Chapter 4
Physiologic Methods of Assessment Relevant to Circadian Rhythm Sleep-Wake Disorders

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Introduction

Various physiologic tools are available to aid in the assessment of circadian rhythms. Some measures are available primarily for use in research protocols, but are increasingly becoming available to sleep clinicians. The measures at our disposal are devised to objectively estimate variables such as circadian phase position, periodicity, and circadian amplitude, which when taken in conjunction with a careful history and physical exam allow for the assessment of essential aspects of circadian biology and behavior by the sleep clinician or researcher.

The physiologic methods that are described hereafter are wrist or body actigraphy, ambulatory light monitoring, melatonin monitoring, ambulatory core body temperature monitoring, polysomnography, multichannel ambulatory monitoring, and circadian gene expression in peripheral cells. Each of these methods has advantages and caveats; the type of circadian measure should be individualized to optimize cost-effectiveness, precision, level of acceptable invasiveness, and labor/time intensiveness. Confounding variables, such as narcotic/hypnotic or activating medication use, comorbid sleep disorders, comorbid psychiatric or medical conditions, preceding sleep duration and timing, and even the measuring tools themselves, can also influence, and be influenced by, these various techniques. As such, the astute clinician or researcher must select the measurement method with discretion. Those planning to assess circadian rhythms and/or circadian rhythm sleep-wake disorders

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may benefit from consulting with a sleep specialist and reviewing the latest guidelines in the International Classification of Sleep Disorders [1].

There are other factors, both intrinsic and extrinsic to the individual, that can affect the precision of circadian measurements. This phenomenon is called "masking," because the true endogenous circadian rhythm can be "masked" by physiologic processes unrelated to the output of the central circadian pacemaker. Examples of this, which will be discussed in more detail below, are the "masking" effects of sleep, food, movement, medications, and the menstrual cycle on the circadian rhythm of core body temperature. Masking effects can be mitigated using the constant routine (CR) protocol, as described in Chap. 2. Examples of CR are constant dim and/or long wavelength light, constant wakefulness in a recumbent posture, and feeding with frequent, regular, and identical small meals and liquid portions.

A final consideration in circadian rhythm measurement is the number of times that the parameter of interest is assessed. Measurement at a single time point provides a snapshot of internal circadian rhythms in the context of sleep timing, light-dark exposure, and other contributing factors close to the time of assessment. It is often of greater interest, however, to obtain serial measurements. In this scenario, the first measure is used to establish the baseline status and in some cases to determine if and when a circadian intervention should be initiated. Subsequent evaluations are used to track changes from baseline to measure any shift in the circadian rhythms.

Actigraphy

The most commonly used method of assessment is actigraphy, which is used for research, clinical, and, with the advent of wearables, personal home monitoring. An actigraph is a noninvasive ambulatory monitor – typically worn on the nondominant wrist and resembling a watch – that provides information about rest and activity patterns. Actigraphically measured estimates of rest and activity correlate well with gold-standard measures of sleep and wakefulness (i.e., polysomnography). Because it is not physically burdensome and time-consuming and is relatively inexpensive, actigraphy data are typically collected for multiple days and nights. The major drawback of wrist actigraphy is its inability to distinguish between true sleep and absence of motion; hence, in disorders of insomnia, where motionless wakefulness is not uncommon, actigraphy may overestimate sleep. Similarly, in disorders of abnormal sleep movement, actigraphy can overestimate wakefulness. Those considering use of wrist actigraphy should consider whether the data may be less reliable in their population of interest.

Actigraphs usually measure nondirectional acceleration. Oftentimes, these may be in arbitrary units (e.g., counts that are specific to the particular actigraph device), as opposed to established measurements of acceleration (meters per second squared or "g"). Common variables measured by actigraphs include duration of movement above a certain threshold ("time above threshold"), timestamps which cross an

arbitrary zero-point ("zero-crossing"), or intensity of movement ("periodic integration") [2]. More recently developed actigraphs have been enhanced to include microelectrical mechanical sensors for three-dimensional acceleration measurement, body temperature measurement, user-input subjective measurements, light level detection, and decibel measurements [2].

Ancoli-Israel et al. described circadian rhythm analysis using actigraphy in a comprehensive review [3]. Actigraphy can identify patterns of sleep and wake that correlate with circadian rhythms of melatonin and core body temperature, particularly in individuals whose circadian rhythms are entrained to typical clock times. Actigraphy is also useful for documenting delayed or advanced sleep phase, disturbed sleep in shift workers, and non-24-hour sleep-wake schedules, especially if the individual being assessed is sleeping at his/her preferred times rather than according to the expected societal schedule [4–7]. Multiple methods to detect and analyze rhythmicity of activity have been proposed, including several variations on the periodogram, an analysis technique that examines time-series data to detect periodic signals. Twenty-four-hour circadian rhythms can be detected in raw actigraphy data using methods such as Fourier spectral analysis, Lomb and Scargle periodogram, Enright periodogram, ANOVA periodogram, and the Cosinor and cosine fit [2, 8–12].

When compared to the gold standard for sleep evaluation, polysomnography (PSG), wrist actigraphy has high accuracy and sensitivity, but relatively low specificity (86%, 96%, and 33%, respectively), at least in the laboratory setting [13]. According to the American Academy of Sleep Medicine (AASM) practice parameters, actigraphy is useful in characterizing and monitoring circadian rhythms not only in healthy adult populations, but also in children and infants; elderly adults and those in nursing facilities, with or without dementia; depressed or schizophrenic patients; and those in inaccessible locations, for example, pilots in spaceflights [14]. Particularly germane to clinicians, actigraphic measurement of sleep patterns is a recommended "guideline" for the evaluation of circadian rhythm sleep-wake phase disorders [15].

Ambulatory Light Monitoring

Photic stimulation is the strongest zeitgeber and provides important information to the internal biological clock. Light entering the eye excites retinal photoreceptors called intrinsically photosensitive retinal ganglion cells (ipRGCs), which then send action potentials via the retinohypothalamic tract to the suprachiasmatic nuclei (SCN) of the hypothalamus. The ipRGCs are specialized to transmit light information rather than to detect and transmit visual information like retinal rod and cone cells. As such, some people with blindness have intact light-dark input through the ipRGCs despite their visual impairment. The SCN function as the internal master clock of the body, and secondary efferents project from the SCN throughout the brain to synchronize physiology and behavior. The SCN govern the timing of melatonin release from the pineal gland, and melatonin secretion is closely linked to the

timing of sleep and darkness. Light exposure patterns are therefore an important circadian measure, and knowledge about daily light-dark exposure can inform measurement of melatonin and other circadian rhythms.

Measured light levels can be used as a proxy measure for zeitgeber strength and provide the researcher with information about an individual's periodicity and circadian phase position. For example, if the amplitude of light exposure is low, it is more difficult for an individual's circadian rhythms to maintain entrainment. Although in rare situations of complete, continuous darkness, the need for a light zeitgeber may be overcome by exposure to social cues and interactions [16], in most settings, cycling periods of light and dark are needed to maintain entrainment. The zeitgeber of light exposure is now more significant as ever in sleep health, as light exposure from smartphones and other electronic devices has been shown to suppress melatonin release by up to 11% in a darkened room and up to 36% when used in a bright room [17].

The effects of disrupted sleep, both among inpatients and ambulatory subjects, can be monitored via actigraphy, as described above, but the amount of light to which a person is exposed has been a bit more elusive. In the research setting, various light meters have shown promise. Both wearable and stand-alone light meters can reliably capture light fluctuations in inpatient settings, and there is high congruence between a light meter that is set up in a room apart from a patient and one that is worn at wrist level [18]. Future work in this area could ultimately lead to home and workplace interventions regarding the timing of light exposure and personalized light-dark prescriptions that enhance circadian entrainment in individuals with circadian disorders [19].

Melatonin Rhythm Measurement

The hormone melatonin conveys information about the external light-dark cycle to the brain and the rest of the body. In entrained individuals, melatonin levels are low during the day, rise acutely in the evening (as long as environmental light levels are dim), remain high through the night, and decline to nearly undetectable levels in the morning. In the laboratory setting, the evening onset of melatonin secretion, termed the "dim light melatonin onset" (DLMO), is a well-studied circadian marker. The DLMO is considered the most accurate marker of circadian phase, as secretion of melatonin from the pineal gland is controlled directly by the suprachiasmatic nuclei. Although melatonin production can be masked by bright light, it is relatively robust in its resistance to the masking effects of other external stimulation, such as carbohydrate ingestion [20]. Given its reliability, robustness, and verifiability, it is often used as a "gold standard" measurement with which other assessments are compared [21].

The DLMO is of great utility to circadian and sleep researchers and clinicians as it can be measured noninvasively, most commonly using salivary or urinary samples [22, 23]. Typically, melatonin levels rise in the 2–3 hours preceding nocturnal sleep.

For this reason, sampling for DLMO determination requires a relatively short sampling window, which makes it an accessible tool [22]. Although most easily obtained via saliva, urinary and plasma analyses are also available. For all, the typical unit of measurement is picograms per milliliter.

Salivary sampling for DLMO takes place under dim light (fewer than 30 lux) every one-half to one hour. Samples should be collected at least 1 hour prior to and through the expected rise in melatonin. Although this method is portable and individuals can collect samples at home, this approach requires attention to several protocol considerations including the necessity to remain in dim light, the importance of accurate sample timing, and taking measures to avoid contaminating saliva samples with food or blood.

Urinary 6-sulfatoxymelatonin (aMT6s) is the primary urinary metabolite of melatonin. Collection of urine samples across 24 hours allows estimation of the rhythm of aMT6s excretion rate. This method does not require disruption of sleep as the overnight excretion rate can be measured using the first morning void. During the day, urine should be collected every 2–8 hours. The phase of the excretion rhythm is estimated from the peak of the cosine fitted curve, termed the "acrophase." Individual ability to void and compliance with collection are both limiting factors for this method of measurement.

Melatonin secretion can also be measured in plasma. In this method, blood is sampled at frequent intervals, most commonly via an intravenous catheter to avoid multiple phlebotomy sessions. The intravenous catheter should be inserted at least 2 hours prior to sampling to avoid alteration in melatonin levels associated with a painful stimulus and concurrent adrenergic surge. Midline intravenous access, central catheterization, or peripherally inserted central catheterization (PICC) may allow frequent sampling without frequent disruption of sleep, but does come with the potential risk of an invasive catheterization, such as local injury/discomfort, infection, or cardiac arrhythmia. Salivary acrophase correlates well with plasma acrophase of melatonin, albeit at a level of 30 percent that of the plasma level [24]. Plasma analysis may therefore allow for greater sensitivity and determination of circadian phase, due to the relatively higher melatonin levels present in plasma, and may be useful in individuals who secrete low levels of melatonin.

Absolute values, relative values (commonly a percentage of maximum or daily average) and curve fitting have all been used to determine circadian phase from the pattern of melatonin secretion. DLMO and its corollary, dim light melatonin offset (DLMOff), as well as the termination of melatonin synthesis ("Synoff"), are variables that can be measured as well to describe melatonin physiology. Synoff represents the transition from maximal nocturnal production to the morning decline, and DLMoff represents the return to daytime melatonin levels. Intraindividual melatonin rhythms, timing, and amplitudes are typically quite stable. However, interindividual differences can be quite large. Thus, measuring these markers of physiological transition as opposed to absolute values may be most reliable in group analyses.

DLMO and other melatonin measurements are not yet largely accessible to the sleep clinician, but there is limited evidence that DLMO measurement has utility in the diagnosis of circadian rhythm sleep disorders. For example, Rahman and

colleagues demonstrated average DLMO was ~2.5 hours later in patients with delayed sleep-wake phase disorder (DSWPD) compared to non-DSWPD patients, whereas sleep latency was only ~40 minutes later. In this study, DLMO had a sensitivity and specificity of 90.3% and 84%, respectively, for diagnosis of DSWPD [25]. Although no melatonin assays are yet approved by the Food and Drug Administration in the United States for the diagnosis and management of circadian rhythm sleep disorders, several assays are commercially available in Europe [26].

Cost and frequency of sampling are the two biggest practical limitations of the melatonin assay [23]. Purchasing the reagents and monoclonal antibodies for each assay, which cost on the order of 12 dollars to 20 euros per sample, may be prohibitive to widespread adoption of these tools in the clinical realm [23, 26].

Core Body Temperature

Core body temperature variation is a well-known circadian rhythm, thought to be due to the interplay of heat production, derived from the metabolic activity of the viscera and the brain, which produces approximately 70% of the resting metabolic rate of the body, and heat loss, brought about by evaporation, conduction, convection, and radiation. This phenomenon was documented as early as 1842, with Gierse's study observing his own oral temperature reaching a maximum in the early evening and a minimum in the early morning hours [27]. More intricate studies under constant routine protocols revealed that distal skin temperature rises in the evening, but heat production, proximal skin temperature, and core body temperature decrease. In the morning hours, the pattern is reversed [28].

Core body temperature as a means of circadian monitoring has been studied in a wide variety of clinical research settings. A pitfall is that it can be masked by a variety of exogenous and endogenous factors. For example, in reproductive-age women, the temperature mesor (mean value) is higher in the luteal phase and lower in the pre-ovulatory phase and during menses when compared to the follicular phase. Consequently, the amplitude of the temperature circadian rhythm is reduced in the luteal phase. The period of circadian temperature variations is not affected by menstruation in the ambulatory population [29]. In stroke patients, body temperature demonstrates infradian rhythms, which is thought to reflect impaired consciousness post-stroke as well as decreased mobility and ambulation [30]. Patients with affective disorders exhibit a smaller core temperature rhythm amplitude than controls without a mood disorder, which is driven by higher nocturnal core temperature among those with affective disorders [31].

Core body temperature can be gathered via invasive methods, such as catheter insertion via the esophagus, rectum, or urinary bladder [32]. Esophageal and bladder temperature monitoring typically occur only in critical care settings and have been shown to be most similar to core body temperature measured via a pulmonary

artery catheter [33]. For obvious reasons, these interventions are not pragmatic for the ambulatory setting, and peripheral body temperature readings are preferable. Wrist skin temperature has been shown to correlate well with DLMO; however, the sensitivity of peripheral thermometry is limited compared to core body temperature measures. Indeed, a recent systematic review concluded that peripheral thermometers do not have clinically acceptable accuracy when precise measurements of body temperature are required [34]. With that said, peripheral thermometry may be acceptable for assessing circadian changes as opposed to absolute values. Various technologies exist for ambulatory core body temperature monitoring. Wearable thermometry can be simple to install and use. Forehead dual heat flux methods [35] and iButton ® skin temperature monitors [36] have been studied as effective means of ambulatory monitoring. Other studies have shown utility with ingestible temperature monitors [29].

Although the constant routine is ideal for measuring the circadian rhythm of core body temperature, CRs are impractical for day-to-day or long-term recording. Thus, algorithms and filters associated with other vital sign changes, such as heart rate, have been devised to "unmask" the effects of physical activity and environmental factors and increase the precision of circadian temperature measurement [37].

Circadian Gene Expression in Peripheral Cells

Although advances in the basic science of molecular genetics in circadian rhythm sleep disorders continue to excite sleep researchers and clinicians, there are still limited applications in clinical practice. Early researchers in circadian rhythms observed the presence of an endogenous timekeeper. Later studies in model organisms such as *Neurospora*, *Drosophila*, *Caenorhabditis elegans*, and rodents revealed homologs and analogs of timekeeping genes, such as *per*, *tim*, *clock*, and *cry*. The first characterization of a Mendelian circadian trait in humans was in the landmark phenotypic study of a family with early sleep onset and offset, thus identifying an autosomal dominant trait with high penetrance for familial advanced sleep-wake phase disorder (FASP) [38, 39]. Ultimately, this was found to be a missense mutation in the human cry2 gene [40]. Since the discovery of FASP, other human circadian phenotypes have been identified and attributed to heredity, including familial natural short sleep (FNSS) [41, 42].

In the research realm, the noninvasive collection and measurement of expression of clock genes has been shown to be feasible. In a study by Akashi et al., circadian rhythmicity of hair follicle cells of the human scalp was measured via real-time polymerase chain reaction (PCR) with [43]. Although this technology has yet to reach clinical medicine, ongoing research may prove these strategies useful in evaluating circadian disorders.

Polysomnography

Polysomnography (PSG) is the gold standard for recording sleep and diagnosing many sleep disorders, e.g., obstructive sleep apnea or narcolepsy. Its utility in circadian rhythm sleep disorders is limited, however, because PSG is usually performed during a single sleep-wake cycle.

PSG utilizes electroencephalogram (EEG), electrooculogram, electromyogram, electrocardiogram, air flow, breathing effort, limb and body movements, and pulse oximetry. EEG frequency and other electrical phenomena recorded with EEG define the various stages of sleep. Sleep is divided into rapid-eye-movement (REM) and non-rapid-eye-movement (NREM) sleep, with stages N1 and N2 considered "light" sleep, N3 as deep or slow wave sleep, and REM sleep as a separate state of consciousness characterized by rapid eye movements, decreased muscle tone, and dream mentation. In general, deeper sleep is characterized by slower EEG frequencies.

In its comprehensive practice parameters for the evaluation of circadian rhythms, the AASM noted that polysomnography is not routinely indicated for the diagnosis of circadian rhythm sleep-wake disorders, but may be indicated to rule out another primary sleep disorder [15]. Polysomnography does have utility in some circadian research, however. For example, in forced desynchrony protocols (see Chap. 2), PSG measurements provide important information about sleep propensity at various points in the circadian cycle.

Multiple Channel Ambulatory Monitoring

Reliable detection, measurement, and interpretation of circadian rhythms and phase shifts using noninvasive ambulatory measurements, especially among individuals living at home and conducting their day-to-day activities, have proven difficult. Nevertheless, there are promising research protocols that may prove useful in clinical settings in the future. One such method is multichannel ambulatory monitoring, which combines several measures described above without relying on measurements of core body temperature or melatonin levels or requiring constant routine laboratory conditions. In one study of this approach [44], research subjects wore multichannel ambulatory monitors for 1 week without alteration to their daily routine, followed by a 32-hour constant routine procedure in the laboratory. Multiple regression techniques were applied to reduce the confounding effects of ambulatory measurements and revealed that the multiple channel approach had statistically significant improvement of variance of prediction error when compared to single predictors (actigraphy or a sleep diary). Compared to core body temperature, the multiple channel method also improved the range of prediction errors and showed a nonsignificant reduction in variance [44]. Multiple channel ambulatory monitoring has shown good accuracy with regard to temporal association with DLMO [21] and

may be a promising new tool for making circadian phase estimates in an ambulatory setting, given its simplicity, applicability to clinical situations, and good reliability in early studies. With the advent of wearables and physiologic monitoring devices marketed directly to consumers for self-assessment, multichannel ambulatory monitors are likely to be used more commonly in future research and clinical assessments.

Heart Rate and Electrocardiographic Monitoring

Electrocardiogram (ECG or EKG) is a useful measurement in sleep medicine and sleep research and provides multiple parameters, e.g., heart rate variability, that have been used for everything from detecting sleep apnea to characterizing sleep stages. ECG is less commonly used to assess circadian rhythms, but this may change in the future. For example, core body temperature can be estimated using heart rate, and cardiac telemetry could be a practical and effective means to estimate core body temperature. R-R interval and mean heart rate have a strong correlation with core body temperature [45]. Further work is needed to fully understand phase differences between ECG and other circadian rhythms. For instance, Krauchi and colleagues showed that heart rate, among other variables, is phase advanced with regard to rectal temperature [46]. Nevertheless, with ambulatory ECG recordings that can run for several weeks, ECG may prove to be an important circadian measure in the coming years.

References

1. American Academy of Sleep Medicine, editor. International classification of sleep disorders. 3rd ed. Darien: American Academy of Sleep Medicine; 2014.
2. Roebuck A, Monasterio V, Gederi E, Osipov M, Behar J, Malhotra A, et al. A review of signals used in sleep analysis. Physiol Meas. 2014;35(1):R1–57.
3. Ancoli-Israel S, Cole R, Alessi C, Chambers M, Moorcroft W, Pollak CP. The role of actigraphy in the study of sleep and circadian rhythms. Sleep. 2003;26(3):342–92.
4. Youngstedt SD, Kripke DF, Elliott JA, Klauber MR. Circadian abnormalities in older adults. J Pineal Res. 2001;31:264–72.
5. Cole RJ, Smith JS, Alcala YC, Elliott JA, Kripke DF. Bright-light mask treatment of delayed sleep phase syndrome. J Biol Rhythms. 2002;17(1):89–101.
6. Carskadon MA, Acebo C, Richardson GS, Tate BA, Seifer R. An approach to studying circadian rhythms of adolescent humans. J Biol Rhythms. 1997;12(3):278–89.
7. Carskadon MA, Wolfson A, Acebo C, Tzischinsky O, Seifer R. Adolescent sleep patterns, circadian timing, and sleepiness at a transition to early school days. Sleep. 1998;21(8):871–81.
8. Scargle JD. Studies in astronomical time series analysis. II – statistical aspects of spectral analysis of unevenly spaced data. Astrophys J. 1982;263:835–53.
9. Refinetti R, Lissen GC, Halberg F. Procedures for numerical analysis of circadian rhythms. Biol Rhythm Res. 2007;38(4):275–325.
10. Enright J. The search for rhythmicity in biological time-series. J Theor Biol. 1965;8(3):426–68.
11. Sokolove PG, Bushell WN. The chi square periodogram: its utility for analysis of circadian rhythms. J Theor Biol. 1978;72(1):131–60.

12. Shono M, Shono H, Ito Y, Muro M, Maeda Y, Sugimori H. A new periodogram using one-way analysis of variance for circadian rhythms. Psychiatry Clin Neurosci. 2000;54(3):307–8.
13. Marino M, Li Y, Rueschman MN, Winkelman JW, Ellenbogen JM, Solet JM, Dulin H, et al. Measuring sleep: accuracy, sensitivity, and specificity of wrist actigraphy compared to polysomnography. Sleep. 2013;36(11):1747–55.
14. Littner M, Kushida CA, Anderson WM, Bailey D, Berry RB, Davila DG, et al. Standards of Practice Committee of the American Academy of Sleep Medicine. Practice parameters for the role of actigraphy in the study of sleep and circadian rhythms: an update for 2002. Sleep. 2003;26(3):337–41.
15. Morgenthaler TI, Lee-Chiong T, Alessi C, Friedman L, Aurora N, Boehlecke B, et al. Standards of Practice Committee of the AASM. Practice parameters for the clinical evaluation and treatment of circadian rhythm sleep disorders. Sleep. 2007;30(11):1445–59.
16. Aschoff J, Fatranská M, Giedke H, Doerr P, Stamm D, Wisser H. Human circadian rhythms in continuous darkness: entrainment by social cues. Science. 1971;171(3967):213–5.
17. Oh JH, Yoo H, Park HK, Do YR. Analysis of circadian properties and healthy levels of blue light from smartphones at night. Sci Rep. 2015;5:11325.
18. Higgins PA, Winkelman C, Lipson AR, Guo SE, Rodgers J. Light measurement in the hospital: a comparison of two methods. Res Nurs Health. 2007;30(1):120–8.
19. Mason IC, Boubekri M, Figueiro MG, Hasler BP, Hattar S, Hill SM, et al. Circadian health and light: a report on the National Heart, Lung, and Blood Institute's workshop. J Biol Rhythms. 2018;33(5):451–7.
20. Kräuchi K, Cajochen C, Werth E, Wirz-Justice A. Alteration of internal circadian phase relationships after morning versus evening carbohydrate-rich meals in humans. J Biol Rhythms. 2002;17(4):364–76.
21. Bonmati-Carrion MA, Middleton B, Revell V, Skene DJ, Rol MA, Madrid JA. Circadian phase assessment by ambulatory monitoring in humans: correlation with dim light melatonin onset. Chronobiol Int. 2014;31(1):37–51.
22. Benloucif S, Burgess HJ, Klerman EB, Lewy AJ, Middleton B, Murphy PJ, et al. Measuring melatonin in humans. J Clin Sleep Med. 2008;4(1):66–9.
23. Molina TA, Burgess HJ. Calculating the dim light melatonin onset: the impact of threshold and sampling rate. Chronobiol Int. 2011;28(8):714–8.
24. Voultsios A, Kennaway DJ, Dawson D. Salivary melatonin as a circadian phase marker: validation and comparison to plasma melatonin. J Biol Rhythms. 1997;12(5):457–66.
25. Rahman SA, Kayumov L, Tchmoutina EA, Shapiro CM. Clinical efficacy of dim light melatonin onset testing in diagnosing delayed sleep phase syndrome. Sleep Med. 2009;10(5):549–55.
26. Keijzer H, Smits MG, Duffy JF, Curfs LM. Why the dim light melatonin onset (DLMO) should be measured before treatment of patients with circadian rhythm sleep disorders. Sleep Med Rev. 2014;18(4):333–9.
27. Gierse A. Quaeniam sit ratio caloris organici, M. D. Thesis. 1842. Halle.
28. Kräuchi K. How is the circadian rhythm of core body temperature regulated? Clin Auton Res. 2002;12(3):147–9.
29. Coyne MD, Kesick CM, Doherty TJ, Kolka MA, Stephenson LA. Circadian rhythm changes in core temperature over the menstrual cycle: method for noninvasive monitoring. Am J Physiol Regul Integr Comp Physiol. 2000;279(4):R1316–20.
30. Takekawa H, Miyamoto M, Miyamoto T, Yokota N, Hirata K. Alteration of circadian periodicity in core body temperatures of patients with acute stroke. Psychiatry Clin Neurosci. 2002;56(3):221–2.
31. Carpenter JS, Robillard R, Hermens DF, Naismith SL, Gordon C, Scott EM, et al. Sleep-wake profiles and circadian rhythms of core temperature and melatonin in young people with affective disorders. J Psychiatr Res. 2017;94:131–8.
32. Lilly JK, Boland JP, Zekan S. Urinary bladder temperature monitoring: a new index of body core temperature. Crit Care Med. 1980;8(12):742–4.
33. Lefrant JY, Muller L, de La Coussaye JE, Benbabaali M, Lebris C, Zeitoun N, et al. Temperature measurement in intensive care patients: comparison of urinary bladder, oesophageal, rectal,

axillary, and inguinal methods versus pulmonary artery core method. Intensive Care Med. 2003;29(3):414–8.
34. Niven DJ, Gaudet JE, Laupland KB, Mrklas KJ, Roberts DJ, Stelfox HT. Accuracy of peripheral thermometers for estimating temperature: a systematic review and meta-analysis. Ann Intern Med. 2015;163(10):768–77.
35. Huang M, Tamura T, Tang Z, Chen W, Kanaya S. A wearable thermometry for core body temperature measurement and its experimental verification. IEEE J Biomed Health Inform. 2017;21(3):708–14.
36. Hasselberg MJ, McMahon J, Parker K. The validity, reliability, and utility of the iButton® for measurement of body temperature circadian rhythms in sleep/wake research. Sleep Med. 2013;14(1):5–11.
37. Nakano T, Koyama E, Imai T, Hagiwara H. Circadian rhythm estimation by core body temperature filtered with simultaneously recorded physiological data. Methods Inf Med. 1997;36(4–5):306–10.
38. Jones CR, Campbell SS, Zone SE, Cooper F, DeSano A, Murphy PJ, et al. Familial advanced sleep-phase syndrome: a short-period circadian rhythm variant in humans. Nat Med. 1999;5(9):1062–5.
39. Jones CR, Huang AL, Ptáček LJ, Fu YH. Genetic basis of human circadian rhythm disorders. Exp Neurol. 2013;243:28–33.
40. Hirano A, Shi G, Jones CR, Lipzen A, Pennacchio LA, Xu Y, et al. A Cryptochrome 2 mutation yields advanced sleep phase in humans. Elife. 2016;5:pii:e16695.
41. He Y, Jones CR, Fujiki N, Xu Y, Guo B, Holder JL Jr, et al. The transcriptional repressor DEC2 regulates sleep length in mammals. Science. 2009;325(5942):866–70.
42. Zhang L, Jones CR, Ptacek LJ, Fu YH. The genetics of the human circadian clock. Adv Genet. 2011;74:231–47.
43. Akashi M, Soma H, Yamamoto T, et al. Noninvasive method for assessing the human circadian clock using hair follicle cells. Proc Natl Acad Sci U S A. 2010;107:15643–8.
44. Kolodyazhniy V, Späti J, Frey S, Götz T, Wirz-Justice A, Kräuchi K, et al. Estimation of human circadian phase via a multi-channel ambulatory monitoring system and a multiple regression model. J Biol Rhythms. 2011;26(1):55–67.
45. Sim SY, Joo KM, Kim HB, Jang S, Kim B, Hong S, et al. Estimation of circadian body temperature rhythm based on heart rate in healthy, ambulatory subjects. IEEE J Biomed Health Inform. 2017;21(2):407–15.
46. Kräuchi K, Wirz-Justice A. Circadian rhythm of heat production, heart rate, and skin and core temperature under unmasking conditions in men. Am J Physiol. 1994;267(3 Pt 2):R819–29.