Estimating Dim Light Melatonin Onset (DLMO) Phase in Adolescents Using Summer or School-Year Sleep/Wake Schedules

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Study Objectives: This analysis examined associations between the salivary dim light melatonin onset (DLMO) phase and self-selected sleep/wake schedules in groups of children and adolescents during summer vacation and during the school year to determine the degree to which sleep/wake patterns can estimate salivary DLMO phase.

Design And Setting: Participants slept at home on self-selected schedules for 5 consecutive nights and reported their bedtime and wake-up time via daily telephone messages. Salivary melatonin was sampled in the laboratory on one evening every 30 minutes in dim light (<50 lux) to determine DLMO phase. Within group bivariate regressions between sleep pattern measures (bedtime, wake-up time, and midsleep time) and DLMO phase were computed.

Participants: One group, ages 9 to 17 years (mean age=12.5, SD=2.3 years, 74 males, 75 females) contributed 149 DLMO phase and sleep/wake pattern measures while on a school year schedule (“school group”). A separate group, ages 9 to 16 years (mean age=13.1, SD=1.3 years, 30 males, 29 females) contributed 59 DLMO phase and sleep/wake pattern measures while on a summer schedule (“summer group”).

Results: Bedtime, midsleep time, and wake-up time were positively correlated with DLMO phase in both groups. Although all correlation coefficients for the summer group were statistically greater compared to the school group, the regression equations predicted DLMO phase within ±1 hour of the measured DLMO phase in ~80% for both groups.

Conclusions: DLMO phase can be estimated using self-selected sleep/wake patterns during the school year or summer vacation in healthy children and adolescents.

Keywords: Adolescent, sleep patterns, DLMO phase, melatonin, circadian rhythms

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INTRODUCTION

CIRCADIAN PHASE ASSESSMENT OF HUMANS HAS BECOME IMPORTANT FOR DESCRIPTIVE, EXPERIMENTAL, AND CLINICAL APPLICATIONS. FOR EXAMPLE, the effects of manipulated light-dark (LD) patterns or administration of exogenous melatonin have been examined using circadian phase markers.1,4 Furthermore, a number of groups use circadian phase to provide a context for neurobehavioral data, such as sleepiness and motor vigilance tasks.1,5-7 Knowledge of circadian phase may also be important for understanding the timing of sleep, developmental differences in sleep behavior,8 and sleep disorders such as delayed sleep phase syndrome (DSPS).9,10 The need for an accurate means of determining circadian phase in a reliable, noninvasive, and reasonably inexpensive manner is driven by such applications.

A common technique currently used to determine circadian phase assesses timing of the secretory pattern of the pineal hormone melatonin. Melatonin has a distinct daily pattern in which circulating melatonin concentration is low during the day, abruptly increases close to habitual bedtime, remains high throughout the night, and decreases to low daytime levels close to wake-up time. The relatively rapid rise and fall of melatonin production provide distinct points on the curve for circadian phase markers. Because light can suppress melatonin levels,11 measures are taken in dim light conditions. The time of the onset of melatonin secretion is called the dim light melatonin onset (DLMO) phase.12,13

Sleep and waking states are regulated by intrinsinc brain mechanisms and are manifested in behavioral patterns. Several models, largely based on Borbély’s Two-Process Model,14 describe these mechanisms as Process S and Process C. The homeostatic sleep/wake-dependent process (Process S) accumulates during wake and dissipates during sleep.14 The circadian system (Process C) is independent of prior waking and sleep; however, it provides intrinsic signals that dictate the timing of sleep and wake.15 We are interested in the degree to which self-reported sleep/wake behavior patterns can estimate intrinsic parameters of Process C in children and adolescents.

Previous studies have demonstrated that measures reflecting the timing of the sleep/wake schedule are correlated with salivary DLMO phase and can estimate phase within approximately ±1 hour for the majority of young adults examined.16-19 Martin and Eastman18 reported that DLMO phase was significantly correlated with reported unrestricted nocturnal sleep onset, midpoint of sleep, and wake-up time averaged over five weekdays. A similar study19 showed DLMO phase was significantly correlated with controlled (fixed) wake-up time and midpoint of sleep time derived from sleep diary reports; however, unlike the unrestricted sleep schedule, the fixed bedtime was not significantly correlated with DLMO phase. Thus, DLMO phase in young adults may be estimated more accurately by sleep patterns when participants are on unrestricted sleep schedules than when sleep timing is strictly regulated. Indeed, this finding was the principal outcome of a third report by Burgess and Eastman,16 which indicated that
unrestricted sleep/wake times showed a greater association with DLMO phase compared to the fixed sleep/wake times.

Fixed and unrestricted sleep schedules are not exclusive to experimental manipulations; they also occur due to social constraints. For example, a young adult in the typical “9-to-5” workplace has some constraints over wake-up time on weekday mornings, whereas a college student may experience fewer restrictions governing his/her wake-up time on weekday mornings. A pattern of greater and lesser sleep/wake control also occurs for children and adolescents throughout the calendar year. During the year, school-night sleep schedules are constrained by school start times; whereas daily sleep/wake patterns during vacations are unrestrained by school schedules.

The purpose of the current analysis was to examine the association between sleep/wake schedule timing and DLMO phase during the school year and during summer vacation in two separate samples. Furthermore, the current analysis aimed to investigate whether sleep schedule variables during the summer are better able to estimate DLMO phase compared to sleep schedule variables during the school year. Based on previous studies in adults, we hypothesized that DLMO phase would be associated with sleep/wake timing. Furthermore, we expected that summer sleep/wake schedule measures (unrestricted) would estimate DLMO phase better than the more constrained sleep/wake schedule measures during the school year.

MATERIALS AND METHODS

Participants

Data were taken from young people who participated in four studies with similar methodology between 1995 and 2002. Some of these data have been previously reported.\textsuperscript{20-23} One group of participants (N=130) ages 9 to 17 years (mean age=12.5, SD=2.3 years) contributed 149 DLMO phase and sleep/wake pattern measures while on a school year schedule (“school group”). A separate group (N=50) ages 9 to 16 years (mean age=13.1, SD=1.3 years) contributed 59 DLMO phase and sleep/wake pattern measures while on a summer schedule (“summer group”). A single participant could contribute more than one data point by taking part in a different study at another time. Age did not differ between groups [t (206)=1.92, P=.056], and the proportion of boys to girls was similar (74.75 in the school group; 30.29 in the summer group).

Standard procedures included an initial telephone screening interview with the participant and one parent, the Sleep Habits Survey\textsuperscript{24} completed by the participant, and a Sleep, Medical, Educational, and Family History Form completed by a parent. The following exclusion criteria were assessed with these tools: variation of self-reported sleep schedules more than 3 hours across a week; chronic insufficient sleep accompanied by signs of excessive sleepiness (e.g., falling asleep inappropriately); personal or parental history of sleep problems or disorders; a history of narcolepsy in a first-degree relative; chronic major illness (e.g., cancer or diabetes) or current illness (e.g., fever or symptoms of respiratory infection); psychopathology personally or in a first-degree relative, including major depression, bipolar disorder, or psychosis in parents or siblings; use of prescription medications or over-the-counter medications that may influence the sleep/wake cycle, alertness/sleepiness, or suppress melatonin; a history of head trauma, brain injury, or physical handicaps (e.g., blindness) that would interfere with study measures; evidence of learning disability (e.g., special education classes or diagnosis) or a diagnosis of ADD or ADHD; and insufficient knowledge of reading and writing of the English language. All but one study excluded individuals who reported morningness/eveningness (M/E) preference (measured using the Morning Scale of Smith and colleagues\textsuperscript{25}) greater than 2 standard deviations from mean values of a normative data pool. The remaining study, which contributed 72 participants in the school group, did not exclude participants based on M/E preference.

The E.P. Bradley Hospital or Lifespan Institutional Review Board approved each protocol. Each child’s parent gave informed consent, participants cosigned to indicate their assent to participate, and each child received monetary compensation for participating.

PROCEDURES

Participants documented their home sleep pattern for about one week in a diary. Participants (and parents, presumably) chose the times at which they slept (“self-selected” schedule), and they were instructed to sleep at home alone and not remain awake all night (no “all-nigh ters”). Participants called the laboratory’s time stamped answering machine every morning to report the previous night’s bedtime, the morning’s wake-up time, and whether they were going to attend school that day. Participants were grouped into the school or summer groups based on these telephone reports and school calendars. Data collection for the school group occurred between January and June or between September and November; data collection for the summer group occurred between June and August.

After sleeping at home for an average of one week, DLMO phase was assessed in the lab during one evening using the serial saliva sampling method. Saliva (~1 mL) was collected in 30-minute intervals in dim light (< 50 lux) using untreated Salivettes (Starstedt, Germany). Participants were seated during the five minutes before each sample to minimize postural effects on melatonin concentration. Approximately 12 to 14 samples were collected per participant, typically beginning 5 hours before his/her average bedtime (computed from daily telephone messages) and ending 30 minutes after average bedtime. If participants ingested anything except water before the saliva sample, they rinsed their mouths and brushed their teeth with water. Ingestion of caffeine, chocolate, non-steroidal anti-inflammatory drugs (NSAIDs), and alcohol were prohibited during the home portion of the study and during saliva collection. Saliva samples were frozen and later assayed for melatonin by another laboratory (Lipid Research Laboratory, Miriam Hospital, Providence, RI) using radioimmunoassay (RIA) test kits (APLCO Diagnostics, Windham, NH). The lower limit of detection of the assay was 0.2 pg/mL.

Sleep/Wake and Circadian Phase Measures

Four sleep/wake schedule variables were obtained from the daily morning telephone messages: reported bedtime and wake-up time, midsleep time (the midpoint between bedtime and wake-up time), and time in bed (total duration of time between bedtime and wake-up time). These variables were computed as the average for the 5 consecutive nights (weekday and weekend) preceding DLMO phase assessment for each participant. Eleven participants in the school group and 10 participants in the summer group were missing one night, and one participant in the summer
group was missing two nights. The average number of pre-DLMO nights chosen for this analysis (~5 nights) was similar to previous adult studies.

Deacon and Arendt,\textsuperscript{26} using simultaneous plasma and salivary melatonin, showed that saliva melatonin concentration values are approximately 40% of plasma levels. Therefore, our lab uses a threshold of 4 pg/mL,\textsuperscript{20} which is in parallel with the 10 pg/mL plasma level threshold introduced by Lewy and colleagues.\textsuperscript{13} The time of the DLMO phase was determined by linear interpolation between the time points before and after the melatonin concentration increased and stayed above the 4 pg/mL threshold. We also computed the interval between DLMO phase and the average reported bedtime and wake-up time (phase angles of entrainment).

**Statistical Analysis**

Groups were subdivided by age (9-12 and 13-16 years) and by sex to provide normative sleep/wake schedule, DLMO phase, and phase angle data during the summer and school year. Sleep/wake variables, DLMO phase, and phase angle differences were assessed using multivariate analysis of variance with group (summer vs. school), age (9-12 and 13-16 year olds), and sex as between subject factors, followed by post hoc t-tests to identify sources of significant interactions. Alpha was set at .05.

The primary aim of this study was to examine the linear association of DLMO phase and sleep/wake schedules and to derive an equation to estimate DLMO phase during the school year and during the summer. Within group bivariate regressions were computed using bedtime, wake-up time, and midsleep time as independent measures (predictor variables) and DLMO phase as the dependent measure. We assessed differences among correlation coefficients following the procedures of Cohen and Cohen.\textsuperscript{37}

**RESULTS**

**Sleep and Circadian Timing Descriptive Analysis**

Analyses of variance revealed significant main effects for group and age for time in bed, bedtime, midsleep time, wake-up time, DLMO phase, and DLMO phase to bedtime interval. The interaction effect for group by age was significant for time in bed, bedtime, wake-up time, and DLMO phase to bedtime interval. Data and statistical results are presented in Tables 1 and 2.

On average, total time in bed was 19 minutes longer in the summer group compared to the school group and 69 minutes longer in the younger group compared to the older group. Post hoc t-tests indicated that the significant group-by-age interaction was due to the older participants in the school group who reported the least amount of time in bed.

Bedtime was 56 minutes later in the summer compared to the school group and 54 minutes later in the older group compared to the younger group. Post hoc t-tests indicated that the significant group-by-age interaction was due to younger participants in the school group reporting the earliest bedtime, and older participants reporting the latest bedtimes in the summer group.

Midsleep time was 66 minutes later for the summer group compared to the school group and was 40 minutes later, on average, in the older participants compared to the younger participants. The group-by-age interaction for midsleep time was nonsignificant.

Reported wake-up time was 76 minutes later in the summer compared to the school group and 26 minutes later in the older participants compared to the younger participants. Post hoc tests revealed that the significant group-by-age interaction was due to the older participants in the summer group reporting the latest wake-up times, and a greater difference between summer and school wake-up times for the older group compared to the younger group. Bedtime, midsleep time, and wake-up time showed a greater range in the summer group (~5.5-7 hours) compared to the school group (~2.5-3.5 hours).

Circadian variables were also compared between groups and ages (Table 2). DLMO phase was on average 70 minutes later for the summer group compared to the school group and 27 minutes later in the older participants compared to the younger participants. The group-by-age interaction for DLMO phase was nonsignificant. DLMO phase also showed a greater range in the summer group (~5 hours) compared to the school group (~3.5 hours).

The time interval from DLMO phase to reported bedtime averaged 15 minutes longer for the school group compared to the summer group and 27 minutes longer for the older participants than the younger participants. Post hoc tests indicated that the significant group-by-age interaction was due to the older participants in the school group who showed the longest time interval between DLMO phase and bedtime. DLMO phase to reported wake-up time did not differ statistically between the summer and school year or between age subgroups.

The main effect for sex was significant for time in bed, wake-up time, and DLMO phase to wake-up time. Across both the summer and school groups, average reported wake-up time for boys (mean = 7:19, SD = 57 minutes) was earlier compared to girls (mean = 7:30, SD = 62 minutes) \([F_{1,120} = 2.64, P < .05}\). Boys also reported less time in bed, on average (mean = 537, SD = 52 minutes) compared to girls (mean = 560, SD = 43 minutes) \([F_{1,120} = 5.64, P < .01}\). The interval from DLMO phase to wake-up time was also shorter for boys (mean = 623, SD = 46 minutes) compared to girls (mean = 641, SD = 45 minutes) \([F_{1,120} = 4.06, P < .01}\). No interaction effects that included sex were significant.

**Linear Association Between DLMO Phase and Sleep Schedule Measures**

A bivariate linear regression was computed to determine the associations between sleep/wake schedule measures (bedtime, midsleep time, and wake-up time) and DLMO phase within each group (summer or school). The results are shown in Figure 1. Within each group, 16% to 64% of DLMO phase variance (determined using \(R^2\)) was accounted for by sleep/wake schedule variables. Overall, youngsters with later sleep schedule times had a later DLMO phase.

For the summer group, DLMO phase was correlated with bedtime, midsleep, and wake-up time. The correlation coefficients for DLMO phase with wake-up time did not differ from that of midsleep time \([t(56) = -.66, P > .05]\) or bedtime \([t(56) = -1.07, P > .05]\). On the other hand, the correlation coefficient for DLMO phase with midsleep time was significantly greater compared to that of bedtime \([t(56) = -2.62, P < .05]\).

In the school group, DLMO phase was correlated with bedtime, midsleep time, and wake-up time. The correlation coefficients for DLMO phase with bedtime did not differ from that of midsleep time \([t(146) = -1.77, P > .05]\) or wake-up time \([t(146) = .98, P > .05]\). In contrast to the summer group, the correlation

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coefficient for DLMO phase with midsleep time was significantly greater compared to that of wake-up time \([t(146) = -2.21, P<.05]\) rather than bedtime.

Correlation coefficients for the summer group were significantly greater compared to the school group for bedtime \([z=6.65, P<.001]\), wake-up time \([z=12.25, P<.001]\), and midsleep time \([z=10.25, P<.001]\).

Estimating DLMO Phase With Sleep Schedule Measures

The derived regression equations to estimate DLMO phase using sleep/wake times within summer and school groups are shown in Table 3. The \textit{estimated} DLMO phase for each participant was computed using these equations and was plotted against their \textit{measured} DLMO phase in Figure 2. Within the summer group, estimated DLMO phase was within \(\pm 1\) hour (bold solid lines) of the measured DLMO phase for 78\% of the group using bedtime, 86\% of the group using midsleep time, and 80\% of the group using wake-up time. Within the school group, estimated DLMO phase was within \(\pm 1\) hour of the measured DLMO phase for 82\% of the group using bedtime, 82\% of the group using midsleep time, and 81\% of the group using wake-up time.

Regression equations derived from one group were applied to the other group’s data, a cross-validation technique that allowed us to assess external validity. The summer regression equation using school bedtime, midsleep time, and wake-up time estimated 80\%, 84\%, and 76\% to within \(\pm 1\) hour of the measured DLMO phase, respectively. The school regression equation using summer bedtime, midsleep time, and wake-up time estimated 53\%, 80\%, and 76\% of participant’s DLMO phases within \(\pm 1\) hour of the measured DLMO phase, respectively.

The range of differences between each \textit{measured} DLMO phase

<table>
<thead>
<tr>
<th>Time in Bed(^b(^c)) (mins)</th>
<th>Summer(^1)</th>
<th>School(^1)</th>
<th>Summer &amp; School</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years):</td>
<td>Mean (SD)(^2)</td>
<td>Minimum</td>
<td>Maximum</td>
</tr>
<tr>
<td>9-12</td>
<td>556 (56)</td>
<td>430</td>
<td>644</td>
</tr>
<tr>
<td>13-16</td>
<td>569 (55)</td>
<td>459</td>
<td>656</td>
</tr>
<tr>
<td>Grand Total</td>
<td>562 (56)</td>
<td>430</td>
<td>656</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Bedtime(^b(^c)) (24-h time)</th>
<th>Summer(^1)</th>
<th>School(^1)</th>
<th>Summer &amp; School</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years):</td>
<td>Mean (SD)(^2)</td>
<td>Minimum</td>
<td>Maximum</td>
</tr>
<tr>
<td>Grand Total</td>
<td>22:56 (61)</td>
<td>20:08</td>
<td>1:32</td>
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<table>
<thead>
<tr>
<th>Midsleep Time(^b(^c)) (24-h time)</th>
<th>Summer(^1)</th>
<th>School(^1)</th>
<th>Summer &amp; School</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years):</td>
<td>Mean (SD)(^2)</td>
<td>Minimum</td>
<td>Maximum</td>
</tr>
<tr>
<td>9-12</td>
<td>3:21 (63)</td>
<td>0:53</td>
<td>5:55</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Wake-up Time(^b(^c)) (24-h time)</th>
<th>Summer(^1)</th>
<th>School(^1)</th>
<th>Summer &amp; School</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years):</td>
<td>Mean (SD)(^2)</td>
<td>Minimum</td>
<td>Maximum</td>
</tr>
<tr>
<td>9-12</td>
<td>8:00 (74)</td>
<td>4:28</td>
<td>10:19</td>
</tr>
<tr>
<td>13-16</td>
<td>8:39 (73)</td>
<td>6:33</td>
<td>11:14</td>
</tr>
<tr>
<td>Grand Total</td>
<td>8:19 (76)</td>
<td>4:28</td>
<td>11:23</td>
</tr>
</tbody>
</table>

\(^1\)n=30 for 9-12 year olds in the summer group; n=29 for 13-16 year olds in the summer group; n=95 for 9-12 year olds in the school group; n=54 for 13-16 year olds in the school group.

\(^2\)All SD values are shown in minutes.

\(^b\)Significant group effect:
- Time in Bed: \(F_{1,200} = 14.58, P < .001\)
- Bedtime: \(F_{1,200} = 44.80, P < .001\)
- Midsleep time: \(F_{1,200} = 91.35, P < .001\)
- Wake-up Time: \(F_{1,200} = 104.81, P < .001\)

\(^c\)Significant age effect:
- Time in Bed: \(F_{1,200} = 6.45, P < .05\)
- Bedtime: \(F_{1,200} = 32.23, P < .001\)
- Midsleep time: \(F_{1,200} = 25.08, P < .001\)
- Wake-up Time: \(F_{1,200} = 10.73, P < .01\)

\(^\star\)Significant group-by-age interactions; significant differences from post hoc tests:
- Time in Bed: \(F_{1,200} = 20.52, P < .001\); 9-12 school > 13-16 school, 13-16 school < 13-16 summer, 9-12 summer > 13-16 school.
- Bedtime: \(F_{1,200} = 4.47, P < .05\); 9-12 school < 13-16 school, 9-12 school < 9-12 summer, 13-16 school < 13-16 summer, 9-12 school < 13-16 summer.
- Wake-up Time: \(F_{1,200} = 4.49, P < .05\);
- 9-12 summer < 13-16 summer, 9-12 school < 9-12 summer, 13-16 school < 13-16 summer, 9-12 school < 13-16 summer, 9-12 summer > 13-16 school.
and the corresponding estimated DLMO phase is shown in Figure 2 (dashed lines). Within both groups, DLMO phase estimated from sleep/wake times was within about ±1.5 hours of the measured DLMO phase, except for summer bedtime. Summer bedtime showed a larger range of differences primarily due to one participant whose estimated DLMO phase was 2 hours and 26 minutes later than her measured DLMO phase. This participant’s average reported bedtime was 22:59 and her measured DLMO phase was 19:19; this time duration between DLMO phase and bedtime (phase angle) was the largest of the summer group (more than two standard deviations above the mean). Other variables included in this analysis did not appear to explain her wide phase angle.

Studies of young adults demonstrate that those with late circadian phases go to bed and wake-up at an early circadian time. By contrast, those with early circadian phases sleep at a late circadian time.28,29 To determine if this association manifests in this age group, we computed a Pearson’s correlation between DLMO phase and the time interval from DLMO phase to bedtime, as well as between DLMO phase and the time interval from DLMO phase to wake-up time in both groups. In the summer group, the interval from DLMO phase to bedtime showed a significant negative correlation with DLMO phase ($R = -.51$). The interval from DLMO phase to wake-up time was not associated with DLMO phase in the summer group ($R = -.17$). In the school group, DLMO phase showed a significant negative correlation with the interval from DLMO phase to bedtime ($R = -.51$) and the interval from DLMO phase to wake-up time ($R = -.77$). Overall, later DLMO phase was associated with smaller intervals; in other words, youngster who showed a late circadian phase tend to sleep at an earlier circadian time compared to those who show an early circadian phase.

**DISCUSSION**

Reported sleep/wake schedules were later during summer vacation compared to the school year in these samples of youngsters and reported time in bed during the summer was longer compared to the school-year; however, the magnitude of difference depended on age. Midsleep time and DLMO phase were later during the summer compared to the school-year and for older compared to younger participants. The DLMO phase to bedtime interval phase angle was longer in the older participants in the school group. Furthermore, sleep schedules during summer vacation showed a higher statistical correlation with DLMO phase in children and adolescents compared to the sleep schedule during the school year. Nonetheless, the ability of the derived regression equations to estimate DLMO phase from sleep/wake schedule measures did not appear to differ between school and summer groups, since

Table 2—Descriptive Statistics for Circadian Measures in School and Summer Groups by Age

<table>
<thead>
<tr>
<th></th>
<th>Summer&lt;sup&gt;1&lt;/sup&gt;</th>
<th>School&lt;sup&gt;1&lt;/sup&gt;</th>
<th>Summer &amp; School</th>
</tr>
</thead>
<tbody>
<tr>
<td>DLMO Phase&lt;sup&gt;a,b&lt;/sup&gt; (24-h time)</td>
<td>Mean (SD)&lt;sup&gt;2&lt;/sup&gt; Minimum Maximum</td>
<td>Mean (SD)&lt;sup&gt;2&lt;/sup&gt; Minimum Maximum</td>
<td>Mean (SD)&lt;sup&gt;2&lt;/sup&gt; Minimum Maximum</td>
</tr>
<tr>
<td>DLMO Phase to Bedtime Interval&lt;sup&gt;a,b,c&lt;/sup&gt; (mins)</td>
<td>Age (years): 9-12 78 (57) -42 220</td>
<td>71 (43) -12 186</td>
<td>72 (47)</td>
</tr>
<tr>
<td></td>
<td>13-16 66 (45) -49 168</td>
<td>116 (50) -2 204</td>
<td>99 (54)</td>
</tr>
<tr>
<td>Grand Total</td>
<td>72 (52) -49 220</td>
<td>87 (51) -12 204</td>
<td></td>
</tr>
<tr>
<td>DLMO Phase to Wake-Up time Interval (mins)</td>
<td>Age (years): 9-12 635 (49) 554 725</td>
<td>632 (42) 538 730</td>
<td>632 (43)</td>
</tr>
<tr>
<td></td>
<td>13-16 637 (48) 526 712</td>
<td>627 (52) 542 723</td>
<td>630 (51)</td>
</tr>
<tr>
<td>Grand Total</td>
<td>636 (48) 526 725</td>
<td>630 (46) 538 730</td>
<td></td>
</tr>
</tbody>
</table>

<sup>1</sup>n=30 for 9-12 year olds in the summer group; n=29 for 13-16 year olds in the summer group; n=95 for 9-12 year olds in the school group; n=54 for 13-16 year olds in the school group.

<sup>2</sup>All SD values are shown in minutes.

<sup>a</sup>Significant group effect: DLMO Phase: $F_{1,200} = 72.32, P < .001$

<sup>b</sup>Significant age effect: DLMO Phase: $F_{1,200} = 8.37, P < .01$

<sup>c</sup>Significant group-by-age interactions; significant differences from post hoc tests: DLMO Phase to Bedtime Interval: $F_{1,200} = 15.09, P < .001$; 9-12 school < 13-16 school, 13-16 school > 13-16 summer, 9-12 summer < 13-16 school.

Table 3—Regression equations to estimate DLMO phase from self-report sleep times

<table>
<thead>
<tr>
<th></th>
<th>Summer</th>
<th>School</th>
</tr>
</thead>
<tbody>
<tr>
<td>DLMO = .75 x (bedtime) + 4.46</td>
<td>DLMO = .44 x (bedtime) + 10.78</td>
<td></td>
</tr>
<tr>
<td>DLMO = .86 x (midsleep) + 18.62</td>
<td>DLMO = .70 x (midsleep) + 18.77</td>
<td></td>
</tr>
<tr>
<td>DLMO = .69 x (wake) + 15.95</td>
<td>DLMO = .60 x (wake) + 16.34</td>
<td></td>
</tr>
</tbody>
</table>

Note: all sleep variable times are in decimal hours.
estimates captured essentially the same percentage of participants within a given range.

**Association Between Sleep/Wake and Circadian Timing**

Sleep/wake schedule measures were associated with DLMO phase in both groups, but the summer group showed a greater statistical correlation. These findings are consistent with those in young adults, if we presume the summer group is comparable to unrestricted sleep/wake patterns and the school group is comparable to an experimentally fixed sleep/wake schedule. Burgess and Eastman showed that salivary DLMO phase was significantly correlated with unrestricted and fixed sleep/wake schedules in young adults, with a weaker association for the fixed schedule.

The current findings are also consistent with previous studies of children and adolescents. In a study by Carskadon and colleagues, young participants kept an unrestricted (“self-selected”) sleep schedule followed by a fixed (“entrained”) sleep schedule, and salivary DLMO phase was assessed after each sleep condition by serial dim-light saliva samples. Actigraphy estimates of sleep onset and sleep offset over 4 days were significantly correlated with DLMO phase during the unrestricted condition (R =...
.76 and R = .72, respectively); however, these sleep/wake measures were not correlated with DLMO phase during the fixed condition. Consistency of this finding may arise because imposing fixed sleep/wake schedules may not produce the same phase relationship between sleep and circadian timing for every individual. This is supported by our finding that the older participants in this sample reported going to bed at a later circadian phase compared to the younger participants during the school year but not during the summer (see DLMO phase to bedtime phase angle in Table 1).

The difference in correlation coefficients between school and summer groups is also likely due to the statistical nature of the regression analysis. Specifically, the school group had a restricted range of times for sleep/wake pattern and DLMO phase measures, especially for wake-up time (see Figure 1 and Table 1). Wake-up times for the school group ranged about 2.5 hours (5:47
to 8:19), whereas wake-up times during the summer ranged about 7 hours (4:28 to 11:23). Moreover, the range for DLMO phase was more limited in the school group (see Figure 1 and Table 2). Thus, DLMO phase ranged about 3.5 hours (18:45 to 22:23) in the school group, whereas DLMO phase ranged about 5 hours (19:13 to 0:11) in the summer group. The correlation coefficient may be lower in the school group because less variance overall was available to allow an association between DLMO phase and sleep/wake patterns in the school group compared to the summer group. A similar caveat applies to the results of young adults. In the study of Burgess and Eastman16 fixed wake-up time showed a smaller range (~4.5 h) compared to unrestricted wake-up time (~6 h; see Figure 2 in reference 16).

On average, the time interval from DLMO phase to wake-up time is similar to that of young adults (~10-10.5 h).16,18,19 By contrast, the DLMO phase to bedtime interval is descriptively different from the interval reported in young adults. After fixed sleep conditions, the DLMO phase to bedtime interval in adults is between approximately 2 and 2.5 h.16,17 After unrestricted sleep, this interval is about 2 to 3 h.16,18 In our sample of youngsters, the DLMO phase to bedtime interval averaged about 1 h during the summer and about 1.5 h during the school year. The biological underpinning of this observation is unknown; however, we could speculate that this difference may be attributed to a longer circadian period in adolescents compared to adults.30 A long circadian period is associated with a shorter phase angle between DLMO phase and habitual sleep time.31 Other external factors such as parental control over sleep times may also contribute to these differences.

Estimated DLMO Phase From Regression Equations

Further examination of the error terms (residuals) for each regression analysis showed that sleep/wake times in both groups estimated a similar percentage (~80%) of DLMO phases within ±1 hour of the measured DLMO phase (see Figure 2). Therefore, although the correlation coefficients for the summer group were statistically higher compared to the school group, the ability to estimate DLMO phase within ±1 hour of the measured DLMO phase did not differ. Furthermore, when one set of equations was applied to the other group’s data, DLMO phase was still estimated to within ±1 hour in about 75% to 80% of the sample. The one exception was the school equation’s relatively poor estimate using bedtimes for the summer sample in which only 52.5% of DLMO phases were estimated within ±1 hour of the measured DLMO phase. This disparity indicates that the summer regression equation has a greater external validity when using bedtime as an estimate of DLMO phase compared to the school bedtime equation.

Additional Sources of Variance

Estimates of DLMO phase that deviated by more than 1 hour of the measured DLMO phase (see Figure 2) indicates that the relationship between the sleep and DLMO phase for these individuals differed from the others in the sample. Overall, those whose estimated DLMO phase fell outside of the ±1 hour range did not show any demographic characteristics that would account for this greater deviation. The source of this residual error is unknown; however, we could speculate about some factors that may influence the variability not accounted. First, DLMO phase is not an exact measure. The computation required a linear interpolation between two time points 30 minutes apart. Because the measure itself is not exact, it will inevitably have some variability that cannot be explained by an environmental, behavioral, or demographic variable.

Variability or error in estimating DLMO phase from sleep/wake patterns may also result from variability of the intrinsic circadian period among individuals. Recently, Wright and colleagues31 reported that the phase relationship (phase angle) between sleep timing under entrained conditions and circadian phase in humans differs depending on an individual’s intrinsic circadian period; a shorter intrinsic period was associated with a wider phase angle of entrainment between DLMO phase and sleep time. Furthermore, for every 6-minute difference in intrinsic period, the phase relationship differed by 24 minutes, on average.31 Therefore, small differences of intrinsic period may have introduced large variation in the phase relationship between sleep/wake patterns and salivary DLMO phase that we were unable to account for in the regression models.

The current analysis studied children and adolescents during the entire calendar year and therefore, the sample varied with respect to solar day length. Previous human studies have shown changes in the melatonin secretory pattern related to long or short photoperiods32 implemented in the laboratory. Yet environmental photoperiod may not significantly alter the pattern of melatonin secretion in humans, because electric lighting allows us to control our photoperiod; essentially, humans are exposed to the same photoperiod year round. To test whether photoperiod could account for the current study’s findings, we ran a multiple linear regression with each sleep schedule variable and photoperiod on the day in which DLMO phase was measured. The association between DLMO phase and each sleep/wake measure (bedtime, midsleep time, and wake-up time) did not significantly change in either the school group or in the summer group with photoperiod accounted for. Therefore, we are skeptical that photoperiod would account for a substantial amount of additional variance associated with DLMO phase in the current samples.

Implications, Limitations, and Future Directions

The regression equations may help limit the expense of time and money by narrowing the time frame needed to collect saliva samples and reducing the number of saliva samples needed to compute DLMO phase. These equations may also be used in research methodology to control for circadian phase when circadian parameters are not directly measured because they may not be the primary focus of a research project or because the procedure is not feasible (i.e., in a field study). An estimate of circadian phase may benefit the analysis and interpretation of the results.

Sleep/wake schedule data used in this analysis was based on daily telephone messages. Therefore, the reliability of these sleep/wake times may not be as accurate as objective measures, such as actigraphy or polysomnography (PSG). Nevertheless, we used daily telephone messages because we reasoned that a remote procedure via telephone could be implemented in a research study in the field or a clinical setting to estimate DLMO phase.

Studies are needed that focus on investigating the association between sleep/wake schedules and DLMO phase in clinical samples of young people. A validated regression equation may serve as a useful and simple tool for clinicians who treat sleep and circadian disorders, specifically in adolescents. Disorders
such as delayed sleep phase syndrome (DSPS), advanced sleep phase syndrome (ASPS), and seasonal affective disorder (SAD) are often treated with bright light therapy (chronotherapy), the timing of which relative to the circadian cycle (DLMO phase) is important for effective treatment. In the case of treatment with exogenous melatonin for developmental circadian delays and circadian disorders such as DSPS, the timing is also critical to the effectiveness of the treatment.

CONCLUSIONS

In summary, these analyses indicate that self-reported sleep/wake pattern measures may provide a good estimate for salivary DLMO phase in children and adolescents when direct measures are not possible. The association between sleep/wake and DLMO phase measures differs depending on sleep/wake variability among subjects when these parameters are assessed. Nevertheless, the regression equations derived from these samples approximate a large percentage of measured DLMO phases to within a ±1 hour error margin. Finally, it is premature to apply these regression equations to clinical settings without further research.

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