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 desynchrony, 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 (Cryptochrome) 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. Similarly, 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, 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. In Cry1 −/− cry2 −/− mice, restricted feeding partially restored oscillations of certain nutrient-regulated genes that were blunted when fed ad libitum. 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. This results in an increased circadian amplitude. Interestingly, substrate phosphorylation by AMPK follows a diurnal rhythm, linking nutrient status and the clock machinery. The cellular NAD(P)+/NAD(P)H ratio is another marker of metabolic status. Fasting, by increasing the cellular content of NAD⁺, stimulates the activity of the NAD⁺-dependent histone deacetylase sirtuin (SIRT) 1, which then interacts with PPAR-gamma coactivator (PGC)-1α to enhance the gluconeogenic pathway (Figure 2). SIRT1 counterregulates the histone acetyl transferase activity of CLOCK/Bmal1 target genes, as previously mentioned, PGC1α and Rev-erbα also regulate de novo glucose synthesis in human HepG2 cells, 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. 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. Conversely, heme binding to Rev-erbα results in repression of PGC1α and δ-aminolevulinic acid synthase-1 gene expression in vitro. 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, and the fatty acid elongase elovl3. 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. Although CYP7A1 expression was not affected in staggerer mice, RORα regulates the expression of the oxysterol 7α-hydroxylase (CYP7B1), an enzyme of the alternative bile acid synthesis pathway.

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, enzymes involved in lipid and cholesterol synthesis. 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.

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 and in obese humans. As previously mentioned, clockΔ19-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. 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, although adipogenesis is impaired in vitro in Bmal1-deficient embryonic fibroblasts. Although these data appear contradictory, such a difference between the in vivo and in vitro situation has been frequently observed for other adipogenic factors. RORα overexpression in 3T3L1 cells also blocks the adipogenic process. Interestingly, REV-ERBα regulates this process in a subtle manner because the REV-ERBα protein must increase and then decrease to allow proper differentiation of fibroblasts into mature adipocytes.

Human data are still scarce. However, a few studies demonstrate that clock genes, including Rev-erbα and RORα, are expressed in human adipose depots. A study conducted in lean, overweight, or obese subjects demonstrates that the expression of Rev-erbα, RORα, and Bmal1, Npas2, Cry1, Pgc1α, and Pparγ 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. RORα modulates plasma lipids, and low plasma HDL-cholesterol levels contribute to the atherosclerosis susceptibility of staggerer mice. 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-erbα dampens PAI-1 oscillations, suggesting it may affect the expression and rhythmicity of PAI-1 and the fibrinolysis cascade in a circadian manner. Rev-erbα and RORα are present in vascular wall cells, including macrophages in which they influence the inflammatory response. In rat vascular smooth muscle cells, Rev-erbα upregulates the expression of interleukin 6 and cyclooxygenase 2. 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.

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. 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, whereas Cry1−/− mice have hypertension. 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 RORα in normal contractile function of smooth muscle cells. In the same line, PPARγ 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. A cardiomyocyte clock-mutant mouse model in which the clockΔ19 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. 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. 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 glycolgen and triglyceride metabolism). However, these variations are lost in the cardiomyocyte clock-mutant heart, and fatty acid oxidation remains constant and abnormally high. PPARα, which plays an important role in fatty acid use by the heart, intervenes in its timing. Indeed, expression of pyruvate dehydrogenase kinase 4, a PPARα target gene, peaks in the middle of the night, and PPARα activation induces pyruvate dehydrogenase kinase 4 gene expression to a larger extent during the night. PPARα is also necessary for food entrainment of the clock in the mouse heart. Altered cardiac fatty acid use and cardiac function may result from perturbed circadian PPARα 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 PPARα 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. In humans, the PPARα 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-erb. In addition, glycogen synthase kinase (GSK)β-mediated stabilization of Rev-erb 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-erb ligands will ultimately depend on cyclic Rev-erb 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 rhythm abundance of the targeted nuclear receptor for obtaining maximal efficacy of the drug.

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None.

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