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Laboratory Validation of an In-Home Method for Assessing Circadian Phase Using the Dim Light Melatonin Onset (DLMO)

Rebecca E. Pullman, B.A.¹, Stephanie E. Roepke, B.A.¹, and Jeanne F. Duffy, M.B.A., Ph.D.^{1,2,*}

¹Division of Sleep Medicine, Department of Medicine, Brigham and Women's Hospital, Boston MA USA

²Division of Sleep Medicine, Harvard Medical School, Boston MA USA

Abstract

Objective—To determine whether an accurate circadian phase assessment could be obtained from saliva samples collected by patients in their home.

Methods—Twenty-four individuals with a complaint of sleep initiation or sleep maintenance difficulty were studied for two evenings. Each participant received instructions for collecting 8 hourly saliva samples in dim light at home. On the following evening they spent 9h in a laboratory room with controlled dim (<20 lux) light, where hourly saliva samples were collected. Circadian phase of dim light melatonin onset (DLMO) was determined using both an absolute threshold (3pg/mL) and a relative threshold (2 standard deviations above the mean of 3 baseline values).

Results—Neither threshold method worked well for one participant who was a 'low-secretor'. In four cases the participant's in-lab melatonin levels rose much earlier and/or were much higher than their at-home levels, and one participant appeared to take the at home samples out of order. Overall, the at-home and in-lab DLMO values were significantly correlated using both methods, and differed on average by 37 (± 19) minutes using the absolute threshold and by 54 (± 36) minutes using the relative threshold.

Conclusions—The at-home assessment procedure was able to determine an accurate DLMO using an absolute threshold in 62.5% of the participants. Thus, an at-home procedure for assessing circadian phase could be practical for evaluating patients for circadian rhythm sleep disorders.

Keywords

DLMO; circadian phase; melatonin; circadian rhythm sleep disorders

Introduction

Current practice parameters for the diagnosis of circadian rhythm sleep disorders (CRSD) recommend the use of sleep logs, actigraphy and/or PSG recordings, but do not include any direct measure of circadian phase¹. A recent study demonstrated that among a group of

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*Address for Correspondence: Jeanne F. Duffy Division of Sleep Medicine 221 Longwood Avenue, BLI438 Boston, MA 02115
Phone: 617-732-7995 Fax: 617-732-4015 jduffy@hms.harvard.edu.

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patients with well-documented delayed sleep timing, not all had delayed circadian rhythms. In fact, approximately half showed normal circadian phase timing as assessed by the dim light melatonin onset (DLMO) despite their late sleep timing². Assessment of circadian phase in patients with altered sleep-wake timing should improve diagnostic specificity, which can then be used to determine the most appropriate course of treatment². In addition, such assessments may be useful in evaluating patients with conventional sleep timing but complaints of sleep initiation or maintenance problems, as they may have a mismatch between their sleep timing and their underlying circadian rhythm of sleep propensity.

While saliva samples can be used to determine the circadian phase of DLMO, the patient must remain in dim light conditions for several hours. Dim lighting conditions are not difficult to achieve in the laboratory or clinic, although collecting serial saliva samples in the clinic or lab is expensive and inconvenient. At-home sampling is performed routinely by some clinics³, but how well the DLMO collected at home compare to DLMO from a well-controlled setting is unknown. The present study aimed to determine whether patients could collect saliva samples in dim light in their home, and how well a DLMO measured at home would correspond to a DLMO collected in controlled laboratory conditions.

Methods

The study was reviewed and approved by the Partners Health Care Human Research Committee, and was conducted in accordance with the principles outlined in the Declaration of Helsinki.

Participants

Study advertising targeted working adults age 20 or older who complained of difficulty falling asleep and/or difficulty getting up in the morning and adults age 50 or older who complained of falling asleep too early, waking too early, and/or not being able to remain asleep.

Participants came for a screening visit during which written informed consent was obtained. We excluded individuals with acute or unstable medical conditions, current or prior psychiatric diagnosis, current indication of depression based on a standardized questionnaire (Beck Depression Inventory or Geriatric Depression Scale), a BMI greater than 30, a diagnosis of any sleep disorder other than one of sleep timing, and the use of any medications known to interfere with melatonin secretion or sleep. All participants scored 5 or higher on the Pittsburgh Sleep Quality Index⁴ and/or the Epworth Sleepiness Scale⁵.

Protocol

Participants who passed the screening visit were scheduled for a laboratory visit approximately 1–2 weeks later. They received an at-home saliva sampling kit and instructions for its use on the evening prior to their laboratory visit. They were instructed to maintain their usual sleep-wake schedule between the screening and laboratory visit, were given a wrist activity monitor to wear, and were given a sleep diary to document their sleep-wake timing. In addition, participants were instructed to call in to a voicemail call-in line before bedtime and after wake time each night.

At-home saliva sample collection

On the evening prior to their laboratory visit, the participant was instructed to remain in dim light and take an hourly saliva sample from 7 hours before until 1 hour after their regular bedtime (as reported at their screening visit). Participants were provided with a night light and a low wattage lamp, and were given a pair of dark goggles in case they unexpectedly

needed to enter an area of their home where the lighting was not dim. They were instructed not to eat or drink anything or to brush their teeth within 20 minutes of taking a sample, to document each sample time on a sample log, and to store the samples in their freezer until they returned for their laboratory visit the next day.

The average bedtime from the at-home monitoring period was determined from the voicemail call-in times, and included all available nights except the at-home sampling night.

Laboratory saliva sample collection

The participant was instructed to arrive at the laboratory 9 hours before their usual bedtime. The participant then entered the light-controlled study room (<20 lux in any direction of gaze) where hourly saliva samples were taken beginning 8 hours before and ending 1 hour after their average bedtime from the week of monitoring. Posture was not controlled and participants could move about their study room, take brief naps between samples, and watch videos, television or use a computer or hand-held electronic device if it emitted <20 lux of light. The participant was provided with a dinner and snack, but not within 20 minutes of a scheduled saliva sample. Saliva samples were kept on ice for up to 1 hour before being frozen. After the final saliva sample was collected, the study was complete.

Saliva samples were assayed for melatonin by SolidPhase, Inc. (Portland, ME) using the Bühlmann Direct Saliva Melatonin RIA kit (ALPCO Diagnostics, Windham, NH), which has an analytical sensitivity of 0.2 pg/mL. Time of the Dim Light Melatonin Onset (DLMO) was determined by linear interpolation between adjacent saliva samples using both a fixed and a relative threshold for both sampling nights. The fixed threshold was 3 pg/mL, and the relative threshold was the value at which the level exceeded 2 standard deviations of the average of 3 baseline values⁶.

Comparisons between at-home and in-lab DLMO were done using a Pearson Correlation, and were performed separately for the 2 DLMO assessment methods.

Results

Thirty-four participants were initially enrolled in the study, six were excluded at the screening visit, and four withdrew consent between the screening visit and laboratory visit. Twenty-four participants completed the study. There were 15 participants in the “trouble falling asleep/trouble waking” group, ranging in age from 21 to 31 years. The “falling asleep too early/waking too early” group consisted of nine participants ranging in age from 50 to 72 years.

Bedtimes were significantly earlier in the older group ($p < 0.01$; see Table 1), as was one of the four DLMO measures (in-lab relative DLMO, $p = 0.06$; see Table 1). No other outcome differed between the two groups, so data from all 24 participants were combined for subsequent analyses.

For two participants, we were unable to determine an at-home DLMO using either method. One participant was a ‘low secretor’, while the other participant appeared to have mixed up the sampling tubes on the at-home night, as DLMO was detected on the in-lab night. Of the remaining 22 participants, we were unable to determine an at-home DLMO using the absolute threshold in four because melatonin levels on the at-home night did not rise above 3pg/mL. It was possible to determine a DLMO using the relative threshold in 21 of those remaining 22 cases, as their melatonin levels, while remaining below 3pg/mL, did show a rise above the baseline values. However, there was evidence from the in-lab assessments

that in four participants the at-home melatonin levels might have been suppressed (see below).

For the in-lab DLMO assessment, we were unable to determine a DLMO using the absolute threshold in three participants (three of the same participants in whom the at-home absolute threshold method failed, whose melatonin levels never rose above 3pg/mL), but could determine DLMO using the relative threshold for two of those three. Thus, we could determine an in-lab DLMO in 87.5% of participants using the absolute threshold method, and in 95.8% using the relative threshold method.

When we compared the at-home and in-lab DLMO timing, the difference averaged 37 ± 19 minutes using the absolute threshold method ($n=18$, range 4 minutes to 76 minutes), and 54 ± 36 minutes using the relative threshold method ($n=20$, range 3 to 121 minutes). In four participants the in-lab melatonin levels rose much (~60 minutes) earlier and/or rose much higher than on the at-home night, raising the possibility that the in-home levels were suppressed by light and therefore inaccurate; thus, we consider that these four cases reduced the overall success rate of the at-home DLMO assessment to 62.5% (15 of 24) using the absolute threshold and 75% (18 of 24) using the relative threshold. Overall, the at-home and in-lab DLMO measurements were significantly correlated. When assessed using the absolute threshold of 3pg/mL the correlation was 0.85 ($p=0.0001$), while using the relative threshold of 2SD above the baseline mean the correlation was 0.68 ($p=0.0009$).

Discussion

We observed a significant correlation between the at-home and in-lab DLMO assessments, although the strength of the correlation depended on the method used for determining DLMO. Thus, we see this study as an initial step in the process of further developing a practical method for evaluating circadian rhythm timing in patients with circadian rhythm sleep disorders (CRSD).

The average difference in DLMO between the at-home and in-lab assessments was greater for the relative threshold method than for the absolute method, and using the relative threshold measurement the range was four hours (from two hours earlier to two hours later). The relative threshold method, while allowing for a DLMO to be determined for individuals who are low melatonin secretors, produces greater variability and can be difficult to determine if an insufficient number of baseline samples are collected. Therefore, in developing and refining a method for determining DLMO outside the laboratory/clinic setting, a fixed threshold for determining DLMO may be more robust.

While some of the at-home vs. in-lab DLMO difference may have been due to assay variability or true biological variability⁷, we suspect that much of this was due to differences in behavior or environment between the two assessments. The at-home sampling protocol required participants to remain awake ~1h later than usual, and staying up late on the at-home night may have in turn led to the in-lab DLMO being later⁸. However, this cannot explain why the in-lab DLMO was earlier for some participants, and for four participants the in-lab melatonin levels rose an hour or more earlier and/or much higher than the at-home levels did. This may have been due to suppression of melatonin by light on the at-home night, or to differences in activity levels or posture between the two nights⁹, although we cannot confirm this. Thus, in addition to the two individuals whose at-home DLMO were unsuccessful (one “low secretor” and one who appeared to mix up the tube order), we consider the at-home DLMO assessments in those four additional participants to also be unsuccessful.

While our findings indicate that an at-home DLMO assessment will not work in every patient, our success rate is similar to that reported recently by a sleep clinic in the Netherlands, who were able to determine DLMO in 76% of nearly 2,000 patients with suspected delayed sleep phase disorder who were assessed in 2008³.

While this preliminary study indicates that this method may be clinically useful, further development and refinement will be required. The laboratory validation of the at-home method should be tested on a larger group of patients, on patients who meet all diagnostic criteria for CRSD, and during all seasons, and posture and light exposure data should be collected to determine whether at-home vs. in-lab differences can be attributed to such factors. Those studies should include older adults, who are more likely to be low melatonin secretors, in order to determine whether an absolute threshold measure is feasible in that population. Data from non-CRSD individuals should also be collected using the same methods as a comparison group. In addition, data on the night-to-night variability of DLMO (and DLMO-to-bedtime interval) should be compiled to determine whether patients have abnormal absolute or relative circadian rhythm timing^{7,8}.

These preliminary findings indicate that an at-home saliva sampling protocol could be further developed as a viable means of assessing circadian phase in patients with CRSDs as well as in patients with sleep onset insomnia who sleep at conventional times. Information about circadian phase, in addition to sleep timing, could aid in the diagnosis and appropriate treatment of such patients.

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Figure 1.

Timing of dim light melatonin onset (DLMO) on the at-home vs. the in-laboratory night. Open symbols: young participants with a complaint of difficulty falling asleep and/or difficulty waking; filled symbols: older participants with a complaint of difficulty falling asleep too early and/or waking too early; dashed line: line of unity. Upper panel: DLMO was determined using a relative threshold, the value at which the level exceeded 2 standard deviations of the average of 3 baseline values. The two measures were highly correlated ($r=0.68$, $n=20$, $p<0.001$). Lower panel: DLMO was determined using a fixed threshold of 3 pg/mL. The two measures were highly correlated ($r=0.85$, $n=18$, $p<0.0001$).

Table 1

Demographic information and results from the overall group of participants, as well as from the two sub-groups (young adults with a complaint of difficulty falling asleep and/or difficulty waking, and older adults with a complaint of difficulty falling asleep too early and/or waking too early).

Overall Group	N	Mean	Standard Deviation
Age	24	38.13	16.71
Male/Female	8/16		
PSQI	24	9.00	2.69
ESS	24	7.75	5.10
MEQ	23	48.78	9.69
Average bed time	24	23:39	1:18
Variability of bed time	24	0:45	0:20
Home DLMO 3pg/mL	18	21:48	1:21
Home DLMO 2SD	21	21:00	1:29
Lab DLMO 3pg/mL	21	22:01	1:19
Lab DLMO 2SD	22	21:12	1:17

Trouble Falling Asleep / Trouble Waking Up Group	N	Mean	Standard Deviation
Age	15	26.07	3.03
Male/Female	3/12		
PSQI	15	8.73	3.03
ESS	15	6.60	4.52
MEQ	14	43.50	8.10
Average bed time	15	0:15	1:05
Variability of bed time	15	0:45	0:20
Home DLMO 3pg/mL	13	22:03	1:24
Home DLMO 2SD	13	21:20	1:31
Lab DLMO 3pg/mL	15	22:16	1:17
Lab DLMO 2SD	15	21:33	1:16

Falling Asleep Too Early / Waking Too Early Group	N	Mean	Standard Deviation
Age	9	58.22	7.71
Male/Female	5/4		
PSQI	9	9.44	2.07
ESS	9	9.67	5.70
MEQ	9	57.00	5.19
Average bed time	9	22:39	1:00
Variability of bed time	9	0:42	0:21
Home DLMO 3pg/mL	5	21:07	1:05
Home DLMO 2SD	6	20:27	1:19
Lab DLMO 3pg/mL	6	21:24	1:18
Lab DLMO 2SD	7	20:27	1:00

PSQI=Pittsburgh Sleep Quality Index score; ESS=Epworth Sleepiness Scale score; MEQ=Morningness-Eveningness Questionnaire score; DLMO=dim light melatonin onset; DLMO 3pg/mL=DLMO determined by the time at which melatonin levels exceeded 3pg/mL; DLMO 2SD=DLMO determined by the time at which melatonin levels exceeded 2 standard deviations of the average of 3 baseline values.