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HIGHLIGHTED TOPIC | *The Role of Clock Genes in Cardiometabolic Disease*

## Clock genes and metabolic disease

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**Marcheva B, Ramsey KM, Affinati A, Bass J.** Clock genes and metabolic disease. *J Appl Physiol* 107: 1638–1646, 2009. First published August 6, 2009; doi:10.1152/jappphysiol.00698.2009.—The circadian system is a key integrator of behavior and metabolism that synchronizes physiological processes with the rotation of the Earth on its axis. In mammals, the clock is present not only within the central pacemaker neurons of the hypothalamus, but also within extra-suprachiasmatic nucleus (SCN) regions of brain and nearly all peripheral tissues. Recent evidence suggests that the complex feedback networks that encompass both the circadian and metabolic systems are intimately intertwined and that disruption of either system leads to reciprocal disturbances in the other. We anticipate that improved understanding of the interconnections between the circadian and metabolic networks will open new windows on the treatment of sleep and metabolic disorders, including diabetes mellitus and obesity.

metabolic syndrome; circadian rhythms; sirtuins; sleep disorders; diabetes mellitus

## CLOCKS, METABOLISM, AND DISEASE

DIVERSE ORGANISMS, ranging from unicellular fungi to plants and vertebrates, have evolved an internal molecular timekeeping mechanism to synchronize endogenous systems with the 24-h environmental light-dark cycle (hence the term circadian, which derives from *circa diem*, or “about a day”). In vertebrates, the circadian clock synchronizes cycles of fuel acquisition, storage, and utilization in anticipation of the daily sleep-wake cycle that corresponds with periods of fasting and feeding. While the existence of an internal clock was originally proposed based on studies of circadian flowering patterns in the mimosa plant more than 200 years ago, the molecular principles of the clock have only recently emerged with the powerful application of forward genetics in flies, plants, and mammals. Positional cloning has revealed that the cellular oscillator is programmed by a conserved transcription-translation feedback loop generated by the rhythmic and opposing action of a set of transcriptional activators and repressors (90). This endogenous timekeeping mechanism is expressed in master pacemaker neurons and nearly all peripheral tissues where it maintains synchrony of diverse processes with ~24-h precision.

It has been recognized for years that many aspects of human health and disease exhibit marked variation across the day and night. Heart attacks, stroke, flash pulmonary edema, and hypertensive crises tend to peak at particular times of the day (17, 59). Association studies have revealed that shift workers, night workers, and sleep-deprived individuals have an increased risk of developing symptoms of the metabolic syndrome (18, 26,

39, 86). Furthermore, there is well-established rhythmicity in many metabolic processes. Lipid and carbohydrate metabolism, hormone release, blood pressure, and the production of coagulation factors all exhibit circadian oscillations. In particular, the cyclical nature of blood glucose regulation has long been established (30). In humans, blood glucose levels peak right before the onset of the activity period (4, 8), and oral glucose tolerance is impaired in the afternoon and evening compared with morning hours, coinciding with a decrease in insulin secretion and altered insulin sensitivity in the evening. Interestingly, these daily cycles of insulin secretion and sensitivity are lost in diabetic patients (7), and diurnal variations of corticosterone and locomotor activity are also abolished in diabetic rats (69, 97). While these studies indicate that there is a critical relationship between metabolic processes and the time of day, the recent availability of genetic and molecular tools has transformed our understanding of the function of the clock transcription network in these processes.

## MOLECULAR BASIS OF THE CIRCADIAN OSCILLATOR

The first mammalian circadian gene *Clock* (*circadian locomotor output cycles kaput*) was discovered through an unbiased mutagenesis screen in the early 1990s (3, 40, 98). Rapid advances in the field in the ensuing years have revealed that the molecular machinery controlling circadian timekeeping consists of a core transcription/translation feedback loop that produces 24-h rhythmic patterns of gene transcription (Fig. 1). CLOCK and its partner BMAL1, both bHLH-PAS (basic helix-loop-helix-Period-Arnt-Single-minded) transcription factors, comprise the positive limb of the circadian oscillator (11, 37). In extra-suprachiasmatic nucleus (extra-SCN) tissues, neuronal PAS domain protein 2 (NPAS2) functions as a CLOCK orthologue (74). Loss-of-function mutations in *Clock* are com-

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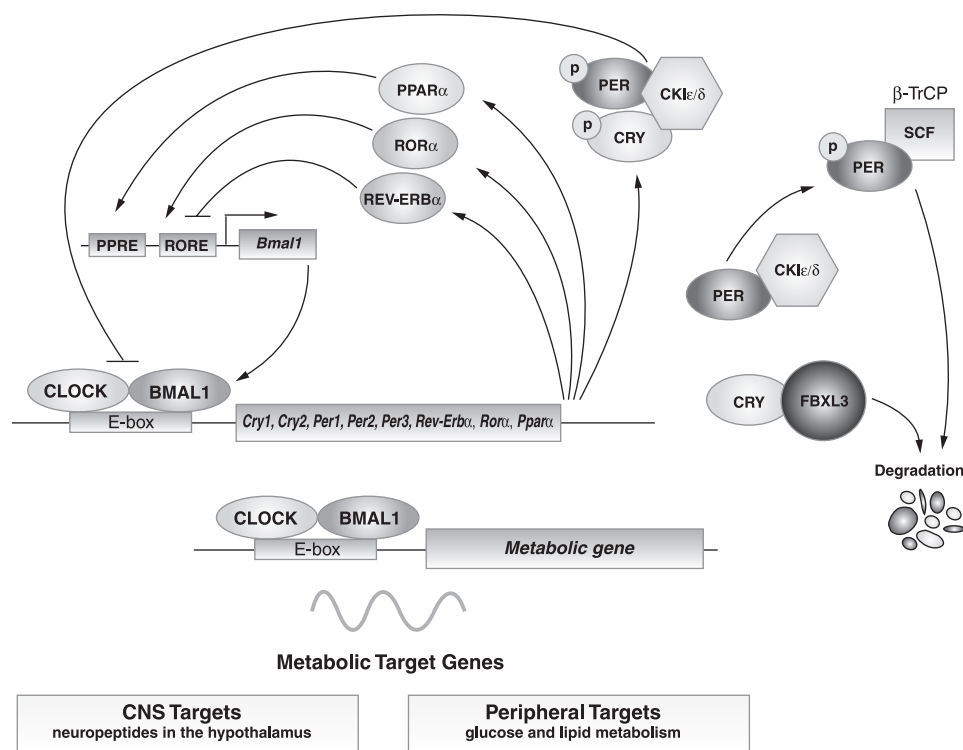


Fig. 1. Core circadian clock network. The core molecular clock machinery is encoded by interlocking transcription-translation feedback loop that oscillates with a 24-h periodicity. The core mammalian clock comprises transcription factors CLOCK and BMAL1, which heterodimerize to drive the transcription of downstream target genes containing E-box enhancer elements. Among these, the period (PER) and cryptochrome (CRY) proteins then multimerize and inhibit the action of the CLOCK:BMAL1 complex, resulting in a rhythmic oscillation of the core clock genes and many of their downstream targets. In addition, the CLOCK:BMAL1 heterodimer also drives the transcription of metabolic target genes in the central nervous system (CNS) and in the peripheral tissues. CKI $\epsilon/\delta$ , casein kinase I-epsilon and -delta; ROR, retinoid-related orphan receptors; RORE, ROR-binding element; PPAR $\alpha$ , peroxisome proliferator-activated receptor- $\alpha$ ; SCF, Skp/Cullin/F-box.

compensated by *Npas2* in SCN, giving rise to normal locomotor activity rhythms in *Clock*-deficient mice, although oscillation of clock genes within peripheral tissues is not fully compensated in the absence of CLOCK (23, 24). CLOCK is necessary and sufficient to produce circadian oscillation in neuronal cells (108). CLOCK:BMAL1 functions as a heterodimer to activate the rhythmic transcription of genes containing E-box enhancer sequences (60). The period genes *Per1*, *Per2*, and *Per3* and the cryptochrome genes *Cry1* and *Cry2* are downstream targets of CLOCK:BMAL1 and encode components of the negative-feedback loop. On translation, the PER and CRY proteins form heterodimers that translocate to the nucleus and repress the transcriptional activity of CLOCK:BMAL1 (32, 82, 95, 111). Phosphorylation of PER and CRY proteins by casein kinase I-epsilon and -delta (CKI $\epsilon/\delta$ ) and the subsequent proteasomal degradation of PERs is an important step in the generation of circadian rhythmicity (1). Recent discovery that mutation of the F-box protein FBXL3, a component of the E3 ubiquitin ligase complex, results in a period lengthening in mice suggests that steps in the degradative pathway also play a key role in generating overt rhythmicity (13, 33, 84). Other interlocking loops further modulate the function of CLOCK and BMAL1. For example, CLOCK and BMAL1 activate transcription of the orphan nuclear receptors *Rev-Erb $\alpha$*  and *Ror $\alpha$* , which in turn comprise a short feedback loop that modulates *Bmal1* transcription. REV-ERB $\alpha$  represses, while ROR $\alpha$  activates, *Bmal1* transcription through shared ROR-binding elements (ROREs) within the *Bmal1* promoter (72).

Studies of mutations in core clock genes have demonstrated their functional roles in the generation of approximately 24-h periodicity of behavior. Mice lacking *Bmal1* or *Per2*, or mice expressing a dominant negative *Clock* mutation, become arrhythmic in constant darkness (3, 11, 109). However, knockout of *Per1*, *Per3*, *Cry1*, or *Cry2* leads to either shortening or

lengthening of circadian period, but not to arrhythmicity, suggesting functional redundancy among the components of the clock machinery (95, 109).

In vertebrates, the master clock mechanism was initially identified through lesioning studies in the SCN in the hypothalamus (87). The core clock machinery was later detected in other brain regions and in the periphery, including tissues important for normal cardiometabolic function (21, 107). The SCN is still considered to be the master circadian pacemaker, driving rhythmic behavior and coordinating peripheral tissue clocks. The function of most peripheral clocks remains to be defined, although recent studies have begun to elucidate the role of peripheral clocks in maintenance of energy balance and in metabolic homeostasis.

#### INTEGRATION OF MOLECULAR CLOCK AND METABOLIC TRANSCRIPTION NETWORKS

Understanding the mechanisms by which the molecular clock controls metabolic transcription networks, and ultimately energy homeostasis, has emerged as an exciting and challenging area. Mounting evidence suggests that various metabolic networks are under circadian control. Reportedly, 3% to 20% of all gene transcripts display a 24-h variation in mRNA levels in both the SCN and peripheral tissues. The pervasive effect of circadian regulation of gene expression in the periphery is demonstrated by the rhythmic transcription of numerous metabolic genes in liver, skeletal muscle, brown and white adipose tissue, heart, and vasculature (2, 66, 70, 78, 88, 105, 110). Among the rhythmic genes identified, many play key roles in lipid and cholesterol biosynthesis, carbohydrate metabolism and transport, oxidative phosphorylation, and detoxification pathways. The observation that key rate-limiting enzymes involved in these processes are under circadian control high-

lights the pervasive role of circadian clocks in normal organismal function (70). Of note, only a small subset of key metabolic genes are direct targets of the core clock genes (65, 76). Many of these targets are transcription factors or other modulators of transcription and translation that, in turn, impart rhythmicity on downstream metabolic genes (70). For example, the core circadian machinery induces daily oscillations in *D-site binding protein (Dbp)*, a transcription factor regulating key gluconeogenic and lipogenic genes (29, 49, 76). Mutation of core circadian genes abolishes rhythmic expression and/or shifts the phase of oscillation of a large number of metabolic genes (43, 53, 67, 70).

Importantly, the period and amplitude of oscillation and the level of expression of each of these metabolic genes vary among different tissues, suggesting a physiologically meaningful role of peripheral clocks for normal cellular function. Interestingly, only a limited number of genes are rhythmically expressed in multiple peripheral tissues (88). In fact, the pattern of gene expression in each peripheral tissue seems to correlate with physiological function. For example, a large portion of rhythmic genes in the muscle show a nadir of expression in the middle of the subjective night, coinciding with the peak of physical activity (53). Many genes in fat peak in the middle of the light period, several hours after the initial bout of feeding, and are possibly associated with nutrient storage (41, 88). Rhythmic genes in the liver peak either at the beginning of day or onset of night, likely reflecting the alternating requirements for lipogenic and lipid catabolic genes across the light-dark feeding-fasting cycle (55, 70). A major question remains as to whether the rhythmic pattern of gene expression arises due to intrinsic clock expression in brain or peripheral tissue, or rather is secondary to the feeding rhythm, although studies in the ultradian vole and mice suggest that certain rhythmic patterns occur independently of the feeding rhythm (96).

A series of recent studies on nuclear hormone receptors (NHRs) further support the extensive interconnections between the circadian clock and energy metabolism. NHRs sense fat-soluble hormones, vitamins, and lipids and regulate various aspects of lipid and carbohydrate metabolism (19). Intriguingly, many NHRs, including *Rev-erba*, *Rora*, and *Ppara*, exhibit a tissue-specific 24-h pattern of gene expression and are directly regulated by the CLOCK:BMAL1 heterodimer (105). In addition to affecting metabolic gene expression, all three of these NHRs participate in circadian feedback loops and regulate the core clock genes. REV-ERB $\alpha$ , an inhibitor of *Bmal1* expression, suppresses hepatic gluconeogenic gene transcription and affects glucose output (106). Furthermore, REV-ERB $\alpha$  is required for adipogenesis, adipocyte differentiation, and lipid metabolism (100). The orphan nuclear receptor ROR $\alpha$ , a positive regulator of *Bmal1*, is also involved in lipogenesis and lipid storage (80). The rhythmically expressed peroxisome proliferator-activated receptor- $\alpha$  (PPAR $\alpha$ ) further induces *Bmal1* transcription (16). Importantly, on binding to endogenous free fatty acids, PPAR $\alpha$  also regulates transcription of genes involved in lipid and glucose metabolism (25). Furthermore, the PPAR transcriptional coactivator PGC-1 $\alpha$  regulates adaptive energy metabolism, is rhythmically expressed, and stimulates *Bmal1* transcription (50–52). Mice lacking *Pgc1 $\alpha$*  display altered locomotor activity rhythm (52).

In sum, the NHRs participate in the reciprocal interaction between circadian and metabolic regulatory networks.

Although the aforementioned studies demonstrate that extensive interactions exist between the core clock machinery and metabolic networks, it is not yet clear as to what factors link the regulation of metabolic and circadian systems. Research into how important metabolic sensors interact with the molecular circadian clock is an active area of investigation.

#### NEW ROLE FOR CHROMATIN MODIFICATION PATHWAY AND NAD

One of the early clues to explain the interdependence of circadian and metabolic processes stemmed from the finding by Rutter et al. (79) suggesting that changes in the cellular redox status of the cell, represented by nicotinamide adenine dinucleotide cofactors NAD(H) and NADP(H), regulate the transcriptional activity of CLOCK and its homolog NPAS2. First, the reduced forms of these redox cofactors enhance DNA binding of CLOCK:BMAL1 and NPAS2:BMAL1 heterodimers, while the oxidized forms inhibit binding. Second, it had been reported that the presumptive cytokine *Pbef*, or pre-B-cell colony enhancing factor, displayed a circadian pattern of gene expression in both liver and heart (70, 88). Interestingly, this gene was later identified as *Nampt*, or nicotinamide phosphoribosyltransferase, and was demonstrated to encode the rate-limiting enzyme in the NAD<sup>+</sup> biosynthesis pathway (75). Increasing the dosage of *Nampt* increases both the total cellular NAD<sup>+</sup> levels and the transcriptional activity of SIRT1, an NAD<sup>+</sup>-dependent deacetylase that has been shown to regulate both histone modification and a host of physiological processes, including glucose homeostasis, fat metabolism, insulin secretion, and apoptosis (9, 57, 75). SIRT1 catalyzes a unique reaction in which hydrolysis of NAD<sup>+</sup> is coupled with protein deacetylation, resulting in the formation of nicotinamide, *O*-acetyl-ADP-ribose, and deacetylated protein substrates. Because SIRT1 relies on NAD<sup>+</sup> for its enzymatic activity, NAD<sup>+</sup> biosynthetic pathways play a critical role in the regulation of SIRT1 function. Of note, *Nampt* was one of only 37 genes that showed identical phases of peak expression in both liver and heart (88), suggesting that circadian regulation of NAMPT-mediated NAD<sup>+</sup> biosynthesis and SIRT1 activity may represent a central core mechanism in the generation or maintenance of circadian patterns in multiple tissues.

A negative-feedback loop linking both the NAMPT-mediated NAD<sup>+</sup> biosynthesis pathway and SIRT1 with the core circadian clock circuitry has recently been described by several groups (5, 61, 62, 73) (Fig. 2). CLOCK and BMAL1 directly regulate the transcription of *Nampt*, which oscillates at both the RNA and protein levels in a circadian fashion, peaking around zeitgeber (ZT) 14. The circadian oscillation of NAMPT directly translates to daily oscillations in NAD<sup>+</sup> levels in liver, and the peak in NAMPT and NAD<sup>+</sup> corresponds with the reported peak in SIRT1 activity around ZT15. SIRT1 then physically interacts with the positive limb of the core clock feedback loop (CLOCK and BMAL1) and is recruited to the promoters of clock target genes. Genetic and pharmacologic manipulation of the NAD<sup>+</sup> biosynthetic pathway and SIRT1 reveal that SIRT1 negatively regulates CLOCK and BMAL1 activity. Because both NAMPT and SIRT1 are acutely sensitive to the metabolic state of the cell, the NAMPT/SIRT1/

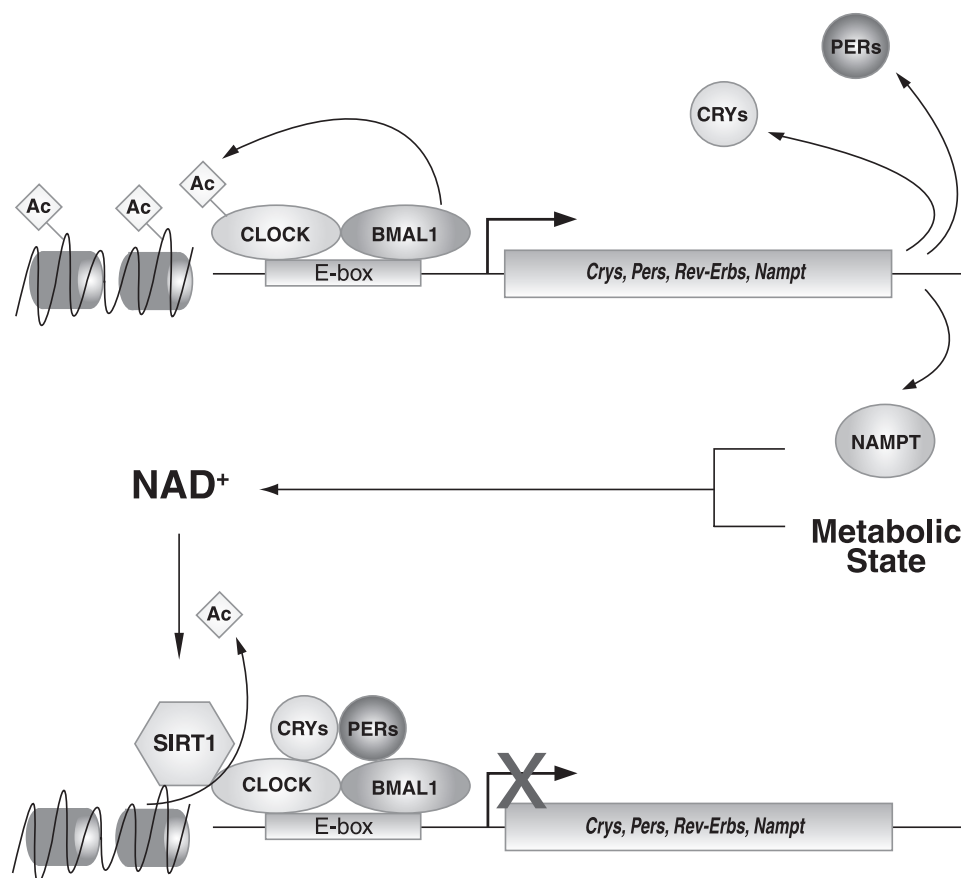


Fig. 2. Negative-feedback loop linking  $\text{NAD}^+$ /SIRT1 with the Clock network. NAMPT, the rate-limiting enzyme in NAD biosynthesis, oscillates in a circadian pattern under the transcriptional control of the CLOCK:BMAL1 heterodimer. Alterations in NAMPT drive oscillation in NAD biosynthesis, which in turn modulates the nutrient responsive deacetylase SIRT1. SIRT1 plays an important role in energy homeostasis and has recently been discovered to function in the control of the circadian clock. SIRT1 activity, dependent on the metabolic state of the cell, inhibits CLOCK:BMAL1 activity and prevents the transcription of both circadian and metabolic downstream target genes. The CLOCK:BMAL1-NAMPT-SIRT1 pathway comprises a novel metabolic feedback that may integrate the daily cycles of activity, feeding, and energy homeostasis. Ac, acetyl.

CLOCK:BMAL1 feedback loop is key to understanding how the circadian clock is able to maintain synchrony of the metabolic oscillators in peripheral tissues in response to nutritional input. Furthermore, the rhythmic production of  $\text{NAD}^+$  is also likely to play a critical role in a number of downstream metabolic pathways, as well as chromatin regulation and potentially aging.

#### EXPERIMENTAL GENETIC EVIDENCE FOR CROSSTALK BETWEEN CIRCADIAN AND METABOLIC TRANSCRIPTION NETWORKS

A major transformation in our understanding of peripheral clocks originated with the demonstration of cell-autonomous circadian gene oscillation in cultured fibroblasts (6). Prior to this work the prevailing model held that circadian oscillation was a unique feature of the neuron. Subsequent creation of biological reporter mice expressing luciferase driven by the promoters of cycling clock genes has enabled real-time monitoring of circadian oscillators from live cell explants of both SCN and peripheral tissues. Studies in luciferase reporter mice have demonstrated the existence of self-sustained oscillators in a variety of peripheral tissues, including liver and muscle (102, 103, 107). Bioluminescence imaging has even enabled detection of persistent circadian oscillation rhythms at the level of single cells (101). Interestingly, the period and phase of expression of key circadian genes vary between tissues of the same animal, indicating presence of local factors that modify function of the core clock loop.

Analysis of circadian mutant animals has begun to provide insight into the metabolic role of clock function in the periphery (Table 1). For example, the dominant *Clock* gene mutation  $\Delta 19$  causes hyperlipidemia, hyperleptinemia, hyperglycemia, and hypoinsulinemia (94). In addition to altered rhythms of locomotor activity, loss of BMAL1 impairs adipogenesis, adipocyte differentiation, and hepatic carbohydrate metabolism (43, 77, 83). BMAL1 also exerts profound effects on skeletal muscle function (53). PER2 deficiency abolishes glucocorticoid rhythmicity and causes alterations in bone density (28, 104). Lack of both CRY1 and CRY2 impairs body growth, changes patterns of circulating growth hormone, and modifies expression of lipogenic and steroidogenic pathways (12). Furthermore, transgenic overexpression of mutant CRY1 results in polydipsia, polyuria, and hyperglycemia, all symptoms of diabetes mellitus (68). Mutations downstream of the core clock, the so-called clock-controlled genes, have also been shown to impact metabolism. For example, ablation of the circadian deadenylase *Nocturnin*, which is involved in posttranscriptional regulation of rhythmic gene expression, alters glucose tolerance, peripheral tissue insulin sensitivity, and response to high-fat diet (35).

Recent evidence suggests that circadian regulation of metabolic processes involves both global and cell-autonomous signals to maintain temporal coordination of feeding cycles and metabolic function. Tissue-specific circadian gene knockout and transgenic animals offer unique opportunities to further elucidate the role of cell autonomous clock function in metab-

Table 1. Behavioral and metabolic phenotypes of circadian gene mutants

Gene Disruption	Mutation	Circadian Phenotype	Metabolic Phenotype	Reference
<i>Clock</i>	Systemic dominant negative mutation	Arrhythmicity in DD	Hypertriglyceridemia, hypercholesterolemia, hyperglycemia, hyperleptinemia	94
<i>Bmal1</i>	Systemic KO	Arrhythmicity in DD	Impaired gluconeogenesis, adipogenesis, adipocyte differentiation; hyperlipidemia, glucose intolerance	77, 83 43
<i>Per2</i>	Systemic KO	Shortening of period	Absent glucocorticoid rhythm, absent diurnal feeding rhythm, obesity, alternations in leptin-dependent bone density	104 28
<i>Cry1</i>	Systemic KO	Shortening of period in DD		99
<i>Cry2</i>	Systemic KO	Lengthening of period in DD		99
<i>Cry1/Cry2</i>	Systemic double KO	Arrhythmicity in DD	Impaired body growth; feminized patterns of growth hormone and metabolic genes in liver	12, 99
Nocturnin	Systemic KO	WT	Resistance to diet-induced obesity, hepatic steatosis, impaired glucose tolerance, increased insulin sensitivity	35
<i>Bmal1</i>	Liver-specific KO	WT	Resting hypoglycemia, exaggerated glucose clearance	43

KO, knockout; DD, constant darkness.

olism and energy balance. Recent liver-specific knockout studies have found that *Bmal1* ablation leads to alterations in glucose clearance and hypoglycemia during the rest period, as well as loss of oscillation of hepatic metabolic genes (43). Conditional liver-specific overexpression of REV-ERB $\alpha$  also abolishes rhythmic expression of most oscillating liver genes (42).

Transgenic overexpression of *Clock* <sup>$\Delta 19$</sup>  in cardiomyocytes has revealed a role of the circadian gene network in heart rate variability, contractility, and responsiveness of the heart to changes in afterload (10). Some or all of the physiological deficits in myocardial function of these mice have been linked to alterations in cardiac fuel handling. Collectively, these findings support the hypothesis that the cardiomyocyte clock enables the heart to anticipate and respond appropriately to rhythmic variations in external stimuli, such as increased workload.

It is important to note that core circadian clock components may play distinct metabolic roles in different tissues, in addition to their function in regulating circadian rhythms. For example, a muscle-specific *Bmal1* rescue, which results in constitutive overexpression, revealed that BMAL1 function in the muscle is important for activity, body weight maintenance, and longevity (54). Further understanding of the role of individual tissue oscillators and their relationship to the central oscillator will provide important insight into the mechanism underlying metabolic disease phenotypes of circadian mutants.

#### ROLE OF SLEEP IN THE INTERACTION BETWEEN CIRCADIAN AND METABOLIC SYSTEMS

Interconnections between circadian, sleep, and metabolic systems have received increased attention in clinical studies over the past decade (please see Refs. 45 and 71 for a more comprehensive review). Cross-sectional studies have repeatedly indicated that lack of sleep is an independent risk factor for obesity and hypertension (31). Chronic short sleep duration is associated with increased BMI and incidence of type 2 diabetes (34, 36, 64, 89). Furthermore, poor sleep quality, as observed in patients with obstructive sleep apnea, is also associated with type 2 diabetes and cardiovascular disease (91). Clinical studies have found that even short periods of

sleep restriction can negatively affect energy metabolism. Partial sleep loss leads to increased glucose levels, decreased insulin sensitivity, and reduced glucose tolerance (86). Short periods of reduced sleep are also associated with decreased leptin and augmented ghrelin levels, leading to increased appetite and food consumption that can potentially cause excess weight gain (85, 89).

The precise mechanisms underlying the interconnection between sleep time and energy balance are still uncertain. In addition, controversy remains concerning the role of the circadian system in sleep, although mounting evidence suggests that the SCN contributes to more than just the timing of sleep and wakefulness (27, 56). Indeed, sleep deprivation per se induces marked changes in the electrical activity of SCN neurons, suggesting that SCN also receives input from cortical structures in addition to photic input to gauge sleep phase (22). Interestingly, key clock genes such as *Clock* and *Bmal1* also influence baseline sleep architecture and quality (44, 63). Additionally, mutations in *Per2* are associated with advanced phase sleep syndrome in humans, although it is not yet known whether these mutations might exert adverse metabolic effects (92). Equally surprising is the recent observation that ablation of melanopsin, the retinal photopigment crucial for light entrainment of SCN, also impacts sleep homeostasis (93). An important output of SCN involves autonomic projections to the pineal gland where melatonin is produced (56). Recent genomewide association studies in human subjects have uncovered a strong association between variation in the melatonin type 1b receptor and glucose in humans (58), pointing toward melatonin as an additional candidate in the linkage between sleep, rhythms, and metabolic control.

Energy homeostasis, sleep, and circadian rhythmicity appear to be interrelated at multiple levels. The energy hypothesis for sleep posits a role for neuronal regeneration following the depletion of key intermediary metabolites adenosine and glycogen during periods of wakefulness (further reviewed in Ref. 81). An additional area of investigation involves the role of non-rapid eye movement (NREM) sleep as an intermediate stage during entry into torpor. Whether and how conditions that trigger torpor may affect sleep architecture is still not known (48). Of special interest will be the identification of

molecular and/or hormonal signals that may coordinate sleep with energy balance and the integration of energy-sensing networks with regulation of sleep. Leptin deficient *ob/ob* mice and leptin-resistant *db/db* mice, which are severely obese and show symptoms of metabolic dysregulation, also exhibit changes in sleep architecture and diurnal rhythmicity (46, 47). Altered sleep architecture is also found in patients with type 1 diabetes (38). Similarly, high-fat feeding in mice alters both behavioral and molecular circadian function and sleep (41).

Finally, altered sleep/activity patterns can reciprocally affect the function of the central and peripheral oscillators and, ultimately, metabolism. Irregular sleep patterns or sleep loss can lead to arrhythmic exposure to light and constant resetting of the central oscillator. Extended periods of wakefulness and fragmented sleep schedule may in turn alter normal feeding patterns and desynchronize peripheral oscillators in metabolic tissues, such as liver and pancreas. Altered patterns of physical activity, such as exercise in the evening, can also lead to a phase shift of normal circadian rhythms (14, 15). Finally, nocturnal eating has separately been associated with increased body mass index (BMI) (20). Overall, dysregulation of sleep and activity can result in misalignment of the central and peripheral oscillators and desynchronization of behavior, metabolic gene expression, and hormone release. Further studies are needed to extend our understanding of the interplay between circadian systems and sleep and to unravel the neural networks and molecular signals through which these systems impact metabolic homeostasis.

#### CONCLUSIONS AND FUTURE DIRECTIONS

Recent advances in mammalian experimental genetics have implicated the circadian system as a key integrator of behavior and metabolism. Compelling new evidence reviewed above has uncovered that, in addition to altering activity, perturbations of normal circadian gene function may lead to impairment of metabolic health. Furthermore, energy homeostasis may be modulated by the alignment of sleep and activity behaviors with the normal timing of metabolic functions. Recent evidence indicates that both networks are integrated through a series of feedback loops, many of which are convergent with nutrient signaling pathways. Consideration of the pervasive role of circadian oscillation at the cell and molecular levels offers new opportunities in the search for mechanism-based therapies including type 2 diabetes mellitus and obesity.

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