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The Circadian Axis and Cardiometabolic Syndrome

Boglárka Varga[1,2], Cristian Norbert Ionescu[2,3], Monica Iudita Maria Szabo[2,4]

[1] Clinic of Diabetology, Emergency County Clinic, Târgu Mureș, Romania
[2] "George Emil Palade" University of Medicine, Pharmacy, Science and Technology, Târgu Mureș, Romania
[3] Clinic of Neurosurgery, Emergency County Clinic, Târgu Mureș, Romania
[4] Department of Internal Medicine, Clinic of Diabetology, Emergency County Clinic, Târgu Mureș, Romania

CORRESPONDENCE

Boglárka Varga
Str. Gheorghe Marinescu nr. 50
540136 Târgu Mureș, Romania
Tel: +40 771 513 001
E-mail: varga.f.boglarka@gmail.com

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Cristian Norbert Ionescu • Str. Gheorghe Marinescu nr. 50, 540136 Târgu Mureș, Romania. Tel: +40 747 780 531, E-mail: cristi.umf@gmail.com

Monica Iudita Maria Szabo • Str. Gheorghe Marinescu nr. 50, 540136 Târgu Mureș, Romania. Tel: +40 265 215 551, E-mail: sztamo@gmail.com

ABSTRACT

Circadian rhythm refers to the daily physiologically fluctuating patterns of systemic processes that occur within a circa 24-hour timeframe, independently of external factors. There is evidence that in time, external and internal cycle misalignment leads to severe health consequences, resulting in the development of cardiometabolic disturbances. Desynchronized hormonal fluctuations along with daily specific macronutrient utilization patterns are also discussed, which by consequence, are all predictors of metabolic syndrome. The aim of this paper is to provide insight on the circadian clock's organization throughout the human body and to explain the underlying genetic background. By understanding these well-established molecular mechanisms and processes, we believe this paper will provide accuracy regarding the importance of the circadian clock's integrity and will highlight its role in the etiopathology of cardiometabolic syndrome.

Keywords: circadian rhythm, cardiometabolic syndrome, diabetes, clock gene, hormonal fluctuation

THE CIRCADIAN AXIS

Central clock: nucleus suprachiasmaticus

The circadian rhythm is regulated mainly by the nucleus suprachiasmaticus (NSC) of the hypothalamus, called the 'master' clock, but clock genes have also been identified in the arcuate (AN) and paraventricular (PVN) hypothalamic nuclei.[1–3] These structures form an essential neuronal network and maintain metabolic health on a whole-body level.[4] The NSC integrates rhythmic oscillations of clock gene expression only if intracellular calcium and cyclic adenosine monophosphate (cAMP) levels are sufficient for membrane depolarization.[4]

By its pacemaker function, the NSC allows these cellular oscillations to be 'entrained', by which a consistent and distinctly timed output is provided to peripheral tissues, even in constant darkness.[4,5]

It has long been proven in animal models that the abolishment of circadian rhythmicity causes the significant alteration of several behavioral and endocrine functions.[6,7] For instance, the transplantation of fetal hamster NSC tissue to replace previously damaged components was found to restore the daily rhythmicity of locomotor activity.[8] This was also observed in Circadian locomotor output cycles kaput (CLOCK) mutant or mCry1/mCry2 double knockout mice, where by grafting, daily behavioral rhythm was reestablished.[9] In all of the cases, circadian behavior and cycle period length were determined by the donor's genotype and not the host's, which sustains the prior regulatory role of the NSC.[10]

External time givers

Besides daily hormonal oscillations, body temperature variations, and behavioral changes, one of the most evident daily rhythms that defines circadian rhythmicity is the sleep/wake cycle.

In the absence of external cues, the circadian clock system performs its 'daily program' freely, which is not completely aligned with the conventional 24-hour cycle.[11]

In order to align with environmental factors and to optimize synchronization, it compiles information from time givers or so called 'zeitgebers', which are: light input, nutrient intake, physical activity, and social engagement.[12–14]

Light input, the most significant time giver of the master clock, is an indirect regulator applied to peripheral clocks throughout the central nervous system (CNS). Signal from the melanopsin-expressing photoreceptor cells in the retina is conducted via the retinohypothalamic tract to the central clock.[4] If light and feeding cycles deviate, a slow reset starts in the periphery, until the new feeding rhythm is achieved.[8]

The most effective time giver for peripheral tissues is the *rhythmic feeding behavior*.

In rodents, restricting food access only during the inactive phase results in a complete phase shift of circadian gene expression in numerous peripheral tissues (heart, pancreas, adipose tissue, kidneys, liver), whereas centrally, it remains identical.[9] For instance, the liver clock maintains circadian rhythmicity in the absence of an intact autonomic hepatic input if either direct adrenal control or rhythmic feeding behavior is present.[10] The connection between the CNS and peripheral clock mechanisms is coordinated through numerous factors, as seen in Figure 1.

Peripheral clocks

The liver is the most studied peripheral circadian oscillator. Here, 20% of the gene expression is believed to function in conformity with daily rhythmicity and the enzymes needed for pathway mechanisms.[15] The vast majority of circadian

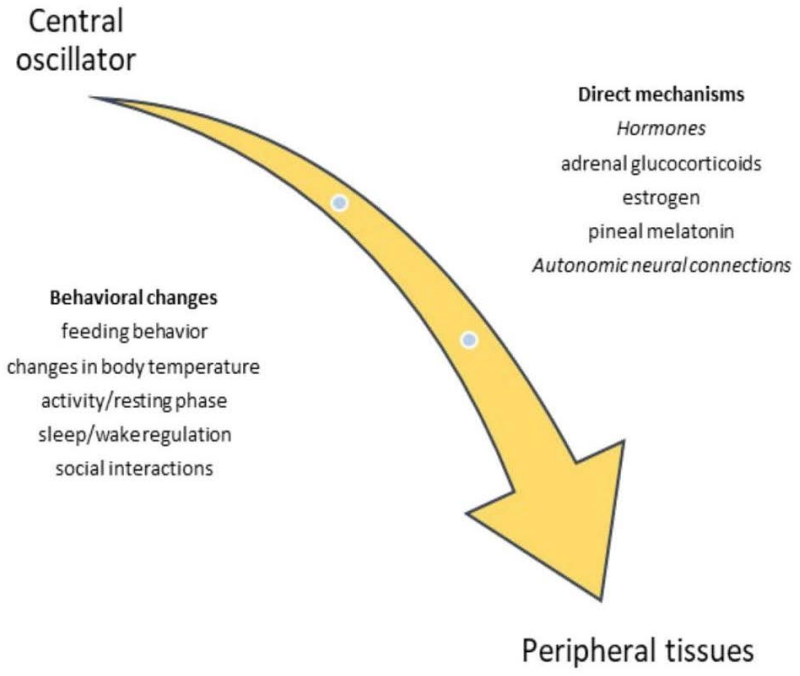

FIGURE 1. Signals connecting central and peripheral oscillators[11–13]

gene transcripts are controlled by the liver clock itself, and only a small subset is influenced by systemic signals.[16] Daily physiologic processes are constantly balanced in order to use nutrients in the most energy-efficient manner. Carbohydrates are fast energy sources, protein-coding genes are expressed when metabolic requirements are the highest, whereas ineffective metabolic cycles are silenced.[15]

A bidirectional relationship between the *gut microbiome* and the circadian clock system has been described, and it is known as the 'gut microbiota-circadian clock axis'. This peripheral oscillator is constantly supervised by the central clock, but its main regulator is the individual feeding behavior.[17] Daily alterations are present in the composition and functioning of the microbiome, also regulated by the rhythmic feeding schedule and nutritional content.[18] Circadian misalignment in time results in dysbiosis, which leads to altered glucose tolerance and obesity. Studies have shown that after fecal transplantation from Period 1/2 knockout (Per1/2−/−) mice to germ-free mice, daily rhythmicity was reestablished only one week after the intervention.[18]

The direct link between *abdominal adiposity* and circadian rhythm disruption was best highlighted in sleep loss studies. Caloric intake is increased during sleep loss due to changes in appetite hormones (detailed below) and higher energy expenditure. Hunger and appetite ratings via visual analog scores (VAS) showed a significant increase in appetite in the evening (8 PM) and decline in the morning (7:50 AM).[19] Rhythm in appetite was observed mainly for high-energy foods (sweets, starchy and salty foods, fruits, meat), which is a result of brain activity changes mainly in the nucleus accumbens (NA), showed by MRI scans.[19,20] Observational studies highlighted the inverse relationship between the length of the sleeping period, body mass index (BMI), and waist circumference.[21] Body fat distribution, mainly in the visceral area, predisposes to increased risk of numerous metabolic and cardiovascular diseases.

As a peripheral tissue, the *skeletal muscle* is supervised by the central clock and influenced mostly by daily external factors such as daily scheduled physical activity.[22] RT-QPCR and microarray analysis of skeletal muscle biopsies revealed that 14.5% of transcripts presented a day-night fluctuation of gene expression, one third of which were protein-coding transcripts involved in mitochondrial dynamics.[23] For instance, MyoD (myogenic differentiation 1 mRNA), a circadian transcriptome specific to muscle cells with a prior role in myogenesis, was shown to be activated by CLOCK and BMAL1 genes.[22] The oxidative capacity of the muscle tissue showed obvious day-night rhythmicity with a peak at the end of the day.[24]

By consequence, a disrupted circadian rhythm will lead to insufficient metabolic flexibility, which in time will easily elicit the development of cardiometabolic disorders.[25]

GENETIC BACKGROUND

Gene expression is coordinated by a 24-hour periodicity in nearly all cells of the human body. This is executed by a transcription/translation feedback loop, which is the main defining element of the mammalian clock.[26] During transcriptional regulation, the heterodimeric transcription factor complex CLOCK and BMAL1 (Brain and Muscle ARNT-Like 1) dimerizes and attaches to a specific site of the DNA called E-box enhancer elements, which automatically activates or inhibits the transcription of downstream Period (Per 1,2,3) and Cryptochrome (Cry 1,2) genes. Transcriptomes interact, form complexes, and translocate to the nucleus to inhibit CLOCK gene expression by interacting with the CLOCK-BMAL1 dimer.[26] Elimination of the repression structure (by degradation or ubiquitination) will restart this process.[27] The most studied interlocking auxiliary feedback loops include the orphan nuclear receptors REV-ERBα and RORα, which will also lead to rhythmic BMAL1 expression.[28]

Several other core clock genes were identified, which facilitate important cardiometabolic disturbances by diminished expression: (1) **BMAL1** – hypotension and increased adipogenesis,[29,30] (2) **PPAR** – decreased day-night blood pressure oscillation,[31] (3) **CRY** – hypertension and excess in aldosterone secretion,[32] (4) **HDAC3** – liver steatosis and lipid metabolism disorders,[33] (5) **PER** – advanced sleep-wake phase disorder (ASWPD) and delayed sleep-wake phase disorder (DSWPD),[34,35] (6) **SIRT1** – fat burning alteration during sleep phases, insulin secretion and gluconeogenesis, adipocyte differentiation.[36–38]

CIRCADIAN FLUCTUATION OF METABOLICALLY SIGNIFICANT HORMONES

Cortisol and *melatonin* both have pleiotropic effects on several tissues and are interrelated, being in an inverted phase relationship. The pineal gland, supervised by the NSC through a polysynaptic network, produces melatonin in order to facilitate sleep in a rhythmic manner. It reaches its peak at the midpoint of the sleeping period, then bright light firmly decreases melatonin levels and promotes cortisol production by the adrenocorticotropic hormone (ACTH) via an independent sympathetic mechanism.[39]

The consequence of the activation of the hypothalamic-pituitary-adrenal axis is a daily rhythmic production of

TABLE 1. The independent effects of behavioral and circadian cycle on metabolically significant hormones[41]

Hormones	Independent effects of the behavioral cycle		Independent effects of the circadian cycle
	Peak	Nadir	Peak
Leptin	After last meal	Around breakfast	No endogen rhythm
Glucose	Depending on meal timing		Biological night (10:30 PM – 6:30 AM)
Insulin	Depending on meal timing		No endogen rhythm
Epinephrine	Wake period	Sleep episode	Biological day (2:30 PM – 6:30 PM)
Norepinephrine	Wake period	Sleep episode	No endogen rhythm
Cortisol	After awakening	Onset of sleep episode	End of biological night

ACTH and a slow decrease during the day, reaching its nadir at the beginning of the sleeping phase. An increasing pattern between 2 AM and 4 AM is shown to lead to a maximal hormonal rise from the adrenal gland in the morning, which provokes vigilance and initiates catabolic processes in adipose and muscle cells.[40] The rhythmic pattern of cortisol secretion is maintained even in the lack of external signals.[41]

Interestingly, during circadian disruption, time interval difference between the beginning of inactive cortisol secretion and the starting point of melatonin production will remain the same (1 hour 25 minutes ± 27 minutes) regardless of the consequent fluctuation of melatonin levels. Therefore, this pattern is useful in identifying circadian phases.[42]

Leptin is mainly produced by adipocytes and in the stomach in order to suppress appetite during the night and to promote nocturnal fasting and sleep.[43,44] Leptin levels show an increase in the first part of the night, peaking at around 4 AM with a decreasing tendency afterwards.[45]

Ghrelin provides a link between peripheral and central clock systems. Produced in the pancreas, stomach, and hypothalamus,[46,47] it stimulates the appetite through its action on neuropeptide Y in the lateral hypothalamus.[48] In vitro studies have highlighted that its fluctuation can change clock behavior by having a direct effect on the NSC.[49] High levels were found during the early hours of the night, with a decrease before awakening and elevated levels one hour prior to meals.[50] The daily variations of this hormone are modulated by nutrient intake, but sleep deprivation maintains high levels of ghrelin leading to increased hunger and consequently to obesity.[51] In order to find out what happens exactly during a regular and a misaligned day, we need to individually analyze the hormonal fluctuations linked to behavioral (fasting/feeding, sleep/wake episodes) and circadian cycles separately, as shown in Table 1 below.[52]

A significant interaction was observed between the two distinct cycles of leptin levels. During behavioral cycle desynchronization, leptin levels were significantly diminished, especially when a shift of maximum 12 hours occurred from the normal cycle. This suggests that leptin can be considered to be a *short-term regulator of energy homeostasis if behavioral misalignment occurs*.[45] A decline in leptin levels leads to increased appetite and low energy expenditure.[53]

The complete inversion of the *cortisol profile* contributes to hyperglycemia and insulin resistance.[54,55] Additionally, during disruption of both behavioral and circadian bases, the *level of melatonin* is maintained, but its rhythm is considerably dampened.[56] The chronic mistiming of daily meals, especially when melatonin levels peak, will lead to metabolic diseases. *Phase angle* is a term mostly used in clinical trials to quantify circadian disharmony. It stands for the activation of circadian factors, such as melatonin onset, under dim light conditions (DLMO) and minimum core body temperature – both are factors of circadian coordination, which are compared to the timepoints of the sleep/wake cycle.[57]

MACRONUTRIENT UTILIZATION WITH CIRCADIAN PATTERN

Macronutrient intake shows circadian periodicity in animal models. At the beginning of the active phase, due to low glycogen stores and the increase of neuropeptide Y in the paraventricular nucleus of the hypothalamus, the elective source of nutrients are carbohydrates. Whereas, by the start of the passive period, there is a switch in macronutrient utilization towards lipids, with a constant slow release of energy throughout the day.[58] At this point, genes playing part in de novo lipogenesis are upregulated, while fatty acid oxidation is reduced. Without parallel lipolysis in adipose tissues, this variation favors fatty acid dissolution in the morning and lipogenesis at the end of the behavioral cycle in the evening.[23]

Glucose utilization

Human studies have demonstrated that in concordance with the *glucostatic theory*, glucose tolerance is increased in the morning due to better β-cell responsiveness. This elicits fast and easy assimilation of carbohydrates, for which insulin response is prompt when fasting glucose levels are stable.[59] Conversely, obese patients have a better glucose tolerance later throughout the day. Increased glucose levels have been observed after waking hours, and glucose and insulin rhythms were dampened and phase-shifted by 1.5–2 hours.[60] Muscle cells and hepatic glycogen stores have their own specific insulin sensitivity patterns with peaks during the evening, whereas the subcutaneous adipose tissue shows 54% intensification in insulin sensitivity around noon, compared to midnight.[61,62] This function is known to be missing or completely reversed in type 2 diabetes mellitus (T2DM). The peak of insulin sensitivity occurs at around 7 PM and decreases in the morning hours, resulting in a well-known mechanism in patients with diabetes, called the 'dawn phenomenon'.[63,64]

Insulin secretion ratio varies across the daily cycle, with total insulin level peaking between 12 PM and 6 PM. During nighttime, its production is decreased, reaching nadir between midnight and 6 AM.[65] The insulin secretory rhythm in diabetic patients is completely absent, which can be additionally explained by the changed characteristics of cortisol secretion.[66]

Insulin clearance is increased importantly during nighttime by 30–40%, mainly during sleeping periods between 11 PM and 3 AM in comparison with morning wake periods. Hepatic insulin extraction is decreased around noon.[67] The uptake of glucose by insulin-independent mechanisms or glucose effectiveness is increased in the morning.[68]

Lipid metabolism

Fifteen percent of lipid metabolites in the plasma and the saliva show daily rhythmicity, out of which 80% are lipid compounds with peaking levels between mid-morning and noon.[69] Almost two thirds of triglycerides show daily fluctuating levels without any consensus regarding the definition of phases.[70–72] Men have a more robust daily variation of triglyceride levels compared to women, due to the effect of estrogen on these compounds. After the consumption of the same meal, the increase in triglyceride level in men is double compared to women;[73] there are no significant daily variations in HDL-C and total cholesterol levels.[74] Cholesterol synthesis is more likely to be influenced by behavioral changes and external aspects, which significantly define its fluctuation.[75] Interindividual variability has been observed regarding lipid rhythms. This finding allows to cluster subjects concerning rhythmicity intensity, amplitude, and timing, concluding the fact that there are separate circadian metabolic phenotypes.[76]

CIRCADIAN RHYTHM DYSFUNCTION IN METABOLIC SYNDROME

Misalignment stands for the state of being in the wrong position compared to something else or being improperly adjusted.[77] The most significant phase shifts concerning circadian rhythm are: sleep/wake cycle misalignment disrespecting the biological night, internal central vs. peripheral phase shift, rhythm of nutrient intake vs. sleep/wake or light/dark cycles.[57] A significant percentage of the risk factors for developing cardiovascular disease is covered by the well-known elements of metabolic syndrome: hypertension, dyslipidemia, elevated plasma glucose, and obe-

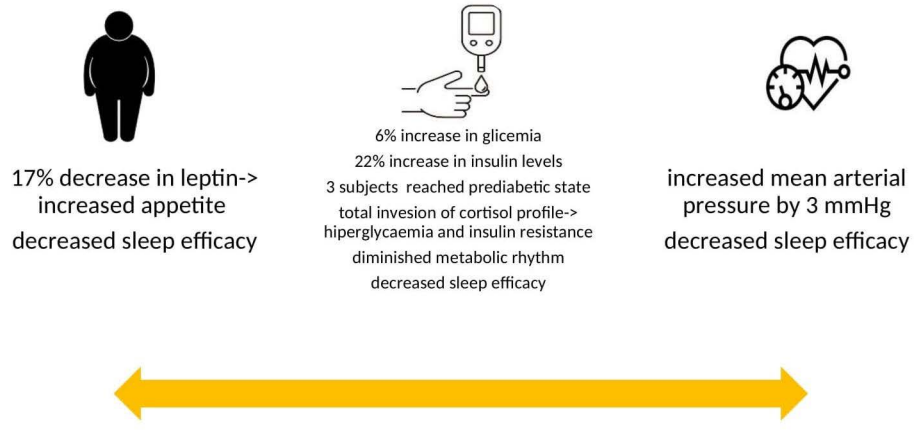

FIGURE 2. The effects of circadian misalignment on the components of metabolic syndrome[73]

sity.[78] During recent years, evidence has proven the link between circadian rhythm disturbances and these conditions.[79–81] This link was best defined by circadian misalignment protocols.[57] For instance, in a study conducted by Frank *et al*., 10 subjects went through 10 days of laboratory protocol, consisting of lengthened daily cycles (28-hour days). After gradually shifting behavioral cycle compared to daily circadian rhythm, a 12-hour difference was obtained. The detrimental effects of circadian misalignment were visible shortly after desynchrony occurred with changes highlighted in Figure 2.[82]

Numerous epidemiological studies found a substantial link between shift work and the emerging risk for developing the components of metabolic syndrome.[83–85] Not only in working-age adults but also in community-dwelling older populations, irregular daily activities measured by actigraphy increased the prevalence of metabolic syndrome. In contrast, balanced daily activity rhythms were linked to lower prevalence of cardiovascular disease.[86] A link with several other conditions, such as sleep disturbances,[87] depression,[88,89] cognitive decline,[90] steatohepatitis,[91] has been demonstrated as well.

A more complete understanding of the pathophysiological changes concerning metabolic syndrome has been offered by epigenetic findings.[92,93] DNA methylation processes are crucial factors in epigenetic alteration, coordinating tissue-specific gene expressions which demonstrated *the harmful effects of only one night of sleep loss*.[94] This was observed in the case of T2DM and obesity, in which '*metabolic memory*' and histone alterations defined gene expression involved in the development of *diabetes complications*.[95]

KEY CARDIOMETABOLIC FACTORS AND OUTCOMES INFLUENCED BY CIRCADIAN CLOCK MACHINERY

Cardiovascular implications

Several studies outlined the major consequence of circadian desynchronization in cardiovascular pathologies. Hypertension,[96] lack of nocturnal drops in blood pressure values, raised blood pressure variability, and altered daily rhythm of cardiac output were all associated to circadian misalignment.[97,98] The high incidence of myocardial infarction (MI) between 6 AM and 12 PM is in close connection with abnormal daily blood pressure patterns. Moreover, a circadian phenotype was recognized in patients with MI linked with BMAL1, CLOCK, and PER1 clock gene alterations.[99] The prevalence of life-threatening arrhythmias (ventricular fibrillation, ventricular tachycardia, sudden cardiac death) after awakening can be explained by the direct impact of the NSC on the electrophysiology of the heart via neurohormonal mechanisms and by local clock mechanisms through ion channel modifications.[100]

Metabolic profile

Preclinical studies of β-cell function in rodents evidenced the fact that several cellular mechanisms, such as decreased insulin exocytosis, altered mitochondrial function, and inadequate response to oxidative stress, are linked to pancreatic islet clock desynchronization. As a result, the size and function of the β-cell mass is decreased, leading to

TABLE 2. Comparison between advanced and delayed sleep-wake phase disorders[106]

Phenotype	Advanced sleep-wake phase disorder (ASWPD)	Delayed sleep-wake phase disorder (DSWPD)
Epigenetic variation	PER2 and CKI synergy[34,114]	PER3 H4 haplotype[115]
Characteristics	"Early bird"	"Night owl"
Wake-up time	Starting from 4 AM	After 10 AM
Bedtime	7 PM	2 AM
Sleep quality and length	Optimal	Optimal
Symptoms	Late afternoon fatigue Decreased work productivity Increased risk of accidents in the late afternoon[116]	Difficulty falling asleep and awakening on time Daytime fatigue Affected daytime function
Health consequences	Mood disorders, depression[116]	Mood disorders: insomnia, depression Psychiatric disorders: hyperactivity disorder, schizophrenia[116] T2DM, hypertension, low fasting and total LDL cholesterol[117]

PER2– Period 2 gene; CKI – casein kinase I; Per3 H4 – H4 haplotype of Period 3 gene

a worsened glycemic control.[101] The raising prevalence of T2DM is also determined by the dampened glucose tolerance throughout the day and the shifting daily fluctuation of glucose levels due to disorganized macronutrient intake.[102] Furthermore, individuals with T2DM and obesity show a distinct rhythm in circadian regulation of metabolic pathways influencing lipolysis, by decreased daily clock function and metabolic gene expression in the subcutaneous adipose tissue.[103]

Nonalcoholic fatty liver disease (NAFLD)

NAFLD is the most commonly associated chronic liver disease linked to metabolic syndrome; 25% of the population of all ages suffers from this condition during the current pandemic of obesity.[104] The accumulation of hepatic triglycerides along with oxidative stress, inflammation, and mitochondrial dysfunction are all in close connection with clock dysfunction, although the clear etiology has not yet been defined.[80,91]

Mental health

Chronic sleep disharmony has its own expression later in life, after several decades, resulting in diminished sleep quality, chronic fatigue, emergence of obesity, T2DM, and increased all-cause mortality.[105–108] Depression, cognitive decline, and other affective disorders caused by sleep/wake cycle disturbances are not only risk factors but also comorbidities and consequences of metabolic changes.[94,109,110] Light therapy and complementary methods were shown to restore the synchronization of the central clock and consequently the underlying peripheral clocks.[111]

Regarding epigenetic variations, there are two distinct disorders in which the starting and ending point of daily activities are off-axis, namely advanced sleep-wake phase disorder (ASWPD) and delayed sleep-wake phase disorder (DSWPD).[112]

Sleep duration is not as significant as the adjustment of starting point of the scheduled sleep episodes. In these conditions, if daily habitual sleeping rhythm of a person is suddenly disturbed by external factors, health consequences occur.[112]

Eveningness on its own, as shown in Table 2, leads to disharmony, even if ordinary day-night rhythm is conducted.[112] The important increase of HbA1c levels is aligned with delayed mid-sleep time and greater amount of food consumed during late night hours, leading to reduced glycemic control.[113]

CONCLUSION

All in all, the circadian machinery is a well-designed and structured system, coordinated by the nucleus suprachiasmaticus, the 'master clock', which converts external signals, divides them on a whole-body level, and integrates them into a 24-hour cycle. In understanding the complexity of both central and peripheral systems and their connection, we must search for signal disruption at an individual level by identifying probable causes of cardiometabolic disorders, which frequently relate closely to circadian misalignment.

CONFLICT OF INTEREST

Nothing to declare.

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