Article

Sleep in Myopic and Non-Myopic Children

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Purpose: To examine differences in sleep between myopic and non-myopic children.

Methods: Objective measurements of sleep, light exposure, and physical activity were collected from 91 children, aged 10 to 15 years, for two 14-day periods approximately 6 months apart. Sleep parameters were analyzed with respect to refractive error, season, day of the week, age, and sex.

Results: Myopic children exhibited differences in sleep duration by day of the week ($P < 0.001$) and season ($P = 0.007$). Additionally, myopic children exhibited shorter sleep latency than non-myopic children ($P = 0.04$). For all children, wake time was later ($P < 0.001$) and sleep duration was longer ($P = 0.03$) during the cooler season compared with the warmer season. On weekends, children went to bed later ($P < 0.001$), woke up later ($P < 0.001$), and had increased sleep duration ($P < 0.001$) than on weekdays. Younger children exhibited earlier bedtime ($P = 0.005$) and wake time ($P = 0.01$) than older children. Time spent outdoors was positively associated with sleep duration ($P = 0.03$), and daily physical activity was negatively associated with wake time ($P < 0.001$).

Conclusions: Myopic children tended to have more variable sleep duration and shorter latency than non-myopic children. Sleep patterns were influenced by season, day of the week, age, time outdoors, and activity.

Translational Relevance: Myopic children tended to have more variable sleep duration and shorter latency than non-myopic children, which may reflect previously reported differences in environmental and behavioral factors between refractive error groups.

Introduction

The prevalence of myopia has been increasing, and with it, associated pathologies and economic burden are expected to rise.[1] Efforts have increased to understand risk factors contributing to myopia so that effective interventions can be developed to prevent myopia and slow its progression. Myopia is attributed to a complex interaction between genetic and environmental factors. Evidence suggests that outdoor time is protective against some aspects of myopia.[2–5] Other potential environmental and behavioral influences include near work, physical activity,[6,7] nutrition,[8] and urbanization.[9] Recent literature suggests that circadian rhythms may also play a role in the regulation of eye growth and refractive error development.[10] The body's circadian clocks help regulate daily rhythms of sleep, alertness, heart rate, body temperature, metabolism, hormone secretion, and many other physiological processes. Circadian rhythms have been demonstrated in several ocular structures,[11–15] and these rhythms may have implications in eye growth and myopia.[10] Animal studies show that when circadian rhythms are disrupted by interrupting the normal light/dark cycle, refractive errors develop.[16–19]

Light is the most potent zeitgeber, or cue, for circadian rhythm entrainment and regulation of sleep/wake cycles.[20] Light information is transmitted by intrinsically photosensitive retinal ganglion cells to the suprachiasmatic nucleus (the master clock of the body), in which higher order pathways control diurnal release of various neurotransmitters and hormones, including melatonin.[21–23] Melatonin is released from the pineal gland and plays a key role in coordinat-

ing sleep/wake patterns. A recent study reported that young adult myopes exhibit higher serum melatonin concentrations than non-myopes, suggesting that links exist between light exposure, circadian rhythm, and myopia.[24]

Daily sleep patterns play an important role in maintaining normal human health. Sleep is crucial for health and well-being, and is known to have roles in promoting growth, learning, and cognitive development.[25] Several recent studies have investigated sleep quality with respect to refractive error.[26–29] In Korean children, a significant inverse relationship was found between sleep duration and myopia.[27] Ayaki et al.[26] found an association between later bedtimes, shorter sleep duration, and poorer sleep quality with increased myopic refractive error. However, other studies have found no association between sleep duration and myopia in Chinese children.[30,31] These studies utilized subjective measures to assess various sleep parameters, which may contribute to the inconsistent findings between studies. Questionnaires and sleep diaries have been found to be less accurate than objective measures, such as polysomnography and actigraphy.[32] Although a number of studies have used objective measures to evaluate sleep in children, there are no studies to date that have selected or classified subjects based on their ocular or refractive characteristics.

With known associations between light exposure and sleep, and potential relationships between sleep and myopia, it is of interest to understand objectively measured habitual sleep patterns in myopic and non-myopic children. In this article, we provide a detailed report of sleep patterns of the myopic and non-myopic children enrolled in the Role of Outdoor Activity in Myopia (ROAM) Study[3,33] and examine the influence of a variety of factors, including season, age, and refractive error, on these data.

Methods

The ROAM study was a prospective, longitudinal examination of light exposure and eye growth in myopic and non-myopic children.[3,33] The ROAM study participants and protocol have been described in detail previously. Briefly, 102 children aged from 10 to 15 years were enrolled, and participation involved four study visits (every 6 months) over an 18-month period in which a series of ocular measurements were collected. Data collection for the first study visit occurred between May and November 2012. At each study visit, measures of visual acuity, subjective refraction (non-cycloplegic), and ocular biometry

(Lenstar LS 900 optical biometer; Haag Streit AG, Koeniz, Switzerland) were collected. In addition to the data collected during the four study visits, objective measurements of ambient light exposure, physical activity, and sleep were also collected using a wrist-worn actigraph device with light sensor (Actiwatch 2; Philips Respironics, Bend, OR). These behavioral measurements were collected twice for each child in the first 12 months of the study. Each measurement period involved 14 days of sensor-wear (i.e., a total of 28 days of measurements), with the two measurement periods being conducted approximately 6 months apart. The study was approved by the Queensland University of Technology Human Research Ethics Committee and adhered to the tenets of the Declaration of Helsinki. All parents provided written informed consent, and children provided written assent prior to participation.

All children enrolled in the study exhibited best corrected visual acuity of 0.00 (logMAR) or better in each eye, no history or evidence of significant ocular disease, and no hyperopic refractive errors (non-cycloplegic) greater than +1.25 diopters sphere (DS). Eligible subjects were classified based on their non-cycloplegic spherical equivalent subjective refractive error at their first study visit as being either myopic (average spherical equivalent refractive error from right and left eyes ≤ –0.50 DS, with at least one eye exhibiting 0.75 DS or more myopia) or non-myopic (average spherical equivalent refractive error from right and left eyes +1.25 to –0.50 DS, with neither eye exhibiting 0.75 DS or more myopia). Subjects were further classified as either being in the younger age group (10 to <13 years of age) or the older age group (13 to <16 years of age).

For objective measurements of ambient light exposure, physical activity, and sleep parameters obtained using the Actiwatch 2, the first 14-day wear period occurred between the first and second study visit (between July and December 2012), and the second wear period between the second and third study visit (between February and August 2013). The Actiwatch 2 is small, lightweight (16 g), and waterproof (for up to 30 minutes in water), and provides measures of visible light illuminance, measuring over a wavelength range from 400 to 900 nm. The light sensor has a peak sensitivity of 570 nm and dynamic range from 5 to 100,000 lux. The device's in-built memory and battery life allow measurements of light exposure to be collected every 30 seconds, 24 hours a day over each 14-day period. Along with the light exposure measurements, the Actiwatch 2 simultaneously provides measures of physical activity and sleep, measured via a solid-state piezoelectric accelerometer.

All measurements in this study were collected during the school term, and children were instructed

to wear the light sensor device on their non-dominant wrist, continuously (24 hours a day) throughout each 14-day measurement period, ensuring that the device was not covered by clothing. If the watch had to be removed for any reason during wear (e.g., when swimming for longer than 30 minutes, or for activities in which wearing a watch was not permitted), children were asked to complete a diary to document the type of activity that was performed and whether they were indoors or outdoors when the watch was removed. The average daily climate/weather conditions (minimum and maximum daily temperatures and amount of rainfall) and day length (number of hours from sunrise to sunset) in Brisbane, Australia from each child's two 14-day periods of light exposure measurements were also determined based on data from the Australian Bureau of Meteorology.

Data Analyses

Following each 14-day period of Actiwatch 2 wear for each child, the data from each device were downloaded using Actiware software version 5.70.1 (Philips Respironics, Bend, OR), and the raw data were exported for further analysis. Data were screened to exclude any invalid data in which there was evidence that the sensor was removed or covered by clothing (any continuous period of 15 minutes or longer during the day with data exhibiting complete inactivity and/or complete darkness). These data were excluded from analysis, unless the period of sensor removal was accounted for by the child in a diary entry, in which case the light levels and physical activity during this period were estimated based on the description provided by the participant in their diary, using a method described in detail elsewhere.[3] All sleep parameters reported here were derived from objective measures from the Actiwatch and automatically determined using the Actiware software. Any days in which there was evidence that the Actiwatch was removed by the children, or in which there were apparent errors in the automatic rest period detection by the Actiware software, were not included in the analysis. Across the entire study, the mean ± SD number of days of valid sleep data from the Actiwatch included in the analysis per child was 23.6 ± 4.8 days out of a possible 28 days.

Detailed analyses of light exposure data with refractive error and eye growth are presented elsewhere.[3,33] Primary outcome measures examined here were bedtime, wake time, sleep duration, sleep latency (minutes to fall asleep), and sleep efficiency (percentage of time asleep while in bed). For each epoch of data, the Actiware software sleep-wake algorithms

objectively determine whether the particular time point is classified as "wake" or "sleep" based on whether the activity levels of the current epoch and the data adjacent to the current epoch are above a certain threshold of activity. In our analyses, the "medium" wake threshold was used, which has been shown to demonstrate the least over- or underestimation of total sleep time in healthy children.[34] Automatic detection of a single major "rest period" by the Actiware software was employed for each day, which detects the longest period of low activity in the 24 hours of greater than 3 hours to define the rest period.

A masked observer inspected the actigraphy data from each subject to identify any days in which there were errors in the automatically detected rest period (e.g., the start of the rest period did not coincide with a reduction in activity level and/or drop in light levels, and the end of the rest period did not coincide with an increase in activity and/or an increase in light levels), and these days were excluded from analysis. These errors in rest period demarcation generally coincided with periods of time when the Actiwatch was removed by the children. The sleep statistics were automatically computed by the Actiware software based on the wake/sleep data within the rest period each day. The first time point of the rest interval was defined as the bedtime, and the last time point in the rest interval as the wake time. Sleep onset was defined as the first 10-minute period in which all but one epoch of data were scored as immobile. For sleep offset, the algorithm identified the last 10-minute period within the rest period for which all epochs but one were scored as immobile. Sleep latency was defined as the time between the start of the rest interval and sleep onset. Sleep efficiency was defined as the percentage of time scored as sleep within the rest period. Sleep duration was the total time within the rest interval scored as sleep.

To examine the circadian rest-activity cycles of the myopic and non-myopic children, analysis of the raw physical activity data exported by the Actiwatch devices for each subject was conducted to determine the interdaily stability (IS) and the intradaily variability (IV) of the rest-activity cycle. The IS parameter varies from 0 to 1 and quantifies how well synchronized these rhythms are to external zeitgebers, such as the light-dark cycle (with higher IS values indicating stronger synchronization and more stable rhythms), whereas the IV parameter provides an assessment of the degree of fragmentation of the rest-activity patterns (with a value of 0 indicating a perfect sign wave rhythm and a value of two indicating Gaussian noise) with higher values considered to be markers of sleep-wake disturbances.[35,36]

The Actiwatch has been shown to have >90% sensitivity for detecting sleep in adolescents,[37] with previous validation studies reporting no significant differences in sleep parameters between the Actiwatch and polysomnography.[38,39] Note that the sleep duration reported here was the time that the software determined the child to be asleep, and not just in bed; therefore sleep duration is not the same as the duration between bedtime and wake time. Each parameter was averaged across all measurement days, as well as separately for weekdays (i.e., school nights, Sunday–Thursday nights) and weekends (Friday–Saturday nights). The influence of time spent outdoors and physical activity on sleep was examined. Time spent outdoors was calculated as mean minutes per day that were spent in light levels >1000 lux (as determined from the Actiwatch 2 illuminance measurements). Physical activity was analyzed as the average counts per minute per day. Only days including at least 90% of valid data were included in this aspect of the analysis.

Behavioral data were collected across all four seasons of the calendar year. To allow an analysis of the seasonal variations in sleep of the children in the study, the climate and day length data from the first and second 14-day periods were derived from the Australian Bureau of Meteorology and used to classify each period of wear from each child by season, as being either from "longer/warmer days" or "shorter/cooler days." Data classified as originating from the longer/warmer days included data collected in February, March, October, November, and December (i.e., summer, early autumn, and late spring), whereas data classified as originating from the shorter/cooler days included measurements from April, May, June, July, August, and September (i.e., winter, late autumn, and early spring). These climate data also confirmed that there was no significant difference in the day length, temperature, or rainfall experienced by myopic and non-myopic children during the time periods when behavioral data were collected ($P > 0.05$ for all comparisons).

Statistical analysis was performed in SPSS (IBM Corp., Armonk, NY). A repeated measures ANOVA was used to examine the influence of season (long vs. short) and day of the week (weekend vs. weekday; within-subject factors) on sleep parameters, as well as age group, sex, and refractive error group (between-subject factors). A repeated measures ANOVA was also used to examine the influence of season, refractive error, age, and sex on IS and IV. Bonferroni adjusted pairwise comparisons were used to examine differences for any statistically significant ($P < 0.05$) main effects and interactions. Multiple linear regression analysis was used to assess the influence of time spent outdoors and physical activity on sleep, considering age and sex as independent variables. Partial correlations were used to look at relationships between each sleep parameter with refractive change and with axial elongation over the study period, while controlling for sex, age group, and refractive error group.

Results

Subjects

Of the 102 subjects, 11 children were excluded from the analysis; 1 was lost to follow-up before the second measurement period, 1 Actiwatch device experienced a battery failure during the second period of wear, 1 participant developed signs of a retinal dystrophy, and 8 had insufficient behavioral data for 1 or more recording period. Therefore data from 91 children (mean age 13.02 ± 1.37 years) were included in all further analyses. All children reported being in good general health, with a small number reporting mild asthma (non-myopes $N = 9$, myopes $N = 3$).

Thirty-six children were classified as myopic (mean subjective spherical equivalent refractive error –2.44 $\pm$ 1.52 D, range –7.56 to –0.63 D) and 55 children were classified as non-myopic (mean subjective spherical equivalent refractive error +0.33 $\pm$ 0.30 D, range –0.38 to +1.13 D). The myopic and non-myopic children were well-matched for both age (mean age 13.01 ± 1.57 years in the myopes and 13.03 ± 1.24 years in the non-myopes) and sex (53% of the myopes and 53% of the non-myopes were girls). Children were classified based on their age at enrolment as being either younger (10 to <13 years, $n = 41$) or older (13 to <16 years, $n = 50$). Representative raw actigraph traces are shown for four days each for a myopic child and a non-myopic child in Supplementary Figure S1.

Children participating in the study were from 42 different schools in Brisbane, Australia. The mean school start time was 8:47 AM $\pm$ 15 minutes for non-myopes and 8:39 AM $\pm$ 13 minutes for myopes ($P < 0.05$), and the mean school finish time was 3:01 PM $\pm$ 10 minutes for non-myopes and 3:04 PM $\pm$ 6 minutes for myopes ($P > 0.05$). On average, daily time spent at school was 12 minutes less per day for non-myopes (6 hours 13 minutes $\pm$ 18 minutes) compared with myopes (6 hours 25 minutes $\pm$ 16 minutes, $P < 0.05$).

Climate Conditions

Compared with shorter/cooler days, the longer/warmer measurement period provided on average 2.0 ± 1.0 more hours of available daylight and

Table 1. Sleep Parameters (mean ± SD) over all Measurement Days for Non-Myopes ($N = 55$) and Myopes ($N = 36$). Parameters Include Mean Daily Bedtime, Wake Time, Sleep Duration (minutes), Sleep Latency (minutes), and Sleep Efficiency (%); *P* Value Shown for Bonferroni Corrected Pairwise Comparisons

Sleep Parameter	Non-Myopic	Myopic	*P* Value
Bedtime	10:02 PM ± 48 min	10:16 PM ± 61 min	0.38
Wake time	6:51 AM ± 37 min	7:06 AM ± 37 min	0.07
Sleep duration	7 hrs 34 min ± 38 min	7 hrs 44 min ± 48 min	0.06
Sleep latency	15.25 ± 12.22 min	11.41 ± 8.0 min	0.04[*]
Sleep efficiency	85.97% ± 4.5%	87.48% ± 4.75%	0.05

[*]Significance at Bonferroni corrected level of 0.05.

Table 2. Sleep Parameters (mean ± SD) for Non-Myopic ($N = 55$) and Myopic ($N = 36$) Children by Day of the Week. Parameters Include Mean Daily Bedtime, Wake Time, Sleep Duration (minutes), Sleep Latency (minutes), and Sleep Efficiency (%); *P* Value Shown for Bonferroni Corrected Pairwise Comparisons

Sleep Parameter	Non-Myopic		Myopic	
	Weekday	Weekend	Weekday	Weekend
Bedtime	9:50 PM ± 44 min	10:37 PM ± 67 min	10:01 PM ± 61 min	10:44 PM ± 66 min
		$P < 0.001$[*]		$P < 0.001$[*]
Wake time	6:34 AM ± 37 min	7:38 AM ± 57 min	6:44 AM ± 30 min	8:01 AM ± 71 min
		$P < 0.001$[*]		$P < 0.001$[*]
Sleep duration	7 hrs 32 min ± 41 min	7 hrs 40 min ± 51 min	7 hrs 35 min ± 54 min	8 hrs 9 min ± 53 min
		$P = 0.33$		$P < 0.001$[*]
Sleep latency	14.35 ± 11.91 min	17.6 ± 16.24 min	12.25 ± 9.12 min	8.77 ± 6.36 min
		$P = 0.03$[*]		$P = 0.05$
Sleep efficiency	86.2% ± 4.56%	85.35% ± 4.99%	87.45% ± 5.19%	87.82% ± 5.01%
		$P = 0.03$[*]		$P = 0.51$

[*]Significance at Bonferroni corrected level of 0.05.

+5.4° ± 3.8°C and +4.2° ± 2.5°C warmer minimum and maximum temperatures, respectively (all $P < 0.001$). The warmer days also had significantly greater average daily rainfall compared with the cooler days (on average 3.0 ± 5.4 mm greater daily rainfall, $P < 0.001$).

Sleep Parameters

Table 1 provides a summary of sleep parameters observed in the study for all children across all measurement days by refractive error group. Table 2 shows sleep parameters by refractive error and day of the weeks, and Table 3 by refractive error and season (longer/warmer days and shorter/cooler days).

Bedtime

For all children, mean daily bedtime was 10:08 PM ± 53 minutes. Bedtime was found to vary significantly by day of the week ($F_{(1,83)} = 117.3$, $P < 0.0001$), but not by season ($P = 0.27$). Post hoc Bonferroni corrected pairwise comparison showed that bedtime was significantly later by 45.4 minutes on weekends compared with weekdays ($P < 0.0001$). Between-subjects effects were significant for age group ($F_{(1,83)} = 8.2$, $P = 0.005$), but not for sex ($P = 0.91$) or refractive error group ($P = 0.38$). Younger children went to bed 34.4 minutes earlier than older children. A significant season by refractive error group interaction was also observed ($F_{(1,83)} = 4.8$, $P = 0.03$), with a significantly later bedtime on shorter/cooler days by 14.0 minutes compared with longer/warmer days observed in the emmetropic children ($P = 0.012$), but no significant seasonal differences observed in the myopic children ($P = 0.48$).

Wake Time

Daily wake time across all measurement days for all subjects was 6:57 AM ± 38 minutes. Wake time was

Table 3. Sleep Parameters (mean ± SD) for Non-Myopic (*N* = 55) and Myopic (*N* = 36) Children by Season. Parameters Include Mean Daily Bedtime, Wake Time, Sleep Duration (minutes), Sleep Latency (minutes), and Sleep Efficiency (%); *P* Value Shown for Bonferroni Corrected Pairwise Comparisons

Sleep Parameter	Non-Myopic		Myopic	
	Shorter/Cooler Days	Warmer/Longer Days	Shorter/Cooler Days	Warmer/Longer Days
Bedtime	10:07 PM ± 48 min	9:53 PM ± 53 min	10:14 PM ± 65 min	10:17 ± 61 min
	P = 0.01[*]		*P* = 0.48	
Wake time	6:58 AM ± 39 min	6:44 AM ± 43 min	7:11 AM ± 40 min	7:02 AM ± 40 min
	P = 0.004[*]		*P* = 0.06	
Sleep duration	7 hrs 36 min ± 44 min	7 hrs 32 min ± 42 min	7 hrs 43 min ± 56 min	7 hrs 35 min ± 52 min
	P = 0.87		*P* = 0.007[*]	
Sleep latency	16.23 ± 14.51 min	14.27 ± 13.92 min	12.43 ± 10.94 min	10.39 ± 7.5 min
	P = 0.51		*P* = 0.28	
Sleep efficiency	85.97% ± 5.04%	85.97% ± 4.74%	88.04% ± 5.21%	86.93% ± 6.05%
	P = 0.92		*P* = 0.08	

[*]Significance at Bonferroni corrected level of 0.05.

found to vary significantly with season ($F_{(1,83)} = 11.31$, $P = 0.001$) and by day of the week ($F_{(1,83)} = 171.62$, $P < 0.0001$). Children woke up 14.2 minutes later during shorter/cooler days compared with longer/warmer days, and 69.2 minutes later on weekends compared with weekdays. There was also a significant day of the week by age group interaction ($F_{(1,83)} = 6.64$, $P < 0.01$). Although both age groups woke up later on weekends compared with weekdays, older children woke up significantly later than the younger children on weekends; the younger children woke up 55.6 minutes later on weekends than weekdays ($P < 0.001$), and the older children woke up 82.9 minutes later on weekends than weekdays ($P < 0.001$). Between-subjects effects were not significant for sex ($P = 0.55$) or refractive error group ($P = 0.07$).
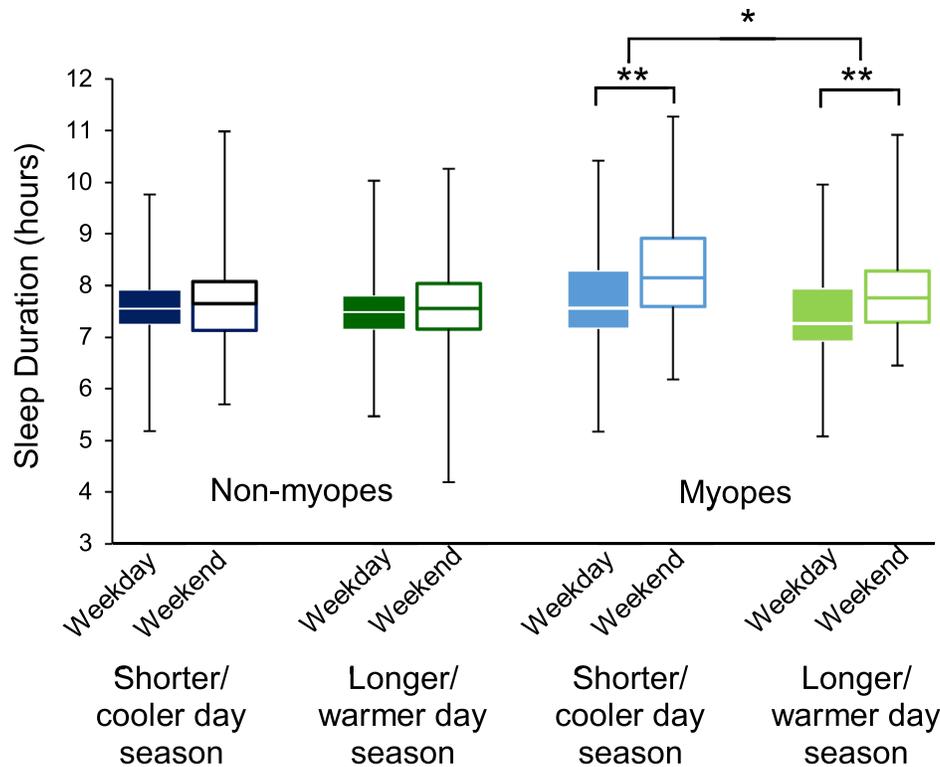
Sleep Duration

Sleep duration across all measurement days for all subjects was 7 hours 38 minutes ± 43 minutes. There were significant main effects of season ($F_{(1,83)} = 4.86$, $P = 0.03$) and day of the week ($F_{(1,83)} = 15.61$, $P < 0.001$) on sleep duration, with children sleeping 11.5 minutes longer on shorter/cool days compared with longer/warmer days, and 19.0 minutes longer on weekends compared with weekdays. There was a significant season by refractive error group interaction ($F_{(1,83)} = 3.98$, $P = 0.05$), with myopes sleeping 22.0 minutes more on shorter/cooler days compared with longer/warmer days ($P = 0.007$), and no difference in sleep duration by season for non-myopes ($P = 0.87$) (Fig.). Myopes slept 28.0 minutes more than non-

myopes on shorter/cooler days ($P = 0.01$). There was a significant day of the week by age group interaction ($F_{(1,83)} = 4.98$, $P = 0.03$), with the older children sleeping 29.7 minutes more on the weekends than weekdays ($P < 0.001$), but no significant differences in sleep duration between days of the week for the younger children ($P = 0.25$). There was a significant day of the week by sex interaction ($F_{(1,83)} = 13.26$, $P < 0.001$), with girls sleeping 36.5 minutes longer on weekends compared with weekdays ($P < 0.001$), but no significant difference in sleep duration between days for boys ($P = 0.83$). Finally, there was a significant day of the week by refractive group interaction ($F_{(1,83)} = 7.15$, $P = 0.01$), with myopes sleeping 31.9 minutes more on weekends compared with weekdays ($P < 0.001$), but no significant difference between days for non-myopes ($P = 0.33$) (Fig.). Myopes slept 30.4 minutes more than non-myopes on weekends ($P = 0.006$).

Sleep Latency

Sleep latency across all measurement days for all subjects was 13.73 ± 10.87 minutes. There were no main effects of season ($P = 0.21$) or day of the week ($P = 0.90$) on sleep latency, nor were there between-subjects effects for age group ($P = 0.70$) or sex ($P = 0.50$). However, there was a significant effect of refractive error ($F_{(1,83)} = 4.45$, $P = 0.04$); for all days considered together, myopes demonstrated a sleep latency of 11.41 ± 8.0 minutes, whereas non-myopes demonstrated a sleep latency of 15.25 ± 12.22 minutes. There was also a significant day of the week by refractive error group interaction ($F_{(1,83)} = 8.88$, $P = 0.004$).

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Figure. Sleep duration (hours per night) for shorter/cooler days (*blue*) and longer/warmer days (*green*) on weekdays (*filled bars*) and weekends (*open bars*) for non-myopic (*darker*) and myopic (*lighter*) children. *$P < 0.01$, **$P < 0.001$.

Non-myopic children had an increased latency on weekends ($P = 0.03$), whereas myopic children had a decreased latency on weekends ($P = 0.05$). A significant difference in latency between refractive groups was only observed on weekends (non-myopes: 17.60 ± 16.2 minutes; myopes: 8.77 ± 6.36 minutes, $P = 0.005$).

Sleep Efficiency

Across all days for all subjects, sleep efficiency was $86.57\% \pm 4.75\%$, which is comparable to a previous report in similarly aged children using the Actiwatch 2.[40] There were no main effects of season ($P = 0.20$) or day of the week ($P = 0.35$) on sleep efficiency, and there were no between-subjects effects for age group ($P = 0.06$), sex ($P = 0.33$), or refractive error group ($P = 0.051$).

Rest-Activity Rhythms

The IS of the rest-activity cycle for all subjects for the shorter/cooler day season was 0.56 ± 0.09, and for the longer/warmer day season was 0.56 ± 0.08. There were no significant differences in IS across season ($P = 0.36$) or by refractive error group ($P = 0.54$) or sex ($P = 0.24$). The younger children had a significantly higher

IS than the older children (0.60 ± 0.09 vs. 0.53 ± 0.08, respectively, $P < 0.001$), indicating more stable daily rest-activity cycles in the younger children. The IV of the rest-activity cycle for the shorter/cooler day season was 0.73 ± 0.13, and for the longer/warmer day season was 0.75 ± 0.15. There were no significant differences in IV across season ($P = 0.32$) or by refractive error group ($P = 0.11$), age ($P = 0.19$), or sex ($P = 0.83$).

Sleep, Outdoor Time, and Physical Activity

The relationships between the five sleep parameters with time spent outdoors and mean daily physical activity, considering sex, age group, and refractive error group as independent variables, are summarized in Table 4. The model revealed that time spent outdoors was significantly associated with sleep duration ($\beta = 0.31$, $t = 2.25$, $P = 0.03$), and that mean daily physical activity was significantly associated with wake time ($\beta = -0.44$, $t = -3.66$, $P < 0.001$). There were no significant associations between bedtime, latency, or efficiency with time spent outdoors or with physical activity. For the other parameters in the model (age group, sex, and refractive error), the only significant association was age group with bedtime ($\beta = 0.21$, $t = 2.02$, $P < 0.05$).

Table 4. Multiple Linear Regression Analysis for Each Sleep Parameter with Time Outdoors and Activity (coefficient β and P values shown). Other Parameters Included in the Model were Refractive Error Group, Age Group, and Sex, with the Only Significant Association Being Age Group with Bedtime ($\beta = 0.21$, $P < 0.05$)

	Time Outdoors		Activity	
Bedtime	$\beta = -0.24$	$P = 0.07$	$\beta = -0.19$	$P = 0.13$
Wake time	$\beta = 0.03$	$P = 0.80$	$\beta = -0.44$	$P < 0.001^*$
Sleep duration	$\beta = 0.31$	$P = 0.03^*$	$\beta = -0.26$	$P = 0.06$
Latency	$\beta = -0.12$	$P = 0.34$	$\beta = -0.10$	$P = 0.47$
Efficiency	$\beta = 0.04$	$P = 0.79$	$\beta = -0.18$	$P = 0.17$

*Significance at level of 0.05.

Sleep, Refractive Error Change, and Axial Elongation

The relationships between the five sleep parameters with refractive error change and axial elongation over 1 year was also assessed using partial correlations, while controlling for sex, age group, and refractive error group as independent variables. Neither refractive error change nor axial elongation were correlated with any of the sleep parameters tested (all $P > 0.05$).

Discussion

This study provides a detailed analysis of the typical daily patterns of sleep using objective measurement techniques in a population of 10 to 15 year old Australian non-myopic and myopic children during two seasons. Findings showed that sleep latency is significantly shorter for myopic children compared with non-myopic children. Additionally, sleep duration and bedtime showed significant interactions with refractive error by season and day of the week. Time spent outdoors was positively associated with sleep duration, and daily physical activity was negatively associated with wake time.

We were interested in whether sleep parameters vary between non-myopic and myopic children. There were no main effects of refractive error on bedtime, wake time, sleep duration, or sleep efficiency. However, there was a significant main effect of refractive error on sleep latency, with myopic children demonstrating a shorter sleep latency than non-myopic children by approximately 3.8 minutes. Furthermore, there was a significant interaction between refractive error group and day of the week, with non-myopic children exhibiting longer latencies on the weekend compared with weekdays, and myopic children exhibiting shorter latencies on the weekend compared with weekdays.

Sleep latency refers to the duration of time between when the lights are turned off and when the child fell asleep. Shorter sleep latencies have been associated with greater sleep debt and sleep deprivation.[41] Previous reports of actigraph-measured sleep latencies for healthy children range from 7 to 20 minutes.[40,42] Despite differences observed here in sleep latency between refractive error groups, all children were within the normal range. Further research may help to elucidate whether these findings have implications on sleep quality or eye growth.

We found that children's sleep patterns significantly varied by day of the week, when comparing weekdays (i.e., school days) versus weekend days (i.e., days with no school). On weekdays, children went to bed earlier, woke up earlier, and had a shorter sleep duration. Of interest, myopic children in this study showed significantly different sleep duration across days, as well as seasons, whereas non-myopic children showed more consistent sleep durations. Daily variability in sleep duration is referred to as night-to-night variability, which contributes to "social jetlag," and has been shown to be common among school age children.[43,44] Although the within-subject variability calculated across all days and seasons was not significantly different between myopic and non-myopic children, the myopic children exhibited greater variability in sleep duration between weekdays and weekends and between seasons. Weekday to weekend sleep differences are attributed to several factors, including early school start times, evening homework, and extracurricular activities.[45] Accumulating evidence suggests that night-to-night sleep variability may have adverse effects on daytime functioning, mental health, and behavior in children.[46] However, the literature is conflicting, with some studies reporting positive effects of extended sleep duration on weekends,[47,48] and others reporting deleterious effects of sleep variability on cognitive performance and inflammatory regulation, such as in circadian cortisol rhythm.[49]

Although myopic children in this study showed more variability in sleep duration across days and seasons than non-myopic children, there were no overall differences in sleep duration between refractive error groups. Previous studies using questionnaires have reported associations between sleep duration and myopia. Jee et al.[27] found that children ages 12 to 19 years with myopia were more likely to have a shorter sleep duration than those without myopia. Similarly, Gong et al.[50] found that children with less than 7 hours of sleep per night had a 3.37 times higher risk of myopia than those with greater than 9 hours of sleep, and Ayaki et al.[26] reported that children with high myopia had the shortest sleep durations compared with children with no myopia or mild myopia. However, other studies also using questionnaires have reported no differences in sleep duration in children and young adults with myopia compared with those without myopia.[30,51] Future studies utilizing objective measures of sleep with larger sample sizes and refractive error range may help to further understand whether sleep duration plays a role in eye growth.

Similar to previous reports, we found that sleep parameters vary with age,[52] with older children having a later bedtime than younger children. Overall, sleep duration was similar between the younger and older age groups. Studies suggest that with age, adolescents tend to delay bedtime such that sleep duration is shorter on school days, resulting in a sleep debt.[53] Although we did find a longer sleep duration in older children on the weekends compared with weekdays, their weekday sleep duration was similar to the younger children, suggesting that the older children in this study demonstrated more night-to-night sleep variability than the younger children. Previous studies have linked sleep variability in adolescents to intrinsic circadian delays associated with puberty,[52] which may have played a role in the increased sleep variability in the older age group observed here.

Compared across seasons, children woke up significantly later by 14 minutes and slept longer by 11.5 minutes on shorter/cooler days than on longer/warmer days. These small, but significant, differences in wake time and sleep duration between seasons may be attributed to the shorter photoperiod and cooler temperatures that defined the shorter/cooler day season in this study. Melatonin is known to decrease with light exposure in the morning, helping to promote wakefulness.[54,55] A later sunrise and shorter period of daylight affects patterns of diurnal melatonin concentration and likely contributed to the later wake time and longer sleep duration observed here. Additionally, cooler temperatures are known to increase sleep.[56]

Core body temperature decreases during sleep, and a cooler air temperature may further help to modulate body temperature and enhance sleep. The climate in Brisbane, Australia, the location of the current study, exhibited small but significant variations across seasons. Future research in geographic locations that exhibit larger seasonal variations in day length and climate will be of interest to provide further understanding of how seasonal variations influence light exposure and sleep in children across different refractive groups.

Previous studies have shown that physical activity and bright-light therapy improve sleep quality and increase duration in adults.[57,58] Our findings in children show that objectively measured mean daily time outdoors was associated with an increased sleep duration, and physical activity was associated with an earlier morning wake time. Time outdoors and activity were not associated with other sleep parameters, including latency, efficiency, and bedtime. Considering the significant effects on sleep duration and wake time, time outdoors and physical activity may be important meditators of sleep in children.

We examined the IS (stability of the rest-activity cycle) and IV (fragmentation of activity) using the non-parametric circadian analysis procedure.[36] For all subjects, IS and IV were similar to previous studies in healthy teenagers, with no significant differences between refractive error groups. Consistent with previous reports,[59] IS showed significant age-related changes, with older children demonstrating a lower IS than younger children, suggesting that the rest-activity cycle becomes less stable with age.

The small but significant observed differences in sleep parameters between refractive error groups may be due to several mechanisms relating to either physiological processes or social factors. The myopic children in this study had less light exposure than non-myopic children, as reported previously.[3,33] With known relationships between light exposure, dopamine, and melatonin, light exposure patterns may have led to downstream effects on sleep. Factors, such as academic pressure and stress, may also have contributed to sleep patterns, which is supported by previous observations that myopic children have greater academic achievement than non-myopic children.[60] Significant differences in time spent in school may have also contributed to the observed differences in sleep patterns between refractive error groups; myopes were found to have spent 12 minutes more at school per day than non-myopes.

The gold standard for sleep assessment is polysomnography, which takes place in a sleep laboratory. Polysomnography consists of many components,

such as electroencephalogram, electrocardiogram, electrooculogram, and several other measures.[32] In this study, we utilized the wrist actigraphy for objective measures of sleep, which allows for measurements in children's habitual environments, and has been shown to provide highly sensitive measures of sleep.[61,62] We captured, on average, 24 days of sleep data between two seasons for each subject, which would not be feasible using polysomnography. Although actigraphy provides a good estimate of total sleep time in adolescents,[63] this method tends to overestimate sleep time in patients who lie motionless while awake in bed, as well as overestimate "wake after sleep onset," showing poor specificity to detect "wake" in children and adolescents.[61,64] Additionally, the Actiwatch has been shown to have a wide variation in sleep latency compared with polysomnography.[40] A sleep diary indicating when the participant went to bed intending to sleep would have been beneficial to minimize this potential discrepancy. Because wrist actigraphy cannot determine sleep phase, this parameter was not considered here. Finally, the Actiwatch may not accurately reflect light exposure emitted from electronic devices, such as computers, tablets, and handheld phones; here, we did not control for electronic device use after light offset.

Another limitation in this study is that only non-cycloplegic refraction was measured, which increases variability in refractive error data in children.[65] To address this, subjective refraction with maximum plus was performed, which allowed us to group subjects by refractive error, being non-myopic or myopic. Additionally, axial length measurements used here are not likely to be influenced by cycloplegia.[66]

We did not measure systemic melatonin concentration here, which may have provided further insight into subject's diurnal rhythms. Melatonin (5-methoxy-N-acetyltryptamine) concentration is correlated with light exposure, demonstrating concentrations 3 to 10 times higher in the daily dark phase than in the light phase.[14,15,67] With known relationships between melatonin, sleep, and light exposure, and links between light exposure and myopia, it would be of interest to explore relationships between melatonin and refractive error in children to further investigate a potential role of circadian rhythms in myopia. A previous study found that morning serum melatonin concentration is significantly higher in myopic adults compared with non-myopic adults, suggesting that links exist between light exposure, circadian rhythm, and refractive status in humans.[24] Assessing associations between melatonin and refractive error in children may help to elucidate mechanisms for previously observed associations in adults.

Conclusions

The day of the week, season, and age significantly influenced sleep in adolescents. Myopic children tended to demonstrate more night-to-night variability in sleep duration than non-myopic children. Further investigation is warranted to understand the impact and mechanism of associations between myopia and sleep.

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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;123:1036–1042.

2. Wang Y, Ding H, Stell WK, et al. Exposure to sunlight reduces the risk of myopia in rhesus monkeys. *PloS One*. 2015;10:e0127863.

3. Read SA, Collins MJ, Vincent SJ. Light exposure and physical activity in myopic and emmetropic children. *Optom Vis Sci*. 2014;91:330–341.

4. Rose KA, Morgan IG, Ip J, et al. Outdoor activity reduces the prevalence of myopia in children. *Ophthalmology*. 2008;115:1279–1285.

5. Wu PC, Tsai CL, Wu HL, Yang YH, Kuo HK. Outdoor activity during class recess reduces myopia onset and progression in school children. *Ophthalmology*. 2013;120:1080–1085.

6. Jones-Jordan LA, Mitchell GL, Cotter SA, et al. Visual activity before and after the onset of juvenile myopia. *Invest Ophthalmol Vis Sci*. 2011;52:1841–1850.

7. Guggenheim JA, Northstone K, McMahon G, et al. Time outdoors and physical activity as predictors of incident myopia in childhood: a prospective cohort study. *Invest Ophthalmol Vis Sci*. 2012;53:2856–2865.

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8. Lim LS, Gazzard G, Low YL, et al. Dietary factors, myopia, and axial dimensions in children. *Ophthalmology*. 2010;117:993–997.e4.

9. Guo Y, Liu LJ, Xu L, et al. Outdoor activity and myopia among primary students in rural and urban regions of Beijing. *Ophthalmology*. 2013;120:277–283.

10. Chakraborty R, Ostrin LA, Nickla DL, Iuvone PM, Pardue MT, Stone RA. Circadian rhythms, refractive development, and myopia. *Ophthalmic Physiol Opt*. 2018;38:217–245.

11. Stone RA, Quinn GE, Francis EL, et al. Diurnal axial length fluctuations in human eyes. *Invest Ophthalmol Vis Sci*. 2004;45:63–70.

12. Chakraborty R, Read SA, Collins MJ. Diurnal variations in axial length, choroidal thickness, intraocular pressure, and ocular biometrics. *Invest Ophthalmol Vis Sci*. 2011;52:5121–5129.

13. Burfield HJ, Patel NB, Ostrin LA. Ocular biometric diurnal rhythms in emmetropic and myopic adults. *Invest Ophthalmol Vis Sci*. 2018;59:5176–5187.

14. Burfield HJ, Carkeet A, Ostrin LA. Ocular and systemic diurnal rhythms in emmetropic and myopic adults. *Invest Ophthalmol Vis Sci*. 2019;60:2237–2247.

15. Ostrin LA, Jnawali A, Carkeet A, Patel NB. Twenty-four hour ocular and systemic diurnal rhythms in children. *Ophthalmic Physiol Opt*. 2019;39:358–369.

16. Bartmann M, Schaeffel F, Hagel G, Zrenner E. Constant light affects retinal dopamine levels and blocks deprivation myopia but not lens-induced refractive errors in chickens. *Vis Neurosci*. 1994;11:199–208.

17. Stone RA, Lin T, Desai D, Capehart C. Photoperiod, early post-natal eye growth, and visual deprivation. *Vision Res*. 1995;35:1195–1202.

18. Gottlieb MD, Fugate-Wentzek LA, Wallman J. Different visual deprivations produce different ametropias and different eye shapes. *Invest Ophthalmol Vis Sci*. 1987;28:1225–1235.

19. Nickla DL, Totonelly K. Brief light exposure at night disrupts the circadian rhythms in eye growth and choroidal thickness in chicks. *Exp Eye Res*. 2016;146:189–195.

20. Brainard GC, Hanifin JP. Photons, clocks, and consciousness. *J Biol Rhythms*. 2005;20:314–325.

21. Hannibal J, Fahrenkrug J. Melanopsin: a novel photopigment involved in the photoentrainment of the brain's biological clock? *Ann Med*. 2002;34:401–407.

22. Freedman MS, Lucas RJ, Soni B, et al. Regulation of mammalian circadian behavior by non-rod, non-cone, ocular photoreceptors. *Science*. 1999;284:502–504.

23. Hattar S, Liao HW, Takao M, Berson DM, Yau KW. Melanopsin-containing retinal ganglion cells: architecture, projections, and intrinsic photosensitivity. *Science*. 2002;295:1065–1070.

24. Kearney S, O'Donoghue L, Pourshahidi LK, Cobice D, Saunders KJ. Myopes have significantly higher serum melatonin concentrations than non-myopes. *Ophthalmic Physiol Opt*. 2017;37:557–567.

25. Bruce ES, Lunt L, McDonagh JE. Sleep in adolescents and young adults. *Clin Med (Lond)*. 2017;17:424–428.

26. Ayaki M, Torii H, Tsubota K, Negishi K. Decreased sleep quality in high myopia children. *Sci Rep*. 2016;6:33902.

27. Jee D, Morgan IG, Kim EC. Inverse relationship between sleep duration and myopia. *Acta Ophthalmol*. 2016;94:e204–e210.

28. Xu C, Pan C, Zhao C, et al. Prevalence and risk factors for myopia in older adult east Chinese population. *BMC Ophthalmol*. 2017;17:191.

29. Abbott KS, Queener HM, Ostrin LA. The ipRGC-driven pupil response with light exposure, refractive error, and sleep. *Optom Vis Sci*. 2018;95:9.

30. Zhou Z, Morgan IG, Chen Q, Jin L, He M, Congdon N. Disordered sleep and myopia risk among Chinese children. *PLoS One*. 2015;10:e0121796.

31. Wei SF, Li SM, Liu L, et al. Sleep duration, bedtime, and myopia progression in a 4-year follow-up of Chinese children: The Anyang Childhood Eye Study. *Invest Ophthalmol Vis Sci*. 2020;61:37.

32. Ibanez V, Silva J, Cauli O. A survey on sleep assessment methods. *PeerJ*. 2018;6:e4849.

33. Read SA, Collins MJ, Vincent SJ. Light exposure and eye growth in childhood. *Invest Ophthalmol Vis Sci*. 2015;56:6779–6787.

34. Ward TM, Lentz M, Kieckhefer GM, Landis CA. Polysomnography and actigraphy concordance in juvenile idiopathic arthritis, asthma and healthy children. *J Sleep Res*. 2012;21:113–121.

35. Blume C, Santhi N, Schabus M. 'nparACT' package for R: a free software tool for the non-parametric analysis of actigraphy data. *MethodsX*. 2016;3:430–435.

36. Goncalves BS, Cavalcanti PR, Tavares GR, Campos TF, Araujo JF. Nonparametric methods in actigraphy: an update. *Sleep Sci*. 2014;7:158–164.

37. Lee XK, Chee N, Ong JL, et al. Validation of a consumer sleep wearable device with actigraphy and polysomnography in adolescents across sleep

opportunity manipulations. *J Clin Sleep Med*. 2019;15:1337–1346.

38. Pigeon WR, Taylor M, Bui A, Oleynk C, Walsh P, Bishop TM. Validation of the sleep-wake scoring of a new wrist-worn sleep monitoring device. *J Clin Sleep Med*. 2018;14:1057–1062.

39. Kushida CA, Chang A, Gadkary C, Guilleminault C, Carrillo O, Dement WC. Comparison of actigraphic, polysomnographic, and subjective assessment of sleep parameters in sleep-disordered patients. *Sleep Med*. 2001;2:389–396.

40. Meltzer LJ, Wong P, Biggs SN, et al. Validation of actigraphy in middle childhood. *Sleep*. 2016;39:1219–1224.

41. Goel N, Rao H, Durmer JS, Dinges DF. Neurocognitive consequences of sleep deprivation. *Semin Neurol*. 2009;29:320–339.

42. Gaina A, Sekine M, Chen X, Hamanishi S, Kagamimori S. Validity of child sleep diary questionnaire among junior high school children. *J Epidemiol*. 2004;14:1–4.

43. Sun W, Ling J, Zhu X, Lee TM, Li SX. Associations of weekday-to-weekend sleep differences with academic performance and health-related outcomes in school-age children and youths. *Sleep Med Rev*. 2019;46:27–53.

44. Bei B, Allen NB, Nicholas CL, Dudgeon P, Murray G, Trinder J. Actigraphy-assessed sleep during school and vacation periods: a naturalistic study of restricted and extended sleep opportunities in adolescents. *J Sleep Res*. 2014;23:107–117.

45. Hansen M, Janssen I, Schiff A, Zee PC, Dubocovich ML. The impact of school daily schedule on adolescent sleep. *Pediatrics*. 2005;115:1555–1561.

46. Bei B, Wiley JF, Trinder J, Manber R. Beyond the mean: a systematic review on the correlates of daily intraindividual variability of sleep/wake patterns. *Sleep Med Rev*. 2016;28:108–124.

47. Kuula L, Pesonen AK, Martikainen S, et al. Poor sleep and neurocognitive function in early adolescence. *Sleep Med*. 2015;16:1207–1212.

48. Wing YK, Li SX, Li AM, Zhang J, Kong AP. The effect of weekend and holiday sleep compensation on childhood overweight and obesity. *Pediatrics*. 2009;124:e994–e1000.

49. Simpson NS, Diolombi M, Scott-Sutherland J, et al. Repeating patterns of sleep restriction and recovery: do we get used to it? *Brain Behav Immun*. 2016;58:142–151.

50. Gong Y, Zhang X, Tian D, Wang D, Xiao G. Parental myopia, near work, hours of sleep and myopia in Chinese children. *Health*. 2014;6:7.

51. Qi LS, Yao L, Wang XF, et al. Risk factors for incident myopia among teenaged students of the experimental class of the air force in China. *J Ophthalmol*. 2019;2019:3096152.

52. Randler C, Fassl C, Kalb N. From lark to owl: developmental changes in morningness-eveningness from newborns to early adulthood. *Sci Rep*. 2017;7:45874.

53. Owens JA, Weiss MR. Insufficient sleep in adolescents: causes and consequences. *Minerva Pediatr*. 2017;69:326–336.

54. Brainard GC, Rollag MD, Hanifin JP. Photic regulation of melatonin in humans: ocular and neural signal transduction. *J Biol Rhythms*. 1997;12:537–546.

55. Cajochen C, Krauchi K, Wirz-Justice A. Role of melatonin in the regulation of human circadian rhythms and sleep. *J Neuroendocrinol*. 2003;15:432–437.

56. Harding EC, Franks NP, Wisden W. The temperature dependence of sleep. *Front Neurosci*. 2019;13:336.

57. Montgomery P, Dennis J. A systematic review of non-pharmacological therapies for sleep problems in later life. *Sleep Med Rev*. 2004;8:47–62.

58. Murray K, Godbole S, Natarajan L, et al. The relations between sleep, time of physical activity, and time outdoors among adult women. *PLoS One*. 2017;12:e0182013.

59. Rensen N, Steur LMH, Wijnen N, van Someren EJW, Kaspers GJL, van Litsenburg RRL. Actigraphic estimates of sleep and the sleep-wake rhythm, and 6-sulfatoxymelatonin levels in healthy Dutch children. *Chronobiol Int*. 2020 Mar 4;1–13, doi:10.1080/07420528.2020.1727916. Online ahead of print.

60. Morgan IG, Ohno-Matsui K, Saw SM. Myopia. *Lancet*. 2012;379:1739–1748.

61. Meltzer LJ, Walsh CM, Traylor J, Westin AM. Direct comparison of two new actigraphs and polysomnography in children and adolescents. *Sleep*. 2012;35:159–166.

62. Weiss AR, Johnson NL, Berger NA, Redline S. Validity of activity-based devices to estimate sleep. *J Clin Sleep Med*. 2010;6:336–342.

63. Johnson NL, Kirchner HL, Rosen CL, et al. Sleep estimation using wrist actigraphy in adolescents with and without sleep disordered breathing: a comparison of three data modes. *Sleep*. 2007;30:899–905.

64. Martin JL, Hakim AD. Wrist actigraphy. *Chest*. 2011;139:1514–1527.

65. Choong YF, Chen AH, Goh PP. A comparison of autorefraction and subjective refraction with

and without cycloplegia in primary school children. *Am J Ophthalmol*. 2006;142:68–74.

66. Huang J, McAlinden C, Su B, et al. The effect of cycloplegia on the lenstar and the IOLMaster biometry. *Optom Vis Sci*. 2012;89:1691–1696.

67. Selmaoui B, Touitou Y. Reproducibility of the circadian rhythms of serum cortisol and melatonin in healthy subjects: a study of three different 24-h cycles over six weeks. *Life Sci*. 2003;73:3339–3349.