Title: Myopia, or near-sightedness, is associated with delayed melatonin circadian timing and lower melatonin output in young adult humans

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Abstract

Study objectives: Myopia, or near-sightedness, is the most common refractive vision disorder and predisposes the eye to many blinding conditions in adulthood. Recent research has suggested that myopia is associated with increased endogenous melatonin production. Here we investigated the differences in melatonin circadian timing and output in young adult myopes and non-myopes (or emmetropes) as a pathogenesis for myopia.

Methods: Eighteen myopic (refractive error [mean ± standard deviation] -4.89 ± 2.16 dioptres) and 14 emmetropic participants (-0.09 ± 0.13 dioptres), aged 22.06 ± 2.35 years were recruited. Circadian timing was assessed using salivary dim light melatonin onset, collected half-hourly for 7 hours, beginning 5 hours before and finishing 2 hours after individual average sleep onset in a sleep laboratory. Total melatonin production was assessed via aMT6s levels from urine voids collected from 6 pm and until wake-up time the following morning. Objective measures of sleep timing were acquired for a week prior to the sleep laboratory visit using an actigraphy device.

Results: Myopes (22:19 ± 1.8 hrs) exhibited a dim light melatonin onset phase-delay of 1 hr 12 min compared to emmetropes (21:07 ± 1.4 hrs), p=0.026, d = 0.73. Urinary aMT6s melatonin levels were significantly lower among myopes (29.17 ± 18.67) than emmetropes (42.51 ± 23.97, p=0.04, *d* = 0.63). Myopes also had a significant delay in sleep onset, greater sleep onset latency, shorter sleep duration and more evening-type diurnal preference than emmetropes (all p<0.05).

Conclusions: These findings suggest a potential association between circadian rhythms and myopia in humans.

Keywords: melatonin, circadian rhythms, myopia, sleep, dim light melatonin onset, urinary 6-sulphatoxymelatonin, refractive error, axial eye length

Statement of Significance

Myopia is the most common vision disorder among children and young adults. It predisposes the eye to many blinding conditions in adulthood. Recent research has suggested that myopia is associated with increased endogenous melatonin production. In this study, we found that young adult myopes have significantly delayed melatonin circadian timing and lower melatonin output compared to normal sighted non-myopes. Furthermore, myopes also exhibited delayed and reduced sleep, and more evening-type diurnal preference than non-myopes. These findings suggest the importance of studying circadian rhythms in myopia development. Future studies on young myopic children are needed to ascertain whether delays in the melatonin circadian rhythm and preferred timing of sleep and wakefulness during pubertal development are associated with the onset of myopia.

Introduction

Myopia, or near-sightedness, is the most common refractive vision disorder among children and young adults, and represents the highest prevalence of all refractive errors globally.[1] In normally sighted individuals, or emmetropes, distant images distinctly focus at the retina. Due to elongated eyes in myopic individuals, distant images appear blurry as they focus in front of the retina. Generally, myopia first occurs in school-aged children around puberty, but can appear at any age from early childhood.[2-4] In most cases, it stabilises once the body is fully grown, usually in the early twenties. The prevalence of myopia is increasing globally and is more common in economically developed, urban regions of the world.[5-9] Myopia, especially in severe cases, is a leading cause of visual impairment because of its association with a number of vision threatening eye diseases such as retinal tear and detachment, glaucoma and cataract.[10] Despite much research, the underlying mechanisms and causes for the high and increased prevalence of myopia are unknown.

In recent years, there has been a growing interest in understanding the role of circadian rhythm disruption in myopia development (see review).[11] This is based on a large body of research suggesting that the disruption of the daily light/dark cycle, hence natural circadian rhythms, perturbs normal eye growth and leads to refractive errors in laboratory animals.[12-14] For instance, rearing chicks under constant light enlarges their eyes while flattening the cornea, resulting in hyperopia or far-sightedness.[12,13] Exposure to a minimum of 4 h darkness per day, given at the same time each day without interruption inhibits this response, suggesting a circadian effect.[15] These effects are believed to be mediated by alterations in natural circadian rhythms of the eye,[16] and changes in retinal dopamine[17,18] and melatonin[19,20] levels and their endogenous rhythms that regulate eye growth.[21-23] These intrinsic circadian rhythms are also essential for structural changes, metabolic and neurochemical activities, and gross function of the retina and the eye.[24-27]

More recent studies have investigated the link between circadian dysregulation and refractive error by examining the relationship between myopia and sleep quality.[28-31] One study found that children with high myopia exhibited the shortest sleep duration and latest bedtime, contributing to the poor self-reported sleep quality.[30] Similarly, another study reported an inverse relationship between myopia and sleep duration in Korean adolescents aged 12 - 19 years.[29] On the contrary, some studies found insufficient evidence of poor sleep in myopes.[31,32] Differing results, lack of comprehensive data on objective measures of sleep outcomes, and poor understanding of the

biological link between poor sleep and myopia warrant further investigation into the relationship between the two variables.

Melatonin, a neurohormone synthesised in the brain (by the pineal gland), mainly at night, helps to regulate sleep and alertness, and circadian rhythms that are ubiquitous to all biological functions in the body.[33] A specialised set of retinal photoreceptors known as intrinsically photosensitive retinal ganglion cells (ipRGC's) relay the environmental light information to the suprachiasmatic nuclei in the hypothalamus, which in turn regulates the endogenous release of melatonin.[34,35] Melatonin levels are elevated during the biological night and are negligible during the day.[33,36] Changes in systemic melatonin concentrations could influence ocular growth by entering into the eye through retinal or choroidal vasculature. For instance, systemic administration of melatonin has been shown to induce thinning of the choroid in chicks with experimental myopia, indicating worsening of myopia.[37]

Despite evidence of poor sleep in myopes and modulation of ocular growth through changes in melatonin concentrations, only a handful of human studies have examined the relationship between melatonin and refractive error. One recent study reported a significant positive association between morning serum melatonin concentration and the magnitude of myopia, with myopes demonstrating up to three times greater serum melatonin concentration than emmetropes.[38] In this study, fasting blood samples were collected at a single timepoint, between 8:30 am – 10:00 am, to analyse melatonin concentration. A cross-sectional melatonin profile analysis only represents the relapsing or descending phase of the melatonin curve,[39] and not necessarily the differences in total melatonin production between myopes and emmetropes. Therefore, the higher serum melatonin level in myopes may be a result of myopic individuals having a delayed timing of the melatonin circadian rhythm, greater melatonin output in total, or both. Another study by Abbott et al did not observe differences in the morning melatonin concentration between myopes and emmetropes, based on analysis of a single saliva sample collected between 9:00 am – 11:00 am.[28] In another recent study, Burfield and colleagues[40] found no differences in the peak circadian timing of melatonin in adult myopes and non-myopes (mean peak timing for both groups, 3:19 am), based on saliva samples collected every 4 hours over a period of 24 hours. However, none of these measurements represent the endogenous starting point of melatonin secretion, also known as the Dim-Light Melatonin Onset (DLMO),[41,42] which is considered as the gold-standard assessment of melatonin and circadian rhythm timing.

In this study, we investigated the differences in the timing of the melatonin circadian rhythm (i.e., DLMO timing), total melatonin output and sleep outcomes between myopic and emmetropic young adults. Moreover, we examined whether differences in melatonin output, timing, and sleep outcomes are associated with degree (or severity) of myopia. We hypothesized that:

a) the timing of the melatonin circadian rhythm, as measured by salivary DLMO, would be significantly delayed in myopic individuals compared to emmetropic individuals;
b) myopic individuals would have a higher melatonin output compared to emmetropic individuals;
c) and that due to delayed circadian timing, myopes would exhibit a significant delay in sleep onset time and more evening-type diurnal preference than emmetropes.

Methods

Participants

Eighteen myopic and 14 emmetropic (n = 17, 53% female) were recruited for the study via recruitment flyers posted on the University campus and the University online newsletter. The mean age of 21.14 ± 1.61 and 22.78 ± 2.62 years for emmetropic and myopic groups respectively were not significantly different from one another (Mann-Whitney rank sum test, p=0.089). There were no significant differences in gender between groups, $\chi^2(1) = 0.15$, p=0.70. Ethics approval was obtained from the Southern Adelaide Clinical Human Research Ethics Committee. Participants were treated in accordance with the Declaration of Helsinki and the project adhered to the National Statement on Ethical Conduct in Human Research guidelines (updated in May 2015).

Inclusion/exclusion criteria

To meet inclusion criteria, participants were required to be 18 to 25 years old. Myopes with refractive error of -1.50 dioptres or greater and emmetropes within ±0.5 dioptres of zero error, with a logMAR visual acuity of 0.00 or better were recruited.

Exclusion criteria enabled screening of behavioural, medical or psychological variables known to interfere with circadian rhythms or the interpretation of eye measurements, including: (a) self-reported or diagnosed sleep disorders; (b) use of medication affecting melatonin or dopamine levels

(e.g., melatonin supplements, anti-inflammatory drugs); (c) history of major eye disease or corrective refractive surgery; (d) a difference in power of > 1.00 dioptres between the two eyes; (e) a refractive error other than myopia; (f) a cylindrical refraction or astigmatism > 1.00 dioptres; (g) were diagnosed with clinical depression or anxiety; (h) smoke; (i) were habitual high consumers of alcohol (>7 drinks/week) or caffeine (>400mg of caffeine/day); (j) indicated a history of substance abuse in the last 6 months; (k) worked night shift in the past two months (defined as a work period between the hours of 22:00 and 08:00); (l) undertook trans-meridian travel of ≥ 2 time zones in the last two months; or (m) were pregnant or lactating.

Materials

Online self-report questionnaires: Interested and consenting candidates' eligibility was screened using a structured, online battery of self-report questionnaires including a general health and medical questionnaire designed by the authors to specifically examine inclusion and exclusion criteria. The questionnaire battery also consisted of validated questionnaires including the Pittsburgh Sleep Quality Index (PSQI)[43] and the Morningness-Eveningness Questionnaire[44] to measure general sleep behaviour and differences in sleep quality between conditions, as well as diurnal preference, respectively.

Ocular measurements: Those who continued to meet inclusion criteria attended a vision and eye health examination with an experienced optometrist (RC), at the Flinders Health2Go Clinic to further assess ocular eligibility. All ocular measurements were performed using standard clinical instruments commonly used in optometry clinics. To determine the optical power of the eye or spherical equivalent refraction (SER), an undilated autorefraction using a Zeiss i.Profiler plus (Carl Zeiss Vision, Germany; "Zeiss i.Profiler plus") was performed.[45] An IOL Master 500 (Carl Zeiss, Jena, Germany; "Zeiss IOLMaster 500") was used to measure the axial length of the eye.[46,47] A Cirrus High-Definition Optical Coherence Tomography (Cirrus HD-OCT 5000, Carl Zeiss Meditec Inc., Dublin, CA, USA; "Cirrus HD-OCT 5000") was used to examine retinal structure and eye posterior.[48] Visual acuity was measured using the standard Snellen visual acuity chart, and the mean of the representative value of both eyes was used for analyses. Ocular biometry measurements were taken between 09:00 - 12:00 to minimise circadian diurnal effects.[49]

Information on parental myopia was obtained from a validated refractive status questionnaire and categorised as either '0 parents myopic', '1 parent myopic' or '2 both parents myopic'.[50] Because myopia is strongly associated with greater amounts of time spent on reading and near activities,[51,52] the amount of near work performed during different visual activities was quantified using a variable called dioptre-hours (Dh).[50,51] Using a modified questionnaire, the Dh was calculated as: Dh = 3 x [time spent on intense near reading for an assignment + writing an assignment on a notebook + reading for pleasure (e.g. reading magazines or books everyday as hobby) + hand-held video games + intensely working on mobile/tablets + casually browsing on mobile/tablets (e.g. Facebook, Twitter etc.)] + 2 x [time spent working on computer or laptop for an assignment + casually browsing on computer or laptop (e.g. Facebook, Twitter etc.) + playing video-games (e.g. X-box, PlayStation etc.)] + 1 x (watching television).

Outcome measures

Actigraphy: Sleep measures were derived from a wrist worn activity device that was used to infer sleep during periods of inactivity. Participants were provided with a Philips Respironics Actiwatch 2 with an inbuilt light sensor, to wear on the dominant hand during the seven days leading up to their sleep laboratory stay. Participants were informed that Actwatch 2 are waterproof for < 30 minutes, thus could generally be worn at all times. They were also instructed to depress an event marker on the side of the watch to indicate the time they attempted sleep at night and got out of bed in the morning, enabling objective timestamping of exact moments when sleep opportunities commenced and ceased each night.

Philips Actiware (version 6.0.9, Philips Respironics, Bend, OR) software was used to initialize collection of activity and light data in 30-second epochs, which was later downloaded and scored in the same software program. Trained personnel verified and manually scored the actigraphy data using event marker and habitual bedtime from the sleep-wake diary to infer the start and end of rest periods for all participants. The software algorithm calculated the sleep periods using default algorithms and medium sensitivity threshold. The software algorithm then automatically calculated objective sleep parameters (i.e., sleep onset, sleep onset latency, frequency and length of awakenings after sleep onset, total sleep time, wake up time and sleep efficiency). Actigraphy has a

high sensitivity of detecting sleep (97%) but low specificity of detecting wake (33%) compared to polysomnography.[53] Nevertheless, it is a reliable method for recording sleep/wake patterns.[54]

Sleep-wake diary: In conjunction with the actigraphy device, participants were also provided with a pen-paper version of a sleep-wake diary to complete during the seven days leading up to their sleep laboratory stay.[55] Participants were instructed to complete the sleep-wake diary each morning immediately upon getting out of bed. To measure habitual sleep and daily activities, participants reported bedtime, time lights were turned off to attempt sleep, sleep onset time, nocturnal awakenings, wake up time, out of bed time (Supplemental Table 1). In addition to their sleep habits, participants also recorded daytime activities know to affect sleep such as nap time and duration, as well as timing and frequency of caffeine, alcohol and food intake. In addition to measuring general sleep and activity patterns, habitual bedtime from the sleep-wake diary (confirmed by actigraphy) was used to estimate optimal times of saliva collection for dim light melatonin onset measurements.

Salivary dim light melatonin onset (DLMO) measurement: DLMO was derived from saliva samples collected using Salivettes® (Cat. No. 51.1534; Sarstedt Australia Pty Ltd, Mawson Lakes, SA). Fifteen half hourly saliva samples were collected for 7 hours, starting 5 hours before and continuing to 2 hours after participants' sleep diary-estimated typical bedtime. Participants remained in a dim environment (< 10 lux) and refrained from food consumption from 17:00 (when they entered the sleep laboratory) until after their final saliva sample collection. Participants were permitted to drink water up to 5 minutes prior to their next sample. During collection, cotton swabs were saturated with saliva by rolling the swab in the mouth for > 1 minute to ensure sufficient saliva saturation for assay analysis and maintain consistent sample timing between participants.[56]

Samples were stored in the sleep laboratory freezer at -20°C and were sent to the Adelaide Research Assay Facility (Robinson Research Institute, University of Adelaide) for double antibody radioimmunoassay using standards and reagents supplied by Bühlmann Laboratories (RKDSM-2, Bühlmann Laboratories AG, Schönenbuch, Switzerland). The assay was based on the Kennaway G280 anti-melatonin antibody[56], used $^{[125I]}$2-iodomelatonin as the radioligand and followed the protocol provided by Bühlmann Laboratories. Saliva samples were assayed in duplicate using a sensitivity of 4.3 picomoles (pM). The intra-assay coefficient of variation from our assays was

determined to be 7.4%. The inter-assay coefficient of variation of the low and high concentration quality control was 4.8% and 6.7% respectively.

Urinary melatonin measurement: Participants' last uncollected urine void occurred at 18:00. After this time, urine secretion was collected in a 3.0 L urine container for each individual participant (Cat. No. 77.575.001; Sarstedt Australia Pty Ltd, Mawson Lakes, SA), with the final urine void made prior to participants' discharge from the laboratory on the following morning. This measurement reflected the cumulative amount of circulating melatonin corresponding to the period between the initial and final urine void. An assayed concentration of an aliquot sample was multiplied by the total urine volume to calculate the total melatonin metabolite output for the night for each participant. Urinary 6-Sulphatoxymelatonin (aMT6s) was detectable in all urine samples.

The aMT6s levels[57] were analysed at the Adelaide Research Assay Facility by double antibody radioimmunoassay, using standards and reagents supplied by Stockgrand Ltd. (aMT6s-HU-K200, Stockgrand Ltd., Guildford, Surrey, UK). This assay was derived from the method developed by Aldhous and Arendt.[58] 500µl of 1:250 dilution urine samples were incubated overnight at 4°C with 100µl of a 1:20,000 dilution solution of anti-aMT6s antibody and 50µl [125I]aMT6s radioligand (diluted to ~5000 total counts per minute). The following day the antibody-bound fraction of aMT6s was precipitated by adding 50µl ice cold anti sheep/goat SacCel (IDS, Bolton, UK), incubating at 4°C for 20 min and then centrifuging at 4000 rpm for 20 min. The supernatant containing unbound radioligand was removed, and the radioactivity of the precipitate was determined in a Wizard 2 gamma counter (Perkin Elmer, Glen Waverley, Victoria, Australia). The aMT6s levels were calculated by interpolation from the standard curve. The sensitivity of our assay was 0.5ng/ml. Samples were assayed in duplicate and the intra-assay coefficient of variation of our assay was 7.1%.

Procedure

Screening and intake: Participants providing informed consent were sent a unique identification number and a link to online self-report screening questionnaires. Those who met general sleep, medical, and health inclusion criteria were invited to attend an appointment at the Flinders Health2Go Clinic for a brief eligibility screening of their vision, refractive error and eye health with an optometrist. Those who were deemed eligible, underwent a comprehensive eye examination as

described above. After the eye examination, eligible participants were given the sleep-wake diary to complete and Actiwatch to wear during the 7-days prior to their sleep laboratory overnight session.

Laboratory protocol: On the morning of their experimental overnight, participants had their last meal prior to arrival and no later than 17:00. They were instructed not to eat cheese, bananas, chocolate, asparagus and drinking alcohol or caffeine drinks, which are known to affect salivary melatonin concentration.[59] During saliva collections for DLMO determination, participants remained awake and engaged in sedentary activities (e.g., watching television, laptop work). The ambient lighting and electronic device remained <10 lux at all times, to ensure that melatonin secretion would not be inhibited by light exposure and interfere with a valid measure of DLMO.[60] At 18:00 participants made their last uncollected urine void and all subsequent urine voids were collected. Urine containers were kept refrigerated in between voids. Saliva samples were collected half-hourly beginning 5 hr before and continued for 2 hr after their baseline sleep onset time. After the final saliva sample was collected, sleep was permitted, and participants slept in until they naturally woke the following morning to ensure all melatonin metabolite output was collected. Participants made a final urine void before laboratory discharge.

Statistical analysis

The left and right eye SER and axial length values were used to calculate the average SER and axial length. DLMO timing was defined when the salivary melatonin reached a threshold of 12.9 pM and remained elevated for a minimum of two samples (i.e., 1 hour) following this time.[61] The estimated DLMO timing was calculated manually by simple linear interpolation between the pre-and post-DLMO value of interest for all participants. In one participant, melatonin output was detected in the urine analysis, however salivary melatonin levels remained < 4.3 pM throughout saliva collection suggesting that saliva sampling did not occur late enough to catch DLMO. For this individual, DLMO was conservatively estimated as the final saliva sample time. The aMT6s concentration values were multiplied by the total urine volume to calculate the total aMT6s excreted values per participant.

All reported sleep measures including, sleep onset time (hours: minutes), sleep onset latency (minutes), wake after sleep onset (minutes), total sleep time (hours), total time in bed (hours), wake

up time (hours: minutes) and sleep efficiency (%) were based on objective actigraphy estimates, except for bedtime and lights-out time that were determined from self-reported sleep diary data.

The differences in ocular (SER, axial length, parental myopia and Dh), melatonin (DLMO and the total urinary aMT6s) and sleep profiles between the two refractive error groups (myopic, emmetropic) were determined using independent samples t-tests, or when normality failed, Mann-Whitney tests for independent samples. A linear regression analysis was performed using the least-squares approach to investigate the association between melatonin and sleep variables and the two primary independent variables (i.e. SER and axial length) across the two refractive groups combined. For this analysis, both groups were combined to study the association between circadian rhythms and the entire range of refractive errors, from emmetropia to high myopia. All statistical analyses were performed using commercial software SigmaStat 3.5 (Aspire Software International, Ashburn, VA). A p-value of <0.05 was considered to be statistically significant.

Results

Ocular profile

The mean differences in ocular, melatonin and sleep characteristics between the myopic and emmetropic groups, as well as their effect sizes (Cohen's *d*) are shown in Table 1. The optical power of the eye or SER was significantly more negative for the myopic group (mean ± standard deviation [SD], -4.89 ± 2.16 dioptres [D]) than the emmetropic group (-0.09 ± 0.13 D, Mann-Whitney rank sum test, p<0.001, Figure 1a). Consistent with the negative SER, the axial length of the eye was ~1.84 mm longer in myopes compared to emmetropes [t (30 degrees of freedom or df) = -5.937, p<0.001, Figure 1b]. Furthermore, the myopic group also had a greater number of myopic parents (0 myopic parents, 16.67%, 1 myopic parent, 50%; 2 myopic parents, 33.33%) than the emmetropic group (0 myopic parents, 85.71%, 1 myopic parent, 14.29%; 2 myopic parents, 0%) ($\chi^2(2) = 15.60$, p<0.001). Myopes also spent significantly more time doing reading and near work (i.e. Dh) compared to the emmetropic group [t (30 df) = -2.070, p=0.023]. The effect sizes of Dh and parental myopia were medium to large; *d* = 0.74 and 1.76, respectively (Table 1).

Melatonin profile

It was hypothesized that myopic individuals would exhibit higher urinary melatonin output compared to emmetropic individuals. The total amount of urine secreted overnight was not different between the two refractive groups [t (30 df) = -0.558, p=0.291, Table 1]. However, the aMT6s levels derived from the total overnight volume of urine were significantly lower in myopes (29.17 ± 18.67) compared to emmetropes (42.51 ± 23.97, Mann-Whitney rank sum test, p=0.042, Figure 2a). The effect size of the mean difference was moderate, *d* = 0.63

Consistent with our hypothesis, the myopic group exhibited a significant phase-delay of 1 hour and 12 minutes, in the salivary DLMO in comparison with the emmetropic group [t (29 df) = 2.023, p=0.026, Figure 2b]. The effect size was moderate, d = 0.73.

Sleep profile

Both objective and subjective measures of sleep showed overall poor sleep quality in myopes (Table 1). Consistent with phase-delayed DLMO in myopes, myopic individuals exhibited a significant delay in sleep onset time by 56 minutes compared to emmetropes [t (30 df) = -2.149, p=0.019, Figure 3]. Myopes also exhibited a greater sleep onset latency (i.e. time taken to fall sleep after lights-out), shorter total time in bed and shorter total sleep time than emmetropes (all $p<0.05$, Figure 3). However, bedtime, lights-out time, midsleep point, wake up time, wake after sleep onset, and sleep efficiency were all similar between the two refractive groups (all $p>0.05$, Figures 3 and 4). The effect sizes of all measured sleep variables were generally medium to large, as shown in Table 1.

The global PSQI score of subjective sleep quality over the past month was not statistically different between the two refractive groups [t (30 df) = -1.509, p=0.070, Figure 5a]. Both groups were generally intermediate types with MEQ scores between 42 and 58 (emmetropes, 53.21 ± 8.47; myopes, 47.44 ± 5.99); however, the difference in the MEQ score was statistically significant, indicating that myopes were relatively more ‘evening type’ than normal sighted emmetropic individuals [t (30 df) = -2.258, p=0.015, Figure 5b]. The effect sizes of PSQI and MEQ measures were moderate to large; *d* = 0.54 and 0.80, respectively (Table 1).

Association between variables

Because there was considerable variation of refractive error and axial length across the two refractive groups it was informative to complement the analysis of differences between groups with a regression analysis (Table 2).

Figure 6 shows the association between the two dependent melatonin variables and the two independent ocular variables, SER and axial length, across the two refractive groups. DLMO (SER, r^2 = 0.22; axial length, r^2 = 0.17), urinary aMT6s levels (SER, r^2 = 0.15; axial length, r^2 = 0.16), MEQ score (SER, r^2 = 0.19; axial length, r^2 = 0.13) and sleep onset latency (SER, r^2 = 0.14; axial length, r^2 = 0.20) were all significantly associated with both SER and axial length (all $p<0.05$). A significant negative association was observed between sleep onset time and SER (r^2 = 0.15, p=0.03) and a significant positive association was observed between total sleep time and SER (r^2 = 0.22, p=0.007) and total time in bed and SER (r^2 = 0.20, p=0.011). None of the other sleep variables were significantly correlated with either SER or axial length (Table 2).

Discussion

Myopia is the most common refractive disorder in younger people.[1] In this study, we showed that young adult myopes have significantly delayed melatonin circadian timing as measured by DLMO, lower melatonin secretion as measured by urinary aMT6s levels, delayed sleep timing (as indicated by delayed sleep onset time, greater sleep onset latency and shorter sleep duration) and more evening-type diurnal preference than emmetropes.

Clinical research suggests that myopia represents a “complex” disorder with both environmental and genetic origins.[51,62,63] Numerous studies have shown that many factors, including family history, increased near work, educational pressure, socioeconomic status and less time spent outdoors are associated with myopia.[51,64-66] The rapid increases in the prevalence of myopia warrants further research into other factors that may affect its onset and progression. This study may provide important insights into the role of sleep and melatonin rhythms in the pathogenesis of myopia.

Our study adds to the growing evidence of a potential association between circadian dysregulation and myopia development.[11] Previously, a transcriptome analysis of the chick retina following 6 hours of experimentally induced myopia identified several classes of differentially expressed circadian clock genes, including *Opn4* (melanopsin)*, Clock* (circadian locomotor out-put

cycles kaput), *Cry1* (cryptochrome 1), *Npas2* (neuronalpas domain protein 2), *Per3* (period homolog 3) and *Mtnr1a* (melatonin receptor 1A). In another recent study, retinal-specific knockout of the clock gene *Bmal1* induced myopia in mice.[67] More recently, a meta-analysis of genome-wide association studies (GWAS) involving 542,934 European participants identified genetic factors that regulate circadian rhythms, to be associated with myopia.[68]

A novel finding from this study is that melatonin circadian timing was delayed by ~1 hour in young adult myopes compared to normally sighted emmetropes. In addition, the delay in DLMO was positively correlated with the degree of myopia, that is, severe or high myopes exhibited greater delay than low myopes and emmetropes. In a recent study, Burfield et al measured circadian timing of melatonin in adult myopes and emmetropes (age 21 - 41 years) by collecting saliva samples every 4 hours over a period of 24 hours, beginning at 8:00 am.[40] In this study, the acrophase of melatonin at 3:19 am was not significantly different between the two refractive groups. Due to the nature of the study where the participants were exposed to normal indoor lighting until 11:00 pm at night and infrequent sampling of data in the evening, this study missed the gold-standard assessment of melatonin and circadian rhythm timing, the DLMO. This study used a more robust methodology to assess melatonin circadian timing in individuals with myopia.

A delayed circadian rhythm timing is a major etiological factor for Delayed Sleep-Wake Phase Disorder (DSWPD).[41,69-71] Individuals with DSWPD exhibit a delay of ~ 3 - 4 hours in core body temperature and melatonin rhythms, compared to normal sleepers.[71] Micic at al showed that DLMO in people with normal sleep time occurs at approx. 9:00 pm but the DLMO in DSWPD occurs approx. 2.5 hours later at 11:30 pm.[41] In our study, DLMO in emmetropes occurred almost at the same time as normal sleepers (i.e. 9:07 pm), but the myopes showed a mild phase-delay in melatonin rhythm timing with DLMO occurring at 10:19 pm. Consequently, myopic participants also had delayed sleep onset and greater sleep latency with moderate to large effect size (discussed below). Whether myopes have other pathophysiological features of DSWPD, such as a longer period length,[72,73]and an abnormal phase-response curve to light[74,75] warrant further research. Interestingly, the onset of myopia[2,3] and delayed sleep and diurnal preference[76] in young teenagers both occur at around puberty. Future research will answer whether psychosocial and biological factors that contribute to delays in the preferred timing of sleep and wakefulness in adolescents during pubertal development also contribute to the development of myopia.

In this study, we found that overnight melatonin secretion or output, as measured by urinary aMT6s levels, was significantly lower in myopes compared to emmetropes. In addition, there was a linear reduction in melatonin output with increasing severity of myopia. These findings are in contrast to an important study by Kearney and colleagues that found morning serum melatonin levels to be approx. three times higher in myopes than age-matched emmetropes.[38] Kearney and colleagues' melatonin measurements were based on analysis of a single fasting blood sample, collected between 8:30 - 10:00 am, and analysed using liquid chromatography/mass spectrometry (LC/MS). In normal sleepers, the peak of melatonin secretion occurs around 2:00 - 3:00 am at night, and the melatonin levels in the morning only represents the relapsing or descending phase of the melatonin curve.[39] Given the phase-delayed DLMO in myopes as observed in our study, the blood samples collected in the morning would reflect different stages of the melatonin rhythm for the two refractive groups. For emmetropes, a blood sample taken between 8:30 am - 10:00 am would reflect melatonin offset and hence more likely to show a lower melatonin concentration. For myopes, a sample taken during this time would show a higher melatonin concentration, reflecting residual night-time secretion due to delayed melatonin onset. Therefore, the difference in melatonin concentration observed by Kearney et al might be confounded by delayed melatonin circadian rhythms in myopes. On the contrary, the urinary aMT6s levels in the current study represented the total melatonin produced overnight (i.e. the melatonin secreted between 6:00 pm and ~ 8.00 am in the morning), which is arguably a better measure of the total overnight melatonin production for the two refractive groups. Some differences might be attributed to different methods of melatonin quantification adopted by the two studies (LC/MS vs ELISA in the current study).[77]

Consistent with delayed melatonin circadian timing, myopes showed a significant delay of 56 minutes in sleep onset time compared to emmetropes. Myopic participants also had a greater sleep onset latency and shorter sleep duration with moderate to large effect size, indicating overall poor sleep quality in myopes. These findings are consistent with previous reports of poor sleep in myopes, particularly in high myopes.[29,30] Ayaki et al found worst subjective sleep score, shortest sleep duration and latest bedtime in young adult high myopes (10 - 19 years) with the PSQI questionnaire.[30] Similarly, using data from the Korean National Health and Nutrition Examination Survey, Jee et al found a significant relationship between shorter sleep duration and myopia in Korean adolescents age 12 - 19 years.[29] Ayaki et al attributed decreased sleep in myopes to high academic demands (88%

university students in our cohort), distress over poor vision, and increased night-time light exposure,[30] all leading to delayed and disrupted circadian rhythms. Increased time in indoor mesopic light is associated with myopia.[78] Interestingly, despite delayed sleep onset and greater sleep latency in myopes, the wake up time was similar between the two groups (approx. 8:00 am for both groups) contributing to the shorter sleep duration in myopes. The total sleep durations of 8.46 and 7.18 hours in emmetropes and myopes, respectively, were consistent with previously reported sleep durations in adult myopes and non-myopes.[28-30] In our small cohort, these findings suggest that young myopes forcefully wake up in the morning to carry on with their work and academic commitments despite shorter sleep. Future studies are needed to determine how myopes in non-academic occupations and/or different age groups cope with poor sleep.

The MEQ score was significantly lower in myopes with a reasonably large effect size (0.80), suggesting that myopes were more evening-types compared to emmetropes. This could be due to myopes spending greater amounts of time on computers and studying in the evening before bedtime, as indicated by significantly greater Dh and delayed sleep onset compared to their emmetropic counterparts. Whilst we did not collect information about the time of the visual activity, it is possible that myopes, being more evening type, feel their best in the evening to meet their academic demands. Myopia has previously been strongly associated with better academic performance and greater near work.[51,52]

It is known that age-related changes in the crystalline lens[79] and changes in the ipRGC function[80] could influence circadian rhythms. However, all participants in this study were young, between the ages of 19 and 25 years (mean age, 22.06 ± 2.35), with no significant pathological changes in the eye. None of the participants had any age-related changes in the crystalline lens that could reduce light transmittance through the eye and impact circadian photoreception and sleep.[79] Both myopes (0.224 ± 0.041 mm) and emmetropes (0.235 ± 0.035 mm) had normal macular retinal thickness for this age group ($p=0.247$).[81] Importantly, none of the participants from either refractive group had any significant retinal changes that could influence signal transduction through the ipRGCs. Along the same line, previous studies have found no significant effect of refractive error on ipRGC function in young human subjects.[28,82]

The exact mechanisms underlying circadian dysregulation in myopes remain unclear at present. One possibility is that disruptions in circadian rhythms may alter the natural diurnal (or

circadian) rhythms of the eye that are essential for normal ocular growth, which may lead to myopia.[83-85] In addition to the pineal gland, melatonin is also synthesized in the retina[19,20,24] (and other structures)[86] within the eye by a circadian clock.[24] The retina and brain exhibit similar circadian rhythms, with some degree of interaction between the two (see review).[20] So, another possibility is that changes in circadian rhythms may alter retinal dopamine[17] and melatonin[19,20] levels and their endogenous rhythms that influence eye growth[21-23] and many other retinal intrinsic circadian rhythms that are integral to several structural, neurochemical and functional changes in the retina and the eye.[24-27] Given the visual signalling for eye growth is primarily regulated at the retina,[87] changes in local dopamine and melatonin signalling in the retina may result in the development of refractive errors, as shown in laboratory animals (see review).[88,89]

Strengths of the present study include the assessment of total overnight melatonin secretion and circadian timing using standardised methods, and objective and subjective assessment of sleep and chronotype in myopes and non-myopes. However, our study also had limitations. The main weakness was that it was conducted on a relatively small sample size that largely consisted of university students. Therefore, the results may not be necessarily applicable to the wider population with myopia. Secondly, the data collection was performed for almost 11 months, between November 2018 and October 2019, which straddles across two daylight saving (DLS) changes on 7th April 2019 (end of DLS) and 6th October 2019 (beginning of DLS), respectively. Previous studies have shown that the full circadian adjustment following DLS changes can take up to 5 days.[90] Our study commenced on 22nd November 2018, which was already more than 6 weeks into the beginning of DLS on 7th October 2018. There was only one emmetropic participant for whom the DLMO and urinary melatonin output was measured within 2 weeks of DLS transition. However, contrary to our observation in emmetropes, this participant had similar DLMO timing (9:10 pm) and lower melatonin output (32.18) compared to the average DLMO timing of 9:07 pm and melatonin output of 42.51 in the emmetropic group. This emmetropic participant also had the actigraphy data collected across the DLS transition time; however the sleep onset (11:00 pm) and wake up (8:25 am) times were not substantially different from the average habitual sleep timing for emmetropes (10:48 pm and 8:00 am, respectively). For one myopic participant, actigraphy data collection begun 5 days after the commencement of DLS and had no major effect on habitual sleep times. Therefore, the DLS time changes had no major influence of circadian function measured in this study.

In conclusion, this study suggests that young adult myopes have significantly delayed circadian timing compared to normal sighted emmetropes. Myopes also have lower melatonin output, delayed and reduced sleep and evening diurnal preference compared to emmetropes. Based on our findings of delayed melatonin circadian rhythm in myopes, morning bright light therapy and/or early evening exogenous melatonin[91] could be administered to phase-advance circadian timing and normalise sleep patterns in myopes. Whether such treatment would have any short-term or long-term effect on degree of myopia remains an empirical question. Overall, these findings suggest the importance of studying circadian rhythm disruption in refractive error development. Future studies on young myopic children are needed to ascertain whether delays in the melatonin circadian rhythm as well as the preferred timing of sleep and wakefulness during pubertal development are associated with the onset of myopia, and how these factors affect the progression of myopia in childhood.

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References

1. Holden BA, Fricke TR, Wilson DA, et al. Global Prevalence of Myopia and High Myopia and Temporal Trends from 2000 through 2050. Ophthalmology. 2016; 123 (5): 1036-1042.

2. Braun CI, Freidlin V, Sperduto RD, Milton RC, Strahlman ER. The progression of myopia in school age children: data from the Columbia Medical Plan. Ophthalmic epidemiology. 1996; 3 (1): 13-21.

3. Zhao J, Mao J, Luo R, Li F, Munoz SR, Ellwein LB. The progression of refractive error in school-age children: Shunyi district, China. American journal of ophthalmology. 2002; 134 (5): 735-743.

4. Paritsis N, Sarafidou E, Koliopoulos J, Trichopoulos D. Epidemiologic research on the role of studying and urban environment in the development of myopia during school-age years. Annals of ophthalmology. 1983; 15 (11): 1061-1065.

5. Wu HM, Seet B, Yap EP, Saw SM, Lim TH, Chia KS. Does education explain ethnic differences in myopia prevalence? A population-based study of young adult males in Singapore. Optom Vis Sci. 2001; 78 (4): 234-239.

6. Wong TY, Foster PJ, Hee J, et al. Prevalence and risk factors for refractive errors in adult Chinese in Singapore. Invest Ophthalmol Vis Sci. 2000; 41 (9): 2486-2494.

7. Edwards MH, Lam CS. The epidemiology of myopia in Hong Kong. Ann Acad Med Singapore. 2004; 33 (1): 34-38.

8. Vitale S, Sperduto RD, Ferris FL, 3rd. Increased prevalence of myopia in the United States between 1971-1972 and 1999-2004. Arch Ophthalmol. 2009; 127 (12): 1632-1639.

9. Jung SK, Lee JH, Kakizaki H, Jee D. Prevalence of myopia and its association with body stature and educational level in 19-year-old male conscripts in seoul, South Korea. Invest Ophthalmol Vis Sci. 2012; 53 (9): 5579-5583.

10. Saw S. How blinding is pathological myopia? In: BMJ Publishing Group Ltd; 2006.

11. Chakraborty R, Ostrin LA, Nickla DL, Iuvone PM, Pardue MT, Stone RA. Circadian rhythms, refractive development, and myopia. Ophthalmic and Physiological Optics. 2018; 38 (3): 217-245.

12. Li T, Troilo D, Glasser A, Howland HC. Constant light produces severe corneal flattening and hyperopia in chickens. Vision Res. 1995; 35 (9): 1203-1209.

13. Jensen LS, Matson WE. Enlargement of avian eye by subjecting chicks to continuous incandescent illumination. Science. 1957; 125 (3251): 741.

14. Smith EL, 3rd, Bradley DV, Fernandes A, Hung LF, Boothe RG. Continuous ambient lighting and eye growth in primates. Invest Ophthalmol Vis Sci. 2001; 42 (6): 1146-1152.

15. Li T, Howland HC, Troilo D. Diurnal illumination patterns affect the development of the chick eye. Vision Res. 2000; 40 (18): 2387-2393.

16. Nickla DL. Ocular diurnal rhythms and eye growth regulation: where we are 50 years after Lauber. Exp Eye Res. 2013; 114: 25-34.

17. Witkovsky P. Dopamine and retinal function. Doc Ophthalmol. 2004; 108 (1): 17-40.

18. Iuvone PM, Galli C, Garrison-Gund C, Neff NH. Light stimulates tyrosine hydroxylase activity and dopamine synthesis in retinal amacrine neurons. Science. 1978; 202 (4370): 901-902.

19. Leino M. 6-Methoxy-tetrahydro-β-carboline and melatonin in the human retina. Experimental eye research. 1984; 38 (3): 325-330.

20. Wiechmann AF. Melatonin: parallels in pineal gland and retina. Experimental eye research. 1986; 42 (6): 507-527.

21. Stone RA, Lin T, Laties AM, Iuvone PM. Retinal dopamine and form-deprivation myopia. Proc Natl Acad Sci U S A. 1989; 86 (2): 704-706.

22. Schaeffel F, Bartmann M, Hagel G, Zrenner E. Studies on the role of the retinal dopamine/melatonin system in experimental refractive errors in chickens. Vision research. 1995; 35 (9): 1247-1264.

23. Stone RA, Pardue MT, Iuvone PM, Khurana TS. Pharmacology of myopia and potential role for intrinsic retinal circadian rhythms. Exp Eye Res. 2013; 114: 35-47.

24. Tosini G. Melatonin circadian rhythm in the retina of mammals. Chronobiology international. 2000; 17 (5): 599-612.

25. Tosini G, Dirden JC. Dopamine inhibits melatonin release in the mammalian retina: in vitro evidence. Neuroscience letters. 2000; 286 (2): 119-122.

26. Storch KF, Paz C, Signorovitch J, et al. Intrinsic circadian clock of the mammalian retina: importance for retinal processing of visual information. Cell. 2007; 130 (4): 730-741.

27. Tosini G, Pozdeyev N, Sakamoto K, Iuvone PM. The circadian clock system in the mammalian retina. Bioessays. 2008; 30 (7): 624-633.

28. Abbott KS, Queener HM, Ostrin LA. The ipRGC-Driven Pupil Response with Light Exposure, Refractive Error, and Sleep. Optom Vis Sci. 2018; 95 (4): 323-331.

29. Jee D, Morgan IG, Kim EC. Inverse relationship between sleep duration and myopia. Acta Ophthalmol. 2016; 94 (3): e204-210.

30. Ayaki M, Torii H, Tsubota K, Negishi K. Decreased sleep quality in high myopia children. Scientific reports. 2016; 6: 33902.

31. Pan CW, Liu JH, Wu RK, Zhong H, Li J. Disordered sleep and myopia among adolescents: a propensity score matching analysis. Ophthalmic Epidemiol. 2019; 26 (3): 155-160.

32. Zhou Z, Morgan IG, Chen Q, Jin L, He M, Congdon N. Disordered sleep and myopia risk among Chinese children. PloS one. 2015; 10 (3): e0121796.

33. Claustrat B, Geoffriau M, Brun J, Chazot G. [Melatonin in humans: a biochemical marker of the circadian clock and an endogenous synchronizer]. Neurophysiol Clin. 1995; 25 (6): 351-359.

34. Brainard GC, Hanifin JP, Greeson JM, et al. Action spectrum for melatonin regulation in humans: evidence for a novel circadian photoreceptor. J Neurosci. 2001; 21 (16): 6405-6412.

35. Ruby NF, Brennan TJ, Xie X, et al. Role of melanopsin in circadian responses to light. Science. 2002; 298 (5601): 2211-2213.

36. Claustrat B, Brun J, Borson-Chazot F. [Melatonin and circadian rhythm]. Rev Neurol (Paris). 2001; 157 (11 Pt 2): S121-125.

37. Rada JAS, Wiechmann AF. Melatonin receptors in chick ocular tissues: implications for a role of melatonin in ocular growth regulation. Investigative ophthalmology & visual science. 2006; 47 (1): 25-33.

38. Kearney S, O'donoghue L, Pourshahidi LK, Cobice D, Saunders KJ. Myopes have significantly higher serum melatonin concentrations than non-myopes. Ophthalmic and Physiological Optics. 2017; 37 (5): 557-567.

39. Zhdanova IV, Wurtman RJ, Balcioglu A, Kartashov AI, Lynch HJ. Endogenous melatonin levels and the fate of exogenous melatonin: age effects. J Gerontol A Biol Sci Med Sci. 1998; 53 (4): B293-298.

40. Burfield HJ, Carkeet A, Ostrin LA. Ocular and Systemic Diurnal Rhythms in Emmetropic and Myopic Adults. Invest Ophthalmol Vis Sci. 2019; 60 (6): 2237-2247.

41. Micic G, Lovato N, Gradisar M, et al. Nocturnal melatonin profiles in patients with delayed sleep-wake phase disorder and control sleepers. Journal of biological rhythms. 2015; 30 (5): 437-448.

42. Pandi-Perumal SR, Smits M, Spence W, et al. Dim light melatonin onset (DLMO): a tool for the analysis of circadian phase in human sleep and chronobiological disorders. Progress in Neuro-Psychopharmacology and Biological Psychiatry. 2007; 31 (1): 1-11.

43. Buysse DJ, Reynolds CF, 3rd, Monk TH, Berman SR, Kupfer DJ. The Pittsburgh Sleep Quality Index: a new instrument for psychiatric practice and research. Psychiatry Res. 1989; 28 (2): 193-213.

44. Horne JA, Ostberg O. A self-assessment questionnaire to determine morningness-eveningness in human circadian rhythms. Int J Chronobiol. 1976; 4 (2): 97-110.

45. Leube A, Tabernero J, Ohlendorf A, Wahl SJIOVS. Relationship between the subjectively and objectively determined depth of focus of the human eye using defocus curves. 2015: 6016-6016.

46. Carkeet A, Saw SM, Gazzard G, Tang W, Tan DT. Repeatability of IOLMaster biometry in children. Optom Vis Sci. 2004; 81 (11): 829-834.

47. Sheng H, Bottjer CA, Bullimore MA. Ocular component measurement using the Zeiss IOLMaster. Optom Vis Sci. 2004; 81 (1): 27-34.

48. Moderiano D, Do M, Hobbs S, et al. Influence of the time of day on axial length and choroidal thickness changes to hyperopic and myopic defocus in human eyes. Exp Eye Res. 2019; 182: 125-136.

49. Chakraborty R, Read SA, Collins MJ. Diurnal variations in axial length, choroidal thickness, intraocular pressure, and ocular biometrics. Invest Ophthalmol Vis Sci. 2011; 52 (8): 5121-5129.

50. Zadnik K, Satariano WA, Mutti DO, Sholtz RI, Adams AJ. The effect of parental history of myopia on children's eye size. Jama. 1994; 271 (17): 1323-1327.

51. Mutti DO, Mitchell GL, Moeschberger ML, Jones LA, Zadnik K. Parental myopia, near work, school achievement, and children's refractive error. Invest Ophthalmol Vis Sci. 2002; 43 (12): 3633-3640.

52. Saw SM, Chua WH, Hong CY, et al. Nearwork in early-onset myopia. Invest Ophthalmol Vis Sci. 2002; 43 (2): 332-339.

53. Marino M, Li Y, Rueschman MN, et al. Measuring Sleep: Accuracy, Sensitivity, and Specificity of Wrist Actigraphy Compared to Polysomnography. Sleep. 2013; 36 (11): 1747-1755.

54. Ancoli-Israel S, Cole R, Alessi C, Chambers M, Moorcroft W, Pollak CP. The role of actigraphy in the study of sleep and circadian rhythms. Sleep. 2003; 26 (3): 342-392.

55. Carney CE, Buysse DJ, Ancoli-Israel S, et al. The consensus sleep diary: standardizing prospective sleep self-monitoring. Sleep. 2012; 35 (2): 287-302.

56. Voultsios A, Kennaway DJ, Dawson D. Salivary melatonin as a circadian phase marker: validation and comparison to plasma melatonin. J Biol Rhythms. 1997; 12 (5): 457-466.

57. Bojkowski CJ, Arendt J, Shih MC, Markey SP. Melatonin secretion in humans assessed by measuring its metabolite, 6-sulfatoxymelatonin. Clin Chem. 1987; 33 (8): 1343-1348.

58. Aldhous ME, Arendt J. Radioimmunoassay for 6-sulphatoxymelatonin in urine using an iodinated tracer. Annals of Clinical Biochemistry. 1988; 25 (3): 298-303.

59. Peuhkuri K, Sihvola N, Korpela RJF, research n. Dietary factors and fluctuating levels of melatonin. 2012; 56 (1): 17252.

60. Figueiro MG, Rea MSJBrn. Preliminary evidence that light through the eyelids can suppress melatonin and phase shift dim light melatonin onset. 2012; 5 (1): 221.

61. Molina TA, Burgess HJ. Calculating the dim light melatonin onset: the impact of threshold and sampling rate. Chronobiology international. 2011; 28 (8): 714-718.

62. Morgan IG. The biological basis of myopic refractive error. Clin Exp Optom. 2003; 86 (5): 276-288.

63. Farbrother JE, Kirov G, Owen MJ, Pong-Wong R, Haley CS, Guggenheim JA. Linkage analysis of the genetic loci for high myopia on 18p, 12q, and 17q in 51 U.K. families. Invest Ophthalmol Vis Sci. 2004; 45 (9): 2879-2885.

64. French AN, Ashby RS, Morgan IG, Rose KA. Time outdoors and the prevention of myopia. Experimental eye research. 2013; 114: 58-68.

65. Morgan IG, French AN, Rose KA. Intense schooling linked to myopia. In: British Medical Journal Publishing Group; 2018.

66. Sapkota YD, Adhikari BN, Pokharel GP, Poudyal BK, Ellwein LB. The prevalence of visual impairment in school children of upper-middle socioeconomic status in Kathmandu. Ophthalmic epidemiology. 2008; 15 (1): 17-23.

67. Stone RA, McGlinn AM, Chakraborty R, et al. Altered ocular parameters from circadian clock gene disruptions. PloS one. 2019; 14 (6): e0217111-e0217111.

68. Hysi PG, Choquet H, Khawaja AP, et al. Meta-analysis of 542,934 subjects of European ancestry identifies new genes and mechanisms predisposing to refractive error and myopia. Nature Genetics. 2020.

69. Oren DA, Turner EH, Wehr TA. Abnormal circadian rhythms of plasma melatonin and body temperature in the delayed sleep phase syndrome. Journal of neurology, neurosurgery, and psychiatry. 1995; 58 (3): 379.

70. Saxvig IW, Wilhelmsen-Langeland A, Pallesen S, et al. Objective measures of sleep and dim light melatonin onset in adolescents and young adults with delayed sleep phase disorder compared to healthy controls. Journal of sleep research. 2013; 22 (4): 365-372.

71. Micic G, Lovato N, Gradisar M, Burgess HJ, Ferguson SA, Lack L. Circadian Melatonin and Temperature Taus in Delayed Sleep-wake Phase Disorder and Non-24-hour Sleep-wake Rhythm Disorder Patients: An Ultradian Constant Routine Study. J Biol Rhythms. 2016; 31 (4): 387-405.

72. Campbell SS, Murphy PJ. Delayed sleep phase disorder in temporal isolation. Sleep. 2007; 30 (9): 1225-1228.

73. Micic G, de Bruyn A, Lovato N, et al. The endogenous circadian temperature period length (tau) in delayed sleep phase disorder compared to good sleepers. Journal of sleep research. 2013; 22 (6): 617-624.

74. Czeisler CA, Richardson GS, Coleman RM, et al. Chronotherapy: resetting the circadian clocks of patients with delayed sleep phase insomnia. Sleep. 1981; 4 (1): 1-21.

75. Aoki H, Ozeki Y, Yamada N. Hypersensitivity of melatonin suppression in response to light in patients with delayed sleep phase syndrome. Chronobiology international. 2001; 18 (2): 263-271.

76. Carskadon MA, Vieira C, Acebo C. Association between puberty and delayed phase preference. Sleep. 1993; 16 (3): 258-262.

77. de Almeida EA, Di Mascio P, Harumi T, et al. Measurement of melatonin in body fluids: standards, protocols and procedures. Childs Nerv Syst. 2011; 27 (6): 879-891.

78. Landis EG, Yang V, Brown DM, Pardue MT, Read SA. Dim Light Exposure and Myopia in Children. Invest Ophthalmol Vis Sci. 2018; 59 (12): 4804-4811.

79. Yan S-S, Wang W. The effect of lens aging and cataract surgery on circadian rhythm. Int J Ophthalmol. 2016; 9 (7): 1066-1074.

80. Paul KN, Saafir TB, Tosini GJRie, disorders m. The role of retinal photoreceptors in the regulation of circadian rhythms. 2009; 10 (4): 271-278.

81. Wakitani Y, Sasoh M, Sugimoto M, Ito Y, Ido M, Uji Y. MACULAR THICKNESS MEASUREMENTS IN HEALTHY SUBJECTS WITH DIFFERENT AXIAL LENGTHS USING OPTICAL COHERENCE TOMOGRAPHY. RETINA. 2003; 23 (2).

82. Adhikari P, Pearson CA, Anderson AM, Zele AJ, Feigl B. Effect of age and refractive error on the melanopsin mediated post-illumination pupil response (PIPR). Scientific reports. 2015; 5: 17610.

83. Nickla DL, Wildsoet C, Wallman J. The circadian rhythm in intraocular pressure and its relation to diurnal ocular growth changes in chicks. Exp Eye Res. 1998; 66 (2): 183-193.

84. Nickla DL, Wildsoet CF, Troilo D. Diurnal rhythms in intraocular pressure, axial length, and choroidal thickness in a primate model of eye growth, the common marmoset. Invest Ophthalmol Vis Sci. 2002; 43 (8): 2519-2528.

85. Nickla DL, Wildsoet CF, Troilo D. Endogenous rhythms in axial length and choroidal thickness in chicks: implications for ocular growth regulation. Invest Ophthalmol Vis Sci. 2001; 42 (3): 584-588.

86. Acuna-Castroviejo D, Escames G, Venegas C, et al. Extrapineal melatonin: sources, regulation, and potential functions. Cell Mol Life Sci. 2014; 71 (16): 2997-3025.

87. Wallman J. Retinal control of eye growth and refraction. Progress in retinal research. 1993; 12: 133-153.

88. Chakraborty R, Pardue MT. Molecular and biochemical aspects of the retina on refraction. Progress in Molecular Biology and Translational Science. 2015.

89. Crewther DP. The role of photoreceptors in the control of refractive state. Prog Retin Eye Res. 2000; 19 (4): 421-457.

90. Monk TH, Folkard SJN. Adjusting to the changes to and from Daylight Saving Time. 1976; 261 (5562): 688-689.

91. Auger RR, Burgess HJ, Emens JS, Deriy LV, Thomas SM, Sharkey KM. Clinical practice guideline for the treatment of intrinsic circadian rhythm sleep-wake disorders: advanced sleep-wake phase disorder (ASWPD), delayed sleep-wake phase disorder (DSWPD), non-24-hour sleep-wake rhythm disorder (N24SWD), and irregular sleep-wake rhythm disorder (ISWRD). An update for 2015: an American Academy of Sleep Medicine clinical practice guideline. Journal of clinical sleep medicine: JCSM: official publication of the American Academy of Sleep Medicine. 2015; 11 (10): 1199.

Figure Captions

Figure 1. Box and whisker plots of optical power of the eye or spherical equivalent refraction (SER) in dioptres (D) (A) and axial length of the eye in millimeters (mm) (B) between emmetropes and myopes.

Figure 2. Box and whisker plots of aMT6s (ng/ml) levels derived from total overnight urine volume (A) and salivary dim light melatonin onset (DLMO) time (h:min) (B) between emmetropes and myopes.

Figure 3. Comparison of average actigraphy-derived habitual sleep timing (confirmed by sleep-wake diaries) between emmetropes (blue, top) and myopes (red, bottom). Sleep onset time (p=0.019), sleep onset latency (shaded in light red and calculated from bedtime until sleep onset, p=0.020), total sleep time (p=0.001) and total time in bed (calculated from bedtime until wake up time, p=0.002) were significantly different between groups.

Figure 4. Box and whisker plots of wake after sleep onset (minutes) at night (A) and sleep efficiency (%) (B) between emmetropes and myopes.

Figure 5. Box and whisker plots of global Pittsburgh Sleep Quality Index (PSQI) scores indicating subjective sleep quality over the past month (A) and Morningness-Eveningness (MEQ) Scores indicating diurnal preference (B) between emmetropes and myopes.

Figure 6. Associations between the two dependent melatonin variables (dim light melatonin onset and urinary aMT6s levels) and the two independent ocular variables (spherical equivalent refraction and axial length) across emmetropes and myopes.

Table 1. Group differences in ocular, melatonin and sleep profiles between myopes and emmetropes.

Category	Variables (units)	Refractive group		t-value	*df*	p-value	Effect size (Cohen's *d*)[a]
		Emmetropes (n=14)	Myopes (n=18)				
Ocular profile	SER (D)¶	-0.09 ± 0.13	-4.89 ± 2.16			<0.001**	2.95
	Axial length (mm)	23.54 ± 0.69	25.39 ± 0.99	-5.937	30	<0.001**	2.12
	Dh of near work	26.01 ± 5.36	31.84 ± 9.40	-2.070	30	0.023*	0.74
Melatonin profile	Total urine volume (l)	1.20 ± 0.63	1.33 ± 0.66	-0.558	30	0.291	0.20
	aMT6s urinary melatonin¶	42.51 ± 23.97	29.17 ± 18.67			0.042*	0.63
	DLMO (h:min)	9.07 ± 1.43 (9:07 pm)	10.19 ± 1.76 (10:19 pm)	2.023	29	0.026*	0.73
Sleep profile	Bedtime (h:min)	10.32 ± 1.11 (10:19 pm)	10.94 ± 1.17 (10.56 pm)	-1.529	30	0.068	0.54
	Lights-out time (h:min)	10.53 ± 1.22 (10:32 pm)	11.09 ± 1.19 (11:05 pm)	-1.305	30	0.101	0.47
	Sleep onset time (h:min)	10.80 ± 1.25 (10:48 pm)	11.74 ± 1.20 (11:44 pm)	-2.149	30	0.019*	0.77
	Sleep onset latency (m)	23 ± 17	41 ± 25	-2.147	30	0.020*	0.78
	Midsleep time (h:min)	15.41 ± 1.04 (3:25 am)	15.73 ± 0.84 (3:44 am)	-0.985	30	0.166	0.34
	Wake up time (h:min)¶	20.01 ± 1.18 (8:00 am)	19.73 ± 0.68 (7:44 am)			0.595	0.30
	Total time in bed (h)	9.21 ± 1.26 (9 hours and 13 minutes)	7.99 ± 0.99 (7 hours and 59 minutes)	3.070	30	0.002	1.08
	Total sleep time (h)	8.46 ± 1.15 (8 hours and 28 minutes)	7.19 ± 1.08 (7 hours and 11 minutes)	3.230	30	0.001**	1.09
	Wake after sleep onset (m)	44.67 ± 15.66	48.15 ± 12.60	-0.698	30	0.245	0.25
	Sleep efficiency (%)¶	83.41 ± 5.30	79.57 ± 6.19			0.050	0.66
	PSQI score	3.64 ± 1.91	4.72 ± 2.08	-1.509	30	0.070	0.54
	MEQ score	53.21 ± 8.47	47.44 ± 5.99	2.258	30	0.015*	0.80

Means, standard deviations, t-value, degree of freedom (df) and p-values from independent samples t tests are presented. Due to the low sample size, effect sizes (Cohen's *d*) are provided to demonstrate effects when significance is not obtained. Differences in objective sleep timing based on actigraphy monitors are indicated, which were further confirmed by sleep/wake diary.

SER = spherical equivalent refraction; D = dioptre; Dh = dioptre hour; aMT6s = 6-Sulphatoxymelatonin; DLMO = dim-light melatonin onset; PSQI = Pittsburgh Sleep Quality Index; MEQ = Morningness-Eveningness Questionnaire.

a. Size of effect: d > .20 = small; d > .50 = moderate; d > .80 = large.

¶Output variables where Mann-Whitney rank sum test was applied for statistical comparison due to failed normality test.

*Significant group differences indicated at <0.05. **Significant group differences indicated at <0.001.

Table 2. Association between melatonin and sleep variables and the two primary ocular variables, SER and axial length, across the two refractive groups (n=32).

Melatonin and sleep variables	**Ocular variables**					
	SER (D)			**Axial length (mm)**		
	Slope	*r2*	*p-value*	*Slope*	*r2*	*p-value*
DLMO (h:min)	-0.277	0.22	0.007*	0.553	0.17	0.020*
aMT6s urinary melatonin	2.900	0.15	0.030*	-6.850	0.16	0.025*
Bedtime (h:min)	-0.115	0.08	0.114	0.098	0.01	0.567
Lights-out time (h:min)	-0.102	0.06	0.181	0.070	0.01	0.693
Sleep onset time (h:min)	-0.171	0.15	0.030*	0.240	0.06	0.196
Sleep onset latency (m)	-0.050	0.14	0.032*	0.134	0.20	0.011*
Midsleep time (h:min)	-0.075	0.05	0.199	0.083	0.01	0.538
Wake up time (h:min)	0.021	0.004	0.719	-0.073	0.01	0.585
Total time in bed (h)	11.489	0.20	0.011*	-18.806	0.10	0.079
Total sleep time (h)	12.277	0.22	0.007*	-20.534	0.12	0.056
Wake after sleep onset (m)	-0.787	0.03	0.368	1.728	0.03	0.390
Sleep efficiency (%)	0.522	0.06	0.167	-0.763	0.03	0.383
PSQI score	-0.143	0.04	0.265	0.099	0.004	0.740
MEQ score	1.140	0.19	0.013*	-2.146	0.13	0.046*

Slope, *r2* and p-values from linear regression analysis using the least-squares approach are presented. Differences in objective sleep timing based on actigraphy monitors are indicated, which were further confirmed by sleep/wake diary.
SER = spherical equivalent refraction; D = dioptre; aMT6s = 6-Sulphatoxymelatonin; DLMO = dim-light melatonin onset; PSQI = Pittsburgh Sleep Quality Index; MEQ = Morningness-Eveningness Questionnaire.
*Significant association indicated at <0.05.

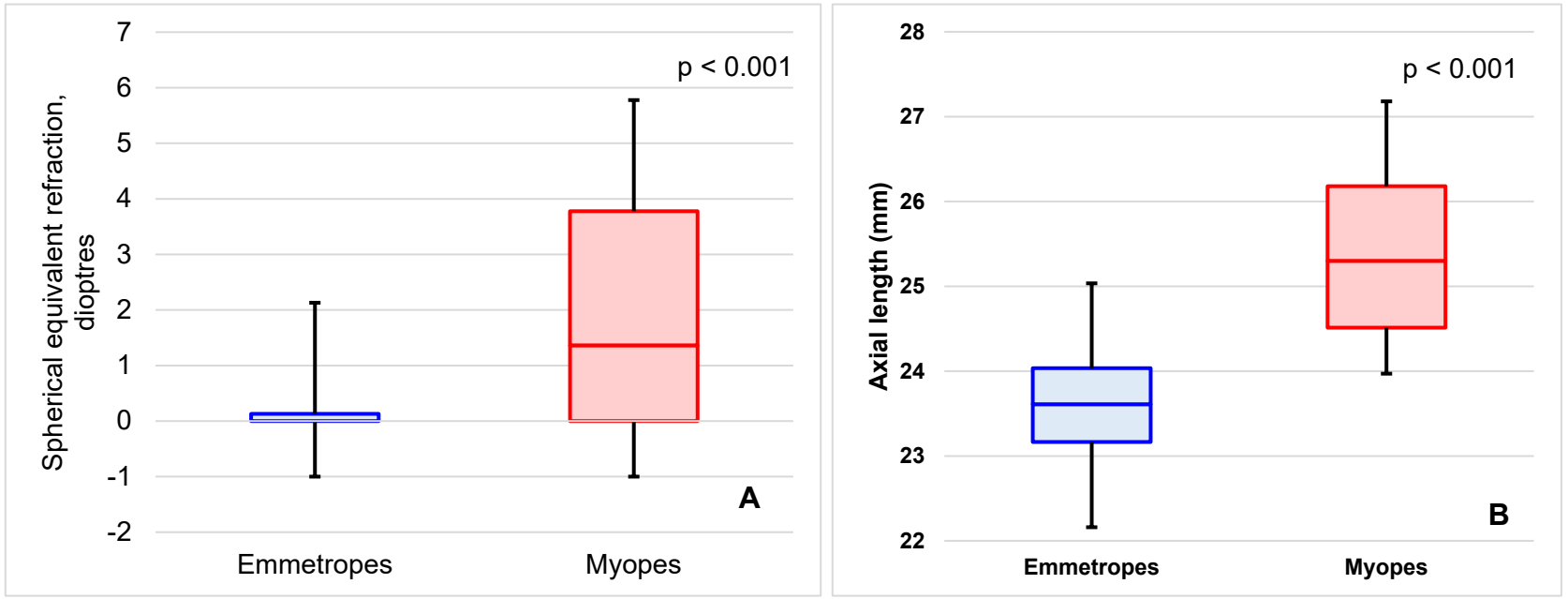

Figure 1. Box and whisker plots of optical power of the eye or spherical equivalent refraction (SER) in dioptres (D) (A) and axial eye length in millimeters (mm) (B) between emmetropes and myopes.

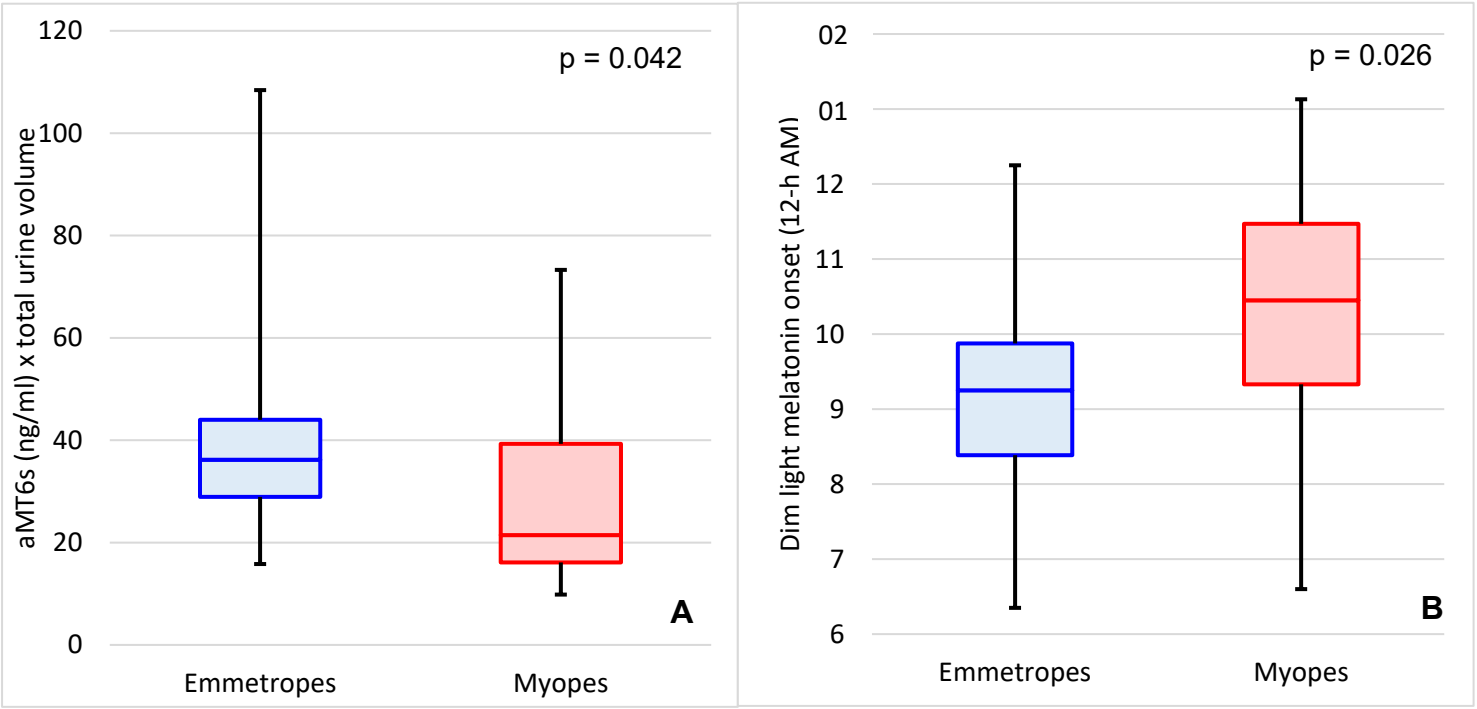

Figure 2. Box and whisker plots of aMT6s (ng/ml) levels derived from total overnight urine volume (A) and salivary dim light melatonin onset (DLMO) time (h.mm) (B) between emmetropes and myopes.

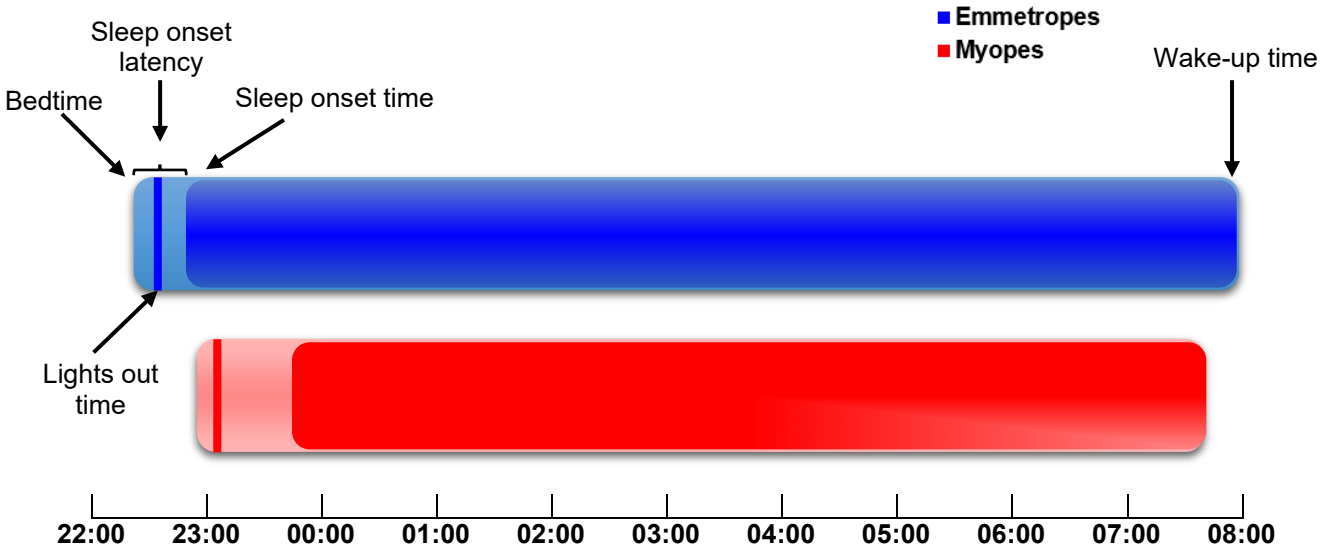

Figure 3. Comparison of average actigraphy-derived habitual sleep timing (confirmed by sleep-wake diaries) between emmetropes (blue, top) and myopes (red, bottom). Sleep onset time ($p = 0.019$), sleep latency (shaded in light red and calculated from bedtime until sleep onset, $p < 0.05$), total sleep time ($p = 0.002$) and total time in bed (calculated from bedtime until wake-up time, $p < 0.05$) were significantly different between groups.

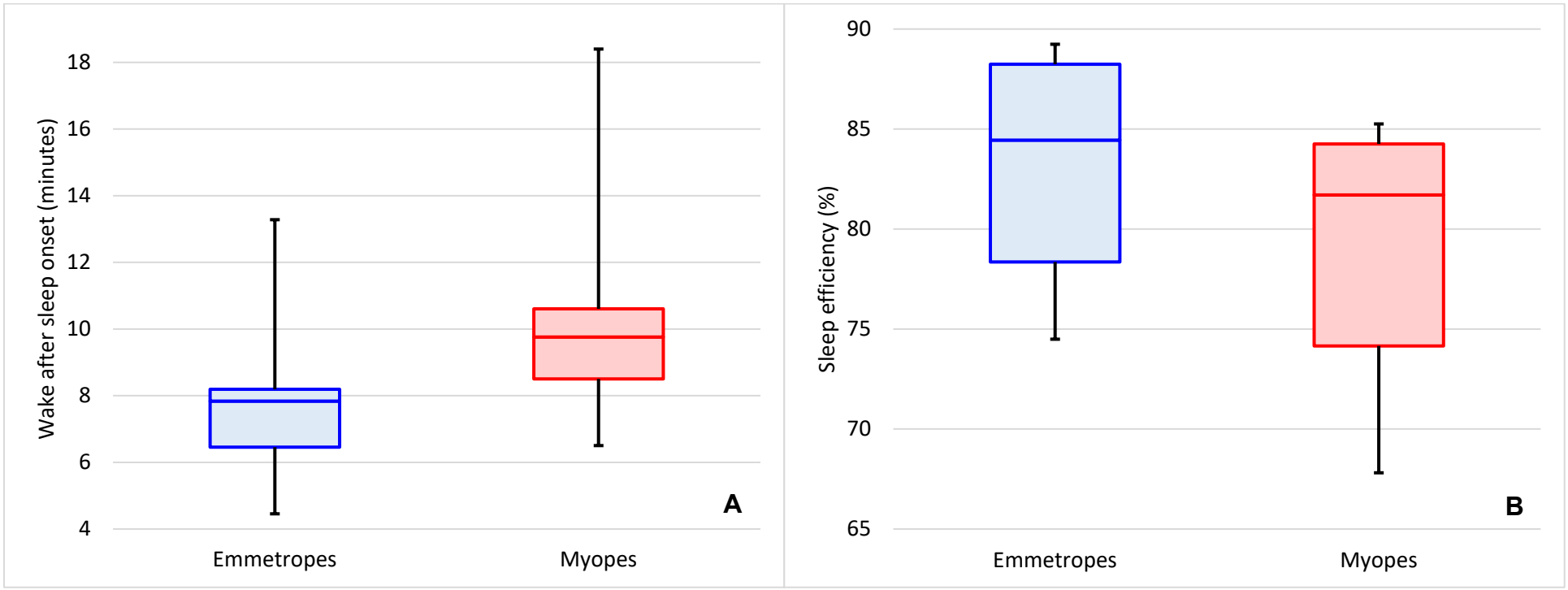

Figure 4. Box and whisker plots of wake duration (minutes) at night (A) and sleep efficiency (%) (B) between emmetropes and myopes.

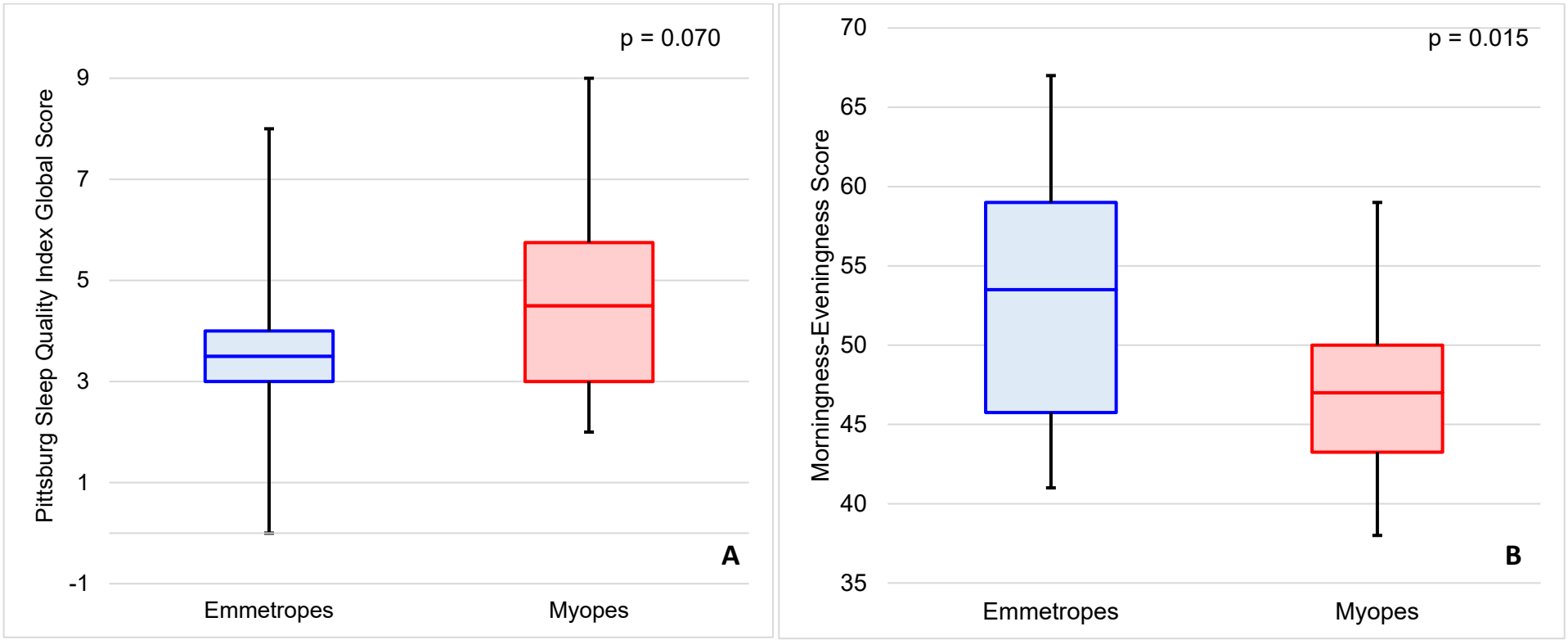

Figure 5. Box and whisker plots of global Pittsburgh Sleep Quality Index (PSQI) scores indicating subjective sleep quality over the past month (A) and Morningness-Eveningness (MEQ) Scores indicating diurnal preference (B) between emmetropes and myopes.

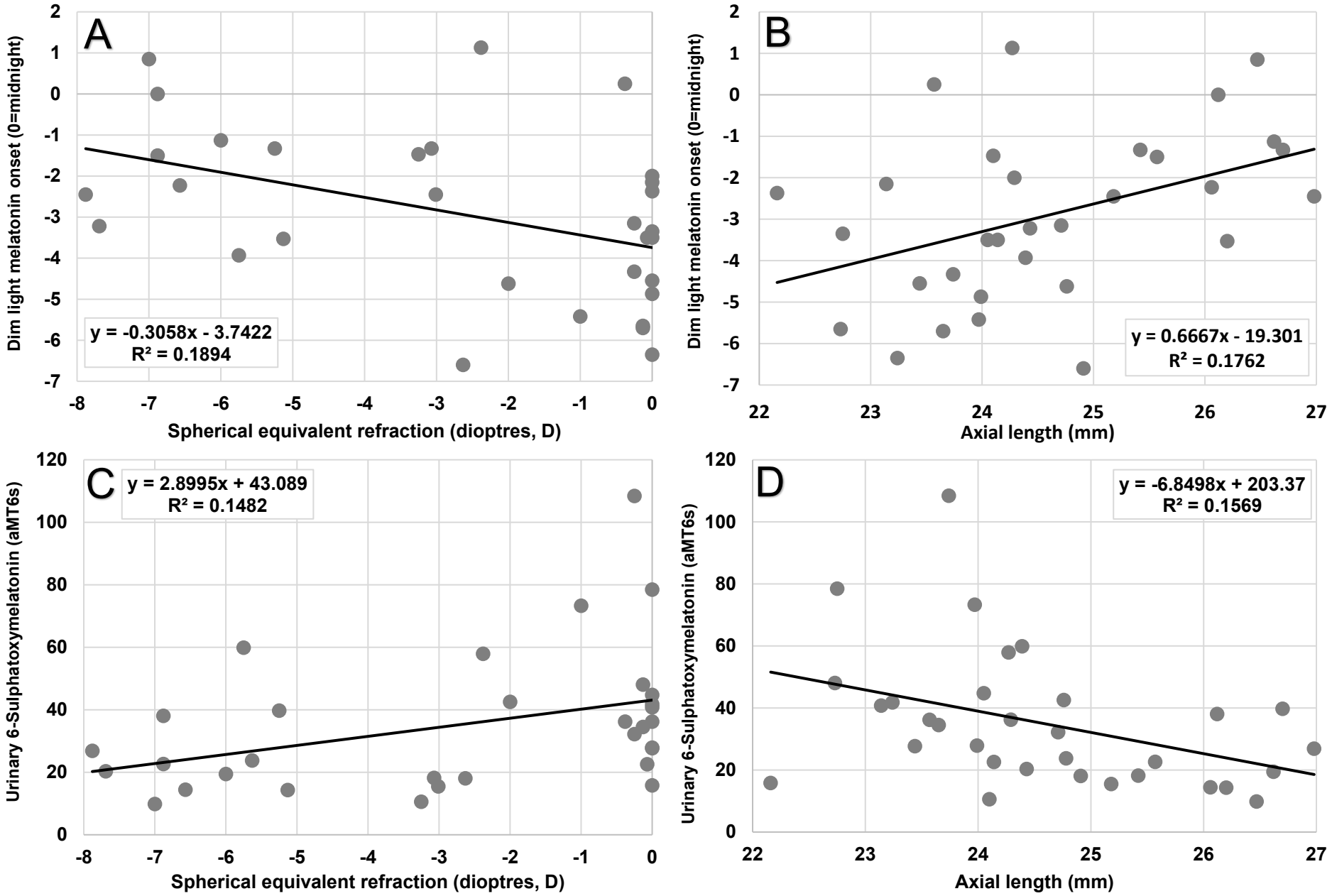

Figure 6. Associations between the two dependent melatonin variables (dim light melatonin onset and urinary aMT6s levels) and the two independent ocular variables (spherical equivalent refraction and axial length) across emmetropes and myopes.

Title: Myopia, or near-sightedness, is associated with delayed melatonin circadian timing and lower melatonin output in young adult humans

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Supplemental table 1. Group differences in subjective sleep profiles derived from sleep-wake diary between myopes and emmetropes.

Variables (units)	**Refractive group**		**t-value**	***df***	**p-value**	**Effect size (Cohen's *d*)**[a]
	Emmetropes (n=14)	Myopes (n=18)				
Bedtime (h:min)	10.32 ± 1.11 (10:19 pm)	10.94 ± 1.17 (10.56 pm)	-1.529	30	0.068	0.54
Lights-out time (h:min)	10.53 ± 1.22 (10:32 pm)	11.09 ± 1.19 (11:05 pm)	-1.305	30	0.101	0.47
Sleep onset time (h:min)	10.91 ± 1.24 (10:55 pm)	11.64 ± 1.18 (11:38 pm)	-1.713	30	0.048*	0.61
Sleep onset latency (m)	26 ± 10	33 ± 14	-1.634	30	0.066	0.56
Wake up time (h:min)	19.72 ± 1.12 (7:43 am)	19.56 ± 0.83 (7:34 am)	0.453	30	0.327	0.17
Time out of bed (h:min)	19.76 ± 1.30 (7:47 am)	19.74 ± 1.13 (7:44 am)	-0.055	30	0.478	0.02
Total time in bed (h)¶	9.45 ± 1.19 (9 hours and 27 minutes)	8.80 ± 0.83 (8 hours and 48 minutes)			0.124	0.65
Total sleep time (h)	8.81 ± 1.20 (8 hours and 49 minutes)	7.92 ± 0.98 (7 hours and 55 minutes)	2.327	30	0.013*	0.82
Wake after sleep onset (m)¶	35.21 ± 19.43	32.89 ± 12.03			0.939	0.15
Sleep efficiency (%)¶	94.10 ± 14.83	90.09 ± 7.70			0.372	0.35

Means, standard deviations, t-value, degree of freedom (df) and p-values from independent samples t tests are presented. Due to the low sample size, effect sizes (Cohen's *d*) are provided to demonstrate effects when significance is not obtained.

a. Size of effect: d > .20 = small; d > .50 = moderate; d > .80 = large.

¶Output variables where Mann-Whitney rank sum test was applied for statistical comparison due to failed normality test.

*Significant group differences indicated at <0.05.