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Twenty-four hour ocular and systemic diurnal rhythms in children

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Abstract

Purpose: Ocular diurnal rhythms have been implicated in myopia, glaucoma, diabetes, and other ocular pathologies. Ocular rhythms have been well described in adults; however, they have not yet been fully examined in children. The goal of this study was to investigate ocular and systemic diurnal rhythms over 24 h in children.

Methods: Subjects, ages 5 to 14 years (n = 18), wore a light, sleep, and activity monitor for one week to assess habitual sleep/wake patterns, then underwent diurnal measurements every 4 h for 24 h. Measurements included blood pressure, heart rate, body temperature, intraocular pressure (IOP), ocular biometry, and optical coherence tomography imaging. Saliva was collected for melatonin and cortisol analysis. Mean ocular perfusion pressure was calculated from IOP and blood pressure. Central corneal thickness, corneal power, anterior chamber depth, lens thickness, vitreous chamber depth, and axial length were determined from biometry. Total retinal thickness, retinal pigment epithelium (RPE) + photoreceptor outer segment thickness, photoreceptor inner segment thickness, and choroidal thickness were determined for a 1 mm diameter centred on the fovea. Subjects' amplitude and acrophase of diurnal variation for each parameter were determined using Fourier analysis, and mean acrophase was calculated using unit vector averaging.

Results: Repeated measures analysis of variance (ANOVA) showed that all parameters except anterior chamber depth exhibited significant variations over 24 h ($p \leq 0.005$ for all). Axial length underwent diurnal variation of 45.25 ± 6.30 μm with an acrophase at 12.92 h, and choroidal thickness underwent diurnal variation of 26.25 ± 2.67 μm with an acrophase at 1.90 h. IOP was approximately in phase with axial length, with a diurnal variation of 4.19 ± 0.50 mmHg and acrophase at 11.37 h. Total retinal thickness underwent a significant diurnal variation of 4.09 ± 0.39 μm with an acrophase at 15.04 h. The RPE + outer segment layer was thickest at 3.25 h, while the inner segment layer was thickest at 14.95 h. Melatonin peaked during the dark period at 2.36 h, and cortisol peaked after light onset at 9.22 h.

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Conflict of interest

The authors report no conflicts of interest and have no proprietary interest in any of the materials mentioned in this article.

Conclusions: Ocular and systemic diurnal rhythms were robust in children and similar to those previously reported in adult populations. Axial length and IOP were approximately in phase with each other, and in antiphase to choroidal thickness. These findings may have important implications in myopia development in children.

Keywords

diurnal rhythms; circadian rhythms; choroidal thickness; axial length; melatonin; cortisol

Introduction

Circadian rhythms are intrinsic variations in physiological parameters that undergo a cycle of approximately 24 h. Circadian rhythms are entrained to the 24 h solar day by several cues, or zeitgebers, the most potent of which is light.[1] Information about light exposure is relayed from the intrinsically photosensitive retinal ganglion cells to the suprachiasmatic nucleus in the hypothalamus, which is considered the master clock of the body.[2,3] When circadian rhythms are synchronised to the 24 h solar day, they are considered diurnal rhythms. Advances in non-contact low coherence optical biometry and optical coherence tomography[4] have made the evaluation of ocular diurnal changes in humans feasible. Diurnal rhythms have been demonstrated in humans for several ocular parameters, including central corneal thickness,[5,6] intraocular pressure,[7] axial length,[8] and retinal[9] and choroidal thicknesses.[10]

Axial length was first shown to undergo diurnal fluctuations in chicks,[11,12] then later demonstrated in humans.[13] The majority of studies examining axial length rhythms in humans has been performed in adults, with findings showing that axial length is longest in the morning and shortest during the night.[7,8,13] It is now well established that the choroid, the vascular structure providing oxygen and nutrients to the outer retina, also undergoes diurnal variations in thickness in animal models, including the chick[14] and marmoset,[15] and in humans.[10,16–19] Evidence suggests that the choroid relays information from the retina to the sclera, acting as a regulator of scleral growth.[20] Diurnal rhythms in axial length and choroidal thickness have been implicated in eye growth control.[21,22] In chick eyes that are developing refractive errors, the phase and amplitude of axial length and choroidal thickness rhythms are altered.[12,14,21] In humans, the amplitude of choroidal thickness diurnal variation has been shown to be correlated with axial length.[10,18] However, studies have shown that the relationship between axial length rhythms and choroidal thickness rhythms are similar in adult emmetropes and myopes.[10,16] These 24 h rhythms have not yet been examined in children, which is when myopia typically develops.[23] Evaluating diurnal rhythms in axial length and choroidal thickness in children is imperative to understand the potential significance of these factors in myopia development and progression.

Diurnal variations in intraocular pressure (IOP) are known to have an important role in glaucoma,[24] and may also be important in myopia.[25] Diurnal IOP can be assessed through repeated tonometry in a laboratory,[7,26] or via home monitoring using hand held tonometry[27] or continuously measuring contact lenses.[24,28] IOP is known to be the greatest in the morning, and lowest before bed-time, with healthy adult subjects showing diurnal variations

of approximately 4–5 mmHg.[26] Recently, hand held portable instruments have been utilised to assess diurnal variations in children in their home, with data collection performed by children's parents.[29–31] Flemmons, *et al.*, reported findings for 11 healthy children, and showed that, similar to adults, children's IOP was greatest in the morning and lowest at nighttime, when assessed from 6:00 am to 9:00 pm.[29] To the best of our knowledge, IOP measurements have not yet been reported for a full 24 h period in children.

Investigations of diurnal variations in corneal parameters have also been performed in adults.[6,32] Studies show that the cornea is thickest in the morning, immediately after eye opening, and decreases throughout the day. Daily fluctuations in corneal thickness have implications in IOP measurement, which has been shown to vary with corneal thickness depending on the type of tonometer used.[33,34] An understanding of corneal diurnal rhythms is also important when considering the effects of contact lens wear, including soft,[35] scleral, and orthokeratology contact lenses,[36] on the ocular surface and in ocular perfusion.

Ocular diurnal rhythms have been implicated in myopia,[22,37] glaucoma,[24] diabetes,[38] and other ocular pathologies.[39] Ocular rhythms have not yet been fully examined in children. We sought to understand if school-age children demonstrate similar 24 h ocular diurnal rhythms as previously reported in adult populations.

Methods

Healthy subjects ages 5–14 years participated ($n = 18$). Subjects provided assent and parents provided permission after the purpose of the study and the risks were explained. The study was approved by the Committee for Protection of Human Subjects at the University of Houston and followed the tenets of the Declaration of Helsinki.

The protocol is outlined in Figure 1. Subjects underwent a screening to determine ocular and systemic health. Non-cycloplegic autorefraction (WAM-5500 Binocular Accommodation Auto Ref/Keratometer, www.grandseiko.com) and axial length (LenStar biometer, www.haag-streit.com) were measured in both eyes. All subjects had best corrected visual acuity of 20/25 (0.1 logMAR, 6/7.5, 0.8 decimal) or better. Exclusion criteria were ocular disease, the use of melatonin or other pharmacological sleep aids, or travel outside of two time zones in the month before the experiment. Following screening and enrolment, an Actiwatch Spectrum device (www.actigraphy.com/) was dispensed for subjects to wear for 1 week. The Actiwatch provided continuous, objective measurements of each subject's habitual light exposure, sleep, and physical activity. Data obtained from the Actiwatch included minutes per day spent exposed to high intensity outdoor light (>1000 lux), mean daily light exposure (lux), activity (counts per minute) and sleep duration (hours), and sleep and wake times.

After 1 week, subjects arrived at the lab at 8:00 am for the first set of measurements, which were collected every 4 h for 24 h. From 8:30 am to 10:00 pm, subjects were free to go about their daily activities and return to the lab at each time point for data collection. From 10:00 pm to 7:00 am, subjects slept in the lab with all lights off, along with a parent or guardian, and were woken at 12:00 am and 4:00 am for measurements. Measurements during the night

were performed under dim red illumination. Measurements took approximately 20–30 min at each time point.

All measurements were collected with subjects in a seated position. At each time point, except for the two during the dark period, subjects first underwent a distance viewing period of 10 min to help standardise conditions under which measurements were recorded. During this time, subjects sat quietly and viewed a television screen at 4 metres under controlled laboratory illumination of approximately 400 lux (LX1330B Digital Illuminance Light Meter, www.drmeter.com).

At each time point, subjects collected 1 mL of saliva into a vial for melatonin and cortisol analysis. Samples were immediately placed into a −20°C freezer and analysed within 1 month of collection using an ELISA salivary control kit (www.salimetrics.com). Each sample was analysed in duplicate.

Heart rate and blood pressure were measured three times, separated by approximately 1 min each, using an OMRON electronic cuff (www.omronhealthcare.com/). Body temperature was measured three times using a Welch Allyn under-the-tongue digital thermometer with disposable probe cover (www.welchallyn.com/en.html). Intraocular pressure (IOP) was measured in the right eye using an Icare® rebound tonometer (www.icaretonometer.com/). Three readings were recorded, each an average of six measures. From the diastolic (DPB) and systolic (SBP) blood pressure and IOP, mean ocular perfusion pressure (MOPP) was calculated for each time point using Equation 1.

$$\mathrm{MOPP} = 2/3([\mathrm{DBP}+1/3(\mathrm{SBP}-\mathrm{DBP})] - \mathrm{IOP}) \quad (1)$$

Spectral domain optical coherence tomography (SD-OCT) was performed with a Spectralis OCT (www.heidelbergengineering.com) to assess retinal and choroidal thicknesses. At each time point, two high resolution, six line 30° radial scans, centred on the fovea and with enhanced depth imaging, were acquired. For noise reduction, B-scan averaging was set at 16 frames, and scans with less than 24 dB quality were repeated. The first image at the first time point (8:00 am) was set as the reference for each subject, with the instrument's tracking function utilised for subsequent imaging. OCT B-scans were exported and analysed with custom written software in MATLAB (www.mathworks.com) using a semi-automated process. Lateral magnification for each scan was determined using a three surface schematic eye, constructed using optical biometry obtained axial length, corneal curvature, anterior chamber depth, and lens thickness, and assuming a spherical retinal surface.[40–42] The internal limiting membrane, external limiting membrane, inner segment/outer segment border, and Bruch's membrane (identified by the retinal pigment epithelium (RPE) and Bruch's membrane junction) were automatically segmented based on A-scan intensity profiles, and errors made in border detection were manually corrected. OCT images were compensated, and sclera/choroid border was manually segmented. A representative b-scan with segmentation is shown in Figure 2. The distance from Bruch's membrane to the internal limiting membrane was calculated as the total retinal thickness. The distance from Bruch's membrane to the inner segment/outer segment border was calculated as the retinal pigment epithelium (RPE) + photoreceptor outer segment thickness. The distance from the

inner segment/outer segment border to the external limiting membrane was calculated as the photoreceptor inner segment thickness. The distance from Bruch's membrane to the posterior choroid was calculated as the choroidal thickness. Axial thickness for each layer was determined for 1536 points along each of the six scan lines. Data are presented as an average for the two images at each time point for the central 1 mm diameter.

Lastly, a non-contact low coherence optical biometer (LenStar, https://www.haag-streit.com) was used to measure central corneal thickness, corneal power, anterior chamber depth, lens thickness, vitreous chamber depth, and axial length. Five measurements were recorded from the right eye and averaged at each time point.

Statistical analyses were performed with Microsoft Excel and MedCalc (https://www.medcalc.org). Data are expressed as mean ± standard error unless otherwise noted. A critical value < 0.05 is considered statistically significant. Normality was confirmed with the Shapiro-Wilk test. For analysis of diurnal measurements, data for the first and last time points (8:00 am on the first and second day) were averaged. Diurnal changes for each parameter were normalized to the average measurement across 24 h for each subject. Repeated measures ANOVA was performed for time-of-day (within-subjects factor) to identify significant diurnal variations. For axial length, central 1 mm choroidal and retinal thicknesses, IOP, and MOPP, relationships were evaluated using cosinor analysis,[43] which uses the least squares method, in this case minimising least-squared residuals between subjects, to fit a sine wave to a time series, and is often used in the analysis of biologic time series that demonstrate predictable rhythms. Note that amplitudes of the diurnal change appear attenuated in cosinor fits due to variations in individual subject's acrophase being averaged together.

To estimate the acrophase and amplitude of diurnal variation for each parameter for each subject, Fourier analysis was used.[44] The fundamental cosine was determined using equation 2,

$$y = \text{diurnal mean} + \frac{\text{Amplitude}}{2}\cos\left(\frac{2\pi}{24}(t - \text{Acrophase})\right) \quad (2)$$

where t is time of measurement (on a 24 h clock), Acrophase is the time where the fitted cosine reaches its peak, and Amplitude is the difference between maximum and minimum y values in the fitted cosine. Acrophase was averaged across all subjects using circular statistical methods,[45] averaging of the unit vectors on a 24 h clock with the resultant phase to give average acrophase and a circular standard deviation S calculated using equation 3,

$$\text{S(hours)} = \frac{24}{2\pi}\sqrt{[2(1 - \text{r})]} \quad (3)$$

where r is the average of the unit vectors. Standard error was calculated as S divided by the square root of the count.

Results

Mean subject age was 10.06 ± 2.53 years (range 5.79 to 14.18) and included 10 females and 8 males. Spherical equivalent refraction of right eyes was +0.35 ± 0.38 D (range +3.44 to −2.38 D) and of left eyes was +0.33 ± 0.35 D (range + 3.13 to −2.12 D). Right and left eyes were not significantly different ($p = 0.81$), and only right eyes are considered further. Twelve subjects were non-myopic (+3.44 to −0.12 D), and six subjects were myopic (−1.00 to −2.38 D).

All subjects were compliant wearing the Actiwatch and did not take the device off at any time during the week. However, three of the watches underwent an error during the recording period; therefore, actigraph and light exposure data are reported for 15 subjects. Subjects demonstrated regular sleep/wake patterns the week before the experiment, i.e. one sleep period per 24 h occurring during the night. Mean daily sleep duration was 8.39 ± 0.21 h, with a mean wake time of 6:41 am ± 11 min and mean sleep time of 9:30 pm ± 46 min. Objectively measured time spent outdoors per day (minutes exposed to >1000 lux) was 62.51 ± 9.59 min. Daily average white light exposure during the day was 831.58 ± 111.77 lux, and during the night was 0.50 ± 0.26 lux. Light exposure during the night was calculated as average lux during the time the Actiwatch detected that the subject was sleeping. Mean daily physical activity during wake periods was 485.26 ± 27.53 counts per minute.

Ocular rhythms

Repeated measures ANOVA for time-of-day showed that central corneal thickness, corneal power, vitreous chamber depth, and axial length exhibited significant diurnal variation over the 24 h measurement period (Table 1). Mean central corneal thickness was 541.13 ± 8.26 μm and exhibited a diurnal variation of 12.22 ± 1.39 μm with an acrophase during the early morning dark period at 4.27 h ($p < 0.001$). On the other hand, corneal power (mean 43.86 ± 0.38 D), was greatest in the afternoon at 13.92 h, with a diurnal variation of 0.20 ± 0.03 D ($p = 0.005$). Mean vitreous chamber depth was 15.16 ± 0.17 mm, with a diurnal variation of 0.086 ± 0.01 mm and acrophase 15.91 h ($p = 0.002$). Mean axial length was 23.25 ± 0.18 mm. Axial length demonstrated a diurnal variation of 0.045 ± 0.006 mm, with an acrophase in the afternoon at 12.92 h ($p < 0.001$). Tukey outlier analysis detected that two subjects exhibited extreme values (greater than 10 times the mean) for amplitude of anterior chamber depth and lens thickness diurnal variations. When these two subjects were removed from analysis, the lens thickness exhibited diurnal variation of 0.041 ± 0.006 mm with an acrophase at 0.80 h ($p < 0.001$), and variations in anterior chamber depth were not significant ($p = 0.36$). The timing of the minimum vitreous chamber depth corresponded approximately to the timing of the thickest lens and choroid.

For the central 1 mm diameter centred on the fovea, total retinal thickness, RPE + outer segment thickness, and inner segment thickness demonstrated significant diurnal variations ($p < 0.001$ for all). Cosinor analysis for these parameters is shown in Figure 3. Mean total retinal thickness was 277.49 ± 4.52 μm, with a diurnal variation of 4.09 ± 0.39 μm and acrophase at 15.04 h. Mean RPE + outer segment thickness was 59.04 ± 0.47 μm, with a

diurnal variation of 1.69 ± 0.18 μm and acrophase at 3.25 h. Mean inner segment thickness was 28.15 ± 0.37 μm, with a diurnal variation of 2.62 ± 0.15 μm and acrophase at 14.95 h.

Mean choroidal thickness over the central 1 mm diameter centred on the fovea was 355.65 ± 13.17 μm. Choroidal thickness demonstrated a significant diurnal variation of 26.25 ± 2.67 μm with an acrophase at 1.90 h ($p < 0.001$).

Mean intraocular pressure was 12.95 ± 0.52 mmHg, and demonstrated a significant diurnal variation of 4.19 ± 0.50 mmHg with an acrophase at 11.37 h ($p < 0.001$). Mean MOPP was 36.24 ± 1.27 mmHg, with a diurnal variation of 7.92 ± 0.89 mmHg and acrophase at 23.60 h ($p < 0.001$). Cosinor analysis showing the relationship between diurnal variations in axial length, choroidal thickness, IOP, and MOPP is shown in Figure 4. Axial length acrophase occurred 1.54 h after IOP acrophase, and 11.01 h after choroidal thickness acrophase. IOP and MOPP were in antiphase with each other, with acrophases 11.77 h apart.

Systemic rhythms

Body temperature, heart rate, and mean arterial pressure all demonstrated significant diurnal variations ($p \leq 0.002$ for all, Table 2). Mean body temperature was 37.12 ± 0.06°C, with a diurnal variation of 1.07 ± 0.10°C and acrophase minute, with a diurnal variation of 12.53 ± 1.46 beats per minute and acrophase at 19.53 h. Mean arterial pressure was 83.43 ± 0.88 mmHg, with a diurnal variation of 7.17 ± 1.07 mmHg and acrophase at 23.55 h.

One subject (age 7 years) was unable to provide a sufficient saliva sample at the 12:00 am time point; therefore, salivary melatonin and cortisol analyses include only 17 subjects. Additionally, one subject was identified as an extreme outlier for melatonin concentration and was excluded. Melatonin and cortisol concentrations demonstrated significant diurnal variations ($p < 0.001$ for both, Figure 5). Melatonin maintained a low concentration during the lights on period, and increased during the lights off period. The mean amplitude of melatonin diurnal variation was 29.40 ± 3.15 pg mL^{-1}, with an acrophase at 2.36 h. Cortisol peaked in the morning after lights on, with a mean amplitude of variation of 0.26 ± 0.05 μg dL^{-1} and an acrophase at 9.22 h.

Discussion

This study shows that children demonstrate ocular and systemic diurnal rhythms similar to those previously reported in adult populations. Corneal thickness and power, axial length, retinal thickness, choroidal thickness, intraocular pressure, and mean ocular perfusion pressure undergo robust diurnal variations in children over a 24 h period. Diurnal variations in body temperature and melatonin and cortisol concentrations reported here are consistent with previous studies.[46–49]

Axial length diurnal variations observed here in children are comparable to previous reports in adults. Studies in adults have found amplitudes of diurnal variations of 19–46 lm, with acrophases in the range of 11–13 h.[7,10,16] Our findings show that children's axial length underwent significant diurnal variation of 45.24 ± 0.06 μm, with an acrophase at 12.92 h. Stone, *et al.*, assessed diurnal variations in axial length over 24 h in subjects ages 7 to 53

years old.[13] In a re-analysis of their data including only the children (n = 10, ages 7–17 years), six of ten showed significant diurnal variation in axial length, with a mean amplitude of approximately 29 μm, and acrophase at 12.28 h.

In this study, subjects showed a mean choroidal thickness of 355.65 ± 13.17 μm, with a diurnal variation of 26.25 ± 2.67 μm that peaked at 1.90 h. Previous studies examining a pediatric population have shown that the mean subfoveal choroidal thickness ranges from 227 to 359 μm, with thinner choroids in myopic compared to non-myopic children.[50–53] The amplitude and acrophase of choroidal thickness variation found here is similar to previous reports in adults, with variations of 26–34 lm, with acrophases ranging from 23.5 to 3.0 h.[10,16–18,54,55]

Children's axial length was greatest around noon, then decreased throughout the evening and night. The choroid was thickest during the night, approximately 11 h earlier than axial length acrophase, making the two rhythms in approximate antiphase. In adult subjects, we previously observed a similar pattern, with axial length and choroidal thickness rhythms being out of phase by approximately 10.5 h.[10] The relationship between axial length and choroidal thickness diurnal rhythms may play an important role in emmetropization and myopia development and progression.[37] Nickla showed that normal chicks exhibit diurnal oscillations in axial length and choroidal thickness that are out of phase.[12,14,21] Specifically, axial length and choroidal thickness rhythms in normal emmetropizing chick eyes were out of phase by approximately 9 h. In chick eyes that were growing faster and developing myopia, axial length and choroidal thickness rhythms shifted to 12 h out of phase.[12] In chick eyes that were undergoing decreased eye growth rates from prior form deprivation myopia or by wearing of positive spectacle lenses, the rhythms shifted so that axial length and choroidal thickness were in phase with each other.[12] In our subject population, children were likely past the emmetropization phase, and at an age when childhood myopia typically onsets and progresses.[23] We only measured refraction on one occasion; therefore, we were unable to determine if subjects were undergoing active myopic axial elongation. Future studies examining rhythms in children who are emmetropes versus progressing myopes may help to clarify the relationship between axial length and choroidal thickness rhythms in human eye growth.

Similar to findings in adults,[10] we found that the total retinal thickness, in the 1 mm diameter centred on the fovea, underwent diurnal changes of about 4 lm. The RPE + photoreceptor outer segments showed a diurnal variation of approximately 1.7 μm with an acrophase at 3.25 h, and the photoreceptor inner segments showed a diurnal variation of approximately 2.6 μm with an acrophase at 14.95 h. This antiphase relationship could represent differing functions of the inner and outer segments, with the inner segment containing the mitochondria and other organelles of the cell, and the outer segments containing membranous discs. We speculate that the observed diurnal rhythm in the RPE + photoreceptor outer segments was a function of cone outer segment membranous disc shedding and renewal.[56] In the 1 mm diameter surrounding the fovea, the primary photoreceptor types are medium and long wavelength cones.[57] Disc shedding has been shown to be rhythmically controlled by an intrinsic circadian oscillator that uses endogenous dopamine and melatonin as its light and dark signal, respectively,[58] which likely contributed

to the observed diurnal variations in the outer retina. Cone outer segment shedding is known to largely occur at night, following light offset, which is in phase with melatonin rhythms.[59]

Our findings indicate that children's anterior segments also undergo diurnal biometric variations. We found that the cornea was thickest in the early morning during the dark period, and corneal power was greatest in the afternoon, similar to previous findings in adult populations.[6,60] Thickening of the cornea during the night is likely a result of enema caused by a reduced oxygen supply in a closed-eye environment.[61] Decreased corneal power in the night and early morning may be due to flattening of the cornea by the closed eyelid. Lens thickness also demonstrated significant diurnal variation, following removal of two subjects that exhibited lens thickness changes that were identified as outliers, while variations in anterior chamber depth did not reach statistical significance. Previous studies in adults have been conflicting with respect to diurnal anterior chamber depth and lens thickness rhythms, with some showing significant variations,[7] and others showing no diurnal change.[10] It is possible that children did not show significant diurnal variations in anterior chamber depth because younger lenses exhibit greater accommodative range,[62] and therefore greater variability in anterior segment measures.

As discussed, several optical components of the eye demonstrated diurnal variation, including corneal power, lens thickness, and axial length, which could potentially influence refraction and visual quality throughout the day. However, taken together, it is unlikely that these factors would result in a clinically significant change in vision. For example, axial length is greatest in the morning by approximately 45 μm, equivalent to an increase in power of approximately 0.1 D, while corneal power is less in the morning (and greatest in the afternoon), by about 0.2 D. Given the depth of focus of the human eye of about 0.3–0.4 D,[63] large accommodative amplitude of children which can compensate for defocus,[64] and the rhythms of axial length and corneal power being in approximate antiphase, diurnal variations in optical components would not be expected to impact vision.

Intraocular pressure was highest late in the morning and decreased throughout the day and into the night, consistent with previous studies.[29,30] Aqueous production is known to decrease at nighttime, which may contribute to a lower IOP.[65] Intraocular pressure is also known to be influenced by posture, with higher IOP observed when the body is in a supine position due to increased episcleral venous pressure.[66,67] When diurnal IOP is measured in a supine position, IOP is highest during the night with acrophases ranging from 2 to 7.5 h.[68,69] Here, all measurements were recorded with subjects in an upright position, and the IOP acrophase was observed at 11.37 h, approximately in phase with axial length rhythms. It is likely that nighttime IOP would have been higher if subjects were in a supine position for the measurement, which would then shift the acrophase to an earlier hour. A previous study noted that IOP diurnal variations are observable for both upright and supine positions, with no significant differences in rhythm based on body position.[70] However, future studies should consider capturing nighttime IOP in a supine position to understand natural nocturnal IOP. Speculation exists whether axial length rhythms are a result of rhythms in IOP.[71,72] It would be informative to understand if a shift in IOP acrophase in a supine position would dissociate IOP rhythms from axial length rhythms. While evidence suggests that diurnal increases in IOP are not causative for axial length increases, dissociating the rhythms would

further prove that axial length rhythms are not a passive, biomechanical consequence of IOP rhythms.

We were interested in measuring body temperature, melatonin, and cortisol concentrations because they are well known markers of systemic circadian rhythms.[47,73] Additionally, recent investigations have suggested they may be altered with myopia.[10,74] Therefore, establishing these rhythms with respect to ocular rhythms in children, when myopia typically onsets, is important for future studies. Melatonin is a neurohormone synthesised and secreted from the pineal gland in darkness, mediated by input from intrinsically photosensitive retinal ganglion cells and regulated by the suprachiasmatic nucleus.[75] Cortisol is a glucocorticoid secreted from the adrenal gland and plays a role in the stress response.[48] As expected, body temperature was highest in the afternoon, melatonin peaked during the dark period, and cortisol peaked in the morning, just after the end of the dark period, similar to previous studies in humans.[48,49]

In conclusion, we have demonstrated that significant diurnal variations in multiple ocular and systemic parameters are observable over a 24 h period in children. Axial length and choroidal thickness rhythms were in approximate antiphase with each other. With increasing evidence that ocular diurnal rhythms play an important role in emmetropization and myopia development,[22] these findings may have important implications for eye growth in children.

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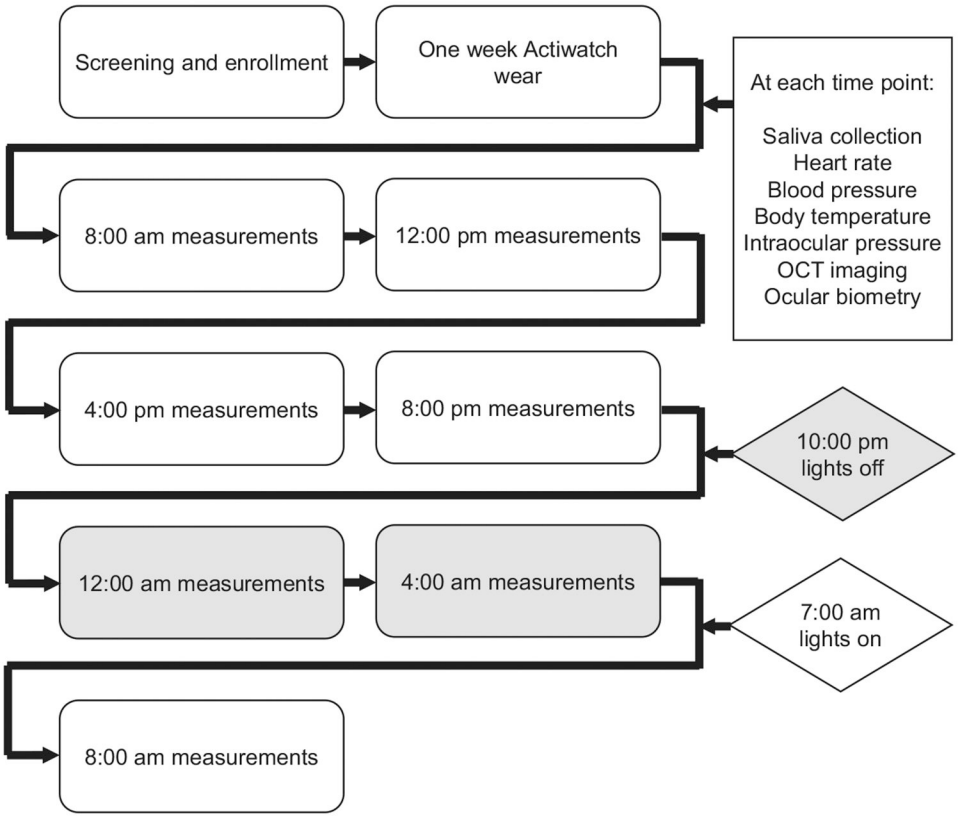

Figure 1.

Protocol. Following screening and enrolment, subjects wore an Actiwatch for one week, then underwent ocular and systemic measurements every 4 h for 24 h. Grey areas represent the dark period.

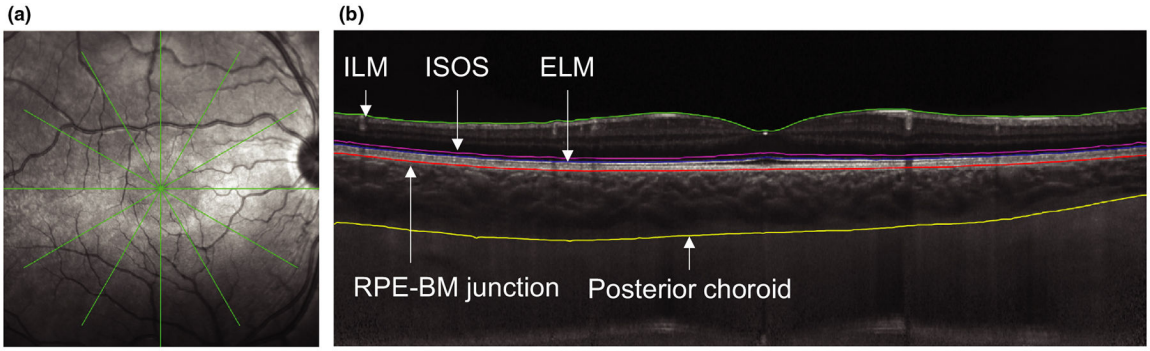

Figure 2.

a) Representative infrared image showing the radial scan pattern and b) b-scan with segmentation as follows: inner limiting membrane (ILM, green), inner segment/outer segment border (ISOS, magenta), external limiting membrane (ELM, blue), retinal pigment epithelium/Bruch's membrane junction (RPE-BM, red), and posterior choroid (yellow).

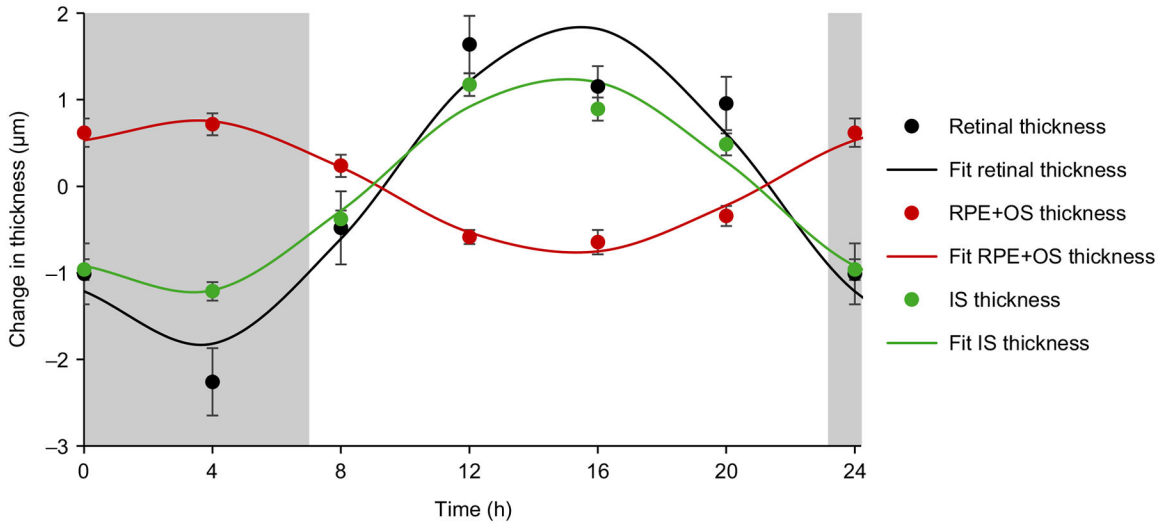

Figure 3.

Mean (± standard error) 24 h change in total retinal thickness (µm, black), retinal pigment epithelium + outer segment thickness (RPE+OS µm, red), and inner segment thickness (IS µm, green) for all subjects (n = 18); solid lines are cosinor fits to the data; grey areas represent the dark period.

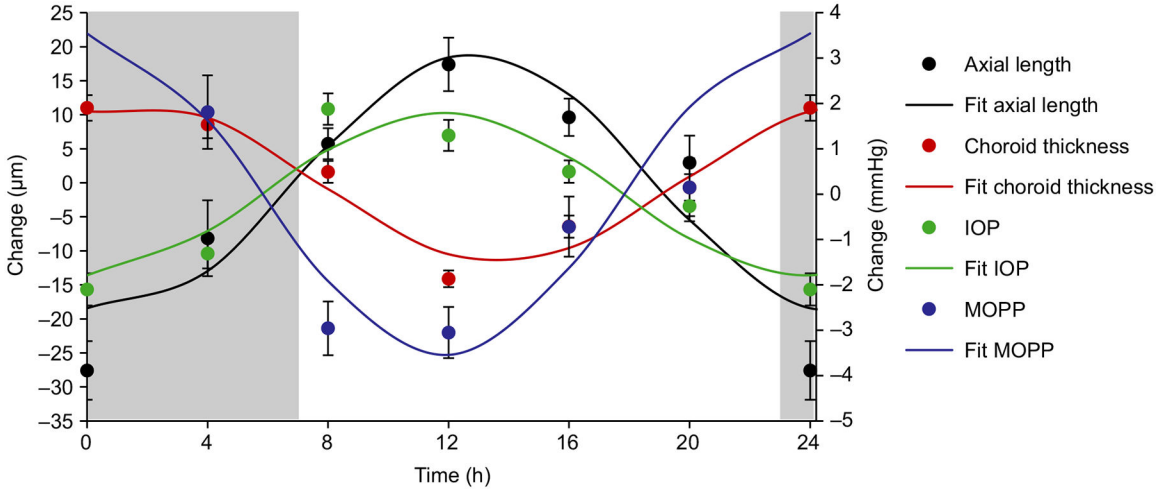

Figure 4.

Mean (± standard error) 24 h change in axial length (µm, black), choroidal thickness (µm, red), intraocular pressure (IOP mmHg, green), and mean ocular perfusion pressure (MOPP mmHg, blue) for all subjects (n = 18); solid lines are cosinor fits to the data; grey areas represent the dark period.

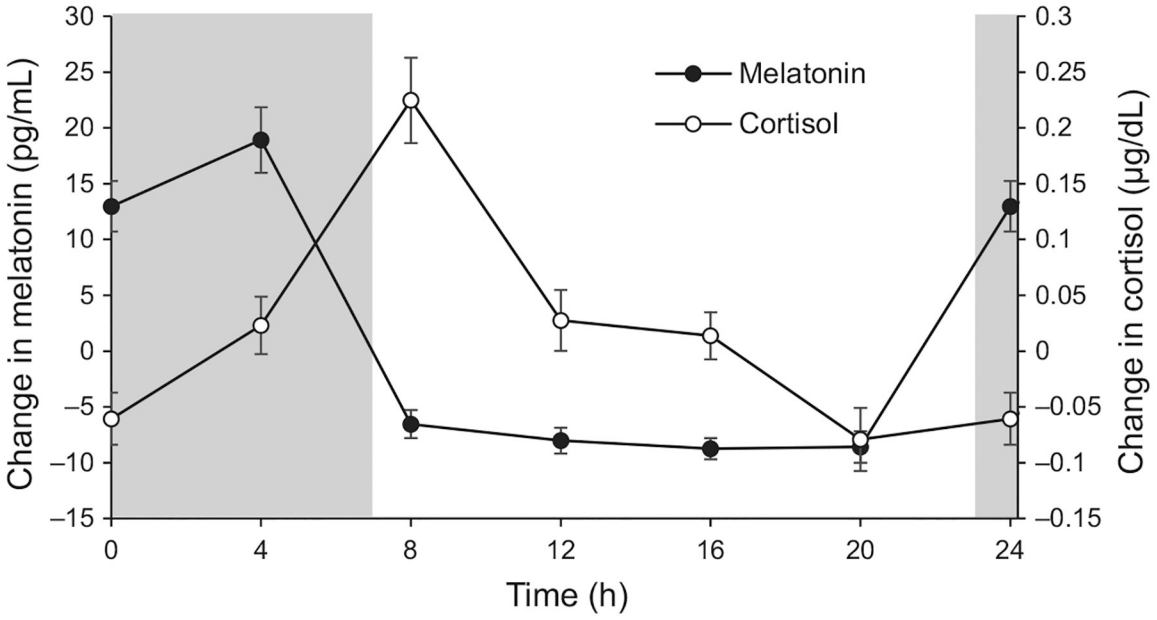

Figure 5.

Mean (± standard error) change in melatonin concentration (filled symbols) and cortisol concentration (open symbols) across 24 h for all subjects (melatonin $n = 16$, cortisol $n = 17$); grey areas represent the dark period.

Table 1.

Group mean (± standard error), amplitude of diurnal variation, and acrophase for each ocular parameter for all subjects ($n = 18$); F statistic and p values are reported for repeated measures ANOVA for time-of-day

Parameter	Group mean	Amplitude	Acrophase	F statistic	p value
Central corneal thickness (μm)	541.13 ± 8.26	12.22 ± 1.39	4.27 ± 0.38 h	27.15	<0.001 *
Corneal power (D)	43.86 ± 0.38	0.20 ± 0.03	13.92 ± 0.80 h	3.63	0.005 *
Anterior chamber depth (mm)	3.77 ± 0.06	0.041 ± 0.005	9.43 ± 1.09 h	0.46	0.36 †
Lens thickness (mm)	3.55 ± 0.04	0.041 ± 0.04	0.80 ± 0.64 h	5.93	<0.001 *,†
Vitreous chamber depth (mm)	15.16 ± 0.17	0.086 ± 0.01	15.91 ± 3.98 h	4.26	0.002 *
Axial length (mm)	23.25 ± 0.18	0.045 ± 0.006	12.91 ± 0.64 h	13.51	<0.001 *
Retinal thickness (central 1 mm, μm)	277.49 ± 4.52	4.09 ± 0.39	15.04 ± 0.53 h	15.84	<0.001 *
RPE + outer segment thickness (μm)	59.04 ± 0.47	1.69 ± 0.18	3.25 ± 0.37 h	18.60	<0.001 *
Inner segment thickness (μm)	28.15 ± 0.37	2.62 ± 0.15	14.95 ± 0.29 h	57.36	<0.001 *
Choroidal thickness (central 1 mm, μm)	355.65 ± 13.17	26.25 ± 2.67	1.90 ± 0.42 h	23.98	<0.001 *
Intraocular pressure (mmHg)	12.95 ± 0.52	4.19 ± 0.50	11.37 ± 0.57 h	17.95	<0.001 *
Mean ocular perfusion pressure (mmHg)	36.24 ± 1.27	7.92 ± 0.89	23.60 ± 0.58 h	16.78	<0.001 *

* Significance at $p < 0.05$.

† Two outliers not included.

Table 2.

Group mean (± standard error), amplitude of diurnal variation and acrophase for each systemic parameter for all subjects ($n = 18$); F statistic and p values are reported for repeated measures ANOVA for time-of-day

Parameter	Group mean	Amplitude	Acrophase	F statistic	p value
Body temperature (°C)	37.12 ± 0.06	1.07 ± 0.10	14.65 ± 0.45	14.96	<0.001 *
Heart rate (bpm)	88.03 ± 2.38	12.53 ± 1.46	19.53 ± 0.87	4.25	0.002 *
Mean arterial pressure (mmHg)	83.43 ± 0.88	7.17 ± 1.07	23.55 ± 0.74	5.69	<0.001 *
Melatonin (pg mL^{-1}) †	12.55 ± 1.29	29.40 ± 3.15	2.36 ± 0.28	40.43	<0.001 *
Cortisol (μg dL^{-1}) ‡	0.15 ± 0.03	0.26 ± 0.05	9.22 ± 0.34	15.38	<0.001 *

* Significance at $p < 0.05$.

† $n = 16$.

‡ $n = 17$.