Nuclear Receptors Linking Circadian Rhythms and Cardiometabolic Control

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Abstract—Many behavioral and physiological processes, including locomotor activity, blood pressure, body temperature, sleep (fasting)/wake (feeding) cycles, and metabolic regulation display diurnal rhythms. The biological clock ensures proper metabolic alignment of energy substrate availability and processing. Studies in animals and humans highlight a strong link between circadian disorders and altered metabolic responses and cardiovascular events. Shift work, for instance, increases the risk to develop metabolic abnormalities resembling the metabolic syndrome. Nuclear receptors have long been known as metabolic regulators. Several of them (ie, Rev-erbα, RORα, and peroxisome proliferation–activated receptors) are subjected to circadian variations and are integral components of molecular clock machinery. In turn, these nuclear receptors regulate downstream target genes in a circadian manner, acting to properly gate metabolic events to the appropriate circadian time window. (Arterioscler Thromb Vasc Biol. 2010;30:1529-1534.)

Key Words: circadian rhythm ▪ cardiometabolic disorders ▪ nuclear receptors ▪ Rev-erbα RORα PPAR ▪ PGC1α ▪ biological clock ▪ metabolic syndrome ▪ diabetes mellitus ▪ gene expression ▪ insulin resistance ▪ metabolism

Circadian Rhythms in Physiology

Circadian rhythms are variations that occur with a period of approximately 1 day (“circa diem”) and allow the organism to anticipate and optimize its metabolic, hormonal, and locomotor activity to predictable environmental daily changes.1 In mammals, a central clock resides in the suprachiasmatic nuclei of the hypothalamus and synchronizes physiology to day/night cycles. In metabolic organs, output signals from the suprachiasmatic nuclei clock, conveyed by peripheral oscillators, are combined to additional circadian cues, such as food availability, information concerning local fuel availability, and the hormonal milieu to drive circadian rhythms in intermediary metabolites (eg, AMP/ATP or oxidized nicotinamide-adenosine dinucleotide (NAD+) /reduced nicotinamide adenine dinucleotide ratios) and enzymes involved in local physiology. Consequently, many metabolic functions, including lipid and carbohydrate metabolism, and hormone secretion follow circadian variations.2 The circadian clock also synchronizes the cardiovascular system. The heart and vasculature have an autonomous circadian pacemaker to anticipate physiological demand in heart fuel use and contractile function.3 For instance, blood pressure displays marked circadian variations, increasing in the morning and decreasing at night. Heart beat and blood flow, vascular tone, fibrinolytic activity, and endothelial function are all naturally subjected to diurnal variations.4 Interestingly, the incidence of acute myocardial infarction, sudden cardiac death, and ischemic stroke is highest early in the morning.

Adverse Cardiometabolic Consequences of Altered Circadian Rhythms: Clinical Evidence

The metabolic syndrome comprises a constellation of abnormalities, including dyslipidemia, high fasting blood glucose level, and hypertension.5 It is precipitated by central obesity and increases the risk for type 2 diabetes mellitus and cardiovascular complications. In addition to genetic risk factors, numerous environmental factors (eg, increased food intake and physical inactivity) contribute to the etiology of the metabolic syndrome. Chronic circadian derangement, experienced by shift workers, also increases the risk of developing features of the metabolic syndrome (Figure 1).6,7 Interestingly, in humans subjected to a progressive forced desynchronization, circadian misalignment increased blood glucose despite increased insulin, suggestive of decreased insulin sensitivity and increased blood pressure, with a maximal disturbance during maximal misalignment (ie, 180° phase shift).8 A decrease in sleep duration and poor-quality sleep, although not circadian disorders per se, are often seen in nightshift workers, travelers, and patients with obstructive sleep apnea. Sleep curtailment results in reduced glucose tolerance and
insulin sensitivity and increased hunger and appetite. These data suggest that long-term sleep restriction may have deleterious effects on glucose homeostasis and body weight. Indeed, the prevalence of type 2 diabetes and higher body mass index is increased in self-reported short sleepers.

In humans, polymorphisms in different genes belonging to the clock machinery are linked to the development of features of the metabolic syndrome. Indeed, brain and muscle ARNT-like protein 1 (Bmal1) is associated with type 2 diabetes in humans, and several polymorphisms in the clock (circadian locomotor output cycles kaput) gene are associated with body weight and increased susceptibility to obesity and weight loss in response to dietary intervention. Similarly, polymorphisms in per2 and npas genes are linked to a high fasting blood glucose level and hypertension, respectively.

Biological Circadian Clock

Molecular Organization of the Clock

In mammals, CLOCK, Bmal1, and the CLOCK paralog NPAS2 form the positive limb that activates the transcription of target genes, including the per (Period) and cry (Cryochrome) genes (Figure 2). In turn, the proteins Per and Cry repress CLOCK/Bmal1-mediated gene transactivation. The nuclear receptors Rev-erbα and RORα form an additional regulatory loop. Rev-erbα gene transcription is activated by CLOCK/Bmal1, resulting in daily fluctuations of Rev-erbα, which, in turn, represses Bmal1. RORα competes with Rev-erbα for the binding to the Bmal1 promoter through a common RORE/RevRE site and activates its transcription. The nuclear receptors peroxisome proliferator–activated receptor (PPAR) α and γ bind to Rev-erbα and Bmal1 promoters and upregulate their expression. Finally, PPARγ coactivator (PGC) 1α potentiates RORα transcriptional activity and enhances Rev-erbα and Bmal1 transcription.

Moreover, (post)translational modifications of the clock components, via phosphorylation, sumoylation, ubiquitination, and acetylation, dictate the clock components’ stability and, thus, the appropriate timing of the circadian period to nearly 24 hours.

Peripheral Clocks

Circadian variations are observed in the expression of 10% to 20% of the transcriptome in metabolic tissues. Mice harboring a dysfunctional hepatic molecular clock display a nearly complete...
dampening of circadian variations of the hepatic transcriptome,\textsuperscript{16} suggesting that local peripheral pacemakers are able to elicit and sustain local circadian variations. Feeding time is a dominant zeitgeber for peripheral clocks, and changes in the time of food availability entrain a new schedule in peripheral rhythms of body temperature, behavior (locomotor activity), and clock gene expression independently of the master suprachiasmatic nuclei clock.\textsuperscript{17–19} In Cry1\textsuperscript{−/−}cry2\textsuperscript{−/−} mice, restricted feeding partially restored oscillations of certain nutrient-regulated genes that were blunted when fed ad libitum.\textsuperscript{20} Thus, the “nutritional” and “circadian” network somehow superimpose at the regulatory level. Recent reports have revealed that several “nutrient sensors” and intermediary metabolites couple metabolic and circadian regulation (Figure 2). AMP kinase (AMPK) is a nutrient sensor that is activated on food deprivation and phosphorylates and destabilizes CRY1.\textsuperscript{21} This results in an increased circadian period and derepression of CLOCK/BMAL1 target genes, as evidenced by an increased Rev-erb\textsubscript{α} circadian amplitude. Interestingly, substrate phosphorylation by AMPK follows a diurnal rhythm, linking nutrient status and the clock machinery. The cellular NAD(P)\textsubscript{+}/NAD(P)H ratio is another marker of metabolic status. Fasting, by increasing the cellular content of NAD\textsuperscript{+}, stimulates the activity of the NAD\textsuperscript{+}-dependent histone deacetylase sirtuin (SIRT) 1, which then interacts with PPAR-gamma coactivator (PGC)-1\alpha to enhance the gluconeogenic pathway (Figure 2). SIRT1 counterregulates the histone acetyl transferase activity of CLOCK and drives cyclic expression of Bmal1, Per2, and CRY1.\textsuperscript{22,23} In turn, CLOCK/Bmal1 regulates the expression of nicotinamide phosphoribosyltransferase, the rate-limiting enzyme of the NAD\textsuperscript{+} synthetic pathway, also known as visfatin.\textsuperscript{24,25} Interestingly, SIRT1 activity and NAD\textsuperscript{+} metabolism are modulated by AMPK, and the concerted action of AMPK and SIRT1 through PGC1\alpha likely connects cellular energy status and the circadian clock.

**Circadian Control of Energy Homeostasis**

**Circadian Control of Glucose and Lipid Metabolism by Clock Genes and Nuclear Receptors**

Blood concentrations of glucose and many hormones (ie, insulin, ghrelin, and leptin) exhibit circadian variations in animals and humans. Daily fluctuations are also observed in insulin sensitivity.\textsuperscript{26} For instance, glucose tolerance decreases during the day, whereas the glucose-stimulated increase in insulin is higher in the morning. These variations are lost in obese subjects and in patients with type 2 diabetes.\textsuperscript{26,27}

Clock genes impinge on metabolic pathways and body weight control. Indeed, clock\textsubscript{Δ19}-mutant mice are hyperphagic, become obese, and develop hyperlipidemia and hyperglycemia.\textsuperscript{28} In contrast, clock-mutant mice on an ICR genetic background may be protected against diet-induced obesity because of reduced intestinal fat absorption.\textsuperscript{29} In addition, a clock mutation specifically in the liver and muscle results in a modest sex-dependent effect on glucose tolerance and insulin sensitivity.\textsuperscript{30} Whole-body deletion of Bmal1 results in blunted gluconeogenesis, as revealed by the result of a pyruvate tolerance test.\textsuperscript{31} A more detailed comparison of total versus liver-specific Bmal1 deletion revealed that total Bmal1 deficiency leads to increased fat mass, impaired glucose tolerance, and decreased insulin sensitivity and secretion (with a normal resting glycemia level); whereas deletion of this gene specifically in the liver results in hypoglycemia during the inactive phase and altered hepatic circadian expression of genes involved in glucose metabolism.\textsuperscript{32} Thus, although some discrepancies exist between the different reports, these data suggest that whole-body and/or organ-specific alterations in the clock machinery result in compromised energy homeostasis. In the same line, overexpression of a mutant CRY1 in mice results in altered glucose homeostasis.\textsuperscript{33} In vitro, 7α-hydroxycholesterol modulates glucose output and G6Pase and phosphoenolpyruvate carboxykinase (PEPCK) expression in a RORα-dependent manner.\textsuperscript{34} Rev-erbα, whose activity is modulated by heme,\textsuperscript{35,36} also regulates de novo glucose synthesis in human HepG2 cells,\textsuperscript{36} although the expression of gluconeogenic genes remains unaltered and glucose tolerance appears normal in Rev-erbα-deficient and Rev-erbα–overexpressing mice. As previously mentioned, PGC1α enhances Rev-erbα transcription through enhanced RORα transcriptional activity.\textsuperscript{37} PGC1α also regulates the expression of heme/δ-aminolevulinic acid synthase-1, the rate-limiting enzyme in the heme synthesis pathway, indicating cross talk between PGC1α and Rev-erbα.\textsuperscript{38} Conversely, heme binding to Rev-erbα results in repression of PGC1α and δ-aminolevulinic acid synthase-1 gene expression in vitro.\textsuperscript{39} The in vivo physiological meaning of these observations remains to be determined. Rev-erbα and RORα also play a crucial role in vivo in the control of lipid metabolism by regulating the expression of liver apolipoproteins,\textsuperscript{40} sterol regulatory element–binding protein,\textsuperscript{41,42} and the fatty acid elongase elovl3.\textsuperscript{43} Both Rev-erbα–deficient and staggerer mice, which harbor a natural nonfunctional mutation in the RORα gene, are dyslipidemic. Rev-erbα also regulates bile acid metabolism by downregulating Cyp7a1 expression through indirect mechanisms.\textsuperscript{41,44} Although CYP7A1 expression was not affected in staggerer mice, RORα regulates the expression of the oxygenase 7α-hydroxylase (CYP7B1), an enzyme of the alternative bile acid synthesis pathway.\textsuperscript{45}

PPARα is also rhythmically expressed in liver and regulates diurnal variations in the expression of fatty acid synthase and 3-hydroxy-3-methylglutaryl–coenzyme A reductase, 2 enzymes involved in lipid and cholesterol synthesis.\textsuperscript{46} In addition, PPARα participates in the circadian variations of fibroblast growth factor (FGF)-21. When compared with day time injection, nighttime injection of the PPARα ligand bezafibrate has a more pronounced effect on FGF21 expression. In addition, PPARα plays a role in the entrainment by food of peripheral pacemakers.\textsuperscript{47}

These data indicate that the clock components and nuclear receptors integrate signals from both intermediary metabolism and the circadian clock to optimize fuel use or storage across the light/dark cycle.

**Circadian Control of Adipose Tissue Physiology**

Adipose tissue physiology demonstrates circadian variations. For instance, genes encoding proteins involved in lipid storage are highly expressed at feeding time. In addition, the expression of adipokines (eg, adiponectin) and plasminogen
activator inhibitor (PAI)-1 displays diurnal variations. However, these circadian variations are blunted in obese and diabetic animals\(^{48}\) and in obese humans.\(^{49,50}\)

As previously mentioned, clock\(^{\Delta}19\)-mutant mice become obese on feeding a high-fat diet, at least in part because of increased food intake and an altered circadian pattern in locomotor activity.\(^{28}\) Other reports have highlighted the role of Bmal1 in adipogenesis and the development of diet-induced obesity. Indeed, Bmal1-deficient mice display increased fat content,\(^{32}\) although adipogenesis is impaired in vitro in Bmal1-deficient embryonic fibroblasts.\(^{51}\) Although these data appear contradictory, such a difference between the in vivo and in vitro situation has been frequently observed for other adipogenic factors. ROR\(\alpha\) overexpression in 3T3L1 cells also blocks the adipogenic process.\(^{52}\) Interestingly, REV-ERB\(\alpha\) regulates this process in a subtle manner because the REV-ERB\(\alpha\) protein must increase and then decrease to allow proper differentiation of fibroblasts into mature adipocytes.\(^{52,53}\)

Human data are still scarce. However, a few studies demonstrate that clock genes, including Rev-erb\(\alpha\) and ROR\(\alpha\), are expressed in human adipose depots. A study\(^{55}\) conducted in lean, overweight, or obese subjects demonstrates that the expression of Rev-erb\(\alpha\), ROR\(\alpha\), and Bmal1, Npas2, Cry1, Pgc1\(\alpha\), and Ppar\(\gamma\) in subcutaneous tissue correlates with body mass index in young subjects, suggesting they may interfere with adipocyte function. This affects the timing of alterations of diverse processes (eg, lipid storage/lipolysis), which may participate in the long-term deleterious effects of circadian disorders on body mass index control.

Circadian Control of the Cardiovascular System

Animal Studies Identifying Clock Genes as Major Players in Cardiovascular Physiology

The clock machinery directly influences risk factors predisposing to vascular diseases and cardiac dysfunction. ROR\(\alpha\) modulates plasma lipids, and low plasma HDL-cholesterol levels contribute to the atherosclerosis susceptibility of staggerer mice.\(^{56}\) PAI-1 is an important inhibitor of the fibrinolysis cascade that may promote the development of atherothrombosis. Its expression oscillates in a circadian manner with a zenith in the early morning in humans, a time that coincides with acute thrombotic and cardiovascular events, such as myocardial infarction. Rev-erb\(\alpha\) dampens PAI-1 oscillations, suggesting it may affect the expression and rhythmicity of PAI-1 and the fibrinolysis cascade in a circadian manner.\(^{57}\) Rev-erb\(\alpha\) and ROR\(\alpha\) are present in vascular wall cells, including macrophages in which they influence the inflammatory response.\(^{58,59}\) In rat vascular smooth muscle cells, Rev-erb\(\alpha\) upregulates the expression of interleukin 6 and cyclooxygenase 2.\(^{58}\) In human macrophages, it represses the induction of toll-like receptor 4, the receptor of lipopolysaccharide, thereby diminishing the production of cytokines in response to lipopolysaccharide.\(^{59}\)

Clock-mutant and Bmal1-deficient mice display impaired vascular remodelling, pronounced intimal hyperplasia, and thrombosis associated with increased expression of PAI-1 after surgical ligation of the left carotid artery.\(^{60}\) Both models exhibit endothelial dysfunction, as revealed by an impaired relaxation in response to acetylcholine. In addition, Bmal1\(^{-/-}\) mice are hypotensive and have blunted circadian rhythms when blood pressure is measured,\(^{61}\) whereas Cry1\(^{-/-}\) mice have hypertension.\(^{62}\) Staggerer mice also display a lower mean arterial blood pressure, altered vascular function in mesenteric arteries, and attenuated response to vasoconstrictors, indicating a role for ROR\(\alpha\) in normal contractile function of smooth muscle cells.\(^{63}\) In the same line, Ppar\(\gamma\) ablation in either endothelial or smooth muscle cells results in attenuations of circadian variations when blood pressure is measured and higher heart rate and blunted circadian variations in the aortic expression of clock genes.\(^{64}\) A cardiomyocyte clock-mutant mouse model in which the clock\(^{\Delta}19\) gene is expressed specifically in cardiomyocytes displays an altered circadian response to epinephrine, attenuated circadian variations in heart rate with a decrease during the dark phase, and signs of bradycardia in isolated hearts.\(^{65}\) In wild-type mice, the infarct size after experimental ischemia is greatly influenced by the time of the day of ischemia infliction. A 3.5-fold increase in infarct size, fibrosis, and adverse remodeling were observed in mice subjected to ischemia at the sleep-to-wake transition compared with the wake-to-sleep transition.\(^{66}\) Cardiomyocyte clock-mutant mice exhibited attenuated time-of-day variations in these different parameters and significantly reduced infarct size irrespective of the time of the day, indicating that the cardiomyocyte circadian machinery plays an important role in the response to ischemic injury.

Clock Genes and Nuclear Receptors Control the Circadian Control of Cardiometabolism

Fatty acids are the major source of energy for the heart, and disruption in their circadian use may alter cardiac function. Cardiomyocytes display circadian oscillations in numerous transcriptional programs involved (eg, in glycogen and triglyceride metabolism).\(^{65,67}\) However, these variations are lost in the cardiomyocyte clock-mutant heart, and fatty acid oxidation remains constant and abnormally high. Ppar\(\alpha\), which plays an important role in fatty acid use by the heart, intervenes in its timing. Indeed, expression of pyruvate dehydrogenase kinase 4, a Ppar\(\alpha\) target gene, peaks in the middle of the night, and Ppar\(\alpha\) activation induces pyruvate dehydrogenase kinase 4 gene expression to a larger extent during the night.\(^{68}\) Ppar\(\alpha\) is also necessary for food entrainment of the clock in the mouse heart.\(^{47}\) Altered cardiac fatty acid use and cardiac function may result from perturbed circadian Ppar\(\alpha\) signaling.

Modulation of the Circadian Control of Metabolism

Together, these data indicate that tight temporal control is required for normal cardiometabolic function. They also suggest that metabolic abnormalities, resulting from circadian disorders, may be modulated by pharmacologically manipulating the activity and expression of clock genes and nuclear receptors. Interestingly, the administration of the Ppar\(\alpha\) ligand bezafibrate during the night phase increases fibroblast growth factor 21 and pyruvate dehydrogenase kinase 4 to a larger extent when compared with daytime administration.\(^{69}\) In humans, the Ppar\(\alpha\) ligand fenofibrate only lowers blood
pressure during sleep. Moreover, dexamethasone, a glucocorticoid receptor ligand, potently induces a phase shift in fibroblasts in vitro and in peripheral mouse tissues in vivo. Glucocorticoids inhibit the phase adjustment of the peripheral clock in response to restricted feeding in the light phase. In contrast, glucocorticoid receptor–deficient mice adapt more rapidly to food restriction. Similarly, a synthetic Rev-erbβ ligand induces a phase resetting in primary lung fibroblasts and lung slices, and the resulting shift (advance versus delay) depends on the rhythmic expression profile of Rev-erbβ. In addition, glycogen synthase kinase (GSK)3β–mediated stabilization of Rev-erbβ appears to be a crucial event for circadian rhythm initiation, maintenance, and synchronization after serum shock. Thus, it is likely that the response to Rev-erbβ ligands will ultimately depend on cyclic Rev-erbβ abundance and might be affected by the individual chronotype.

In conclusion, modulating nuclear receptor activity is an interesting possibility for affecting physiological processes altered by a circadian challenge. However, more studies are necessary to better understand the influence of the time of administration of a ligand and its formula (rapid versus extended release) and the rhythmic abundance of the targeted nuclear receptor for obtaining maximal efficacy of the drug.

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Disclosures

None.

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