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# **External validity of the Japanese version of the reduced morningness-eveningness questionnaire (rMEQ) score using dim light melatonin onset and sleep-wake behavior in young adults.**

Taisuke Eto<sup>a,b</sup>, Yuki Nishimura<sup>c</sup>, Hiroki Ikeda<sup>c</sup>, Taiki Kunieda<sup>d</sup>, Minori Enomoto<sup>d</sup>, Tomohide Kubo<sup>c</sup>, Ana Adan<sup>e,f</sup>, and Shingo Kitamura<sup>b</sup>

<sup>a</sup>Research Fellow of the Japan Society for the Promotion of Science, Kodaira, Japan

<sup>b</sup>Department of Sleep-Wake Disorders, National Institute of Mental Health, National Center of Neurology and Psychiatry, Kodaira, Japan

<sup>c</sup>National Institute of Occupational Safety and Health, Japan Organization of Occupational Health and Safety, Kawasaki, Japan

<sup>d</sup>Department of Medical Technology, School of Health Sciences, Tokyo University of Technology, Ota-ku, Japan

<sup>e</sup>Department of Clinical Psychology and Psychobiology, University of Barcelona, Barcelona, Spain

<sup>f</sup>Institute of Neurosciences, University of Barcelona, Barcelona, Spain

\*Correspondence: Shingo Kitamura

E-mail address: [s-kita@ncnp.go.jp](mailto:s-kita@ncnp.go.jp)

## **External validity of the Japanese version of the reduced morningness-eveningness questionnaire (rMEQ) score using dim light melatonin onset and sleep-wake behavior in young adults.**

Reduced Morningness-Eveningness Questionnaire (rMEQ), a five-item version of the 19-item MEQ, is a practical tool for assessing circadian typology or “morningness” and “eveningness” preference. Although we previously validated the Japanese version of rMEQ with MEQ, external validation against an established standard was lacking. This study aimed to additionally validate the Japanese rMEQ in young adults by assessing the dim light melatonin onset (DLMO), a marker of circadian phase, and subjective/objective sleep-wake habits. Twenty healthy young adults (mean age:  $23.0 \pm 1.9$  years) participated in this study and were assessed the circadian typology by rMEQ and MEQ. The sleep-wake habits were measured by sleep diary, actigraphy and the Pittsburgh Sleep Quality Index (PSQI). Salivary melatonin samples were collected to determine DLMO. Results showed significant correlations between rMEQ scores and DLMO, as well as sleep variables based on sleep diary, actigraphy and PSQI. Correlations between rMEQ scores and these variables were consistently stronger than those for MEQ. These findings highlight the validity of the Japanese rMEQ and that the rMEQ more accurately reflects circadian typology and internal circadian phases compared to MEQ, at least in Japanese young adults. The Japanese rMEQ could be a valuable tool for efficiently assessing circadian typologies in Japanese young adults.

Keywords: Circadian typology, Morningness, Eveningness, rMEQ, Validation, DLMO, Circadian phase

## Introduction

Circadian typology or morningness-eveningness has been known as the phenotype of the individual biological clock that is expressed in human rest and activity cycles (Adan et al. 2012; Di Milia et al. 2013). For example, morningness individuals tend to spontaneously wake up early in the morning, be active, and go to bed early in the evening, while eveningness individuals wake up and are active later timing compared to morningness and intermediate individuals (Carrier et al. 1997; Taillard et al. 1999). Each circadian typology has potential strengths: Morningness individuals may be suitable for early schedules because they show better athletic performance in the morning time (Vitale and Weydahl 2017); Eveningness individuals might be suited for night work because they show high self-monitoring ability of cognitive performance under sleep deprivation (Nishimura et al. 2025); and intermediate individuals may exhibit flexibility to adapt to variable time demands. Among them, Eveningness individuals tend to accumulate sleep debt on workdays, as their own sleep-wake cycle is likely to separate from the social cycle, which is more aligned with the morningness (Roepke and Duffy 2010; Kitamura et al. 2010). Therefore, several studies have highlighted the eveningness preference as a risk factor for various health problems, such as cardiovascular (Makarem et al. 2020), metabolic disorders (Kianersi et al. 2023), mental health problems (Zou et al. 2022), and mortality (Hublin and Kaprio 2023).

The morningness-eveningness preferences vary with age, and young adults generally demonstrate a strong tendency toward delayed sleep behavior (Roenneberg et al. 2004; Kennaway 2023). Notably, eveningness preference has been linked to an increased risk of cardiometabolic (Li et al. 2023) and mental health problems (Walsh et al. 2022; Wang et al. 2022; Qu et al. 2023), even within this age group. Given that young adults are a predominantly eveningness population, the appropriate and accurate assessment of circadian typology in young adults is crucial to understanding the real situation, establishing proper sleep hygiene, and aiding in the effective prevention and treatment of diseases.

The Morningness-Eveningness Questionnaire (MEQ) is widely used for circadian typology evaluation (Horne and Ostberg 1976). However, the MEQ has 19 response items, which is a heavy burden on subjects and is difficult to handle in field and large-scale surveys.

In 1991, Adan and Almirall proposed a reduced MEQ (rMEQ) with only five items from the MEQ (Adan and Almirall 1991). The rMEQ can evaluate circadian typology in one dimension, as it uses a correspondence analysis for the MEQ to extract only questions related to the morningness-eveningness factor. The rMEQ has been translated into many languages, and we developed the Japanese version of the rMEQ and evaluated psychometric properties, validity and reliability (Eto et al. 2024). However, in our previous study, we did not include external validation against sleep-wake habits that was carried out in the study of the Italian version for young adults (Natale et al. 2006a; 2006b) and for children and adolescents (Tonetti et al. 2024), or in the study of the German version for adolescents (Paciello et al. 2022). In addition, given that the rMEQ is an effective tool to assess the phenotype of the circadian clock, it is crucial to determine whether it reflects an individual's endogenous circadian phase, but this has not been verified in any language version.

In this study, we aimed to evaluate the convergent validity of the Japanese version of rMEQ in young adults using sleep-wake behavior and dim light melatonin onset (DLMO), a well-established marker of the circadian rhythm phase (Benloucif et al. 2008). Additionally, we compared the correlations between the rMEQ and MEQ with the external parameters.

## **Methods**

### ***Participants***

Twenty healthy young adults (mean age  $\pm$  standard deviation (SD):  $23.0 \pm 1.9$  years old, age range: 21–28 years, six females and fourteen males) participated in this study. We adhere to the definition of young adult in accordance with the US Census Bureau definition as ages 18 to 31 (Vespa 2017). Participants were recruited using email-distributed flyers, personal referrals (word of mouth), and through a professional recruitment agency specializing in research volunteers (Souken, Tokyo, Japan). The inclusion criteria were that participants were between the ages of 18 and 30 at the time consent was obtained. The exclusion criteria were participants who 1) have a history of pre-existing medical conditions; cardiovascular, liver, endocrine, brain/neurological, psychiatric disorders, or taking therapeutic drugs for those disorders, 2)

have engaged in travel across time zones or shift work at least 6 months prior to the experiment, 3) are dependent on alcohol or have excessive drinking and smoking habit. Applicability to the exclusion criteria was confirmed by the participant's self-report. An oral and paper-based explanation of the study was provided to all participants before the experiment. All participants provided written informed consent to participate in this study, which was approved by the Ethical Committee of the National Center of Neurology and Psychiatry (NCNP), Japan. This study was conducted in accordance with the principles of the Declaration of Helsinki.

### ***Measurements***

- *Morningness-Eveningness questionnaire*

Participants were asked to respond to a questionnaire that included the Japanese version of the MEQ (Horne and Ostberg 1976; Ishihara et al. 1984) and rMEQ (Adan and Almirall 1991; Eto et al. 2024) and demographic information such as gender and age. The MEQ comprises 19 questions related to circadian typologies, where higher total scores signify morning preference and lower scores indicate evening preference. The total MEQ score ranges 16–86. The validity and reliability of the Japanese version of MEQ have been confirmed with significant correlations between MEQ score and sleep variables, such as bedtime, and 0.815 of Chronbach's  $\alpha$ , respectively (Inomata et al. 2014). The rMEQ, which is a reduced scale of MEQ, was composed of five items: questions 1, 7, 10, 18, and 19—from the MEQ (Adan and Almirall 1991). The total rMEQ score ranges 4–25. It has been shown that the Japanese version of rMEQ score significantly correlated with MEQ score ( $\rho = 0.88$ ) and achieved the 0.618 of Chronbach  $\alpha$  (Eto et al. 2024).

- *Sleep-wake habits*

Sleep-wake habits were monitored by a subjective sleep diary and actigraphy for a week. The participants were instructed to record light-off time, bedtime, rise time, and frequency and total duration of wake after sleep onset every morning during the sleep-wake habits monitoring. For each question for assessment of the light-off time, bedtime, and rise time were “What time did you turn the light off?”, “What time did you fall asleep?” and “What time did you get up and get out of bed?”, respectively. Mid-sleep point in the sleep diary was defined as the mid-point

time between bedtime and rise time. For the sleep-wake habits monitoring, the participants wore either a wrist-worn actigraph FS-760 (ACOS Co., Ltd., Nagano, Japan) (Nakazaki et al. 2014) or a wrist-worn actigraph MotionWatch 8 (CamNtech Ltd., Cambridgeshire, UK) (Elbaz et al. 2012). 17 participants wore a FS-760 and 3 participants wore a MotionWatch 8. Sleep variables: sleep onset time, waketime and mid-sleep point, which is a mid-point time between sleep onset time and waketime, were extracted from the activity data obtained from each actigraph using dedicated software (FS-760 data: SleepSign Act, MotionWatch8 data: MotionWare Software). In addition, participants were asked to respond to the Japanese version of the Pittsburgh Sleep Quality Index (PSQI) (Buysse et al. 1989; Doi et al. 2000). We also extracted the participant's sleep-wake habits from PSQI over the previous month: bedtime, sleep onset time, waketime and mid-sleep point, which is a mid-point time between bedtime and waketime. The validity and reliability of the Japanese version of PSQI have been confirmed in a previous study (Doi et al. 2000).

- *Salivary melatonin onset*

The internal circadian phase was determined by salivary melatonin secretion onset time in the evening, which is known as dim light melatonin onset: DLMO (Lewy and Sack 1989; Benloucif et al. 2008). The DLMO assessments were performed in an experimental laboratory. The illuminance of the lab was set to dim ( $< 2$  lx) in the angle gaze when participants were seated and looking straight ahead. Saliva samples were collected to determine melatonin concentration every hour using the Salivette saliva collection device with a plain cotton plug (Salivette, Sarstedt AG & CO. KG, Nümbrecht, Germany) from 4 hours before to an hour after each participant's habitual bedtime. Participants were instructed to sit and rest in a chair until finishing the saliva sample collection procedure and not to drink anything 15 minutes before each saliva sampling. The Salivette with saliva-absorbed cotton plug was centrifuged for 5 min at 1000 g. Extracted saliva was decanted into a plastic tube and stored at  $-30^{\circ}\text{C}$  until assayed. Salivary melatonin concentrations were quantified by using a radioimmunoassay kit (RK-DSM2-U, Novolytix GmbH, Witterswil, Switzerland). The time of DLMO was determined by linear interpolation between two time points at which melatonin concentration crossed the 3.0 pg/mL threshold (Benloucif et al. 2008). MEQ, rMEQ and PSQI were obtained on the same

day as DLMO measurement for all participants. Although 10 of the 20 participants had their DLMO measured immediately after the monitoring period, the remaining 10 participants, due to scheduling constraints of the experiment, sleep diary and actigraph data were collected several months prior to DLMO measurement.

### ***Data analysis***

MEQ scores were categorized into three types: evening (16–41 points), neither (42–58 points), and morning (59–86 points), following standard criteria (Horne and Ostberg 1976). Similarly, the rMEQ scores were categorized into three types: evening (4–11 points), neither (12–17 points), and morning (18–25 points), in line with the original and our previous work (Adan and Almirall 1991; Eto et al. 2024). The sleep variables obtained by sleep diary and actigraph were to be averaged over a week, but due to participants forgetting to wear the actigraph and problems in conducting the experiment, nine participants were averaged over a five-day period and one participant was averaged over a four-day period in the actigraph data. Correlation between MEQ, rMEQ scores and DLMO or each sleep variable from sleep diary (bedtime, rise time and mid-sleep point), actigraphy (sleep onset time, waketime and mid-sleep point) and PSQI (bedtime, sleep onset time, waketime and mid-sleep point), was evaluated using Spearman's correlation coefficient. Statistical analyses were performed using R 4.2.2 (R Core Team). A  $p < 0.05$  was considered statistically significant in all analyses.

### **Results**

The sleep diary and actigraphy data showed that two participants had extremely short sleep durations and irregular sleep habits during the sleep-wake habits monitoring period; therefore, the following results were obtained with the remaining 18 participants ( $23.0 \pm 1.9$  years old, six females and twelve males) in the analysis basically. However, three participants and a participant were further excluded from the analysis for correlation of DLMO, i.e.,  $n = 15$ , and sleep variables based on sleep diary, i.e.,  $n = 17$ , respectively. This is because there was no melatonin secretion until the end of saliva sampling time in the three participant, and a

participant did not record the sleep diary. Table 1 shows the median values and interquartile range (IQR) of all variables for the remaining participants. The breakdown of the categorized circadian typology of the participants was five evening, twelve neither and a morning type in rMEQ and six evening, ten neither and two morning type in MEQ. Circadian typologies were consistent between rMEQ and MEQ in 14 participants (77.8%), which is in line with our previous study (Eto et al. 2024). However, one evening type in rMEQ was classified as neither type in MEQ, and three neither types in rMEQ were classified as two evening types and one morning type in MEQ. According to the answers to the questionnaires, 10 of 18 participants were engaged in regularly structured activities, such as work and school. The mean data for the sleep diary and actigraphy included data for free days.

Figure 1 shows the relationship between rMEQ / MEQ score and DLMO. The rMEQ score significantly negatively correlates with DLMO ( $\rho = -0.59$ , Fig 1A), indicating that the individual who has a high rMEQ score, i.e., morningness preference, exhibits an earlier internal circadian phase and the individual who has a low rMEQ score, i.e., eveningness preference, exhibits later internal circadian phase, while the MEQ score does not significantly correlate with DLMO ( $\rho = -0.20$ , Fig 1B).

Table 2 shows results for the correlation between rMEQ / MEQ scores and DLMO or sleep variables obtained from sleep diary, actigraphy and PSQI. The bedtime, rise time, and mid-sleep point based on the sleep diary were significantly and negatively correlated with the rMEQ score, and also with the MEQ score. For the actigraphy-based sleep variables, i.e., sleep onset time, waketime and mid-sleep point, all sleep variables were significantly negatively correlated with the rMEQ scores. In contrast, the waketime and mid-sleep point based on the actigraph were significantly and negatively correlated with the MEQ score, while there is no significant correlation between the sleep onset time and MEQ score. All correlation coefficients of rMEQ with sleep variables based on sleep diary and actigraphy were larger than those of MEQ. For the sleep variables based on PSQI, which means habitual sleep-wake behavior the previous month, the bedtime, sleep onset time, wake time and mid-sleep point were significantly negatively correlated with rMEQ score. Similarly, the sleep variables based on PSQI were significantly correlated with MEQ score. All correlation coefficients of rMEQ with

sleep variables based on PSQI were larger than those of MEQ, similar to the results for the sleep variables based on sleep diary and actigraph.

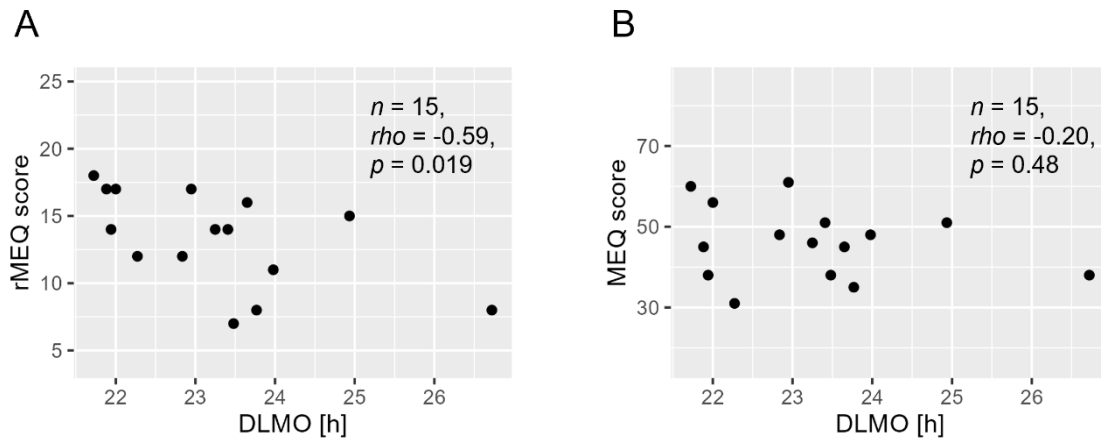


Figure 1. Correlation relationship between DLMO and (A) rMEQ, (B) MEQ score. DLMO: Dim Light Melatonin Onset, MEQ: Morningness-Eveningness Questionnaire, and rMEQ: reduced MEQ.

## Discussion

We demonstrated that the Japanese version of rMEQ score significantly correlated with the endogenous circadian phase, i.e., DLMO, and sleep-wake habit variables based on the sleep diary, actigraphy and PSQI in young adults. In addition, all correlation coefficients of DLMO and each sleep variable with rMEQ score were higher than that with MEQ score. These results highlight that the Japanese version of the rMEQ can accurately assess circadian typology and could be an appropriate tool for assessing that compared with MEQ, at least in Japanese young adults.

The rMEQ score significantly correlated with DLMO, a well-established marker of circadian phase, with a relatively strong correlation coefficient. Conversely, the MEQ score did not show a significant correlation with DLMO. This discrepancy may be attributed that the rMEQ consists of questions specifically related to the morningness-eveningness, as previously described (Adan and Almirall 1991). While some previous studies have reported correlations between MEQ score and endogenous melatonin rhythm (Liu et al. 2000; Martin and Eastman

2002; Kitamura et al. 2014; Kantermann et al. 2015), including one non-significant finding (Kitamura et al. 2014), their reported correlation coefficients (ranging from  $-0.36$  to  $-0.70$ ) are generally weaker than the correlation observed in our study between the rMEQ score and DLMO, with the exception of one study (Kantermann et al. 2015). In addition, a review article reported by Kennaway (Kennaway 2023) indicated that the MEQ scores were not significantly related to DLMO in nearly half (14/29) of the data sets analyzed. This may suggest that the rMEQ may be a more sensitive indicator of the internal circadian phase than the MEQ.

All sleep variables based on sleep diary, actigraphy and PSQI significantly negatively correlated with rMEQ scores consistently with the Italian version of the rMEQ study (Natale et al. 2006b). Natale et al. demonstrated that the score of the Italian version of the rMEQ was significantly negatively correlated with sleep onset time, wake-up time, and the midpoint of sleep, which was measured by actigraphy. Our results suggest the validity of the Japanese version of rMEQ as a tool for assessing sleep-wake cycles exhibited on the basis of an individual circadian typology. We also show that there are significant negative correlations between the MEQ scores and sleep variables, which are consistent with previous studies (Zavada et al. 2005; Inomata et al. 2014). The study by Inomata et al. has reported the correlation between MEQ scores and sleep variables (bedtime, waketime and mid-sleep point) based on actigraphy in the Japanese participants as well as our study, and the correlation coefficients (bedtime:  $r = -0.55$ , waketime:  $r = -0.55$ , mid-sleep point:  $r = -0.59$ ) in the previous study are almost comparable with our correlation coefficient, even though there is a difference between the bedtime or SOT. Interestingly, all correlation coefficients of rMEQ scores and sleep variables except for RT in the sleep diary were greater than that of MEQ scores. This would highlight the high sensitivity of rMEQ to circadian typology, similar to the results of the correlation with DLMO. The only slight difference in correlation coefficient between rMEQ and MEQ in RT based on sleep diary and WT based on actigraphy may be due to the fact that wake-up time tends to be determined by social restriction, such as starting time of work and school, rather than individual circadian typology.

Young adulthood is the life stage when eveningness is most pronounced (Roenneberg et al. 2004; Kennaway 2023). Previous studies on university students have shown that

eveningness is associated with an increased risk of mental health problems (Cheung et al. 2023), e.g., in Canada (Walsh et al. 2022) and China (Wang et al. 2022; Qu et al. 2023). Especially in the Chinese study, evening types had a 3.9 times higher odds for mild depression compared to morning type (Qu et al. 2023). Additionally, young adulthood is also the period when sex differences in circadian typology become most evident, with males exhibiting a stronger tendency toward eveningness (Roenneberg et al. 2004; Fischer et al. 2017). Since our study did not control for sex distribution, the applicability of the rMEQ may be somewhat limited. However, the rMEQ, which is sensitive to detecting the intrinsic circadian phase, could serve as an early screening tool for circadian typology. Early identification and intervention may help prevent sleep and mental health issues.

Although we showed the validity of the Japanese version of rMEQ in young adults, the sample sizes were small, especially in correlation analysis with DLMO, even though the effect sizes, i.e., *rho*-values, showed sufficiently high values and were comparable to previous studies. Additionally, the samples were not representative of the population due to the lack of systematic selection. As this study targeted young adults, further investigation is needed to determine whether the external validity of the Japanese version of the rMEQ is also demonstrated for other age groups. Finally, in half of the participants, there were temporal gaps between the sleep habit assessments by sleep diary/actigraphy and the DLMO measurements. To address this limitation, we conducted an exploratory correlation analysis within this subgroup only. The results showed no significant correlations between sleep indices from the sleep diary/actigraphy and the rMEQ score. Similarly, we found no significant correlation between rMEQ and PSQI-derived sleep indices, even though the PSQI was completed on the same day as the rMEQ. This finding suggests that the interval itself had little, if any, contribution to the lack of correlation, given that both sleep habits (with a several-month interval) and PSQI (with no interval) showed similarly poor correlations with rMEQ scores in this subgroup. Therefore, the existence of this temporal gap is considered to have a limited impact on the overall conclusions of this study. Given that the participants were university students, their sleep habits may have been influenced by external factors such as academic or social obligations, leading to discrepancies between behavioral sleep patterns and their

underlying circadian typology. Nonetheless, the significant association between rMEQ scores and DLMO supports the validity of the rMEQ as a tool for assessing circadian typology.

In summary, we demonstrated the validity of the Japanese version of rMEQ by using the DLMO and subjective/objective sleep-wake habits variables as external references in young adults, and this is the first study showing that the rMEQ may reflect in the internal circadian phase. In addition, our results highlight that the rMEQ seems to have greater sensitivity for the assessment of circadian typology than the MEQ. The Japanese version of rMEQ could be a valuable tool for the convenient and efficient assessment of circadian typologies in the Japanese young adult population.

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### **Declaration of Interest statement**

The authors declare that they have no competing interests.

### **Availability of data and materials**

The datasets analyzed in this study are not publicly available because of the privacy policy but are available from the corresponding author upon reasonable request.

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## Tables

Table 1. Median and interquartile range (IQR) values of all variables.

Variables	<i>n</i>	Median [Q1-Q3]
<b>Age</b>	18	22.5 [22.0-23.0]
<b>MEQ score</b>	18	47.0 [38.0-51.0]
<b>rMEQ score</b>	18	14.0 [11.3-16.0]
<b>DLMO</b>	15	23.2 [22.1-23.7]
<b>Sleep diary</b>	17	
Bedtime		24.9 [24.2-25.1]
Rise time		7.79 [7.43-8.56]
Mid-sleep point		4.30 [3.82-5.17]
<b>Actigraphy</b>	18	
Sleep onset time		25.3 [24.5-25.7]
Waketime		7.88 [7.42-8.58]
Mid-sleep point		4.68 [3.94-5.22]
<b>PSQI</b>	18	
Bedtime		25.0 [24.1-25.0]
Sleep onset time		25.4 [24.7-25.9]
Waketime		8.75 [7.63-9.50]
Mid-sleep point		4.75 [4.00-5.24]

Table 2. Correlation between MEQ, rMEQ and DLMO or sleep variables obtained from sleep diary (bedtime, rise time and mid-sleep point), actigraphy (sleep onset time, waketime and mid-sleep point) and PSQI (bedtime, sleep onset time, waketime and mid-sleep point). MEQ: Morningness-Eveningness Questionnaire, rMEQ: reduced MEQ, DLMO: Dim Light Melatonin Onset, and PSQI: Pittsburgh Sleep Quality Index.

Variables	<i>n</i>	rMEQ		MEQ	
		<i>rho</i> -value	<i>p</i> -value	<i>rho</i> -value	<i>p</i> -value
<b>DLMO</b>	15	-0.595	0.019	-0.196	0.484
<b>Sleep diary</b>	17				
Bedtime		-0.849	< 0.001	-0.682	0.003
Rise time		-0.623	0.008	-0.605	0.010
Mid-sleep point		-0.723	0.001	-0.649	0.005
<b>Actigraphy</b>	18				
Sleep onset time		-0.580	0.012	-0.310	0.210
Waketime		-0.649	0.004	-0.632	0.005
Mid-sleep point		-0.711	<0.001	-0.573	0.013
<b>PSQI</b>	18				
Bedtime		-0.841	< 0.001	-0.744	< 0.001
Sleep onset time		-0.669	0.002	-0.623	0.006
Waketime		-0.585	0.011	-0.508	0.031
Mid-sleep point		-0.795	< 0.001	-0.681	0.002

### **Figure legends**

Figure 1. Correlation relationship between DLMO and (A) rMEQ, (B) MEQ score. DLMO: Dim Light Melatonin Onset, MEQ: Morningness-Eveningness Questionnaire, and rMEQ: reduced MEQ.