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 as well as 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. Shiftwork, 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, PPARs) are subjected to circadian variations and are integral components of the 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.

I. Introduction: circadian rhythms in physiology

Circadian rhythms are variations which occur with a period of approximately one 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 (SCN) of the hypothalamus and synchronizes physiology to day/night alternances. In metabolic organs, output signals from the SCN clock conveyed by peripheral oscillators are combined to additional circadian cues such as food availability and information concerning local fuel availability and the hormonal milieu to drive circadian rhythms in intermediary metabolites (such as the AMP/ATP or NAD+/NADH 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 cardio-vascular system. The heart and vasculature have an autonomous circadian pacemaker to anticipate physiological demand in heart fuel utilization and contractile function [3]. For instance, blood pressure displays marked circadian variations, rising in the morning hours 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.

II. Adverse cardiometabolic consequences of altered circadian rhythms: clinical evidences

The metabolic syndrome comprises a constellation of abnormalities including dyslipidemia, high fasting blood glucose and hypertension [5]. It is precipitated by central obesity and increases the risk for type 2 diabetes and cardiovascular complications. Beside genetic risk factors, numerous environmental factors such as 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 to develop features of the metabolic syndrome (Figure1) [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, travellers and patients suffering from obstructive sleep apnea. Sleep curtailment results in reduced glucose tolerance and insulin sensitivity, and increased hunger and appetite [9]. 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 BMI is increased in self-reported short sleepers [10].

In humans, polymorphisms in different genes belonging to the clock machinery are linked to the development of features of the metabolic syndrome. Indeed, *Bmal1* is associated to type 2 diabetes in humans [11] and several polymorphisms in the *clock* gene are associated with body weight and increased susceptibility to obesity [12,13], and weight loss in response to dietary intervention [14]. Similarly, polymorphisms in *per2* and *npas* genes are linked to high fasting blood glucose and hypertension, respectively [15].

III. The biological circadian clock

A. Molecular organization of the clock

In mammals, Circadian Locomotor Output Cycles Kaput (CLOCK), Brain and Muscle ARNT like protein 1 (BMAL1), and the CLOCK paralog NPAS2 form the positive limb which activates the transcription of target genes including the *per* (*Period*) and *cry* (*Cryptochrome*) genes (Figure2). In turn, the proteins PER and CRY repress CLOCK/BMAL1-mediated gene transactivation [2]. 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 [2]. The nuclear receptors Peroxisome Proliferator-Activated Receptor (PPAR)α and γ bind to the *Rev-erb*α and *Bmal1* promoters and up-regulate their expression. Finally, PPARγ co-activator (PGC)1α potentiates RORα transcriptional activity and enhances *Rev-erb*α 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 24h.

B. Peripheral clocks

Circadian variations are observed in the expression of 10-20% 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 [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 rhytms of body temperature, behavior (locomotor activity), and clock gene expression independently of the master SCN clock [17-19]. In *Cry*1-/-*cry*2-/- mice, restricted feeding partially restored oscillations of certain nutrient-regulated genes which were blunted when fed ad libitum [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 (Figure2). Adenosine monophosphate-activated protein kinase (AMPK) is a nutrient sensor which is activated upon food deprivation and phosphorylates and destabilizes CRY1 [21]. This results in an increased circadian period and de-repression of CLOCK/BMAL1 target genes as evidenced by an increased Rev-erbα 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 the 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 PGC1α to enhance the gluconeogenic pathway (Figure2). SIRT1 counter-regulates the histone acetyl transferase activity of CLOCK and drives cyclic expression of *Bmal1*, *Per2* and *Cry1* [22,23]. In turn, CLOCK/BMAL1 regulates the expression of nicotinamide phosphoribosyltransferase (NAMPT), the rate-limiting enzyme of the NAD^+ synthetic pathway also known as visfatin [24,25]. Interestingly, SIRT1 activity and NAD^+

metabolism are modulated by AMPK, and concerted action of AMPK and SIRT1 through PGC1α likely connect cellular energy status and the circadian clock.

IV. Circadian control of energy homeostasis

A. Circadian control of glucose and lipid metabolism by clock genes and nuclear receptors

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

Clock genes impinge on metabolic pathways and body weight control. Indeed, clockΔ19 mutant mice are hyperphagic, become obese and develop hyperlipidemia and hyperglycemia [28]. By contrast, others have reported that clock mutant mice on a ICR genetic background are protected against diet-induced obesity because of a reduced intestinal fat absorption [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 [30]. Whole-body deletion of *Bmal1* results in blunted gluconeogenesis as revealed by a pyruvate tolerance test [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, but normal resting glycemia, whereas deletion of this gene specifically in the liver results in hypoglycemia during the inactive phase, as well as altered hepatic circadian expression of genes involved in glucose metabolism [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, over-expression of a mutant CRY1 in mice results in altered glucose homeostasis [33]. In vitro, 7α-hydroxycholesterol modulates glucose output and G6Pase and PEPCK expression in a RORα-dependent manner [34]. Rev-erbα, whose activity is modulated by heme [35,36], also regulates *de novo* glucose synthesis in human HepG2 cells [36],

although the expression of gluconeogenic genes remains unaltered, and glucose tolerance appears normal in Rev-erbα-deficient and Rev-erbα over-expressing mice. As mentioned earlier, PGC1α enhances *Rev-erbα* transcription through enhanced RORα transcriptional activity [37]. PGC1α also regulates the expression of heme/δ-aminolevulinic acid synthase (ALAS)-1, the rate limiting enzyme in the heme synthesis patway, indicating a cross-talk between PGC1α and Rev-erbα [38]. Conversely, heme binding to Rev-erbα results in a repression of PGC1α and ALAS1 gene expression in vitro [39]. The in vivo physiological meaning of these observations remains to be determined. REV-ERBα and RORα play also a crucial role i*n vivo* in the control of lipid metabolism by regulating the expression of liver apolipoproteins [40], sterol regulatory element binding protein (SREBP) [41,42] and the fatty acid elongase *elovl3* [43], and both REV-ERBα-deficient and staggerer mice, which harbor a natural non-functional mutation in the RORα gene, are dyslipidemic. REV-ERBα also regulates bile acid metabolism by down-regulating *Cyp7A1* expression through indirect mechanisms [41,44]. Whereas 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 [45].

PPARα is also rhythmically expressed in liver and regulates diurnal variations in the expression of fatty acid synthase (FAS) and 3-hydroxy-3-methylglutaryl-CoA reductase (HMG-CoAR), two enzymes involved in lipid and cholesterol synthesis [46]. In addition, PPARα participates in the circadian variations of fibroblast growth factor (FGF)-21 and administration of the PPARα ligand bezafibrate at night, compared to day time injection, has a more pronounced effect on FGF21 expression. In addition, PPARα plays a role in the entrainment by food of peripheral pacemakers [47].

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

B. 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 the feeding time. In addition, expression of adipokines (such as adiponectin) and plasminogen

activator inhibitor (PAI)-1 display diurnal variations. These circadian variations are however blunted in obese and diabetic animals [48] and obese humans [49,50].

As mentioned earlier, *clock*Δ19 mutant mice become obese upon feeding a high fat diet, at least in part due to 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. RORα over-expression in 3T3L1 cells also blocks the adipogenic process [52]. Interestingly, REV-ERBα regulates this process in a very subtle manner since the REV-ERBα protein must increase and then fall to allow proper differentiation of fibroblasts into mature adipocytes [53,54].

Human data are still scarce but a few studies demonstrate that clock genes, incl. 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, as well as Bmal1, NPAS2, Cry1, PGC1α, and PPARγ in subcutaneous tissue correlates with BMI in young subjects [55], suggesting they may interfere with adipocyte function, thereby affecting the timing of alternance of diverse processes such as lipid storage/lipolysis, which may participate in the long-term deleterious effects of circadian disorders on BMI control.

V. Circadian control of the cardio-vascular system

A. Animal studies identifying clock genes as major players in cardio-vascular physiology

The clock machinery directly influences risk factors predisposing to vascular diseases and cardiac dysfuntion. RORα 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 which 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 affect the fibrinolysis cascade in a circadian manner [57]. Rev-erbα and RORα are present in vascular wall cells including macrophages where they influence the inflammatory response [58,59]. In rat vascular smooth muscle cells, Rev-erbα up-regulates the expression of interleukin (IL)-6 and cyclooxygenase-2 [58]. In human macrophages, it represses the induction of *toll like receptor (TLR)-4*, the receptor of lipopolysaccharide (LPS), thereby diminishing the production of cytokines in response to LPS [59].

clock mutant and *Bmal1*-deficient mice display impaired vascular remodelling, pronounced intimal hyperplasia, and thrombosis associated to 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 in blood pressure [61], whereas Cry-/- mice suffer from hypertension [62]. Staggerer mice also display 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 [63]. In the same line, PPARγ ablation in either endothelial or smooth muscle cells results in attenuations of circadian variations in blood pressure and higher heart rate, and blunted circadian variations in the aortic expression of clock genes [64]. A cardiomyocyte clock mutant (CCM) 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 [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 remodelling was observed in mice subjected to ischemia at the sleep-to-wake transition compared to the wake-to-sleep transition [66]. CCM 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.

B. 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 utilization may alter cardiac function. Cardiomyocytes display circadian oscillations in numerous transcriptional programs involved, for instance, in glycogen and triglyceride metabolism [65,67]. However, these variations are lost in the CCM heart, and fatty acid oxidation remains constant and abnormally high. PPARα which plays an important role in fatty acid utilization by the heart, intervenes in its timing. Indeed, expression of pyruvate dehydrogenase kinase (PDK)-4, a PPARα target gene, peaks in the middle of the night, and PPARα activation induces *PDK-4* gene expression to a larger extent during the night [68]. PPARα is also necessary for food entrainment of the clock in the mouse heart [47]. One can hypothesize that altered cardiac fatty acid utilization and cardiac function may result from perturbed circadian PPARα signalling.

VI. Modulation of the circadian control of metabolism

Together these data indicate that a 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, administration of the PPARα ligand bezafibrate during the night phase increases FGF21 and PDK4 to a larger extent as compared with daytime administration [69]. In humans, the PPARα ligand fenofibrate only lowers blood pressure during sleep [70]. Moreover, dexamethasone, a glucocorticoid receptor (GR) ligand potently induces a phase shift in fibroblasts in vitro as well as in peripheral mouse tissues in vivo [71]. Glucocorticoids inhibit the phase adjustment of the peripheral clock in response to a restricted feeding to the light phase [72]. By contrast, GR-deficient mice adapt more rapidly to food restriction. Similarly, a synthetic REV-ERBα ligand induces a phase resetting in primary lung fibroblasts and lung slices [73], and the resulting shift (advance vs delay) depends on the rhythmic expression profile of Rev-erbα. In addition, GSK3β-mediated stabilization of REV-ERBα appears a crucial event for circadian rhythm initiation, maintenance and synchronisation after serum shock [74].

Thus, it is likely that the response to REV-ERBα ligands will ultimately depend on cyclic REV-ERBα abundance, and might be affected by the individual chronotype.
In conclusion, modulating nuclear receptor activity is an interesting possibility to affect 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 vs extended release,..), as well as the rhythmic abundance of the targeted nuclear receptor to obtain a maximal efficacy of the drug.

Figure legends:

Figure 1: Circadian disruption may arise from genetic (clock gene mutations) or environmental (shiftwork,..) factors, and contributes to the development of behavioural and cardio-metabolic disorders.

Figure 2: The core clock machinery consists of a series of interlocked transcriptional/translational loops which generate and maintain circadian rhythms, and post-translational modifications (dashed lines) ensure the proper timing of the clock. Intracellular metabolism (through NAD+/SIRT1 and ATP/AMP-AMPK) and nutrients (through binding to nuclear receptors) impinge on the clock machinery (doted lines).

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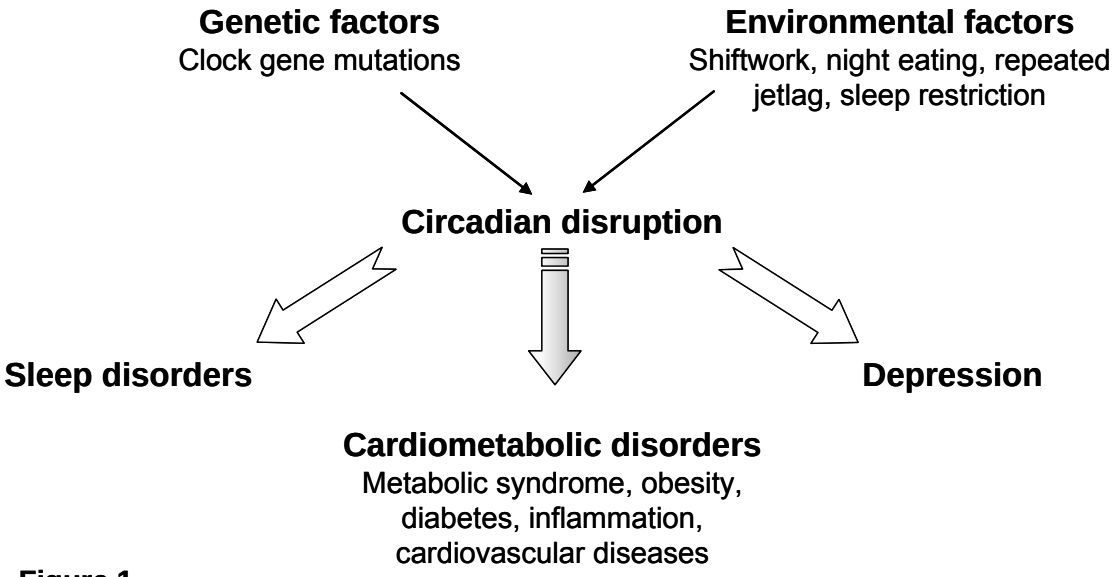

Figure 1

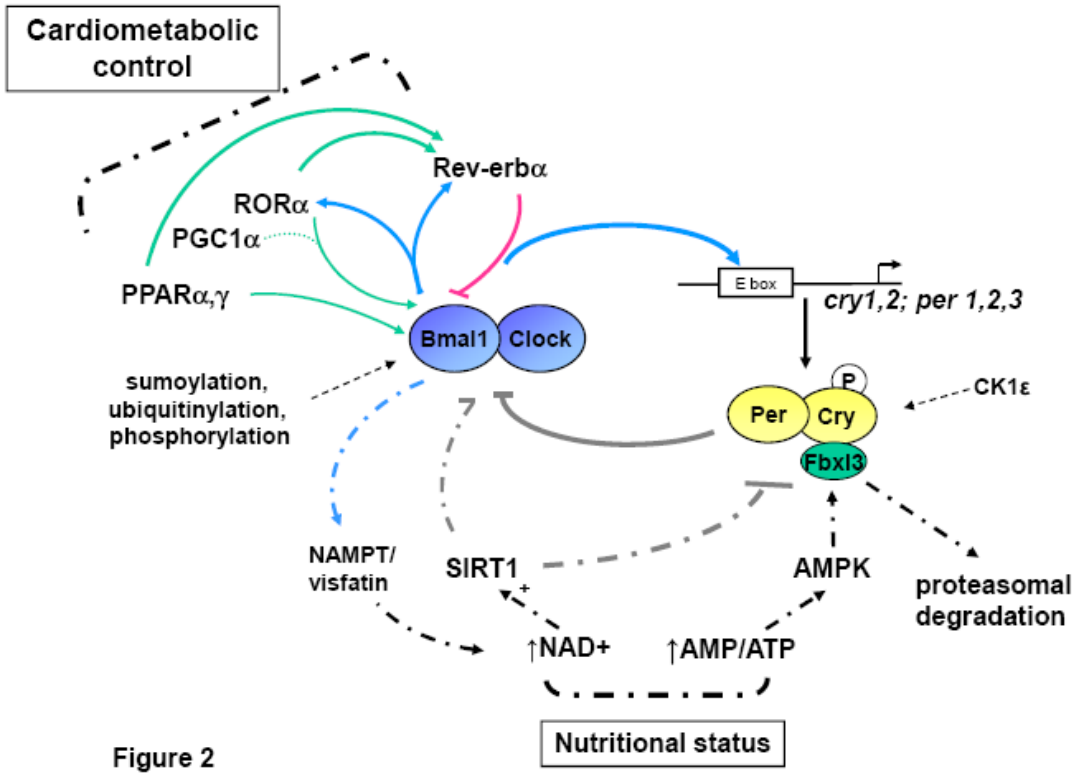

Figure 2