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SHORT REPORT

Evidence of circalunar rhythmicity in young children's evening melatonin levels

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Summary

In adults, recent evidence demonstrates that sleep and circadian physiology change across lunar phases, including findings that endogenous melatonin levels are lower near the full moon compared to the new moon. Here, we extend these results to early childhood by examining circalunar fluctuations in children's evening melatonin levels. We analysed extant data on young children's circadian rhythms ($n = 46$, aged 3.0–5.9 years, 59% female). After following a strict sleep schedule for 5–7 days, children completed an in-home, dim-light circadian assessment (<10 lux). Salivary melatonin was assessed at regular 20- to 30-min intervals until 1 h past each child's scheduled bedtime. Melatonin levels varied significantly across lunar phases, such that melatonin was lower in participants assessed near the full moon as compared to near the new moon. Significant differences were observed at 50 min ($mean_{full} = 2.5$ pg/ml; $mean_{new} = 5.4$ pg/ml) and 10 min ($mean_{full} = 7.3$ pg/ml; $mean_{new} = 15.8$ pg/ml) before children's scheduled bedtime, as well as at 20 min ($mean_{full} = 15.5$ pg/ml; $mean_{new} = 26.1$ pg/ml) and 50 min ($mean_{full} = 19.9$ pg/ml; $mean_{new} = 34.3$ pg/ml) after bedtime. To our knowledge, these are the first data demonstrating that melatonin secretion, a process regulated by the human circadian system, is sensitive to changes in lunar phase at an early age. Future research is needed to understand the mechanisms underlying this association (e.g., an endogenous circalunar rhythm) and its potential influence on children's sleep and circadian health.

KEYWORDS
children, circadian, development, lunar phase, melatonin, moon

INTRODUCTION

The impact of the moon on human biology and behaviour has long been a curiosity among scientists, with researchers examining circalunar changes in outcomes such as seizures, gout, and aneurysms (Chakraborty, 2014). The evidence on whether such associations exist remains conflicted (Foster & Roenneberg, 2008); however, in the past decade, data from several innovative studies indicate that sleep and circadian physiology fluctuate across lunar phases in adults (Cajochen et al., 2013; Dergaa et al., 2021; Smith, Croy, & Waye, 2014; Turányi et al., 2014). Cajochen et al. (2013) first observed that electroencephalography (EEG)-assessed total sleep duration and endogenous melatonin decreased near the full moon in a light-controlled sleep laboratory. These findings were replicated and extended in a recent study in which blood samples were collected from healthy adult males in the morning and evening of both the full moon and new moon under laboratory conditions (Dergaa et al., 2021). These results confirmed that participants had significantly lower melatonin levels at the full moon compared to the new moon at both times of day.

Research examining the impact of the lunar cycle on children's physiology is scarce. In one study with school-aged children, significantly lower insulin sensitivity and higher blood pressure occurred when assessed on mornings near the full moon compared to the new moon (Sjödin et al., 2015). Differences in accelerometry-derived sleep duration across lunar phases were also reported in school-aged children (Chaput et al., 2016; Sjödin et al., 2015), although the findings were inconsistent. Finally, data from two previous studies with adolescents failed to show associations between the lunar cycle and objective measures of physical activity or explosive physical performance (Smith, Standl, Schulz, & Heinrich, 2017; Yousfi et al., 2018). Whether the influence of the moon on human physiology extends to the maturing circadian system is currently unknown; thus, we examined the association between lunar phase and endogenous melatonin levels in early childhood. We utilised extant data from healthy, good-sleeping children who completed an in-home circadian assessment under well-controlled, dim-light conditions. Salivary melatonin was collected throughout the evening and retrospectively examined for differences in melatonin concentration across the lunar cycle.

METHODS

Participants

Analysis included data from two previous studies on children's circadian rhythms (Akacem, Wright Jr., & LeBourgeois, 2018; Hartstein et al., 2022). Data were collected solely during the summer months (mid-May to mid-September) to control for seasonal variation in photoperiod. Participants were 46 children aged 3.0–5.9 years (mean [SD] 4.3 [0.7] years, 59% female) recruited from the greater Boulder, Colorado area. Strict eligibility criteria were employed to ensure that only healthy children with no sleep, circadian, or ophthalmological disorders were enrolled (See Akacem et al., 2018; Hartstein et al., 2022 for full eligibility criteria). All study procedures were approved by the University of Colorado Boulder Institutional Review Board. We obtained written informed consent from parents and provided compensation for participation.

Protocol

Before the start of the protocol, researchers covered windows in each child's bedroom with black tarps and removed any bedside lamps or other small light sources, which ensured children received no light exposure between scheduled bedtime and wake time throughout the study. For the first 5–7 days, children followed a strict parent-selected sleep schedule (bedtime and wake time) in accordance with the child's habitual sleep/wake times, which was verified by wrist-worn actigraphy (Figure 1). An in-home, dim-light circadian assessment was then performed. Researchers covered all windows and confirmed that light levels throughout the home remained at <10 lux at the child's angle of gaze. Children entered the dim-light 4 h and 20 min before their scheduled bedtime and provided saliva samples at 20- to 30-min intervals until 1 h past bedtime. Researchers were in the home with the child during waking hours of the protocol. Between saliva samples, children interacted with the researchers, playing with a variety of age-appropriate toys, doing crafts, or reading storybooks. Children remained in a seated posture for 5 min before and during each saliva sample and refrained from eating or drinking for 15 min before samples. During each sample, light intensity was measured by holding

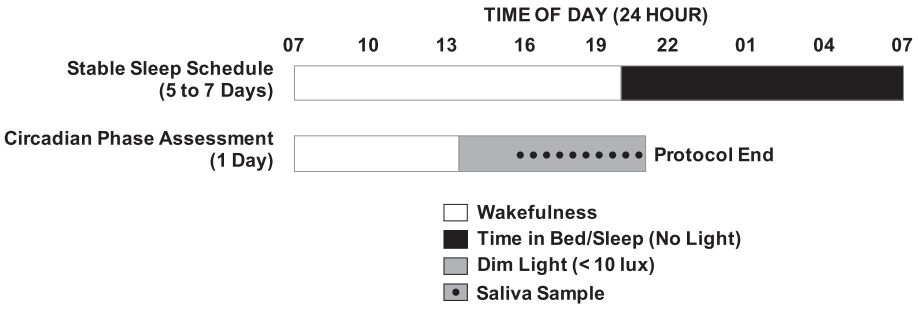

FIGURE 1 Sample study protocol. For 5–7 days, children followed a parent-selected sleep schedule. They then completed an in-home, dim-light circadian assessment anchored to their scheduled bedtime. The timing of wake, sleep, and circadian phase assessment in the figure are provided as an example

TABLE 1 Means and standard deviations of sample characteristics

	Lunar category			
Variable	Lunar 1 Full Moon	Lunar 2	Lunar 3 New Moon	Statistics
N	17	17	12	χ^2 (2, $N = 46$) = 1.09, $p = 0.58$
Sex, % female	58.8	47.1	75.0	χ^2 (2, $N = 46$) = 2.27, $p = 0.32$
Mean (SD):				
Age, years	4.42 (0.46)	4.16 (0.64)	4.46 (0.97)	F (2, 43) = 0.89, $p = 0.42$
Bedtime, clock time (h:min)	8:05 p.m. (0:26)	8:04 p.m. (0:35)	8:19 p.m. (0:43)	F (2, 43) = 0.87, $p = 0.43$
DLMO, clock time (h:min)	7:36 p.m. (0:34)	7:24 p.m. (0:44)	7:32 p.m. (1:03)	F (2, 43) = 0.31, $p = 0.73$

Note: DLMO, dim-light melatonin onset.

a photometer ~5 cm from the child's eye and pointed in the approximate angle of gaze. To collect saliva, children chewed a braided cotton roll for ~2 min. The samples were immediately centrifuged, stored in coolers with ice packs on-site, and then transferred to a −20°C laboratory freezer. Samples were later assayed off-site (Solid Phase, Inc.).

Analysis

Researchers first identified the number of days between the circadian assessment and the nearest full moon (https://www.timeanddate.com/moon/phases/usa/boulder). Consistent with prior research (Cajochen et al., 2013), data were categorised into one of three corresponding groups (Lunar Category): Lunar 1 (0–4 days from the nearest full moon), Lunar 2 (5–9 days), and Lunar 3 (10–14 days).

Melatonin samples were assayed using radioimmunoassay (Bühlmann Laboratories AG). The limits of detection were 0.50 and 50.0 pg/ml. Dim-light melatonin onset (DLMO) was determined as the time at which melatonin levels crossed 4 pg/ml (LeBourgeois et al., 2013).

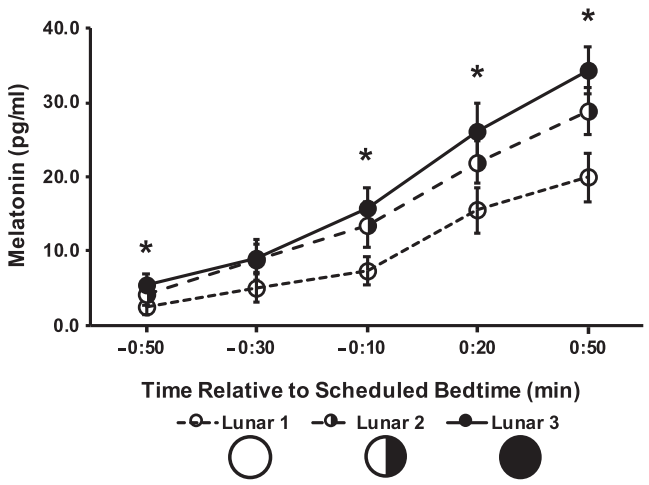

FIGURE 2 Average group melatonin levels. Melatonin levels were significantly lower 50 and 10 min before scheduled bedtime, as well as 20 and 50 min after bedtime for children assessed near the full moon (Lunar 1) compared with those assessed near the new moon (Lunar 3). Error bars denote standard error. Asterisks indicate a $p \leq 0.05$

A chi-squared test was used to examine the distribution of sex across the three groups. One-way analysis of variance (ANOVA) with lunar category as the independent variable was utilised to test for differences in age, scheduled bedtime (lights out time), and DLMO. Mann–Whitney tests were used to examine sex differences in children's melatonin levels. We used Kruskal–Wallis ANOVA to examine differences in melatonin levels across lunar phases, as Kolmogorov–Smirnov tests revealed significantly non-normal distributions. A Bonferroni correction was applied to all pairwise comparisons. Effect sizes for pairwise comparisons are presented as *r* values. Significance testing was performed with an α level of 0.05.

RESULTS

The mean (SD) light intensity near the child's eye was 0.61 (0.49) lux during saliva samples. The average scheduled bedtime was 8:09 p.m. (SD = 0:34 min). DLMO ranged from 5:48 p.m. to 9:16 p.m. (mean = 7:31 p.m., SD = 0:46 min), occurring at a mean (SD) of 38 (36) min before bedtime. The demographic, sleep, and circadian characteristics across each lunar category are presented in Table 1. There were no significant differences in the distribution of sex, age, bedtime, or DLMO across lunar categories.

Figure 2 depicts average melatonin levels for each lunar category collected throughout the evening of the dim-light circadian assessment. Melatonin levels did not differ between males and females (all $p > 0.14$). Kruskal–Wallis ANOVAs revealed that children's melatonin levels varied significantly across lunar phases when assessed at 50 and 10 min before scheduled bedtime, as well as 20 and 50 min after. Pairwise comparisons indicated that melatonin levels were significantly lower at each time point (all $p \leq 0.05$) in participants assessed 0–4 days from the full moon (Lunar 1) than those assessed 10–14 days from the full moon (Lunar 3). Group means and SDs by lunar phase for each time-point are reported in Table 2.

DISCUSSION

To our knowledge, this is the first study examining the association between lunar phase and circadian physiology in children. Consistent with prior work in adults, we observed significantly lower evening

TABLE 2 Means and standard deviations of melatonin levels by lunar phase

Time relative to scheduled bedtime, min	Lunar 1	Lunar 2	Lunar 3	Statistics	Effect size, *r*
−50	2.48 (4.35)	4.27 (6.11)	5.43 (5.33)	$\chi^2(2, N = 45) = 6.25, p = 0.04$*	−0.39
−30	5.01 (7.83)	8.85 (10.60)	9.09 (6.10)	$\chi^2(2, N = 44) = 4.84, p = 0.09$	−0.36
−10	7.31 (7.93)	13.55 (12.29)	15.75 (9.75)	$\chi^2(2, N = 46) = 7.29, p = 0.03$*	−0.45
+20	15.52 (12.64)	21.97 (11.83)	26.12 (12.93)	$\chi^2(2, N = 46) = 7.23, p = 0.03$*	−0.43
+50	19.90 (13.46)	28.80 (12.68)	34.25 (11.02)	$\chi^2(2, N = 45) = 8.88, p = 0.01$*	−0.50

*values statistically significant at $p < 0.05$.

melatonin levels when assessed near the full moon (Lunar 1) compared with near the new moon (Lunar 3) under well-controlled in-home conditions (Cajochen et al., 2013; Dergaa et al., 2021).

Several mechanisms are proposed to underlie the association between the lunar cycle and sleep and circadian physiology (Vyazovskiy & Foster, 2014). Although light is the strongest zeitgeber to the circadian clock, it is unlikely that exposure to moonlight (~0.25 lux at full moon) could affect melatonin levels. During the 5–7 days before the circadian assessment, researchers covered windows in the child's bedroom and melatonin collection occurred in a highly-controlled dim-light environment (<10 lux). Similar findings in adults were likewise collected under light-controlled laboratory conditions (Cajochen et al., 2013; Dergaa et al., 2021). Additionally, a placebo effect has been hypothesised, in which knowledge of the lunar phase and its purported effect contributes to the expected alterations in behaviour (Röösli et al., 2006). This does not apply to the present study given the age of the subjects and the strict control of sleep schedules and photoperiods. Finally, it has been proposed that fluctuations of human biology with lunar phase represent an endogenous circalunar clock with a period of ~29.5 days, as has been observed across a wide range of organisms (Vyazovskiy & Foster, 2014). Determining the molecular mechanisms underlying this rhythm and its adaptive function in humans requires further examination (Andreatta & Tessmar-Raible, 2020).

An association between lunar cycle and sleep duration was reported in several studies (Cajochen et al., 2013; Röösli et al., 2006; Smith et al., 2014; Turányi et al., 2014). In adults, EEG-derived sleep duration is 20–25 min shorter near the full moon (Cajochen et al., 2013; Smith et al., 2014). In two studies with school-aged children, waist-worn accelerometry revealed a significant association between lunar phase and sleep duration (Chaput et al., 2016; Sjödin et al., 2015); however, the direction was inconsistent, and the magnitude of the difference observed in each study was small (4–5 min). We were unable to examine the association between lunar phase and sleep duration via actigraphy due to the rigid parent-selected sleep schedule protocols. Given the differences observed in melatonin, a hormone that helps prepare the body for sleep, young children's readiness for sleep, timing, and quality of sleep may similarly vary throughout the lunar cycle and should be examined in future research.

These data were collected under rigorous conditions in the home environment, controlling for several confounding variables that could have masked lunar rhythmicity. First, before the circadian assessment, children followed a strict sleep schedule and received no light exposure between bedtime and wake time. This ensured there were no light cues from the moon and that children were synchronised to a consistent light/dark pattern. Second, data were collected exclusively during the summer months in one geographical location, allowing for consistency in season and photoperiod across all participants. Finally, data collection and processing were unlikely influenced by implicit biases, as this research question was developed post hoc. However, some limitations of our data should be noted. The strict eligibility criteria employed during recruitment resulted in a homogenous population of healthy children, limiting the generalisability of these findings. Additionally, only between subjects' data were examined and therefore individual differences in melatonin secretion or lunar rhythmicity cannot be determined. In summary, we present evidence that young children's circadian physiology changes across the lunar cycle. Future research should explore the mechanisms underlying this association and whether it plays a role in children's sleep and circadian health.

AUTHOR CONTRIBUTIONS

All authors contributed to protocol design, data collection, and data processing. Lauren E. Hartstein conceived the study question, performed the analyses, and drafted the manuscript. Lameese D. Akacem, Kenneth P. Wright, Cecilia Diniz Behn, and Monique K. LeBourgeois contributed to manuscript revisions.

CONFLICT OF INTEREST

Lameese D. Akacem has no financial or personal conflicts to declare. Lauren E. Hartstein reports receiving research support from the National Institutes of Health (NIH), outside the submitted work. Cecilia Diniz Behn reports receiving research support from the NIH, the National Science Foundation, and LumosTech, outside the submitted work. Kenneth P. Wright reports research support/donated materials: DuPont Nutrition & Biosciences, Grain Processing Corporation, and Friesland Campina Innovation Centre and reported being a consultant to and/or receiving personal fees from Circadian Therapeutics, Inc., Circadian Biotherapies, Inc., Philips, Inc., and US Army Medical Research and Materiel Command - Walter Reed Army Institute of Research, outside the submitted work. Monique K. LeBourgeois reports receiving travel funds from the Australian Research Council and research support from the NIH, beyond the submitted work.

DATA AVAILABILITY STATEMENT

Research data are not shared.

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