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ORIGINAL ARTICLE

A modified at-home methodology for measuring dim light melatonin onset timing in healthy adults

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ABSTRACT

The goal of this study was to investigate whether a modified at-home dim light melatonin onset (DLMO) assessment would be comparable to the in-laboratory DLMO assessment. Fifty-five participants underwent the at-home DLMO, while 55 age- and sex-matched participants underwent the in-laboratory DLMO assessment. Each participant underwent 14 d of actigraphy monitoring with sleep diary, one overnight polysomnography (PSG), followed by another night for DLMO assessment, either in the laboratory or at home. The at-home DLMO and the in-laboratory DLMO were correlated to chronotype and habitual sleep wake time. DLMOs, compliance with the sampling time and dim lighting were compared between the two groups. The DLMOs were similar between at-home and in-laboratory assessments (Absolute threshold: 22:14 h at home and 22:30 h at in-laboratory, $p = 0.18$; Relative threshold: 21:42 h at home and 22:19 h at in-laboratory, $p = 0.17$). The earlier at-home DLMO and earlier in-laboratory DLMO were both moderately correlated to earlier chronotype and earlier sleep wake time. The compliance with the scheduled sampling time was slightly lower than that in the laboratory, while the compliance with dim lighting was comparable between the at-home group and the in-laboratory group. Our modified at-home assessment of DLMO is a feasible and valid alternative to the in-laboratory assessment.

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Introduction

Dim light melatonin onset (DLMO) assessment is currently the most reliable method to determine the endogenous circadian phase (Benloucif et al. 2008). It is extensively used in research to estimate individuals' circadian timing, which could be in aid of the diagnosis of circadian phase disorder, and to optimize the benefits of chronotherapies (e.g. bright light therapy) (Benloucif et al. 2008). Typically, the secreted melatonin progressively increases 2–3 h before habitual sleep time under a dim light environment (<20 lux), which allows serial sampling starting from a 6-h time window before sleep in the standard DLMO testing protocol (Pandi-Perumal et al. 2007). Salivery sampling is the most practical method because of its non-invasiveness and convenience for frequent sampling, as compared to plasma and urinary sampling (Benloucif et al. 2008). While DLMO can be measured accurately in the laboratory setting with strict control over dim lighting and sampling time, the implementation of in-laboratory DLMO assessment is labour intensive and costly (Molina and Burgess 2011). In addition, some participants/patients are reluctant to undergo such testing in the in-laboratory setting. For example, it typically requires participants/patients' extensive commitment and may disrupt one's daily routines due to

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its requirement for participants/patients to arrive at the laboratory 6–8 h before habitual bedtime. Besides, participants/patients are required to remain in a laboratory with a dim light environment throughout the night (Pandi-Perumal et al. 2007), which may further induce additional psychological burden, such as discomfort or anxiety associated with being in an unfamiliar, dark room (Burgess et al. 2015). Moreover, measuring DLMO at home could be cost-effective since it eliminates the need for laboratory facilities, thereby reducing time and financial burden on both patients and clinic sites (Murray et al. 2024). Thus, further evidence in supporting a valid protocol for measuring circadian phase at home environment could provide advantages for the convenience of participants, potentially cost-saving and help to promote ambulatory medicine in a scalable manner.

Given these limitations, several studies have attempted to validate the at-home DLMO tests (Burgess et al. 2015, 2016). However, these studies have reported varying levels of reliability and validity for at-home DLMO assessments (Burgess et al. 2015, 2016; Pullman et al. 2012; Vlasac et al. 2023). For example, an earlier study reported that the DLMO measured at home was 37 min in average later than that in the laboratory within individuals when dim light and sampling time compliance were not ensured (Pullman et al. 2012). To address the compliance issues, some studies tried to improve the procedures by monitoring dim light and sampling time through objective measures during the test (Burgess et al. 2015, 2016). By implementing these measures, they reported a satisfactory agreement between at-home DLMO and in-laboratory DLMO in healthy adults and patients with delayed sleep phase disorder (Burgess et al. 2015, 2016). However, there remain some methodological issues as there is a lack of real-time supervision of sampling (Burgess et al. 2015, 2016), and some participants may fail to adhere to the designated sampling time, resulting in a decrease in the overall success rate of home DLMO assessment. Previous studies with the at-home setting could only assess sampling compliance data after participants had completed the at-home test (Burgess et al. 2015, 2016). A previous study additionally included a pre-recorded video to provide instructions on the dim lighting and delivery of reminder messages of the sampling time to participants for at-home DLMO assessment (Vlasac et al. 2023). However, detailed data regarding the reliability and compliance of at-home DLMO assessments were not reported in that study (Vlasac et al. 2023). It remains unclear whether at-home DLMO assessments with such modified procedures would be a feasible and valid alternative to in-laboratory DLMO assessments.

Thus, we aimed to examine whether the DLMO measured by the modified at-home assessment procedures would have comparable circadian outcomes to DLMO measured in the laboratory settings. We modified the at-home assessment of DLMO by complementing on-site monitoring and control of dim light environment in participants' home, instructing participants to self-report sampling time and providing reminder messages to ensure compliance during the at-home DLMO assessment. We investigated the compliance, DLMO timing, and melatonin secretion level of modified at-home DLMO assessments as compared to the in-laboratory DLMO assessments.

Materials and Methods

Participants

Participants recruitment and screening were conducted in the Department of Psychology, University of Hong Kong and Li Chiu Kong Family Sleep Assessment Unit, Department of Psychiatry, the Chinese University of Hong Kong, between 2020 and 2023. The present study was approved by the Joint Chinese University of Hong Kong-New Territories East Cluster Clinical Research Ethics Committee (No. 2020.249) and the Institutional Review Board of the University of Hong Kong (No. EA1909038). The initial screening included a clinical interview to assess psychiatric disorders (The Structural Clinical Interview for DSM-5, SCID-CV) (Shabani et al. 2021) and sleep and circadian disorders (The Diagnostic Interview for Sleep Patterns and Disorders, DISP) (Merikangas et al. 2014). The study recruited healthy adult participants aged between 21 and 45 y who met the following criteria: they were not taking any medication, not on a shift work schedule, and did not have any current or lifetime psychiatric, sleep, or circadian rhythm disorders.

After the screening interview, participants were invited to complete a battery of online questionnaires that measured demographics, lifestyles (caffeine and alcohol consumption), sleep and circadian rhythm features. Subjective circadian preference was measured by the Horne-Östberg Morningness-Eveningness Questionnaire (MEQ) (Cheung et al. 2022). Morning- and evening-chronotypes were defined as MEQ score ≥59 and MEQ score ≤41, respectively, while intermediate chronotype was defined as a score on MEQ between 42 and 58 (Cheung et al. 2022). Subjective sleep quality was measured by the Insomnia Severity Index (ISI). Subclinical insomnia was defined as ISI score ≥10 (Morin et al. 2011).

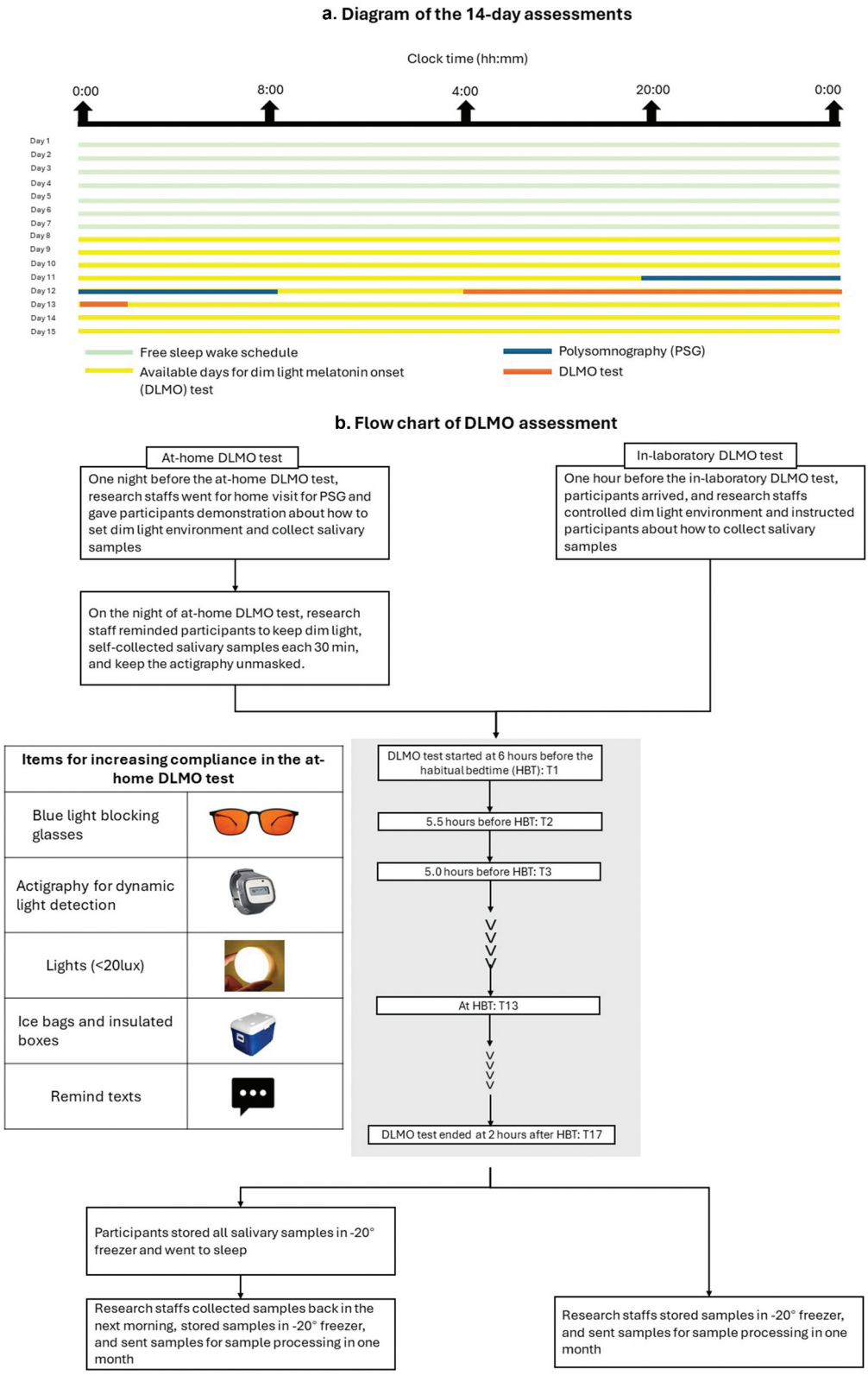

Figure 1. Flowchart of subject recruitment and DLMO methodology.

Protocol

Figure 1a shows the diagram of sleep and circadian rhythm measurements in this study. Participants were instructed to wear the actigraphy on their nondominant wrist for 24 h per day (apart from when bathing or swimming) and press the button to generate the event marker when they were ready to get asleep and upon awakening. They also completed self-reported sleep

wake timing in sleep diary upon awake in the morning (e.g. bedtime, sleep onset time, wake time after sleep onset, sleep offset and rising time) for 14 d. After actigraphy for at least 7 d, participants were scheduled to undergo polysomnography (PSG) and the dim light melatonin onset (DLMO) assessment, either in the laboratory or at home.

Sleep Monitoring: Actigraphy and Polysomnography (PSG)

To examine whether potential differences in sleep architecture (e.g. slow wave sleep) would be a probable confounding factor for the outcome of DLMOs (Ukraintseva et al. 2020; Zisapel 2018), we compared PSG-measured sleep architecture between the at-home and the in-laboratory group (Yoon et al. 2019). We also compared actigraphy-measured sleep wake pattern between both groups, to minimize potential confounding effect of circadian preference on outcomes of DLMO measured at home and in the laboratory. Actigraphy data were recorded in 1-min epoch. The wake threshold was set to medium sensitivity (40 counts per min), and the time of inactivity for estimating sleep onset and offset timing was set to 10 min (Actiware version 6.0, Philips Respironics Inc.). The start and end epochs of major rest intervals were manually scored according to self-report data from sleep diaries (Smith et al. 2018). The actigraphy variables included bedtime, getup time, sleep onset latency, wake time after sleep onset, and sleep efficiency. During the DLMO assessment, actigraphy was also used to monitor light intensity, and all participants were instructed to keep the actigraphy uncovered throughout the DLMO night. To detect indoor dim light, the light sensor was adjusted to detect illuminance levels above 0.1 lux.

The general sleep architecture was measured by PSG in the laboratory (Compumedics Grael PSG system) or ambulatory portable PSG at home (Nox A1 PSG System). Both PSGs recording montage included electroencephalogram (EEG) consisting of F3/M2, F4/M1, C3/M2, C4/M1, O1/M2, and O2/M1 deviation, bilateral electrooculogram (EOG), electrocardiogram (ECG) and electromyogram (EMG) of mentalis muscle and bilateral anterior tibialis muscles. PSG data were visually scored by two well-trained researchers according to the American Academy of Sleep Medicine (AASM) 2017 guidelines (Berry et al. 2020). Inter-rater reliability of scoring ranged between 0.86 and 0.92 in this study.

Protocol of the Dim Light Melatonin Onset (DLMO) Assessment

Each participant's habitual bedtime was calculated based on their sleep diary. The salivary collection period was scheduled to start 6 h before and end 2 h after the average habitual bedtime. All participants were instructed to collect 1–1.8 ml salivary sample every 30 min using the passive drool method into a 2 ml polypropylene vial and remain seated as much as possible in a dark environment (<20 lux). All participants were advised to prepare food and take a bath ahead of time, and must not consume any alcohol, caffeine, or any fruits that might affect the circulation of melatonin (e.g. strawberry, banana) 24 h prior to the assessment. All participants were required to rinse and brush their teeth with water if they had consumed food or drink before salivary sample collections. Figure 1b shows the flowchart of the at-home DLMO and the in-laboratory DLMO assessments.

The at-home DLMO assessment. Our research staff visited participants' home to set up the ambulatory PSG on the night prior to the DLMO assessment. During this visit, participants were provided with detailed instructions and demonstrations of dim lighting. The indoor dim light environment was prepared by closing all blinds and curtains, dimming all indoor lights, and minimizing the light intensity of electronic media (e.g. cell phones, televisions, computers, and iPads) to the lowest level possible for participants' reference. When giving the dim light environment demonstration, light intensity was controlled below 20 lux and measured around the assessment room and at eye level, in the direction of gaze, approximately 30–40 cm from the eyes using the TENMARS TM-201 Light Meter. Participants were instructed to roll up sleeves or wear short sleeve shirts to ensure that the light sensor of the actigraphy would not be covered. Each participant was provided with a home salivary collection kit, which included 17 collection tubes with prepared label informing the corresponding sequence (e.g. "T1" for 6 h before HBT, "T2" for 5.5 h before HBT), a pack of straws, a pair of blue-light blocker glasses, several removable ice packs and an insulation box. Night lights were provided to participants to assist in dimming the room, and blue light blocker glasses were also provided to participants only for exceptional circumstances when participants had to enter a common area where lighting was unavoidable (e.g. washroom).

To ensure sampling time compliance, participants were instructed to send text messages to research staff about the actual sample collecting time immediately after they had collected the salivary samples. Our research staff would also send reminder messages to participants if they did not self-report sample collections within 5 min. After collecting all salivary samples, the participants were

instructed to immediately store them in the freezer (−20°C – −4°C) until researchers collected them back the next day.

The in-laboratory DLMO assessment. The in-laboratory DLMO was performed in the Sleep Research Clinic and Laboratory at the University of Hong Kong. The laboratory is equipped with a controllable light system for each bedroom. Participants were invited to arrive at least 15 min before the DLMO assessment started. When the laboratory assessment started, participants were instructed to remain awake and in the same position as much as possible under dim light (<20 lux, at eye level, in the direction of gaze, 30–40 cm from eyes). During the assessment period, participants were prompted by laboratory staff to give salivary samples every 30 min. The salivary sample collection protocol was the same as the at-home protocol. After collection, all samples were immediately stored in −20°C freezer.

DLMO Preprocessed Data for Analysis

Salivary melatonin samples were analyzed using Liquid Chromatography-Mass Spectrometry (LCMS). The methodology of sample assay used positive electrospray ionization (Waters Acquity Xevo TQ-XS system, Waters Corporation, Milford, MA, USA). The method had a lower limit of quantitation at 4.65 pmol/L (1.08 pg/mL) and a linear analytical range up to 2000 pmol/L (Cheung et al. 2022). The inter-assay coefficients of variation ranged between 4.2% and 5.1%, and the intra-assay coefficients of variation ranged between 3.0% and 4.3% (Cheung et al. 2022).

DLMO was determined by absolute and relative thresholds. When using the absolute threshold, the melatonin onset time was calculated when the melatonin level reached the threshold of 3 pg/mL and kept above the threshold for the next 2 h (Pandi-Perumal et al. 2007). When using the relative threshold, the melatonin onset time was calculated when the melatonin level reached the average of the first three melatonin data points plus two standard deviations of the same three timepoints (Voultsios et al. 1997). Missing melatonin data in the first three timepoints were not included in the relative threshold calculation. To calculate the overall melatonin secretion intensity, the area under the curve (AUC) of melatonin level between 6 h before and 2 h after habitual bedtime was calculated using the trapezoid method (Pandi-Perumal et al. 2007). Samples with the melatonin level lower than 1.08 pg/mL were imputed as 1.08 pg/ml for AUC calculation.

Regarding the compliance in DLMO assessments, we used actigraphy-based light intensity to document dim light compliance, because the actiwatch may capture a more diverse source of ambient light in the room (from multiple sources, e.g. by cell phone/iPad/laptops). Poor dim light compliance was documented by light intensity >50 lux for 1 min, 3 min, and 10 min (Burgess et al. 2015; Chang et al. 2012). The sampling compliance was documented by categorizing those collected samples within 5 min from the scheduled sampling time (Burgess et al. 2015).

Statistical Analysis

To control for potential confounding effect, age and sex were matched, and data from 110 participants were included in statistical analysis. Description of demographic, actigraphy-measured sleep wake timing and circadian parameters, PSG-measured sleep architecture, seasons in DLMO assessment, compliance with dim light and sampling time were compared in both groups by Chi-square test and Mann–Whitney U-test, wherever appropriate. DLMO determined by different thresholds and the melatonin levels were compared with potential confounders (age, sex, chronotype, and seasons) adjusted by the Analysis of Covariance (ANCOVA). The covariate selections were based on previous studies that reported associations of DLMO with age, sex, seasonal variations, and chronotype (Reiter et al. 2021; Zerbini et al. 2021). Correlations of DLMO by different thresholds with other circadian measures (MEQ, habitual bedtime, habitual getup time) were calculated in the at-home and in-laboratory groups by the Spearman correlational analyses. All analyses were performed using R studio software (Version: 2023.09.1 + 494). Statistical significance was determined at $p < 0.05$.

Results

Age- and sex-matched participants from both groups were included in the analysis (1782 valid sample data; $N = 110$; At-home: $n = 55$; Mean age: 30.85 y, range: 23–48 y, 67.3% female; In-laboratory: $n = 55$; Mean age: 29.58 y, range: 24–45 y, 67.3% female).

As shown in Table 1, the at-home group had more morning chronotype (21.8% at home >9.1% in the laboratory, $p < 0.01$) and less evening chronotype (7.3% at home <30.9% in the laboratory, $p < 0.01$) as compared to the in-laboratory group. Sleep quality indicated by the percentage of self-reported subclinical insomnia problem (ISI ≥ 10) was comparable between both groups. Sleep architectures, in particular, the NREM stage 3 sleep that may affect dim light melatonin

Table 1. Demographic and psychological characteristics of the study population.

	At-home n = 55	In-laboratory n = 55	*p* value
Demographic and sleep characteristics			
Age (y)	30.85 (5.76)	29.58 (4.71)	0.21
Sex, female	37 (67.3)	37 (67.3)	1.00
Marital status, married	22 (40.0)	7 (12.7)	0.02
Housing type, house ownership	49 (89.1)	33 (60.0)	<0.01
Employment status, employed	35 (63.6)	42 (76.4)	0.15
Educational level, ≥Bachelor	53 (96.4)	54 (98.0)	0.56
Family income, ≥20,000 hKD/month	36 (65.4)	50 (90.9)	0.04
Frequently drink tea, ≥ 3 times/week	10 (18.2)	16 (29.1)	0.43
Frequently drink coffee, ≥ 3 times/week	20 (36.4)	19 (34.5)	0.95
Chronotype			
Morning	12 (21.8)	5 (9.1)	<0.01
Intermediate	39 (70.9)	33 (60.0)	
Evening	4 (7.3)	17 (30.9)	
Subclinical insomnia (ISI ≥10)	4 (7.3)	8 (14.5)	0.22
Polysomnography-measured sleep architecture			
Total sleep time (h)	7.25 (1.17)	7.18 (1.64)	0.92
Sleep onset latency (min)	8.20 (10.10)	10.85 (10.35)	0.22
Sleep efficiency (%)	92.10 (6.15)	92.15 (6.55)	0.60
Wake time after sleep onset (min)	26.05 (31.78)	27.15 (26.25)	0.70
NREM stage 1 duration (min)	21.75 (12.12)	25.50 (31.13)	0.08
NREM stage 1 percentage (%)	4.95 (3.50)	6.30 (7.98)	0.05
NREM stage 2 duration (min)	233.25 (54.50)	229.25 (59.75)	0.97
NREM stage 2 percentage (%)	52.85 (9.20)	52.55 (27.17)	0.88
NREM stage 3 duration (min)	60.25 (33.62)	61.50 (29.62)	0.35
NREM stage 3 percentage (%)	15.05 (6.85)	14.00 (7.83)	0.38
REM stage duration (min)	110 (27.38)	110.25 (62.13)	0.59
REM stage percentage (%)	26.3 (7.33)	24.75 (13.22)	0.26
Actigraphy-measured sleep wake timing			
Weekly bedtime (hh:mm)	23:58 (1:20)	00:43 (1:07)	0.42
Weekly getup time (hh:mm)	08:07 (1:26)	08:10 (1:17)	0.64
Weekly Sleep onset latency (min)	8.50 (10.47)	5.60 (6.75)	<0.01
Weekly WASO (min)	79.07 (46.81)	44.56 (35.21)	<0.01
Weekly sleep efficiency (%)	79.63 (8.1)	85.97 (11.53)	<0.01
Weekly Time in Bed (h)	7.97 (1.24)	7.68 (0.90)	0.40
Weekly Total Sleep Time (h)	6.30 (1.25)	6.46 (1.22)	0.11

Note: *p* values were calculated by Chi-square test and Mann–Whitney U-tests. Descriptive statistics were shown as median (interquartile range) or n (%) as appropriate. WASO: Wake time after sleep onset. NREM: Non-rapid eye movement sleep. REM: Rapid eye movement sleep. ISI: Insomnia severity index.

onset timing (Mongrain et al. 2006), also did not differ between both groups. Results of the weekly sleep wake pattern, as calculated by actigraphy, showed similar bedtime and getup time between both groups (Bedtime: 23:27 h at home vs 00:21 h in laboratory, $p = 0.42$; Getup time: 8:15 h at home vs 8:08 h in laboratory, $p = 0.64$). However, the at-home group showed longer sleep onset latency, longer wake time after sleep onset and lower sleep efficiency as compared to the in-laboratory group (All $p < 0.01$).

Comparisons of DLMO-related variables between the at-home and the in-laboratory groups are shown in Table 2. Percentages of receiving light >50 lux for more than 1 min and more than 3 min were comparable between the at-home and the in-laboratory groups (>50 lux more than 1 min: 14.3% at home vs 11.5% in the laboratory, $p = 0.68$; >50 lux more than 3 min: 6.1% at home vs 1.9% in the laboratory, $p = 0.28$). None of the participants at home and laboratory settings received light > 50 lux for more than 10 min. Compliance with the scheduled sampling time was higher in the in-laboratory group. About 69.1% in the at-home group collected all salivary samples within 5 min, while a higher percentage, 89.1% in the in-laboratory group collected all samples within 5 min ($p = 0.01$) was observed. Six participants in the in-laboratory group did not start the test on time because of traffic jam and late off-duty hour, and 17 participants in the at-home group delayed collecting sample in 5–10 min of the scheduled time in the middle of the test.

To compare DLMO timing and dim light melatonin levels between both groups, potential confounding factors including age, sex, chronotype and seasons were adjusted in comparisons (Table 2). Results showed that DLMO timing was determined as 22:14 h at home, and 22:30 h in laboratory, when using the absolute threshold ($p = 0.18$). When using the relative threshold, DLMO timing was determined as 21:42 h at home and 22:19 h in the laboratory ($p = 0.17$). The dim light melatonin level at each timepoint and the accumulated dim light melatonin level during the whole assessment period did not differ between the two groups (All $p \geq 0.05$).

Table 2. Comparisons of dim light melatonin between the in-laboratory group and the at-home group.

	At-home n = 55	In-laboratory n = 55	P value
Compliance to dim light environment, n (%)			
>50 lux more than 1 min	7 (14.3)	6 (11.5)	0.68
>50 lux more than 3 min	3 (6.1)	1 (1.9)	0.28
Compliance to sample collection, n (%)			
All samples collected within 5 min of the scheduled time	38 (69.1)	49 (89.1)	0.01
With missing samples	21 (38.2)	15 (27.3)	0.22
Seasons, n (%)			
Spring	15 (27.3)	8 (14.5)	0.17
Summer	10 (18.2)	19 (34.5)	
Autumn	20 (36.4)	18 (32.7)	
Winter	10 (18.2)	10 (18.2)	
Dim light melatonin onset (DLMO, hh:mm)†, mean (standard error)			
DLMO (Absolute threshold)	22:14 (0:08)	22:30 (0:08)	0.18
DLMO (Relative threshold) (Missing: *n* = 13)	21:42 (1:24)	22:19 (1:08)	0.17
Dim light melatonin level (pg/ml), mean (standard error) †			
6.0 h before HBT	1.13 (0.02)	1.07 (0.02)	0.05
5.5 h before HBT	1.69 (0.53)	1.55 (0.53)	0.85
5.0 h before HBT	1.37 (0.31)	1.55 (0.30)	0.68
4.5 h before HBT	1.17 (0.05)	1.14 (0.05)	0.76
4.0 h before HBT	1.35 (0.12)	1.22 (0.12)	0.45
3.5 h before HBT	1.99 (0.32)	1.48 (0.32)	0.28
3.0 h before HBT	2.77 (0.43)	2.17 (0.43)	0.34
2.5 h before HBT	4.10 (0.70)	4.22 (0.70)	0.90
2.0 h before HBT	6.74 (0.96)	7.51 (0.96)	0.58
1.5 h before HBT	9.88 (1.23)	11.28 (1.23)	0.43
1.0 h before HBT	12.61 (1.44)	14.61 (1.43)	0.34
0.5 h before HBT	16.83 (1.75)	17.87 (1.71)	0.68
At HBT	18.93 (1.90)	20.73 (1.87)	0.51
0.5 h after HBT	24.65 (2.50)	22.80 (2.50)	0.61
1.0 h after HBT	24.63 (2.65)	25.55 (2.54)	0.81
1.5 h after HBT	28.02 (3.26)	26.90 (2.84)	0.80
2.0 h after HBT	31.78 (3.67)	28.44 (3.13)	0.50
Accumulated melatonin level (AUC, pg*h/ml, mean (standard error) †			
AUC 5.5 h before HBT	0.71 (0.10)	0.52 (0.11)	0.23
AUC 5.0 h before HBT	1.51 (0.28)	1.18 (0.29)	0.43
AUC 4.5 h before HBT	2.08 (2.92)	1.95 (1.46)	0.59
AUC 4.0 h before HBT	2.80 (0.37)	2.48 (0.38)	0.56
AUC 3.5 h before HBT	3.64 (0.40)	3.16 (0.42)	0.42
AUC 3.0 h before HBT	4.89 (0.53)	4.06 (0.55)	0.29
AUC 2.5 h before HBT	6.69 (0.80)	5.66 (0.83)	0.38
AUC 2.0 h before HBT	9.52 (0.18)	8.50 (1.23)	0.56
AUC 1.5 h before HBT	13.70 (1.69)	13.21 (1.75)	0.85
AUC 1.0 h before HBT	19.23 (2.29)	19.30 (2.37)	0.98
AUC 0.5 h before HBT	27.70 (3.03)	26.92 (3.10)	0.86
AUC at HBT	37.68 (3.84)	36.28 (3.88)	0.80
AUC 0.5 h after HBT	49.88 (4.78)	45.41 (4.89)	0.53
AUC 1.0 h after HBT	63.30 (6.12)	57.13 (5.97)	0.48
AUC 1.5 h after HBT	75.49 (8.11)	69.05 (7.24)	0.56
AUC 2.0 h after HBT	92.52 (10.07)	83.03 (9.08)	0.50

Note: †: *p* values were adjusted for age, sex, chronotype (MEQ score), and seasons. DLMO: Dim light Melatonin Onset. HBT: Habitual bedtime. AUC: Area under the curve. Data are presented as n (%), or mean (standard error) whenever appropriate.

Figure 2 shows the correlations between DLMO and the other circadian measures in the at-home and the in-laboratory groups. Both the at-home and the in-laboratory DLMOs were correlated to MEQ, habitual bedtime, and habitual getup time (At-home: DLMO using absolute threshold: with MEQ: $\rho = -0.34$, $p = 0.01$; with habitual bedtime: $\rho = 0.46$, $p < 0.001$; with habitual getup time: $\rho = 0.47$, $p < 0.001$; DLMO using relative threshold: with MEQ: $\rho = -0.38$, $p = 0.008$; with habitual bedtime: $\rho = 0.60$, $p < 0.001$; with habitual getup time: $\rho = 0.44$, $p < 0.001$; In-laboratory: DLMO using absolute threshold: with MEQ: $\rho = -0.37$, $p = 0.005$; with habitual bedtime: $\rho = 0.63$, $p < 0.001$; with habitual getup time: $\rho = 0.66$, $p < 0.001$; DLMO using relative threshold: with MEQ: $\rho = -0.66$, $p = 0.013$; with habitual bedtime: $\rho = 0.62$, $p < 0.001$; with habitual getup time: $\rho = 0.63$, $p < 0.001$). Figure 3 shows that the dim light melatonin level at each timepoint and the accumulated dim light melatonin level were comparable between the two groups.

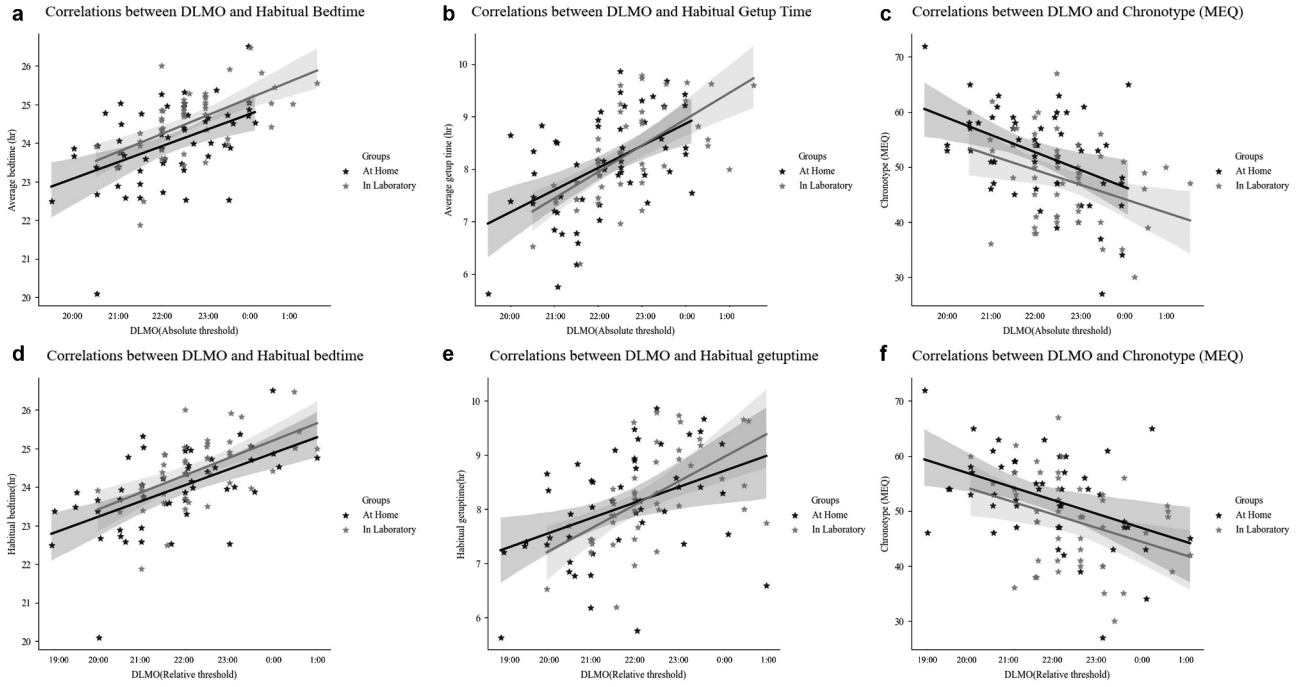

Figure 2. Correlations of DLMOs with chronotype and habitual sleep wake time.

Discussion

In this study, we applied the modified procedures to measure at-home DLMO using a protocol similar to that in the laboratory settings. Based on actigraphy light monitoring and salivary sample collection protocols reported by earlier studies (Burgess et al. 2015, 2016), we further modified the at-home DLMO protocol by incorporating time reminder messages and providing an in-person demonstration of dim lighting environment. It is anticipated that these additional measures could further enhance timely sampling and dim lighting compliance. The DLMOs measured at home and in the laboratory were both correlated to earlier chronotype and earlier sleep wake time. The overall compliance with dim light was comparable between the at-home and in-laboratory groups. The compliance with the scheduled sampling time was 69.1% in the at-home group. Albeit it was reasonably satisfactory in the home assessment, it was 20% lower than the in-laboratory group. In brief, the results of this study support a feasible and valid home DLMO assessment with good compliance in dim light setting and sampling schedule.

Overall, we determined the absolute threshold of DLMO in all 8 h melatonin profiles, possibly attributed to the time window ending at 2 h after habitual bedtime that captured all the rise of dim light melatonin (Crowley et al. 2016). However, 11.8% (13 out of 110) of DLMO by relative threshold could not be calculated due to the missing samples in the first three timepoints. We also observed a high variation of DLMO using the relative threshold, which may result in spurious estimates in the circadian phase (Crowley et al. 2016). Taken together, the 3 pg/mL absolute threshold may be preferred when calculating DLMO from a melatonin profile with an 8-h sampling time window and a 30-min sampling rate.

The correlations of the at-home DLMOs with chronotype and sleep wake timing were comparable to those in the in-laboratory group, suggesting that DLMO measured by our home assessment methods is feasible to determine a timing that reflect circadian phase of an individual. Several approaches in our modified home DLMO assessment are highlighted. First, the remote supervision of sampling time through participants' self-reported text messages, and the timely text reminders may effectively address compliance to the scheduled sampling time. Thus, 69.1% of participants reported sampling time within 5 min of the scheduled time in the at-home group, which was reasonably high compared with the reported 56% in previous home study (Burgess et al. 2015). Nonetheless, the percentage of compliance with the scheduled sampling time at home was still significantly lower compared to the in-laboratory, suggesting that the methods used to ensure compliance with the sampling protocol may need further improvement. Second, the approach of providing in-person demonstrations of constructing dim light

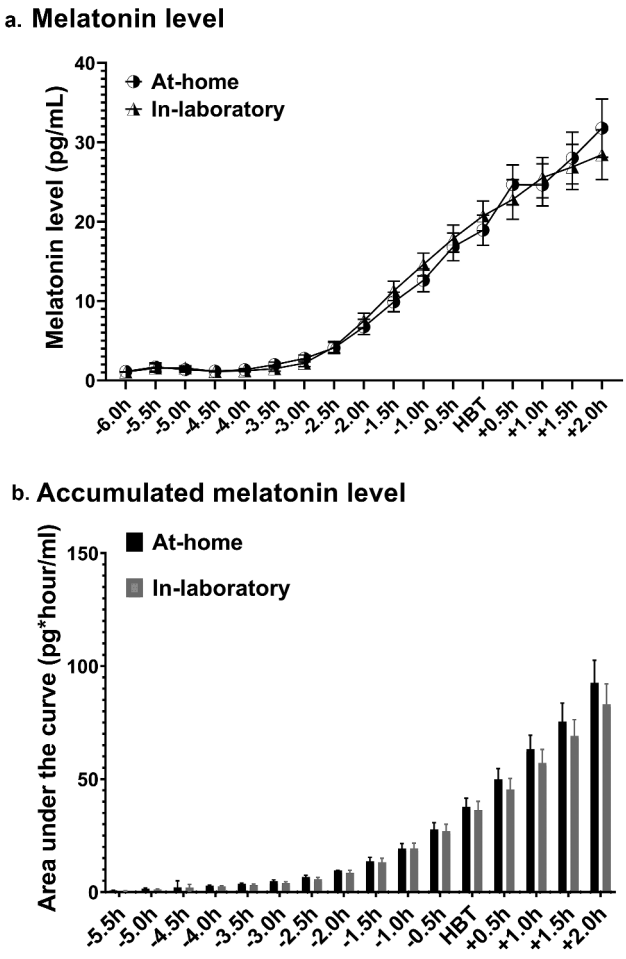

Figure 3. Melatonin level between the at-home and the in-laboratory groups.

environment at home may further enhance compliance with the dim lighting requirements, especially when combined with actigraphy monitoring of light intensity (Burgess et al. 2015, 2016). We observed that 14.3% in the at-home group performed poor compliance with dim light (>50 lux for more than 1 min) at the home assessment, while 11.5% in the laboratory group showed similar poor dim light compliance. It is likely that the repeated prompts of sampling time in the at-home group might have ensured an optimal dim light control. Together, our study provides evidence that incorporating an on-site monitoring and control of dim light environment, repetitive text messaging and reminders of sampling time, and additionally providing blue light blockers might enhance compliance with home DLMO assessments. It is advisable for future studies to consider integrating these approaches into the standardized home circadian phase assessments.

This study had notable strengths, such as rigorous procedures for home DLMO assessments. The enhanced procedures reduced discrepancy of DLMO measured at home as compared to that in the laboratory. While the successful rate of capturing DLMOs and compliance were generally high, there were certain limitations to consider. First, a major limitation of this study was related to employment of two different samples between the at-home and in-laboratory groups. Nonetheless, the subjects from both groups were age and sex matched and had comparable demographic features and sleep wake timing. The second limitation pertains to the fact that all our participants were in good health. The absence of any clinical sleep disorder left it unclear as to whether the same home procedures for measuring DLMOs would yield similar results in clinical populations. The third limitation was that our participants were young and highly educated, enabling themselves to comprehend the details of home DLMO procedures well. To address these three limitations, future studies should validate our modified home procedures in a more diverse population including clinical and patients at both younger and older spectrum. Fourth, there was a lack of consideration on the individual light sensitivity, a factor that may also confound consistency in dim light melatonin onset between the at-home group and the in-laboratory group (Stone et al. 2020). Fifth, although the procedure of remote supervision by research staff largely improved compliance with sampling time at home, it remains a laborious task for staff to remotely monitor the entire assessment period. Future studies that aim at conducting large-scale studies for measuring circadian rhythm-related problems could consider automatic reminders during home DLMO assessments. For instance, the development of an AI-chatbot could assist participants in calculating habitual bedtime (Singh et al. 2023), generating individualized DLMO sampling time windows, and sending auto-reminder messages throughout the assessment period. Lastly, the at-home procedures may be further applied and studied at younger adolescents and older subjects as the circadian disturbances are increasingly found across age and clinical disorders, for example, circadian disturbances in high-risk offspring of parents with bipolar affective disorder (Feng et al. 2022; Lei et al. 2024), and circadian disturbances in neurodegenerative disorders (Feng et al. 2020).

Conclusions

These findings suggest that our modified procedure for at-home assessments has comparable feasibility and results to in-laboratory measurements when determining dim light melatonin onset in healthy young adults. The at-home DLMO procedures may facilitate easy access and wider applicability of large-scale ambulatory

assessment of circadian phase for both research and clinical use in general population.

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Disclosure Statement

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Author Contributions

Conceptualization: C.X.C., R.W., F.T.W.C., N.Y.C., J.W.Y. C., W.K.H., S.Y.Y., Y.L., and Y.K.W.; Methodology: C.X. C., R.W., F.T.W.C., N.Y.C. and J.W.Y.C.; Writing-original draft: C.X.C.; Writing-review and editing: C.X.C., F.T.W. C., N.Y.C., J.W.Y.C., C.S.H., S.X.L., T.M.C.L, and Y.K.W.; Melatonin assays: A.W.Y.H and C.S.H; Data curation: C. X.C. and R.W. All authors have read and agreed to the published version of the manuscript.

Data Availability Statement

The data presented in this study are available from the corresponding author upon reasonable request.

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