 3:17	7:03 – 15:20	0.0010
CCT	0.013 ± 0.007	0.001 – 0.023	4:49 ± 2:15	0:53 – 11:00	0.00002
ACD	0.048 ± 0.026	0.015 – 0.099	19:13 ± 4:04	12:32 – 2:58	0.0077
ACV	10.56 ± 6.15	2.11 – 22.06	15:04 ± 3:39	3:19 – 18:32	0.0001