The circadian clock and metabolism

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ABSTRACT

Mammals have developed an endogenous circadian clock located in the SCN (suprachiasmatic nuclei) of the anterior hypothalamus that responds to the environmental light–dark cycle. Human homoeostatic systems have adapted to daily changes in a way that the body anticipates the sleep and activity periods. Similar clocks have been found in peripheral tissues, such as the liver, intestine and adipose tissue. Recently it has been found that the circadian clock regulates cellular and physiological functions in addition to the expression and/or activity of enzymes and hormones involved in metabolism. In turn, key metabolic enzymes and transcription activators interact with and affect the core clock mechanism. Animals with mutations in clock genes that disrupt cellular rhythmicity have provided evidence to the relationship between the circadian clock and metabolic homoeostasis. The present review will summarize recent findings concerning the relationship between metabolism and circadian rhythms.

INTRODUCTION: THE CIRCADIAN CLOCK IN MAMMALS

Most organisms on Earth are capable of predicting the light–dark phases and restricting their activity to certain hours throughout the 24-h cycle. By developing an endogenous circadian (circa—about and dies—day) clock, which is entrained to external stimuli, animals ensure that physiological processes are performed at the optimal time [1]. In mammals, the central circadian clock is located in the SCN (suprachiasmatic nuclei) of the anterior hypothalamus in the brain. The SCN clock is composed of multiple, single-cell circadian oscillators, which, when synchronized, generate co-ordinated circadian outputs that regulate overt rhythms [2–5]. Light is the most potent synchronizer for the SCN [6]. Light is perceived by the retina and the signal is transmitted via the RHT (retinohypothalamic tract) to the SCN [7–9]. Similar clock oscillators have been found in peripheral tissues, such as the liver, adipose tissue, intestine, heart and retina [9–15]. The circadian clock influences nearly all aspects of physiology and behaviour, including sleep–wake cycles, cardiovascular activity, endocrine system, physiology of the gastrointestinal tract and hepatic metabolism [9,16]. The SCN sends signals to peripheral oscillators in order to prevent the dampening of circadian rhythms in these tissues. The SCN accomplishes this task via neuronal connections or circulating humoral factors [17]. The fraction of cyclically expressed transcripts in each peripheral tissue ranges between 5% and 20% of the total population and the vast majority of these genes are tissue-specific [12,13,16,18–24]. These findings emphasize the circadian control over a large portion of the transcriptomes in peripheral tissues. For a peripheral tissue, signals from the central SCN clock or the local endogenous clock may control rhythmic gene expression [25,26].

Key words: circadian rhythm, clock, metabolism, nutrition, obesity, suprachiasmatic nuclei (SCN).
Abbreviations: AMPK, AMP-activated protein kinase; ARC, arcuate nucleus; BMAL1, brain and muscle Arnt-like protein-1; CLOCK, circadian locomotor output cycles kaput; CKIr, casein kinase 1r; CRY, CRYPTOCHROME; NAMPT, nicotinamide phosphoribosyltransferase; NEFA, non-esterified ‘free’ fatty acid; PPAR, peroxisome-proliferator-activated receptor; PER, PERIOD; PGC-1α, PPARγ co-activator 1α; REV-ERBα, reverse erythroblastosis virus α; ROR, retinoic acid receptor-related orphan receptor; ROER, ROR response element; SCN, suprachiasmatic nuclei; SIRT1, sirtuin 1; vmARC, ventromedial ARC.
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Recently, there has been a growing interest in the relationships between metabolism and circadian rhythms as it has been found that many hormones involved in metabolism, such as insulin, glucagon, adiponectin and corticosterone, exhibit circadian oscillation [27–30]. Leptin also exhibits striking circadian patterns in both gene expression and protein secretion, with peaks during the sleep phase in humans [31]. Neither feeding time nor adrenalectomy affected the rhythmicity of leptin release. However, ablation of the SCN has been shown to eliminate leptin circadian rhythm in rodents, suggesting that the central circadian clock regulates leptin expression [32]. In addition to the endocrine control, the circadian clock has been reported to regulate metabolism and energy homeostasis in peripheral tissues [33,34] by mediating the expression and/or activity of certain metabolic enzymes and transport systems [35,36]. Also, a large number of nuclear receptors involved in lipid and glucose metabolism has been found to exhibit circadian expression [37].

**THE MOLECULAR MECHANISM OF THE CIRCADIAN CLOCK**

The circadian clock is an intracellular mechanism sharing the same molecular components in SCN neurons and peripheral cells [38]. Concerted co-expression of specific clock genes is the heart of the core clock mechanism. CLOCK (circadian locomotor output cycles kaput) and BMAL1 (brain and muscle Arnt-like protein-1) are capable of activating transcription upon binding to E-box (5′-CACGTG-3′) sequences [9]. The proteins PERIODs (PER1, PER2 and PER3) and two CRYPTOCHROMEs (CRY1 and CRY2) serve as negative regulators [5,39,40] (Figure 1). Thus CLOCK:BMAL1 heterodimers bind to E-box sequences and mediate transcription of a large number of genes including those of the negative feedback loop Pers and Crys. When PERs and CRYs are produced in the cytoplasm, they oligomerize and translocate to the nucleus to inhibit CLOCK:BMAL1-mediated transcription (Figure 1). As part of this cyclical process, the stability of PERs and CRYs is tightly controlled by CKIs (casein kinase Is) and the F-box protein FBXL3 respectively [41–45]. All the aforementioned clock genes exhibit a 24-h rhythm in SCN cells and peripheral tissues, except for Clock that has been shown not to oscillate in the SCN [41]. Recent studies have demonstrated that CLOCK has intrinsic histone acetyltransferase activity, suggesting that rhythmic activation of chromatin remodelling may underlie the clock transcriptional network [46,47].

Other transcriptional loops involve Bmal1 expression. Bmal1 expression is negatively regulated by the transcription factor REV-ERBα (reverse erythroblastosis virus α) [48], which recruits HDAC (histone deacetylase) complexes [49]. Bmal1 expression is positively regulated by RORα (retinoic acid receptor-related orphan receptor α) and RORγ [50] via the RORE (ROR response element) [51] (Figure 1). This alternating promoter occupancy by RORs and REV-ERBα occurs because Rev-erbα and Rev-erbα expression is regulated by CLOCK:BMAL1 [48]. CKIs also phosphorylates and partially activates the transcription factor BMAL1 [52].

**KEY METABOLIC FACTORS ARE LINKED TO THE CORE CLOCK MECHANISM**

Recently, several key metabolic factors have been shown to be closely associated with the core clock mechanism. These findings further emphasize the tight control of the circadian clock over metabolism.

**PPARα (peroxisome-proliferator-activated receptor α)**

PPARα is a nuclear receptor family member that regulates the transcription of genes involved in lipid and glucose metabolism upon binding of endogenous NEFAs (non-esterified ‘free’ fatty acids). The circadian rhythmicity of PPARα provides an example of a reciprocal link between circadian and lipid metabolic processes. The CLOCK:BMAL1 heterodimer mediates transcription of PPARα, which subsequently binds to the PPRE (PPAR-response element) and activates transcription of Bmal1 [53–55] (Figure 1). Bmal1 has also been shown to be regulated by PPARγ in cells of the aorta [56].

**REV-ERBα**

REV-ERBα, a pro-adipogenic transcription factor whose levels increase dramatically during adipocyte differentiation [57], is a negative regulator of Bmal1 expression [48], as mentioned above (Figure 1). REV-ERBα exhibits striking diurnal variations in expression in murine adipose tissue [58] and rat liver [59]. During adipocyte differentiation, REV-ERBα has been shown to act downstream of the differentiation factor PPARγ by facilitating gene expression of PPAR target genes [60,61]. Ectopic REV-ERBα expression in 3T3L1 pre-adipocytes promotes their differentiation into mature adipocytes [60].

**RORα**

RORα, which regulates lipogenesis and lipid storage in skeletal muscle, is a positive regulator of Bmal1 expression [50,62,63] (Figure 1). Mice deficient in RORα or REV-ERBα have impaired circadian locomotor activity and gene expression [48,50].

**SIRT1 (sirtuin 1)**

CLOCK and its homologue NPAS2 can bind efficiently to BMAL1 and consequently to E-box sequences
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**Figure 1** Interaction among metabolic factors and the circadian core mechanism

High NADPH levels promote CLOCK:BMAL1 binding to E-box sequences leading to the acetylation of BMAL1 and expression of Pers, Cry1s and other clock-controlled metabolic genes. The negative feedback loop, PERs:CRYs, binds to CLOCK:BMAL1 and consequently PERs are acetylated. When AMPK is activated by high AMP levels, it phosphorylates CKIε, leading to PER phosphorylation, increases NAD⁺ levels leading to the deacetylation of PERs and BMAL1 by SIRT1, and phosphorylates CRYs. Consequently, PERs:CRYs repression is relieved, phosphorylated PERs and CRYs are degraded and another cycle begins. NAMPT whose expression is controlled by CLOCK:BMAL1, increases synthesis of NAD⁺. RORα stimulates and REV-ERBα inhibits Bmal1 transcription, acting through ROREs. Expression of Bmal1 is also positively regulated by PPARα. RORα needs a co-activator, PGC-1α, which is phosphorylated by AMPK. In parallel, AMPK activation leads to an increase in NAD⁺ levels, which, in turn activate SIRT1. SIRT1 activation leads to PGC-1α deacetylation and activation. Ac-ADP-r, acetyl ADP ribose; NAM, nicotinamide.

In the presence of reduced nicotinamide adenine dinucleotides (NADH and NADPH) (Figure 1). The oxidized forms, NAD⁺ and NADP⁺, inhibit DNA binding of CLOCK/NPAS2:BMAL1 [64,65]. As the NADP⁺/NADPH redox equilibrium depends on the metabolic state of the cell, this ratio could dictate the binding of CLOCK/NPAS2:BMAL1 and result in phase-shifts of gene expression. SIRT1, an NAD⁺-dependent histone deacetylase involved in transcriptional silencing, genome stability and a key factor in the longevity response to caloric restriction [66,67], interacts directly with CLOCK and deacetylates BMAL1 and PER2 [68,69] (Figure 1). After binding to E-box, CLOCK and CBP/p300 acetylate histones H3 and H4 [46] and BMAL1 [70]. BMAL1 acetylation potentiates its binding by the repressive PER/CRY complex [70] and, as a result, PER2 is acetylated [68]. When acetylated, PER2 [68] and possibly BMAL1 [69] are more stable (Figure 1). SIRT1 then becomes activated and starts deacetylating BMAL1, PER2 and histones [71]. Deacetylated PER2 is further phosphorylated and degraded and a new cycle begins. It has also been shown that CLOCK:BMAL1 heterodimer regulates the circadian expression of NAMPT (nicotinamide phosphoribosyltransferase), a rate-limiting enzyme in the NAD⁺ salvage pathway. SIRT1 is recruited to the Nampt promoter and contributes to the circadian synthesis of its own coenzyme [72,73].

**AMPK (AMP-activated protein kinase)**

High levels of AMP signal low energy in the cell. Interestingly, AMPK, an important nutrient sensor, phosphorylates CKIε, resulting in increased CKIε activity and degradation of PER2. PER2 degradation leads to a phase advance in the circadian expression pattern of clock genes [74]. In addition, the expression profile of clock-related genes, such as Per1 and Cry2, in skeletal muscle in response to AICAR (5-amino-4-imidazole-carboxamide riboside), an AMPK activator, as well as the diurnal shift in energy utilization, is impaired in AMPKγ3 subunit knockout mice [75]. AMPK also enhances SIRT1 activity by increasing cellular NAD⁺ levels, resulting in the deacetylation and modulation of the activity of downstream SIRT1 targets [76] (Figure 1). AMPK has also been shown to phosphorylate and destabilize CRY1 in mouse fibroblasts, leading to altered circadian rhythms [77].

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**PGC-1α (PPARγ co-activator 1α)**

PGC-1α, a transcriptional co-activator that regulates energy metabolism, is rhythmically expressed in the liver and skeletal muscle of mice. PGC-1α stimulates the expression of Bmal1 and Rev-erba, through co-activation of the ROR family of orphan nuclear receptors [78,79] (Figure 1). Mice lacking PGC-1α show abnormal diurnal rhythms of activity, body temperature and metabolic rate due to aberrant expression of clock genes and those involved in energy metabolism. Analyses of PGC-1α-deficient fibroblasts and mice with liver-specific knockdown of PGC-1α indicate that it is required for cell-autonomous clock function [78]. PGC-1α is activated by AMPK phosphorylation and SIRT1 deacetylation [80] (Figure 1).

Further research of the mechanisms, in which the aforementioned metabolic regulators are involved, is warranted. As some of these regulators can be modulated by medications, the effect of the medications should be tested for their ability to alter circadian rhythms.

**EFFECT OF METABOLIC HORMONES ON THE CIRCADIAN CLOCK**

It seems that leptin could be the bridge between energy homeostasis and circadian control, due to its circadian oscillation and expression of its receptor in several hypothalamic regions. Obese leptin-deficient ob/ob mice have a strong ultradian, rather than a circadian activity pattern and show increased activity during the light period compared with controls, suggesting disrupted rhythms [81,82]. Receptors for leptin and ghrelin are present on SCN cells [83–85], so it is possible that these hormones bind directly to SCN neurons, similarly to their effect on NPY/AgRP (neuropeptide Y/Agouti-related protein) neurons in the ARC (arcuate nucleus). Activation of vmARC (ventromedial ARC) neurons by systemic administration of the ghrelin mimetic GH (growth hormone)-releasing peptide-6 combined with SCN tracing showed that vmARC neurons transmit feeding-related signals to the SCN [84]. This injection induced Fos in the vmARC and resulted in attenuation of light-induced phase delay in mice and light-induced Fos expression in the SCN in rats [86]. Administration of ghrelin to SCN slices or SCN explants in vitro caused phase shifts in Per2::lac reporter gene expression. However, administration of ghrelin to wild-type mice only caused phase shifts after 30 h of food deprivation, whereas intraperitoneal injection of ghrelin did not cause phase shifts in wild-type mice fed ad libitum [87]. Thus it seems that ghrelin and leptin may affect the SCN. However, further research is required to determine whether this effect is direct or via the ARC.

**METABOLIC DISORDERS GO HAND IN HAND WITH DISRUPTION OF CIRCADIAN RHYTHMS**

Recent studies demonstrate that disruption of circadian rhythms may lead to manifestations of the metabolic syndrome [88–90]. Circadian control of glucose metabolism is implicated by the variation in glucose tolerance and insulin action across the day [91,92]. Evidence suggests that loss of circadian rhythmicity of glucose metabolism may contribute to the development of metabolic disorders, such as Type 2 diabetes, in both rodents [93–95] and humans [92,96]. For example, daily cycles of insulin secretion and glucose tolerance are lost in patients with type 2 diabetes [92,97], as are daily variations in plasma corticosterone levels and locomotor activity in streptozotocin-induced diabetic rats [93,94]. In addition, some clock genes exhibited altered expression in the liver, heart and kidney in diabetic animals [13,98,99]. These findings indicate that a critical relationship exists between endogenous circadian rhythms and diabetes. Interestingly, the oscillations of clock and adipokine genes were mildly suppressed in the adipose tissue of obese KK mice and greatly suppressed in the adipose tissue of obese diabetic (KK-AV) mice compared with wild-type mice [30]. Similarly, obese diabetic mice exhibited circadian oscillation of most genes in the liver, but some genes had attenuated, but still rhythmic, expression [100]. In addition, in type 1 diabetes patients, lipolysis increased earlier in the evening than in healthy controls and remained elevated throughout the night, indicating that lipolysis shows a distinct circadian rhythm that is altered in type 1 diabetes patients [101]. These findings point to the tight relationship between disruption of circadian rhythms and metabolic disorders. In addition, resetting circadian rhythms may help to alleviate some of the symptoms seen in these disorders.

**KNOCKOUTS OR MUTATIONS IN CLOCK GENES LEAD TO METABOLIC DISORDERS**

The most compelling evidence that the circadian clock controls metabolism and that circadian disruption is associated with multiple negative metabolic manifestations, is demonstrated by clock gene mutant mouse models.

**Clock**

Homozygous C57BL/6J ClockΔ19 mice, with a truncated exon 18 and deleted exon 19 of the Clock gene, have an attenuated diurnal feeding rhythm, are hyperphagic and obese, and develop a metabolic syndrome of hyperlipidaemia, hepatic steatosis and hyperglycaemia [102]. In addition, ClockΔ19 mice (C57BL/6J) had altered gluconeogenesis. Insulin administration caused significantly greater hypoglycaemia in ClockΔ19 mutant
mice than in wild type mice [103]. Increased insulin sensitivity was also seen in \( \text{Clock}^{\Delta 19} \) mutant, melatonin producing mice of the BALB/c/CBA background together with fasting hyperglycaemia in young adult males, fasting hyperglycaemia in older females, and substantially impaired glucose tolerance overall [104]. In \( \text{Clock}^{\Delta 19} \) on a Jcl:ICR background, serum levels of triacylglycerol (triglyceride) and NEFAs were significantly lower than in wild-type control mice, whereas total cholesterol and glucose, insulin and leptin levels did not differ [105]. Unlike C57BL/6J \( \text{Clock}^{\Delta 19} \) mutant mice [102], neither male nor female Jcl:ICR \( \text{Clock}^{\Delta 19} \) mutant mice were obese, and they mostly had low or normal fasting plasma glucose and low plasma NEFAs. Combination of the \( \text{Clock}^{\Delta 19} \) mutation (Jcl:ICR) with the leptin knockout (ob/ob) resulted in significantly heavier mice than the \( \text{ob}/\text{ob} \) phenotype [106]. However, in Jcl:ICR \( \text{Clock}^{\Delta 19} \) mutant mice, a high-fat diet amplified the diurnal variation in glucose tolerance and insulin sensitivity and obesity was attenuated through impaired dietary fat absorption [105]. Triacylglycerol content in the liver was significantly less increased in Jcl:ICR \( \text{Clock}^{\Delta 19} \) mutant mice fed on a high-fat diet compared with wild-type mice. Jcl:ICR \( \text{Clock}^{\Delta 19} \) mutant mice had attenuated daily rhythms of \( \text{Acc}4 \) (acyl-CoA synthetase long-chain 4) and \( \text{Fabp}1 \) (fatty acid binding protein 1) gene expression in the liver under both normal and high-fat diet conditions compared with wild-type mice, which could have led to the attenuated accumulation of triacylglycerols in the liver under a high-fat diet [107]. In \( \text{Clock}^{\Delta 19} \) mutant, melatonin producing mice of the BALB/c/CBA background, relative weight of epigonadal fat compared with body weight was not significantly different between male wild-type and mutant mice fed on a high-fat diet [104]. Although the effects on metabolism were variable, due to strain differences, overall, it seems that disruption of \( \text{Clock} \) leads to altered metabolic pathways. Analyses of other mouse strains or other rodent species may aid in getting a clearer picture as to the effect of the \( \text{Clock} \) mutation.

**Bmal1**

\( Bmal1^{-/-} \) knockout mice, similarly to C57BL/6J \( \text{Clock}^{\Delta 19} \) mutant mice, exhibited suppressed diurnal variations in glucose and triacylglycerols as well as abolished gluconeogenesis [103]. Liver-specific deletion of \( Bmal1 \) showed a direct effect of the liver clock on glucose metabolism, as exhibited by hypoglycaemia during fasting, exaggerated glucose clearance and loss of rhythmic expression of hepatic glucose regulatory genes [108]. Embryonic fibroblasts from \( Bmal1^{-/-} \) knockout mice failed to differentiate into adipocytes. Loss of BMAL1 expression led to a significant decrease in adipogenesis and gene expression of several key adipogenic/lipogenic factors, such as PPARγ2, adipocyte fatty aP2 (acid-binding protein 2), SREBP-1a (sterol-

regulatory-element-binding protein 1a) and FAS (fatty acid synthase). Furthermore, overexpression of BMAL1 in adipocytes increased lipid synthesis activity. These results indicate that BMAL1, a master regulator of circadian rhythm, also plays important roles in the regulation of adipose differentiation and lipogenesis in mature adipocytes [109].

**Per2**

\( \text{Per2}^{-/-} \) mice exhibit no glucocorticoid rhythm even though the corticosterone response to hypoglycaemia is intact. In addition, the diurnal feeding rhythm is absent in \( \text{Per2}^{-/-} \) mice. Although food consumption is similar during the light and dark periods on a high-fat diet, \( \text{Per2}^{-/-} \) mice develop significant obesity [110]. In addition, \( m\text{Per2}^{-/-} \) mice also exhibit increased bone density in mice [111]. As bone and adipose tissue share a common ontogeny, it is possible these findings may also have implications for adipogenesis [112].

Analyses of other clock gene mutants will help to understand better the mechanism by which the circadian clock controls metabolism.

**CONCLUSIONS**

The prominent influence of the circadian clock on human physiology is demonstrated by the temporal and pronounced activity of a plethora of systems, such as sleep–wake cycles, feeding behaviour, metabolism and physiological and endocrine activity. Disrupted circadian rhythms might lead to attenuated feeding rhythms, disrupted metabolism and obesity. Disruptions of rhythms together with genetic background increase the risk to develop these health complications. Findings in murine models show the strong link between genetic background and circadian rhythm disruption in determining the severity of metabolic disorders. However, further study is required in order to fully understand the intricate relationships between the circadian clock and metabolism. This understanding may lead to the resetting of the circadian clock leading, in turn, to better functionality of physiological systems, prevention of metabolic disorders and promotion of well-being.

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